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THE ROLE OF PHOSPHORUS IN DIET-INDUCED THERMOGENESIS OF LEAN MALE SUBJECTS

by
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AN ABSTRACT OF THE THESIS OF

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Energy Expenditure (EE) is the sum of resting metabolic rate (RMR), diet-induced thermogenesis (DIT) and energy expended on physical activity. Diet-induced thermogenesis is the increase that takes place in EE after ingestion of food, noting that this increase is above the RMR and accounts for 5% to 15% of total EE. This increase can be largely related to the increased production of ATP which is used to cover the cost of digestion, absorption, transport and storage of the ingested food. Proteins have the highest thermic effect (20%-30%) compared with carbohydrates (5%-10%) and fats (0%-3%). Moreover, proteins contain high quantity of phosphorus that is required for energy production through the synthesis of ATP. However, it is not clear whether the high thermogenesis of proteins is affected by their content of phosphorus. Thus, the aim of the present study was to explore the impact of phosphorus ingestion on DIT and substrate oxidation of healthy lean male subjects consuming two isocaloric meals, a normal protein-low phosphorus meal and a high protein-low phosphorus meal using a low phosphorus containing protein, egg white.

This is a single-blind, randomized, and placebo-controlled crossover study that was conducted on healthy lean male subjects who were randomly allocated into two experiments, 12 subjects each, a normal-protein meal experiment (15% of total E from protein) and a high-protein meal experiment (50% of total E from protein). Overnight fasted subjects undertook 2 sessions separated by a minimum of one week and were asked to consume the appropriate meal with phosphorus (500 mg) or placebo tablets in a random order. Energy expenditure, % fat oxidation, % carbohydrate oxidation and respiratory quotient (RQ) were measured at baseline and for the next four hours following meal consumption in a period of 15 minutes interval with 15 minutes break, using a ventilated hood and canopy system (COSMED QUARK CPET UNIT) for indirect calorimetry measurement. Urine was collected at the end of the experimental period in both experiments and the urinary parameters phosphorus, creatinine, and urine urea nitrogen (UUN) were measured using Vitros 350 analyzer.

Postprandial EE increased following the ingestion of the different meals (Time: P=0.000); however, postprandial EE of the phosphorus supplemented group was found to be significantly higher than that of the control especially from 60 minutes and onward in the normal-protein meal experiment (Phosphorus: P=0.001) and from 120 minutes and onward in the high-protein meal experiment (Phosphorus: P=0.003). The

increase in postprandial EE was not associated with significant differences in fat and carbohydrate oxidation between the control and phosphorus treatments in both experiments. Additionally, phosphorus supplementation had no significant effect on urinary nitrogen excretion in both experiments.

Phosphorus was able to significantly increase and prolong postprandial EE in both the normal-protein meal and the high-protein meal experiments; therefore, it is reasonable to conclude that phosphorus is involved in DIT, and the high thermic effect of proteins is partially attributed to their high phosphorus content. In line with that, 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), Adenosine triphosphate (ATP), Normal protein, High protein.

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ABBREVIATIONS

%	Percent
/	Per
&	And
<	Less than
>	Greater than
=	Equal to
±	Plus or minus
Δ	Difference
ºC	Degree Celsius
ATP	Adenosine triphosphate
AUB	American University of Beirut
AUBMC	American University of Beirut Medical Center
BMI	Body mass index
cm	Centimeter
CO	Carbohydrate oxidation
CO_2	Carbon Dioxide
d	Day
DIT	Diet-induced thermogenesis
dL	Deciliter
DRI	Dietary reference intake
E	Energy
EE	Energy expenditure
et al.	(and others)
FO	Fat oxidation
g	Gram
GFR	Glomerular filtration rate
HP	High protein
IRB	Institutional review board
Kcal	Kilocalorie
Kg	Kilogram
m^2	Square meter
mg	Milligram
min	Minute
n	Number of subjects
NP	Normal protein
O_2	Oxygen
P	Phosphorus
P/Cr	Phosphorus to creatinine ratio
PTH	Parathyroid hormone
RDA	Recommended dietary allowance
REE	Resting energy expenditure
RMR	Resting metabolic rate
rpm	Rounds per minute
RQ	Respiratory quotient

r.t.	Room temperature
SEM	Standard error of the mean
TEE	Total energy expenditure
TEF	Thermic effect of food
TW- ANOVA	Two-way analysis of variance
UUN	Urine urea nitrogen
UUN/Cr	UUN to creatinine ratio

CHAPTER I

INTRODUCTION

Energy expenditure is related to three major components: resting (basal) metabolic rate (RMR), diet-induced thermogenesis (DIT) and energy expended on physical activity. Diet-induced thermogenesis is mainly related to ATP production that is needed to cover the cost of digestion, absorption, transport and storage of the ingested food, and it contributes to 5% - 15% of total energy expenditure. Several studies have reported a reduced DIT among obese subjects as compared with lean subjects (Segal *et al.,* 1985; Segal *et al.,* 1990; Segal *et al.,* 1990; Thorne *et al.,* 1989; Thorne *et al.,* 1990); however, other studies showed no difference (D'Alessio *et al.,* 1988; Ravussin & Swinburn, 1996; Tentolouris *et al.,* 2008). Moreover, these differences in DIT seem to diminish following weight loss (Thorne *et al.,* 1989; Thorne *et al.,* 1990) indicating that DIT is not a causal factor for obesity.

It has been suggested that phosphorus status is inversely related to body weight due to the fact that all food sources that cause weight gain are low in phosphorus such as refined cereals, sweeteners, and oils. On the other hand, high-protein foods that are high in phosphorus have a major and successful role in weight loss. Additionally, several studies have reported a correlation between high dairy consumption and decreased body weight that could not be explained by calcium (Wagner *et al.,* 2007; Yanovski *et al.,* 2009), and since dairy products contain high amounts of phosphorus, the decrease in body weight reported could be explained by the involvement of phosphorus. In line with that, it was reported that body weight and plasma phosphorus

are inversely related to each other (Haap *et al.,* 2006; Haglin *et al.,* 2001; Kalaitzidis *et al.,* 2005; Lind *et al.,* 1993; Lindgärde & Trell, 1977). Moreover, a lot of evidence in animals supports an inverse relationship between eating behavior and hepatic ATP levels. In these studies, a decrease in hepatic ATP is transmitted by the neural afferents to the central nervous system resulting in an increase in food intake (Friedman, 2007). While the fundamental evidence for this mechanism has been tested primarily in animals, human studies indirectly support a potential role for hepatic ATP in energy and body weight regulations (Cortez-Pinto *et al.,* 1999; Nair *et al.,* 2003). In addition, data from our laboratory showed that the ingestion of phosphorus preload was associated with a 30% decrease in energy intake at a subsequent meal (Obeid *et al.,* 2010).

High-protein diets have been promoted as a successful strategy for weight loss due to the fact that dietary protein promotes satiety and thus reduces energy intake at subsequent meals (Leidy *et al.,* 2015), and dietary protein is also associated with increased thermogenesis (Paddon-Jones *et al*., 2008). High-protein foods are rich sources of phosphorus; however, less is known about the involvement of phosphorus in the high thermogenesis of proteins.

The overall hypothesis of the present study is that phosphorus ingestion by healthy lean male subjects would be associated with an increase in DIT, and that the high thermogenesis of protein is partially related to its high phosphorus content. The present study will, therefore, clarify the effect of phosphorus ingestion on DIT and substrate oxidation of healthy lean male subjects consuming two isocaloric meals, a normal protein-low phosphorus meal and a high protein-low phosphorus meal.

CHAPTER II

LITERATURE REVIEW

A. Diet-Induced Thermogenesis (DIT)

Total energy expenditure (TEE) is the sum of resting energy expenditure (REE), thermic effect of food (TEF) or DIT, and energy expended on physical activity. Resting energy expenditure is the largest component and accounts for 65-75% of TEE (Paddon-Jones *et al*., 2008). Diet-induced thermogenesis is the most difficult and the least reproducible component of total daily energy expenditure because it is affected by the meal size and composition, the palatability of the food, the time of the meal, the consumption frequency, the duration of measurements, and the position of the participants in addition to other factors that are difficult to control, including subject's genetic background, age, physical fitness, and sensitivity to insulin (Tentolouris *et al.,* 2008). Meal thermogenesis is enhanced by food palatability. For instance, the ingestion of a palatable meal (parmesan fondue, spaghetti with meat balls and chocolate éclair) increased DIT by almost 50% more than if the same meal was blended, desiccated and consumed as a tasteless biscuit (Johnston *et al.,* 2002). Palatability has been shown to influence the cephalic phase of DIT which occurs within the first 40 minutes of meal ingestion, and a low palatability is associated with a lower DIT. Moreover, it has been shown that the inclusion of a moderate amount of carbohydrate to a low-calorie high protein liquid meal-replacement shake can augment DIT (Scott & Devore, 2005).

Diet-induced thermogenesis is the main form of thermogenesis in humans and accounts for 5% to 15% of TEE (Tentolouris *et al.,* 2008). It is the increase in energy

expenditure above resting after consumption of food and can be largely related to the increase production of ATP which is used to cover the cost of digestion, absorption, transport, and storage of the ingested food. The increase in DIT after consumption of a meal is strongly related to the energy content and macronutrient composition of the meal. The greater the energy content, the greater the DIT (Scott & Devore, 2005), and in terms of macronutrient content, proteins have the highest DIT (20%-30%), followed by carbohydrates (5%-10%) and fats (0%-3%) (Tentolouris *et al.,* 2008). Accordingly, the main determinant of DIT is the energy content followed by the protein fraction of the food (Westerterp, 2004).

The high thermic effect of protein is affected by the protein source and digestibility. For instance, animal proteins have a higher thermogenesis than vegetable proteins, and rapidly digested protein, such as whey, induces a higher thermogenesis than slowly digested protein, such as casein (Westerterp-Plantenga *et al.,* 2006). Studies have shown that milk proteins are digested and absorbed at different rates. Moreover, whey protein has a higher thermic effect than does protein composed of casein or soy. It has been shown that whey and hydrolyzed proteins are rapidly digested and absorbed and they improve nitrogen and protein retention. Additionally, animal proteins have a higher thermic effect than do vegetable proteins because animal proteins influence protein turnover and favor protein synthesis (Acheson *et al.,* 2011) since animal proteins are complete proteins having all the essential amino acids and thus influence DIT to a greater extent than do lower-quality proteins such as vegetable proteins (Westerterp-Plantenga *et al.,* 2009). Another determinant of the high thermogenesis of protein is the amino acid composition of the protein. The number of amino groups that undergoes conversion to urea in the urea cycle, at a cost of four ATP, ranges from one

for an amino acid such as proline or alanine, to three for histidine. Therefore, taking into account the stoichiometry of amino acid catabolism and urea synthesis, the calculated energy expenditure to produce ATP is ranging from 153 KJ/ATP for cysteine, to 99 KJ/ATP for glutamate. For glucose, this value is 91 KJ/ATP (Westerterp-Plantenga *et al.*, 2006).

Several explanations have been proposed for the high thermogenesis of protein. It has been suggested that increased protein turnover accounts for most (68%) of the thermogenic effect of protein. The body has no storage capacity of excess protein and therefore has to oxidize or eliminate excess amino acids resulting in increased thermogenesis (Paddon-Jones *et al*., 2008). The high ATP costs of postprandial peptide bond and protein synthesis and the high cost of urea production and gluconeogenesis are the main reasons for the high thermic effect of protein (Halton & Hu, 2004). In addition, high-protein meals may increase thermogenesis via an up-regulation of uncoupling proteins. For instance, an increase in protein intake was associated with an increase in uncoupling protein-2 in the liver and uncoupling protein-1 in adipose tissue in animal models, changes that are positively correlated with energy expenditure (Paddon-Jones *et al*., 2008). High-protein meals may increase the proton-pump activity in liver membranes and thereby induce uncoupled respiration resulting in a higher thermogenesis (Mikkelsen *et al.,* 2000). Since dietary protein accounts for a significant portion of phosphorus, it is reasonable to assume that phosphorus might be involved in the high thermogenesis of protein through increasing ATP production.

A reduced DIT may contribute to the development of obesity; however, studies examining the differences in DIT between lean and obese subjects have found conflicting results. Some studies have shown a reduced DIT among obese subjects

while other studies have found no differences in DIT between lean and obese subjects (Tentolouris *et al.,* 2008). Even though DIT is the smallest component of energy expenditure, it may play a role in the development and/or maintenance of obesity (Westerterp, 2004).

B. Protein Functions, Sources and Requirements

Proteins are composed of amino acids linked by peptide bonds. The sequence of the amino acids determines the structure and function of proteins in the body. Primary roles of proteins include structural protein, enzymes, hormones, transport and immunoproteins (Mahan *et al.,* 2012). Dietary protein contains most of the 20 amino acids needed for protein synthesis. Amino acids are classified based on the body's ability to synthesize the amino acid from other carbon sources as indispensable (essential), conditionally indispensable, and dispensable (nonessential). Dietary proteins differ in their amino acid content. For instance, lysine is the limiting amino acid in cereals and methionine is the limiting amino acid in legumes. Therefore, protein quality is important when considering protein requirements (Arentson-Lantz *et al.*, 2015).

Rich sources of protein are obtained from animal flesh and animal products such as milk and eggs, and from plant foods such as legumes and beans. Animal protein is more efficient than vegetable protein because the latter is encased in carbohydrates and is less available to digestive enzymes. Further, animal protein has a high biological value since it constitutes the essential amino acids needed for protein synthesis. The quality of dietary protein can be improved by combining protein sources with different limiting amino acids (Mahan *et al.,* 2012). Dietary sources of protein account for a

significant portion of phosphorus with meat, poultry and fish being the major sources (Sherman & Mehta, 2009).

Protein requirement is defined as the amount of protein needed to achieve growth in neonates, children and pregnant women, and maintenance in adults and the elderly (Arentson-Lantz *et al.*, 2015). The amount of protein needed is based on the minimum daily requirement to maintain nitrogen balance. Sufficient quantity of high quality protein is required to prevent catabolism of own protein stores (Astrup *et al.*, 2015). A healthy adult human requires 0.8 g of protein per kilogram of healthy body weight and this could be obtained when dietary protein makes up approximately 10% to 15% of total energy intake; however, protein requirements increase during conditions of stress and disease (Mahan *et al.*, 2012). The dietary reference intakes (DRIs) established for dietary protein are shown in Table 1.

Table 1. Dietary Reference Intakes (DRIs) for Protein in g/Kg/day According to Age Group

Age Group	**DRI (g/Kg/day)**
Infants	1.5
1-3 years	1.1
4-13 years	0.95
14-18 years	0.85
Healthy Adults	0.8
Pregnant and lactating women	1.1

C. Protein and Body Weight

Over the past 20 years, high-protein diets have been promoted as a successful strategy for weight loss. Greater weight loss, fat mass loss, preservation of lean mass, reductions in triglycerides, blood pressure, and waist circumference were reported after higher-protein energy-restriction diets than after lower-protein energy-restriction diets (Leidy *et al.,* 2015).

Protein is a macronutrient with the most pronounced thermogenic and satiating potential compared with carbohydrates and fats, and the most promising nutrient in preserving lean body mass. A moderate consumption (25-35 g) of high-quality protein during each meal has been shown to stimulate muscle protein synthesis, promote muscle health and preserve lean body mass with increasing age. Moreover, an increase in the proportion of dietary protein (about 25% Kcal) during a hypo-energetic diet regimen induced body weight/fat loss and prevented weight regain in obese individuals (Arentson-Lantz *et al.*, 2015).

The mechanisms by which dietary protein regulates body weight are multifactorial. Proteins promote satiety to a greater extent than carbohydrates and fats, and thus reduce energy consumption at subsequent meals. In addition, dietary proteins are associated with increased thermogenesis and maintenance of lean body mass (Paddon-Jones *et al*., 2008). Diets with increased protein have been shown to be beneficial for the treatment or prevention of obesity, osteoporosis, type 2 diabetes, Metabolic Syndrome, heart disease, and sarcopenia. Replacing carbohydrates with protein reduces daily energy intake by ~ 200 kcal according to studies of energy regulation for weight management. The mechanism for this satiety could be mediated

by intestinal hormones or by reducing peak post-prandial insulin response (Layman, 2009).

Evidence has shown that high-protein diets increase total weight loss and increase the percentage of fat loss. A 6-month randomized trial of 60 overweight and obese subjects comparing the effects of a high-protein diet (25% energy; 128-139 g/d) and a medium-protein diet (12% energy; 76-80 g/d) showed that fat loss was almost as twice in subjects receiving the high-protein diet. The benefits of high-protein diets have also been demonstrated in long-term studies. A 12-mo study assessing the long term effects of a high-protein diet (25% energy) in 50 overweight and obese subjects found that weight loss was greater in the high-protein group compared with the normal-protein group (12% energy) (-9.4 kg versus -5.9 kg) (Paddon-Jones *et al.*, 2008). Research has proven that high-protein diets protect the metabolically active lean tissues during weight loss (<15% loss) compared with high carbohydrate low fat low protein diets (30% to 40% loss) and therefore, result in long-term successful weight loss (Layman, 2009).

It has been hypothesized that there is an inverse association between calcium, the major mineral component in milk, and body weight. A daily increase of 300 mg of calcium or approximately one serving of dairy was associated with a yearly reduction of ~ 1 kg of body fat in children and 2.5 to 3.0 kg of body weight in adults. However, dairy products exert a more significant anti-obesity effect than supplemental calcium suggesting that there are other components in dairy responsible for weight loss (Anderson & Moore, 2004). Diets high in proteins or dairy products are also rich sources of phosphorus (Sherman & Mehta, 2009); therefore, the associations between such diets and weight loss could be explained by the involvement of phosphorus.

