DOCTORAL RESEARCH PROPOSAL

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The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet Induced Thermogenesis

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Abstract:

Phosphorus is known to have an indispensable role in carbohydrate and energy metabolism. It is responsible for intracellular uptake and trapping of glucose, the most vital step in the energy production pathway. Phosphorus is also required for the synthesis of adenosine triphosphate (ATP), and consequently plays an imperative role in protein turnover and postprandial thermogenesis. Any compromise in postprandial phosphorus levels is therefore expected to delay postprandial cellular uptake of glucose, impair phosphorylation, slow down ATP production, and eventually hinder carbohydrate metabolism and overall energy production. Although postprandial thermogenesis is the smallest contributor to total energy expenditure (TEE), it nevertheless plays a major role in the onset and development of obesity. Hence, on the metabolic level, a decrease in postprandial phosphorus availability can induce several metabolic conditions that favor the development of different components of the metabolic syndrome including glucose intolerance and obesity. The overall objective of this research study is to measure the independent impact of dietary phosphorus on carbohydrate metabolism and diet induced thermogenesis in a continuous real-time mode, via the continuous monitoring of blood glucose levels and core body temperature. To entirely serve this objective, macronutrient composition and phosphorus content of standardized meals will be manipulated and altered in the course of three sequential experiments. Changes in post prandial glucose levels, body temperature, and heat flux will subsequently be measured and compared among three different interventions.

1. Background & Rational

Over the past few decades, the world has been witnessing gradual but significant changes in eating behaviors in light of nutritional transitions from traditional diets rich in complex carbohydrates to diets high in refined sugars, simple carbohydrates, and electrolyte-free commodities (Popkin, 2006). These overall dietary changes were associated with a global increase in the prevalence of metabolic syndrome and other chronic diseases (Saklayen, 2018). The increased consumption of refined cereals and electrolyte-free commodities such as, sugars, oils, and sweeteners resulted in substantial reductions in the intake of micronutrients, especially that of phosphorus, hence compromising its postprandial status. Phosphorus is known to have an indispensable role in both carbohydrate and energy metabolism. Hence, on the metabolic level, such a compromise in postprandial phosphorus levels can induce several metabolic conditions that favor the development of different components of the metabolic syndrome such as glucose intolerance and obesity (Obeid, Hachem, & Ayoub, 2014). It is thereby reasonable to deduce that preserving or boosting the status of phosphorus in food -within tolerable limits- would ameliorate such metabolic deviations.

Phosphorus plays an essential role in carbohydrate metabolism via phosphorylation of glucose to glucose-6-phosphate; an essential step for cellular glucose uptake and intracellular glucose trapping (Bouché, Serdy, Kahn, & Goldfine, 2004). The need for phosphorus is therefore highest during the postprandial period, as indicated by its reduced level after glucose ingestion and by the improvement in insulin sensitivity following its addition (Khattab, Abi-Rashed, Ghattas, Hlais, & Obeid, 2015). Apart from its role in the intracellular trapping of glucose, which is a vital step in the energy production pathway, phosphorus is also required for the synthesis of adenosine triphosphate (ATP), the energy currency of all cells (Bonora, et al., 2012). Hence, the low availability of phosphorus is expected to delay postprandial cellular uptake of glucose, impair phosphorylation, and eventually hinder carbohydrate metabolism and energy production (Kalaitzidis, Tsimihodimos, Bairaktari, Siamopoulos, & Elisaf, 2005).

Daily total energy expenditure (TEE) consists of three components: basal metabolic rate (BMR), diet-induced thermogenesis (DIT) and the energy cost of physical activity (AA) (Westerterp, 2004). Diet induced thermogenesis -also referred to as postprandial energy expenditure (PEE) - is the increase in energy expenditure above basal resting rate that occurs after the ingestion of food components and accounts for 5%–15% of total energy expenditure, depending on type of macronutrients, micronutrients, overall body weight, and insulin sensitivity (Westerterp, 2004). Protein rich foods retain the highest DIT, owing to the fact that protein metabolism -in terms of protein biosynthesis and breakdown- is vastly dependent on ATP availability and supply (Pesta & Samuel, 2014). Protein biosynthesis, in specific, is a very energy-intensive process, whereby 4 ATP equivalents are required for the formation of every peptide bond, or the equivalent of 0.67 kcal/1 g of protein synthesized (Hammoud, Jabbour, Tawil, Ghattas, & Obeid, 2017). This process is highly reliant on phosphorus availability, as revealed and

