AMERICAN UNIVERSITY OF BEIRUT

WORKLOAD OF WATER POLO PLAYERS FOLLOWING A PHOSPHORUS SUPPLEMENTED HIGH CARBOHYDRATE MEAL.

by
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Special thanks are for my family, friends, professors, colleagues, and teammates.

AN ABSTRACT OF THE THESIS OF

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Phosphorus supplementation has been recommended as an ergogenic aid by previous studies some of which reported a significant improved cardiac capacity in athletes. The literature on the topic is however ambivalent, with as many studies finding no improvement in performance. Most of the studies researched the chronic effect following a loading period. The current study aimed to research the acute effect of phosphorus supplementation, taken concomitantly with a carbohydrate load.

When a sample of 14 male water polo players was supplemented with 400 mg of phosphorus (P) concomitant with a glucose solution, they displayed a significant increase in heartrate difference between phosphorus and placebo during exercise ($p<0.001$), yet supplementation left unaffected the exercise efficiency. The breathing rate per minute was unchanged, which may be interpreted as an improvement in VO_2 max since the heart rate increase would have normally necessitated a higher breath count. This interpretation resonates with previous research on ergogenic effects of phosphorus which found no improvement in time trials but an increase in VO_2 max.

In our trial, we calculated the change in work efficiency while controlling for muscle glycogen by asking the participants to observe an overnight fast, and by depleting the remaining reserves through a 25 mins bike run before meal intake. We then measured the change in work output 3 hours after a 400Kcal glucose solution following a 20 mins run at a constant cadence of 85 rpm and an incremental power output protocol. It was a crossover design with a phosphorus intervention at a dose of 1 mg/kcal.

Keywords: Glycogen Supercompensation, Phosphorus, Sports Nutrition.

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ABBREVIATIONS LIST

ATP: adenosine triphosphate
BIA: bioelectrical impedance analysis
Ca^{2+}: calcium
CHO: carbohydrate
CPET: Cardio Pulmonary Exercise Test
ECG: electro cardiac screening
EEm: Energy Expenditure per minute
FFM: fat free mass
FGF: fibroblast growth factor
Glu-6-P: glucose-6-phosphate
GPa: glycogen phosphorylase
GSa: glycogen synthase
HR: heartrate
HRavg: average heartrate
HRmax: maximal heartrate
HRR: heart rate reserve
HPO_4^-: hydrogen phosphate
H_2PO4^-: dihydrogen phosphate
Kcal: kilocalorie
Kg: kilogram
NS: non-significant
P: phosphorus
PCT: proximal convoluted tubule
PTH: parathyroid hormone
RBC: red blood cell
RPM: revolutions per minute
RQ: respiratory quotient
SD: standard deviation
VO_2 max: maximal oxygen consumption
2.3-DPG: 2.3 diphosphoglycerate

PHOSPHORUS IS HESPERUS

CHAPTER I

INTRODUCTION

The use of phosphorus as ergogenic aid has been widely reported and researched in recent reviews (Buck, Wallman, Dawson, & Guelfi, 2013). Most of the research highlighted its chronic intake effect, comprising a dose of 2-3 grams of phosphate for a loading period of 3-6 days (Kopec, Dawson, Buck, & Wallman, 2015). The benefits of phosphate supplementation on athletic performance have been attributed to several potential factors, like increased maximal oxygen uptake and improved cardiac output (Folland, Stern, & Brickley, 2008). The underlying mechanisms were hypothesized to be the increased plasma content in 2.3-disphosphoglycerate (2.3-DPG) which may be a factor due to its role in decreasing the oxygen affinity of hemoglobin (Di Caprio, Stokes, Higgins, & Schonbrun, 2015). Subsequently researchers suggest an easier release of oxygen in the exercising tissue. Another line of investigation was based on the effect of hypophosphatemia on red blood cell (RBC) metabolism (Lichtman, Miller, Cohen, & Waterhouse, 1971), where decreased levels of 2.3-DPG and glycolysis in RBC lead to reduced oxygen delivery. A beneficial effect of phosphate supplementation is attributed to a possible role in increasing the rate of glycogenolysis and subsequently adenosine triphosphate (ATP) resynthesizing (Chasiotis, 1988; Hargreaves & Richter, 1988), because its higher extracellular concentration may explain the increased activity of phosphorylase A required for glycogenolysis.

We test the hypothesis of whether such benefits take place in the context of acute intake, in comparison to several-day loading that we mostly encounter in literature. One investigation, which found no effect on athletic performance upon acute phosphate supplementation, was done using calcium phosphate (Galloway, Tremblay, Sexsmith, & Roberts, 1996).

Their trial was done following an overnight fast, and the phosphorus was administered without a meal, which may have reduced the glycogen contribution to subsequent work output. The existing link between serum phosphate, calcium and PTH may also be a confounding factor (Deroisy et al., 1997; Silverberg et al., 1986), therefore the acute effect of potassium phosphate on athletic performance may reveal a different aspect of phosphate supplementation. Recent work on the acute effect of phosphorus on postprandial lipemia (Hazim et al., 2014), and higher blood glucose clearance (Khattab et al., 2015) are indications that its acute metabolic effects are complex and warrant further investigation (figure 2). In our trial we aimed to detect the effect of mild (400 mg) acute phosphate supplementation on subsequent work output and energy efficiency. If an improvement in the phosphorus supplemented group's work output is detected, as a significant difference in efficiency would indicate, it could be interpreted as a result of a higher glycogen formation (Cannon, White, Andriano, Kolkhorst, & Rossiter, 2011; Martin & Tomescu, 2017), likely due to its role in muscle signaling (Ivy, 1991; Rauch, St Clair Gibson, Lambert, & Noakes, 2005) and possibly resulting from higher net glycogen breakdown (Hespel & Richter, 1992).

