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DETERMINING THE EFFECT OF 3-MONTHS PREMEAL PHOSPHORUS SUPPLEMENTATION ON ENERGY INTAKE, BODY WEIGHT, AND LIPID PROFILE IN OVERWEIGHT/OBESE INDIVIDUALS

by
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AN ABSTRACT OF THE THESIS OF

Jennifer Joseph Ayoub for Master of Science
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Title: Determining the Effect of 3-Months Premeal Phosphorus Supplementation on Energy Intake, Body Weight, and Lipid Profile in Overweight/Obese Individuals

The present study is based on previous data showing that an intake of 500 mg phosphorous (P) preload is able to reduce subsequent food intake by 27-33%. It is also known that P ingestion efficiently increases insulin sensitivity after 60 minutes of its intake. It is also important to note that both P and insulin are involved in food intake as well as in lipid metabolism. Assuming that the ingestion of 375 mg P supplements three times daily with each main meal, in addition to the consumption of P from regular food, the total daily P intake would be about 3g/day which is 25% less than the upper limit (4g/day). Since changes in body weight require 3 months, using this variable as the main outcome would provide robust information on the role of P. Therefore, the objective of this study is to investigate the medium-term effect of 375 mg P ingested three-times-daily in overweight and obese adults. Primary outcome variables are body weight, BMI, waist circumference (WC), serum lipids, GLP-1 and subjective appetite scores.

This is a double-blind, randomized, placebo-controlled study. A sample of 49 overweight and obese subjects (18 men and 31 women) of average age 30±3 years and a BMI of 31 ±1.3 kg/m^2 (mean±SEM) participated. Subjects were randomized to receive daily placebo (cellulose) or potassium phosphate (375mg) tablets with each main meal (breakfast, lunch, and dinner) for a period of 3 months. Body weight, BMI, WC, GLP-1, serum lipids, and appetite scores were collected at baseline and after 3 months.

The current intervention resulted in a significantly ($p<0.05$) decreased body weight (-0.44±0.53 kg), BMI (-0.16±0.18 kg/m2) and WC (-3.48±0.60 cm) as compared with placebo (1.13± 0.45 kg, 0.42±0.18 kg/m2 and 0.38±0.4 kg/m2, respectively). The change in GLP-1 and serum lipids (i.e. total cholesterol, LDL-C, HDL-C and TG) did not differ between groups. Subjective scores of appetite, quantity of food to reach fullness, hunger, and number of snacks significantly decreased within the phosphorous group after 6 and 12 weeks of supplementation.

P supplementation over a period of 3 months was significantly associated with decreased body weight, BMI, waist circumference, and subjective nutritional appetite scores. However, there was no significant effect on serum GLP-1 and lipid parameters. These findings support a promising role of the mineral P in treating obesity, especially abdominal adiposity. The exact mechanisms of action and longer term effects still need to be clarified.

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ABBREVIATIONS

%	Percent
/	Per
<	Lower than
>	Higher than
±	Plus or minus
^{o}C	Degree Celsius
2,3-DPG	2,3 diphosphoglycerate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	ANALYSIS OF VARIANCE
Apo	Apolipoprotein
ATP	Adenosine triphosphate
AUB	American University of Beirut
AUBMC	American University of Beirut Medical Center
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
cm	centimeter
CM	Chylomicron
CRP	C-reactive protein
CRU	Clinical Research Unit
CVD	Cardiovascular disease
dL	Deciliter
ELISA	Enzyme-Linked Immunosorbent Assay
et al.	(and others)
FA	Fatty Acid
FFA	Free fatty acid
FGF-23	Fibroblast growth factor-23
FPG	Fasting plasma glucose
g	Gram
G6P	Glucose-6-phosphate
GFR	Glomerular filtration rate
GLP-1	Glucose-like peptide 1
$H_2PO_4^-$	Dihydrogen phosphate
HbA1c	Glycosylated Hemoglobin
HDL-C	High-density lipoprotein cholesterol
HFCS	High fructose corn syrup
HPO_4^{2-}	Monohydrogen phosphate
HSL	Hormone-sensitive lipase
IRB	Institutional Review Board
K2HPO4	Potassium phosphate dibasic
Kcal	Kilocalorie
Kg	Kilogram
KH2PO4	Potassium phosphate monobasic
L	Liter
LDL-C	Low-density lipoprotein cholesterol
LpL	Lipoprotein Lipase

m	Meter
MetS	Metabolic syndrome
mg	Milligram
min	Minute
mL	Milliliter
mmol	Millimol
n	Number of subjects
O_2	Oxygen
OGTT	Oral glucose tolerance test
P	Phosphorus
Pi	Inorganic phosphorus
PL	Phospholipid
pM	Picomoles
PO4	Phosphate
PTH	Parathyroid hormone
RDA	RECOMMENDED DAILY ALLOWANCE
RPM	Rounds per minute
SD	Standard deviation
SEM	Standard error of the mean
SFA	Saturated fatty acids
S-P	Serum phosphorus
SREBP1c	Sterol Regulatory Element-Binding Protein 1 c
T_0	Time 0 minutes
T_{2hr}	Time after 2 hours
TC	Total cholesterol
TG	Triglyceride
TMD	Tetra Methyl Benzidine
TRLs	Triglyceride-rich lipoproteins
U	Unit
VLDL	Very low-density lipoproteins
Vs.	Versus
WC	Waist circumference
W/H	Waist-to-hip ratio
WHO	World Health Organization
β	Beta
µ	Micro
µIU	Micro-International Unit

CHAPTER I

INTRODUCTION

Overweight and obesity are global health problems, reaching dangerous levels in many high, medium, and low-income countries (Hossain *et al*., 2007; Santos *et al*., 2012; Tesauro *et al*., 2013). In 2008, 1.5 billion adults were overweight, and more than 1 in 10 were obese (WHO, 2008). High levels of obesity are also present in the Eastern Mediterranean region (Sibai *et al*., 2010). In Lebanon, the rates have doubled in 2009 compared to 1997, with a yearly increase rate of 5.2% (Nasreddine *et al*., 2012). This trend alerts the association between obesity and metabolic syndrome (MetS), which are known risk factors for cardiovascular disease (CVD) as well (Tesauro *et al*., 2013).

Several factors are linked to obesity such as environmental, genetic, and behavioral ones (Popkin *et al*., 2012). In addition, modernization has aided in several dietary modifications, which might also be a reason behind changes in weight status. For example, the consumption of high carbohydrate foods has been reported to have increased; this type of diet happens to be low in phosphorus and positively associated with weight gain (Haglin, 2001; Obeid, 2013; Santos *et al*., 2012); whereas a high protein diet, on the other hand, is rich in phosphorus and has been positively linked with lower energy intake and consequent weight loss (Halton & Hu, 2004).Thus, the mineral phosphorus might be correlated with weight-related issues.

When studying energy metabolism, a negative relationship between phosphorus and energy intake is presented. For instance, the administration of this mineral as different preloads significantly decreases subsequent food intake. This might possibly be linked to the involvement of phosphorus in the postprandial increase of

hepatic adenosine triphosphate (hepatic ATP) synthesis, which in turn triggers earlier satiation and consequent reduction in energy intake (Obeid *et al.*, 2010). Furthermore, the role of phosphorus in enhancing resting metabolic rate, postprandial thermogenesis (Belin & He, 2007; Jaedig *et al.,* 1994) and oxidative reactions (Obeid, 2013) has been discussed, which positively associates this mineral with increased energy expenditure.

Phosphate status is also correlated with MetS (Bohannon *et al*., 1989; Haglin, 2001; Riley *et al*., 1979). For instance, hypophosphatemia has been positively associated with increased body weight, BMI, and disrupted fat distribution; it has also been interrelated with insulin resistance, hyperglycemia, dyslipidemia, and hypertension in obese individuals (DeFronzo & Lang, 1980; Ditzel & Lervang, 2010; Haglin, 2001).

In addition, phosphorus is essential in carbohydrate metabolism (Kjeldsen *et al,*. 1988). Thus, when insulin is released, glycolysis and cellular uptake of serum inorganic phosphate (Pi) are stimulated. Phosphorus is required for the phosphorylation of carbohydrate compounds intracellularly in the liver and skeletal muscles; therefore, an increased insulin level helps trigger a rapid decrease in serum phosphate (S-P) concentrations due to elevated cellular uptake (Kalaitzidis *et al*., 2005; Marshall *et al*., 1979; Weisinger *et al*., 1998).

Furthermore, some evidence support a positive relationship between hypophosphatemia, lipogenesis, and hypertriglyceridemia, through the role of insulin in fat metabolism (Haglin, 2001; Knochel, 1977). To elaborate, phosphorus helps improve insulin sensitivity (Khattab *et al*., 2011; Nowicki *et al*., 1996) which in turn might aid in decreasing postprandial triglycerides (TGs) (Garg, 1996) and affect triglyceride-rich lipoproteins (TRLs) (Hazim *et al*., 2014). Moreover, the effect of phosphorus on

decreasing energy intake highlights its importance in weight loss (Obeid *et al*., 2010), which in turn helps improve the lipid profile (Layman *et al*., 2003).

Biological mechanisms behind the previously mentioned correlations are still not clearly stated. In addition, some documented data has been controversial, particularly those associated with serum lipid parameters. Therefore, this triggers our attention to investigate the effect of pre-meal phosphorus supplementation (over a period of 3-months) on food intake, appetite, body weight, waist circumference, and serum lipids in overweight/obese individuals.

CHAPTER II

LITERATURE REVIEW

A. Distribution of Phosphorus in the Body

Phosphorus is an essential element that makes up around 0.65 to 1.1 percent of the adult body (Aloai *et al.*, 1984; Diem, 1970) and is usually found in the phosphate form (Amanzadeh & Reilly, 2006). The majority of phosphorus (85%) is present in the skeleton as hydroxyapatite; the remaining is in soft tissues (14%) and the extracellular fluid (1%) (Diem, 1970; Farrow & White, 2010).

Intracellularly, phosphate mainly exists as organic compounds, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), creatine phosphate, and nicotinamide adenine dinucleotide (Baker *et al.*, 2002; Takeda *et al*., 2012); whereas in the extracellular medium, phosphorus is generally inorganic (Amanzadeh & Reilly, 2006; Levi & Popovtzer, 1999) and is physiologically regulated within a narrow range of 2.5-4.5 mg/dl (0.80-1.44 mmol/L) (Bansal, 1990;Kuro, 2010; Levi & Popovtzer, 1999; Takeda *et al.*, 2012). Inorganic phosphorus (Pi) represents a minor fraction of the total; however, it is highly important and may reflect the status of stores in the body (Bansal, 1990; Takeda *et al.*, 2012). About 85% of serum phosphate (S-P) is in the form of free ions ($H_2PO_4^-$,HPO_4^{2-} and PO_4^{3-})and less than 15% is bound to protein (Levi & Popovtzer, 1999).

B. Phosphorus Intake and Requirements

Total phosphate intake depends on both natural and processed foods. Protein-rich sources (e.g.: meat, milk, cheese, poultry, and fish) and cereal grains have high content of this mineral (Takeda *et al.*, 2012); however, the presence of phytates in grains decreases the bioavailability of phosphorus (Uribarri, 2007). In addition, the use of phosphorus as a food additive has increased its amounts in products such as processed meats and some beverages including sodas, juices, and sports drinks (Calvo & Park, 1996; Calvo & Uribarri, 2013; Murphy-Gutekunst, 2007). Moreover, Pi may be present in dietary supplements, enriched/fortified foods, some over-the-counter (OTC) medications, and water (Calvo & Uribarri, 2013). On the other hand, items such as oils, high fructose corn syrup (HFCS), sugars, and sweeteners contain negligible phosphorus amounts; additionally, refined cereal commodities lose around 70% of their phosphorus content due to the refinement process (Obeid, 2013).

During the past few decades, changes in dietary habits and modernization have led to the increased intake of low phosphorus products, which are currently contributing to more than 50% of the food supply (Obeid, 2013). This has caused a decrease in the daily phosphorus ingestion to about 1- 1.5 g d^{-1} (Ervin *et al.*, 2004; Gattineni & Baum, 2012) as compared to our ancestor's intake of 2.5 g d^{-1} (based on the intake of primarily raw, unprocessed foods with a 2,500 kcal d^{-1} diet and around 1 mg P/kcal) (Food and Nutrition Board, 1997). The current intake level is still above that of the recommended daily allowance (RDA) (700 mg); however, the latter is based on the lower end of the normal adult serum Pi. Furthermore, both ancestral and current intakes are lower than the upper limit (UL) (4 g d^{-1}) for adult males and females (Food and Nutrition Board, 1997).

It is important to note that low phosphorus food sources are inversely associated with low socioeconomic status (Popkin, 2006), where people with such incomes greatly consume dietary choices which are energy-dense but nutrient poor due to their more affordable prices (Darmon & Drewnowski, 2008; Drewnowski, 2009). This may help explain the increased prevalence of obesity among these populations (Drewnowski, 2009; Molarius *et al*., 2008), and a possible assumption behind this increased weight status could be attributed to the decreased phosphorus intake.

C. Functions of Phosphorus in the Body

Phosphorus is important for many biological processes, and it functions both intracellularly and extracellularly. It is essential for cell structure (e.g. phospholipids, nucleotides, phosphoproteins), oxygen-carrying capacity of hemoglobin (2,3-diphosphoglycerate (2,3-DPG)), bone mineralization, acid-base homeostasis (urinary buffering), and phosphorylated intermediates including those involved in cellular signaling. Phosphorus is also crucial for the generation of energy as ATP via several enzymatic processes implicated in glycolysis, gluconeogenesis, and oxidative phosphorylation (Baker *et al.*, 2002; Bansal, 1990; Takeda *et al.*, 2012). Thus, disturbed phosphate levels might alter the activity of several metabolic pathways (Levi & Popovtzer, 1999).

D. Factors Affecting Phosphorus Homeostasis

In order for phosphorus to function properly, it is necessary to keep its serum level controlled within the normal range. This regulation can be maintained via several processes including the following: intestinal absorption (16 mg/kg/day), renal reabsorption and excretion (13 mg/kg/day), and skeletal storage (3 mg/kg/day) (Berndt & Kumar, 2009). Furthermore, factors such as parathyroid hormone (PTH), calcitriol, phosphate transporters, and phosphatonins are chief regulators of phosphate status (Marks *et al.*, 2010; Penido & Alon *et al.*, 2012; Shaikh *et al.*, 2008). In addition, dietary Pi intake, dopamine, adrenergic activity, and blood pH can also influence plasma Pi concentrations (Shaikh *et al.*, 2008). Figure 1 represents phosphorus homeostasis in humans, and figure 2 shows the interaction between PTH and vitamin D for phosphate regulation.

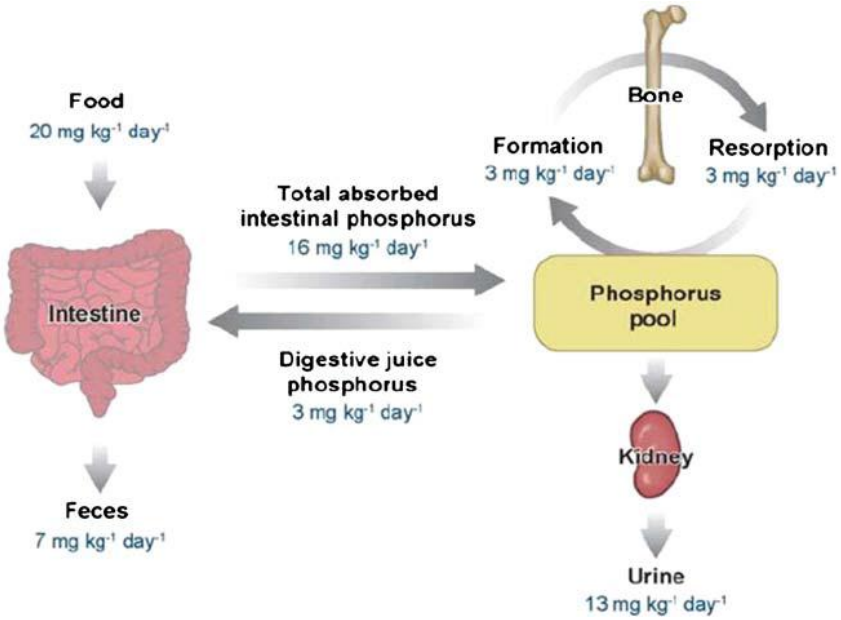

Figure 1: Phosphorus Homeostasis in Humans

Berndt TJ, Schiavi S, Kumar R (2005) "Phosphatonins" and the regulation of phosphorus homeostasis. Am J Physiol Renal Physiol, 289:F1170–F1182.