confirmed by various experimental studies (Hammoud, Jabbour, Tawil, Ghattas, & Obeid, 2017). Although DIT is the smallest contributor to TEE, it nevertheless plays a major role in the onset and development of obesity, particularly because almost more than 50% of an average person's time is spent in the postprandial state (Freckmann, et al., 2007). Numerous clinical and observational studies have confirmed that obese individuals, especially those who are diabetics have a blunted or diminished DIT, which tends to normalize after weight loss (Segal, Edano, Blando, & Pi-Sunyer, 1990); (Thörne & Wahren, 1990); (Golay, et al., 1982). Hence, it can be deduced that insulin resistance undertakes an important role in postprandial ATP synthesis (Felig, 1984), possibly by decreasing the peripheral cellular uptake of substrates required for its synthesis – primarily glucose and phosphorus (Campillo, Aguayo, Pages, Castillo, & Osorio, 1982). On the flip side, phosphorus depletion also induces insulin resistance and glucose intolerance, thereby activating a vicious chain (Xie, Tran, Finegood, & van de Werve , 2000). On another front, hepatic postprandial ATP production has been strongly associated with signaling satiety, via means of the hepatic vagal afferent activity which detects and transmits to the central nervous system any changes in hepatic energy status (Friedman, 2007). It can therefore be concluded that apart from its role in promoting insulin sensitivity and thermogenesis, phosphorus's effect on satiety and food intake, further validates the reported inverse relationship between postprandial phosphorus status, obesity, and metabolic syndrome (Haap, et al., 2006); (Kalaitzidis, Tsimihodimos, Bairaktari, Siamopoulos, & Elisaf, 2005); (Håglin, Lindblad, & Bygren, 2001).

The above established phosphorus associations may explain several related experimental observations. For example, the phosphorus in dairy products may have contributed to the observed inverse association between dairy product intake and metabolic syndrome (Lutsey, Steffen, & Stevens, 2008), especially given that calcium alone failed to explain such an association (Yanovski, et al., 2019). Moreover, the inverse relationship between increased intake of whole grains and the risk of the different components of metabolic syndrome (Ye, Chacko, Chou, Kugizaki, & Liu, 2012) may be partially explained by their high phosphorus content, since added cereal fiber failed to induce such an effect and was proposed to be a marker of other components of whole grains that impart health advantages (Jenkins, et al., 2002). This has been also confirmed and proven in previous work, whereby phosphorus enrichment and supplementation of refined carbohydrates significantly reduced postprandial glucose and triglycerides levels in healthy individuals (El Khoury, et al., 2020). Comparable results were reported in rats, through which thermogenesis was increased by increasing uncoupling protein-1 (UCP-1) expression in brown adipose tissue, following a four week administration of a high phosphorus diet (Abuduli, et al., 2016). In humans however, very few studies investigating the outcomes of phosphorus supplementation on DIT have been conducted. In light of all the above, and since postprandial phosphorus status is directly affected by dietary phosphorus content of a meal (Khattab, Abi-Rashed, Ghattas, Hlais, & Obeid, 2015), it is worth investigating further the independent and continuous impact of dietary phosphorus ingestion on postprandial carbohydrate metabolism and diet induced thermogenesis, simultaneously.

2. Objectives:

The overall objective of this study is to measure the impact of dietary phosphorus on carbohydrate metabolism and diet induced thermogenesis via the continuous monitoring of blood glucose levels and core body temperature. To entirely serve this objective, macronutrient composition and phosphorus content of standardized meals will be manipulated and altered in the course of three sequential experiments. Changes in post prandial glucose levels, body temperature, and heat flux will subsequently be measured and compared among the three different interventions.

Specific aim of Experiment 1: to determine the magnitude of carbohydrate metabolism on post prandial energy expenditure, in the presence of phosphorus.

Subjects will be asked to consume a standardized refined carbohydrate meal with an oral phosphorus supplement, and a standardized refined carbohydrate meal with a phosphorus-free placebo capsule, on two separate visits. The refined carbohydrate meal shake will consist of granulated sugar, corn oil, dried egg white powder, water and a few drops of artificial flavor. Throughout both interventions, serum glucose levels and core body temperature will be continuously monitored over a period of 10 days, for carbohydrate metabolism determination and DIT calculation, as illustrated in Figure 1.

Expected outcome: meals given with phosphorus supplementation will attain lower postprandial glucose levels due to an improved glucose cellular uptake and a more optimized intracellular glucose trapping. Through processes of cellular respiration, namely glycolysis and oxidative phosphorylation, glucose's energy will be harvested as ATP, thereby expecting an increase in energy production and expenditure. The increase in postprandial exogenous phosphorus levels is also expected to boost postprandial ATP synthesis and therefore accelerate DIT as compared to non-phosphorus containing meals.

Specific aim of Experiment 2: to indirectly measure the effect of dietary phosphorus on protein turnover and consequently on postprandial energy expenditure, in addition to investigating the independent impact of dietary phosphorus found in dairy foods on postprandial glycaemia of healthy individuals.

Subjects will be asked to consume a standardized dairy meal (which is typically rich in protein and phosphorus) with a phosphorus-free placebo capsule, and a standardized dairy meal with a phosphate binder medication (Renvela), The dairy meal shake will consist of full fat powdered milk, granulated sugar, corn oil, dried egg white powder, water and a few drops of artificial flavor. *Renvela*®, dosage used is 800 mg/meal for phosphorus binding, (Sanofi-Aventis LLC, 2017) on two separate visits. Throughout both interventions, serum glucose levels and core body temperature will be continuously monitored over a period of 10 days, for carbohydrate metabolism determination and DIT calculation, as illustrated in Figure 1.

Expected outcome: meals given without phosphate binders will attain lower postprandial glucose levels and higher protein turnover rates, given that protein synthesis and degradation are highly dependent on phosphorus availability. This experiment might as well reveal the importance of phosphorus contribution in the widely known glycemic lowering ability of milk and dairy foods.

