PREMEAL PHOSPHORUS SUPPLEMENTATION FOR REDUCING ENERGY INTAKE AND BODY WEIGHT

by
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PREMEAL PHOSPHORUS SUPPLEMENTATION FOR REDUCING ENERGY INTAKE AND BODY WEIGHT

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Title: Premeal Phosphorus Supplementation for Reducing Energy Intake and Body Weight

Background: The present study is based on accumulating evidence in animals supporting an inverse relationship between eating behavior and hepatic Adenosine TriPhosphate (ATP) levels, that are known to be dependent on Phosphorous availability. In line with that, an inverse relationship between plasma phosphorus and body weight has been reported. Furthermore, common foods typically associated with weight gain, such as refined cereals, sweeteners, and oils, are low in phosphorus.

Objective: Preliminary data have previously demonstrated that pre-meal Phosphorus (P) intake reduces immediate subsequent food intake by 27-33%. This study aims to investigate the acute effect of increasing doses of phosphorus, on satiety, and subsequent food intake in overweight/obese subjects, and the chronic effect (3 months) of phosphorus supplementation on food intake, bodyweight, waist circumference, HbA1c, insulin sensitivity and glucose tolerance of overweight and obese subjects. We hypothesize that P supplementation suppresses satiety and food intake and subsequently reduces body weight, waist circumference and HbA1c, which will improve insulin sensitivity and glucose tolerance. Such effects would be expected to reduce morbidity and mortality.

Design: The study is divided into two experiments in which the acute and chronic effects of phosphorus supplementation have been studied. The acute study is a randomized crossover study. Healthy overweight/obese women (n=12) with a BMI of 31.0±1.3kg/m² and a mean age of 29.75±2.36 years were recruited. Subjects consumed different phosphorus or placebo (potassium phosphate or cellulose) doses with 0mg, 125mg, 250mg, 375mg, or 500mg randomly on different test days for 5 weeks with a washout out period of 1 week between test days. Visual analog scales rated hunger and satiety for 0min, 15min, 30min, 45min, 60min, 75min, and food intake was measured at an ad libitum lunch of pizza and water 80 minutes after consuming the supplements.

As for the chronic effect of phosphorus, it is a double-blind, randomized, placebo-controlled study. Overweight and obese subjects (n=49) (18 men and 31 women) with a BMI of 31.0±1.3 kg/m² and age of 30±3.0 years 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. Weight, BMI, waist circumference (WC), HbA1c, fasting and 2h OGTT glucose, insulin and GLP-1 were collected at baseline and 3 months after supplementation.
Results: Responses to satiety-related questions did not differ among treatments for the acute effect of phosphorus. Food intake was also indistinguishable among doses.

After 3 months P supplementation, the change in weight (-0.44± 0.53 kg), BMI (0.16± 0.18 kg/m²) and WC (-3.48±0.60cm) was significantly (p<0.05) lower compared with placebo (1.13± 0.45 kg, 0.42±0.18 kg/m² and 0.38±0.4kg/m², respectively). The change in blood glucose, insulin, GLP-1, and HbA1c did not differ between groups.

Conclusion: for the acute phase of phosphorous supplementation satiety and food intake did not change in a dose-dependent manner after subjects consumed 0, 125, 250, 375, and 500 mg of phosphorus. However, phosphorous supplementation over a period of 3 months was significantly associated with decreased body weight,BMI, and waist circumference. On the other hand, there was no significant effect on HbA1c, glucose, insulin and GLP-1. The 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 elucidated.
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# ABBREVIATIONS

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<th>Full Form</th>
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<tr>
<td>2,5-AM</td>
<td>2,5 Anhydro-D-Mannitol</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine DiPhosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine MonoPhosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis Of Variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine TriPhosphate</td>
</tr>
<tr>
<td>AUBMC</td>
<td>American University of Beirut Medical Center</td>
</tr>
<tr>
<td>B-GLU</td>
<td>Blood Glucose</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CA</td>
<td>Calcium</td>
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<tr>
<td>CR</td>
<td>Creatinine</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
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<td>CRU</td>
<td>Clinical Research Unit</td>
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<tr>
<td>DDP IV</td>
<td>Dipeptidyl Peptidase IV</td>
</tr>
<tr>
<td>ECF</td>
<td>Extra Cellular Fluid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>HFCS</td>
<td>High Fructose Corn Syrup</td>
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<tr>
<td>G-6-P</td>
<td>Glucose-6-Phosphate</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon Like Peptide -1</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis Model Assessment</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>MRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>PI</td>
<td>Inorganic Phosphate</td>
</tr>
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<td>PO₄</td>
<td>Phosphate</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Daily Allowance</td>
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<td>RPM</td>
<td>Round Per Minute</td>
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<tr>
<td>S-GLU</td>
<td>Serum Glucose</td>
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<td>S-INS</td>
<td>Serum Insulin</td>
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<tr>
<td>S-P</td>
<td>Serum Phosphorus</td>
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<tr>
<td>TCA</td>
<td>TriCarboxylic Acid</td>
</tr>
<tr>
<td>TNF-</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>UTP</td>
<td>Uridil TriPhosphate</td>
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<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
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<td>WHR</td>
<td>Waist to Hip Ratio</td>
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CHAPTER I
INTRODUCTION

The incidence of obesity has increased dramatically over the past 50 years and is associated with many metabolic diseases including diabetes and cardiovascular disease.

It was recently proposed that disturbance in phosphate metabolism may represent a key feature of the metabolic syndrome (Friedman, 2007). Reduced hepatic adenosine triphosphosphate (ATP) stores are more prevalent in overweight and obese subjects than in lean subjects (Nair et al., 2003). In addition, the recovery from hepatic ATP depletion becomes progressively less efficient as body mass increases in healthy controls and is severely depleted in patients with obesity-related nonalcoholic steatohepatitis (Pinto et al., 1999). It has been shown that hepatic ATP is inversely correlated with body mass index (BMI) (Bohannon, 1989). Phosphate is also involved directly in carbohydrate metabolism (DeFronzo and Lang, 1980); (Kalaitzidis et al., 2005); (Marshall et al., 1978) and hypophosphatemia can result in impaired insulin resistance, and hyperinsulinemia (Paula et al., 1998). According to this model, reduced phosphate levels may contribute directly to the development of obesity, hypertension, and dyslipidemia that characterize metabolic syndrome (Haglin, 2001); (Ljunghall and Hedstrand, 1979). Phosphate depletion may result from decreased dietary intake or reduced intestinal absorption, increased urinary excretion, and internal redistribution (Haglin, 2001). Lower phosphate concentrations in patients with metabolic syndrome may result at least in part from reduced dietary intake (Haglin, 2001). It has been proposed that an unbalanced diet,
characterized by low phosphate and high carbohydrate consumption, may lead to reduced serum phosphate levels in patients at risk for the development of metabolic syndrome (Haglin, 2001). Finally, reduced phosphate levels in the metabolic syndrome may represent the consequence of increased transfer of phosphate from the extracellular to the intracellular compartment. Increased insulin levels in patients with the metabolic syndrome could be a major determinant of this process (Bohannon, 1989); (DeFronzo and Lang, 1980); (Riley et al., 1979). Especially that insulin levels correlated negatively with phosphate concentrations (Bohannon, 1989); (DeFronzo and Lang, 1980); (Riley et al., 1979).

Therefore, the objectives of this proposal are to extend and confirm these findings by determining the conditions under which phosphorus ingestion decreases food intake, and to conduct preclinical testing to determine if phosphorus supplementation with meals results in weight loss in overweight/obese individuals. We hypothesize that meal phosphorus supplementation of 375mg will decrease subsequent food intake and lead to a reduction in body weight, body mass index, waist circumference, HbA1c, improve insulin sensitivity and glucose tolerance of overweight and obese subjects. Such effects would be expected to reduce morbidity and mortality of the subjects.
A. Role of Phosphorus

Phosphorus is one of the most abundant elements in the human body. Most phosphorus in the body is complexed with oxygen as phosphate (PO$_4$). Its homeostasis is essential for bone mineralization and for tissue growth; it is a component of the hydroxyapatites, of several energy compounds (ATP, ADP, AMP, UTP...) and of phospholipids. Additionally, it is involved in aerobic and anaerobic energy metabolism (Landsman, 2001). A depletion of the diet in phosphorus has been associated with “Pi appetite” as it increases appetite for search of phosphorus (Ohnishi et al., 2007). Normal blood phosphorus values without any disease state are 2.6-4.4 mg/dL (www.hosp.uky.edu/Clinlab/report.pdf).

B. Phosphorus Intake

It is well accepted that the increase in obesity during the past few decades is associated with several changes in dietary habits and thus nutrient intake, so it is important to determine whether phosphorus may be part of this change. Dietary phosphorus content has decreased with increased food processing over this time period.

Current daily phosphorus intake is about 1.4 g/d (Ervin et al., 2004), although concern has been raised regarding the contribution of phosphorus containing additives, cola beverages (~16 mg/100 mL), etc., as well as the bioavailability of phosphorus from different sources (Calvo and Uribarri, 2013). This intake is lower than the predicted
intake when consuming a diet with 1 mg P/kcal, but is above the present recommended daily allowance (RDA) of 700 mg. It should be mentioned that RDA is based on the lower end of the normal adult serum inorganic phosphate (Pi) and that this would have been 2,100mg if it had been based on the middle of the normal range (Food and Nutrition Board, Institute of Medicine, 1997).
CHAPTER III
LITERATURE REVIEW

A. Phosphorus, CRP, and Obesity

Figure 1 depicts the vicious cycle of the “obese metabolism”. It begins with an inflammation inducing factor, which activates Tumor Necrosis Factor (TNF)-alpha and Interleukin-1 (IL-1). As a result, the tricarboxylic acid (TCA) cycle is inhibited leading to lower energy production and more food intake. This overeating and limited energy spending increase the amount of fat tissue. This process is further worsening the metabolism since adipocytes have the ability to amplify TNF-alpha and IL-1 (Hotamisligil et al., 1995); (Kern et al., 2001). Therefore, the more adipose tissue one has, the higher level of these cytokines would be present in the body further increasing oxidative stress and fuelling the damaging process (Wlodek and Gonzales, 2003).
Figure 1: Vicious circle of events leading to obesity. Closing of the circle is caused by the ability of adipose tissue to produce TNF-alpha and IL-1 (Wlodek and Gonzales, 2003). It begins with an inflammation inducing factor, which activates TNF-alpha and IL-1. As a result TCA cycle is inhibited leading to lower energy production and more food intake which leads to an increase the amount of fat tissue. This process is further worsening the metabolism since adipocytes have the ability to amplify TNF-alpha and IL-1 and as a result increasing oxidative stress and fuelling the damaging process.
Adipose tissue is an important source of cytokines, and adiposity contributes to the proinflammatory milieu (Ahima and Flier, 2000); (Yudkin et al., 1999). Serum cytokine levels are elevated in humans and animals with excess adiposity (Samad and Loskutoff, 1996); (Yudkin et al., 1999), and there are differences in the level of messenger ribonucleic acid (mRNA) encoding proteins secreted by adipocytes between human subcutaneous and visceral adipose tissues (Dusserre et al., 2000); (Eriksson et al., 2000); (Fried et al., 1998); (Giacchetti et al., 2002). Adipocytes secrete Interleukin-6 (IL-6) (Fried et al., 1998); (Kern et al., 2001), one of the chief inducers of C-reactive protein (CRP) production by the liver (Castelle et al., 1990); (Papanicolaou et al., 1998). Approximately, 30% of circulating IL-6 is estimated to be from adipose tissue (Mohamed et al., 1998). Moreover, some reports have suggested that visceral adipose tissue secretes more IL-6 than subcutaneous adipose tissue (Fried et al., 1998). IL-6 concentration has been reported to correlate more strongly with waist to hip ratio (WHR) than with BMI, although CRP concentration was correlated more strongly with BMI than with WHR (Yudkin et al., 1999).

