EFFECTS OF DIETARY FATS AND CARBOHYDRATE ON INSULIN SECRETION, INFLAMMATION AND GASTRO-INTESTINAL PEPTIDES IN ABDOMINALLY OBESE INDIVIDUALS: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Dietary recommendations promote low-fat diet but not saturated fat. This has led to increased refined carbohydrate-intake which can potentially be disadvantageous to cardiovascular-risk. To investigate the controversy surrounding present dietary guidelines, this study compared the effects of substitution of high refined carbohydrate or monounsaturated fatty acids (MUFA) for saturated fatty acids (SAFA) on insulin secretion and inflammation in abdominally-obese subjects (waist circumference: ≥80 cm for women, ≥90cm for men; 20-60 years old). Using a crossover design, randomized controlled trial in 54 subjects, we compared the effects of substitution of 7% energy as carbohydrate or MUFA for SAFA for a period of 6 weeks each where the control (SAFA) diet consisted of 15% en protein, 53% en carbohydrate and 32% en fat (12% en SAFA, 13% en MUFA). Subjects were provided three meals during weekdays and test oil for home cooking within study guidelines during weekends. Blood samples were collected at fasting, 15 and 30 min and hourly intervals thereafter till 6 hours in response to mixed meal challenge (muffin and milkshake) with SAFA or MUFA (872.5 kcal, 50 g fat, 88 g carbohydrate) or CARB (881.3 kcal, 20 g fat, 158 g carbohydrate) enrichment corresponding to the background diets. As expected, postprandial non-esterified fatty acid suppression and elevation of C-peptide, insulin and glucose secretion were the greatest with high-carbohydrate (CARB) meal. Interestingly, CARB meal attenuated postprandial insulin secretion corrected for glucose response and disposition index ($P < 0.05$) however the insulin sensitivity was not affected ($P > 0.05$). SAFA and MUFA had similar effects on all markers except for fasting glucose-dependent insulinotropic peptide (GIP) concentrations which increased after MUFA but not SAFA when compared with CARB ($P < 0.05$). No significant differences in fasting inflammatory and thrombogenic factors (interleukin (IL)-6, IL-1β, C-reactive protein, E-selectin, plasminogen activator inhibitor-

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1 (PAI-1) and D-dimer) were noted between diets ($P > 0.05$). As indicated by incremental area under the curve during postprandial state, CARB meal was found to increase plasma IL-6 while MUFA meal elevated postprandial plasma D-dimer significantly more compared with SAFA meal ($P < 0.05$). Comparing the 3 meals, there were similar postprandial reductions in augmentation index and pressure. CARB diet was found to reduce HDL_3 by 7.8% and increase small dense HDL (sdHDL) by 8.6% compared with SAFA diet ($P < 0.05$). SAFA diet increased large HDL subfractions compared with both CARB and MUFA diets by 4.9% and 6.6% ($P < 0.05$), respectively. In conclusion, a 6-week lower-fat/higher-carbohydrate (increased by 7% refined carbohydrate) diet may have greater adverse effect on insulin secretion corrected for glucose and disposition index compared with isocaloric higher-fat diets. In contrast, exchanging MUFA for SAFA at 7% energy had no appreciable adverse impact on insulin secretion. Overall, the evidence presented in this study suggests that the replacement of SAFA with MUFA or carbohydrates may not improve inflammatory and thrombogenic markers in abdominally obese individuals. Indeed increased high refined carbohydrate consumption adversely impacts HDL subfractions compared to high SAFA intake, hence may increase coronary heart disease risk.

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ABSTRAK

Panduan pemakanan mempromosikan diet rendah lemak tetapi tidak mengglakkan pengambilan lemak tepu (SAFA). Ini mengakibatkan peningkatan dalam pengambilan karbohidrat halus namun ini tidak bermanfaat terhadap risiko kardiovaskular. Untuk menyiasat ketidakpastian mengenai panduan pemakanan ini, kajian ini membanding kesan penggantian karbohidrat halus atau asid lemak monotaktepu (MUFA) dengan SAFA terhadap rembesan insulin dan keradangan dalam subjek yang gemuk abdomen (lilitan pinggang: $\geqslant$ 80cm bagi wanita, $\geqslant$ 90cm bagi lelaki; umur: 20-60 tahun). Dengan menggunakan reka bentuk rawak, terkawal dan crossover pada 54 subjek, kajian ini membandingkan kesan penggantian 7% tenaga daripada karbohidrat atau MUFA dengan SAFA dalam diet terkandung 2000 kcal selama 6 minggu yang mana diet kawalan (SAFA) mengandungi 15% tenaga protein, 53% tenaga karbohidrat dan 32% tenaga lemak (12% tenaga SAFA, 13% tenaga MUFA). Subjek dibekalkan 3 hidangan setiap hari kerja dan minyak masak berserta garis panduan untuk masakan di rumah pada hari minggu. Sampel darah dikumpul pada waktu berpuasa dan 15, 30 min serta setiap jam sehingga 6 jam selepas makan hidangan campuran (muffin dan susu kocak) kaya dengan SAFA atau MUFA (872.5 kcal, 50 g lemak, 88 g karbohidrat) atau CARB (881.3 kcal, 20 g lemak, 158 g karbohidrat) sepadan dengan diet rawatan. Seperti yang dijangka, didapati rembesan asid lemak bebas paling rendah dan kepekatan C-peptida, insulin dan glukosa paling tinggi selepas hidangan karbohidrat (CARB). Hidangan CARB mengurangkan rembesan insulin diperbetulkan glukosa dan indeks disposisi ($P < 0.05$) selepas makan namun sensitiviti insulin tidak dipengaruhi ($P > 0.05$). SAFA dan MUFA mempunyai pengaruh yang seiras terhadap semua petunjuk kecuali kepekatan peptida insulinotopik glukosa-bergantungan sewaktu berpuasa yang meningkat selepas diet MUFA berbanding dengan CARB ($P < 0.05$). Tiada perbezaan signifikan dalam faktor-faktor radang dan

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trombogenik (interleukin (IL)-6, IL-1β, protein C-reaktif, E-selectin, plasminogen pengaktif perencat-1 (PAI-1) and D-dimer) diperhatikan antara diet dalam keadaan berpuasa ($P > 0.05$). Peningkatan plasma IL-6 selepas hidangan CARB dan peningkatan plasma D-dimer selepas hidangan MUFA adalah lebih tinggi berbanding dengan hidangan SAFA ($P < 0.05$). Pengurangan indeks dan tekanan augmentasi selepas makan tidak menunjukkan perbezaan signifikan ($P > 0.05$) antara hidangan. Diet CARB mengurangkan HDL_3 sebanyak 7.8% dan meningkatkan HDL berkepadatan kecil sebanyak 8.6% berbanding dengan diet SAFA ($P < 0.05$). Diet SAFA meningkatkan HDL berkepadatan tinggi berbanding dengan diet CARB dan MUFA sebanyak 4.9% dan 6.6% ($P < 0.05$), masing-masing. Kesimpulannya, pengambilan diet rendah lemak/tinggi karbohidrat yang mengandungi 7% tenaga karbohidrat halus lebih tinggi selama 6 minggu mungkin mengakibatkan kesan buruk yang lebih besar terhadap rembesan insulin diperbetulkan glukosa dan indeks disposisi berbanding diet tinggi lemak yang berkalori sama. Sesungguhnya peningkatan penggunaan karbohidrat halus memberi kesan buruk terhadap subfraksi HDL dan boleh mencetuskan tindak balas pro-radang selepas makan. Sebaliknya, penggantian MUFA dengan SAFA sebanyak 7% tenaga tidak memberi kesan buruk yang ketara pada rembesan insulin. Secara keseluruhan, kajian ini menunjukkan bahawa penggantian SAFA dengan MUFA atau karbohidrat halus tidak meningkatkan penanda radang dan trombogenik dalam individu yang mempunyai obesiti abdomen. Pengambilan karbohidrat halus yang tinggi mengakibatkan kesan buruk terhadap subfraksi HDL berbanding dengan pengambilan SAFA, maka meningkatkan risiko penyakit jantung.

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University of Malaya

TABLE OF CONTENTS

University of Malaya

University of Malaya

University of Malaya

LIST OF FIGURES

University of Malaya

University of Malaya

LIST OF TABLES

University of Malaya

University of Malaya

University of Malaya

LIST OF SYMBOLS AND ABBREVIATIONS

%	percentage
<	less than
>	more than
~	approximately
° C	degree Celsius
®	registered propriety name
C	carbon
cm	centimeter
g	gram
kg	kilogram
kcal	kilocalorie
ml	millilitre
L	litre
mmol	millimole
P	probability
vs	versus
h	hour
ANOVA	Analysis of variance
Apo A1	Apolipoprotein A1
Apo B100	Apolipoprotein B100
BMI	Body mass index
CARB	Carbohydrate-enriched
CCK	Cholecystokinin
CRP	C-reactive protein
DI	Disposition index
en	Energy

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ELISA	Enzyme-linked immunoassay
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization of the United Nations
GC	Gas chromatography
GI	Glycemic index
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide
GM	Geometric mean
HDL	High density lipoprotein
HDL-C	High density lipoprotein-cholesterol
HOMA2-IR	Homeostatic model assessment 2-insulin resistance
HOMA2-%S	Homeostatic model assessment 2-insulin sensitivity
HOMA2-%B	Homeostatic model assessment 2-beta cell function
IFG	Impaired fasting glucose
iAUC	Incremental area under the curve
IRS	Insulin receptor substrate
IVGTT	Intravenous glucose tolerance test
KANWU	Kuopio, Aarhus, Naples, Wollongong and Uppsala
LIPGENE	Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis
IL-1β	Interleukin-1β
IL-6	Interleukin-6
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein-cholesterol
Lp (a)	Lipoprotein (a)
MCP-1	Monochemoattractant protein-1

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MUFA	Monounsaturated fatty acid
MPOB	Malaysian Palm Oil Board
NEFA	Non-esterified fatty acids
NFG	Normal fasting glucose
PYY	Peptide YY
rQUICKI	Revised quantitative insulin senstitivity
RISCK	Reading, Imperial, Surreys, Cambridge and King's
SAFA	Saturated fatty acid
TAG	Triacylglycerol
WHO	World Health Organization

University of Malaya

LIST OF APPENDIX

University of Malaya

University of Malaya

CHAPTER 1: INTRODUCTION

1.1 Overview of Study

The prevalence of obesity has skyrocketed over the past decades. This phenomenon is worrying since the progression of insulin resistance, Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) are especially related to abdominal obesity. Excess fat deposition results in 'adiposopathy' which is linked to abnormal increased production of pro-inflammatory cytokines and decreased release of anti-inflammatory adiponectin (Bays et al., 2013). Such phenomena contribute to the progression of T2DM and CVD development owing to obesity-induced chronic inflammation (Y. S. Lee et al., 2011).

Dietary habits appear to be the pivotal determinant in obesity development. Dietary guidelines were hence amended for the purpose of preventing obesity and alleviating the risk of obesity related morbidity. World Health Organization (WHO) suggests that total fat should be remain at 30-35% energy (% en) while saturated fatty acids (SAFA) intake should not be more than 10% en and should be replaced with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (WHO, 2011). SAFA is not favored based on its elevating effect on low density lipoprotein-cholesterol (LDL-C) which is linked to higher CVD risk (Mensink & Katan, 1992). However, increased SAFA intake does not lead to deleterious impact on total cholesterol: high density lipoprotein-cholesterol (HDL-C) (Mensink, Zock, Kester, & Katan, 2003). A recent meta-analysis of observational studies and randomized controlled trials reported insufficient evidence to support the current guidelines recommending high PUFA consumption and low SAFA intake (Chowdhury et al., 2014). In addition, increased refined carbohydrate intake as the replacement for reduced SAFA intake poses no beneficial effects on CVD risk since both LDL-C and HDL-C levels are lowered on this

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dietary plan (Jakobsen et al., 2009). In fact, the consumption of fatty acids plays a role in modulating CVD risk mediated through biological mechanisms beyond mere lipid profile measurements, such as subclinical inflammation, endothelial dysfunction, thrombotic tendency, oxidative stress, blood pressure and insulin sensitivity (Hu & Willett, 2002; Kleemann, Zadelaar, & Kooistra, 2008). Taken together, the conventional nutritional guidelines on dietary fats may require reappraisal based on new evidence beyond lipid profile evaluation.

The Multi-Ethnic Study of Atherosclerosis Associations reported that the association between SAFA and incident CVD depend on the food source (de Oliveira Otto et al., 2012). The study demonstrated that the consumption of dairy SAFA is inversely associated while that of meat SAFA is positively associated with CVD risk. No association were found between plant- and butter-derived SAFA, however the low intakes of these categories limited the statistical power. Palm olein – a plant source of SAFA, is widely applied for domestic use in South East Asian countries. Similar to other palm oil producing countries, Malaysia was identified as one of the world's highest SAFA consuming countries in the year 2010 (Micha et al., 2014). While WHO recommends <10% en SAFA intake per day, the average daily SAFA intake in Malaysian adults was 20.3% en (Micha et al., 2014). Health authorities such as the American Heart Association encourage replacement of tropical oils (e.g. palm olein) with cooking oils rich in MUFA (e.g. high oleic sunflower oil) or PUFA (e.g. soybean oil). Given Malaysia is one of the top countries with highest palm olein consumption, it is important to investigate the necessity of the substitution of dietary SAFA with MUFA in the abdominally obese Malaysian/Asian population.

The trend for healthy diet pattern has moved towards low-fat diet for the prevention of chronic degenerative diseases development such as cardiovascular disease and diabetes. Decreasing fat intake in the Malaysian population might inadvertently lead

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to increased carbohydrate consumption at the expense of fat-intake given the fact that protein calorie sources are more expensive than carbohydrate calorie sources. On the other hand, the Food and Agriculture Organization of the United Nations (FAO) and WHO pointed out that the people in western countries need to find ways to reduce their fat intake while residents in Asia, Africa and South America should incorporate more fat into their carbohydrate-rich (CARB) diet (FAO, 2010). Given these conflicting recommendations, it is important to investigate the necessity of the substitution of carbohydrate with fat in Malaysian's diet in which the mean daily carbohydrate intake of Malaysian adults in 2003 was 59% en (Mirnalini et al., 2008). Recent evidence showed that replacement of SAFA with CARB (especially sugar) in food products has increased the risk of metabolic syndrome (Malhotra, 2013). Taken together, it is of our interest to evaluate substitution of CARB for SAFA in Malaysian subjects.

In light of the fact that abdominally obese individuals, in particular Asians, are susceptible to the development of T2DM (WHO, 2004), our research also explores the effects of fat quantity and quality on glucose homeostasis and insulin secretion. Conflicting findings were reported by three large scale interventional studies. The Reading, Imperial, Surreys, Cambridge and King's (RISCK) (Jebb et al., 2010) and the Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis (LIPGENE) (Tierney et al., 2011) studies found no significant difference in insulin sensitivity and acute insulin response to glucose in both subjects at risk of developing and with metabolic syndrome administered SAFA, carbohydrate or MUFA diets for 24 or 12 weeks respectively. These two aforementioned studies exchanged MUFA for SAFA at around 7% en whereas around 10% en exchange was done between fat and carbohydrate. The KANWU (Vessby et al., 2001) study reported a borderline statistically significant difference between SAFA and MUFA diets at 9% en exchange (P = 0.0534) in insulin sensitivity but not in insulin secretion and first-phase

University of Malaya

insulin response following a 12-week diet exposure in healthy subjects, where SAFA diet impaired insulin sensitivity but no changes on MUFA diet as compared to baseline. However the finding was limited by the absence of insulin response to glucose measurement and *ad libitum* diet intake which resulted in wide variation of total fat intake. The KANWU study further revealed that the adverse effect of SAFA on insulin sensitivity only applied to the participants with <37% en fat intake.

Gastro-intestinal peptides such as glucagon-like peptide (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), ghrelin, peptide YY (PYY) and cholecystokinin (CCK) are short-term signals for satiety feedback to the brain hence regulating glucose homeostasis (Blatnik & Soderstrom, 2011; Holzer & Farzi, 2014). Disruption of the synchronicity of the brain-gut-adipose tissue circuit can lead to metabolic disorders such as obesity and diabetes (Yi & Tschop, 2012). For example lipid-induced CCK release was found to inhibit hepatic glucose production. High-fat overfeeding was found to impair the gut-brain-liver neuronal mechanism hence leading to hyperglycemia (C. Y. Lee, 2013). Growing clinical evidence reports that levels of gastro-intestinal peptides are compromised in overweight (Mittelman et al., 2010), impaired glucose tolerance (Ahren, Larsson, & Holst, 1997) and T2DM patients (Theodorakis, Carlson, Muller, & Egan, 2004). Fasting concentrations of GLP-1 and PYY, for example, are lower in obese individuals (Brennan et al., 2012; Ranganath et al., 1996; Zwirska-Korczala et al., 2007). Thomsen et al. reported that the ingestion of MUFA-enriched meal resulted in higher GIP (Thomsen et al., 1999) and GLP-1 (Thomsen, Storm, Holst, & Hermansen, 2003) concentrations compared with SAFA-enriched meal in both overweight with T2DM and lean, healthy subjects whose test meals contained same content of total fat and carbohydrate. In this context, the type of dietary fat, in particular triglycerides with differing degrees of saturation may exert differential impact on incretins, i.e. GIP and GLP-1, which play a role in insulin secretion and satiety.

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Postprandial state is a continuous metabolic state in humans as we consume at least 3 meals a day. For instance, it takes 6 to 12 hours (h) for the digestion, absorption and metabolic disposal of dietary fat. Even though fasting measurements of biomarkers are commonly used to predict risk of disease development, yet postprandial lipemia and postprandial glycemia have been implicated in the aetiology of chronic metabolic diseases such as T2DM and complications such as CVD. Therefore, both fasting and postprandial effects are of interest. Other studies making similar comparisons such as S. J. van Dijk et al. (2009), Bos et al. (2010) and Due, Larsen, Mu, et al. (2008) have not examined the postparandial effects of an acute metabolic challenge with meals of differing fat composition in terms of fat quality and quantity. We have performed a metabolic postprandial challenge test which reflected the dietary composition of the chronic background dietary intervention at the end of each dietary intervention, in order to assess postprandial effects of an acute metabolic challenge.

Due to the limited information available on the aforementioned, our study is the first in the South East Asia region to evaluate the **fasting and postprandial** effects on insulin secretion, insulin sensitivity, glucose homeostasis, inflammatory responses, endothelial and vascular functions and gastro-intestinal peptides release of the consumption of isocaloric SAFA-, CARB- or MUFA-enriched mixed meals after a 6-week exposure to the respective intervention diets in individuals with central obesity. Using a cross-over design, we conducted a randomized controlled trial comparing the effects of substitution of 7% energy as refined carbohydrate (CARB) or MUFA for SAFA for a period of 6 weeks each. Fasting and postprandial blood samples in response to corresponding CARB, MUFA or SAFA enriched meal-challenges were collected after 6 weeks on each diet-treatment for the assessment of outcomes. Uniquely, the present study was conducted under free-living conditions more reflective of the real-world, although

University of Malaya

closely supervised for compliance and used blended palm olein as a source of plant SAFA and blended high oleic sunflower oils as a source of MUFA.

1.2 Objectives

The current study was conducted to:

1) investigate the effect of fat quantity (SAFA- and MUFA diets (high-fat) *vs* CARB diet (low-fat)) on insulin secretion and sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptides release in individuals with abdominal obesity under fasting and postprandial conditions.

2) investigate the effect of fat quality (compare SAFA *vs* MUFA diets) on insulin secretion and sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptides release in individuals with abdominal obesity under fasting and postprandial conditions.

1.3 Hypotheses

1. Replacing fat with carbohydrate in a 6-week habitual diet will affect **fasting** state's insulin secretion and sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptides release in individuals with abdominal obesity.

2. Increased carbohydrate will adversely influence **postprandial** insulin secretion and sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptides release in individuals with abdominal obesity when compared with high fat intake.

3. A plant-source SAFA-enriched (palmitic acid from palm olein) diet will be comparable with MUFA (oleic acid from high oleic sunflower oil) with regards to its 6-week dietary effects on **fasting** state's insulin secretion and sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptides release in individuals with abdominal obesity.

University of Malaya

4. Replacing SAFA (palmitic acid from palm olein) with MUFA (oleic acid from high oleic sunflower oil) will not influence **postprandial** state's insulin secretion and sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptides release in individuals with abdominal obesity.

Keynotes

-Abnormal increased production of pro-inflammatory cytokines by adipose tissues contributes to the progression of T2DM and CVD development in abdominally obese individuals.

-Current dietary guidelines recommend the replacement of SAFA with CARB or MUFA based on evidence from resultant serum lipid profile alone.

-Decreasing fat intake leads to increased carbohydrate consumption, which reportedly poses no beneficial effect on cardiovascular risk.

-Malaysia is one of the top countries with highest palm olein (rich source of SAFA) consumption.

-Gastro-intestinal peptides are short-term signals for satiety feedback to the brain hence playing a role in glucose homeostasis.

-The chronic effects of the mentioned replacements of CARB for fat and SAFA for MUFA on insulin secretion, insulin sensitivity, inflammatory and hemostatic responses, and gastro-intestinal peptide release in individuals with abdominal obesity are unclear, hence are of interest of this study.

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3.2 Diet Composition Analysis

Before the start of each dietary intervention and for 15 days during each intervention, food samples were collected during test cooking and analyzed to determine if the fatty acid composition, energy and macronutrient composition of the intervention diet were at the prespecified target percentages. The food samples from SAFA, CARB and MUFA diets were analyzed on a daily basis; the average results of 15-days per intervention were then calculated.

3.2.1 Handling of Food Samples

Each meal and drink from the same day menu inclusive of breakfast, lunch and dinner was collected and weighed prior to homogenization (Waring (R) Commercial Heavy Duty Blender, USA). A measured volume of water was added to the mixture as and when necessary. The actual mass of sample homogenate (W1) after the correction for the mass of added water was then determined. A portion of the homogenate was sampled into a Petri dish with pre-determined weight (W2), in replicate. The mass of the Petri dish with the aliquot of the homogenate (W3) was weighed and the mass of the aliquot was calculated by subtracting W2 from W3.

3.2.2 Determination of Moisture

The Petri dish with the homogenate aliquot was then dried overnight in a convection oven (Ecocel MMM Medcenter, USA) at 105 °C. The drying procedure was completed when the mass of the Petri dish with the dried aliquot (W4) reached a constant value. The moisture content was determined by the difference between W3 and W4. Moisture content was converted to equivalents of the total fresh mass of the meal sample as follows:

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$$\text{Moisture content of aliquot (W3-W4) (g)} \times \frac{\text{Total fresh mass of sample (W1) (g)}}{\text{Mass of frash aliquot (W3}-\text{W2) (g)}}$$

3.2.3 Determination of Total Dry Mass

The difference between the mass of the Petri dish and the dried aliquot (W4) and the empty Petri dish (W2) determines the total dry mass of the aliquot. The calculation of the total dry matter in the meal sample is:

$$\text{Dried mass of aliquot (W4-W2) (g)} \times \frac{\text{Total fresh mass of sample (W1) (g)}}{\text{Mass of freash aliquot (W3}-\text{W2) (g)}}$$

3.2.4 Determination of Fat Content

Soxhlet extraction (Hitchcock and Hammond 1980) was applied for the extraction of total lipids from the dried aliquot harvested as described in Section 3.2.1.2. A total of 10 g of the ground dried aliquot (W5) was transferred into a pre-weighed thimble which was then plugged with cotton wool. The thimble with dried aliquot was placed into the Soxhlet extractor. Around 200 mL of petroleum ether was poured into a pre-weighed 250 mL round-bottomed flask to which was added 5 anti-bumping granules (W6). After the assembling of the flask and extractor on the Soxhlet apparatus, the extraction was performed on a heating mantle (Ross (R) Mantle, USA) for 2 h.

At the end of a 30 min rest period, after the completion of extraction, the flask with the extraction product was assembled in a rotary evaporator (RV06-ML, IKA (R) Werke, GMBH & Co, KG, Germany) in order to vaporize petroleum ether from the solution with extracted lipids. The flask with extracted lipids was then left for a while and weighed (W7) until a constant value was obtained. The lipid content in the aliquot of

University of Malaya

dried sample was determined by the difference between W7 and W6. The percentage of fat content in the aliquot of dried food sample was calculated as follows:

$$\frac{\text{Mass of extracted lipids (W7}-\text{W6) (g)}}{\text{Mass of dried aliquot of food (W5) (g)}} \text{ x } 100\%$$

3.2.5 Determination of Fatty Acid Composition

The extracted lipids left in the flask as described in Section 3.2.1.4 were dissolved with a small volume of chloroform and transferred into a labeled vial. The sample was dried with hydrogen gas and stored for determination of fatty acid composition. Free fatty acid was first converted from oil to methyl ester (FAME) using the modified rapid method as described in Nang et. al. (2010). 50 µl of oil was added to 1 mL of hexane in a 2 mL centrifuge tube and the mixture was vortexed for 15 seconds. 100 µl of 3 N sodium methoxide was added into the mixture and then vortexed for another 15 seconds. The mixture was left to stand for 15 minutes and the upper layer of the separation was withdrawn and transferred into a labeled vial. The vial was tightly sealed and stored at 4 °C until analysis of fatty acid composition. The stored FAME was dissolved with 50 µl hexane and then 1 µl of the aliquot was automatically injected for 30 min-GC on a BPX 70 column (25 m length x 220 µm (i.d) x 0.25 µm film thickness; SGE Sarl, Courtabœuf, France) using the Perkin Elmer Autosystem (Perkin Elmer, Norwalk, CT). Gaseous oxygen was used for ignition whereas hydrogen gas was the carrier gas. The oven temperature was adjusted to 160 °C and held at that level for 4 min, then increased 10 °C/min up to 200 °C and held at that temperature for 10 min, then elevated 40 °C/min to 240 °C and held for 10 min. The detector temperature and carrier gas flow were respectively set to 250 °C and 1 ml/min. With reference to the retention time of 37 FAME standards (Sigma-Aldrich, Australia), the results were displayed as a percentage composition.

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3.2.6 Determination of Protein Content

Protein content was determined following the Kjeldahl method. About 0.5 g of ground dried food sample as obtained from the procedures as described in Section 3.2.1.2 was weighed and placed into a labeled 500 mL Kjeldahl digestion tube to which was added 2 tablets of selenium catalyst and 10 mL of concentrated sulfuric acid. The digestion tube was assembled to the Buchi-Kjeldahl system (Buchi 435 Digestion Unit, Switzerland) for 2 h-controlled heating digestion performed in the fume hood.

After the digested sample was left to cool for an hour, the automated distillation process was performed with a Buchi Distillation apparatus (B-323, Buchi Distillation Unit, Switzerland) where 60 mL of 32% sodium hydroxide and 40 mL of distilled water were required for the 7-minute distillation. The liberated ammonia during the distillation was trapped in 100 mL of 2% boric acid contained in a Erlenmeyer flask.

The mixture of boric acid and ammonia was had added to it 2 drops of Tashiro's indicator solution and was then titrated against the standard sulfuric acid. End point was reached when the mixture turned from green to permanent pink color. The titre volume was adjusted against a blank titration with 100 mL of 2% boric acid incorporated with 2 drops of indicator. The mean of two titrations was calculated as the final titre volume. The percentage of protein content in the aliquot of dried food sample was calculated by use of the following formula:

$$\frac{\text{Titre volume (mL) x Normality of titrant}}{\text{Mass of dried aliquot of food (mg)}} \text{ x Equivalent weight of nitrogen x 6.25 x 100\%}$$

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3.2.7 Determination of Energy Content

Around 0.5 g of the dried and ground food samples collected from the process as described in Section 3.2.1.2 were used to determine the gross calorific value using an automated bomb calorimeter (C5000 control IKA-Calorimeter system, IKA (R) WERKE, KG, Germany). The automated system of the calorimeter enables the combustion of food samples to take place under precisely defined conditions. The decomposition vessel was filled with pure (99.95%) oxygen and charged with a weighed food sample. Following the ignition of the sample, the system calculated specific gross calorific value from heat capacity of the calorific system, weight of the food sample and increase in temperature of the water inside the inner vessel of the measurement cell. The calorific value obtained was then used to calculate the total calorie content of the daily diet via following formula:

$$\frac{\text{Calorie per sample } (\frac{\text{J}}{\text{g}})}{\text{Mass of dried aliquot of food (mg)}} \text{ x Equivalent weight of nitrogen}$$

3.2.8 Determination of Carbohydrate Content

Proportion of carbohydrate content was not determined by chemical analysis but estimated by subtracting the percentage of fat and protein (as determined in Sections 3.2.1.4 and 3.2.1.6) from 100%, indicated by the following formula:

100% - percentage of fat content (%) – percentage of protein content (%)

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CHAPTER 2: LITERATURE REVIEW

2.1 Obesity

The striking growth of obesity prevalence rates is a worldwide epidemic. Owing to sedentary lifestyle, positive energy balance and unhealthy nutrition, 69% of adults in the United States are overweight or obese (Flegal, Carroll, Kit, & Ogden, 2012). The prevalence of obesity in Malaysia increased rapidly from year 1996 to 2008 and the number of obese subjects has almost tripled. The percentage of obesity was 4.0%, 10.0% and 14.1% in Malaysian men and 7.6%, 17.4% and 22.5% in Malaysian women, respectively in the year 1996, 2006 and 2008 (IPH, 1997; Kee et al., 2008; Mohamud et al., 2011). In the year 2014, the national prevalence of overweight and obesity was 32.4% and 18.5% respectively and 20% of Malaysians were having abdominal obesity (IPH, 2014). Such a sky-rocketing increase in prevalence of obesity in Malaysia over the past 20 years is indeed worrying.

2.1.1 Definition of Obesity

World Health Organization previously defined overweight as a body mass index (BMI) ≥ 25 kg/m^2 while obesity is ≥ 30 kg/m^2. There has been increasing debate as to whether BMI cut-off points for Asians should be reviewed and two previous attempts (WHO/IASO/IOTF, 2000) were made to establish new BMI cut off points for Asian and Pacific populations. The reasons for this are 1) Asians tend to have high risk of T2DM and CVD at BMI lower than 25 kg/m^2 and 2) associations between BMI, percentage of body fat, and body fat distribution differ across populations (WHO, 2004). However, the WHO expert consultation committee eventually decided not to make any changes to the BMI cut-off points for Asian populations but suggested additional categories to indicate

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health risk, i.e. BMI < 18·5 kg/m^2, underweight; 18·5–23 kg/m^2, increasing but acceptable risk; 23–27·5 kg/m^2, increased risk; and ⩾27·5 kg/m^2, high risk (WHO, 2004).

2.1.2 Obesity Paradox

The conventional belief in the correlation between obesity and CVD, T2DM was challenged when epidemiological studies reported the new insights of "obesity paradox". This phenomenon describes the observation that higher BMI was related to improved survival rates in patients with diseases such as coronary heart disease, hypertension, heart failure, T2DM, kidney and pulmonary diseases (Gruberg et al., 2002; Hainer & Aldhoon-Hainerova, 2013; Romero-Corral et al., 2006). This may suggest that BMI may not be a relevant tool to assess chronic disease risk. However, the paradox is still under debate and remains inconclusive after more than 10 years, given that selection or survival bias and treatment bias may exist in the previous findings (Standl, Erbach, & Schnell, 2013).

2.1.3 Generalized Obesity *versus* Central Obesity

Measures of central obesity may be more relevant than BMI. Coutinho et al. (2011) indicated that assessment of central obesity as measured by waist circumference and waist to hip ratio is directly associated with mortality in coronary artery disease (CAD) patients, whereas BMI is not. A large cohort study reported that elevated BMI in the absence of central obesity did not impose a detrimental effect on survival in patients with CAD, however normal BMI with concurrent central obesity was associated with the lowest survival rate compared with other patterns of obesity (Coutinho et al., 2013). These findings may help to explain the phenomenon of the 'obesity paradox'.

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Compared with generalized obesity (as measured by BMI), there is increasing evidence demonstrating body fat mass, also known as abdominal, visceral or central obesity, to be a better predictor of abdominal obesity-related morbidities (WHO, 2000). Although not conclusive, WHO (2011) reviewed data from most cross-sectional studies and suggested that waist circumference or waist-hip ratio (measurement of abdominal obesity) is superior to BMI in predicting T2DM risk; however BMI, waist circumference or waist-hip ratio are all similarly correlated to the risk of CVD. In terms of practicality, the use of waist-hip ratio is limited due to the difficulty in measuring hip circumference compared with waist circumference. In addition, measuring waist circumference is more accurate than weight and height (WHO, 2011). Hence, waist circumference is the preferred obesity variable compared to hip circumference and waist-hip ratio. Indeed, Seidell (2010) reported that waist circumference alone is an all-cause mortality predictor ahead of waist-hip ratio and BMI.

2.1.4 Recommendations on Cut-off Points

World Health Organization, National Cholesterol Education Program and International Diabetes Federation (IDF) recommend different cut-off points for risk assessment of central obesity, as summarized in Table 2.1. Only IDF has specified the range according to population and geography. There is no standardized recommendation for worldwide use; with some countries even establishing their own recommendations (WHO, 2011).

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Table 2.1: Cut-off Points for Risk Assessment of Central Obesity as Recommended by World Health Organization, National Cholesterol Education Program and International Diabetes Federation

Indicator	Cut-off points	Risk of metabolic complications
WHO		
WC	> 94 cm (M); > 80 cm (W)	Increased
WC	> 102 cm (M); > 88 cm (W)	Substantially increased
Waist-hip ratio	≥ 0.90cm (M); ≥ 0.85 cm (W)	Substantially increased
NCEP		
WC	> 102 cm (M); > 88 cm (W)	Not specified
IDF		
WC for Europids	> 94 cm (M); > 80 cm (W)	Not specified
WC for South Asians, Chinese and Japanese	> 90 cm (M); > 80 cm (W)	Not specified

WHO, World Health Organization; WC, waist circumference; NCEP, National Cholesterol Education Program; IDF, International Diabetes Federation.

Anand et al. (2011) reported that South Asians have less subcutaneous space for excess fat and therefore their excess fat is stored in ectopic compartments. It was also found that the accumulation of abdominal fat tissue is greater in South Asians and Chinese compared with Europeans (Lear, Humphries, Kohli, & Birmingham, 2007). Owing to the differences in fat distribution among populations, IDF waist circumference population and ethnicity cut-off points should be used in the Malaysian population (> 90 cm (men); > 80 cm (women)) since our population is genetically and geographically more related to South Asians, Chinese and Japanese populations. This is in line with the 'harmonized criteria' a joint consensus statement issued by the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity in 2009 which attempted to unify criteria by advocating the use of population- and country-specific definitions of waist circumference for the diagnosis of Metabolic Syndrome (Alberti et al., 2009).

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2.2 Role of Adipose Tissue in Inflammation

Genetic predeposition and positive energy balance will lead to excess adipose tissue formation and hence increase the risk of obesity development (Bays et al., 2013). A growing body of evidence indicates that adipose tissue is not merely an energy storage site but has various functions. In fact, adipose tissue is an endocrine organ secreting cytokines (adipocytokines) and therefore plays an important role in low grade inflammation. Increased fat accumulation as found in obese individuals results in adipocyte proliferation (hyperplasia) and enlargement (hypertrophy) which can lead to adipocyte and adipose tissue dysfunction (adiposopathy) (Bays et al., 2013; Van de Voorde, Pauwels, Boydens, & Decaluwe, 2013). Adiposopathy is related to oxygen depletion in adipose tissue due to excess fat accumulation therefore causing adipocyte cell injury, death and inflammation (Anghel & Wahli, 2007; Kalupahana, Claycombe, & Moustaid-Moussa, 2011). Hence, this leads to organellar dysfunction, hormone dysregulation, impaired storage of fatty acids, increased circulating fatty acids and lipotoxicity to nonadipose tissue organs (Bays et al., 2013). Increased circulating fatty acids may be delivered to visceral depots and even nonadipose tissue organs, contributing to ectopic fat deposition for example, fatty liver and increased VLDL particles. This can lead to dyslipidemia a characteristic feature of metabolic syndrome and T2DM (Bays et al., 2013).

Adiposopathy is also related to oxidative stress, abnormal increased production of reactive oxygen species, cytokines such as monochemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α); and decreased production of anti-inflammatory adiponectin (Bays et al., 2013; Teng, Chang, Chang, & Nesaretnam, 2014). Elevated levels of pro-inflammatory adipocytokines including TNF-α and IL-6 have been shown to be present in obese, insulin

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resistant and T2DM individuals (Bays et al., 2013; Dandona, Aljada, & Bandyopadhyay, 2004). It has been demonstrated *in vitro* and *in vivo* that the chronic activation of pro-inflammatory pathways within insulin target cells impair insulin signalling in the obese state (Y. S. Lee et al., 2011; Schaeffer, Rusnak, & Amson, 2008). The release of unhealthy adipocytokines such as IL-6, TNF-α and leptin favor the development of cardiovascular dysfunction including endothelial dysfunction, hypertension, atherosclerosis and heart diseases (Van de Voorde et al., 2013).

Traditional risk factors such as dyslipidemia, diabetes mellitus, high blood pressure and physical inactivity do not fully explain incident CVD (Balagopal et al., 2011). Non-traditional risk factors for CVD include chronic inflammation, hemostasis and endothelial dysfunction (Balagopal et al., 2011). Combined assessment of traditional and non-traditional risk factors, respectively represented by LDL-C and CRP factor provides better CVD risk prediction rather than either alone (Ridker, Rifai, Rose, Buring, & Cook, 2002). Figure 2.1 summarizes this new concept in the pathophysiology of CVD.

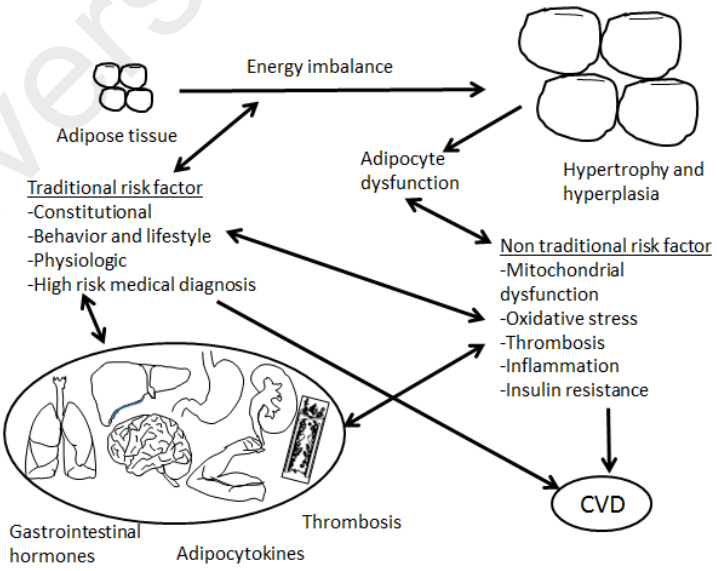

Figure 2.1: Overall Conceptual Framework of New Concepts in the Pathophysiology of Cardiovascular Disease (CVD) (Balagopal et al., 2011).

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Multiple lines of evidence point to the pivotal role of chronic inflammation in the pathogenesis of CVD. Emergent evidence indicates the involvement of inflammation in all stages of atherosclerosis (an early precursor of CVD) including plaque development, disruption and thrombosis (Balagopal et al., 2011). Examples of inflammatory biomarkers include IL-6, IL-1β and CRP. IL-6 and IL-1β are cytokines secreted by macrophages and monocytes. IL-1β activates local endothelial cells and hence induces vasodilation which increases the permeability of blood vessels. This recruits more leukocytes and other proinflammatory markers to the site of infection (Akdis et al., 2011). Any factors that can cause inflammatory response were found to stimulate the secretion of IL-6, for example IL-1β, TNF-α, lipopolysaccahride (a bacterial product), viral infection and products released by necrotic cells (Naugler & Karin, 2008). IL-6 amplifies the proinflammatory state by stimulating the production of other proinflammatory cytokines such as PAI-1, IL-1 and MCP-1 (Kleemann et al., 2008). Both IL-1β and IL-6 stimulate hepatocytes to produce acute phase protein such as CRP. It has been reported that CRP may be a stronger CVD risk predictor than LDL-C (Ridker et al., 2002). CRP catalyzes M1 macrophage polarization and triggers plaque formation through macrophage infiltration of both adipose tissue and atherosclerotic lesions (Yousuf et al., 2013).

Inflammation is highly interrelated with hemostasis. Hemostasis refers to the maintenance of the fibrinolysis/coagulation system which changes the vascular endothelium. The imbalance of the fibrinolysis/coagulation system leads to the development of atherosclerosis. The relationship between these two pathophysiological processes is bidirectional, i.e. inflammation activates hemostasis which in turn promotes inflammatory response, and the development of CVD. IL-6, IL-1β and TNF-α induce hemostasis through several mechanisms including increased platelet activation and endothelial cell dysfunction, suppressed fibrinolytic activity, tissue factor mediated

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activation of the plasma coagulation cascade and impaired function of physiologic anticoagulant pathways (Margetic, 2012). Inflammation disturbs the function of endothelial cells in maintaining the physiological balance involved in the control of the hemostatic system. Activated endothelial cells therefore produce more procoagulant or antifibrinolytic components such as thromboxane A2, von Willebrand factor, plasminogen activator inhibitor (PAI-1), and reduce the secretion of components with anticoagulant and profibrinolytic properties (Verhamme & Hoylaerts, 2009). In addition, activated endothelial cells also express tissue factor and adhesion molecules at their surface, for example E-selectin, P-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) (Margetic, 2012). The expression of E-selectin enables the recruitment of leukocytes to the site hence promotes inflammation, and also regulates the interactions between certain blood cells and endothelial cells (Verhamme & Hoylaerts, 2009). The inappropriate activation of the hemostatic system will lead to the formation of fibrin-platelet clot which contributes to the progression of atherosclerosis. The local or systemic activation of coagulation will release fibrin degradation products such as D-dimer. D-dimer elevation was reported to be associated with a two times increased risk of near-term cardiovascular events (Kleinegris, ten Cate, & ten Cate-Hoek, 2013).

2.3 Obesity-induced Chronic Inflammation

Chronic inflammation (systemic inflammation) occurs when the innate immune system is chronically activated and pro-inflammatory cytokines are released from immune-related cells. The findings of macrophage infiltration into adipose tissue bring a mechanistic insight into the obesity-induced chronic inflammation. Obesity-induced inflammatory state involves a phenotypic switch in adipose tissue macrophage from M2

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to M1 state. The M2 state is the default state producing antiinflammatory macrophages such as IL-10 and IL-Ra whereas the M1 state comprises proinflammatory macrophage populations for example TNF-α and IL-6 secreting cells.(Olefsky & Glass, 2010).

Chemokines are a class of cytokines that attract white blood cells to site of infection. In the development of adiposopathy, MCP-1 (a chemokine) stimulates the recruitment of macrophages into adipocytes via MCP-1/ CC chemokine receptor 2 (CCR2) cascade (de Luca & Olefsky, 2008; Weisberg et al., 2003). Instead of converting monocytes to M2 macrophages, migration of monocytes from the circulation to the macrophage clusters surrounding dead adipocytes switches M2 to M1 state. This phenotypic switch of M2 to M1 is characterized by a reduction in IL-10 and arginase production, in conjunction with an increase in pro-inflammatory TNF-α production (Kalupahana et al., 2011; Lumeng, Bodzin, & Saltiel, 2007).

2.4 Inflammation and Insulin Resistance

Insulin resistance is a physiological state where insulin target cells fail to respond to normal insulin action. This condition is characterized by a higher than normal level of insulin needed to maintain the normal level of glucose in the circulation. The disruption of insulin signal transduction pathways, either directly or indirectly, leads to insulin resistance (Hirabara et al., 2012). Glucose uptake is initiated with the binding of insulin to insulin receptor which results in tyrosine phosphorylation of insulin receptor. This will then be followed by the binding of insulin receptor with intracellular docking proteins, for instance insulin receptor substrate (IRS), Scr homology collagen (SHC) and associated protein substrates. IRS is the most prominent docking protein involved in insulin signalling (Hirabara et al., 2012). These proteins are tyrosine phosphorylated on the C-terminal region generating specific binding sites for protein containing Src

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Homology-2 (SH2) domains, e.g. phosphatidylinositol-3 kinase (PI-3K) (Gual, Le Marchand-Brustel, & Tanti, 2005). This subsequently activates kinases including protein kinase B (PKB) (or known as Akt) and protein kinase C (PKC). These protein kinases promote peripheral glucose uptake by causing translocation of glucose transporter type 4 (GLUT4) to the plasma membrane (Gual et al., 2005; Jakobsen et al., 2009).

There are many potential pathways involved in inflammation-modulated insulin resistance (Cruz-Teno et al., 2012; Gorjao, Takahashi, Pan, & Massao Hirabara, 2012). A major mechanism by which proinflammatory mediators impede insulin signalling is via phosphorylation of certain serine kinases on IRS proteins (Gual et al., 2005; Hotamisligil et al., 1996). Examples of serine kinases playing an integral role in both inflammatory pathway and insulin signalling are JNK and IKKβ (Aguirre, Uchida, Yenush, Davis, & White, 2000; Cai et al., 2005; Jiao et al., 2009). These serine kinases phosphorylate transcription factors including AP1 and NF-κB, which then activate overlapping inflammatory markers (Olefsky & Glass, 2010). This eventually leads to impaired insulin signalling via serine phosphorylation.

Another possible mechanism linking inflammation to insulin resistance involves suppression of cytokine signalling 3 (SOCS-3) expression by cytokines. The binding of SOCS-3 to phosphorylated Tyr960 of the insulin receptor was reported to inhibit insulin induced Stat5B activation, therefore leading to decreased IRS-1 tyrosine phosphorylation and suppressed insulin signaling (Emanuelli et al., 2001).

Numerous *in vitro* and *in vivo* studies have demonstrated that chronic activation of proinflammatory pathways within insulin target cells impair insulin signalling in the obese state (Cai et al., 2005; Y. S. Lee et al., 2011; Schaeffler et al., 2008). In line with this, elevated levels of the proinflammatory adipocytokines including IL-1β, IL-6 and TNF-α have been shown to present in obese, insulin resistant and T2DM individuals

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(Dandona et al., 2004). The increased levels of IL-1β, IL-6 and TNF-α may leak into the circulation and impair insulin sensitivity in distal tissues through endocrine effects (Olefsky & Glass, 2010).

2.5 Role of Gastro-intestinal Peptides in Glucose Homeostasis

It is well known that the maintenance of glucose balance is controlled by insulin which stimulates glucose uptake in skeletal muscle and adipose tissue, plus inhibits endogenous glucose production. Other glucoregulatory hormones such as glucagon, catecholamines, cortisol and growth hormone also play a substantial role in glucose homeostasis. In addition, a growing body of evidence demonstrating that the central nervous system plays a key role in glucose homeostasis via central pathways that overlap with the regulation of food intake and body weight (C. Y. Lee, 2013) . Due to the common origin of the parasympathetic ganglia of both the brain and gut during embryogenesis, many gastro-intestinal hormone and peptide, and their receptors are found in the brain (Yi & Tschop, 2012). Energy homeostasis and glucose metabolism are regulated by the synchronization achieved by neuronal and humoral interaction between brain and gut (Heijboer et al., 2006). Gastro-intestinal hormones that trigger satiety such as GLP-1, PYY and CCK are secreted after nutrient digestion and absorption, whereas the hunger gastro-intestinal hormone, i.e. ghrelin initiates the feeding episode (Hameed, Dhillo, & Bloom, 2009).

GLP-1 is synthesized by enteroendocrine L cells mainly located in the mucosa of the distal part of the small intestine and colon, whereas GIP is secreted by K cells, mainly found in the mucosa of the duodenum and the jejunum of the gastro-intestinal tract (Seino & Yabe, 2013). Fat, protein and carbohydrate in the lumen of the gut have been shown to stimulate the secretion of GLP-1 and GIP which are also known as insulinotropic hormones (incretins) (Del Prete, Iadevaia, & Loguercio, 2012). Incretins

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bind to the respective receptors which then activate and increase the level of intracellular cyclic adenosine monophosphate in pancreatic β cells, the activation thereby stimulates insulin secretion dependent of glucose changes (Seino, Fukushima, & Yabe, 2010). Besides its insulinotropic effect, glucose metabolism is regulated by GLP1 via other mechanisms: 1) by controlling food consumption and body weight, hence affecting insulin sensitivity; 2) by suppression of glucagon secretion and hence reducing endogenous glucose production 3) by affecting tissue specific insulin sensitivity i.e. glucose uptake; 4) by modulating gastric emptying and gut motility, and thereby meal-related fluctuations in glucose levels (Heijboer et al., 2006).

Both GLP-1 and GIP have similar effects on the β-cell but whereas GLP-1 inhibits glucagon secretion by the alpha cell, GIP is a weak stimulant of glucagon secretion (Holst, 2004). GLP-1 delays gastric emptying and reduces satiety but GIP has no extra-islet effects. There is divided opinion as to the relative importance of GLP1 and GIP with regards to the incretin effect. There are some that believe that both the incretin hormones contribute to the incretin effect equally (Vilsboll, Krarup, Madsbad, & Holst, 2003). Others believe that of the 2 hormones GLP-1 is the peptide of major physiologic importance and accounts for 70 to 80% of the incretin response while, GIP accounts for 20-30% of the response (Mikhail, 2006).

Ghrelin is mainly synthesized by a subset of stomach cells, hypothalamus and pituitary (Delporte, 2013). This hormone increases blood glucose concentration by stimulating liver glycogenolysis and neoglucogenesis (Delporte, 2013). On the other hand, elevation of CCK in the duodenum was found to lower glucose production insulin-independently involving the activation of the gut CCK-A receptor and a gut-brain-liver neuronal axis (Cheung, Kokorovic, Lam, Chari, & Lam, 2009). The release of CCK is particularly stimulated by fat and protein and is found to stimulate PYY release and suppress ghrelin level. PYY is known to be co-secreted from intestinal L-cells with GLP-

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1, yet new evidence shows that other gastro-intestinal peptides such as CCK and GIP may also be co-secreted with PYY (Egerod et al., 2012). PYY is expressed in pancreas and GI tract, plays a role in glucose homeostasis through regulation of insulin secretion (S. Manning & Batterham, 2014).

Growing clinical evidence reports that the levels of gastro-intestinal peptides are compromised in overweight (Mittelman et al., 2010), impaired glucose tolerance (Ahren et al., 1997) and T2DM patients (Mazzaferri, Starich, & St Jeor, 1984; Theodorakis et al., 2004). Fasting concentrations of GLP-1 and PYY, for example, are lower in obese individuals (Brennan et al., 2012; Zwirska-Korczala et al., 2007). Disruption of the synchronicity of the brain-gut-adipose tissue circuit can lead to metabolic disorders such as obesity and diabetes (Yi & Tschop, 2012).

2.6 Dietary Fatty Acids

Triacylglycerols (TAG), free fatty acids, phospholipids and sterol esters are molecules found in oils, lipids or fats; and triacylglycerol is the main component (Fahy et al., 2005). A triacylglycerol molecule comprised a glycerol backbone attached to 3 fatty acid chains. The 3 fatty acids consist of similar or different fatty acids, be it saturated, monounsaturated or polyunsaturated fatty acid which can be further differentiated by chain length and the number of double bonds. The variations in configuration hence determine the characteristics of a fat, oil or lipid. Figure 2.2 displays the structure of a triacylglycerol molecule.

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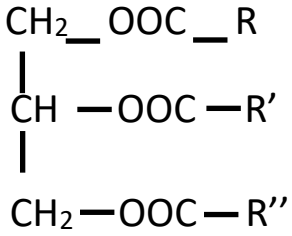
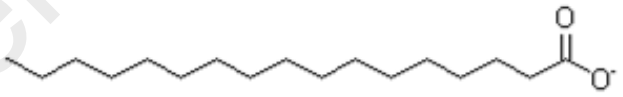
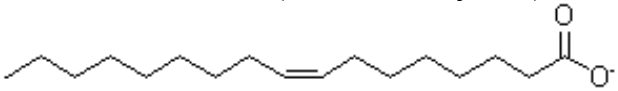


Figure 2.2: Chemical Structure of A Triacylglycerol Molecule. RCO_2H, $R' CO_2H$, and $R'' CO_2H$ Represent Molecules of Either Similar or Different Fatty Acids.

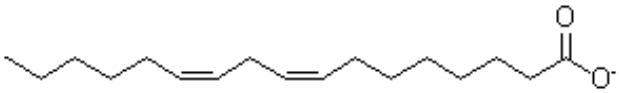
Figure 2.3 showed a few examples of the structure of fatty acids. SAFA have all carbon atoms on the glycerol backbone saturated with hydrogen atoms. The abbreviation of SAFA includes the number of carbon atoms and a zero after the colon, for example C18:0 or 18:0. SAFA are more stable and have a longer shelf life compared to unsaturated fatty acids owing to the least chemical reactivity. MUFA contain one double bond in the carbon chain and are abbreviated as the number of carbon atoms followed by a one after the colon, such as C16:1 or 16:1. Polyunsaturated fatty acids (PUFA) contain more than one double bond in the carbon chain. The abbreviation for PUFA is the number of carbon atoms followed by the number of double bond after the colon, for example C18:2 or C18:3. Dietary sources of different type of fatty acids are shown in Table 2.2.



Stearic acid, C18:0 (Saturated fatty acid)



Oleic acid, C18:1 n-9 (Monounsaturated fatty acid)



Linoleic acid, C18:2 n-6 (Polyunsaturated fatty acid)

Figure 2.3: Examples of the Structure of Fatty Acids.

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Table 2.2: Fatty Acids in Fats and Oils Sources, (Ratnayake & Galli, 2009)

Common name	Abbreviation	Typical Sources
Lauric acid	C 12:0	Coconut oil, palm kernel oil
Myristic acid	C 14:0	Dairy fat, coconut oil, palm kernel oil
Palmitic acid	C 16:0	Most fats and oils
Stearic acid	C 18:0	Most fats and oils
Palmitoleic acid	C 16:1 n-9	Marine oils, macadamia oil, most animal and vegetable oils
Oleic acid	C 18:1 n-9	All fats and oils, especially high oleic sunflower oil, olive oil, canola oil and safflower oil
Linoleic acid	C 18:2 n-6	Most vegetable oils
α-linoleic acid	C 18:3 n-3	Flaxseed oil, soybean oil, perilla oil and canola oil
γ-linoleic acid	C 18:3 n-6	Evening primrose oil, borage and blackcurrent seed oils
Arachidonic acid	C 20:4 n-6	Animal fats, liver, egg lipids and fish
Eicosapentaenoic acid	C 20:5 n-3	Fish, especially oily fish such as salmon, anchovy, herring, smelt mackerel
Dososapentaenoic acid	C 22:5 n-6	Very minor component in animal tissues
Docosahexaenoic acid	C 22:6 n-3	Fish, especially oily fish such as salmon, anchovy, herring, smelt mackerel

2.7 Impact of Dietary Fats on Inflammation and Insulin Resistance

SAFA has been labeled as the purported culprit in inflammatory response based on mounting *in vitro* and *in vivo* evidence (Ajuwon & Spurlock, 2005; Bradley, Fisher, & Maratos-Flier, 2008). It was hypothesized that SAFA stimulate inflammatory response through toll like receptor-4 (TLR-4) dependent or TLR-4 independent pathway. TLR are a class of pattern recognition receptors that play a crucial role in the innate immune system (reviewed in (Takeda, Kaisho, & Akira, 2003)). TLR-4 activates pro-inflammatory pathways by stimulating the expression of transcription factors, including IKKβ and NF-κB (Amyot, Semache, Ferdaoussi, Fontes, & Poitout, 2012; Kim & Sears, 2010). The levels of TLR-4 were found to be higher in the obese state whereby the expression of transcription factors was exhibited in insulin target tissues i.e. adipose tissue, muscle, liver, brain, vasculature and pancreatic β-cells (Kim & Sears, 2010). Given that insulin resistance is modulated by pro-inflammatory pathways, diminished effect of

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insulin resistance was demonstrated on mice in the absence of TLR where no inflammation happens (Amyot et al., 2012; Saberi et al., 2009).

SAFA is the acyl component of lipopolysaccharides (LPS), which is a ligand of TLR-4. It has been reported that the stimulation of TLR-4 requires the binding of SAFA to TLR-4 in the presence of glycoproteins e.g. myeloid differentiation protein 2 (MD2) and a cluster of differentiation 14 (CD14) (J. Y. Lee et al., 2003). In addition, SAFA have been reported to promote dimerization of TLR-4 so as to induce TLR-4-dependent gene expression and hence activate the receptor (Wong et al., 2009). The stimulatory effects of SAFA, lauric, palmitic and stearic acids in particular, have been found to increase IL-6 gene expression in the macrophage via the TLR-4 dependent pathway (Shi et al., 2006).

Beyond the TLR-dependent pathway, SAFA also stimulate pro-inflammatory mechanisms through the TLR-independent pathway by producing reactive oxygen species (ROS). ROS activate nucleotide-binding domain, leucine-rich repeat containing family, pyrin domain-containing 3 (NLRP3) inflammasome (Dostert et al., 2008) that forms a complex with apoptotic speck protein (ASC) (Zhou, Yazdi, Menu, & Tschopp, 2011). This NLRP3-ASC inflammasome complex controls the release of interleukin-1β (IL-1β) from pro-IL-1β (Franchi, Eigenbrod, Munoz-Planillo, & Nunez, 2009). The release of IL-1β decreases insulin signalling in insulin target cells, providing a possible SAFA-mediated inflammatory response leading to insulin resistance (Jager, Gremeaux, Cormont, Le Marchand-Brustel, & Tanti, 2007).

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2.8 Clinical Evidence of Impact of Dietary Fats on Indices of Insulin Secretion and Insulin Sensitivity

The following subsections summarize published literature on the 1) acute effect of dietary fat quantity (Table 2.3), 2) chronic effect of dietary fat quantity (Table 2.4), 3) acute effect of dietary fat quality (Table 2.5) and 4) chronic effect of dietary fat quality (Table 2.6), on indices of insulin secretion and insulin sensitivity.

2.8.1 Acute Effects of Dietary Fat Quantity on Insulin Secretion and Insulin Sensitivity

Postprandial 2 h glucose was reported to be an independent predictor of diabetes. The duration of the rise in postprandial glucose and the magnitude of the glucose increment are the two components of acute glucose variations that determine dysglycaemia (Blaak et al., 2012). As summarized in Table 2.3, an early study (Collier & O'Dea, 1983) compared the consumption of 50 g boiled potato (carbohydrates) alone with that of additional 63 g butter (567 kcal) in 8 healthy lean subjects. Higher insulin corrected for glucose and lower first hour-incremental glucose response were reported with the consumption of carbohydrates together with additional fat. The findings however may be confounded by the additional calories contributed by butter. Another study similarly compared higher-calorie (1079 kcal) high-fat meal with low-fat meal (630 kcal) in the setting of an 18-h period of closed-loop insulin delivery method of glucose control in 7 type 1 diabetes patients (Wolpert, Atakov-Castillo, Smith, & Steil, 2013). Both meals contained similar carbohydrate content (96 g) and fat content was 50 g higher in high-fat meal compared with low-fat meal (80 g *vs* 30 g fat). High-fat meal led to more hyperglycemia (> 6.7 mmol/L) from 0 to 5 h despite requiring more total insulin delivery,

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in addition to higher insulin concentrations from 5 to 10 h. Carbohydrates-to-insulin ratio was lower for the high-fat meal reflecting higher insulin requirement and lower insulin coverage/efficacy. The comparison was limited by the study design which did not allow head-to-head comparison between fat and carbohydrates. High-fat dinner contained higher total calorie (1079 *vs* 630 kcal) contributed from the additional 50 g fat whereas the reduced fat content in low-fat dinner was not compensated for with increased carbohydrates content to enable comparisons at equal calorie intake.

A recent well-designed study (Gibbons et al., 2013) compared high-fat (50.3% en carbohydrate, 38.0% en fat) and high-carbohydrate (83.6% en carbohydrate, 3.2% en fat) mixed meals in an isocaloric manner where yoghurt, honey, fruit and coffee or tea were served to overweight/obese subjects (n = 16). As expected, high-carbohydrate meal containing 33% en higher carbohydrate content increased insulin and glucose response when compared to high-fat ingestion. The findings were in concordance with the following two studies comparing 30% en exchange between fat and carbohydrate. Glucose and C-peptide responses (iAUC) were found to be higher following high-carbohydrate meal in both healthy (n = 6) and T2DM (n = 6) groups in a 2-h isocaloric mixed meal challenge (Gutniak, Grill, & Efendic, 1986). The study supplied subjects with whole milk, crisp bread, butter, ham and cheese *vs* low-fat milk, white bread, dark bread, butter and jam. Tannous dit El Khoury, Obeid, Azar, and Hwalla (2006) recruited 10 healthy subjects who were tested with liquid meals containing 50 or 20% en fat which provided 30% of subjects' resting energy expenditure. The isocaloric meals consisted of Ensure Complete Balanced Nutrition®, soy/whey protein, sugar, sunflower and water. Significant increments of insulin and glucose concentrations were found at 15 and 30 min. High-carbohydrate meal displayed higher glucose peak than high-fat meal but there was no difference in time to peak glucose. Another study comparing 60 and 20% en fat intake however did not find significant differences in glucose peak between isocaloric meals

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(van der Klaauw et al., 2013), instead the study reported earlier glucose peak (30 *vs* 60 min) and lower insulin peak on high-fat meal compared with high-carbohydrate meal. The test meal consisted of pancakes and bacon served with either cheddar cheese or maple syrup. The different observations in glucose peaking pattern as found in this study and the aforementioned study (Tannous dit El Khoury et al., 2006) which found that there was no difference in timing of glucose peak, is likely a reflection of variability of glucose absorption due to different food composition of the test meals (Blaak et al., 2012).

On the whole, acute glucose and insulin response was affected by the alteration of macronutrient composition in isocaloric meals where high carbohydrate intake results in higher postprandial glucose and/or insulin responses when compared to high-fat intake in healthy lean, healthy overweight/obese, T2DM non-obese and type 1 diabetes. However none of the above studies used insulin corrected for glucose or disposition index (DI) as a measure of insulin secretion.