D. Distribution of Phosphorus in the Body

Phosphorus is an essential biologic element that is required by all cells for normal function and it is a critical component of all living organisms. It is a multivalent nonmetal element naturally found in inorganic PO_4 rocks, and is never found as a free element in nature because of its high reactivity but it is present in the anion form PO_4 (Kalantar-Zadeh *et al.,* 2010). Phosphorus is the second most abundant element in the human body after calcium (Pravst, 2011). The average total phosphorus content in a 70 kg man is approximately 700 g, of which 85% is in the bone and teeth (in the form of hydroxyapatite), 14% in soft tissues and only 1% in the extracellular space. Phosphorus-containing compounds are essential for cell structure (cell membrane and nucleic acids), cellular metabolism (generation of ATP), regulation of subcellular processes (phosphorylation of key enzymes), and maintenance of acid-base homeostasis (urinary buffering) (Amanzadeh & Reilly, 2006). Intracellular phosphorus is found mainly in the form of organic compounds such as creatinine phosphate, ATP, nucleic acids, phospholipids, and phosphoproteins. Phosphorus in the plasma is found in both organic and inorganic forms, and the normal serum phosphorus concentration ranges between 3.0-4.5 mg/dl (Uribarri, 2007). Several enzymes, hormones, and intracellular signaling molecules are activated by phosphorylation (Kalantar-Zadeh *et al.,* 2010).

E. Phosphorus Sources and Requirements

Since phosphorus is present in all living organisms, it is almost found in all foods (Kalantar-Zadeh *et al.,* 2010). The main sources of dietary phosphorus are organic, from animal and vegetable proteins, and inorganic mainly from food additives. Protein-rich foods are the main sources of organic phosphorus including dairy products,

fish, meat, and chicken, and plant foods such as legumes, nuts, and chocolates (Noori *et al.,* 2010). According to the Food and Nutrition Board of the Institute of Medicine, the recommended dietary allowance of phosphorus is 700 mg/d in healthy adults. An allowance of up to 1250 mg/d has been suggested in older children and pregnant women (Kalantar-Zadeh *et al.,* 2010). The recommended dietary allowance (RDA) of phosphorus according to age group is shown in table 2.

Table 2. Recommended Dietary Allowance (RDA) in mg/day for Phosphorus by Age Group (National Academy of Science, 1997)

Age Group	**RDA (mg/day)**
1-3 years	460
4-8 years	500
9-18 years	1250
19-70 years	700
>70 years	700

Digestibility of phosphorus from animal foods is higher than from plant foods (Kalantar-Zadeh *et al.,* 2010). A large fraction of phosphorus (about 75%) in plant-based foods is mainly found in the form of phytate, and the human small intestine does not secrete the enzyme phytase needed to degrade phytate and release phosphorus; therefore, phytate phosphorus is not bioavailable in humans unless the food is treated to release phosphorus (Uribarri, 2007). The bioavailability of phosphorus from plant-based foods depends on the method of preparation (Calvo & Uribarri, 2013). For instance, leavening bread with phytase-containing yeast makes phytate phosphorus more

bioavailable (Uribarri, 2007). Boiling can remove phytate, also soaking or rehydration of dried beans and other legumes make phytate phosphorus more available (Calvo & Uribarri, 2013). In contrast, phosphorus from animal-based foods such as meat is well absorbed since it is mostly found as organic compounds that are hydrolyzed in the gastrointestinal tract releasing inorganic phosphorus (Uribarri, 2007). Only 40% to 60% of organic dietary phosphorus is absorbed. Inorganic phosphorus such as phosphorus additives are not bound to proteins as phosphorus present naturally in foods. They are salts that are highly absorbed in the intestinal tract, usually > 90%. Common sources include certain beverages, enhanced or restructured meats, frozen meals, cereals, snack bars, processed or spreadable cheeses, instant products, and refrigerated bakery products (Kalantar-Zadeh *et al.,* 2010).

There is a strong positive correlation between dietary protein and phosphorus intake. In a study that was done on patients with chronic kidney disease, a regression equation that shows the relationship between dietary protein and phosphorus intake was developed: Dietary P (mg) = 128 mg P + (dietary protein in g) x 14 mg P/ g protein (Kalantar-Zadeh *et al.,* 2010).

Egg white, a rich source of high biologic value protein, is almost devoid of phosphorus and has one of the lowest P-to-protein ratios (Kalantar-Zadeh *et al.,* 2010). Phosphorus and protein contents of selected food items including phosphorus to protein ratios, and the phytate phosphorus content of selected plant-based foods are shown in tables 3 and 4, respectively.

Table 3. Phosphorus and Protein Content of Selected Foods*

Food Item	P (mg/100g)	Protein (g/100g)	P/Protein (mg/g)	P/Calorie (mg/Kcal)
Meats				
Chicken Breast	213	22.5	9.5	1.8
Lamb	160	16.9	9.5	0.6
Ground Beef	175	19.4	9.0	0.9
Fish	174	20.3	8.6	1.3
Crab	133	18.5	7.2	1.5
Breads, Cereals, and Nuts				
White Bread	96.7	8.9	10.9	0.4
Almonds	479.7	21.2	22.6	0.8
Pistachio	490.3	20.1	24.4	0.9
Walnuts	345.7	15.2	22.7	0.5
Cereals	431	13.7	31.5	0.9
Milk, Dairy, and Eggs				
Egg white	15.1	10.9	1.4	0.3
Egg whole	198	12.6	15.7	1.4
Mozzarella Cheese	352.7	22.2	15.9	1.2
Yogurt	95.1	3.5	27.2	1.6
Whole Milk	84	3.1	27	1.4
Legumes and Rice				
Peas	108.3	5.4	20	1.3
Beans	38	4.8	7.9	1.1
Lentils	179.8	9	20	1.5
White Rice	43	2.7	15.9	0.3
Brown Rice	103	2.7	38.1	0.8

*Individual values taken from the USDA National Nutrient Database.

Table 4. Total and Phytate Phosphorus Content of Selected Plant-Based Food Items (Ravindran *et al.,* 1994)

Food Item	Phosphorus (g/100g)		Phytate-P (% of Total)
	Total	Phytate-P	
Maize	0.26	0.22	84.6
Brown Rice (unpolished)	0.38	0.28	73.7
Rice (polished)	0.31	0.17	54.8
Wheat Bran	1.15	1.03	76.9
Cassava	0.16	0.04	25
Sweet Potato	0.21	0.05	23.8
Potato	0.24	0.05	20.8
Soy Bean	0.6	0.37	61.7
Lentils	0.31	0.2	64.5

F. Phosphorus Absorption and Homeostasis

Phosphate is absorbed in the small intestine by both passive paracellular diffusion along an electrochemical gradient and active transport by the luminal sodium phosphate cotransporter type 2b (Uribarri, 2007). The net absorption of dietary phosphorus through the intestinal tract is approximately 40% to 80%, based on the type of the diet and the effect of hormones such as vitamin D (calcitriol), which increases the intestinal absorption of phosphorus. In individuals with normal kidney function, > 95% of the absorbed phosphorus is excreted through urine. Approximately, 70% to 90% of the phosphorus filtered by the glomeruli is reabsorbed by the renal tubular cells, which is controlled by PTH and fibroblast growth factor 23, both of which decrease the tubular phosphorus reabsorption. Therefore, high dietary phosphorus intake rarely leads to major changes in serum phosphorus concentrations in people with normal or partly

attenuated kidney function (Kalantar-Zadeh *et al.,* 2010). Unlike calcium, intestinal absorption of phosphorus is linearly related to phosphorus intake over the range of 4-30 mg/kg/day. Hence, the main determinants of intestinal phosphorus absorption are the amount of phosphorus present in the diet, its bioavailability, and the presence of phosphorus binders. Phosphorus homeostasis is determined by the interactions among intestinal absorption, renal excretion, and exchanges with bone and the intracellular space (Figure 1) (Uribarri, 2007).

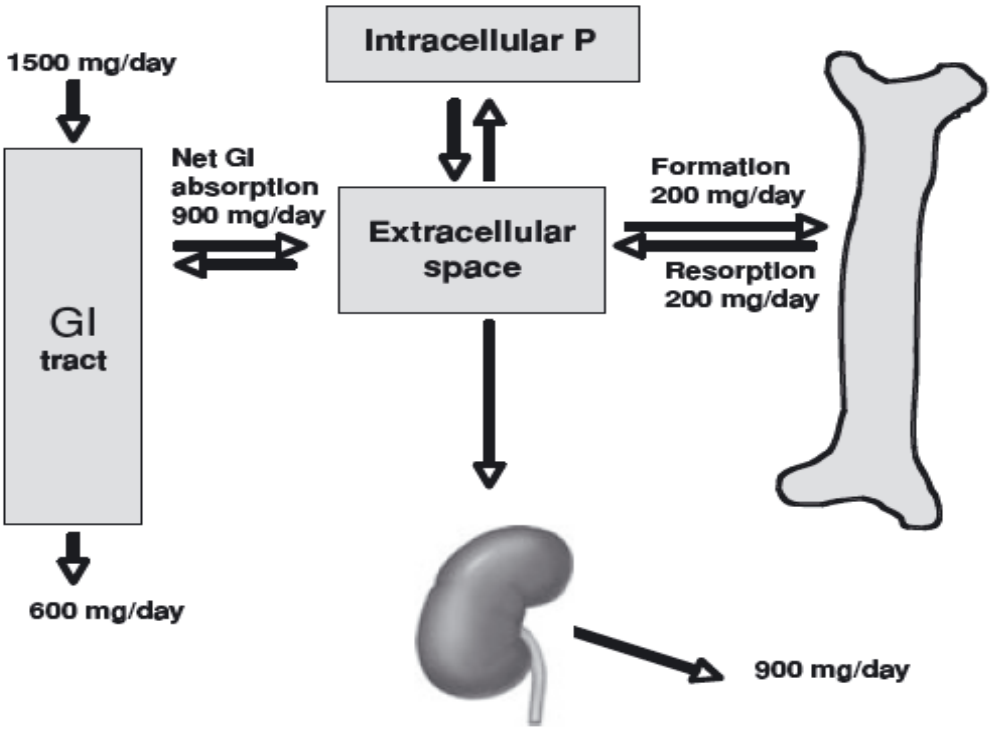

Figure 1. Phosphorus Homeostasis

G. Phosphorus, ATP, Food Intake

The physiological regulation of food intake acts mainly at the central level and is partially governed by signals produced originally from the liver via hepatic postprandial metabolism involving ATP production (Friedman, 2007; Langhans & Scharrer, 1992). A lot of evidence has supported a relationship between declining hepatic ATP levels and increasing food intake. This decline in hepatic ATP production is thought to transduce changes in hepatic energy status into neural signals or hepatic vagal afferent activity that is transmitted to the central nervous system (Freidman, 2007; Hong *et al.,* 2000; Oberhaensli *et al.,* 1986; Rwason & Freidman, 1994; Riquelme *et al.,* 1984). In line with that, several studies that were conducted on animals and humans reported several abnormalities in hepatic ATP of obese individuals (Chavin *et al.,* 1999; Cortez *et al.,* 1999). Additionally, in the human liver, ATP status was reported to vary with hepatic ATP store (Nair *et al.,* 2003) and recovery from hepatic ATP depletion (using fructose infusion) (Cortez *et al.,* 1999) being inversely related to body mass index (BMI). Moreover, an analysis of the metabolic data using the Knowledge Discovery in Databases have concluded that decreased energy levels or ATP deficiency were strongly linked to the development of obesity which is due to driving of overeating and conserving energy (Wlodek & Gonzales, 2003). Accordingly, Obeid *et al.* (2010) found that the addition of 500 mg of phosphorus to different preloads led to substantial reduction in *ad libitum* subsequent energy intake (27-33%).

H. Factors Affecting ATP Production

ATP production, including hepatic ATP, depends on the availability of phosphorus (Morris *et al.,* 1978; Solomon & Kirby, 1990) along with two other factors.

First, there are only limited quantities of free phosphate stored within the cells, and the metabolic phosphate of most tissues depend on the extracellular fluid (ECF) inorganic phosphate (P_i). When ECF P_i levels are low, this will be followed by cellular dysfunction. Second, there is almost constant phosphorus absorption across a wide range of intake (Food and Nutrition Board, 1997) suggesting a lack of the adaptive mechanism that usually improves phosphorus absorption during low intakes which can occur with other micronutrients as well.

ATP production is affected by fructose since it has a "phosphate-sequestering" capacity. Unlike glucose, fructose is not subjected to a feedback mechanism for fructose phosphorylation resulting in the accumulation of fructose-1-phosphate (F-1-P) in the liver. Consequently, phosphorus will be unavailable for other important metabolic reactions such as the regeneration of ATP (Bizeau & Pagliassotti, 2005; Karczmar *et al.,* 1989; Morris *et al.,* 1978; Oberhaensli *et al.,* 1986). In addition, the release of insulin after carbohydrate "glucose" ingestion increases the intracellular uptake of glucose along with phosphorus and other several electrolytes besides phosphorylation of different compounds. Therefore, under conditions of fructose or high-glucose/low-phosphorus, there will be a competition for phosphorus between phosphorylation of some compounds and ATP production. Moreover, there is a link between phosphorus and different metabolic situations related to increased body weight or energy intake. For instance, the consumption of high-fructose corn syrup (HFCS) and obesity (Bray *et al.,* 2004), increased protein intake, which is high in phosphorus, and the subsequent decrease in energy intake (Halton & Hu, 2004; Latner & Schwartz, 1999), and the inverse association between dairy product, which is high in phosphorus, and body weight provided that calcium failed to explain such an association (Teegarden, 2005;

Wagner *et al.,* 2007; Yanovski *et al.,* 2009). Conversely, an increase in calcium intake whether from diet or from supplements reduces the availability of phosphorus by impairing phosphorus absorption (Heaney & Nordin, 2002), not forgetting to mention that high doses of calcium carbonate are used as phosphate binders.

Obesity is characterized by insulin resistance which in turn predisposes to the development of impaired glucose tolerance which is known to decrease the peripheral uptake of both glucose and phosphorus (Campillo *et al.,* 1982) leading to the reduction in the capacity of ATP production thus altering DIT (Felig, 1984).

I. Phosphorus and Body Weight

Obesity has increased in the past few decades due to changes in dietary habits and nutrient intake, and this was strongly associated with the increase in the consumption of sugar, oils, and sweeteners such as HFCS which contain small amounts of phosphorus. In addition, refined cereals have small amount of phosphorus due to the refinement process which reduces phosphorus content by about 70%. The Food Balance Sheet of the FAO (FAO, 2010) reveals that these commodities contributed to approximately 56% and 59% of the food supply (kcal/capita/day) in the USA and Lebanon, respectively. Furthermore, starchy foods such as potatoes are low in phosphorus. The socioeconomic status has a strong effect on the consumption of these commodities. For instance, people of low socioeconomic status highly consume starchy foods due to the reason that these foods have high energy density (kcal/g food) and low energy cost (US$/1000 kcal). This could explain the high prevalence of overweight and obesity among people of low socioeconomic status (Drewnowski, 2009). In line with that, it is reasonable to assume that body weight and phosphorus intake are inversely

related to each other given that the prevalence of overweight and obesity is strongly correlated with the increase in the consumption of foods that have low levels of phosphorus.

The phosphorus content of several major raw nonrefined food commodities such as meat, cereals, pulses is around 1 mg P/kcal, so a person consuming 2,500 kcal per day, would be getting about 2.5 g of phosphorus. However, the daily phosphorus intake nowadays is about 1.4 g/day (Ervin *et al.,* 2004) which is above the present RDA but much lower than the predictable intake. Both the ancestral and the current intakes of phosphorus are in fact lower than the upper intake limit which is 4g/day (Food and Nutrition Board, 1997).

Several studies have found a relationship between body weight and phosphorus intake, but conflicting findings were reported (Alonso *et al.,* 2010; Beydoun *et al.,* 2008; Elliott *et al.,* 2008). Some studies have found that serum phosphate is inversely related to body weight (Haap *et al.,* 2006; Haglin *et al.,* 2001; Kalaitzidis *et al.,* 2005; Lind *et al.,* 1993; Lindgärde & Trell, 1977), and hypophosphatemia was assumed to be involved in the development of metabolic syndrome, including increased BMI (Haglin, 2001). Due to the similarity in the phosphate fractional excretion rate between lean, overweight, and obese subjects, the reduction in serum phosphate is mainly due to a reduction in dietary intake rather than problems with excretion (Kalaitzidis *et al.,* 2005).

In summary, ATP production strongly depends on the availability of phosphorus and it is important for several processes including eating behavior and energy expenditure (Figures 2 & 3). Increase in the consumption of refined cereals, sugars (fructose), potatoes, and oils negatively influence the availability of phosphorus in our body. The release of insulin will stimulate phosphorylation of many compounds compromising the availability of phosphorus needed for ATP production given that ATP can act as a phosphate donor (Karczmar *et al.,* 1989). Consequently, an increase in insulin release under a low phosphorus diet will impair the situation. Additionally, an impaired glucose tolerance reduces the peripheral uptake of phosphorus (Campillo *et al.,* 1982) and will therefore affect thermogenesis (Felig, 1984).