CHAPTER II

LITERATURE REVIEW

A. Glycogen: function and metabolism

As the only form of glucose storage in the human body, glycogen- a term to mean "sugar former," holds a critical position in blood glucose regulation (Greenberg, Jurczak, Danos, & Brady, 2006). Xu et al. in their model on glycogen regulation (2011) situate the glucose-based chain as the primary currency in a large exchange scheme where a swift change from the fasted state to the fed state partly defines homeostasis. Upon uptake into the cell, glucose gets phosphorylated and then depending on the enzymatic intracellular medium it would either undergo glycolysis or is stored through glycogenesis (Rines, Sharabi, Tavares, & Puigserver, 2016). During the first 12-24 hours following post-digestive glycogenesis, glycogen becomes the main reserve supplementing our glucose requirements. The various enzymes influencing different pathways are swayed by an intricate signaling matrix encompassing the normoglycemic hormonal balance. Multifactorial vectors like insulin, glucagon, epinephrine, and leptin result in glycemic regulation through glycogen synthesis or breakdown according to homeostatic requirements (K. Xu et al., 2011). The aforementioned review also lists metabolite interaction as factor in glycogen homeostasis, be it the lactate transfer from erythrocytes and muscle into the liver to generate glucose, which leads to less glycogen breakdown, or intracellular elevation of cyclic-AMP resulting from pancreatic glucagon secretion or from stress which in contrast, leads to increased glycogen breakdown. When glycogenesis takes place in the muscle, glycogen is stored in different compartments of the muscle cell (Marchand et al., 2002) . Glycogen depletion

which has been linked to the inability to sustain muscular effort was reported to differ depending on the localization of the depleted stores (Joachim Nielsen, Holmberg, Schrøder, Saltin, & Ørtenblad, 2011). Intermyofibrillar stores situated between the myofibrils (figure-1), showed a relation to calcium ion (Ca^{2+}) reuptake, or half relaxation time, while the stores inside the myofibrils between the contractile filaments, or intramyofibrillar, seemed to correlate to Ca^{2+} release from the sarcoplasmic reticulum, or fatigue and in both cases, independent of the myoplasmic energy level, or ATP and phosphocreatine concentration (Kabbara, Nguyen, Stephenson, & Allen, 2000; Joachim Nielsen, Farup, Rahbek, de Paoli, & Vissing, 2015; J. Nielsen, Schroder, Rix, & Ortenblad, 2009).

The enzyme glycogen phosphorylase (GPa) catalyzes Glycogen breakdown to Glucose, while glycogen synthase (GSa) favors the linking of glucose-6-phosphate (Glu-6-P) units into a glycogen chain (Hers, De Wulf, & Stalmans, 1970). Each of these enzymes in concerted association with the digestive, metabolic and consciousness states will push in the direction required to keep glycaemia within physiologic range (Holst, Gribble, Horowitz, & Rayner, 2016; Scharf, Naidoo, Zimmerman, & Pack, 2008). Glycogen depletion in astrocytes, for instance, affects sleep regulation as wakefulness depletes the brain's glycogen reserves (Benington & Heller, 1995; Petit, Burlet-Godinot, Magistretti, & Allaman, 2015). When digestion is taking place, the glucose requirements are provided by the gut and glycogen synthesis is favored, while in the fasting and fasted states and during exercise, glycogen degradation is favored (Bonjorn, Latour, Belanger, & Lavoie, 2002; K. Xu et al., 2011).