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Table 2.3: Acute Effects of Dietary Fat Quantity on Indices of Insulin Secretion and Insulin Sensitivity

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Collier & O'Dea, 1983)	Healthy lean; 8 (4/4)	21.5 ± 1.5	Randomized, controlled, crossover	0, 15, 30, 60, 90, 120, 180, 240 min	1. Carbohydrate 2. Carbohydrate + Fat 3. Protein 4. Protein + Fat	Unequal calorie test meal <u>Carbohydrate</u> 50 g carbohydrate *50 g whole, unpeeled, boiled potato* <u>Carbohydrate + Fat</u> 50 g carbohydrate and 50 g fat *50 g whole, unpeeled, boiled potato with 63 g butter* <u>Protein</u> 50 g protein *250 g veal* <u>Protein + Fat</u> 50 g protein and 50 g fat *250 g veal with 63 g butter*	$iAUC_{glucose(0\text{-}60\ min)}$: Carbohydrate ↑↑ *vs* Carbohydrate + Fat; NS between Protein *vs* Protein + Fat $iAUC_{insulin(0\text{-}60\ min)}$: NS between Carbohydrate *vs* Carbohydrate + Fat; NS between Protein *vs* Protein + Fat $iAUC_{insulin:glucose}$: Carbohydrate ↓↓ *vs* Carbohydrate + Fat
(Gutniak et al., 1986)	Healthy non-obese; 6 (-/-) T2DM non-obese; 6 (-/-)	Healthy: 39.8 ± 2.3 T2DM non-obese: 55.2 ± 2.3	Randomized, controlled, crossover	20-min intervals for 120 min after meal	1. Low-carbohydrate meal (LCM) 2. High-carbohydrate meal (HCM)	Isocaloric (300 kcal) breakfast <u>LCM</u> 51% F, 23% P, 26% C *200 ml whole milk, 1 slice of crisp bread, 5 g butter, 20 g ham, 20 g cheese* <u>HCM</u> 22% F, 16% P, 62% C *150 ml low-fat milk, 20 g white bread, 25 g dark bread, 5 g butter, 15 g ham, 22 g jam*	$iAUC_{glucose\ and\ C\text{-}peptide\ (0\text{-}120\ min)}$ in healthy and T2DM subjects: HCM ↑↑ *vs* LCM $iAUC_{insulin\ (0\text{-}120\ min)}$ in healthy subjects: HCM ↑↑ *vs* LCM $iAUC_{insulin\ (0\text{-}120\ min)}$ in T2DM subjects: NS $iAUC_{insulin\ and\ C\text{-}peptide\ (0\text{-}120\ min)}$ of LCM: Healthy ↓↓ *vs* T2DM $iAUC_{insulin\ and\ C\text{-}peptide\ (0\text{-}120\ min)}$ of HCM: NS $iAUC_{glucose\ (0\text{-}120\ min)}$ of LCM and HCM: Healthy ↓↓ *vs* T2DM

n, sample size; M, men; W, women; y, year; F, fat; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; AUC, area under the curve; ↑↑, significantly higher; ; *vs*, compared to; ↓↓, significantly lower; NS, no significant difference between diets

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'Table 2.3, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Wolpert et al., 2013)	Type 1 diabetes; 7 (5/2)	55 ± 12	Randomized, controlled, crossover, without washout Closed-loop glucose control periods starting with HFD or LFD and ending before next lunch.	18 h Glucose was sampled every 20–30 min during closed-loop control. Insulin levels were obtained every 20–60 min during closed-loop control.	1) High-fat dinner (HFD) 2) Low-fat dinner (LFD)	Standardized low-fat lunch and breakfast were respectively provided 6 h prior or 14 h after dinner meal. Breakfast: 688 kcal, 106 g C Lunch: 676 kcal, 106 g C The dinners provided standardized protein and carbohydrate (similar glycemic index) but differed in fat content and calories depending on subjects' energy requirement. HFD 1079 kcal, 60 g F (predominantly SAFA) High-fat day: 2444 kcal, 30% F, (80 g F, 96 g C) *Grilled cheese sandwich, green salad with added cheese, croutons, and grilled chicken and orange slices.* LFD 630 kcal, 10 g F Low-fats day: 1995 kcal, 14% F, 19% C (30 g F, 96 g C) *Grilled chicken breast, rice, broccoli, carrots, green salad, and grapes.*	After dinner Total insulin delivered (1 to 5 h), $AUC_{glucose}$ > 6.7 mmol/L (1 to 5 h) and $AUC_{insulin\ (5\text{-}10\ h)}$: HFD ↑↑ *vs* LFD Carbohydrate-to insulin ratio: HFD ↓↓ *vs* LFD After breakfast Total insulin delivered, $AUC_{glucose}$ >6.7 mmol/L and $AUC_{insulin}$: NS
(Gibbons et al., 2013)	Overweight/obese ; 16 (5/11)	45.6 ± 6.2	Randomized, crossover, single-blind	3 h Bleeding time: 10, 20, 30, 60, 90, 120 and 180 m in	1. High-fat meal (HFM) 2. High-carbohydrate meal (HCM)	Isocaloric 590 kcal mixed meal consisted of yogurt, honey, fruit, and coffee or tea HFM 50.3% F: 11.7% P: 38.0 % C HCM 3.2% F: 13.2% P: 83.6% C	Glucose and insulin: HCM ↑↑ *vs* HFM

n, sample size; M, men; W, women; y, year; h, hour; F, fat; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; AUC, area under the curve; ↑↑, significantly higher; *vs*, compared to; ↓↓, significantly lower; NS, no significant difference between diets

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'Table 2.3, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Tannous dit El Khoury et al., 2006)	Healthy; 10 (10/0)	22.2 ± 1.28	Randomized, controlled, crossover Run-in: 3 days	3 h Bleeding time: 15, 30, 60, 120, and 180 min	1. High-fat meal (HFM) 2. High-protein meal (HPM) 3. High-carbohydrate meal (HCM)	Liquid meal (mean 615 kcal) provided 30% of subjects' REE *Ensure Complete Balanced Nutrition®, soy/whey protein, sugar, SFO and water* <u>HFM</u> 50% F, 20% P, 30% C <u>HPM</u> 20% F, 50% P, 30% C <u>HCM</u> 20% F, 20% P, 60% C	Changes in insulin from baseline: HCM ↑↑ *vs* HFM and HPM at 15 and 30 min; HFM and HPM ↑↑ *vs* HCM at 180 min. Glucose: All meals ↑ at 15 and 30 min, returned to baseline at 60 min; HCM ↑↑ (peaked) *vs* HFM and HPM Changes in glucose from baseline: HCM ↑↑ *vs* HFM and HPM at 15 and 30 min. Insulin and glucose increase: HCM ↑↑ *vs* HFM and HPM
(van der Klaauw et al., 2013)	Healthy; 8 (3/5)	Mean: 32	Randomized. controlled, single blinded, crossover	4 h Bleeding time: 0, 30, 60, 90, 120, 180, 240 min	1. High-fat meal (HFM) 2. High-protein meal (HPM) 3. High-carbohydrate meal (HCM)	Test meal provided 20% daily energy requirement of each subject. <u>HFM</u> 60% F, 20% P, 20% C *Wholemeal pancakes were served with bacon and grated cheddar cheese* <u>HPM</u> 20% F, 60% P, 20% C *Pancake made up of high-P pancake mix and served with no-sugar maple syrup and full-fat Greek yoghurt.* <u>HCM</u> 20% F, 20% P, 60% C *Buckwheat pancakes served with bacon and maple syrup*	Glucose peak: NS Peaking time for glucose: 30 min for HFM and HPM, 60 min for HCM Insulin peak: HCM ↑↑ (2 times greater) *vs* HPM and HFM

n, sample size; M, men; W, women; y, year; h, hour; REE, resting energy expenditure; F, fat; P, protein; C, carbohydrate; %, % energy; ↑↑, significantly higher; *vs*, compared to; ↑, higher compared to baseline; ↓↓, significantly lower; NS, no significant difference between diets

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2.8.2 Chronic Effects of Dietary Fat Quantity on Insulin Secretion and Insulin Sensitivity

Growing evidence suggests the nutritional approach of chronically modulating fat/carbohydrate quantity even in the absence of significant weight loss plays an essential role in mediating insulin and glucose regulation differentially. Evidence is available for comparisons between 1) high-fat/high-MUFA and high-carbohydrate diet, and 2) high-fat/high-MUFA, high-fat/high-SAFA and high-carbohydrate diet.

In a non-randomized controlled crossover study (Bonanome et al., 1991; Chandler-Laney et al., 2014)), 19 T2DM subjects who were on hypoglycemic drugs but did not receive insulin therapy were fed high-carbohydrate diet (25% en fat, 10% en MUFA, 60% en carbohydrate) during the first and third dietary regimen whereas high-fat/high-MUFA diet (40% en fat, 25% en MUFA, 45% en carbohydrate) was given during the second dietary treatment. No appreciable difference in fasting C-peptide, insulin and glucose was reported after adaptation to each diet for 2 months. The diet allocation sequence however, was not randomized and this limited the findings. The observations were in line with the findings reported by Chandler-Laney et al. (2014). Adaptation to the 4-week background diet (39 *vs* 27% en fat) in this study also did not result in significant difference in fasting insulin and glucose concentrations, in overweight but healthy subjects (n = 35 in high-fat group, n = 29 in high-carbohydrate group). Significant differences, however, were found during the postprandial mixed meal challenge on the last day of dietary intervention where macronutrient compositions of the mixed meals and background diets were similar. The high-fat meal comprised oatmeal, rye bread, English muffins and pastry filled with scrambled eggs and cheese sauce; meanwhile the high-carbohydrate meal was made up of cereals and pancakes or waffles with syrup. Although the difference in fat amount did not exert significant effects on the markers at the fasting

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state, high-carbohydrate mixed meal increased postprandial insulin response and induced higher insulin peak together with earlier glucose peaking compared with the high-fat meal. However, the insulin response was not corrected for glucose and insulin sensitivity was not measured in that study, hence we could not interpret the dietary effect on β-cell responsiveness. It is still noteworthy that significant differences observed at postprandial although not at fasting state will increase T2DM risk because repetitive exposure to insulin hypersecretion may lead to β-cell dysfunction (Aston-Mourney, Proietto, Morahan, & Andrikopoulos, 2008). The reason for the non-significant difference at the fasting state exhibited in the two studies discussed above (Bonanome et al., 1991; Chandler-Laney et al., 2014) may be the moderate differences in fat and carbohydrates amount, i.e. 15 and 12% en. When higher amount of fat (MUFA) was substituted by the same portion of complex carbohydrates for 4 weeks, i.e. 25% en, plasma and urinary glucose concentrations and insulin requirements were increased in 10 hospitalized T2DM male patients (Garg, Bonanome, Grundy, Zhang, & Unger, 1988). The subjects received 1) high-carbohydrate diet (25% en fat) prepared using palm oil and corn oil; and 2) high-MUFA diet (50% en fat) enriched with olive oil, in a crossover randomized manner. Interpretation of these results is also confounded by the fact that not only fat quantity is altered but also fat quality.

The aforementioned findings however were found contradictory that high-fat/high-MUFA diet displayed detrimental effects compared to high-carbohydrate diet in two parallel design studies utilizing similar dietary treatments and macronutrient composition (Goree et al., 2011; Gower et al., 2012). The first one supplied daily eucaloric high-fat/high-MUFA (n = 34, 39% en fat, 43% en carbohydrate) and high-carbohydrate (n = 29, 27% en fat, 55% en carbohydrate) diets to healthy subjects for 8 weeks each. Lower fasting glucose change from baseline was observed on high-carbohydrate diet compared to high-fat diet. Furthermore, the chronic adherence to high-

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carbohydrate diet resulted in higher insulin secretion corrected for glucose after a standard liquid meal (Carnation Instant Breakfast and whole milk) at week 8 compared to baseline. B-cell responses during basal condition and after glucose stimulus, insulin release after glucose stimulus as well as basal insulin secretion rate were calculated as well but there was no significant difference. The other study by Gower et al. (2012) demonstrated that the similar high carbohydrate intake in 27 overweight or obese individuals with IFG resulted in lower fasting glucose concentration when compared to high-fat diet; furthermore β-cell response to glucose after a standard liquid meal was higher at week 8 compared to baseline whereas no difference was found in insulin sensitivity determined by calculation. On the other hand, insulin sensitivity was found higher on high-carbohydrate diet in another 42 healthy overweight or obese individuals however glucose concentration and postprandial β-cell response to glucose were similar between diets in the same study (Gower et al., 2012). The findings from the two studies suggest that replacement of fat with carbohydrate increased β-cell responsiveness accompanied by weaker glucose tolerance. The deterioration of β-cell responsiveness may be secondary to the lipotoxic effect of high fat intake on islet β-cell function.

Interesting results were reported when varied fats were compared with high-carbohydrate diet. A 4-week dietary intervention compared the consumption of 38% en fat (rich in SAFA or MUFA) *vs* 28% en fat from isocaloric diets in 59 healthy individuals (Perez-Jimenez et al., 2001). The study reported higher fasting insulin but not glucose on high-fat/SAFA-enriched diet compared to high-carbohydrate diet and high-fat/MUFA-enriched diet. Furthermore, high-fat/SAFA-enriched diet increased mean glucose in the modified insulin-suppression test and decreased both basal and insulin-stimulated 2-deoxyglucose uptake in peripheral monocytes. The findings indicated lower insulin sensitivity on high-fat/SAFA-enriched diet. One limitation in this study was the assignment of high-SAFA diet for all subjects during the first dietary intervention phase

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whereas the other two diets were randomly allocated for the subsequent intervention phases. Hence, the effect of SAFA may be confounded by non-randomized diet sequence allocation. The same research group conducted another study (Paniagua et al., 2007) with similar dietary treatments and duration in abdominally obese, insulin resistant subjects (*n* = 11). The high-SAFA and high-MUFA diets contained 38% en fat whereas the high-carbohydrate diet provided 28% en fat. Chronic high-SAFA consumption resulted in higher fasting glucose concentration compared with high-MUFA and high-carbohydrate intake; while high-MUFA diet contributed to higher insulin sensitivity and proinsulin level compared to others. On the other hand, higher postprandial glucose and insulin response and decreased glucagon concentration were reported with a breakfast meal challenge comprised of 200 mL skim milk, 70 g bread and 75 g marmalade (high-carbohydrate meal), in comparison to the meal sets consisting of 200 mL whole milk, 50 g bread and 25 g butter (high-SAFA meal) or 27 g olive oil (high-MUFA meal). Besides, lower insulin secretion was detected after high-SAFA challenge meal compared to the other meals however insulin corrected for glucose was not measured. The findings suggest that fat type may confound the differential effects of modulating fat amount on insulin and glucose dynamics.

The detrimental effects of high-fat/SAFA-enriched diet compared to high-carbohydrate diet were not seen in the other studies. A validated supermarket model for free food collection was applied in two controlled, parallel, randomized studies. The subjects achieved $\geqslant$10% weight loss following an 8 weeks energy restriction (800-1000 kcal) diet before participating in the 6-month studies. Subjects in both aforementioned studies collected free food products from the supermarket under *ad libitum* condition to achieve 35% en fat, 50% en carbohydrate; 40% en fat, 45% en carbohydrate; 25% en fat, 60% carbohydrate respectively for high-SAFA, high-MUFA or high-carbohydrate diet. Energy intake was aimed for weight maintenance over the 6 months period. Decreased

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fasting insulin and improved insulin resistance (HOMA-IR) from baseline were demonstrated on high-MUFA diet compared with high-SAFA diet and high-carbohydrate diet in 131 obese and overweight subjects (Due, Larsen, Mu, et al., 2008). Conflicting results were reported in another study examined 20 obese men and 26 premenopausal women (Due, Larsen, Hermansen, et al., 2008) where insulin sensitivity (Matsudas index), fasting glucose and insulin, and also insulin and glucose responses during an oral glucose tolerance test were found to be similar between both high-SAFA and high-MUFA with high-carbohydrate diets. This finding was in keeping with the results demonstrated in the multi-center RISCK study which recruited 548 subjects at risk of developing metabolic syndrome. The parallel study design employed by the RISCK investigators provided evidence that substitution of SAFA with MUFA (7% en) and CARB (10% en) for 24 weeks did not result in significant differences in insulin sensitivity between the 3 diet groups (Jebb et al., 2010). LIPGENE, another multicenter study (Tierney et al., 2011) supported the aforementioned findings. The LIPGENE trial was a randomized, parallel arm study that compared high-fat/high-SAFA or MUFA diet with high-carbohydrate diet in 417 subjects who had metabolic syndrome. After 12 weeks chronic exposure the fasting insulin, glucose and insulin sensitivity were not significantly different between 39 and 29% en fat consumption in this study population.

In summary, no clear conclusion can be drawn as to whether chronic carbohydrate or fat consumption is the culprit responsible for the down-regulation of β-cell responsiveness, worsening insulin sensitivity, and impaired insulin and glucose regulation. The observations and comparisons of trial results are often confounded by the type of fat used, source of fat, quantity of fat, study design (cross-over *vs* parallel arm, absence of controls etc), different methods of assessing postprandial response (mixed solid meal *vs* OGTT *vs* standardized liquid meal) and subject populations of different phenotypes and risk categories. Not all studies assessed insulin secretion corrected for

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glucose and insulin sensitivity (i.e. DI) thus weakening the validity of conclusions of reduced β-cell responsiveness. In addition, the total fat content for the high-fat diet protocols discussed here, was relatively high, i.e. more than 37% en. Such high fat content is unlikely to reflect the dietary fat intake for Asian populations, which tend to consume more carbohydrate. It would therefore be interesting to utilize an experimental protocol with high-fat diet intervention of less than 37% en total fat in comparison with a high-carbohydrate diet. Studies should also include larger sample populations and be of longer duration. Comparisons between lean controls and different phenotypes such as the nondiabetic overweight /obese group or T2DM group are also required.

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Table 2.4: Chronic Effects of Dietary Fat Quantity on Indices of Insulin Secretion and Insulin Sensitivity

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Bonanome et al., 1991)	T2DM without receiving insulin therapy; 19 (10/9)	55 ± 6	Non-randomized, controlled, crossover No wash-out	2 months	1. High-carbohydrate diet (HCD) 2. High-MUFA diet (HMD) Treatment sequence: HCD → HMD → HCD	Subjects received 3 isocaloric diets providing 15% en exchange between CARB and MUFA. <u>HCD</u> 25% F, 15% P, 60% C (44% complex, 16% simple); 10% SAFA, 10% MUFA, 5% PUFA <u>HMD</u> 40% F, 15% P, 45% C (36% complex, 9% simple); 10% SAFA, 25% MUFA, 5% PUFA *Olive oil*	Glucose, insulin, glycosylated hemoglobin and fructosamine: NS
(Chandler-Laney et al., 2014)	Overweight but healthy; HFD: 35 LFD: 29	21 – 50	Randomized, controlled, crossover	4 wk PP bleeding time: 0, 15, 60, 90, 120, 180, and 240 min.	1. High-fat diet (HFD) 2. Low-fat diet (LFD)	Eucaloric diets were provided by research center. <u>HFD</u> 39% F, 18% P, 43% C; GL ≤45 points/1000 kcal Test meal: 570 kcal; 38.1% F, 16.4% P, 47.2% C; 14.6% SAFA, 15.0% MUFA, 5.5% PUFA *Oatmeal, rye bread, English muffins, and Pillsbury Toaster Scramblers®.* <u>LFD</u> 27% F, 18% P, 55% C; GL ≥75 points/1000 kcal Test meal: 548 kcal; 23.8% F, 16.6% P, 62.3% C; 8.3% SAFA, 9.1% MUFA, 4.6% PUFA *Cereals and pancakes or waffles with syrup.*	Fasting glucose and insulin: NS <u>Postprandial measurements</u> Time of highest recorded glucose, 3 h glucose and 4 h glucose: HFD ↑↑ *vs* HCD Insulin peak and $iAUC_{insulin}$: HFD ↓↓ *vs* HCD Time of lowest recorded glucose, time of highest recorded insulin, time of lowest recorded insulin, glucose peak, glucose nadir, $iAUC_{glucose}$, insulin nadir, 3 h insulin and 4 h insulin: NS

n, sample size; M, men; W, women; T2DM, T2DM; y, year; wk, week; PP, postprandial; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; iAUC, incremental area under the curve; ↑↑, significantly higher; ↓↓, significantly lower; *vs*, compared to; NS, no significant difference between diets NS, no significant difference between diets

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Perez-Jimenez et al., 2001)	Healthy; 59 (30/29)	23.1 ± 1.8	Randomized, controlled, crossover Modified insulin suppression test on the last day of intervention: a continuous somatostatin (to inhibit endogenous insulin secretion), insulin and glucose were infused into vein.	4 wk Bleeding time during insulin suppression test: 150,160, 170, 180 min	1. High-SAFA diet (HSD) 2. High-MUFA/ Mediterranean diet (HMD) 3. High-carbohydrate diet (HCD) HSD → HMD/ HCD → HCD/ HMD	Daily meals were provided to subjects. <u>HSD</u> 2580 kcal; 37.7% F, 18.1% P, 44.2% C 22.6% SAFA, 10.1% MUFA, 5% PUFA *Palm oil and butter* <u>HMD</u> 2580 kcal; 38.4% F, 17.5% P, 44.1% C 9.2% SAFA, 24.4% MUFA, 5.2% PUFA *Olive oil* <u>HCD</u> 2531 kcal; 27.9 % F, 17.6% P, 54.5% C 9.2% SAFA, 13.5% MUFA, 4.8% PUFA *Biscuits, bread and jam*	<u>Fasting measurements</u> Insulin: HSD ↑↑ *vs* HMD and HCD Glucose: NS <u>Glucose uptake in monocyte</u> Basal glucose uptake and insulin-stimulated glucose uptake: HSD ↓↓ *vs* HMD and HCD <u>Modified insulin suppression test</u> Mean glucose: HSD ↑↑ *vs* HMD and HCD Mean insulin: NS

n, sample size; M, men; W, women; T2DM, T2DM; y, year; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; AUC, area under the curve; ↑↑, significantly higher; *vs*, compared to; ↓, lower compared to baseline; ↓↓, significantly lower; NS, no significant difference between diets

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Paniagua et al., 2007)	Abdominally obese with insulin resistance; 11 (4/7)	62 ± 9.4	Randomized, controlled, crossover	4 wk PP bleeding: 0, 15, 30, 60, 90, 120, 150, 180 min	1) High-SAFA diet (HSD) 2) High-MUFA Mediterranean diet (HMD) 3) High-carbohydrate diet (HCD)	Subjects adhered to ad libitum isocaloric diet intervention, followed by a 443 kcal breakfast meal for postprandial challenge. <u>HSD</u> 38% F, 15% P, 47% C; 23% SAFA, 9% MUFA, 6% PUFA *Test meal: 200 ml whole milk, 50 g bread, and 25 g butter.* <u>HMD</u> 38% F, 15% P, 47% C; 9% SAFA, 23% MUFA, 6% PUFA 75% MUFA was provided by extra virgin olive oil. *Test meal: 200 ml skim milk, 50 g bread, and 27 g olive oil* <u>HCD</u> 20% F, 15% P, 65% C; 6% SAFA, 8% MUFA and 6% PUFA *Test meal: 200 ml skim milk, 70 g bread, and 75 g marmalade*	Glucose: HMD and HCD ↓↓ *vs* HSD HOMA-IR : HMD ↓↓ *vs* HCD and HSD Insulin: NS Glucagon: HCD ↑↑ *vs* HSD and HMD Proinsulin: HMD ↓↓ *vs* HSD and HCD <u>PP measurements</u> Acute insulin response: HSD ↓↓ *vs* HCD and HMD $AUC_{glucose}$ and $AUC_{insulin}$: HCD ↑↑ *vs* HSD and HMD Glucagon: HCD ↓↓ *vs* HSD and HMD Proinsulin to insulin ratio at 30 and 60 min: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; HOMA-IR, homeostatic model assessment-insulin resistance; AUC, area under the curve; ↓↓, significantly lower; ↑↑, significantly higher; *vs*, compared to; NS, no significant difference between diets

University of Malaya

'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Garg et al., 1988)	T2DM receiving insulin therapy; 10(10/0)	56 ± 2	Randomized, controlled, crossover 7 – 22 days wash-out Daylong responses were measured at 2-h intervals in 8 subjects, on the last day of diet intervention.	4 wk 7-day run in	1. High-CARB diet (HCD) 2. High-MUFA diet (HMD)	Subjects were hospitalized in metabolic ward. <u>HCD</u> 25% F, 15% P, 60% C (47% complex, 13% simple); 9% SAFA, 9% MUFA, 7% PUFA *Palm oil and corn oil* <u>HMD</u> 50% F, 15% P, 35% C (22.5% complex, 12.5% simple); 10% SAFA, 33% MUFA, 7% PUFA *Olive oil*	<u>Fasting measurements</u> Plasma glucose: MUFA and CARB ↓, MUFA ↓↓ *vs* CARB Urinary glucose and insulin requirement: =, MUFA ↓↓ *vs* CARB Glycosylated hemoglobin: MUFA and CARB ↓, NS <u>Day long responses</u> $AUC_{glucose,}$ $AUC_{free\ insulin:}$ MUFA ↓↓ *vs* CARB

n, sample size; M, men; W, women; T2DM, T2DM; y, year; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; AUC, area under the curve; ↑↑, significantly higher; *vs*, compared to; ↓, lower compared to baseline; ↓↓, significantly lower; NS, no significant difference between diets

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Goree et al., 2011)	Healthy overweight; HFD: 34 HCD: 29	HFD: 35.6 ± 8.5 HCD: 34.6 ± 8.1	Randomized, parallel, single-blind Run-in: 3 days	8 wk Bleeding time Solid meal test: 0, 15, 60, 90, 120, 180 and 240 min Liquid meal test: 5 min from time zero to 30 min, every 10 min from 30 to 180 min, and at 210 and 240 min.	1.High-fat diet (HFD) 2.High-carbohydrate diet (HCD)	Eucaloric (2500 kcal/d) diets were allocated for daily consumption. HFD 39% F: 18% P: 43% C; < 10% SAFA; higher in Omega-3 and oleic acid GL ≤ 45 points / 1000 kcal HCD 27% F:18% P:55% C; GL ≥ 75 points / 1000 kcal Solid meal test (week 4) *Normal breakfast as given in the background diet* Liquid meal test (baseline and week 8) 7 kcal/kg body weight; 24% F, 17% P, 59% C (glucose intake range: 36 - 78 g) *Carnation Instant Breakfast (Nestle) and whole milk.*	Fasting measurements Glucose change from baseline: HFD ↑↑ *vs* HCD Insulin and insulin sensitivity: NS Solid meal test Peak insulin and $AUC_{insulin}$: HFD ↓↓ *vs* HCD Liquid meal test ϕ S: HFD ↓↓ *vs* HCD ϕ B, ϕ D, X0, SRB: NS Significant correlation between ϕ S and $AUC_{glucose}$
(Gower et al., 2012)	Overweight/obese; 69 (31/38) {NGT; 42 (12M/30F), IFG; 27 (19M/8F)} *6 drop-out; n=63	NGT: 32.9 ± 8.3 IFG: 38.6 ± 7.2	Randomized, parallel, single-blind	8 wk	1.High-fat diet (HFD) 2.High-carbohydrate diet (HCD)	Eucaloric (2500 kcal/d) diet; 12% en fat exchange with CARB HFD 39% F:18% P:43% C; GL ≤ 45 points / 1000 kcal *Whole-wheat bread, fruits and high-fiber vegetable.* HCD 27% F:18% P:55% C GL 75 points / 1000 kcal *White bread, mashed potatoes and rice.* Liquid meal test (baseline and week 8) *As referred to Goree et al., 2011	Glucose NGT: NS IFG: HFD ↑↑ *vs* HCD Insulin sensitivity NGT: HFD ↓↓ *vs* HCD IFG: NS PhiD NGT: NS IFG: HFD ↓↓ *vs* HCD

n, sample size; M, men; W, women; y, year; wk, week; CARB, carbohydrate diet; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; GL, glycemic load; PP, postprandial; ↑↑, significantly higher; *vs*, compared to; ↓↓, significantly lower; NS, no significant difference between diets; ϕ S, amount of insulin secreted for a given amount of glucose during nonbasal conditions; ϕ B, β cell response to glucose during the basal condition; ϕ D, response of the β cell to an increase in glucose; X0, measure of the amount of insulin released immediately after the glucose stimulus; SRB, measure of the basal insulin secretion rate; AUC, area under the curve; NGT, normal glucose tolerance; IFG, impaired fasting glucose; PhiD, dynamic (postprandial) β-cell response to glucose.

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Jebb et al., 2010)	At risk of developing metabolic syndrome; 548 (230/318) HS/HGI; 85 HM/HGI; 111 HM/LGI; 116 LF/HGI; 116 LF/LGI; 121	M:52 ± 10 W:51± 9	Randomized, controlled, parallel, non-blinded Intravenous glucose tolerance test (3 h) after diet intervention	6 months 4 wk run-in	1. HS/HGI 2. HM/HGI 3. HM/LGI 4. LF/HGI 5. LF/LGI	Isocaloric (2050kcal/d) diet varied in type of fat and glycemic index; ~7% en exchange between SAFA and MUFA (HS/HGI *vs* HM/HGI and HM/LGI); ~10% en exchange between SAFA and CARB (HS/LGI *vs* LG/HGI and LF/LGI); GI different at 8 points (HGI *vs* LGI). *Subjects were provided with key sources of fat (e.g. spreads, cooking oils and margarine) and carbohydrates (e.g. bread, pasta, rice and cereals)* HS/HGI 37.5% F, 20.5% P, 42% C 16% SAFA, 11.5% MUFA, 5.8% PUFA *Palm olein and milk fat* HM/HGI 35.6% F, 19.5% P, 44.9% C 9.5% SAFA, 16.2% MUFA, 6.6% PUFA *Spread, margarine rich in oleic acid derived from HOS and olive oil, HOS (additional source in Australia centre).* HM/LGI 35.7% F, 19.7% P, 44.6% C 9.6% SAFA, 16.3% MUFA, 6.9% PUFA LF/HGI 27.5% F, 21.4% P, 51.1% C 9.2% SAFA, 9.8% MUFA, 5.2% PUFA LF/LGI 26.1% F, 22.4% P, 51.5%C 8.3% SAFA, 9.7% MUFA, 5.1% PUFA	Insulin sensitivity, Sg, AIR_G, RQUICKI: NS

n, sample size; M, men; W, women; wk, week; HS, high-saturated fatty acids; HGI, high glycemic index; HM, high-monounsaturated fatty acids; LGI, low glycemic index; LF, low-fat; y, year; F, fat; P, protein; C, carbohydrate; %, % energy; ; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; HOS, high-oleic sunflower oil; Sg, glucose effectiveness; AIR_G, an indicator of endogenous insulin secretion; RQUICKI, Revised Quantitative Insulin Sensitivity Check Index; NS, no significant difference between diets

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Due, Larsen, Mu, et al., 2008)	Obese and overweight; 131 (55/76) No. of subjects completing dietary intervention: HMD; 39 LFD; 43 Control; 24	HMD: 29.2 ± 4.5 LFD: 27.3 ± 4.9 Control: 27.6 ± 5.1	Randomized, controlled, parallel	6 months 3 wk run-in * Recruited subjects were those who achieved ≥10% weight loss following an 8-week energy restriction (800-1000 kcal)	1. High-MUFA diet (HMD) 2. Low-fat diet (LFD) 3. Typical western diet (Control)	A validated supermarket model was used to allow subjects to collect all free foods where dietary intake was under *ad libitum* condition, with the aim to maintain weight. Moderate alcohol consumption was allowed. Food selection either from supermarket or outside was altered by trained personnel referring to dietary composition from database. <u>HMD</u> 40% F, 15% P, 45% C <10% SAFA, >20% MUFA, 5-10% PUFA *More whole-grain foods, nuts, and legumes* <u>LFD</u> 25% F, 15% P, 60% C <10% SAFA, 10% MUFA, 5-10% PUFA <u>Control</u> 35% F, 15% P, 50% C >15% SAFA, 10% MUFA, 0-10% PUFA	Changes in fasting glucose: NS Changes in fasting insulin and HOMA-IR: Significant difference between MUFA and LFD *vs* control (MUFA ↓; LFD and control ↑)(Due, Larsen, Hermansen, et al., 2008).

n, sample size; M, men; W, women; y, year; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; ↑↑, significantly higher; *vs*, compared to; NS, no significant difference between diets; HOMA-IR, homeostatic model assessment-insulin resistance; ↓, lower compared to baseline; ↑, higher compared to baseline; =, no changes from baseline.

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Due, Larsen, Hermansen, et al., 2008)	Obese men (20/0) Premenopausal women (0/26) Subjects allocation: HMD; 16 LFD; 18 Control; 12	28.0 ± 0.7	Randomized, controlled, parallel	6 months 3 wk run-in OGTT bleeding time: 30, 60, 90, 120 min * Recruited subjects were those who achieved an ≥8% weight loss following an 8-week energy restriction (800-1000 kcal)	1. High-MUFA diet (HMD) 2. Low-fat diet (LFD) 3. Typical western diet (Control)	A validated supermarket model was used to allow subjects to collect all free foods where dietary intake was under *ad libitum* condition, with the aim to maintain weight. Moderate alcohol consumption was allowed. Food selection either from supermarket or outside was altered by trained personnel referring to dietary composition from database. <u>HMD</u> 40% F, 15% P, 45% C <10% SAFA, >20% MUFA, 5-10% PUFA *More whole-grain foods, nuts, and legumes* <u>LFD</u> 25% F, 15% P, 60% C <10% SAFA, 10% MUFA, 5-10% PUFA <u>Control</u> 35% F, 15% P, 50% C >15% SAFA, 10% MUFA, 0-10% PUFA	Changes in fasting glucose and insulin: Significant difference between MUFA and control (MUFA ↓; control ↑); NS between MUFA *vs* LFD and control *vs* LFD Changes in Matsudas index (indication of insulin sensitivity), $AUC_{glucose}$ and $AUC_{insulin}$: NS <u>OGTT</u> Glucose and insulin change compared to baseline: NS

n, sample size; M, men; W, women; y, year; wk, week; OGTT, oral glucose tolerance test; F, fat; %, % energy; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; ↓, lower compared to baseline; ↑, higher compared to baseline; NS, no significant difference between diets; *vs*, compared to; AUC, area under the curve.

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'Table 2.4, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Tierney et al., 2011)	Metabolic syndrome; HSD: 100 HMD: 111 HCD: 106 HCn3D: 100	Mean 55	Randomized, controlled, parallel	12 wk	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. High-Carbohydrate diet (HCD) 4. High-Carbohydrate supplemented with n-3 PUFA (HCn3D)	Subjects maintained their habitual diet and consume exchangeable fat from supplied spread, cooking oil, baking fats, mayonnaise and biscuits. HSD 2070 kcal/d; 39.8 F%, 17.4% P, 41.2% C; 17.5% SAFA, 12.7% MUFA, 6.3% PUFA *Full-fat dairy foods, and replaced one snack product usually eaten with a HSFA cookie daily.* HMD 2070 kcal/d; 38.9% F, 16.9% P, 42.1% C; 10.4% SAFA, 18.8% MUFA, 6.7% PUFA *Low-fat dairy foods, high-MUFA mayonnaise or a handful of hazelnuts or cashew nuts and replaced normally consumed snack product with HMUFA biscuit.* HCD 1965.7 kcal/d; 29.6 F%, 19.2% P, 49.4% C; 8.6% SAFA, 11.8% MUFA, 6% PUFA *Reduced the intake of high-fat snacks, consumed 2 extra portions of complex CARB daily and took the supplied capsules daily.* HCn3D 1975.3 kcal/d; 29.1% F, 18.3% P, 50.2% C; 8.7% SAFA, 11.4% MUFA, 5.7% PUFA *Reduced the intake of high-fat snacks, consumed 2 extra portions of complex CARB daily and took the supplied capsules daily.*	SI, fasting insulin, glucose concentrations and HOMA-IR: NS

n, sample size; M, men; W, women; y, year; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; NS, no significant difference between diets; SI, insulin sensitivity; HOMA-IR, homeostatic model assessment-insulin resistance

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2.8.3 Acute Effects of Different Types of Dietary Fat on Insulin Secretion and Insulin Sensitivity

The acute effects of SAFA *vs* MUFA on insulin and glucose regulation were compared in studies using different types of test meals. In two crossover double blind studies (Poppitt et al., 2006) investigators fed the subjects with a mixed meal consisting of a drink and a muffin incorporated with test fats. Poppitt et al. (2006) supplied the mixed meal to 18 healthy subjects where high-SAFA or high-MUFA butter was baked into the muffin. The amount of butter was 5.3 g per 239 kcal according to subjects' daily intake. No significant difference in the 6 h insulin change was detected after the consumption of a blueberry muffin, a milk- and sugar-free decaffeinated hot beverage and/or a glass of cold water. High-SAFA meal was 15.6% en richer in SAFA whereas high-MUFA meal was enriched with additional 10% en of MUFA, as compared to the other meal. In this experiment, the failure to achieve PUFA content standardization between meals and also head-to-head comparison of test fatty acids limited interpretation of the findings. A study by Filippou, Berry, Baumgartner, Mensink, and Sanders (2014) strictly controlled the energy exchange of test fatty acids, where SAFA meal and MUFA meal were respectively 20% en richer in SAFA or MUFA. The test meals comprised a strawberry flavored milkshake and a muffin baked with 50 g palm olein or high oleic sunflower oil. No significant differences were found in postprandial glucose, insulin, C-peptide and insulin corrected for glucose in 50 healthy subjects. Women had a lower AUC increase in glucose in the first hour and higher AUC increase in insulin and C-peptide at early phase when compared to men, however insulin corrected for glucose was not evaluated, Clegg et al. (2012) compared the effects of SAFA *vs* MUFA on glucose response to 3 pancakes and 200mL of water in 10 healthy subjects. The meal included 50 g of carbohydrate and 202 kcal test fat, namely 26.9 g butter (SAFA) and 22.4 g olive oil (MUFA) respectively. No

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difference between the effects of SAFA and MUFA was found over the course of the 2 h postprandial evaluation. In summary, the 3 aforementioned studies reported consistent findings that SAFA (from palm olein or butter) does not impair glucose or insulin regulation in healthy subjects, when challenged with a test meal in the form of a muffin alone, muffin + shake or pancakes. Similar investigations are lacking in subjects with the insulin resistance phenotype.

3 other studies looked at the response solely to liquid test meals where test fats were infused into a drink or soup for subjects' consumption (Beysen et al., 2002; Thomsen et al., 2003; P. R. van Dijk et al., 2012). In a randomized crossover, non-blinded study (Thomsen et al., 2003), 12 overweight T2DM subjects were fed white bread, water and energy free soup to which was added butter, olive oil or blank (control). During the 8-h measurement, postprandial glucose and insulin excursions were not significantly different between SAFA and MUFA meals. In concordance with Thomsen et al. (2003), another randomized crossover study (P. R. van Dijk et al., 2012) also reported similar impact of SAFA and MUFA at 40% en on insulin and glucose response in 50 subjects (lean healthy, obese healthy and obese T2DM) who consumed strawberry-flavored shakes mixed with 95 g palm olein or high oleic sunflower oil. However, conflicting findings were reported in a study conducted by Beysen et al. (2002) involving 8 healthy subjects. In that study the test drink was prepared with 50 g palm stearin, olive oil or water (control) added into 45.5 g water with sweetener, emulsifier and cocoa powder. The palm stearin and olive oil were rich in respectively 59% SAFA or 69% MUFA, leading to differing amounts of test fatty acid in the comparison. Heparin infusion (to increase lipolysis) was given from 90 to 300 min; and a hyperglycemic clamp was carried out at 210 to 300 min to assess insulin secretion in response to intravenous glucose. No significant differences were detected in pre-clamp insulin secretion rate, pre-clamp glucose, efficacy of insulin conversion from proinsulin during the first 10 min of clamp, insulin secretion after the

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clamp, mean insulin after heparin and insulin sensitivity after clamp. However, the acute MUFA exposure was associated with higher total C-peptide and insulin during the hyperglycemic clamp; and higher first phase 210- 220 min proinsulin during the clamp. The small sample size and the unequal % test fatty acid in the 2 treatments limited the application of findings. Further, the results contradict another study in 11 subjects with T2DM but not receiving insulin therapy (Shah, Adams-Huet, Brinkley, Grundy, & Garg, 2007). Increased insulin response as measured by rank transformation of insulin values after subtraction of baseline was found on the meal richer in 38% en SAFA instead of MUFA, whereas no difference in glucose response. The study compared 51 g palm oil and olive oil in a set of farina, egg substitute, ham, white bread, skim milk and orange juice. The strength of this acute study was a standardization of 3 day-background diet prior to the meal test. The conflicting results found in the 2 previously discussed studies may be due to the different methods of assessment and the types of food in the test meal. The results were also limited by the small sample size (n = 8 *vs* 11).

In short, there is no clear evidence of the detrimental effect of high-SAFA meal on insulin and glucose regulations compared to high-MUFA meal. Most studies (5 out of 7) reported similar postmeal impact of SAFA and MUFA on insulin secretion after the consumption of liquid meal or mixed meal. Further research is required to explore possible differential effects of SAFA and MUFA on insulin and glucose regulation in subjects who are abdominally obese, those with T2DM and metabolic syndrome.

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Table 2.5: Acute Effects of Different Types of Dietary Fat on Indices of Insulin Secretion and Insulin Sensitivity

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Clegg et al.. 2012)	Healthy; 10 (2/8)	26.4 ± 3.9	Randomized, balanced, single-blind	Finger pricks at 0, 15, 30, 45, 60, 90 and 120 min	1. Medium chain triglycerides (MCT) 2. Sunflower oil (SO) 3. Olive oil (OO) 4. Butter 5. Control (no oil)	Test food consisted of 3 pancakes and 200 mL of water, containing 50 g of available carbohydrate and 202 kcal of either: 1. 24.3 g MCT 2. 22.4 g SO 3. 22.4 g OO 4. 26.9 g butter 5. control	$iAUC_{glucose}$: Control ↑↑ *vs* OO and MCT; NS for control *vs* SO and butter; NS between all test fats
(Shah et al., 2007)	T2DM and not receiving insulin therapy; 11 (11/0)	54.6 ± 12.2	Randomized, controlled, crossover	PP bleeding: 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min Run-in: 3 – 4 days	1. Palmitic acid 2. Oleic acid 3. Linoleic acid 4. EPA and DHA	Each test meal provided 1000 kcal, made up of 50% F, 15% P and 35% C. Test meal contained farina, egg substitute, ham with 5% fat, white bread, skim milk, orange juice, and 51 g of test oil. <u>Palmitic acid</u> 51.3% SAFA, 38.9% cis-MUFA, 9.6% n-6 PUFA and 0.2% n-3 PUFA *Palm oil* <u>Oleic acid</u> 13.8% SAFA, 77.1% cis-MUFA, 8.3% n-6 PUFA and 0.6% n-3 PUFA *Olive oil* <u>Linoleic acid</u> 6.5% SAFA, 15.0% cis-MUFA, 78.0% n-6 PUFA *Safflower oil* <u>EPA and DHA</u> 17.6% SAFA, 29.4% cis-MUFA, 2.3% n-6 PUFA and 38.6% n-3 PUFA *Salmon oil*	Glucose response, peak insulin time, $AUC_{insulin}$ and $AUC_{glucose}$: NS Insulin response: Palmitic acid and linoleic acid ↑↑ *vs* oleic acid and EPA and DHA. Insulin after subtraction of baseline value: Linoleic acid ↑↑ *vs* oleic acid and EPA and DHA; palmitic acid ↑↑ *vs* EPA and DHA Peak insulin concentration: Linoleic acid ↑↑ *vs* oleic acid and EPA and DHA.

n, sample size; T2DM, Type 2 Diabetes Mellitus; M, men; W, women; y, year; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; %, % energy; NS, no significant difference between diets; ↑↑, significantly higher; *vs*, compared to.

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'Table 2.5, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(P. R. van Dijk et al., 2012)	Lean nondiabetic, obese nondiabetic and obese type 2 diabetes; 50 (50/-)	50-70	Randomized , controlled, crossover Wash out: ≥1 week	PP bleeding: 2 and 4 h	1. SAFA 2. MUFA 3. n-3 PUFA	987 kcal strawberry flavour shake consisting 87% F, 4% P, 9% C was prepared with low-fat yoghurt, low-fat milk, 7.5 g of sugar and 95 g of the test fat. 165 mg Tocoblend L50 (Vitamin E) was added to prevent lipid oxidation. <u>SAFA</u> 47% SAFA, 34% MUFA, 6% PUFA *95 g palm oil* <u>MUFA</u> 7% SAFA, 72% MUFA, 7% PUFA *95 g high oleic sunflower oil* <u>n-3 PUFA</u> 29% SAFA, 23% MUFA, 35% PUFA *40 g palm oil + 40 g Marinol D-40 of which 40% was DHA*	Increase in insulin: n-3 PUFA ↓ *vs* SAFA and MUFA at 2h Glucose response: NS Higher insulin response in obese diabetic *vs* lean and obese non diabetic.
(Poppitt et al., 2006)	Lean, healthy; 18 (18/0)	23 ± 4.2	Randomized. controlled, cross-over, double-blind	Bleeding time: 0, 1, 3, 6 h	1. High SAFA diet (HSD) 2. Low-SAFA diet (LSD).	Lipid content based upon subjects' total daily energy intake (5.3 g butter per 239 kcal of daily intake). 70.8% F,4.9% P, 23.2% C; Mean calorie:747.6 kcal; Mean butter fat: 59 g from 73 g butter. *A blueberry muffin incorporated with butter and a milk- and sugar-free decaffeinated hot beverage and/or glass of cold water* HSD: 70.5% SAFA, 22.1% MUFA, 3.0% PUFA; 31.5% palmitic acid, 18.6% oleic acid, 1.2% linoleic acid LSD: 54.4% SAFA, 32% MUFA, 10.5% PUFA; 18.8% palmitic acid, 30.0% oleic acid, 7.2% linoleic acid	Insulin change over 6 h: NS Insulin peak: 1 h

n, sample size; M, men; W, women; y, year; h, hour; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; %, % energy; ↓, significantly lower; *vs*, compared to.

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'Table 2.5, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Filippou, Berry, et al., 2014)	Healthy; 50 (25/25)	24.9	Randomized, crossover, double-blind	PP bleeding: 0, 15, 30, 60, 90 and 120 min	1. HOS 2. PO 3. IPO 4. Lard	846 kcal mixed meal consisted of a muffin and a milkshake providing 53% F: 40% C: 6.6% P. 50 g test oils were baked into muffins. HOS 2.3% PA:1.5% SA: 42.8% OA 0.6% PA at sn-2 position PO 22.6% PA: 2.1% SA: 21.1% OA 9.2% PA at sn-2 position IPO 22.6% PA: 2.1% SA: 21.1% OA 39.1% PA at sn-2 position Lard 15.5% PA: 8.6% SA: 20.9% OA 70.5% PA at sn-2 position	$iAUC_{insulin/glucose(0\text{-}120\ min)}$, glucose, insulin and C-peptide: NS Increase in glucose at the first h: women ↓↓ *vs* men Increase in insulin and C-peptide at the early phase: women ↑↑ *vs* men
(Thomsen et al., 2003)	Overweight with T2DM; 12 (7/5)	64 ± 4	Randomized, crossover, non-blinded	Bleeding in 30 min intervals between 0 – 480 min	1.SAFA 2.MUFA 3.Placebo	An energy free soup added with test oil/placebo, consumed together with white bread providing 50 g C and 250 mL tap water SAFA 1004 kcal; 71.7% fat; 51.6% SAFA *100 g unsalted butter consisted of 20% water* MUFA 1004 kcal; 71.7% fat; 51.3% MUFA *80 g olive oil* Placebo 261 kcal *Blank*	The highest glucose: Placebo Glucose and insulin peak: Earlier after placebo *vs* SAFA and MUFA PP glucose, insulin and $AUC_{insulin/glucose}$: NS between SAFA and MUFA $iAUC_{glucose}$: MUFA ↓↓ *vs* placebo; NS for SAFA *vs* placebo; NS for SAFA *vs* MUFA $iAUC_{insulin}$: NS for MUFA *vs* SAFA; Placebo ↓↓ vs SAFA and MUFA

n, sample size; M, men; W, women; y, year; T2DM, Type 2 Diabetes Mellitus; HOS, high oleic sunflower oil; PO, palm olein; IPO, interesterified palm olein; F, fat; P, protein; C, carbohydrate; PA, palmitic acid; SA, stearic acid; OA, oleic acid; %, % energy; iAUC, incremental area under the curve; *vs*, compared to; NS, no significant difference between diets; ↓↓, significantly lower

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'Table 2.5, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Beysen et al., 2002)	Healthy; 8 (4/4)	21-54; median 29	Randomized, crossover, non-blinded Wash out: 2 wk	PP Bleeding time: 0, 30, 60, 90, 120, 150, 180, 225, 240, 255, 270, 285, 300 min Hyperglycaemic clamp at 210 – 300 min to raise blood glucose concentrations to 10 mmol/l	1. SAFA 2. MUFA 3. PUFA 4. Water + saline	100 g hot chocolate-flavoured drink consisted 50 g of oil or water (for control), 0.5 g emulsifier, 1 g of sweetener, 3 g cocoa powder and 45.5 g of water. The drink is carbohydrate and protein free. <u>SAFA</u> 59% palmitic acid *Palm stearin* <u>MUFA</u> 69% oleic acid *Olive oil* <u>PUFA</u> 74% linoleic acid *Refined safflower oil* *A bolus of heparin (500 IU) was given at 90 min and this was immediately followed by a 0.4 IU•kg–1•min–1 heparin infusion (from 90 min until end of experiment) to increase the action of lipoprotein lipase for the alteration of NEFA pool. Control (water) was not given heparin but saline.*	Pre-clamp insulin secretion rate, pre-clamp glucose, glucose after clamp, insulin after heparin infusion (90 – 210 min), second phase to: first phase insulin ratio, insulin clearance (C-peptide: insulin ratio) at 210 min, efficacy of insulin conversion (proinsulin: insulin ratio) during the first 10 min of clamp: NS Insulin secretion rate after clamp: All ↑; SAFA, MUFA and PUFA ↑↑ *vs* water; NS for SAFA, MUFA and PUFA Mean insulin after heparin infusion (90 – 210 min): SAFA, MUFA and PUFA ↑↑ *vs* water Total insulin, total proinsulin and second phase (220-300 min) C-peptide during clamp: MUFA ↑↑ *vs* PUFA Total insulin, total C-peptide and first phase (210-220 min) proinsulin during clamp: MUFA ↑↑ *vs* SAFA Total insulin and total proinsulin: PUFA ↓↓ *vs* SAFA Insulin sensitivity (at 230 and 245 min): NS for SAFA, MUFA and PUFA *vs* water

n, sample size; M, men; W, women; wk, week; y, year; HOS, high oleic sunflower oil; PO, palm olein; IPO, interesterified palm olein; F, fat; P, protein; C, carbohydrate; %, % energy; iAUC, incremental area under the curve; vs, compared to; NS, no significant difference between diets; ↑, higher compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower

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2.8.4 Chronic Effects of Different Types of Dietary Fat on Insulin Secretion and Insulin Sensitivity

Two early studies in healthy subjects reported significant differences between the impact of SAFA and MUFA on insulin sensitivity (Lovejoy et al., 2002; Vessby et al., 2001) as measured by intravenous glucose tolerance test (IVGTT). The Kuopio, Aarhus, Naples, Wollongong and Uppsala (Vessby et al.) study was a multicenter, crossover study with large sample size (n = 162). The healthy subjects were instructed to consume a 3-month isocaloric *ad libitum* diet consisting of 37% en fat, test fats were allocated to subjects for meal preparation at home. Fat sources used for the 9% en test fatty acid-enrichment were butter and margarine for the SAFA diet or high oleic sunflower oil and olive oil for MUFA diet. Although the study reported similar body weight throughout the intervention and compatible fatty acid changes of serum phospholipids and cholesterol esters as the measurement of dietary compliance, 95% of the subjects consumed 29-45% en fat despite the target fat intake of 37% en. No significant difference was found between diets in terms of insulin sensitivity although SAFA reduced by 10% and MUFA improved by 2% insulin sensitivity compared to baseline. Furthermore, no difference was observed with regards to fasting insulin and glucose; and first-phase insulin response during the IVGTT. DI was not measured in this study. The subgroup analysis however reported significantly lower insulin sensitivity with SAFA enrichment compared to MUFA in the subgroup consuming less than 37% en total fat. Nevertheless, the finding is limited by the unreported sample size in the mentioned subgroups and the results may be confounded by the wide range of total fat intake in the *ad libitum* diet. Based on the study by Lovejoy et al. (2002), it was however demonstrated that fasting plasma glucose, insulin, glucose effectiveness, acute insulin response to glucose, DI, insulin secretion and insulin sensitivity were not affected by enrichment of SAFA or MUFA when total fat intake is

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less than 30% en in 25 healthy overweight and lean subjects. This randomized, crossover, double-blind study provided daily meals that contained 28% en fat, utilizing various vegetable oils and decholesterolized butter. However the fat source for each diet was not specified in this study. Subgroup analysis was done between lean and overweight groups. The enrichments of 9% en palmitic acid impaired insulin sensitivity reducing it by 24% from baseline, in a subgroup of 7 overweight subjects. The difference was however not statistically significant. Taken together, the two studies reported that SAFA, notably from dairy fat and unknown types of vegetable oils and margarine, impaired insulin sensitivity compared to baseline. The studies also suggested that detrimental effect of SAFA on insulin sensitivity may be more evident when total fat intake is between 30–37% en. This however is in conflict with findings by Jebb et al. (2010), Vega-Lopez, Ausman, Jalbert, Erkkila, and Lichtenstein (2006) and Filippou, Teng, Berry, and Sanders (2014) whose studies utilized a total of 37, 28 and 30% en fat, respectively and found no significant difference in fasting insulin, glucose and insulin sensitivity.

The subjects in the parallel-arm design KANWU study (Jebb et al., 2010) who were at risk of developing metabolic syndrome, were allocated a high-SAFA/high-glycemic index (GI), high-MUFA/high-GI or high-MUFA/low-GI diet for 24 weeks with a targeted 8% en exchange of SAFA for MUFA. No significant differences were found in insulin sensitivity (as measured by RQUICKI), glucose effectiveness and insulin secretion as measured by IVGTT between SAFA and MUFA. Fat sources used for SAFA diet included palm oil and milk fat while those for MUFA diet were olive oil and high oleic sunflower oil. In this trial, one limitation is that the free-living subjects on SAFA diet in actual fact consumed additional 6.5% en SAFA in exchange for 4.6% en MUFA compared with MUFA diet, although the study had actually aimed for an 8% en exchange between SAFA and MUFA. The randomized crossover 5-week study that was performed in 15 postmenopausal women who were hypercholesterolmic (Vega-Lopez et al. (2006)

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also had a similar limitation where 8.4% en SAFA was exchanged for 4.5% en MUFA when comparisons were made between diets enriched with palm oil (SAFA) and canola oil (MUFA). This study reported no significant differences in fasting insulin, glucose, and homeostatic model assessment-insulin resistance (HOMA-IR). In line with the two studies discussed above, a 6-week randomized crossover study (Filippou, Teng, et al., 2014b) involving 41 healthy subjects reported SAFA and MUFA exert similar effects on fasting glucose, insulin, C-peptide and homeostatic model assessment 2-insulin resistance (HOMA2-IR). The test diets allowed 8% en exchange between SAFA and MUFA sourced from respectively palm olein and high oleic sunflower oil. During the standard meal challenge upon the completion of each dietary intervention where a palm oil-baked muffin and a glass of milkshake were ingested as a standardized test meal across all diet groups, similar effects of SAFA and MUFA were shown with regards to postprandial insulin and glucose response.

Significant differences were reported when a blend of palm olein and butter (SAFA) was compared with Mediterranean diet (MUFA) which emphasized fruits, vegetables, nuts, grains and olive oil, in a randomized crossover study (Perez-Jimenez et al., 2001). The 59 healthy subjects were provided SAFA meals followed by MUFA meals. The 6-week dietary interventions contained 38% en total fat where SAFA and MUFA differed by 14% en in both diets. The adherence to high-SAFA diet caused reduced insulin sensitivity, as indicated by 1) an elevation in fasting insulin, 2) reductions in both basal and insulin-stimulated 2-deoxyglucose uptake in peripheral monocytes, and 3) a decrease in mean plasma glucose concentrations during the insulin suppression test. However, the incomplete randomization of the intervention sequence may be a confounding factor. Future studies are warranted to investigate if the beneficial properties of MUFA-enriched/Mediterranean diet were contributed by the other food components but not

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solely by MUFA in Mediterranean diet, such as polyphenols in olive oil that own anti-inflammatory properties and hence prevent the progression of insulin resistance.

In contrast with the preceding studies, the total fat quantity consumed by subjects in the following three studies amounts to more than 37% en. LIPGENE was a large multi-centre, parallel arm study (Tierney et al., 2011) in metabolic syndrome subjects who consumed 39% en fat daily. Sample size for the SAFA and MUFA group was respectively 100 and 111. The subjects were instructed to follow a 12-week *ad libitum* SAFA diet (n=100) comprising full-fat dairy foods or MUFA diet (n=111) enriched with low-fat dairy foods, mayonnaise and hazelnut. High-SAFA or high-MUFA biscuits were distributed to subjects to replace daily snacks. 7% en exchange between SAFA and MUFA did not result in significant differences in fasting insulin, fasting glucose, insulin resistance (calculated by HOMA-IR); insulin response to glucose and insulin sensitivity as measured by IVGTT; DI was not reported. The study conducted by Uusitupa et al. (1994) assigned 10 healthy subjects to a 3-week *ad-libitum* diet intervention, where total fat intake was 40% en. The subjects were provided butter and dairy products during SAFA intervention (20% en SAFA, 12% en MUFA, 4% en PUFA) ; and low erucic acid rapeseed oil margarine for MUFA diet (9% en SAFA, 19% en MUFA, 10% en PUFA). There were no significant differences in glucose disappearance rate and insulin response as evaluated by IVGTT. However, both overall glucose response and glucose level between 30 to 70 min were found lower on MUFA diet compared to SAFA diet. The finding was however limited as DI and insulin sensitivity was not measured in this study and the non-standardization of PUFA content leading to unequal % en exchange of test fatty acids. Paniagua et al. (2007) recruited 11 abdominally obese, insulin resistant subjects to follow a 4-week *ad libitum* diet. The diet contained 38% en fat, fat source for SAFA diet was unspecified while that for MUFA diet was virgin olive oil. Fasting glucose, insulin and glucagon were found to be similar after both diets. However, HOMA-IR,

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fasting glucose and pro-insulin concentrations were significantly lower on MUFA diet compared to SAFA diet. A mixed meal challenge was performed after the dietary intervention. Whole milk and butter; or skim milk and olive oil were respectively incorporated into SAFA and MUFA test meals. Acute insulin response on SAFA meal were significantly lower compared to MUFA meal, but no significant difference was detected in postprandial insulin and glucose response.

The beneficial properties of olive oil as observed in the study conducted by Paniagua et al. (2007) were not obviously displayed in another randomized parallel study utilizing refined olive oils enriched with 9% en SAFA or MUFA in which total fat intake was not reported (S. J. van Dijk et al., 2009). In this 8-week exposure, MUFA as compared to SAFA did not increase insulin sensitivity evaluated by hyperinsulinemic-euglycemic clamp in 20 abdominal overweight subjects, who were provided with 90% daily meals and allowed to choose some low-fat products. A randomized parallel arm study conducted by Bos et al. (2010) compared 8-week high-SAFA/Western style (37% en fat), high-MUFA/refined olive oil (40% en fat) and Mediterranean/extra virgin olive oil (40% en fat) diets in 57 mildly abdominally obese subjects, where the diets supplied 9% en exchange between SAFA and MUFA. The study reported significantly lower insulin concentration and insulin sensitivity as measured by HOMA-IR after high-SAFA diet compared to baseline, whereas there were no changes in either parameter on the other diets. Nevertheless, the changes were not significantly different between dietary intervention arms. Fasting insulin, C-peptide and glucose were also similar in all 3 arms. Hyperinsulinemic-euglycemic clamp was conducted in a subgroup of 30 subjects during the last 3 weeks of dietary intervention; no difference was detected in insulin sensitivity between diets. These findings echo those of another study in which obese men and premenopausal women consumed 6-month SAFA (n = 12) and MUFA (n = 16) diets (Due, Larsen, Hermansen, et al., 2008) in a randomized parallel arm study. The subjects were

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advised to select foods available in a validated supermarket model, for a 10% en exchange between SAFA and MUFA. SAFA diet provided 35% en fat while MUFA diet contained 40% en fat. Food selection was altered referring to dietary composition and those who were on MUFA treatment consumed more whole-grain food, nuts and legumes. Compared to baseline, MUFA diet lowered while SAFA diet increased fasting insulin and glucose concentrations. However, no significant differences were found in insulin and glucose response, and insulin sensitivity measured during a 2-h oral glucose tolerance test between the intervention arms. Interpretation of these results is confounded by the differing proportions of fat and carbohydrate in the 2 diets.

Beyond the evidence showing beneficial effect of MUFA compared to SAFA on insulin sensitivity, there is also research demonstrating that the higher is the MUFA content, the higher is the insulin concentration and insulin resistance (HOMA-IR) (Gill et al., 2003). These results were reported in a randomized crossover study involving 35 moderately hypercholesterolemic subjects. During the 6-week diet intervention, the subjects consumed from low-fat foods and baked products prepared with margarine and spread from unknown source. MUFA content in the 3 dietary treatments were respectively 13.7, 10.3, and 7.8% en. The conflicting effects of MUFA on insulin sensitivity were yet to be clarified by future studies.

On the whole, chronic exposure to different types of fatty acid appear to influence the regulation of insulin and glucose and insulin sensitivity in healthy and obese individuals. However, the evidence demonstrating the detrimental effects of both SAFA and MUFA are not consistent and clear. Beneficial effects of MUFA have been demonstrated but vary with MUFA source: with ingestion of extra virgin olive oil but not canola demonstrating metabolic improvements. Two large studies of prolonged exposure to SAFA or MUFA utilizing reference methods such as the IVGTT to evaluate insulin sensitivity have demonstrated no significant difference in insulin sensitivity between diets

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in healthy subjects as found in the KANWU study (9% en exchange, 37% en total fat diet) (Vessby et al., 2001) and those with metabolic syndrome in the RISCK study (7% en exchange, 39% total fat en) (Tierney et al., 2011). The KANWU study (Vessby et al., 2001) however did find a longitudinal reduction in insulin sensitivity in the SAFA group but not the MUFA group. However some studies with larger en exchange of 14% for example in the paper by Perez-Jimenez et al. (2001) as part of a total fat 38% en diet demonstrated that SAFA significantly reduced insulin sensitivity as measured by deoxyglucose uptake and the modified insulin suppression test which are less accurate measures of insulin sensitivity. The study with the largest sample population of those with metabolic syndrome and longest exposure of 6 months i.e. RISCK study however found that the achieved 6.5% en exchange of SAFA for 4.6% en MUFA in a total fat 35-37% en diet had no significant effect on insulin sensitivity as measured by IVGTT. It could be argued however that this relatively small energy exchange would have no effect. Further investigations are needed to examine the effect of the quantum of % en exchange SAFA and MUFA in the context of normal and high total fat diets on insulin sensitivity and secretion utilizing reference methods that can evaluate DI such as the IVGTT. In addition, future studies should consider having larger sample size, longer duration, and better study design to reduce confounding factors such as different proportion of fat and carbohydrates between diets, and unequal % en exchange of test fatty acids. The issue of differential impact of the dietary source of MUFA and SAFA on metabolism should also be studied.

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Table 2.6: Chronic Effects of Different Types of Dietary Fat on Indices of Insulin Secretion and Insulin Sensitivity

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Lovejoy et al., 2002)	Healthy overweight and lean; 25 (12/13)	28.0 ± 2.0	Randomized, controlled, crossover, double-blind IVGTT at the end of intervention	4 wk; 2 wk wash-out	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. High-trans fat diet (HTD)	Meals and snacks were provided to subjects where all diets comprised 28% F, 15% P, 57% C. *Fat blends were prepared for each diet using various vegetable oils and decholesterolized butter fat, and were used to provide 15% fat.* HSD 9% palmitic acid HMD 9% cis-oleic acid HTD 9% trans-oleic acid	Glucose, insulin, insulin sensitivity, disposition index, glucose effectiveness, acute insulin response to glucose; basal, mean and total insulin secretion: NS Insulin sensitivity: overweight (n=7) ↓; NS between overweight and lean
(Vega-Lopez et al., 2006)	LDL-C ≥130 mg/dL; 15 (5/10) *Postmenopausal women	63.9 ± 5.7	Randomized, controlled, crossover	5 wk	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. High-PUFA diet (HPD)	Subjects were provided 2065 kcal/d (for females) or 3050 kcal/d (for males) diet containing energy from 28% F, 15% P and 57% C. Test fat provided 20% F. HSD *Palm oil* HMD *Canola oil* HPD *Soybean oil*	Insulin, glucose, and HOMA-IR: NS

n, sample size; M, men; W, women; y, year; wk, week; LDL-C, low density lipoprotein-cholestorol; F, fat; %, % energy; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; NS, no significant difference between diets.