Specific aim of Experiment 3: to indirectly determine the impact of dietary phosphorus supplementation on protein turnover and postprandial energy expenditure, under conditions of phosphorus restriction.

Subjects will be asked to consume a standardized dried egg white meal with an oral phosphorus supplement, and a standardized dried egg white meal with a phosphorus-free placebo capsule, on two separate visits. The dried egg white meal shake will consist of dried egg white powder, granulated sugar, corn oil, water and a few drops of artificial flavor. Egg whites typically contain all essential amino acids and hold negligible amounts of phosphorus. Throughout both interventions, serum glucose levels and core body temperature will be continuously monitored over a period of 10 days, for carbohydrate metabolism determination and DIT calculation, as illustrated in Figure 1.

Expected outcome: egg white meals administered with phosphorus supplements will retain higher energy expenditure levels, despite the initial phosphorus restriction. Exogenous phosphorus supplementation will accelerate ATP production required for protein synthesis and turnover.

3. Research Design and Methods:

Sample size and allocation

Based on data from (Westerterp-platenga, Wouters, & Ten Hoor, 1990), the mean postprandial elevation in body temperature is 0.8°C ± 0.35 (mean ± SD). Accordingly, each of the three experiments requires a sample size of 16 participants (8 males and 8 females). The probability that this study would detect a treatment difference at a two-sided 0.05 significance level is 90 percent; if the true difference between treatments is 0.3°C (a difference of 40% between groups or treatments). This is based on the assumption that the within-patient standard deviation of the response variable is 0.35.

The method of randomization applied in this study is block randomization, following the paper-based, jar model method. Participants' names will be coded, written on separate papers, and dropped in two jars (categorized by gender). Whenever a subject is allocated, a paper will be randomly selected, without returning it back to the jar (random allocation without replacement). Hence, the assignment of each subject to his/her respective control or experimental group is random, and the order of the treatments is also random. It is worth noting that participants may choose to participate in one or more of the three sub-experiments.

Statistical Analysis

Data about food intake, hunger, appetite, and physical activity will be collected. Continuous glucose monitoring, core body temperature and heat flux will be uploaded from synchronized devices and further analyzed. These synchronized scores will yield individual food intake curves, difference from baseline curves, glycemic and thermal curves (comprising incremental area under the curve, peaks, dips, and duration to restore baseline levels). All data will be analyzed using SPSS V.25 (Chicago, Illinois, United States). Weight, BMI, blood glucose levels, core body temperature and heat flux, will be analyzed as continuous variables; while age, gender, treatment groups, appetite scores, hunger scores, will be analyzed as categorical variables. Within-subject variation (for supplement effect) will be assessed using paired t-test. Additionally, one-way ANOVA will be used to assess between-subjects variations and two-way ANOVA will be used to check time-related differences. Pearson or Spearman (based on the data distribution) correlations will also be performed to correlate all continuous variables together. Statistical significance will be set at a p-value of <0.05.

Study Participants

Generally healthy males and females between the ages of 18 and 64 years, with normal BMI levels, recruited from the Lebanese population will be enrolled in this study. Participants will be recruited from among the AUB community, via means of emails to departmental mailing lists and posters located in University departments. Potential subjects who are interested in participating will undergo a detailed eligibility assessment, where they will be asked questions regarding their overall health status, possible use of medications, and dietary restrictions, using a pre-screening questionnaire performed by study staff. The estimated time for the pre-screening questionnaire administration is about 20-30 minutes. Candidates meeting preliminary inclusion criteria will then be asked to show up for an initial screening visit. During the screening visit, study staff will measure and log candidates' anthropometric measurements and body composition analyses. Study staff will also have to perform a finger-prick blood glucose test to rule out for any pre-diabetic/ diabetic conditions, and a urine test to rule out any cases of renal failure or insufficiency. The estimated time for the completion of the screening visit is around 20-30 minutes. Subjects will be clearly informed about the study's timeline and protocol, and consequently sign a written informed consent. Interested and eligible subjects may choose to participate in one or more of the three experiments. Throughout the entire study course, subjects will be asked to maintain their regular dietary and physical activity habits, avoid alcohol consumption as well as any unusual strenuous exercise 24 hours prior to each experimental session.

Inclusion criteria:

- Have a BMI within the healthy range (19.5-24.5 kg/m^2)
- Willing and able to give informed consent for participation in the study
- Not taking any medications

- Willing to maintain his/her regular dietary and physical activity habits throughout entire study period
- Have a smartphone and capable of understanding how to use the continuous glucose monitoring and core body temperature devices along with their synchronized mobile applications
- Participants with HIP coverage
- Participants with no intentions of traveling during the 10 days study period, due to daily research commitments

Exclusion criteria:

- Have any significant medical disease, including pre-diabetes, diabetes, or renal failure
- Have lactose intolerance or allergies to milk, eggs and/or dairy foods
- History of any bariatric surgeries
- History of kidney stones
- History or current psychiatric illness
- Having experienced a weight loss of 3% or more in the preceding 3 months
- Pregnancy or lactation

Study Protocol

This study will include three different interventional experiments; each being a single-blinded, randomized, crossover experiment, where participants serve as their own control. The macronutrient composition and phosphorus content of standardized test meals is what distinctively classifies each of the experiments. The duration of each experiment is 10 days. To each experiment, there will be two phases, an experimental phase and a control/placebo phase, assigned in random order and separated by a washout period of 2 days, to minimize any carryover effects. Each of the phases will start off with a baseline visit, followed by two days of interventional visits, where participants will have to consume standardized breakfast meals, Figure 1.