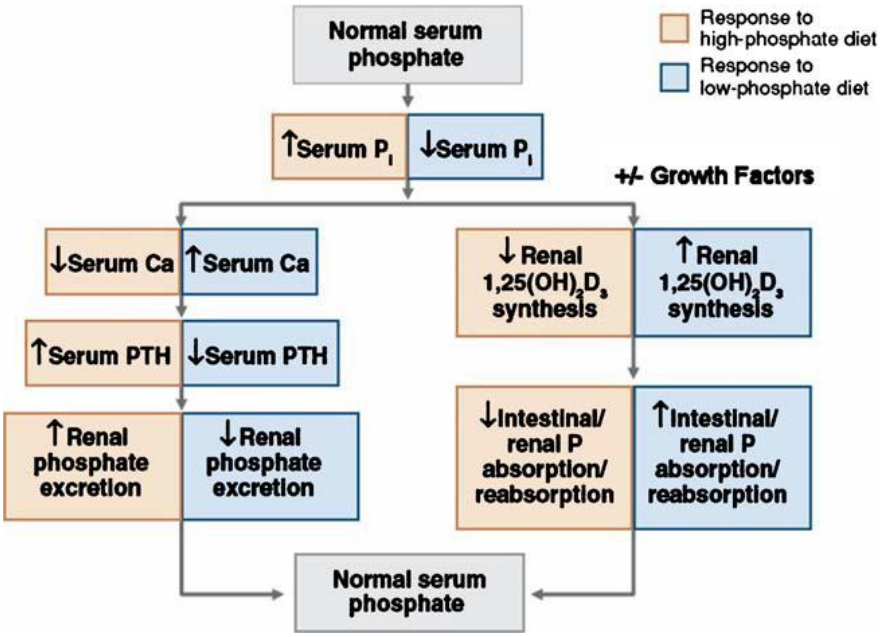

Figure 2: The Interaction between Parathyroid Hormone and Vitamin D-Endocrine System in the Regulation of Phosphorus Homeostasis
Shaikh, A., Berndt, T., & Kumar, R. (2008).Regulation of phosphate homeostasis by the phosphatonins and other novel mediators. Pediatric Nephrology, 23(8), 1203-1210.

1. Intestinal Absorption

Dietary phosphorus absorption occurs along the line of the small intestine via (1) a transcellular process, which is mediated by sodium–phosphate type IIb co-transporters, and (2) by a paracellular process, which requires passive diffusion and is greatly dependent on the concentration of phosphorus present in the lumen. Recent studies have proposed the capability of the intestine to "sense" the level of phosphorus, where an increased absorption has been associated with higher intakes; however, little is known about the saturation of this process (Shaikh *et al.*, 2008). Furthermore, factors such calcitriol can affect intestinal absorption by 30% (Wilz *et al.*, 1979), and some minerals such as calcium, sodium, and magnesium might also influence the absorption process though nutrient interaction (Xu *et al.*, 2001).

2. Renal Reabsorption

The kidney is the main organ for phosphorus balance in the body (Shils *et al.*,2006), where type IIa (Forster *et al*., 2006) and type III (Urer *et al.*,2004) sodium–phosphate co-transporters are required for Pi filtering at the level of the glomerulus and reabsorption in the proximal tubule (Forster *et al*., 2006; Urer *et al.*,2004). Additionally, PTH and fibroblast growth factor 23 (FGF-23) are among the hormones that also influence renal reabsorption (Farrow & White, 2010). Other factors may include insulin, growth hormone, and thyroid hormone which stimulate this process (Bringhurst & Leder, 2006; Murer *et al*., 2000). However, calcitonin, glucagon, and glucocorticoids induce renal excretion of phosphorus (Murer *et al*., 2000).

3. Bone

Bone remodeling has been shown to be part of serum Pi control through factors such as osteoblasts, osteoclasts, and the alkaline phosphatase enzyme. Moreover, FGF-23, which has a role in protecting bones from high levels of phosphorus, also helps regulate serum concentration by its hypophosphatemic effect (Tiosano & Hochberg, 2009).

4. Hormones

a. Parathyroid hormone (PTH)

The primary physiological role of PTH is the regulation of serum calcium concentration (Kemper *et al.*, 1974; Penindo & Alon, 2012). Another important role is its effect on the regulation of S-P levels, where high concentrations of the latter up-regulate PTH synthesis and production by the parathyroid gland (Bergwitz & Jüppner,

2010). In turn, PTH acts on several organs to decrease S-P levels back to normal, mainly through inhibiting phosphate reabsorption at the level of the kidneys (Forster *et al.,* 2006; Kalaitzidis *et al.,* 2005).

b. Calcitriol or 1,25-$(OH)_2D_3$

PTH is a strong enhancer of vitamin D production, where the former induces the enzyme responsible for 1-alpha-hydroxylation (Boullion, 2006); however, high levels of 1,25-$(OH)_2D_3$ suppress PTH (Bergwitz & Jüppner, 2010). Therefore, activated vitamin D has a role in phosphate homeostasis either (1) directly by stimulating dietary phosphorus absorption at the level of enterocytes (Boullion, 2006) or (2) indirectly via suppressing PTH which thus enhances reabsorption at the level of the kidneys (Penindo & Alon, 2012). Additionally, 1,25-$(OH)_2D_3$ can stimulate resorption of osteoclasts causing the release of phosphorus into the blood (Penindo & Alon, 2012).

c. Phosphatonins: Fibroblast Growth Factor 23 (FGF-23)

Phosphatonins are peptides which have recently been identified in phosphorus homeostasis (Penindo & Alon, 2012); among them are the following: fibroblast growth factor-7 (FGF-7), secreted frizzled related protein-4 (sFRP-4), matrix extracellular phosphoglycoprotein (MEPE), and fibroblast growth factor-23 (FGF-23) (Penindo & Alon, 2012; Shaikh *et al.,* 2008).

FGF-23 has a hypophosphatemic effect, where it can suppress the kidneys' proximal tubular reabsorption of phosphorus (as seen with PTH). This phosphatonin can also regulate S-P in an indirect manner by enhancing the expression of 24α-hydroxylase enzyme rather than 1α-hydroxylase in the kidneys; consequently, the synthesis of 1,25-

$(OH)_2D_3$ is prevented and thus intestinal absorption of phosphorus is decreased (Berndt *et al.*, 2007; Kuro-o, 2010).

E. Hepatic Adenosine Triphosphate (Hepatic ATP) and Food Intake

Despite the diversified roles of the liver in the body, this organ has a well-established function in the control of feeding behavior. When changes in its energy status occur, feedback responses to the central nervous system are signaled via vagal sensory neurons which regulate food intake; thus, when hepatic ATP is low, messages that induce overeating are sent (Friedman, 2007; Langhans & Scharrer, 1992). This data is based on research that was studying the fructose analog called 2,5-anhydro-D-mannitol (2,5-AM) which has a phosphate-trapping capacity. This molecule was shown to cause a decline in the amount of Pi, resulting in lower liver ATP production and a triggered feeding response (Rwason & Friedman, 1994). Hence, the dependency of ATP production on adequate phosphorus supply is concluded (Morris *et al*., 1978; Solomon & Kirby, 1990). In addition, it is known that an increased prevalence/sustainability of obesity is associated with low hepatic ATP level because of its effect on inducing food intake (Friedman, 2007; Langhans & Scharrer, 1992; Nair *et al.,* 2003; Wlodek & Gonzales, 2003); thus, this also further explains the involvement of phosphorus in weight changes.

F. Factors Affecting Phosphate Availability for ATP Production

Phosphate loading inhibits both the reduction in liver ATP and the increase in food intake (Morris *et al.,* 1978), but several factors may compromise phosphorus availability for ATP production.

1. Fructose

Dietary intake of fructose is greatly increasing due to the elevated consumption of soft drinks and foods prepared with major caloric sweeteners such as HFCS (Baciano *et al.,* 2005; White, 2013). The increased ingestion of such foods is significantly linked with accelerating rates of obesity, insulin resistance, hypertension, and dyslipidemia particularly hypertriglyceridemia (Elliot *et al.,* 2002).

These correlations can be explained by the following mechanisms: (1) unlike glucose, fructose does not stimulate postprandial insulin production, which in turn hinders the release of the insulin-dependent hormone, leptin. Knowing that both insulin and leptin have hypophagic functions, ingesting high levels of fructose would cause a combined effect on lowering the levels of both hormones in the blood; this in turn would trigger an increase in food intake and its associated metabolic complications (Elliot *et al.,* 2002). (2) Fructose metabolism bypasses the limiting enzyme phosphofructokinase, which usually controls glucose metabolism. Hence, the lack of such negative feedback makes fructose and its metabolites (glycerol-3-phosphate and acetyl CoA) unregulated sources for lipogenesis in the liver, enhancing adverse metabolic alterations as explained in figure 3 (Tappy *et al.,* 2010). In addition, the mass uptake and phosphorylation of fructose in the liver could largely degrade ATP to AMP and uric acid (Tappy *et al.,* 2010).

Furthermore, as previously mentioned, fructose is known to have a "phosphate-sequestering" capacity. When fructose-1-phosphate (F 1-P) accumulates in the liver due the absence of negative feedback mechanisms, phosphorus becomes unavailable for other essential metabolic pathways such as the regeneration of ATP (Rwason & Friedman, 1994).

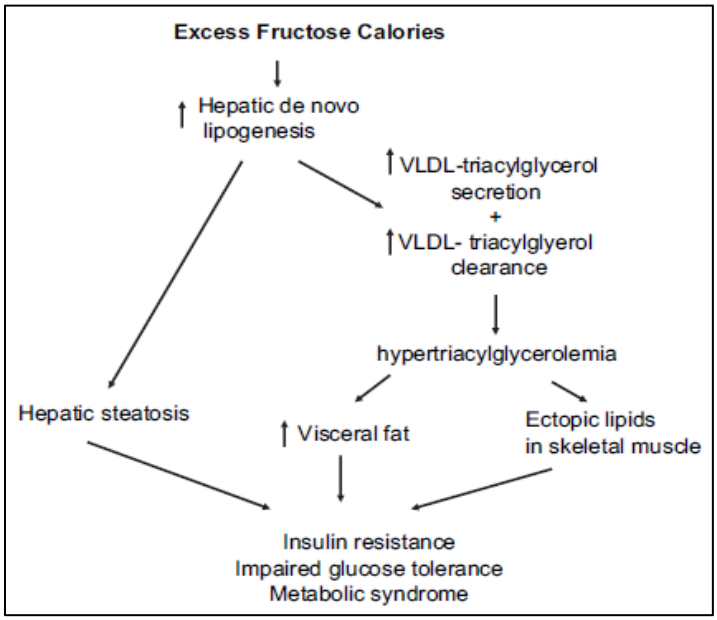

Figure 3: Mechanisms Linking Excess Fructose Consumption to Metabolic Disorders
Tappy, L., Lê, K. A., Tran, C., & Paquot, N. (2010). Fructose and metabolic diseases: new findings, new questions. Nutrition, 26(11), 1044-1049.

2. *Calcium*

Calcium, either from the diet or from supplements, has the ability to complex with phosphate in the intestinal lumen. Thus, in case of excessive calcium intake without a corresponding increase in that of phosphorus, the absorption of the latter falls. In parallel, the ratio of calcium to phosphate might increase upon the use of supplements or food fortificants that consist of non-phosphate calcium salts; this has been shown to negatively affect the availability of phosphorus for absorption in addition to increasing the risk of phosphorus insufficiency (Heaney & Nordin, 2002).

3. *Dairy Products and Proteins*

It has been suggested that high protein diets are associated with weight loss, possibly by promoting less energy intake and increasing energy expenditure (Pioli *et al.*, 2013). Similarly, dairy foods have been shown to assist in both weight and fat loss (Due

et al., 2004), independent of the mineral calcium (Yanovski *et al.,* 2009). In addition, data has revealed that, when comparing the consumption levels of dairy products among overweight individuals, people ingesting high amounts of such diets had significantly lower risks of MetS (Pereira *et al.,* 2002). According to Takeda *et al*. (2012), diets high in proteins or dairy products provide rich sources of phosphorus; therefore, the mentioned associations between these diets and weight loss could be explained by the involvement of phosphorus.

G. Causes of Hypophosphatemia

Several factors might decrease S-P concentration, among which are the following: an unbalanced diet, trans-cellular shift, and/or increased renal excretion (Haglin, 2001). However, when studying the relationship between hypophosphatemia and the activity of the kidneys, no association was found since excretion levels of phosphorus were similar among individuals with MetS as compared to controls. On the other hand, data has related decreased S-P levels to the consumption of diets low in phosphate and high in carbohydrates and/or to an increased transfer of S-P to the intracellular space (Kalaitzidis *et al.,* 2005).

The shift of extracellular phosphate is mainly stimulated by an elevated insulin level; in addition, this increased transfer could also be due to an activated sympathetic nervous system as seen with MetS or due to the resulting increment in serum catecholamine levels. To elaborate, both insulin and catecholamines stimulate glycolysis, which is accompanied by an increase in the formation of phosphorylated carbohydrate compounds in the liver and skeletal muscle cells. As a consequence, S-P

concentrations may rapidly decrease favoring a state of hypophosphatemia (Kalaitzidis *et al.*, 2005).

Furthermore, some other factors such as the use of medications (diuretics or antacids), trauma (surgery or infections), diabetic ketoacidosis, or malnutrition could also enhance hypophosphatemia (Haglin, 2001).

H. Phosphorus and Carbohydrate Metabolism

The literature has associated low S-P with disturbed metabolism, particularly that of carbohydrates (Çelik & Andiran, 2011; Haglin, 2001; Park *et al*., 2009).

1. Serum Phosphate (S-P) and Glucose Tolerance

Hypophosphatemia was first associated with impaired glucose tolerance in 1926, after which it was correlated with disturbances in glucose utilization, decreased insulin sensitivity, and hyperinsulinemia in individuals with MetS (Gudmundsdottir *et al.*, 2008). These relationships were also seen in healthy non-diabetic people, where low S-P was linked with increased glycemia and reduced insulin sensitivity 2-hours postprandially (Haap *et al*., 2006).

2. Mechanisms Relating Phosphate and Glucose Metabolism

There are several possible mechanisms that relate phosphate levels to glucose metabolism. (1) The infusion of electrolytes/minerals, including phosphate, has been shown to increase postprandial thermogenesis and carbohydrate phosphorylation (Jaedig *et al*., 1994). Hence, an impaired phosphate status (especially if chronic) limits the phosphorylation of carbohydrate intermediates in glycolysis and glycogenesis,

consequently decreasing the utilization of glucose by peripheral cells (Haglin, 2001; Kalaitzidis *et al.*, 2005; Xie *et al*., 2000). (2) Hypophosphatemia has been shown to cause insulin resistance and secondly hyperinsulinemia (Haap *et al*., 2006; Haglin, 2001). This effect could be explained by the fact that, when S-P is low, PLs which are majorly found in cell membranes get affected; thus, cell membrane structure might be altered, in turn hindering the binding of insulin to its receptors (Anderson & Moore, 2004). Furthermore, a low phosphate status might disturb the phosphorylation of serine and tyrosine residues at the level of insulin receptors; in consequence, the insulin signaling cascade might be interrupted, leading to insulin resistance (Lizcano & Alessi, 2002). Consequently, serum insulin levels might increase as a compensatory mechanism to the reduced insulin sensitivity (Haap *et al*., 2006; Haglin, 2001). Furthermore, insulin has a hypophospatemic effect by transporting phosphate from the extracellular to the intracellular space; thus, hyperinsulinemia would cause a decrease in S-P concentrations. Therefore, these interrelations could lead to the progression of a viscous cycle which may promote MetS (Gudmundsdottir *et al.*, 2008; Haglin, 2001; Kalaitzidis *et al.*, 2005). (3) Studies on rats have shown that a diet deficient in phosphate upregulates microsomal glucose-6-phosphatase (G6Pase) activity in the liver. Accordingly, hepatic glucose may be overproduced, leading to glucose intolerance and insulin resistance, which are metabolic complications documented in various diseases characterized with hypophosphatemia (Çelik & Andiran, 2011).

