AMERICAN UNIVERSITY OF BEIRUT

THE ROLE OF PHOSPHORUS IN POSTPRANDIAL ENERGY EXPENDITURE OF HEALTHY MALE SUBJECTS

by
RIM IBRAHIM BAALBAKI

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Nutrition and Food Science
of the Faculty of Agricultural and Food Sciences
At the American University of Beirut

Beirut, Lebanon
September 2019

AMERICAN UNIVERSITY OF BEIRUT

THE ROLE OF PHOSPHORUS IN POSTPRANDIAL ENERGY EXPENDITURE OF HEALTHY MALE SUBJECTS

by

RIM IBRAHIM BAALBAKI

Approved by:

Dr. Omar Obeid, Professor
Nutrition and Food Sciences — Advisor

Dr. Elie-Jacques Fares, Assisstant Professor
Nutrition and Food Sciences — Member of Committee

Dr. Hala Ghattas, Associate Research Professor
Epidemiology and Population Health — Member of Committee

Date of thesis defense: September 4th, 2019

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name: Baalbaki (Last) Rim (First) Ibrahim (Middle)

⊗ Master's Thesis ○ Master's Project ○ Doctoral Dissertation

✗ I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

I authorize the American University of Beirut, to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes

after : **One ---- year from the date of submission of my thesis, dissertation, or project.**

Two ---- years from the date of submission of my thesis, dissertation, or project.

Three ---- years from the date of submission of my thesis, dissertation, or project.

Rim 16/9/2019

Signature Date

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my advisor dr. Omar Obeid for his trust, for the opportunity he gave me to participate in this research study, and for his valuable help.

My special thanks go to Dr. Elie-Jacques Farres for his constant assistance in the experimental work and the data analysis. Thank You!

I am also grateful to my third committee member, Dr. Hala Ghattas, for her precious time and cooperation.

I would like to express my sincere thanks to the research assistants Ms. Carla el Mallah, Ms. Nehmat El Helou, Ms. Marie Elisabeth Ragy and Ms. Dima Wattar for their valuable help whenever needed.

I am also thankful to my participants, for their time and trust.

I am deeply grateful to my beloved parents and husband for their continuous support. I cannot express my love and respect for you. Thank you for always helping and pushing me to pursue my goals.

To my kids, you have been my ultimate motivation, through all the ups and downs. I will be forever thankful for being your mother.

AN ABSTRACT OF THE THESIS OF

Rim Ibrahim Baalbaki for Master of Science
Major: Nutrition

Title: The Role of Phosphorus in Diet-Induced Thermogenesis of Healthy Male Subjects

Diet induced thermogenesis is the increase in postprandial energy expenditure above the basal metabolic rate divided by the energy content of the food consumed during the meal, and it is usually expressed as a percentage (Westerterp, 2004). Each macronutrient has a specific DIT range, with the proteins having the highest DIT (20-30% of the energy content), following by the carbohydrates (5-15%), and then the fats with the lowest DIT of (0-3%) (Westerterp et al, 1999). Phosphorus availability is an important determinant of DIT, since it is needed for ATP production. Refined carbohydrates and high sugar foods in addition to high saturated and trans-fats containing foods, that are linked to an increased obesity risk are low in phosphorus. Whereas protein rich foods (like meats, fish, poultry and dairy products) in addition to plant based foods such as nuts, unrefined grains, and beans, which are all related to decreased risk of obesity, are high in phosphorus (Halton et al, 2004). Previous studies have been done to measure the impact of phosphorus supplementation on DIT after consuming either a high protein meal (Abdouni et al, 2018) or a high carbohydrates meal (Assaad et al, 2018), and both studies concluded that phosphorus does increase PEE. Thus, in line with the previous studies, the present study is conducted to measure the involvement of phosphorus on DIT and respiratory quotient (RQ) of healthy male subjects consuming a high saturated fat meal.

This study is a double-blind, randomized, and placebo-controlled crossover clinical trial. Twelve healthy male subjects with an age range between 19 and 35 years and with a body mass index ranging from 19.2-34.6 Kg/m^2 were recruited through direct approach. They were asked to come to the testing room after an overnight fast at 8:00 am. Anthropometric measurements (weight and height) were taken, then a finger prick experiment was done to determine the HbA1c blood level using the DCA VANTAGE SIEMENS MACHINE, and urine was collected to determine creatinine concentration in urine using VITROS 350 CHEMISTRY SYSTEM. Then the subjects were asked to rest for

30 min on a couch in a supine position, the resting REE including VO2 and VCO2, and RQ was measured for 30 min using the ventilated hood and canopy system (COSMED QUARK CPET UNIT, Italy) for indirect calorimetry measurement. Subjects had 15 min to consume the high saturated fat meal with the appropriate supplement. The supplements were either 500 mg of phosphorus tablets or placebo tablets that were given in a random order each on one visit. Finally, the postprandial REE and RQ were measured for 4 hours, divided into a 15min measurement, followed by a 15min break. Subjects undertook 2 sessions in a random order over 2 different days which were separated by a minimum of one week as a washout period.

The addition of phosphorus to the high saturated fat meal significantly increased the percent change from baseline in PEE; suggesting an increase in the production of ATP that depends primarily on the availability of phosphorus. Moreover, there was a significant difference between the two treatments in RQ, but there was no significant difference in the percent change in RQ for the phosphorus treatment compared to the control, suggesting no difference in fat oxidation when supplementing phosphorus to the high saturated fat meal compared to the control. When calculating the AUC for each variable, we only found a significant difference in the AUC for the RQ of phosphorus treatment compared to the control.

In line with the previous studies (Abdouni et al 2018, Assaad et al, 2018), phopshporus was able to increase the percent change from baseline in PEE, suggesting that phosphorus is involved in DIT. Moreover, results from this study may prove to be of vital importance for future work on the potential use of phosphorus supplements for weight reduction.

Keywords: Phosphorus, Diet-induced thermogenesis (DIT), Post-Prandial Energy Expenditure (PEE), Adenosine triphosphate (ATP), Respiratory Quotient (RQ), High saturated Fat.

CONTENTS

ILLUSTRATIONS

TABLES

ABBREVIATIONS

%	Percent
/	Per
&	And
<	Less than
>	Greater than
=	Equal to
±	Plus or Minus
Δ	Difference
βAR	Beta Adrenergic Receptors
AA	Arachidonic Acid
ALA	α-linolenic acid
AMDR	Acceptable Macronutrient Distribution Range
ATP	Adenosine Triphosphate
AUB	American University of Beirut
AUC	Area Under the Curve
BMI	Body Mass Index
CHD	Coronary heart disease
cm	Centimeter
CVD	Cardiovascular disease
CO_2	Carbon Dioxide
DHA	Docosahexaenoic acids
DIT	Diet Induced Thermogenesis
dL	Deciliter
DM II	Type 2 diabetes
DRI	Dietary Reference Intake
E	Energy
EE	Energy Expenditure
EPA	Eicosapantaenoic acids
Et al.	And Others
FAO	Food and Agriculture Organization
g	Gram

HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HSF	High saturated fat
IBD	Inflammatory Bowel Disease
IRB	Institutional Review Board
Kcal	Kilocalorie
Kg	Kilogram
LA	Linoleic acids
LDL-c	Low-density lipoprotein Cholesterol
m^2	Square meter
mg	Milligram
min	Minute
MUFA	Monounsaturated fatty acids
n	Number of subjects
O_2	Oxygen
P	Phosphorus
PEE	Post-prandial Energy Expenditure
PPAR	Peroxisome proliferator-activated receptor
PTH	Parathyroid Hormone
PUFA	Polyunsaturated Fatty Acids
RDA	Recommended dietary allowance
REE	Resting energy expenditure
RMR	Resting metabolic rate
RQ	Respiratory quotient
SEM	Standard error of the mean
SFA	Saturated Fatty Acids
TC	Total Cholesterol
TEE	Total energy expenditure
TEF	Thermic effect of food
TW-ANOVA	Two-way analysis of variance
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Total energy expenditure consists of three components: Basal metabolic rate, which is the energy required by the body when at rest to ensure vital functions; energy cost of physical activity; and diet induced thermogenesis.

Post-prandial energy expenditure (PEE) is the increase in metabolic rate above the basal metabolic rate that occur after ingestion of food, it is the energy required for the digestion, absorption, transport and storage of food. Although DIT consists only of 5-15% of total energy expenditure, it can play a major role in the development of obesity. Many studies were conducted to determine the major determinants of DIT, a study (Westerterp, 2004) found that DIT is mostly affected by the energy content of the food, and the protein and the alcohol fraction of the diet.

Another important determinant of DIT is phosphorus availability; Evidence has shown that the obesogenic, energy dense, and low nutrients diets are also low in phosphorus. The decreased phosphorus intake accompanied by an increase in carbohydrates intake will lead to an increased insulin secretion, to promote the uptake of carbohydrates and phosphorus by the peripheral cells; leading to a decreased availability of phosphorus for ATP generation, essentially needed postprandially for DIT.

A low DIT has been closely linked to obesity. The decreased hepatic ATP lead to signals transmission to the nervous system to increase energy intake. Moreover, the reduced phosphorus status will affect the oxygen carrying capacity of hemoglobin, which will lead to a decrease in physical activity. All of those factors will cause an overall decrease in energy expenditure and an increase in energy intake, and will eventually increase the risk of obesity (Obeid, 2013).

Phosphorus is mainly found in protein rich foods such as meats, fish, poultry, dairy products and also in plant based foods such as nuts, unrefined grains, and beans. Thus, weight loss caused by having a high protein diet for increased satiety and better insulin sensitivity (by limiting refined carbohydrates and sugars, and saturated and trans fats), may also be due to the fact that protein rich foods are also high in phosphorus. (Halton et al, 2004).

Two studies were conducted to determine the impact of ingestion of phosphorus on the DIT of lean and obese subjects. The first study (Abdouni et al, 2018) was to determine the impact of ingestion of phosphorus with a high protein-low phosphorus meal compared to normal protein-low phosphorus meal. they compared the DIT between the respective meals supplemented by either phosphorus or placebo, and reported a significant increase in postprandial energy expenditure with phosphorus supplementation independent from the protein content of the meal. The second study (Assaad et al, 2018) was to determine the impact of ingestion of phosphorus with a high carbohydrate meal. They compared the DIT between supplementation of either phosphorus or placebo with meals, and reported a significant increase in EE with the ingestion of Phosphorus. Therefore, it was reasonable to conclude that phosphorus is involved in DIT.

In line with the previous studies, the present study is conducted to measure the involvement of phosphorus on DIT of male subjects consuming high saturated fat meals. The overall hypothesis of this study, is that phosphorus ingestion by healthy subjects would be associated with an increase in DIT.

CHAPTER II

LITERATURE REVIEW

A. Diet induced thermogenesis and obesity

Diet induced thermogenesis can be defined as the increase in postprandial energy expenditure above the basal metabolic rate divided by the energy content of the food consumed during the meal, and it is usually expressed as a percentage (Westerterp, 2004). Each macronutrient has a specific DIT range, with the proteins having the highest DIT (20-30% of the energy content), following by the carbohydrates (5-15%), and then the fats with the lowest DIT of (0-3%) (Westerterp et al, 1999).

Many studies were conducted to find the determinants that mostly affects DIT. There are biological determinants of the individuals, and determinants that are related to the food and habits. One of the biggest biological determinants was found to be insulin sensitivity and the sympathetic nervous activity; a study (Watanabe et al, 2006) found a negative correlation between thermic effect of food (TEF) and a suppressed insulin sensitivity, and a positive correlation between TEF and the sympathetic nervous activity. They concluded that low insulin sensitivity decreases the response in the sympathetic nervous activity, which will lead to a low postprandial energy expenditure, hence low thermic effect of food. Moreover, βARs were found to be essentials in the process of the sympathetic nervous signaling. In a study conducted on mice (Bachman et al, 2002), β-less mice were found to have a 16% lower metabolic rate then β-with mice, and had increased leptin levels consistent with an increase in fat mass. On the other hand, UCP1 was found to be essential for the diet-induced adrenergic thermogenesis in mice, and its ablation induces obesity and abolishes DIT (Feldmann et al, 2008). In addition to the biological determinants, a big determinant of TEF was found to be diet composition. Westerterp et al (1998) found that macronutrients have a direct effect on DIT, with the proteins having the highest thermic response, and fats having a diminished thermic response. Moreover Westerterp (2004) found that the main determinant of DIT is the energy content of the food and the protein fraction of the diet. Since DIT is related to the activation of energy requiring processes of absorption, initial metabolism and storage of food prior to their oxidation, energy content of the food is a big determinant of DIT. Moreover, the high DIT of proteins is thought to regulate body

weight by increasing satiety (Westerterp, 2004). Another important determinant of DIT is meal pattern, irregular meal pattern lead to a lower DIT compared to regular meal pattern (Farshchi et al, 2004). Finally, duration of the meal and the number of shews were also shown to affect DIT (Hamada et al, 2014). More shews and higher duration of the meal increase DIT.

Fats were shown to have the lowest DIT in macronutrients, with protein having a 3-fold higher DIT than fats (Tentolouris et al, 2007). However, the different types of fats themselves were shown to have different DIT. Unsaturated fatty acids were shown to have higher thermogenic effect as compared to saturated fatty acids (Takeushi et al, 1995), the proposed mechanism was an observed lower sympathetic nervous activity in BAT of rats when fed SFA than PUFA. Similar results were shown for humans, with a higher DIT when consuming high PUFA/SFAs ratio over a prolonged period compared to low PUFA/SFA ratio (Lichtenbelt et al, 1997; Clevenger et al, 2014). The possible mechanism suggested by Clevenger et al (2014) was the greater ability of PUFAs to activates PPARs compared with SFA, in addition to the enzymes responsible for the unsaturated fatty acids oxidations (enoyl co-A isomerase and 2,4-dienoyl-coA reductase) that are involved in DIT. Comparing SFA and MUFA, Piers et al (2002) found no difference in DIT, but rather found a higherw DIT for high waist circumference subjects than low waist circumference subjects after consumption of olive oil (MUFA), but this difference was not seen for subjects who consumed cream (SFA). The possible mechanism suggested is a higher activation of the sympathetic nervous system by MUFA, and a higher response to catecholamines by the abdominal adipocytes than the gluteo-femoral adipocytes. Same results were demonstrated by Soares et al (2003).