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(S. J. van Dijk et al., 2009)	Abdominal overweight; 20 (10/10)	SAFA: 52.0 ± 6.3 MUFA: 58.9 ± 5.3	Randomized, controlled, parallel, non-blinded Euglycemic-hyperinsulinemic clamp (baseline, run-in and in the final 2 week of intervention)	8 wk 2 wk run-in	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD)	Isocaloric diets where 90% en supplied by diet, 10% en limited choice of low-fat product. HSD 19% SAFA and 11% MUFA *Refined olive oil* HMD 11% SAFA and 20% MUFA *Refined olive oil*	Insulin sensitivity: NS
(Vessby et al., 2001)	Healthy with normal or overweight BMI; SAFA: 83 MUFA: 79	SAFA: 48.9 ± 7.5 MUFA: 48.2 ± 8.1	Randomized , controlled, parallel, single-blind Intravenous glucose tolerance test (glucose dose: 300 mg/kg; 0.03 U/kg insulin injected 20 min after glucose dosing)	3 months 2 wk run-in	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD)	Isocaloric (2140 kcal/d) diet consisted of average 37% F, 15% P, 45% C; 9% en exchange between SAFA and MUFA. Subjects prepared their diets with supplied test fat and staple foods during intervention and consumed 6 capsules of either fish oil or placebo daily. HSD 17% SAFA, 14% MUFA, 6% PUFA *Butter, margarines* HMD 8% SAFA, 23% MUFA, 6% PUFA *Spread, margarine rich in oleic acid derived from HOS and olive oil, HOS (additional source in Australia centre).*	Insulin sensitivity index, first-phase insulin response, insulin, glucose: NS Insulin sensitivity when fat intake >37% en: NS Insulin sensitivity when fat intake <37% en: HSD ↓↓ *vs* HMD Insulin sensitivity: NS (between fish oil and placebo)

n, sample size; M, men; W, women; wk, week; BMI, body mass index; y, year; F, fat; %, % energy; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HOS, high oleic sunflower oil; NS, no significant difference between diets; ; ↓, lower compared to baseline.

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Bos et al., 2010)	Mild abdominally obese; HSD: 19(8/11) HMD: 18(8/10) MRD: 20(8/12)	HSD: 52.0 ± 7.2 HMD: 58.9 ± 5.1 MRD: 58.9 ± 5.1	Randomized, parallel, controlled, double-blind Euglycemic-hyperinsulinemic clamp in subgroup *n* = 30 (baseline, run-in and the last 3 wk)	8 wk; 2 wk of HS diet run-in	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. Mediterranean diet (MRD)	~2500 kcal meals were provided at research center on working days while take-home meals were allocated for weekend consumption, where SAFA and MUFA was exchanged for 9% en between diets. The received foods covered 90% of energy needs and subjects were free to choose food items low in fat and fibre for the remaining 10% en. <u>HSD</u> 36.8% F, 13.5% P, 47.3% C; 19.2% SAFA, 10.7% MUFA, 5.4% PUFA *Western-style diet high in SAFA* <u>HMD</u> 39.9% F, 11.4% P, 46.4% C; 10.9% SAFA, 20.3% MUFA, 7.0% PUFA *Rich in refined olive oil and 200 g vegetables, 1 serving of fruits, 84 g meat and 360 g dairy products* <u>MRD</u> 40.2% F, 14.5% P, 41.1% C; 10.7% SAFA, 21.4% MUFA, 6.5% PUFA *Rich in extra-virgin olive oil and 250 g vegetables, 3 servings of fruits, 64 g meat and 224 g dairy products*	Insulin and HOMA-IR: HSD ↓, MRD and HMD =; NS C-peptide, glucose, HDL-C: =, NS Insulin sensitivity in the subgroup clamp study: NS between HSD vs HMD; NS between MRD vs HMD

n, sample size; M, men; W, women; wk, week; BMI, body mass index; y, year; F, fat; %, % energy; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HOS, high oleic sunflower oil; ↓, lower compared to baseline; =, no changes from baseline; NS, no significant difference between diets.

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Filippou, Teng, et al., 2014)	Healthy; 41 (31/10)	M: 28.6 ± 6.3 W: 29.3 ± 8.0	Randomized, crossover, double-blind Run-in: 2 week	6 wk PP bleeding: 0, 10, 20, 30, 60, 90, 120 min	1. PO 2. IPO 3. HOS	Isocaloric (2000 kcal/d) diet consisted of 30% F, 15% P, 55% C. Daily meals were provided to subjects during weekdays. <u>PO</u> 12% SAFA, 12% MUFA, 4% PUFA <u>IPO</u> 12% SAFA, 12% MUFA, 4% PUFA <u>HOS</u> 4% SAFA, 20% MUFA, 4% PUFA <u>PP challenges</u> Standardized test meal prepared with PO (at week 6) 845 kcal; 50 g F, 14 g P, 85 g C	Fasting glucose, insulin, C-peptide, HOMA2-IR: NS PP iAUC $_{0\text{-}120min}$ for C-peptide and glucose: NS PP glucose at 2 h: IPO ↓ vs PO and HOS PP NEFA: NS
(Uusitupa et al., 1994)	Healthy ; 10(0/10)	23 ± 1.6	Randomized, controlled, crossover IVGTT was performed after diet (n=9)	3 wk; washout 2 wk	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD)	Subjects followed 1600, 1800 or 2000 kcal diet providing 40% F, 15% P and 45% C. <u>HSD</u> 20.4% SAFA, 12.0% MUFA, 3.9% PUFA *Butter and dairy products* <u>HMD</u> 8.5% SAFA, 19.0% MUFA, 10.3% PUFA *Low-erucic acid rapeseed oil margarine* .	AUC $_{glucose}$: HMD ↓↓ vs HSD Glucose between 30 to 70 min: HMD ↓↓ vs HSD Insulin area and glucose disappearance rate: NS

n, sample size; M, men; W, women; wk, week; IVGTT, intravenous glucose tolerance test; y, year; F, fat; %, % energy; P, protein; C, carbohydrate; PO, palm olein; IPO, interesterified palm olein; HOS, high oleic sunflower oil; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PP, postprandial; HOMA2-IR, homeostatic model assessment 2-insulin resistance; AUC, area under the curve; iAUC, incremental area under the curve; NS, no significant difference between diets; ↓, lower compared to baseline; vs, compared to; ↓↓, significantly lower.

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Gill et al., 2003)	Moderately hypercholesterole-mic; 35 (17/18)	55.0 ± 5.6	Randomized, crossover,	6 wk Wash-out 8 weeks	1. Low-MUFA diet (LMD) 2. Moderate-MUFA diet (MMD) 3. High-MUFA diet (HMD)	Subjects consumed test fat incorporated into margarine or spread, baked products according to a daily target for which provided 30% en fat. Low-fat ready meals and some fat-free foods were also provided; consumption of lean white meats was permitted. *Baked products: cakes, cookies and scones.* *Fat-free foods: most cereals, fruit, vegetables, rice, low-fat soups, and skim milk.* *Lean white meats: chicken breast without skin, lean pork and lean ham.* <u>LMD</u> 2397 kcal; 33.1% F, 12.4% P, 52.2% C 14.7% SAFA, 7.8% MUFA, 6.1% PUFA <u>MMD</u> 2474 kcal; 32.7% F, 12.2% P, 52.5% C 11.2% SAFA, 10.3% MUFA, 7.2% PUFA <u>HMD</u> 2490 kcal; 31.9% F, 12.3% P, 53.2% C 7.3% SAFA, 13.7% MUFA, 6.8% PUFA	Insulin and insulin resistance (HOMA-IR): ↑↑ with increasing MUFA ratio Glucose: NS

n, sample size; M, men; W, women; BMI, body mass index; y, year; wk, week; F, fat; %, % energy; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ↑↑, significantly higher; NS, no significant difference between diets.

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Paniagua et al., 2007)	Abdominally obese with insulin resistance; 11 (4/7)	62 ± 9.4	Randomized, controlled, crossover	4 wk PP bleeding: 0, 15, 30, 60, 90, 120, 150, 180 min	1) High-SAFA diet (HSD) 2) High-MUFA Mediterranean diet (HMD) 3) High-carbohydrate diet (HCD)	Subjects adhered to *ad libitum* isocaloric diet intervention, followed by a 443 kcal breakfast meal for postprandial challenge. <u>HSD</u> 38% F, 15% P, 47% C; 23% SAFA, 9% MUFA, 6% PUFA *Test meal: 200 ml whole milk, 50 g bread, and 25 g butter.* <u>HMD</u> 38% F, 15% P, 47% C; 9% SAFA, 23% MUFA, 6% PUFA *75% MUFA was provided by extra virgin olive oil.* *Test meal: 200 ml skim milk, 50 g bread, and 27 g olive oil* <u>HCD</u> 20% F, 15% P, 65% C; 6% SAFA, 8% MUFA and 6% PUFA *Test meal: 200 ml skim milk, 70 g bread, and 75 g marmalade*	Glucose: HMD and HCD ↓↓ vs HSD HOMA-IR and proinsulin : HMD ↓↓ vs HCD and HSD Insulin: NS Glucagon: HCD ↑↑ vs HSD and HMD <u>PP measurements</u> Acute insulin response: HSD ↓↓ vs HCD and HMD $AUC_{glucose}$ and $AUC_{insulin}$: HCD ↑↑ vs HSD and HMD Glucagon: HCD ↓↓ vs HSD and HMD Proinsulin to insulin ratio at 30 and 60 min: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; HOMA-IR, homeostatic model assessment-insulin resistance; AUC, area under the curve; ↓↓, significantly lower; ↑↑, significantly higher; vs, compared to; NS, no significant difference between diets

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Jebb et al., 2010)	At risk of developing metabolic syndrome; 548 (230/318) HS/HGI; 85 HM/HGI; 111 HM/LGI; 116 LF/HGI; 116 LF/LGI; 121	M:52 ± 10 W:51± 9	Randomized, controlled, parallel, non-blinded Intravenouse glucose tolerance test (3 h) after diet intervention	6 months 4 weeks run-in	1. HS/HGI 2. HM/HGI 3. HM/LGI 4. LF/HGI 5. LF/LGI	Isocaloric (2050kcal/d) diet varied in type of fat and glycemic index; ~7% en exchange between SAFA and MUFA (HS/HGI vs HM/HGI and HM/LGI); ~10% en exchange between SAFA and CARB (HS/LGI vs LG/HGI and LF/LGI); GI different at 8 points (HGI vs LGI). *Subjects were provided with key sources of fat (e.g. spreads, cooking oils and margarine) and carbohydrates (e.g. bread, pasta, rice and cereals)* <u>HS/HGI</u> 37.5% F, 20.5% P, 42% C 16% SAFA, 11.5% MUFA, 5.8% PUFA *Palm olein and milk fat* <u>HM/HGI</u> 35.6% F, 19.5% P, 44.9% C 9.5% SAFA, 16.2% MUFA, 6.6% PUFA *Spread, margarine rich in oleic acid derived from HOS and olive oil, HOS (additional source in Australia centre).* <u>HM/LGI</u> 35.7% F, 19.7% P, 44.6% C 9.6% SAFA, 16.3% MUFA, 6.9% PUFA <u>LF/HGI</u> 27.5% F, 21.4% P, 51.1% C 9.2% SAFA, 9.8% MUFA, 5.2% PUFA <u>LF/LGI</u> 26.1% F, 22.4% P, 51.5%C 8.3% SAFA, 9.7% MUFA, 5.1% PUFA	Insulin sensitivity, Sg, AIR_G, RQUICKI: NS

n, sample size; M, men; W, women; wk, week; HS, high-saturated fatty acids; HGI, high glycemic index; HM, high-monounsaturated fatty acids; LGI, low glycemic index; LF, low-fat; y, year; F, fat; P, protein; C, carbohydrate; %, % energy; ; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; HOS, high-oleic sunflower oil; Sg, glucose effectiveness; AIR_G, an indicator of endogenous insulin secretion; RQUICKI, Revised Quantitative Insulin Sensitivity Check Index; NS, no significant difference between diets

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Tierney et al., 2011)	Metabolic syndrome; HSD: 100 HMD: 111 HCD: 106 HCn3D: 100	Mean 55	Randomized, controlled, parallel	12 wk	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. High-Carbohydrate diet (HCD) 4. High-Carbohydrate supplemented with n-3 PUFA (HCn3D)	Subjects maintained their habitual diet and consume exchangeable fat from supplied spread, cooking oil, baking fats, mayonnaise and biscuits. HSD 2070 kcal/d; 39.8 F%, 17.4% P, 41.2% C; 17.5% SAFA, 12.7% MUFA, 6.3% PUFA *Full-fat dairy foods, and replaced one snack product usually eaten with a HSFA cookie daily.* HMD 2070 kcal/d; 38.9% F, 16.9% P, 42.1% C; 10.4% SAFA, 18.8% MUFA, 6.7% PUFA *Low-fat dairy foods, high-MUFA mayonnaise or a handful of hazelnuts or cashew nuts and replaced normally consumed snack product with HMUFA biscuit.* HCD 1965.7 kcal/d; 29.6 F%, 19.2% P, 49.4% C; 8.6% SAFA, 11.8% MUFA, 6% PUFA *Reduced the intake of high-fat snacks, consumed 2 extra portions of complex CARB daily and took the supplied capsules daily.* HCn3D 1975.3 kcal/d; 29.1% F, 18.3% P, 50.2% C; 8.7% SAFA, 11.4% MUFA, 5.7% PUFA *Reduced the intake of high-fat snacks, consumed 2 extra portions of complex CARB daily and took the supplied capsules daily.*	SI, fasting insulin, glucose concentrations and HOMA-IR: NS

n, sample size; M, men; W, women; y, year; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; NS, no significant difference between diets; SI, insulin sensitivity; HOMA-IR, homeostatic model assessment-insulin resistance

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Due, Larsen, Hermansen, et al., 2008)	Obese men (20/0) Premenopausal women (0/26) Subjects allocation: HMD; 16 LFD; 18 Control; 12	28.0 ± 0.7	Randomized, controlled, parallel	6 months 3 wk run-in OGTT bleeding time: 30, 60, 90, 120 min * Recruited subjects were those who achieved an ≥8% weight loss following an 8 week energy restriction (800-1000 kcal)	1. High-MUFA diet (HMD) 2. Low-fat diet (LFD) 3. High-SAFA/typical western diet (HSD)	A validated supermarket model was used to allow subjects to collect all free foods where dietary intake was under *ad libitum* condition, with the aim to maintain weight. Moderate alcohol consumption was allowed. Food selection either from supermarket or outside was altered by trained personnel referring to dietary composition from database. HMD 40% F, 15% P, 45% C <10% SAFA, >20% MUFA, 5-10% PUFA *More whole-grain foods, nuts, and legumes* LFD 25% F, 15% P, 60% C <10% SAFA, 10% MUFA, 5-10% PUFA HSD 35% F, 15% P, 50% C >15% SAFA, 10% MUFA, 0-10% PUFA	Changes in fasting glucose and insulin: Significant difference between HMD and control (HMD ↓; HSD ↑); NS between HMD vs LFD, NS between HSD vs LFD Changes in Matsudas index (indication of insulin sensitivity), $AUC_{glucose}$ and $AUC_{insulin}$: NS

n, sample size; M, men; W, women; y, year; wk, week; OGTT, oral glucose tolerance test; F, fat; %, % energy; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; ↓, lower compared to baseline; ↑, higher compared to baseline; NS, no significant difference between diets; vs, compared to; AUC, area under the curve.

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'Table 2.6, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Perez-Jimenez et al., 2001)	Healthy; 59 (30/29)	23.1 ± 1.8	Randomized, controlled, crossover Modified insulin suppression test on the last day of intervention, where a continuous somatostatin, insulin and glucose were infused into vein.	4 wk Bleeding time during insulin suppression test: 150,160, 170, 180 min	1. High-SAFA diet (HSD) 2. High-MUFA/ Mediterranean diet (HMD) 3. High-carbohydrate diet (HCD) HSD → HMD → HCD or HSD → HCD → HMD	Daily meals were provided to subjects. <u>HSD</u> 2580 kcal; 37.7% F, 18.1% P, 44.2% C 22.6% SAFA, 10.1% MUFA, 5% PUFA *Palm oil and butter* <u>HMD</u> 2580 kcal; 38.4% F, 17.5% P, 44.1% C 9.2% SAFA, 24.4% MUFA, 5.2% PUFA *Olive oil* <u>HCD</u> 2531 kcal; 27.9 % F, 17.6% P, 54.5% C 9.2% SAFA, 13.5% MUFA, 4.8% PUFA *Biscuits, bread and jam*	<u>Fasting measurements</u> Insulin: HSD ↑↑ vs HMD and HCD Glucose: NS <u>Glucose uptake in monocyte</u> Basal glucose uptake and insulin-stimulated glucose uptake: HSD ↓↓ vs HMD and HCD <u>Modified insulin suppression test</u> Mean glucose: HSD ↑↑ vs HMD and HCD Mean insulin: NS

n, sample size; M, men; W, women; y, year; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; HOMA-IR, homeostatic model assessment-insulin resistance; AUC, area under the curve; ↓↓, significantly lower; ↑↑, significantly higher; vs, compared to; NS, no significant difference between diets. Note: Somatostatin inhibits endogenous insulin secretion.

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2.9 Impact of Dietary Fats on Inflammatory and Thrombogenic Responses

The following subsections summarize published literature on the 1) acute effect of dietary fat quantity (Table 2.7), 2) chronic effect of dietary fat quantity (Table 2.8), 3) acute effect of dietary fat quality (Table 2.9) and 4) chronic effect of dietary fat quality (Table 2.10), on inflammatory and thrombogenic responses.

2.9.1 Acute Effects of Dietary Fat Quantity on Inflammatory and Thrombogenic Responses

The continual post-meal inflammatory status leads to the development of metabolic disorders such as T2DM and cardiovascular diseases. Excessive postprandial increment in lipids has been demonstrated to stimulate the release of pro-inflammatory markers. Miglio et al. demonstrated that the consumption of 82 g fat in a high-fat mixed meal induced postprandial IL-6 and TNF-α elevation over 8 h in 13 male and 2 female overweight individuals (Miglio et al., 2013). The elevation of IL-6 and TNF-α occurred respectively from 2 h or 30 min respectively until 8 h after the meal. The high-fat mixed meal consisted of fried eggs, fried potatoes, Italian rose-shaped dinner rolls, Emmenthal cheese and a 500 ml of low sugar content beverage. Contradictory findings were reported in another 8 h acute study conducted by Blackburn et al. (2006). In this study, 38 abdominally obese males ingested a high-fat mixed meal consisting of cheese, eggs, toast, peanut butter, whipped cream, milk and peaches. The total fat intake was 60 g fat/m^2 body surface. The high-fat meal resulted in an increment of IL-6 at 8 h and a decrement of TNF-α at 4 h but CRP remained unchanged. The discrepancies between these two studies could be due to the different time points of blood sampling. Blackburn et al. (2006) started blood sampling at 4 h after meal ingestion and thus may have missed detection of

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early elevation of IL-6 and TNF-α as observed by Miglio et al. (2013) whose group sampled blood at 0, 0.5, 1, 2, 4, 6 and 8 h. Interpretation of the findings from these two studies is difficult as the studies did not include a control group. Esser et al. (2013) compared the effects of high-fat meal with effects of a control meal (average breakfast) in 20 healthy men in a randomized, double-blinded, crossover study. The subjects consumed 500 ml shakes which provided either 95 g fat with the high-fat meal or 14.5 g fat with the average breakfast. The high-fat meal contained fresh cream, water and sugar (954 kcal); while the average breakfast was prepared with full cream milk, full cream, lemonade, fantomalt and wheat fiber (500 kcal). Ingestion of the high-fat meal resulted in higher IL-8, central pulse pressure and radial systolic blood pressure; lower sVCAM-1 and serum amyloid A compared with the average breakfast. The study did not however find significant differences between the meals with regards to their effect on radial diastolic blood pressure, central systolic blood pressure, augmentation index, IL-6, CRP, sICAM-1, E-selectin, P-selectin, thrombomodulin, sICAM-3, IL-1β and TNF-α. One limitation of this study was that the two meals were not isocaloric wherewith the high-fat meal containing 554 kcal higher than average breakfast.

Further, it was suggested that more profound pro-inflammatory response is found in individuals with insulin resistance and obesity. In conjunction with this, Blackburn et al. (2006) performed subgroup analysis in the aforementioned study. It was reported that delayed elevation of IL-6 and significant reduction of TNF-α were found in insulin sensitive subjects compared to the insulin resistant group. Patel et al. (2007) also supported this hypothesis that the obese subjects in their study had higher NF-kB binding activity increment at 3 h compared to lean subjects. Increased ROS generation at 2 h was however detected in both obese and lean subjects. The high-fat meal provided 60 g fat and comprised Big Mac, large French fries, a large Coke, and apple pie. However, plasma

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cytokines were not measured in this study and the postprandial duration were rather short (3 h).

P. J. Manning et al. (2008) reported that ingestions of both high-fat and low-fat meals increased IL-6 but did not affect TNF-α and CRP concentrations in 15 obese and 14 lean female subjects. The quantity of fat did not impose differential effects on the inflammatory markers however different inflammatory responses were found between lean and obese subjects. The overall IL-6 response, as shown by iAUC, was higher in obese compared to lean individuals. Obese subjects showed prolonged IL-6 elevation, i.e. from 4 to 6 h, compared to lean subjects whereas elevated IL-6 concentration was only found at 6 h in the lean. The subjects consumed 3 types of high-fat meals and 2 types of low-fat meals in a crossover manner. The high-fat meals were 2 eggs and 200 g instant mashed potato enriched with cream (high SAFA, 71 g fat), olive oil (high-MUFA, 72 g fat) or canola oil (high PUFA, 72 g fat); whereas the low-fat meals consisted of 2 eggs served with 200 g mashed potato and 40 ml trim milk (high-starch, 12 g fat); or all bran (high-fiber, 13 g fat). The mean calorie intake of high-fat meals in obese subjects (850 kcal) was 200 kcal higher than lean subjects (650 kcal), based on the setting that the fat intake was in proportion with body weight. Besides, the high-fat and low-fat meals (330 kcal) were also not isocaloric as the fat content in the low-fat meals was 300 or 500 kcal lower than the high-fat meals in obese or lean subjects, respectively. The unstandardized caloric intake may complicate the results interpretation hence more well-controlled studies are required. A randomized, parallel study in 10 lean, obese and T2DM male subjects (Phillips et al., 2013) supports the findings by P. J. Manning et al. (2008). No discernable changes in IL-6, TNF-α and CRP were found after the high-fat meal compared with water (control). The 30 men consumed 57.5 g fat (with 29 g saturated fat) derived from bacon, egg muffin, 2 hash browns with a glass of caramel-flavored milk

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drink. However, this study did not support the hypothesis that fat quantity causes more pronounced inflammatory response in obese or T2DM subjects than in lean individuals.

Taken together, high-fat meal may increase the level of inflammatory markers, in particular IL- 6, ROS generation and NF-kB binding activity. Besides, the evidence also showed more profound pro-inflammatory response in obese and T2DM subjects. However, these findings may be limited due to the absence of control and unstandardized energy content between meals. Future acute studies on fat amount are needed based on well study design.

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Table 2.7: Acute Effects of Dietary Fat Quantity on Inflammatory and Thrombogenic Responses

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Blackburn et al., 2006)	Abdominally obese; 38 (38/0)	44 ± 10	No control included	8 h PP bleeding time: 0, 240 and 480 min	1. High-fat meal (HFM)	60 g fat/m^2 body surface *Eggs, cheese, toast, peanut, whipped cream, peaches and milk*	CRP: =; NS IL-6: ↑ at 480 min; ↑ at 480 min in insulin sensitive; ↑ at 240 min, 480 min in insulin resistant TNF-α: ↓ at 240 min, = at 480 min; ↓ at 240 min in insulin sensitive; = at 240 min in insulin resistant
(Miglio et al., 2013)	Overweight; 15 (13/2)	45 ± 8	No control included	8 h PP bleeding time: 0, 30, 60, 120, 240, 360, 480 min	1. High-fat meal (HFM)	82 g fat (36.9 g SAFA) *Fried potatoes, fried eggs, Emmenthal cheese, Italian rose-shaped dinner rolls, with 500 ml of low sugar content beverage*	IL-6, TNF-α: ↑ over time
(Patel et al., 2007)	Lean: 10 (5/5); Obese: 8 (5/3)	30.5 ± 6.5	No control included	3 h PP bleeding time: 0, 60, 120, 180 min	1. High-fat meal (HFM)	60 g fat *Big Mac, large French fries, a large Coke, and apple pie*	ROS generation: ↑ at 120 min in lean and obese NF-κB binding activity: ↑ at 120 min in lean; ↑ at 120, 180 min in obese; Obese ↑↑ vs lean for difference between 180 and 120 min

n, sample size; M, men; W, women; T2DM, type 2 diabetes mellitus; y, year; h, hour; PP, postprandial; %, % energy; F, fat; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; IL-6, interleukin-6; ↑, ↑, increased compared to baseline; vs, compared to; ↓, decreased compared to baseline; TNF-α, tumor necrosis factor-α; =, no changes compared to baseline; ROS, reactive oxygen species; NF-kB, nuclear factor-κB; NS, no significant difference between diets; HMW, high molecular weight.

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'Table 2.7, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(P. J. Manning et al., 2008)	Abdominal obesity; (0/15) Lean; (0/14)	AO:54±9 Lean:53±10	Randomized, controlled, cross over, single-blind Wash-out:1 week	6 h PP bleeding time: 0, 60, 240, 360 min	1. High SAFA meal (HSM) 2. High MUFA meal (HMM) 3. High PUFA meal (HPM) 4. High carbohydrates/high starch meal (HCSM) 5. High carbohydrates/high fiber meal (HCFM)	<u>HSM</u> Obese: 869.3 kcal, 73.5 % F, 9.7% P, 17.9% C; 18.6% PA, 19.7% OA Lean: 677.8 kcal, 67.7% F, 11.8% P, 21.8% C; 17.3% PA, 18.6% OA *200 g mashed potato, 2 eggs and 1.6 g cream/kg body weight (3 g fiber)* <u>HMM</u> Obese: 847.3 kcal, 76.5% F, 8.5% P, 16.1% C Lean: 656 kcal, 70% F, 11% P, 20.7% C; 9.6% PA, 43.9% OA *200 g mashed potato, 2 eggs and 0.6 g olive oil/kg body weight (3 g fiber)* <u>HPM</u> Obese: 847.3 kcal, 76.5% F, 8.5% P, 16.1% C; 5.3% PA, 40.4% OA Lean: 656 kcal, 70% F, 11.0% P, 20.7% C; 5.5% PA, 35.7% OA *200 g mashed potato + 2 eggs+0.6 g canola oil/kg body weight (3 g fiber)* <u>HCSM</u> 307.4 kcal, 35.1% F, 23.4% P, 44% C, 8.8% PA, 11.7% OA, 3 g fiber *200 g mashed potato + 2 eggs (3 g fiber)* <u>HCFM</u> 334.4 kcal, 35% F, 22.7% P, 45.5% C, 8.8% PA, 10.8% OA, 19 g fiber *All-Bran, 40 ml trim milk and 2 eggs (9 g fiber)*	Plasma IL-6: ↓ at 1 h and ↑ at 4 h in obese; ↑ at 6 h in obese and lean ; NS between HSM, HMM and HPM $iAUC_{IL-6}$: Obese ↑↑ vs lean; NS between HCSM vs HSM, HMM and HPM TNF-α and IL-8: =; NS between lean and obese $iAUC_{TNF-\alpha}$: HCFM ↓↓ vs HCSM in obese

n, sample size; M, men; W, women; y, year; AO, abdominal obesity; h, hour; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; PA, palmitic acid; OA, oleic acid; IL-6, interleukin-6; ; =, no changes compared to baseline; ↑, increased compared to baseline; ↓, decreased compared to baseline; NS, no significant difference between diets; iAUC, incremental area under the curves; ↑↑, significantly higher; ↓↓, significantly lower ; TNF-α; tumor necrosis factor-α; IL-8, interleukin-8.

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'Table 2.7, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Esser et al., 2013)	Healthy; 20 (20/0)	22 ± 2	Randomized, controlled, double-blind, crossover Wash out: 1 week	6 h PP bleeding time: 0, 180, 360 min	1. High-fat meal (HFM) 2. Average breakfast (control)	A 500 ml shakes were provided to subjects. <u>HFM</u> 954 kcal, 89.6% F,2.5 % P,9.2% C 95 g fat (54 g SAFA) *53% fresh cream, 3% sugar, 44% water* <u>Control</u> 400 kcal, 32.6% F, 17 % P, 49.5% C 14.5 g fat (9 g SAFA), 2.3 g fiber *43% full cream milk, 48% full cream, 4% lemonade, 4% fantomalt, 1% wheat fiber*	Radial diastolic pressure, central systolic blood pressure, augmentation index, CRP, sICAM-1, P-selectin, sICAM-3, IL-1β and TNF-α: =; NS IL-6: both ↑; NS E-selectin and thrombomodulin: both ↓; NS Central pulse pressure and radial systolic blood pressure and IL-8: HFM ↑↑ vs Control SAA and sVCAM-1: HFM ↓↓ vs Control
(Phillips et al., 2013)	Lean; 10 (10/0) Obese; 10 (10/0) T2DM; 10 (10/0	28.8 ± 9.1	Randomized, controlled, single-blind, parallel Wash out: 1 week	6 h PP bleeding time: 0,60, 120, 180, 240, 300,360 min	1. High-fat meal (HFM) 2. Water	<u>HFM</u> 987.9 kcal, 51.5% F, 14.5% P, 34% C 57.5 g F (29 g SAFA) *A bacon and egg muffin, 2 hash browns, caramel flavored milk drink (250 ml of 4% fat milk, 4 teaspoons skim milk powder and 1 teaspoon caramel flavor* <u>Water</u> *50 ml room temperature water each h*	TNF-α: =; NS IL-6: both meals ↑, NS; ↑↑ in T2DM vs lean after water; NS between groups after HFM hsCRP : ↓ in lean, ↑ in obese; ↑ in all groups after HFM, NS; ↑ in lean and T2DM but ↓ in obese after water; Total adiponectin: HFM ↑; NS HMW adiponectin: =; NS

n, sample size; M, men; W, women; y, year; h, hour; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; CRP, C-reactive protein; sICAM, soluble intercellular adhesion molecule; IL-1β, interleukin-1β; TNF-α; tumor necrosis factor-α; =, no changes compared to baseline; IL-6, interleukin-6; ↓, decreased compared to baseline; sVCAM, soluble vascular adhesion molecule; SAA, serum amyloid A; NS, no significant difference between diets; iAUC, incremental area under the curves; ↑↑, significantly higher; ↓↓, significantly lower ; =, no changes from baseline; HMW, hihg molecular weight

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2.9.2 Chronic Effects of Dietary Fat Quantity on Inflammatory and Thrombogenic Responses

Saturated fats are negatively viewed as 'bad fats' and purportedly contribute to CVD development. Reducing dietary saturated fats appears to be a trend as this is perceived as a healthier food choice. However, increased risk of cardiovascular diseases has been reported when the energy content derived from dietary fat is mainly been replaced by carbohydrates, especially refined carbohydrates. The hypothesis may need further confirmation as contradictory results with regards to inflammatory response were found in clinical studies of chronic exposure. A large sample size and rigorously well-designed multicenter study, the RISCK study (Jebb et al., 2010), compared the 6-month consumption of 5 isocaloric diets (2050 kcal) rich in SAFA, MUFA (high- or low GI) or carbohydrates (high- or low-GI) in 548 subjects who were at risk of developing metabolic syndrome. This parallel arm study provided key food sources to subjects, i.e. palm olein and milk fat for high-SAFA diet; margarine enriched with high oleic sunflower and olive oil for high-MUFA diet; and bread, pasta, rice and cereals for high-carbohydrates diet. The high-fat and high-carbohydrates diets (36 vs 26% en fat) did not result in differential effects on inflammatory and endothelial response as measured by CRP, fibrinogen, ICAM-1, PAI, FVIIc. Sanders, Lewis, Goff, Chowienczyk, and Group (2013) performed a subgroup analysis (n = 112) of the RISCK study that compared the high-SAFA, high-MUFA and high-carbohydrates diets containing high GI carbohydrate focusing on endothelial function and arterial stiffness. No significant difference between diets was detected with regards to arterial stiffness, augmentation index, flow-mediated dilation, 8-isoprostane F2α-III, supine diastolic and systolic blood pressure. However, the 6-month duration of the study may be insufficient for detecting changes in arterial stiffening, and longer intervention period may be required. In agreement with the two studies, Bladbjerg

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et al. (2011) reported that 6-month consumption of high-carbohydrates diet was comparable with high-SAFA or high-MUFA diet in regard to the fasting concentration of inflammatory biomarkers, such as IL-6, CRP, ICAM, von Willebrand factor and tissue factor pathway inhibitor. The overweight subjects were provided the high-SAFA/high-GI, high-MUFA and high-carbohydrates/mixed-GI diets comprising respectively 32, 38 or 24% en fat. However, the finding of this study may be of limited value due to the unequal distribution of the sample size among groups, i.e. n = 26, 54 and 51 following high-SAFA, MUFA and carbohydrates diet. Contradictory findings were reported by a recent randomized, parallel arm study (Jonasson, Guldbrand, Lundberg, & Nystrom, 2014). The study observed significantly higher fasting IL-6 and IL-Ra concentrations after the 2-year *ad libitum* adherence to high-carbohydrates diet compared to high-fat diet in T2DM subjects. However, no significant change was reported for CRP, TNF receptor 1 and TNF receptor 2. The high-carbohydrates diet contained 29% en fat, whereas the high-fat diet replaced 20% en carbohydrates with 9% en SAFA, 7% en MUFA and 3% en PUFA. The fat sources for each diet however were not stated. The discrepant findings with regards to IL-6 between this study and the aforementioned study may be due to the difference in metabolic characteristics of subjects, i.e. T2DM *vs* overweight; and the control of diet adherence (free living condition *vs* strict control on food intake).

The adverse effects of carbohydrates on inflammation and endothelial function were more obvious when compared to high-MUFA Mediterranean diet, as found by 3 sub-studies of PREvencio´n con DIeta MEDiterra´nea (PREDIMED) study. This randomized, parallel arm study assessed the effects of high-MUFA diets supplemented with virgin olive oil or nuts compared with a high-carbohydrate diet. The subjects at high risk of CVD were advised to enrich their Mediterranean diet with either i) 1 L/week virgin olive oil or ii) 30 g/d mixed nuts; or to reduce all types of fat and follow AHA guidelines in the high-carbohydrate diet. The first sub-study was conducted by Mena et al. (2009)

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where 106 subjects were randomized evenly into the 3 diet intervention groups. The high-MUFA diets and high-carbohydrates diet respectively supplied 37 vs 34% en fat. IL-6, CRP, sICAM-1 and sVCAM-1 concentrations increased after the 3-month consumption of the high-carbohydrates diet, whereas no changes were observed on E-selectin and P-selectin. Another sub-study (Urpi-Sarda et al., 2012) with higher statistical power (n = 516) and longer study duration (1 year) compared the fat intake of 41 and 37% en fat respectively between the high-fat and low-fat groups. The study observed that the levels of IL-6, ICAM-1, TNFR-60 and TNFR-80 decreased in the high-carbohydrates diet group compared to the 2 high-MUFA diet groups. Casas et al. (2014) conducted a 1-year parallel dietary intervention in 164 subjects. Plasma IL-6, IL18/IL10 ratio and P-selectin decreased following the consumption of both high-MUFA diets, however the diets increased CRP concentration. On the other hand, consumption of high-carbohydrates diet elevated plasma IL-6 and sICAM. There were no appreciable difference between diets in the changes of plasma IL-18, IL-10, E-selectin, VCAM, MMP-9, TGF-β1 and MMP/TMP-1 ratio from baseline. Overall, there were some noteworthy limitations of these 3 studies. The *ad libitum* diets were not isocaloric among groups where in the high-carbohydrate diet contained around 200 kcal less than that of the 2 high-fat diets. This limitation could be a covariate in the studies. Also, the studies were limited by the difficulty of ensuring adherence to diet, leading to only 3 to 4% en difference in total fat intake between high-fat and high-carbohydrates diets. Other than MUFA, mixed nuts are rich sources of PUFA whereas virgin olive oil is a rich source of α-tocopherol and polyphenols such as tyrosol, oleuropein, oleocanthal and hydroxytyrosol (Urpi-Sarda et al., 2012). The aforementioned contents possess anti-inflammatory properties which reduce the risk of CVD occurrence. Hence, the beneficial effects of high-MUFA/Mediterranean diets with regards to CVD risk were more likely attributable to supplemental virgin olive oil and mixed nuts rather than the influence of fat quantity.

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Three studies incorporated n-3 PUFA into the high-carbohydrates diet, and observed conflicting results. Delgado-Lista et al. (2008) and Fuentes et al. (2008) submitted 20 healthy men to a 4-week dietary intervention. The fat content in the two high-fat diets and the high-carbohydrates diet were respectively 38 and 30% en fat. The supplementation of 4% en PUFA (walnuts) during high-carbohydrates diet did not promote a better hemostatic profile (FVIIc, PAI-1, D-dimer, tPA and TBxB2) as compared to high-SAFA and high-MUFA (virgin olive oil) diet (Delgado-Lista et al., 2008). On the other hand, the fasting level of the inflammatory marker, sVCAM-1 decreased on high-MUFA and high-carbohydrates diet compared to high-SAFA diet (Fuentes et al., 2008). However, sVCAM-1 levels were found to be higher on the high-carbohydrates diet compared to the high-fat diets at 4 and 6 h during the standard meal challenge. Furthermore, high-carbohydrates and high-SAFA diet exhibited lower ischemic reactive hyperaemia during the postprandial challenge, indicating attenuated endothelium-mediated vasodilatation as compared to high-MUFA diet. On the other hand, the multicenter LIPGENE study reported conflicting results (Petersson et al., 2010). The study compared high-fat/high-SAFA, high-fat/high-MUFA, high-carbohydrates and high-carbohydrates/n-3 PUFA diets in subjects who had metabolic syndrome (n = 417). The high-fat and high-carbohydrates diets contained 40 vs 27% en fat. The study did not observe any significant difference in regard to the oxidative stress and inflammatory biomarkers such as urinary 8-Iso-PGF2α, urinary keto-dihydro-PGF2α and plasma CRP. Meneses et al. (2011) recruited 39 subjects in a substudy of the LIPGENE study. This study measured plasma cytokines and expression of inflammatory genes which characterize chronic low-grade inflammation. The authors reported similar effects of the 4 diets on adipose tissues inflammatory genes such as IL-1β, MCP-1, IKBβ2, IKBα and p65 during the fasting and postprandial state. It is to be noted that the postprandial state, irrespective of fat quantity and quality, induced the gene expression of MCP-1 and IL-1β

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in adipose tissue but not in plasma. This discrepant observation was explained by the authors as being due to the fact that the synthesis and secretion processes do not happen simultaneously, and/or there are additional regulatory mechanisms acting on the secretory pathway.

As a whole, the chronic effect of fat quantity on inflammatory status and endothelial function are uncertain based on the limitations of current evidence in the literature. Future studies with longer study duration may provide more reliable evidence, given that detection of changes in endothelial function may require more than 6 months. Besides, a control group should be included in studies comparing high-MUFA diet vs high-carbohydrates diet to ensure that the findings are not confounded by fat quality.

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Table 2.8: Chronic Effects of Dietary Fat Quantity on Inflammatory and Thrombogenic Responses

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Delgado-Lista et al., 2008	Healthy; 20 (20/0)	23.3±2.2	Randomized, controlled, cross over, double-blind Wash out: 1 week	28 days PP bleeding time: 0, 60, 120, 180, 240, 300, 360 min	1. High SAFA meal (HSM) 2. High MUFA meal (HMM) 3. High carbohydrate/high PUFA meal (HCPM)	Subjects received diet followed by PP meal challenge. Test meal consisted of 60% F, 15% P, 25% C (1 g fat/kg body weight). <u>HSM</u> 38% F, 15% P, 47% C; 22% SAFA, 12% MUFA, 4% PUFA *Butter(48 g/d)* Test meal: 35% SAFA, 22% MUFA, 4%PUFA <u>HMM</u> 38% F, 15% P, 47% C; 10% SAFA, 24% MUFA, 4% PUFA *Virgin olive oil and spread (70 ml/d)* Test meal: 22% SAFA, 38% MUFA, 4% PUFA <u>HCPM</u> 30% F, 15% P, 55% C; 10% SAFA, 12% MUFA, 8% PUFA *Biscuits (25 g/d), jam (12 g/d), and bread, walnuts (35 g/d)* Test meal: 20% SAFA, 24% MUFA, 16% PUFA	<u>Fasting measurements</u> FVIIc, PAI-1, D-dimer, tPA and TBX B2: NS <u>Changes from preprandial value</u> FVIIc: HMM and HSM ↓; HMM ↓↓ vs HSM PAI-1:HMM ↓, HSM ↑; HMM ↓↓ vs HSM D-dimer, tPA, tPA/PAI-1and TBX B2: =; NS

n, sample size; M, men; W, women; y, year; h, hour; PP, postprandial; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FVIIc, factor VII coagulant activity; PAI-1, plasminogen activator inhibitor-1; tPA, tissue-type plasminogen activator; TBX B2, thromboxane B2; NS, no significant difference between diets; ↓, decreased compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower; =, no changes compared to baseline.

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Meneses et al., 2011	Metabolic syndrome; 39 (14/25)	Mean 56.6	Randomized, controlled, parallel	12 wk PP bleeding time: 0, 120, 240 min	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. Low-fat high complex carbohydrate diet + HOS supplement (LFD) 4. Low-fat high complex carbohydrate diet + n-3 PUFA supplement (LFn3D)	Subjects consumed ad-libitum diet followed by a postprandial challenge. Test meals consisted of 65% F, 10% P, 25% C. *Skimmed milk, white bread, eggs, egg yolks* <u>HSD</u> 40.3% F, 19.2% P, 38.3% C; 17.9% SAFA, 12.8% MUFA, 6.1% PUFA Test meal: 38% SAFA, 21% MUFA, 6% PUFA; *Butter, tomato* <u>HMD</u> 40.2% F, 19.2% P, 40.7% C; 9.1% SAFA, 21.1% MUFA, 5.7% PUFA Test meal: 12% SAFA, 43%, MUFA 10% PUFA; *Olive oil, tomato* <u>LFD</u> 27.1% F, 21.2% P, 51.2% C; 6.6% SAFA, 11.5% MUFA, 5.3% PUFA Test meal: 21% SAFA, 28% MUFA, 16% PUFA; *Butter, olive oil, walnuts* <u>LFn3D</u> 26.5% F, 18.8% P, 54.1% C; 6.4% SAFA, 11.1% MUFA, 5.0% PUFA Test meal: 21% SAFA, 28% MUFA, 16% PUFA *Butter, olive oil, walnuts*	<u>Fasting</u> IkBα, IkBβ2, MCP1, IL-1β and p65 expression: NS <u>PP challenge</u> p65 and IkBα gene expression: NS MCP-1, IL-1β gene expression in adipose tissue: ↑; NS IkBα mRNA, IkBβ2 mRNA, p65 mRNA, MCP1 mRNA, IL-6 mRNA and IL-1β: NS Plasma IL-6 and MCP-1: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MCP-1, monocyte chemoattractant protein-1; IL-1β, interleukin-1β; IL-6, interleukin-6; ↑, increased compared to baseline; NS, no significant difference between diets

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Jebb et al. 2010	At risk of developing metabolic syndrome; 548 (230/318) HS/HGI; 85 HM/HGI; 111 HM/LGI; 116 LF/HGI; 116 LF/LGI; 121	M:52 ± 10 W:51± 9	RCT, parallel, non-blinded	6 months 4 wk run-in	1. High SAFA/ high GI diet (HS/HGI) 2. High MUFA/ high GI diet (HM/HGI) 3. High MUFA/ low GI diet (HM/LGI) 4. Low-fat/ high GI diet (LF/HGI) 5. Low-fat/ low GI diet (LF/LGI)	Isocaloric (2050kcal/d) diet varied in type of fat and glycemic index; ~7% en exchange between SAFA and MUFA (HS/HGI vs HM/HGI and HM/LGI); ~10% en exchange between SAFA and CARB (HS/LGI vs LG/HGI and LF/LGI); GI different at 8 points (HGI vs LGI). Subjects were provided with key sources of fat (e.g. spreads, cooking oils and margarine) and carbohydrates (e.g. bread, pasta, rice and cereals) HS/HGI 37.5% F, 20.5% P, 42% C 16% SAFA, 11.5% MUFA, 5.8% PUFA Palm olein and milk fat HM/HGI 35.6% F, 19.5% P, 44.9% C 9.5% SAFA, 16.2% MUFA, 6.6% PUFA Spread, margarine rich in oleic acid derived from HOS and olive oil, HOS (additional source in Australia centre). HM/LGI 35.7% F, 19.7% P, 44.6% C 9.6% SAFA, 16.3% MUFA, 6.9% PUFA LF/HGI 27.5% F, 21.4% P, 51.1% C 9.2% SAFA, 9.8% MUFA, 5.2% PUFA LF/LGI 26.1% F, 22.4% P, 51.5%C 8.3% SAFA, 9.7% MUFA, 5.1% PUFA	Fibrinogen, CRP, ICAM-1, PAI, FVIIc: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1; PAI, plasminogen activator inhibitor; FVIIc, factor VIIc; NS, no significant difference between diets

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Bladbjerg et al., 2010	Overweight; HMD: 54 (22/32) HCD: 51 (22/29) Control: 26 (11/15)	HMD: 29.2 ± 4.5 HCD: 27.3 ± 4.9 Control: 27.6 ± 5.1	Randomized, controlled, parallel	6 months	1. High MUFA diet (HMD) 2. High carbohydrate diet (HCD) 3. Danish diet (Control)	Subjects who lost >8% of body weight were recruited. Foods were provided to subjects. <u>HMD</u> 2748 kcal, 38.4% F, 15.3% P, 43.3% C; 7.1% SAFA, 20.2% MUFA, 7.8% PUFA *High intake of vegetable oils, whole-grain food, nuts and legumes* <u>HCD</u> 2508 kcal, 23.6% F, 15.8% P, 57.6% C; 7.9% SAFA, 8.4% MUFA, 5.2% PUFA *Low in fat, high in mixed-GI carbohydrates* <u>Control</u> 2603 kcal, 32.1% F, 15.9% P, 49.8% C; 15.1% SAFA, 10.4% MUFA, 4.0% PUFA *Moderate in fat, high in SAFA, moderate in high-GI carbohydrates, low in fibre*	ICAM, vWF, TFPI: =; NS CRP: all ↓; NS IL-6: HMM and HCM ↓, Control =; NS

n, sample size; M, men; W, women; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ICAM-1, intercellular adhesion molecule-1; vWF, von Willebrand factor; TPFI, tissue factor pathway inhibitor; CRP, C-reactive protein; IL-6, interleukin-6; ↓, decreased compared to baseline; =, no changes compared to baseline NS, no significant difference between diets

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Casas et al., 2014	High risk for CVD; EVOO: 55 Nuts: 55 HCD: 54	EVOO: 68.1±6 Nuts: 67.6±6 HCD: 67.4±6	Randomized, controlled, parallel	1 y	1. Mediterranean diet + extra virgin olive oil (EVOO) 2. Mediterranean diet + nuts (Chowdhury et al.) 3. High carbohydrate diet (HCD)	Subjects were advised to adjust their *ad libitum* daily diets. No total calorie restriction was advised, nor was physical activity promoted. <u>EVOO</u> Increase the intake of vegetables (≥ 2 servings/d), fresh fruit (≥ 3servings/d), legumes, nuts, fish or seafood (≥ 3 servings/wk), and the use of olive oil for cooking and dressing *50 mL/d of extra virgin olive oil* <u>Nuts</u> Increase the intake of vegetables (⩾ 2 servings/d), fresh fruit (⩾ 3 servings/d), legumes, nuts, fish or seafood (⩾ 3 servings/wk), and the use of olive oil for cooking and dressing *30 g/d of walnuts, almonds and hazelnuts; olive oil for meal preparation* <u>HCD</u> Reduce all types of fat, with particular emphasis on recommending the consumption of lean meats, low-fat dairy products, cereals, potatoes, pasta, rice, fruits and vegetables.	sVCAM: EVOO and Nuts ↓, HCD=; NS sICAM: EVOO ↓ and HCD ↑, Nuts=; p <0.05 sE-selectin: EVOO and HCD =, Nuts ↓; NS sP-selectin: EVOO and Nuts ↓, HCD=; p <0.05 IL-6: EVOO and Nuts ↓, HCD ↑; p <0.05 CRP: EVOO and Nuts ↑; HCD =; p <0.05 IL-18: Nuts ↓, EVOO and HCD =; NS IL-10, TIMP-1: All =; NS IL-18/IL-10 ratio: EVOO and Nuts ↓, HCD =; p <0.05 MMP-9, MMP-9/TIMP-1 ratio and TGF-β1: EVOO and Nuts =,HCD ↑; NS Correlationship between MUFA diet intake with all markers: NS •No post-hoc test was conducted

n, sample size; M, men; W, women; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; sVCAM, soluble vascular cell adhesion molecule; sICAM-1, soluble intercellular adhesion molecule-1; IL-6, interleukin-6; CRP, C-reactive protein; IL-18, interleukin-18; IL-10, interleukin-10; MMP-9, matrix metallopeptidase-9; TIMP-1, tissue inhibitors of metalloproteinases; TGF-β1, transforming growth factor-β1; ↑, increased compared to baseline; ↓, decreased compared to baseline; =, no changes compared to baseline NS, no significant difference between diets

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Jonasson et al. 2014	T2DM; HCD: 31 (13/18) HFD: 20 (14/16)	HCD: 63 ± 11 HFD: 61 ± 9.5	Randomized, controlled, parallel	2 y	1. High carbohydrate diet (HCD) 2. High-fat diet (HFD)	Subjects followed *ad libitum* traditional Swedish diets. Isocaloric 1600 kcal/day for women and 1800 kcal/day for men. <u>HCD</u> 29% F, 20% P, 49% C 11% SAFA, 11% UFA, 5.1% PUFA <u>HFD</u> 49% F, 23% P, 25% C 20% SAFA, 18% UFA, 7.7% PUFA	CRP, TNFR1, TNFR2: =; NS IL-1Ra: HCD =, HFD↓; HCD ↑↑ vs HFD IL-6: HCD↑, HFD =; HCD↑↑ vs HFD
Mena et al., 2009	T2DM or 3 CVD risk factors; VOO: 35 Nuts: 35 HCD: 36	VOO: 66±11 Nuts: 66± 7 HCD: 69± 6	Randomized, controlled, parallel	3 months	1. Mediterranean diet + virgin olive oil (VOO) 2. Mediterranean diet + nuts (Chowdhury et al.) 3. High carbohydrate diet (HCD)	Subjects followed *ad libitum* diets. <u>VOO</u> 1979 kcal, 35.6%F, 17.8% P, 40.9% C; 9.6% SAFA, 17.6% MUFA, 5.6% PUFA *VOO (1L/wk)* <u>Nuts</u> 2106 kcal, 37.7% F, 16.3% P, 41.6% C; 9.0% SAFA, 16.7% MUFA, 8.7% PUFA *Mixed nuts (30 g/d, as 15 g walnuts, 7.5 g almonds, and 7.5 g hazelnuts)* <u>HCD</u> 1807 kcal, 34.2% F, 19.3% P, 42.4% C; 9.4% SAFA, 15.5% MUFA, 6.6% PUFA *Reduce intake of all types of fat and followed AHA guidelines*	E-selectin and P-selectin: NS sICAM-1 and IL-6: VOO and Nuts ↓, HCD ↑ sVCAM-1 and CRP: VOO ↓, Nuts =, HCD ↑

n, sample size; M, men; W, women; T2DM, type 2 diabetes mellitus; CVD, cardiovascular diasease; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CRP, C-reactive protein; TNFR, tumor necrosis factor receptor; IL-1Ra, interleukin-1 receptor antagonist; sVCAM, soluble vascular cell adhesion molecule; sICAM-1, soluble intercellular adhesion molecule-1; IL-6, interleukin-6; ↑, increased compared to baseline; ↓, decreased compared to baseline; =, no changes compared to baseline NS, no significant difference between diets

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Sanders et al., 2013	At risk of developing metabolic syndrome; HSD: 30 (12/18) HMD: 44 (14/30) HCD: 38 (14/24)	51	Randomized, controlled, parallel Run-in: 1 month HSD	6 months	1. High SAFA diet (HSD) 2. High MUFA diet (HMD) 3. High carbohydrate diet (HCD)	<u>HSD</u> 1886 kcal, 35.7% F, 17.8% P, 42.8% C; 14.6% SAFA, 11.3% MUFA, 5.7% PUFA *Full-fat milk (3.8 g/100 mL) and cheese (35 g/100 g); standard salad dressing* <u>HMD</u> 1908 kcal, 34.4 % F, 16.2% P, 47.2% C; 9.4% SAFA, 15.0% MUFA, 6.5% :PUFA *Skim milk (0.1 g fat/100 g) and half-fat cheese (18 g/100 g); high MUFA salad dressing* <u>HCD</u> 1941 kcal, 26.1% F, 17.8% P, 53.1% C 10% SAFA, 57% MUFA, 33% PUFA *Skim milk (0.1 g fat/100 g) and half-fat cheese (18 g/ 100 g); additional portions of bread, potatoes, and rice; reduced-fat salad dressing.*	PWV_{c-f} arterial stiffness, central and peripheral augmentation index, flow-mediated dilation, 8-isoprostane $F_{2\alpha}$-III, supine diastolic and systolic BP: NS

n, sample size; M, men; W, women; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PWV_{c-f}: carotid to femoral pulse wave velocity; NS, no significant difference between diets.

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Fuentes et al., 2008	Healthy; 20 (20/0)	23.3 ± 1.5	Randomized, controlled, crossover	4 wk PP bleeding time: 0, 120, 240, 360 and 480 min	1. High SAFA diet (HSD) 2. High MUFA diet (HMD) 3. High carbohydrate/ PUFA diet (HCD)	Subjects were provided daily meals. A PP challenge was conducted after dietary intervention. PP meal consisted of 50 – 66% daily energy intake. <u>HSD</u> 38% F, 15% P, 47% C; 22% SAFA, 12% MUFA, 4% PUFA, 0.4% ALA PP meal: 60% F, 15 % P,25% C 35% SAFA, 22% MUFA, 4% PUFA, 0.7% ALA *Butter* <u>HMD</u> 38% F, 15% P, 47% C; <10% SAFA, 24% MUFA, 4% PUFA, 0.4% ALA PP meal: 60% F, 15 % P,25% C 22% SAFA, 38% MUFA, 4% PUFA, 0.7% ALA *Extra virgin olive oil* <u>HCD</u> <30% F, 15% P, 55% C; <10% SAFA, 12% MUFA, 8% PUFA, 2% ALA PP meal: 60% F, 15 % P,25% C; 20% SAFA, 24% MUFA, 16% PUFA, 4% ALA *Walnuts*	<u>Fasting measurements</u> sVCAM-1: HSM ↑↑ vs HMM and HCM sICAM-1: NS <u>PP meal</u> sVCAM-1 at 4 h: HSM and HMM ↓↓ vs HCM sVCAM-1 at 6 h: HSM ↓↓ vs HCM IRH at 4 h and 6 h: HSM and HCM ↓↓ vs HMM

n, sample size; M, men; W, women; wk, week; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ALA, alpha linolenic acid; sVCAM, soluble vascular cell adhesion molecule; sICAM-1, soluble intercellular adhesion molecule-1; IRH, ischaemic reactive hyperaemia; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significant difference between diets.

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'Table 2.8, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Urpi-Sarda et al., 2012	At risk for CHD; VOO: 178 (83/95) Nuts: 175 (89/86) HCD: 163 (82/81)	67 ± 6	Randomized, controlled, parallel, single blind	1 y	1. Mediterranean diet with VOO (VOO) 2. Mediterranean diet with nuts (Chowdhury et al.) 3. High carbohydrates diet (HCD)	Subjects consumed *ad libitum* diet. Mediterranean foods were provided to the 2 Mediterranean diet groups. <u>VOO</u> 2423.5 kcal, 41.2% F, 15.7% P, 39.9% C; 9.7% SAFA, 21.9% MUFA, 6.3% PUFA *Increased intakes of vegetables (≥2 servings/d), fresh fruit (≥3 servings/d), legumes, nuts, fish or seafood (≥3 servings/wk) and the use of OO. 1 L/wk VOO was provided.* <u>Nuts</u> 2534.7 kcal, 41.5% F, 15.5% P, 39.3% C; 9.2% SAFA, 21.3% MUFA, 7.8% PUFA *Increased intakes of vegetables (≥2 servings/d), fresh fruit (≥3 servings/d), legumes, nuts, fish or seafood (≥3 servings/wk) and the use of OO. 15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts were provided for daily consumption.* <u>HCD</u> 2189.5 kcal, 37% F, 16.4% P, 44.6% C; 10.7% SAFA, 18.5% MUFA, 6.2% PUFA *Followed the AHA guidelines (reducing all type of fat intake)*	IL-6: VOO and Nuts ↓, HCD =; VOO and Nuts ↓↓ vs HCD TNFR60, TNFR80: VOO and Nuts ↓, HCD ↑; VOO and Nuts ↓↓ vs HCD ICAM-1: VOO and Nuts =, HCD ↑; VOO and Nuts ↓↓ vs HCD

n, sample size; M, men; W, women; y, year; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; d, day; wk, week; TNFR, tumor necrosis factor receptor; ↑, significantly higher than baseline; ↓, significantly lower than baseline; ↓↓, significantly lower; NS, no significant difference between diets.

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2.9.3 Acute Effects of Different Types of Dietary Fat on Inflammatory and Thrombogenic Responses

The effect of type of fatty acids on acute low grade inflammation is still uncertain. It has been suggested that SAFA may not cause unfavorable acute inflammatory response in overweight or obese individuals (Teng et al., 2014). The hypothesis is in conjunction with a crossover study in 4 men and 6 women who had overweight or obesity (Peairs, Rankin, & Lee, 2011). The subjects were randomly assigned to 12.8 kcal/kg milkshake enriched with refined palm oil, refined olive oil or refined olive oil plus 4 g n-3 PUFA. The 6-h postprandial challenge showed that SAFA was comparable with MUFA and PUFA in their acute effects on CRP, TNF-α, VCAM-1 and ICAM-1. Further, SAFA was found to lower NF-kβ activation compared with PUFA. The authors explained that the suppressed NF-kβ activation may be caused by the high palmitate content in palm oil, which was in accordance with in vitro evidence (Ajuwon & Spurlock, 2005). Another similar study (P. R. van Dijk et al., 2012) provided milkshake to 18 lean non diabetic, 18 obese non diabetic and 6 obese T2DM men in a randomized manner. The test fat added into the milkshake was refined palm oil, refined olive oil, and refined olive oil plus 4 g of n-3FA from 8 g fish oil supplement pills as n-3 PUFA. Null effect on plasma IL-1β and TNF-α concentration was observed, irrespective of type of fat consumed. However, the study reported lower peripheral blood mononuclear cell (PBMC) MCP-1 and IL-8 concentrations after SAFA compared with MUFA and n-3 PUFA high-fat meals at 4 h. A possible explanation for this induction might be that unsaturated fatty acids have higher tendency of oxidation compared to SAFA, and hence trigger oxidative stress and affect inflammatory status.

Indistinguishable postprandial changes between palm oil and olive oil were also found with other types of test meal. Stonehouse, Brinkworth, and Noakes (2015)

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conducted a 5 h postprandial challenge in 28 T2DM men. The isocaloric test meal was chicken strips fried in 40 g palm olein or olive oil, served with lightly fried bread and salad. No appreciable differences were found between meals in VCAM-1, ICAM-1, E-selectin, PAI-1 and tissue plasminogen activator. Another acute study (Teng, Nagapan, Cheng, & Nesaretnam, 2011) recruited 10 healthy men and compared 50 g palm oil, olive oil and lard (as SAFA) in an isocaloric mixed meal of 60 g mashed potato, 50 ml skim milk, 200 ml orange juice. The mixed meals did not cause any difference in the 4 h postprandial change in IL-6, TNF-α, IL-1β and leptin. The results indicated that either plant or animal source of SAFA (palm oil vs lard) was comparable with MUFA in their effects on the measured cytokines. However, other sources of SAFA may cause different results.

Cocoa butter is a plant source of SAFA, a major ingredient in chocolate production. Palmitic acid and stearic acid are the major fatty acids contained in cocoa butter. Tholstrup, Teng, and Raff (2011) compared cocoa butter (48% en SAFA) with refined olive oil (12% en SAFA) in 10 healthy men fed with mashed potato. The isocaloric meals did not cause significant difference in IL-6 and high sensitivity CRP at 4 and 6 h. The limitations of this study include the small sample size and restricted time points for blood sampling. On the other hand, Poppitt et al. (2008) conducted a trial where SAFA from animal source i.e. butter and cream was also compared with olive oil. A double blind, crossover study tested an average of 73 g high-SAFA and high-MUFA butter in 18 healthy lean men where fat intake was based upon subjects' total daily energy intake (5.3 g butter per 239 kcal of daily intake) (Poppitt et al., 2008). The test meal was a sweet blueberry muffin, a milk- and sugar-free decaffeinated hot beverage, and/or cold water. It was concluded that an acute increase in SAFA content had no adverse effect on IL-6, TNF-α and high sensitivity CRP compared with high MUFA content. However, differential effects between high-SAFA and high-MUFA meals were observed when

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butter (SAFA) was compared with extra virgin olive oil (MUFA) in 8 healthy males (Bellido et al., 2004). Besides inducing higher sICAM response, SAFA from butter was found to increase NF-kβ activation at 3 h compared with MUFA. The result contradicts the aforementioned finding reported by Peairs et al. (2011), where SAFA from palm oil reduced NF-kβ activation. The reason for the differential effect may be due to the difference in physical characteristics of the test fats. Butter contains considerable amount of medium chain triglycerides which leads to better absorption compared with palm oil. This may explain why butter, which is also a rich source of palmitic acid, did not suppress NF-kβ activation as found with palm oil. Manning et al. (2004 & 2008) observed that all high-fat meals regardless of type of fats imposed similar postprandial impact on plasma IL-6, IL-8 and TNF-α concentration when cream was compared with olive oil. The studies were performed in i) 14 lean vs 15 abdominal obese women and ii) 7 male and 11 female subjects who had T2DM, where 200 g mashed potato and 2 cooked eggs were given as test meals, in a non-isocaloric manner.

Evidence to-date suggests that there is no clear adverse effect of the consumption of SAFA-rich meals on acute inflammatory and endothelial responses. However, different changes in NF-kβ activation were observed after the consumption of different types of SAFA.