3. Phosphorous and Insulin Response

Studies positively correlate hypophosphatemia with elevated fasting plasma glucose (FPG), high postprandial blood glucose, and impaired insulin sensitivity (Haap *et al.,* 2006; Haglin, 2001; Park *et al*., 2009). In addition, when comparing S-P concentrations, lower levels were seen in patients with MetS as opposed to healthy individuals (Kalaitzidis *et al*., 2005). Moreover, glucose intolerant subjects, who were also hypophosphatemic, have shown a significantly improved glucose response after phosphate supplementation (Wittmann & Nagy, 1997). More recently, the inclusion of phosphorus in oral glucose load has been demonstrated to improve insulin sensitivity (Khattab *et al.,* 2011); a proposed mechanism could be related to phosphate's capacity of trapping glucose intracellularly after phosphorylating it.

I. Phosphorus and Lipid Metabolism

1. Postprandial Lipid Metabolism

After a meal, ingested fat is hydrolyzed by pancreatic enzymes and absorbed into the enterocytes, after which chylomicrons (CM) are formed and secreted into the lymph and then the blood. In circulation, lipoprotein lipase (LpL) breaks down the TGs in the CM, modifying the latter into a remnant which is then cleared from circulation by the hepatocytes (Riccardi *et al.,* 2006). In the liver, the delivered fatty acids are re-assembled in very low-density lipoproteins (VLDLs), which are rich in TGs and characterized by the apo-B apolipoprotein, and released into the blood (Klop *et al.*, 2011). Subsequently, endothelium-bound lipoprotein lipase (LpL) hydrolyses the TGs in the VLDLs into glycerol and fatty acids. However, in the postprandial state, LpL

availability is limited; thus, because of the competition occurring at the level of this enzyme, lipoproteins may accumulate (Klop *et al.*, 2011).

2. Phospholipids

In the body, lipids occur as either storage or structural form, where the latter is mainly constituted of phospholipids (PLs). Additionally, PLs function in cellular metabolism and digestion, and the most abundant one is lecithin (phosphatidylcholine), which is predominantly found in the membrane lipid bilayer and in different lipoproteins (i.e. very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), high-density lipoproteins (HDLs)) (Mahan & Escott-Stamp, 2000).

3. *Phosphorus, Insulin Resistance, and Lipid Metabolism*

a. Insulin and Lipid Metabolism

Insulin is critical for the regulation of lipid metabolism since it stimulates fatty acid (FA) and TG synthesis and promotes fat storage in adipocytes. This may be explained by the role of insulin in activating LpL enzyme, enhancing cellular glucose uptake that constitutes the glycerol portion of the TG molecule, and converting acetyl CoA into TGs (Dimitriadis *et al.*, 2011; McMurray & Hackney, 2005). Concomitantly, insulin suppresses lipolysis by inhibiting hormone-sensitive lipase (HSL) (Burén, 2003; McMurray & Hackney, 2005), in addition to cyclic adenosine monophosphate and protein kinase A (McMurray & Hackney, 2005). However, the consumption of high fat foods, particularly those rich in saturated fatty acids (SFAs), induces visceral obesity and decreases insulin sensitivity (Manco *et al.*, 2004; Park *et al.*, 2001).

b. Insulin Resistance and Lipid Metabolism

Evidence has linked insulin resistance with impaired carbohydrate and fat metabolism; as a result, circulating glucose and free fatty acid (FFA) concentrations might increase (Holloway *et al.*, 2011). To add, insulin resistance has been directly associated with an increased risk of 'atherogenic dyslipidemia', which is characterized by an elevated VLDL-TG, decreased HDL-C, and the presence of small dense LDL particles. These characteristic changes in lipid profile may be related to the role of insulin resistance in assembling and producing VLDL, leading to an elevated serum TG level which in turn might decrease HDL-C and help generate small dense LDL particles (D'Agostino *et al.*, 2004). Lipoprotein abnormalities and lipemia have also been documented in the postprandial state of patients with low insulin sensitivity (Annuzzi *et al.*, 2004; Riccardi *et al.*, 2006); this may be related to the fact that insulin has been shown to affect the production, clearance and degradation of TRLs (Adiels *et al.*, 2008; Duez *et al.*, 2008). Besides the risk of decreasing TG clearance and overproducing VLDLs, insulin resistance might also increase *de novo* lipogenesis by activating the fatty acid synthase (FAS) enzyme and SREBP1c (Nguyen *et al*., 2008).

c. Phosphorus and Lipid Metabolism

Data has shown that phosphorus is essential for various lipid components in the body, particularly PLs and chief enzymes (Mahan & Escott-Stamp, 2000) such as glucose-6-phosphatase (G6P) which is involved in lipogenesis (Park *et al.*, 2005). Therefore, disturbances in phosphate status might deteriorate these functions and disrupt the lipid balance. In addition, as previously mentioned, hypophosphatemia is positively

associated with insulin resistance, a state in which the lipid profile might be impaired (D'Agostino *et al.,* 2004).

However, there is controversial data regarding the association between S-P and the different components of the lipid profile. For instance, according to Park *et al*. (2009), S-P is positively correlated with total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and lipoprotein (a). On the other hand, Lippi *et al*. (2009) associated increasing S-P levels with decreased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and TC/HDL-C; whereas both Park *et al.* and Lippi *et al.* inversely associated S-P with triglyceride levels. Moreover, Farhangi *et al.* (2011) documented a weak positive relationship between phosphorus and total cholesterol and a weak negative relationship with PTH; in addition, subjects with increased S-P levels had significantly higher levels of TG concentrations than their controls. Additionally, a recent study has shown that supplementing meals with 500 mg of phosphorus seems to affect the different components of postprandial lipemia; results have shown a significant increase in postprandial levels of apolipoprotein B48 (ApoB48) and a significant decrease in apolipoprotein B100 (ApoB100), where ApoB48 and Apo B100 are characteristic components of CMs and VLDLs respectively (Hazim *et al.,* 2014). Furthermore, some data has shown a positive correlation between serum FGF-23, which as previously explained has a hypophosphatemic effect, and TG levels (Holecki *et al*., 2011).

d. Mechanisms Linking Phosphorus and Lipid Metabolism

The correlation between low S-P and dyslipidemia can be explained by mechanisms linking phosphate, lipid, and glucose metabolisms. For instance, S-P affects phospholipid metabolism involved in the synthesis of cholesterol in the liver (Haglin, 2001). Additionally, in cases of hypophosphatemia, blood glucose and serum TG are closely related (Haglin, 2001; Park *et al.*, 2009), where in a hyperglycemic state, TG production augments. Studies have also shown that individuals with high risk of hypophosphatemia and hepatic phosphate depletion have elevated serum TG and low serum HDL levels, as seen in diabetic patients (Foley *et al.*, 2009; Haglin, 2001; Park *et al.*, 2009). Moreover, when S-P is high, the increased PTH concentration may diminish the activity of lipoprotein lipase, which consequently leads to abnormal lipid status (Querfeld *et al*., 1999). Another mechanism linking phosphorus to lipid metabolism is via insulin responses; phosphate has been shown to improve insulin sensitivity (Khattab *et al*., 2011; Nowicki *et al*., 1996), which in turn aids in decreasing post-prandial TGs and may affect TRLs (Adiels *et al.*, 2008; Duez *et al*., 2008; Garg, 1996).

J. Phosphorus and Body Weight

Many studies have consistently supported the positive correlation between hypophosphatemia and increased body weight (Haglin, 2001; Obeid, 2013) and vice versa (Foley *et al.,* 2009). Proposed mechanisms relate phosphorus with controlling food intake, energy expenditure, and capacity of physical activity (Obeid, 2013). Another factor which might contribute to obesity is insulin resistance and its association with hypophosphatemia (Gudmundsdottir *et al.,* 2008).

1. Food Intake

As explained earlier, low phosphate levels may decrease ATP production and diminish energy status in the liver. Consequently, decreased hepatic ATP levels tend to increase food intake (Friedman, 2007; Langhans & Scharrer, 1992; Nair *et al.*, 2003; Wlodek & Gonzales, 2003). Whereas premeal phosphorus ingestion stimulates postprandial hepatic ATP synthesis which, in turn, leads to increased satiation. For instance, upon the administration of 500 mg phosphorus to different carbohydrate preloads, meal size significantly decreased by 27-33% in subsequent *ad libitum* energy intake (Obeid *et al.*, 2010).

2. Energy Expenditure

a. Diet-Induced Thermogenesis (DIT)

Several studies have shown that obese subjects have lower diet-induced thermogenesis (DIT); however, this is normalized when weight is lost. Therefore, DIT can be excluded as being a causal factor for weight gain. To add, insulin has been shown to be implicated in this process via stimulating peripheral uptake of glucose and phosphorus (Obeid, 2013). Moreover, the addition of phosphorus has been reported to increase resting metabolic rate and postprandial thermogenesis (Belin & He, 2007; Jaedig *et al.*, 1994) possibly by enhancing insulin sensitivity (Obeid, 2013). Therefore, imbalances in phosphate levels may have a role in reducing metabolic rate, and the inverse correlation between S-P and glycemia might explain the associated increase in BMI (Haglin, 2001).

b. Oxidative Reactions and Energy Expenditure

Plasma phosphorus is essential for the production of 2,3-DPG, the molecule which functions in the oxygen-carrying capacity of hemoglobin (Bremner *et al*., 2002; Ditzel *et al.,* 2010). Hence, a low phosphate status might decrease oxygen delivery, which would then compromise its availability for oxidative reactions. In turn, these decreased processes would cause lower physical activity and hinder energy expenditure (Obeid, 2013).

Furthermore, Holecki *et al*. (2011) has revealed that obese individuals have 42% higher FGF-23 levels compared to non-obese, thus favoring a state of lower physical activity (Dalal *et al.,* 2011). FGF-23 is also known to have a hypophosphatemic effect; therefore, its increased levels could deteriorate the phosphate status even more (Holecki *et al*., 2011) and worsen energy metabolism as well.

Evidence further supports these findings, where a review article documented the presence of physical fatigue in obese subjects; however, phosphate loading at different timings helped enhance physical performance (Obeid, 2013).

3. Insulin Resistance

As previously stated, there is widespread acceptance in the literature that S-P and insulin resistance are inversely associated (DeFronzo & Lang, 1980; Gudmundsdottir *et al.,* 2008; Park *et al.,* 2009). To add, insulin resistance can create a vicious cycle which further disrupts pancreatic beta-cell function, worsens insulin resistance, and increases weight gain (Russell-Jones & Khan, 2006). Therefore, hypophosphatemia may be negatively associated with BMI via its effect on increasing insulin resistance.

K. Phosphorus, Body Fat, and Waist Circumference

While some studies have linked phosphorus to BMI, others have also related phosphorus with variations in fat distribution rather than obesity as such. For instance, Lind *et al.* (1993) has shown that S-P is inversely associated with both BMI and waist-to-hip ratio (W/H). More specifically, hypophosphatemia has also been correlated with elevated central obesity or waist circumference (WC) (Lind *et al.*, 1993; Park *et al.*, 2009).

In addition, the relationship between phosphate and fat distribution can be explained indirectly through serum FGF-23 levels on one hand and protein-rich diets on the other hand. (1) High levels of FGF-23 have been positively linked with WC and W/H ratio. Therefore, since FGF-23 has a phosphate lowering effect (Tiosano and Hochberg, 2009), it may be assumed that phosphorus*per se* negatively associates with WC and W/H. (2) Furthermore, the consumption of a diet rich in proteins, which is a high source of phosphorus (Takeda *et al.*, 2012), has been shown to help achieve greater overall weight loss while preserving fat-free mass (Krieger *et al.*, 2006; Westerterp-Platenga & Lejeune., 2005).

L. Phosphorus and Glucose-like Peptide 1 (GLP-1)

Glucose-like peptide 1 (GLP-1) is an incretin hormone produced by the intestine in response to food consumption; it stimulates pancreatic secretion of insulin and suppresses that of glucagon (Weaver *et al.*, 2008). GLP-1 has been shown to have an effect on reducing food intake, regulating hunger, and decreasing gastric emptying (Holst, 2009). Its level has also been positively correlated with that of intracellular cyclic adenosine monophosphate (cAMP) (Simpson *et al.*, 2007). To add, phosphate is

an integral part of cAMP; therefore, the availability of this mineral might increase the production of cAMP and thus enhance GLP-1 secretion.

M. Phosphorus and Other Metabolic Disorders

1. Refeeding Syndrome

Refeeding syndrome is a state of hormonal and metabolic changes that may occur in malnourished patients upon aggressive rehabilitation. This syndrome is complex and is characterized by several severe changes that may include the following: hypophosphatemia, sodium retention, alterations in the metabolism of macronutrients especially that of glucose, thiamine deficiency, and low serum potassium and magnesium levels (Mehhanna *et al.,* 2008).

These modifications may result in serious clinical disorders and have been linked to elevated insulin production, which occurs after the consumption of high carbohydrate foods during the refeeding process. Accordingly, the increased insulin secretion creates a state of anabolism which stimulates the cellular uptake of glucose and macrominerals mainly phosphorus, potassium, and magnesium. Hence, on one hand, serum levels of macrominerals may rapidly decrease; on the other hand, insulin resistance which is characterized by hyperinsulinemia and hyperglycemia might occur, along with its further complications (Obeid *et al.,* 2014).

Refeeding syndrome has been compared to today's elevated intake of the "Western diet", which happens to be low in the previously mentioned macrominerals and is associated with several metabolic complications resembling those seen in MetS (Obeid *et al.,* 2014).

2. Metabolic Syndrome (MetS)

The metabolic syndrome (MetS) is defined as the presence of three or more of the following criteria: (1) central obesity with WC ≥102 cm or ≥88 cm in males and females, respectively; (2) TG level ≥150 mg/dl (1.7 mmol/L); (3) HDL-C <40 mg/dl (1.03 mmol/L) in males and <50 mg/dl (1.3 mmol/L) in females; (4) elevated blood pressure of ≥130/≥85 mmHg and (5) fasting plasma glucose (FPG) level ≥100 mg/dl (5.55 mmol/L) (Flechtner-Mors *et al.*, 2010).

As previously discussed, phosphorus is an essential element involved in various metabolic reactions, where its low serum level has been associated with several metabolic complications including hyperglycemia, insulin resistance, dyslipidemia, and hypertension (DeFronzo & Lang, 1980; Gudmundsdottir *et al.*, 2008; Haglin, 2001; Kalaitzidis *et al.*, 2005; Park *et al.*, 2009). In addition, hypophosphatemia has been correlated with BMI in an inverse manner via alterations in food intake, energy expenditure, and body weight (Obeid *et al.*, 2014). Therefore, the literature has supported the positive association between low S-P and the components of MetS.

3. Diabetes

Healthy individuals respond to dietary carbohydrate intake by secreting optimal amounts of insulin that regulates blood glucose and Pi within normal physiological ranges. However, this response is jeopardized in type 1 and type 2 diabetic individuals, who happen to have hyperglycemia and hypophosphatemia (Vorum & Ditzel, 2014).

The presence of hypophosphatemia in diabetics could be explained by several proposed mechanisms as follows: (1) Pi and glucose have the same reabsorptive pathway in the kidneys, but the latter has a higher capacity of binding to be reabsorbed;

this might cause decreased renal reabsorption of phosphate; (2) diabetics have higher hemoglobin A1c concentrations which require more 2,3-DPG for proper oxygen release capacity (Ditzel & Lervang, 2009); (3) hyperinsulinemia shifts S-P to the intracellular space to be used for phosphorylating glucose and other metabolites, thus leading to a rapid decrease in S-P levels (Kalaitzidis *et al.*, 2005).

Furthermore, hypophosphatemia *per se* might increase the risk of diabetes via its association with hyperglycemia, hyperinsulinemia, decreased insulin sensitivity, and other components of the MetS (DeFronzo & Lang, 1980; Gudmundsdottir *et al.*, 2008; Park *et al.*, 2009).