A signal for control of food intake tied to changes in energy metabolism, perhaps associated with ATP production, may serve to link changes in energy expenditure and storage to those in energy intake. At the physiologic level, maintenance and alterations in energy balance are accomplished through changes in the partitioning of metabolic fuels. Because mechanisms of fuel partitioning determine whether and in which tissues metabolic fuels are oxidized, shifts in the flux of fuels could affect eating behavior by altering the metabolic signal that controls food intake (Friedman, 1990); (Friedman, 1991). In general, partitioning affects intake indirectly by altering the
oxidation of fuels in the tissue or cells that generate the signal for appetite control. The following figures 2 and 3 illustrate normal conditions of ATP produced in the liver and a decrease in ATP production in the liver respectively. A decrease in ATP production would stimulate eating behavior whereas the increase in ATP suppresses it.

Under normal conditions (Figure 2) there is equilibrium between fuel storage, mobilization, and utilization resulting in the unchanged fuel supply for oxidation and the eating behavior signal stays the same and energy intake would also remain unchanged (Friedman, 1995).

Hyperphagia associated with the development of obesity is accompanied by a metabolic state that fosters the deposition of fat in adipose tissue. This shift in fuel partitioning toward storage is independent of and occurs before the change in food intake in nearly every animal model studied (Friedman, 1991); (Friedman, 1976). Overeating results because fuels that would otherwise be oxidized to produce ATP are redirected into fat stores (Figure 3). Thus, hyperphagia is both caused by and contributes to the increased deposition of fat and the development of obesity. Overeating appears to be an appropriate response to a need for energy created not by the lack of food outside the body, but by the sequestration of energy in stores within the body (Friedman, 1995).
Figure 2: Hypothetical relation between fuel partitioning and control of energy intake under normal or steady state conditions (Friedman, 1995). Under normal conditions there is equilibrium between fuel storage, mobilization, and utilization resulting in the unchanged fuel supply for oxidation and the eating behavior signal stays the same and energy intake would also remain unchanged.

Figure 3: Hypothesized mechanism for increased energy intake associated with increased energy deposition during the development of obesity (Friedman, 1995). This shift in fuel partitioning toward storage is independent of and occurs before the change in food intake. Overeating results because fuels that would otherwise be oxidized to produce ATP are redirected into fat stores. Thus, hyperphagia is both caused by and contributes to the increased deposition of fat in the development of obesity.
B. Phosphorus, Adenosine Triphosphate and Energy Balance

The production of ATP, especially hepatic ATP, depends upon adequate sources of phosphorus (P) (Morris et al., 1978); (Solomon and Kirby, 1990). Cells can store a limited amount of free phosphate and most tissues depend on extracellular fluid (ECF) Pi for their metabolic phosphate. Cellular dysfunction results when there are low ECF Pi levels. There is a lack of an adaptive mechanism that improves phosphorus absorption at low intakes, as can occur with some other micronutrients (Food and Nutrition Board, Institute of Medicine, 1997). Therefore, phosphorus availability in food becomes an important factor that governs phosphorus levels in the circulation and in turn its availability for ATP production. Thus, low phosphorus intake would be expected to reduce ATP production, which is believed to affect food intake and energy expenditure. Evidence supports a relationship between declining hepatic ATP levels and increasing food intake; this decline is thought to transduce changes in hepatic energy status into neural signals or hepatic vagal afferent activity that is transmitted to the central nervous system (Friedman, 2007); (Hong et al., 2000); Langhans and Scharrer, 1992); (Nair et al. 2003); (Rawson and Freidman, 1994); (Riquelme et al., 1984).

The currency of free energy in living organisms is ATP. Nutrients from food are metabolized to fuel and next, either to ATP- and only then they become equivalents of energy- or become precursors of fat or proteins. Fat and proteins may be metabolized to ATP but this is not an automatic conversion and involves several metabolic steps. Fat is the storage of fuel and only after it is metabolized to ATP does it become energy (figure 4). As a result obese individuals have a deficit of energy in the form of ATP with simultaneous overproduction of fat (Wlodek and Gonzales, 2003).
Figure 4: Fuel distribution between energy productive (ATP) and fat synthesis: (a) in normal metabolism; (b) in obesity. Dotted lines indicate decreased efficiency, large arrow increased efficiency of a process as compared to normal metabolism (Wlodek and Gonzales,2003). The currency of free energy in living organisms is ATP. Nutrients from food are metabolized to fuel and next, either to ATP- and only then they become equivalents of energy- or become precursors of fat. Fat may be metabolized to ATP but this is not an automatic conversion and involves several metabolic steps. Fat is the storage of fuel and only after it is metabolized to ATP does it become energy. Thus obese individuals have a deficit of energy in the form of ATP with simultaneous overproduction of fat.
C. Phosphorus and Food Intake

The supply of energy is the most basic requirement of every cell and every organism. Inadequate energy supply in the body will be transformed into increased appetite. In the experiments on rats, treating animals with metabolic inhibitors, 2,5-anhydro-d-mannitol or/and methyl palmoxirate, controlled the ATP levels in liver cells. These experiments revealed a direct correlation between eating habits and the level of ATP, ATP/ADP ratio and the phosphorylation potential in liver cells only (Oberhaensli et al., 1986); (Wlodek and Gonzales, 2003). These experiments indicate that there is an integrated metabolic control of food intake with liver ATP levels acting as a major sensor of energy status in the body (Wlodek and Gonzales, 2003). In obese people the levels of hepatic ATP are decreased and hepatic ATP content is inversely related to BMI, decreasing steadily with increasing BMI (Nair et al., 2003).

Another appetite signalling mechanism involves leptins (Friedman, 1998). These proteins are produced by adipocytes and are known to decrease appetite and food intake. Thus, the more adipose tissue is in the body, the stronger the signal decreasing appetite would be. However, most obese people have elevated levels of leptins as compared to lean people (Considine et al., 1996); (Szymczak and Laskowska-Klita, 2005). In spite of that, their appetite is not decreased (Widdowson et al., 1997). This effect is called leptin resistance and it is one of the puzzles about obesity. The signalling leading to leptin resistance is presented schematically in Figure 5 (Wlodek and Gonzales, 2003). Considering the interaction of the two signaling mechanisms: leptins and liver-ATP, gives an explanation of the puzzle. In obesity both of these mechanisms send signals to the brain but the signals are opposite: leptins to decrease the
appetite and low ATP levels to increase the appetite (Wlodek and Gonzales, 2003). Since the energy supply is the most basic and important need of every living organism, supplying energy would be the priority signal and would override any others. Thus the final outcome would be an increase in appetite. Accepting liver signaling as the most important regulator of appetite helps to understand why it is so difficult for obese individuals to comply with low calorie diets (Wlodek and Gonzales, 2003). Liver is an energy distributor in the body and low levels of ATP in liver indicate that all of the energy sources of the body are used up. Thus, the message coming to the brain from liver cells is an “emergency” signal difficult to ignore (Wlodek and Gonzales, 2003).

Figure 5: Possible mechanism of leptin resistance. Signals triggered by low ATP levels in liver cells have higher priority and override “do not eat” signal coming from leptins. The final result is an increase in appetite. Dotted arrows indicate less efficient processes, large arrows—more efficient processes than in normal metabolism (Wlodek and Gonzales, 2003). Considering the interaction of the two signaling mechanisms: leptins and liver-ATP, gives an explanation of the increase food intake and obesity. In obesity both of these mechanisms send signals to the brain but the signals are opposite: leptins to decrease the appetite and low ATP levels to increase the appetite. Since the energy supply is the most basic and important need of every living organism, supplying energy would be the priority signal and would override any others. Thus the final outcome would be an increase in appetite.
D. GLP-1

Glucagonlike Peptide-1 (GLP-1), secreted by L-cells of the distal small intestine, presents the most important incretin hormone in treatment of type 2 diabetes as it stimulates insulin gene expression, promotes insulin biosynthesis, indirectly increases insulin sensitivity, stimulates the proliferation of existing β-cells, stimulates the maturation of new β-cells and inhibits their apoptosis (Nauck, 2011). In patients with type 2 diabetes, infusion of endogenous GLP-1 has been shown to provide not only improved glycemic control but also beneficial effects on β-cell function, weight, and cardiovascular risk factors (Drucker and Nauck, 2006). Moreover, GLP-1 shows positive effects, both in the treatment of obesity and type 2 diabetes, making GLP-1 especially attractive for obesity diabetes (Gault et al., 2011); (Nauck, 2011). However, GLP-1 is not very effective due to its rapid degradation in blood by the enzyme Dipeptidyl-Peptidase IV (DPP-IV). Previous studies have shown a link between an increased protein intake and an increase in satiety to a higher extent than does carbohydrate and fat and this was due to an increase in GLP-1 (Lejeune et al., 2006).

E. Phosphorus, Hyperglycemia and Insulin Resistance

Phosphate is needed for ATP generation and therefore seems to be an important component of energy metabolism (Haap et al., 2006); (Massry et al., 1992); (Thompson and Kemp, 1995). Reduction of serum phosphate could theoretically lead to disturbances of energy metabolism resulting in insulin resistance and impaired glucose tolerance (IGT). Low serum phosphate levels inhibit the phosphorylation of carbohydrate intermediates in glycolysis and gluconeogenesis (Haap et al., 2006); (Xie et al., 2000).
Hypophosphataemia, present in almost one-fifth of severely obese patients, may be involved in the development of an impaired metabolism. Overconsumption of carbohydrate (an unbalanced diet) and disturbed metabolism in obesity, are all connected with some risk of hypophosphataemia, which in turn could account for the hyperglycaemia, hypertension and dyslipidaemia presented in the literature as metabolic syndrome or insulin resistance syndrome (Figure 6) (Haglin, 2001).

Figure 6. A causal-model of hypophosphataemia (Haglin, 2001). In obesity, overeating generally and overconsumption of carbohydrates in particular could cause the decrease in SP levels, thus explaining the high risk of developing type II diabetes. Many obese persons suffer from hyperinsulinaemia, hypertriglyceridaemia, hypertension and low S-HDL, which are indicative of the metabolic syndrome.
In obesity, overeating generally and overconsumption of carbohydrates in particular could cause the decrease in serum phosphorus (S-P) levels, thus explaining the high risk of developing type II diabetes. Inverse correlations between S-P and BMI, and blood glucose(B-glu) have been reported earlier (Lind et al., 1993); (Lindgarde and Trell, 1977); (Ljunghall et al.,1979).

A low S-P level limits the phosphorylation of carbohydrate intermediates in glycolysis and glycogenesis, while chronic (but not acute) hypophosphataemia inhibits glucose transport (Davis et al.,1979); (Haglin, 2001); (Marshall et al., 1978). Hypophosphataemia might cause insulin resistance and a compensatory increase in insulin secretion. Hyperinsulinaemia, if present, could have increased S-P transit from extra to intracellular space and could have increased the resorption of phosphate in the kidney tubules (Ritz et al., 1980). Moreover, chronic phosphate depletion may affect ATP regeneration in the pancreatic beta cells, partly by raising the cytosolic calcium level, which causes an interruption in insulin production (Levi et al., 1992). This might explain why type II diabetic patients become insulin dependent.