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Table 2.9: Acute Effects of Different Types of Dietary Fat on Inflammatory and Thrombogenic Responses

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Tholstrup et al., 2011	Healthy; (10/0)	38.2±10.7	Randomized, controlled, cross over, single-blind	6 h PP bleeding time: 0, 240, 360 min	1. Cocoa butter 2. Refined olive oil	1 g test fat and 1 g mashed potato powder per kg body weight; mixed with 3 ml of boiling water per g of mashed potato powder. 621.4 kcal, 76.1 % F, 2.6% P, 21.3% C. <u>Refined olive oil</u> 11.8% SAFA, 56.5% MUFA, 7.8% PUFA *Stearic acid (34.7% wt)* <u>Cocoa butter</u> 47.7% SAFA, 26.1% MUFA, 2.3% PUFA *Oleic acid (34.1% wt)*	IL-6, hsCRP: NS
Bellido et al., 2004	Healthy; 8(8/0)	*not specified (medical student)	Randomized, controlled, crossover Wash out: 1 week Run in : 4 wk	9 h PP bleeding time: 0, 180, 360, 540 min	1. High SAFA meal (HSM) 2. High MUFA meal (HMM) 3. High PUFA meal (HPM)	Test meals consisted of 1 g fat/kg body weight. 60% F,15% P,25% C <u>HSM</u> 38% SAFA, 22% MUFA, 4% PUFA, 0.7% ALA Butter <u>HMM</u> 22% SAFA, 38% MUFA, 4% PUFA, 0.7% ALA Extra virgin olive oil <u>HPM</u> 20% SAFA, 24% MUFA, 16% PUFA, 4% ALA Walnuts	NF-kβ activation: HSM ↑↑ vs HMM at 180min; HPM ↑↑ vs HMM at 540 min sICAM: HMM and HPM ↓↓ vs HSM

n, sample size; M, men; W, women; y, year; w, week; h, hour ; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; IL-6, interleukin-6; hsCRP, high sensitivity C-reactive protein; TNF-α; NF-kβ, necrosis factor-kappa β; sICAM, soluble intercellular adhesion molecule; NS, no significant difference between diets

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'Table 2.9, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Manning et al., 2008	Abdominal obesity; (0/15) Lean; (0/14)	AO:54±9 Lean:53±10	Randomized, controlled, cross over, single-blind Wash-out:1 week	6 h PP bleeding time: 0, 60, 240, 360 min	1. High SAFA meal (HSM) 2. High MUFA meal (HMM) 3. High PUFA meal (HPM) 4. High carbohydrates/high starch meal (HCSM) 5. High carbohydrates/high fiber meal (HCFM)	<u>HSM</u> Obese: 869.3 kcal, 73.5 % F, 9.7% P, 17.9% C; 18.6% PA, 19.7% OA Lean: 677.8 kcal, 67.7% F, 11.8% P, 21.8% C; 17.3% PA, 18.6% OA *200 g mashed potato, 2 eggs and 1.6 g cream/kg body weight (3 g fiber)* <u>HMM</u> Obese: 847.3 kcal, 76.5% F, 8.5% P, 16.1% C Lean: 656 kcal, 70% F, 11% P, 20.7% C; 9.6% PA, 43.9% OA *200 g mashed potato, 2 eggs and 0.6 g olive oil/kg body weight (3 g fiber)* <u>HPM</u> Obese: 847.3 kcal, 76.5% F, 8.5% P, 16.1% C; 5.3% PA, 40.4% OA Lean: 656 kcal, 70% F, 11.0% P, 20.7% C; 5.5% PA, 35.7% OA *200 g mashed potato + 2 eggs+0.6 g canola oil/kg body weight (3 g fiber)* <u>HCSM</u> 307.4 kcal, 35.1% F, 23.4% P, 44% C, 8.8% PA, 11.7% OA, 3 g fiber *200 g mashed potato + 2 eggs (3 g fiber)* <u>HCFM</u> 334.4 kcal, 35% F, 22.7% P, 45.5% C, 8.8% PA, 10.8% OA, 19 g fiber *All-Bran, 40 ml trim milk and 2 eggs (9 g fiber)*	Plasma IL-6: ↓ at 1 h and ↑ at 4 h in obese; ↑ at 6 h in obese and lean ; NS between HSM, HMM and HPM $iAUC_{IL-6}$: Obese ↑↑ vs lean; NS between HCSM vs HSM, HMM and HPM TNF-α and IL-8: =; NS between lean and obese $iAUC_{TNF-α}$: HCFM ↓↓ vs HCSM in obese

n, sample size; M, men; W, women; y, year; AO, abdominal obesity; h, hour; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; PA, palmitic acid; OA, oleic acid; IL-6, interleukin-6; ; ↑, increased compared to baseline; ↓, decreased compared to baseline; NS, no significant difference between diets; iAUC, incremental area under the curves; ↑↑, significantly higher; ↓↓, significantly lower ; TNF-α; tumor necrosis factor-α; IL-8, interleukin-8; =, no changes compared to baseline.

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'Table 2.9, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Manning et al., 2004	Type 2 diabetes with diet control ; 7/11	61±10	Randomized, controlled, cross over, single-blind Wash out: 1 week	6 h PP bleeding time: 0, 1, 2, 6 h	1. Control 2. High SAFA meal (HSM) 3. High MUFA meal (HMM)	Control *200 g instant mashed potatoes plus two cooked eggs alone* HSM *200 g instant mashed potatoes, two cooked eggs and 0.6 g cream/kg body weight* HMM *200 g instant mashed potatoes, two cooked eggs and 0.6 g olive oil/kg body weight*	IL-6: ↓ after all meals, then returned to baseline; NS TNF-α: ↓ at 4 h and 6 h after all meals; NS .
Stonehouse et al., 2015	Type 2 diabetes with diet control ; 28 (28/0)	56.8	Randomized, controlled, cross over, double-blind Wash out: 1 week	5 h PP bleeding time: 0, 1, 2, 3, 4, 5 h	1. High SAFA meal (HSM) 2. High MUFA meal (HMM)	Fried lean chicken strips in 40 g test oil, served with lightly fried bread and salad (20 g lettuce, 10 g tomato and 10 g cucumber). 667 kcal, 58% F, 30% P, 11% C. HSM 21.7% SAFA, 27.1% MUFA, 6.7% PUFA *40 g palm olein* HMM 9.6% SAFA, 44.2% MUFA, 4.2% PUFA *40 g olive oil*	Plasma PAI-1 and tPA : ↓ ;NS Plasma VCAM-1: ↑ after 1 h , and return to baseline; NS Serum ICAM-1: =; NS Serum E-selectin: ↓ after 4 h; NS

n, sample size; M, men; W, women; y, year; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; IL-6, interleukin-6; TNF-α; tumor necrosis factor-α; ↑, increased compared to baseline; ↓, decreased compared to baseline; NS, no significant difference between diets; =, no changes compared to baseline; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; VCAM-1, vascular cellular adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.

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'Table 2.9, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Poppitt et al., 2008	Healthy lean; 18 (18/0)	19–33 y	Randomized, controlled, cross over, double-blind Wash out: 3 days	24 h PP bleeding time: 0, 1, 3, 6 and 10 h Run-in: 3 days for each test	1. High SAFA meal (HSM) 2. High UFA meal (HUM)	Test meal (0 h): A sweet blueberry muffin, a milk and sugar-free decaffeinated hot beverage, and/or a glass of cold water. *73 g dairy butter* Lunch (6 h): 3.1 g of fat, comprising vegetarian pasta, bread roll, orange juice. Afternoon snack (8 h): 0.7 g of fat, comprising fruit cake, apple juice. Dinner (10 h): 1.3 g of fat, comprising vegetarian risotto, raspberry desert, carbonated beverage. <u>HSM</u> SAFA:UFA ratio is 71:29 <u>HMM</u> SAFA:UFA ratio is 55:45	IL-6: ↑ over 6 h on both meals; NS TNF-α: ↓ over 6 h on HSM; NS hs-CRP: =; NS
Peairs et al., 2011	Obese and overweight; 10 (4/6)	31.3 ± 3.3	Randomized, controlled, cross over Run in before each test: 3 days	6 h PP bleeding time: 0, 60, 120, 240,360 min	1. High SAFA meal (HSM) 2. HighMUFA meal (HMM) 3. High-PUFA meal (HPM)	The fat source was blended with 1% milk, strawberry flavored syrup, low-fat frozen yogurt, and non-fat dry milk powder. The milkshake provided 12.8 kcal/kg body weight: average 1267 kcal with the PUFA adding 36 kcal; 59% F, 11% P, 30% C <u>HSM</u> Refined palm oil <u>HMM</u> Refined olive oil <u>HPM</u> Refined olive oil plus 4 g of n-3 PUFA from 8 g fish oil supplement pills (300 mg EPA, 200 mg DHA per 1 g)	CRP: All ↑, NS TNF-α: All ↓, NS VCAM-1: All tended to ↓, NS ICAM-1: NS AUC $_{ICAM-1}$: HSM tended to be ↑↑ vs HMM AUC $_{NF-kB:}$ HPM ↑↑ vs HSM

n, sample size; M, men; W, women; y, year; h, hour; PP, postprandial; %, % energy; SAFA, saturated fatty acids; UFA, unsaturated fatty acids; IL-6, interleukin-6; TNF-α; tumor necrosis factor-α; hs-CRP, high sensitivity C-reactive protein; ↑, increased compared to baseline; ↓, decreased compared to baseline; =, no changes compared to baseline; NS, no significant difference between diets.

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'Table 2.9, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Van Dijk et al. 2012	Lean nondiabetc, obese nondiabetic and obese type 2 diabetes; 50 (50/-)	50-70	Randomized, controlled, cross over Wash out: ≥1 week	4 h PP bleeding time: 120 and 240 min	1. High SAFA meal (HSM) 2. High MUFA meal (HMM) 3. High PUFA meal (HPM)	Shakes containing low-fat yoghurt, low-fat milk, strawberry flavour, 7.5 g of sugar and 95 g of the test fat. 165 mg Tocoblend L50 (source of Vitamin E) was added to PUFA shake to prevent lipid oxidation and to SAFA and MUFA shakes for standardization. 987 kcal consisted of 87% F, 4% P, 9% C. <u>HSM</u> 47% SAFA, 34% MUFA, 6% PUFA *95 g palm oil* <u>HMM</u> 7% SAFA, 72% MUFA, 7% PUFA *95 g HOS* <u>HPM</u> 29% SAFA, 23% MUFA, 35% PUFA *40 g palm oil + 40 g Marinol D-40 of which 40% was DHA*	IL-1β and TNF-α: =; NS PBMC MCP-1 and IL-1β: MUFA and n-3PUFA ↑↑ vs SAFA at 4h IL-1β: Obese diabetic ↑↑vs lean and obese at fasting state TNF-α: Lean ↓↓vs obese and obese diabetic after postprandial challenge
Teng et al., 2011	Healthy; 10 (10/0)	21.9 ± 0.7	Randomized, controlled, single-blind, crossover Wash out: 1 week	4 h PP bleeding time: 0, 30, 60, 120, 180 and 240 min	1. Virgin olive oil (VOO) 2. Palm olein (PO) 3. Lard	Test meal consisted of 60 g mashed potatoes, 50 mL skim milk, 200 mL orange juice and 50 g test fat. 683 kcal, 60% F, 7% P, 33% C. <u>VOO</u> 45% MUFA <u>PO</u> 24% SAFA (21% palmitic acid) <u>Lard</u> 29% SAFA (15% palmitic acid, 10% stearic acid)	IL-6, TNF-α, IL-1β and leptin: NS

n, sample size; M, men; W, women; y, year; h, hour; PP, postprandial; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α; tumor necrosis factor-α; =, no changes compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower;

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2.9.4 Chronic Effects of Different Types of Dietary Fat on Inflammatory and Thrombogenic Responses

SAFA is often linked to pro-inflammatory effects and cardiovascular dysfunction via modulation of the production of cytokines and chemokines. In order to identify the individual effect of dietary fatty acids, Silver et al. (2014) conducted a parallel-arm, double blind study where 91 obese female subjects were allocated to the supplementation of 9 capsules containing either 9 g stearic acid (SAFA), oleic acid (MUFA), linoleic acid (PUFA) or control (edible silicon dioxide powder). The supplementation resulted in minimal change in most chemokines measured, i.e. IL-1α, IL-1β, IL-6, IL-10, IL-17, PAI-1 antigen, TNF-β, LAEI, SAEI and tPA antigen, irrespective of the types of fatty acids supplement. However, oleic acid was found to reduce TNF-α, IL-12 and tPA activity whereas stearic acid lowered the level of IFN-γ. Furthermore, both stearic and oleic acids diminished flow mediated dilatation. This study did not show clear evidence of the detrimental effect of stearic acid on inflammation status. However, Baer, Judd, Clevidence, and Tracy (2004) reported the pro-inflammatory effect of stearic acid relative to oleic acid in a 5-week high-calorie, high-fat diet (3100 kcal) intervention involving 50 healthy men. The replacement of 8% en oleic acid with stearic acid elevated the concentrations of IL-6, E-selectin and fibrinogen but not CRP.

A study conducted by Lithander et al. (2008) investigated the effect of increased SAFA intake. The 18 mildly hyperlipidemic subjects were supplied with a 5-day rotational diet (3176 kcal) containing 18 or 13% en SAFA for 3 weeks. The consumption of high-SAFA and low-SAFA diets did not result in significant difference in TNF-α, IL-6, hs-CRP and adiponectin. However, the study did not report the source of test fats used. It is noteworthy that the effect on inflammation may vary based on the type of fatty acid and fat source. Teng, Voon, Cheng, and Nesaretnam (2010) compared 2300 kcal-diets

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enriched with high oleic palm olein, palm stearin or partially hydrogenated soybean oil, which were respectively rich in oleic acid, palmitic acid or trans fatty acid. Weekday-meals were supplied to 41 subjects in a crossover manner. The authors found increments in IL-8 concentration, but not IL-6, IL-1β, hsCRP and TNF-α concentration, after the consumption of a 5-week high-palmitic acid diet compared to high-oleic acid diet. One limitation of this study was that PUFA content was not standardized among diets and hence may confound the determination of the impact of the individual fatty acids, i.e. 8% en in high-oleic palm olein diet and 4% en in the others. The multicenter RISCK study (n = 548) compared isocaloric (2050 kcal) high-SAFA, high-MUFA/high-GI and high-MUFA/low-GI diets with standardized PUFA contents (Jebb et al., 2010). The sources for the high-SAFA diet (16% en SAFA) were palm olein and milk fat while the high-MUFA diets (9.5% en MUFA) were prepared with spread and margarine derived from high oleic sunflower oil. The type of fatty acid did not appear to modulate the inflammatory status as observed from the fasting levels of fibrinogen, CRP, ICAM-1, PAI-1 and FVIIc.

Two studies compared the effect of SAFA and MUFA on the inflammatory response where each study utilized similar fat sources containing different fatty acid compositions. In a parallel study involving 20 abdominal overweight subjects, S. J. van Dijk et al. (2009) compared two 8-week isocaloric diets prepared with refined olive oil, respectively comprised of 19 or 11% en SAFA. The levels of adiponectin, complement 3 and RANTES/CCL5 did not vary according to the type of fatty acids. However, this finding may be limited as the sample size in this study was relatively small. Another comparison of high-SAFA and low-SAFA diets was performed by adjusting the intake of dairy products in 112 subjects with metabolic syndrome (Sanders et al., 2013). The changes in arterial stiffness, augmentation index, flow-mediated dilation, 8-isoprostane F2α-III, supine diastolic and systolic blood pressure were not significantly different

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between diets. Longer study duration may be required for future investigations as the 6-month duration of this study may not be sufficient for the detection of arterial stiffening.

Health authorities such as the American Heart Association recommend reducing SAFA intake to 5 to 6% en of daily energy intake and replacing it with PUFA and MUFA sources, i.e. olive oil. To ascertain the beneficial effect of olive oil attributed to the rich MUFA and antioxidant content, studies compared diets enriched with olive oil vs SAFA sources such as butter, milk or palm olein. Two similar studies (Delgado-Lista et al., 2008; Fuentes et al., 2008) allocated 20 healthy men to follow *ad libitum* diets enriched with virgin olive oil/spread (10% en SAFA) or butter (22% SAFA) in a crossover manner. A standard test meal challenge was conducted after the 4-week diet intervention. Both diets did not induce significant differences in hemostatic and endothelial function during fasting state, as measured by FVIIc, PAI-1, D-dimer, tPA, thromboxane B2 and sICAM-1. However, sVCAM-1 concentration increased after high-SAFA diet rather than high-MUFA diet. Moreover, the consumption of high-SAFA meal elevated the postprandial FVIIc and PAI-1 and attenuated endothelium-mediated vasodilatation (as measured by ischaemic reactive hyperaemia) relative to high-MUFA meal. The two studies showed that olive oil is more cardio-protective compared to butter. However, butter did not exhibit adverse effect when compared to soybean oil products (rich in MUFA and PUFA) in a 7-week diet intervention (Lichtenstein et al., 2003). The 36 subjects with hypercholesterolemia were provided meals supplemented with butter, soybean oil, semi-liquid margarine, soft margarine, shortening or traditional stick margarine (2114 kcal in women and 2792 kcal in men). This randomized, crossover study reported that butter and soybean oil did not result in significant differences in fasting hsCRP, HDL-2 and HDL-3. However, one major limitation in study was that the PUFA content among diets was not standardized. Therefore this study design led to the comparisons based on dietary fats quantity as a whole and prevented the head-to-head comparison of individual fatty acids

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per se. Voon, Ng, Lee, and Nesaretnam (2011) reported that the usage of palm olein, olive oil and coconut oil in meal preparation caused similar inflammatory response during the fasting and postprandial states, i.e. IL-6, IL-1β, IL-8, hsCRP, TNF-α, IFN-γ and total hemocysteine. The study achieved 7% en exchange between SAFA with MUFA for palm olein and olive oil; and 13% en exchange between SAFA and MUFA for coconut oil and olive oil diet groups. Weekday-meals were catered to the 45 healthy subjects, and standard breakfast was given during the 2-h postprandial challenge. It is notable that the duration of blood sampling for the postprandial challenge was relatively short (2 h) and hence may preclude the detection of inflammatory changes at later time.

The Mediterranean diet is favored for its cardio-protective effect due to its rich content of MUFA and antioxidant components. The beneficial effect of the Mediterranean diet was demonstrated in 180 patients with metabolic syndrome by Esposito et al. (2003) in a 2-year randomized, parallel study. The study showed that Mediterranean diet increased endothelial function score and decreased plasma hsCRP, IL-6, IL-7 and IL-8 compared to high-SAFA diet. The favorable effects of Mediterranean diet may be credited to the increased intake of total fruit, vegetables, nuts, whole grain and olive oil. However, Bladbjerg et al. (2011) reported conflicting findings. The study assigned 54 subjects to the high-MUFA Mediterranean diet group and 26 subjects to the high-SAFA Danish diet group. Subjects were advised to increase intake of vegetable oils, whole grain food, nuts and legumes in the high-MUFA diet group; whereas those who adapted to high-SAFA diet consumed food containing increased SAFA and moderate high-GI carbohydrate, low fiber. The beneficial effect of Mediterranean diet was not shown in the overweight subjects as compared to Danish diet with regards to inflammatory biomarkers such as IL-6, CRP, ICAM-1, von Willebrand factor and tissue factor pathway inhibitor. However, it is to be noted that the unequally distributed sample size and the unstandardized total fat intake (38.4% en in MUFA diet group and 32.1% en in SAFA diet group) may confound

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these observations. Petersson et al. (2010) compared the consumption of some high-MUFA elements found in Mediterranean diet (n = 111), i.e. olive oil and nuts, with full fat dairy foods (n = 100) in the LIPGENE study. This randomized, parallel study lasted 12 weeks and recruited subjects with metabolic syndrome. The consumption of the *ad libitum* diets did not result in significant differences in plasma CRP, urinary 8-Iso-PGF2α and urinary keto-dihydro-PGF2α. A substudy of the LIPGENE study recruited 39 subjects who were given skim milk, white bread, eggs and tomato enriched with olive oil and butter during the mixed meal challenge (Meneses et al., 2011). The background diets and the mixed meals did not alter the plasma IL-6, MCP-1 and adipose tissues inflammatory genes such as IL-1β, MCP-1, IKBβ2, IKBα and p65. The observations from the studies discussed above suggest that the food components in the Mediterranean diet rather than the type of fat may play a more important role in giving the beneficial effect over high-SAFA diet.

Based on the emergent evidence discussed above, consumption of diets enriched with SAFA cannot be concluded to be determining factor in pro-inflammatory response. Instead, fat source and diet pattern may play a more important role in the modulation of chemokines release. Rigorously well-designed studies with longer duration and larger sample size are required to improve understanding of the role played by type of dietary fatty acids (fat quality) in inflammation and endothelial function.

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Table 2.10: Chronic Effects of Different Types of Dietary Fat on Inflammatory and Thrombogenic Responses

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Silver et al., 2014	Obese yet healthy; HSD: 23 (0/23) HMD: 22 (0/22) HPD: 26 (0/26) Placebo: 20 (0/20)	Placebo: 37.0 ± 7.3 HSD: 36.4 ± 7.5 HMD: 37.3 ± 6.4 HPD: 36.1 ± 6.1	Randomized, controlled, parallel, double blind Run in: 2 wk	14 wk	1. High SAFA diet (HSD) 2. High MUFA diet (HMD) 3. High PUFA diet (HPD) 4. High-fat diet (Placebo)	Subjects followed daily menu with 9 fatty acid capsules supplementation (81 kcal/d). Daily caloric intake was REE x 1.2 (sedentary) activity factor. Oils, spreads, nuts and seeds were provided for meal preparation. 1906 kcal, 48.9% F, 21.3% P, 30.2% C; 30.6% SAFA, 35.1% MUFA, 34.2% PUFA HSD *Stearic acid* HMD *Oleic acid* HPD *Linoleic aicd* Control *Food grade silicon dioxide powder*	IL-1α, SBP, DBP and PAI-1 antigen: HSD, HMD and HPD ↓, Placebo =; NS IL-1β and TNF-β: Placebo ↓, HSD, HMD and HPD =; NS IL-6, , LAEI and SAEI: All =; NS IL-10: Placebo and HPD ↓; NS IL-17: HSD, HMD and placebo, HPD =; NS tPA antigen: HSD =, HMD, HPD and placebo ↓; NS TNF-α: All =; HMD ↓↓ vs placebo IL-12: Placebo, HMD and HPD ↓, HSD =; HMD ↓↓ vs placebo IFN-γ: All ↓; HSD ↓↓ vs placebo FMD: HSD ↓, HMD, HPD and placebo =; HSD and HMD ↓↓ vs placebo tPA activity: All ↑; HMD ↓↓ vs placebo

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; IL: interleukin; TNF, tumor necrosis factor; SAEL, small artery elasticity index; LAEL, large artery elasticity index; tPA, tissue plasminogen activator; IFN, interferon; FMD, flow mediated dilatation; ↑, significantly higher than baseline; ↓, significantly lower than baseline; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significant difference between diets

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Delgado-Lista et al., 2008	Healthy; 20 (20/0)	23.3±2.2	Randomized, controlled, cross over, double-blind Wash out: 1 week	28 days PP bleeding time: 0, 60, 120, 180, 240, 300, 360 min	1. High SAFA meal (HSM) 2. High MUFA meal (HMM) 3. High carbohydrate/high PUFA meal (HCPM)	Subjects received diet followed by PP meal challenge. Test meal consisted of 60% F, 15% P, 25% C (1 g fat/kg body weight). <u>HSM</u> 38% F, 15% P, 47% C; 22% SAFA, 12% MUFA, 4% PUFA *Butter(48 g/d)* Test meal: 35% SAFA, 22% MUFA, 4%PUFA <u>HMM</u> 38% F, 15% P, 47% C; 10% SAFA, 24% MUFA, 4% PUFA *Virgin olive oil and spread (70 ml/d)* Test meal: 22% SAFA, 38% MUFA, 4% PUFA <u>HCPM</u> 30% F, 15% P, 55% C; 10% SAFA, 12% MUFA, 8% PUFA *Biscuits (25 g/d), jam (12 g/d), and bread, walnuts (35 g/d)* Test meal: 20% SAFA, 24% MUFA, 16% PUFA	<u>Fasting measurements</u> FVIIc, PAI-1, D-dimer, tPA and TBX B2: NS <u>Changes from preprandial value</u> FVIIc: HMM and HSM ↓; HMM ↓↓ vs HSM PAI-1:HMM ↓, HSM ↑; HMM ↓↓ vs HSM D-dimer, tPA, tPA/PAI-1and TBX B2: =; NS

n, sample size; M, men; W, women; y, year; h, hour; PP, postprandial; %, % energy; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FVIIc, factor VII coagulant activity; PAI-1, plasminogen activator inhibitor-1; tPA, tissue-type plasminogen activator; TBX B2, thromboxane B2; NS, no significant difference between diets; ↓, decreased compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower; =, no changes compared to baseline.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Fuentes et al., 2008	Healthy; 20 (20/0)	23.3 ± 1.5	Randomized, controlled, crossover	4 wk PP bleeding time: 0, 120, 240, 360 and 480 min	1. High SAFA diet (HSD) 2. High MUFA diet (HMD) 3. High carbohydrate/ PUFA diet (HCD)	Subjects were provided daily meals. A PP challenge was conducted after dietary intervention. PP meal consisted of 50 – 66% daily energy intake. <u>HSD</u> 38% F, 15% P, 47% C; 22% SAFA, 12% MUFA, 4% PUFA, 0.4% ALA PP meal: 60% F, 15 % P,25% C 35% SAFA, 22% MUFA, 4% PUFA, 0.7% ALA *Butter* <u>HMD</u> 38% F, 15% P, 47% C; <10% SAFA, 24% MUFA, 4% PUFA, 0.4% ALA PP meal: 60% F, 15 % P,25% C 22% SAFA, 38% MUFA, 4% PUFA, 0.7% ALA *Extra virgin olive oil* <u>HCD</u> <30% F, 15% P, 55% C; <10% SAFA, 12% MUFA, 8% PUFA, 2% ALA PP meal: 60% F, 15 % P,25% C 20% SAFA, 24% MUFA, 16% PUFA, 4% ALA *Walnuts*	<u>Fasting measurements</u> sVCAM-1: HSM ↑↑ vs HMM and HCM sICAM-1: NS <u>PP meal</u> sVCAM-1 at 4 h: HSM and HMM ↓↓ vs HCM sVCAM-1 at 6 h: HSM ↓↓ vs HCM IRH at 4 h and 6 h: HSM and HCM ↓↓ vs HMM

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ALA, alpha linolenic acid; sVCAM, soluble vascular cell adhesion molecule; sICAM-1, soluble intercellular adhesion molecule-1; IRH, ischaemic reactive hyperaemia; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significant difference between diets.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Baer et al. 2004	Healthy; 50 (50/-)	25-60	Randomized, controlled, parallel, double-blind	5 wk	1. High carbohydrate (HCD) 2. Oleic acid (OL) 3. Lauric: Myristic: Palmitic = 0.3:1.4:8.3 (LMP) 4. Stearic acid (STE) 5. Trans-fatty acid (Ruth et al.) 6. TFA+STE	Daily meals were provided to subjects. <u>HCD</u> 3086 kcal, 30.6% F, 15% P, 54.6% C; 9.7% LMP, 2.9% STE, 10.4% OL, 0.2 TFA, 3.7% linoleic acid <u>OL</u> 3126 kcal, 38.3% F, 15.1% P, 46.6% C; 9.7% LMP, 2.9% STE, 17.6% OL, 0.1 TFA, 3.8% linoleic acid <u>LMP</u> 3129 kcal, 39.7% F, 15% P, 45.4% C; 18.0% LMP, 2.7% STE, 10.5% OL, 0.2 TFA, 4.2% linoleic acid <u>STE</u> 3122 kcal, 39.8% F, 14.9% P, 45.2% C; 10% LMP, 10.9% STE, 10.5% OL, 0.3% TFA, 4.4% linoleic acid <u>TFA</u> 3117 kcal, 39.6% F, 14.9% P, 45.7% C; 10.1% LMP, 2.8% STE, 10.6% OL, 8.3 TFA, 4.0% linoleic acid <u>TFA + STE</u> 3098 kcal, 39.5% F, 14.8% P, 45.6% C; 10% LMP, 6.9% STE, 10.6% OL, 4.2 % TFA, 4.3% linoleic acid	Fibrinogen: STE ↑↑ vs HCD, OL, TFA, LMP CRP: TFA ↑↑ vs HCD, OL, TFA+STE IL-6: STE, LMP, TFA ↑↑ vs OL E-selectin: TFA ↑↑ vs all others; OL ↓↓vs TFA, STE, TFA+STE, LMP

n, sample size; M, men; W, women; y, year; w, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ALA, alpha linolenic acid; sVCAM, soluble vascular cell adhesion molecule; sICAM-1, soluble intercellular adhesion molecule-1; IRH, ischaemic reactive hyperaemia; ↑↑, significantly higher; ↓↓, significantly lower.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Petersson et al., 2010	Metabolic syndrome; HSD: 100 (46/54) HMD: 111 (46/65) LFD: 106 (59/47) LFn3D: 100 (46/54)	Mean 56.6	Randomized, controlled, parallel	12 wk PP bleeding time: 0, 120, 240 min	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. Low-fat high complex carbohydrate diet + HOS supplement (LFD) 4. Low-fat high complex carbohydrate diet + n-3 PUFA supplement (LFn3D)	Subjects consumed *ad libitum* diet followed by a postprandial challenge. HSD 39.8% F, 17.1% P, 41.9% C; 17.6% SAFA, 12.5% MUFA, 6.4% PUFA HMD 39.5% F, 16.6% P, 40.7% C; 9.1% SAFA, 21.1% MUFA, 5.7% PUFA LFD 29.2% F, 18% P, 49.1% C; 6.6% SAFA, 11.5% MUFA, 5.3% PUFA LFn3D 28.2% F, 17.9% P, 51.6% C; 6.4% SAFA, 11.1% MUFA, 5.0% PUFA	Urinary 8-Iso-$PGF_{2\alpha}$ and 15-Keto-dihydro-$PGF_{2\alpha;}$ plasma CRP: NS Subgroup analysis Habitual fat intake, smoking, sex: NS Total fat intake above median in LFHCCn-3 (28.2% F): Urinary 8-Iso-$PGF_{2\alpha}$ ↑ Total fat intake above median in HMD (39.5% F): Urinary 15-Keto-dihydro-$PGF_{2\alpha;}$ ↓
Van Dijk et al. 2009	Abdominal overweight; 20 (10/10)	SAFA: 52.0 ± 6.3 MUFA: 58.9 ± 5.3	Randomized, controlled, parallel, non-blinded	8 wk Run-in: 2 wk	1. High SAFA diet (HSD) 2. High MUFA diet (HMD)	Diets with similar macronutrient were provided to subjects. 90% en fat was supplied by diet, 10% en fat was obtained from limited choice of low-fat product HSD 19% SAFA and 11% MUFA *Refined olive oil* HMD 11% SAFA and 20% MUFA *Refined olive oil*	Adiponectin: SAFA ↓; MUFA =; NS C3: SAFA and MUFA =; NS RANTES/CCL5: SAFA and MUFA ↓; NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CRP, C-Reactive Protein; C3, Complement 3; RANTES/CCL5, regulated on activation normal T cell expressed and secreted/chemokine (C-C motif) ligand 5;hs CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor; IL-6, interleukin-6; ↑, significantly higher than baseline; ↓, significantly lower than baseline; NS, no significant difference between diets.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Lichtenstein et al. 2003	LDL-C > 130 mg/dl; 36 (18/18)	M: 60 ±7 W:67 ±4	Randomized, controlled, crossover double-blind	7 wk	1. Soybean oil 2.Semi-liquid margarine 3.Soft margarine 4.Traditional stick margarine 5. Butter	Subjects were provided meals. 2114 kcal in women and 2792 kcal in men. 30% F, 17% P, 51% C. <u>Soybean oil</u> 12.87% SAFA, 55.87% MUFA, 29.5% PUFA; 67 mg cholesterol/1000 kcal <u>Semi-liquid margarine</u> 16.03% SAFA, 59.68% MUFA, 23.35% PUFA; 67 mg cholesterol/1000 kcal *Soybean oil* <u>Soft margarine</u> 17.6% SAFA, 46.84% MUFA, 33.53% PUFA; 67 mg cholesterol/1000 kcal *Soybean oil and cottonseed oil* <u>Shortening</u> 17.39% SAFA, 36.91% MUFA, 44.63% PUFA; 67 mg cholesterol/1000 kcal *Soybean oil* <u>Traditional stick margarine</u> 16.88% SAFA, 26.48% MUFA,55.19% PUFA; 121 mg cholesterol/1000 kcal *Soybean oil* <u>Butter</u> 61.65% SAFA, 3.67% MUFA, 26.88% PUFA	hsCRP: NS HDL-2 and HDL-3: NS

n, sample size; M, men; W, women; wk, week; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; hs CRP, high sensitivity C-reactive protein; HDL, high density lipoprotein; NS, no significant difference between diets.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Sanders et al., 2013	At risk of developing metabolic syndrome; HSD: 30 (12/18) HMD: 44 (14/30) HCD: 38 (14/24)	51	Randomized, controlled, parallel Run-in: 1 month HSD	6 months	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3.High-carbohydrate diet (HCD)	HSD 1886 kcal, 35.7% F, 17.8% P, 42.8% C; 14.6% SAFA, 11.3% MUFA, 5.7% PUFA *Full-fat mik (3.8 g/100 mL) and cheese (35 g/100 g); standard salad dressing* HMD 1908 kcal, 34.4 % F, 16.2% P, 47.2% C; 9.4% SAFA, 15.0% MUFA, 6.5% :PUFA *Skim milk (0.1 g fat/100 g) and half-fat cheese (18 g/100 g); high MUFA salad dressing* HCD 1941 kcal, 26.1% F, 17.8% P, 53.1% C SAFA:MUFA:PUFA: 10:57: 33; *Skim milk (0.1 g fat/100 g) and half-fat cheese (18 g/ 100 g); additional portions of bread, potatoes, and rice; reduced-fat salad dressing.*	PWV arterial stiffness, central and peripheral augmentation index, flow-mediated dilation, 8-isoprostane $F_{2\alpha}$-III, supine diastolic and systolic BP: NS
Lithander et al. 2008	Mildly hyperlipidemia; 18 (18/0)	39.7 ± 13.9	Randomized. controlled, cross over, double-blinded Wash out: 4 wk Blood collection: 0, 1, 7, 14, 21, 22 days	3 week	1. High SAFA diet (HSD) 2. Low-SAFA diet (LSD)	Subjects followed isocaloric (3176 kcal) 5-day rotation diet consisted of 34% F, 13% P and 53% C. Foods and snacks were provided. HSD 18% SAFA, 10% MUFA, 7% PUFA *Unknown source* LSD 13% SAFA, 12% MUFA, 8% PUFA *Unknown source*	TNF-α, IL-6,hs-CRP, adiponectin: NS

n, sample size; M, men; W, women; wk, week; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PWV_{c-f}: carotid to femoral pulse wave velocity; TNF-α, tumor necrosis factor; IL-6, interleukin-6; NS, no significant difference between diets.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Meneses et al., 2011	Metabolic syndrome; 39 (14/25)	Mean 56.6	Randomized, controlled, parallel	12 wk PP bleeding time: 0, 120, 240 min	1. High-SAFA diet (HSD) 2. High-MUFA diet (HMD) 3. Low-fat high complex carbohydrate diet + HOS supplement (LFD) 4. Low-fat high complex carbohydrate diet + n-3 PUFA supplement (LFn3D)	Subjects consumed ad-libitum diet followed by a postprandial challenge. Test meals consisted of 65% F, 10% P, 25% C; *Skim milk, white bread, eggs, egg yolks* <u>HSD</u> 40.3% F, 19.2% P, 38.3% C; 17.9% SAFA, 12.8% MUFA, 6.1% PUFA Test meal: 38% SAFA, 21% MUFA, 6% PUFA; *Butter, tomato* <u>HMD</u> 40.2% F, 19.2% P, 40.7% C; 9.1% SAFA, 21.1% MUFA, 5.7% PUFA Test meal: 12% SAFA, 43%, MUFA 10% PUFA; *Olive oil, tomato* <u>LFD</u> 27.1% F, 21.2% P, 51.2% C; 6.6% SAFA, 11.5% MUFA, 5.3% PUFA Test meal: 21% SAFA, 28% MUFA, 16% PUFA; *Butter, olive oil, walnuts* <u>LFn3D</u> 26.5% F, 18.8% P, 54.1% C; 6.4% SAFA, 11.1% MUFA, 5.0% PUFA Test meal: 21% SAFA, 28% MUFA, 16% PUFA *Butter, olive oil, walnuts*	<u>Fasting</u> IkBα, IkBβ2, MCP1, IL-1β and p65 expression: NS <u>PP challenge</u> p65 and IkBα gene expression: NS MCP-1, IL-1β gene expression in adipose tissue: ↑; NS IkBα mRNA, IkBβ2 mRNA, p65 mRNA, MCP1 mRNA, IL-6 mRNA and IL-1β: NS Plasma IL-6 and MCP-1: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; TNF-α; tumor necrosis factor-α; vs, compared to; ↑, significantly higher than baseline; ↓↓, significantly lower; NS, no significant difference between diets

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Jebb et al. 2010	At risk of developing metabolic syndrome; 548 (230/318) HS/HGI; 85 HM/HGI; 111 HM/LGI; 116 LF/HGI; 116 LF/LGI; 121	M:52 ± 10 W:51± 9	RCT, parallel, non-blinded	6 months 4 wk run-in	1. High SAFA/ high GI diet (HS/HGI) 2. High MUFA/ high GI diet (HM/HGI) 3. High MUFA/ low GI diet (HM/LGI) 4. Low-fat/ high GI diet (LF/HGI) 5. Low-fat/ low GI diet (LF/LGI)	Isocaloric (2050kcal/d) diet varied in type of fat glycemic index; ~7% en exchange between SAFA MUFA (HS/HGI vs HM/HGI and HM/LGI); ~10% exchange between SAFA and CARB (HS/LGI LG/HGI and LF/LGI); GI different at 8 points (HG LGI). *Subjects were provided with key sources o (e.g. spreads, cooking oils and margarine) carbohydrates (e.g. bread, pasta, rice and cereals)* HS/HGI 37.5% F, 20.5% P, 42% C 16% SAFA, 11.5% MUFA, 5.8% PUFA *Palm olein and milk fat* HM/HGI 35.6% F, 19.5% P, 44.9% C 9.5% SAFA, 16.2% MUFA, 6.6% PUFA *Spread, margarine rich in oleic acid derived from HOS and olive oil, HOS (additional source in Australia centre).* HM/LGI 35.7% F, 19.7% P, 44.6% C 9.6% SAFA, 16.3% MUFA, 6.9% PUFA LF/HGI 27.5% F, 21.4% P, 51.1% C 9.2% SAFA, 9.8% MUFA, 5.2% PUFA LF/LGI 26.1% F, 22.4% P, 51.5%C 8.3% SAFA, 9.7% MUFA, 5.1% PUFA	Fibrinogen, CRP, ICAM-1, PAI, FVIIc: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1; PAI, plasminogen activator inhibitor; FVIIc, factor VIIc; NS, no significant difference between diets

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Esposito et. al., 2004	Metabolic syndrome; HMD: 90 (44/46) HSD: 90 (45/45)	HMD: 44.3 ± 6.4 HSD: 43.5 ± 5.9	RCT, parallel, single-blind	2 years	1. Mediterranean diet (HMD) 2. High SAFA diet (HSD)	Subjects consumed *ad libitum* diets and increased their level of physical activity <u>HMD</u> 2065 kcal, 28% F, 14% P, 58% C; 8% SAFA, 12.4% MUFA, 7.6% PUFA *Total fruit, vegetable, and nuts intake (274 g/d), whole grain intake (103 g/d), and olive oil consumption (8 g/d).* <u>HSD</u> 2184 kcal, 30% F, 13.5% P, 57.1% C; 13.7% SAFA, 9.6% MUFA, 6.7% PUFA	Endothelial function score: HMD ↑, HSD =; HMD ↑↑ vs HSD hs-CRP, IL-6, IL-7 and IL-18: HMD ↓, HSD =; HMD ↓↓ vs HSD
Bladbjerg et al., 2011	Overweight; HMD: 54 (22/32) HCD: 51 (22/29) Control: 26 (11/15)	HMD: 29.2 ± 4.5 HCD: 27.3 ± 4.9 Control: 27.6 ± 5.1	Randomized, controlled, parallel	6 months	1. High MUFA diet (HMD) 2. High carbohydrate diet (HCD) 3. Danish diet (Control)	Subjects who lost >8% of body weight were recruited. Foods were provided to subjects. <u>HMD</u> 2748 kcal, 38.4% F, 15.3% P, 43.3% C; 7.1% SAFA, 20.2% MUFA, 7.8% PUFA High intake of vegetable oils, whole-grain food, nuts and legumes <u>HCD</u> 2508 kcal, 23.6% F, 15.8% P, 57.6% C; 7.9% SAFA, 8.4% MUFA, 5.2% PUFA Low in fat, high in mixed-GI carbohydrates <u>Control</u> 2603 kcal, 32.1% F, 15.9% P, 49.8% C; 15.1% SAFA, 10.4% MUFA, 4.0% PUFA Moderate in fat, high in SAFA, moderate in high-GI carbohydrates, low in fiber	ICAM, vWF, TFPI: =; NS CRP: all ↓; NS IL-6: HMM and HCM ↓, Control =; NS

n, sample size; M, men; W, women; y, year; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ICAM-1, intercellular adhesion molecule-1; vWF, von Willebrand factor; TPFI, tissue factor pathway inhibitor; CRP, C-reactive protein; IL, interleukin; ↑, increased compared to baseline; ↓, decreased compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significant difference between diets; =, no changes compared to baseline.

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'Table 2.10, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Teng et al., 2010	Healthy; 41 (8/33)	28.8 ± 9.1	Randomized, controlled, single-blind, crossover Wash out: 1 week	5 wk *Results were means of week 4 and 5.	1. High oleic palm olein diet (HOPO) 2. Partially hydrogenated soybean oil diet (PHSO) 3. Palm stearin diet (PST)	Subjects were provided meals during weekdays and test fats for home meal preparation during weekends. <u>HOPO</u> 2221.3 kcal, 33.5% F, 19.7% P, 47.2% C; 10.8% SAFA, 15.3% MUFA, 8.6% PUFA; 15% OA, 9% PA <u>PHSO</u> 2125.7 kcal, 32.3% F, 19.3% P, 48.5% C; 10.4% SAFA, 17.1% MUFA, 4.2% PUFA; 7% OA, 10% TFA <u>PST</u> 2292.9 kcal, 31.7% F, 19.2% P, 49.0% C; 16.4% SAFA, 10.7% MUFA, 3.8% PUFA; 11% OA, 14% PA	IL-6, IL-1β and TNF-α: NS hsCRP: HOPO and PST ↓↓ vs PHSO IL-8: PST ↑↑ vs HOPO and PHSO
Voon et al., 2011	Healthy; 45(9/36)	30.1±8.3	Randomized, controlled, cross over, single-blind Run-in: 3 wk	5 wk PP bleeding time: 7 times in 2 h	1. Olive oil 2. Coconut oil 3. Palm olein	Meals were provided during weekdays. Diets comprised 30% F, 20% P, 50% C. A bowl of rice or noodle, 2 meats (chicken or fish), 1 vegetable) <u>Olive oil</u> 30.1% F, 23.1% P, 46.8% C; 6.6% SAFA, 20.1% MUFA, 4.4% PUFA <u>Coconut oil</u> 30.6% F, 23.1% P, 46.3% C; 20.6% SAFA, 6.9% MUFA, 2.9% PUFA <u>Palm olein</u> 30.6% F, 22.7% P, 46.7% C; 12.5% SAFA, 13.0% MUFA, 4.9% PUFA PP challenge (at week 4) Standard breakfast (composition unspecified)	Fasting and PP challenge tHcy, TNF-α, IL-1β, IL-6, hsCRP, IFN-γ, IL-8: NS

n, sample size; M, men; W, women; y, year; wk, week; PP, postprandial; %, % energy; F, fat; P, protein; C, carbohydrate; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OA, oleic acid; PA, palmitic aicd; TFA, trans fatty acid; IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α; tumor necrosis factor-α; hsCRP, high sensitivity C-reactive protein; IL-8, interleukin-8; tHcy, total homocysteine; IFN-γ, interferon-γ; NS, no significant difference between diets

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2.10 Impact of Dietary Fats on Gastro-intestinal Peptides

The following subsections summarize published literature on the 1) acute effect of dietary fat quantity (Table 2.11), 2) chronic effect of dietary fat quantity (Table 2.12), 3) acute effect of dietary fat quality (Table 2.13) and 4) chronic effect of dietary fat quality (Table 2.14), on gastro-intestinal peptides.

2.10.1 Acute Effects of Dietary Fat Quantity on Gastro-intestinal Peptides Release

A review (Cummings & Overduin, 2007) pointed out that suppression of ghrelin occurs upon meal consumption, with greater suppression by carbohydrate compared to protein followed by fat at isocaloric conditions. In agreement with the aforementioned findings, in part, Tannous dit El Khoury et al. (2006) reported that the greatest magnitude of suppression of acylated ghrelin over the postprandial 3 h period (iAUC not measured) was found with high-carbohydrate meal compared with high-fat and high protein meals. However, the study by Tannous dit El Khoury et al. (2006) also found that high-protein meal suppressed acylated ghrelin longer than the high-fat and high-carbohydrate meals. The isocaloric meals given to these 10 healthy men were based on 30% en of their resting energy expenditure (mean 615 kcal) utilizing a liquid meal prepared with Ensure Complete Balanced Nutrition, soy/whey protein, sugar, sunflower oil and water, giving a 30% en exchange between fat, carbohydrate and protein. Another trial utilizing 3-h meal tolerance test (Brennan et al., 2012) showed conflicting findings. In this study, the 16 lean and 16 obese men consumed a standard breakfast before they were fed an isocaloric test meal containing 30% en of the subjects' resting energy expenditure (mean 215 kcal) as either fat, carbohydrate or protein, in a crossover design. The isocaloric meal consisted of tomato sauced-pasta, lean beef mince, onion, olive oil, mixed herbs and a vanilla yoghurt

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dessert. Contrary to the aforementioned study by Tannous dit El Khoury et al. (2006), this experiment revealed suppression of ghrelin in both lean and obese groups was the greatest by protein, followed by fat then carbohydrate at certain time points (iAUC was not measured). Furthermore, the suppression of ghrelin was found longer on high-fat and high-protein meals. The inconsistency of results for ghrelin suppression generated in these two studies may be explained by the study design. The first difference between the two studies was the biomarker: acylated ghrelin vs total ghrelin. Total ghrelin is the sum of 10% de-acylated ghrelin and 90% acylated ghrelin. In addition, the study design also differed with regards to the form of test meals (liquid vs solid meal) as well as the energy content of test meal (615 vs 215 kcal). On the other hand, no significant difference in ghrelin suppression was reported in a study comparing carbohydrate and fat, but not protein by Gibbons et al. (2013), in which 16 overweight or obese subjects were exposed to isocaloric (590 kcal) high-fat meal (50.3% en fat) or high-carbohydrate meal (3.2% en fat), in a random order. Both meals comprised yoghurt, honey, fruit and coffee or tea. The absence of high-protein meal as one of the treatments may have caused the different findings.

The aforementioned study that found that protein was a stronger stimulant of CCK elevation at the late phase of postprandial challenge (iAUC was not measured) (Brennan et al., 2012). Higher CCK level after protein *vs* carbohydrate ingestion occurred between 120-180 min in lean subjects. Protein also elevated CCK to a higher degree compared to carbohydrate as well as fat between 150-180 min in the obese group. The CCK elevating action of protein lasted longer than that of fat and carbohydrate in both groups. No other relevant acute study compared the effects fat quantity.

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Brennan et al. (2012) also reported that higher PYY level after protein *vs* carbohydrate was observed between 90-120 min and 180 min in obese subjects. The greater increase in PYY with protein in obese subjects was in agreement with findings of van der Klaauw et al. (2013), in healthy subjects which are elucidated below. van der Klaauw et al.'s 4-h study provided 8 healthy subjects, pancakes with different trimmings such as bacon and grated cheddar cheese for high-fat meal; no-sugar maple syrup and full-fat Greek yoghurt for high-protein meal; and bacon and maple syrup for high-carbohydrate meal. The isocaloric test meals provided 20% daily energy requirement to subjects and each meal supplied standardized fat, protein or carbohydrate exchange at 40% en. GLP-1 and the co-secreted PYY were found to be higher on the high-protein meal compared to the other two meals.

In contrast with the aforementioned findings, Helou, Obeid, Azar, and Hwalla (2008) observed that fat but not carbohydrate was more effective in stimulating the significant rise in PYY compared to protein at 15 and 30 min in subjects who consumed a meal with caloric content of 30% of subjects' resting energy expenditure (mean 477 kcal). PYY peaked at 30 min for fat, earlier than that for carbohydrate and protein respectively at 60 and 120 min. However, the PYY elevation after fat returned to baseline at 120 min whereas PYY was still significantly increased compared to baseline at the end of challenge (180 min). The finding is however limited as iAUC was not measured. In this study, each of the 8 obese hyperinsulinemic women were fed a liquid mixed meal consisting of Ensure®, soy/whey protein, sugar, sunflower oil and water. The discrepancies observed with regards to PYY in these studies could be attributed to the divergence in test meals: 1) food composition (assorted food across treatments *vs* solid food items varying in composition *vs* liquid meal varying in composition), 2) % en exchange (40 *vs* 47% *vs* 30% en between fat and carbohydrate) and 3) energy content (20% daily energy requirement *vs* 590 kcal *vs* 30% of resting energy expenditure). (Gibbons et

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al., 2013) did not include protein in the treatments and reported that fat was a more potent stimulant for GLP-1 and PYY compared to carbohydrate (study design was described as above).

Ranganath et al. (1996) compared GLP-1 and GIP responses to isocaloric (340 kcal) meals between 6 lean *vs* 6 obese women. The high-fat meal was 37.8 g Sainsbury's Double Cream and the high-carbohydrate meal was 100 g Hycal, Smith-Kline Beecham; both diluted 1:1 by volume with water. However, the macronutrient composition was not reported. Higher GLP-1 response in response to high-carbohydrate meal over 3 h was found in obese compared to lean subjects. In both subject groups, GLP-1 was found to be lower at 30 min after the administration of intravenous bolus of heparin for 10000 units at 120 min which was used for lipoprotein lipase activity assessment. However, no significant difference in GIP response was found between groups. Collier and O'Dea (1983) investigated the impact of coingestion of fat with carbohydrate or protein on GIP. The carbohydrate meal comprised 50 g whole, unpeeled, boiled potato whereas protein meal was 250 g veal. The additional fat was from 63 g butter. Elevation in GIP level was sustained until the end of the study (4 h) after both carbohydrate or protein meals coingested with fat, whereas GIP level returned to baseline after both meals without fat. In addition, the GIP response over the first 60 min after the meals with fat was higher than that of the meals without fat. Also, coingestion of carbohydrate with fat resulted in higher GIP response compared to meals comprised of protein combined with fat. Nevertheless, the strength of the finding may be limited by the unequal caloric content of the test meals.

In the main, acute gut hormone responses were affected by alteration in macronutrient composition. However, no particular differential effect of fat and carbohydrate can be concluded based on the conflicting findings from the evidence available. The sample size in these studies was small; two with sample size n=16 while

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the others used 10 or less subjects. Furthermore, most of the studies did not include the measurement of iAUC leading to difficulty in interpreting the differential effects of each macronutrient over the postprandial period.

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Table 2.11: Acute Effects of Dietary Fat Quantity on Gastro-intestinal Peptides Release

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Tannous et al. 2006	Healthy; 10 (10/0)	22.2 ± 1.28	Randomized. controlled, cross-over Run-in: 3 days Washout: 2 wk	3 h Bleeding time: 15, 30, 60, 120, and 180 min	1. High-fat meal (HFM) 2. High-protein meal (HPM) 3. High-carbohydrate meal (HCM)	Liquid meal (mean 615 kcal) providing 30% of subjects' REE *Ensure Complete Balanced Nutrition®, soy/whey protein, sugar, SFO and water* <u>HFM</u> 50% F, 20% P, 30% C <u>HPM</u> 20% F, 50% P, 30% C <u>HCM</u> 20% F, 20% P, 60% C	Acylated ghrelin: ↓ at 60 min after all meals, HFM and HCM returned to baseline while HPM ↓ at 180 min. Changes in acylated ghrelin from baseline: HPM ↓↓ *vs* HFM and HCM at 180 min; HCM ↓↓ *vs* HPM at 30 and 60 min. Acylated ghrelin suppression : HCM ↑↑ *vs* HFM and HPM
van der Klaauw et al. 2013	Healthy; 8 (3/5)	Mean: 32	Randomized. controlled, single blinded, cross-over	4 h Bleeding time: 0, 30, 60, 90, 120, 180, 240 min	1. High-fat meal (HFM) 2. High-protein meal (HPM) 3. High-carbohydrate meal (HCM)	Breakfast test meal providing 20% daily energy requirement which 60 % was from test nutrient. <u>HFM</u> 60% F, 20% P, 20% C *Whole meal pancakes were served with bacon and grated cheddar cheese* <u>HPM</u> 20% F, 60% P, 20% C *Pancake made up of high-P pancake mix and served with no-sugar maple syrup and full-fat Greek yoghurt.* <u>HCM</u> 20% F, 20% P, 60% C *Buckwheat pancakes served with bacon and maple syrup*	Ghrelin: NS PYY and GLP-1: HPM ↑↑ *vs* HFM and HCM

n, sample size; M, men; W, women; y, year; wk, weeks; REE, resting energy expenditure; h, hour; SFO, sunflower oil; F, fat; P, protein; C, carbohydrate; %, % energy; ↑, increased compared to baseline; *vs*, compared to; ↓, decreased compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significance between diets; PYY, peptide YY; GLP-1, glucagon-like peptide-1.

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'Table 2.11, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Helou, Obeid, Azar, & Hwalla, 2008	Obese hyperinsulinemic; 8 (0/8)	28.60 ± 2.29	Randomized. controlled, cross-over Run-in: 3 days Wash-out: 1 month	3 h Bleeding time: 0, 15, 30, 60, 120 and 180 min	1. High-fat meal (HFM) 2. High-protein meal (HPM) 3. High-carbohydrate meal (HCM)	Liquid meal providing 1 kcal/ml was given based on 30% of subjects' resting energy expenditure where mean calorie was 477 kcal. *Ensure®, soy/whey protein, sugar, SFO, water* <u>HFM</u> 50% F, 20% P, 30% C; 42.6% UFA *18.73 g SFO* <u>HPM</u> 50% F, 20% P, 30% C; 14.5 % UFA *2.8 0 g SFO* <u>HCM</u> 20% F, 20% P, 60% C; 17.4% UFA *0.58 g SFO*	Peaking pattern of PYY_{3-36} after HCM: ↑ immediately and peaked at 60 min (45% ↑); 30% ↑ at 15, 30, 120 and 180 min Peaking pattern of PYY_{3-36} after HFM: ↑ immediately and peaked at 30 min (35% ↑); returned to baseline at 120 min. Peaking pattern of PYY_{3-36} after HPM: = until 30 min, NS ↑ at 60 min; peaked at 120 min (30% ↑); ↑ at 180 min PYY: HFM ↑↑ *vs* HPM at 15 and 30 min; HFM ↑↑ *vs* HPM and HCM at 30 min. Increase in PYY_{3-36} from baseline: HFM ↑↑ *vs* HPM at 15 and 30 min; HFM ↓↓ *vs* HPM at 120 min
Ranganath et al.1 996	Obese; 6 (0/6) Lean; 6 (0/6)	Obese: 30.2 ± 10.7 Lean: 31.0 ± 9.5	Randomized, controlled, crossover Wash-out: 2 days	3 h Bleeding time: -30, 0, 15, 30, 45, 60, 90, 120, 135, 150 and 180 min	1. High-carbohydrate meal (HCM) 2. High-fat meal (HFM) Intravenous bolus of heparin (10 000 units) was administered 2 h after meal	Isocaloric (340 kcal) test meal. (macronutrient composition not reported) <u>HCM</u> Hycal 100 g, Smith-Kline Beecham diluted 1:1 by volume with water <u>HFM</u> Sainsbury's Double Cream, 37.8 g diluted 1:1 by volume with water	Fasting GLP-1: Obese ↓↓ *vs* lean GLP-1 peak : 15 min in obese, 90 min in lean iAUC GLP-1: HC ↓↓ in obese *vs* lean GLP-1: ↓↓ at 150 min *vs* at 120 min (heparin infusion) in both groups $iAUC_{GIP}$: NS in both groups

n, sample size; M, men; W, women; h, hour; F, fat; P, protein; C, carbohydrate; %, % energy; ↑, increased compared to baseline; *vs*, compared to; ↓, decreased compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significance between diets; PYY, peptide YY; GLP-1, glucagon-like peptide-1, iAUC, incremental area under the curve

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'Table 2.11, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Collier and O'Dea, 1983	Healthy lean; 8 (4/4)	21.5 ± 1.5	Randomized, controlled, crossover	4 h Bleeding time: 0, 15, 30, 60, 90, 120, 180, 240 min	1. Carbohydrate 2. Carbohydrate + Fat 3. Protein 4. Protein + Fat	Test meals containing unequal calorie was served as breakfast <u>Carbohydrate</u> 50 g carbohydrate *50 g whole, unpeeled, boiled potato* <u>Carbohydrate + Fat</u> 50 g carbohydrate and 50 g fat *50 g whole, unpeeled, boiled potato with 63 g butter* <u>Protein</u> 50 g protein *250 g veal* <u>Protein + Fat</u> 50 g protein and 50 g fat *250 g veal with 63 g butter*	$iAUC_{GIP\ (0\text{-}60\ min)}$: Carbohydrate + Fat ↑↑ *vs* Carbohydrate; Protein + Fat ↑↑ *vs* Protein; Carbohydrate + Fat ↑↑ *vs* Protein + Fat GIP at 4 h: Carbohydrate + Fat and Protein + Fat ↑; Carbohydrate and Protein =
Gibbons et al. 2013	Overweight/obese; 16 (5/11)	45.6 ± 6.2	Randomized, crossover, single-blind	180 min Bleeding time: 0, 15, 30, 60, 90, 120, and 180 min	1. High-fat meal (HFM) 2. High-carbohydrate meal (HCM)	<u>Breakfast</u> Isocaloric 590 kcal mixed meal consisted of yogurt, honey, fruit, and coffee or tea <u>HFM</u> 50.3% F: 11.7% P: 38.0 % C <u>HCM</u> 3.2% F: 13.2% P: 83.6% C	Ghrelin: NS GLP-1 and PYY: HF ↑ *vs* HC

n, sample size; M, men; W, women; h, hour; ↑, increased compared to baseline; *vs*, compared to; ↑↑, significantly higher; ↓↓, significantly lower; GIP, Glucose-dependent Insulinotropic Polypeptide; PYY, peptide YY; =, no changes from baseline

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'Table 2.11, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Brennan et al. 2012	Healthy, lean;16 Obese; 16	Healthy: 29 ± 2 Obese: 33 ± 0.5	Randomized, controlled, crossover Wash out: 3-7 days	3 h Bleeding at 0, 15, 30, 60, 90, 120, 150, 180 min	1. High-fat meal (HFM) 2. High-protein meal (HPM) 3. High-carbohydrate meal (HCM)	Standard breakfast consisting of a cup of white coffee with 1 teaspoon of sugar, a glass of orange juice, and of whole-meal toast with butter and jam. Test meal consisting of conventional pasta, with a tomato-based sauce, lean beef mince, onion, olive oil, mixed herbs, and a vanilla yoghurt dessert. The energy content was 30% of subjects' estimated daily energy requirement. <u>HFM</u> 224.5 kcal; 55 F: 15 P: 30 C *Pure cream* <u>HPM</u> 212.1 kcal; 25 F: 45 P: 30 C *Whey protein isolate* <u>HCM</u> 213 kcal; 30 F: 10 P: 60 C *Corn flour and raw sugar*	<u>Lean subjects</u> CCK: HP ↑↑ *vs* HC at 120-180 min; HF and HC ↓ at 180 min PYY: HF ↑↑ *vs* HC at 90 min; HP ↑ at 180 min; Ghrelin: HP ↓↓ *vs* HC at 120-180 min; HF ↓↓ *vs* HC at 150-180 min; HP ↓↓ *vs* HF at 90-180 min; HP and HF ↓ at 180 min <u>Obese subjects</u> CCK: HP ↑↑ *vs* HF and HC at 150- 180 min; HF and HC ↓ at 180 min PYY: HP ↑↑ *vs* HC at 90-120 and 180 min; HF ↑↑*vs* HC at 150 min; HP, HC and HF = at 180 min Ghrelin: HP ↓↓ *vs* HC at 120-180 min; HF ↓↓ *vs* HC at 180 min; HP ↓↓ *vs* HF at 150 min; HP and HF ↓ at 180 min <u>Comparison between lean and obese subjects</u> CCK and ghrelin: NS in baseline or in response to test meals between groups PYY: Baseline was slightly lower in obese; NS in response to test meals between groups

n, sample size; M, men; W, women; F, fat; P, protein; C, carbohydrate; %, % energy; ↑, increased compared to baseline; *vs*, compared to; ↓, decreased compared to baseline; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significance between diets; CCK, cholecystokinin; PYY, peptide YY; ; =, no changes from baseline.

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2.10.2 Chronic Effects of Dietary Fat Quantity on Gastro-intestinal Peptides Release

In recent years, exploration in the influence of circulating gut hormones on food intake regulation and prevention of obesity has gained much attention. While awaiting the positive findings to be confirmed, there was novel suggestion that determination of fasting gut hormones could be a tool to evaluate development of metabolic syndrome and comorbidities of obesity (Zwirska-Korczala et al., 2007). It was also reported that GIP antagonized the glucagon suppressing effect of GLP-1 in T2DM patients (Mentis et al., 2011). The alteration in macronutrient content in habitual diet following recent nutritional guidelines to lower fat intake may trigger the nutritional response of gut hormones. Evidence from animal studies showed that high-fat diet caused hyperplasia in K cells and hence resulted in oversecretion of GIP (Gniuli et al., 2010). A human study by (Numao et al., 2012) supported the hypothesis. This study provided 9 healthy subjects with Japanese normal-fat diet *vs* high-fat diet which differed by 47% en of fat (22 *vs* 69% en fat) for 3 days. Significantly higher fasting concentration of GIP was observed compared to baseline and between diets after the short adherence to the extremely high fat intake. However, the postprandial GIP responses to oral glucose tolerance test (75 g liquid glucose) performed at the end of each diet treatment did not exhibit significant difference. The significance of the fasting GIP increment was not explained by the authors. However, it is noteworthy that the exceedingly high fat intake at 69% en is not common in a daily diet. The finding should be confirmed in further investigations using more reasonable energy content from fat consumed for a longer period. In this study however, (Numao et al., 2012) also observed unvaried fasting GLP-1 concentration at the end of the diet intervention and between diets. Yet, the high fat intake resulted in higher circulating GLP-1 concentration between 30 to 90 min and GLP-1 response over 120 min in response to

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75 g glucose. The authors hypothesized that the increased circulating GLP-1 was an adaptive response to glycogen depletion after high-fat diet. Another study (Paniagua et al., 2007) comparing a relatively lower % en of fat exchange (22 *vs* 38% en fat) for a longer period further supports the findings increased postprandial GLP-1 after high fat diet by Numao et al. (2012). This 4-week crossover dietary intervention compared high-fat diets (38% en fat, rich in either 23% en SAFA or MUFA) and high-carbohydrate diet (20% en fat) in a group of abdominally obese subjects with insulin resistance. The 14% en exchange between SAFA and MUFA was achieved by utilizing common food-stuffs such as butter, whole milk, stuffed turkey (SAFA) *vs* extra virgin olive oil and skimmed milk (MUFA). Marmalade and sugar are examples of foods used to replace fat with carbohydrate at an exchange of 18% en. At the end of the intervention, fasting blood sample collection was followed by a meal challenge in which bread was provided for all test meals, accompanied with butter and whole milk for SAFA meal; skimmed milk and olive oil for MUFA meal; and skimmed milk and marmalade for carbohydrate meal. After 4 weeks exposure, fasting GLP-1 did not differ between all 3 diets whereas postprandial GLP-1 response over 3 h (AUC) and GLP-1 concentration at 60 and 120 min were higher following both high-fat diets; while measurements of fasting and postprandial GIP were not done in this study.