4. Cardiovascular Diseases (CVD)

Phosphorus has been shown to have a U-shaped association with CVD (Park *et al*., 2009). Some data has documented that hyperphosphatemia, which is defined as serum Pi concentrations greater than 5mg/dl (1.61 mmol/l), is positively associated with cardiovascular disease, especially in individuals having impaired kidney function (Caudarella *et al.*, 2007; Park *et al.*, 2009). Findings have also related hypophosphatemia with MetS (Park *et al*., 2009), which is a known risk factor of CVD. Other studies have shown that even within normal ranges, high S-P levels favor cardiovascular risks. Such an association is aggravated in individuals with a history of myocardial infarction (Lippi *et al.*, 2009).

The biological mechanisms are still not clearly stated, but few propositions have been made. High S-P has the ability to stimulate the phenotypic transformation of vascular smooth muscle cells into osteoblasts capable of producing a pro-mineralizing milieu. In addition, when phosphate enhances PTH secretion and 1,25-$(OH)_2$ D

formation, it may also stimulate vascular calcifications, which at the level of the myocardium increases the risk of tachyarrhythmias and cardiovascular disturbances, especially in hemodialysis patients (Lippi *et al.*, 2009; Park *et al*., 2009). Furthermore, as presented earlier, some data associates hypophosphatemia with an increased risk of lipid abnormalities, where disturbances such as elevated LDL-C, increased triglycerides, and low HDL-C levels have been shown to increase cardiovascular diseases (Miller, 2009). The effect of phosphorus on the development of atheroma is not well understood; supplementing meals with this mineral reduces ApoB100 and increases ApoB48, where both are involved in the process of atherogenesis especially ApoB48 (Hazim *et al.*, 2014).

5. Hypertension

The acute infusion of the hormone epinephrine has been reported to have a hypophosphatemic effect in men, regardless of their blood pressure status; this might be due to the ability of epinephrine to shift S-P from the extracellular to the intracellular medium. In addition, data has shown a positive association between low S-P and increased sympathetic tone in essential hypertension (Gudmundsdottir *et al.*, 2008); a negative correlation between phosphate and systolic (Foley *et al.*, 2009) and diastolic blood pressures (Park *et al*., 2009) has also been documented.

6. Inflammation

"C-reactive protein (CRP) is a marker of inflammation and a predictor of cardiovascular risk" (Choi *et al.*, 2013). It has been positively correlated with BMI, explaining the association between inflammation and obesity (Choi *et al.*, 2013; Visser *et al.*, 1999). In addition, when linking CRP to the mineral phosphorus, an inverse connection was documented (Park *et al*., 2009); this data is further supported by the negative association between phosphorus and BMI in relation to CRP.

N. Phosphorus and Gender Differences

Experimental and clinical studies have reported variation in the metabolism of phosphate among genders. For instance, according to Haglin *et al.* (2001), higher S-P concentrations are seen in women as compared to men, but a more severe reaction is documented in women when S-P status is low. Furthermore, when correlating S-P levels with age, a positive association and a negative one have been reported in women and men respectively. The significantly different concentration of phosphorus among genders might be a factor behind the protection of women (but not men) from CVD throughout middle age, at least in the obese state.

CHAPTER III

MATERIALS AND METHODS

A. Study Design

This study is double-blinded, randomized, and placebo-controlled. The protocol was approved by the Institutional Review Board (IRB) at the American University of Beirut (AUB), under the code NUT.00.11. Written informed consents (Appendices A and B) were obtained from all participants.

B. Study Population

The study was conducted at the Clinical Research Unit (CRU) at the American University of Beirut Medical Center (AUBMC) between June and December 2013. Adult subjects were recruited either by advertisement with posters or by direct approach, where individuals were informed about the study and its general inclusion criteria.

Out of the total 63 Lebanese volunteers (20 males and 43 females) who consented, 14 dropped for personal reasons, and 49 subjects (18 males and 31 females) completed the study. Participants were either overweight or obese with a body mass index (BMI) $\geq$25 kg/m^2, and their ages ranged between 18 and 45 years. Subjects were divided into two groups based on the type of randomly allocated supplement; 21 individuals were in the placebo group and 28 were in the potassium phosphate group. Figure 4 shows the flow of recruitment of subjects.

As for exclusion criteria, they were as follows: glomerular filtration rate (GFR) <60, presence of any significant medical disease, pregnancy or lactation, regular

administration of drugs that affect body weight, and weight loss of 3% or more in the preceding 3 months.

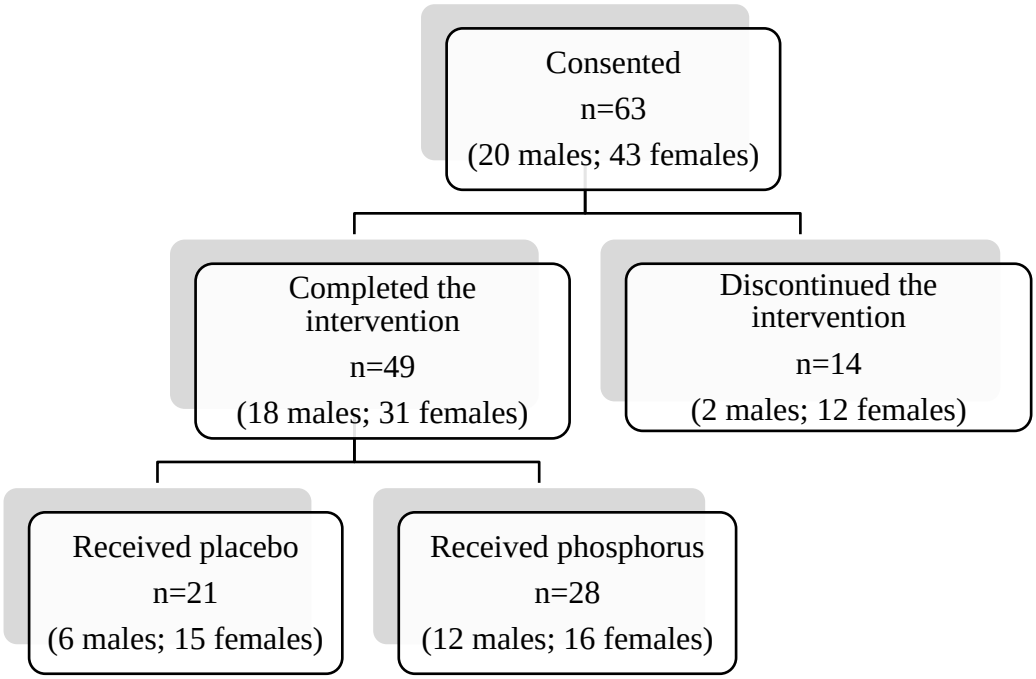

Figure 4: Flow of Recruitment of Subjects

C. Anthropometric Assessment

While subjects had light clothes and no shoes, weight was measured to the nearest 0.1 kg using a calibrated Seca balance, and height was measured via a portable stadiometer with a movable head piece. BMI was calculated as the ratio of weight (Kg) to height $(m)^2$. Waist circumference (cm) was measured at the umbilicus level using a flexible, non-stretchable measuring tape.

D. Protocol

Participation in the study was over a period of 3 months, during which the randomly allocated supplements were ingested. The tablets were constituted of either cellulose (placebo) or potassium phosphate as shown in tables 1 and 2.

Table 1: Ingredients of the Potassium Phosphate Tablet

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

Table 2: Ingredients of the Placebo Tablet

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

The study was divided into four visits, and the timeline is summarized in figure 5.

1. Visit 1

The purpose and the protocol of the study were explained to the volunteers. Subjects were then screened for inclusion and exclusion criteria via a screening questionnaire (Appendix C), which included information related to general health, weight loss, medications, and physical activity.

2. Visit 2

Eligible subjects were asked to arrive to the CRU after an overnight fasting. Anthropometric measurements including weight, height and WC were taken, and urine samples were collected. Using a 22 gauge needle, venous blood samples were withdrawn (t = 0 min) by a registered nurse. A heplock was used to keep the needle locked for a period of 2 hours in order to avoid pricking the subjects again. Then the individuals were asked to drink a 75 g anhydrous glucose solution within 2 minutes in order to perform the oral glucose tolerance test (OGTT). After 120 minutes, the second blood sample was withdrawn. No heparin was added, and water saline was infused between withdrawals to prevent the vein from obstructing. A total of 10 ml blood volume was collected, where part of the sample was presented to the AUBMC laboratory for the analysis of baseline HbA1c (glycosylated hemoglobin). The remaining withdrawn blood was centrifuged for 15 minutes at 3500 rpm at 3^{o}C; the separated serum was then stored in aliquots at -80^{o}C to be used later on for measuring the levels of phosphorus, triglycerides, total cholesterol, LDL-C, HDL-C, CRP, insulin, glucose, and GLP-1. As for the collected urine samples, they were aliquoted into four labeled tubes with 9 ml of urine in each; the tubes were then stored at -20^{o}C to be used later for measuring concentrations of calcium, phosphorus, and creatinine.

Furthermore, individuals were asked to complete a subjective appetite score questionnaire (Appendices D and E) in addition to that related to general health, medications, and physical activity. Moreover, in order to maintain a double-blinded research, the names of the supplements were replaced by letters, either A or B; those letters were equally placed in a bowl from which the subjects randomly selected one type of supplementation. Thus, at the end of the visit, participants were given a 6-

weeks' supply of the chosen tablets. The individuals were requested to take 3 tablets (a dose of 375 mg in the case of the phosphate group) with each main meal (breakfast, lunch, and dinner). They were also asked to maintain their regular dietary and physical activity habits during the entire study course and avoid alcohol consumption as well as any strenuous exercise 24 hours prior to their CRU visit.

3. Visit 3

After 6 weeks, a third visit was scheduled with the subjects where the previously mentioned questionnaires were completed again. Additionally, the remaining tablets were collected from the participants in order to assess their compliance, and another supply of the same type of tablet was given to them to be used in the subsequent 6 weeks.

4. Visit 4

When a total of 12 weeks had passed, the procedures of visit 2 were applied again in order to measure weight and WC, collect blood and urine samples, perform an OGTT, and complete the formerly mentioned questionnaires. Furthermore, the remaining tablets were re-collected in order to assess participants' adherence.

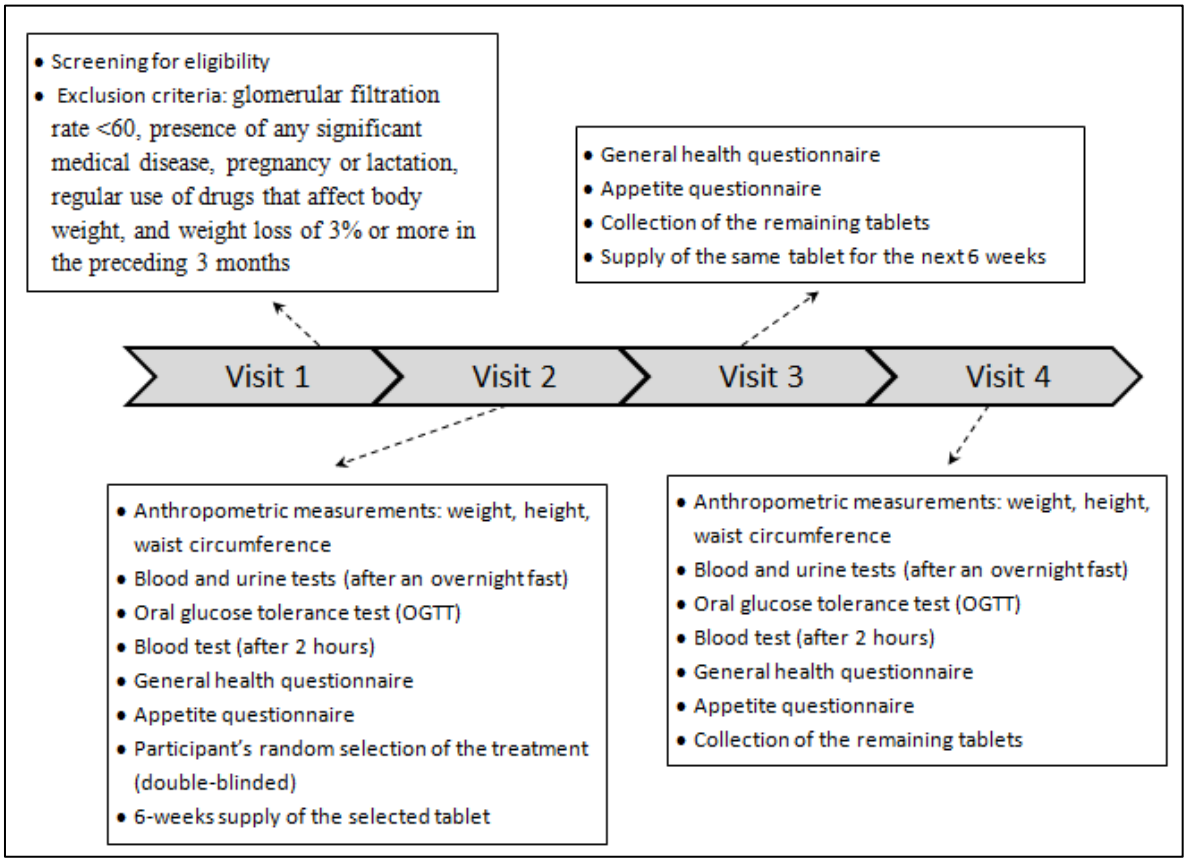

Figure 5: Timeline of the 3-Months Double-Blinded Placebo Controlled Study

E. Biochemical Measurements

1. Serum Parameters

a. Triglycerides, Total Cholesterol, HDL-C, LDL-C, Phosphorus, Glucose, and CRP

Concentrations of serum TG, total cholesterol, HDL-C, LDL-C, phosphorus, and glucose were determined via an enzymatic colorimetric method using the Vitros 350 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson, 50-100 Holmers Farm Way, High Wycombe, Buckighamshire, HP 12 4DP, United Kingdom). Levels of CRP were measured by a fixed-point immune-rate method of the Vitros.

b. Insulin and GLP-1

Concentrations of serum insulin and GLP-1 were measured by the use of the ELISA (Enzyme-Linked Immunosorbent Assay) kit from Diametra Millipore, USA. The test was performed by means of a microwell plate using two highly specific antibodies that bind to the analyte tested. After that, a coloring agent, Tetra Methyl Benzidine (TMD), was added followed by a stop solution. A coloring then appeared; its strength is proportional to the quantity of the analyte present in the sample. Subsequently, a spectrophotometer was used to measure the absorbance, and a formula was derived based on the equation of the curve obtained from the controls in the kit. The concentration of the analyte was then calculated using the derived formula.

c. Glycosylated Hemoglobin (HbA1c)

The Department of Pathology and Laboratory Medicine at AUBMC performed the analysis of baseline HbA1c using the BioRad Variant Hemoglobin Analyzer, via the high-performance liquid chromatography method.

2. Urinary Parameters: Calcium, Phosphorus, Creatinine

Using EPPENDORF Centrifuge 5810R, urine specimens were centrifuged for 10 minutes at 3500 rpm at 20^{o}C. Urinary creatinine levels were measured by the use of the Vitros 350 analyzer. In addition, samples were acidified to measure urinary concentrations of calcium and phosphorus. The colorimetric procedure was used to test for the concentrations of phosphorus and calcium, and the two-point rate method was used to measure creatinine levels.

F. Statistical Analyses

All calculations were made using the Mini-Tab statistical software version 16. The two-sample t-test was used when comparing the variation of a parameter between the two study groups. When considering results within the same group, the paired t-test was used for comparing a certain variable at two different points of collection, such as evaluating baseline parameters and those after 2 hours of the OGTT, or when comparing variables at the beginning of the study opposed to those after 3-months of supplementation. As for the ordinal or ranked variables of the appetite questionnaire scores, the Kruskal-Wallis test was used to compare data between the two study groups. One-way ANOVA followed by Fisher comparison was applied to analyze the variation of appetite scores within the same group at different periods of time (week 0, week 6, and week 12).

CHAPTER IV

RESULTS

A. Subject Characteristics

A total of 49 subjects, with an average age of 30.00±3.00 years, completed the study. Participants were divided as follows: 21 subjects (42.86%) in the placebo group versus 28 subjects (57.14%) in the phosphorus group. The majority of individuals were females who represented 71.43% and 57.14% of the placebo and phosphorus groups, respectively. The baseline characteristics of the study population, including age, sex, anthropometric variables, serum parameters, as well as urinary parameters are presented in table 3.