Finally, comparing MUFA and PUFA, K. R. Polley et al (2018) and Jones et al (2008) found greater DIT following MUFA rather than PUFA consumption. On the other hand, Clevenger et al (2014) found a greater DIT for PUFA rather than MUFA, but this contradicting result may be due to the fact that Clevenger et al's (2014) study population were premenopausal women, and there are potential metabolic differences between males and females causing this difference as mentioned by K. R. Polley et al (2018).

Obesity is an epidemic that has been on the rise worldwide (Abuduli et al, 2016). In 2008, WHO reported that more than 1 in 10 of the world population were obese. Obesity has been linked to several serious diseases like diabetes mellitus and cardiovascular diseases. And efforts to counteract it were deemed ineffective, mainly because of the relapse after weight loss treatments (Carneiro et al, 2016). Obesity is mainly due to an imbalance between energy intake and energy expenditure that lead to an accumulation of fat storage. Low energy

expenditure may be due to low REE, low energy from physical activity, and low DIT or a combination of those three (Roberts, 1995). The association between DIT and obesity has been studied extensively and have led to contradictory findings. In a study comparing DIT for obese and non-obese men, Segal et al (1990) found DIT to be significantly lower for obese participants for the 3h after meal consumption. Another study by Schutz et al (1984) showed similar results; comparing obese and non-obese women, they found a lower DIT for obese women; and a negative correlation between DIT and body fat percentage, they attributed the low DIT to a defective sympathetic nervous system response for obese subjects. Another study however, found no correlation between leanness or obesity and DIT, but rather found a positive relation between DIT and the caloric content of the meal (D'Alessio et al, 1988). Some studies investigated the relation between obesity and PEE by employing a weight loss regimen (Granata et al, 2002). Ravussin et al (1983) found a positive relationship between thermic effect of food and weight loss. Weight loss significantly increased PEE in noninsulin dependent obese subjects. Moreover, the change in PEE with weight loss was shown to be dependent on the body type (gluteo-femoral obesity or abdominal obesity), den Besten et al (1988) reported an increase in in PEE following weight loss for gluteo-femoral obese women and not in abdominally obese women. Other studies however (Armellini et al, 1999), have shown that PEE for obese subjects was independent of the regional fat deposition; but rather the low PEE in obese subjects could be due to a large inactive fat mass (total adipose tissue) that leads to hyperinsulinemia and insulin resistance and reduced glucose uptake and storage.

B. Fats Functions, sources and requirements

Dietary fats can be defined as the lipids in plants or animals that can be consumed as food (FAO, 2010). Triglycerides mostly compose glycerolipids, accompanied by little amounts of phospholipids, monoacylglycerol, diacylglycerol, and sterols and sterol esters. Fatty acids mainly compose these entities and are required by humans for energy, and structural and metabolic activities. Those fatty acids can be divided into three classes, depending on the presence and number of double bonds: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Most of the double bonds of the naturally occurring unsaturated fatty acids are in the cis position, which means the hydrogen atoms on the double bonds are on the same side; when they occur in the opposite side, the

double bond will be in the trans orientation (FAO, 2010).

SFA are mainly provided by animal fats (butyric, caproic, caprilic and capric acids…), but can also be provided by tropical oils like peanuts (arachidic, behenic, and lignoceric acids…), coconuts (caprylic, capric, lauric, and myristic acids) or palm oils (lauric, myristic and palmitic acids…). MUFAs occur mainly in the cis-form oleic acid that is essentially provided by olive oil, canola oil, and high oleic sunflower and safflower oil. PUFAs on the other hand, mostly occur in two forms, either in n-6 family (linoleic acids (LA) and arachidonic acids (AA)) or n-3 family (α-linolenic acid (ALA), eicosapantaenoic acids (EPA) and docosahexaenoic acids (DHA)). Linoleic acids' typical sources are vegetable oils, whereas arachidonic acids sources are animal fats, liver, egg lipids and fish. α-linolenic acid mainly comes from vegetable oils like flaxseed oil, perilla oil, canola oil, and soybean oil. EPA and DHA on the other hand, come from fish, especially oily ones like salmon, herring, anchovy… (FAO, 2010).

SFAs have different roles and effects in the human body. Long chain saturated fatty acids (LCSFA) are essentially used as energy sources that can be stored in the adipocytes in the form of triacylglycerol, Medium chain saturated fatty acids (MCSFA) on the other hand, are more readily oxidized in the mitochondria (Beermann et al, 2003). Actually, oxidation of SFA decreases with increasing the carbon chain length(laurate> myristate> palmitate> stearate) (FAO, 2010). Moreover, MUFAs and PUFAs are more readily oxidized than SFA (Piers et al, 2002). High MUFA diet was shown to have a cholesterol lowering effect especially when substituting a high SFA diet (Kris-Etherton et al, 1999; Kaseb et al, 2013). PUFAs on the other hand, have different physiological functions depending on their type. They have an essential role in regulating inflammatory processes. AA from LA is responsible for producing pro-inflammatory eicosanoids, that are involved in inflammation, vasoconstriction and platelet aggregation. EPA and DHA from ALA are responsible for producing anti-inflammatory eicosanoids and docosanoids, that are mostly vasodilatators and anti-aggregators. EPA and DHA when taken in high quantities as fish oil supplements, compete with AA and reduce their inflammatory cytokines production (Calder, 2006). Another biological role of PUFAs is gene regulation. PUFAs activate genes responsible for fatty acids oxidation and mobilization (like peroxisome proliferators activated receptors PPARα) and inhibit genes responsible for de novo lipogenesis (like sterol regulatory element binding protein SREBP-1). Thus, PUFAs shift fatty acids metabolism from synthesis and

storage towards transport and oxidation. They are primarily stored in cell membrane phospholipids and can modify membrane fluidity and thickness (FAO, 2010).

The acceptable macronutrient distribution range (AMDR) for total fat intake ranges between 20-35%. With 15% being the lower value for AMDR, to ensure adequate intake of essential fatty acids, and an absorption of fat soluble vitamins. And 35% being the upper limit, that is usually recommended for those engaged in high physical activity (FAO, 2010). However, fat intake should be in moderation, considering the quality of diet and subject's anthropometric measurements. The Dietary recommended intake of the different types of fats for adults, infants and children, and pregnant and lactating women are summarized in the bellow tables.

Table 1: Recommended dietary intake for total fat and fatty acid intake for adults (FAO, 2010)

			Level of Evidence			
Fat/FA	**Measure**	**Numeric amount**	**Convincing**	**Probable**	**Possible**	**Insufficient**
Total fat	AMDR U-AMDR L-AMDR	20–35%E 35%E 15%E		No relation with CHD events, fatal CHD, total cancer, or cancer subtypes		Risk of diabetes, metabolic syndrome components, body weight/adiposity
SFA	U-AMDR	10%E	C12:0–16:0 ↑ LDL and total/HDL ratio in comparison to cis MUFA or PUFA; ↑ LDL but no effect on total/HDL in comparison to carbohydrate		↑ risk of diabetes	Risk of hypertension, body weight/adiposity
MUFA	AMDR	By difference [a], [b]	↓ LDL and total/HDL ratio when substituting SFA (C12:0–16:0)		↓ risk of metabolic syndrome components	Risk of diabetes, body weight/adiposity, CHD events, total cancer or cancer subtypes
Total PUFA	AMDR (LA + ALA + EPA + DHA) U-AMDR L-AMDR AI	6–11%E 11%E 6%E 2.5–3.5%E	See above, for exchange of SFA for PUFA Essential (LA, ALA) ↓ risk of CHD events when PUFA replace SFA		↓ risk of metabolic syndrome components, diabetes ↑ lipid peroxidation with high consumption, especially when tocopherol intake is low Specific minimum to prevent deficiency unclear	Risk of body weight/adiposity, total cancer or cancer subtypes
n-6 PUFA	AMDR (LA) EAR AI	2.5–9%E 2%E (SD of 0.5%) 2–3%E	See above, for exchange of SFA for PUFA Essential (LA)	↓ risk of metabolic syndrome components, diabetes	Specific minimum to prevent deficiency unclear	Risk of body weight/adiposity, total cancer or cancer subtypes
n-3 PUFA	AMDR (n-3[c]) L-AMDR (ALA) AMDR (EPA + DHA)	0.5–2%E > 0.5%E 0. 250–2* g/day	↓ risk of fatal CHD events (EPA+DHA) Essential (ALA)		↓ risk of total CHD events, stroke Specific minimum to prevent deficiency unclear	
TFA[d]	UL	<1%E	↓ HDL and ↑ total/HDL ratio in comparison to SFA (C12:0–C16:0), cis MUFA or PUFA ↑ risk of CHD events	↑ risk of fatal CHD and sudden cardiac death ↑ risk of metabolic syndrome components, diabetes		Risk of body weight/adiposity, diabetes, total cancer or cancer subtypes

[a] Total fat [%E] – SFA [%E] – PUFA [%E] – TFA [%E] [b] can be up to 15 – 20 %E, according to total fat intake [c] ALA + n-3 long-chain PUFA [d] total TFA from ruminant and industrially-produced sources
* for secondary prevention of CHD

Table 2: Recommended dietary intake for total fats and fatty acids for infants (0-24 months) and children (2-18 years) (FAO, 2010)

Fat/FA	Age Group	Measure	Numeric Amount	Level of Evidence
Total fat	0-6 mo	AMDR	40-60%E	Convincing
		AI	based on composition % of total fat in HM,	Convincing
	6-24 mo	AMDR	gradual reduction, depending on physical activity, to 35%E[a]	Convincing
	2-18 yr	AMDR	25-35%E*	Probable
SFA	2-18 yr	U-AMDR	8%E* Children from families with evidence of familiar dyslipidemia (high LDL cholesterol) should receive lower SFA but not reduced total fat intake	Probable
MUFA	2-18 yr	AMDR	total fat [%E] - SFA [%E] - PUFA [%E] - TFA [%E]	Probable
Total PUFA	6-24 mo	U-AMDR	<15%E	Probable
	2-18 yr	U-AMDR	11%E	Probable
LA & ALA	0-24 mo	Comment	essential and indispensable	Convincing
n-6 PUFA				
AA	0-6 mo	AI	0.2-0.3%E[b]	Convincing
		U-AMDR	Based on HM composition as %E of total fat	Convincing
LA	0-6 mo	AI	HM composition as %E of total fat	Convincing
	6-12 mo	AI	3.0-4.5%E	Convincing
	6-12 mo	U-AMDR	<10%E	Probable
	12-24 mo	AI	3.0-4.5%E	Convincing
	12-24 mo	U-AMDR	<10%E	Probable
n-3 PUFA				
ALA	0-6 mo	AI	0.2-0.3%E[b]	Convincing
	6-24 mo	AI	0.4-0.6%E	Probable
	6-24 mo	U-AMDR	<3%E	Probable
DHA	0-6 mo	AI	0.1-0.18%E[b]	Convincing
	0-6 mo	U-AMDR	no upper value within the HM range up to 0.75%E	Convincing
	0-6 mo	Comment	conditionally essential due to limited synthesis from ALA	Probable
	6-24 mo	AI	10-12 mg/kg	Probable
	0-24 mo	Comment	critical role in retinal and brain development	Convincing
EPA+DHA	2-4 yr	AI	100-150 mg (age adjusted for chronic disease prevention)[c]	Probable
	4-6 yr	AI	150-200 mg (bridged from an infant value of 10 mg/kg)	Probable
	6-10 yr	AI	200-250 mg (to the adult value assigned at age 10 years)	Probable
TFA[d]	2-18 yr	UL	<1%E	Convincing

C. Fats, body weight, and diseases

Dietary fats play a major role in controlling body weight. Many diets have been proposed to induce weight loss and reduce cardiac risk factor. Diets varied from being low-carbs high fats diet (like Atkins diet), or very low fats diet (like ornish diets). Both diets induced weight loss after one year, but there was a problem with adherence to the diet for a long term, since they were considered too extreme (Dansinger et al, 2005). In an attempt to investigate whether a high polyunsaturated fats diet could attenuate the effect of a high

saturated fat diet, Crescenzo et al (2015) assessed body composition and energy balance after two weeks of feeding hamsters a high fat diet rich either in lard or safflower/linseed oil. They concluded that PUFAs decrease the obesogenic effect of a high fat diet and ameliorates blood lipid profile; it also decreases hepatic steatosis and mitochondrial oxidative stress. Same results were obtained for Yang et al (2017), when feeding a high MUFA and PUFA/SFA ratio to obese hamsters. This diet led to a decrease in body fat accumulation and a decrease in blood cholesterol and higher insulin levels. They attributed the lower obesogenic profile of hamsters fed high MUFA and PUFA diet to an increase in lipolytic enzyme activities. Maffeis et al (2001) investigated whether a fat meal is a risk factor for fat gain in children. They fed obese and non-obese children a high fat and a low-fat meal and took anthropometric measurements and indirect calorimetry after meal ingestion. They concluded that a high fat meal lead to a lower thermogenesis and a higher positive fat balance than a low-fat meal.

Considering fats and diseases, as evidence has shown, different types of fats have different effects on body's metabolism and blood lipid profile. SFAs have detrimental effects on body's fat accumulation and blood lipid profile. Consumption of SFAs have been linked to increased coronary heart disease risk, by increasing LDL-c and decreasing insulin sensitivity (Li et al, 2019; Siri-Tarino et al, 2010). This has led to a recommendation to decrease SFAs intake to less than 10% of energy intake, to decrease the risk of CVD. Replacing SFAs with MUFAs in animal studies has led to a decrease in LDL cholesterol without lowering the level of HDLs, which has led to a lower LDL/HDL ratio. Replacing SFAs with PUFAs on the other hand, had been shown to decrease both LDL and HDL cholesterol, but the decrease in LDL would be to a greater extend, thus leading to a lower LDL/HDL ratio (Siri-Tarino et al, 2010). In a meta-analysis of RCTs, Mozaffarian et al (2010) concluded that replacing SFAs by PUFAs decreased the incidence of CHD by 19%, each 5% increase in energy from PUFAs decreased CHD risk by 10%. The possible suggested mechanism was that this replacement decreased LDL-c without a reduction in HDL-c, leading to a lower TC/HDL ratio.