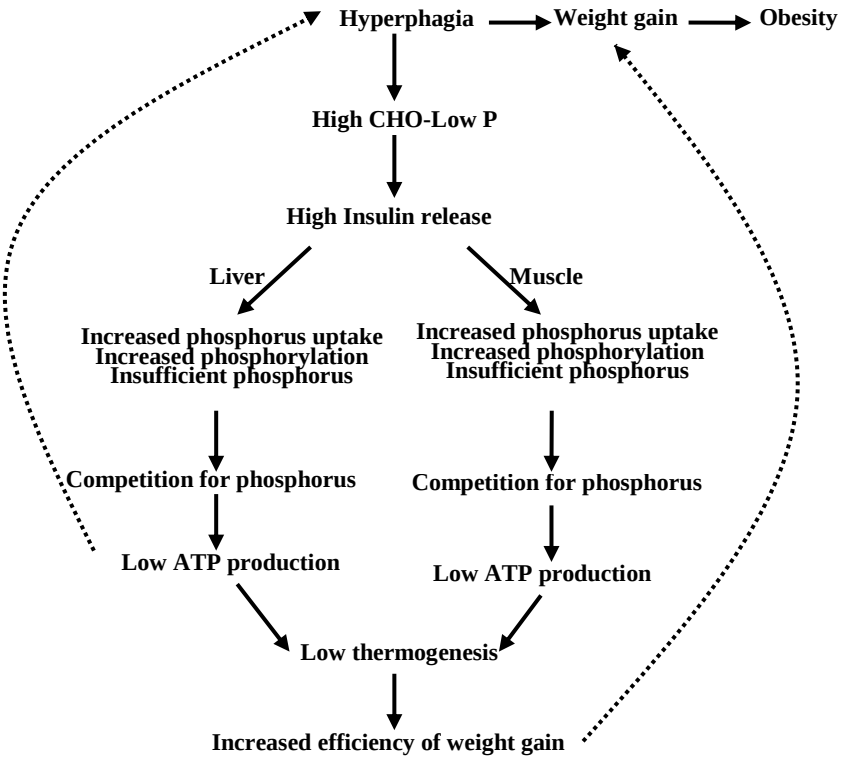

Figure 2. Proposed Interaction among Phosphorus, ATP Production and Obesity

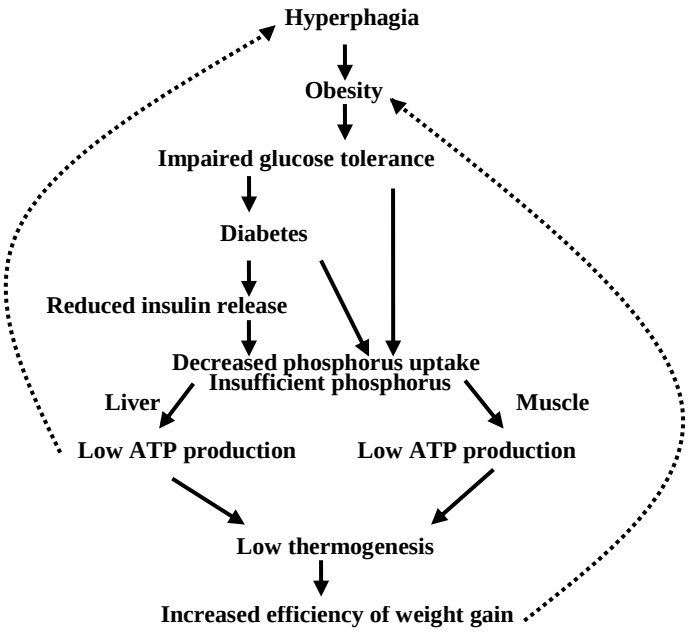

Figure 3. Proposed Interaction among Phosphorus, Impaired Glucose Tolerance, Thermogenesis and Obesity

The objective of the present study was to determine the effect of phosphorus supplementation on diet-induced thermogenesis and substrate oxidation, including % fat oxidation, % carbohydrate oxidation and respiratory quotient, of healthy lean male subjects consuming two isocaloric meals (554 Kcal), a normal protein-low phosphorus meal and a high protein-low phosphorus meal, using egg white powder as the main source of protein.

The study was divided into two experiments:

1. Effect of Phosphorus Supplementation on Diet-Induced Thermogenesis of Lean Male Subjects Consuming a Normal Protein-Low Phosphorus Meal
2. Effect of Phosphorus Supplementation on Diet-Induced Thermogenesis of Lean Male Subjects Consuming a High Protein-Low Phosphorus Meal

CHAPTER III

MATERIALS AND METHODS

A. Study Design

This study is a single-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 September 2015 and June 2016. Healthy lean male subjects with an age range between 19 and 35 years and with a normal body mass index (BMI: 18.5-24.9 Kg/m^2) were recruited through direct approach. The purpose and the protocol of the study were explained to the volunteers. Interested subjects were asked to fill a general health screening questionnaire (Appendices I and II) to determine feasibility for participation in the study. Subjects who fulfilled the general entrance requirements signed an informed consent (Appendices III, IV, V and VI) and were referred for a fasting blood test to determine their blood glucose, creatinine and estimated glomerular filtration rate (Est. GFR) at the American University of Beirut Medical Center (AUBMC). Exclusion criteria included GFR<60 $ml/min/1.73m^2$ and Creatinine<1.2 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. Moreover, 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 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 and the use of nutritional supplements one day prior to the study.

C. Experimental Protocol

The present study was divided into two experiments. A total of 12 healthy lean male subjects were allocated in each experiment in such a way that 7 subjects participated in both experiments due to feasibility of participation; however, 5 new subjects were allocated in each experiment.

Experiment 1: Effect of Phosphorus Supplementation on Diet-Induced Thermogenesis of Lean Male Subjects Consuming a Normal Protein-Low Phosphorus Meal

A total of 12 healthy lean male subjects participated in this experiment. Participants were given a 554 Kcal normal protein-low phosphorus meal (15% of total energy from protein).

Experiment 2: Effect of Phosphorus Supplementation on Diet-Induced Thermogenesis of Lean Male Subjects Consuming a High Protein-Low Phosphorus Meal

A total of 12 healthy lean male subjects participated in this experiment. Participants were given a 554 Kcal high protein-low phosphorus meal (50% of total energy from protein).

In both experiments, overnight fasted subjects (>8 hours) were asked to attend the research room (fixed r.t. at 22ºC) on 2 visits separated by a minimum of one week washout period at around 8:00 am. Anthropometric measurements including weight, height, waist and hip circumference were taken. Subjects were then asked to rest for around 30 minutes on a couch, then resting energy expenditure (REE), % fat oxidation (FO), % carbohydrate oxidation (CO) and respiratory quotient (RQ) were measured for 30 minutes using a ventilated hood and canopy system (COSMED QUARK CPET UNIT, Italy) for indirect calorimetry measurement. The rate of flow of air pumped through the hood was maintained by keeping CO_2 levels constant in the hood between 1.00 and 1.20%. Subjects were then given the appropriate meal (normal-protein meal/ high-protein meal) with 500 mg of phosphorus tablets (potassium phosphate) on one visit or with placebo (cellulose) tablets (Tables 5 & 6) on the other visit in a random order. Subjects were blinded on the types of the tablets and they were asked to consume the meal within 15 minutes. Postprandial energy expenditure (EE), % FO, % CO and RQ were then measured for the next four hours during a period of 15 minute intervals with 15 minute breaks. During measurements, subjects were asked to sit in a fixed position with as minimal movement as possible, while during the breaks they were allowed to move and they were provided with water when needed. Urine was collected at the end of the experimental period in both visits and the whole volume was recorded. Urine samples were then aliquoted into three labeled tubes with 10 ml of urine in each and stored in a freezer at -20˚C for later use to measure the levels of phosphorus, creatinine, and urine urea nitrogen (UUN) using the Vitros 350 analyzer. Study design is shown in figure 4.

Table 5. 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 6. 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

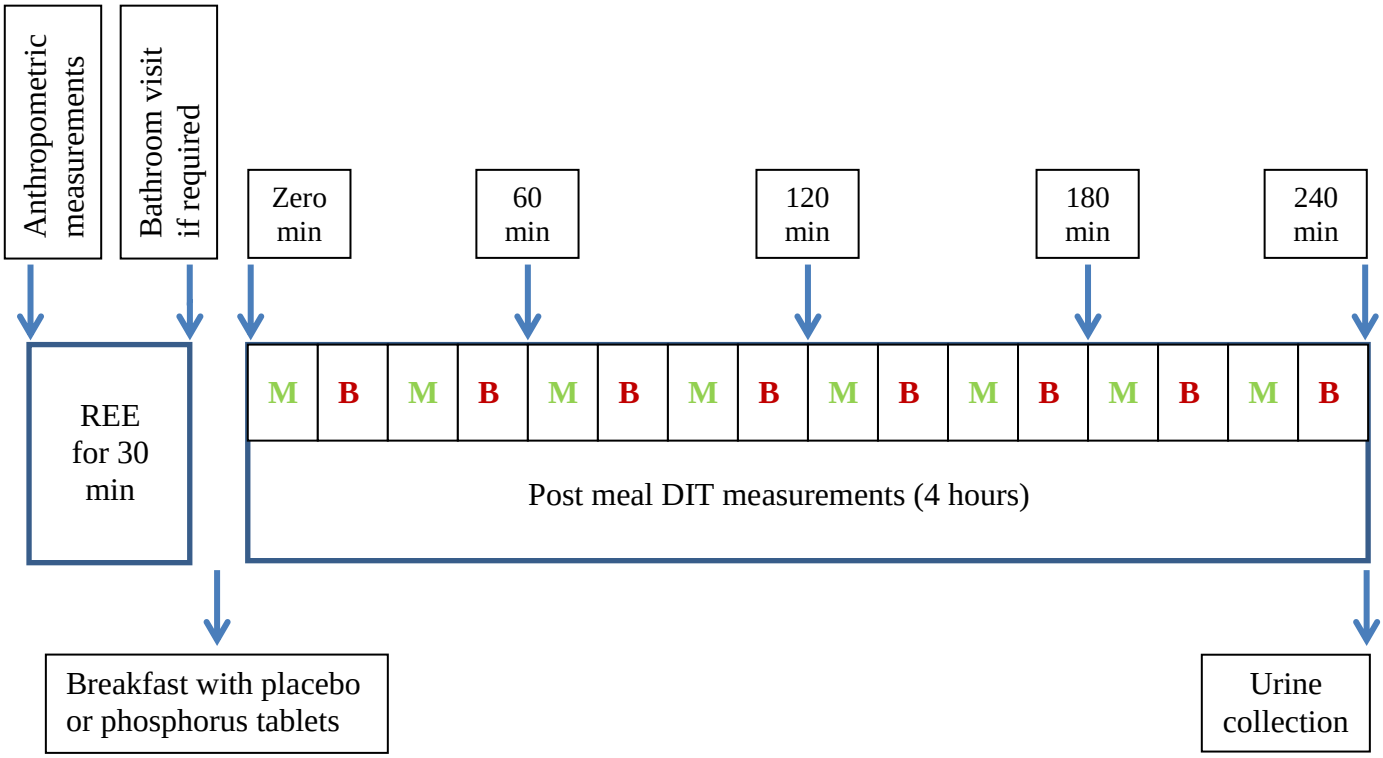

Figure 4. Procedure for Diet-Induced Thermogenesis Test Days. M represents the measurements for 15 minutes on the COSMED machine; and B represents the break for 15 minutes

D. Anthropometric Measurements

Weight (Kg) was measured using a calibrated Seca balance while subjects were wearing light clothes and without shoes, and height (cm) was measured using a portable stadiometer with a movable head piece. Waist circumference (cm) was measured at the umbilicus level, and hip circumference (cm) at the widest part of the buttocks using a flexible, non-stretchable measuring tape.

E. Experimental Meals

The normal-protein meal and the high-protein meal had isocaloric content (554 Kcal), and were prepared using the same ingredients but with varied levels of macronutrient distribution (Table 7). The experimental meals comprised of toast, butter and protein shake that was prepared using egg white powder, sugar and unsweetened cocoa powder with the addition of vanilla and raspberry flavors to increase the acceptability of the shake. A spray-dried egg white powder was used as the main source of protein due to the fact that egg white protein is a complete protein having all the essential amino acids and is almost devoid of phosphorus (Kalantar-Zadeh *et al.*, 2010). The polysaccharide xanthan gum was used as a thickening agent and a taste enhancer in the protein shake preparation.

The normal-protein meal provided 21 grams of protein (15% of total energy), 73 grams of carbohydrates (53% of total energy) and 20 grams of fat (32% of total energy). On the other hand, the high-protein meal provided 70 grams of protein (50% of total energy), 40 grams of carbohydrates (29% of total energy) and 13 grams of fat (21% of total energy).

Table 7. Composition of the Normal-Protein and High-Protein Meals

	Normal-Protein Meal (Low in P)	**High-Protein Meal** (Low in P)
Toast *P: 103 mg/100 g*	65 grams	30 grams
Butter *P: 24 mg/100g*	1.5 Tbsp. (21.3 grams)	1 Tbsp. (14.2 grams)
Egg white protein powder *P: 89mg/100g*	18 grams	82 grams
Unsweetened Cocoa Powder *P: 734mg/100g*	½ Tbsp. (2.7 grams)	½ Tbsp. (2.7 grams)
Sugar	3 Tbsp. (37.8 grams)	4 tsp. (16.8 grams)
Xanthan Gum	0.1 grams	0.2 grams
Water	330 ml	330 ml
Vanilla and Raspberry Flavors	Few drops	Few drops
Carbohydrates		
Grams:	72.88 grams	39.6 grams
Calories:	291.52 Kcal	158.4 Kcal
Percentage:	**52.61%**	**28.61%**
Proteins		
Grams:	21.06 grams	69.81 grams
Calories:	84.24 Kcal	279.24 Kcal
Percentage:	**15.20%**	**50.43%**
Fats		
Grams:	19.81 grams	12.89 grams
Calories:	178.29 Kcal	116.01 Kcal
Percentage:	**32.17%**	**20.95%**
Total Calories	**554 Calories**	**554 Calories**
P (mg)	**109 mg**	**143 mg**

F. Measurement of Baseline and Postprandial EE, %FO, %CO and RQ

The machine measures O_2 consumption and CO_2 production by the machine's own O_2 and CO_2 analyzers, and it calculates the difference between the expired air in the hood and room air. In addition, the machine automatically calculates energy expenditure, % fat oxidation, % carbohydrate oxidation and respiratory quotient. Absolute values for each variable were plotted against time, and difference from baseline was calculated for each variable by subtracting the absolute value at each time point from the average baseline value, and they were plotted against time. Respiratory quotient is an indication of substrate oxidation and it is expressed as the ratio of CO_2 produced to O_2 consumed while food is being metabolized ($RQ=CO_2$ produced/O_2 consumed), and it ranges from 0.7-1.0 (Fats: 0.7, Proteins: 0.8, Carbohydrates: 1.0).

G. Urinary Parameters: Phosphorus, Creatinine, Urine Urea Nitrogen

Urine specimens were thawed at room temperature and were then centrifuged for 5 minutes at 3500 rpm at 20ºC using EPPENDORF Centrifuge 5810R. Urinary levels of phosphorus, creatinine, and urea nitrogen were measured using Vitros 350 analyzer.

H. 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 urinary parameters between the control and phosphorus treatments. Statistical significance was set at $P < 0.05$.

CHAPTER IV

RESULTS

1) EXPERIMENT 1: EFFECT OF PHOSPHORUS SUPPLEMENTATION ON DIET-INDUCED THERMOGENESIS OF LEAN MALE SUBJECTS CONSUMING A NORMAL PROTEIN-LOW PHOSPHORUS MEAL

A. Subject Characteristics

A total of 12 healthy lean male subjects completed experiment 1. Baseline characteristics including age, anthropometric measurements and serum parameters are presented in table 8. All participants had normal BMI, normal waist circumference and hip circumference, and normal fasting blood glucose levels, blood creatinine and estimated glomerular filtration rate.

Table 8. Baseline Characteristics of the Normal-Protein Group

Variable	Mean ± SEM
Age (years)	23.08 ± 1.45
Anthropometric Variables	
Weight (Kg)	73.65 ± 1.90
Height (cm)	178.09 ± 1.60
BMI (Kg/m²)	23.279 ± 0.698
WC (cm)	77.79 ± 1.92
HC (cm)	98.36 ± 1.60
Serum Parameters	
FBG (mg/dL)	89.67 ± 2.43
Creatinine (mg/dL)	0.92 ± 0.04
Est. GFR (mL/min/1.73mý)	114.92 ± 4.67

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; FBG, fasting blood glucose; Est. GFR, estimated glomerular filtration rate.

B. Postprandial Energy Expenditure

Resting energy expenditure (REE) of the lean male subjects was almost similar in both treatments, starting at 1.26 Kcal/minute in the control and 1.22 Kcal/minute in the phosphorus treatments. Postprandial energy expenditure then increased similarly in both the control and phosphorus treatments following meal ingestion till it reached its maximum at 60 minutes (1.56 Kcal/minute) with phosphorus supplementation; however, this increase was significant according to time only (P=0.000) and there was no significant difference according to treatment (P=0.949). Afterwards, postprandial energy expenditure started to decline equally in both treatments reaching a minimum of 1.39 Kcal/minute, but it failed to go back to baseline (Table 9 & Figure 5).

A similar increase in Δ postprandial energy expenditure was observed in both treatments in the first 30 minutes following meal ingestion. This was followed by a significant increase in Δ postprandial energy expenditure with phosphorus supplementation according to time (P=0.000) and treatment (P=0.001), and it reached its maximum at 60 minutes (0.34 Kcal/minute). Δ Postprandial energy expenditure started to decrease after 60 minutes in both treatments reaching a minimum of 0.13 Kcal/minute and 0.17 Kcal/minute in the control and phosphorus treatments, respectively, but it failed to go back to baseline (Table 10 & Figure 6).