The more carbohydrate the diet contains, the more phosphate is needed to maintain an optimal metabolism, with synthesis of ATP and glucose-6-phosphate (G-6-P). Hypophosphataemia in obese subjects could account for the pathophysiology of glucose intolerance, and concomitantly the increased risk of type II diabetes in connection with obesity (Lind et al., 1993).
Both experimental and clinical studies have revealed that hypophosphataemia/phosphate depletion disturb carbohydrate metabolism, with consequent reduced glucose transport, hyperglycaemia and hyperinsulinaemia (Davis et al., 1979); (DeFronzo and Lang, 1980); (Haglin, 2001); (Harter et al., 1976).
CHAPTER IV

ACUTE EFFECT OF PHOSPHORUS

MATERIALS AND METHODS

A. General procedure/Participants

This work was approved by the Institutional Review Board (IRB) of the American University of Beirut (AUB) and written informed consent was obtained from all subjects (Appendix I and II). All forms (consent, health questionnaire, three factor eating questionnaire, palatability questionnaire, and visual analogue scales) were translated from English to Arabic and approved by the IRB since most subjects are not English educated. 15 female subjects (Table 1), 18-45 years old (mean age 29.75±2.36), had a BMI ≥ 25.0kg/m² (mean BMI: 31.98±1.37) were recruited during the period from October 2012- till February 2013 by posters or by direct approaching. Three participants dropped out for personal reasons and 12 continued the study. In brief, participants who had stable body weight for the last 3 months and were unrestrained eaters as assessed by the three-factor eating questionnaire were selected (Stunkard and Messick, 1985) (Appendix IV and V). All subjects were regular breakfast consumers and were asked to maintain their regular dietary and physical activity habits throughout the course of the study. Exclusion criteria included: any significant medical diseases, pregnancy or lactation; regular use of medication that affects body weight; and a weight loss of 3% or more in the preceding 3 months. Subjects were asked to avoid alcohol consumption, as well as any unusual strenuous exercise 24 h before the study. A randomized, cross-over design was used. At baseline, participants completed a health questionnaire (Appendix
III) and three factor eating questionnaire (Appendix IV and V), height and weight were measured using “SECA” balance, BMI calculated using the formula weight in kilograms divided by height in meters squared (Kg/m²). During the test (a minimum of 1 week wash out period was required for each test day) periods, participants reported to the Faculty of Agriculture and Food Sciences/Department of Nutrition/Organooleptics study room at the AUB after a 12 hour overnight fast where their weight was taken and they consumed their normal breakfast meal. During the 4 hours following the breakfast, additional food intake was to be avoided, except for water that was allowed up to one hour prior to the study time. Appetite ratings were collected prior to consuming the supplements using a 100-mm visual analogue scale (VAS) (Appendix VI and VIII), and a palatability questionnaire (Appendix VII and IX) was filled out immediately after taking the supplements. The supplements received were placebo (cellulose), or potassium phosphate (each pill containing 125mg of phosphorus). At each visit a total of 4 pills were taken and they were randomly sampled in which each participant on every test day received a different dose of phosphorous (0, 125, 250, 375, and 500 mg) or placebo supplements were given to ensure blinding. Thus, each experiment used a within-subject design, wherein each participant served as her own control. Following that, participants rated their appetite sensation on the VAS at regular time intervals at 15, 30, 45, 60 and 75 min after taking the supplements. Subsequently, ad libitum pizza and water were offered at 80 minutes. The pizza was produced by Food Engineers (www.foodengineers.com.lb) and each pizza had a weight of 410g and provided 700 calories. Subjects were asked to eat freely until they felt ‘comfortably full’; both food and water intakes were measured. Water intake was measured by volume (mL) and food
intake by weight (g) was obtained from the overall weight of pizza consumed. This was repeated every week, for 5 weeks with a minimum of 1 week wash out period.

Table 1: Characteristics of participants

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>12</td>
</tr>
<tr>
<td>Age (y)</td>
<td>29.75±2.36</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6±0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.37±3.39</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m^2)</td>
<td>31.98±1.37</td>
</tr>
</tbody>
</table>

**B. Statistical Analysis**

The data were analyzed using the software Minitab 16 and one way analysis of variance (ANOVA) was used. Specific comparisons were made using Fisher’s pairwise comparisons. All values are expressed as mean±SEM. A probability of p<0.05 was considered to be statistically significant.

**C. Visual Analogue Scales**

Satiety was evaluated using questions from a previously validated 100 mm VAS (Flint et al., 2000)(Appendix VI and VIII). The VAS was translated to Arabic by the NFSC department at AUB. Questions were taken directly from the original citation:

hunger—How hungry do you feel? I am not hungry at all (0 mm) vs I have never been more hungry (100 mm); satisfaction—How satisfied do you feel? I am completely empty (0 mm) vs I cannot eat another bite (100 mm); fullness—How full do you feel? Not at all
full (0 mm) vs totally full (100 mm); prospective food intake—How much do you think you can eat? Nothing at all (0 mm) vs a lot (100 mm); desire for specific food types—Would you like to eat something sweet? Yes very much (0 mm) vs no not at all (100 mm); would you like to eat something salty? Yes very much (0 mm) vs no not at all (100 mm); would you like to eat something savoury? Yes very much (0 mm) vs no not at all (100 mm); would you like to eat something fatty? Yes very much (0 mm) vs no not at all (100 mm).

Five characteristics were used to assess the palatability of the supplements (Appendix VII and IX). Visual appeal, smell, taste, aftertaste and palatability were scored as good (0 mm) vs bad (100 mm).
CHAPTER V
ACUTE EFFECT OF PHOSPHORUS

RESULTS

A. Food and Water Intake

The ad libitum pizza and water intake was measured on the days of the experiment and the average food and water intake is presented in Tables 2 and 3 respectively. There was no significant difference with respect to the amount of pizza consumed ($p = 0.505$) or water intake ($p=0.653$) with respect to different phosphorus dosages.

Table 2: Comparing weight of pizza (g) consumed 80 minutes after different phosphorus dosage supplementation

<table>
<thead>
<tr>
<th>Load of Phosphorus (mg)</th>
<th>Weight of pizza consumed (g)</th>
<th>Anova P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>477.1±46.6</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>500.5±46.5</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>466.8±39.2</td>
<td>0.505</td>
</tr>
<tr>
<td>275</td>
<td>410.7±38.1</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>502.3±30.3</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Comparing the total intake of water (mL) intake 80 minutes after different phosphorus dosage supplementation

<table>
<thead>
<tr>
<th>Load of Phosphorus (mg)</th>
<th>Amount of water consumed (ml)</th>
<th>Anova P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>658±107</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>747±151</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>895±124</td>
<td>0.653</td>
</tr>
<tr>
<td>275</td>
<td>915±182</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>746±117</td>
<td></td>
</tr>
</tbody>
</table>

B. Visual Analogue Scale

Our results did not show any significant difference with respect to the different phosphorus dosage in all eight questions at different time intervals of the VAS (satiety and hunger scores). The table below (Table 4) shows the appetite score using VAS score at different time intervals along with the p value with respect to different loads and questions. With respect to the question ‘how hungry do you feel?’ there was no significant difference with respect to the different phosphorus loads at 0, 15, 30, 45, 60, and 75 min (p=0.948; 0.962; 0.90; 0.881; 0.941; and 0.897 respectively). Similar results were seen for ‘how satisfied do you feel?’ at 0, 15, 30, 45, 60, and 75 min (p=0.881; 0.854; 0.949; 0.587; 0.646; and 0.586 respectively); ‘how full do you feel?’ at 0, 15, 30, 45, 60, and 75 min (p= 0.976; 0.974; 0.654; 0.890; 0.711; and 0.826); ‘how much do you think you can eat?’ the p values at the different time intervals were 0.862; 0.879; 0.943; 0.979; and 0.991; as for the questions for the desire for specific food types at the different time intervals 0, 15, 30, 45, 60, and 75 min were as follows: p=0.916; 0.877; 0.993; 0.895; 0.911; and 0.965 for ‘would you like to eat something sweet?’; for ‘would you like to eat something salty?’ p=0.847; 0.985; 0.933; 0.956; 0.971; and
0.992. As for ‘would you like to eat something savory?’ $p=0.943; 0.945; 0.996; 0.955;$ 0.992; and 0.997. Similar non-significant results for ‘would you like to eat something fatty?’ $p=0.849; 0.934; 0.970; 0.966; 0.996;$ and 0.969 respectively.

Table 4: Evaluating satiety and hunger scores using the VAS at different timings after different dosage of phosphorus supplementation. (Potassium Phosphate dosage: 0mg (cellulose-placebo), 125mg, 250 mg, 375mg, 500mg)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>VAS</th>
<th>How Hungry do you feel?</th>
<th>How Satisfied do you feel?</th>
<th>How full do you feel?</th>
<th>How much you think you can eat?</th>
<th>Would you like to eat something sweet?</th>
<th>Would you like to eat something salty?</th>
<th>Would you like to eat something savory?</th>
<th>Would you like to eat something fatty?</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.962</td>
<td>0.854</td>
<td>0.974</td>
<td>0.879</td>
<td>0.877</td>
<td>0.985</td>
<td>0.985</td>
<td>0.934</td>
<td>0.934</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.9</td>
<td>0.949</td>
<td>0.654</td>
<td>0.943</td>
<td>0.993</td>
<td>0.933</td>
<td>0.996</td>
<td>0.97</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>0.881</td>
<td>0.587</td>
<td>0.89</td>
<td>0.979</td>
<td>0.895</td>
<td>0.895</td>
<td>0.955</td>
<td>0.966</td>
<td>0.966</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.941</td>
<td>0.646</td>
<td>0.711</td>
<td>0.991</td>
<td>0.911</td>
<td>0.911</td>
<td>0.992</td>
<td>0.996</td>
<td>0.996</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.897</td>
<td>0.586</td>
<td>0.826</td>
<td>0.916</td>
<td>0.965</td>
<td>0.965</td>
<td>0.997</td>
<td>0.969</td>
<td>0.969</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: VAS, Visual Analogue Scale,

* $P < 0.05$. 

24
CHAPTER VI

CHRONIC EFFECT OF PHOSPHORUS

MATERIALS AND METHODS

A. Study Design:

This is a double-blind, randomized, placebo-controlled study. It was approved by the Institutional Review Board committee of the American University of Beirut, under the code NUT.00.11. Written informed consents to participate in the study were read and signed by all subjects (Appendix X and XI).

B. Subjects:

63 Lebanese overweight/obese subjects (20 males and 43 females) with a body mass index (BMI) ≥25 kg/m² were recruited by posters or by direct approaching. The age range was chosen to be between 18 and 45 years. Subjects were screened using a questionnaire to determine their eligibility for participation (Appendix XIII). All forms that were filled out were translated from English to Arabic at the NFSC department at AUB for subject convenience. 14 subjects dropped out for personal reasons and 49 continued the study (31 females and 18 males). The participants had a mean age of 30.00±3.00 years and a mean BMI of 31.00±1.30kg/m². Exclusion criteria included: glomerular filtration rate <60 ml/min² or any significant medical diseases, pregnancy or lactation; regular use of medication that affects body weight; and a weight loss of 3% or more in the preceding 3 months.
C. Anthropometric Measurements:

Weight, waist circumference (WC) were taken, and BMI was calculated at each visit. Height in meters was measured on the first visit. BMI was calculated using the formula weight in kilograms divided by height in meters squared (Kg/m²), and waist circumference (cm) using a flexible and non-stretchable measuring tape was taken around the umbilicus.