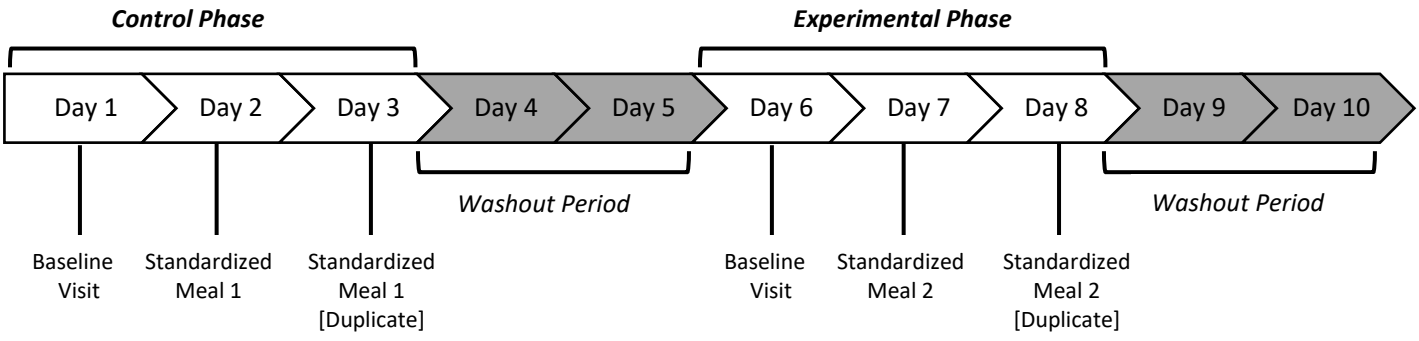


Figure 1. Timeline diagram of each of the three interventional experiments.

For every baseline and interventional visit, subjects will be asked to come following a 12-hour overnight fast. Subjects will be instructed to refrain from engaging in any strenuous physical activity and alcohol consumption, one day prior to each visit.

Baseline Clinic Visit

Upon arrival, subjects will be taken to the testing facility [Faculty of Agriculture and Food Sciences/Department of Nutrition or the Central research unit (CRU)/ American University of Beirut] where both glucose monitoring device (on day 1) and continuous core body temperature device (on days 1 and 6) will be installed. Study staff will instruct each participant in a one-to-one session on the use of the wearable devices, mobile apps, and overall compliance. Participants will also be trained on logging their complete dietary intake over the 10-day study period, along with the exact time of ingestion, ingredient quantities, and physical activity records, so that compliance could be closely tracked by study staff.

Interventional Clinic Visits

On each of their interventional visits (days 2, 3, 7, and 8), participants will consume their randomly-assigned breakfast meals while wearing the continuous glucose and temperature monitoring devices. Participants will be asked to stay stationary and fast for 3 hours post ingestion of every breakfast meal, after which they may eat ad libitum, while recording their entire daily intake. As illustrated in Figure 1, on days 3 and 8, subjects will have a duplicate of the same meal ingested the day before.

Standardized Test Meals

Standardized breakfast meals will be previously prepared and packaged in the Pilot Plant [Department of Nutrition and Food Sciences/ American University of Beirut]. The breakfast meals offered during the experimental and control phases will be isovolumetric, isocaloric, will undergo same method of preparation, and will have equivalent macronutrient composition. The only difference in experimental versus control meals, will be in their phosphorus content, as determined by the use of phosphate supplements, phosphate binders, or placebo capsules, Table 1.

Table 1. Standardized Breakfast Meal Composition:

	Control Meal	***Experimental Meal***
Experiment 1	Refined carbohydrate meal with a phosphorus-free placebo capsule.	Refined carbohydrate meal with an oral phosphorus supplement.
Experiment 2	Dairy meal with a phosphorus-free placebo capsule.	Dairy meal with a phosphate binder.

Experiment 3	Dried egg white meal with a phosphorus-free placebo capsule.	Dried egg white meal with an oral phosphorus supplement.

The standardized meals will be composed of the following:

- For one serving of the refined carbohydrate shake:
  - 120 mL of water
  - 75g of white granulated sugar
  - 50g of egg white powder (brand ABIAD FOODS)
  - 13.5 g of corn oil
  - Few drops of strawberry flavor (brand Foster Clark's)

- For one serving of the dairy protein shake:
  - 120 mL of water
  - 37.5g of white granulated sugar
  - 36g of powdered milk
  - 21.5g of egg white powder (brand ABIAD FOODS)
  - 4g of corn oil
  - Few drops of coconut and vanilla flavors (brands Foster Clark's and Vahiné respectively)

- For one serving of the egg white shake:
  - 120 mL of water
  - 50g of white granulated sugar
  - 75g of egg white powder (brand ABIAD FOODS)
  - 13.5 g of corn oil
  - Few drops of vanilla flavor (brand Vahiné)