At baseline, no significant difference was found when comparing the different variables between the two study groups, except for serum phosphorus (S-P) concentration after 2 hours of the OGTT (p=0.010, table 3). Furthermore, on average, individuals were obese having a mean BMI>30 kg/m^2; they also had normal fasting S-P levels within the range of 2.5-4.5mg/dl. Referring to the guidelines of the Adult Treatment Panel III (ATP III), the results of serum lipids were as follows: normal fasting triglyceride levels in the phosphorus group (<150 mg/dl) and borderline high in the placebo group (150-199 mg/dl), borderline high total cholesterol and LDL-C in both groups (200-239 mg/dl and 130-159 mg/dl, respectively), and normal HDL-C in both groups (40-60 mg/dl). In addition, mean levels of fasting plasma glucose, glucose after 2 hours of the OGTT, and HbA1C were normal in both groups (<100 mg/dl, <140 mg/dl and <5.7%, respectively) according to the cutoff points of the American Diabetes Association (ADA).

Table 3: Baseline Characteristics of the Study Population (n=49)

Variable	Placebo Group	Phosphorus Group	P-Value
N	21 (42.86)	28 (57.14)	
Age (years)	36.67±2.10	34.40±2.10	0.450
Sex			
Female	15 (71.43)	16 (57.14)	
Male	6 (28.57)	12 (42.86)	
Anthropometric variables			
Weight (kg)	92.33±3.27	87.73±3.51	0.342
Height (m)	1.65±0.02	1.67±0.02	0.603
BMI (kg/m^2)	33.73±0.84	31.40±0.86	0.060
WC (cm)	109.43±2.16	105.66±2.36	0.245
Serum parameters (mg/dl)			
Phosphorus T_0	4.11±0.15	4.35±0.10	0.193
Phosphorus T_{2hr}	3.41±0.12	3.83±0.10	0.010*
TG T_0	161.50±15.10	140.50±15.40	0.335
TG T_{2hr}	147.30±14.60	118.90±10.50	0.122
TC	221.85±9.09	215.89±7.94	0.624
HDL-C	46.30±2.46	42.37±2.26	0.245
LDL-C	143.29±6.81	145.71±6.44	0.797
CRP	9.82±1.23	9.80±0.98	0.986
Glucose T_0	95.30±2.40	101.40±6.20	0.357
Glucose T_{2hr}	117.20±10.00	119.40±13.00	0.892
HbA1C (%)	5.44±0.09	5.63±0.18	0.358
Insulin T_0 (µIU/mL)	11.41±1.74	7.50±1.47	0.093
Insulin T_{2hr} (µIU/mL)	54.74±6.50	38.48±5.69	0.067
GLP-1 T_0 (pM)	36.83±3.90	43.86±6.53	0.361
GLP-1 T_{2hr} (pM)	27.24±3.51	35.80±5.42	0.192
Urinary parameters (mg/dl)			
Calcium (Ca)	9.56±1.20	10.17±1.50	0.752
Creatinine (Cr)	177.90±20.30	206.00±23.20	0.367
Ca/Cr	0.07±0.02	0.06±0.01	0.385
Phosphorus (P)	79.99±9.29	88.38±9.94	0.540
P/Cr	0.47±0.04	0.48±0.03	0.817

NOTE: Values are expressed as mean±SEM or n(%). Abbreviations: BMI, body mass index; Ca: calcium; cm, centimeter; Cr: creatinine; CRP: C-reactive protein; GLP-1, Glucose-like peptide 1; HbA1C, glycated hemoglobin; HDL-C: high-density lipoprotein cholesterol; kg, kilogram; LDL-C: low-density lipoprotein cholesterol; m, meter; n, number of subjects; P: phosphorus; T_0: time of blood withdrawal at fasting; T_{2hrs}: time of blood withdrawal after 2 hours of OGTT; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group using two-sample t-test

B. Body Weight and Body Mass Index (BMI)

Body weight and BMI were measured at baseline and after 3 months of supplementation. At each period of time, results were compared between the two groups on one hand and within the same study group on the other hand. Table 4 shows the results of these variables.

Table 4: Body Weight and BMI at Baseline and After 3 months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
Weight (Kg)	Placebo	92.33±3.27	93.46±3.31	1.13±0.45	0.020ǂ
	Phosphorus	87.73±3.51	87.28±3.54	-0.44±0.53	0.408
	P-Value	0.342	0.208	0.028*	
BMI (Kg/m^2)	Placebo	33.73±0.84	34.14±0.85	0.42±0.18	0.028ǂ
	Phosphorus	31.40±0.86	31.24±0.88	-0.16±0.18	0.372
	P-Value	0.060	0.022*	0.026*	

NOTE: Values are expressed as mean±SEM. Abbreviations: BMI, body mass index; kg, kilogram; m, meter

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

At baseline, no significant difference was found between the body weights of the two study groups (p=0.342). Similarly, BMI was not significantly different either (p=0.060), and individuals were obese with a mean BMI of 33.73±0.84 kg/m^2 and 31.40±0.86 kg/m^2 in the placebo and phosphorus groups, respectively.

After 3 months of the intervention, comparing body weight as such showed no significant difference between the two groups (p=0.208), but variations occurred within each group as follows: body weight significantly increased from 92.33±3.27 kg to 93.46±3.31 kg (p=0.020) in the placebo group, and it decreased from 87.73±3.51 kg to 87.28±3.54 kg (p=0.408) in the phosphorus group. This change led to a significant difference, where body weight increased by 1.13±0.45 kg in the placebo group, whereas it decreased by 0.44±0.53 kg in the phosphorus group with p=0.028. Figure 6 and table 4 show this significant variation in body weight between groups.

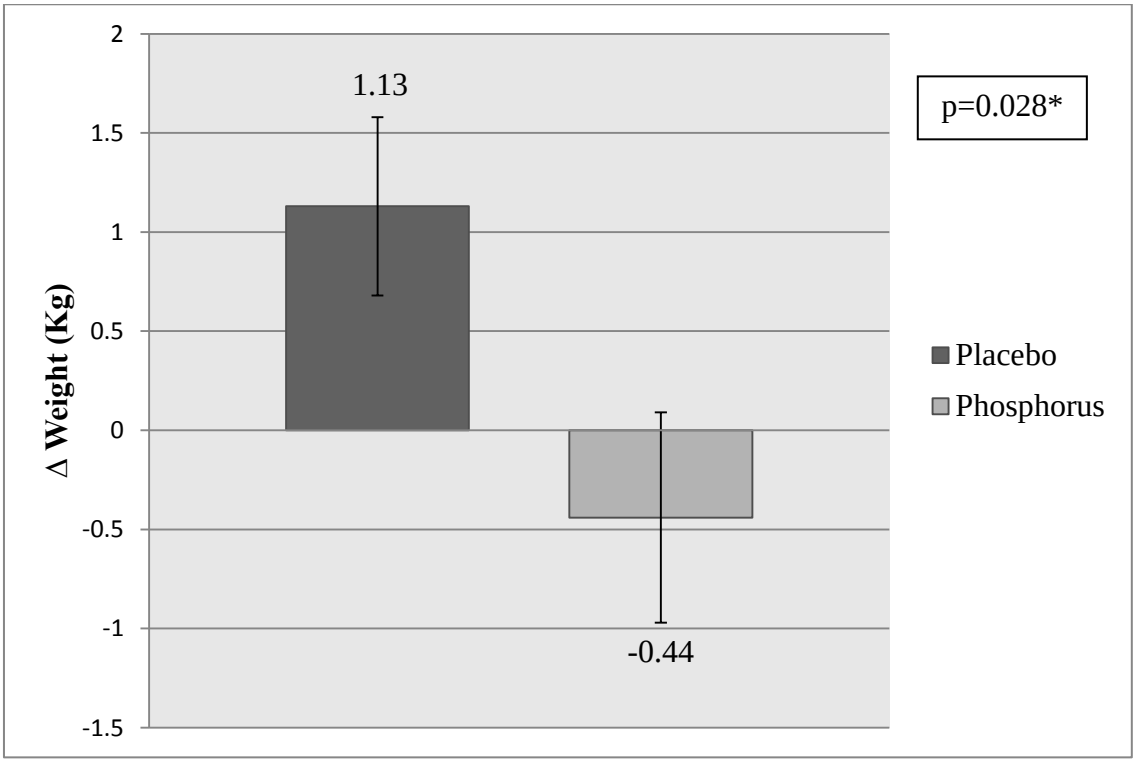

Figure 6: Comparing the Difference in Body Weight between the Placebo and the Phosphorus Groups after 3 Months of Supplementation
Abbreviations: Δ Weight, difference in body weight; kg, kilogram
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group

As for BMI, levels significantly varied after 3 months of supplementation (p=0.022), and comparing the increase of 0.42±0.18 kg/m^2 in the placebo group to the decrease of 0.16±0.18 kg/m^2 in the phosphorus group also showed significant results (p=0.026). Figure 7 and table 4 show this significant variation in BMI between groups.

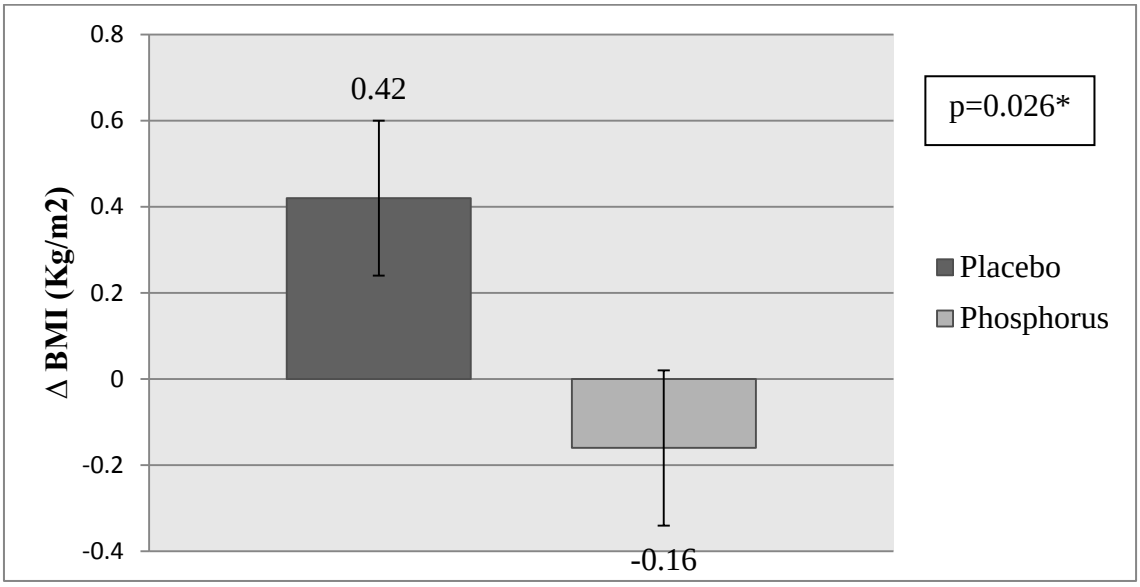

Figure 7: Comparing the Difference in Body Mass Index between the Placebo and the Phosphorus Groups after 3 Months of Supplementation
Abbreviations: Δ BMI, difference in body mass index; kg, kilogram; m, meter
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group

C. Waist Circumference (WC)

Waist circumference (WC) was measured at baseline and after 3 months of the randomly selected treatment, where values were compared either between groups or within the same study group as shown in table 5.

At baseline, no significant difference was present between the WC of the two groups ($p=0.245$). However, after 3 months of the intervention, WC significantly varied between the groups ($p=0.026$); this is explained by the increased of WC from 109.43±2.16 cm to 109.81±2.22 cm in the placebo treatment ($p=0.346$) versus the significant decrease from 105.66±2.36 cm to 102.18±2.46 cm ($p<0.001$) in the phosphorus treatment. The significant change in WC between the two groups was due an increase of 0.38±0.39 cm in the placebo group vs. a decrease of 3.48±0.60 cm in the phosphorus group with $p<0.001$; this data is also presented in figure 8.

Table 5: Waist Circumference at Baseline and after 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
WC (cm)	Placebo	109.43±2.16	109.81±2.22	0.38±0.39	0.346
	Phosphorus	105.66±2.36	102.18±2.46	-3.48±0.60	<0.001ǂ
	P-Value	0.245	0.026*	<0.001*	

NOTE: Values are expressed as mean±SEM. Abbreviations: cm, centimeter; WC, waist circumference
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test
ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

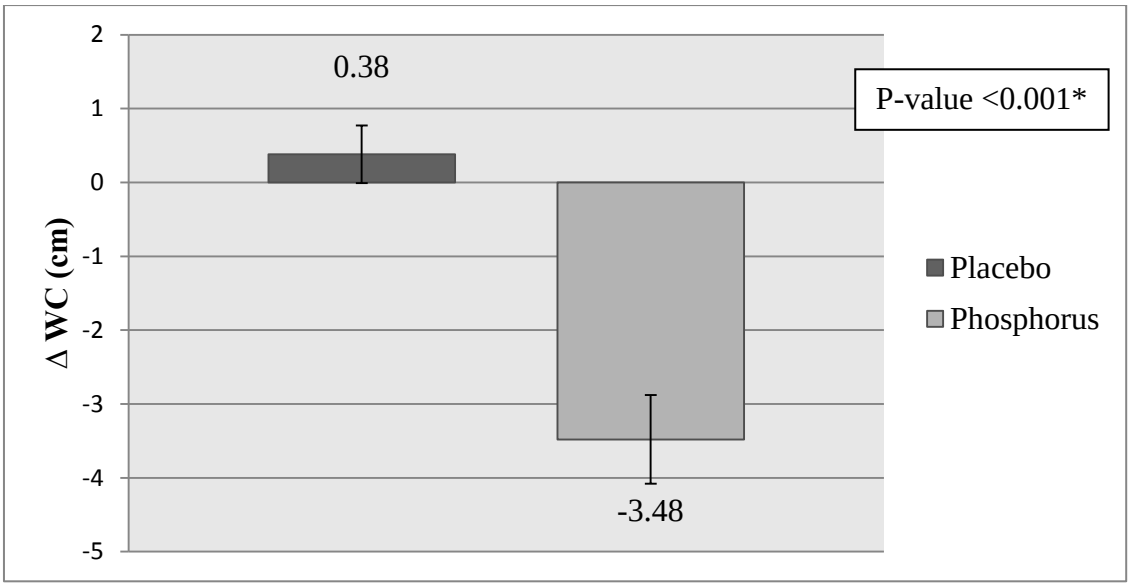

Figure 8: Comparing the Difference in Waist Circumference between the Placebo Group and the Phosphorus Group after 3 Months of Supplementation
Abbreviations: Δ WC, difference in waist circumference; cm, centimeter
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group

D. Serum Phosphorus (S-P)

S-P was measured at fasting levels (T_0) and after 2 hours of the OGTT (T_{2hr}), both at baseline and after 3 months of supplementation as shown in table 6.

S-P levels at T_0 were not significantly different between the two groups neither at baseline (p=0.193) nor after 3 months of the study (p=0.837), and concentrations in both groups were within the normal physiological range of 2.5-4.5mg/dl. In addition, when measuring variations within each group, no significant difference was shown either (p=0.428 and p=0.082, respectively). Similarly, there were no significant results when comparison their differences between groups (p=0.089).

Furthermore, serum levels after 2 hours of the OGTT (T_{2hr}) was significantly different between the two groups at the beginning of the study, but not after 3 months of supplementation (p=0.010 and p=0.445, respectively). When comparing results within each group separately, variations were significant in the placebo group (p=0.016) but not in the phosphorus group (p=0.237). This variation was significant when assessing the differences between the two groups at the end of the study (p=0.017).

Table 6: Serum Phosphorus Levels (Fasting and After 2 Hours of OGTT) at Baseline and after 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
Serum P T_0 (mg/dl)	Placebo	4.11±0.15	4.22±0.13	0.11±0.14	0.428
	Phosphorus	4.35±0.10	4.16±0.10	-0.19±0.10	0.082
	P-Value	0.193	0.837	0.089	
Serum P T_{2hr} (mg/dl)	Placebo	3.45±0.12	3.61±0.12	0.16±0.06	0.016‡
	Phosphorus	3.83±0.10	3.72±0.07	-0.11±0.09	0.237
	P-Value	0.010*	0.445	0.017*	

NOTE: Values are expressed as mean±SEM. Abbreviations: dl, deciliter; mg, milligram; P, phosphorus; T_0: time of blood withdrawal at fasting; T_{2hrs}: time of blood withdrawal after 2 hours of OGTT

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

‡ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

E. Serum Triglyceride (TG)

Serum triglyceride (TG) was measured at fasting levels (T_0) and after 2 hours of the OGTT (T_{2hr}), both at baseline and after 3 months of supplementation (table 7).