Comparing the benefits of a high MUFA diet to a low-fat diet, a high MUFA diet have been linked to a lower CVD risk, since it lowers blood cholesterol and blood TG (Kris-Etherton et al, 1999). Another risk factor for CVD is high blood pressure. MUFA was shown to decrease systolic and diastolic blood pressure compared to SFA, at total fat intake of 37% energy, but this effect was lost when having a higher fat intake (Rasmussen et al, 2006). In a

meta-analysis of prospective cohort studies, Yang et al (2016) concluded that long chain n-3 PUFAs, especially DHAs, are associated with a decrease in the incidence of high blood pressure.

Fat diets are also associated with inflammation that leads to insulin resistance and predispose individuals to DM II (Harford et al, 2011). SFAs lead to inflammation by activating inflammatory markers (like NFκB, TNF-α, interleukine-6), MUFAs on the other hand, do not lead to an activation of inflammatory markers. SFA and trans-FA alter insulin sensitivity whereas n-3 PUFAs improve it. The possible suggested mechanism is that SFAs impair insulin signaling pathways required for glucose uptake, and reduce adiponectin secretion (Siri-Tarino et al, 2010). Moreover, as have previously been shown, PUFAs can have inflammatory or anti-inflammatory properties depending on their type. n-6 PUFA are considered pro-inflammatory because of AA coming from LA that activates inflammatory markers such as leukotrienes and prostaglandins. n-3 PUFAs on the other hand, are anti-inflammatory. ALA is considered an essential FA, because humans cannot synthesize it. ALA produces EPA and DHA that mainly act as competitive substrate for AA. n-3 PUFAs can also inhibit NFκB pathway in inflammatory conditions. The significance of the anti-inflammatory properties of n-3 PUFAs can be of particular importance in controlling inflammatory diseases like IBD (Marion-Letellier et al, 2015).

Moreover, SFAs also may lead to atherogenesis by inducing a selective uptake of cholesterol by the capillary wall (Siri-Tarino et al, 2010). n-3 PUFAs decrease the risk of CVD by acting as vasodilatators and anti-aggregators, n-6 PUFAs on the other hand, increase the risk of CVD by having a contradictory effect, that is vasoconstriction and pro-platelet aggregation (FAO, 2010).

D. Distribution of phosphorus in the body

Phosphorus is a mineral that is naturally found in nature as an inorganic apatite form. It has a variety of oxidation state that ranges from PH-3 to P2O5. Phosphorus is second only to calcium among body inorganic elements. 85% of Phosphorus are found in skeletal tissues, 14% in soft tissues and only 1% in blood and body fluids. 70% of the phosphorus in human plasma is found as organic phospholipids. The remaining 30% inorganic phosphorus

will be either free (83%) or protein bound (17%). The majority of the free inorganic phosphorus found in plasma (80%) is in the form of HPO_4^{2-}, 20% is in the form of $H_2PO_4^-$, and less than 0.01% is found as PO_4^{3-}. Phosphorus consist of 1% of body weight, which means that a 70 Kg man will have 700g of phosphorus. The phosphorus in skeletal tissues are found as calcium phosphate at a ratio of 1:2 (P:Ca ratio). The phosphorus in the body have structural and metabolic roles. It can be resorbed from bones depending on body's needs by calcitriol and PTH, it is a component of genetic materials (DNA and RNA), of phospholipds (structural component of membranes) and phosphoproteins (cell proliferation and differentiation). Phosphorus' major metabolic functions are ensuring acid-base balance, being a major anion within body cells, and is essential to replenish energy compounds like ATP. Other important metabolic functions also exist. It is used for phosphorylation and dephosphorylation of compounds of substrates (like in glycogen synthesis and glycolysis), it delivers oxygen through 2,3 diphosphoglycerate, and is a component of growth factors or cytokines.

E. Phosphorus sources and requirements

Dietary phosphorus is mainly found in animal products that contain protein like dairy products, meat, fish, poultry and eggs; or in foods containing PO_4 additives. It is mainly absorbed in its inorganic form, so food additives increase phosphorus absorption drastically (Kalantar-Zadeh et al, 2010). Phosphorus is also found in lesser extent in cereal grains and many vegetables, including legumes (Anderson, 2013). Since it is highly abundant in the food supply, deficiency is highly rare. As per the National Academies Press (1997), the adequate intake (AI) and the recommended dietary allowance (RDA) of phosphorus are as such:

AI for infants for the first 6 months is 100mg/day

AI for infants from 7 to 12 months is 275mg/day

RDA for Children 1 through 3 years is 460 mg/day

RDA for Children 4 through 8 years is 500 mg/day

RDA for boys and girls 9 through 18 years is 1250 mg/day

RDA for men and women >19 years is 700 mg/day
RDA for pregnancy and lactation: 14 through 18 years is 1250mg/day

19 through 50 years is 700 mg/day

On the other hand, the tolerable upper limit (UL) was set to be 4g for adults, adolescents and lactating women, 3g for children and older adults, and 3.5g for pregnancy.

Phosphorus in most food sources exhibit good bioavailability, except when it is present as phytic acids in grains and plant seeds (like in nuts, cereal, beans and peas). Human's digestive system doesn't contain phytase, thus it cannot hydrolyze phytic acid, making phosphorus not bioavailable. However, some plants naturally contain phytase, making phosphorus bioavailable. Also yeasts degrade phytate, which means that whole grains that are used to make leavened bread have a higher bioavailable phosphorus content than when it is used to make unleavened bread. Moreover, phosphorus in human milk have higher efficiency of absorption than from cow's milk. The efficiency of absorption from infant's soy milk is the lowest since phosphorus is present as phytic acid (National Academies Press, 1997).

F. Phosphorus absorption and homeostasis

As mentioned previously, phosphate is more readily absorbed as inorganic phosphate at a rate of 65-75% in adults and at even higher rate in children. Phosphate will be absorbed in the small intestine by cotransport with Na^+. Serum phosphate increases an hour after ingestion. This increase stimulates the secretion of PTH, which acts on the kidneys to favor phosphate excretion. However, serum phosphorus is not regulated as tightly as serum calcium, serum phosphorus is mostly affected by cellular release of phosphorus and dietary intake (Anderson, 2013).

Phosphorus concentration in blood ranges between 2.7–4.5 mg/dl. Several hormones control phosphorus concentration to keep it in this range including PTH, 1,25 $(OH)_2$ vitamin D, insulin, glucagon, calcitonin… In contrast to calcium that is primarily regulated by vitamin D, phosphorus is primarily regulated by PTH, that controls its excretion via the kidneys (by blocking its reabsorption by the proximal convoluted tubule), and increases bone resorption of phosphorus and calcium ions, and enhances the absorption of phosphorus and calcium by the small intestine by the 1,25 $(OH)_2$ vitamin D. The hormone Fibroblast growth

factor 23 (FGF 23) secreted by skeletal cells was also shown to decrease serum phosphorus concentration, by decreasing renal reabsorption and intestinal absorption of phosphorus. In addition, FGF 23 reduces renal vitamin D production, and thus decreases intestinal absorption of calcium. The net balance of phosphorus in adults remains zero because of the role of PTH in controlling organs responsible of keeping phosphorus concentration within the normal range. For older adults however, phosphorus balance will become negative if the kidneys are functioning well, because of very low dietary intake of phosphorus. Older adults usually also have low intake of calcium. The low intake of both minerals is linked to osteoporosis. But scenarios of a low intake of calcium with adequate intake of phosphorus are also linked to bone fragility, because of an increased PTH secretion. The phosphorus balance and homeostasis are summarized in figure 1 (Anderson, 2013).

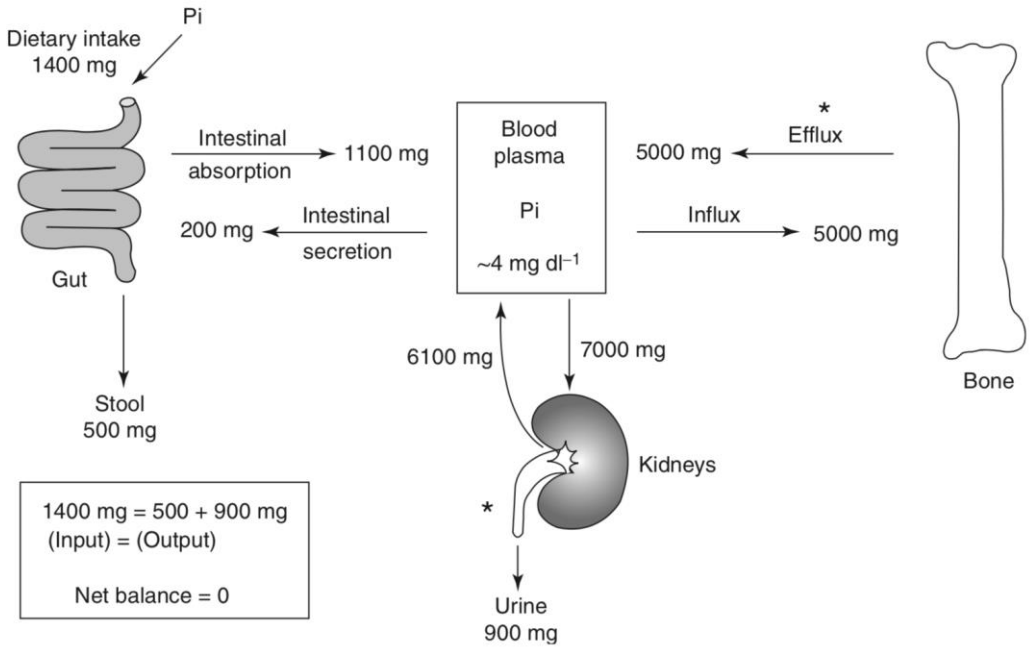

Figure 1: Phosphorus balance and homeostasis (Anderson, 2013)

G. Phosphorus, ATP and food intake

Hepatic ATP depends on adequate dietary sources of phosphorus, since the body lack an adaptive mechanism to increase absorption of phosphorus upon low intake, and there is a relatively low amount of free phosphate stored within cells, and most tissues depend upon ECF phosphorus for their metabolic function. The obesogenic diets that are high in fats and refined carbs, are also low in phosphorus, which will lead to low ATP production, thus affecting energy balance and food intake. The possible mechanism is that low hepatic ATP

(changes in hepatic energy status) is thought to induce hepatic vagal afferent signals to the central nervous system to increase energy intake and decrease energy expenditure (Obeid, 2013). On the other hand, the ratio of ATP to ADP is thought to regulate food intake. A high hepatic ATP production promotes satiety, and the faster this increase, the lower the energy intake.

High carbohydrate intake is thought to decrease phosphorus available for ATP production, by stimulating insulin secretion, which promotes uptake of phosphorus, along with carbs, to use it for substrate phosphorylation (Figure 2). Thus, it is reasonable to conclude, that an addition of phosphorus supplements to the meal will help promote satiety and decrease energy intake, by providing phosphorus for both phosphorylation of substrates and hepatic ATP production (Obeid et al, 2010).

In addition, high fructose diets (like having high fructose corn syrup in the diet) also lead to a decrease in serum phosphate, since it needs to be phosphorylated to fructose-1-P, and unlike glucose, it doesn't have a feedback mechanism to stop producing fructose-1-P when it accumulates, and fructose will compete with ATP production for phosphorus utilization (Obeid, 2013). A high fructose consumption lead to hepatic ATP depletion and impairs recovery of this depletion, thus it may also be a risk factor for the development of non-alcoholic fatty liver disease (NAFLD) (Abdelmalek et al, 2012). An accumulation of uric acid and an inhibition of AMP- activate protein kinase (AMPK) that stimulates production of ATP, by high fructose consumption, may be the cause of fat accumulation in liver, thus leading to NAFLD (Bawden et al, 2015).

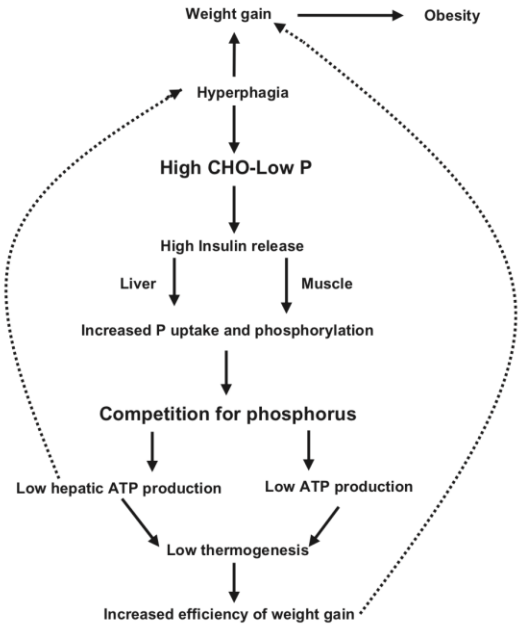

Figure 2: Proposed interaction among phosphorus, adenosine triphosphate (ATP) production and obesity (Obeid, 2013).

H. Factors affecting ATP production

As have previously been mentioned, fructose consumption negatively affects ATP production by utilizing phosphorus for the formation of fructose-1-P, and inhibiting AMPK. High refined carbs and high fats diets that are usually low in phosphorus, also compete with ATP production by increasing phosphorus uptake for substrate phosphorylation. Another factor that was known to affect ATP production is insulin sensitivity. Insulin is secreted by the pancreas to induce carbohydrates and phosphorus uptake by the cells. In case of insulin resistance for elderly patients with T2DM, there is a low peripheral uptake of glucose and phosphorus, leading to low ATP production in the muscles and liver. The prolonged hyperinsulinemia and hyperglycemia with an increased hepatic lipid accumulation, with a chronic insulin resistance, could lead to an oxidative stress that affects the hepatic mitochondrial flexibility. This is not the case for obese non-diabetic subjects who, on the contrary, have higher hepatic ATP production, suggesting that insulin resistance alone cannot impair muscle and liver ATP production for young obese subjects with no NAFLD (Fritsch et al, 2015).

On the other hand, Kahl et al (2018), found other factors that affect hepatic ATP production, independent of insulin sensitivity. For adults with normal insulin sensitivity, glucose tolerance and hepatic lipids (HCL), there was a positive association between

phosphate and non-esterified fatty acids (NEFA). NEFA may increase hepatic phosphorus and stimulate energy generating processes. In addition, this study found a positive relation between ATP and some essential amino acids (leucine). Leucine was shown to improve mitochondrial performance in healthy humans, leading to higher ATP production.