Table 9. Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	1.26 ± 0.05	1.22 ± 0.04
30	1.49 ± 0.05	1.47 ± 0.06
60	1.53 ± 0.05	1.56 ± 0.06
90	1.53 ± 0.06	1.53 ± 0.05
120	1.49 ± 0.05	1.49 ± 0.05
150	1.48 ± 0.05	1.48 ± 0.04
180	1.43 ± 0.06	1.44 ± 0.05
210	1.39 ± 0.06	1.41 ± 0.05
240	1.39 ± 0.06	1.39 ± 0.05

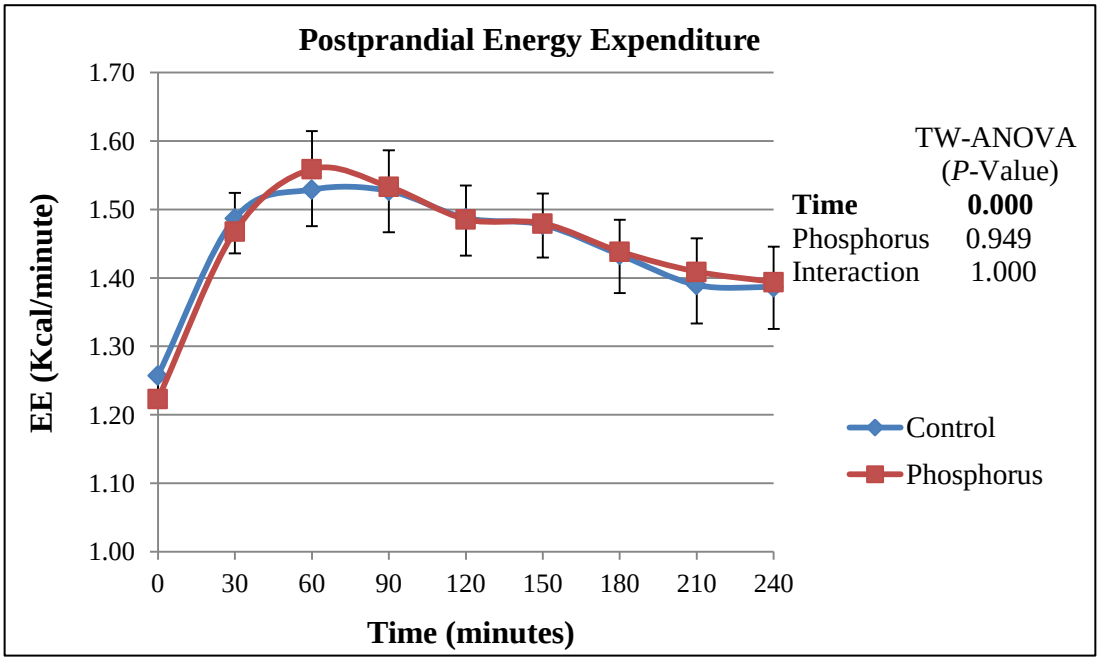

Figure 5. Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Table 10. Changes in Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.00 ± 0.00	0.00 ± 0.00
30	0.23 ± 0.02	0.24 ± 0.03
60	0.27 ± 0.02	0.34 ± 0.03
90	0.27 ± 0.03	0.31 ± 0.02
120	0.23 ± 0.03	0.26 ± 0.02
150	0.22 ± 0.02	0.26 ± 0.02
180	0.18 ± 0.02	0.22 ± 0.02
210	0.13 ± 0.02	0.19 ± 0.02
240	0.13 ± 0.03	0.17 ± 0.03

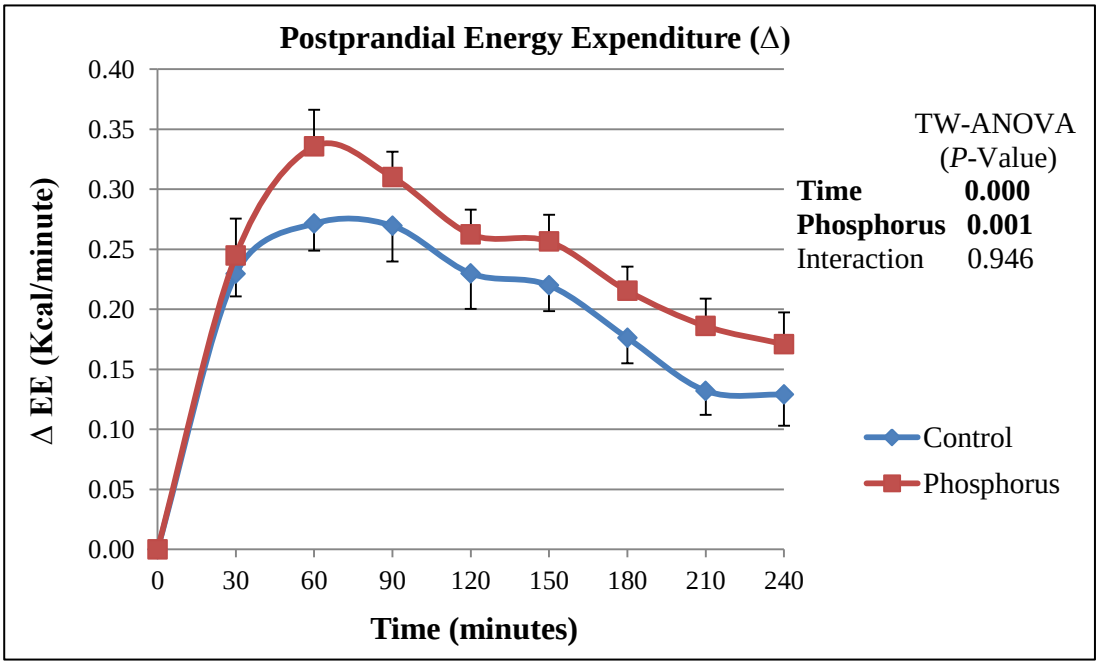

Figure 6. Changes in Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

C. Postprandial Fat Oxidation

Baseline fat oxidation started at 62.1% in the control and 65.2% in the phosphorus treatments. Postprandial fat oxidation then decreased in both treatments in the first 30 minutes following meal ingestion reaching a minimum of 56.0% in the control and 59.0% in the phosphorus treatments. Afterwards, it started to increase in both treatments till the end of the experimental period reaching a maximum of 81.0% and 81.1% in the control and phosphorus treatments, respectively, and there was a significant difference between both treatments according to time (P=0.000) and treatment (P=0.040) (Table 11 & Figure 7).

A similar trend was observed in Δ postprandial fat oxidation. There was a decrease in Δ postprandial fat oxidation in the first 30 minutes after meal ingestion reaching a minimum of -4.7% in the control and -6.3% in the phosphorus treatments, followed by an increase that persisted till the end of the experimental period reaching a maximum of 16.7% in the control and 15.9% in the phosphorus treatments. However, there was a significant difference between both treatments according to time only (P=0.000), and there was no significant difference according to treatment (P=0.442) (Table 12 & Figure 8).

Table 11. Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	62.1 ± 5.21	65.2 ± 2.99
30	56.0 ± 4.63	59.0 ± 5.02
60	58.7 ± 4.77	65.3 ± 2.87
90	68.4 ± 3.39	74.2 ± 2.37
120	73.5 ± 2.63	74.0 ± 2.47
150	73.4 ± 2.87	76.5 ± 2.13
180	76.2 ± 1.71	79.2 ± 2.03
210	79.0 ± 1.68	80.8 ± 1.55
240	81.0 ± 1.02	81.1 ± 1.19

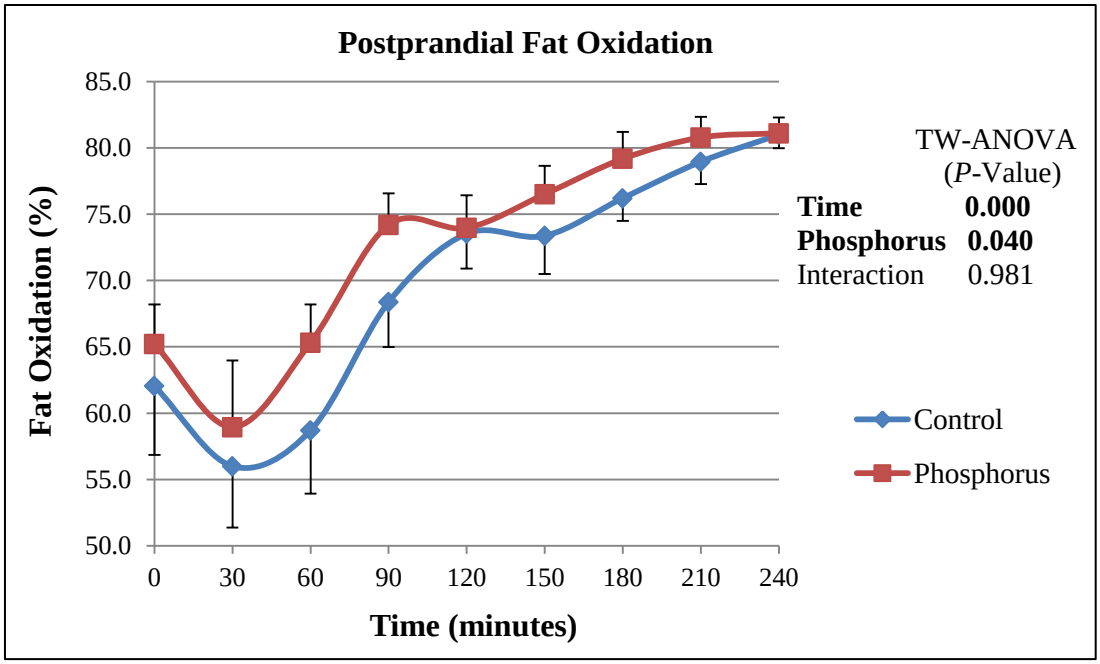

Figure 7. Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Table 12. Changes in Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.0 ± 0.00	0.0 ± 0.00
30	-4.7 ± 3.70	-6.3 ± 3.42
60	-3.5 ± 2.96	0.1 ± 2.62
90	4.0 ± 4.41	9.0 ± 1.87
120	9.2 ± 4.34	8.8 ± 1.97
150	9.2 ± 3.67	11.3 ± 1.92
180	11.8 ± 4.55	14.0 ± 2.52
210	14.6 ± 5.23	15.6 ± 2.54
240	16.7 ± 5.57	15.9 ± 2.47

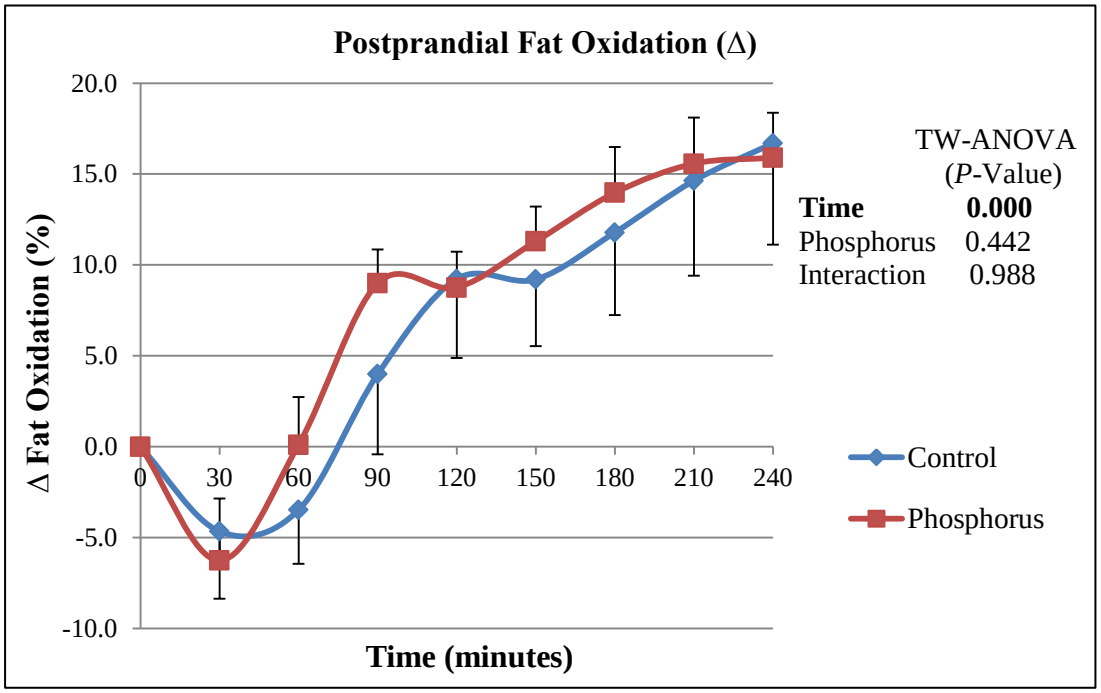

Figure 8. Changes in Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

D. Postprandial Carbohydrate Oxidation

Baseline carbohydrate oxidation started at 19.3% in the control and 15.6% in the phosphorus treatments. Postprandial carbohydrate oxidation then increased in the first 30 minutes following meal ingestion in both treatments reaching a maximum of 28.3% and 25.1% in the control and phosphorus treatments, respectively. Afterwards, it started to decrease in both treatments till the end of the experimental period reaching a minimum of 1.9%. There was a significant difference between both treatments according to time (P=0.000) and treatment (P=0.030) (Table 13 & Figure 9).

Similar differences were observed in Δ postprandial carbohydrate oxidation in both treatments. Δ Postprandial carbohydrate oxidation increased in the first 30 minutes following meal ingestion reaching a maximum of 9.0% and 9.5% in the control and phosphorus treatments, respectively, followed by a decrease in Δ postprandial carbohydrate oxidation in both treatments that persisted till the end of the experimental period reaching a minimum of -17.4% in the control and -13.7% in the phosphorus treatments. However, the difference between both treatments was significant according to time only (P=0.000), and there was no significant difference according to treatment (P=0.667) (Table 14 & Figure 10).

Table 13. Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	19.3 ± 4.89	15.6 ± 2.87
30	28.3 ± 4.45	25.1 ± 4.92
60	26.2 ± 4.71	19.6 ± 2.65
90	16.2 ± 3.38	10.5 ± 2.23
120	10.7 ± 2.56	10.2 ± 2.27
150	10.7 ± 2.62	7.7 ± 1.96
180	7.4 ± 1.59	4.4 ± 1.97
210	4.1 ± 1.33	2.4 ± 1.56
240	1.9 ± 0.80	1.9 ± 1.14

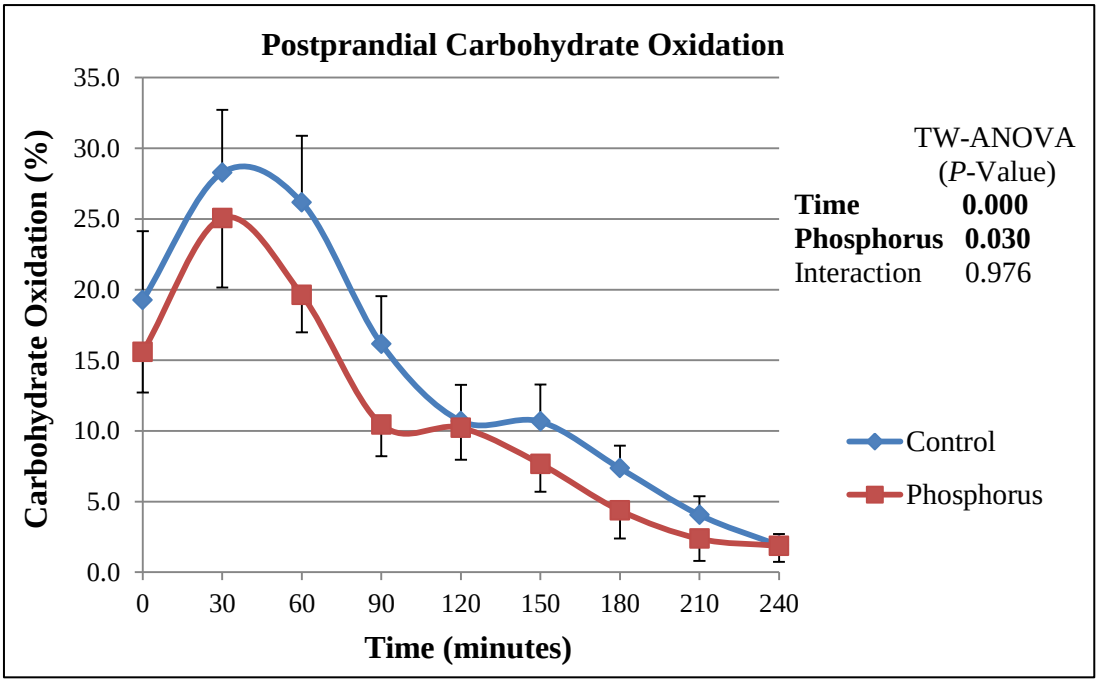

Figure 9. Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Table 14. Changes in Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.0 ± 0.00	0.0 ± 0.00
30	9.0 ± 3.62	9.5 ± 3.59
60	6.9 ± 2.96	4.0 ± 2.63
90	-3.1 ± 4.08	-5.1 ± 1.86
120	-8.6 ± 3.67	-5.4 ± 1.99
150	-8.6 ± 3.08	-7.9 ± 1.94
180	-11.9 ± 3.97	-11.2 ± 2.59
210	-15.2 ± 4.60	-13.2 ± 2.62
240	-17.4 ± 4.99	-13.7 ± 2.47

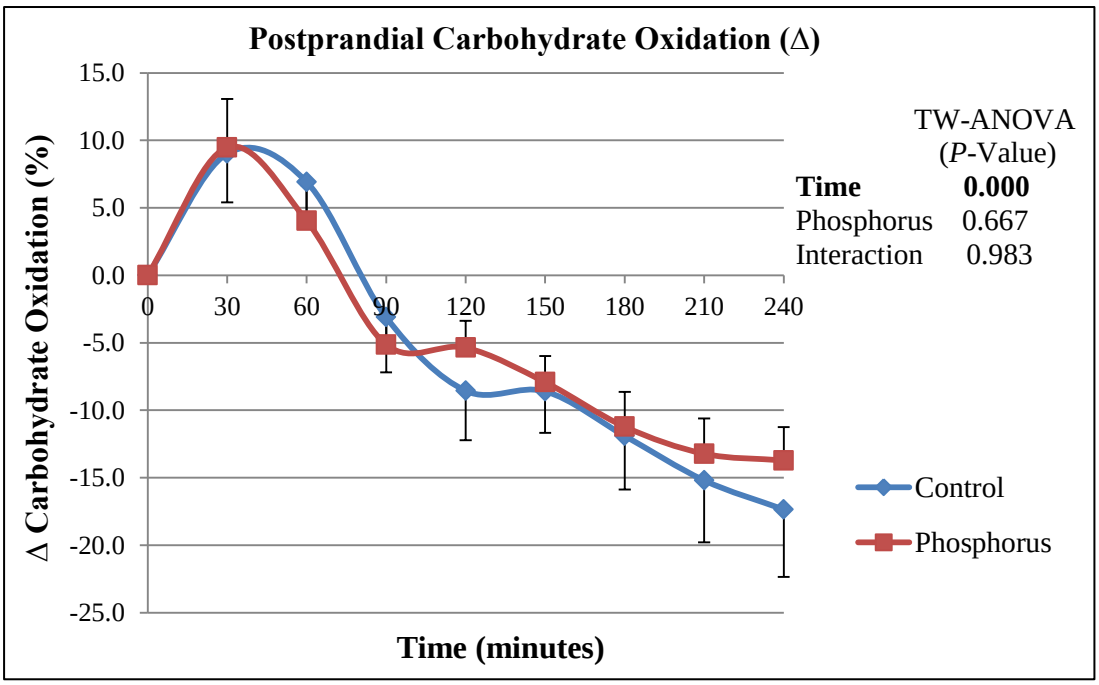

Figure 10. Changes in Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without phosphorus

E. Postprandial Respiratory Quotient (RQ)

Baseline RQ was 0.78 in the control and 0.75 in the phosphorus treatments. Postprandial RQ then increased in the first 30 minutes following meal ingestion in both the control and phosphorus treatments reaching a maximum of 0.81 and 0.79 in the control and phosphorus treatments, respectively, then it started to decrease in both treatments till the end of the experimental period reaching a minimum of 0.67 in the control and 0.65 in the phosphorus treatments. There was a significant difference between both treatments according to time (P=0.000) and treatment (P=0.003) (Table 15 & Figure 11).