Fasting levels were not significantly different at both periods of time (p=0.335 and p=0.855, respectively). The change in levels after 3 months of the study was also insignificant, either among each group alone (p=0.063 for the placebo group and p=0.911 for the phosphorus group) or when comparing between the two groups (p=0.157).

In addition, serum TG measured at T_{2hr} showed no significant difference between the groups, neither at baseline (p=0.122) nor after 3 months (p=0.761). When comparing the differences, results were also insignificant within each group alone (p=0.063 and p=0.513 for the placebo and phosphorus groups, respectively) or between groups (p=0.052).

Additionally, when assessing the difference between TG levels after 2 hours of the OGTT (T_{2hr}- T_0**)**, no significant results were shown (p=0.605 and p=0.898, respectively).

Table 7: Serum Triglyceride Levels (Fasting and After 2 Hours of OGTT) at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
Serum TG T_0 (mg/dl)	Placebo	161.50±15.10	141.30±10.80	-20.30±10.30	0.063
	Phosphorus	140.50±15.40	139.50±14.30	-0.96±8.58	0.911
	P-Value	0.335	0.855	0.157	
Serum TG T_{2hr} (mg/dl)	Placebo	147.30±14.60	127.80±10.10	-19.60±9.94	0.063
	Phosphorus	118.90±10.50	123.20±11.00	4.25±6.41	0.513
	P-Value	0.122	0.761	0.052	
Serum TG Difference (T_{2hr}- T_0)	Placebo	-14.15±6.24	-13.50±4.27		
	Phosphorus	-19.70±8.66	-14.79±8.97		
	P-Value	0.605	0.898		

NOTE: Values are expressed as mean±SEM. Abbreviations: dl, deciliter; mg, milligram; T_0: time of blood withdrawal at fasting; T_{2hrs}: time of blood withdrawal after 2 hours of OGTT; TG, triglyceride

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

F. Total Serum Cholesterol

Total serum cholesterol (TC) was measured at baseline and after 3 months of the intervention, and results are presented in table 8.

Serum cholesterol levels were borderline high in both study groups, and no significant difference was observed neither at baseline (p=0.624) nor after 3 months (p=0.725). Similarly, when studying the variation of this variable within each group, there was no statistical significance (p=0.861 in the placebo group and p=0.749 in the phosphorus group). Similar results were also seen when comparing the differences in levels between the two groups (p=0.725).

Table 8: Total Serum Cholesterol at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	**Baseline**	**After 3 months of supplementation**	**Difference (after – before)**	**P-Value**
Total Serum Cholesterol (mg/dl)	Placebo	221.85±9.09	220.85±9.75	-1.00±5.65	0.861
	Phosphorus	215.89±7.94	217.70±7.25	1.81±5.60	0.749
	P-Value	0.624	0.725	0.725	

NOTE: Values are expressed as mean±SEM. Abbreviations: dl, deciliter; mg, milligram
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test
ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

G. Serum HDL-C

Serum HDL-C levels at baseline and after 3 months of the allocated intervention are compared in table 9.

There was no significant difference between the groups neither at baseline (p=0.245) nor after 3 months of supplementation (p=0.145), and variations showed no statistical significance throughout the study when considering each group separately (p=0.439 in the placebo group and p=0.963 in the phosphorus group). Additionally, when comparing the differences between the two groups, data was not statistically significant (p=0.612).

Table 9: Serum HDL-C at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
Serum HDL-C (mg/dl)	Placebo	46.30±2.46	47.25±2.68	0.95±1.20	0.439
	Phosphorus	42.37±2.26	42.30±2.15	-0.07±1.60	0.963
	P-Value	0.245	0.145	0.612	

NOTE: Values are expressed as mean±SEM. Abbreviations: dl, deciliter; HDL-C, high-density lipoprotein cholesterol; mg, milligram

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

H. Serum LDL-C

Serum LDL-C levels at baseline and after 3 months of the placebo and phosphorus treatments are presented in table 10.

No significant difference was shown among the two study groups at both phases, baseline and after 3 months of supplementation (p=0.797 and p=0.900, respectively); even their differences were not statistically significant (p=0.952). Likewise, when considering variations within each group, results were not significant either (p=0.695 in the placebo group and p=0.680 in the phosphorus group).

Table 10: Serum LDL-C at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
Serum LDL-C (mg/dl)	Placebo	143.29±6.81	145.66±7.94	2.37±5.96	0.695
	Phosphorus	145.71±6.44	147.63±6.17	1.92±4.59	0.680
	P-Value	0.797	0.900	0.952	

NOTE: Values are expressed as mean±SEM. Abbreviations: dl, deciliter; LDL-C, low-density lipoprotein cholesterol; mg, milligram

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

I. CRP

The comparison of CRP levels in the placebo and phosphorus groups is shown in table 11; results were measured at baseline and after 3 months of supplementation.

No significant difference was shown when comparing CRP levels between the two groups, neither at baseline nor after 3 months (p=0.986, p=0.455, respectively); that was also the case when comparing their differences (p=0.327). Additionally, when observing each group separately, no significant variations were found, where p=0.185 for the placebo group and p=0.983 for the phosphorus group.

Table 11: Serum CRP at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	Difference (after – before)	P-Value
CRP (mg/dl)	Placebo	9.82±1.23	11.07±1.47	1.25±0.91	0.185
	Phosphorus	9.80±0.98	9.81±1.04	0.02±0.85	0.983
	P-Value	0.986	0.455	0.327	

NOTE: Values are expressed as mean±SEM. Abbreviations: CRP, C-reactive protein; dl, deciliter; mg, milligram

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

J. Serum GLP-1

Serum GLP-1 was measured at fasting levels and after 2 hours of the OGTT, where samples were withdrawn at baseline and after 3 months of supplementation. No significant differences were shown (table 12).

Table 12: Serum GLP-1 Levels (Fasting and after 2 Hours of OGTT) at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation
GLP-1 T_0 (pM)	Placebo	36.83±3.90	37.29±4.05
	Phosphorus	43.86±6.53	37.72±3.14
	P-Value	0.361	0.934
GLP-1 T_{2hr} (pM)	Placebo	27.24±3.51	26.76±2.85
	Phosphorus	35.80± 5.42	28.76±2.80
	P-Value	0.192	0.621
GLP-1 Difference (T_{2hr}- T_0)	Placebo	-9.60±2.61	-10.53±3.76
	Phosphorus	-8.06±3.36	-8.96±2.81
	P-Value	0.719	0.741

NOTE: Values are expressed as mean±SEM. Abbreviations: dl, deciliter; mg, milligram; P, phosphorus; T_0: time of blood withdrawal at fasting; T_{2hrs}: time of blood withdrawal after 2 hours of OGTT

* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test

ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

K. Urinary Parameters

The following urinary parameters were measured: calcium, creatinine, phosphorus, calcium to creatinine ratio (Ca/Cr,), and phosphorus to creatinine ratio (P/Cr). No significant differences were observed between the two groups when assessing parameters at baseline or after 3 months of the study; similarly, variations within each group were also not significant (table 13).

Table 13: Urinary Parameters at Baseline and After 3 Months of the Placebo and Phosphorus Treatments

	Group	Baseline	After 3 months of supplementation	P-Value
Ca (mg/dl)	Placebo	9.56±1.20	13.44±2.38	0.166
	Phosphorus	10.17±1.50	9.31±1.44	0.471
	P-Value	0.752	0.147	
Cr (mg/dl)	Placebo	177.90±20.30	187.60±17.20	0.709
	Phosphorus	206.00±23.20	173.70±15.30	0.095
	P-Value	0.367	0.550	
Ca/Cr	Placebo	0.07±0.02	0.09±0.02	0.561
	Phosphorus	0.06±0.01	0.05±0.01	0.817
	P-Value	0.385	0.111	
P (mg/dl)	Placebo	79.99±9.29	76.93±8.09	0.819
	Phosphorus	88.38±9.94	85.67±8.29	0.792
	P-Value	0.540	0.454	
P/Cr	Placebo	0.47±0.04	0.43±0.04	0.424
	Phosphorus	0.48±0.03	0.54±0.04	0.238
	P-Value	0.817	0.069	

NOTE: Values are expressed as mean±SEM. Abbreviations: Ca, calcium; Cr, creatinine; P, phosphorus
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at each time interval using two-sample t-test
ǂ p value <0.05, values are significantly different between baseline and after treatment time intervals within the same study group using paired t-tests

L. Appetite Questionnaire

The appetite questionnaire was used at three phases of the study as follows: at baseline (week 0), half-way (week 6), and after three 3 months (week 12). The appetite scores were ranked from 1 till 5, with the latter being the highest (table14).

Table 14: The Variables of the Appetite Questionnaire (Based on Wilson *et al.*, 2005)

Variable		**Score**
Appetite	**My appetite is**	
	Very poor	a=1
	Poor	b=2
	Average	c=3
	Good	d=4
	Very good	e=5
Quantity of food to reach fullness	**When I eat**	
	I feel full after eating only a few mouthfuls	a=1
	I feel full after eating about a third of a meal	b=2
	I feel full after eating over half a meal	c=3
	I feel full after eating most of the meal	d=4
	I hardly ever feel full	e=5
Hunger	**I feel hungry**	
	Rarely	a=1
	Occasionally	b=2
	Some of the time	c=3
	Most of the time	d=4
	All of the time	e=5
Taste of food	**Food tastes**	
	Very bad	a=1
	Bad	b=2
	Average	c=3
	Good	d=4
	Very good	e=5
Number of main meals	**Normally I eat**	
	Less than one meal a day	a=1
	One meal a day	b=2
	Two meals a day	c=3
	Three meals a day	d=4
	More than three meals a day	e=5
Number of snacks	**How often do you snack?**	
	Less than once a day	a=1
	Once a day	b=2
	Twice a day	c=3
	Three times a day	d=4
	More than three times a day	e=5

Table 15 presents the assessment of appetite scores. On one hand, the results within each group were compared throughout the study. On the other hand, data were compared between the two study groups at week 0, week 6, and week 12 separately.

Table 15: Appetite Scores of the Placebo and Phosphorus Groups at Baseline, after 6 Weeks, and after 12 Weeks

	Group	Week 0 (w_0)	Week 6 (w_6)	Week 12 (w_{12})	P-Value
Appetite	Placebo	3.81±0.81	3.48±1.03	3.57±0.75	0.448
	Phosphorus	4.25±0.89	3.25±1.14	3.46±1.17	0.002ǂ
	P-Value	0.098	0.425	0.762	
Quantity of food to reach fullness	Placebo	3.76±0.83	3.19±0.93	3.43±1.03	0.146
	Phosphorus	3.96±0.64	3.07±0.94	3.14±1.04	<0.001ǂ
	P-Value	0.525	0.657	0.369	
Hunger	Placebo	3.43±0.87	2.81±1.17	3.10±1.04	0.161
	Phosphorus	2.96±0.92	2.29±0.90	2.29±0.98	0.009ǂ
	P-Value	0.063	0.098	0.012*	
Taste of food	Placebo	3.76±0.94	3.76±0.89	3.71±0.90	0.981
	Phosphorus	4.18±0.86	3.82±0.91	3.82±0.82	0.208
	P-Value	0.149	0.770	0.606	
Number of main meals	Placebo	3.76±0.54	3.91±0.44	3.95±0.50	0.433
	Phosphorus	3.54±1.00	3.61±0.63	3.57±0.57	0.940
	P-Value	0.518	0.189	0.063	
Number of snacks	Placebo	2.71±1.19	2.52±0.75	2.62±0.87	0.811
	Phosphorus	3.21±1.45	2.46±1.35	2.46±1.35	0.070#
	P-Value	0.233	0.499	0.402	

NOTE: Values are expressed as mean±SD.
* p value <0.05, values are significantly different for the placebo group versus the phosphorus group at a specific period of time using Kruskal-Wallis test
ǂ p value <0.05, values are significantly different between the different periods of time within the same study group using one-way ANOVA
value is statistically significant within the group using Fisher comparison

Data variation within the phosphorus group (table 15 and figure 9) showed significant difference throughout the study when assessing the level of appetite ($p=0.002$), quantity of food to reach fullness ($p<0.001$), and hunger ($p=0.009$); results decreased significantly between week 0 on one hand and weeks 6 and 12 on the other hand. Concerning the number of snacks, ANOVA failed to detect significance; however,

the subgroup analysis using Fisher method showed significant difference between week 0 as compared to weeks 6 and 12. Additionally, the taste of food and the number of main meals did not significantly differ throughout the weeks. As for the placebo group, none of the variables showed significant variation.

When comparing the results of each variable between groups, data showed no significant difference except for hunger which was significantly lower in the phosphorus group as opposed to the placebo group, when measured at week 12 of participation (p=0.012; table 15).

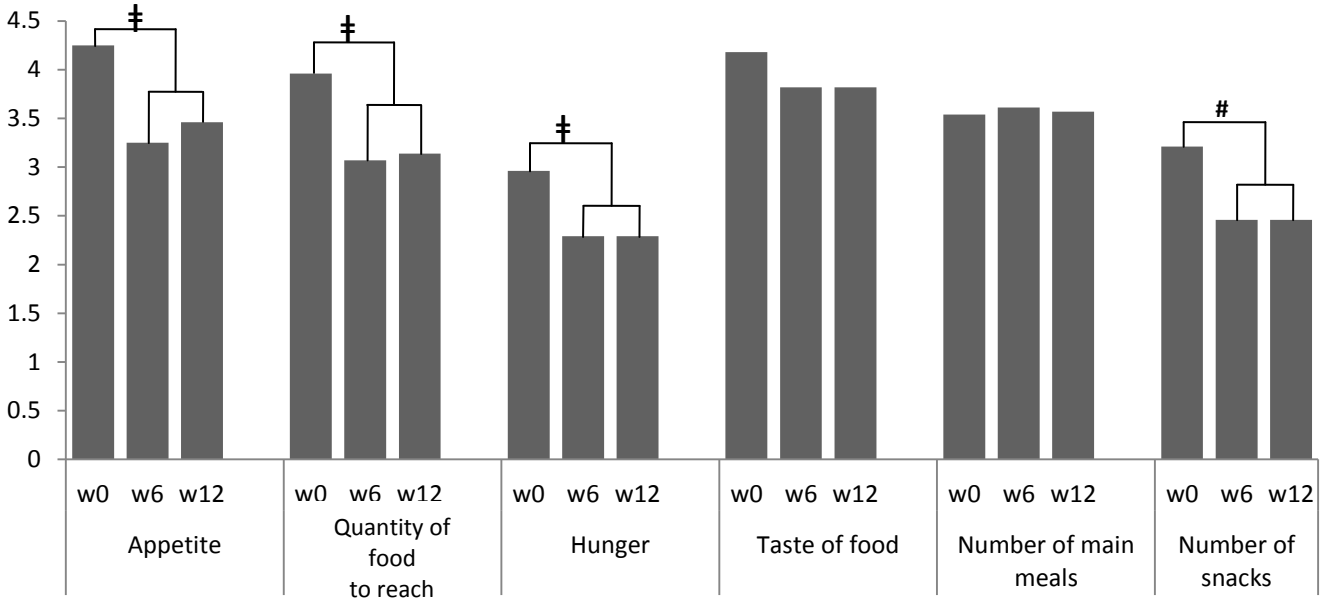

Figure 9: Comparing the Variation in Appetite Scores of Subjects in the Phosphorus Group

Abbreviations: w0, week 0 (baseline); w6, week 6 (half-way through the study); w12, week 12 (at the end of the study)

‡ p value <0.05, values are significantly different for that particular question within the phosphorus group

statistically significant via Fisher comparison

CHAPTER V

DISCUSSION

The present study investigated the medium-term effect of premeal phosphorus ingestion on some factors of the MetS including body weight, BMI, WC, serum lipids and other blood parameters, in addition to subjective appetite scores in overweight/obese individuals. Our objective is based on the fact that phosphorus enhances the status of ATP in the body, in particular hepatic ATP which has been shown to be an important appetite regulator (Obeid *et al.*, 2010). Additionally, phosphorus is known to increase insulin sensitivity, where the ingestion of 500 mg of this mineral significantly affects insulin after 60 minutes of intake (Khattab *et al.*, 2011). The biological mechanisms of such associations are not clearly understood, and data is controversial especially that related to the effect of phosphorus on serum lipids.