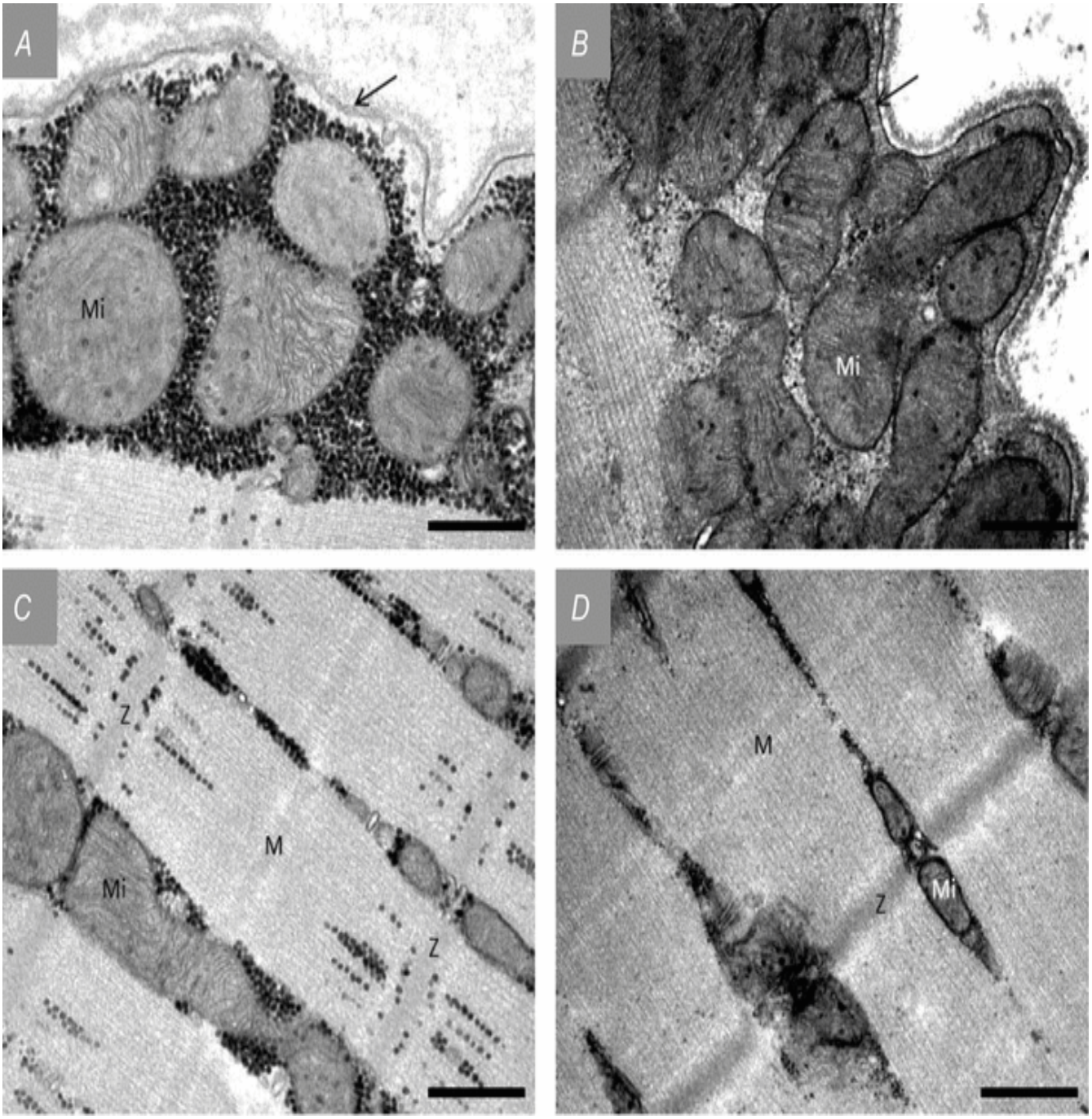

Figure 1: Localization of glycogen in the muscle

Representative TEM images of the subsarcolemmal (A and B) and myofibrillar (C and D) regions pre-exercise (A and C) and post (B and D) approximately 1 h of exhaustive exercise

All images originate from an arm type I fibre. Glycogen is visualized as black dots. Mi, mitochondria; Z, Z-line; M, M-band. The arrows indicate the sarcolemma. Scale bar = 0.5 μm.

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B. Phosphorus

1- Role and metabolism

Finding its way from apatite ores to the food cycle and then to human bone, enamel, dentin, as well as plasma, phosphorus (P) is presently acquired via fertilizers used in feed production, for the most part. Through food, it is integrated into metabolic and physiologic homeostasis (Ciobota et al., 2014; El-Taher & Khater, 2016; Li et al., 2015; Liu & Lal, 2014). Plasma P is tightly regulated through the parathyroid hormone action (PTH), gut absorption, exchange with intracellular milieu, reabsorption at the proximal convoluted tubule (PCT) of the kidney, and excretion under the effect of PTH. Keenly kept between 0.8 and 1.5 mmol/liter (or 2.5-4.5 mg/dl) serum phosphorus deviations from this range are indicative of elevated risk of kidney damage. Hazard ratios significantly increase for each increment of 1 mg/dl in populations with glomerulonephritis (Blaine, Chonchol, & Levi, 2014; Da et al., 2015; Dawson-Hughes, Harris, & Dallal, 1991; D. Xu, Lv, Wang, Zhang, & Zhang, 2016).

Phosphorylation, can be defined as a type of post-translational modification that modulates protein interaction (with nucleic acids for instance), it takes place in nearly all cells (Thapar, 2014). The adenosine triphosphate (ATP) which provides the phosphoryl group during the phosphorylation process is a ubiquitous molecule found in all eukaryotes (Cohen, 2002).

Phosphate is often described as the most abundant anion in the human body, intracellularly stored for the most part (14% of total P) and making up about 1% of total body weight (Penido & Alon, 2012), or around 11-14 gr per kg of the fat-free mass (Buck et al., 2013). Most of the P is found in the bone (85%), where it partakes in its structural integrity (Marks, Debnam, & Unwin, 2010). Less than 1% of the body stores are exchangeable in between serum and intracellular

media, and intake is quickly compensated (within 60 mins) through limiting reabsorption in the kidneys (Biber, Hernando, & Forster, 2013). Out of the 1600mg provided by a typical diet, and the 3 mg/kg/day secreted into the intestine in addition to the pancreatic and biliary contributions, 7mg/kg/day exit in feces and 13 mg/kg/day are absorbed by the proximal intestine ending up in the extracellular pool then depending on the rate of bone remodeling, are exchanged with plasma leading to a 13mg/kg/day excretion in urine at the zero metabolic state (Berndt, Schiavi, & Kumar, 2005). Intestinal absorption of phosphate seems to be modulated by a family member of the klotho transmembrane protein transporters. The bone derived klotho proteins, which also partake in kidney function, combine with the fibroblast growth factor (FGF) receptors to modulate mineral homeostasis. The klotho family comprises of 3 members: α, β, and γ klothos. The Phosphate related counterpart from the FGF family is FGF number 23 (FGF23), α-klotho combines with FGF23's receptor conducing to phosphate homeostasis, in concert with calcium and vitamin D metabolism. (Bian, Xing, & Hu, 2014; Razzaque, 2009; Y. Xu & Sun, 2015)

2- Use as ergogenic aid

Phosphate supplementation is safe, affordable and its benefits to athletes have been explored since the beginning of the 20th century, albeit with inconclusive results (Buck et al., 2013; West, Ayton, Wallman, & Guelfi, 2012). The explanations of positive effects are attributed to several possible factors like enhanced myocardial efficiency, better ATP synthesis, improved buffering capacity, or increased 2,3-diphosphoglycerate (2,3-DPG) concentration (Fukuda, Smith, Kendall, & Stout, 2010). Typical supplementation regimens found in literature (table-2) had consisted of several day loading by 1-4 grams of sodium phosphate, with inconsistent gains in workload or endurance (Brewer, Dawson, Wallman, & Guelfi, 2014; Brown & Keith, 1993; Goss et al., 2001; Tremblay, Galloway, & Sexsmith, 1994; Williams, 2005).

When detected, the increase in VO_{2max} ranged between 5-12%, (table-1) the increased power output between 10-17%, the reduction in time to complete time trial between 3-20% (Czuba, 2008; Czuba, Zajac, Poprzecki, Cholewa, & Woska, 2009; Folland et al., 2008; Kreider et al., 1992; Kreider, Miller, Williams, Somma, & Nasser, 1990; Stewart, McNaughton, Davies, & Tristram, 1990).