Two studies however have reported contrasting findings on GLP-1. In a study allocating non-isocaloric diets to 12 healthy men, the high-fat diet (4806 kcal, 42% en fat) provided additional 2134 kcal from 190 g fat compared to the high-carbohydrate diet (2672 kcal, 11% en fat) for 14 days (Boyd et al., 2003). The diets did not result in appreciable differences in postprandial GLP-1 and CCK in response to a 90-min duodenal lipid infusion (6.28 kJ/min) administered on the test day after the completion of each diet treatment, whereas fasting measurements were not made. The limitation of the findings may be the differences in total energy and protein intake between diets. Nevertheless, a

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well-designed randomized parallel study by Ellis et al. (2012) revealed comparable findings in overweight subjects. In this study, 61 sedentary, overweight subjects underwent intervention exposure to either high-fat diet (n = 27; 39% en fat) or high-carbohydrate diet (n = 34; 27% en fat) in an isocaloric manner for 8 weeks. Besides fasting measurements at the study end, a liquid test meal challenge was performed at baseline and after treatment. Carnation Instant Breakfast and whole milk were given as the test meal with caloric content of 7 kcal/kg. Both diets did not cause variation in GLP-1 and ghrelin between diets at fasting state furthermore no significant difference in the postprandial levels of both markers compared with baseline. Besides the larger sample size, the lower and more reasonable % en exchange between diets in this study at 12% may explain the differing results with regards to GLP-1 by Numao et al. (2012) and Paniagua et al. (2007) as discussed previously, i.e % en exchange was respectively 47% and 18%. In a well-powered study (Beasley et al., 2009), 164 subjects with prehypertension or stage 1 hypertension consumed the 3 OMNI-Heart diets, where fat and carbohydrate was exchanged for 10% en. In agreement with Ellis et al. (2012), this study found that fasting ghrelin did not change in comparison with baseline and there was no significant difference between diets after a 6-week diet rich in protein, carbohydrate or unsaturated fat (predominantly MUFA). Fasting obestatin level was found to be higher following diets high in carbohydrate and unsaturated fats *vs* protein. Fasting leptin was found to be lower than baseline for all diets, where high protein intake lowered fasting leptin level to a greater extent compared to high carbohydrate consumption.

Taken together, there is no consistent differential effect of a longer duration of high-fat and high-carbohydrate intake on gut hormones based on the limited evidence available, although short duration of very high fat intake increases fasting GIP and postprandial GLP1 and some studies report increased postprandial GLP1 with longer exposure to increased fat intake. Further studies are warranted to confirm the findings, in

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particular if postprandial GLP-1 would be altered by a certain minimal % en exchange between fat and carbohydrate.

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Table 2.12: Chronic Effects of Dietary Fat Quantity on Gastro-intestinal Peptides Release

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Paniagua et al. 2007	Abdominally obese with insulin resistance; 11 (4/7)	62 ± 9.4	Randomized, controlled, crossover	4 wk PP bleeding time: 0, 15, 30, 60, 90, 120, 150, 180 min	1) High-SAFA diet (SAFA) 2) High-MUFA Mediterranean diet (MUFA) 3) High-carbohydrate diet (CARB)	Subjects adhered to *ad libitum* isocaloric diet intervention, followed by a 443 kcal breakfast meal for postprandial challenge. <u>SAFA</u> 2285 kcal; 38% F, 15% P, 47% C; 23% SAFA, 9% MUFA, 6% PUFA *Butter, stuffed turkey, whole milk* Breakfast meal: 200 ml whole milk, 50 g bread, and 25 g butter. <u>MUFA</u> 2290 kcal; 38% F, 15% P, 47% C; 9% SAFA, 23% MUFA, 6% PUFA *Extra virgin olive oil, skim milk* Breakfast meal: 200 ml skim milk, 50 g bread, and 27 g olive oil <u>CARB</u> 2275 kcal; 20% F, 15% P, 65% C; 6% SAFA, 8% MUFA and 6% PUFA *Marmalade* with sugar, skim milk with sugar, sunflower oil Breakfast meal: 200 ml skim milk, 70 g bread, and 75 g marmalade	<u>Fasting measurement</u> GLP-1: NS <u>PP measurements</u> AUC $_{GLP-1}$: CARB ↓↓ *vs* SAFA and MUFA

n, sample size; M, men; W, women; wk, weeks; PP, postprandial; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ; F, fat; P, protein; C, carbohydrate; %, % energy; *vs*, compared to; ↓↓, significantly lower; NS, no significance between diets; GLP-1, glucagon-like peptide-1

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'Table 2.12, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Beasley et al. 2008	Prehypertension or stage 1 hypertension; 164 (71/73)	53.6 ± 10.8	Randomized, controlled, crossover double-blinded 6-day run-in	6 wk	1. Protein 2. Carbohydrate 3. Unsaturated Fats (OMNI-Heart treatments)	Isocaloric US diet (2100 kcal) were provided to subjects, different in 10% en test nutrient. <u>Protein</u> 27% F, 25% P, 48% C; GL 177; 6% SAFA, 13% MUFA, 8% PUFA *Plant based protein* <u>Carbohydrates</u> 27% F, 15% P, 58 C; GL 242; 6% SAFA, 13% MUFA, 8% PUFA; <u>Unsaturated Fats</u> 37% F, 15% P, 48,C; GL 183; 6 % SAFA, 21% MUFA, 10% PUFA *Olive oil spread, olive oil and canola oil*	<u>Fasting measurement</u> Obestatin: =; Carbohydrates and Unsaturated ↑↑ *vs* Protein Ghrelin: =; NS Leptin: Protein, Carbohydrates and Unsaturated Fats ↓; Protein ↓↓ *vs* carbohydrate
Numao et al. 2012	Healthy; 9 (9/0)	27 ± 1	Randomized, controlled, crossover	3 days OGTT bleeding time: 0, 15, 30, 45, 60, 90 and 120 min	1. High-fat diet (HFD) 2. Low-fat diet (LFD)	Isocaloric Japanese diet was supplied to subjects. OGTT was conducted after dietary intervention. <u>HFD</u> 2793 kcal; 69% F, 11% P, 20% C <u>LFD</u> 2824 kcal; 22%F, 11% P, 67% C <u>OGTT</u> 75 g liquid glucose	Fasting GIP: HFD ↑, LFD = Fasting GLP-1: =, NS PP GLP-1: HFD ↑↑ *vs* LFD at 30, 45, 60 and 90 min AUC $_{GLP\text{-}1(0\text{-}120\ min)}$: HFD ↑↑ *vs* LFD PP GIP: NS

n, sample size; M, men; W, women; wk, week; OGTT, oral glucose tolerance test; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ; F, fat; P, protein; C, carbohydrate; %, % energy; *vs*, compared to; ↓↓, significantly lower; NS, no significance between diets; ↑↑, significantly higher; ↑, higher compared to baseline; ↓, lower compared to baseline; ; =, no changes from baseline; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide

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'Table 2.12, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Ellis et al. 2012	Sedentary overweight; HFD: 34 (18/16) LFD: 27 (13/14)	HFD: 35.6 ± 8.5 LFD: 34.6± 8.1	Randomized, controlled, parallel 3-day run-in	8 wk Bleeding time during liquid test meal challenge: 0, 15, 60, 90, 120, 180, 240 min	1) High-fat diet(HFD) 2) Low-fat diet(LFD)	Isocaloric (2500 kcal/d) diet; 12% en fat exchange with CARB. Foods were allocated for daily consumption including weekends. <u>HFD</u> 39% F, 18% P, 43% C <u>LFD</u> 27% F, 18% P, 55% C <u>Liquid test meal</u> Carnation Instant Breakfast and whole milk 7 kcals/kg; 24.0% F, 17.4% P, 58.6% C	<u>Fasting measurements</u> Ghrelin and GLP-1: = for both diets <u>Changes from fasting values during challenge</u> Ghrelin and GLP-1: = for both diets $AUC_{ghrelin}$ and AUC_{GLP-1}: = for both diets
Boyd et al. 2003	Healthy; 12 (12/0)	26.7 ± 1.7	Randomized, controlled, crossover, single-blinded	14 days Bleeding time: -15, 0, 15, 30, 45, 60, 75 and 90 min	1. High-fat diet (HFD) 2. Low-fat diet (LFD) On test day, a duodenal lipid infusion was administered at a rate of 6.28 kJ/min for 90 min	Non-isocaloric daily meal plans, including snacks, were designed for each subject. Both diets incorporated similar foods, with standardized protein content. Subjects received weighed and repackaged commercial foods. <u>HFD</u> 4806 kcal; 42% F, 10% P, 46% C *Net excess of ~190 g fat vs LFD* <u>LFD</u> 2672 kcal; 11% F, 18% P, 69% C	CCK: Both meals peaked at 15 min; NS GLP-1: Both meals rose steadily; NS

n, sample size; M, men; W, women; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; vs, compared to; ↓↓, significantly lower; NS, no significance between diets; ↑↑, significantly higher; ↑, higher compared to baseline; ↓, lower compared to baseline; =, no changes compared to baseline; AUC, area under the curve; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin

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2.10.3 Acute Effects of Different Types of Dietary Fat on Gastro-intestinal Peptides Release

Two similar studies (Thomsen et al., 1999; Thomsen et al., 2003) compared the incretin response to SAFA and MUFA (refer to Table 2.13). The meal was an energy free soup, white bread containing 50 g carbohydrate and 250 mL tap water. 100 g unsalted butter, 80 g olive oil or blank was respectively added into the soup for SAFA meal, MUFA meal and control. The studies recruited 12 overweight subjects with T2DM (Thomsen et al., 2003) and 10 healthy, lean subjects (Thomsen et al., 1999). Both SAFA and MUFA meals increased $iAUC_{GIP}$ to an equal extent whereas the increment was significantly higher when compared to control meal in T2DM subjects (Thomsen et al., 2003). In healthy subjects, similar findings were found with regards to the GIP increment during the initial 3 h however MUFA increased GIP more than SAFA and control after 180 min (Thomsen et al., 1999). Further , incremental GLP-1 response to MUFA over 8 h was the highest and the response to the control meal was the lowest in T2DM subjects (Thomsen et al., 2003). A different pattern of GLP-1 changes was observed in healthy subjects (Thomsen et al., 1999). The elevation after MUFA was higher compared to control meal during the initial 180 min. SAFA and MUFA increased GLP-1 more than the control meal after 180 min. Consistent with Thomsen et al. (2003), Beysen et al. (2002) reported that MUFA was more effective in increasing GLP-1 levels compared to SAFA between 30-300 min. In particular, SAFA from palm stearin was found to be more slowly absorbed and hence did not elevate GLP-1 before heparin infusion. After heparin infusion where more fatty acids were released, all types of fats raised GLP-1 compared to water. The test meal consumed by the 8 healthy subjects was 100 g hot chocolate-flavoured drink incorporated with 50 g test oil (palm stearin (SAFA), olive oil (MUFA), refined safflower oil (PUFA)) or water (for control).

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Kozimor, Chang, and Cooper (2013) reported that consumption of liquid meal high in MUFA instead of SAFA and PUFA resulted in lower PYY response over 5 h, in 14 healthy women. The meal consisted of one test oil added to Ensure® with soy lecithin and Nesquik®: sunflower oil and flaxseed oil (for PUFA), canola oil and extra virgin olive oil (for MUFA) or butter, coconut oil and red palm oil (for SAFA). However in another study, similar effects on ghrelin and leptin over a day was observed when comparing a high-fat breakfast enriched with high-SAFA or low-SAFA/high-MUFA or high-PUFA in 18 lean, healthy men (Poppitt et al., 2006). The meal was a blueberry muffin baked with butter and a decaffeinated hot beverage without sugar and milk and/or a glass of cold water. Fat-free food was given for lunch, snack and dinner.

Interesterified fat is a type of fat undergone interesterification process to rearrange position of the 3 fatty acids attached to the glycerol backbone. Such process produces new fat species and is usually done to change the physical characteristic of a fat/oil such as to modify the melting point, change the saturated fat content and extend the shelf life (Karupaiah & Sundram, 2007). In a recent well-powered study (Filippou, Berry, et al., 2014), an isocaloric (846 kcal) mixed meal comprising a milkshake and muffin incorporated with test oil was ingested by 50 healthy subjects. This study utilized interesterified palm olein and palm olein respectively owned 39% and 9% palmitic acid (SAFA) in the sn-2 position while the fatty acid compositions of both oils were similar. High oleic sunflower oil and palm olein stimulated higher postprandial GIP increment from baseline as compared to interesterified palm olein and lard, other gastro-intestinal peptides was not measured. The maximal postprandial increment of GIP concentration from baseline (Δ) was compared and significant differences were found between palm olein *vs* interesterified palm olein and lard. Differential effects were displayed although SAFA and MUFA contents were similar across palm olein, lard and interesterified palm olein. The possible factor explaining the different effects of the meals may be the

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different % en of palmitic acid at sn-2 position of triacylglycerol backbone in the isocaloric 846 kcal mixed meal (a muffin and a milkshake), i.e. 0.6, 9.2, 39.1 and 70.5 of high oleic sunflower oil, palm olein, interesterified palm olein and lard, respectively. In other words, higher GIP increment as found in high oleic sunflower oil and palm olein may be due to the lower % composition of palmitic acid at sn-2 position. The discernable differences in GI hormone secretion may also be a result of differing physical characteristics of the oil in the 4 meal challenges such as solid fat content or melting point which have an effect on absorption rate (Berry, Miller, & Sanders, 2007). It is possible that in addition to type of fatty acids, the actual food fat source may play a role in gut hormone secretion.

In balance, on reviewing the literature, MUFA may be a more potent stimulant for GLP-1 secretion as compared to SAFA. Aside from type of fatty acid, fat source which can affect fatty acid content at sn-2 position and hence physical characteristics that impact absorption is also an important consideration that can modulate gastro-intestinal peptides kinetics. Future research is needed to investigate the impact of different fat sources of SAFA. In addition, more research on the relationship between sn-2 position and gut hormone changes is required to bridge the gap of information.

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Table 2.13: Acute Effects of Different Types of Dietary Fat on Gastro-intestinal Peptides Release

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Thomsen et al. 2003	Overweight with T2DM; 12 (7/5)	64 ± 4	Randomized, crossover, non-blinded	8 h Bleeding in 30 min intervals between 0 – 480 min	1. SAFA 2. MUFA 3. Control	An energy free soup added with test oil/blank, consumed together with white bread providing 50 g C and 250 mL tap water <u>SAFA</u> 1004 kcal; 72% SAFA 100 g unsalted butter <u>MUFA</u> 1004 kcal; 74% MUFA 80 g olive oil <u>Control</u> 261 kcal *Blank*	PP GLP-1 during the initial 6h: Control ↓↓ *vs* SAFA and MUFA; NS for SAFA and MUFA iAUC $_{GLP-1:}$ MUFA ↑↑ *vs* SAFA and blank; SAFA ↑↑ *vs* control PP GIP and iAUC$_{GIP}$: SAFA and MUFA ↑↑ *vs* blank; NS for SAFA and MUFA
Kozimor et al. 2013	Healthy normal BMI; 14 (0/14)	23.5 ± 4.0	Randomized, crossover, single-blind	5 h	1. PUFA 2. MUFA 3. SAFA	~716 kcal high-fat liquid meal consisted of test oil added in Ensure® with soy lecithin and Nesquik® providing 70% en F: 25% en C: 5% en P. <u>PUFA</u> 732 kcal, 76.8% SAFA, 15.9% MUFA, 42.3% PUFA Sunflower oil and flaxseed oil <u>MUFA</u> 726 kcal, 6.9% SAFA, 42.4% MUFA: 16.1% PUFA Canola oil and extra virgin olive oil <u>SAFA</u> 691kcal, 44.9% SAFA, 13.7% MUFA, 4.9% PUFA Butter, coconut oil and red palm oil	PYY: PUFA ↑↑ *vs* MUFA at 150, 180, 240 and 300 min; SAFA ↑↑ *vs* MUFA at 240 and 300 min; SAFA *vs* PUFA:NS AUC PYY (0-300 min): MUFA ↓↓ *vs* SAFA and PUFA; SAFA *vs* PUFA: NS

n, sample size; M, men; W, women; T2DM, Type 2 Diabetes Mellitus; h, hour; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids;%, % energy; F, fat; P, protein; C, carbohydrate; *vs*, compared to; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significance between diets; GLP-1, glucagon-like peptide-1, iAUC, incremental area under the curve

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'Table 2.13, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Thomsen et al. 1999	Healthy, lean; 10 (5/5)	23 ± 2	Randomized, crossover, single-blinded Washout: ≥ 1 week	8 h 0 – 360 min for blank; 0- 480 min for SAFA and MUFA Bleeding time: every 30 min	1. MUFA 2. SAFA 3. Control	An energy free soup added with test oil/blank, consumed together with white bread providing 50 g C and 250 mL tap water <u>SAFA</u> 1004 kcal; 72% SAFA 100 g unsalted butter <u>MUFA</u> 1004 kcal; 74% MUFA 80 g olive oil <u>Control</u> 261 kcal *Blank*	GIP at 0-180 min: SAFA and MUFA↑↑ *vs* control; NS for SAFA and MUFA; GIP at 180-480 min: MUFA ↑↑ *vs* SAFA and control GLP-1 at 0-180 min: MUFA ↑↑ *vs* control; NS for SAFA *vs* MUFA and control GLP-1 at 180-480 min: SAFA and MUFA ↑↑ *vs* control; NS for SAFA and MUFA
Filippou et al. 2014	Healthy; 50 (25/25)	24.9	Randomized, crossover, double-blind	8 h	5. HOS 6. PO 7. IPO 8. Lard	846 kcal mixed meal consisted of a muffin and a milkshake providing 53% en F: 40% en C: 6.6% en P. Test oils were baked into muffins. <u>HOS</u> 2.3% PA:1.5% SA: 42.8% OA 0.6% PA at sn-2 position <u>PO</u> 22.6% PA: 2.1% SA: 21.1% OA 9.2% PA at sn-2 position <u>IPO</u> 22.6% PA: 2.1% SA: 21.1% OA 39.1% PA at sn-2 position <u>Lard</u> 15.5% PA: 8.6% SA: 20.9% OA 70.5% PA at sn-2 position	Maximal increment in GIP from baseline; and increment in GIP from baseline in the first 30 min: HOS and PO ↑ *vs* IPO and lard

n, sample size; M, men; W, women; h, hour; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids;%, % energy; C, carbohydrate; *vs*, compared to; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significance between diets; GLP-1, glucagon-like peptide-1, GIP, glucose-dependent insulinotropic polypeptide; PA, palmitic acid; SA, stearic acid; OA, oleic acid.

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'Table 2.13, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Poppit et al. 2006	Lean, healthy; 18 (18/0)	23 ± 4.2	Randomized. controlled, cross-over, double-blinded Breakfast: 0 h Lunch: 6.5 h Snack: 8 h	24 h Bleeding time: 0, 1, 3, 6, 10, 24 h	1. High SAFA diet (HSD) 2. Low-SAFA diet (LSD).	<u>High-fat breakfast</u> 70.8% F,4.9% P, 23.2% C; Mean calorie:747.6 kcal; Mean butter fat:59 g from 73 g butter. Lipid content based upon subjects' total daily energy intake (5.3 g butter per 239 kcal of daily intake) *A blueberry muffin incorporated with butter and a milk- and sugar-free decaffeinated hot beverage and/or glass of cold water* <u>HSD</u> 70.5% SAFA, 22.1% MUFA, 3.0% PUFA; 31.5% palmitic, 18.6% oleic, 1.2% linoleic <u>LSD</u> 54.4% SAFA, 32% MUFA, 10.5% PUFA; 18.8% palmitic, 30.0% oleic, 7.2% linoleic <u>Fat-exclusion lunch</u> 3.4% F, 14.2% P, 78.8% C; 3.1 g F *Vegetarian pasta, bread roll, orange juice* <u>Fat-exclusion snack</u> 1.8 % F, 7.2% P, 90.6% C; 0.7 g F *Fruit cake and apple juice* <u>Fat-exclusion dinner</u> 2.8 % F, 9.9% P, 85.1% C; 2.4 g F *Vegetarian risotto, raspberry dessert, carbonated beverage*	Ghrelin over 1 and 6 h:Both =; NS Ghrelin at 10 h: Both ↓ Leptin over 6 h: Both ↓; NS

n, sample size; M, men; W, women; h, hour; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids;%, % energy; F, fat; P, protein; C, carbohydrate; *vs*, compared to; ↑, significantly higher from baseline; ↓, significantly lower from baseline; =, no changes from baseline; NS, no significance between diets; GLP-1, glucagon-like peptide-1, GIP, glucose-dependent insulinotropic polypeptide, AUC, area under the curve

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'Table 2.13, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Beysen et al, 2002	Healthy; 8 (4/4)	21-54; median 29	Randomized, crossover, non-blinded Wash out: 2 wk Hyperglycaemic clamp at 210 – 300 min to raise blood glucose concentrations to 10 mmol/l	6 h Bleeding time for GLP-1 samples: -30, 0, 30, 60, 90, 120 ,150, 180, 210, 220, 240, 270, 300, 330 and 360 min	1. SAFA 2. MUFA 3. PUFA 4. Water	100 g hot chocolate-flavoured drink consisted 50 g of oil/water, 0.5 g emulsifier, 1 g of sweetener, 3 g cocoa powder and 45.5 g of water. The drink is carbohydrate and protein free. SAFA Palm stearin, 59% palmitic acid MUFA Olive oil; 69% oleic acid PUFA Refined safflower oil; 74% linoleic acid *A bolus of heparin (500 IU) was given at 90 min and this was immediately followed by a 0.4 IU•kg–1•min–1 heparin infusion (from 90 min until end of experiment) to increase the action of lipoprotein lipase for the alteration of NEFA pool. Control (water) was not given heparin but saline.*	GLP-1 before heparin infusion: MUFA and PUFA ↑↑ *vs* water; NS for SAFA *vs* water GLP-1 from 30- 300 min: SAFA, MUFA and PUFA ↑↑ *vs* water; MUFA ↑↑ *vs* SAFA GLP-1 after clamp: SAFA, MUFA and PUFA dropped; water remained constant

n, sample size; M, men; W, women; wk, weeks; h, hours; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; %, % energy; *vs*, compared to; ; ↑↑, significantly higher; ↓↓, significantly lower; NS, no significance between diets; GLP-1, glucagon-like peptide-1

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2.10.4 Chronic Effects of Different Types of Dietary Fat on Gastro-intestinal Peptides Release

The potential differential effect chronic exposure of dietary fats of differing in fat saturation on gut hormone release has been scantily investigated when compared to trials on the effects of fat quantity. Among the 5 published trials conducted so far (Table 2.14), 4 studies reported non-significant differences in the gut hormone secretion. In a randomized double-blind cross over study, (Lithander et al., 2008) evaluated two isocaloric (3176 kcal) diets, one rich in SAFA (18% en SAFA, 10% en MUFA and 7% en PUFA) and another rich in unsaturated fats (13% en SAFA, 12% en MUFA and 8% en PUFA) in 18 subjects with mild hyperlipidemia over 3 weeks. Fasting ghrelin and leptin levels on day 7, 14, 21 and 22 did not change significantly from the baseline value, irrespective of the type of fat consumed. However, this finding may need further confirmation as the % en of MUFA and PUFA replaced for SAFA did not account for head-to-head comparison of fatty acid composition per se, i.e. 5% en SAFA was replaced with 2% en MUFA and 1% en PUFA in unsaturated fat diet *vs* SAFA diet. The inappreciable differences in gut hormone changes after chronic exposure to dietary fats differing in fat saturation was further confirmed in the following 3 studies with head-to-head comparison of different types of fatty acids. Brynes et al. (2000) fed 9 overweight subjects with T2DM more than 3 slices/day of carrot cake incorporated with either corn oil (for MUFA diet) or olive oil (for PUFA diet) to allow 10% en exchange of MUFA *vs* PUFA between diets. The 4-week dietary intervention did not result in a significant change in fasting GLP-1 concentration. Aside from that, the authors observed non-significant differences between diets in GLP-1 in response to a standard test meal (i.e. a supplement drink) at the end of the dietary interventions. Two studies comparing SAFA *vs* MUFA revealed similar findings. Paniagua et al. (2007) reported that fasting GLP-1

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concentration did not change in response to alterations in fat saturation over 4 weeks in 11 abdominally obese subjects with insulin resistance. SAFA (from common food stuffs) was exchanged at 14% en with MUFA originating principally from extra virgin olive oil. There was a similar GLP-1 response to 2 preloads of equal caloric and macronutrient content but divergent food sources, i.e. whole milk, butter and bread (SAFA meal) *vs* skim milk, olive oil and bread (MUFA meal). The considerably large % en exchange between SAFA and MUFA may have increased the strength of this finding. Nevertheless, the small sample size and short study duration may limit the validity of the results. A recent well-powered longer duration study (Filippou, Teng, Berry, & Sanders, 2014) corroborates the aforementioned findings, although % en exchange between SAFA *vs* MUFA was at a lower quantum, i.e. 8% en. An isocaloric (2000 kcal/d) diet for 7 weeks, prepared with either palm olein, interesterified palm olein (both for SAFA diet), or high oleic sunflower oil (for MUFA diet) was allocated to 41 healthy subjects, in a cross over manner. This study reported that GIP and GLP-1 levels did not differ between mixed meal challenges consisting of muffin and milkshake rich in either SAFA or MUFA.

Contrasting results with regards to GIP were reported by a shorter term study (Itoh et al., 2014). The subjects consumed in succession a 7-day high-SAFA diet, followed by another 7-day low-SAFA diet. A standard Japanese meal with macronutrient composition echoing that of background diet was given as test meal at the end of each diet. The 7-day background diet consumption of 6% en higher in SAFA led to increased fasting GIP concentration compared to PUFA (butter *vs* soybean oil). In addition, similar finding was observed where the sum postprandial GIP concentrations at 0, 30 and 120 min after SAFA meal was significantly higher compared to PUFA meal. The postprandial increments of GIP and GLP-1 however were non-significant between the two meals. Nevertheless, the strength of the results generated by this study may be limited by the small sample size (n=11) and the possible bias caused by the non-randomized study design.

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Taken together based on the evidence discussed above, most studies reported long term alteration of dietary fat quality may not influence gastro-intestinal peptides release. However, the negative finding requires confirmation by more well-powered and well-designed studies.

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Table 2.14: Chronic Effects of Different Types of Dietary Fat on Gastro-intestinal Peptides Release

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Itoh et al. 2014	Healthy nondiabetic; 11 (0/11)	23.6 ± 1.7	Non-randomized, controlled Wash-out:3 wk Day 1 (LFD) → Day 2 (LFD PP challenge; LSD/HSD) → Day 3 to 7 (LSD/HSD) → Day 8 (LSD/HSD PP challenge) Treatment sequence: LSD → HSD	7 days Bleeding time: 0, 30, 60 ,120 min Bleeding time for GIP sample collection: 0, 30, 120 min	1) High SAFA diet (HSD) 2) Low-SAFA diet (LSD)	Standard Japanese meals (~1680 kcal) differed in cooking oil were provided <u>HSD and test meal</u> 30% F, 16% P, 54% C; 15% SAFA, 12% MUFA, 3% PUFA *47 g butter* <u>LSD and test meal</u> 30% F, 16% P, 54% C %; 9% SAFA, 12% MUFA, 9% PUFA *40 g soybean oil*	<u>Calculations</u> Σ_{GIP} was the sum of the values at 0, 30 and 120 min <u>Fasting measurements</u> GIP: HSD ↑↑ *vs* LSD <u>PP measurements</u> $\Sigma_{GIP:}$: HSD ↑↑ *vs* LSD GIP: HSD and LSD ↑ sharply at 30 min
Filippou et al. 2014	Healthy; 41 (31/10)	M: 28.6 ± 6.3 W: 29.3 ± 8.0	Randomized, crossover, single-blinded Run-in: 2 week	7 wk PP bleeding: 0, 10, 20, 30, 60, 90, 120 min	1. Palm olein (PO) 2. Interesterified palm olein (IPO) 3. High oleic sunflower oil (HOS)	Iso-caloric (2000 kcal/d) diet consisted of 30% F, 15% P, 55% C; 6% en exchange of palmitic for oleic acid. Daily meals were provided to subjects during weekdays. PO SAFA:MUFA:PUFA= 12:12:4 IPO SAFA:MUFA:PUFA= 12:12:4 HOS SAFA:MUFA:PUFA= 4:20:4 Standardized test meal 845 kcal; 50 g F, 14 g P, 85 g C Muffin made with PO and a milkshake	PP GIP and GLP-1: NS

n, sample size; M, men; W, women; wk, week; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; F, fat; P, protein; C, carbohydrate; %, % energy; *vs*, compared to; ↑↑, significantly higher; ↑, higher compared to baseline; GIP, glucose-dependent insulinotropic polypeptide; GI, glycemic index; NS, no significant difference between diets; =, no changes compared to baseline; GLP-1, glucagon-like peptide-1

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'Table 2.14, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
(Paniagua et al. 2007)	Abdominally obese with insulin resistance; 11 (4/7)	62 ± 9.4	Randomized, controlled, crossover	4 wk PP bleeding time: 0, 15, 30, 60, 90, 120, 150, 180 min	1) High-SAFA diet (SAFA) 2) High-MUFA Mediterranean diet (MUFA) 3) High-carbohydrate diet (CARB)	Subjects adhered to *ad libitum* isocaloric diet intervention, followed by a 443 kcal breakfast meal for postprandial challenge. <u>SAFA</u> 2285 kcal; 38% F, 15% P, 47% C; 23% SAFA, 9% MUFA, 6% PUFA *Butter, stuffed turkey, whole milk* Breakfast meal: 200 ml whole milk, 50 g bread, and 25 g butter. <u>MUFA</u> 2290 kcal; 38% F, 15% P, 47% C; 9% SAFA, 23% MUFA, 6% PUFA *Extra virgin olive oil, skim milk* Breakfast meal: 200 ml skim milk, 50 g bread, and 27 g olive oil <u>CARB</u> 2275 kcal; 20% F, 15% P, 65% C; 6% SAFA, 8% MUFA and 6% PUFA *Marmalade with* sugar, skim milk with sugar, sunflower oil Breakfast meal: 200 ml skim milk, 70 g bread, and 75 g marmalade	<u>Fasting measurement</u> GLP-1: NS <u>PP measurements</u> AUC $_{GLP-1}$: CARB ↓↓ *vs* SAFA and MUFA

n, sample size; M, men; W, women; wk, week; PP, postprandial; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ; F, fat; P, protein; C, carbohydrate; %, % energy; *vs*, compared to; ↓↓, significantly lower; NS, no significance between diets; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide

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'Table 2.14, continued'

Reference	Health Status; n (M/W)	Age (y)	Design	Duration	Treatment	Dietary Intervention	Outcomes
Lithander et al. 2008	Mildly hyperlipidemia; 18 (18/0)	39.7 ± 13.9	Randomized. controlled, cross over, double-blinded Wash out: 4 wk Blood collection: 0, 1, 7, 14, 21, 22 days	3 wk	1. High SAFA diet (HSD) 2. Low-SAFA diet (LSD)	Subjects followed isocaloric (3176 kcal) 5-day rotation diet consisted of 34% F, 13% P and 53% C. Foods and snacks were provided. <u>HSD</u> 18% SAFA, 10% MUFA, 7% PUFA *Unknown source* <u>LSD</u> 13% SAFA, 12% MUFA, 8% PUFA *Unknown source*	Ghrelin and leptin: both diets =; NS
Brynes et al. 2000	Overweight with T2DM; 9 (5/4)	56.0 ± 5.3	Randomized. controlled, double blinded, cross-over SITT on day 1 and 23: 0.05 U/kg bolus of insulin Bleeding at -15, 0, 3, 4, 6, 8, 10, 12, 14, and 15 min STM on day 2 and 24: -15, 0, 5, 15, 30, 45, 60, 90, 120, 150, and 180 min	4 wk Wash-out: min 2 wk	1. MUFA (mainly oleic acid) 2. PUFA (mainly linoleic)	Isocaloric (2300 kcal) diet substituting MUFA or PUFA for high-GI carbohydrate. Carrot cake incorporated with MUFA/ PUFA was portioned at least 3 pieces/day to provide additional 10% en above habitual intake. <u>MUFA</u> 39.1% F, 13.5% P, 47.2% C; 20.3% MUFA, 4.2% PUFA, 8% SAFA Corn oil <u>PUFA</u> 41.1% F, 12.4% P, 46.5% C 11.7% MUFA, 13.4% PUFA, 9.2% SAFA Olive oil <u>Standard test meal (STM)</u> 597 kcal, 30% (20 g) F, 16.7% (25 g) P, 53.6% (80 g) C	Fasting GLP-1: =; NS <u>STM measurements</u> Integrated AUC for GLP-1: NS

n, sample size; M, men; W, women; wk, week; F, fat; P, protein; C, carbohydrate; %, % energy; GI, glycemic index; *vs*, compared to; NS, no significant difference between diets; =, no changes compared to baseline; AUC, area under the curve; GLP-1, glucagon-like peptide-1

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Keynotes

Summarizing the literatures available, there is no clear evidence showing detrimental effects of SAFA compared to MUFA and CARB on the measured markers given that conflicting results may be influenced by confounding factors as follows:

- Free-living basis dietary intervention without strict monitoring of compliance

- Comparison of unequal energy exchange of test fat/CARB between diets

- Lack of standardization of food sources or even menus across treatments

-Various sources of test fat in one treatment (e.g. milk, butter and palm olein for SAFA diet)

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CHAPTER 3: METHODOLOGY

3.1 Research Design

The study protocol was approved by the Medical Ethics Committee of University of Malaya Medical Centre (reference no. 871.5) and registered under ClinicalTrials.gov (identifier: NCT01665482). The study intervention was carried out from early March through mid-July 2012 at the research institute of the Malaysian Palm Oil Board (MPOB), Bangi.

3.1.1 Participants

Eligible participants with abdominal obesity (Asian cut off points: waist circumference ≥ 80 cm for women and ≥ 90 cm for men) aged 20-60 years old were recruited through flyers (APPENDIX A), posters, emails and phone calls. Exclusion criteria were BMI ≤ 18.5 kg/m^2; medical history of cardiovascular disease, diabetes, dyslipidemia; other diagnosed chronic illness; current use of antihypertensive or lipid lowering medication; plasma total cholesterol (TC) > 6.5 mmol/L, triacylglycerol (TAG) > 4.5 mmol/L; alcohol intake exceeding > 28 units per week; pregnancy, smoking, breastfeeding. The flow of subject recruitment is shown in the CONSORT diagram (Figure 3.1). The study was open to public's participation and targeted to recruit volunteers working near the area of Pusat Latihan Bangi where MPOB was located. Distribution of flyers was carried out in the distance range of 6 km from MPOB, for example INFRA, POSLAJU, RISDA and UPM. The travel distance to study research centre has been taken into consideration for subject recruitment. In addition, circular emails were sent through MPOB internal email system and invitations were made through phone call to the volunteers of previous MPOB's researches.

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Interested volunteers were briefed fully on the nature of the study (APPENDIX B) and thereafter gave written consent (APPENDIX C); they were then required to fill up a study questionnaire (APPENDIX D) and to self-report if they had any diagnosed medical illness and medical history. Besides, they were required to attend a screening clinic to further evaluate eligibility for the study (APPENDIX E).

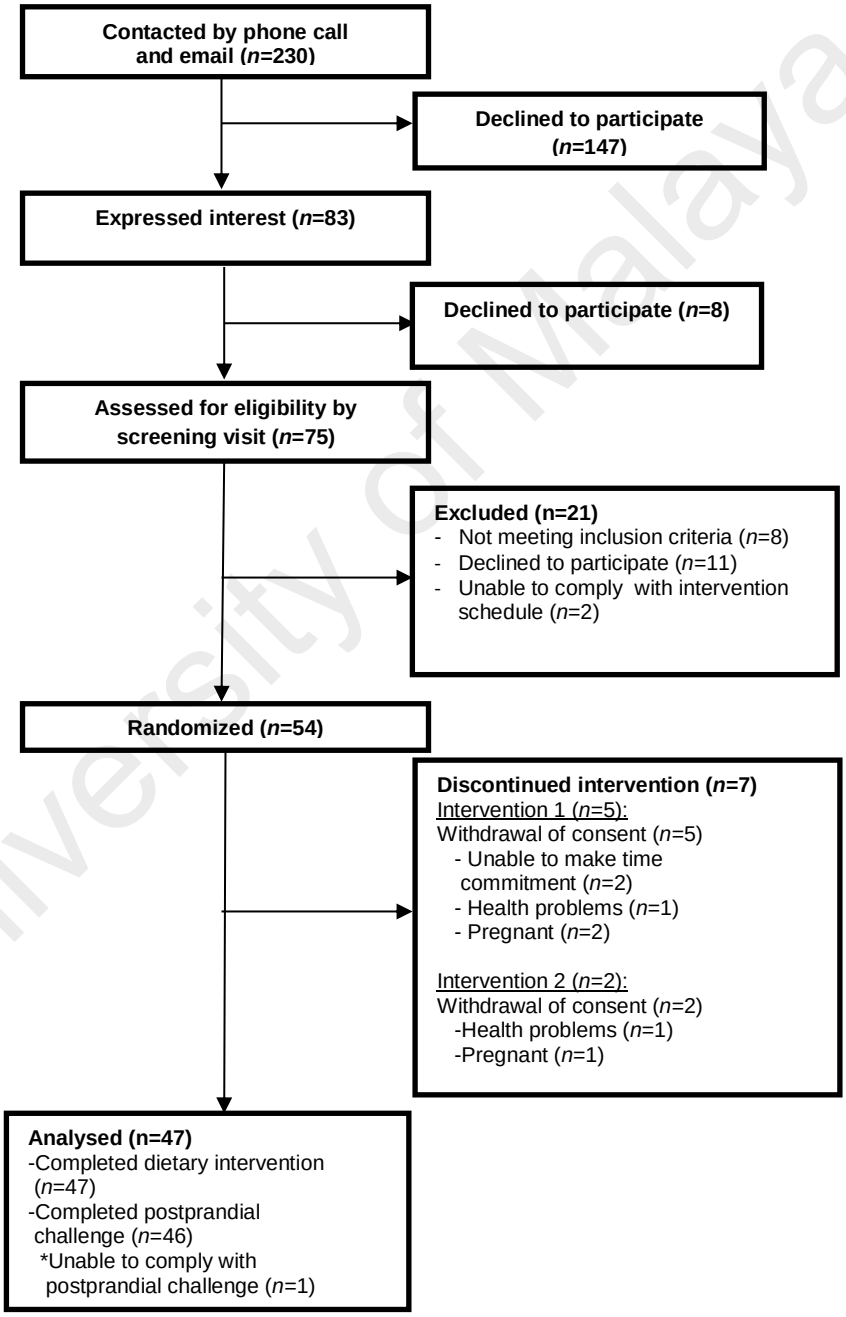

Figure 3.1: Consort Diagram.

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After a 10-h overnight fast, blood samples of the volunteers were collected for the following laboratory tests: lipids, glucose, insulin, liver function, kidney function and hematological indices. Physical and anthropometric measurements such as height, weight, BMI, body fat % and waist circumference were recorded. A total of 54 subjects were assigned to the randomized dietary interventions, the baseline characteristics of subjects are shown in Table 3.1.

Table 3.1: Baseline Characteristics of the Study Participants Completing the 3 Consecutive 6-week Dietary Interventions

	All* (n = 47)
Physical characteristics	
Gender	
Male (*n*)	12
Female (*n*)	35
Age (years)	32.8 ± 8.7
Weight (kg)	74.2 ± 14.6
BMI (kg/m^2)	28.7 ± 4.1
Waist (cm)	94.8 ± 10.2
Body fat (%)	36.6 ± 7.9
Systolic BP (mmHg)	124.5 ± 12.4
Diastolic BP (mmHg)	81.8 ± 10.7
Fasting biochemical profile	
Plasma glucose (mmol/L)	5.8 ± 0.8
Serum insulin (mU/L)	16.50 ± 10.29
Serum C-peptide (nmol/L)	0.63 ± 0.26
rQUICKI	0.149 ± 0.015
HOMA2-%B$^{\alpha}$	104.7 ± 34.89
HOMA2-%S$^{\alpha}$	70.7 ± 34.81
HOMA2-IR$^{\alpha}$	1.898 ± 1.184
HOMA2-%B$^{\gamma}$	89.49 ± 22.72
HOMA2-%S$^{\gamma}$	81.40 ± 32.52
HOMA2-IR$^{\gamma}$	1.462 ± 0.656
Serum total cholesterol (mmol/L)	5.0 ± 0.8
Serum HDL cholesterol (mmol/L)	1.3 ± 0.3
Serum TAG (mmol/L)	1.2 ± 0.7
Total: HDL cholesterol	4.0 ± 0.8
Serum NEFA (mmol/L)	0.71 ± 0.16
Dietary intake †	
Energy (kcal/day)	2051.8 ± 545.3
Protein (% en)	16.3 ± 2.8
Carbohydrate (% en)	57.3 ± 7.2
Fat (% en)	26.9 ± 6.2

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'Table 3.1, continued'

Values are means ± SD. * Ethnicity: Malay *n*=47. † Determined from 3-day weighed diet record using Nutritionist Pro™ software (AXXYA Systems LLC. Texas, USA).
[α] Calculated from fasting glucose and insulin concentrations.
[γ] Calculated from fasting glucose and C-peptide concentrations.

3.1.2 Sample Size and Outcomes

The first primary outcome was the difference in the change of postprandial C-peptide secretion (β-cell function) from preprandial baseline between the 3 types of dietary interventions (measured as $iAUC_{C\text{-}peptide}$) in response to a test meal after 6-week background diet exposure). A sample size of 44 subjects has a 90% power to detect a difference between means of 302.46 with a significance level (alpha) of 0.05 (two-tailed) in serum concentrations of C-peptide between groups. This estimate was based on a mean plasma C-peptide increment of 1049 IU with a within subject SD of 490 for a healthy, adult population. The second primary outcome was the difference in fasting plasma IL-6 concentration between the 3 types of dietary interventions. A sample size of 48 subjects would have a 80% power to detect a difference of 0.5 SD unit change of IL-6 at $P = 0.01$. We aimed to recruit a total of 54 healthy participants, allowing for approximately 10% drop-out rate.

The following secondary outcomes were specified:

1) Differences in QUICKI index and HOMA2-%S index of insulin sensitivity, serum glucose, insulin and NEFA concentrations
2) Differences in endothelial and inflammation markers: serum CRP, plasma IL-1β and E-selectin
3) Differences in hemostatic markers: plasma PAI-1 and D-dimer
4) Differences in gut hormones: plasma GIP, GLP-1, ghrelin, PYY and CCK

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All other outcomes will be regarded as exploratory:

1) Differences in systolic and diastolic blood pressure
2) Differences in lipid profile
3) Differences in pulse wave analysis

Measures of compliance:

1) Differences in body weight and percentage body fat
2) Differences in fatty acid composition of plasma lipid and erythrocyte membrane phospholipid
3) Differences in meal appreciation

3.1.2 Study Design

This was a prospective randomized, controlled, single-blind crossover trial to examine the fasting and postprandial effects of 3 isocaloric diets (plant-source SAFA, CARB, MUFA) in 54 abdominally obese individuals. We studied the effects of substitution of 7% energy as refined carbohydrate or MUFA for SAFA for a period of 6 weeks each. Each participant underwent all three 6-week dietary interventions in a randomly allocated fashion. The nutrient composition of each diet in brief was: SAFA diet - total fat 32% en, SAFA 12% en, MUFA 13% en, carbohydrate 55% en; CARB diet - total fat 24% en, SAFA 5% en, MUFA 14% en, carbohydrate 62% en; and MUFA diet - total fat 32% en, SAFA 5% en, MUFA 21% en, carbohydrate 55% en (Table 3.3). Fasting and postprandial blood samples in response to corresponding SAFA, CARB or MUFA enriched meal-challenges were collected after 6 weeks on each diet-treatment for the assessment of outcomes. The subjects were prescribed eucaloric (2000 kcal) diets instead of low-calorie diets to maintain their body weight throughout the intervention. The maintenance of body

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weight across the duration of the trial was meant to avoid confounding factors such as changes in body weight and metabolism that would influence study outcomes. In the case of SAFA diet (control), palmitic acid contributed to 12% en of total energy intake which was similar to that of another similar chronic study conducted in the Malaysian population (Filippou, Teng, et al., 2014) and this level was also comparable to the average palmitic acid intake by the Malaysian population. The use of this particular composition of control SAFA diet which reflects the common man's diet allowed the investigation of the effects of the application of levels of total fat and palmitic acid intake within the Malaysian population on various outcomes. There was no run-in period prior to the commencement of diet interventions. This was because most of the subjects had previous experience in dietary clinical trial participation and were familiar with similar trial protocols; furthermore with this trial design weekday meals were supplied to study volunteers. Each 6-week background diet exposure was followed by a 6-h postprandial challenge; subjects then continued with the next dietary intervention without washout between treatments. Unlike those trials testing therapeutic drugs, it was impossible for the subjects to stop consuming cooking oil (the treatment/intervention in this study) in their habitual diets for washout purposes. It was possible to replace our test oils with some other cooking oil if a washout period were to be included; however that cooking oil may become a confounding factor. Therefore a longer 6-week intervention duration without washout between treatments was chosen to in order to accommodate changes in insulin sensitivity, inflammation, gastro-intestinal peptides and lipids produced by the previous dietary phase of the trial. Insulin sensitivity and inflammatory response were significantly different between diets after 4-week interventions in similar crossover studies using healthy subjects (Fuentes et al., 2008; Perez-Jimenez et al., 2001). High-fat overfeeding for only 5 days significantly changed fasting gastro-intestinal peptides in healthy men (Brons et al., 2009). In addition it is a fact that changes in plasma lipids are observed to plateau

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following dietary modification for 3 weeks. In addition the absence of a washout between diets in this study shortened the trial period considerably, enabling us to avoid confounding factors related to seasonal changes, i.e. festivals and school holidays which might have occurred with a longer trial incorporating washout. In addition, the continuous dietary exposure allowed a more homogenous experience for all treatments in terms of compliance and participant motivation.

The diet intervention was performed from early March through mid-July 2012 at the research institute of the MPOB. Subjects were assigned evenly to three consecutive 6-week dietary interventions rich in SAFA (A), MUFA (B) and CARB (C) in treatment sequence ABC, BCA or CAB (Figure 3.2). The random selection of subjects' treatment sequence was performed using a list of random numbers auto-generated using Microsoft Excel software, where sequences ABC, BCA and CAB were labeled number 1, 2 and 3 respectively. Women were stratified according to menstrual cycle to avoid the concurrence of menstruation and blood sample collection. In addition, men and women were equally distributed among the treatment sequence groups to minimize effects of hormonal changes upon certain endpoints.

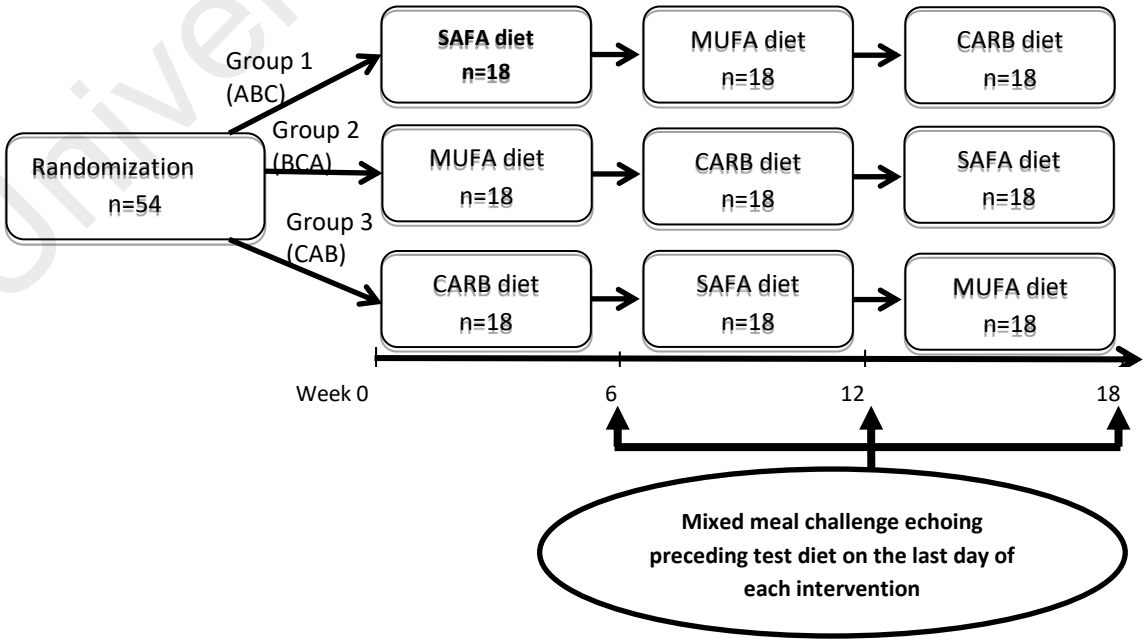

Figure 3.2: Allocation of Subjects into 3 Consecutive 6-week Dietary Interventions in Randomized Order.

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Time points at which blood was collected are summarized in Figure 3.3 and Table 3.2. Fasting blood samples for C-peptide, insulin, glucose, NEFA, IL-6, IL-1β, CRP, E-selectin, PAI-1, D-dimer, TC, HDL-C, low density LDL-C, triacylglycerol, apolipoprotein A1 (apo A1), apolipoprotein B100 (apo B100), lipoprotein (a) (Lp (a)), low density lipoprotein subfractions (LDLs), high density lipoprotein subfractions (HDLs), were collected **at baseline (study enrolment), as well as, week 5 and 6 of each intervention.** The mean value of 2 fasting samples of C-peptide, insulin, glucose and NEFA 5 minutes apart and the mean of fasting samples at week 5 and 6 (for all other markers) were used in the statistical analysis to control for random measurement error and regression dilution bias.

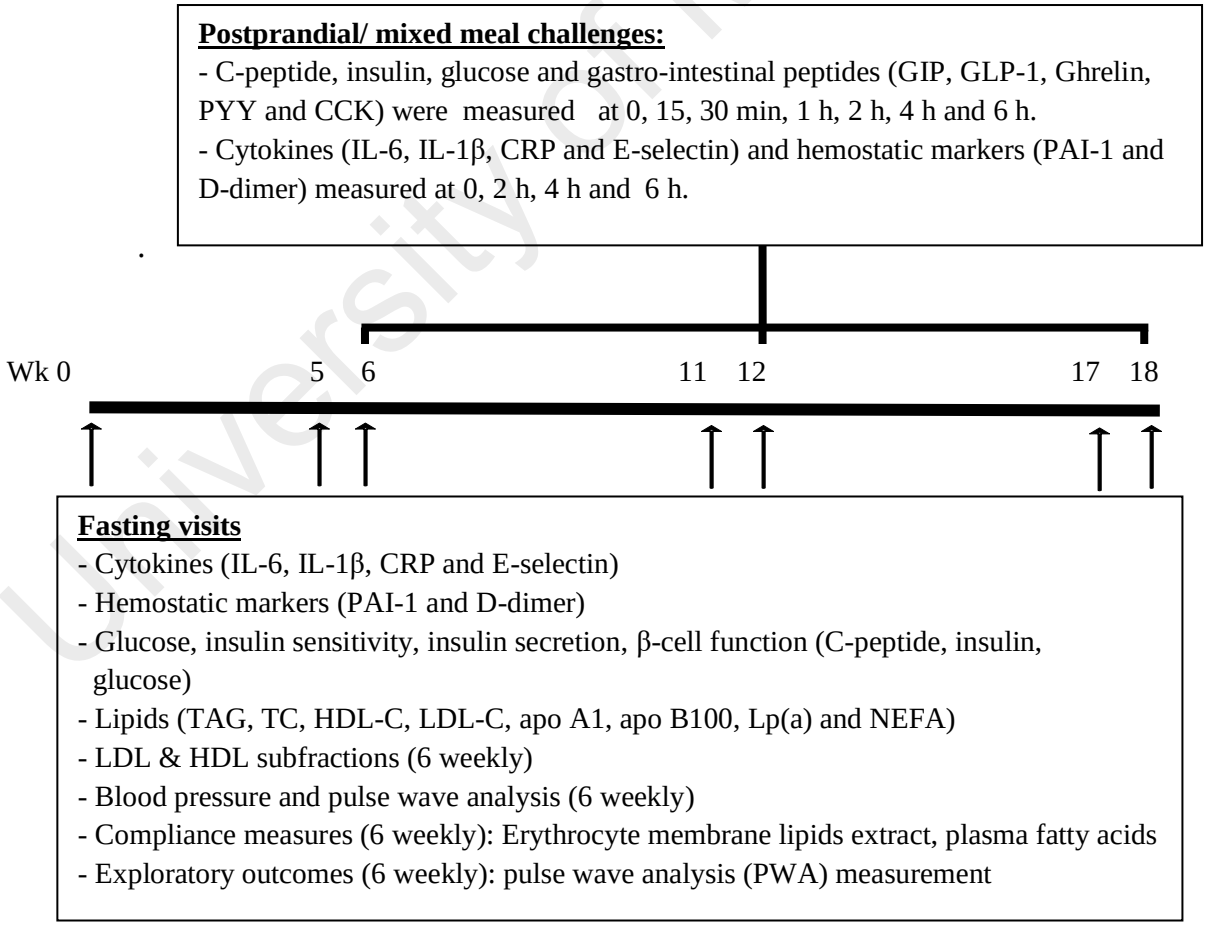

Figure 3.3: Blood Collection Time-Points and Measurement of Biomarkers.

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Following the fasting sample collection at week 6, subjects underwent a mixed meal challenge echoing the nutrient composition of the preceding 6 weeks. After the consumption of the test meal, blood samples were drawn over 6 h at 0, 15 and 30 min, 1, 2, 4 and 6 h for evaluation of C-peptide, insulin, glucose, NEFA, IL-6, IL-1β, CRP, E-selectin, PAI-1, D-dimer, triacylglycerol, GIP, GLP-1, ghrelin, PYY and CCK levels.

Table 3.2: Blood Sample Collection and Sample Volume in Each Vial

Markers	Sample	Fasting		Postprandial	
		Time point (week)	Sample volume (µL)	Time point (min)	Sample volume (µL)
C-peptide and insulin	Serum	0, 5, 6, 11, 12, 17, 18	300*	15, 30, 60, 120, 240, 360	300
Glucose	Plasma	0, 5, 6, 11, 12, 17, 18	1000*	15, 30, 60, 120, 240, 360	1000
NEFA	Serum	0, 5, 6, 11, 12, 17, 18	300*$^{\gamma}$	15, 30, 60, 120, 240, 360	300$^{\gamma}$
IL-6 and IL-1β	Plasma	0, 5, 6, 11, 12, 17, 18	500$^{\gamma}$	120, 240, 360	500$^{\gamma}$
PAI-1, D-dimer and E-selectin	Plasma	0, 5, 6, 11, 12, 17, 18	250$^{\gamma}$	120, 240, 360	250$^{\gamma}$
CRP	Serum	0, 5, 6, 11, 12, 17, 18	150	120, 240, 360	150
Gastro-intestinal peptides	Plasma	0, 6, 12, 18	150$^{\gamma}$	15, 30, 60, 120, 240, 360	150$^{\gamma}$
TAG	Serum	0, 5, 6, 11, 12, 17, 18	150	60, 120, 180, 240, 300, 360	150
Lipid markers	Serum	0, 5, 6, 11, 12, 17, 18	600	-	-
Plasma fatty acid	Plasma	0, 6, 12, 18	500$^{\gamma}$	-	-
Erythrocyte fatty acid	Erythrocytes after plasma removal	0, 6, 12, 18	1000$^{\gamma}$	-	-

* Duplicate measurement at 0 and 5 min; $^{\gamma}$ duplicate analysis at the same time points.
NEFA, Non-esterified fatty acids; IL, interleukin; CRP, C-reactive protein; TAG, triacylglycerol

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3.1.3 Dietary Interventions

The subjects adhered to all 3 dietary intervention in a crossover manner. All 3 intervention diets provided subjects with ~2000 kcal energy per day. Macronutrient composition in both the SAFA- and MUFA diets included 31% en fat, 14% en protein and 55% en CARB whilst the low-fat/high-CARB diet consisted of 24% en fat, 14% en protein and 61% en CARB (Table 3.3). Protein and PUFA content were standardized at 14% en and 6% en respectively, across all 3 diets. The control, SAFA diet, contained 12% en SAFA, 13.1% en MUFA and 6.4% en PUFA. The 7% en derived from SAFA in the control, SAFA diet, was replaced with 7% en CARB or MUFA respectively in the CARB and MUFA diets.

Table 3.3: Composition of Experimental Diets

	SAFA diet	CARB diet	MUFA diet
Total calories (kcal)	**2054.9 ± 205.7**	**2081.2 ± 198.1**	**2054.9 ± 205.7**
Protein (% en)	**13.8 ± 2.2**	**14.3 ± 2.3**	**13.8 ± 2.2**
Carbohydrate (% en)	**54.7 ± 4.7**	**61.4 ± 3.8**	**54.7 ± 4.7**
Fat (% en)	**31.5 ± 4.3**	**24.3 ± 2.7**	**31.5 ± 4.3**
SAFA (% en)	**12.0 ± 0.4**	**4.5 ± 0.5**	**5.0 ± 0.8**
C12:0 (% en)	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
C14:0 (% en)	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C16:0 (% en)	10.2 ± 0.4	3.2 ± 0.5	3.3 ± 0.7
C18:0 (% en)	1.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
C20:0 (% en)	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
C22:0 (% en)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
MUFA (% en)	**13.1 ± 0.3**	**14.0 ± 0.5**	**20.5 ± 1.0**
C16:1 n-7 (% en)	0.3 ± 0.1	0.4 ± 0,2	0.2 ± 0.1
C18:1 n-9 (% en)	12.7 ± 0.3	13.6 ± 0.6	20.2 ± 1.1
C20:1 n-9 (% en)	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
PUFA (% en)	**6.4 ± 0.6**	**5.8 ± 0.5**	**6.0 ± 0.5**
C18:2 n-6 (% en)	6.0 ± 0.6	5.4 ± 0.4	5.7 ± 0.5
C18:3 n-3 (% en)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
C20:5 n-3 (% en)	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
C22:6, n-3 (% en)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Fiber (g)	11.3 ± 3.7	12.8 ± 3.7	11.3 ± 3.7

All values are expressed as mean ± SD. Abbreviations: SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

The basal diets supplied 22 g fat (derived from food components) for all 3 treatment groups. For the SAFA- and MUFA diets 49 g test oil were added (SAFA: palm

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olein blend, MUFA: high oleic sunflower oil blend) during food preparation. 34 g high oleic sunflower oil blend was used to prepare CARB diet with an additional 28 g of simple sugar supplied as sweetened drinks such as barley drink, honey drink and rose syrup drink. The drinks with mean glycemic index (GI) 35.5 and mean glycemic load (GL) 21.6 were provided on alternate days to achieve desired medium GI content in the CARB diet, which represents the typical South East Asian menu. The estimation of GI and GL for the additional simple and complex carbohydrates supplied in the CARB diet was calculated as per Atkinson, Foster-Powell, and Brand-Miller (2008).

Table 3.4: **Fatty Acid Composition of Test Oils**

	Palm Olein Blend (for SAFA diet)	High Oleic Sunflower Oil Blend (for MUFA diet)	High Oleic Sunflower Oil Blend (for CARB diet)
C 12:0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C 14:0	0.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C 16:0	34.3 ± 0.1	3.9 ± 0.1	4.1 ± 0.0
C 18:0	3.7 ± 0.0	2.8 ± 0.1	2.8 ± 0.1
C 20:0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
C 22:0	0.1 ± 0.0	0.6 ± 0.0	0.4 ± 0.0
C 24:0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
SAFA	**39.3 ± 0.1**	**7.5 ± 0.2**	**7.3 ± 0.1**
C 16:1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C 18:1	41.0 ± 0.1	73.7 ± 0.3	67.3 ± 0.6
C 20:1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
MUFA	**41.2 ± 0.1**	**73.8 ± 0.3**	**67.4 ± 0.6**
C 18:2	19.2 ± 0.2	18.1 ± 0.6	25.2 ± 0.7
C 18:3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
PUFA	**19.4 ± 0.1**	**18.2 ± 0.6**	**25.2 ± 0.7**

Test oils comprised 69% of total fat; while the remaining total fat was derived from invisible fat as found in food ingredients. Since PUFA content might be a confounding variable, the test oils were blended with sunflower oil to achieve the standardization of PUFA content in all diets. Refined, bleached and deodorised palm olein with iodine value 56 (MOI Food Malaysia Sdn. Bhd., Malaysia) was blended with sunflower oil (Sunlico®, Yee Lee Edible Oils, Malaysia) in the proportion of 83:17 for

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the preparation of test oil used in SAFA diet. High oleic sunflower oil (Neuvida®, Yee Lee Edible Oils, Malaysia) and sunflower oil (Sunlico®, Yee Lee Edible Oils, Malaysia) was blended following the ratio of 68:32 for CARB diet and 76:24 for MUFA diet. The fatty acid composition of test oils used for preparation of daily meals, snacks and postprandial challenge mixed meal preparation is listed in Table 3.4.

3.1.4 Menu Design and Preparation of Intervention Diets

Three 5-day cycle menus (Appendix F) in each intervention were designed based on calculation of total calorie and macronutrient composition requirements using *Nutritionist Pro* software (AXXYA Systems LLC., Stafford, TX, USA). The menus were rotated twice in each intervention so as to provide food variety. The typical test diets consisted of a serving of white rice, fish or chicken, fruit and drink; and two servings of vegetable. Total calorie for the test meal was adjusted during menu designing based on the mean energy requirement of all subjects. Therefore, the subjects were required to complete a 3-day weighed food record (Appendix G) with a kitchen weighing scale on 2 weekdays and one weekend day in order to determine the energy requirement which varied among subjects. Energy requirement of each subject was then estimated based on the food record through *Nutritionist Pro* software (AXXYA Systems LLC., Stafford, TX, USA). In order to satisfy participants' caloric requirement or satiety, the subjects were free to request snacks (Malay pancake (*kuih bakar*) and cupcakes in strawberry, vanilla and orange flavors) with a macronutrient composition (Table 3.5) similar to their randomized dietary allocation. The snacks were prepared monthly by trained bakery staff of our selection (Zie Azie Bakery & Class, Kajang). Each snack was packaged in a zipper bag and stored in the freezer at -20 °C. Recipes for and nutritional composition of the snacks are included in Appendix H and Appendix I.