In the present study, subjects were asked to ingest 375 mg of phosphorus with each main meal (breakfast, lunch, and dinner) for a period of 3 months. This intervention showed that phosphorus can significantly improve body weight, BMI, and WC when compared to placebo, in addition to significantly decreasing appetite scores that were reported by the participants in the phosphorus group. Moreover, this study showed that a dose of 375 mg three times a day has no effect on serum lipid parameters of individuals with mild dylipidemia.

Our data supports studies that have correlated phosphorus to decreased body weight and visceral fat, particularly via the effect of phosphorus on food intake. For instance, in the postprandial state, insulin production increases, which triggers the transfer of serum phosphorus (S-P) into the intracellular space to be utilized for the

phosphorylation of several metabolites. This shift can lead to a rapid decrease in S-P levels (Kalaitzidis *et al.,* 2005), hence decreasing the availability of phosphorus for hepatic ATP production (Obeid, 2013). However, the ingestion of premeal phosphorus supplements provides a surplus of this mineral, thus improving its status in the liver and increasing hepatic ATP production; this would consequently lead to earlier satiation and decreased food intake (Friedman, 2007; Nair *et al.,* 2003; Wlodek and Gonzales, 2003). These findings are also supported by a report that documented a significant decrease in meal size after the administration of 500 mg of phosphorus to different carbohydrate preloads (Obeid *et al.,* 2010).

In addition, the effect of phosphorus on energy expenditure might be a suggested mechanism underlying the association between phosphorus and reduced body weight, BMI, and WC observed in the current study. A review paper done by Obeid (2013) explains the positive correlation of phosphorus with diet-induced thermogenesis and oxidative reactions, thereby enhancing energy expenditure. Our results are also consistent with previous studies that have examined the effects of a high protein diet on weight loss and have associated it with decreased energy intake and higher energy expenditure (Pioli *et al.,* 2013). In addition, the increased consumption of dairy products among overweight individuals has revealed significantly lower risks of MetS (Pereira *et al.,* 2002). In fact, it is important to note that diets high in proteins or dairy products are also rich sources of phosphorus (Takeda *et al*., 2012); therefore, further supporting our findings.

Moreover, another underlying mechanism could be related to the effect of phosphorus on insulin response in the body, where the ingestion of this mineral was shown to improve insulin sensitivity (Khattab *et al.,* 2011). This, in turn, would help

stimulate protein synthesis and the subsequent increase in lean body mass (Wilcox, 2005). Therefore, resting energy metabolism and energy expenditure would be enhanced (Byrne *et al.*, 2003), aiding in decreasing visceral fat or WC measures (Lind *et al*., 1993).

Furthermore, insulin is also involved in lipid metabolism; therefore, phosphorus ingestion could affect serum lipid parameters through the improvement of insulin sensitivity (Hazim *et al*., 2014). Nevertheless, findings regarding this relationship are controversial. For example, Lippi *et al*. (2009) positively associated phosphorus with decreased levels of total cholesterol, LDL-C, TG, and the ratio of total cholesterol to HDL-C; whereas Park *et al.* (2009) correlated phosphorus with increased total cholesterol, HDL-C, and lipoprotein (a) but decreased TG levels. Additionally, Fanhanji *et al.* (2011) showed a significant positive relation between phosphate and TG; Hazim *et al.* (2014) documented a reduction in ApoB100 and an increase in ApoB48, which are characteristic components of VLDL and CM respectively. As for the results of the current study, there was no correlation between the ingestion of 375 mg of phosphorus three times a day over a period of 3 months and the studied serum lipid variables (i.e. total cholesterol, LDL-C, HDL-C, and TG).

In fact, the controversial results documented by the different studies might be due to the variation in the level of phosphorus being assessed. Therefore, it may be proposed that phosphorus and lipid parameters could be interrelated via a dose-dependent process. Moreover, high phosphorus levels were reported to enhance risks of all-causes of cardiovascular death (Caudarella *et al*., 2007; Park *et al.*, 2009); for instance, hyperphosphatemia was shown to be associated with endothelial dysfunction by decreasing flow-mediated dilatation (Shuto *et al.*, 2009) and enhancing vascular

calcification (Lippi *et al.*, 2009; Park *et al*., 2009). On the other hand, low phosphate status was positively associated with MetS which is a known risk factor for CVD (Haglin, 2001; Park *et al.*, 2009) probably via decreased insulin sensitivity. The differences in results might also be due to the variation in lipid status or general health of the study participants. For instance, subjects in the current study are mildly dyslipidemic, which might explain the absence of significant variation in their lipid parameters. In addition, even though the physiological mechanisms behind phosphate and TG associations are not fully understood, Park *et al.* (2009) explained that S-P can affect phospholipids in the liver, especially in cases of hyperglycemia where the production of TG is increased. However, the correlation between blood glucose and serum TG is stronger in diabetic patients (Haglin, 2001), which is not the case of our study participants who have normal HbA1C. Furthermore, the duration of the intervention was relatively short, which is another possible reason behind the absence of significant variation in serum lipid parameters.

As for the appetite scores, subjects of the phosphorus group reported a significant decrease in the variables related to appetite, quantity of food to reach fullness, hunger, and number of snacks in weeks 6 and 12 as compared to baseline. No variation in the taste of food or in the number of main meals was shown. Therefore, a dosage of 375 mg of phosphorus taken three times a day with the meals is capable of significantly reducing the previously mentioned variables. In fact, phosphate absorption peaks at around one hour after ingestion of a meal (Anderson, 1998) which could also explain the association between phosphorus supplementation and the decrease in subsequent food intake reported in the appetite scores. These results are in line with the

observed significant decrease in body weight, BMI, and WC of the subjects in the phosphorus group as compared to the placebo.

However, the measurement of serum GLP-1 showed no significant difference, although appetite scores were significantly reduced. A possible explanation could be the small sample size. Another probable reason could be the timing at which serum GLP-1 was measured; for instance, in the current study, blood samples were withdrawn at 120 minutes postprandially, which could have missed the peak of GLP-1 that was observed at about 15 to 30 minutes (Hassan *et al.,* 2013).

As for CRP, some data has shown its inverse correlation with phosphorus (Park *et al*., 2009). Previous studies have also correlated CRP levels with BMI, presenting a link between inflammation and obesity (Choi *et al.,* 2013; Visser *et al.,* 1999). For instance, weight loss was shown to be associated with a significant decrease in CRP levels in obese postmenopausal women (Tchernof *et al.,* 2002) and in patients who had undergone gastric bypass surgeries (Zaqorski *et al*., 2005). However, the BMI level of the subjects in those studies was much higher than that of the participants in the current study (who happen to be at the lower range of class I obesity). Therefore, this might be an explanation to the absence of significant difference in CRP levels.

Another variable of interest is the fasting level of S-P, where no significant variation was observed when comparing the subjects ingesting phosphorus supplements to those having the placebo. This observation could be used as added evidence to previous studies that have shown S-P levels to be reflective of kidney function (Hsu and Chertow, 2002) rather than to dietary phosphorus intake. To elaborate, studies have shown that excess dietary phosphorus ingestion leads to more phosphorus excretion in the urine without significantly influencing S-P concentration; this is due to the presence

of regulatory hormones that keep a specific S-P level regardless of dietary intake (Palomino *et al*,. 2013). In addition, concerning the level of fasting S-P at baseline, individuals in both groups had normal physiological concentrations; this is another explanation to the lack of significant increase in S-P concentration when comparing the phosphorus group to the placebo. On the other hand, S-P after 2 hours of the OGTT was shown to be significantly lower in the phosphorus group as compared to the placebo; a suggested mechanism could be an improvement in insulin sensitivity where insulin stimulates the uptake of phosphorus into the intracellular membrane (Kalaitzidis *et al*., 2005).

The main strength of this study is its design being a randomized controlled trial, where it provides strong evidence by allowing the measurement of the impact or effect of our intervention on the outcomes studied. Thus, our study provides conclusions on causality. In addition, randomization helps avoid the pitfalls of selection bias and minimize the risks of bias by confounding; our groups were similar in all aspects except their exposure to the type of intervention they had chosen. Furthermore, double blinding helps minimize possible bias occurring in the responses of participants and in outcome measurements.

Some potential limitations of this study should be taken into account. First is the small sample size. Another limitation is the absence of analyzing body composition of the subjects. Furthermore, the participants' dietary intake, quantity and content, was not accurately monitored. For instance, supplements could have been ingested with a meal rich in proteins, which already contains high phosphorus levels; the tablets could have also been ingested with carbohydrate rich meals, which happen to be relatively low in phosphorus and high in glucose. Moreover, it would have been interesting to measure

the concentration of parathyroid hormone (PTH) which is one of the chief regulators of phosphate balance (Bergwitz and Jüppner, 2010) that also has the ability to lower the activity of lipoprotein lipase involved in lipid metabolism (Querfeld *et al*., 1999). Likewise, we could have measured the level of fibroblast growth factor-23 (FGF-23), which has been recently identified in the control of phosphorus homeostasis (Penindo and Alon, 2012). The assessment of vitamin D and calcitriol, which are strongly connected to phosphate metabolism, could have been performed as well (Boullion, 2006; Penindo and Alon, 2012).

Additionally, our subjects have normal HbA1C, mild dyslipidemia, and no severe obesity; thus, future research including diabetic or severely obese participants could be applied.

CHAPTER VI

CONCLUSION AND RECOMMEDATIONS

The literature has shown the importance of maintaining an appropriate phosphate balance in the body. On one hand, the deficiency of this mineral has been associated with the progression of the metabolic syndrome (MetS) (DeFronzo and Lang, 1980; Haglin, 2001; Kalaitzidis *et al.*, 2005; Gudmundsdottir *et al.*, 2008; Park *et al.*, 2009). On the other hand, an elevated S-P level has been correlated with increased risks of CVD, although there is limited data examining such correlation in generally healthy individuals (Park *et al.*, 2009).

Our results have shown that the ingestion of 375 mg of phosphorus supplements, taken three times per day with each main meal over a period of 3 months, significantly decreases weight, BMI, and WC which are components of the MetS. In addition, subjective appetite scores were reported to be significantly lower among individuals in the phosphorus group. These results are consistent with the findings of previous studies that have shown a positive association between phosphorus and decreased risks of MetS.

Additionally, our intervention showed no significant effect on serum lipid parameters such as total cholesterol, LDL-C, HDL-C, and TG. The literature has reported controversial findings regarding this issue; thus, the difference in results among studies might be due to the presence of a dose-dependent relation between phosphorus and serum lipids.

It is important to note that our findings support a promising role of the mineral phosphorus in treating obesity, especially abdominal adiposity. Therefore, after

extensive investigations, phosphorus utilization could be considered for the future development of weight reduction supplements or implementing fortification of flour. Additional research is warranted to examine the exact mechanisms of actions and longer term effects of phosphorus, particularly in diabetic individuals.

APPENDIX

A. CONSENT FORM (ENGLISH)

SBS NUTRITION CONSENT
AUB

Title of Research Study: Premeal phosphorus supplementation for reducing energy intake and body weight.

Title of experiment: Determining the medium-term (3-month) effect of phosphorus preload on body weight, subjective satiety and hormonal status in overweight/obese individuals (placebo-controlled trial).

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut]
Co-Investigator: Sami Azar, Sani Hlais, Maya Bassil
Researchers: Murielle Abou-Samra, Darine Shatila
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 or the Central research unit (CRU). American University of Beirut Medical Center.

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 Study**: Phosphorus is a mineral that is naturally present in our foods and is required by our body for normal function. It has been found that phosphorus supplementation taken before meals has the potential to reduce meal size. However its long term effect has not been measured yet. It is well accepted that changes in body weight require about 3 months. Using body weight as the outcome, which is the ultimate outcome of weight loss approaches, would provide robust information on the role of phosphorus. 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. The significant new finding of this study will be conveyed to subjects.

B. **Project/Procedures Description:** Subjects' recruitment will be done either by posters or direct approaching. This is a double-blind, randomized, placebo-controlled study. Overweight and obese subjects (18-45 years; BMI $\geq$ 25 kg/m^2) will be randomized to receive either placebo (cellulose) or potassium phosphate tablets (375mg) with each main meal (breakfast, lunch, and dinner) for a period of 3 months. A total of 75 subjects will be needed in each group to complete the study.
In this study, (Screening Visit) you will first be screened for eligibility. Exclusion criteria include: glomerular filtration rate < 60 or any significant medical diseases; pregnancy or lactation; regular use of medication that affects body weight; a weight loss of 3% or more in the preceding 3 months.
Visit 1: Eligible subjects will be asked to fast for about 12 hour (overnight), before attending the testing facility [Faculty of Agriculture and Food Sciences/Department of Nutrition or the Central research unit (CRU)/ American University Hospital]. Blood and urine samples will be taken in the fasted state and subjects will be given 75g of glucose to drink and blood sample will be collected 1 and 2 hours later (OGTT). At the same time, anthropometric assessment (age, weight, height, and waist and hip circumference), body composition (Using Inbody), blood pressure will be performed. In addition, you will be asked to fill several forms: a seven-day food and physical activity record forms, hunger score form. Subjects taking any nutritional supplements will be asked to ingest with or after the meal to avoid interaction with the phosphorus supplement.

You will be given a supply of 6 weeks of the allocated supplement (phosphorus or placebo) and asked to take 3 tablets with each meal (breakfast, lunch and dinner). You will be asked to maintain your 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 each visit.

Visit 2: you will be asked to visit the testing site (as above), anthropometric measurement and urine sample will be taken. You will be asked to fill out questionnaires related to your adherence to the tablet regimen, general health, and self-reports of mood, stress, physical activity and appetite score
You will be given the remaining supply of the supplement.

Visit 3 (final visit). Same as in visit 1 in which OGTT will be performed. You will be asked to fill out questionnaires related to your adherence to the tablet regimen, general health, and self-reports of mood, stress, physical activity and appetite score

C. Duration: The estimated time to complete this study is approximately three months. You will have to visit the testing facility 4 times (Screening, visit 1, 2 and 3). Each visit will require you to stay for a period of 3 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. You will rest comfortably in a chair and an intravenous needle will be placed in a vein of your forearm by a qualified nurse. There are no risks involved in this procedure. Blood samples (10ml) will be taken in fasting, and after 1 and 2 hours of having 75g of glucose, and urine samples will be taken at baseline and at end of the experiment. Fasting blood glycated hemoglobin (HbA1c) will be determined. Plasma samples will be analyzed for lipid profile, glucose, insulin, appetite hormones etc will be analyzed at baseline and at 3 months. Urine samples will be analyzed for creatinine, calcium, phosphorus, magnesium, and alpha helical peptide (a bone resorption marker).

The foreseeable risks and discomforts associated with the study are as follows:

- The following measures will be taken:
- Before we start, the procedure and measurement techniques will be reviewed with you to ensure that you are comfortable with the protocol.
- All tests will be carried as per the standard clinical procedures.
- The skin area will first be sterilized with alcohol. A qualified nurse will place the needle, ensure its correct operation and collect the blood samples. All blood samples will be taken with sterilized instruments. Once the needle is removed, the wound is cleaned with alcohol. A sterile bandage is then applied. You will be asked to report any unusual discomfort or discoloration of the skin.

The procedure followed in the study will not cause any major risk other than discomfort from the needle prick for blood withdrawal as mentioned in the procedures above. However, there may be unforeseen risks. We have conducted several experiments using the same dose of Phosphorus and received no complaints of adverse effects or discomfort.
You receive no direct benefits from participating in this research; the primary outcomes expected are changes in body weight and body fat mass (at 3 months). 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.
You will get specific nutritional advices at the end of the study.

E. **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.

F. Compensation/Incentive: No costs have to be paid by you. All participants will receive free water and meal for lunch. There will neither be anticipated expenses for participating nor will additional costs for transportation, parking etc that be reimbursed.