Author	Phosphate Amount	Regimen	Performance Criteria	Significance or Smallest Worthwhile Change
Brewer et al., 2014	1-2 gr. Sodium Phosphate	Daily for 5 days, with food	Time to completion	No
			Power output	No
Buck et al., 2014	0.5-5 gr. Sodium Phosphate	Daily for 6 days, with food	Time to completion	No
			Power output	No
Galloway et al., 1996	22.2 gr. Calcium Phosphate	Acutely, without food	Time to exhaustion	No
			VO_2	No
			2,3-DPG	No
Czuba et al., 2009	1-2 gr. Sodium Phosphate	6-21 days, without food (2 gr. glucose)	Power output	Yes
			VO_2	Yes: 5.3% increase
			HRmax decrease	Yes: 2.7% decrease
			2,3-DPG	No
Folland et al., 2008	4 gr. Sodium Phosphate	6 days without food	Power output	Yes: 9.8% increase
			Time to completion	Yes: 3% decrease
			HR decrease	No

Table 1: Different findings on ergogenic improvement subsequent to Phosphate supplementation: Specifying the type of phosphate used and the quantity, as well as the performance criteria where phosphorus' effect was detected when present.

Study	Supplement	Dose	Washout period (days)	Double-blind crossover	Participants (VO_2max, $ml \cdot kg^{-1} \cdot min^{-1}$)	Exercise test(s)	Ergogenic effect
Cade et al.	Sodium phosphate	4 × 1 g per day, for 3 days.	7	Yes	10 male runners (56)	Treadmill VO_2max	Increased VO_2max 6–12%.
Bredle et al.	Dicalcium phosphate	176 mmol per day, for 3/4 days.	14	Yes	11 male runners (63)	Treadmill VO_2max and 70% VO_2max to exhaustion	No performance changes.
Kreider et al.	Tribasic sodium phosphate	4 × 1 g per day, for 3/6 days	14	Yes	7 male runners (74)	Treadmill VO_2max and 5 mile time-trial	Increased VO_2max 9%; no effect on 5 mile time
Mannix et al.	Dicalcium phosphate	5 g single dose	7	Yes	10 healthy males (47)	Cycle ergometer response to 60% VO_2max	No effect on CV parameters.
Stewart et al.	Sodium phosphate	6 × 0.6 g per day, for 3 days	7	Yes	8 male cyclists (49)	Cycle ergometer VO_2max	Increased VO_2max 11%.
Kreider et al.	Tribasic sodium phosphate	4 × 1 g per day, for 3/4 days	17	Yes	6 male cyclists (70)	Cycle ergometer VO_2max test and 40 km time-trial	Increased VO_2max 9%; and mean power (17%) in 40km time-trial.
Galloway et al.	Calcium phosphate	22.2 g 90 min before	7	Yes	6 high fitness (63) 6 low fitness (44)	Cycle ergometer VO_2max test	No effect

Table 2: Phosphate supplementation, endurance exercise, and performance: studies specifying the type of phosphate used and the quantity, the intervention style, the lung capacity of participants, as well as the performance criteria where phosphorus' effect was detected when present.

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C. Interaction of phosphate and glycogen

Since both substrates play a physiologic role in athletic performance, it could be useful to explore the common context of their effects, and possibly their interaction.

1- Glycogen supercompensation

The term supercompensation describes a process of carbohydrate (CHO) intake following exercise with the aim of replenishing the muscles' glycogen stores to levels above baseline, in order to gain an advantage in the subsequent performance (Jensen & Richter, 2012). Several approaches were developed to reach those ends, based on either totally depleting the glycogen stores (prior to reloading) through intense exercise at maximum heartrate (HR_{max}), or the method of partial depletion through exercise at 65% of $VO_{2\,max}$ (Arnall et al., 2007; Galy et al., 2014; Goforth et al., 2003). It is estimated that 500 gr CHO intake could lead to the optimal glycogen storage of 80-100 μmol/g wet weight. Glycogen synthesis seems correlated to glycogen synthase activation by glucose-6-phosphate and which transfers the uridine diphosphate glucose to the amylose chain. All the aforementioned processes are additionally controlled by phosphorylation-dephosphorilation reactions (Bouskila et al., 2010; Ivy, 1991; Soderling, Srivastava, Bass, & Khatra, 1979). Phosphate availability has been therefore proposed as a limiting factor at this juncture (Chasiotis, 1988).

2- Available explanations of Phosphate's ergogenic benefits

The major explanations of phosphate's ergogenic effect are reported to be increased myocardial efficiency, improved ATP synthesis, increased 2,3-DPG in plasma, and enhanced buffering capacity.

Increased myocardial efficiency was suspected due to a significant increase in $VO_{2\ max}$ along with a decrease in maximal heart rate and no change in lactate concentration or 2,3-DPG, after 6 days supplementation of 25 mg of sodium phosphate per kg of fat free mass (FFM) per day (Czuba et al., 2009). The improvement in ATP availability has been a classical interpretation (Morris, Nigon, & Reed, 1978; Xie, Tran, Finegood, & van de Werve, 2000), where inorganic phosphate at the active site of phosphorylase was a limiting factor in glycogen synthesis (Chasiotis, 1988). The buffering capacity improvement is explained by the ability of compensating for the lactic acid flux resulting from exercise by providing ample dihydrogen phosphate ($H_2PO_4^-$) when hydrogen phosphate (HPO_4^-) acting as a weak acid and accepting the lactic acid protons by turning into $H_2PO_4^-$ (Horswill, 1995; K. Sahlin, 2005; Kent Sahlin, 2014). The enhanced 2,3-DGP interpretation is based on the observed reduction in oxygen affinity when upon phosphate supplementation, erythrocytes 2,3-DPG level is increased leading to higher oxygen release in exercising tissues (Bremner, Bubb, Kemp, Trenell, & Thompson, 2002; Cade et al., 1984).