Similar changes were observed in Δ postprandial RQ in both treatments; the same increase in the first 30 minutes reaching a maximum of 0.03 and 0.04 in the control and phosphorus treatments, respectively, and the same decrease was observed afterwards that persisted till the end of the experimental period reaching a minimum of -0.11 in both treatments. There was a significant difference between both treatments according to time only (P=0.000), and there was no significant difference according to treatment (P=0.664) (Table 16 & Figure 12).

Table 15. Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.78 ± 0.02	0.75 ± 0.02
30	0.81 ± 0.02	0.79 ± 0.02
60	0.79 ± 0.02	0.77 ± 0.01
90	0.76 ± 0.01	0.73 ± 0.01
120	0.73 ± 0.01	0.73 ± 0.01
150	0.73 ± 0.01	0.72 ± 0.01
180	0.72 ± 0.01	0.69 ± 0.02
210	0.70 ± 0.01	0.66 ± 0.02
240	0.67 ± 0.01	0.65 ± 0.01

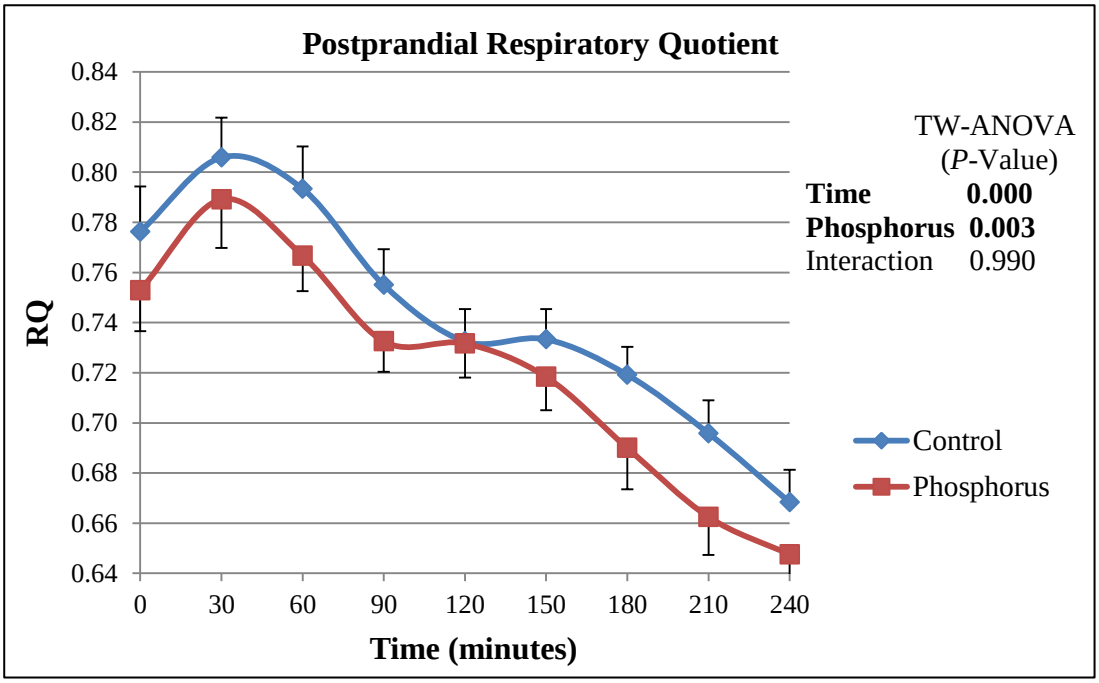

Figure 11. Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Table 16. Changes in Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.00 ± 0.00	0.00 ± 0.00
30	0.03 ± 0.01	0.04 ± 0.01
60	0.02 ± 0.01	0.01 ± 0.01
90	-0.02 ± 0.02	-0.02 ± 0.01
120	-0.04 ± 0.01	-0.02 ± 0.01
150	-0.04 ± 0.01	-0.03 ± 0.01
180	-0.06 ± 0.02	-0.06 ± 0.01
210	-0.08 ± 0.02	-0.09 ± 0.01
240	-0.11 ± 0.02	-0.11 ± 0.01

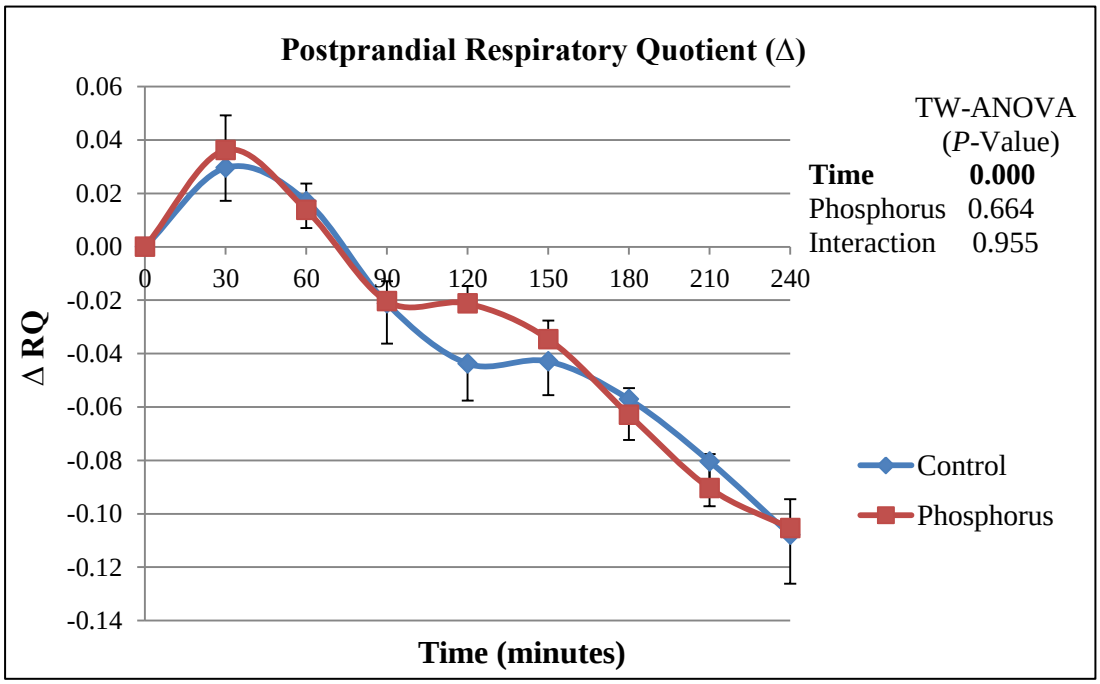

Figure 12. Changes in Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the Normal-Protein Meal with or without Phosphorus

F. Urinary Parameters

Urine was collected at the end of the experimental period in each visit and the whole volume was recorded. Using Vitros 350 analyzer, the following urinary parameters were measured: total phosphorus, total creatinine, and total urine urea nitrogen (UUN). Moreover, phosphorus to creatinine ratio (P/Cr), and UUN to creatinine ratio (UUN/Cr) were calculated.

Phosphorus to creatinine ratio (P/Cr) was significantly higher with phosphorus supplementation (*P*=0.027); however, no significant differences were observed in the other urinary parameters between the control and phosphorus treatments (Table 17).

Table 17. Urinary Parameters of the Control and Phosphorus Treatments of the Normal-Protein Group

Variable	Control	Phosphorus	*P*-Value
	Mean ± SEM	Mean ± SEM	
V of Urine (ml)	460.8 ± 68.6	354.2 ± 48.7	-
Total P (mg)	167.7 ± 33.2	236.6 ± 25.6	0.121
Total UUN (mg)	3468 ± 248	3287 ± 246	0.621
P/Cr	0.31 ± 0.05	0.50 ± 0.05	**0.027***
UUN/Cr	6.96 ± 0.53	7.02 ± 0.25	0.897

Abbreviations: V, volume; P, phosphorus; UUN, urine urea nitrogen; Cr, creatinine.
**P*-Value<0.05, values that are significantly different between the control and the phosphorus treatments using paired t-tests.

2) EXPERIMENT 2: EFFECT OF PHOSPHORUS SUPPLEMENTATION ON DIET-INDUCED THERMOGENESIS OF LEAN MALE SUBJECTS CONSUMING A HIGH PROTEIN-LOW PHOSPHORUS MEAL

A. Subject Characteristics

A total of 12 healthy lean male subjects completed experiment 2. Baseline characteristics including age, anthropometric measurements and serum parameters are presented in table 18. All participants had normal BMI, normal waist circumference and hip circumference, and normal fasting blood glucose levels, blood creatinine and estimated glomerular filtration rate.

Table 18. Baseline Characteristics of the High-Protein Group

Variable	Mean ± SEM
Age (years)	22.33 ± 1.30
Anthropometric Variables	
Weight (Kg)	73.85 ± 2.40
Height (cm)	180.18 ± 1.46
BMI (Kg/m²)	22.733 ± 0.573
WC (cm)	78.99 ± 1.78
HC (cm)	98.63 ± 1.84
Serum Parameters	
FBG (mg/dL)	88.83 ± 1.94
Creatinine (mg/dL)	0.88 ± 0.03
Est. GFR (mL/min/1.73mý)	121 ± 2.86

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; FBG, fasting blood glucose; Est. GFR, estimated glomerular filtration rate.

B. Postprandial Energy Expenditure

Resting energy expenditure (REE) of the lean male subjects was the same in both visits (1.20 Kcal/minute). Postprandial energy expenditure then increased equally in both the control and phosphorus treatments in the first 90 minutes following meal ingestion. Afterwards, the increase was more pronounced with phosphorus supplementation starting from 120 minutes and onward reaching its maximum at 120 minutes (1.51 Kcal/minute), and there was a significant difference between both treatments according to time (P=0.000) and treatment (P=0.024). In both treatments, postprandial energy expenditure failed to go back to baseline and it remained at a high level in the control (1.40 Kcal/minute) and in the phosphorus (1.45 Kcal/minute) treatments till the end of the experimental period (Table 19 & Figure 13).

The same trend was observed in Δ postprandial energy expenditure. Δ Postprandial energy expenditure increased similarly in both the control and phosphorus treatments in the first 90 minutes following meal ingestion. This was followed by a significant increase in Δ postprandial energy expenditure with phosphorus supplementation starting from 120 minutes and onward according to time (P=0.000) and treatment (P=0.003), and it reached its maximum at 120 minutes (0.31 Kcal/minute). Moreover, Δ postprandial energy expenditure failed to go back to baseline and it remained at a high level till the end of the experimental period in both the control (0.21 Kcal/minute) and phosphorus (0.24 Kcal/minute) treatments (Table 20 & Figure 14).

Table 19. Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	1.20 ± 0.02	1.20 ± 0.04
30	1.39 ± 0.05	1.40 ± 0.05
60	1.44 ± 0.04	1.43 ± 0.04
90	1.45 ± 0.04	1.48 ± 0.05
120	1.44 ± 0.04	1.51 ± 0.05
150	1.42 ± 0.04	1.48 ± 0.03
180	1.40 ± 0.04	1.47 ± 0.04
210	1.36 ± 0.04	1.46 ± 0.04
240	1.40 ± 0.05	1.45 ± 0.04

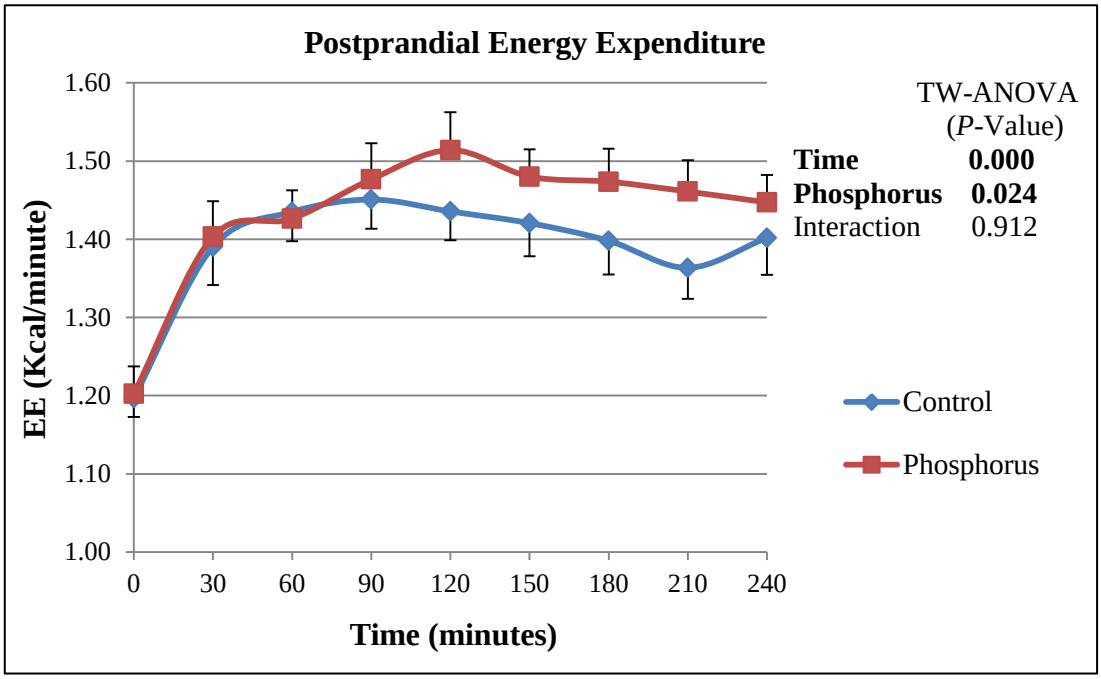

Figure 13. Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Table 20. Changes in Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.00 ± 0.00	0.00 ± 0.00
30	0.19 ± 0.03	0.20 ± 0.02
60	0.24 ± 0.03	0.22 ± 0.02
90	0.25 ± 0.03	0.27 ± 0.04
120	0.24 ± 0.03	0.31 ± 0.04
150	0.22 ± 0.03	0.28 ± 0.02
180	0.20 ± 0.03	0.27 ± 0.02
210	0.17 ± 0.02	0.26 ± 0.03
240	0.21 ± 0.03	0.24 ± 0.03

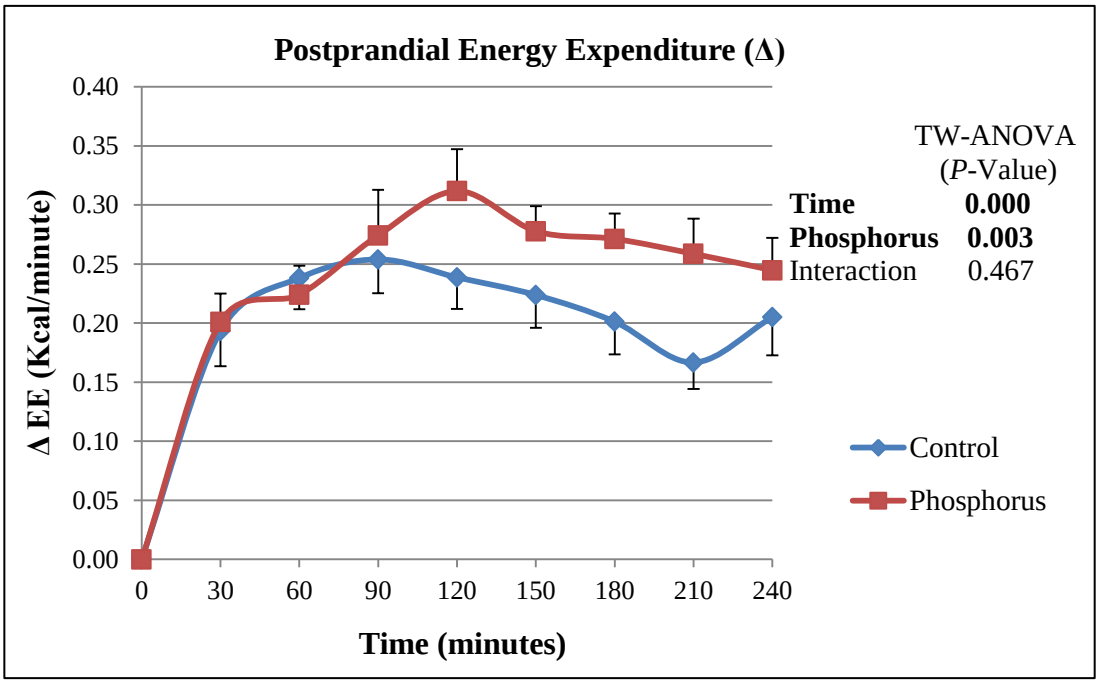

Figure 14. Changes in Postprandial Energy Expenditure (Kcal/minute) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

C. Postprandial Fat Oxidation

Baseline fat oxidation started at 59.7% and 58.9% in the control and phosphorus treatments, respectively. Postprandial fat oxidation then increased directly following meal ingestion in both treatments, and it remained increasing till the end of the experimental period reaching a maximum of 81.5% in the control and 80.0% in the phosphorus treatments. However, there was a significant difference between both treatments according to time only (P=0.000), and there was no significant difference according to treatment (P=0.120) (Table 21 & Figure 15).

Similar differences were observed in Δ postprandial fat oxidation in both treatments. Δ Postprandial fat oxidation increased directly following meal ingestion, and it remained increasing till the end of the experimental period reaching a maximum of 21.9% and 21.1% in the control and phosphorus treatments, respectively. There was no significant difference in Δ postprandial fat oxidation between both treatments according to treatment (P=0.328), but there was a significant difference according to time (P=0.000) (Table 22 & Figure 16).