I. Phosphorus and body weight

Obesity is among the biggest causes of morbidity and mortality in the western world (Crescenzo et al, 2015). Obesity is associated with high refined carbohydrate diets (Assaad et al, 2018) and high saturated fats diets (Crescenzo et al, 2015). Both diets are low in micronutrients, especially phosphorus, which is involved in ATP production. ATP is required for all energy requiring mechanisms (DIT, REE, physical activity…) that play a role in the development of obesity. High protein diets on the other hand are associated with lower obesity risk. Increasing proteins in diets also means increasing dietary phosphorus (Kremsdorf et al, 2013). Thus, it is reasonable to assume that phosphorus in proteins also play a role in decreasing the risk of obesity, besides proteins' high satiety effect. In a trial to investigate whether the lower obesogenic effect of high protein diets is also affected by protein's phosphorus content, Abdouni et al (2018) fed participants either high or low protein diets (egg whites that are deficient in phosphorus), with or without phosphorus; and measured the postprandial energy expenditure (PEE) for the next four hours. The investigators found a similar PEE for both diets that was significantly increased by phosphorus ingestion. This result shows the important role that phosphorus may play in the development of obesity. Another study done by Assaad et al (2018), comparing the PEE after the addition of phosphorus to a refined carbs meal with the PEE after having the refined carbs meal with placebo, showed similar results with an increase in PEE with phosphorus ingestion.

Phosphorus was also shown to improve insulin sensitivity, playing a role in decreasing CVD risk. Since insulin sensitivity play a role in lipid metabolism, Hazim et al (2014) investigated whether phosphorus can alter postprandial lipemia. Subjects were given high fats meal with or without phosphorus, and postprandial blood samples were taken every hour for six hours after the meal ingestion. There were no changes in serum insulin, non-esterified fatty acids (NEFA), and triglycerides (TG). However, phosphorus supplementation increased the postprandial concentration of apoB48 and decreased apoB100. Both

apolipoproteins have an atherogenic effect, so the effect of this phosphorus' manipulation on the development of CVD is not clear.

In conclusion, phosphorus in our body is affected by the type of dietary intake. Fructose have a phosphorus sequestering effect. High refined carbs and oils diets are low in phosphorus, and decrease phosphorus availability for ATP; by stimulating insulin secretion that promotes phosphorus uptake by peripheral cells. High proteins diets are also high in phosphorus, and their high thermogenic effect may also be due in part to their high phosphorus content. Phosphorus' effect on postprandial lipemia is not clear, since it was shown to increase ApoB48 and decrease ApoB100. Thus, the effect of phosphorus on lipid metabolism, especially lipids' DIT, is of special importance; to determine the potential role of phosphorus on the development of obesity related to high fats meal.

The objective of the present study was to determine the effect of phosphorus supplementation on diet induced thermogenesis and respiratory quotient, of healthy male subjects consuming a high saturated fat meal (79% of the Kcal as fat, 270Kcal), using unsalted spreadable butter as the main source of fat.

The overall hypothesis of this proposal is that phosphorus ingestion by healthy subjects would be associated with an increase in diet-induced thermogenesis when taking a high fat meal. Our specific aim is as follows:

Specific Aim: To assess the impact of phosphorus ingestion on PEE of healthy male subjects consuming high saturated fat meals.

CHAPTER III

MATERIALS AND METHODS

A. Study Design

This study is a double-blind, randomized, and placebo-controlled crossover clinical trial. The protocol was approved by the Institutional Review Board (IRB) at the American University of Beirut (AUB), under the code NUT.00.22. The trial is registered with Clinical Trial.gov, NCT02482142.

B. Study Population

The study was conducted at the Department of Nutrition and Food Sciences at the American University of Beirut (AUB) between June 2017 and January 2019. Healthy male subjects with an age range between 19 and 35 years and with a body mass index ranging from 19.2-34.6 Kg/m^2 were recruited through direct approach. The purpose and the protocol of the study were explained to the volunteers. Subjects who fulfilled the general entrance requirements (do not have any of the exclusion criteria) signed an informed consent (Appendices I and II) and were referred to do a finger prick and a urine test to determine their HbA1c and albumin and creatinine levels, respectively, to make sure that they have normal glucose levels and kidney functions. Subjects with abnormal values will be asked to contact their doctor. Exclusion criteria included HbA1c level >5.5 and urine creatinine level >300 mg/dl, subjects with diabetes, cardiovascular, cerebrovascular, pulmonary, renal, hepatic, or endocrinological (PTH) diseases in addition to the presence of any other significant medical disease. Subjects who were on regular use of medication that affects body weight and subjects with a weight loss ≥ 3% in the preceding 3 months were also excluded. Subjects were advised to take a weight maintenance diet which contained 250-300 grams of carbohydrate per day 3 days prior to the study, and were asked to avoid any intense physical activity or the use of nutritional supplements one day prior to the study.

C. Experimental Protocol

Experiment: The impact of phosphorus ingestion on PEE of healthy subjects consuming high saturated fat meal (12 subjects).

In this experiment, overnight fasted subjects (> 8 hours) were requested to attend the testing room at around 8:00 am. Anthropometric measurements (weight and height) were taken, then a finger prick experiment was done to determine the HbA1c blood level using the DCA VANTAGE SIEMENS MACHINE (origin: UK, manufacturer: Siemens Healthcare Diagnostics, Inc, NY, USA), and urine was collected to determine creatinine concentration in urine using VITROS 350 CHEMISTRY SYSTEM (Ortho-Clinical Diagnostics, NY, USA). Then the subject was asked to rest for 30 min on a couch in a supine position, the resting REE including VO2 and VCO2, and RQ was measured for 30 min using the ventilated hood and canopy system (COSMED QUARK CPET UNIT, Italy) for indirect calorimetry measurement. Subjects had 15 min to consume the high saturated fat meal with the appropriate supplement. The supplements were either 500 mg of phosphorus tablets (see table 4) or placebo tablets (see table 5) that were given in a random order each on one visit. Finally, the postprandial REE and RQ were measured for 4 hours, divided into a 15min measurement, followed by a 15min break (see figure 3). Subjects undertook 2 sessions in a random order over 2 different days which were separated by a minimum of one week as a washout period.

Session 1: Ingestion of phosphorus tablets (4 tablets) with the high saturated fat meal (table6).

Session 2: Ingestion of placebo tablets (4 tablets) with the high saturated fat meal (table 6).

Table 3: Ingredients of the Potassium Phosphate Tablets*

125 mg phosphorus from:
189.4 mg of Potassium Phosphate Monobasic (KH2PO4) 22.76%
349.5 mg of Potassium Phosphate Dibasic (K2HPO4) 17.78%
108 mg of Dicalcium Phosphate 19%
50 mg of Micro Crystalline Cellulose
50 mg Stearic Acid
10 mg Magnesium Stearate
10 mg Croscarmellose Sodium
5 mg Silicon Dioxide
QS Pharmaceutical Glaze
772 mg Total Theoretical Weight

*A TCC Company

Table 4: Ingredients of the Placebo Tablets*

300 mg of Micro Crystalline Cellulose
200 mg Calcium Carbonate
160 mg Stearic Acid
15 mg Magnesium Stearate
20 mg Croscarmellose Sodium
5 mg Silicon Dioxide
QS Pharmaceutical Glaze
700 mg Total Theoretical Weight

*A TCC Company

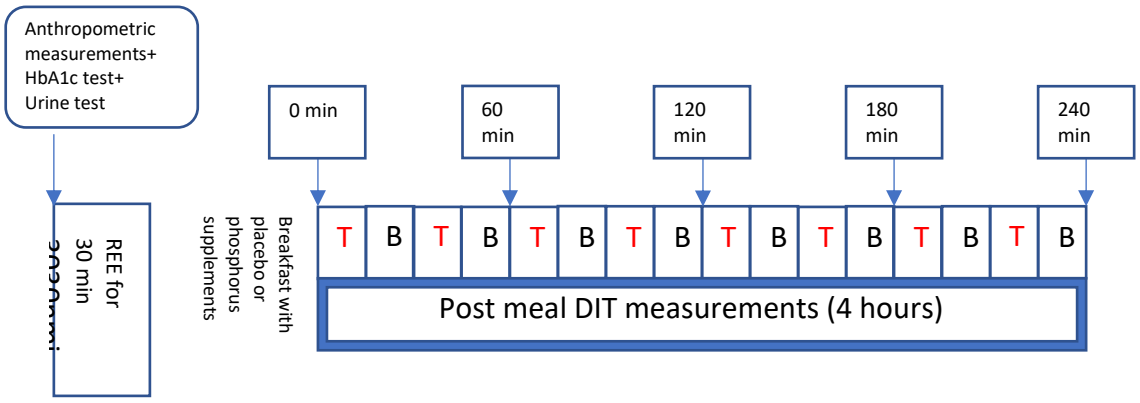

Figure 3: Procedure for Diet-induced thermogenesis (DIT) test days. T represents the measurements for 15 minutes on the COSMED machine; and B represents the break for 15 minutes.

D. Anthropometric measurements

Weight (Kg) was measured using an InBody machine while subjects were wearing light clothes and without shoes, and height (cm) was measured using a portable stadiometer with a movable head piece.

E. Experimental meals

The experimental meal comprised a toast and unsalted spreadable butter. The meal

provided 23.68g of fats (79% of total Kcal), including 14.527g saturated fats and 7.235g unsaturated fats, 11.97g of carbs (18% of total Kcal), and 2.18 g of proteins (3% of total Kcal). The meal had a total calorie of 270 Kcal with a total Phosphorus of 38.1 mg (see table 5).

	High saturated fat meal (low in P)
Toast ***P: 103 mg/100 g***	30 grams
Butter ***P: 24 mg/100g***	6 tsp – 2tbsp 30 grams
CHO - **grams** - **Calories** - **percentage**	 - 11.97 grams - 48 Calories - 18%
PROTEIN - **grams** - **Calories** - **percentage**	 - 2.18 grams - 9 Calories - 3%
FAT - **grams** - **Calories** - **percentage**	 - 23.68 grams - 213 Calories - 79%
Saturated Fat **- grams**	14.527 grams
Unsaturated Fat **-grams**	7.235 grams
Total calories	270 Calories
P (mg)	**38.1 mg**

Table 5: Composition of the High Saturated Fat Meal

F. Measurement of Baseline and Postprandial EE and RQ

The COSMED machine measures VO2 consumption and VCO2 production, and it calculates the difference between the expired air in the hood and room air. In addition, the machine calculates energy expenditure and respiratory quotient. Absolute values for each variable were plotted against time, and the percent difference from baseline was calculated for each variable by subtracting the value at each time point from the average baseline value divided by the baseline value times 100, and they were plotted against time. Respiratory quotient is an indication of substrate oxidation and it is expressed as the ratio of CO2 produced to O2 consumed while food is being metabolized (RQ=CO2 produced/O2 consumed), and it ranges from 0.65-1.0 (Fats: 0.65-7, Proteins: 0.8, Carbohydrates: 1.0).

G. Statistical Analysis

Data are presented as means ± SEM of all values. Data analysis was performed using the MINITAB 16 software program. Results were analyzed by two-way analysis of variance (ANOVA) with treatment (phosphorus or control) and time as the main variables. The paired t-test was used to compare area under the curve between the control and phosphorus treatments. Statistical significance was set at $P < 0.05$.

CHAPTER IV

RESULTS

A. Subjects characteristics

Baseline characteristics including age, anthropometric measurements and fasting blood glucose and urine parameters are presented in table 6. Subjects were 12 healthy men, ranging in age from 21 to 35 and varying between normal adiposity to mild obesity (BMI 19.2 – 34.6 Kg/m^2). All participants had normal blood HbA1c levels and urine creatinine level.

Table 6: Baseline Characteristics of the participants

Variable		**Mean ± SEM**
Age (years)		24.83 ± 1.27
Anthropometric Variables	Weight (kg)	85.83 ± 4.31
	Height (m)	178.50 ± 1.46
	BMI (Kg/m^2)	26.99 ± 1.38
Serum Parameters	HbA1c (%)	5.25 ± 0.09
Urine Parameters	Creatinine (mg/dl)	258.11 ± 20.56

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c.

B. Postprandial Energy Expenditure

Resting energy expenditure (REE) of the subjects was the same for the control and the phosphorus treatment (1.3 Kcal/min). Postprandial energy expenditure (PEE) then increased similarly in both the control and phosphorus treatments for approximately two third of the time after meal ingestion (the first 150 min following meal ingestion), afterwards the increase tended to be higher when given phosphorus at 180 min after meal ingestion (1.43 for phosphorus treatment vs 1.38 for control); but this increase wasn't significantly different according to treatment (P=0.057) but was only significant according to time (P=0.001). Then, at the last time point (240 min), the phosphorus treatment and the control had an almost similar PEE with 1.45 Kcal/min for the phosphorus treatment and 1.43 Kcal/min for the control. Both failed to go back to the REE (Table 7 and Figure 4).

Changes in PEE, began at 0% and increased similarly for both treatments until 60 min, afterwards the phosphorus treatment had a higher difference from baseline as compared to the control except at time 120 min when it was close (7.1% for the control and 7.2% for the phosphorus treatment). The highest difference between the two treatments was seen at 180 min (3.5% for the control and 7.8% for the phosphorus treatment). At 240 min, there was still a significant difference between the two treatments (6.9% for the control and 9.6% for the phosphorus treatment). The increase in the percent change from baseline for both treatment was significantly different according to time (P Value = 0.001) and according to treatment (P Value = 0.028).