3- Hypothesis of current experiment

Our hypothesis is that an acute phosphorus dose could lead to higher work efficiency. This finding would be explained by higher glycogen compensation which the subsequent exercise help us detect (Ivy, 1991; Rauch et al., 2005). Our reasoning follows a previous study on phosphate supplementation's effect on glucose uptake (Khattab et al., 2015) where phosphorus co-ingestion showed a faster postprandial plasma glucose drop than pre-ingestion (Figure.2), suggesting a likely role for exogenous P in glucose uptake. This experiment uses a relatively small amount of potassium phosphate to investigate its acute supplementation, in comparison

with chronic several-day supplementation at higher doses (Cade et al., 1984; Czuba, 2008; Czuba et al., 2009; Kreider et al., 1992; Kreider et al., 1990). We will administer 400 mg of potassium phosphate accompanied by 100 gr glucose three hours prior to the trial, compared to 2-10 gr per day for 4-6 days prior to trial.

Previous experiment with acute supplementation using calcium phosphate at 22.2 gr doses did not produce any ergogenic effects (Galloway et al., 1996). As far as we know this was the only acute supplementation trial with phosphate, but it was administered after a nightlong fast and did not contain any CHO source. We reasoned that the lack of ergogenic benefit may have been due to the lack of glycogen. Other studies noted that glycogen buildup, specifically, affects muscle signaling regardless of myoplasmic energy (ATP and Phosphocreatine) level (Joachim Nielsen et al., 2011). Another trial using a 4-day calcium phosphate supplementation regimen with 176 mmol/d (5.5 gr Pi) resulted in increased plasma Pi, with no significant increase in $VO_{2\ max}$ but a decrease in stroke volume and cardiac output, suggesting cardiovascular function alteration (Bredle, Stager, Brechue, & Farber, 1988).

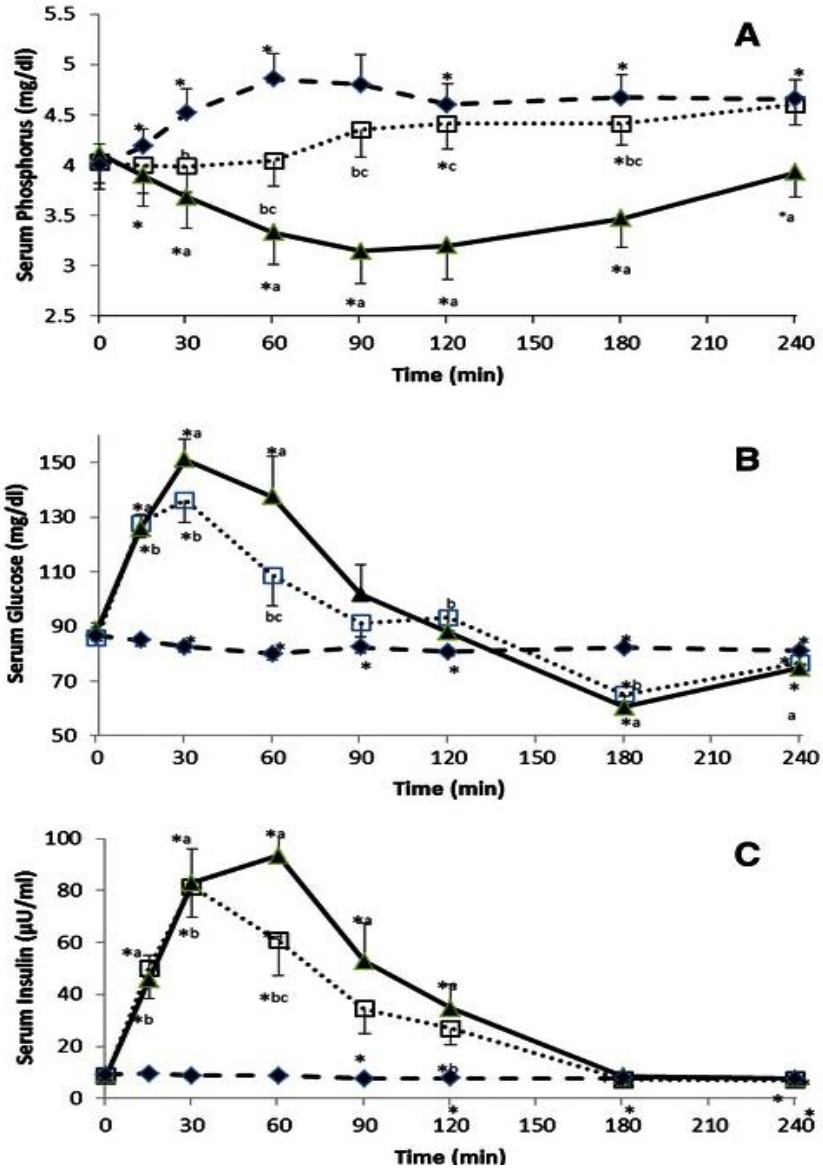

Figure 2: Phosphorus and glucose variation upon simultaneous intake.

Changes in serum phosphorus (**a**), glucose (**b**) and insulin (**c**) levels after the ingestion of 500 mg phosphorus (-♦-), 75 g glucose (-▲-) or co-ingestion glucose + phosphorus (75 g glucose + 500 mg of phosphorus) (..□..).

(Khattab, Abi-Rashed, Ghattas, Hlais, & Obeid, 2015)

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CHAPTER III

METHODOLOGY

A. Choice of subjects

1- Inclusion criteria

American University of Beirut (AUB) and Saint Joseph University (USJ) water polo players who are between the age of 18 and 25 years old, were included in the study. A group of 13 male athletes all members of AUB's water polo varsity team, and 2 from USJ's water polo varsity team, having similar energy requirements and exercise patterns, were given either placebo or a supplement of potassium phosphate in a crossover blinded fashion, after a glycogen depletion session two hours earlier and an overnight fast. The experimental protocol was approved by the American University of Beirut's Institutional Review Board.

2- Risk assessment

It should be noted that the university requires a clearance from Family Medicine following a general health and electro cardiac screening (ECG) for inclusion on a varsity team, which indicates that the trial includes no increased risk for the participating athletes (University-Sports, 2015) The health survey filled by the Family Medicine department physician includes presence of allergies and previous medical conditions.