Potassium phosphate tablets (125 mg of potassium phosphate per tablet) will be used as the source of oral phosphorus supplementations. Phosphorus-free placebo capsules will be composed of cellulose. Sevelamer carbonate (*Renvela*®) will be used for phosphate binding. The dosage that will be used for this study is 800mg of Sevelamer per meal. *Sevelamer* is an orally administered pharmaceutical, widely approved for the treatment of hyperphosphatemia in patients with end-stage renal disease. It consists of crosslinked polymeric amine which chelates dietary phosphate in the gastrointestinal tract, subsequently preventing its absorption, and enhancing its fecal excretion (Biswas, Sultan, BK, & Bachar, 2014). Although calcium-based and aluminum-based phosphate binders effectively reduce serum phosphorus levels, these agents can lead to hypercalcemia, increased vascular calcification, and have been associated with several hematological, skeletal, and neurological toxicity (Biswas, Sultan, BK, & Bachar, 2014). Sevelamer, however, is a non-metal containing phosphate binder, efficacious in reducing serum phosphate levels without causing any adverse metabolic effects, or changes in bone turnover or mineralization. Mounting evidence from randomized trials and observational studies confirmed

good efficacy and safety of generic drug Sevelamer carbonate, in terms of its binding capacity constants (K2) and affinity constants (K1), without eliciting any gastrointestinal side effects or discomfort (Biswas, Sultan, BK, & Bachar, 2014; Spaia, 2011).

Continuous Glucose Monitoring

Glucose levels will be measured using *Dexcom G6 Systems*. The Dexcom G6 System is an integrated continuous monitoring system (iCGM) that provides continuous glucose readings which are updated every 5 minutes, providing glucose levels and trends, in conjunction with receivers to detect glucose readings, which will be provided to the participants. The Dexcom G6 is a 10-day wear-sensor device that is designed to be water resistant (Dexcom, Inc., 2019). Monitors will be inserted and fitted by trained clinical practitioners at participants' baseline clinical visit (on day 1). CGMs will be worn throughout the whole experimental period (9 days) and removed on day 10. The iCGM System consists of three main components: a sensor, a Bluetooth Low Energy (BLE) transmitter, and a BLE enabled receiver. Dexcom G6 monitors glucose activity via the use of a slim sensor, which gets inserted beneath the abdomen's skin, by a one-touch auto-applicator. The slim sensor continuously measures glucose levels and sends data wirelessly to a compatible smart phone device, via a transmitter. Users can then view glucose data on the G6 CGM Application, installed on the receiver, since the mobile application is not compatible with all types of phone software. (Dexcom, Inc., 2019). The sensor insertion process is simple and pain-free, whereby 100% of Dexcom users have reported that the auto-applicator was "very easy" to use, and 84% reported no pain or mild pain (Shah, Laffel, Wadwa , & Garg, 2018). The Dexcom G6 System features an exceptional accuracy, with an overall mean absolute relative difference (MARD) of 9.0% (Wadwa, Laffel, Shah, & Garg , 2018), and was proven to demonstrate consistent performance, even when glucose levels may be rapidly falling or rising (Shah, Laffel, Wadwa , & Garg, 2018). The FDA cleared the use of Dexcom G6 continuous glucose monitoring system, as a replacement to finger-stick glucose testing for diabetes treatment decisions, and permitted its use as a stand-alone glucose determining device for both adults and children aged two years and above (Dexcom, Inc., 2019).

Continuous Body Temperature Monitoring

Body temperature will be monitored using *CORE Systems,* by *greenTEG*. CORE is a non-invasive wearable device that continuously measures and delivers real-time core body temperature data, via the use of thermal energy transfer sensors. This innovative sensor technology is built into a compact waterproof device that can be comfortably worn and attached to a strap around the chest area. The CORE body temperature monitoring device features a 3-days battery life of continuous transmission, and is validated with a high accuracy of ±0.21 °C. Monitors will be fitted by trained clinical practitioners at participants' baseline clinical visits (on days 1 and 6). CGMs will be worn throughout the 3-days experimental phase and the 3-days control phase sequentially, and will be removed during washout periods. CORE features an integrated technology to be used in conjunction with compatible smart phone devices - iOS and Android- (CORE Body Temperature Monitor, 2020).

4. ***Outcomes***

This trial aims to examine the impact of dietary phosphorus in predicting individual postprandial responses in context of different meals consumed. For primary outcome reporting, changes in baseline and post prandial glucose levels, body temperature, and heat flux will be measured. Glucose incremental area under the curve (0 to 2 hours) will also be deduced. Secondary main outcomes will include additional data collected over the 10-days study course, such as daily food intake logs, and physical activity records.