Table 7: Postprandial Energy Expenditure (Kcal/minute) of the subjects following the ingestion of the high saturated fat meal with or without phosphorus

Time (minutes)	Control Mean ± SEM	Phosphorus Mean ± SEM
0	1.32 ± 0.06	1.34 ± 0.05
30	1.38 ± 0.05	1.37 ± 0.05
60	1.41 ± 0.06	1.42 ± 0.06
90	1.39 ± 0.06	1.43 ± 0.06
120	1.43 ± 0.06	1.42 ± 0.04
150	1.42 ± 0.06	1.44 ± 0.05
180	1.38 ± 0.07	1.43 ± 0.05
210	1.44 ± 0.07	1.44 ± 0.05
240	1.43 ± 0.07	1.45 ± 0.06

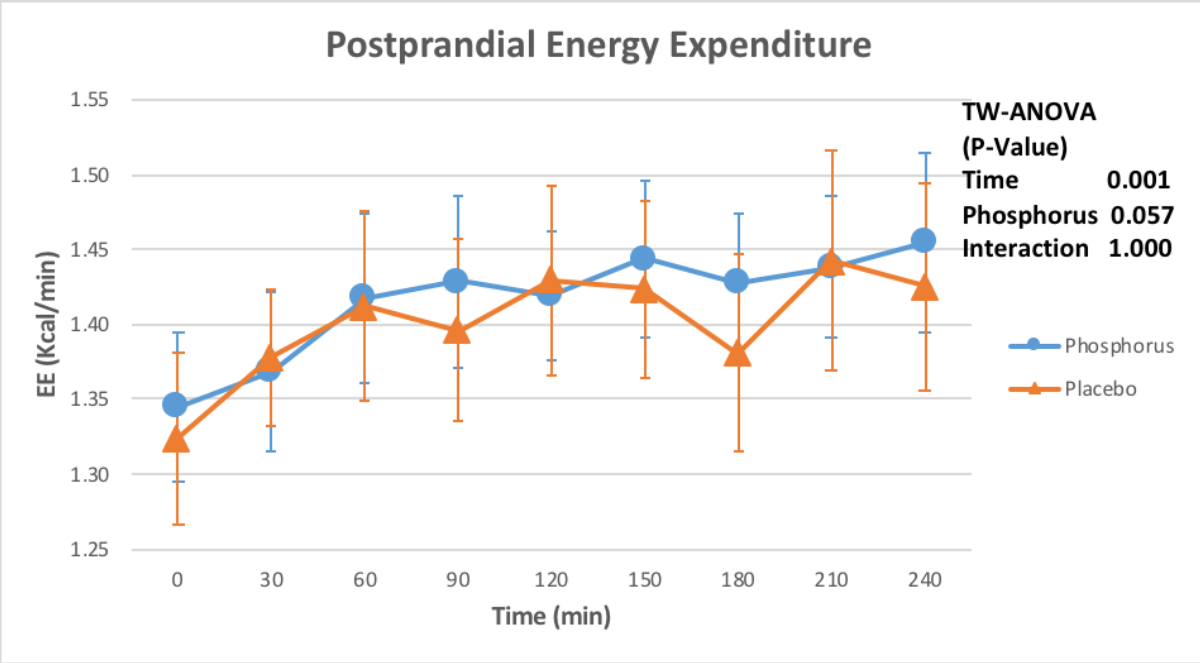

Figure 4: Postprandial Energy Expenditure (Kcal/minute) of the Subjects Following the Ingestion of the High Saturated Fat Meal with or without Phosphorus

Table 8: Changes in Postprandial Energy Expenditure (Kcal/minute) of the subjects following the ingestion of the high saturated fat meal with or without phosphorus

Time (minutes)	Control Mean ± SEM	Phosphorus Mean ± SEM
0	0.00 ± 0.00	0.00 ± 0.00
30	3.6 ± 1.14	2.9 ± 1.83
60	5.7 ± 1.45	6.5 ± 1.94
90	4.5 ± 1.68	7.2 ± 1.54
120	7.1 ± 1.59	7.2 ± 2.76
150	6.9 ± 2.36	9.3 ± 4.21
180	3.5 ± 2.52	7.8 ± 3.06
210	7.9 ± 2.65	8.7 ± 3.61
240	6.9 ± 3.01	9.6 ± 3.27

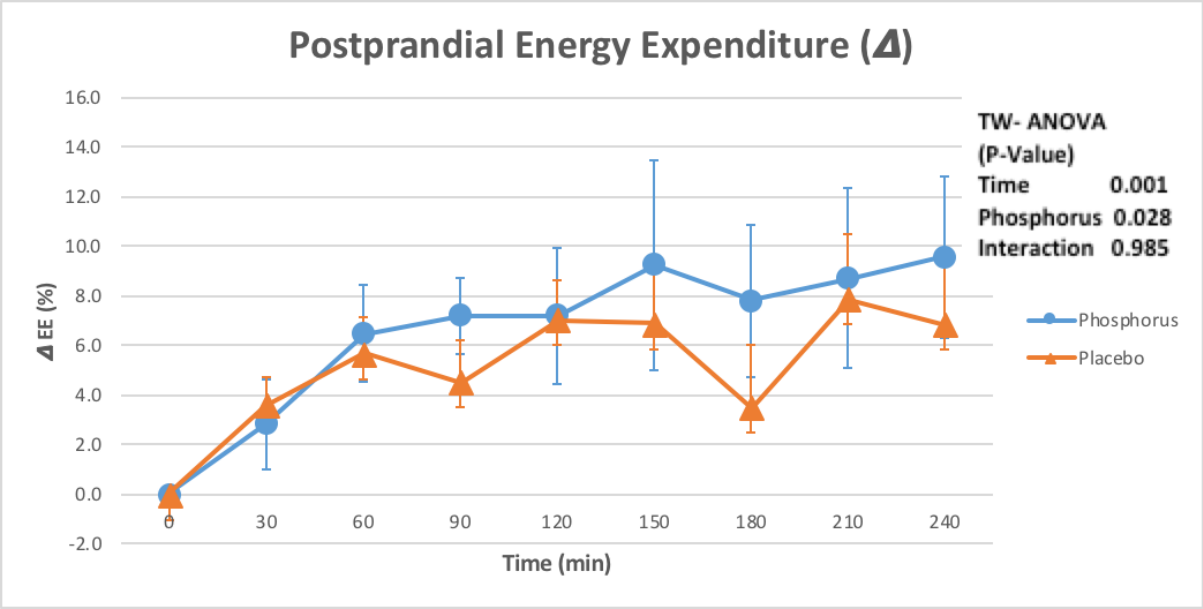

Figure 5: Changes in Postprandial Energy Expenditure (%) of the Subjects Following the Ingestion of the High Saturated Fat Meal with or without Phosphorus

C. Postprandial Respiratory Quotient (RQ)

Baseline RQ was 0.85 in the control and 0.79 in the phosphorus treatments. Postprandial RQ then stayed relatively stable in the first 120 min following meal ingestion in both the control (varying between 0.84 and 0.83) and phosphorus treatments (varying between 0.79 and 0.78). Afterwards, the RQ started decreasing in both treatments reaching a minimum of 0.8 and 0.75 in the control and phosphorus treatments, respectively, at the last time point (240 min). There was a significant difference between both treatments according to time (P=0.000) and treatment (P=0.000) (Table 9 & Figure 6).

Changes in postprandial RQ (%) was very similar for both treatments. It began at 0% for both treatments, then it decreased similarly for both treatments until time 60 min when it reached -2.5% for the control and -2.2% for the phosphorus, afterwards it increased to a maximum of -1.2% for the control and -0.4% for the phosphorus treatment at 90 min, then it started decreasing to reach a minimum of -5.5% at 180 min for the control and -5.7% at time 210 min for the phosphorus treatment, then increased slightly to be -5.3% for the control and -5.5% for the phosphorus treatment at the end of the experiment (240 min). There was a significant difference between both treatments according to time (P=0.000) but not according to treatment (P=0.372) (Table 10 & Figure 7).

Table 9: Postprandial Respiratory Quotient (RQ) of the Subjects Following the Ingestion of the High Saturated Fat Meal with or without Phosphorus

Time (minutes)	Control Mean ± SEM	Phosphorus Mean ± SEM
0	0.85 ± 0.02	0.79 ± 0.02
30	0.83 ± 0.02	0.79 ± 0.02
60	0.83 ± 0.02	0.78 ± 0.02
90	0.84 ± 0.02	0.79 ± 0.02
120	0.83 ± 0.02	0.78 ± 0.02
150	0.82 ± 0.02	0.76 ± 0.02
180	0.8 ± 0.02	0.76 ± 0.02
210	0.8 ± 0.02	0.75 ± 0.02
240	0.8 ± 0.01	0.75 ± 0.02

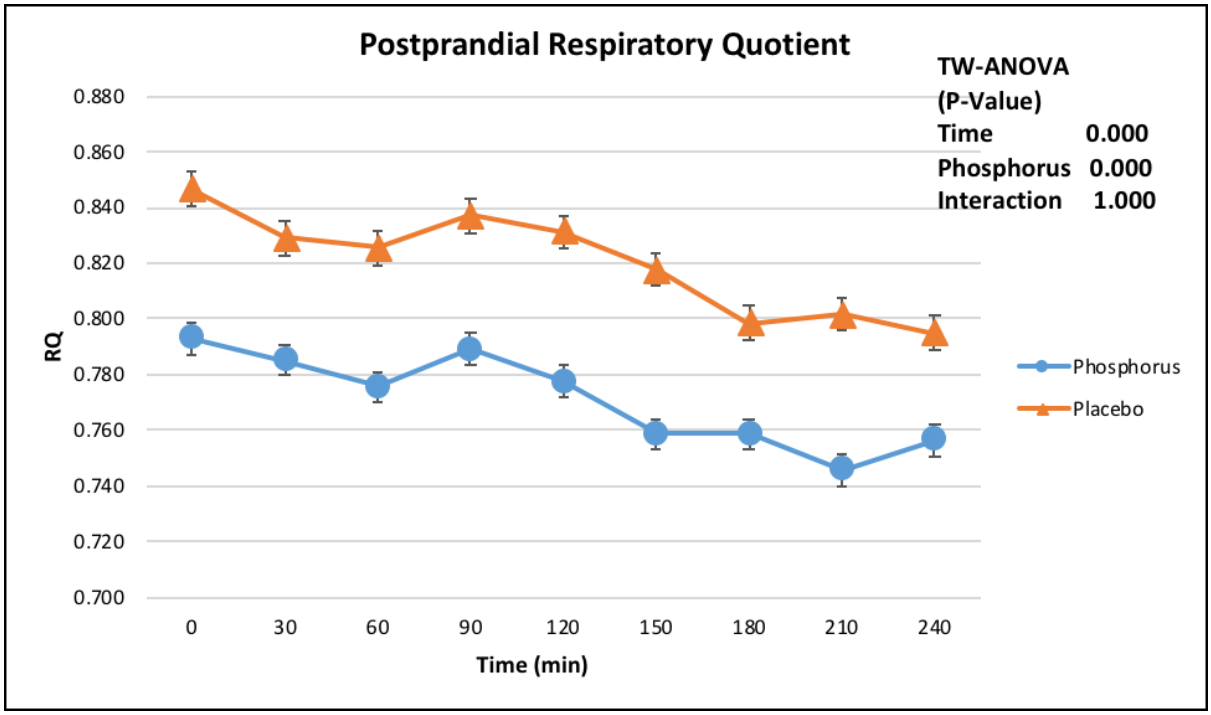

Figure 6: Postprandial Respiratory Quotient (RQ) of the Subjects Following the Ingestion of the High Saturated Fat Meal with or without Phosphorus

Table 10: Changes in Postprandial Respiratory Quotient (RQ) of the Subjects Following the Ingestion of the High Saturated Fat Meal with or without Phosphorus

Time (minutes)	Control Mean ± SEM	Phosphorus Mean ± SEM
0	0.00 ± 0.00	0.00 ± 0.00
30	-2.1 ± 0.88	-0.9 ± 0.89
60	-2.5 ± 0.99	-2.2 ± 1.23
90	-1.2 ± 0.83	-0.4 ± 1.16
120	-1.7 ± 1.17	-1.8 ± 1.64
150	-3.4 ± 0.95	-4.1 ± 1.19
180	-5.5 ± 1.45	-4.1 ± 1.24
210	-5.1 ± 1.63	-5.7 ± 1.55
240	-5.3 ± 2.1	-5.5 ± 1.32

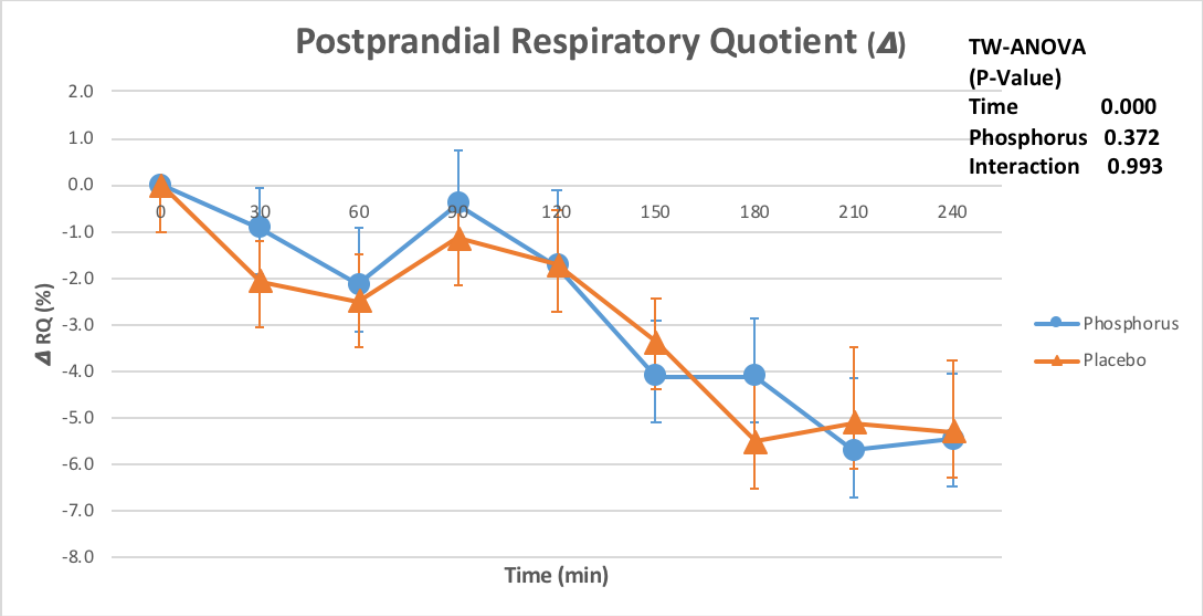

Figure 7: Changes in Postprandial Respiratory Quotient (RQ) of the Subjects Following the Ingestion of the High Saturated Fat Meal with or without Phosphorus

D. Area Under the Curve (AUC)

The area under the curve for the REE, percent change in REE, RQ, and percent change in RQ was calculated using the GraphPrism software. Total and net AUC for each variable are shown in table 12, for the control and the phosphorus treatment.
The AUC of the RQ was significantly lower with phosphorus supplementation than the control, with a P-Value of 0.007. The total and net AUC of the RQ were the same for the control (194.95) and the phosphorus treatment (183.27). there was no significant difference for any other AUC variables (table 11).