G. 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.

H. **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:
Social & Behavioral Sciences Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5440 or 5445

I. 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?

J. 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 (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

_____ _____
Printed name of subject Signature of participant

_____ AM/PM
Date and time

Investigator/Research Staff
I have explained the research to the parent or legal guardian of the child subject/participant before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the parent/legal guardian of the child participant/subject.

_____ _____
Printed name of person obtaining permission Signature of person obtaining permission

Date and time _____ AM/PM

Institutional Review Board
American University of Beirut
2 9 APR 2013
APPROVED

APPENDIX

B. CONSENT FORM (ARABIC)

Institutional Review Board
American University of Beirut

13 MAY 2013

RECEIVED

موافقة للإشتراك في البحث العلمي

عنوان البحث: تأثير التناول المسبق لمكملات الفسفور على الحد من كمية التناول اللاحق للطاقة عند البالغين و على الوزن.
عنوان الاختبار: تحديد تأثير المدة المتوسطة (3 أشهر) على التناول المسبق لمكملات الفسفور على الوزن, الشعور الشخصي بالشبع و مستوى الهرمون عند الاشخاص الذين يعانون من زيادة الوزن أو سمنة . (دراسة مراقبة-وهمية)
إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.
الباحثين المساعدين: سامي عازار, ساني حليس, مايا باسيل.
منسقي البحث: موريل أبو سمرا, دارين شاتيلا.
العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان 01-350000
مكان إجراء البحث: الجامعة الأميركية في بيروت /قسم التغذية أو مركز البحوث\ أو وحدة البحوث المركزية (CRU) / مستشفى الجامعة الأميركية/ المركز الطبي.

أنت مدعو(ة) للمشاركة ببحث علمي سيجرى في الجامعة الأميريكية في بيروت. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. هذا البيان يصف الهدف، والإجراءات، والفوائد، والمخاطر، والمضايقات، والاحتياطات في هذه الدراسة. بالاضافة الى وصف الطرق البديلة اذا توافرت لديك. وكذلك حقك في الانسحاب من الدراسة في أي وقت. يجب أن لا تتردد في طرح أي أسئلة قد تكون لديكم.

أ) هدف هذا البحث: الفوسفور هو من المعادن التي تتواجد بشكل طبيعي في الأطعمة و هومطلوب من قبل الجسم لأداء وظيفته العادية. وقد وجد أن مكملات الفوسفوراذا أخذت قبل الوجبات لديه القدرة على تقليل حجم الوجبة. لكن تأثيرها على المدى البعيد لم يحدد بعد. أي تغيير يحصل للوزن يحتاج الى 3 اشهر على الاقل و هو أمر متفق عليه. وباستخدام وزن الجسم كنتيجة، حيث تكون النتيجة النهائية هي فقدان الوزن وبالتالي يمكن تقديم معلومات قوية حول دور الفوسفور. إن هدف البحث أطروحة وستنشر في صحيفة طبية ومن الممكن تقديمها في المؤتمرات الأكاديمية. سيتم الاخبار عن نتائج هذا البحث في نهاية الدراسة

ب) وصف الإجراءات والمشروع: ستتم عملية إختيار المشاركين في الدراسة عن طريق ملصقات أو اتصال مباشر .هذه الدراسة هي دراسة مزدوجة التعمية عشوائية مراقبة-وهمية. سوف يتم تقسيم الاشخاص الذين يعانون من وزن زائد أو سمنة (الاعمار تتراوح بين 18-45 سنة,مؤشر كتلة الجسم ≤ 25 كجم/م 2) عشوائيا لأخذ اما حبوب وهمية (سيلولوز) أو بوتاسيوم فوسفات (375مغ) مع كل وجبة رئيسية (الفطور,الغداء, العشاء) لمدة 3 أشهر. و سيشارك 75 شخص في كل مجموعة في هذا البحث.
في هذا البحث (**زيارة المعاينة**) سيتم أولا التأكد اذا كان بامكانك المشاركة أو لا. من لا يحق له المشاركة: من يعاني من نسبة التفلتر الكلوي <60 أو أي أمراض مزمنة أخرى، النساء الحوامل و الرضع, الاستعمال المزمن للادوية التي تؤثر على وزن الجسم،خسارة ما يقارب 3% أو أكثر من الوزن خلال 3أشهر المسبقة.
الزيارة الاولى: سوف يطلب من الاشخاص الذين تم اختيارهم للمشاركة في هذا البحث للصوم مدة 12 ساعة قبل القدوم إلى مكان الاختبار (كلية الزراعة والغذاء العلوم/قسم التغذية أو مركز البحوث\ أو وحدة البحوث المركزية (CRU) / مستشفى الجامعة الأميركية/ المركز الطبي.). سوف تؤخذ عينات الدم و البول على الريق و من ثم سوف يُطلب منك شراب مشروب حلو يحتوي على ٧٥غ من مادّة الجلوكوز و سوف يتم أخذ عينات الدم بعد ساعة وساعتين من تناول الشراب نتابع حيث يتم فحص تقبّل الجلوكوز. و في الوقت عينه سوف يتم أخذ قياساتك (العمر,الطول، الوزن ومحيط الخصر و الورك), و سيتم فحص تكاوين الجسم بالاضافة الى قياس مستوى ضغط الدم. كما سيطلب منك أن تملا عدة استمارات: جدول بما أكلته خلال 7 ايام و استمارة النشاط البدني أو استمارة تحديد مستوى الجوع. سوف يطلب من المشاركين الذين يتناولون مكملات غذائية أن تأخذ مع الوجبة او بعدها لمنع أي تفاعل مع مكمل الفسفور.سوف يعطى لكل مشارك كمية من المكملات المحددة له (وهمية أو فسفور) لمدة تكفيه خلال 6 أسابيع و سيطلب تناول 3 حبات مع كل وجبة رئيسية (الفطور,الغداء, العشاء). خلال هذا البحث، سوف نطلب منك أن تتابع تناولك للطعام بشكل طبيعي ونشاطك البدني أيضاً خلال مدة الدراسة وتفادى تناول الكحول والنشاطات الكثيفة قبل 24 ساعة قبل القدوم الى كل زيارة.

NUT.OO.11 March 9, 2013

Institutional Review Board
American University of Beirut

29 APR 2013

APPROVED

الزيارة الثانية: سوف يطلب منك زيارة مكان البحث المذكور أعلاه حيث سوف يتم قياس الوزن و الطول و محيط الخصر و الورك و جمع عينات البول و ملئ استمارة التزامك بتناول الحبوب و استمارة تحديد الجوع و عن الصحة بشكل عام و عن الحالة الذهنية و التوتر و النشاط البدني. و سوف يتم اعطاؤك كمية من المكملات المتبقية.

الزيارة الثالثة و الاخيرة: سيعاد ما تم في الزيارة الاولى حيث يتم فحص ثقبّل الجلوكوز في كل زيارة سوف يطلب منك أن تملأ استبيانات لها علاقة ب مدى التزامك بتناول الحبوب, و عن الصحة بشكل عام, وعن الحالة الذهنية و التوتر و النشاط البدني و الشعور الشخصي بالشبع.

ج) **المدة**:إن الوقت المقدر لانهاء البحث هو تقريبا ثلاثة أشهر. مطلوب زيارة موقع الدراسة أربع مرات (المعاينة، الزيارة الاولى و الثانية و الثالثة) .مدة كلّ زيارة 3 ساعات تقريباً.يمكنك الانسحاب من البحث في أي وقت. إن أردت التوقف عن المشاركة، ما من عقوبة تفرض عليك ولن تخسر أي من الفوائد التي تملكها وقرارك لن يؤثر على أي علاقة مستقبلية مع الجامعة الأمريكية في بيروت.

د) **المخاطر والمضايقات والفوائد**:مشاركتك في هذه الدراسةقد يكون لها اثار جانبية ثانوية. سوف نطلب منك أن تسترخ بشكل مريح على سرير. تضع ممرضة مؤهلة حقنة مثبتة (أي أنبوبة بلاستيكية صغيرة) في وريد الساعد و سوف تؤخذ عينات 10 مل من دم على الريق و بعد ساعة وثم ساعتين من تناول 75غ غلوكوز. كما ستؤخذ عينات البول قبل البدء بالاختبار و بعد انتهاءالاختبار. سيتم تحليل جزء من عيّنات الدم في مختبر المركز الطبّي: أي معدّل الكوليسترول، الدهون الثلاثية، السكر، السكر الهموغلوبيني (HbA1c) والانسولين قبل البدء بالاختبار و بعد 3 أشهر. عينات البول سوف تحلل للكرياتينين و الكالسيوم و الفسفور و المغنيزيوم و ألفا هليكل بيبتيد (مؤشر خسارة العظم).

قد تكون هناك مخاطر لا يمكن التنبؤ بها.

المخاطر التي يمكن التنبؤ بها:

- سيتم شرح تفصيلي لبروتوكول الدراسة والفحوصات التي ستخضع لها عند المقابلة لضمان راحتك.
- ستجرى جميع الفحوصات حسب البروتكول الطبي.
- لتخفيف المخاطر المتعلّقة باستخدام الحقنة، سوف تعقّم منطقة الجلد جيّداً بالسبيرتو قبل وضعها من قبل ممرضة مؤهلة. سوف تتأكد الممرّضة من وضعها الصحيح وتقوم بجمع عيّنات الدم بالطريقة السليمة. ستكون جميع الأدوات التي تستعمل معقّمة. ستُسحب الحقنة فقط من الممرضة. عندما يتم سحب الحقنة ، يُنظّف الجرح بالسبيرتو والبيروكسيد ثم تُوضع لزقة معقّمة. سيُطلب منك الإبلاغ عن أي إزعاج أو تغيير لون في جلدك.

الإجراء المتبع في هذه الدراسة لا يسبب أي مخاطر رئيسية أخرى غير عدم الراحة من وخز الإبرة لسحب الدم كما ذكر في الإجراءات المذكورة أعلاه. ومع ذلك، قد يكون هناك مخاطر غير متوقعة. لقد أجرينا عدة تجارب باستخدام نفس الجرعة من الفوسفور ولم نتلقى أية شكاوى عن الآثار السلبية أو عدم الراحة.

لن تتقاضى أي أجر لهذه الدراسة، التغيرات المتوقعة بعد 3 أشهر هو الوزن و الكتلة الدهنية في الجسم . قد يكون للفسفور دورا في ضبط الشهية و محاربة السمنة اذا وجهنا نتائج هذا البحث لتطوير مكملات تحتوي على الفسفور. سيمكننا ذلك من ايجاد طرق لتغيير نظامنا الغذائي عن طريق زيادة تناولنا للفسفور.

سيتم إعطائك بعض النصائح الغذائية في نهاية الدراسة.

ه) **السرية:** لتأمين سرية إجاباتك، إسمك والمعرفات الأخرى لن تكون معلقة مع أجوبتك لضمان السرية. جميع المعلومات والمدونات ستحفظ في غرفة مغلقة أو حاسوب لديه رمز سري. الوصول إلى المعلومات مسموحٌ فقط للباحث الأساسي والباحين الذين يعملون مباشرةً على الدراسة. جميع المعلومات ستدمر بشكل مسؤول من بعد 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 السرية. مثلاً يمكن للمعلومات الشخصية المتعلقة باشتراكك أن تعطى لمجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت إن طُلبت و للجان الأخلاق المهنية المستقلة، ومفتشين من للإدارات الحكومية المنظمة، مكتب حماية البحث الإنساني للولايات المتحدة أو أي وكالة تنظيمية فدرالية أو دولية أخرى، أو راعي البحث، إن وجد أو أي وكالة تسند البحث.

و) **التعويض / الحافزة:**لن تتقاضى أي أجر لهذه الدراسة، الوجبة المقدمة (مياه و وجبة غداء) مجانية ولن تتقاضى أجر التنقل أو كلفة موقف السيارة الخ.

ز) **الدفع للإصابات ذات صلة بالبحث:** ما من تغطية لحصول الحوادث الغير متوقعة. إن تعرضت إلى إصابة جراء البحث، أو لأي سؤال عن الإصابات المتعلقة بالبحث، يرجى الاتصال بالدكتور عمر عبيد 350000 (01) مقسم 4440، email: oo01@aub.edu.lb

ح) أسئلة ومعلومات الاتصال

١) لأي أسئلة أو أي مخاوف حول البحث، 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.

٢) لأي أسئلة أو أي مخاوف حول حقك كمشارك في هذا البحث يمكنك الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية للعلوم السلوكية والاجتماعية
أو 5445 مقسم 350000 (01) الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان
email: irb@aub.edu.lb، 5440

ح) حقوق المشاركين:

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

أ. الاتصال في المستقبل

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

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

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناء عليه فانني، حرا مختارا، أجيز إجراء هذا البحث و اوافق على الإشتراك فيه.

إسم المشترك

التاريخ و الوقت:

توقيع المشترك

الباحثون:

لقد شرحت كل التفاصيل التي تتعلق بهذا البحث لأهل الطفل المشارك أو للوصي الشرعي قبل الحصول على امضاء الأخير. لا يوجد فراغات في هذه الوثيقة، و قد تم اعطاء نسخة لأهل الطفل المشارك أو للوصي الشرعي.

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:

إمضاء الشخص المأذون للموافقة: التاريخ و الوقت:

Institutional Review Board
American University of Beirut
29 APR 2013
APPROVED

APPENDIX

C. SCREENING (GENERAL HEALTH) QUESTIONNAIRE

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_____)

1/2

APPENDIX

D. APPETITE QUESTIONNAIRE (ENGLISH)

Simplified Nutritional Appetite Questionnaire (modified)

Name: _____ **Sex (circle):** Male / Female
Age: _____ **Date**: _____

1. My appetite is
a. very poor
b. poor
c. average
d. good
e. very good

2. When I eat
a. I feel full after eating only a few mouthfuls
b. I feel full after eating about a third of a meal
c. I feel full after eating over half a meal
d. I feel full after eating most of the meal
e. I hardly ever feel full

3. I feel hungry
a. rarely
b. occasionally
c. some of the time
d. most of the time
e. all of the time

4. Food tastes
a. very bad
b. bad
c. average
d. good
e. very good

5. Normally I eat
a. less than one meal a day
b. one meal a day
c. two meals a day
d. three meals a day
e. more than three meals a day

6. How often do you snack?
a. less than once a day
b. once a day
c. two times a day
d. three times a day
e. more than three times a day

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APPENDIX

E. APPETITE QUESTIONNAIRE (ARABIC)

Simplified Nutritional Appetite Questionnaire (modified)

الاسم: _____ **الجنس:** ذكر \ أنثى
العمر: _____
التاريخ: _____

١ - شهيتي:
أ. ضعيفة جدًا
ب. ضعيفة
ت. متوسطة
ث. جيدة
ج. جيدة جدًا

٢ - عندما أتناول الطعام:
أ. أشعر بالشبع بعد تناول فقط كمية قليلة من الطعام
ب. أشعر بالشبع بعد تناول ثلث الوجبة
ت. أشعر بالشبع بعد تناول أكثر من نصف الوجبة
ث. أشعر بالشبع بعد تناول معظم الوجبة
ج. نادراً ما أشعر بالشبع

٣ - أشعر بالجوع:
أ. نادراً
ب. بين الحين و الآخر
ت. في بعد الأوقات
ث. معظم الأوقات
ج. كلّ الوقت

٤ - مذاق الطعام:
أ. سيئ للغاية
ب. سيئ
ت. عادي
ث. جيد
ج. جيد جدًا

٥ - عادةً أتناول:
أ. أقل من وجبة يومياً
ب. وجبة واحدة يومياً
ت. وجبتين يومياً
ث. ثلاث وجبات يومياً
ج. أكثر من ثلاث وجبات يومياً

٦- كم مرة تتناول الطعام بين الوجبات الأساسية:
أ. أقل من مرة يومياً
ب. مرة يومياً
ت. مرتين يومياً
ث. ثلاث مرات يومياً
ج. أكثر من ثلاث مرات يومياً

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APPENDIX

F. TABLES OF DATA ENTRY

Name	subject numb	phone numb	Age	Gender (M/F)	Date (Start)	weight (start) kg	Height (start) cm	BMI (start) kg/m2	WC (start) cm	SAMPLE A/B

weight (end) kg	Date (end)	Urine analysis (beginning)	glucose 2 hrs post 75 g of OGTT (beginning of exp)	glucose time zero (beginning of exp)	HbA1c beginning of experiment	Health questionnaire	Average daily caloric intake (Cal)

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