D. Protocol:

The study was performed at the Clinical Research Unit (CRU) at the American University of Beirut Medical Center (AUBMC) where the individuals were scheduled for 2 visits, separated by a period of 3 months during which they were randomly given either potassium phosphate or placebo (cellulose) tablets. The composition of the tablets is shown in tables 5 and 6 respectively.

Table 5: Composition of Potassium Phosphate Tablet

<table>
<thead>
<tr>
<th>125 mg phosphorus from:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>189.4 mg of potassium phosphate monobasic (KH2PO4) 22.76%</td>
<td></td>
</tr>
<tr>
<td>349.5 mg of potassium phosphate dibasic (K2HPO4) 17.78%</td>
<td></td>
</tr>
<tr>
<td>108 mg of dicalcium phosphate 19%</td>
<td></td>
</tr>
<tr>
<td>50 mg of micro crystalline cellulose</td>
<td></td>
</tr>
<tr>
<td>50 mg stearic acid</td>
<td></td>
</tr>
<tr>
<td>10 mg magnesium stearate</td>
<td></td>
</tr>
<tr>
<td>10 mg croscarmellose sodium</td>
<td></td>
</tr>
<tr>
<td>5 mg silicon dioxide</td>
<td></td>
</tr>
</tbody>
</table>
Table 6: Composition of Cellulose (Placebo) Tablet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mg of micro crystalline cellulose</td>
<td></td>
</tr>
<tr>
<td>200 mg calcium carbonate</td>
<td></td>
</tr>
<tr>
<td>160 mg stearic acid</td>
<td></td>
</tr>
<tr>
<td>15 mg magnesium stearate</td>
<td></td>
</tr>
<tr>
<td>20 mg croscarmellose sodium</td>
<td></td>
</tr>
<tr>
<td>5 mg silicon dioxide</td>
<td></td>
</tr>
</tbody>
</table>

Participants were first screened for eligibility, taking into account the exclusion criteria mentioned above. Then on each CRU visit, the same procedure was followed. The subjects came to the unit 10-12 hours after overnight fast. Upon arrival, anthropometric measurements (height, weight, and waist circumference) were taken, and subjects were weighed in light clothes barefoot using the SECA balance. Then a urine sample was collected in a urine cup, after which fasting blood was withdrawn by a 22 gauge needle inserted into the anticubital vein by a registered nurse (t=0 min). A heparlock was used to keep the needle locked for a period of 2 hours. No heparin was added. Afterwards, an oral glucose tolerance test (OGTT) was performed where subjects were asked to drink a 75 g anhydrous glucose solution in a period of 2 minutes. Blood was withdrawn later after 120 minutes from the timing of the consumption of the glucose solution. The heparlock was kept until the second blood withdrawal to avoid pricking the subjects again. Water saline was infused between withdrawals to prevent the obstruction of the vein. A total of 10 ml was collected on each visit. One collected blood sample was presented for the AUBMC laboratory for HbA1c analysis, and the other blood samples were stored in a refrigerator at 4°C (until the end of the experiment) then centrifuged for 15 minutes at 3500 RPM at 3°C for serum and plasma separation.
The serum separated was stored in aliquots at -80°C freezer to be used to measure serum glucose, insulin, GLP-1, total phosphate, and CRP. Collected urine samples were aliquoted into 4 labeled tubes and stored at -20°C to be then used to measure calcium, phosphorous, and creatinine. Baseline characteristic of subjects are found in tables 7 and 8.

Questionnaires (in English and Arabic) related to their adherence to the tablet regimen, general health, self-reports of mood, stress, physical activity, and appetite scores were filled out by the subjects during the first CRU visit, 6 weeks after taking the tablets, and during the second CRU visit (Appendix XIV and XV).

At the first visit, subjects were randomly given a supply of 6 weeks of the allocated supplement (either phosphorous or placebo) and were asked to take 3 tablets (375 mg in the case of phosphate tablet) 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, avoid alcohol consumption as well as any strenuous exercise 24 hours prior to the CRU visit. After 6 weeks, the remaining supplements were collected from the subjects in order to consider their adherence to the tablets; then they were given the second supply of supplements to be taken for the subsequent 6 weeks. After completing the second 6 weeks, the same procedure was followed in the CRU as visit 1, and the supplements were re-collected in order to evaluate their compliance. Another means to assess compliance to the supplement regimen is determining the phosphorous content of urine (indicator).
E. Analytical Procedure:

1. Insulin and GLP-1

Serum insulin and GLP-1 levels were tested for each blood collection using the ELISA kit from Millipore Corporation, Billerica, MA, USA.

- The insulin test was done in a microwell plate. The test is based on two monoclonal antibodies that bind to insulin: one which is immobilized in the plate and the second which is a horseradish peroxidase conjugate. After the insulin in the serum binds to the antibodies, 3,3',5,5'-Tetramethylbenzidine followed by a stop solution are added whereby a yellow color forms and thus the absorbance is read via a spectrophotometer. To obtain the concentration levels in the serum, the concentration of the controls (which are present in the kit) and the measured absorbance of the control (tested in the microwell plate) are used to derive an equation for calculating the concentrations.

- GLP-1 was also tested in a microwell plate. Two antibodies were used, one which is a monoclonal antibody and the other which is a polyclonal antibody, to capture the molecule. Then, the horseradish peroxidase conjugate was used in the presence of 3,3',5,5'-Tetramethylbenzidine, followed by a stop solution whereby the results (absorbance) were read using a spectrophotometer. To obtain the concentration levels in the serum, the concentration of the controls (which are present in the kit) and the measured absorbance of the control (tested in the microwell plate) are used to derive an equation for calculating the concentrations.
2. HbA1c

HbA1c was measured at time zero at each of the two CRU visits. The blood withdrawn was analyzed at the Department of Pathology and Laboratory Medicine at AUBMC using the BioRad Variant Hemoglobin Analyzer which uses the high-performance liquid chromatography method.

3. Calcium, Phosphorus, Creatinine

Urine specimens were centrifuged using EPPENDORF Centrifuge 5810R on 20\(^\circ\)C, for 10 minutes at 3500 ppm speed.

Calcium, Phosphorus, and Creatinine were measured using Vitros 350 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson, 50-100 Holmers Farm Way, High Wycombe, Buckinghamshire, HP 12 4DP, United Kingdom) at the NFSC department (AUB).

F. Statistical Analysis:

The software used for data analysis is Minitab 16. The data were analyzed using paired T-test within the group and Specific comparisons were made between the treatments using unpaired T-test. All values are expressed as mean±SEM. A probability of $p<0.05$ was considered to be statistically significant.
Table 7: Characteristics of Participants at Baseline

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo</th>
<th>Potassium Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N (49)</strong></td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>30.00±3.00</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>F(15)</td>
<td>F(16)</td>
</tr>
<tr>
<td></td>
<td>M(6)</td>
<td>M(12)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>92.30±3.20</td>
<td>87.70±3.50</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.65±0.02</td>
<td>1.67±0.02</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td>109.43±2.20</td>
<td>105.66±2.40</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>33.73±0.84</td>
<td>31.40±0.86</td>
</tr>
<tr>
<td><strong>HbA1c</strong></td>
<td>5.44±0.09</td>
<td>5.63±0.18</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; WC, waist circumference, HbA1c, hemoglobin A₁c

Characteristics of participants at baseline before taking the supplements.

*P <0.05
### Table 8: Serum and Urine Values of Participants at Baseline

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo</th>
<th>Potassium Phosphate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S-Glu (mg/dL) T₀</strong></td>
<td>95.30±2.40</td>
<td>101.40±6.20</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>S-Glu (mg/dL) T₂hr</strong></td>
<td>117.20±10.00</td>
<td>119.40±13.00</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>S-Ins (µU/mL) T₀</strong></td>
<td>11.41±1.70</td>
<td>7.50±1.50</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>S-Ins (µU/mL) T₂hr</strong></td>
<td>54.70±6.50</td>
<td>38.50±5.70</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>S-P (mg/dL) T₀</strong></td>
<td>4.11±0.15</td>
<td>4.35±0.54</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>S-P (mg/dL) T₂hr</strong></td>
<td>3.41±0.12</td>
<td>3.83±0.10</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>GLP 1 T₀</strong></td>
<td>36.80±3.90</td>
<td>43.90±6.50</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>GLP 1 T₂hr</strong></td>
<td>27.20±3.50</td>
<td>35.80±5.40</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>CRP (mg/dL)</strong></td>
<td>9.82±1.20</td>
<td>9.80±0.98</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**Urine**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ca</strong></td>
<td>9.56±1.20</td>
<td>10.17±1.50</td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>177.90±20.00</td>
<td>206.00±23.00</td>
</tr>
<tr>
<td><strong>Ca/Cr</strong></td>
<td>0.07±0.02</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>80.00±9.30</td>
<td>88.40±9.90</td>
</tr>
<tr>
<td><strong>P/Cr</strong></td>
<td>0.47±0.04</td>
<td>0.47±0.34</td>
</tr>
</tbody>
</table>

Abbreviations: S-Glu, serum glucose; S-Ins, serum insulin; S-P, serum phosphorus; GLP1, Glucagon-like-peptide 1; CRP, C-reactive protein; Ca, calcium; Cr, creatinine; P, phosphorus; T₀, time zero; T₂hr, time 2 hours

Serum and urine values of participants at baseline before taking the supplements.

*P <0.05
CHAPTER VII

CHRONIC EFFECT OF PHOSPHORUS

RESULTS

A. Body Weight and Body Mass Index

At the beginning of the experiment the two groups did not have a significant
difference with respect to body weight and BMI ($p=0.34$ and $P=0.06$ respectively)
(Table 7). However what can be noticed is that the placebo containing group has a
slightly higher weight (92.30±3.20 kg)and BMI (33.73±0.84 kg/m$^2$)than the potassium
phosphate group (87.70±3.50kg; 31.40±0.86kg/m$^2$), but this was not significant. The
experimental duration was of 3 months/person. By the end of the experiment in terms of
body weight and BMI between the two groups there was a significant difference with a
greater reduction in weight and BMI in the phosphorus supplemented as compared to
Placebo group (Table 9). The placebo group had an increase in weight of1.13±0.45 kg
and for the phosphorus supplemented group a reduction of 0.44±0.53 kg was noted after
3 months treatment with a $p$ value of 0.03. For BMI similar results were noted. There
was an increase in BMI of 0.42±0.18kg/m$^2$ in the placebo group and a drop in BMI was
noted of0.16±0.18kg/m$^2$ in the phosphorus supplemented group with a $p$ value of 0.03
(Table 9).

B. Waist Circumference

At baseline, no significant difference was noted between the two groups with
respect to waist circumference $p= 0.25$ (Table 7). However, at the end of the experiment
a highly significant decrease in waist circumference was noticed in the phosphorus
supplemented as compared to Placebo group (Table 9). There was an increase in the waist circumference of 0.38±0.40 cm in the placebo group after 3 months and a reduction of 0.35±0.60 cm in the phosphorus supplemented group with a p value of 0.00 (Table 9).