Table 21. Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	59.7 ± 5.72	58.9 ± 4.43
30	66.3 ± 4.03	64.8 ± 3.78
60	69.1 ± 4.37	65.3 ± 3.02
90	71.9 ± 3.21	67.2 ± 3.71
120	75.2 ± 2.66	73.0 ± 3.15
150	77.7 ± 2.21	75.0 ± 2.65
180	80.5 ± 1.43	77.3 ± 2.41
210	80.4 ± 1.26	79.8 ± 1.32
240	81.5 ± 1.11	80.0 ± 1.88

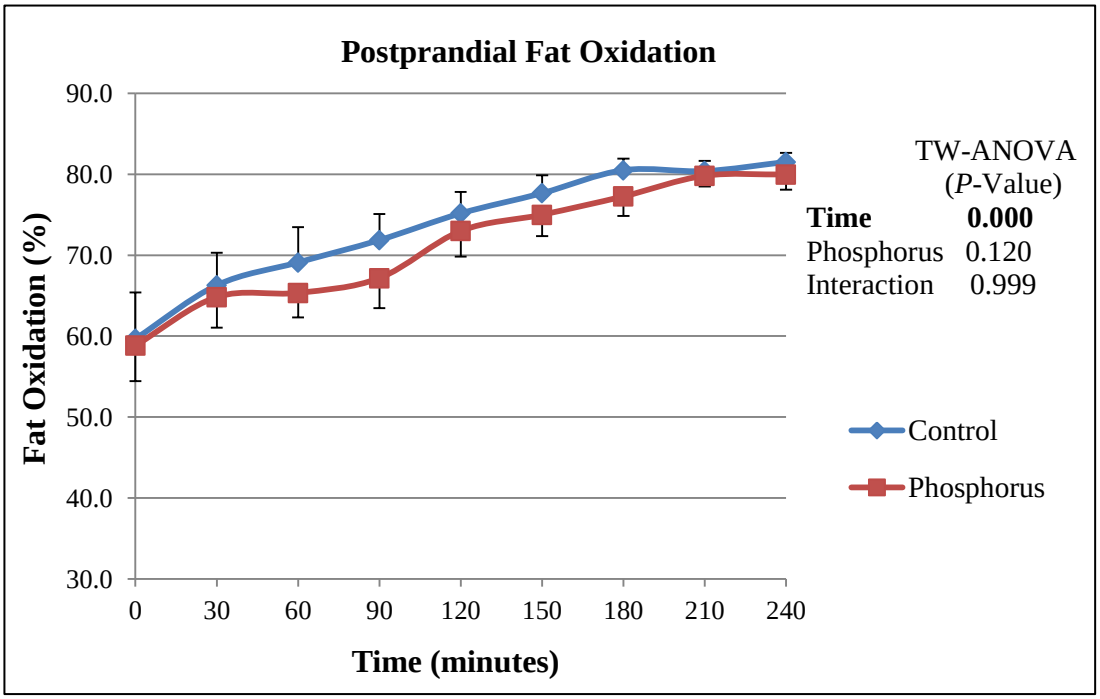

Figure 15. Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Table 22. Changes in Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.0 ± 0.00	0.0 ± 0.00
30	6.6 ± 3.51	6.0 ± 1.84
60	9.4 ± 1.72	6.5 ± 2.52
90	12.2 ± 3.28	8.3 ± 2.38
120	15.5 ± 3.88	14.1 ± 1.87
150	18.0 ± 4.04	16.2 ± 2.34
180	20.8 ± 4.88	18.4 ± 2.46
210	20.7 ± 5.11	21.0 ± 3.45
240	21.9 ± 5.51	21.1 ± 3.46

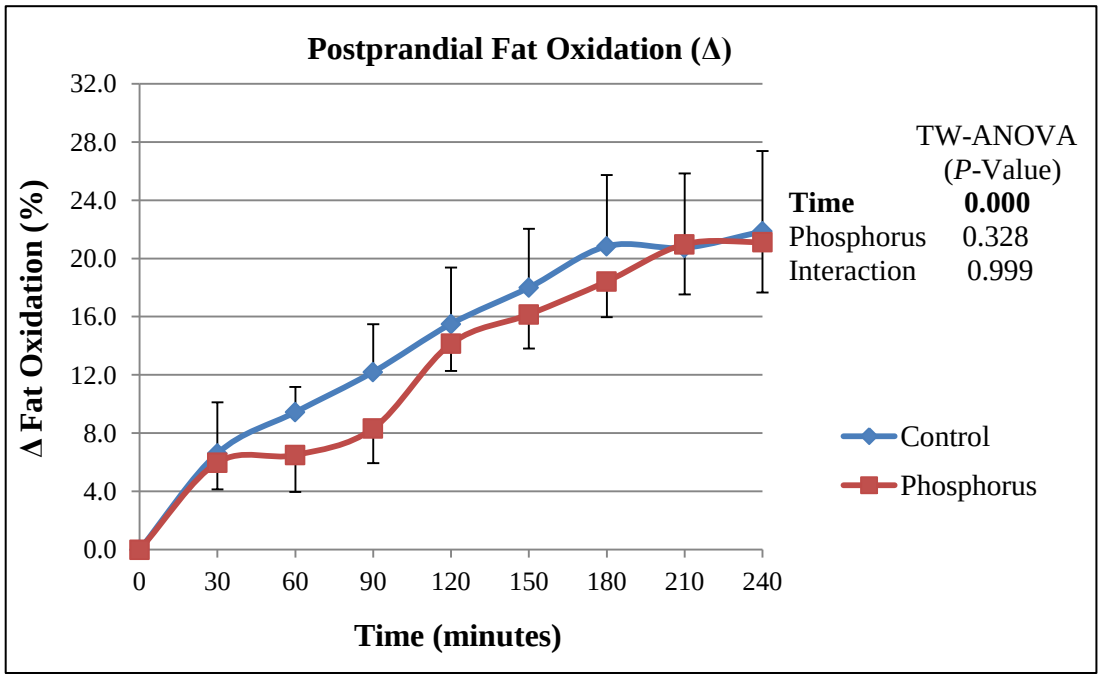

Figure 16. Changes in Postprandial Fat Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

D. Postprandial Carbohydrate Oxidation

Baseline carbohydrate oxidation started at 20.9% in the control and 21.6% in the phosphorus treatments. Afterwards, postprandial carbohydrate oxidation decreased in both the control and phosphorus treatments directly following meal ingestion, and it remained decreasing till the end of the experimental period reaching a minimum of 1.7% and 3.8% in the control and phosphorus treatments, respectively. There was a significant difference between both treatments according to time only (P=0.000), and there was no significant difference according to treatment (P=0.059) (Table 23 & Figure 17).

A similar trend was observed in Δ postprandial carbohydrate oxidation. Δ Postprandial carbohydrate oxidation decreased in both treatments directly following meal ingestion, and it remained decreasing in both treatments till the end of the experimental period till it reached a minimum of -19.2% and -17.8% in the control and phosphorus treatments, respectively. There was a significant difference between both treatments according to time only (P=0.000), and there was no significant difference according to treatment (P=0.188) (Table 24 & Figure 18).

Table 23. Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	20.9 ± 5.44	21.6 ± 4.26
30	16.9 ± 3.94	18.4 ± 3.74
60	14.6 ± 4.25	18.3 ± 3.04
90	12.0 ± 3.13	16.8 ± 3.87
120	8.6 ± 2.57	11.5 ± 3.20
150	5.9 ± 2.13	9.2 ± 2.54
180	2.8 ± 1.09	6.8 ± 2.31
210	2.5 ± 1.19	4.1 ± 1.06
240	1.7 ± 0.87	3.8 ± 1.86

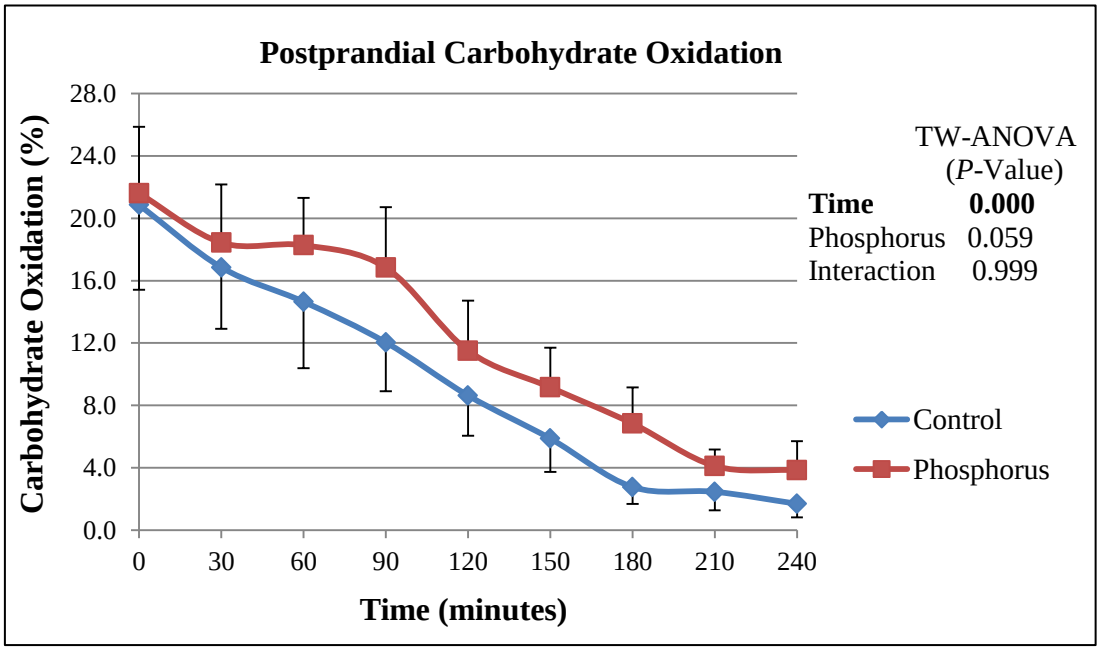

Figure 17. Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Table 24. Changes in Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.0 ± 0.00	0.0 ± 0.00
30	-4.0 ± 3.59	-3.2 ± 1.81
60	-6.2 ± 1.62	-3.3 ± 2.32
90	-8.8 ± 3.27	-4.8 ± 2.30
120	-12.2 ± 3.83	-10.1 ± 1.76
150	-15.0 ± 3.95	-12.4 ± 2.31
180	-18.1 ± 4.80	-14.8 ± 2.50
210	-18.4 ± 5.09	-17.5 ± 3.54
240	-19.2 ± 5.44	-17.8 ± 3.43

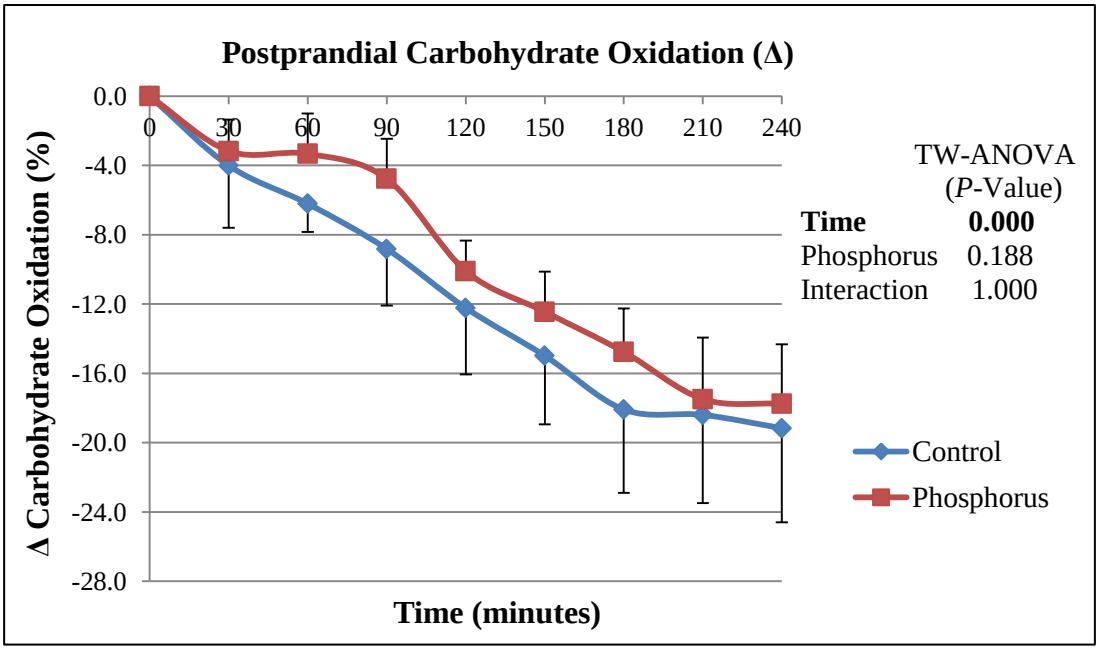

Figure 18. Changes in Postprandial Carbohydrate Oxidation (%) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

E. Postprandial Respiratory Quotient (RQ)

Baseline RQ was the same in both visits starting at 0.78. Postprandial RQ then decreased following meal ingestion in both treatments, and it continued to decrease till the end of the experimental period reaching a minimum of 0.66 and 0.68 in the control and phosphorus treatments, respectively. There was a significant difference between both treatments according to time (P=0.000) and treatment (P=0.008) (Table 25 & Figure 19).

Similar changes were observed in Δ postprandial RQ in both the control and phosphorus treatments. Δ Postprandial RQ decreased in both treatments following meal ingestion, and it remained decreasing till the end of the experimental period reaching a minimum of -0.13 and -0.10 in the control and phosphorus treatments, respectively. There was a significant difference in both treatments according to time (P=0.000) and treatment (P=0.003) (Table 26 & Figure 20).

Table 25. Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.78 ± 0.02	0.78 ± 0.02
30	0.76 ± 0.02	0.76 ± 0.02
60	0.75 ± 0.02	0.77 ± 0.01
90	0.74 ± 0.01	0.76 ± 0.02
120	0.72 ± 0.01	0.74 ± 0.01
150	0.71 ± 0.01	0.73 ± 0.01
180	0.68 ± 0.01	0.71 ± 0.01
210	0.67 ± 0.02	0.69 ± 0.01
240	0.66 ± 0.02	0.68 ± 0.01

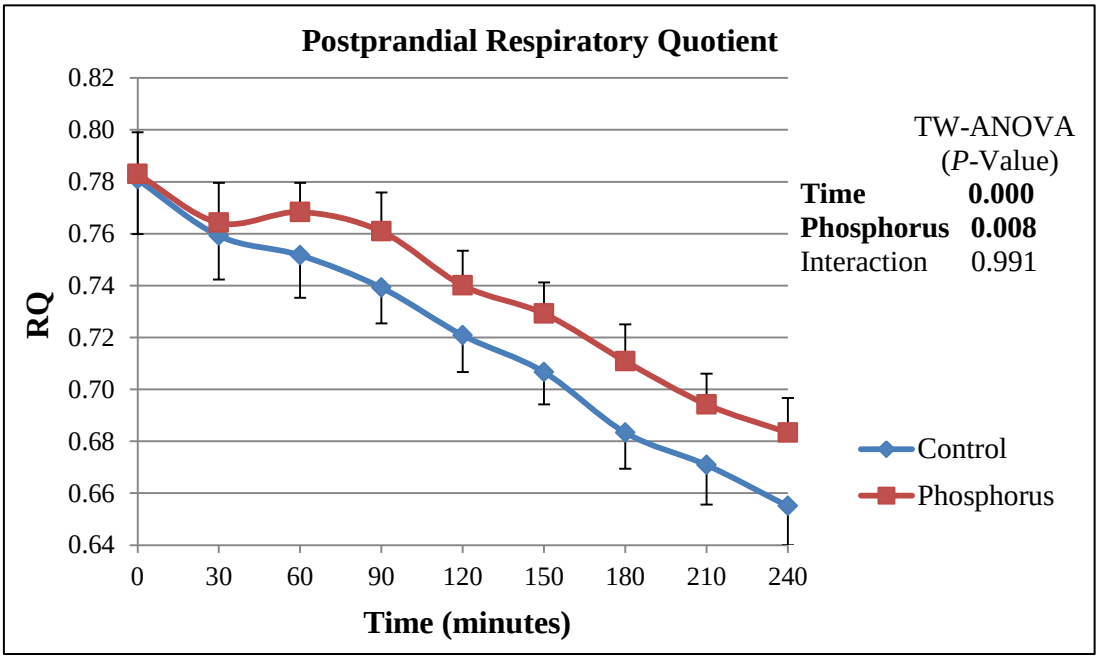

Figure 19. Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Table 26. Changes in Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

Time (minutes)	Control	Phosphorus
	Mean ± SEM	Mean ± SEM
0	0.00 ± 0.00	0.00 ± 0.00
30	-0.02 ± 0.01	-0.02 ± 0.01
60	-0.03 ± 0.01	-0.01 ± 0.01
90	-0.04 ± 0.01	-0.02 ± 0.01
120	-0.06 ± 0.01	-0.04 ± 0.01
150	-0.07 ± 0.01	-0.05 ± 0.01
180	-0.10 ± 0.02	-0.07 ± 0.01
210	-0.11 ± 0.02	-0.09 ± 0.01
240	-0.13 ± 0.02	-0.10 ± 0.01

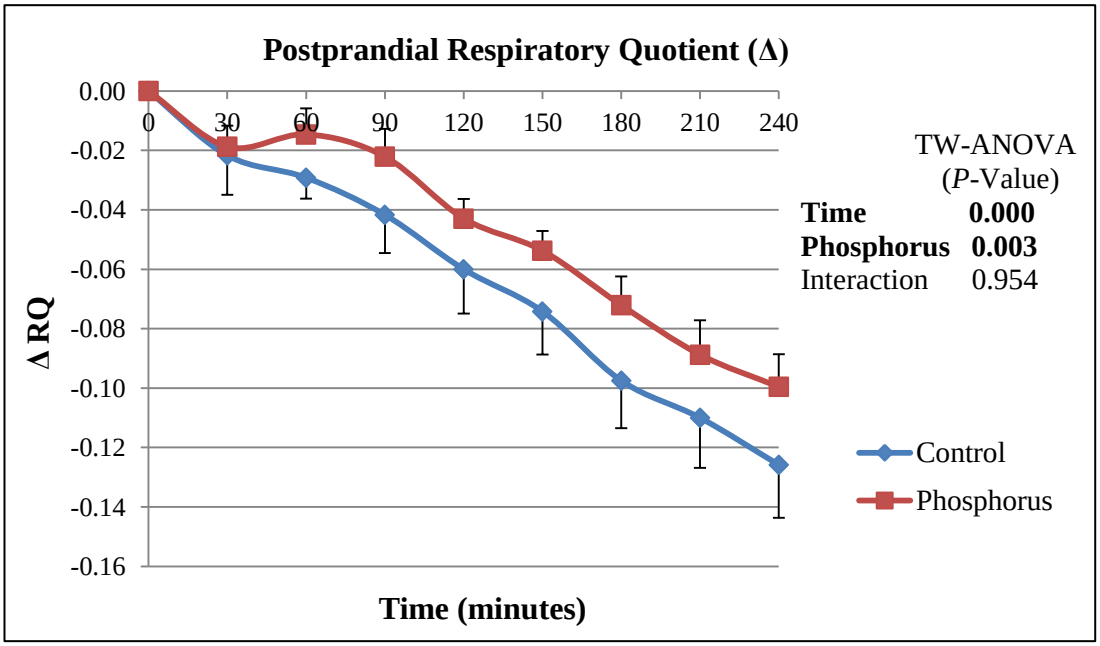

Figure 20. Changes in Postprandial Respiratory Quotient (RQ) of the Lean Subjects Following the Ingestion of the High-Protein Meal with or without Phosphorus

F. Urinary Parameters

Urine was collected at the end of the experimental period in each visit and the whole volume was recorded. Using Vitros 350 analyzer, the following urinary parameters were measured: total phosphorus, total creatinine, and total urine urea nitrogen (UUN). Moreover, phosphorus to creatinine ratio (P/Cr), and UUN to creatinine ratio (UUN/Cr) were calculated.