B. Design and protocol

1- Rational for design

To detect any potential benefit of phosphate supplementation, hypothesized to occur due to an advantage in glycogen synthesis, we aimed to isolate the effect of supplementation from confounders like cardiac benefits which mostly require chronic phosphorus supplementation.

The aforementioned processes (myocardial efficiency, erythrocyte affinity) usually require an adaptation period judging from the design of the chronic intake experiments, while a co-ingestion of glucose and phosphorus will allow a direct evaluation of the effect of phosphorus on exercise efficiency, in acute setting.

2- Nondepletion protocol

We opted to induce a decrease in muscle glycogen, with a 20 mins exercise at 65% $VO_{2\ max}$, compared to a regimen of exercise to exhaustion and several bouts of sprinting at 120% $VO_{2\ max}$ (Arnall et al., 2007; Goforth et al., 2003). The glycogen replenishment did not seem to vary significantly between these options. The estimation of maximal heart rate (HR_{max}) during a water polo practice was done using a water resistant heart rate monitor, as will be described in detail in the Instruments section. During the depletion protocol, the participants were asked to keep their heart rate between 72-78% of maximal heart rate value (or 75% of what's usually obtained through heart monitoring devices during training) in order to keep at 60-70% $VO2_{\ max}$ (Lounana, Campion, Noakes, & Medelli, 2007). In addition, the participants were asked to observe an overnight fast, which favors glycogen depletion (Izumida et al., 2013).

3- Efficiency as estimate for Glycogen supercompensation

Power output and muscle glycogen content seem to be correlated (Ørtenblad, Westerblad, & Nielsen, 2013; Rauch et al., 2005). Muscle glycogen may be considered the critical factor

influencing muscle signaling (Churchley et al., 2007; Rapoport, 2010). We therefore opted to test the difference in exercise efficiency to infer improvement in muscle glycogen (Fares et al., 2017; Reger, Peterman, Kram, & Byrnes, 2013; Santalla, Naranjo, & Terrados, 2009). Work efficiency served as an indirect measure of the effect of phosphorus supplementation on glycogen accumulation.

4- Experiment protocol

i. Following an overnight fast, the participants were taken to the testing facility [Faculty of Agriculture and Food Sciences/Department of Nutrition] where: anthropometric measurements were taken (height, weight), in addition to a body composition analysis using bioelectrical impedance analysis (BIA) InBody brand 770 model.
ii. The participant were asked to rest for 30 minutes, wearing the heart rate monitor and gas exchange mask using the Quark Cardio Pulmonary Exercise Testing (CPET) Cosmed brand, while their resting energy expenditure is calculated, and then to sit for 5 minutes on the cycle ergometer for 5 minutes without pedaling
iii. After a total of 25 minutes resting participants cycled on the ergometer for 20 minutes at an average of 75% of the maximal heartrate during training, wearing the mouthpiece, to be familiar with the process, and the Borg scale was introduced
iv. Power was increased by 25W from the starting 75W to familiarize the participants with the load of the ergometer, and determine the suitable protocol in case the used power load was not suitable, instead 2 increments of 25 W and 1 increment of 10W were used
v. After 20 minutes of pedaling they were served a flavored drink containing 100 gr of glucose dissolved in 296 ml of water, the solution was provided by the American University of Beirut Medical Center (AUBMC) Glucose-100 Tolerance Drink made by Azer Scientific, with either 4 pills each containing 100 mg (total 400 mg) of phosphorus or placebo, and asked return to the lab 3 hours after the glucose ingestion.
vi. The participants were asked to return to the lab 3 hours later to allow the absorption of the glucose. Then asked to cycle on the ergometer for 25 minutes following the protocol of increasing 25 W every 5 minutes from the start at 75 W and at an average of 85 revolutions per minute (RPM) while wearing the breathing mask, except in cases where

the participants were unable to reach 150 W, where a different increase load was devised in the first morning phase.

The perception of fatigue was recorded at the last minute of each 5 minutes stage.

C. Instruments

1- Body composition

Body composition analysis was estimated by bioelectrical impedance analysis (BIA) using the InBody720 body composition analyzer which runs an electric current through the body in order to determine its composition (bones, fat, muscles, water, and their specific distributions). It was found to be an accurate method of assessing body composition, and to produce a smaller error in males (Faria, Faria, Cardeal, & Ito, 2014; Volgyi et al., 2008) .

2- Maximum heart rate in water

Estimates of heartrate (HR) during training were previously done using a water resistant heart rate monitor from the brand Swimovate using their model called PoolMateHR. A typical training session includes warm-up, drills and a water polo game. Waterproof heart rate monitors use a separate sensor worn around the chest, this method is often used in studies involving water polo (Botonis, Toubekis, & Platanou, 2016; Galy et al., 2014; Lupo, Capranica, Cugliari, Gomez, & Tessitore, 2016; Platanou & Geladas, 2006). The submersion in water has been reported to associate with a decreased heart rate, by around 15 beats/minute during maximal effort (Lupo et al., 2016), the heart rate limit used during the trial did exceed 85% of the standard maximal heart rate value during training. Cadence was kept between 85-90 RPM, tentatively keeping HR 75% of the heartrate reserve (HRR) as by the Karvonen formula which uses the difference between HR_{max} and HRR multiplied by the desired percentage and added to HRR (Camarda et al., 2008).

3- Efficiency calculation from ergometer

Work efficiency was calculated using Energy Expenditure data in the COSMED brand Modular Ergometer (Cyclergometer) and Cardio Pulmonary Exercise Test (CPET).

The ergometer designed by the COSMED is equipped with a gas exchange analysis module. It enabled us to calculate the energy expenditure while detecting the gas exchange ratio to make sure the desired range of 60% and 80% of $VO2_{max}$. The device and its modules have been validated by several studies (Nieman, Austin, Dew, & Utter, 2013; Nieman et al., 2007; Vandarakis, Salacinski, & Broeder, 2013). Efficiency was calculated from the slope of the energy expenditure curve, using the formula Efficiency= 1/slope * 1.414 (Fares et al., 2017).