C. HbA1c

Our subjects were not diabetic therefore they had normal HbA1c levels and there was no significant difference between the two groups at the beginning of the experiment p= 0.36 (Table 7). After supplementation for 3 month period there was no significant difference and no significant decrease in HbA1c was also noted (p=0.97) (Table 9).

Table 9: Comparision of weight, WC, BMI, HbA1c after 3 months treatment

<table>
<thead>
<tr>
<th>Group Baseline</th>
<th>Group After 3 months</th>
<th>Difference Baseline- 3 months</th>
<th>P at baseline</th>
<th>P After 3 months treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Potassium Phosphate</td>
<td>Placebo Potassium Phosphate</td>
<td>Placebo Potassium Phosphate</td>
<td>Placebo Potassium Phosphate</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.30±3.20</td>
<td>87.70±3.50</td>
<td>93.46±3.31</td>
<td>87.28±3.54</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>109.43±2.20</td>
<td>105.66±2.40</td>
<td>109.8±2.20</td>
<td>102.2±2.50</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.73±0.84</td>
<td>31.40±0.86</td>
<td>34.14±0.85</td>
<td>31.24±0.88</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.44±0.09</td>
<td>5.63±0.18</td>
<td>5.25±0.10</td>
<td>5.44±0.15</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; WC, waist circumference, HbA1c, hemoglobin A₁c
Comparison of anthropometric measurements (weight, wc), BMI, and HbA1c between the two groups (placebo vs phosphorus) at baseline and 3 months (at end of study) post supplementation.
*P <0.05

D. Serum Glucose
At baseline serum glucose was normal among the subjects indicating that they were not diabetic. Since both groups were not diabetic, no significant difference was noted at T₀ (p=0.36) or T₂hr post OGTT (p=0.89)(Table 8) and also at the end of the treatment no significant change was apparent in the blood glucose value from baseline at T₀(p=0.72) and T₂hr (p= 0.74) post OGTT (Table 10).

E. Serum Phosphorus

With respect to serum phosphorus, participants in both groups had the mean serum phosphorus levels at baseline within the normal range of 2.5-4.5mg/dL(Table 8) and there was no significant difference among the group at baseline T₀. However after the OGTT after 2 hours serum phosphorus levels slightly dropped but remained within the normal range p=0.01 (Table 8) and the drop was slightly more in the placebo group. Similar result were noted after 3 months of treatment (Table 10) with also a slight drop of phosphorus after 2 hours post OGTT p=0.02.
### Table 10: Serum analysis after 3 months treatment

<table>
<thead>
<tr>
<th></th>
<th>Group at Baseline</th>
<th>Group After 3 months</th>
<th>Difference Baseline - 3 months</th>
<th>P at baseline</th>
<th>P after 3 months treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Potassium Phosphate</td>
<td>Placebo</td>
<td>Potassium Phosphate</td>
<td></td>
</tr>
<tr>
<td><strong>S-Glu (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_0)</td>
<td>95.30 ±2.40</td>
<td>101.40 ±6.20</td>
<td>93.90 ±2.32</td>
<td>101.43 ±5.98</td>
<td>-1.35 ±2.21</td>
</tr>
<tr>
<td>(T_{2hr})</td>
<td>117.20 ±10.00</td>
<td>119.40 ±13.00</td>
<td>114.00 ±9.05</td>
<td>113.30 ±14.00</td>
<td>-3.15 ±6.69</td>
</tr>
<tr>
<td><strong>S-P (mg/dL)</strong></td>
<td>4.11 ±0.15</td>
<td>4.35 ±0.54</td>
<td>4.22 ±0.13</td>
<td>4.18 ±0.10</td>
<td>0.11 ±0.14</td>
</tr>
<tr>
<td>(T_0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_{2hr})</td>
<td>3.41 ±0.12</td>
<td>3.83 ±0.10</td>
<td>3.61 ±0.12</td>
<td>3.72 ±0.07</td>
<td>0.16 ±0.06</td>
</tr>
<tr>
<td><strong>CRP (mg/dL)</strong></td>
<td>9.82 ±1.20</td>
<td>9.80 ±0.98</td>
<td>11.07 ±1.50</td>
<td>9.71 ±1.00</td>
<td>1.25 ±0.91</td>
</tr>
</tbody>
</table>

Abbreviations: S-Glu, serum glucose, S-P, serum phosphorus, CRP, C-reactive protein, \(T_0\), time zero, \(T_{2hr}\), time 2 hours.

Comparison of S-Glu, S-P, and CRP between the two groups (placebo vs phosphorus) at baseline and 3 months (at end of study) post supplementation.

\*P <0.05.

**F. Serum Insulin**

The subjects did not have insulin resistance and there was no significant difference at baseline \(T_0\) and \(T_{2hr}\) =0.09 and \(p=0.07\) respectively, between the two treatment groups(Table 8). After 3 months treatment there was also no significant difference at \(T_0\) and \(T_{2hr}\) =0.56 and \(p=0.12\) respectively(Table 11).
G. Serum GLP-1 and CRP

Similar results were found with respect to serum GLP-1 in that there was no significant difference between the groups at T₀ and 2 hours post OGTT both at baseline and 3 months after treatment, \( p=0.72 \) and 0.74 respectively (Table 11).

The normal reference range for CRP is 0-0.9mg/dL (Paula et al., 1998) and in this study our subjects had elevated CRP levels in both groups (placebo at baseline 9.82±1.20mg/dL and potassium phosphate 9.80±0.98mg/dL), but with no significant difference among the 2 groups at the start of the experiment \( p=0.99 \). There was also no significant difference after 3 months treatment \( p=0.33 \) and CRP was still elevated in both groups (placebo: 11.07±1.50mg/dL; potassium phosphate: 9.71±1.00mg/dL) (Table 10).
Table 11: Serum Insulin and GLP-1 analysis after 3 months treatment

<table>
<thead>
<tr>
<th></th>
<th>Group at Baseline</th>
<th>Group After 3 months</th>
<th>P at baseline</th>
<th>P after 3 months treatment</th>
<th>P Value Difference Baseline - 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Potassium Phosphate</td>
<td>Placebo</td>
<td>Potassium Phosphate</td>
<td></td>
</tr>
<tr>
<td><strong>S- Ins (mg/dL) T₀</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.41 ±1.70</td>
<td>7.50 ±1.50</td>
<td>8.79 ±1.29</td>
<td>10.20 ±1.97</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>S- Ins (mg/dL) T₂hr</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54.70 ±6.50</td>
<td>38.48 ±5.69</td>
<td>48.47 ±6.56</td>
<td>33.84 ±6.34</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>GLP1 T₀</strong></td>
<td>36.83 ±3.90</td>
<td>43.86 ±6.53</td>
<td>37.29 ±4.05</td>
<td>37.72 ±3.14</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>GLP1 T₂hr</strong></td>
<td>27.24 ±3.51</td>
<td>35.80 ±5.42</td>
<td>26.76 ±2.85</td>
<td>28.76 ±2.80</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Abbreviations: S-Ins, serum insulin, GLP1, Glucagon-like-peptide 1, T₀, time zero, T₂hr, time 2 hours
Comparison of S-Gluc, S-P, and CRP between the two groups (placebo vs phosphorus) at baseline and 3 months (at end of study) post supplementation.
*P < 0.05.

### H. Urine

Urine was tested for calcium, creatinine, phosphorus, calcium/creatinine ratio and phosphorus to creatinine ratio. Among all of these no significant difference was observed between the two groups at baseline and no significant change was evident after 3 months of treatment (Tables 8 and 12).
Table 12: Urine analysis after 3 months treatment

<table>
<thead>
<tr>
<th>Group At Baseline</th>
<th>Group After 3 months</th>
<th>P at baseline</th>
<th>P after 3 months treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Potassium Phosphate</td>
<td>Placebo</td>
<td>Potassium Phosphate</td>
</tr>
<tr>
<td>Ca</td>
<td>9.56±1.20</td>
<td>10.17±1.50</td>
<td>13.44±2.38</td>
</tr>
<tr>
<td>Cr</td>
<td>177.90±20.00</td>
<td>206.00±23.00</td>
<td>187.60±17.2</td>
</tr>
<tr>
<td>Ca/Cr</td>
<td>0.07±0.02</td>
<td>0.06±0.01</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>P</td>
<td>80.00±9.30</td>
<td>88.40±9.90</td>
<td>76.93±8.09</td>
</tr>
<tr>
<td>P/Cr</td>
<td>0.47±0.04</td>
<td>0.47±0.34</td>
<td>0.43±0.04</td>
</tr>
</tbody>
</table>

Abbreviations: Ca, calcium; Cr, creatinine; P, phosphorus.
Comparison of urine Ca, Cr, Ca/Cr, P, P/Cr between the two groups (placebo vs phosphorus) at baseline and 3 months (at end of study) post supplementation.

* P <0.05
CHAPTER VII

DISCUSSION

Overconsumption, an unbalanced diet, and interrupted metabolism in obesity, are all connected with some risk of hypophosphataemia, which in turn could account for the hyperglycaemia (Bell, 1993); (Friedlander and Rosenthal, 1926); (Knochel, 1977), hypertension and dyslipidaemia (Standl, 1995) presented in the literature as metabolic syndrome or insulin resistance syndrome (Fox et al., 1964). The low S-P concentration may have been due to phosphate depletion caused by an unbalanced diet, trans-cellular shift, and/or increased phosphate excretion (Bolliger and Hartman, 1925); (DeFronzo and Lang, 1980); (Himsworth, 1935); (Simonson and DeFronzo, 1982). The present hypothesis is based on the well-known relationship between carbohydrate metabolism and phosphorylation in the intermediate metabolism. Dietary composition was correlated to glucose tolerance many years ago (Himsworth, 1935). Early investigations into phosphate metabolism also revealed the participation of phosphate in carbohydrate metabolism and it was shown that insulin transports phosphate and glucose from the extra- to the intracellular spaces (Bolaert et al., 1992); (Bolliger and Hartman, 1925). It was suggested for the first time in 1926 that hypophosphataemia per se may contribute to impaired glucose tolerance (Friedlander and Rosenthal, 1926).

For the acute study all of our subjects were female since the duration for each test day was 5 hours and men were not available due to their work conditions. This factor is a strength in that we were able to compare the effect of phosphorus on a single gender, but it is a drawback since there have been previous experimental and clinical studies in
which gender differences in phosphate metabolism have been reported. Women do have higher S-P levels than men, and there is an increase in S-P with age in women, in contrast to the decrease with age in men. This might be an explanation as to why women are protected from cardiovascular disease during the fifth decade of life whereas men are not (Dominiguez et al., 1976) (Haeglin et al., 2001); (Schwarz et al., 1985). So as a result in this case women might have not been phosphorus deficient to start off with since blood tests were not performed. It is recommended that a study be done on overweight/obese men to be able to compare the effect of phosphorus appetite.