Total phosphorus (P=0.001) and phosphorus to creatinine ratio (P/Cr) (P=0.000) were significantly higher with phosphorus supplementation; however, there was no significant difference in total UUN and UUN to creatinine ratio (UUN/Cr) in both treatments (Table 27).

Table 27. Urinary Parameters of the Control and Phosphorus Treatments of the High-Protein Group

Variable	Control	Phosphorus	*P*-Value
	Mean ± SEM	Mean ± SEM	
V of Urine (ml)	391.7 ± 66.1	414.2 ± 48.6	-
Total P (mg)	120.8 ± 15.5	246.9 ± 25.0	**0.001***
Total UUN (mg)	3002 ± 206	3305 ± 353	0.254
P/Cr	0.27 ± 0.04	0.56 ± 0.05	**0.000***
UUN/Cr	6.75 ± 0.47	7.49 ± 0.64	0.095

Abbreviations: V, volume; P, phosphorus; UUN, urine urea nitrogen; Cr, creatinine.
**P*-Value<0.05, values that are significantly different between the control and the phosphorus treatments using paired t-tests.

CHAPTER V

DISCUSSION

The present study investigated the effect of phosphorus supplementation on DIT and substrate oxidation of healthy lean male subjects consuming two isocaloric meals, a normal protein-low phosphorus meal and a high protein-low phosphorus meal. Our objective is based on the fact that the availability of phosphorus enhances the status of ATP in the body, including hepatic ATP, which is essential for many processes including eating behavior and energy expenditure (Obeid *et al.,* 2010). Thus, low phosphorus intake would be associated with reduced ATP production and therefore, would affect food intake and energy expenditure. Furthermore, insulin production increases in the postprandial state which triggers the transfer of serum phosphorus into the intracellular space to phosphorylate several metabolites. This shift leads to a rapid decrease in serum phosphorus levels (Kalaitzidis *et al.,* 2005) and therefore, decreases the availability of phosphorus for hepatic ATP production (Obeid, 2013). However, phosphorus ingestion provides a surplus of this mineral and thus improves its status in the liver and increases hepatic ATP production, which would consequently lead to an increase in ATP availability which is associated with an increase in DIT. In addition, phosphorus is known to stimulate insulin sensitivity (Obeid, 2013). It has been shown that the ingestion of 500 mg of phosphorus was associated with a significant increase in insulin sensitivity after 60 minutes of intake (Khattab *et al.,* 2011). Moreover, a review paper done by Obeid (2013) has shown a positive correlation between phosphorus and DIT.

The present findings confirm that there is a strong association between phosphorus intake and DIT. There was a significant increase in DIT following phosphorus ingestion (500 mg) with the normal-protein meal and with the high-protein meal. This increase in DIT is associated with an increase in ATP production that depends primarily on the availability of the ingested phosphorus. However, phosphorus ingestion with the normal-protein meal and with the high-protein meal did not significantly affect fat oxidation and carbohydrate oxidation. Moreover, phosphorus supplementation had no significant effect on urinary nitrogen excretion.

Our data support studies that have correlated phosphorus intake to decreased body weight and visceral fat. For instance, a recent study done on overweight and obese adults found that phosphorus supplementation (375 mg) with each main meal (breakfast, lunch, and dinner) for 12 weeks was significantly associated with decreased body weight, BMI, and WC (Ayoub *et al.,* 2015). The mechanism underlying the association between phosphorus intake and reduced body weight, BMI, and WC could be explained by the increase in DIT following the ingestion of phosphorus with each meal. Our results are also consistent with previous studies that have examined the effects of high-protein diets on weight loss. It has been shown that dietary proteins are associated with reduced energy intake (Leidy *et al.,* 2015) and increased thermogenesis (Paddon-Jones *et al*., 2008). It is important to mention that dietary proteins are also rich sources of phosphorus; therefore, further supporting our findings.

It has been shown for many years that the ingestion of dietary proteins causes a rapid increase in postprandial energy expenditure immediately following meal ingestion (Acheson *et al.,* 2011). In addition, recent findings suggest that diets high in protein (HP, >20% of energy from protein) have a higher thermogenic effect than normal-

protein diets (NP, <20% of energy from protein) over the short term as well as the long term (Yang *et al.,* 2014). In line with that, our results have shown that following protein consumption, postprandial energy expenditure rises rapidly and was sustained for as long as four hours post high-protein meal consumption, and to a lower extent post normal-protein meal consumption. However, postprandial energy expenditure of the phosphorus supplemented group was found to be significantly higher than that of the control especially from 60 minutes (0.34 Kcal/minute) and onward following the normal-protein meal consumption (P=0.001), and from 120 minutes (0.31 Kcal/minute) and onward following the high-protein meal consumption (P=0.003). The effect of phosphorus supplementation with the normal-protein meal on DIT was obvious one hour post meal ingestion. This could be explained by the fact that the majority of phosphorus needs 60 minutes to be absorbed (Karczmar *et al.,* 1989; Khattab *et al.,* 2015). However, the effect of phosphorus supplementation with the high-protein meal on DIT was obvious two hours post meal ingestion. This could be explained by the effect of protein-rich meals on delayed gastric emptying.

Postprandial protein synthesis increased with phosphorus supplementation in the normal-protein meal experiment due to the fact that phosphorus availability led to an increase in hepatic ATP production, and protein synthesis is known to be energy expensive with a minimal cost of 4 ATP equivalents needed for the formation of 1 peptide bond (Bender *et al.,* 2012). Moreover, phosphorus is known to stimulate insulin sensitivity therefore, the improvement in insulin sensitivity and the availability of ATP led to an increase in postprandial protein synthesis which is translated into increased thermogenesis. Therefore, the increase in postprandial energy expenditure with phosphorus supplementation in the normal-protein meal experiment was probably due

to an increase in postprandial protein synthesis. Additionally, the increase in DIT with phosphorus supplementation in the high-protein meal experiment could be explained by the energy-requiring pathways of protein metabolism. As mentioned previously, enhanced insulin sensitivity and ATP availability as a consequence of phosphorus supplementation led to an increase in postprandial protein synthesis; however, the body has no storage capacity to cope with high protein intakes and thus, needs to be metabolized immediately (Yang *et al.,* 2014). Therefore, the surplus of amino acids that could not be involved in protein synthesis are directed into other energy-requiring pathways of protein metabolism including gluconeogenesis and ureagenesis. The energy costs of protein synthesis and protein break down have been estimated to be 3.6 and 0.7 kJ/g, respectively (Veldhorst *et al.,* 2009). The high ATP costs of postprandial protein synthesis and the high cost of urea production and gluconeogenesis could therefore explain the increase in postprandial energy expenditure that was sustained for 4 hours in the high-protein meal experiment.

Our results are consistent with previous studies that have examined the effects of normal-protein diets and high-protein diets on postprandial energy expenditure. Postprandial energy expenditure of healthy subjects consuming a high-protein meal (25% of total energy) was significantly higher than postprandial energy expenditure following the consumption of an adequate-protein meal (10% of total energy), and this increase persisted >210 minutes (Smeets *et al.,* 2008). Moreover, a greater DIT resulted from the consumption of a high-protein (62% protein) liquid meal replacement shake compared with a normal-protein (17% protein) liquid meal replacement shake in healthy active subjects (Scott & Devore, 2005). Karst *et al.* (1984) observed an increase in DIT of healthy lean male subjects consuming 1 MJ protein meal in the form of egg white,

and this increase persisted over 4 hours. They suggested that protein degradation definitely played a role in the high DIT; however, it was not the main causal factor. In line with that, it is reasonable to conclude that the higher DIT induced by high-protein meals compared to that of normal-protein meals is attributed to their higher phosphorus content besides the energy-requiring pathways of protein metabolism.

In the overnight postabsorptive state, the body chiefly relies on fat oxidation for energy production following glucose utilization that is derived from endogenous glucose production, which consists of two processes, glycogenolysis (the release of glucose from stored glycogen) and gluconeogenesis (the synthesis of glucose from non-carbohydrate substrates) (Veldhorst *et al.,* 2009). This was clearly revealed by our baseline measurements in which baseline fat oxidation of the lean male subjects was high in both the normal-protein meal and the high-protein meal experiments, control: 62.1%; phosphorus: 65.2%, and control: 59.7%; phosphorus: 58.9%, respectively. On the other hand, baseline carbohydrate oxidation of the lean male subjects was low in both the normal-protein meal and the high-protein meal experiments, control: 19.3%; phosphorus: 15.6%, and control: 20.9%; phosphorus: 21.6%, respectively.

There is an evidence for the existence of hierarchy in macronutrient oxidation rate in the postprandial state with the following sequence: protein > carbohydrate > fat. Additionally, a negative relationship was found between postprandial fat and glucose oxidation in lean subjects (Tentolouris *et al.,* 2008). This comes in line with our results in which postprandial glucose utilization increased in the first 30 minutes following the ingestion of the normal-protein meal with and without phosphorus supplementation, and was accompanied by a decrease in fat oxidation. This could be explained by the action of insulin release which stimulated glucose utilization and blocked fat oxidation.

However, 30 minutes post meal ingestion, the body shifted to fat utilization and glucose utilization was decreased, and this persisted till the end of the experimental period. Furthermore, an increase in postprandial RQ was observed in the first 30 minutes following the normal-protein meal ingestion which is an indication of glucose utilization, and this was followed by a decrease in postprandial RQ which indicates fat oxidation.

Postprandial fat oxidation of the lean male subjects increased directly following the ingestion of the high-protein meal with and without phosphorus supplementation, and was accompanied by a decrease in glucose utilization that persisted till the end of the experimental period. Moreover, a decrease in postprandial RQ was observed directly following meal ingestion which persisted till the end of the experimental period indicating fat oxidation. A possible explanation of the enhanced fat oxidation following a high-protein meal consumption is the low carbohydrate content of such meals (Labayen *et al.,* 2004). Additionally, it is known that increased fat oxidation inhibits glucose utilization via glycolytic and aerobic pathways. The sites of inhibition of glucose utilization are the steps catalyzed by phosphofructokinase, the rate-limiting enzyme in glycolysis, and pyruvate dehydrogenase, the enzyme required for the formation of acetyl coenzyme A from pyruvate. This relationship is known as the glucose-fatty acid cycle which indicates that an elevation in fat oxidation interferes with glucose oxidation (Tentolouris *et al.,* 2008).

Our data are supported by previous findings in which the consumption of a high-protein meal was associated with a higher postprandial fat oxidation as compared with a balanced-protein meal in both lean and obese subjects (Labayen *et al.,* 2004). Moreover, Lejeune *et al*. (2006) found that a high-protein diet, compared with an

adequate-protein diet, was associated with an increased 24-h satiety, thermogenesis, sleeping metabolic rate, protein balance, and increased fat oxidation when consumed in energy balance over 4 days.

Phosphorus supplementation with both the normal-protein meal and the high-protein meal had no significant effect on fat oxidation and carbohydrate oxidation, and on urinary nitrogen excretion. We expected that the levels of urine urea nitrogen (UUN) would be lower with phosphorus supplementation indicating that protein synthesis is enhanced and protein degradation is decreased since urea nitrogen is positively correlated with protein breakdown. The reason behind that could be the short duration of the experimental period and more time could be needed to determine the effect of phosphorus ingestion on urinary nitrogen excretion. In agreement with previous studies (Garrow & Hawes, 1972; Karst *et al*., 1984), the increase in DIT in both the normal-protein meal and the high-protein meal experiments did not affect urinary nitrogen excretion. Additionally, Johnston *et al.* (2002) found no effect of a high-protein diet intervention on both plasma urea nitrogen and urine urea nitrogen concentrations.

The main strength of the present study lies in its design. A randomized controlled trial determines whether a cause-effect relationship exists between an intervention and an outcome, and the process of randomization helps minimize the risk of any possible bias. Additionally, all subjects had similar baseline characteristics, and the content and quantity of the test meals were accurately monitored and the only difference was the exposure to the treatment. Furthermore, blinding helps avoid possible bias occurring in the responses of the participants to the different treatments.

Our study is not without limitations. The first limitation lies in its small sample size. Second, although subjects were under good control, some movements and a few

moments of sleepiness were unavoidable during the experimental period, which might have slightly affected some measurements. Moreover, it would have been interesting to collect subjective appetite scores from the participants, and to measure blood levels of hunger and satiety hormones at baseline and in the postprandial state to examine whether or not phosphorus supplementation exerts any effect.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

Our results have shown that the ingestion of 500 mg of phosphorus with a normal protein-low phosphorus meal and with a high protein-low phosphorus meal was significantly associated with an increase in DIT and thus, the high thermogenesis of proteins is partially attributed to their high content of phosphorus. Phosphorus, therefore, could be the key for weight loss through its effect on increasing DIT. Accordingly, dietary phosphorus intake has a beneficial role in preventing the depletion of intracellular phosphorus and the consequent low ATP production which is translated into low thermogenesis. Additionally, our results have shown no significant effect of phosphorus ingestion on fat and carbohydrate oxidation, and on urinary nitrogen excretion.

In conclusion, our results support a promising role of the mineral phosphorus in weight management through its effect on increasing DIT. Therefore, phosphorus could be considered for future use in the form of weight reducing supplements or implementing fortification of flour. Further research should be considered to examine the exact mechanism of action of phosphorus on DIT and substrate oxidation in the human body.

APPENDIX I

GENERAL HEALTH SCREENING QUESTIONNAIRE

(ENGLISH)

Name: **Subject number:** **Date:**

Height: _____ **Weight:** _____
(Both filled by the investigator after taking the measurements)

Please answer the following questions:

1. **Do you suffer from one or more of the following?**
   a. Diabetes
   b. Heart diseases
   c. Dyslipidemia
   d. Hypertension
   e. Other: _____
2. **Did you undergo any surgery in the last 5 years?**
   ☐ No ☐ Yes (specify :_____)
3. **Did you lose more than 3 Kilograms in the last 3 months?**
   ☐ No ☐ Yes
4. **Are you currently taking any medication?**
   ☐ No ☐ Yes (specify :_____)
5. **Are you a smoker?**
   ☐ No ☐ Yes (specify number of cigarettes per day:_____)
6. **Do you drink alcohol?**
   ☐ No ☐ Yes (specify average number of drinks per week _____)
7. **Have you been dependent on the use of drugs in the past 5 years?**
   ☐ No ☐ Yes
8. **Do you take any nutritional supplement?**
   ☐ No ☐ Yes (please specify _____)
9. **Do you do any exercise?**
   ☐ No ☐ Yes (please specify type, duration and frequency_____)

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APPENDIX II

GENERAL HEALTH SCREENING QUESTIONNAIRE

(ARABIC)

الاسم: **رقم المشترك:** **التاريخ:**

الطول: _____ **الوزن:** _____

(تدون المعلومتين من قبل الباحث بعد أخذ قياسات الوزن و الطول)

الرجاء الاجابة عن الأسئلة التالية:

هل تعاني من واحد أو أكثر من التالي؟

مرض السكري

أمراض في القلب

ارتفاع الشحوم والدهون في الدم

ارتفاع في ضغط الدم

أمراض اخرى: _____

هل خضعت لأية عملية في الخمسة سنوات الماضية؟

☐ كلا ☐ نعم (حدد: _____)

هل خسرت من وزنك أكثر من ثلاثة كيلوغرامات خلال الأشهر الثلاثة الماضية؟

☐ كلا ☐ نعم

حاليا" هل تأخذ أية دواء؟

☐ كلا ☐ نعم (حدد: _____)

هل أنت مدخن؟

☐ كلا ☐ نعم(حدد عدد السجائر في اليوم : _____)

هل تشرب الكحول؟

☐ كلا ☐ نعم (حدد معدل عدد المشروبات في الاسبوع: _____)

هل تعاطيت المخدرات خلال الخمسة سنوات الماضية؟

☐ كلا ☐ نعم

هل تتناول مكملات غذائية؟

☐ كلا ☐ نعم (حدد: _____)

هل تمارس أية رياضة بدنية؟

☐ كلا ☐ نعم (حدد نوع الرياضة و الوقت خلال ممارستها و تكرارها _____)

APPENDIX III

NORMAL PROTEIN 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 3: The impact of phosphorus ingestion on DIT of lean and obese subjects consuming normal protein-low phosphorus meal.

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut]
Co-Investigator: Mariam Assaad, Lina Abdouni
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. Fasting blood test will be conducted to determine glucose level 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 and cup of egg white protein shake + 1/2 cup of orange juice 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 32 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 as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by a third party or governmental insurance.
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.

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

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NUT.00.22 November 10, 2015

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:_____

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APPENDIX IV

NORMAL PROTEIN 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 الاختبار3: تحديد تأثير مادة الفوسفور على "التأثير الحراري للطعام" عند تناول وجبة معتدلة بالبروتينات وقليلة الفوسفور لدى الشخص ذات الوزن الطبيعي والبدين.

إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين: مريم أسعد ولينا عبدوني.

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 الإستمارة 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 سحب الدّم لتحديد مستوى السّكر وتقييم عمل الكلى. يرجى إستشارة الطبيب المختص في حال تبيّن في فحص الدّم خلل في المعدّلات المطلوبة. 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 التغذيةً وحدة الأبحاث في الجامعة الأميركية في بيروت) حيث يتمّ أخذ الطول والوزن ومن ثمّ تملأ إستبانة عن الصّحة.