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Table 3.5: Macronutrient Composition of Snacks

	Cupcake for SAFA or MUFA diet	Cupcake for CARB diet	Malay pancake for SAFA or MUFA diet	Malay pancake for CARB diet
Total calories (kcal)	157.4	157.6	157.4	157.0
Fat (% en)	30.3	22.9	30.2	22.3
Fat (g)	5.3	4.0	5.3	3.9
Protein (% en)	13.4	13.3	14.6	14.7
Protein (g)	5.2	5.3	5.8	5.8
Carbohydrate (% en)	56.3	63.8	55.3	63.1
Carbohydrate (g)	22.2	25.1	21.8	24.8

Determined using Nutritionist Pro™ software (AXXYA Systems LLC. Texas, USA).

Test cooking carried out by the caterer underwent laboratory macronutrient analyses to detect whether the macronutrient/fatty acid composition was similar to the target. The menu had to be adjusted until the food ingredient and amount of test oil contributed to the target macronutrient/fatty acid composition. Gedung Catering & Canopy Services, Seri Kembangan was our selected caterer for the test meal preparation. The cook was trained to prepare meals according to trial specifications, i.e. the food ingredients' weight had to be measured and the utensils should not be cross-contaminated with other test oils. In addition, the amount of test oil needed for daily meal preparation in each intervention was measured and bottled. The bottled oil for everyday cooking was allocated to the caterer weekly. Weekday meals were prepared at the caterer's kitchen located at Seri Kembangan. Breakfast and lunch were served at the dining hall in the study institute, MPOB while dinner-packs were provided for home consumption. A daily project leader was appointed to monitor the food portions served by the waiters in the institute's dining hall. If subjects were found to have significant amounts of unconsumed leftover food at the end of the meal, the project leader would try to find out the reason for this and encourage subjects to try to consume the leftovers as much as possible, according to the macronutrient proportion.

University of Malaya

Subjects had easy access to a nutritionist if they required dietary advice. Consumption of supplements or traditional medicines was prohibited throughout the study for the purpose of securing the treatment effect solely from test fatty acids or carbohydrates. Cooking with supplied test oils was encouraged over the weekends when meals were not provided, yet eating out was not prohibited. Dietary guidelines for meal choices over weekends (Appendix J) were explained to the subjects. Dietary advice was provided from time to time in order to remind subjects adhering to the CARB diet to reduce oil consumption. Bottled test oils were allocated to subjects for meal preparation at home during weekends. At the start of each treatment, 1 kg of the specific test oil was allocated to each subject; replenishment of test oil was available upon request with the condition that the used oil bottles had to be returned. The bottles were also collected at the end of each treatment. Remaining oil in all the collected bottles was weighed. About 4.5 tablespoons (56 g) of test oil should be consumed by subjects adherent to SAFA- and MUFA diets, whereas about 3 tablespoons (39 g) of test oil should be used for meal preparation in CARB diet. Amount of test oil was to be adjusted commensurately if the subject would like to cook for his family members. The test oil supply could be replenished up to a maximum 4 kg per subject in each intervention. Subjects were required to record the test oil usage and food consumption over the weekends. Project leaders provided dietary advice to subjects individually based on the weekly submitted record.

University of Malaya

3.1.6 Compliance

To ensure strict dietary compliance, subjects who failed to collect > 10% meals during each 6-week intervention were considered drop-outs. Subjects were weighed and evaluated bi-weekly using body composition analyzer (TANITA, USA) and % body fat was determined. In order to avoid body weight acting as a covariate, dietary advice was given to subjects with > 2 kg weight fluctuation with regards to either increasing or decreasing their food or snack intake. Visual analogue scale (VAS) forms (Appendix K) were filled up by subjects after the breakfast, lunch and dinner on a Wednesday at the midpoint of each diet intervention. The acceptability and palatability of meals, which may affect diet compliance, were compared among the three diets. In addition, fatty acid composition of plasma and erythrocyte membrane lipid extracts (assayed by gas chromatography (GC)) and serum lipid profile were evaluated as a measure of dietary compliance.

3.1.7 Postprandial Challenge

The SAFA- and MUFA diets were followed by a 6-h high-fat challenge with their respective test fats incorporated into mixed meals echoing background diet intervention, and the CARB diet by a 6-h high-carbohydrate challenge echoing background diet intervention. The postprandial challenge was performed at the end of each intervention at MPOB institute's clinic. Subjects abstained from strenuous exercise, consumed chicken-flavored instant cup porridge containing 180 kcal, 1 g fat (Appendix L) for dinner the previous night and fasted overnight for 10 h on the day before the postprandial test. On the postprandial challenge day, subjects were asked to fill up a questionnaire (Appendix M) upon arrival for the purpose to confirm if their health condition allowed them to participate in the postprandial challenge. Following the measurements of blood pressure,

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body weight and pulse wave analysis after a short rest, a cannula was inserted into the antecubital vein of the forearm and duplicate fasting blood samples were then collected at -5 and 0 min. The isocaloric mixed meal challenge comprised a base of ~170 g vanilla-flavored muffin with a glass of 250 mL strawberry-flavored milkshake supplying a total of ~880 kcal for each isocaloric mixed meal challenge with 15.8 g protein. The SAFA and MUFA meals provided 50.7 g fat and 88.0 g carbohydrates; whereas CARB meal provided 20.8 g fat and 157.6 g carbohydrates. Additional carbohydrate content in CARB meal's muffin and milkshake was supplied by 70 g Valens Caborie® glucose polymers module (Pharm-D, USA). Table 3.6 displays the composition of test meals. Blood samples were collected through cannula in situ at 15, 30 min and hourly up till 6 h after the start of meal consumption (at 0 min) for evaluation of C peptide, insulin, glucose, NEFA, IL-6, IL-1β, CRP, E-selectin, PAI-1, D-dimer, triacylglycerol, GIP, GLP-1, ghrelin, PYY and CCK levels (see Figure 3.3, Table 3.2).

Table 3.6: Composition of Postprandial Test Meals

	SAFA meal	CARB meal	MUFA meal
Total (muffin and milkshake)			
Total calorie, Kcal	**872.1**	**881.3**	**872.1**
Protein, g	**15.8**	**15.8**	**15.8**
Carbohydrate, g	**88.0**	**157.6**	**88.0**
Fat, g	**50.7**	**20.8**	**50.7**
SAFA, g	**19.7**	**1.5**	**3.7**
C12:0, g	0.1	0.0	0.0
C14:0, g	0.4	0.0	0.0
C16:0, g	17.2	0.8	1.9
C18:0, g	1.9	0.6	1.4
C20:0, g	0.1	0.0	0.1
C22:0, g	0.0	0.1	0.3
C24:0, g	0.0	0.0	0.0
MUFA, g	**20.6**	**13.5**	**36.9**
C16:1 n-7, g	0.0	0.0	0.0
C18:1 n-9, g	20.5	13.5	36.8
C20:1, g	0.1	0.0	0.1
PUFA, g	**9.7**	**5.0**	**9.1**
C18:2 n-6, g	9.6	5.0	9.0
C18:3 n-3, g	0.1	0.0	0.0

University of Malaya

'Table 3.6, continued'

	SAFA meal	CARB meal	MUFA meal
Muffin			
Total calorie, Kcal	746.4	556.3	746.4
Protein, g	8.9	8.9	8.9
Carbohydrate, g	63.8	83.7	63.8
Fat, g	50.6	20.6	50.6
Milkshake			
Total calorie, Kcal	125.7	325.0	125.7
Protein, g	6.9	6.9	6.9
Carbohydrate, g	24.2	73.9	24.2
Fat, g	0.2	0.2	0.2

Nutrient composition was determined using Nutritionist Pro™ software (AXXYA Systems LLC. Texas, USA).
Abbreviations: SAFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

3.1.7.1 Preparation of Test Meal

Each mixed meal challenge was comprised of one muffin and a glass of milkshake. The muffins were prepared by the investigators in the kitchen of the study institute in advance and stored in a -20°C freezer. On the evening of the day before postprandial challenge the muffins were removed from the freezer. Once the participant was cannulated the muffin was warmed in the microwave for 30 seconds before serving. Muffins with similar weight were matched for each subject in his all 3 postprandial challenges. The ingredients for baking 20 muffins and preparation of each glass of 250 ml milkshake are as indicated in Table 3.7. Participants were encouraged to consume all of the test meal challenges within 10 minutes and to scrape the sides of the muffin cup as far as possible to prevent any fat waste.

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Table 3.7: Ingredients for Preparation of 20 Muffins and One Glass Milkshake for SAFA- And MUFA Diet Interventions

Ingredients	Quantity	
	SAFA- and MUFA diets	CARB diet
Dry ingredients for 20 muffins		
Cornflour	206 g	206 g
Plain flour	606 g	606 g
Caster sugar	606 g	606 g
Baking powder	42 g	42 g
Dried pasteurised egg white	86 g	86 g
Carborie	0 g	400 g
Wet ingredients for 20 muffins		
Skimmed milk	812 ml	812 ml
Test fat (PO/ HOS)	1100 g	446 g
Vanilla essence	86 ml	86 ml
Wet weight per muffin	**177.2 g**	**164.2 g**
Ingredients for 1 glass of milkshake		
Skim milk	200 ml	200 ml
Nesquik (strawberry)	15 g	15 g
Carborie	0 g	50 g

3.3 Blood Handling

Subjects were required to fast overnight for at least 10 h before the fasting blood sample collection at week 0 and week 5, and 6 (before mixed meal challenge) of each intervention. Trained and certified phlebotomists and medical professionals were hired to perform all phlebotomy and cannulation in compliance with standard protocols.

3.3.1 Collection of Fasting Blood Samples

Fasting blood samples were collected in duplicate at 5 minutes interval via venipuncture at week-0, 5 and 6 of each intervention. Upon subjects' arrival, subjects were asked to fill up a questionnaire (Appendix N). A tourniquet was placed around the arm and the selected site was decontaminated with an alcohol swab prior to blood collection. Venipuncture was performed with a butterfly needle (21G x 0.75 inch needle x 12 inch

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tubing, BD Vacutainer® Safety-Lok™ Blood Collection Set) with the luer adapter screwed into a syringe for blood withdrawal. The blood was then transferred into Vacuette® blood collection tubes (Greiner Bio-One, Germany) containing clot activator (for C-peptide, insulin, NEFA, CRP, TC, HDL-C, LDL-C, triacylglycerol, apo A1, apo B100, Lp(a) plus LDL and HDL subfractions), fluoride oxalate (for glucose), EDTA (for plasma fatty acid, IL-6 and IL-1β), EDTA + citrate (for E-selectin, PAI-1 and D-dimer).

3.3.2 Collection of Postprandial Blood Samples

On the postprandial challenge day which fell on the last day of each intervention (week 6), the forearm of subjects was first secured with a torniquet and a cannula (22G x 1.00 inch (0.9 mm x 25 mm), BD Venflon™ Pro Safety Shielded IV Catheter, German) inserted into the antecubital vein after decontamination. The cannula was then taped in place with support dressings (BD Vecafix™, German). Blood collection was carried out at required time points into a syringe via a 3-way adaptor, and the blood was dispensed into blood collection tubes. The cannula was screwed close with cannula nut between samples. Flushing of the cannula with saline was performed after withdrawal of blood or when necessary.

3.3.3 Blood Sample Processing

25 µL of dipeptidyl peptidase IV (DPP IV) inhibitor (Millipore Corporation, Merck, Middlesex, UK) was added to the 2 ml EDTA blood collection tubes intended for total GIP and total GLP-1 sample collection to prevent degradation of the incretins. 84 µl of AEBSF serine protease inhibitor (R&D Systems, USA) was added to each 2 mL EDTA blood collection tube specified for total ghrelin, total PYY and CCK sample collection.

University of Malaya

After the blood samples were dispensed into the blood collection tubes, the tubes were capped well and inverted gently for 10 times. The tubes with clot activator and with EDTA + citrate were kept at room temperature and the rest were placed into a beaker filled with ice flakes. The blood samples were then brought to processing lab for centrifugation within 20 minutes, on the condition that blood sample in the tubes added with clot activator was clot before centrifugation. Centrifugation of the tubes containing EDTA + citrate was carried out at 20 °C and 3000 rpm for 15 minutes while the rest was done at 4 °C and 3000 rpm for 15 minutes. The separated serum or plasma was aliquoted into cryo vials in duplicate. Snap freezing was performed on the vials containing samples for measurement of inflammatory markers with liquid nitrogen. Serum and plasma were then sampled in aliquots after centrifugation and kept frozen at -80 °C until analysis. Packed erythrocytes remaining after plasma removal after centrifugation were stored in a 4˚C fridge for 3-5 days before treatment according to methods specified in Rose and Oklander (1965). A summary of the blood handling protocol is available in Appendix O.

3.4 Compliances Measures: Determination of Fatty Acid Composition from Erythrocyte Membrane

1mg/ml pentadecanoic acid (C15:0) in methanol served as the internal standard owing to the absence of C15:0 in nature. The stock solution was prepared by adding 375 mL methanol and ~100 mL toluene into a 500 mL volumetric flask, followed by the addition of 50 mL acetyl chloride on ice in a dropwise manner. 100 µL of plasma sample was pipetted into glass screw top tubes and added with 2.2 mL of stock solution followed by a light vortex. The mixture was heated at 60 °C for 2 h. After cooling, 5 mL of 6% sodium carbonate was added into the tubes. The tubes were then vortexed and centrifuged at 2000 rpm for 10 minutes at 10 °C. The upper phase of the separation was removed and kept in

University of Malaya

labeled GC micro vials at 4 °C until fatty acid composition analysis by GC analysis following the procedures described in section 3.2.5.

3.5 Compliance Measures: Determination of Fatty Acid Composition from Erythrocyte Membrane

This procedure begins with lipid extraction from erythrocytes. After plasma is removed from the centrifuged blood tubes for other specified purposes, the remaining erythrocytes are chilled at 4 °C for 3 to 5 days before extraction. On the day of extraction, the erythrocyte samples were removed from the chiller and centrifuged at 4 °C at 2000 rpm for 15 min. The supernatant and buffy coat was then removed, and the packed erythrocytes were decanted into labelled 50 mL graduated centrifuge tubes. The blood tubes were rinsed with 0.89% sodium chloride saline to clear out the erythrocytes tightly packed at the bottom of blood tubes. Packed erythrocytes were washed 3 times with 5 volumes (erythrocytes: 0.89% cold saline = 1:4). Each wash involved gently inverting the capped tubes 2 to 3 times, centrifugation at 4 °C and 2000 rpm for 15 min, and clearance of supernatant layer. An aspirator was used to remove the supernatant layer more precisely at the last wash.

0.5 mL of erythrocytes without any supernatant were pipetted into 25 mL centrifuge tubes using dip pasteur pipette. The tip of the pipette was flushed with 0.5 mL distilled water and the mixture vortexed then chilled at 4 °C for 15 minutes. After that, 5.5 mL chilled isopropanol + 50 mg/L butylated hydroxytoluene was slowly added and vortexed. Following another 15 minutes chill, 3.5 mL chilled chloroform + 50 mg/L butylated hydroxytoluene was added to the mixture then chilled for 30 min. The mixture was vortexed then centrifuged again under the same conditions. The lipid extract formed

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the supernatant layer and the whole upper layer was poured into a labelled glass tube. These extracts were stored at -20 °C for less than 6 months until the next processing.

From the preparation of methyl ester, 2mL of the lipid extracts were thawed then transferred into labelled GC vial. Where sample quantities were insufficient, chloroform-isopropanol mixture was added up to 2 mL to allow comparable concentrations in all samples. The samples were evaporated at 45 °C for 1 h using the Eppendorf concentrator (Eppendorf, Hamburg, Germany). After the samples were redissolved with 100 µL hexane, 50 µL 2 N sodium methoxide in anhydrous methanol (NaOCH3) was added and the mixture was vortexed thoroughly. Following the addition of 1 mL hexane and a spatula of anhydrous calcium chloride the mixture was covered and left for 1 h at room temperature. The samples but not the crystals were then transferred to new vials and evaporated for ~20 min at room temperature using the Eppendorf concentrator (Eppendorf, Hamburg, Germany). The methyl ester yield was redissolved in 50 µL hexane and transferred into inserts in a new GC microvial which were chilled before GC analysis. The fatty acid composition was analyzed using GC following the procedures described in section 3.2.5.

3.6 Biochemical Analyses

3.6.1 Enzyme-linked Immunosorbent Assays (ELISA) for Measurements of Concentrations of Inflammatory and Hemostatic Markers and Gastro-intestinal Peptides

The concentrations of inflammatory markers, haemostatic markers and gastro-intestinal peptides were determined using commercial enzyme-linked immunosorbent assays (ELISA). The assays were performed following the protocol established by manufacturers.

University of Malaya

All samples of a particular subject were assayed in duplicate within the same 96-well assay plate. ELISA kits: Quantikine® High Sensitivity Human IL-6 and Quantikine® High Sensitivity IL-1β Quantikine® and Human sE-selectin/CD62E were manufactured by R&D Systems, USA; IMUCLONE® D-dimer and IMUBIND® Plasma PAI-1 were produced by Sekisui Diagnostics, USA; Human GIP (Total), High Sensitivity GLP-1 Active Chemiluminescent, Human Ghrelin (Total) and Human PYY (Total) were manufactured by EMD Millipore, USA; and Cholecytokinin Octapeptide (CCK) (26-33) (Human, Rat, Mouse) Extraction Free EIA Kit was manufactured by Phoenix Pharmaceuticals, USA.

3.6.2 Lipid Assay and Glycaemic Measurements

Serum lipids (TC, HDL-C, triacylglycerol, apo A1, apo B100 and Lp(a)), plasma C-peptide, insulin, glucose and fructosamine were analyzed using the Siemens Advia 2400 chemistry analyzer at an ISO accredited pathology laboratory, Pathlab (Medical Testing SAMM No. 406), while NEFA samples were analyzed using the ACS-ACOD method (Wako Diagnostics GmbH) at the study laboratory in MPOB using a Hitachi 902 autoanalyzer. Singlet analysis was done for samples sent to Pathlab whereas NEFA samples were analyzed in duplicate. Briefly, determination of total cholesterol utilized an enzymatic method; measurement of HDL-C utilized direct-HDL cholesterol method; quantification of triglyceride was based on the Fossati three-step enzymatic reaction with a Trinder endpoint; determination of apo A1 and apo B was done with polythylene glycol enhanced immunoturbidimetric assay; Lp(a) analysis applied turbidimetric immunoassay method; and measurement of CRP applied latex-enhanced immunoturbidimetric assay. LDL-C was calculated referring to the Friedewald equation.

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Lipoprint System LDL subfractions kit and Lipoprint System HDL subfractions kit (Quantimetrix, USA) were used for separation of lipoproteins to yield LDL subfractions and HDL subfractions by apolyacrylamide gel electrophoresis technique. All samples from a subject were analyzed in the same batch. LDL and HDL subfractionations were performed as instructed in the product insert. Lipoware computer software (Quantimetrix, USA) was used to analyze the LDL and HDL particle according to the scanned image of the dyed gels (Figure 3.4).

Figure 3.4: Scanned Image of Dyed Gel after Completion of Electrophoresis.

3.6.3 Pulse Wave Analysis for Assessment of Endothelial Function

Pulse wave analysis of the radial artery by applanation tonometry was performed using the SphygmoCor CP System (AtCor Medical, Australia). Before commencement of pulse wave analysis, new patient entries were created on the SphygmoCor software version 9 (AtCor Medical, Australia). Subjects underwent measurement of arterial stiffness through this analysis at baseline; fasting state and at 4-h after the meal during the postprandial challenge. Sitting at a comfortable position with the left arm lying horizontal on a table, the tonometer was placed on the wrist perpendicularly and adjusted to a position where a strong and reproducible waveform was detected by the software. Analysis was repeated for those with operator index less than 80. Corrected augmentation index (AIX), aortic

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augmentation pressure, aortic diastolic pressure, aortic systolic pressure and aortic pulse pressure were calculated by the software with entry of the brachial blood pressure and heart rate measurements.

3.7 Calculations

Body Mass Index (BMI) is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). Homeostatic model assessment of insulin resistance (HOMA2-IR), insulin sensitivity (HOMA2-%S) and β-cell function (HOMA2-%B) were calculated based on fasting glucose pairing with either fasting insulin or C-peptide, using the HOMA Calculator version 2.2.2 (http://www.dtu.ox.ac.uk/homacalculator/, accessed June 2014). Revised Quantitative Insulin Sensitivity Check Index (rQUICKI) was calculated as 1/(log fasting insulin (mU/ml) + log fasting glucose (mg/dl) + log fasting non-esterified fatty acids (NEFA) (mmol/l)) (Ijzerman et al., 2009). Incremental areas under the curves (iAUC) were calculated by the conventional trapezoid rule after the subtraction of baseline reading, using postprandial data up to 360 min for IL-6, IL-1β, E-selectin, PAI-1, D-dimer, CRP, NEFA and triacylglycerol; up till 120 min for C-peptide, insulin, glucose; 0-120 min (early phase) and 120-360 min (late phase) for GLP-1, GIP, ghrelin, PYY and CCK. Corrected insulin (for glucose) response is defined as the ratio of $iAUC_{0\text{-}120\ min}$ of insulin divided by $iAUC_{0\text{-}120\ min}$ of glucose. DI was calculated by multiplying corrected insulin (for glucose) response with HOMA2-%S.

3.8 Statistical Analysis

Where duplicate measurements were available, the mean value on treatment was used in the analysis. With regards to postprandial data, the difference from the preprandial fasting

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value was calculated and the mean delta used in statistical analysis. Standard distributional checks were analyzed by D' Agostino & Pearson omnibus normality test; logarithmic and square root transformations were performed where applicable. Geometric means (GM) were presented following the transformation to indicate treatment effect. Non-parametric statistical analyses were performed where data did not conform to a normal distribution following transformation Friedman's test and Dunn's test using Graph Pad Prism software (version 5.02; Graph Pad software, Inc., La Jolla, CA, USA). Incremental area under the curves (iAUC), not including the areas below baseline, was calculated for all postprandial measurements applying the trapezoid rule using Graph Pad Prism software (version 5.02; Graph Pad software, Inc., La Jolla, CA, USA). Individual graph exhibiting postprandial absolute values and the responsive iAUC graph were plotted with Graph Pad Prism software (version 5.02; Graph Pad software, Inc., La Jolla, CA, USA). Where significant meal x time x gender interaction was detected, the individual graph and iAUC bar graph for men and women were included.

As each subject served as his own control, baseline values were not used in the statistical analysis. Instead the fasting and postprandial values at the end of each treatment period were compared between treatments using one way ANOVA with repeated measures and Bonferroni's multiple comparison post hoc test by SPSS software (version 18; SPSS Inc, Chicago, IL). Repeated-measures ANOVA was performed with meal and time as within subject factors, and gender as between subject factor. *P* value of < 0.05 was considered as being of statistical significance. Results were reported as mean/GM (95% CI).

A subgroup analysis of insulin corrected to glucose and disposition index was made according to subjects' fasting glucose concentration at baseline as majority of abdominally obese subjects had elevated fasting glucose level. Subjects were categorized into 3 groups, i.e. normal fasting glucose (NFG) (<5.6 mmol/L), impaired fasting glucose

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(IFG) (5.6-6.9 mmol/L) and T2DM (≥7.0 mmol/L). This subgroup analysis investigated if the diverging effects of CARB vs SAFA and MUFA on insulin secretion was seen mainly in groups with elevated fasting glucose.

Keynotes

-Subjects were randomized into 3 isocaloric consecutive 6-week dietary interventions (without washout) each followed by a mixed meal challenge.

-Three meals and snacks were provided to subjects on working days and subjects had to prepare meals with our test oils during weekends.

-Our study strictly control the compliance of subjects and carefully designed menus that allowed a similar energy exchange at 7% en across treatments.

-Subgroup analysis was performed on insulin secretion indices.

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CHAPTER 4: RESULTS

4.1 Subject Characteristics

A total of 54 abdominally obese yet healthy subjects (mean age 32.8 years) with no known medical illnesses were recruited into this study. 47 of them (35 females and 12 males) successfully completed the 3 x 6-week dietary interventions in which 31 females and 6 males were MPOB staffs. 1 female subject (MPOB staff) was unable to participate in the postprandial challenge. Hence, the sample size used for data analysis was n = 47 for fasting measurements and n = 46 for postprandial measurements. The reasons for non-completion given by the 7 drop-outs included inability to make a time-commitment (n = 2), health problems (n = 2) and pregnancy (n = 3) (Fig. 3.1). Baseline demographic and clinical characteristics of the subjects are shown in Table 4.1.

The mean body mass index was in the overweight (pre-obese) range, i.e. 28.7 kg/m^2 and the percentage of body fat was high (39.9% in women and 27.0% in men). Using cut-offs advocated for Asians, 38.3% of participants were overweight (BMI 23 – 24.9 kg/m^2) and 57.4% (BMI ≥ 25 kg/m^2) obese at baseline. Mean waist circumference (93.2 cm in women and 99.5 cm in men) exceeded the cut-off point for abdominal obesity for Asians recommended by IDF (80 cm in women and 90 cm in men). The habitual dietary intakes analyzed based on the 3-day diet record were typical of an urban Malaysian population providing 26.9% en fat, 16.3% en protein and 57.0% en carbohydrate; energy intake was 1915.4 kcal/d in women and 2449.7 kcal/d in men. The relatively high fasting plasma glucose (5.8 mmol/L) at baseline was close to the cut-off point of impaired fasting glycaemia (6.1- 6.9 mmol/L) ("Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation," 2006). However, 59.6% of the 47 participants had impaired fasting glucose levels (5.6 –

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6.9 mmol/L) and 6.4% had diabetes (fasting blood glucose ⩾7.0 mmol/L) based on American Diabetes Association criteria (Nathan et al., 2007); 40.4% had blood pressure >130/85 mmHG; 38.3% had LDL cholesterol >3.4 mmol/L. 34% fit the criteria for metabolic syndrome as defined by the Harmonized Criteria (Alberti et al., 2009). The baseline mean TC and LDL cholesterol were relatively elevated in the women (5.1 ± 0.8 and 3.2 ± 0.7 mmol/L) but not the men (4.8 ± 0.8 and 3.0 ± 0.7 mmol/L). The higher HDL cholesterol level in the women compared with men (women: 1.4 ± 0.3 vs men: 1.1 ± 0.2 mmol/L) hence contributed to the lower and better total: HDL cholesterol ratio compared with the men (women: 3.9 ± 0.8 vs men: 4.3 ± 0.9). These differences between the genders were not however statistically significant.

Table 4.1: Baseline Demographic and Clinical Characteristics of Study Participants Completing the 3 Dietary Interventions

	All (*n*=47[1])	**Women (***n*=35[1])	**Men (***n*=12[1])
Physical characteristics			
Age (years)	32.8 ± 8.7	34.4 ± 8.7	28.3 ± 7.4
Height(cm)	159.4 ± 7.8	156.5 ± 6.4	167.8 ± 4.7
Weight (kg)	74.2 ± 14.6	71.2 ± 13.5	82.8 ± 12.2
BMI (kg/m^2)	28.7 ± 4.1	28.6 ± 4.3	28.8 ± 3.8
Waist (cm)	94.8 ± 10.2	93.2 ± 10.0	99.5 ± 9.7
Body fat (%)	36.6 ± 7.9	39.9 ± 5.9	27.0 ± 4.5
Systolic BP (mmHg)	124.5 ± 12.4	124.3 ± 13.7	125.3 ± 7.9
Diastolic BP (mmHg)	81.8 ± 10.7	82.2 ± 11.0	80.5 ± 10.1
Biochemical profile (mmol/L)			
Plasma glucose	5.8 ± 0.8	5.8 ± 0.9	5.9 ± 0.4
Serum total cholesterol	5.0 ± 0.8	5.1 ± 0.8	4.8 ± 0.8
Serum HDL cholesterol	1.3 ± 0.3	1.4 ± 0.3	1.1 ± 0.2
Serum LDL cholesterol	3.1 ± 0.7	3.2 ± 0.7	3.0 ± 0.7
Serum TAG	1.2 ± 0.7	1.2 ± 0.7	1.4 ± 0.8
Total: HDL cholesterol	4.0 ± 0.8	3.9 ± 0.8	4.3 ± 0.9
Habitual dietary intake[2]			
Energy (kcal/day)	2051.8 ± 545.3	1915.4 ± 500.0	2449.7 ± 488.6
Protein (% en)	16.3 ± 2.8	16.8 ± 2.9	14.7 ± 1.8
Carbohydrate (% en)	57.3 ± 7.2	56.8 ± 7.2	58.9 ± 7.5
Fat (% en)	26.9 ± 6.2	27.0 ± 6.0	26.6 ± 6.9

Values are means ± SD. [1] Ethnicity: Malay. [2]Determined from 3-day weighed diet record using Nutritionist Pro^{TM} software (AXXYA Systems LLC. Texas, USA).

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4.2 Compliance and Monitoring Measures during Treatments

The details of monitoring measures employed to ensure adequate compliance of dietary intake are delineated in Table 4.2. Body weight, percentage body fat, systolic and diastolic pressure were constant across interventions ($P > 0.05$). Changes observed in plasma fatty acid composition and erythrocyte membrane phospholipid fatty acids composition between diets confirmed good dietary compliance. SAFA diet (26.8 ± 2.3%) had higher plasma palmitic acid levels compared with CARB (26.3 ± 1.8%) and MUFA diets (26.0 ± 2.0%), respectively ($P < 0.05$); whereas SAFA diet (22.8 ± 3.0%) exhibited lower oleic acid content compared with both CARB (24.7 ± 2.7%) and MUFA (25.0 ± 3.3%) diets ($P < 0.05$). As for erythrocyte membrane phospholipids, % oleic acid was reduced after SAFA diet (14.4 ± 0.4%) compared with CARB (15.5 ± 0.3%) and MUFA (15.1 ± 0.2%) diets ($P < 0.05$); in addition MUFA content was lower ($P < 0.05$) after SAFA diet (15.0 ± 0.2%) compared with CARB diet (15.9 ± 0.3%).

Table 4.2: Weight Monitoring and Blood Pressure during the 3 x 6-week Dietary Intervention

	Baseline	SAFA diet	CARB diet	MUFA diet
Weight monitoring				
Weight (kg)	74.2 ± 14.6	73.8 ± 14.4	73.9 ± 14.6	73.9 ± 14.3
Body fat (%)	36.6 ± 7.9	36.5 ± 7.7	36.4 ± 7.9	36.4 ± 7.8
Blood pressure				
Systolic (mmHg)	124.5 ± 12.4	120.1 ± 10.0	120.8 ± 10.9	121.7 ± 11.7
Diastolic (mmHg)	81.79 ± 10.73	78.4 ± 8.7	78.3 ± 10.2	79.3 ± 9.5

Values are mean ± SD; n=47. Repeated measures ANOVA: no significant differences between diets ($P > 0.05$).

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Subjects were equally compliant with the 3 dietary treatments as measured by meal attendance, snack collection, meal appreciation and weekend home oil usage throughout the dietary interventions (Table 4.3). The overall attendance for meal collections on weekdays achieved was 97.1 ± 4.4%, 98.0 ± 3.2% and 97.1 ± 5.1% for SAFA, CARB and MUFA diets respectively, only 2 – 3% meals were missed. No significant differences were detected between the 3 treatments with regards to overall % attendance, % meal attendance of breakfast, lunch and dinner, total % meals missed and % meals missed due to sick leave ($P > 0.05$). Snacks including cupcake and Malay pancake were allocated to those subjects who required higher calorie intake to avoid the interference of weight fluctuation. The number of snacks collected per subject during the 6-week intervention (a total of 30 weekdays) was relatively small at 24.5 ± 24.4 (SAFA diet), 26.6 ± 25.2 (CARB diet) and 25.5 ± 26.2 (MUFA diet). No significant differences in the amount of snacks collected and extra energy intake from daily snacks were found between all treatments ($P > 0.05$).

Little variation and no significant differences were found in the degree of acceptability (i.e. degree to which the food was consumed to meet one's caloric needs) and palatability (i.e. degree of agreeableness of a meal to the taste of the subject) between diets, measured by VAS of 10 cm. The meal appreciation and palatability assessed by VAS were 7.0 ± 1.2, 7.1 ± 1.3, 6.9 ± 1.4 and 7.2 ± 1.3, 7.3 ± 1.3, 7.1 ± 1.4 for SAFA, CARB and MUFA diets respectively ($P > 0.05$).

The consumption of test oil during CARB diet intervention should be lower than that during the other 2 interventions. However the average oil consumption per weekend day (the amount was shared by family members) was 96.5 ± 51.0 g/day during the CARB diet with no significant differences compared with SAFA (93.5 ± 43.5 g/day) and MUFA diets (95.3 ± 42.3 g/day) ($P > 0.05$). Nevertheless, these results may not necessarily indicate that the subjects following CARB diet consumed a higher than targeted amount

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of oil. It should be noticed that the weekend home oil usage shown in Table 4.3 included the portion consumed by the family members rather than the subjects alone.

Table 4.3: Compliance Measurements during the Three 6-week Dietary Interventions.

	SAFA diet	CARB diet	MUFA diet
Meals and snacks			
Meals attended (%)[1]	97.1 ± 4.4	98.0 ± 3.2	97.1 ± 5.1
Breakfast	96.9 ± 4.5	97.9 ± 3.6	97.1 ± 5.1
Lunch	97.2 ± 4.4	97.9 ± 3.6	96.9 ± 5.0
Dinner	97.1 ± 4.7	98.2 ± 3.2	97.3 ± 5.3
Meals missed (%)[1]	2.9 ± 4.4	2.0 ± 3.2	2.9 ± 5.1
Due to sick leave	0.3 ± 1.1	0.4 ± 1.5	0.2 ± 0.8
Total snacks collected (per person)[2]	24.5 ± 24.4	26.6 ± 25.2	25.5 ± 26.2
Strawberry cupcake	4.7 ± 6.1	4.9 ± 6.6	4.6 ± 4.8
Vanilla cupcake	4.2 ± 6.8	5.4 ± 7.1	4.5 ± 8.1
Orange cupcake	5.1 ± 6.0	4.9 ± 5.7	5.2 ± 7.3
Kuih bakar (Malay pancake)	10.5 ± 14.1	11.5 ± 13.4	11.1 ± 14.9
Total calorie from snacks (kcal/day)	128.2 ± 127.8	139.2 ± 131.9	133.2 ± 137.0
Visual analogue scale[3]			
Acceptability (cm)	7.0 ± 1.2	7.1 ± 1.3	6.9 ± 1.4
Breakfast	6.4 ± 1.8	6.7 ± 1.3	6.5 ± 1.9
Lunch	7.2 ± 1.5	7.3 ± 1.7	7.0 ± 1.5
Dinner	7.2 ± 1.5	7.3 ± 1.5	7.2 ± 1.5
Palatability (cm)	7.2 ± 1.3	7.3 ± 1.3	7.1 ± 1.4
Breakfast	6.7 ± 2.0	6.9 ± 1.4	6.6 ± 1.8
Lunch	7.5 ± 1.4	7.5 ± 1.7	7.2 ± 1.5
Dinner	7.4 ± 1.6	7.4 ± 1.3	7.4 ± 1.6
Weekend home oil usage amount			
Issued over 6-weeks (g)	1186.1 ± 509.1	1247.1 ± 599.6	1191.4 ± 513.2
Returned at the end of 6-weeks (g)	64.0 ± 159.8	88.8 ± 166.9	47.4 ± 92.2
Consumed over 6-weeks (g)	1122.1 ± 522.3	1158.4 ± 611.6	1144.0 ± 508.0
Consumed/weekend (g/day)	93.5 ± 43.5	96.5 ± 51.0	95.3 ± 42.3

Values are mean ± SD. n = 47. Repeated measures ANOVA: no significant differences between diets ($P > 0.05$). .

[1]Meals attended and meals missed are presented as % of the total number of meals consumed during the 3 x 6-week dietary intervention.

[2]Snack muffins collected are presented as mean number collected per person during each intervention.

[3]Acceptability and palatability are mean values of breakfast, lunch and dinner.

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4. 3 Postprandial C-peptide, Insulin and Glucose Indices

4.3.1 C-peptide

The analysis of variance of postprandial changes in serum C-peptide revealed significant meal x time interaction ($P < 0.001$) (Figure 4.1). In general, the 3 test meals robustly elevated plasma C-peptide from 0 until 30 min, subsequently peaking at 2.15 (1.92, 2.37) nmol/L (SAFA meal) at 60 min and 3.61 (3.14, 4.09) nmol/L (CARB meal) and 2.28 (1.95, 2.61) nmol/L (MUFA meal) at 120 min. C-peptide levels then decreased and returned to baseline at 360 min. Table 4.4 shows the changes from fasting concentrations (Δ) following the respective test meals at the end of each dietary intervention. The C-peptide changes from baseline after the CARB meal were significantly higher ($P < 0.001$) than the SAFA and MUFA meals at all time points from 30 min onwards. The $iAUC_{C\text{-}peptide\ (0\text{-}120min)}$ were 154.0 (135.4, 172.5) (SAFA), 272.3 (245.6, 299.1) (CARB) and 158.0 (136.6, 179.4) (MUFA): 77% and 72% higher after CARB meal compared with SAFA- and MUFA meals respectively ($P < 0.001$). Women demonstrated a delayed postprandial C-peptide increase at the initial 120 min but sustained a higher level after 120 min compared with men as indicated by meal x time x gender interaction ($P = 0.007$) (Figure 4.2).

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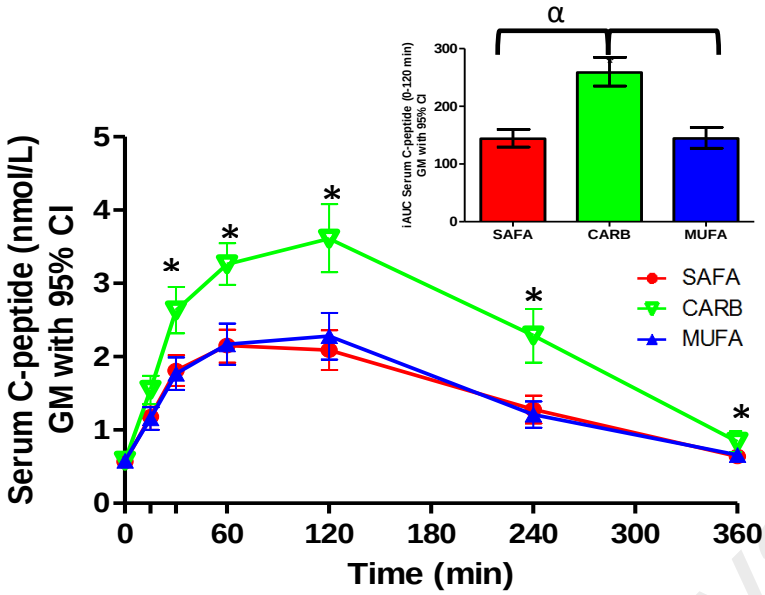

Figure 4.1: Postprandial Serum C-Peptide Concentrations Following Mixed Meal at the End of Each 3 x 6-week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data (0-120 min) were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$, meal x time interaction: $P < 0.001$ and meal x time x gender: $P = 0.007$.

[2]Insert: iAUC over 120 min (GM ± 95% CI) ($n = 46$). Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: $P < 0.0001$

*Change from baseline was significantly different between CARB *vs* SAFA and MUFA meals ($P < 0.001$). [α]Significant difference between diets ($P < 0.001$).

Table 4.4: Changes from Fasting Concentration (Δ) of Serum C-peptide after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	0.57 (0.50, 0.63)	0.58 (0.51, 0.65)	0.58 (0.50, 0.65)
Δ 15	0.61 (0.47, 0.75)	0.96 (0.79, 1.14)	0.58 (0.45, 0.71)
Δ 30	1.24 (1.06, 1.43)	2.05 (1.77, 2.33)*	1.19 (1.01, 1.38)
Δ 60	1.58 (1.40, 1.76)	2.68 (2.44, 2.93)*	1.59 (1.36, 1.81)
Δ 120	1.53 (1.30, 1.76)	3.03 (2.61, 3.46)*	1.70 (1.43, 1.98)
Δ 240	0.71 (0.56, 0.86)	1.70 (1.39, 2.01)*	0.63 (0.50, 0.77)
Δ 360	0.08 (0.03, 0.12)	0.26 (0.16, 0.37)*	0.08 (0.04, 0.12)
iAUC$_{(0\text{-}120min)}$	154.0 (135.4, 172.5)	272.3 (245.6, 299.1)*	158.0 (136.6, 179.4)

Values are GM with 95% CI, expressed in nmol/L; $n = 46$ (women $n = 34$; men $n = 12$).

[1]Changes from fasting data (0-120 min) were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$, meal x time interaction: $P < 0.001$ and meal x time x gender: $P = 0.007$.

[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: $P < 0.0001$.

*Significant difference compared with SAFA and MUFA meals ($P < 0.001$).

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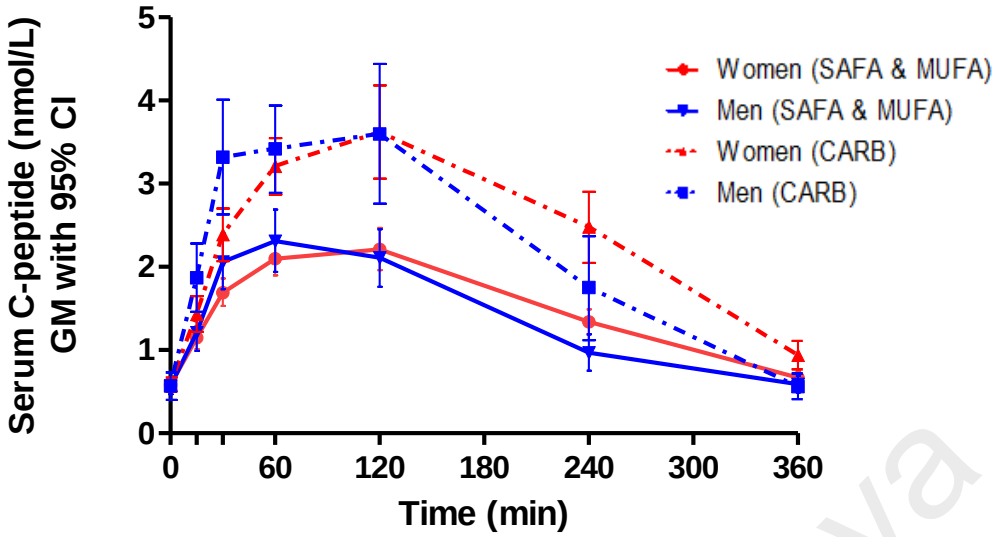

Figure 4.2: Gender x Time Interaction for C-Peptide Concentrations in Women and Men Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention. Data are GM with 95% CI. *n* = 46 (34 women, 12 men). Deviations from preprandial values (0-360 min) were analyzed by repeated measures ANOVA (3 meals, 6 time points): gender x time interaction, $P < 0.001$, meal x time x gender interaction, $P = 0.007$.

4.3.2 Insulin

The changes in postprandial insulin concentration are depicted in Figure 4.3. Insulin showed a significant meal x time interaction ($P < 0.001$), with CARB meal displaying a higher increase compared with SAFA and MUFA meals. Insulin concentrations peaked at 111.7 (91.5, 132.0), 114.3 (92.1, 136.4) mU/L respectively for SAFA and MUFA meals at 30 min whereas they peaked at 205.0 (141.7, 268.3) mU/L for CARB meal at 120 min, with a return to baseline by 360 min in all 3 groups. As referred to Table 4.5, the insulin changes from baseline after the CARB meal were significantly higher than SAFA and MUFA meals at time points from 15 min onwards ($P < 0.05$). iAUC $_{(0\text{-}120\text{min})}$ of CARB meal (18829 (14910, 22748)) was 98% and 86% higher than SAFA (9534 (7563, 11505)) and MUFA meals (10127 (7756, 12498)) ($P < 0.001$) respectively. Insulin response corrected for glucose ($iAUC_{insulin/glucose}$) ($P = 0.048$) and DI ($P = 0.030$) after CARB meal tended to be lower than SAFA and MUFA meals.

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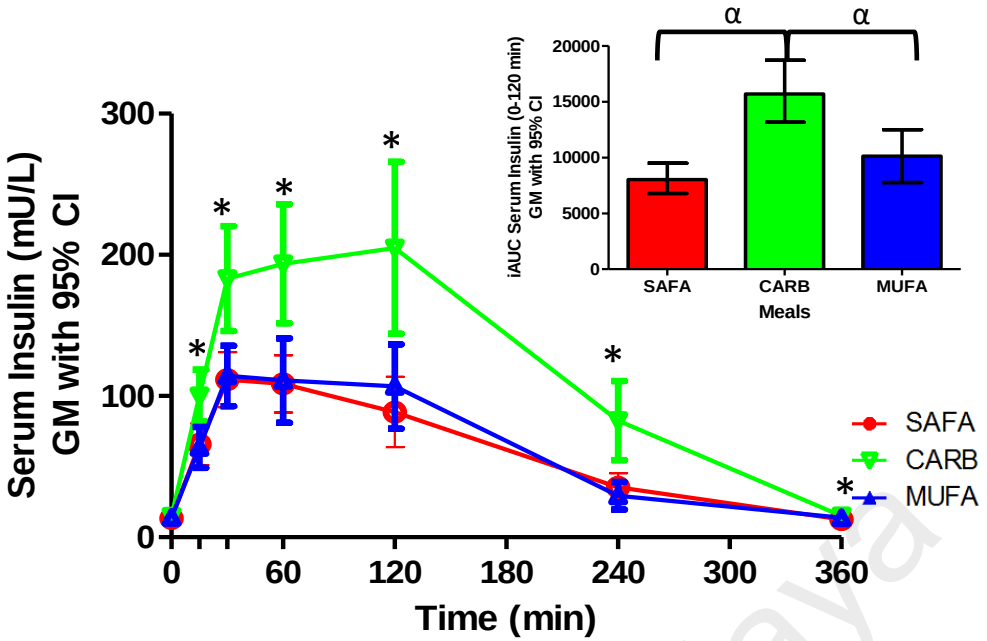

Figure 4.3: Postprandial Serum Insulin Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data (0-120min) were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$, meal x time interaction: $P < 0.001$.

[2]Insert: iAUC over 120 min (GM ± 95% CI) (n = 46). Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: $P < 0.0001$

*Change from baseline was significantly different between CARB *vs* SAFA and MUFA meals ($P < 0.001$). [α]Significant difference between diets ($P < 0.001$).

Table 4.5: Changes from Fasting Concentration (Δ) of Serum Insulin after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	13.47 (11.26, 15.68)	14.81 (11.58, 18.03)	14.19 (10.81, 17.57)
Δ 15	52.32 (38.51, 66.13)	49.34 (35.76, 62.92)*	85.43 (68.12, 102.74)
Δ 30	98.26 (79.65, 116.87)	100.07 (80.18, 119.95)*	168.43 (132.82, 204.04)
Δ 60	95.17 (76.19, 114.15)	96.83 (68.99, 124.66)*	178.90 (137.84, 219.96)
Δ 120	75.17 (51.50, 98.84)	92.58 (64.94, 120.21)*	190.18 (130.47, 249.89)
Δ 240	21.79 (13.11, 30.48)	15.21 (7.72, 22.70)*	67.84 (41.74, 93.95)
Δ 360	-0.79 (-2.22, 0.65)	-0.39 (-2.05, 1.27)*	0.36 (-3.01, 3.73)
$iAUC_{0\text{-}120min}$	9534 (7563, 11505)	18829 (14910, 22748)*	10127 (7756, 12498)
$iAUC_{insulin/glucose}$[2]	77.72 (60.78, 94.66)	64.20 (50.54, 77.86)	72.66 (60.47, 84.84)
$DI_{0\text{-}120\ min}$[3]	5605 (3981, 7229)	4133 (3464, 4803)	4942 (4109, 5774)
$DI_{0\text{-}120\ min}$[4]	6306 (4812, 7801)	4807 (3994, 5621)	5765 (4796, 6734)

Values are GM with 95% CI, expressed in mU/L; n = 46 (women n = 34; men n = 12).

[1]Changes from fasting data (0-120min) were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$, meal x time interaction: $P < 0.001$.

[2]Repeated measures ANOVA of $\log_e$ corrected insulin to glucose ratio $_{(0\text{-}120min)}$: P = 0.0484; Bonferroni's Multiple Comparison Test, $P > 0.05$.

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'Table 4.5, continued'

[3]Calculated from fasting glucose and insulin concentrations. $\log_e$ DI, $P = 0.0949$
[4]Calculated from fasting glucose and C-peptide concentrations. $\log_e$ DI, $P = 0.0297$; Bonferroni's Multiple Comparison Test, $P > 0.05$.
[4]Repeated measures ANOVA of $\log_e$ $iAUC_{(0\text{-}120min)}$: $P < 0.0001$. *Significantly different compared with SAFA and MUFA meals ($P < 0.05$).
Abbreviations – DI, disposition index.

4.3.2.1 Subgroup Analysis of Insulin Secretion

As reported in Table 4.6, no significant difference was found in insulin corrected to glucose and DI in all subgroups of NFG, IFG and T2DM ($P > 0.05$). Although not significantly different, we observed a trend of highest insulin corrected to glucose and DI following SAFA diet in both NFG and IFG groups. On the other hand, SAFA diet exhibited lowest levels for the two afore-mentioned indices in T2DM group. However, it is to be noted that the really small sample size of T2DM group ($n = 3$) limits the power of analysis.

Table 4. 66: Subgroup Analysis of Insulin Secretion Indices Following the Three 6-week Dietary Interventions

	SAFA diet	CARB diet	MUFA diet
Insulin corrected to glucose			
NFG[1]	85.57 (43.40, 127.7)	59.67 (44.44, 74.90)	74.96 (56.29, 93.62)
IFG	78.54 (60.62, 96.45)	70.00 (48.87, 91.12)	75.17 (57.53, 92.80)
T2DM	30.83 (14.83, 46.82)	32.75 (3.987, 61.52)	37.75 (19.16, 56.35)
Disposition index[2]			
NFG	7425 (4181, 10670)	5220 (4111, 6330)	6474 (4884, 8065)
IFG	5114 (3075, 7153)	3871 (3035, 4706)	4525 (3598, 5453)
T2DM	1085 (-798.5, 2968)	1150 (-399.4, 2700)	1164 (-603.7,2931)

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'Table 4.6, continued'

	SAFA diet	CARB diet	MUFA diet
Disposition index[3]			
NFG[1]	7933 (4850, 11015)	5736 (4406, 7067)	7436 (5528, 9343)
IFG[1]	5960 (4148, 7772)	4669 (3591, 5747)	5324 (4239, 6408)
T2DM	1410 (-446.9, 3267)	1448 (-117.5, 3013)	1537 (15.26, 3059)

Values are mean ± SD and GM ± SD[1].
NFG: *n*=15 (women *n*=12; men *n*=3).
IFG: *n*=28 (women *n*=19; men *n*=9)
T2DM: *n*=3 (women *n*=3; men *n*=0)
[2] Calculated from fasting glucose and insulin concentrations.
[3] Calculated from fasting glucose and C-peptide concentrations.
Data was analyzed by repeated measures ANOVA (among 3 treatments in one subgroup). No significant differences between diets ($P > 0.05$) in all subgroups.
Abbreviations – NFG, normal fasting glucose; IFG, impaired fasting glucose; T2DM, Type 2 Diabetes Mellitus

4.3.3 Glucose

Comparison of the postprandial response over 360 min between meals for plasma glucose is shown in Figure 4.4. Repeated measure ANOVA showed significant meal x time interaction ($P < 0.001$). Glucose concentrations after all meals peaked at 30 min and returned to baseline before 360 min. The peak glucose after CARB meal was 25% higher compared with both high-fat meals (9.29 (8.84, 9.73) (CARB) *vs* 7.59 (7.20, 7.98) (SAFA), 7.44 (7.09, 7.79) (MUFA) mmol/L). Glucose concentrations following CARB meal were significantly higher than SAFA and MUFA meals from 15 to 120 min ($P < 0.05$). At 360 min, glucose concentration after CARB meal decreased from baseline more than that of the MUFA meal ($P < 0.05$) (Table 4.7). $iAUC_{(0\text{-}120min)}$ showed that CARB meal (324.0 (282.4, 365.7)) increased plasma glucose at 110 and 107% over 6 h compared with SAFA (154.3 (127.9, 180.7)) and MUFA (156.6 (131.7, 181.5)) meal, respectively ($P < 0.001$).

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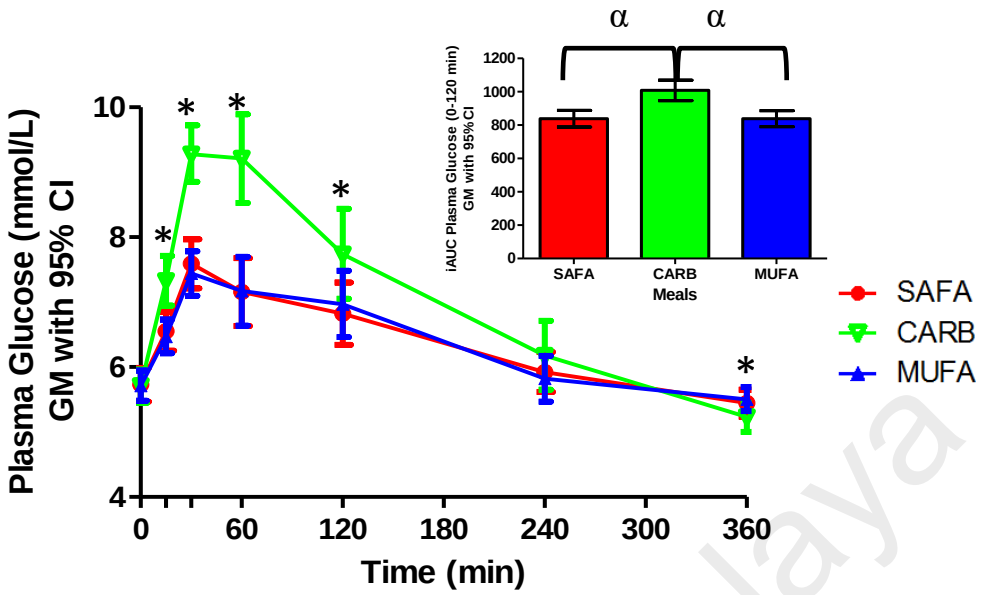

Figure 4.4: Postprandial Serum Glucose Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data (0-120min) were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$, meal x time interaction: $P < 0.001$.

[2]Insert: iAUC over 120 min (GM with 95% CI) ($n = 46$). Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120\text{min})}$: $P < 0.0001$

*Change from baseline was significantly different between CARB *vs* SAFA and MUFA meals ($P < 0.05$). [α]Significant difference between diets ($P < 0.001$).

Table 4.7: Changes from Fasting Concentration (Δ) of Serum Glucose after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	5.73 (5.47 to 5.99)	5.71 (5.45 to 5.96)	5.71 (5.48 to 5.95)
Δ 15	0.83 (0.63 to 1.02)	1.62 (1.32 to 1.92)*	0.75 (0.58 to 0.92)
Δ 30	1.86 (1.60 to 2.13)	3.58 (3.26 to 3.90)*	1.72 (1.50 to 1.95)
Δ 60	1.43 (1.08 to 1.77)	3.50 (2.96 to 4.04)*	1.45 (1.08 to 1.82)
Δ 120	1.09 (0.78 to 1.41)	2.03 (1.50 to 2.57)*	1.26 (0.89 to 1.62)
Δ 240	0.20 (-0.02 to 0.41)	0.47 (0.05 to 0.89)	0.11 (-0.13 to 0.35)
Δ 360	-0.28 (-0.51 to -0.05)	-0.48 (-0.70 to -0.26)*	-0.21 (-0.41 to -0.01)
iAUC$_{(0\text{-}120\text{min})}$	154.3 (127.9, 180.7)	324.0 (282.4, 365.7)*	156.6 (131.7, 181.5)

Values are GM with 95% CI, expressed in mmol/L; $n = 46$ (women $n = 34$; men $n = 12$).

[1]Changes from fasting data (0-120min) were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$, meal x time interaction: $P < 0.001$.

[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120\text{min})}$: $P < 0.0001$.

*Significantly different compared with SAFA and MUFA meals ($P < 0.05$).

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4.4 Fasting C-peptide, Insulin and Glucose Indices

Table 4.8 shows the fasting concentrations of C-peptide, insulin, glucose and NEFA following the 3 dietary interventions. Measurements were made at week 5 and 6 and the mean results were reported. No significant differences were noted in fasting concentrations of C-peptide, insulin, glucose, fructosamine, rQUICKI, HOMA2-%B, HOMA2-%S and HOMA2-IR between diets ($P > 0.05$).

Table 4.8: Fasting C-peptide, Insulin and Glucose Indices in 47 Participants Following the Three 6-week Dietary Interventions

	Baseline†	SAFA diet	CARB diet	MUFA diet
C-peptide (nmol/L)[1]	0.63 (0.37, 0.89)	0.59 (0.36, 0.83)	0.60 (0.36, 0.85)	0.60 (0.35, 0.84)
Insulin (mU/L)[1]	16.5 (13.6, 19.4)	15.0 (12.5, 17.5)	14.7 (12.5, 17.0)	15.1 (12.3, 17.9)
Glucose (mmol/L)[1]	5.78 (5.56, 6.00)	5.80 (5.56, 6.04)	5.77 (5.54, 6.00)	5.76 (5.51, 6.00)
Fructosamine(µmol/L)[1]	313.7 (304.8, 322.5)	304.1 (296.7, 311.6)	309.8 (300.2, 319.4)	313.3 (303.3, 323.3)
rQUICKI	0.193 (0.188, 0.197)	0.195 (0.191, 0.200)	0.195 (0.191, 0.199)	0.195 (0.191, 0.199)
HOMA2-%B[2]	104.7 (94.7.114.7)	100.5 (88.9, 112.1)	99.0 (89.7, 108.2)	100.4 (90.7,110.0)
HOMA2-%S[2]	70.75 (60.80, 80.70)	75.72 (64.19, 87.25)	77.76 (65.27, 90.25)	76.28 (65.78, 86.77)
HOMA2-IR[2]	1.894 (1.555, 2.233)	1.722 (1.438, 2.006)	1.696 (1.435,1.957)	1.738 (1.409, 2.067)
HOMA2-%B[3]	89.49 (83.00, 95.99)	87.19 (78.53, 95.85)	87.94 (80.58, 95.31)	87.97 (81.14, 94.80)
HOMA2-%S[3]	81.40 (72.11, 90.70)	85.28 (75.69, 94.87)	84.93 (74.94, 94.93)	84.29 (75.53, 93.05)
HOMA2-IR[3]	1.462 (1.274,1.649)	1.375 (1.209, 1.541)	1.396 (1.223,1.569)	1.385 (1.208,1.562)

Values are mean ± SD and GM ± SD[1]. n=47 (women n=35; men n=12).
[2] Calculated from fasting glucose and insulin concentrations.
[3] Calculated from fasting glucose and C-peptide concentrations.
Data was analyzed by repeated measures ANOVA (among 3 treatments). No significant differences between diets ($P > 0.05$).
†Baseline data was reported as the reference and not used in data analysis.
Abbreviations – rQUCIKI, Revised Quantitative Insulin Sensitivity Check Index; HOMA2-%β, Homeostatic Model Assessment 2-Pancreatic Beta Cell Function; HOMA2-%S, Homeostatic Model Assessment 2-Insulin Sensitivity; HOMA2-IR, Homeostatic Model Assessment 2-Insulin Resistance.

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4.5 Postprandial Inflammatory and Endothelial Responses

4.5.1 Interleukin-6 (IL-6)

The analysis of variance on plasma IL-6 concentrations following the consumption of the test meals revealed no significant meal x time interaction ($P = 0.245$) but significant time interaction ($P < 0.001$). As shown in Figure 4.5, IL-6 concentrations did not change significantly from 0 to 120 min following all 3 meals and only began to increase from 120 – 360 min. There were no significant changes from baseline between the 3 meals at all time points ($P > 0.05$) (Table 4.9). However, iAUC $_{(0\text{-}360min)}$ after CARB meal (4.86 (2.72, 7.00)) was 66% higher than SAFA meal (2.92 (1.98, 3.86)) ($P < 0.05$) whereas no significant difference as compared to MUFA meal (4.81 (2.87, 6.75)) ($P > 0.05$).

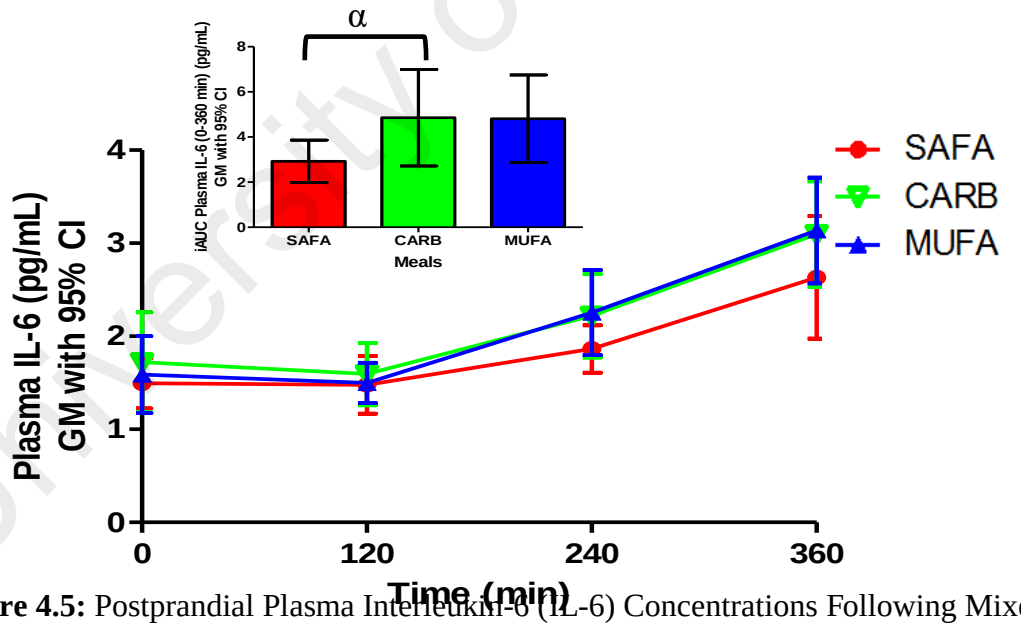

Figure 4.5: Postprandial Plasma Interleukin-6 (IL-6) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; $n = 46$ (women $n = 34$; men $n = 12$).

[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: $P = 0.610$, time effect: $P < 0.001$, meal x time interaction: $P = 0.245$.

[2]Insert: iAUC over 360 min (GM with 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360min)}$: $P = 0.019$.

$^{\alpha}$ Significant difference between diets ($P < 0.05$).

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Table 4.9: Changes from Fasting Concentration (Δ) of Plasma Interleukin-6 after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	1.49 (1.23, 1.76)	1.72 (1.19, 2.26)	1.59 (1.17, 2.00)
Δ 120	-0.02 (-0.20, 0.17)	-0.09 (-0.44, 0.26)	-0.13 (-0.37, 0.12)
Δ 240	0.37 (0.12, 0.62)	0.67 (0.10, 1.24)	0.50 (-0.16, 1.16)
Δ 360	1.14 (0.53, 1.74)	1.55 (0.94, 2.16)	1.38 (0.63, 2.12)
iAUC $_{(0\text{-}360min)}$	2.92 (1.98, 3.86)	4.86 (2.72, 7.00)*	4.81 (2.87, 6.75)

Values are GM with 95% CI, expressed in pg/mL; n = 46 (women n = 34; men n = 12).
[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: P = 0.610, time effect: $P < 0.001$, meal x time interaction: P = 0.245.
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360min)}$: P = 0.019.
*Significantly different compared with SAFA meal ($P < 0.05$).