Another drawback for the acute study was that we were not able to control the breakfast that was consumed by the subjects and each subject had their usual breakfast and this might have had influence on the appetite. This breakfast might have been high in fructose, glucose, or protein. Several studies have shown that an increased consumption of fructose, mainly in the form of high-fructose corn syrup (HFCS), has paralleled the rise in obesity and this has raised a concern over fructose’s involvement in weight gain (Barnard and Wen, 1994); (Bollaert et al.,1992); (Obeid et al., 2010). Fructose is known to have ‘phosphate-sequestering’ capacity, by virtue of which the phosphate attaches covalently to organic molecules, rendering it unavailable to participate in other essential metabolic reactions, including the regeneration of ATP (Bizeau and Pagliassotti, 2005); (Champe and Harvey, 1994); (Mayes,1993); (Obeid et al., 2010). This was demonstrated in humans, in whom the intravenous administration of fructose (250mg per kg of body weight over 5 min) resulted in a 75% reduction in hepatic ATP concentration (Obeid et al., 2010). In addition, fructose administration to rats was reported to reduce plasma Pi, hepatic Pi and ATP, whereas previous phosphate loading attenuated the reductions of
ATP and Pi, and a strong correlation was found between hepatic Pi and ATP (Mayes, 1993). Moreover, insulin release following glucose ingestion is known to increase extrahepatic phosphorus uptake, which would compromise hepatic phosphorus availability. Hence, both glucose and fructose seem to have the potential of reducing phosphorus availability for hepatic ATP production, but the magnitude of such an effect or its implication for energy intake is not clear (Bizeau, 2005). On the other hand the high phosphorus content of protein may be implicated in the reduction of energy intake under conditions of increased protein intake (Halton and Hu, 2004); (Latner and Schwartz, 1999), as well as in the decrease in body weight and fat mass under an isocalorically high-protein diet (Wycherley et al., 2012). In addition, the high phosphorus content of milk may partially explain the inverse association between dairy product intake and body weight, especially given that calcium failed to clarify such an association (Teegarden, 2005); (Wagner et al., 2007); (Yanovski et al., 2009).

Previous human studies indirectly support a potential role for hepatic ATP in energy and body weight regulations (Abdelmalek et al., 2012); (Nair et al., 2003). In line with that a previous study conducted by Obeid et al. has found that the addition of 500 mg phosphorus to different carbohydrate preloads caused a substantial reduction in ad libitum subsequent energy intake (27–33%) (Obeid et al., 2010). However, in comparison to previous results, our data for the acute study did not find any significant results. Taking phosphorus or placebo supplements as a preload once per week 80 minutes prior to pizza consumption did not result in a significant decrease in the amount consumed. There was no significant difference as well between different dosages of phosphorus with respect to food and water intake. One explanation could be that the
supplements in the study were taken 3 hours after consuming breakfast with water and not a carbohydrate preload that might have had an effect on limiting the hepatic uptake of phosphorus. These findings are not in support of the hypothesis that phosphorus content of a preload reduces subsequent food intake in overweight/obese subjects.

For our chronic study we realized that the placebo group was slightly more overweight than the phosphorus supplemented group and this might have had an influence on a greater drop in serum phosphorus after OG GT, and this is in line with previous studies in which obese subjects tend to have lower serum phosphorus levels than the non-obese. And also most of the subjects were women and not men and this also might have had an effect on the serum phosphorus being within the normal range. After a 12 hour fast there is a decrease in urine phosphorus and by performing a spot urine test is not sufficient. It might have been more efficient if a 24-hour urine collection was performed to measure subject compliance with the intake of the supplements.

Our results are in line with the previous study conducted by Obeid et al. in which phosphorus supplementation had an effect on the subsequent weight regulation(Obeid et al., 2010). We had a significant decrease in weight, BMI, and WC in the phosphorus supplemented group as compared the placebo group. There was no significant difference with respect to HbA1c, insulin and glucose. In this case phosphorus may provide a link between different observations associated with increased body weight or energy intake. In the postprandial state, phosphorus is utilized for the phosphorylation of many metabolites and its uptake by extrahepatic tissue is stimulated by insulin release, and thus its availability for hepatic ATP production may be compromised. Therefore, it is
reasonable to postulate that the provision of a surplus amount of phosphorus in one meal would improve the hepatic phosphorus status or would offset any reduction caused by the second meal. This in turn would accelerate hepatic ATP synthesis, leading to an early termination of the eating episode (Hong et al., 2000); (Rawson and Freidman, 1994); (Rawson et al., 1994). And as a result will lead to a reduction in weight, BMI, and WC as was noted in our chronic study.

According to a study conducted by Jaedig et al., (1994) obese (but not lean) individuals respond with an increase in postprandial thermogenesis after treatment with K-Mg-phosphate solutions. This indicates the involvement of phosphate in thermogenesis and in the regulation of the basal metabolic rate in obese people (Reaven, 1988). A reduced metabolic rate due to an imbalance between phosphate and carbohydrates in the diet, indicated by the inverse relation between S-P and B-glu, may have contributed to the increase in body fat and high BMI values (Barnard and Wen, 1994); (Harter et al., 1976); (Nichols, 1997). However with respect to the current study our participants were overweight/obese but did not have hypophosphatemia.

Another study performed by Kalaitzidis et al. (2005) showed that patients with metabolic syndrome have significantly lower phosphate levels compared to healthy individuals. A reduced phosphate level in patients with metabolic syndrome may decrease the peripheral utilization of glucose, thus leading to the development or exacerbation of insulin resistance. Haap et al. (2006) showed that a low serum phosphate level was associated with reduced insulin sensitivity. In a published Coronary Artery Risk Development in Young Adults study (Pereira et al., 2000), it showed that
overweight individuals with a high consumption of dairy products (that contain large quantities of phosphate) had a significantly lower risk of metabolic syndrome compared with those who consumed less dairy (Pereira et al., 2000).

It has also been shown that a carbohydrate meal or infusion (e.g., 5% dextrose) decreases serum phosphorus concentration, because phosphate shifts into intracellular fluid as a result of stimulation of glycolysis and formation of phosphorylated glycolytic intermediates in muscle, liver, and adipose cells (DeFronzo and Lang, 1980); (Haglin, 2001); (Marshall et al., 1978); (Paula et al.; 1998). In contrast, protein intake increases serum phosphorus concentration because of the relatively high phosphorus content of protein-rich diets (Haglin, 2001).

This has been shown in a study conducted by Khattab et.al (2011) that revealed that the inclusion of phosphate into OGTT was able to prevent the reduction in both total and inorganic phosphates, and at the same time, phosphate addition was able to reduce both insulin and HOMA at 60 minutes. They came to a conclusion that phosphate seemed to improve insulin sensitivity after an oral glucose load (Khattab et.al., 2011). However in contrary to these studies, our subjects were not phosphorus deficient to start with. Also food intake was not controlled for the subjects, nor was the supplement specified with what food it needed to be consumed with. Subjects were to take the supplements with their meal, and this meal could have contained enough proteins and as a result enough phosphorus instead of it being a glucose load to enhance phosphorus uptake. In our study there was no significant difference between the control group and the group taking phosphorus with respect to insulin sensitivity or glucose tolerance or
HbA1c after 3 months treatment, even though there was a significant reduction in weight, BMI, and WC with a in the group taking phosphorus as compared to the control group.
CHAPTER IX
CONCLUSION AND RECOMMENDATIONS

The increases in obesity during the past few decades have paralleled modernization (industrialization, globalization of food markets, etc.) and several changes in dietary habits (Drewnowski and Popkin, 1997). These are mainly related to the dramatic increase in the consumption of refined cereals (where refinement reduces phosphorus content by about 70%) and oils, sugars, and sweeteners such as HFCS which contain negligible amounts of phosphorus (Ervin et al., 2004); (Popkin, 2006); (Popkin and Girdib-Larsen, 2004); (Swinburn et al., 2004). Given the increased prevalence of obesity among people consuming high quantities of food with low levels of phosphorus, it is reasonable to postulate that low phosphorus intake may be involved in the development of obesity (Bray et al. 2004).

Based on our acute study results, we cannot conclude that different phosphorus content of a preload reduces subsequent food intake in overweight/obese subjects.

However, we can conclude from our chronic study result that daily phosphorus supplementation of 375mg three times per day with each main meal (Breakfast, Lunch, and Dinner) over a period of 3 months was significantly associated with decreased body weight, BMI, and waist circumference. However, there was no significant effect on HbA1c, glucose, insulin, and GLP-1.
According to the results, one must consider, in subsequent studies, the administration of phosphorus in combination with a carbohydrate preload to test the effect on glucose, insulin, and HbA1c. In addition, it would be interesting to test the effect of phosphorus on pre-diabetic or diabetic subjects.

Following extensive investigations, phosphorus may be a new target for the development of supplements for appetite control and obesity reduction. The 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 elucidated.
BIBLIOGRAPHY


96. www.hosp.uky.edu/Clinlab/report.pdf [Online].


APPENDIX I

SBS NUTRITION CONSENT ENGLISH

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

Title of experiment: Determining the effect of different doses of phosphorus preloads on subsequent food intake.

Principal Investigator: Dr. Omar Obied/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Co-Investigator: Sami Aziz, Sami Hasim
Researchers: Marwa Hassan, Marielle 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 - Faculty of Agriculture and Food Sciences/Department of Nutrition/Organooleptics study room

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. It also describes 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 bodies for normal function. It has been found that phosphorus supplementation taken before meals has the potential to reduce meal size. However, the precise dose that will produce such an effect has not been elucidated yet. Thus, the purpose of the present study is to provide different quantities of phosphorus (tablets or solution) and to compare the effect of the different doses on subsequent food intake. This research study is part of a master thesis and is being conducted with the goal of publication in a scientific journal and possibly presentation at academic conferences.

B. Project/Procedures Description:
Subjects' recruitment will be done either by posters or direct approaching.

In this study subjects 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 the study. Screening criteria include: 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. The only preparation you need to do on your behalf is to come fasting for the last 12 hours and stop the ingestion of any nutritional supplement.

Following a 12 hour (overnight) fast, you will be taken to the testing facility (Faculty of Agriculture and Food Sciences/Department of Nutrition/Organooleptics study room) where anthropometric measurements (height, weight) will be taken, you will be asked to fill the Three-Factor Eating questionnaires and questionnaires about your health. Following that, you will be provided with a standard breakfast 4 hours before consuming the phosphorus supplement or placebo. During the 4 hours following the breakfast, you will be asked to avoid any additional food intake, except for water that will be allowed up to 1 hour prior to the study time. On every test day, you will be taking a different quantity of phosphorus supplement or placebo, however, the amount of phosphorus and placebo supplements will be equal to ensure blinding.

Upon arrival to each session, you will be asked questions related to the compliance with the required fasting time, the routine exercise and alcohol intake during the previous night. If any deviation should occur, we will reschedule for another testing date.
Before consuming the phosphorus supplement or placebo, you will be asked to complete an appetite questionnaire and this will be repeated at 15, 30, 45, 60 and 75 minutes after the tablets ingestion. Also, you will be answering questions relate to the palatability of the supplement or placebo immediately after their ingestion. After 80 min of consuming the phosphorus supplement or placebo, a pizza for lunch plus water will be offered. You will be asked to eat freely until feeling "comfortably full".

After each experiment, weight will be measured again. This study is a randomized control study and a total of 15 subjects (overweight/obese; BMI ≥ 25 kg/m²) would be required for its completion.

C. Duration: The estimated time to complete this study is approximately five weeks. You will have to visit the testing facility (Organoleptic room) located in the Faculty of Agriculture and Food Sciences/Department of Nutrition five times and each visit will require you to stay for a period of about 6 hours. Each visit will be separated by a minimum of 1 week. Participants may wish to go and come back and they will be reminded via SMS to continue the experiment.