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 كمية إستهلاك الأوكسجين وإنتاج ثاني أكسيد الكربون. بعد ذلك،

سيتناول المشترك بـ 10 دقائق وجبة تتضمن التوست مع الزبدة وكوب من مشروب بروتيني (مكوّن من بروتينات بياض البيض) ونصف كوب من عصير الليمون + 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 32 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) و **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**: إذا حصل أي عارض سلبي من جراء المشاركة في هذه الدراسة، لن يكون هناك أي تعويض مالي لتغطية التكاليف في حال لم تغطّيها شركات التأمين الخاصّة أو أي برامج حكومية. 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: _____

NUT.00.22 November 10, 2015

Institutional Review Board
American University of Beirut
14 DEC 2015
APPROVED

APPENDIX V

HIGH PROTEIN 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 protein-low phosphorus meal.

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut]
Co-Investigator: Mariam Assaad, Lina Abdouni
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. Fasting blood test will be conducted to determine glucose level 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 and cup of egg white protein shake + 1/2 cup of orange juice 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 32 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 as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by a third party or governmental insurance.
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.

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
09 MAR 2015
APPROVED

NUT.00.20 November 7, 2014

APPENDIX VI

HIGH PROTEIN 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 بالبروتينات وقليلة الفوسفور لدى الشخص ذات الوزن الطبيعي والبدين.

إسم الباحث: د.عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين: مريم أسعد ولينا عبدوني.

العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان 01-350000

مكان إجراء البحث: الجامعة الأمريكية في بيروت -كلية الزراعة وعلم الغذاء /قسم التغذيةً 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 الإستمارة 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 سحب الدّم لتحديد مستوى السّكر وتقييم عمل الكلى. يرجى إستشارة الطبيب المختص في حال تبيّن في فحص الدّم خلل في المعدّلات المطلوبة. 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 التغذيةً وحدة الأبحاث في الجامعة الأميركية في بيروت) حيث يتمّ أخذ الطول والوزن ومن ثمّ تملأ إستبانة عن الصّحة.

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 وكوب من مشروب بروتيني (مكوّن من بروتينات بياض البيض) ونصف كوب من عصير الليمون + 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 32 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 هذه 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) و **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**: إذا حصل أي عارض سلبي من جراء المشاركة في هذه الدراسة، لن يكون هناك أي تعويض مالي لتغطية التكاليف في حال لم تغطّيها شركات التأمين الخاصّة أو أي برامج حكومية. 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.00.20 November 7, 2014

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APPROVED

BIBLIOGRAPHY

Acheson, K. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Emady-Azar, S., Ammon-Zufferey, C., … & Bovetto, L. (2011). Protein choices targeting thermogenesis and metabolism. *The American Journal of Clinical Nutrition, 93*(3), 525-534.

Alonso, A., Nettleton, J. A., Ix, J. H., De Boer, I. H., Folsom, A. R., Bidulescu, A., Kestenbaum, B. R., Chambless, L. E., Jacobs, D. R. (2010). Dietary Phosphorus, Blood Pressure, and Incidence of Hypertension in the Atherosclerosis Risk in Communities Study and the Multi-Ethnic Study of Atherosclerosis. *Hypertension, 55*(3), 776-784.

Amanzadeh, J., & Reilly, R. F. (2006). Hypophosphatemia: an evidence-based approach to its clinical consequences and management. *Nature Clinical Practice Nephrology, 2*(3), 136-148.

Anderson, G. H., & Moore, S. E. (2004). Dietary Proteins in the Regulation of Food Intake and Body Weight in Humans. *The Journal of Nutrition, 134*(4), 974S-979S.

Arentson-Lantz, E., Clairmont, S., Paddon-Jones, D., Tremblay, A., & Elango, R. (2015). Protein: A nutrient in focus. *Applied Physiology, Nutrition, and Metabolism, 40*(8), 755–761.

Astrup, A., Raben, A., & Geiker, N. (2015). The role of higher protein diets in weight control and obesity-related comorbidities. *International Journal of Obesity, 39*, 721-726.

Ayoub, J. J., Samra, M. J. A., Hlais, S. A., Bassil, M. S., & Obeid, O. A. (2015). Effect of phosphorus supplementation on weight gain and waist circumference of overweight/obese adults: a randomized clinical trial. *Nutrition & Diabetes, 5*(e189), 1-6.

Bender, D. A. (2012). The metabolism of "surplus" amino acids. *British Journal of Nutrition, 108*(S2), S113-S121.

Beydoun, M. A., Gary, T. L., Caballero, B. H., Lawrence, R. S., Cheskin, L.I., & Wang, Y. (2008). Ethnic differences in dairy and related nutrient consumption among US adults and their association with obesity, central obesity, and the metabolic syndrome. *The American Journal of Clinical Nutrition, 87*(6), 1914-1925.

Bizeau, M. E., & Pagliassotti, M. J. (2005). Hepatic adaptations to sucrose and fructose. *Metabolism Clinical and Experimental, 54*(9), 1189-1201.

Bray, G. A., Nielsen, S. J, & Popkin, B. M. (2004). Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *The American Journal of Clinical Nutrition, 79*(4), 537-543.

Calvo, M. S., & Uribarri, J. (2013). Contributions to Total Phosphorus Intake: All Sources Considered. *Seminars in Dialysis, 26*(1), 54-61.

Campillo, J. E., Aguayo, J., Pages, I., Castillo, M., & Osorio, C. (1982). Inorganic phosphate-insulin relationships in normal subjects and in patients with moderate glucose intolerance. *Diabetes and Metabolism, 8*(4), 289-293.

Chavin, K. D., Yang, S., & Lin, H. Z. (1999). Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. *The Journal of Biological Chemistry, 274*(9), 5692-5700.

Cortez-Pinto, H., Chatham, J., Chacko, V. P., Arnold, C., Rashid, A., & Diehl, A. M. (1999). Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA, 282*(17), 1659-1664.

D'Alessio, D. A., Kavle, E. C., & Mozzoli, M. A. (1988). Thermic effect of food in lean and obese men. *The Journal of Clinical Investigation, 81*(6), 1781-1789.

Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005). This report may be accessed via www.nap.edu

Drewnowski, A. (2009). Obesity, diets, and social inequalities. *Nutrition Reviews, 67*(Suppl_1), S36-S39.

Elliott, P., Kesteloot, H., & Appel, L. J. (2008). Dietary Phosphorus and Blood Pressure International Study of Macro- and Micro-Nutrients and Blood Pressure. *Hypertension, 51*(3), 669-675.

Ervin, R. B., Wang, C. Y., Wright, J. D., & Kennedy-Stephenson, J. (2004). Dietary intake of selected minerals for the United States population: 1999-2000. *Advance Data, 27*(341), 1-5.

FAO (2010). Food Balance Sheet. www.fao.org

Felig, P. (1984). Insulin is the mediator of feeding-related thermogenesis: insulin resistance and/or deficiency results in a thermogenic defect which contributes to the pathogenesis of obesity. *Clinical Physiology, 4*(4), 267-273.

Food And Nutrition Board (1997), Institute Of Medicine: "Dietary Reference Intakes for Calcium, Magnesium, Phosphorus, Vitamin D, and Fluoride." Washington, DC: National Academy Press.

Friedman, M. I. (2007). Obesity and the hepatic control of feeding behavior. *Drug News Perspect, 20*(9), 573-578.

Garrow, J. S., & Hawes, S. F. (1972). The role of amino acid oxidation in causing specific dymanic action in man. *British Journal of Nutrition, 27*(1), 211-219.

Haap, M., Heller, E., Thamer, C., Tschritter, O., Stefan, N., & Fritsche, A. (2006). Association of serum phosphate levels with glucose tolerance, insulin sensitivity and insulin secretion in non-diabetic subjects. *European Journal of Clinical Nutrition, 60,* 734-739.

Haglin, L., Lindblad, A., & Bygren, L. O. (2001). Hypophosphataemia in the metabolic syndrome. Gender differences in body weight and blood glucose. *European Journal of Clinical Nutrition, 55,* 493-498.

Halton, T. H., & 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.

Heaney, R. P., & Nordin, B. E. C. (2002). Calcium Effects on Phosphorus Absorption: Implications for the Prevention and Co-Therapy of Osteoporosis. *Journal of the American College of Nutrition, 21*(3), 239-244.

Hong, J., Graczyk-Milbrandt, G., & Friedman, M. I. (2000). Metabolic inhibitors synergistically decrease hepatic energy status and increase food intake. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 278*(6), R1579–R1582.

Johnston, C. S., Day, C. S., & Swan, P. D. (2002). Postprandial Thermogenesis is Increased 100% on a High-Protein, Low-Fat Diet Versus a High-Carbohydrate, Low-Fat Diet in Healthy, Young Women. *Journal of the American College of Nutrition, 21*(1), 55-61.

Kalaitzidis, R., Tsimihodimos, V., Bairaktari, E., Siamopoulos, K. C., & Elisaf, M. (2005). Disturbances of Phosphate Metabolism: Another Feature of Metabolic Syndrome. *American Journal of Kidney Diseases, 45*(5), 851-858.

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

Karczmar, G. S., Kurtz, T., Tavares, N. J., & Weiner, M. W. (1989). Regulation of hepatic inorganic phosphate and ATP in response to fructose loading: an in vivo ^{31}P-NMR study. *Biochimica et Biophysica Acta, 1012*(2), 121-127.

Karst, H., Steiniger, J., Noack, R., & Steglich, H. D. (1984). Diet-Induced Thermogenesis in Man: Thermic Effects of Single Proteins, Carbohydrates and Fats Depending on Their Energy Amount. *Annals of Nutrition and Metabolism, 28*(4), 245-252.

Khattab, M., Abi-Rashed, C., Ghattas, H., Hlais, S., & Obeid, O. (2015). Phosphorus ingestion improves oral glucose tolerance of healthy male subjects: a crossover experiment. *Nutrition Journal, 14*(112).

Khattab, M., Azar, S., Mattar, M., & Obeid, O. (2011). Effect of phosphorus on the oral glucose tolerance test. *Proceedings of the Nutrition Society, 70*(OCE3), E60.

Labayen, I., Diez, N., Parra, D., Gonzalez, A., & Martinez, J. A. (2004). Basal and postprandial substrate oxidation rates in obese women receiving two test meals with different protein content. *Clinical Nutrition, 23*(4), 571-578.

Langhans, W., & Scharrer, E. (1992). Metabolic control of eating. World Rev. *Nutr. Diet, 70,* 1-67.

Latner, J. D., & Schwartz, M. (1999). The effects of a high-carbohydrate, high-protein or balanced lunch upon later food intake and hunger ratings. *Appetite, 33*(1), 119-128.

Layman, D. K. (2009). *Dietary Guidelines* should reflect new understandings about adult protein needs. *Nutrition & Metabolism, 6*(12).

Leidy, H. J., Clifton, P. M., Astrup, A., Wycherley, T. P., Westerterp-Plantenga, M. S., Luscombe-Marsh, N. D., Woods, S. C., & Mattes, R. D. (2015). The role of protein in weight loss and maintenance. *The American Journal of Clinical Nutrition, 101*(6), 1320S-1329S.

Lejeune, M. P., Westerterp, K. R., Adam, T. C., Luscombe-Marsh, N. D., & Westerterp-Plantenga, M. S. (2006). Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *The American Journal of Clinical Nutrition, 83*(1), 89-94.

Lind, L., Lithell, H., Hvarfner, A., Pollare, T., & Ljunghall, S. (1993). On the relationships between mineral metabolism, obesity and fat distribution. *European Journal of Clinical Investigation, 23*(5), 307-310.

Lindgärde, F., & Trell, E. (1977). Serum Inorganic Phosphate in Middle-Aged Men. I. Inverse Relation to Body Weight. *Acta Medica Scandinavica, 202*(1-6), 307-311.

Mahan, L. K., Escott-Stump, S., & Raymond, J. L. *Krause's Food and the Nutrition Care Process.* United States of America: Saunders, 2012. Print.

Mikkelsen, P. B., Toubro, S., & Astrup, A. (2000). Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. *The American Journal of Clinical Nutrition, 72*(5), 1135-1141.

Morris, J. R., Nigon, K., & Reed, E. (1978). Evidence that the severity of depletion of inorganic phosphate determines the severity of the disturbance of adenine nucleotide metabolism in the liver and renal cortex of the fructose-loaded rat. *The Journal of Clinical Investigation, 61*(1), 209-220.

Nair, S., Chacko, V. P., Arnold, C., & Diehl, M. (2003). Hepatic ATP reserve and efficiency of replenishing: comparison between obese and nonobese normal individuals. *The American Journal of Gastroenterology, 98*(2), 466-470.

Noori, N., Sims, J. J., Kopple, J. D., Shah, A., Colman, S., Shinaberger, C. S., …& Kalantar-Zadeh, K. (2010). Organic and Inorganic Dietary Phosphorus and its Management in Chronic Kidney Disease. *Iranian Journal of Kidney Diseases, 4*(2), 89-100.

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

Obeid, O. A., Dimachkie, S., & Hlais, S. (2010). Increased phosphorus content of preload suppresses *ad libitum* energy intake at subsequent meal. *International Journal of Obesity, 34*(9), 1446-1448.

Oberhaensli, R. D., Galloway, G. J., Taylor, D. J., Bore, P. J., & Radda, G. K. (1986). Assessment of human liver metabolism by phosphorus-31-magnetic resonance spectroscopy. *The British Journal of Radiology, 59*(703), 695-699.

Paddon-Jones, D., Westman, E., Mattes, R. D., Wolfe, R. R., Astrup, A., & Westerterp-Plantenga, M. (2008). Protein, weight management, and satiety. *The American Journal of Clinical Nutrition, 87*(5), 1558S-1561S.

Pravst, I. (2011). Risking public health by approving some health claims? – The case of phosphorus. *Food Policy, 36*(5), 726-728.

Ravindran, V., Ravindran, G., & Sivalogan, S. (1994). Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. *Food Chemistry, 50*(2), 133-136.

Ravussin, E., & Swinburn, B. A. (1996). Energy expenditure and obesity. *Diabetes Reviews, 4*, 403-422.

Rawson, N. E., & Freidman, M. I. (1994). Phosphate loading prevents the decrease in ATP and increase in food intake produced by 2,5-anhydro-d-mannitol. *American American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 266*(6), R1792-R1796.

Riquelme, P. T., Wernette-Hammond, M. E., Kneer, N. M., & Lardy, H. A. (1984). Mechanism of action of 2, 5-anhydro-d-mannitol in hepatocytes. *The Journal of Biological Chemistry, 259*(8), 5115-5123.

Scott, C. B., & Devore, R. (2005). Diet-induced thermogenesis: variations among three isocaloric meal-replacement shakes. *Nutrition, 21*(7-8), 874-877.

Segal, K. R., Edano, A., & Blando, L. (1990). Comparison of thermic effects of constant and relative caloric loads in lean and obese men. *The American Journal of Clinical Nutrition, 51,* 14-21.

Segal, K. R., Edano, 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.

Segal, K. R., Gutin, B., Nyman, A. M., & Pi-Sunyer, F. X. (1985). Thermic Effect of Food at Rest, during Exercise, and after Exercise in Lean and Obese Men of Similar Body Weight. *The Journal of Clinical Invest*igation, *76*(3), 1107-1112.

Sherman, R. A., & Mehta, O. (2009). Dietary Phosphorus Restriction in Dialysis Patients: Potential Impact of Processed Meat, Poultry, and Fish Products as Protein Sources. *American Journal of Kidney Diseases, 54*(1), 18-23.

Smeets, A. J., Soenen, S., Luscombe-Marsh, N. D., Ueland, Ø., & Westerterp-Plantenga, M. S. (2008). Energy Expenditure, Satiety, and Plasma Ghrelin, Glucagon-Like Peptide 1, and Peptide Tyrosine-Tyrosine Concentrations following a Single High-Protein Lunch. *The Journal of Nutrition, 138*(4), 698-702.

Solomon, S. M., Kirby, D. F. (1990). The refeeding syndrome: a review. *Journal of Parenteral and Enteral Nutrition, 14*(1), 90-7.

Teegarden D. (2005). The influence of dairy product consumption on body composition. *The Journal of Nutrition, 135*(12), 2749-2752.

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 Clinical and Experimental, 57*(3), 313-320.

Thorne, A., Hallberg, D., & Wahren, J. (1989). Meal-induced thermogenesis in obese patients before and after weight reduction. *Clinical Physiology, 9*(5), 481-498.

Thorne, A., Naslund, I., & Wahren, J. (1990). Meal-induced thermogenesis in previously obese patients. *Clinical Physiology, 10*(1), 99-109.

Uribarri, J. (2007). Phosphorus Homeostasis in Normal Health and in Chronic Kidney Disease Patients with Special Emphasis on Dietary Phosphorus Intake. *Seminars in Dialysis, 20*(4), 295-301.

Veldhorst, M. A. B., Westerterp-Plantenga, M. S., & Westerterp, K. R. (2009). Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *The American Journal of Clinical Nutrition, 90*(3), 519-526.

Wagner, G., Kindrick, S., Hertzler, S., & Disilvestro, R. A. (2007). Effects of Various Forms of Calcium on Body Weight and Bone Turnover Markers in Women Participating in a Weight Loss Program. *Journal of the American College of Nutrition, 26*(5), 456-461.

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

Westerterp-Plantenga, M. S., Luscombe-Marsh, N., Lejeune, M. P. G. M., Diepvens, K., Nieuwenhuizen, A., Engelen, M. P. K. J., … & Westerterp, K. R. (2006). Dietary protein, metabolism, and body-weight regulation: dose-response effects. *International Journal of Obesity, 30*, S16-S23.

Westerterp-Plantenga, M. S., Nieuwenhuizen, A., Tome´, D., Soenen, S., & Westerterp, K. R. (2009). Dietary Protein, Weight Loss, and Weight Maintenance. *The Annual Review of Nutrition, 29*, 21-41.

Wlodek, D., & Gonzales, M. (2003). Decreased energy levels can cause and sustain obesity. *Journal of Theoretical Biology, 225*(1), 33-44.

Yang, D., Liu, Z., Yang, H., & Jue, Y. (2014). Acute effects of high-protein versus normal-protein isocaloric meals on satiety and ghrelin. *European Journal of Nutrition, 53*(2), 493-500.

Yanovski, J. A., Parikh, S. J., Yanoff, L. B., Denkinger, B. I., Calis, K. A., Reynolds, J. C., Sebring, N. G., & Mchugh, T. (2009). Effects of calcium supplementation on body weight and adiposity in overweight and obese adults: a randomized trial. *Annals of Internal Medicine, 150*(12), 821-829.