4.5.2 Interleukin-1β (IL-1β)

Time (P = 0.020) but not meal x time interaction (P = 0.077) was observed for plasma IL-1β over 360 min (shown in Figure 4.6). Plasma IL-1β was found to fall below baseline at 120 min after all meals. The changes from baseline between meals (Table 4.10) were not significantly different at all time points (P > 0.05). iAUC$_{(0\text{-}360min)}$ were 0.28 (0.22, 0.35), 0.27 (0.19, 0.34) and 0.26 (0.20, 0.33) for SAFA, CARB and MUFA respectively, no significant differences were detected between meals (P > 0.05) .

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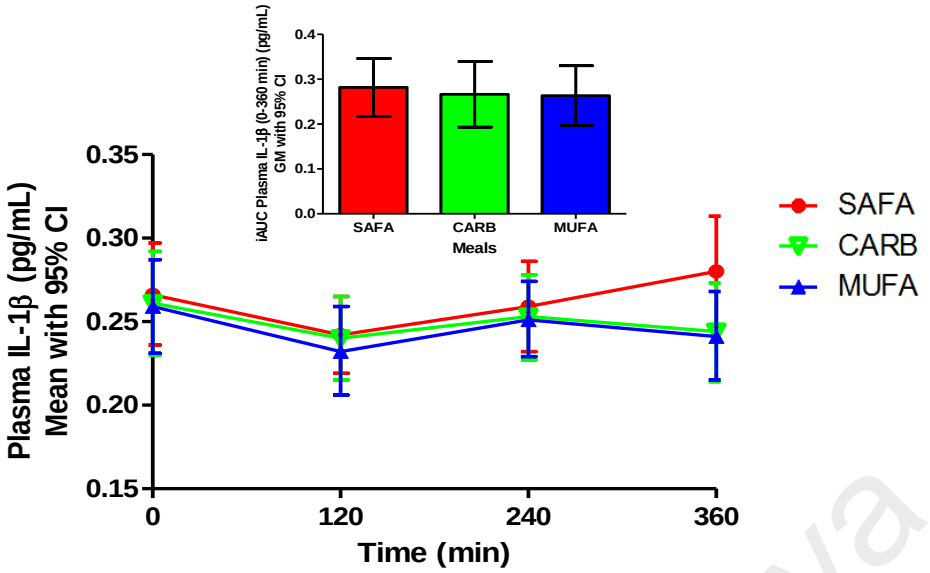

Figure 4.6: Postprandial Plasma Interleukin-1β (IL-1β) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data were $\log_e$ transformed, 191nalysed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: *P* = 0.064, time effect: *P* = 0.020, meal x time interaction: *P* = 0.077.

[2]Insert: iAUC over 360 min (GM ± 95% CI) (*n* = 46): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\ min)}$: *P* > 0.05.

Table 4.10: Changes from Fasting Concentration (Δ) of Plasma Interleukin-1β after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0[1]	0.27 (0.24, 0.30)	0.26 (0.23, 0.29)	0.26 (0.23, 0.29)
Δ 120	-0.02 (-0.05, 0.00)	-0.02 (-0.04, 0.00)	-0.03 (-0.05, 0.00)
Δ 240	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)
Δ 360	0.01 (-0.01, 0.04)	-0.02 (-0.04, 0.01)	-0.02 (-0.04, 0.01)
iAUC$_{(0\text{-}360min)}$	0.28 (0.22, 0.35)	0.27 (0.19, 0.34)	0.26 (0.20, 0.33)

Values are mean[1]and GM with 95% CI, expressed in pg/mL; *n* = 46 (women *n* = 34; men *n* = 12).

[2]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: *P* = 0.064, time effect: *P* = 0.020, meal x time interaction: *P* = 0.077.

[3]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360min)}$: *P* = 0.411.

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4.5.3 C-Reactive Protein (CRP)

There was no significant time (P = 0.358), meal (P = 0.547) and meal x time effect (P = 0.549) on serum CRP responses (Figure 4.7). No change was observed on CRP concentrations over the 6 h. Table 4.11 shows the change from fasting concentration (Δ) following the respective test meals at the end of each dietary intervention. The changes from baseline between the 3 meals were not significantly different between meals at all time points (P > 0.05). In addition, no significant difference was detected on iAUC after all meals (P > 0.05). $iAUC_{(0\text{-}360min)}$ values for SAFA, CARB and MUFA meals were 1.44 (0.82, 2.07), 1.65 (0.91, 2.38) and 1.28 (0.75, 1.81) respectively.

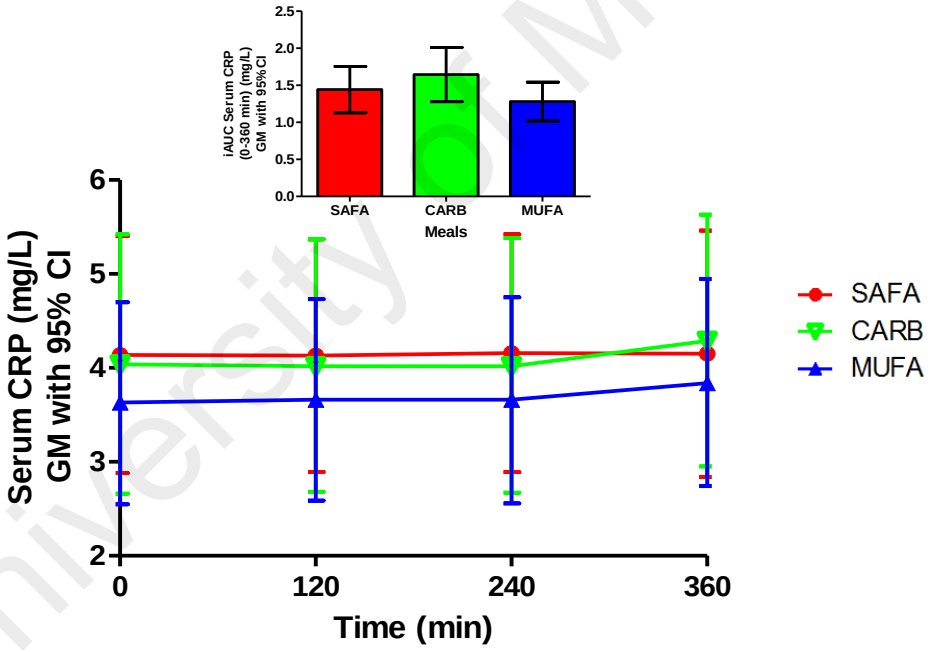

Figure 4.7: Postprandial Serum C-Reactive Protein (CRP) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Values are GM with 95% CI; n=46 (women n=34; men n=12).
[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor; meal effect: P = 0.547, time effect: P = 0.358 and meal x time interaction: P = 0.549.
[2]Insert: iAUC over 360 min (GM ± 95% CI) (n = 46): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360min)}$: P = 0.660.

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Table 4.11: Changes from Fasting Concentration (Δ) of Serum C-reactive Protein after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	4.14 (2.88, 5.40)	4.04 (5.42, 2.66)	3.63 (4.70, 2.55)
Δ 120	-0.02 (-0.04, 0.00)	-0.02 (-0.06, 0.02)	0.03 (0.02, 0.05)
Δ 240	0.01 (0.02, 0.01)	-0.02 (-0.04, 0.01)	0.03 (0.05, 0.02)
Δ 360	0.01 (0.06, -0.04)	0.25 (0.21, 0.29)	0.22 (0.25, 0.19)
$iAUC_{(0\text{-}360min)}$	1.44 (0.82, 2.07)	1.65 (0.91, 2.38)	1.28 (0.75, 1.81)

Values are GM with 95% CI, expressed in mg/L; n = 46 (women n = 34; men n = 12).
[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: P = 0.547, time effect: P = 0.358 and meal x time interaction: P = 0.549.
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360min)}$: P = 0.660.

4.5.4 E-selectin

Repeated measure ANOVA for E-selectin showed significant meal x time interaction (P = 0.035). The 3 meals merely decreased E-selectin concentrations from 0 to 360 min (shown in Figure 4.8). No significant differences (P > 0.05) were detected in the changes from baseline between meals at all time points (Table 4.12). In addition, no significant differences were detected on iAUC $_{(0\text{-}360min)}$ between all meals. A borderline significant value for iAUC between meals (P = 0.051) was found where SAFA meal resulted in the lowest iAUC value (10.68 (7.041, 14.31)) compared to others (11.11 (7.591, 14.62) (CARB) and 16.31 (9.887, 22.73) (MUFA)).

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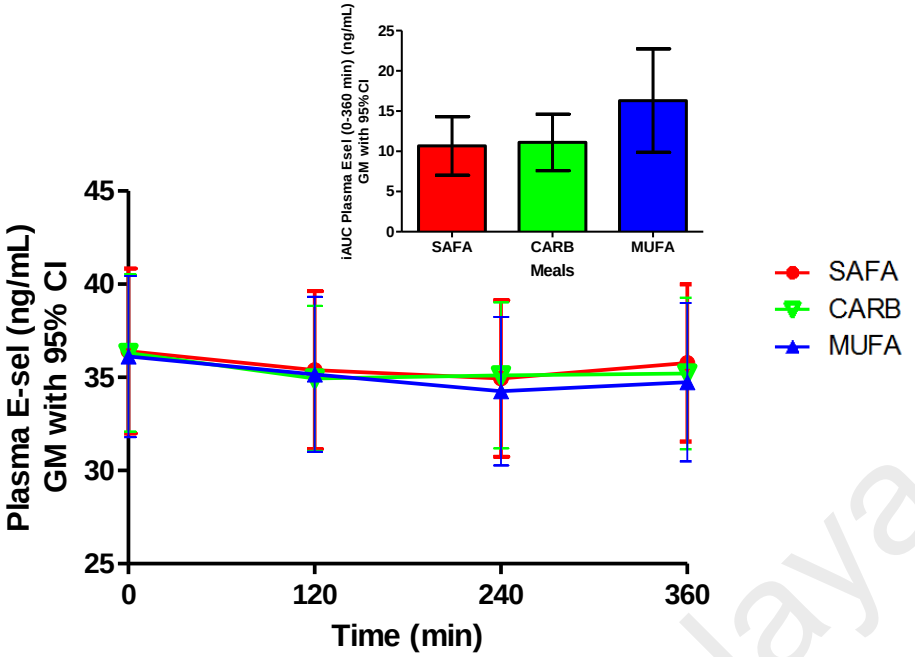

Figure 4.8: Postprandial Plasma E-Selectin Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor; meal effect: $P = 0.350$, time effect: $P = 0.864$ and meal x time interaction: $P = 0.035$.

[2]Insert: iAUC over 360 min (GM ± 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P = 0.051$.

Table 4.12: Changes from Fasting Concentration (Δ) of Plasma E-selectin after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	36.41 (31.99, 40.84)	36.31 (32.09, 40.53)	36.12 (31.79, 40.44)
Δ 120	-1.03 (-1.77, -0.28)	-1.36 (-2.36, -0.36)	-0.95 (-2.56, 0.66)
Δ 240	-1.48 (-2.50, -0.46)	-1.20 (-2.09, -0.31)	-1.86 (-3.41, -0.31)
Δ 360	-0.65 (-1.89, 0.60)	-1.10 (-2.01, -0.19)	-1.37 (-2.93, 0.18)
$iAUC_{0\text{-}360min}$	10.68 (7.041, 14.31)	11.11 (7.591, 14.62)	16.31 (9.887, 22.73)

Values are GM with 95% CI, expressed in ng/mL; $n = 46$ (women $n = 34$; men $n = 12$).

[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: $P = 0.350$, time effect: $P = 0.864$ and meal x time interaction: $P = 0.035$

[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P = 0.051$

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4.5.5 Plasminogen Activator Inhibitor-1 (PAI-1)

There was no significant meal x time interaction (P = 0.348) on plasma PAI-1 responses. The 3 meals shared similar peaking patterns as shown in Figure 4.9. The concentrations gradually decreased from 0 to 240 min and steadily increased towards 360 min (time effect: P < 0.001). The changes from preprandial value were not significantly different (P > 0.05) between meals at all time points (Table 4.13). No significant differences were detected on iAUC$_{(0\text{-}360min)}$ between meals (P > 0.05): 106.60 (88.22, 125.00) (SAFA), 102.70 (81.70, 123.70) (CARB) and 100.40 (77.21, 123.50)) (MUFA).

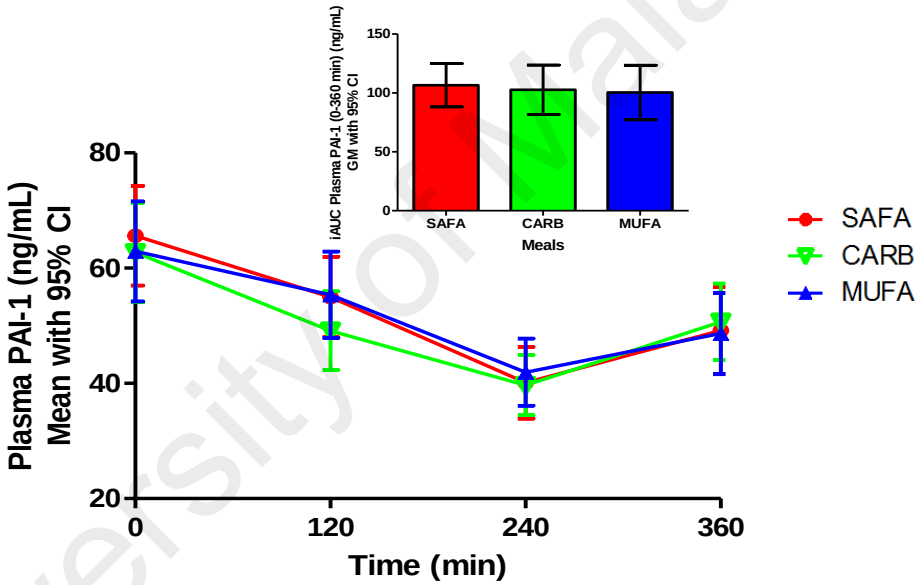

Figure 4.9: Postprandial Plasma Plasminogen Activator Inhibitor-1 (PAI-1) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; n=46 (women n=34; men n=12).

[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor; meal effect: P = 0.579, time effect: P < 0.001 and meal x time interaction: P = 0.348.

[2]Insert: iAUC over 360 min (GM ± 95% CI) (n = 46): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360min)}$: P = 0.423.

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Table 4.13: Changes from Fasting Concentration (Δ) of Plasma Plasminogen Activator Inhibitor-1 after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	65.64 (57.00, 74.29)	62.70 (54.09, 71.31)	62.90 (54.22, 71.58)
Δ 120	-10.64 (-14.70, -6.59)	-7.52 (-13.63, -1.41)	-13.55 (-18.83, -8.28)
Δ 240	-25.53 (-31.65, -19.41)	-20.96 (-27.45, -14.47)	-23.02 (-29.31, -16.72)
Δ 360	-16.49 (-23.69, -9.29)	-14.21 (-21.62, -6.79)	-12.03 (-18.03, -6.02)
iAUC[1]	106.60 (88.22, 125.00)	102.70 (81.70, 123.70)	100.40 (77.21, 123.50)

Values are GM with 95% CI, expressed in ng/mL; n = 46 (women n = 34; men n = 12).
[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: P = 0.579, time effect: P < 0.001 and meal x time interaction: P = 0.348
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: P = 0.423.

4.5.6 D-dimer

The analysis of variance on plasma D-dimer concentrations following the consumption of the test meals revealed no significant meal x time interaction (P = 0.889). As shown in Figure 4.10, plasma D-dimer was found to increase after meals (time effect: P = 0.004). No difference in the change from fasting concentration (Δ) was observed between meals (Table 4.14). iAUC$_{(0\text{-}360\text{min})}$ showed that MUFA meal (201.40 (148.50, 254.20)) increased D-dimer at 43% over 360 min compared with SAFA meal 140.70 (90.81, 190.60) (SAFA) (P = 0.054).

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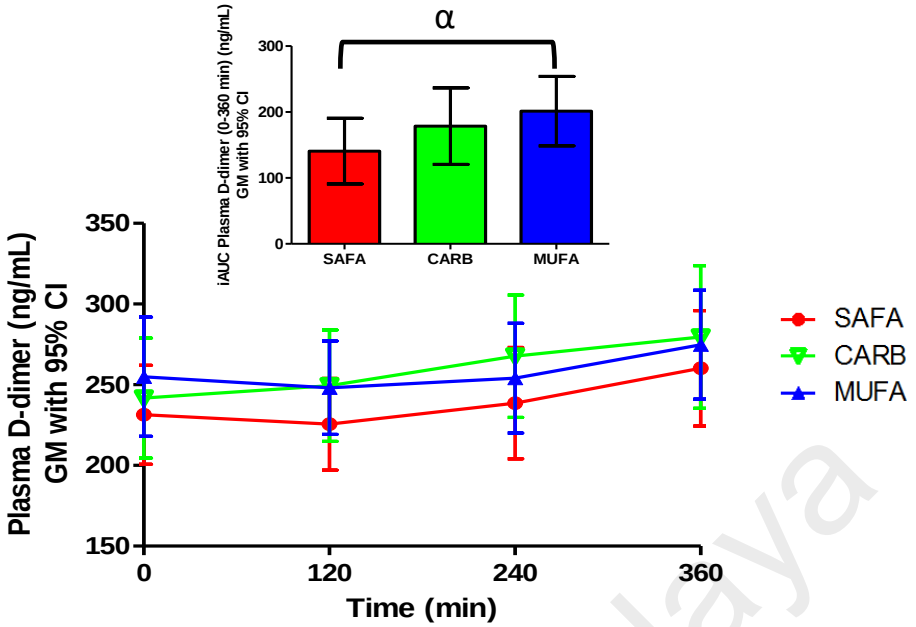

Figure 4.10: Postprandial Plasma D-Dimer Concentrations Following Mixed Meal at the End of Each 3 X 6-Week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor; meal effect: $P = 0.290$, time effect: $P = 0.004$ and meal x time interaction: $P = 0.889$.

[2]Insert: iAUC over 360 min (GM ± 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P = 0.054$.

[α] Significant difference between diets ($P < 0.05$).

Table 4.14: Changes from Fasting Concentration (Δ) of Plasma D-dimer after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	231.44 (262.06, 200.82)	241.75 (204.58, 278.92)	255.00 (218.11, 291.88)
Δ 120	-5.91 (-20.72, 8.90)	7.66 (-5.17, 20.48)	-6.86 (-22.45, 8.73)
Δ 240	7.09 (-12.88, 27.05)	25.92 (3.99, 47.85)	-0.88 (-18.31, 16.56)
Δ 360	28.74 (15.33, 42.16)	37.78 (14.32, 61.24)	19.80 (-2.48, 42.09)
iAUC	140.70 (90.81, 190.60)	178.70 (120.60, 236.70)	201.40 (148.50, 254.20)*

Values are GM with 95% CI, expressed in ng/mL; $n = 46$ (women $n = 34$; men $n = 12$).

[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: $P = 0.290$, time effect: $P = 0.004$ and meal x time interaction: $P = 0.889$.

[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P = 0.054$.

[*]Significantly different compared with SAFA meal ($P < 0.05$)

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4.6 Fasting Inflammatory and Endothelial Responses

Table 4.15 shows the fasting concentration of cytokines and C-reactive protein following randomization to treatments. The mean of the measurements made at week 5 and 6 were reported as the results shown below. No significant differences were found between diets on all measured inflammatory markers ($P > 0.05$).

Table 4.15: Fasting Concentration of Cytokines and Thrombogenic Markers Concentration in 47 Participants Following the Three 6-Week Dietary Interventions

	Baseline†	SAFA diet	CARB diet	MUFA diet
IL-6 (pg/mL)	1.418 (1.183,1.652)	1.523 (1.305, 1.740)	1.562 (1.264,1.861)	1.608 (1.300,1.918)
D-dimer (ng/mL)	253.96 (214.63, 293.29)	245.25 (213.72, 276.78)	260.65 (221.20,300.09)	257.06 (223.16,290.96)
E-selectin (ng/mL)	36.28 (32.42, 40.13)	36.47 (32.30, 40.73)	36.53 (32.40, 40.83)	36.53 (32.40, 40.65)
PAI-1 (ng/mL)[1]	63.24 (54.29, 72.19)	63.85 (55.88, 71.81)	62.48 (54.90, 70.07)	63.91 (56.16, 71.66)
IL-1β (pg/mL)[1]	0.231 (0.200, 0.261	0.262 (0.237, 0.286)	0.253 (0.228, 0.277)	0.253 (0.227, 0.279)
CRP (mg/mL)	4.466 (2.599, 6.333)	4.023 (2.819, 5.227)	3.845 (2.572, 5.117)	3.702 (2.624, 4.780)

Values are mean ± SD[1] and GM ± SD. n=47 (women n=35; men n=12). Data was analyzed by repeated measures ANOVA (among 3 treatments). No significant difference between diets. †Baseline data was reported as the reference and not used in data analysis. Abbreviations - SAFA, saturated fatty acids; CARB, carbohydrate; MUFA, monounsaturated fatty acids; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; IL-1β, interleukin-1β; CRP, C-reactive protein.

4.7 Postprandial Gastro-intestinal Peptides

4.7.1 Glucagon-like Peptide-1 (GLP-1)

The changes in GLP-1 concentrations over 360 min are depicted in Figure 4.11. There were significant changes for GLP-1 after all meals (meal x time interaction: $P < 0.001$). GLP-1 concentration peaked at 30 min after CARB meal 2.876 (2.613, 3.139), which was 17.6% and 21.4% higher than SAFA (2.445 (2.271, 2.620)) and MUFA meals (2.369 (2.133, 2.604)). The changes of GLP-1 concentrations from baseline were higher at 15

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and 30 min but lower at 360 min ($P < 0.05$) after CARB meal compared with both high-fat meals (Table 4.16). The 3 meals did not result in significant difference in iAUC $_{(0\text{-}120\text{min})}$ and iAUC $_{(120\text{-}360\text{min})}$ ($P > 0.05$).

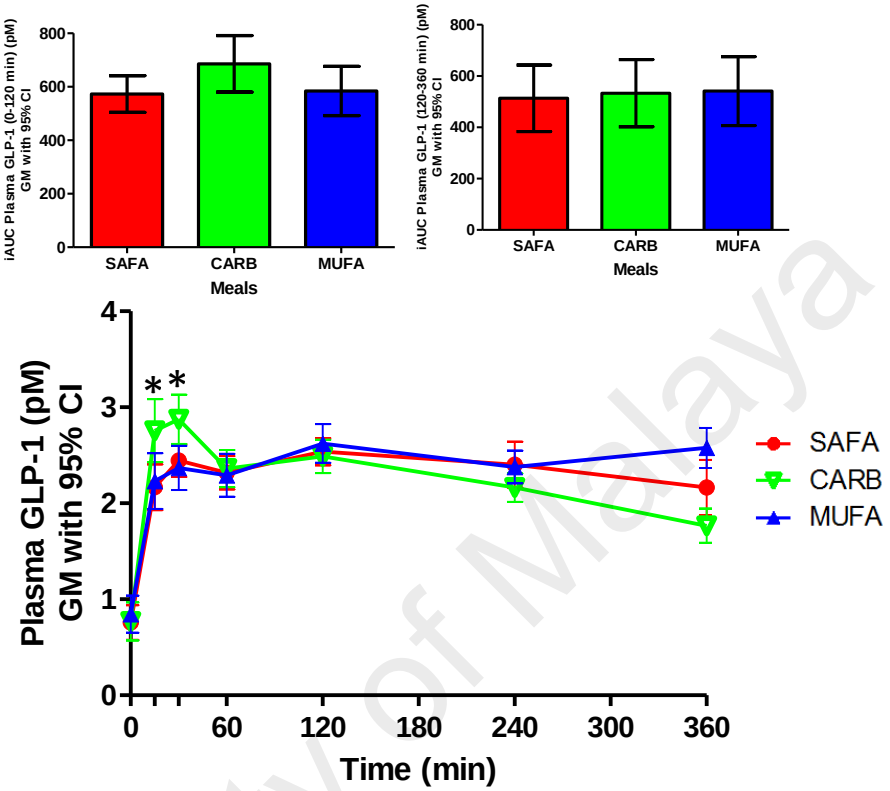

Figure 4.11: Postprandial Plasma Glucagon-Like Peptide-1 (GLP-1) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).
[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor; meal effect: $P = 0.077$, time effect: $P = 0.047$ and meal x time interaction: $P < 0.001$.
[2]Insert: iAUC over 120 min (mean with 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120\text{min})}$: $P = 0.0496$, $\log_e$ iAUC $_{(120\text{-}360\text{min})}$: $P = 0.8661$.
*Significant difference between CARB *vs* SAFA and MUFA meals ($P < 0.05$).

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Table 4.16: Changes from Fasting Concentration (Δ) of Plasma Glucagon-like Peptide-1 after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	1.29 (0.61, 1.96)	1.05 (0.63, 1.46)	1.15 (0.81, 1.49)
Δ 15	4.08 (2.84, 5.31)	7.82 (6.14, 9.49)*	4.83 (3.44, 6.21)
Δ 30	5.03 (3.97, 6.09)	7.99 (6.25, 9.72)*	5.08 (4.02, 6.13)
Δ 60	4.44 (3.35, 5.53)	4.97 (4.07, 5.88)	4.69 (3.79, 5.60)
Δ 120	5.39 (4.56, 6.21)	5.50 (4.62, 6.38)	6.21 (5.21, 7.22)
Δ 240	5.15 (3.77, 6.53)	3.89 (3.23, 4.56)	4.85 (3.96, 5.73)
Δ 360	4.37 (3.10, 5.64)	2.44 (1.81, 3.07)*	6.01 (4.89, 7.12)
$iAUC_{(0\text{-}120min)}$[2]	573.2 (504.5, 641.8)	685.8 (580.2, 791.4)	584.3 (492.5, 676.1)
$iAUC_{(120\text{-}360min)}$[3]	513.5 (383.7, 643.2)	533.2 (402.0, 664.3)	541.1 (407.2, 675.6)

Values are GM with 95% CI, expressed in pM; n = 46 (women n = 34; men n = 12). [1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: P = 0.338, time effect: P < 0.001 and meal x time interaction: P < 0.001. *Significantly different compared with SAFA and MUFA meals (P < 0.05).
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: P = 0.0496.
[3]Repeated measures ANOVA of $\log_e$ iAUC $_{(120\text{-}360min)}$: P = 0.8661.

4.7.2 Glucose-dependent Insulinotropic Peptide (GIP)

Figure 4.12 displays GIP changes over 360 min during postprandial challenge. There was a significant change in plasma GIP over 120 min for the three meals (meal x time effect, P < 0.001). A similar increase observed to GLP-1 at 15 and 30 min after CARB meal compared with SAFA and MUFA meals was mimicked by GIP (P < 0.05). All three meals peaked at similar concentrations at 120 min (P > 0.05), followed by a sharp decrease at 240 – 360 min after CARB meal compared with MUFA meal (P < 0.05) (Table 4.17). Nevertheless, no significant difference was detected on $iAUC_{(0\text{-}120min)}$ and $iAUC_{(120\text{-}360min)}$ between meals (P > 0.05). GIP was found to express a significant gender x time interactions (P = 0.012) (Figure 4.13). Women had higher GIP levels than men following the two high-fat meals however vice versa for the CARB meal.

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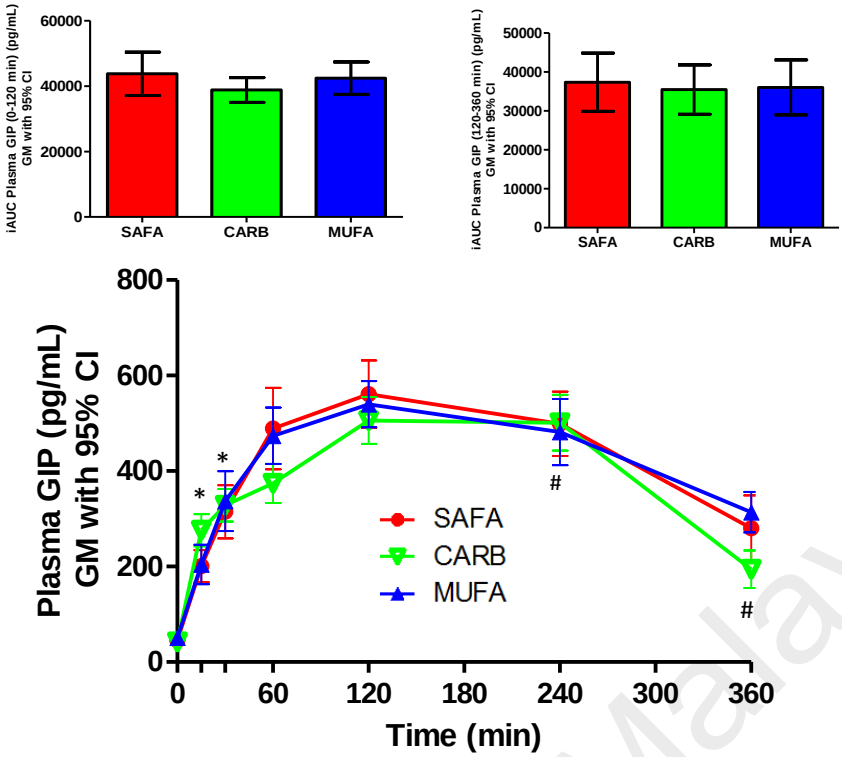

Figure 4.12: Postprandial Plasma Glucose-Dependent Insulinotropic Peptide (GIP) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor; meal effect: $P = 0.338$, time effect: $P < 0.001$ and meal x time interaction: $P < 0.001$.

[2]Insert: iAUC over 120 min (mean with 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120\text{min})}$: $P = 0.5488$, $\log_e$ iAUC $_{(120\text{-}360\text{min})}$: $P = 0.9541$.

*Significant difference between CARB *vs* SAFA and MUFA meals ($P < 0.05$). [#]Significant difference between CARB *vs* MUFA meal ($P < 0.05$).

Table 4.17: Changes from Fasting Concentration (Δ) of Plasma Glucose-Dependent Insulinotropic Peptide after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	45.83 (37.91, 53.76)	42.05 (34.67, 49.43)	51.28 (42.44, 60.12)
Δ 15	154.93 (122.81, 187.05)	234.27 (203.71, 264.82)*	153.06 (113.19, 192.93)
Δ 30	268.94 (215.46, 322.43)	286.12 (256.44, 315.80)*	285.71 (223.91, 347.50)
Δ 60	443.01 (361.10, 524.93)	331.99 (294.68, 369.31)	422.71 (366.25, 479.17)
Δ 120	514.92 (446.57, 583.26)	463.86 (418.87, 508.85)	488.48 (441.97, 534.99)
Δ 240	453.34 (390.13, 516.54)	459.19 (403.87, 514.51)[#]	430.27 (359.90, 500.65)
Δ 360	234.42 (170.48, 298.35)	152.32 (113.52, 191.12)[#]	262.61 (219.78, 305.43)
iAUC$_{(0\text{-}120\text{min})}$[2]	43758 (37129, 50388)	38808 (35038, 42577)	42417 (37480, 47354)
iAUC$_{(120\text{-}360\text{min})}$[3]	37372 (29894, 44849)	35488 (29153, 41824)	36038 (28990, 43085)

Values are GM with 95% CI, expressed in pg/mL; $n = 46$ (women $n = 34$; men $n = 12$).

[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6

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'Table 4.17, continued'

time points) with gender as a between subject factor: meal effect: $P = 0.338$, time effect: $P < 0.001$ and meal x time interaction: $P < 0.001$.

*Significantly different compared with SAFA and MUFA meals ($P < 0.05$). #Significantly different compared with MUFA meal ($P < 0.05$).

[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120\text{min})}$: $P = 0.5488$.

[3]Repeated measures ANOVA of $\log_e$ iAUC $_{(120\text{-}360\text{min})}$: $P = 0.9541$.

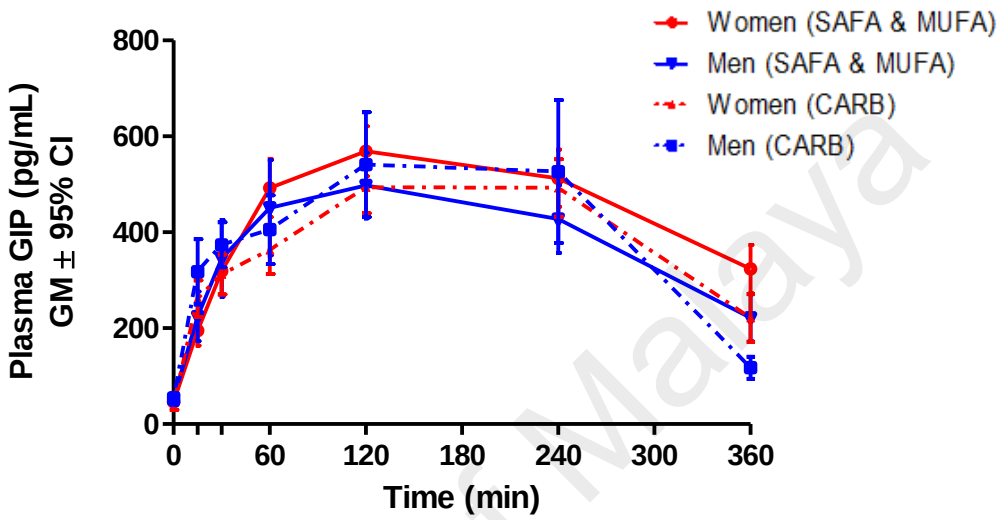

Figure 4.13: Gender x Time Interaction for Glucose-Dependent Insulinotropic Peptide Concentration in Women and Men Following Mixed Meal at the End Of Each 3 x 6-Week Dietary Intervention.

Data are GM with 95% CI. $n = 46$ (34 women, 12 men). Deviations from preprandial values (0-360min) were analyzed by repeated measures ANOVA (3 meals, 6 time points): gender x time interaction, $P = 0.012$, meal x time x gender interaction, $P = 0.469$.

4.7.3 Ghrelin

For ghrelin (Figure 4.14), the meal effect and meal x time interaction were not significant ($P = 0.169$, $P = 0.178$ respectively). There was a significant effect of time ($P < 0.001$) with the meals having a suppressive effect on ghrelin concentrations. The minimum peaks achieved at 120 min were 187.2 (160.1, 214.3) pg/mL for SAFA, 180.5 (154.6, 206.4) pg/mL for CARB and 194.1(163.0, 225.1) pg/mL for MUFA meal. Our study demonstrated that CARB meal sustained larger suppression at 240 min compared to the MUFA meal ($P < 0.05$) however followed by a sudden rebound and return to baseline at 360 min. There was no significant difference between meals when iAUC $_{(0\text{-}120\text{min})}$ and

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iAUC $_{(120\text{-}360\text{min})}$ levels were examined ($P > 0.05$) (Table 4.18). A greater suppression of ghrelin was observed in men compared with women after all meals as shown in Figure 4.15 (gender x time interaction, $P = 0.041$).

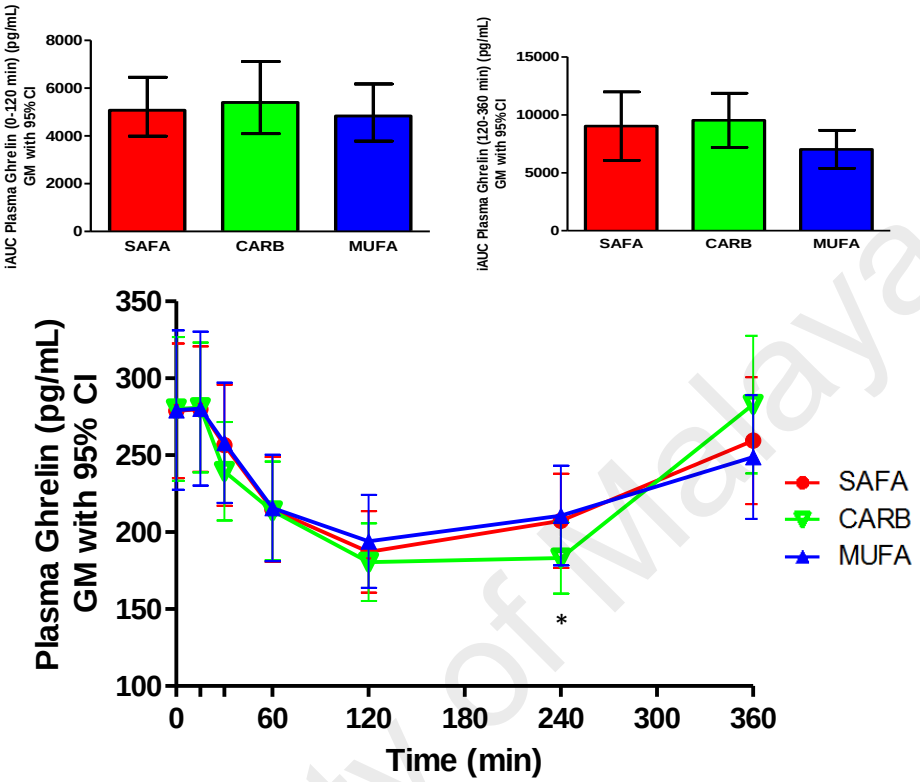

Figure 4.14: Postprandial Plasma Ghrelin Concentrations Following Mixed Meal at the End of Each 3 x 6-week Dietary Intervention**.**

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).
[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor; meal effect: $P = 0.169$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.178$.
[2]Insert: iAUC over 120 min (mean with 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120\text{min})}$: $P = 0.6435$, $\log_e$ iAUC $_{(120\text{-}360\text{min})}$: $P = 0.9656$.
*Significant difference between CARB and MUFA meals.

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Table 4.18: Changes from Fasting Concentration (Δ) of Plasma Ghrelin after SAFA, CARB and MUFA Meal

Time (min)	SAFA meal	CARB meal	MUFA meal
0	278.89 (235.09, 322.70)	280.09 (233.34, 326.84)	279.32 (227.52, 331.12)
Δ 15	1.10 (-11.05, 13.25)	0.93 (-11.14, 13.01)	1.03 (-9.87, 11.92)
Δ 30	-22.35 (-33.61, -11.09)	-40.53 (-64.21, -16.86)	-21.22 (-41.15, -1.28)
Δ 60	-64.13 (-80.96, -47.30)	-66.27 (-86.64, -45.90)	-63.52 (-84.84, -42.20)
Δ 120	-91.70 (-113.34, -70.05)	-99.58 (-126.93, -72.22)	-85.24 (-110.05, -60.43)
Δ 240	-71.46 (-94.57, -48.35)	-96.89 (-126.81, -66.96)*	-68.53 (-95.01, -42.04)
Δ 360	-19.43 (-43.00, 4.13)	2.88 (-25.74, 31.50)	-30.41 (-50.51, -10.32)
$iAUC_{(0\text{-}120min)}$[2]	6692 (5140, 8243)	7796 (5804, 9788)	6728 (4681, 8774)
$iAUC_{(120\text{-}360min)}$[3]	9028 (6075, 11980)	9531 (7188,11873)	7021 (5378, 8665)

Values are GM with 95% CI, expressed in pg/mL; n = 46 (women n = 34; men n = 12).
[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: $P = 0.169$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.178$.
*Significantly different compared with MUFA meals ($P < 0.05$).
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: $P = 0.6435$.
[3]Repeated measures ANOVA of $\log_e$ iAUC $_{(120\text{-}360min)}$: $P = 0.9656$.

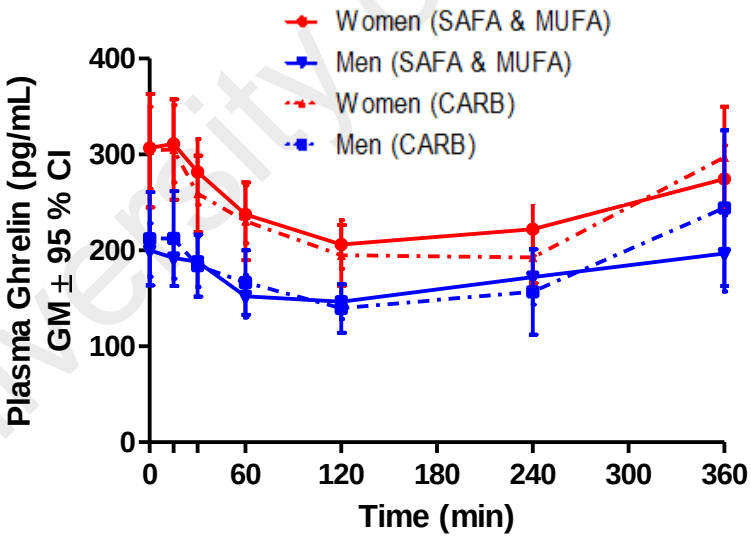

Figure 4.15: Gender x Time Interaction for Ghrelin Concentrations in Women and Men Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Data are GM with 95% CI. n = 46 (34 women, 12 men). Deviations from preprandial values (0-360min) were analyzed by repeated measures ANOVA (3 meals, 6 time points): gender x time interaction, $P = 0.041$, meal x time x gender interaction, $P = 0.387$.

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4.7.4 Peptide YY

The effect of time ($P < 0.001$) and meal x time interaction ($P = 0.002$) were significant for PYY changes over 120 min. As shown in Figure 4.16, all meals sustained elevation of PYY levels over the 360 min. CARB meal caused higher PYY increase from baseline compared to SAFA and MUFA meals at 2 h however lower increase compared to MUFA meal at 360 min ($P < 0.05$) (Table 4.19). No significant differences were detected on $iAUC_{(0\text{-}120min)}$ between all meals in PYY ($P = 0.3723$) however $iAUC_{(120\text{-}360min)}$ after CARB meal was significantly higher compared to MUFA meal.

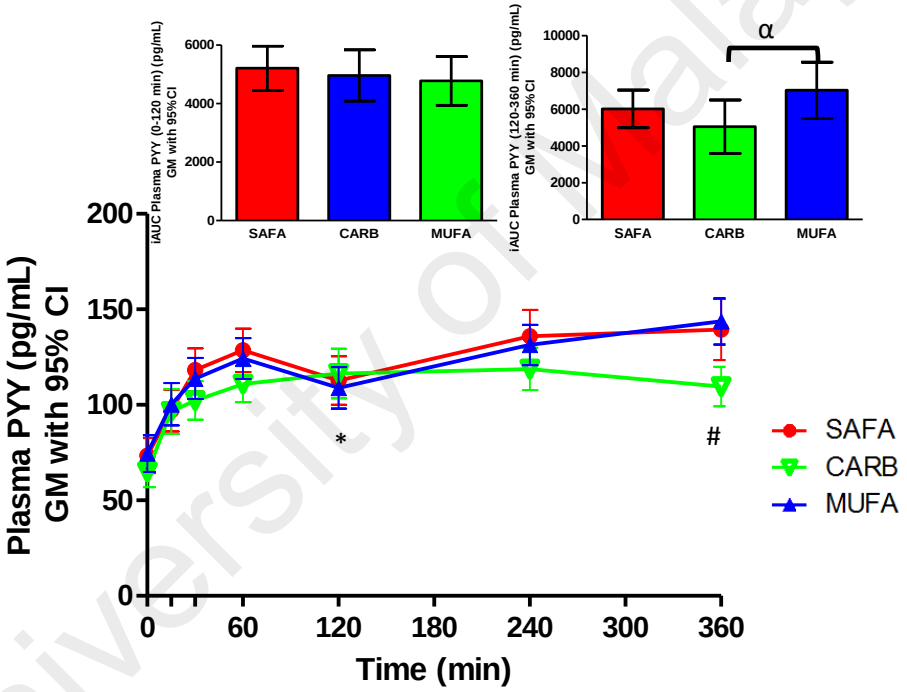

Figure 4.16: Postprandial Plasma Peptide YY Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention**.**

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor; meal effect: $P = 0.539$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.002$.

[2]Insert: Incremental area under the curve (iAUC) over 120 min (mean ± 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: $P = 0.3723$, $\log_e$ iAUC $_{(120\text{-}360min)}$: $P = 0.0087$.

*Significant difference between CARB vs SAFA and MUFA meals ($P < 0.05$).

[#]Significant difference between CARB and MUFA meals ($P < 0.05$).

[α]Significant difference between meals ($P < 0.05$).

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Table 4.19: Changes from Fasting Concentration (Δ) of Plasma Peptide YY after SAFA, CARB and MUFA Meal.

Time (min)	SAFA meal	CARB meal	MUFA meal
0	73.45 (64.25, 82.64)	64.85 (56.85, 72.84)	74.50 (64.84, 84.16)
Δ 15	23.59 (17.03, 30.15)	31.69 (23.04, 40.33)	25.82 (17.69, 33.95)
Δ 30	44.85 (36.91, 52.80)	37.46 (29.92, 45.01)	39.33 (30.09, 48.57)
Δ 60	55.12 (45.26, 64.98)	46.05 (38.10, 54.00)	49.73 (39.29, 60.17)
Δ 120	39.28 (31.20, 47.35)	51.56 (41.04, 62.08)*	34.39 (26.50, 42.28)
Δ 240	62.50 (51.35, 73.64)	53.97 (43.18, 64.76)	56.84 (44.40, 69.28)
Δ 360	65.97 (51.96, 79.97)	44.72 (35.13, 54.31)#	69.17 (56.41, 81.93)
$iAUC_{(0\text{-}120min)}$[2]	5210 (4454, 5967)	4962 (4087, 5836)	4777 (3945, 5609)
$iAUC_{(120\text{-}360min)}$[3]	6014 (4988, 7039)	5046 (3596, 6497)[#]	7028 (5499, 8557)

Values are GM with 95% CI, expressed in pg/mL; n = 46 (women n = 34; men n = 12).
[1]Changes from fasting data (0-120min) as a between subject factor: meal effect: $P = 0.539$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.002$.
*Significantly different compared with SAFA and MUFA meals ($P < 0.05$).
[#]Significantly different compared with MUFA meals ($P < 0.05$).
[2]Repeated measures ANOVA of $\log_e iAUC_{(0\text{-}120min)}$: $P = 0.3723$.
[3]Repeated measures ANOVA of $\log_e iAUC_{(120\text{-}360min)}$: $P = 0.0087$.

4.7.5 Cholecystokinin

There were no significant meal ($P = 0.586$) and meal x time effect ($P = 0.209$) on plasma CCK changes over 120 min. Nevertheless, there was a significant effect of time ($P = 0.014$) with all meals stimulating an immediate rise at 15 min followed by a steady decline reaching the baseline level at 360 min (Figure 4.17). $iAUC_{(0\text{-}120min)}$ and $iAUC_{(120\text{-}360min)}$ between meals were not significantly different ($P = 0.3723$, $P = 0.0715$) (Table 4.20).

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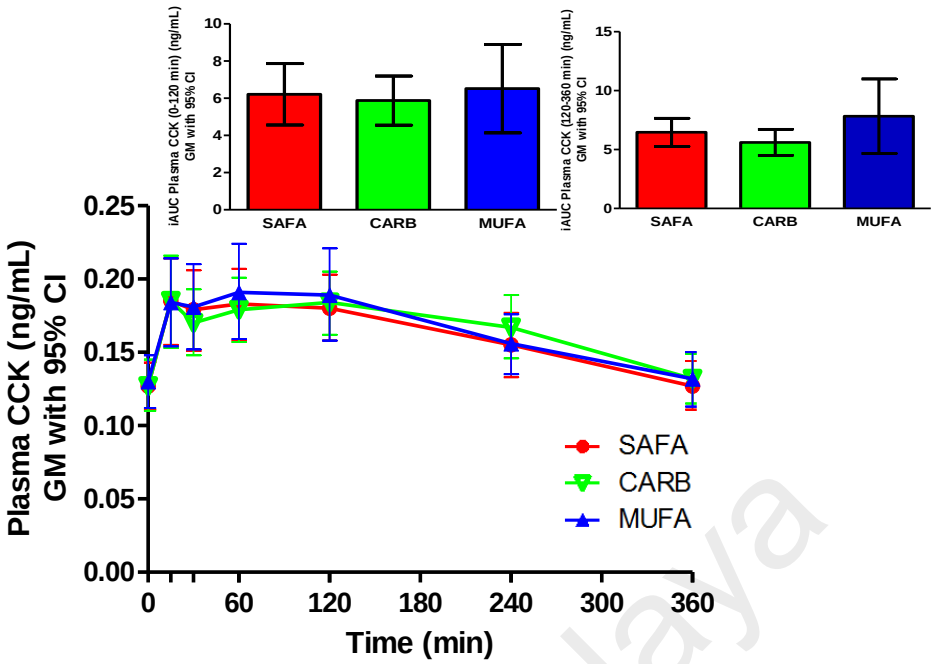

Figure 4.17: Postprandial Plasma Cholecystokinin Concentrations Following Mixed Meal at the End of Each 3 x 6-week Dietary Intervention.

Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).

[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor; meal effect: *P* = 0.586, time effect: *P* = 0.014 and meal x time interaction: *P* = 0.209.

[2]Insert: iAUC over 2 h (mean ± 95% CI) (*n* = 46): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: *P* = 0.4364, $\log_e$ iAUC $_{(120\text{-}360min)}$: *P* = 0.0715.

Table 4.20: Changes from Fasting Concentration (Δ) of Plasma Cholecystokinin after SAFA, CARB and MUFA Meal.

Time (min)	SAFA meal	CARB meal	MUFA meal
0	0.13 (0.11, 0.14)	0.13 (0.11, 0.15)	0.13 (0.11, 0.15)
Δ 15	0.06 (0.04, 0.08)	0.06 (0.04, 0.08)	0.05 (0.04, 0.07)
Δ 30	0.05 (0.03, 0.07)	0.04 (0.03, 0.05)	0.05 (0.03, 0.07)
Δ 60	0.06 (0.04, 0.07)	0.05 (0.04, 0.06)	0.06 (0.04, 0.08)
Δ 120	0.05 (0.04, 0.07)	0.06 (0.05, 0.07)	0.06 (0.04, 0.08)
Δ 240	0.03 (0.02, 0.04)	0.04 (0.03, 0.05)	0.03 (0.01, 0.04)
Δ 360	0.00 (-0.01,0.01)	0.00 (-0.01, 0.02)	0.00 (-0.01, 0.01)
iAUC$_{(0\text{-}120min)}$[2]	6.211 (4.559,7.864)	5.875 (4.557, 7.194)	6.517 (4.139, 8.895)
iAUC$_{(120\text{-}360min)}$[3]	6.463 (5.268,7.658)	5.611 (4.508, 6.714)	7.833 (4.671, 10.99)

Values are GM with 95% CI, expressed in pg/mL; *n* = 46 (women *n* = 34; men *n* = 12).

[1]Changes from fasting data (0-120min) were analyzed by repeated measures ANOVA (3 meals, 4 time points) with gender as a between subject factor: meal effect: *P* = 0.586, time effect: *P* = 0.014 and meal x time interaction: *P* = 0.209.

[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}120min)}$: *P* = 0.4364.

[3]Repeated measures ANOVA of $\log_e$ iAUC $_{(120\text{-}360min)}$: *P* = 0.0715.

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4.8 Fasting Gastro-intestinal Peptides

Table 4.21 shows the fasting concentrations of gastro-intestinal peptides after the three diet exposures. Fasting GIP concentrations were 18.0% lower after CARB diet compared with MUFA diet ($P < 0.05$). No appreciable differences were observed for fasting GLP-1, ghrelin, PYY and CCK between diets ($P > 0.05$).

Table 4.21: Fasting Gastro-intestinal Peptides Concentration in 47 Participants Following the Three 6-Week Dietary Interventions.

	SAFA diet	CARB diet	MUFA diet
GLP-1 (pM)	0.953 (0.663, 1.242)	1.049 (0.633, 1.464)	1.149 (0.807, 1.490)
GIP (pg/mL)	45.83 (37.91, 53.76)	42.05 (34.67, 49.43)[#]	51.28 (42.44, 60.12)
Ghrelin (pg/mL)	278.9 (235.1, 322.7)	280.1 (233.3, 326.8)	279.3 (227.5, 331.1)
PYY (pg/mL)[1]	73.45 (64.25, 82.64)	64.85 (56.85, 72.84)	74.50 (64.84, 84.16)
CCK (ng/mL)[1]	0.127 (0.111, 0.143)	0.127 (0.110, 0.145)	0.130 (0.112, 0.148)

Values are mean ± SD[1] and GM ± SD. *n*=47 (women *n*=35; men *n*=12). Data was analyzed by repeated measures ANOVA (among 3 treatments). No significant difference between diets except where noted.
[#]Significantly different compared to MUFA diet: $P < 0.05$.
Abbreviations - GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic peptide; PYY, peptide YY; CCK, cholecystokinin.

4.9 Postprandial Lipids

4.9.1 Triacylglycerol (TAG)

The analysis of variance on serum TAG concentrations following the consumption of the test meals revealed significant meal x time interaction ($P = 0.002$). After the consumption of test meals, TAG concentrations increased gradually from 0 min and peaked at 120 min then decreased moderately towards 360 min (shown in Figure 4.18). Peak TAG

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concentrations after SAFA and MUFA meals (2.530 (2.187, 2.874), 2.448 (2.154, 2.742) mmol/L) were significantly higher than CARB meal (1.952 (1.726, 2.179) mmo/L). The changes from baseline after CARB meal were significantly lower compared with SAFA and MUFA meals from the 15-360 min time points (P < 0.001) (Table 4.22). As expected, the iAUC for CARB meal (9.89 (8.70, 11.07)) was 7.83% and 8.69% lower than SAFA (11.93 (10.39, 13.48)) and MUFA meals (11.99 (10.62, 13.37)), respectively (P < 0.000).

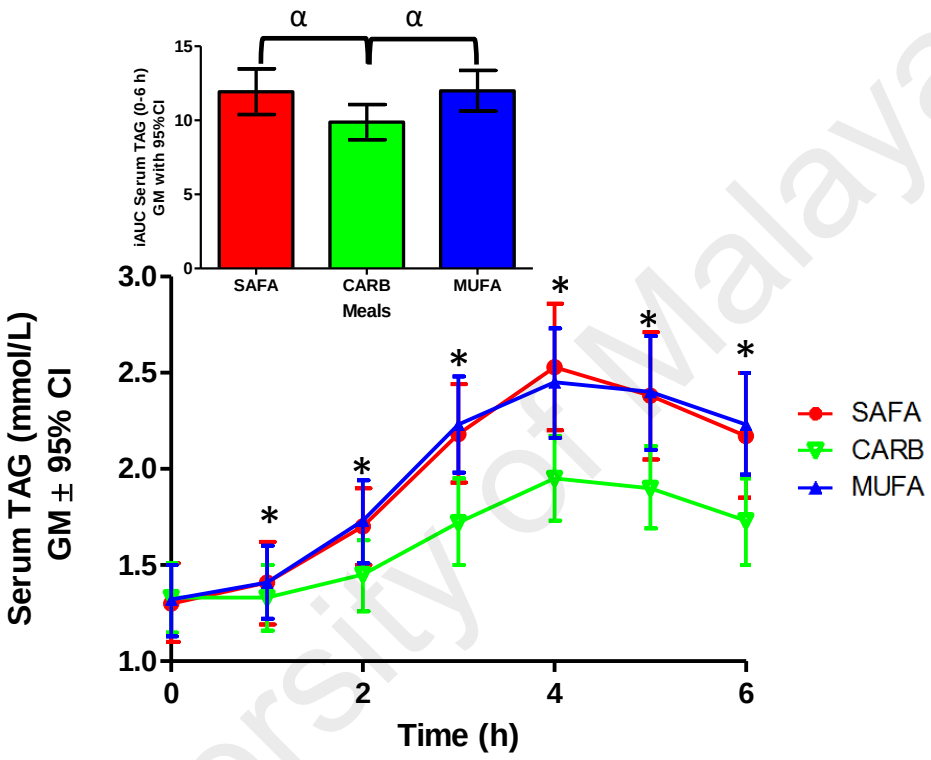

Figure 4.18: Postprandial Serum Triacylglycerol (TAG) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Values are GM with 95% CI; *n* = 46 (wmen *n* = 34; men *n* = 12).
[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor; meal effect: *P* < 0.001, time effect: *P* < 0.001 and meal x time interaction: *P* = 0.002.
[2]Insert: iAUC over 360 min (GM ± 95% CI) (*n* = 46): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: *P* < 0.0001.
*Change from baseline was significantly different between CARB *vs* SAFA and MUFA meals (*P* < 0.001).
[α]Significant difference between diets (*P* < 0.001).

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Table 4.22: Changes from Fasting Concentration (Δ) of Serum Triacylglycerol (TAG) Concentrations after SAFA, CARB and MUFA Meal.

Time (min)	SAFA meal	CARB meal	MUFA meal
0	1.30 (1.10, 1.51)	1.33 (1.15, 1.51)	1.32 (1.13, 1.50)
Δ 60	0.10 (0.03, 0.17)	0.01 (-0.04, 0.05)*	0.10 (0.02, 0.18)
Δ 120	0.39 (0.30, 0.48)	0.12 (0.05, 0.19)*	0.41 (0.30, 0.53)
Δ 180	0.88 (0.74, 1.01)	0.40 (0.29, 0.50)*	0.92 (0.75, 1.09)
Δ 240	1.23 (1.03, 1.42)	0.63 (0.52, 0.73)*	1.13 (0.94, 1.32)
Δ 300	1.08 (0.88, 1.27)	0.58 (0.48, 0.67)*	1.08 (0.87, 1.29)
Δ 360	0.87 (0.68, 1.06)	0.40 (0.27, 0.53)*	0.92 (0.73, 1.11)
iAUC	11.93 (10.39, 13.48)	9.89 (8.70, 11.07)*	11.99 (10.62, 13.37)

Values are GM with 95% CI, expressed in mmol/L; n = 46 (women n = 34; men n = 12).
[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.002$.
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P < 0.001$.
*Significantly different compared with SAFA and MUFA meals ($P < 0.05$).

4.9.2 Non-esterified Fatty Acids (NEFA)

Significant meal x time interaction ($P < 0.001$) was detected with regards to the changes in NEFA levels over the 360 min postprandial period. As shown in Figure 4.19, serum NEFA concentrations decreased from 0 to 120 min followed by a higher rebound after CARB meal compared with the 2 others intervention arms. The CARB meal suppressed NEFA concentrations better than the SAFA and MUFA meals from 60 to 360 min ($P < 0.001$) (Table 4.23). iAUC $_{(0\text{-}360\text{min})}$ of the CARB meal 2.52 (2.32, 2.72) was 15.9 and 15.1% lower ($P < 0.001$) than the SAFA (2.92 (2.71, 3.13)) and MUFA (2.90 (2.71, 3.09)) meals. There was a significant meal x time x gender interaction (P=0.030) between meals for NEFA.

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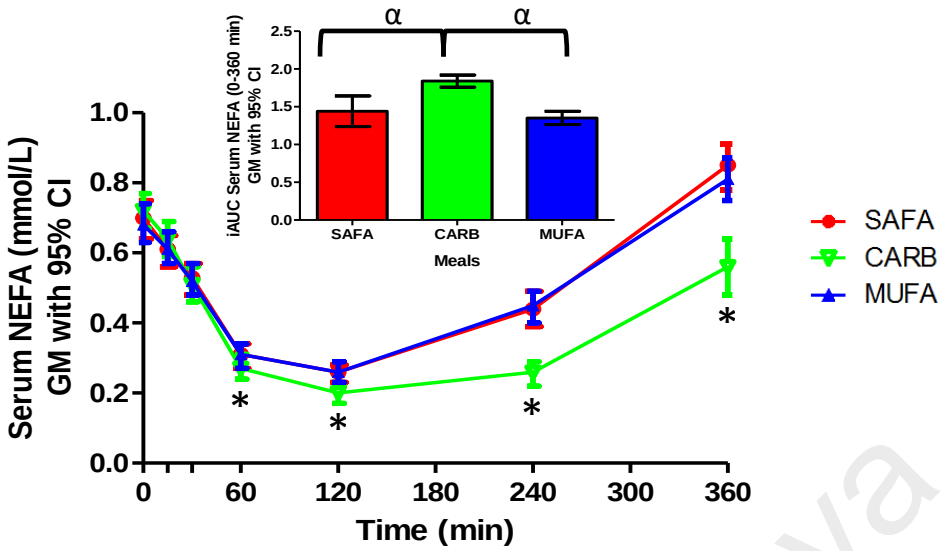

Figure 4.19: Postprandial Serum Non-Esterified Fatty Acids (NEFA) Concentrations Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Values are GM with 95% CI; *n*=46 (women *n*=34; men *n*=12).
[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor; meal effect: $P < 0.001$, time effect: $P < 0.001$ and meal x time interaction: $P < 0.001$.
[2]Insert: iAUC over 360 min (GM ± 95% CI) ($n = 46$): Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P < 0.0001$.
*Change from baseline was significantly different between CARB *vs* SAFA and MUFA meals ($P < 0.001$).
[α]Significant difference between diets ($P < 0.001$).

Table 4.23: Changes from Fasting Concentration (Δ) of Serum Non-esterified Fatty Acid (NEFA) after SAFA, CARB and MUFA Meal.

Time (min)	SAFA meal	CARB meal	MUFA meal
0[α]	0.70 (0.64, 0.75)	0.72 (0.67, 0.77)	0.68 (0.63, 0.74)
Δ 15	-0.09 (-0.12, -0.05)	-0.08 (-0.10, -0.05)	-0.07 (-0.10, -0.05)
Δ 30	-0.17 (-0.21, -0.13)	-0.21 (-0.24, -0.17)	-0.16 (-0.20, -0.12)
Δ 60	-0.39 (-0.44 , -0.34)	-0.44 (-0.48, -0.41)*	-0.38 (-0.43, -0.33)
Δ 120	-0.44 (-0.49, -0.38)	-0.52 (-0.57, -0.47)*	-0.42 (-0.48, -0.37)
Δ 240	-0.25 (-0.32, -0.19)	-0.46 (-0.51, -0.41)*	-0.24 (-0.29, -0.18)
Δ 360	0.15 (0.09, 0.21)	-0.15 (-0.23, 0.08)*	0.12 (0.06, 0.18)
iAUC $_{(0\text{-}360\text{ min})}$	2.92 (2.71, 3.13)	2.52 (2.32, 2.72)*	2.90 (2.71, 3.09)

Values are mean[α] or GM with 95% CI, expressed in mmol/L; $n = 46$ (women $n = 34$; men $n = 12$).
[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 6 time points) with gender as a between subject factor: meal effect: $P < 0.001$, time effect: $P < 0.001$ and meal x time interaction: $P < 0.001$.
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}360\text{min})}$: $P < 0.001$.
*Significantly different compared with SAFA and MUFA meals ($P < 0.05$).

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4.10 Fasting Lipids and Lipoproteins

Table 4.24 shows the fasting serum lipid and lipoprotein profiles at baseline and after 6 weeks of each dietary intervention. Improved reliability of results was achieved by reporting the mean of fasting concentrations measured at week 5 and 6.