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 does not involve any physical risk or emotional risk to you beyond the risks of daily life. You will have to take the phosphorus supplements or placebo and then eat a pizza. The different phosphorus doses that you will be provided with are all below the upper limit of recommendation and are proven to be safe. However, there may be unforeseen risks such as allergic reaction, nausea, vomiting, stomach pain, and diarrhea. We have conducted several experiments using the same dose of Phosphorus and received no complaints of adverse effects or discomfort. Subjects showing any side effect will be excused.

You receive no direct benefits from participating in this research; however, when phosphorous is added to the different meals, it was found to reduce the energy intake in the next meal. Therefore, by investigating the quantity and the durational response of phosphorous on the control of subsequent food intake, 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 phosphorus intake. This significant new finding will be conveyed to subjects.

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 pizza for lunch. There will neither be anticipated expenses for participating and costs for transportation, parking etc will not be reimbursed.

Institutional Review Board
American University of Beirut
NUT.00.11  April 18th, 2012

2 | P a g e
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 oob@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; oob@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 and understand the above information. I agree to participate in the research study.

Participant Name: ___________________________ Date: __________________

Participant Signature: ___________________________

Printed Name of person authorized to consent for subject: ___________________________

Relationship to Subject: ___________________________

Signature of Person authorized to consent: ___________________________ Date: __________________

Documentation of Consent:
Printed Name of Person obtaining Consent: ___________________________

Signature of Person obtaining Consent: ___________________________

Date: ___________ Time: ___________

April 18th, 2012

Institutional Review Board
American University of Beirut
APPENDIX II
SBS NUTRITION CONSENT ARABIC

This text is in Arabic and contains information about institutional review board approval for studies involving human participants. The document is related to nutrition and consent, possibly for dietary studies or interventions. Specific details about the study design, methodology, and ethical considerations for participants are included, ensuring that all necessary permissions and approvals are in place for the research to proceed ethically and legally.
تعتمد هذه الدراسة المدى الزمني للاختبارات على فترات زمنية مختلفة. تم استناد النظر في هذه الدراسة إلى النتائج السابقة، فضلاً عن مجموعة من الدراسات السابقة التي اعتمدت على النتائج الزمنية المختلفة. لقد تم الأخذ في الاعتبار أن تأثيرات الفصول والمواسم مختلفة. حيث أن الدراسة أثبتت أن زيادة الفصول على مساحة الأراضي بالسعة من قبل تقليد جمع الوجبة، التي تحدث فيها تغييرات عبر الفصول، من دون تقديرها بشكل صحيح، قد تكون مكملات تقدير الفصول، لذا فإن استخدام هذه الطرق تحلل توقعات المكملات تحتوي على الفصول، مما سيعمل على تحسين نتائجنا الفائقة عن طريق زيادة تنfdننات الفصول. أي معنى مهما تلت من هذا البحث ستكون مشكلتنا بالمشاركون.

(1) السحالي: تقديم سري إيجابي، يمكن أن يؤدي إلى عديد من المكملات الأخرى التي تكون متعلقة بما يمكن أن يكون للسحالي. جميع المعلومات والمواد متضمنة في عرف نقطة أو مرجعية لديه وزم مثير. الوصول إلى المعلومات مختلفة. فكل النقاط الرئيسية والناجحة التي يعملون مباشرة على الدراسة. جميع المعلومات المتضمنة بشكل عام من قبل الدراسات السابقة، تتعلق بحالة الدراسة. لا يمكن أن يجعل نسخة مبتكرة بكلمات غير دليلية أو قانونية لتكون من المكملات التي تتضمن هذه النتائج، حيث يجب أن يكون السحالي مثيرًا. فلا يمكن أن يكون للسحالي الصناعية المختلفة باتجاهه أن تُطلق على أساس المراجعة الموسوية في الجامعة الأمريكية. في بروتوكول غير ثابتة في النتائج، يمكن أن تكون مكملات تقديمية أو مفيدة أو تجريبية، أو دولة أخرى، أو راعي الدراسة، إذا وجد أي وكالة تهدف إلى البحث، أو (2)ائي أو بيتيًا) مجانية وفقًا للمناقشة:

(3) مقصد للإصابات ذات صلة بالبحث: من خلال تحقيق معلومات عبر مواقف متوقعة. إننا نعترف إلى إعداد جزء البحث، أو لأي سؤال عن الإصابات المتصلة بالبحث، يجري الاتصال بالدكتور عمر عبد 350000 (01) مقطع 4403; email: ooo1@aub.edu.lb

NUT.00.11
April 18th, 2012
Institutional Review Board
American University of Beirut
6.7 MAY 2012
APPROVED

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حقوق المشاركين: المشاركة في هذا البحث طوعية. يمكنك مقادرة البحث في أي وقت من دون أي عقوبة. إن قرارك بعدم المشاركة لا يؤثر على مساعدتك في الجامعة الأمريكية في بيروت. هل لديك أي استفسارات حول المعلومات الواردة أعلاه؟ هل ترغب في المشاركة في هذا البحث؟

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

ملخصاً: للمشاركة الكاملة بإيجاب أو من تابعة مشاركاتك في هذا البحث.

موافقة المشترك:
قد قررت إتمام هذه الحفظة وفقاً على إجراء هذا البحث وافق على الإشراك فيه. إتفاق المشترك

الإسم المطبع للشخص الملون للمواقة من أجل الشخص:

العلاقة بالشخص:

إبضاء الشخص الملون للمواقة:

توقيع الموثقة:

الإسم المطبع للمواكلة التي يطلب الموافقة:

إبضاء الشخص الذي يطلب الموافقة:

التاريخ:

NUT.00.11 April 18th, 2012

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American University of Beirut
6/7 MAY 2012
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APPENDIX III

HEALTH QUESTIONNAIRE

Name: ____________________________  Subject number: ____________________________

Preheat: __________________________

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

Questions on palatability of test meals
Please answer the following questions:

1. Do you suffer from one or more of the following?
   a. Diabetes
   b. Heart disease
   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
Weekly Compliance questionnaire

1. Did you have the required breakfast (man’ousheh) this morning?
   □ Yes    □ No
   If the answer is “No” please inform the investigator

2. At what time did you have the breakfast? ____________

3. Did you eat or drink anything after having breakfast except for water?
   □ Yes    □ No

4. Did you eat or drink anything for the past hour?
   □ Yes    □ No

5. Have you done any kind of any unusual strenuous exercise yesterday?
   □ Yes    □ No

6. Did you drink any kind of alcoholic beverages last night?
   □ Yes    □ No
### APPENDIX IV

### THREE FACTOR EATING QUESTIONNAIRE ENGLISH

#### Three- Factor Eating Questionnaire/Part I

<table>
<thead>
<tr>
<th>Name:</th>
<th>Subject number:</th>
<th>Date:</th>
<th>T</th>
<th>F</th>
<th>Factor Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>I usually eat too much at social occasions, like parties and picnics.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>I am usually so hungry that I eat more than three times a day.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>When I have eaten my quota of calories, I am usually good about not eating any more.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Dieting is so hard for me because I just get too hungry.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>I deliberately take small helpings as a means of controlling my weight.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Sometimes things just taste so good that I keep on eating even when I am no longer hungry.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>When I feel anxious, I find myself eating.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Life is too short to worry about dieting.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Since my weight goes up and down, I have gone on reducing diets more than once.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>I often feel hungry that I just have to eat something.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>When I am with someone who is overeating, I usually overeat too.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>I have a pretty good idea of the number of calories in common food.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Sometimes when I start eating, I just can't seem to stop.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>It is not difficult for me to leave something on my plate.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>At certain times of the day, I get hungry because I have gotten used to eating then.</td>
<td></td>
<td>T</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

Three- Factor Eating Questionnaire/Part 1

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>18.</td>
<td>While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.</td>
</tr>
<tr>
<td>19.</td>
<td>Being with someone who is eating often makes me hungry enough to eat also.</td>
</tr>
<tr>
<td>20.</td>
<td>When I feel blue, I often overeat.</td>
</tr>
<tr>
<td>21.</td>
<td>I enjoy eating too much to spoil it by counting calories or watching my weight.</td>
</tr>
<tr>
<td>22.</td>
<td>When I see a real delicacy, I often get so hungry that I have to eat right away.</td>
</tr>
<tr>
<td>23.</td>
<td>I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.</td>
</tr>
<tr>
<td>24.</td>
<td>I get so hungry that my stomach often seems like a bottomless pit.</td>
</tr>
<tr>
<td>25.</td>
<td>My weight has hardly changed at all in the last ten years.</td>
</tr>
<tr>
<td>26.</td>
<td>I am always hungry so it is hard for me to stop eating before I finish the food on my plate.</td>
</tr>
<tr>
<td>27.</td>
<td>When I feel lonely, I console myself by eating.</td>
</tr>
<tr>
<td>28.</td>
<td>I consciously hold back at meals in order not to gain weight.</td>
</tr>
<tr>
<td>29.</td>
<td>I sometimes get very hungry late in the evening or at night.</td>
</tr>
<tr>
<td>30.</td>
<td>I eat anything I want anytime I want.</td>
</tr>
<tr>
<td>31.</td>
<td>Without even thinking about it, I take a long time to eat.</td>
</tr>
<tr>
<td>32.</td>
<td>I count calories as a conscious means of controlling my weight.</td>
</tr>
<tr>
<td>33.</td>
<td>I do not eat some foods because they make me fat.</td>
</tr>
<tr>
<td>34.</td>
<td>I am always hungry enough to eat at any time.</td>
</tr>
<tr>
<td>35.</td>
<td>I pay a great deal of attention to changes in my figure.</td>
</tr>
<tr>
<td>36.</td>
<td>While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.</td>
</tr>
</tbody>
</table>

Name:  Subject number:  

**Directions**: Please answer the following questions by circling the number above the response that is appropriate to you.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
<th>Factor number</th>
</tr>
</thead>
<tbody>
<tr>
<td>37. How often are you dieting in a conscious effort to control your weight?</td>
<td>1 Rarely</td>
<td>2 Sometimes</td>
</tr>
<tr>
<td>38. Would a weight fluctuation of 2 kg affect the way you live your life?</td>
<td>1 Not at all</td>
<td>2 Slightly</td>
</tr>
<tr>
<td>39. How often do you feel hungry?</td>
<td>1 Only at mealtimes</td>
<td>2 Sometimes between meals</td>
</tr>
<tr>
<td>40. Do your feelings of guilt about overeating help you to control your food intake?</td>
<td>1 Never</td>
<td>2 Rarely</td>
</tr>
<tr>
<td>41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?</td>
<td>1 Easy</td>
<td>2 Slightly difficult</td>
</tr>
<tr>
<td>42. How conscious are you of what you are eating?</td>
<td>1 Not at all</td>
<td>2 Slightly</td>
</tr>
<tr>
<td>43. How frequently do you avoid “stocking up” on tempting foods?</td>
<td>1 Almost never</td>
<td>2 Seldom</td>
</tr>
<tr>
<td>44. How likely are you to shop for low calorie foods?</td>
<td>1 Unlikely</td>
<td>2 Slightly unlikely</td>
</tr>
<tr>
<td>45. Do you eat sensibly in front of others and splurge alone?</td>
<td>1 Never</td>
<td>2 Rarely</td>
</tr>
</tbody>
</table>

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

<table>
<thead>
<tr>
<th></th>
<th>1 Unlikely</th>
<th>2 Slightly likely</th>
<th>3 Moderately likely</th>
<th>4 Very likely</th>
</tr>
</thead>
</table>

47. How frequently do you skip dessert because you are no longer hungry?

<table>
<thead>
<tr>
<th></th>
<th>1 Almost never</th>
<th>2 Seldom</th>
<th>3 At least once a week</th>
<th>4 Almost every day</th>
</tr>
</thead>
</table>

48. How likely are you to consciously eat less than you want?

<table>
<thead>
<tr>
<th></th>
<th>1 Unlikely</th>
<th>2 Slightly likely</th>
<th>3 Moderately likely</th>
<th>4 Very likely</th>
</tr>
</thead>
</table>

49. Do you go on eating binges though you are not hungry?

<table>
<thead>
<tr>
<th></th>
<th>1 Never</th>
<th>2 Rarely</th>
<th>3 Sometimes</th>
<th>4 At least once a week</th>
</tr>
</thead>
</table>

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself?