Figure 3: COSMED Ergometer (Cyclergometer) and CPET Mask (Cardio Pulmonary Exercise Test)

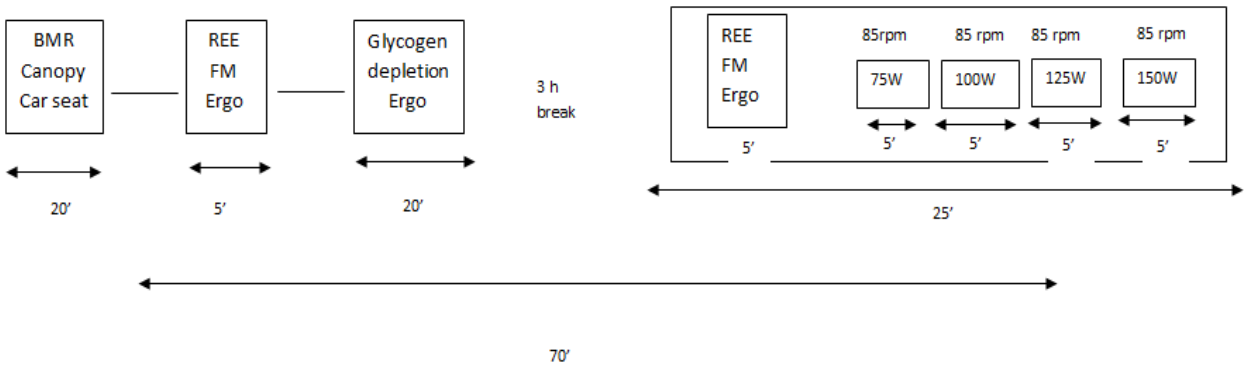

Figure 4: The experimental protocol: 20 mins sitting down relaxed on couch followed by 5 mins on bike without cycling. Glycogen depletion is next for 20 mins, followed 3 hours later by the incremental protocol: 5 mins rest on bike followed by 5 mins at 80 rpm speed at power 75 Watts up to 150.

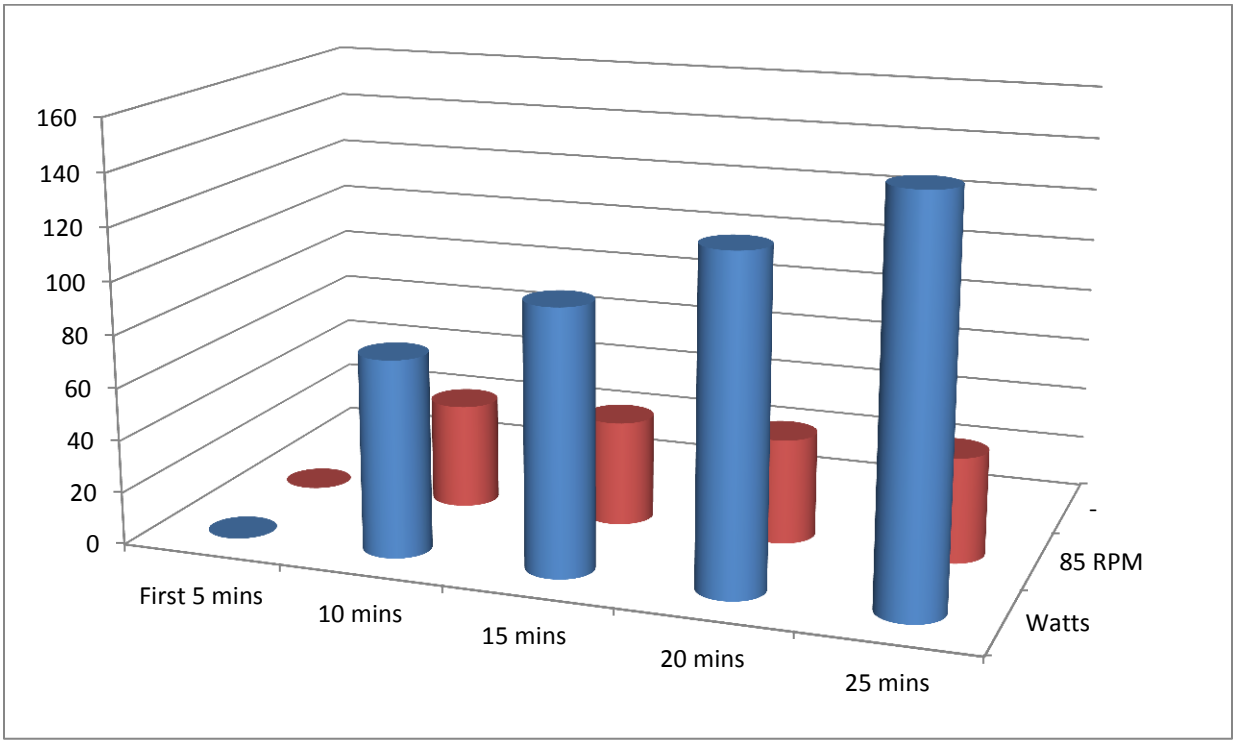

Figure 5: The incremental test with 0 Watts for 5 mins resting to 75 Watts at steady 85 RPM speed and incrementally to 100 Watts then 125 then 150 Watts

D. Statistical design

1- Sample size determination

Sample size was estimated following previous studies, varying from 6-11 participants. We also used the formula for two paired samples: $n \geq (\sigma_d / \delta_d)^2 (Z_\alpha + Z_\beta)^2$ with n= sample size, σ_d = population standard deviation (SD) estimated by pooled sample SD, and δ_d = mean difference, with Z_α corresponding to z-score of type-I error and Z_β the z-score for type-II error (Noordzij et al., 2010; van der Tweel et al., 2012). Since supplementation is relatively safe, especially at the low doses we administer, and because any improvement is valuable, we opted for a Power of 60% ($Z_\beta = 0.84$), and a 10% probability of false positive ($Z_\alpha = 1.64$). Previous power output upon from Phosphorus supplementation (Folland et al., 2008) showed $\sigma_d = 46.27$ W and $\delta_d = 30$ W for Power, and $\sigma_d = 60.05$ s and $\delta_d = 40$ s in time trial reduction, so $n \geq 14$ for a paired sample.