5. ***Literature Review & Related Work***

Phosphorus & Glycemia

Accumulating evidence has confirmed the association between hypophosphatemia and impaired glucose tolerance secondary to insulin insensitivity, through which plasma phosphate level was reported to be synergistically related to glucose tolerance and insulin sensitivity (Håglin, 2001; Lippi, Montagnana, Salvagno, Targher, & Guidi, 2009). In the postprandial state, low serum phosphate levels were reported to be associated with elevated blood glucose and reduced insulin sensitivity (Friedman, Obesity and the hepatic control of feeding behavior, 2007). This is because phosphorus plays a crucial role in carbohydrate metabolism via phosphorylation of glucose to glucose-6-phosphate (Bouché, Serdy, Kahn, & Goldfine, 2004). The need for phosphorus is highest during the postprandial period, as indicated by its reduced level post glucose ingestion and by the improvement in insulin sensitivity following its addition - Figure 2 (Khattab, Abi-Rashed, Ghattas, Hlais, & Obeid, 2015). Hence, the low availability of phosphorus is expected to delay postprandial cellular uptake of glucose, impair phosphorylation, and eventually hinder carbohydrate metabolism and energy production (Kalaitzidis, Tsimihodimos, Bairaktari, Siamopoulos, & Elisaf, 2005). In support of the above, numerous epidemiological studies have confirmed that low serum phosphate levels are associated with high postprandial blood glucose levels and reduced insulin sensitivity, in healthy non-diabetic individuals (Haap, et al., 2006; Khattab, Abi-Rashed, Ghattas, Hlais, & Obeid, 2015). Khattab et al. has found that the addition of 500 mg phosphorus to OGTT was able to significantly improve insulin sensitivity (both insulin concentrations and HOMA at 60 minutes post-ingestion), while preventing the reduction in both total and inorganic phosphates. Additionally, Nowicki et al. has reported that phosphate infusion of healthy subjects significantly improved insulin sensitivity under euglycemic conditions (Nowicki, Fliser, Fode, & Ritz, 1996). These changes are likely to have been attributed to phosphorus capacity to enhance insulin mediated peripheral phosphorylation, which is highly dependent on extracellular phosphorus availability or exogenous (dietary) phosphorus supply (Khattab, Abi-Rashed, Ghattas, Hlais, & Obeid, 2015).

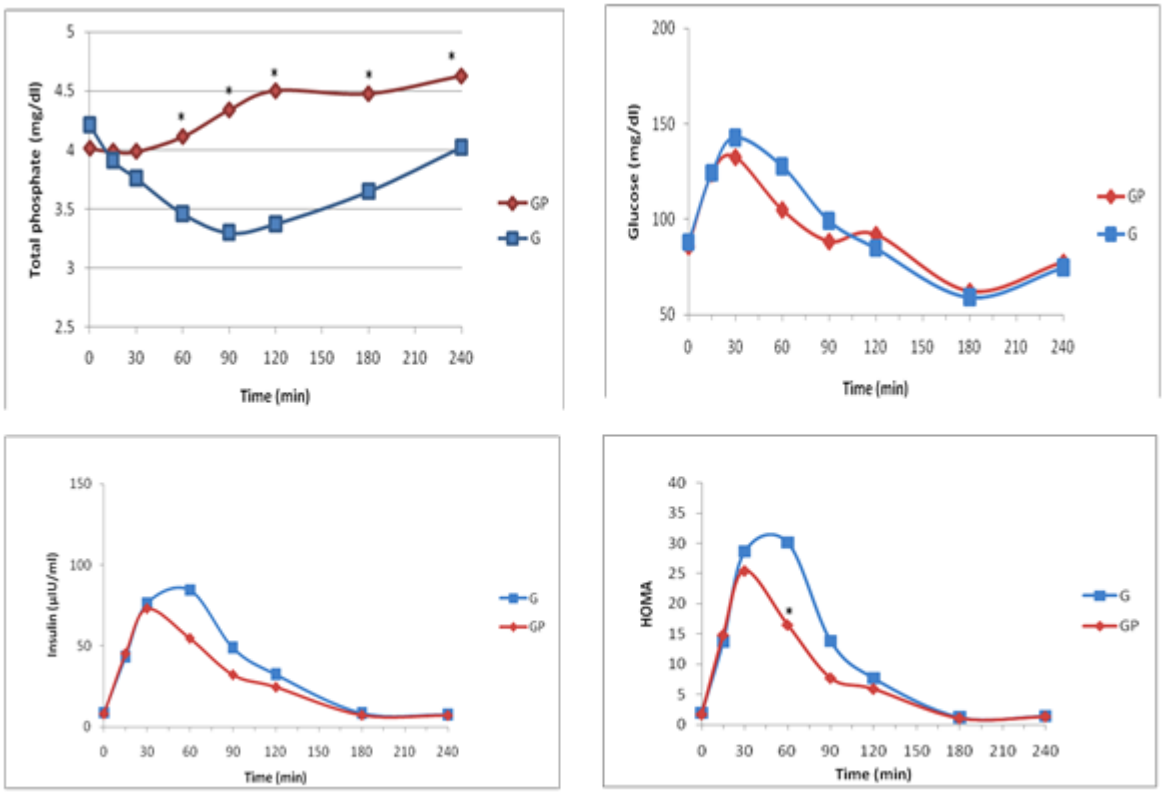


Figure 2. Mean serum total phosphorus (a), glucose (b), insulin (c) and HOMA (d) response following the consumption of G and G+P treatments. *Values are significantly different at p<0.05 as analyzed by paired t-test.