Table 11: Area Under the Curve Parameters of the Phosphorus and Control treatments of the Subjects Following the Ingestion of the High Saturated Fat Meal

AUC-Variables	Control Mean ± SEM	Phosphorus Mean ± SEM	P-Value
AUC_REE_Total	334.2 ± 15.4	337.5 ± 13.2	0.56
AUC_%Dif_REE_Total	1385 ± 323	1956 ± 467	0.243
AUC_%Dif_REE_Net	1269 ± 343	1607 ± 555	0.545
AUC_RQ_Total	194.95 ± 5.09	183.27 ± 5.18	0.007
AUC_%Dif_RQ_Total	1302 ± 334	1253 ± 317	0.869

CHAPTER V

DISCUSSION

The present study investigates the effect of phosphorus supplementation on diet induced thermogenesis and respiratory quotient of healthy male subjects consuming a high saturated fat meal. Phosphorus availability for ATP production is conditioned by different factors. The type of the macronutrient consumed is the most important factor, since glucose consumption stimulates insulin release, which favors glucose and phosphorus uptake by the cells for substrate phosphorylation, thus making phosphorus unavailable for hepatic ATP production (Obeid et al, 2010). In addition, fructose has a "phosphate sequestering action"; when consumed, it needs to be phosphorylated to fructose-1-P, but unlike glucose, it doesn't have a feedback mechanism to stop producing fructose-1-P when it accumulates, thus high fructose consumption will compete with ATP production for phosphorus utilization (Obeid, 2013). On the other hand, high protein diets are usually high in phosphorus, so their high DIT may also be due to the fact that they provide phosphorus for hepatic ATP production. Previous studies have examined the relationship between supplementing phosphorus to meals with different macronutrients composition and the change in DIT. Abdouni et al (2018) fed participants either high or low protein diets, with or without phosphorus; and measured the postprandial energy expenditure (PEE) for the next four hours. The investigators found a significant increase in PEE after phosphorus ingestion compared to the meal with placebo. Moreover, Assaad et al (2018), compared the PEE after the addition of phosphorus to a refined carbs meal with the PEE after having the refined carbs meal with placebo, and showed similar results with a significant increase in PEE with phosphorus ingestion. Thus, it was reasonable to conclude that dietary phosphorus is involved in DIT. In line with the previous studies, the present study is conducted to measure the involvement of phosphorus in DIT of male subjects consuming high saturated fat meals. The effect of phosphorus on lipid metabolism, especially lipids' DIT, is of special importance; to determine the potential role of phosphorus on the development of obesity related to high fats meal.

The present findings confirm that there is a strong association between phosphorus intake and DIT. The addition of phosphorus to the high saturated fat meal significantly increased the percent change from baseline in DIT; suggesting an increase in the production of ATP that depends primarily on the availability of phosphorus. In addition, PEE between

the two treatments tended to be significantly different (P=0.057). Moreover, there was a significant difference between the two treatments in RQ, but there was no significant difference in the percent change in RQ for the phosphorus treatment compared to the control, suggesting no difference in fat oxidation when supplementing phosphorus to the high saturated fat meal compared to the control. When calculating the AUC for each variable, we only found a significant difference in the AUC for the RQ of phosphorus treatment compared to the control.

Our results are compatible with the studies that correlate phosphorus intake to decreased body weight and visceral fats. High protein foods in addition to whole grains contain phosphorus, which may play a partial role in their weight loss effect. In an attempt to investigate if the phosphorus by itself is associated to decreased body weight, independently from the food source, Ayoub et al (2015) gave participants phosphorus supplements or placebo with the three main meals for 12 weeks, and anthropometric measurements and blood profile were recorded. They found a significant decrease in body weight, BMI, waist circumference and subjective appetite scores. Supporting a promising role for phosphorus in weight management and decreasing visceral fats and energy intake.

It has been shown that fats have the lowest DIT among macronutrients (0-3%) (Tentolouris et al, 2007). However, the different types of fats themselves have different DIT. Saturated fatty acids were shown to have the lowest DIT among the fats (Casas-Agustench et al, 2008). In line with this, PEE in the present study increased for both treatments from baseline (1.3 Kcal) until it reached 1.4 Kcal at the end of the experiment, both failing to get back to the REE after 4 hours from meal ingestion. The difference in PEE between the two treatments only tended to be significant (P=0.057). However, the percent change from baseline was significantly different between the two treatments (P= 0.028), it was higher for the phosphorus treatment compared to the placebo at time 90 min and from 150 min and onward, this could be explained by the fact that serum phosphate increases an hour after ingestion (Anderson, 2013), and this result support the hypothesis that phosphorus ingestion with the saturated fat meal actually increase its thermic effect.

Despite the literature showing conflicting results, saturated fatty acids consumption has been linked to insulin resistance and greater BMI, predisposing individuals to type II DM (Lovejoy et al, 2002; Bray et al, 2002). A high RQ means a high oxidation of carbohydrates and a low oxidation of fats, and is usually correlated to weight gain (Bray et al, 2002). A shift from high carbs intake to high fat intake may result in a positive fat balance, because the body needs several days to adjust fat oxidation to match fat intake (Smith et al, 2000). In the

study by Smith et al (2000), a higher baseline RQ was correlated to a higher insulin concentration, suggesting that insulin resistance is correlated to decreased fat oxidation. They showed that fat oxidation is delayed when increasing the percent fat in the diet from a 37% fat diet to a 50% fat diet, suggesting that fat oxidation in the muscles is modulated by the level of physical activity, which increases the enzymes required to oxidize fats (Lipoprotein Lipase and Carnitine L-Palmitoyl Transferase I), and the number of mitochondria.

In the present study, we didn't observe a significant decrease in the RQ following phosphorus supplementation as compared to the placebo, so there was no higher fat oxidation when ingesting phosphorus with the high saturated fats meal. This suggests that phosphorus supplementation did not play a role in increasing fat oxidation. This finding confirms a study by Smith et al (2000) which deemed that fat oxidation is difficult to be adjusted promptly to match fat intake, unless having a high physical activity level. However, we found a significant difference when calculating the AUC of the RQ for the phosphorus treatment as compared to the placebo. There are many determinants that may affect RQ, Toubro et al (1998) found that 24 hour RQ is mostly affected (besides diet's macronutrient composition) by fasting plasma insulin levels, plasma free fatty acids concentration and the two days' diet composition immediately preceding the test date. This means that a slight change in the starting time for the same individual between the two sessions, could reflect in a change in RQ caused by a change in fasting insulin. Also, even though we asked the participants to have a weight maintenance diet three days prior the study date, a failure of compliance to have the same macronutrient composition during the two days prior to each session, could also explain the difference in RQ seen for the same individuals between the two sessions.

In a previous study that investigated the effect of phosphorus on postprandial lipemia (Hazim et al, 2014), subjects were given high fats meal with or without phosphorus, and postprandial blood samples were taken every hour for six hours after the meal ingestion. There were no changes in serum insulin, non-esterified fatty acids (NEFA), and triglycerides (TG). However, phosphorus supplementation increased the postprandial concentration of apoB48 and decreased apoB100. Both lipoproteins are atherogenic, thus this may raise some questions on the advantage of supplementing phosphorus to a high fat meal.

The main strength of the study is its design, it is a double blind randomized controlled trial which maintain the integrity of the study and reduce the potential for bias, by blinding both the researcher and the participant to the supplement ingested randomly in each session; and enable us to determine whether a cause-effect relationship exists between

supplementing phosphorus to the meal and the DIT; in addition, meals were equally and accurately given to all participants.

The weaknesses of the study include the small sample size, and some abrupt movements or moments of sleepiness that affected some measurements, even though the participants were overall under good control. In addition, even though we tried to keep the starting time fixed at 8:00 am, there was still a failure of compliance for some subjects to come at the same time in both sessions, and as we mentioned earlier, this could have affected the results. Moreover, in alignment with Hazim et al (2014), we concluded that fats, to be totally absorbed and metabolized, need more than 4 hours (they conducted the experiment for 6 hours postprandially), thus we can conclude that the duration of the experiment may be another limitation of this study. We should also mention that we would have gotten significant results with the AUC if we have divided it into two intervals (first 120 min and the second 120 min), since fats and phosphorus needs 60-90 min to be absorbed, and the biggest difference was seen after 120 min (150 min and onwards); but since it was a double-blind experiment, this was acknowledged too late, and because of time constraints to submit the thesis we did not divide them. Finally, it would have been interesting if we had blood tests taken at several time points postprandially, to be able to have a clearer picture of the effect of phosphorus supplementation on postprandial lipemia along with PEE and fat oxidation, however, taking blood tests continuously during the experiment, would have affected the PEE results because of the stress it would have imposed to the participants during the test.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

Our results have shown that phosphorus significantly increases the PEE when ingesting it with the high saturated fat meal. Moreover, our results suggest that phosphorus can have a weight reduction effect when consumed with the meal, because of its involvement in increasing DIT. Additionally, our results have shown no significant effect of phosphorus ingestion on the percent change in RQ, suggesting that phosphorus was not able to increase fat oxidation when consumed with a high saturated fat meal.

In conclusion, our results suggest a promising effect of phosphorus on weight reduction through increasing DIT. More research should be done however, to investigate the effect of a long-term phosphorus supplementation, especially when consumed with high fat meals, since it was previously being shown that phosphorus, when consumed with a high fat meal, increased the postprandial concentration of apoB48 and decreased apoB100. Both lipoproteins are atherogenic, so there was no clear effect of the role of phosphorus on improving lipid profile.

APPENDIX I

CONSENT FORM (ENGLISH)

Consent to participate in a research study

Title of Research Study: The role of phosphorus ingestion on diet induced thermogenesis of both lean and obese subjects.

Experiment 1: The impact of phosphorus ingestion on DIT of lean and obese subjects consuming high saturated fat meals.

Experiment 2: The impact of phosphorus ingestion on DIT of lean and obese subjects consuming high unsaturated fat meals.

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Address: American University Beirut, Cairo Street, Hamra, Beirut-Lebanon/01–350 000

Site where the study will be conducted: American University of Beirut- Department of Nutrition and Food Science

We are asking you to participate in a **research study**. Before agreeing to participate in the research, it is important that you read the information below. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. Also described are the alternative procedures, if any, available to you, as well as your right to withdraw from the study at any time. You should feel free to ask any questions that you may have.

A. Purpose of the research: Phosphorus is a mineral that is naturally present in our foods and is required by our bodies for normal function. Phosphorus is known to be involved in energy metabolism. However, it's not clear whether phosphorus ingestion with meal can increase energy expenditure and whether such increase is similar between lean and obese subjects. Thus the purpose of the study is to determine the acute effect of phosphorus ingestion on energy expenditure. This is a cross over study in which the subject acts as his/her control. Subjects (lean or obese) will be given a meal with (in one visit) or without (in another visit) phosphorus and their energy expenditure will be measured for 4 hours. This research study is part of a master thesis and is being conducted with the goal of publication in a scientific journal and possibly presentation at academic conferences.

B. Project/Procedures Description: Subjects' recruitment will be done either by posters or direct approaching. Participants will do a finger prick and a urine test to determine their HbA1c and albumin and creatinine levels, respectively, to make sure that they have normal glucose levels and kidney function. Subjects with abnormal values will be asked to contact their doctor. Eligible subjects will be asked to maintain their regular dietary and physical activity habits during the entire study course, avoid alcohol consumption as well as any unusual strenuous exercise 24 hours prior to the study.

Exclusion criteria include: any significant medical diseases; abnormal kidney function, pregnancy or lactation; regular use of medication that affects body weight; a weight loss of 3% or more in the preceding 3 months. The only preparation you need to do on your behalf is to come fasting for the last 8 hours and stop the ingestion of any nutritional supplement. Following a minimum 8 hour (overnight) fast, you will be taken to the testing facility [Faculty of Agriculture and Food Sciences/Department of Nutrition where: anthropometric measurements (height, weight) will be taken, you will be asked to questionnaires about your health.

You will be asked to relax for about 30 min. After that your energy expenditure will be measured for 30 min using the COSMED cardio pulmonary exercise testing (CPET) machine). Simply you will be seated on a couch and a canopy will be placed over your head in order to measure your oxygen consumption and carbon dioxide production. Then, you will be asked to ingest, within 10 minutes, Then, you will be asked to ingest, within 10 minutes, a meal of toast with butter or olive oil with 4 tablets of placebo in one visit and 4 tablets of phosphorous supplement (500 mg P) on the other visit (knowing that every subject will have to come two visits; separated by minimum of one week). Directly after that, your energy expenditure will be determined for 4 hour on a 15 min interval, with a 15 break between each interval. Urine sample over the experimental period will be collected.

This study is a randomized control study and a total of 64 subjects per group would be required for its completion.

C. Duration: The estimated time to complete this study is approximately two weeks. You will have to visit the testing facility 2 times. The visits will be spaced by a period of one week minimum and you will be asked to stay for a time period of 5 hours.

You may leave the study at any time. If you decide to stop participating, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with AUB.

D. Risks, Discomforts and Benefits: Your participation in this study involves only minimal risks. May experience some discomfort from the needle prick for blood withdrawal. The potential side effects of high phosphorus intake are nausea, diarrhea, or epigastric pain; from our experience the use of this dose was not associated with any of these signs. Subjects will not encounter any potential discomfort from the canopy of the

system. Throughout the process of the study, there may be unforeseen events that might take place.

E. You receive no direct benefits from participating in this research; however, when phosphorous is added to the different meals, it was found to increase the diet induced thermogenesis. Therefore, by investigating the effect of phosphorous on the energy expenditure in human body, phosphorous could be a new target for the development of supplements for appetite control and reduce obesity. Moreover, the results obtained are interested in increasing our knowledge and in the modification of our dietary habits by increasing our phosphorous intake. This significant new finding will be conveyed to subjects.

F. Confidentiality: To secure the confidentiality of your responses, your name and other identifiers will never be attached to your answers. All codes and data will be kept in a locked drawer in a locker room or in a password protected computer that is kept secure. Data access is limited to the Principal investigator and researchers working directly on the project. All data will be destroyed responsibly after the required retention period. Your privacy will be maintained in all published and written data resulting from this study. Your name or other identifying information will not be used in our reports or published papers.

There may be circumstances where your confidential information must be released. For example, personal information regarding your participation may be disclosed if required by the AUB IRB, the U.S. Office of Human Research Protections or other federal or international regulatory agencies, or the sponsor of the study, if any, or agency supporting the study.