- 0 → Eat whatever you want, whenever you want it
- 1 → Usually eat whatever you want, whenever you want it
- 2 → Often eat whatever you want, whenever you want it
- 3 → Often limit food intake, but often "give in"
- 4 → Usually limit food intake, rarely "give in"
- 5 → Constantly limit food intake, never "give in"

51. To what extent does this statement describe your eating behavior? "I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow."

<table>
<thead>
<tr>
<th></th>
<th>Not like me</th>
<th>Little like me</th>
<th>Pretty good</th>
<th>Describes me perfectly</th>
</tr>
</thead>
</table>

### APPENDIX V

#### THREE FACTOR EATING QUESTIONNAIRE ARABIC

Three-Factor Eating Questionnaire/Part I

<table>
<thead>
<tr>
<th>Factor number</th>
<th>Arabic Question</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>عندما كنت أرى عباءة من النسا، أجيم من الصحب، عم الأكل، حتى أو أني قد أنتهيت من كل وجهة نظر.</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>أنا عادة أكل بكثير في الملاهي الاجتماعية، مثل الحفلات والفرزات.</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>أنا عادة أكل بكثير في الوجبات الخفيفة في اليوم.</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>عندما أكون قد أكلت قليل من السعرات الحرارية، فقط للذاتي، بدأ أكل للقول.</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>إذا أكلت حاليا ما كان أكله لكون أكلة، ما إذا كان محبوبًا.</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>الراحة القدرة بدأ عند الطباعة على حليته.</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>16</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>إذا كنت أنا قد أكلت قليل من السعرات الحرارية، فقط للذاتي.</td>
<td>5</td>
</tr>
</tbody>
</table>

### Three-Factor Eating Questionnaire/Part I

<table>
<thead>
<tr>
<th>Question</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. If I eat too much of anything, I try to burn it off.</td>
<td>21</td>
</tr>
<tr>
<td>2. I eat more for pleasure than to satisfy hunger.</td>
<td>22</td>
</tr>
<tr>
<td>3. When I eat, I try to eat as much as possible.</td>
<td>23</td>
</tr>
<tr>
<td>4. I prefer to eat less food, but I feel hungry.</td>
<td>24</td>
</tr>
<tr>
<td>5. I eat less food when I am sleepy.</td>
<td>25</td>
</tr>
<tr>
<td>6. I eat more at breakfast than I eat at night.</td>
<td>26</td>
</tr>
<tr>
<td>7. I eat more when I am sad or depressed.</td>
<td>27</td>
</tr>
<tr>
<td>8. I eat more when I am happy or excited.</td>
<td>28</td>
</tr>
<tr>
<td>9. I eat more when I am tired or nervous.</td>
<td>29</td>
</tr>
<tr>
<td>10. I eat more when I am angry or upset.</td>
<td>30</td>
</tr>
<tr>
<td>11. I eat more when I am hungry.</td>
<td>31</td>
</tr>
<tr>
<td>12. I eat more when I am stressed.</td>
<td>32</td>
</tr>
<tr>
<td>13. I eat more when I am bored.</td>
<td>33</td>
</tr>
<tr>
<td>14. I eat more when I am tired or sleepy.</td>
<td>34</td>
</tr>
<tr>
<td>15. I eat more when I am relaxed.</td>
<td>35</td>
</tr>
<tr>
<td>16. If I eat less food, I feel hungry.</td>
<td>36</td>
</tr>
</tbody>
</table>

---

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Three-Factor Eating Questionnaire/Part II

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Factor Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>هل من المعاد ان تكون تنبيه كوبيلة لان نضيذ وزنك؟</td>
<td>4</td>
</tr>
<tr>
<td>38</td>
<td>هل تقلب وزنك بوجود 2.5 كيلو إثم على طريقة علاج حياتك؟</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>متى تشعر بالجوع؟</td>
<td>4</td>
</tr>
<tr>
<td>40</td>
<td>هل شعورك بالاثد عند الأرطا بأكمل يساعدك على أن تشكي كمية الأكل؟</td>
<td>4</td>
</tr>
<tr>
<td>41</td>
<td>كيف تصرف الجهد بنروف عن الأكل في متساء ورقتية الحناء وعدد الأكل لساعات مديدة قاملة؟</td>
<td>4</td>
</tr>
<tr>
<td>42</td>
<td>ما مدى وعيك للطعام الذي تنتحله؟</td>
<td>4</td>
</tr>
<tr>
<td>43</td>
<td>هل يمكن تجنبك للاطراف بكل الأطعمة المغرفة؟</td>
<td>4</td>
</tr>
<tr>
<td>44</td>
<td>ما مدى احتفال ان تنسام للطعام تحتوي على سرعات حرارية قليلة؟</td>
<td>4</td>
</tr>
<tr>
<td>45</td>
<td>هل تأكل بانتهاء أمام الخنادق وعليك بالذكاء اكل لحم الخنزير؟</td>
<td>4</td>
</tr>
<tr>
<td>46</td>
<td>ما مدى احتفال ان تتنام الأكل ينمو لكر مثقل ليفظ الطعام الذي تنتحله؟</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question</th>
<th>Response Options</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>47. Have you been feeling hungry on the job?</td>
<td>4, 3, 2, 1</td>
<td>4 3 2 1</td>
</tr>
<tr>
<td>48. How often do you eat a meal at home?</td>
<td>4, 3, 2, 1</td>
<td>4 3 2 1</td>
</tr>
<tr>
<td>49. Have you lost weight recently?</td>
<td>4, 3, 2, 1</td>
<td>4 3 2 1</td>
</tr>
<tr>
<td>50. How much do you feel satisfied with your eating habits?</td>
<td>0, 1, 2, 3, 4, 5</td>
<td>-5 -4 -3 -2 -1 0</td>
</tr>
<tr>
<td>51. Do you have a regular sleep pattern?</td>
<td>4, 3, 2, 1</td>
<td>4 3 2 1</td>
</tr>
</tbody>
</table>

## APPENDIX VI

### VISUAL ANALOGUE SCALE ENGLISH

<table>
<thead>
<tr>
<th>Name:</th>
<th>Subject number:</th>
<th>Date:</th>
</tr>
</thead>
</table>

**Preload:**

<table>
<thead>
<tr>
<th>A- Questions on appetite and desire for specific food types</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How hungry do you feel?</strong></td>
</tr>
<tr>
<td>I am not hungry at all</td>
</tr>
<tr>
<td><strong>How satisfied do you feel?</strong></td>
</tr>
<tr>
<td>I am completely empty</td>
</tr>
<tr>
<td><strong>How full do you feel?</strong></td>
</tr>
<tr>
<td>Not at all full</td>
</tr>
<tr>
<td><strong>How much do you think you can eat?</strong></td>
</tr>
<tr>
<td>Nothing at all</td>
</tr>
<tr>
<td><strong>Would you like to eat something sweet?</strong></td>
</tr>
<tr>
<td>Yes, very much</td>
</tr>
<tr>
<td><strong>Would you like to eat something salty?</strong></td>
</tr>
<tr>
<td>Yes, very much</td>
</tr>
<tr>
<td><strong>Would you like to eat something savoury?</strong></td>
</tr>
<tr>
<td>Yes, very much</td>
</tr>
<tr>
<td><strong>Would you like to eat something fatty?</strong></td>
</tr>
<tr>
<td>Yes, very much</td>
</tr>
</tbody>
</table>

APPENDIX VII

PALATABILITY QUESTIONNAIRE ENGLISH

B- Questions on palatability of the test

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Bad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual appeal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aftertaste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palatability</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX VIII

VISUAL ANALOGUE SCALE ARABIC
APPENDIX IX
PALATABILITY QUESTIONNAIRE ARABIC
APPENDIX X
CONSENT FORM ENGLISH

Title of Research Study: Premall 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-Samar, 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 ≥ 25 kg/m²) will be randomized to receive either placebo (cellulose) or potassium phosphate tablets (125 mg) 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.

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.

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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). Phosphorus 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 phosphorus intake. You will get specific nutritional advice 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.

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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
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

NUT.OO.11 March 9th, 2013

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APPENDIX XI
CONSENT FORM ARABIC

Institutional Review Board
American University of Beirut

3 MAY 2013

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لا يمكنني قراءة النص العربي المكتوب بشكل متحجج.

يرجى تقديم نص يمكنني قراءته بشكل طبيعي.

---

نوعية البيانات: غير محدد

تاريخ الخروج: 09/03/2013

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لا يوجد نص يمكن قراءته بشكل طبيعي من الصورة المقدمة.
## APPENDIX XII

DATA COLLECTION OF SUBJECTS

<table>
<thead>
<tr>
<th>Name</th>
<th>Subject</th>
<th>Phone num</th>
<th>Age</th>
<th>Gender</th>
<th>(M/F)</th>
<th>Data (start)</th>
<th>Weight</th>
<th>(start) kg</th>
<th>Height</th>
<th>(start) cm</th>
<th>BMI (start)</th>
<th>WC (start)</th>
<th>CM</th>
<th>SAMPLE</th>
<th>AV/B</th>
</tr>
</thead>
</table>

American University of Beirut

01 Jul 2018

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APPENDIX XIII
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: __________________________)

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APPENDIX XIV

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
APPENDIX XV
APPETITE QUESTIONNAIRE ARABIC

Simplified Nutritional Appetite Questionnaire (modified)

الاسم: 
العمر: 
التاريخ: 

1. شهيء
أ. ضعيف جداً 
ب. ضعيف 
ت. متوسط 
ث. جيد 
ج. جيد جداً 

2. عندما تتناول الطعام:
أ. أشعر بالشبع بعد تناول فطيرة كبيرة من الطعام 
ب. أشعر بالشبع بعد تناول ثلاثة وجبات 
ت. أشعر بالشبع بعد تناول أكثر من نصف الوجبة 
ث. أشعر بالشبع بعد تناول معظم الوجبة 
ج. أشعر بالشبع بعد تناول أقل من نصف الوجبة 

3. أشعر بالجوع:
أ. نادرًا 
ب. بين الحين والآخر 
ت. قبل الوجبات 
ث. كل الوقت 
ج. دائمًا 

4. مكان الطعام:
أ. منزل 
ب. مسجد 
ت. عادي 
ث. جيد 
ج. جيد جداً 

5. عادة الشرب:
أ. أقل من مرة يوميًا 
ب. مرة واحدة يوميًا 
ت. وجبتين يوميًا 
ث. ثلاث وجبات يوميًا 
ج. أكثر من ثلاث وجبات يوميًا 

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