Dairy products & Glycaemia

Dairy products, which are known to be rich sources of dietary phosphorus, differ from other carbohydrate containing foods in that they trigger elevated insulin responses, in defiance of their low glycemic indices (Drehmer, et al., 2015). The intake of dairy products has been found to be significantly associated with a lower risk of type 2 diabetes mellitus in three meta-analyses of large prospective epidemiological studies (O'Connor, et al., 2014; Tong, Dong, Wu, Li, & Qin, 2011; Elwood, Pickering, Givens, & Gallacher, 2010; Aune, Norat, Romundstad, & Vatten, 2013). Regular consumption of dairy products was found to improve glycemia, overall body composition, as well as lower incidence of obesity and metabolic syndrome in both observational and long-term clinical studies (Drouin-Chartier, et al., 2016; Kratz, Baars, & Guyenet, 2013). Although this association has been well established and confirmed, yet the underlying mechanisms behind this insulinotropic effect have not been extensively identified and explained (Bjørnshave & Hermansen, 2014; Tucker, Erickson, LeCheminant, & Bailey, 2015). When testing the effect of pure lactose on insulin response in healthy subjects, the *insulin index* paralleled the *glycemic index*, thereby indicating that the insulinotropic effect of dairy products is not attributed to the lactose fraction, and that some other non-carbohydrate component is behind their favorable insulinotropic effect (Ostman, Elmståhl, & Björck, 2001).

Milk protein, on the other hand, appears to stimulate an increase in postprandial insulin response with a corresponding reduction in postprandial blood glucose levels (Ostman, Elmståhl, & Björck, 2001; Elmståhl & Björck, 2001). Studies exploring the insulinotropic effect of the dairy protein have found that the whey fraction seems to contain the predominating insulinotropic secretagogue (Nilsson, Stenberg, Frid, Holst, & Björck, 2004; Zawadzki, Yaspelkis, & Ivy, 1992; Loon, Kruijshoop, Verhagen, Saris, & Wagenmakers, 2000). The reduction in blood glucose is suggested to occur because of whey protein's rapid digestion and its high content of branched-chain amino acids, which are in turn thought to be insulinogenic (Akhavan, Luhovyy, Brown, Cho, & Anderson, 2010). Peterson et al. explored the glycemic impact of adding escalating doses of a glycemic index lowering peptide fraction (GILP) from whey to a glucose drink, whereby the addition of GILP to an oral glucose bolus have reduced blood glucose iAUC in a dose dependent manner (Petersen, et al., 2009). GILP is a rich source of phosphorus; it can be therefore postulated that the dose dependent reduction in blood glucose iAUC per gram of protein, may be accredited to its phosphorus content, correspondingly (Table 2). Another recent study conducted by Chen et al., demonstrated that co-ingestion of whey protein hydrolysate with milk minerals (rich in calcium and phosphorus) potently stimulates plasma Glucagon-like-peptide-1 (GLP1) availability, which in turn triggers insulin secretion, and regulates post prandial glucose status (Chen, et al., 2020).

Table 2. Composition of different test meals:

Component	Control	5 g GILP	10 g GILP	20 g GILP
Minerals				
Calcium (mg)	0	121	241	482
Phosphorus (mg)	0	63	127	254
Sodium (mg)	0	53	107	214
Potassium (mg)	0	25	50	100
Magnesium (mg)	0	13	25	50
Zinc (ug)	0	15	30	60

Conversely, a very recent study revealed that insulin production was not elevated post consumption of high-protein milks, in contrast with the suggestion that lower glucose levels are due to an increase in blood insulin stimulation by BCAA (Kung, et al., 2020). These findings suggest that the insulinotropic effect of dairy foods may be linked to an improvement in insulin sensitivity rather than insulin synthesis, thereby further backing our hypothesis on the role of dairy phosphorus in enhancing cellular glucose and phosphorylation. Considering that, El Khoury et al. has found that the contribution of dairy products to post-meal glucose control and to satiety was independent of their protein to carbohydrate ratio in healthy males, whereby increasing protein content in yogurt failed to detect a significant effect on post-meal glycaemia (El Khoury, et al., 2014). Clustered together, these findings synergistically support the plausibility of this hypothesis, indicating that the glycemic benefits of dairy foods -previously ascribed to their protein content- may be confounding the favorable role of the macromineral phosphorus in improving glycaemia.

Phosphorus & Postprandial Energy Metabolism

Previous experimental studies found that the ingestion of phosphorus-rich foods (i.e. dairy products and meats) was significantly associated with an increased in postprandial energy expenditure and a decrease in body weight (Mirmiran, Esmaillzadeh, & Azizi, 2005) (Halton & Hu, 2004). It has also been clinically elucidated that phosphorus supplementation after a meal (Uściłko, et al., 1993) or with orange juice (Jaedig, Lindgärde, & Arborelius, 1994) was associated with a significant increase in resting energy expenditure among obese women. Likewise, another study revealed that 3 months phosphorus supplementation improved body weight and waist circumference among overweight and obese individuals (Ayoub, Samra, Hlais, Bassil, & Obeid, 2015). From previous work, we have confirmed that the administration of 500 mg phosphorus to different preloads have successfully resulted in substantial reductions in ad libitum subsequent energy intake (27%–33%) (Obeid, Dimachkie, & Hlais, 2010). This is explained through the essential contribution of phosphorus in the process of ATP synthesis, and the role of postprandial ATP production in signaling satiety via means of the hepatic vagal afferent activity (Friedman, 2007). Similarly, an analysis of the metabolic data via the Knowledge Discovery in Databases revealed that compromised ATP levels were significantly associated with the onset and progression of obesity by signaling overeating and conserving energy (Wlodek & Gonzales , 2003). Another related study revealed that the administration of phosphorus to a glucose solution resulted in an increase in DIT compared to placebo group, in both lean and obese subjects (Bassil & Obeid, 2016). The proposed mechanism behind those results further confirm that phosphorus addition could have stimulated insulin sensitivity and subsequent hepatic substrate uptake of glucose and phosphorous, consequently resulting in an improved rate of ATP production and thermogenesis.