G. Compensation/Incentive: You will be paid 40 $ as a total (20$ in every visit). These are considered as an anticipated expense for participating and as a cost for transportation, parking etc.

H. Payment for Research-related Injury: In case of any adverse event, AUBMC will cover the cost of treating, on its premises, medical adverse events resulting directly from the medication and/or medical procedures of this research study. Otherwise, it will not cover for the costs of medical care for any medical condition or issue.
If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

I. Contact Information and Questions:
1) If you have any questions or concerns about the research you may contact:
Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb;

Institutional Review Board
American University of Beirut

26 APR 2018

APPROVED

2) If you have any questions, concerns or complaints about your rights as a participant in this research, you can contact the following office at AUB:
Biomedical Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5440

J. Participant Rights: Participation in this study is voluntary. You are free to leave the study at any time without penalty. Your decision not to participate is no way influences your relationship with AUB.
Do you have any questions about the above information? Do you wish to participate in this study?

K. Future Contact
Would you like to be contacted for future research? Yes _____ No _____
Please notify that the investigator has the right to end subject's participation in this study.

Participant Consent:
I have read and understand the above information. I agree to participate in the research study.

Participant Name: _____ Date: _____

Participant Signature: _____

Printed Name of person authorized to consent for subject: _____

Relationship to Subject:_____

Signature of Person authorized to consent: _____

Date: _____

Documentation of Consent:

Printed Name of Person obtaining Consent:_____

Signature of Person obtaining Consent:_____

Date:_____ Time:_____

Institutional Review Board
American University of Beirut

NUT.00.22 March, 2017

26 APR 2018

APPROVED

APPENDIX II

CONSENT FORM (ARABIC)

موافقة على المشاركة في البحث العلمي

عنوان البحث: دور تناول مادة الفوسفور في "التأثير الحراري للطعام" عند الشخص ذات الوزن الطبيعي والبدين.

عنوان الاختبار1: تحديد تأثير مادة الفوسفور على "التأثير الحراري للطعام" عند تناول وجبة غنيّة بالدهون المشبّعة لدى الأشخاص ذوي الوزن الطبيعي والبدين.
عنوان الاختبار2: تحديد تأثير مادة الفوسفور على "التأثير الحراري للطعام" عند تناول وجبة غنيّة بالدهون الغير مشبّعة لدى الأشخاص ذوو الوزن الطبيعي والبدين.

إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.
العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان 01-350000
مكان إجراء البحث: الجامعة الأمريكية في بيروت -كلية الزراعة وعلم الغذاء /قسم التغذية وحدة الأبحاث في الجامعة الأميركية.

أنت مدعو(ة) للمشاركة في بحث علمي سيجرى في الجامعة الأميريكية في بيروت. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) المشاركة. تشرح هذه الإستمارة هدف الدراسة، إجراءاتها، فوائدها، مخاطرها، المضايقات الممكن أن تصدر عنها والإحتياطات اللازمة. بالإضاقة، تشرح هذه الإستملرة الإجراءات البديلة فضلا عن حقك في الإنسحاب من الدراسة في أي وقت. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء يمتدّ بصلة لهذه الدراسة.

أ) **هدف هذا البحث**: الفوسفور هو من المعادن التي تتواجد بشكل طبيعي في الأطعمة وهو عنصر أساسي يمكّن الجسم من أداء وظائفه الطبيعيّة. من المتعارف عليه أنّ لمادة الفسفور دورا أساسيا في عمليّة الأيض وإنتاج الطاقة في جسم الإنسان. لكن، فإنه لا يزال غير واضح إن كان تناول الفسفور مع الطعام يرفع نسبة "التأثير الحراري للطعام"، وإذا هذا الإرتفاع هو مماثل عند الشخص ذات الوزن الطبيعي والشخص البدين. بناء على ذلك، تهدف الدراسة إلى تحديد تأثير مادة الفسفور على "التأثير الحراري للطعام". تعتبر هذه دراسة تشقلبية (cross over) بحيث يقارن المشترك لذاته (study control). سيتناول المشترك (ذات الوزن الطبيعي والبدين) وجبة طعام مع الفسفور (في زيارة) ووجبة طعام بدون الفوسفور (في زيارة أخرى)، وبعدها سوف يتم قياس كميّة حرق الطاقة في الجسم لمدة أربع ساعات. إن هذا البحث جزء من أطروحة ماجيستير من الممكن أن ينشر في صحيفة علميّة أو يشارك في مؤتمرات أكاديمية.

ب) **وصف إجراءات الدراسة**: ستتم عمليّة إختيار المشاركين في الدراسة إمّا عن طريق الملصقات والإعلانات أو عبر الإتصال المباشر. سيتم أخذ عيّنة دم صغيرة من الإصبع للتأكد من مستوى مخزون السكر (HbA_1C) وعيّنة بول لتقييم عمل الكلى من خلال تحديد مستوى الألبومين (albumin) والكرياتينين (creatinine). يطلب من المشترك المؤهل الحفاظ على العادات الغذائيّة والنشاط البدني الطبيعي طيلة مدة الدراسة. يطلب أيضا تفادي تناول الكحول والإفراط بممارسة الرياضة قبل 24 ساعة من بدء الدراسة.
تشمل معايير الإستبعاد: الأمراض الطبيّة، مشاكل في وظائف الكلى، الحمل والرضاعة، التناول المنتظم لأي دواء يؤثر على الوزن، خسارة 3% من الوزن أو أكثر في الأشهر الـ 3 السابقة.
يطلب من المشترك الصوم 8 ساعات والكفّ عن تناول أي من المكملّات الغذائيّة قبل المجيء إلى مكان الإختبار (كلية الزراعة وعلم الغذاء/قسم التغذية وحدة الأبحاث في الجامعة الأميركية في بيروت) حيث يتمّ أخذ الطول والوزن ومن ثمّ تملأ إستبانة عن الصحّة.

NUT.00.22 March, 2017

بعد ذلك، يطلب من المشترك الإسترخاء لمدة 30 دقيقة. ومن ثمّ، سيتم قياس كميّة حرق الطاقة، بعدد السعرات الحرارية عند الراحة، لمدة 30 دقيقة من خلال آلة الـ COSMED cardio pulmonary exercise testing (CPET) machine. ببساطة، سوف يجلس المشترك على أريكة وسيتم وضع "canopy" (مثل مظلّة تغلّف الرأس) فوق الرأس لقياس كميّة إستهلاك الأوكسجين وإنتاج ثاني أكسيد الكربون. بعد ذلك، سيتناول المشترك بـ 10 دقائق وجبة تتضمن التوست مع الزبدة أو زيت الزيتون + 4 أقراص (جرعة 500 مغ) من الفسفور في إحدى الزيارات و4 أقراص وهمية في الزيارة الأخرى. بعدها، سيتم مجددا قياس كميّة حرق الطاقة من خلال الآلة عينها لمدّة 4 ساعات (بالتناوب بين 15 دقيقة تحت مظلة الآلة و15 دقيقة من الراحة، الى أن تنتهي الـ 4 ساعات). وسيتم جمع عيّنة بول خلال الفترة الإختبار.
العدد التقريبي للمشاركين في هذه الدراسة : تتضمن هذه الدراسة 64 مشارك. بترتيب عشوائي، ستعيّن الجلستان (جلسة الوجبة مع الفوسفور وجلسة الوجبة بدون الفوسفور) على أن يفصل بين الجلستين أسبوعا كاملا كحد أدنى.

ت) **المدّة**: إن الوقت المقدّر لإنهاء البحث هو أسبوعين. على المشترك زيارة مكان الإختبار (كلية الزراعة والغذاء العلوم/قسم التغذية) مرّتين لمدة 5 ساعات في الزيارة الواحدة. يمكن الإنسحاب من البحث في أي وقت. إن أراد المشترك التوقف عن المشاركة، ما من عقوبة تفرض عليه ولن يخسر أي من الفوائد التي يتملكها، وقراره لن يؤثر على أي علاقة مستقبليّة مع الجامعة الأمريكية في بيروت.

ث) **المخاطر والمضايقات والفوائد**: لا تشكّل المشاركة في هذا الإختبار مخاطر ملموسة. من المحتمل أن يواجه المشترك بعض الإنزعاج من وخز إبرة سحب الدمّ. الآثار الجانبية المحتملة لتناول كميّات عالية من مادة الفسفور هي الغثيان، الإسهال، والألم في المعدة. لقد أجريت مسبقا عدة تجارب إستخدم فيها المقدار نفسه من الفوسفور ولم تسفر هذه الكميّة عن أي تأثيرات جانبية أو إنزعاج. أما بالنسبة للجلوس على الآلة، فليس هناك أيّ خطر أو شعور بالإزعاج. في جميع مراحل الدراسة، من المحتمل مواجهة أحداث غير متوقعة.

المشترك لن يتلقى أي فوائد مباشرة من المشاركة في هذا البحث؛ وجدت بعض الدراسات أنّ إضافة الفوسفور على وجبات الطعام تسبب زيادة "التأثير الحراري للطعام". لذا، من خلال دراسة دور الفوسفور في زيادة حرق الطاقة في الجسم، يمكن للفوسفور أن يكون وسيلة جديدة للسيطرة على الشّهية والحد من الوزن الزائد والسّمنة. بالإضافة إلى ذلك، فإنّ النتائج التي تم الحصول عليها هي مهمة في زيادة معرفتنا وتعديل العادات الغذائية وزيادة تناول كميّة الفوسفور. سيتم إبلاغ المشاركين بالنتائج والمعلومات الهامّة.

ج) **السريّة**: لضمان سريّة المشاركة، الإسمك والمعلومات الأخرى التي تعرّف عن الهويّة لن تكون معلقة مع الإجابات والنتائج. جميع المعلومات والمدونات ستحفظ في غرفة مغلقة أو على حاسوب لديه رمز سري. الوصول إلى المعلومات مسموحٌ فقط للباحث الأساسي والباحين الذين يعملون مباشرةً في هذة الدراسة. ستتلف جميع المعلومات من بعد مرور الوقت المطلوب. سيحافظ على سريتك في جميع المعلومات المكتوبة والمنشورة عن نتائج هذا البحث. لن يتسعمل إسمك أو أي معلومة متعلقة بهويتك في تقاريرنا أو مقالاتنا المنشورة.
من الممكن أن توجد ظروف حيث يجب نشر معلوماتك السريّة. مثلاً يمكن للمعلومات الشخصية المتعلقة باشتراكك أن تعطى لمجلس المراجعة المؤسسية (IRB) في الجامعة الأمريكية في بيروت وللجان الأخلاق المهنية المستقلة مثل مكتب الولايات المتحدة لحماية البحوث الإنسانية، ومفتشين من اللإدارات الحكومية المنظمة، أو أي وكالة تنظيمية فدرالية أو دولية أخرى، أو مموّل البحث، إن وجد أو أي وكالة تسند البحث.

ح) **التعويض/الحافزة**: سيتقاضى المشترك ٤٠ دولار (٢٠ دولار في كل جلسة) كأجر التنقل أو كلفة موقف السيارة الخ .

خ) **تسديد تكاليف الإصابات الناتجة عن البحث:** ان المركز الطبي في الجامعة الأميركية في بيروت سوف يغطي تكاليف العلاج في المركز للعوارض الطبية السلبية الناجمة مباشرة عن اللأدوية و/أو الإجراءات الطبية االخاصة بهذه الدراسة البحثية. في ما عدا ذلك، لن يقوم المركز الطبي بتغطية تكاليف العناية الطبية لأية حالة أو مُشكلة مَرَضية.

إن تعرضت لإصابة جراء البحث، أو لديك سؤال يتعلّق عن الإصابات المحتملة المتعلقة بالبحث، يرجى الاتصال بالدكتور عمر عبيد 350000 (01) مقسم 4440، أو إرسال بريد إلكتروني على العنوان: email: oo01@aub.edu.lb

د) **أسئلة ومعلومات الاتصال:**

1) لأي سؤال أو إستفسار، يمكنك الاتصال بالدكتور عمر عبيد، قسم التغذية وعلم الطعام الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان 350000 (01) مقسم 4440، email: oo01@aub.edu.lb

2) للأسئلة، المخاوف، والشكاوى حول حقك كمشارك في هذا البحث يمكنك الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت: مجلس المراجعة المؤسسية للعلوم الطبية في الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان5440 ext-350000 1 00961 , email: irb@aub.edu.lb

ذ) **حقوق المشاركين:**

المشاركة في هذا البحث إختياريّة. يمكنك مغادرة البحث في أي وقت من دون أي جزاء. إن قرارك بعدم المشاركة لن يؤثر بأي شكل من الأشكال على علاقتك بالجامعة الأمريكية في بيروت. هل لديك أسئلة حول المعلومات الواردة أعلاه؟ هل ترغب في المشاركة في هذه الدراسة؟

ر) **الاتصال في المستقبل:**

هل ترغب بالاتصال بك للمشاركة في أبحاث أخرى في المستقبل؟ نعم ____ لا ____

ملاحظة: للباحث الحق الكامل بايقاف أي مشارك عن متابعة مشاركته في هذا البحث.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناء على ذلك فإنني، حرا مختارا، أجيز إجراء هذا البحث وأوافق على الإشتراك فيه .

إسم المشترك _____ التاريخ _____ توقيع المشترك _____

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص: _____

العلاقة بالشخص: _____

إمضاء الشخص المأذون للموافقة: _____ التاريخ: _____

توثيق الموافقة:

الإسم المطبوع للشخص الذي يطلب الموافقة: _____

إمضاء الشخص الذي يطلب الموافقة: _____

التاريخ: _____ الوقت: _____

Institutional Review Board
American University of Beirut

NUT.00.22 March, 2017

26 APR 2018

APPROVED

BIBLIOGRAPHY

Abdelmalek, M. F., Lazo, M., Horska, A., Bonekamp, S., Lipkin, E. W., Balasubramanyam, A., et al. (2012). Higher dietary fructose is associated with impaired hepatic adenosine triphosphate homeostasis in obese individuals with type 2 diabetes. Hepatology, 56(3), 952-960.

Abdouni, L., Olabi, A., & Obeid, O. (2018). Postprandial energy expenditure of protein is affected by its phosphorus content. *Journal of Thermal Biology, 78*, 214-218.