Consumption of the MUFA diet mildly reduced TC at 3.8% and LDL cholesterol levels at 5.5% compared with the SAFA diet ($P < 0.05$), the concentrations were 4.71 (4.48, 4.94) *vs* 4.89 (4.68, 5.09) mmol/L; 2.89 (2.71, 3.10) *vs* 3.05 (2.89, 3.21) mmol/L respectively. However HDL cholesterol following the CARB diet was 4.2% lower compared with the SAFA diet (1.18 (1.13, 1.24) *vs* 1.23 (1.17, 1.29) mmol/L) ($P < 0.05$). LDL and HDL cholesterol levels after the SAFA diet simultaneously increased, hence total: HDL cholesterol ratio following the SAFA diet was not significantly different from the other 2 diets ($P > 0.05$). There were no significant differences between diets in fasting concentrations of apo-B100, apo-A1, apo-B100: apoA-1, Lp (a) TAG and NEFA ($P > 0.05$). Interestingly, CARB diet was found to reduce HDL_3 at 7.78% and increase % small dense HDL (sdHDL) at 8.57% compared with SAFA diet ($P < 0.05$). SAFA diet increased large HDL subfractions compared with both CARB and MUFA diets at 4.88% and 6.55% ($P < 0.05$), respectively.

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Table 4.24: Fasting Serum Lipid and Lipoprotein Concentrations.

	Baseline[#]	SAFA diet	CARB diet	MUFA diet
Total cholesterol (mmol/L)[1]	5.00 (4.76, 5.23)	4.89 (4.68, 5.09)	4.79 (4.58, 4.99)	4.71 (4.48, 4.94)[†]
LDL cholesterol (mmol/L)[1]	3.14 (2.94, 3.33)	3.05 (2.89, 3.21)	3.00 (2.84, 3.17)	2.89 (2.71, 3.10)[†]
Mean LDL particle size (nm) [1]	270.8 (269.8, 271.8)	270.9 (270.0, 271.9)	270.4 (269.4, 271.3)	270.7 (269.8, 271.5)
Small dense LDL (%)[1]	5.11 (3.46, 6.77)	4.36 (2.87, 5.84)	5.02 (3.38, 6.65)	4.66 (3.24, 6.08)
HDL cholesterol (mmol/L)[1]	1.30 (1.22, 1.37)	1.23 (1.17, 1.29)	1.18 (1.13, 1.24) [†]	1.21 (1.14, 1.28)
HDL-2 (%)[1]	13.33 (12.26, 14.40)	13.62 (12.53, 14.72)	13.09 (12.08, 14.09)	13.06 12.17,13.95)
HDL-3 (%)[1]	9.12 (8.33, 9.91)	9.42 (8.59, 0.24)	8.74 (7.97, 9.51)[†]	8.95 (8.16, 9.74)
Small HDL particle (%)[1]	12.07 (10.80, 13.34)	11.55 (10.22, 12.88)	12.54 (11.23, 13.85)[†]	12.06 (10.93, 13.19)
Intermediate HDL particle (%)[1]	54.38 (52.68, 56.08)	54.07 (52.55, 55.59)	54.73 (53.35, 56.12)	55.75 (54.25, 57.25)[†]
Large HDL particle (%)[1]	33.41 (31.04, 35.78)	34.17 (31.84, 36.49)	32.58 (30.41, 34.76)[†]	32.07 (30.05, 34.09)[†]
Total: HDL cholesterol[1]	3.97 (3.73, 4.21)	4.06 (3.84, 4.28)	4.13 (3.91, 4.36)	4.01 (3.76, 4.25)
Apo-B100 (g/L)[1]	0.92 (0.87, 0.97)	0.91 (0.86, 0.96)	0.91 (0.86, 0.96)	0.90 (0.85, 0.95)
Apo-A1 (g/L)[1]	1.33 (1.28, 1.38)	1.27 (1.22, 1.31)	1.25 (1.21, 1.29)	1.27 (1.22, 1.32)
Apo-B100: Apo-A1[1]	0.70 (0.65, 0.75)	0.72 (0.68, 0.77)	0.74 (0.69, 0.79)	0.72 (0.67, 0.77)
Lp(a) (mg/dL)[2]	15.1 (11.8, 18.4)	16.9 (13.6, 20.2)	17.9 (14.0, 21.7)	17.4 (14.2, 20.7)
TAG (mmol/L)[2]	1.24 (1.03, 1.44)	1.34 (1.13, 1.55)	1.32 (1.14, 1.49)	1.34 (1.13, 1.55)
NEFA (mmol/L) [1]	0.71 (0.66, 0.76)	0.68 (0.64, 0.73)	0.68 (0.64, 0.71)	0.69 (0.65, 0.73)

Values are mean ± SD[1] and GM ± SD[2]. *n*=47 (women *n*=35; men *n*=12). Data was analyzed by repeated measures ANOVA (among 3 treatments) using Bonferroni's multiple comparisons test.

[†]Significantly different from SAFA, $P < 0.05$.

[#]Baseline data was reported as the reference and not used in data analysis.

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4.11 Postprandial Pulse Wave Analysis

4.11.1 Augmentation Index

Repeated measures ANOVA analysis for augmentation index did not show significant meal x time interaction (P = 0.665). Figure 4.20 displays a decline in augmentation index from 0 towards 240 min; however no significant difference in the deviation were detected between the 3 meals (P > 0.05). $iAUC_{(0\text{-}240min)}$ for SAFA, CARB and MUFA meals were respectively 612.4 (460.6, 764.2), 739.0 (557.6, 921.8) and 662.9 (493.3, 832.5), no significant difference was detected between meals (Table 4.25).

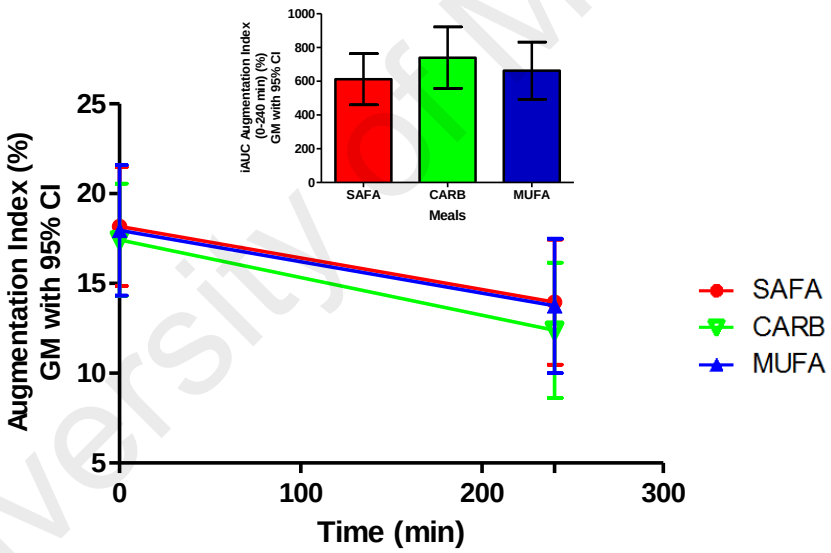

Figure 4.20: Postprandial Augmentation Index Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Values are GM with 95% CI; n=46 (women n=34; men n=12).
[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 1 time point) with gender as a between subject factor; meal effect: P = 0.384, time effect: P < 0.001 and meal x time interaction: P = 0.665.
[2]Insert: iAUC over 240 min (GM ± 95% CI) (n = 46): Repeated measures ANOVA of $\log_e iAUC_{(0\text{-}240min)}$: P > 0.05.

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Table 4.25: Changes from Fasting Augmentation Index after SAFA, CARB and MUFA Meal.

Time (min)	SAFA meal	CARB meal	MUFA meal
0	18.17 (14.77, 21.57)	17.43 (14.21, 20.65)	17.96 (14.23, 21.69)
Δ 240	-4.212 (-2.680, -5.744)	-5.308 (-3.184, -6.893)	-4.216 (-2.442, -5.989)

Values are GM with 95% CI, expressed in %; n = 46 (women n = 34; men n = 12).

[1]Changes from fasting data were loge transformed, analyzed by repeated measures ANOVA (3 meals, 1 time point) with gender as a between subject factor: meal effect: P = 0.384, time effect: P < 0.001 and meal x time interaction: P = 0.665.

[2]Repeated measures ANOVA of $\log_e$ iAUC (0-240min): P > 0.05.

4.11.2 Augmentation Pressure

Similar to postprandial changes in augmentation index, augmentation pressure decreased from baseline over the 240 min after all the meals (Figure 4.21). No significant meal x time interaction (P = 0.344) was detected with regards to the changes. Furthermore, there were no significant differences in deviation from baseline and iAUC between meals (Table 4.26). iAUC $_{(0\text{-}240\text{min})}$ of the SAFA, CARB and MUFA meals were 205.3 (160.6, 250.0), 249.7 (192.7, 306.6) and 211.0 (156.9, 265.2) respectively.

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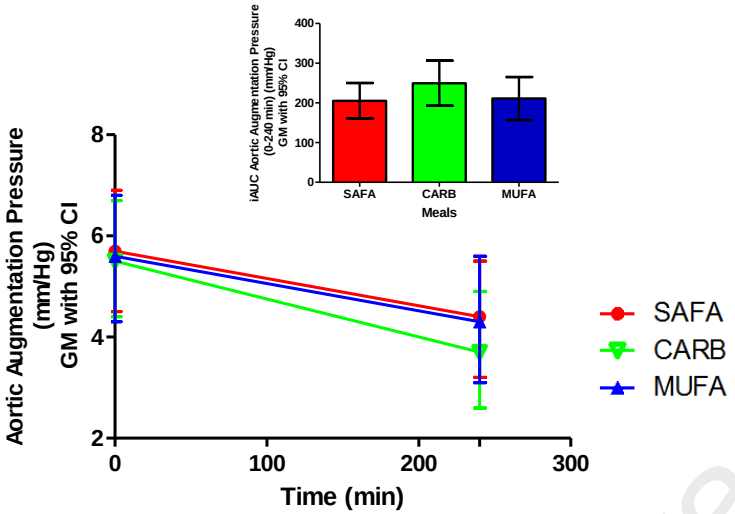

Figure 4.21: Postprandial Augmentation Pressure Following Mixed Meal at the End of Each 3 x 6-Week Dietary Intervention.
Values are GM with 95% CI; n=46 (women n=34; men n=12).
[1]Changes from fasting data were analyzed by repeated measures ANOVA (3 meals, 1 time point) with gender as a between subject factor; meal effect: $P = 0.384$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.665$.
[2]Insert: iAUC over 240 min (GM ± 95% CI) (n = 46): Repeated measures ANOVA of loge iAUC (0-240min): P > 0.05.

Table 4.26: Changes from Fasting Augmentation Pressure after SAFA, CARB and MUFA Meal.

Time (min)	SAFA meal	CARB meal	MUFA meal
0	5.672 (4.435, 6.909)	5.537 (4.343, 6.731)	5.580 (4.297, 6.864)
Δ 240	-1.317 (-1.812, -0.822)	-1.802 (-2.368, -1.237)	-1.252 (-1.839, -0.666)

Values are GM with 95% CI, expressed in %; $n = 46$ (women $n = 34$; men $n = 12$).
[1]Changes from fasting data were $\log_e$ transformed, analyzed by repeated measures ANOVA (3 meals, 1 time point) with gender as a between subject factor: meal effect: $P = 0.394$, time effect: $P < 0.001$ and meal x time interaction: $P = 0.344$.
[2]Repeated measures ANOVA of $\log_e$ iAUC $_{(0\text{-}240\text{min})}$: $P > 0.05$.

4.12 Fasting Pulse Wave Analysis

Results of pulse wave analysis measured at fasting state after the completion of each dietary intervention (week 6) are shown in Table 4.27. No significant differences were found between diets with regards to aortic systolic pressure, aortic diastolic pressure, aortic pulse pressure, aortic augmentation pressure and aortic augmentation index ($P > 0.05$).

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Table 4.27: Fasting Measurements of Pulse Wave Analysis in 47 Participants Following the Three 6-week Dietary Interventions.

	Baseline	SAFA diet	CARB diet	MUFA diet
Aortic systolic pressure (mmHg)	113.4 (109.6,117.3)	109.4 (106.4,112.4)	109.5 (106.4, 112.6)	110.40 (107.0,113.8)
Aortic diastolic pressure (mmHg)	83.28 (80.17, 86.38)	79.73 (77.18, 82.28)	79.38 (76.58, 82.17)	80.68 (77.83, 83.53)
Aortic pulse pressure (mmHg)	30.13 (28.24, 32.02)	29.67 (28.06, 31.29)	30.11 (28.51, 31.72)	29.71 (28.21, 31.21)
Aortic augmentation pressure (mmHg)	6.15 (4.76, 7.54)	5.70 (4.52, 6.87)	5.61 (4.47, 6.75)	5.53 (4.30, 6.76)
Aortic augmentation index (%)	18.69 (15.03, 22.35)	18.22 (14.97, 21.46)	17.59 (14.51, 20.67)	17.83 (14.27, 21.39)

Values are mean ± SD. *n*=47 (women *n*=35; men *n*=12). Data was analyzed by repeated measures ANOVA (among 3 treatments). No significant difference between diets.

Keynotes

-Interestingly, CARB meal attenuated postprandial insulin secretion corrected for glucose response and disposition index however the insulin sensitivity was not affected.

-No significant difference in insulin corrected for glucose and DI was found in subgroups of NFG, IFG and T2DM.

-SAFA and MUFA had similar effects on all markers except for fasting glucose-dependent insulinotropic peptide (GIP) concentrations which increased after MUFA but not SAFA when compared with CARB.

-As indicated by incremental area under the curve during postprandial state, CARB meal was found to increase plasma IL-6 while MUFA meal elevated postprandial plasma D-dimer significantly more compared with SAFA meal; however the meal x time interactions were not significant difference.

-CARB diet was found to reduce HDL_3 by 7.8% and increase small dense HDL (sdHDL) by 8.6% compared with SAFA diet. SAFA diet increased large HDL subfractions compared with both CARB and MUFA diets by 4.9% and 6.6%, respectively.

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CHAPTER 5: DISCUSSION

5.1 Scope

The present study was designed to investigate both fasting and postprandial effects of the 7% en replacement of palm olein derived SAFA with carbohydrate or high-oleic sunflower oil derived MUFA on insulin secretion and sensitivity, glucose homeostasis, gastro-intestinal peptides release, inflammatory and endothelial responses in subjects with central obesity after a 6-week isocaloric dietary intervention. Comparisons between SAFA and MUFA effects were made in the context of total fat content of 31.5% en. Although we recruited abdominally obese subjects with no known medical illness, on evaluation at baseline 59.6% and 6.4% had undiagnosed IFG and T2DM while 34% had metabolic syndrome as defined by the Harmonized Criteria, indicating that this was a group at high risk of developing T2DM and CVD. As seen from our subject population, young Malaysians (mean age of 32) tend to have high percentage of body fat (37%) and vast majority of them are at such high risk for metabolic syndrome that deserves close attention of health authorities. The study was designed to examine the metabolic consequences of changes in macronutrient composition (fat and carbohydrate quantity) and fat quality (SAFA *vs* MUFA) under free-living conditions albeit with close supervision in a setting where study subjects were provided with prepared meals according to study specifications 5 out of 7 days a week for each 6 week dietary intervention. Compliance was strictly monitored with measures of plasma fatty acids and erythrocyte membrane composition, weight change and visual analogue scale. We were successful in ensuring compliance of study volunteers with the dietary interventions with >95% of the 3 main meals/day (5 days a week) prepared according to trial specifications being consumed throughout the study duration. Measures of plasma fatty

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acids and erythrocyte membrane composition also indicated good compliance with the diets. This was achieved with no significant weight change in subjects at the end of each dietary intervention which could confound interpretation of outcomes.

The methodology aimed to fill the gap of unanswered questions posed by the KANWU and LIPGENE studies in which no differences in insulin secretion or sensitivity were detected between SAFA, CARB and MUFA diets but food was consumed in an *ad libitum* manner thus raising issues of whether the en exchanges at study end were on target. These studies were conducted in Caucasian with different genetic risks and carbohydrate and fat food sources compared with Asians. Pasta (dhurrum wheat) and bread utilized in these trials as the carbohydrate component does not reflect the day-to-day diet of most Asians including those from South East Asia, China and India, a substantially large bloc of the global population. The carbohydrate staple of Asians is short grain rice, as utilized as a major source of carbohydrate in our trial. Similarly olive oil as a source of MUFA is expensive and not readily available to most Asians, hence our study used high-oleic sunflower oil. Palm olein is widely used as culinary oil worldwide especially in Asia given that the balanced fatty acid composition of 50% SAFA, 40% MUFA and 10% PUFA makes it a versatile vegetable oil for food formulation. We also wanted to evaluate insulin secretion in the context of background glucose levels and background insulin sensitivity i.e. DI. To date, this is the first such single blind, crossover and randomized human clinical trial in Asia utilizing food sources reflective of daily dietary use in the local Asian population.

5.2 Postprandial Insulin Secretion, Insulin Sensitivity and Glucose Control

As expected, the primary outcome of the study as assessed by the postprandial changes in the absolute C-peptide levels were higher after a high-carbohydrate/low-fat mixed meal

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following a 6-week background diet enriched with 7% en carbohydrate in replacement of SAFA and MUFA. $iAUC_{C\text{-}peptide}$ was also significantly higher after the acute mixed meal challenge corresponding to the higher plasma glucose concentrations after the high-carbohydrate meal, when compared with the two high-fat meals. Interestingly, this is not always the case as shown by two other acute setting studies. Our observations were in contrast with two acute studies (Collier & O'Dea, 1983; Wolpert et al., 2013) providing test meals at different energy content. The high-fat meal in the aforementioned studies contained an extra 450 kcal compared with the high-carbohydrate meal, and resulted in higher insulin response corrected for glucose compared to the high-carbohydrate meal. In parallel with our study, other studies that utilized isocaloric test meals demonstrate that high-carbohydrate meal resulted in higher glucose and insulin or C-peptide concentrations than a high-fat meal (Gibbons et al., 2013; Gutniak et al., 1986; Tannous dit El Khoury et al., 2006). The isocaloric diets and meals used in the present study with the % en from fat being replaced by equal % en from carbohydrate prevented energy content from confounding interpretation of outcomes.

Acute glucose variations after a meal, such as the peaking time and the magnitude of glucose rise are factors that may predict diabetes risk. Despite the exaggerated increase in plasma insulin after CARB meal, peak plasma glucose in our subjects was found to be 19% higher after CARB meal than the normal glycemic response after the meal for healthy subjects (<7.8 mmol/L) as defined by the International Diabetes Federation (Ijzerman et al., 2009). Furthermore, our study noted a delayed insulin peak at 2 h in the South East Asian subjects compared with data obtained from Caucasians consuming similar test meals where insulin peaked at 30 min (Filippou, Berry, et al., 2014), which indicated impaired insulin sensitivity in this population (Goff et al., 2013). We can perhaps infer therefore that the habitual diet consumed by South East Asian populations

University of Malaya

which consists of around 60% en carbohydrate with the majority comprised of refined carbohydrate, predisposes the population to higher incidence of T2DM (FAO, 1998).

The present study also found that the postprandial insulin response corrected for glucose after CARB meal tended to be lower compared with both high-fat meals (P = 0.048). Importantly, insulin secretion corrected for insulin sensitivity using a modified DI tended to be lower with the CARB meal after 6 weeks of higher carbohydrate background diet when compared with SAFA and MUFA interventions (P = 0.030). The DI is thought to be the most valid measure of β-cell function and is a more sensitive predictor of risk for development of dysglycemia than either insulin secretion or sensitivity alone (Bergman, 1989). Given the fact that the relationship between insulin sensitivity and insulin secretion is an inverse one described by a hyperbolic function, failure to evaluate insulin secretion in the context of prevailing insulin sensitivity might lead to misguided conclusions (Bergman, 1989). For example the presence of an elevated fasting insulin in an insulin resistant subject considered in isolation might lead one to assume low risk of glucose intolerance, however dysglycemia can be accurately predicted when that insulin concentration is considered in the context of elevated glucose and reduced insulin sensitivity by a measure known as DI. By convention, DI is calculated by multiplying insulin secretion during the first 10 min of an IVGTT (AIRg) with insulin sensitivity derived from the IVGTT (Bergman, 1989). We did not carry out IVGTT and hence have modified this formula, deriving DI by multiplying $iAUC_{insulin}/iAUC_{glucose(0\text{-}120min)}$ (insulin secretion) with HOMA2-%S (insulin sensitivity) as measured in our study This use of a DI has not been used by most studies, eg. the paper by Perez-Jimenez et al. (2001) which also examined insulin secretion after various dietary interventions did not evaluate DI thus limiting our comparison with their study results. Indeed other studies such as that by Shah et al. (2007) even failed to correct insulin secretion for prevailing glucose further confounding interpretation and comparison.

University of Malaya

We avoided exclusion bias by the recruitment of subjects with elevated fasting glucose as long as they were not diagnosed with T2DM prior to enrolment. The reason for this was to avoid detection bias because of skewed detection efforts, or else the observations made may not represent the real characteristics of abdominally obese population in Malaysia where most of them have remarkably high fasting glucose concomitantly. We found diverging effects of CARB vs SAFA and MUFA in insulin corrected to glucose and DI that were not exclusive to the vulnerable T2DM or IFG groups only. This indicated our significant findings in insulin corrected to glucose and DI were not influenced by statistical bias. On the other hand, the different trends of the effects of SAFA in NFG, IFG groups (better insulin secretion) and T2DM group (attenuated insulin secretion) may indicate different metabolic responses in these groups. However it may also be a by chance observation as the sample size of T2DM group was too small (n=3), hence future research to compare the difference between these groups is warranted.

Similar studies comparing carbohydrate with fat in Caucasian populations such as LIPGENE study (Tierney et al., 2011) in those with metabolic syndrome and RISCK study (Jebb et al., 2010) in subjects at risk of developing metabolic syndrome did not reveal any changes in endogenous insulin secretion using IVGTT, this is possibly because the consumption of diets in an *ad libitum* manner did not accurately achieve target of energy exchanges at study end. Our finding echoes previous reports in healthy subjects (Collier & O'Dea, 1983) which demonstrated that carbohydrate alone when compared to the addition of fat to carbohydrate (albeit in a nonisocaloric manner) is associated with decreased insulin secretion corrected for glucose.

This unique finding that increased carbohydrate attenuates insulin secretion in Asians may indicate increased genetic susceptibility to the adverse impact of higher carbohydrate diets. The amount of carbohydrate administered (158 g) represents the amount of carbohydrate in a typical set meal in South East Asia i.e. fried noodle or fried

University of Malaya

rice with a sweetened drink. Hence our findings imply that if an increase as small as 7% en of moderate GI refined carbohydrate (within a short period of 6 weeks) in centrally obese subjects can attenuate insulin response corrected for glucose, repetitive exposure to insulin hypersecretion may lead to the early exhaustion and apoptosis of β-cells and hence the increased risk of developing T2DM (Aston-Mourney et al., 2008; Wajchenberg, 2007).

Insulin resistance and β-cell dysfunction result in the development of T2DM. It has been thought that insulin hypersecretion occurs in order to compensate the insulin need under the circumstance of insulin resistance. However evidence showed that insulin hypersecretion can present independent of insulin resistance. A study showed more frequent and greater amplitude of postprandial increase in insulin release in obese juvenile compared with normal-weight children, without any changes in insulin sensitivity (Le Stunff and Bougneres, 1994). The β-cell may be simply exhausted by the prolonged need to hypersecrete insulin, i.e. long term high-carbohydrate intake, hence overload the endoplasmic reticulum and also result in oxidative stress that were expected to damage β-cell which can causedefect in insulin secretion seen in T2DM condition (Aston-Mourney et al., 2008).

We could not demonstrate that fat quality modulated insulin secretion. This current study found that after the replacement of 6-week SAFA or MUFA diet (7% en exchange), a 16% en exchange between SAFA and MUFA during a mixed meal challenge reflecting the background diet had no differential impact on postprandial C-peptide, insulin and glucose homeostasis. This was in keeping with another similar study in healthy Malaysian subjects given the same mixed meals prepared with palm olein and high oleic sunflower oil in an acute setting (Filippou, Berry, et al., 2014). Moreover, similar findings of no differential impact of fat quality on insulin secretion were reported when strawberry-flavored shakes providing 40% en exchange between SAFA and MUFA

University of Malaya

were given to lean, obese, obese T2DM subjects (P. R. van Dijk et al., 2012). The study by Paniagua et al. (2007) with a similar study design to ours that tested patients with a mixed meal challenge after a chronic exposure to a similar background diet reported differences in terms of lower acute insulin secretion between SAFA and MUFA at 14% en exchanges. The study however did not measure insulin response corrected for glucose hence limited the comparison. In contrast, a 6-hour standardized mixed meal challenge demonstrated an increased insulin response to a set of farina, egg substitute, ham, white bread, skim milk and orange juice which contained 38% en SAFA instead of MUFA in subjects with T2DM (Shah et al., 2007). This discrepancy between the two studies may be due to the different types of food utilized as test meals.

5.3 Fasting Insulin Secretion, Insulin Sensitivity and Glucose Control

Our study demonstrated that the quality and quantity of fats at 7% en exchange affected neither basal insulin secretion as measured by HOMA2-%B nor sensitivity as measured by HOMA-IR, HOMA2-%S or glucose homeostasis after a 6-week isocaloric dietary intervention in abdominally obese individuals. Our findings differ from two 8-week studies (Goree et al., 2011; Gower et al., 2012) that performed similar diet interventions (same macronutrient composition in all diets) by allocating subjects to high-fat/high-MUFA diet or high-carbohydrate diet with 39 *vs* 27% en fat content respectively. High-fat diet reportedly increased fasting glucose concentration in healthy (Goree et al., 2011) and overweight subjects with IFG (Gower et al., 2012); while decreasing insulin sensitivity in IFG subjects (Gower et al., 2012). The higher total fat content in the aforementioned studies by Goree et al. (2011) and Gower et al. (2012) as compared to our subjects who consumed 31.5% *vs* 24% fat respectively in SAFA or MUFA diet *vs* CARB diet may be the main reason for the deterioration in insulin sensitivity and glucose

University of Malaya

homeostasis they observed. It is however noteworthy that in a different study by Garg et al. (1988) 50% en fat (high-fat/high-MUFA) diet reduced insulin requirement, plasma glucose and urinary glucose concentration in T2DM patients when compared to a 25% en fat (high-carbohydrate) diet after 4-week intervention (Garg et al., 1988). In the study by Garg et al. (1988) however there were no direct measures of insulin secretion and sensitivity. These findings that a higher carbohydrate content may have an adverse impact on insulin sensitivity and glucose homeostasis fit in with the hypothesis that a high-carbohydrate diet is more deleterious than a high-fat diet (Malhotra 2013). Alternatively the aforementioned study (Garg et al., 1988) utilized the large en exchange of MUFA sourced from olive oil containing polyphenols, Vitamin E and other natural antioxidants that own anti-inflammatory properties and may have a benefical effect on preventing the progression of insulin resistance. Studies with a larger sample size and longer duration of exposure are warranted to confirm the conflicting findings.

In accordance with the findings of our previous study in healthy subjects (Filippou, Teng, et al., 2014), the RISCK study in subjects at risk of developing metabolic syndrome (Jebb et al., 2010) and the LIPGENE study in metabolic syndrome subjects (Tierney et al., 2011) subjects, our results showed that diet enrichment with SAFA is comparable with carbohydrate or MUFA with regards to its effect on insulin sensitivity. Both the RISCK (Jebb et al., 2010) and LIPGENE (Tierney et al., 2011) studies found that the substitution of SAFA with carbohydrate (10% en exchange) and MUFA (7% en exchange) did not result in significant differences in insulin sensitivity and acute insulin response corrected for glucose. Another large scale study, the KANWU study (Vessby et al., 2001) in healthy subjects however reported borderline significant difference in treatment effect between SAFA and MUFA at 9% en exchange ($P = 0.053$) where SAFA impaired insulin sensitivity compared to baseline, while no appreciable difference was detected in insulin secretion and first-phase insulin response (acute insulin response

University of Malaya

corrected for glucose was not measured). The wide variation of total fat intake (29–45% en) in the free-living supervised subjects and that the SAFA group subjects have lower insulin sensitivity at baseline compared with those in MUFA group may have confounded the findings of the parallel-arm design KANWU study. Our study avoided such limitations by setting each subject as his own control using the crossover design and by providing centrally prepared meals. Furthermore, we found DI was similar between the SAFA and MUFA groups but lower in the CARB group (P=0.030, however no significant difference between diet groups), while none of the four aforementioned studies evaluated insulin secretion in the context of insulin sensitivity (DI).

In conclusion, we found that a small en exchange of 7% fat on the background of total fat 31.5% en for 6 weeks did not adversely impact on insulin sensitivity as measured by HOMA2-%S or glucose homeostasis in free-living subjects. However the same small en exchange of 7% carbohydrate adversely impacted insulin secretion as evaluated by DI. The first observation that increased fat does not lead to 'lipotoxicity' and hence reduced β-cell function and insulin resistance is at first glance counter-intuitive. We cannot exclude the possibility that larger en exchanges of fat may result in lipotoxicity and adversely impact insulin sensitivity/β-cell secretion. However, there is a substantial body of evidence that the adverse effects of elevated fatty acids are only evident in the presence of elevated glucose i.e. glucolipotoxicity (Prentki, Joly, El-Assaad, & Roduit, 2002). The observations of preserved insulin secretion after exposure to dietary fat in the present study can be explained by the absence of combined glucolipotoxicity in a cohort of nondiabetic patients who were at risk of T2DM. Our centrally obese subjects had only mildly elevated mean fasting glucose at baseline i.e. 5.8 mmol/l that was unchanged at study end, and were hence representative of an early stage in the natural evolution of T2DM. In addition, while total fat in the SAFA and MUFA arms contributed to 31.5% en (recommendation for total fat was 20-35% en), after 6 weeks exposure this did not result

University of Malaya

in evidence of increased fasting NEFA indicating perhaps absence of the development of lipotoxicity in this nondiabetic group. Studies in humans have demonstrated that lipid infusion at euglycemia actually induced improved insulin secretion in healthy subjects but reduced secretory response only in weight-matched T2DM subjects (Boden, Chen, Rosner, & Barton, 1995). Experiments in human subjects with and without diabetes have shown that lipid infusions only impair insulin secretion in the presence of hyperglycaemia (Kashyap et al., 2003; Poitout & Robertson, 2008). Of even more interest is the fact that those in the CARB group exhibited borderline significant reductions in insulin secretion despite lower fat intake. One could postulate that these centrally obese subjects may experience impending β-cell exhaustion as a result insulin hypersecretion secondary to high carbohydrate intake. Another fascinating converse view of our results would be that fat in small to moderate amounts (7% en exchange) enhances insulin secretion in this cohort of non-diabetic patients with elevated risk of T2DM. In addition to the studies by Collier and O'Dea (1983) and Boden et al. (1995), there is good experimental evidence that NEFA is necessary for both basal and glucose-stimulated insulin secretion (McGarry, 2002). Work by McGarry (2002) has shown that reducing NEFA levels to almost zero by infusing nicotinic acid (an antilipolytic agent) reduces insulin secretion in response to glucose as well as arginine/leucine/glyburide, to near undetectable levels. The same findings have been replicated qualitatively in humans (Dobbins, Chester, Daniels, McGarry, & Stein, 1998). Hence the intriguing observation that our 'healthy subjects' on increased-fat diet intervention exhibited a higher DI after 6 weeks exposure compared with the lower fat/increased carbohydrate intervention.

University of Malaya

5.4 Postprandial Inflammatory and Endothelial Responses

The present study reveals that 7% en replacement of SAFA with CARB in a mixed meal after a similar background diet for 6 weeks elevated postprandial $iAUC_{0\text{-}360min}$ of IL-6 to a greater extent compared with replacement of SAFA with carbohydrate. On the other hand, replacing SAFA with MUFA in a high-fat snack after a similar 6 week background diet resulted in elevated D-dimer responses. $iAUC_{0\text{-}360\ min}$ for all other measured inflammatory and endothelial parameters such as IL-1β, E-selectin, PAI-1 and CRP were not significantly different between the 3 diets. PAI-1 and augmentation index were found to decrease from baseline at 4 h after all 3 meal challenges. In our previous published study (Teng, Chang, Kanthimathi, Tan, & Nesaretnam, 2015) on the acute effect of mixed meal challenges with similar dietary composition conducted in metabolic syndrome Asian subjects, postprandial IL-1β, CRP and PAI-1 as in the present study were not different between the 3 meals. However unlike our current study (where postprandial IL-6 and D-dimer were higher after MUFA and CARB meal-challenges respectively), in the acute setting without a prior 6 week background diet exposure of similar composition to the mixed meal, both IL-6/D-dimer were not different between the meal challenges (Teng, Chang, Kanthimathi, Tan, & Nesaretnam, 2015; Vessby et al., 2001). Although postprandial elevation of IL-6 and D-dimer was greater after 6 weeks of CARB and MUFA diet respectively compared with SAFA, we expected to observe a more pronounced effect of 7% increase in dietary fat in overweight subjects in terms of elevation of IL-1β, PAI-1 and CRP levels also as excess central adipose tissue secretes proinflammatory markers and this population is exposed to increased risk of developing chronic diseases. However, in contrast to our expectations, our findings are in concord with our previous study in healthy Asian subjects that 6 weeks exposure to diets high in

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SAFA or MUFA do not differentially affect IL-6, CRP, TNF-α and IL-1β levels after challenge meals (Teng et al., 2011).

It has been reported that food ingestion increases the release of IL-6 from skeletal muscle furthermore acutely elevates IL-6 levels in plasma and adipose tissue (P. J. Manning, Sutherland, de Jong, Ryalls, & Berry, 2013). Our study observed postprandial elevation of plasma IL-6 across the 6-hour challenge. The transient increase of cytokines after nutrients ingestion is known as postprandial inflammation which is a normal metabolic response for energy and immunological homeostasis (Ye & Keller, 2010). It is however noteworthy that postprandial inflammation may lead to chronic low-grade inflammation that increases the risk of disease development given that we are always in continuous postprandial state. The extent of postprandial inflammation may be affected by total calorie, amount of lipids and fatty acid composition, amount of carbohydrates and glycemic index (Margioris, 2009). Specifically, high-fat diet and high-carbohydrate/high-GI diet stimulate the release of IL-6 (Kelly et al., 2011). It has been reported that the ingestion of a minimum of 50 g fat is needed for the detection of postprandial cytokine activation (Nappo et al., 2002). This increase in inflammatory cytokines on high-fat meals however was not seen in our abdominally obese Asian subjects despite the use of 50 g fat in the SAFA and MUFA challenges. Previous studies consistently reported that high-fat meals triggered inflammatory responses (reviewed by our group (Teng et al., 2014)) but this is not the case in our current study where instead, rather unexpectedly and importantly, the high-CARB meal increased IL-6 levels to a greater extent postprandially. Our observation suggests that high-carbohydrate/high-GI intake is a stronger stimulator for postprandial inflammation compared with high-fat intake. This is possibly because both palm olein and high-oleic sunflower oil contain phytochemicals that are found to reduce postprandial inflammation (Burton-Freeman, Talbot, Park, Krishnankutty, & Edirisinghe, 2012). This coupled with the deleterious

University of Malaya

changes in HDL subfractions after the high CARB diet is of significance in the light of the controversy surrounding high-carbohydrate versus high-fat diets. Our work seems to indicate that even a short exposure to a high-CARB diet leads to negative changes in surrogate inflammatory and thrombogenic markers indicative of increased cardiovascular risk in abdominally obese Asian subjects.

In agreement with the previous studies (Delgado-Lista et al., 2008; Teng et al., 2015), our study observed significant increase in D-dimer and reduction in PAI-1 compared to baseline, irrespective of the quantity and type of fat. Our detection of postprandial prothrombotic hemostatic state (higher $iAUC_{(0\text{-}360min)}$ of IL-6 and D-dimer) respectively after CARB and MUFA meal compared to SAFA meal may be observed by chance, given that the meal x time interaction for both markers was not significant. One major determinant of PAI-1 modulation is the concentration of insulin (Delgado-Lista et al., 2008). The hyperbolic relationship between insulin and PAI-1 (Delgado-Lista et al., 2008) was reflected from our observation where postprandial reduction in PAI-1 concentration rebounded when postprandial insulin level returned to baseline. Delgado-Lista et al. (2008) however found that SAFA meal induced higher PAI-1 increase than that of CARB meal. This finding was found conflicting with the results reported by our group (Teng et al., 2015) and our current study. The contradiction may be due to the different fat sources of SAFA utilized in the studies, i.e. butter in Delgado-Lista et al. (2008) and palm olein in the latter studies. In studies using butter as a dietary SAFA source, butter was found to increase cytokines postprandially to a greater extent (Masson & Mensink, 2011) compared with polyunsaturated fats in obese subjects. One possible explanation might be that the medium chain triglyceride content of butter is more rapidly absorbed compared with that of other SAFA sources such as palm oil (Seaton, Welle, Warenko, & Campbell, 1986). On the other hand, it has been reported that the optimal time to evaluate changes in CRP in blood plasma is 24 h postprandially and this explains

University of Malaya

our observations of unchanged CRP levels up to 6 h postprandially (Herieka & Erridge, 2014).

5.5 Fasting Inflammatory and Endothelial Responses

The primary outcome, fasting plasma IL-6 was not different after 6 weeks exposure to the three isocaloric diets, indicating perhaps a neutral effect of 7% en exchange of SAFA, MUFA and CARB on chronic inflammation. Neither was there any substantial impact of dietary modification on other cytokines (IL-1β and E-selectin), CRP, and thrombogenic markers (PAI-1 and D-dimer), arterial stiffness (augmentation index) in the fasting state.

Epidemiological studies suggest that elevated fasting CRP and IL-6 are associated with coronary artery disease (Danesh et al., 2008; Shlipak, Ix, Bibbins-Domingo, Lin, & Whooley, 2008). The substitution of MUFA or CARB for SAFA however did not result in improvement of these parameters in the fasting state in this group of subjects who are vulnerable to disease progression. Our findings may perhaps indicate that plant source SAFA for 6 weeks does not worsen inflammation or result in a prothrombotic state in abdominally obese individuals. In agreement with our findings, Lithander et al. (2008) reported that fasting CRP, IL-6 and TNF-α did not differ between a high SAFA or low-SAFA fat diet differing by 5% en fat after a 3-week crossover trial in mildly hyperlipidemic Caucasian subjects. The RISCK study, a large scale study in a Caucasian cohort of subjects at risk of developing metabolic syndrome also found that altering the composition of SAFA (37.5% en fat, 42% en carbohydrate), MUFA/ high or low GI (35.6% en fat, 44.9% en carbohydrate) coupled with CARB/high or low GI (27% en fat, 51% en carbohydrate) diet for a duration of six months did not alter CRP, sICAMs or PAI-1 (Jebb et al., 2010). The findings from this well-designed and largely sampled study (n = 548) possibly suggest that macronutrients from daily diet have little effect on these risk factors

University of Malaya

for CVD. In contrast with the aforementioned findings, a study in healthy subjects reported larger increase from baseline in fasting CRP and IL-6 after *trans* fatty acids when compared with oleic acid (MUFA) after a 4-week dietary intervention (Baer et al., 2004). The significant difference was caused by the harmful effect of *trans* fatty acids on inflammation.

Casas et al. (2014) reported that Mediterranean diet supplemented with both nuts and extra virgin olive oil (a rich source of MUFA) was found to lower plasma IL-6, CRP, soluble intercellular adhesion molecules (sICAMs) and P-selectin compared with a low-fat diet after a 1-year long-term dietary intervention in subjects at risk of developing cardiovascular diseases. The study suggested that the nutrients in the Mediterranean diet and a long study duration (at 1 year) maybe needed for a pronounced impact of dietary modification on inflammatory biomarkers related to atherosclerosis in at risk subjects as in our study population. A study comparing the 6-week supplementation of n-3 PUFA (1100 mg) from fish oil, which is regarded as anti-inflammatory did not improve subclinical inflammation; subsequent weight loss of 9.4 kg achieved with 8 weeks adherence to low calorie diet after the supplementation however significantly bring down the levels of cytokines (Jellema, Plat, & Mensink, 2004; Plat, Jellema, Ramakers, & Mensink, 2007).

5.6 Postprandial Gastro-intestinal Peptides Release

Our study showed no appreciable differences in postprandial gastro-intestinal peptides responses of GLP-1, GIP, PYY, CCK and ghrelin as evaluated by $iAUC_{(0\text{-}120min)}$ between the test meal challenges reflecting preceding 6 week habitual background diet interventions. After the CARB meal, there were significantly higher incremental spikes of GLP-1 and GIP in the early phase at 15 and 30 min time points (when compared with

University of Malaya

the SAFA and MUFA meals) that occurred in response to noticeably elevated glucose levels which in turn stimulated insulin secretion and inhibited glucagon secretion. Others have observed that high-fat meals result in a greater rise in GLP-1 and PYY when compared with a high-carbohydrate meal over duration of 3 h in healthy adults. Paniagua et al. (2007) reported higher postprandial 3 h AUC for GLP-1 with higher fat content background diet compared with our interventions i.e. 38% en *vs* 31.5% en when compared with CARB diet for a duration of 4 weeks in abdominally obese, insulin resistant subjects. However we did not observe such a difference perhaps because of the 6.5% lower fat content of our dietary interventions. On the other hand, Ellis et al. (2012) when comparing a higher fat diet than ours (39% en fat and 43% en carbohydrate) with a lower carbohydrate content than ours (27% en fat and 55% en carbohydrate) for 8 weeks in sedentary overweight subjects echoing our findings found no difference between ghrelin and GLP-1 postprandial responses after a liquid mixed meal challenge. The impaired GLP-1 and PYY responses after high-fat load may be due to insulin resistance and impaired glucose homeostasis in our obese subjects 66% of whom had newly diagnosed dysglycemia. Interestingly when comparing incremental gastro-intestinal peptides levels at particular time points, we found CARB meal resulted in significantly greater suppression of ghrelin compared with baseline at 4 h followed by a rebound to a nonsignificantly higher ghrelin at 6 h compared with baseline. Concomitantly, GLP-1 at 6 h and GIP at 4 and 6 h were significantly incrementally lower with the CARB meal respectively compared with high-fat meals and MUFA meal. Furthermore, the late phase PYY response indicated by $iAUC_{(120\text{-}360min)}$ was significantly lower after CARB meal compared with MUFA meal. Although we did not measure subjective hunger in our subjects, we may postulate that high-carbohydrate meal may cause higher satiety at 4 h as a result of low ghrelin (orexigenic hormone) but is followed by earlier rebound of hunger at 6 h (secondary to lower anorexigenic hormone levels such as GLP-1) compared

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to high-fat meal and greater hunger from 2 to 6 h (lower $iAUC_{PYY}$) compared to high-MUFA intake. Other studies in healthy subjects reported that carbohydrate results in greater postprandial ghrelin suppression in the first 3 h than fat (Foster-Schubert et al., 2008), our study in abdominally obese subjects however demonstrated blunted ghrelin suppression by carbohydrate (signifantly lower ghrelin at 4 h only). This is pursuant to the finding that postprandial ghrelin response is independent of macronutrient composition in obese subjects (Gibbons et al., 2013; Koliaki, Kokkinos, Tentolouris, & Katsilambros, 2010). Some studies reported that high fat intake induces higher PYY and CCK responses compared with high carbohydrate (Gibbons et al., 2013; Wells, Read, Uvnas-Moberg, & Alster, 1997) but not all (Boyd et al., 2003; van der Klaauw et al., 2013). In line with two other acute studies comparing high-fat and high-carbohydrate meals between lean and obese men (Brennan et al., 2012; Yang et al., 2009), we found that alterations in fat quantity had similar impact on postprandial PYY and CCK in our obese subjects. The lipid-sensing action of PYY and CCK appeared to be less sensitive in overweight or obese subjects. In our study of abdominally obese subjects, we did not observe a differential impact of fat quality i.e. MUFA *vs* SAFA on gastro-intestinal peptides release. the differential effects of fat quality and fat quantity on gastro-intestinal peptides were not seen in our subjects with abdominal obesity with majority of them having impaired fasting glucose and insulin resistance. Since the release of gastro-intestinal peptides is controlled by neuronal and humoral interaction between the brain and gut, the impaired gastro-intestinal peptides secretion may reflect brain-gut miscommunication in this group of population (Yi & Tschop, 2012). The disregulation could cause over-feeding and dis-inhibition of liver glucose production, and thus facilitate the development of obesity and metabolic disturbances (Yi & Tschop, 2012).

University of Malaya

5.7 Fasting Gastro-intestinal Peptides Release

We observed a higher fasting GIP after 6 weeks of MUFA and SAFA diets compared with CARB diet, however only the difference between MUFA diet and CARB diet was statistically significant. There is good *in vivo* evidence that reduced fasting GIP concentrations are associated with improved insulin sensitivity (Nasteska et al., 2014; Yabe & Seino, 2013). Our finding is novel and may indicate that 7% en exchange with fat for a period as short as 6 weeks may result in reduced insulin sensitivity in abdominally obese individuals, although there were no significant differences in HOMA2-%S between the 3 diets. The gold standard measurement of insulin sensitivity is by use of the euglycemic hyperinsulinemic clamp or alternatively by utilizing the frequently stimulated IVGTT It is therefore still possible that our subjects may have become insulin resistant after dietary fat exposure. Numao et al. (2012) demonstrated that 3 days exposure to a very high fat diet (69% en) resulted in elevated fasting GIP compared with a 22% en fat diet and compared with baseline, postulating that this may have resulted from K cell hyperplasia. Our 31.5% en fat diet in the MUFA and SAFA arms may have produced a similar result.

It may also be possible that the increased fasting GIP concentrations as observed after a 6-week MUFA diet compared to CARB diet may not be related to the intrapancreatic actions. This may be explained by the observations of two short term feeding intervention studies which reported that high-fat overfeeding increased fasting GIP concentrations (Brons et al., 2009; Numao et al., 2012) possibly due to fat deposition. It was reported that GIP binds to its receptor on adipose tissue and increases the adipose tissue volume (Seino & Yabe, 2013). The increased fasting GIP levels as observed in MUFA diet group may indicate higher fat deposition (Nasteska et al., 2014) which needs further confirmation. However, we did not observe significant changes in % body fat

University of Malaya

between diets as measured by body composition analyzer. Hence, the difference may also be related to other GIP functions. No different changes were detected on fasting GLP-1, ghrelin, PYY and CCK concentrations irrespective of dietary alterations in fat quality or fat quantity, as reported by others (Beasley et al., 2009; Boyd et al., 2003; Ellis et al., 2012; Lithander et al., 2008; Paniagua et al., 2007; Seino & Yabe, 2013).

5.8 Postprandial Lipid Profile

In agreement with concrete evidence from the work of others on lipaemic response after high-fat *vs* low-fat meals (Lopez et al., 2011; Thomsen et al., 1999; Thomsen et al., 2003), we found that high-fat meals, regardless of type, induced greater extent of TAG elevation and lower NEFA suppression. However, the origin or source of the fat may modulate lipaemic response as shown in a previous study by Teng et al (2011). Two SAFA meals enriched with 1) palm olein and 2) lard were compared with a MUFA meal prepared with olive oil. The palm olein-based SAFA meal (21% en palmitic acid) induced a similar lipaemic response when compared with the olive-oil-based MUFA meal. On the other hand, a significantly lower lipaemic response was displayed after lard-based SAFA meal. The results may indicate fat sources that differ by saturation, origin (plant- or animal-derived) and physical state (solid *vs* liquid fat content) can cause diverse lipaemic effects. It has been postulated that the higher solid fat content of certain lipids at room temperature may decrease the rate of absorption which then lowers the postprandial lipaemic response (Berry et al., 2007).

Our present study found that the NEFA suppression following a high-carbohydrate meal is greater compared with both high-fat meals as greater insulin secretion in response to carbohydrate inhibits lipolysis. In concordance with our findings, other studies have also demonstrated that the higher carbohydrate:fat ratio alleviates

University of Malaya

postprandial NEFA concentrations (Frayn, 2001; Whitley et al., 1997), which could be explained by the NEFA-suppressing effect of insulin overriding NEFA spillover as a consequence of high fat intake (Karpe, Dickmann, & Frayn, 2011). In addition, the suppression of NEFA by a high-carbohydrate meal promotes the storage of body fat.

5.9 Fasting Lipid Profile and Lipid Subfractions

SAFA have been labeled as "bad fats" due to the widely accepted belief that SAFA elevate TC and LDL-C level (Malhotra, 2013; Siri-Tarino, Sun, Hu, & Krauss, 2010). Early research reported a correlation between saturated fats and CAD (Ascherio et al., 1996; Keys et al., 1966) hence health authorities have recommended reducing SAFA intake. However, recent systematic reviews and meta-analyses have reported insufficient evidence to support the association between SAFA and CVD, CHD or stroke (Chowdhury et al., 2014; Siri-Tarino et al., 2010) and suggested that the source of SAFA may be a more important factor (Malhotra, 2013; Sanders, 2013).

The current study compared 7% en exchange between SAFA and MUFA from palm olein and high-oleic sunflower oil. We found that levels of TC and LDL-C were significantly higher on SAFA diet compared with MUFA diet. It is also noteworthy that HDL-C level was higher after SAFA diet as well, when compared with CARB diet. This may suggest the HDL-elevating effect of SAFA neutralizes/offsets the potential adverse effect of elevated LDL secondary to SAFA on CVD risk. These changes in lipids in our subjects were in agreement with the predicted estimation by Mensink's equation (Mensink & Katan, 1992), which indicates good compliance of the study population with the diet treatments. Our finding concurs with those of an 8-week study (S. J. van Dijk et al., 2009) which compared isocaloric diets with an equivalent 9% en exchange between SAFA and MUFA components from diets prepared mainly with refined olive oils where

University of Malaya

PUFA content was standardized. This study demonstrated the positive effects of MUFA over SAFA in lowering TC and LDL-C levels but also demonstrated that MUFA did not elevate HDL-C level in 10 abdominally obese subjects. Furthermore, the RISCK study, a large scale multi-center trial with longer feeding period (6 months) and larger sample size found that SAFA diet (n = 85) increased TC, LDL-C and apo-B concentrations compared with MUFA (n = 227) and CARB diet (n = 237) (Jebb et al., 2010). The diets were isocaloric: palm olein and milk fat were used as SAFA sources while the MUFA diet utilized high oleic sunflower oil and olive oil. Another 3-month large scale controlled trial, the KANWU study (n=83 (SAFA) *vs* n=79 (MUFA)), also provided evidence that TC, LDL-C and apo-B levels were elevated after consuming isocaloric diets rich in SAFA sourced from butter and margarine compared with MUFA based in olive oil and high oleic sunflower oil in healthy subjects (Vessby et al., 2001).

In contrast, others have shown that consumption of diets rich in palm olein however resulted in similar effects on serum lipids when compared with olive oil in healthy subjects (Voon et al., 2011). A study by Voon et al. (2001) compared different cooking oils i.e. palm olein, extra virgin olive oil and coconut oil, instead of making head-to-head comparisons of test fatty acids by standardization of PUFA content across diets. Hence, this lack of standardization for PUFA content may explain the nonsignificant differences in lipid markers, i.e. TC, LDL-C, HDL-C, TAG, TC:HDL-C, apo (A), apo (B) and Lp (a) during fasting and non-fasting states after the 5-week intervention: a limitation of the study design.

Although meat and palm olein generally contain equivalent amounts of SAFA and MUFA (Lawrence, 2013), both have been labeled as sources of SAFA and hence the public has been advised to reduce intake of meat and palm olein. It is important to make a distinction between processed meat and red meat , as it is processed meat and not red meat that has been found to be associated with CAD in a meta-analysis (Micha &

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Mozaffarian, 2010), suggesting that other factors, i.e. preservatives, but not SAFA per se increases CAD risk (Lawrence, 2013). SAFA content of palm olein is double that found in olive oil; while polyunsaturated fatty acid (PUFA) content in palm olein is similar to that in olive oil. Hence, it is possible that the PUFA content of palm olein may have some cardio-protective effect.

In terms of low-fat/high-carbohydrate intake, the RISCK study (Jebb et al., 2010) found that HDL and apo-A1 concentrations decreased in subjects at risk of developing metabolic syndrome on a CARB-enriched diet for 6 months relative to a SAFA and MUFA-enriched diets. In addition, the high CARB diet also increased TC:HDL-C ratio relative to MUFA diet. Our study findings agreed, in part, with the RISCK study, as we also observed lower HDL-C levels after CARB diet compared with SAFA diet but not the MUFA diet. The shorter feeding period (6 weeks) in our study may explain the different observations. Besides the quantity of CARB intake, the type of CARB (i.e. low and high GI) also plays a role in determining the lipid profile. It is interesting that the RISCK study also reported greater reductions in TC and LDL-C concentrations following low GI intake compared with high GI intake (Jebb et al., 2010).

Observational studies report higher sdHDL subfraction and lower large HDL subfractions in the patient groups with the diagnosis of coronary artery disease (Superko et al., 2012; Xu et al., 2015). In addition, HDL_3 which is a small, dense HDL subfraction may predict lower risk for CHD (Superko et al., 2012). The current study did not observe a change in % sdLDL between diets, as reported by other similar studies such as the RISCK study (Jebb et al., 2010). The CARB diet in our study (in comparison with the SAFA diet) however was associated with lower fasting HDL cholesterol. Lipid subfraction analysis showed that the decrease in HDL was due to a reduction in HDL_3 which was associated with a concommitant increase in % sdHDL when compared with SAFA diet ($P < 0.05$). SAFA diet on the other hand increased the large HDL3 subfraction

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compared with both CARB and MUFA diets ($P < 0.05$), respectively. A study comparing soybean oil (MUFA) and butter (SAFA) in hypercholesterolemic Caucasians found no significant difference in HDL-2 and HDL-3 subfractions after a 7-week diet intervention (Coutinho et al., 2011). To our knowledge, no other published studies have compared the effects of high-fat and high-carbohydrate diets on HDL subfractions. Taken together, our study findings imply that high carbohydrate consumption may increase CAD or CHD risk as compared to high SAFA intake as high carbohydrate intake is associated with higher sdHDL subfraction, lower large HDL subfraction and lower HDL3.

While cardiologists recommend low fat consumption for CVD prevention, the resultant increased carbohydrate intake may increase the risks of T2DM and obesity development. This controversy gained much attention after the Dietary Guidelines Advisory Committee urged the 2015 Dietary Guidelines for Americans to remove the upper limit of fat intake (currently 35% en) as evidence concluded that reducing total fat intake does not reduce cardiovascular risk; instead healthful-food diet pattern and optimizing certain types of fatty acids are now the focus of the new policy (Mozaffarian, 2015).

5.10 Pulse Wave Analysis

There have been limited investigations with regards to pulse wave analysis in other similar studies such as Delgado-Lista et al. (2008), Paniagua et al. (2007) and Chang et al. (2015). We found no significant differences between dietary interventions in terms of all component markers of the analysis. Our current findings are in agreement with a large scale long term study with 5% en exchange between SAFA, carbohydrate and MUFA for 6 months in subjects at high risk of developing metabolic syndrome (Sanders et al., 2013). The diet did not exert significant impact on arterial stiffness and peripheral

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augmentation index. It is possible that the short 6 week exposure of our dietary interventions was not sufficient to induce detectable changes in arterial pressure and stiffness. Both augmentation index and augmentation pressure in this study displayed continuous decrement from baseline after the test meal consumption. These findings can be explained by the fact that food consumption, possibly related to insulin release, can alter the contour of the arterial pressure wave (O'Rourke, Pauca, & Jiang, 2001).

5.11 Strengths and Limitations

The present study has limitations. The good compliance of the subjects' participation in this study may be influenced by the fact that majority of them were MPOB staffs and most of them had participated in similar research before. We did not select this particular group on purpose however the nature of this study that subjects had to attend 3 times per working day for meal collection at MPOB has limited the willingness to comply by many other interested volunteers working far away. Furthermore, MPOB staffs were well educated and understood the policy of diet intervention study hence having high confidence in the safeness of the test meals and gave high compliance throughout this 18-week study. Besides, this study might be limited by gender imbalance where 75% of the subjects were females. This may be affected by higher female to male ratio in MPOB; furthermore many male smokers were excluded from participation. However, gender was treated as the within-subject factor in this study which prevented gender bias.

As the study subjects were abdominally obese with the majority having insulin resistance and undiagnosed dysglycemia prior to enrolment despite their young age, the findings from this study may not be generalized to all other populations. We included such subjects with metabolic disorders as it represents the true scenario of abdominally obese Malaysians and to avoid exclusion bias. Besides, we proved the significant

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difference in insulin corrected to glucose and DI was not a statistical bias through subgroup analysis. The subjects were of South East Asian origin and hence they represented a high-risk population who are susceptible to the development of diabetes mellitus (WHO, 2004). Our study utilized South East Asian diets in the dietary intervention, our findings cannot therefore be extrapolated to other populations with diets of differing macronutrient composition even for other Asian diets, i.e. South Asian diet and East Asian diet.

It is to be noted that no power calculations were carried out in relation to the postprandial part of the study, and that power analysis for sample size was not performed based on all markers measured. There might be chances of statistical type 1 errors due to the high number of statistical analyses performed. The consequence might be that some of the statistical differences were only observed by chance (for example the group differences in iAUC for IL-6 and D-dimer; the significant difference in ANOVA but no difference between groups for insulin secretion corrected for glucose and DI). These results need further confirmation in a larger scale study with power calculation.

There are several strengths of the study. Firstly, this study design incorporated a controlled dietary intervention under free-living conditions that reflects South East Asian diets. Another strength of our study is the higher proportion of complex and refined carbohydrate sources utilized which is reflective of the dietary pattern in South East Asia and perhaps even other Asian populations in India and China. In comparison with the Mediterranean and typical American diets, effects of this carbohydrate-heavy diet in Asian subjects which is consumed by a large proportion of the world's population has not been as well delineated in the literature. Besides, we achieved good compliance with the dietary intervention that resulted in expected changes in lipid profile and plasma fatty acid composition, as well as a constant subject body weight across all 3 diets for the entire study duration. The good compliance was also corroborated by the > 97% of the weekday

University of Malaya

meal collection, and the weekend oil usage reflects the commitment of study subjects and vigilance of the investigating team. We provided three cooked meals daily with more than 70% experimental fat exchange, which allowed a head-to-head comparison of fatty acids tested. The changes in insulin secretion and sensitivity were measured at physiological levels, which reflected real life scenarios. The long duration of assessment of the postprandial challenge (up to 6 h) was strength of the study too. Lastly, unlike many studies we also calculated insulin response corrected for glucose and DI which are more valid measures of insulin secretion.

Keynotes

-Long term high-CARB diet may result in defects in insulin secretion secondary to β-cell exhaustion, this may increase risk of T2DM development.

-HDL-elevating effect of SAFA neutralizes/offsets the potential adverse effect of elevated LDL secondary to SAFA on CVD risk.

-High sdHDL subfraction, low large HDL subfraction and HDL3 after CARB diet may have a more deleterious impact on cardiovascular risk.

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CHAPTER 6: CONCLUSIONS

We found that the type of fat consumed i.e. plant-source palm olein SAFA *vs* high oleic sunflower oil sourced MUFA (exchanged at 7% en) did not exert differential impact on glucose metabolism, insulin sensitivity and secretion, inflammatory response, endothelial and vascular functions in abdominally obese Asian individuals during fasting state after 6 weeks exposure. On the other hand, the 7% en exchange of dietary fat with high refined carbohydrate may not be enough to impose a significant change on the aforementioned biomarkers after only 6 weeks. However, it is noteworthy that high carbohydrate consumption for a short duration of 6 weeks with a small increase from 55% to 62% en which is close to the upper limit of the recommendation of 45-65% en by the Dietary Guidelines of America, adversely affected HDL subfractions in this high-cardiovascular risk group of abdominally obese nondiabetic Asian subjects. Plant-source SAFA on the other hand increased beneficial large HDL subfractions. Further confirmatory studies are needed for our novel observation of higher fasting GIP levels after the high MUFA and SAFA intake compared to high refined carbohydrate intake (although only the comparison with the MUFA diet was statistically significant) which might indicate increased insulin resistance with higher fat diets.

The addition of 70 g of carbohydrates to the CARB meal during a postprandial challenge resulted in exaggerated postprandial glycaemia and insulinaemic responses, hence leading to significantly greater $iAUC_{0\text{-}120min}$ for C-peptide, glucose and insulin compared with SAFA and MUFA meals. Importantly, insulin response corrected for glucose ($iAUC_{insulin/glucose(0\text{-}120min)}$) and DI with the CARB meal however tended to be lower than that with the SAFA and MUFA meals. Our findings imply that prolonged exposure to only minimally increased dietary carbohydrate for a short duration and the consumption of high refined carbohydrate resulting in increased insulin hypersecretion

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may cause beta-cell exhaustion and more rapid progression to T2DM in abdominally obese individuals. SAFA increased fasting TC and LDL-C concentrations compared to MUFA but also increased HDL (large subfractions) hence total/HDL cholesterol ratio was not different between diets, MUFA however elevated postprandial D-dimer. In addition to the adverse impact on atherogenic fasting state HDL subfractions, high refined carbohydrate also induced higher postprandial IL-6 response indicative of deleterious inflammation. These findings lend weight to the hypothesis that high carbohydrate intake may have a more deleterious impact on cardiovascular risk in high risk subjects compared with high plant-source SAFA intake. Further confirmation is needed on the impact of high refined carbohydrates and MUFA on proinflammatory and thrombogenic responses postprandially.

Translating the results of our study into daily diet recommendation, there is no harm in using palm olein as culinary oil compared to high-oleic sunflower oil in our daily cooking. The incorporation of palm olein at moderate amount i.e. 56 g/day in our daily diet will not affect insulin secretion and sensitivity, moreover it may carry a cardio-protective effect owing to its rich polyphenols content. On the contrary, it is noteworthy that a lower-fat/higher-carbohydrate diet i.e. 39 g/day high-oleic sunflower oil with increased refined carbohydrate may result in poorer insulin secretion and increased risk of T2DM development besides impaired HDL subfractions which then attenuated cardiovascular function, in the long run. However, it is possible that lower-fat/higher carbohydrate diet with increased low glycemic index carbohydrate or complex carbohydrate intake, for example fruits, vegetables and grains may not cause detrimental health effects. All in all, people should focus on healthful-food balanced diet pattern instead of avoiding or focussing on any single food.

To further enhance our knowledge in this area of research, it is recommended that the following future researches of interest to be conducted:

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1. Future investigation should be carried out on subjects with specific diseases, such as T2DM and CVD as well as thin subjects with fatty liver, and to compare the effects of therapeutic diets on them, for example low-glycemic/complex carbohydrate diet and high-antioxidant diet.
2. Larger sample size and longer background diet duration should be applied in future studies.
3. Power analysis for sample size of future studies should be performed based on fasting GIP, and postprandial insulin secretion, DI, IL-6 and D-dimer to confirm our significant different findings.
4. Responses to dietary treatments should be compared among different subject groups, for example NFG, IFG and T2DM.

Keynotes

-The type of fat i.e. SAFA and MUFA did not exert differential impact on glucose homeostasis, insulin sensitivity, insulin secretion and gastrointestinal peptide release under fasting and postprandial conditions.

-High-CARB meal lowered insulin secretion corrected for glucose and DI, which may be detrimental to β-cell function and increase risk of T2DM in the long run.

-A small 7% en exchange of carbohydrate with SAFA adversely impacts on fasting HDL subfractions and postprandial IL-6 response, indicating that high CARB intake may increase CVD risk compared with plant source SAFA.

-SAFA increased fasting TC and LDL-C concentrations vs MUFA but also increased HDL (large subfraction) hence total/HDL cholesterol ratio similar between diets. Therefore plant source SAFA may have neutral effect on CVD risk.

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