On another front, animal studies confirmed that rats put on a high phosphorus diet for 8 weeks had increased thermogenesis compared to controls, which was manifested by increased UCP-1 expression in brown adipose tissue (BAT) (Abuduli, et al., 2016). A recent study by Hammoud et al. proved that phosphorus supplementation significantly improved food intake, weight gain, total body protein content, and energy efficiency, in rats fed a low-protein diet (Hammoud, Jabbour, Tawil, Ghattas, & Obeid, 2017). This further highlights the essential role of phosphorus in protein metabolism, and confirms phosphorus ability in changing body composition. Another animal study proved that phosphorus supplementation succeeded in reversing lower gastrocnemius muscle tension secondary to a slow rate of ATP synthesis, which was formerly caused by dietary phosphorus restriction (Hettleman, Sabina, Drezner , Holmes , & Swain , 1983). Hence, it can be concluded from both human and animal trials that ATP availability is dependent on exogenous phosphorus replenishment, and that the low availability of phosphorus is expected to delay postprandial cellular uptake of glucose, impair phosphorylation, and eventually hinder carbohydrate metabolism and energy production (Kalaitzidis, Tsimihodimos, Bairaktari, Siamopoulos, & Elisaf, 2005).

6. Significance and Innovation:

This study will be the first interventional study to demonstrate the distinct and independent impact of dietary phosphorus on postprandial glycaemia and thermogenesis in a continuous real-time mode. Unlike a single glucose reading extracted from withdrawn blood samples, a continuous glucose monitoring system provides a more realistic and comprehensive approach on understanding overall glycaemia, while taking into account the body's various metabolic states: baseline, absorptive, and post-absorptive states. The significance of this research project lies in the advances of human nutrition in improving glucose tolerance and ultimately preventing the development of insulin resistance and type II diabetes, which has been classified as a global chronic threat.

7. Future directions

The proposed project represents another major step towards the promising potential of the macro-mineral phosphorus in improving glycaemia and energy metabolism. Overall, the study will broaden our understanding of the glycemic lowering ability of phosphorus, and validate its vital role in ATP production and diet induced thermogenesis. If proven to be true, results deduced from the present study will serve as the backbone for future research work, prospectively investigating the long-term effect of mineral supplementation on glycemic status, and comprehensively generating insights into the prevention and management of type II diabetes, in addition to other components of the metabolic syndrome.

8. Budget:

Budget Summary	**Cost (US$)**
Dexcom sensors	5,168.00
Dexcom transmitters	1,624.00
Dexcom receivers	2,693.00
Core devices	5,000.00
Materials and supplies	1,000.00
Subjects' reimbursment	1,920.00
Miscellaneous	1000.00
Total	**18,405.00**

Budget Justification:

- *Dexcom Sensors*: Dexcom sensor box contains three sensors (with their applicators). Each sensor can be worn by 1 individual and collect plasma glucose data for 10 days. We will need 48 Dexcom sensors (1 sensor per person) and therefore we need a total of 16 boxes, costing 323 USD each. This would sum up to a total of 5,168 USD.
- *Dexcom Transmitters*: Each Dexcom transmitter can be used for three months and therefore, because the monitoring period is 10 days, each transmitter can be used by 8 subjects. Therefore, we need 6 transmitters costing 270.75 USD each. This would sum up to a total of 1,624.5 USD.
- *Dexcom Receivers*: Dexcom sensors will send the data to a receiver. Each receiver costs (945 USD). We have acquired 3 receivers for a total of 2693.1 USD.

  Accordingly, we need a total of 9,485.6 USD for Dexcom G6 apparatus.

- *Continuous Body Temperature Monitoring*: Sensors needed to measure core body temperature are available therefore we don't need to purchase them. We will need three sensors and each sensor will be used by 16 subjects. We have acquired five Core devices for 1000 USD each. This would sum up to a total of 5000 USD.

- *Materials and supplies*: these cover the cost of standardized breakfast meals ingredients, oral phosphorus supplements, and phosphate binders.

- *Subjects' reimbursement*: participants will be given Amazon vouchers of $40 as reimbursements for expenses incurred as a result of their participation in each of the three experiments ($40 per experiment). These shall include transportation costs, parking fees, and lost work time fees. This would sum up to a total of 1920 USD.

Miselleneous: this covers the cost of calls, stationary, printing fees (consent forms, questionnaires, etc).

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