Abuduli, M., Ohminami, H., Otani, T., Kubo, H., Ueda, H., Kawai, Y., et al. (2016). Effects of dietary phosphate on glucose and lipid metabolism. *American Journal of Physiology-Endocrinology and Metabolism, 310*(7), E526-E538.

Anderson, J. (2013). Phosphorus: Physiology, dietary sources, and requirements.

Armellini, F., Zamboni, M., Mino, A., Bissoli, L., Micciolo, R., & Bosello, O. (2000). Postabsorptive resting metabolic rate and thermic effect of food in relation to body composition and adipose tissue distribution. *Metabolism, 49*(1), 6-10.

Assaad, M., El Mallah, C., & Obeid, O. (2019). Phosphorus ingestion with a high-carbohydrate meal increased the postprandial energy expenditure of obese and lean individuals. *Nutrition, 57*, 59-62.

Ayoub, J., Samra, M., Hlais, S., Bassil, M., & Obeid, O. (2015). Effect of phosphorus supplementation on weight gain and waist circumference of overweight/obese adults: A randomized clinical trial. *Nutrition & Diabetes, 5*(12), e189.

Bachman, E. S., Dhillon, H., Zhang, C. Y., Cinti, S., Bianco, A. C., Kobilka, B. K., et al. (2002). betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science (New York, N.Y.), 297*(5582), 843-845.

Bawden, S., Stephenson, M., Ciampi, E., Hunter, K., Marciani, L., MacDonald, I. A., et al. (2016). Investigating the effects of an oral fructose challenge on hepatic ATP reserves in healthy volunteers: A 31P MRS study. *Clinical Nutrition, 35*(3), 645-649.

Beermann, C., Jelinek, J., Reinecker, T., Hauenschild, A., Boehm, G., & Klör, H. (2003). Short term effects of dietary medium-chain fatty acids and n-3 long-chain polyunsaturated fatty acids on the fat metabolism of healthy volunteers. *Lipids in Health and Disease, 2*(1), 10.

Bray, G. A., Lovejoy, J. C., Smith, S. R., DeLany, J. P., Lefevre, M., Hwang, D., et al. (2002). The influence of different fats and fatty acids on obesity, insulin resistance and inflammation. *The Journal of Nutrition, 132*(9), 2488-2491.

Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 75*(3), 197-202.

Carneiro, I. P., Elliott, S. A., Siervo, M., Padwal, R., Bertoli, S., Battezzati, A., et al. (2016). Is obesity associated with altered energy expenditure? *Advances in Nutrition, 7*(3), 476-487.

Casas-Agustench, P., López-Uriarte, P., Bulló, M., Ros, E., Gómez-Flores, A., & Salas-Salvadó, J. (2009). Acute effects of three high-fat meals with different fat saturations on energy expenditure, substrate oxidation and satiety. *Clinical Nutrition, 28*(1), 39-45.

Clevenger, H. C., Kozimor, A. L., Paton, C. M., & Cooper, J. A. (2014). Acute effect of dietary fatty acid composition on postprandial metabolism in women. *Experimental Physiology, 99*(9), 1182-1190.

Crescenzo, R., Bianco, F., Mazzoli, A., Giacco, A., Cancelliere, R., di Fabio, G., et al. (2015). Fat quality influences the obesogenic effect of high fat diets. *Nutrients, 7*(11), 9475-9491.

Crescenzo, R., Bianco, F., Mazzoli, A., Giacco, A., Cancelliere, R., di Fabio, G., et al. (2015). Fat quality influences the obesogenic effect of high fat diets. *Nutrients, 7*(11), 9475-9491.

D'Alessio, D. A., Kavle, E. C., Mozzoli, M. A., Smalley, K. J., Polansky, M., Kendrick, Z. V., et al. (1988). Thermic effect of food in lean and obese men. *The Journal of Clinical Investigation, 81*(6), 1781-1789.

Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., & Schaefer, E. J. (2005). Comparison of the atkins, ornish, weight watchers, and zone diets for weight loss and heart disease risk reduction: A randomized trial. *Jama, 293*(1), 43-53.

Den Besten, C., Vansant, G., Weststrate, J. A., & Deurenberg, P. (1988). Resting metabolic rate and diet-induced thermogenesis in abdominal and gluteal-femoral obese women before and after weight reduction. *The American Journal of Clinical Nutrition, 47*(5), 840-847.

Farshchi, H., Taylor, M., & Macdonald, I. (2004). Decreased thermic effect of food after an irregular compared with a regular meal pattern in healthy lean women. *International Journal of Obesity, 28*(5), 653.

Feldmann, H. M., Golozoubova, V., Cannon, B., & Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metabolism, 9*(2), 203-209.

Fritsch, M., Koliaki, C., Livingstone, R., Phielix, E., Bierwagen, A., Meisinger, M., et al. (2015). Time course of postprandial hepatic phosphorus metabolites in lean, obese, and type 2 diabetes patients. *The American Journal of Clinical Nutrition, 102*(5), 1051-1058.

Granata, G. P., & Brandon, L. J. (2002). The thermic effect of food and obesity: Discrepant results and methodological variations. *Nutrition Reviews, 60*(8), 223-233.

Halton, T. L., & Hu, F. B. (2004). The effects of high protein diets on thermogenesis, satiety and weight loss: A critical review. *Journal of the American College of Nutrition, 23*(5), 373-385.

Hamada, Y., Kashima, H., & Hayashi, N. (2014). The number of chews and meal duration affect diet-induced thermogenesis and splanchnic circulation. *Obesity, 22*(5), E62-E69.

Harford, K. A., Reynolds, C. M., McGillicuddy, F. C., & Roche, H. M. (2011). Fats, inflammation and insulin resistance: Insights to the role of macrophage and T-cell accumulation in adipose tissue.*Proceedings of the Nutrition Society, 70*(4), 408-417.

Hazim, J., Hlais, S., Ghattas, H., Shatila, D., Bassil, M., & Obeid, O. (2014). Phosphorus supplement alters postprandial lipemia of healthy male subjects: A pilot cross-over trial. *Lipids in Health and Disease, 13*(1), 109.

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (1997). Dietary reference intakes. *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride* () National Academies Press (US).

Joint, F. (2010). Fats and fatty acids in human nutrition. report of an expert consultation, 10-14 november 2008, geneva.

Jones, P. J., Jew, S., & AbuMweis, S. (2008). The effect of dietary oleic, linoleic, and linolenic acids on fat oxidation and energy expenditure in healthy men. *Metabolism, 57*(9), 1198-1203.

Kahl, S., Nowotny, B., Strassburger, K., Bierwagen, A., Klüppelholz, B., Hoffmann, B., et al. (2017). Amino acid and fatty acid levels affect hepatic phosphorus metabolite content in metabolically healthy humans. *The Journal of Clinical Endocrinology & Metabolism, 103*(2), 460-468.

Kalantar-Zadeh, K., Gutekunst, L., Mehrotra, R., Kovesdy, C. P., Bross, R., Shinaberger, C. S., et al. (2010). Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clinical Journal of the American Society of Nephrology : CJASN, 5*(3), 519-530.

KANWU Study Group Rasmussen Birthe M Vessby Bengt Uusitupa Matti Berglund Lars Pedersen Eva Riccardi Gabrielle Rivellese Angela A Tapsell Linda Hermansen Kjeld kjeld. hermansen@ as. aaa. dk. (2006). Effects of dietary saturated, monounsaturated, and n− 3 fatty acids on blood pressure in healthy subjects. *The American Journal of Clinical Nutrition, 83*(2), 221-226.

Kaseb, F., Rashidi, M., Afkhami-Ardekani, M., & Fallahzadeh, H. (2013). Effect of olive, almond and walnut oil on cardiovascular risk factors in type 2 diabetic patients. *International Journal of Diabetes in Developing Countries, 33*(2), 115-119.

Kremsdorf, R. A., Hoofnagle, A. N., Kratz, M., Weigle, D. S., Callahan, H. S., Purnell, J. Q., et al. (2013). Effects of a high-protein diet on regulation of phosphorus homeostasis. *The Journal of Clinical Endocrinology & Metabolism, 98*(3), 1207-1213.

Kris-Etherton, P. M., Pearson, T. A., Wan, Y., Hargrove, R. L., Moriarty, K., Fishell, V., et al. (1999). High–monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations.*The American Journal of Clinical Nutrition, 70*(6), 1009-1015.

Kris-Etherton, P. M., Pearson, T. A., Wan, Y., Hargrove, R. L., Moriarty, K., Fishell, V., et al. (1999). High–monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations.*The American Journal of Clinical Nutrition, 70*(6), 1009-1015.

Li, J., & Sun, Q. (2019). Consumption of saturated fatty acids and coronary heart disease risk. *International Journal of Cardiology, 279*, 27-28.

Lovejoy, J. C., Smith, S. R., Champagne, C. M., Most, M. M., Lefevre, M., DeLany, J. P., et al. (2002). Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. *Diabetes Care, 25*(8), 1283-1288.

Maffeis, C., Schutz, Y., Grezzani, A., Provera, S., Piacentini, G., & Tatò, L. (2001). Meal-induced thermogenesis and obesity: Is a fat meal a risk factor for fat gain in children? *The Journal of Clinical Endocrinology & Metabolism, 86*(1), 214-219.

Marion-Letellier, R., Savoye, G., & Ghosh, S. (2015). Polyunsaturated fatty acids and inflammation. *IUBMB Life, 67*(9), 659-667.

Mozaffarian, D., Micha, R., & Wallace, S. (2010). Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: A systematic review and meta-analysis of randomized controlled trials. *PLoS Medicine, 7*(3), e1000252.

Obeid, O. (2013). Low phosphorus status might contribute to the onset of obesity. *Obesity Reviews, 14*(8), 659-664.

Obeid, O., Dimachkie, S., & Hlais, S. (2010). Increased phosphorus content of preload suppresses ad libitum energy intake at subsequent meal. *International Journal of Obesity, 34*(9), 1446.

Piers, L., Walker, K., Stoney, R., Soares, M., & O'dea, K. (2002). The influence of the type of dietary fat on postprandial fat oxidation rates: Monounsaturated (olive oil) vs saturated fat (cream).*International Journal of Obesity, 26*(6), 814.

Polley, K. R., Miller, M. K., Johnson, M., Vaughan, R., Paton, C. M., & Cooper, J. A. (2018). Metabolic responses to high-fat diets rich in MUFA v. PUFA. *British Journal of Nutrition, 120*(1), 13-22.

Ravussin, E., Bogardus, C., Schwartz, R. S., Robbins, D. C., Wolfe, R. R., Horton, E. S., et al. (1983). Thermic effect of infused glucose and insulin in man. decreased response with increased insulin resistance in obesity and noninsulin-dependent diabetes mellitus. *The Journal of Clinical Investigation, 72*(3), 893-902.

Roberts, S. B. (1995). Abnormalities of energy expenditure and the development of obesity. *Obesity Research, 3*(S2), 155s-163s.

Schutz, Y., Bessard, T., & Jequier, E. (1984). Diet-induced thermogenesis measured over a whole day in obese and nonobese women. *The American Journal of Clinical Nutrition, 40*(3), 542-552.

Segal, K. R., Edaño, A., & Tomas, M. B. (1990). Thermic effect of a meal over 3 and 6 hours in lean and obese men. *Metabolism, 39*(9), 985-992.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., & Krauss, R. M. (2010). Saturated fat, carbohydrate, and cardiovascular disease. *The American Journal of Clinical Nutrition, 91*(3), 502-509.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., & Krauss, R. M. (2010). Saturated fatty acids and risk of coronary heart disease: Modulation by replacement nutrients. *Current Atherosclerosis Reports, 12*(6), 384-390.

Smith, S. R., de Jonge, L., Zachwieja, J. J., Roy, H., Nguyen, T., Rood, J. C., et al. (2000). Fat and carbohydrate balances during adaptation to a high-fat diet. *The American Journal of Clinical Nutrition, 71*(2), 450-457.

Soares, M., Cummings, S., Mamo, J., Kenrick, M., & Piers, L. (2004). The acute effects of olive oil v. cream on postprandial thermogenesis and substrate oxidation in postmenopausal women. *British Journal of Nutrition, 91*(2), 245-252.

Takeuchi, H., Matsuo, T., Tokuyama, K., Shimomura, Y., & Suzuki, M. (1995). Diet-induced thermogenesis is lower in rats fed a lard diet than in those fed a high oleic acid safflower oil diet, a safflower oil diet or a linseed oil diet. *The Journal of Nutrition, 125*(4), 920-925.

Tentolouris, N., Pavlatos, S., Kokkinos, A., Perrea, D., Pagoni, S., & Katsilambros, N. (2008). Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. *Metabolism, 57*(3), 313-320.

Toubro, S., Sørensen, T. I., Hindsberger, C., Christensen, N. J., & Astrup, A. (1998). Twenty-four-hour respiratory quotient: The role of diet and familial resemblance. *The Journal of Clinical Endocrinology & Metabolism, 83*(8), 2758-2764.

van Marken Lichtenbelt, W., Mensink, R., & Westerterp, K. (1997). The effect of fat composition of the diet on energy metabolism. *Zeitschrift Für Ernährungswissenschaft, 36*(4), 303-305.

Watanabe, T., Nomura, M., Nakayasu, K., Kawano, T., Ito, S., & Nakaya, Y. (2006). Relationships between thermic effect of food, insulin resistance and autonomic nervous activity. *The Journal of Medical Investigation, 53*(1, 2), 153-158.

Westerterp, K. R. (2004). Diet induced thermogenesis. *Nutrition & Metabolism, 1*(1), 5.

Westerterp, K., Wilson, S., & Rolland, V. (1999). Diet induced thermogenesis measured over 24h in a respiration chamber: Effect of diet composition. *International Journal of Obesity, 23*(3), 287.

Westerterp-Plantenga, M., Rolland, V., Wilson, S., & Westerterp, K. (1999). Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber. *European Journal of Clinical Nutrition, 53*(6), 495.

Yang, B., Shi, M., Li, Z., Yang, J., & Li, D. (2016). Fish, long-chain n-3 PUFA and incidence of elevated blood pressure: A meta-analysis of prospective cohort studies. *Nutrients, 8*(1), 58.

Yang, S., Lin, S., Chang, J., & Chien, Y. (2017). High fat diet with a high monounsaturated fatty acid and polyunsaturated/saturated fatty acid ratio suppresses body fat accumulation and weight gain in obese hamsters. *Nutrients*, *9*(10), 1148.