2- Statistical methods

The gas analyzer measurements of energy expenditure allowed us to compare the workload efficiency of two samples using a t-test, to estimate the effect of acute phosphate supplementation on glycogen replenishment. The data was examined for symmetry, and a t-test was adopted, otherwise non-parametric Wilcoxon test would have been used. The previous studies we examined have shown symmetric data and used t-tests for the phosphorus group vs. non-phosphorus group. The hypothesized increase in workload after phosphate supplementation was interpreted as the result of glycogen signaling leading to higher output as per the suggestion of glycogen signaling experiment (Rauch et al., 2005). SPSS was used for the analysis of results.

CHAPTER IV

RESULTS

A. Data analysis

1- Anthropomorphic measurements and body composition

All participants had their height and weight as well as body composition measured:

	n	Mean
Age (years)	14	20±1.2
Fat Percent (%)	14	15±6.6
Weight (Kg)	14	80.8±11.1
Height (cm)	14	181±5.1

Table 3: Basic characteristics of subjects

2- Variables measured

The gas analyzer software records a wide variety of data, of which we used: Energy Expenditure in calories per minute (EEm), Heart Rate in beats per minute (HR), Revolutions per Minute (RPM), Respiratory Quotient (RQ), Power in Watts, Time, Breath per Minute. The relevant data for our analysis consisted of EEm, HR, RPM, Power and Time. Extra variables were recorded during the protocol, like the 6-20 Borg scale perception of fatigue (Borg & Kaijser, 2006). The protocol divided the trial time into 4 phases for each increment of Power in order to calculate the Efficiency out of 4 points in the EEm curve. The HR was calculated as an average of 4 stages during cycling. The resting heart rates while on the ergometer before cycling were compared but not added to the Average HR (HRavg). This was done to check for the effect of Phosphate on heart rhythm.

3- Comparison of Means and significance

a. Energy Expenditure:

Out of 14 participants, 12 had their Energy Expenditure per minute (EEm) measured following the 4 increments protocol, 1 participant followed two different protocols, and 1 participant had a mask malfunction requiring us to omit his EEm values, and 1 participant did not have a resting EEm measurement: There was a non-significant increase in energy expenditure at through all stages of the time trial:

Energy Expenditure (Kcal/min)

Workload	n	Phosphorus	Placebo	*P*-value
Rest	11	2.063 ±0.316	1.961±0.219	0.212 (NS)
Pair 1 At 75 Watts	12	8.174±1.165	7.916±1.085	0.229 (NS)
Pair 2 At 100 Watts	12	9.586±1.032	9.542±0.993	0.812 (NS)
Pair 3 At 125 Watts	12	11.18±1.018	11.13±1.126	0.832 (NS)
Pair 4 At 150 Watts	12	12.95±1.138	12.73±1.205	0.204 (NS)

Table 4: Energy expenditure (Kcal per minute) 3 hours following the ingestion of a glucose load (100g) without or with phosphorus (400mg).

Energy expenditure was determined at different levels of workload (each 5 minutes duration)

NS= non-significant difference

b. Exercise Efficiency:

13 of the 14 participants had their energy efficiency calculated from 4 different points of the energy expenditure per minute (EEm) curve, each phase of the 4 time trial stages consisted of pedaling for 5 minutes at a constant speed and a constant load. One participant's data was discarded because of a malfunction in the gas analyzer mask. We measured the EEm after the 3rd minute to be sure it reflects the adapted metabolic effort.

No significant difference in energy efficiency was detected in 13 crossover pairs after phosphate supplementation:

Energy Efficiency	n	Phosphorus	Placebo	*P*-value
Efficiency	13	20.990±2.228	21.196±2.548	0.746 (NS)

Table 5: Energy efficiency difference between phosphorus and placebo, in percent units: mean of difference and mean of groups.
13 pairs were tested for significant difference using a paired t-test.

NS= non-significant difference

c. Heartrate:

11 participants' data for heartrate (HR) were analyzed. The HR monitor malfunctioned in one case, and in another two different devices were used so the results were discarded. A third participant followed two different cadences and a comparison therefore could not be completed. A significant increase in average HR during the cycling phase was noted in our sample.

Average HR		n	Phosphorus	Placebo	*P*-value
Pair 1	phosphorus	11	142.04±9.23	135.28±9.40	0.000

Table 6: Average heartrate (beats/minute) difference between phosphorus and placebo, and mean heartrate for phosphorus and placebo groups (crossover).
Showing average heartrate of 11 participants after phosphorus (142 ±9.23) and placebo (135.28 ±9.40)

A non-significant increase was detected in the resting phase before the cycling began, but the sample was further reduced because 3 of the participants did not have HR data during that phase, and the remaining 8 did not show a significant increase.

HR phosphorus- placebo	n	Mean HR difference in beats/min	*P*-value
Pair 1 At rest	9	4.44±6.64	0.080 (NS)

Table 7: Heartrate at Rest Comparison between phosphorus and placebo showing a non-significant difference between heartrate after phosphorus and placebo at rest

NS= non-significant difference

A significant increase in HR was detected throughout the 4 stages of cycling:

Heartrate	n	Phosphorus	Placebo	*P*-value
Pair 1	11	118.93±9.48	110.66±7.83	0.004
At 75 Watts				
Pair 2	11	134.09±8.84	127.55±8.29	0.000
At 100 Watts				
Pair 3	11	148.89±10.19	142.06±10.23	0.000
At 125 Watts				
Pair 4	11	166.18±11.82	159.92±12.88	0.000
At 150 Watts				

Table 8: Heartrate means and means difference in beats per minute during cycling phases at different power rates
Shows the average heartrate of 11 pairs at each stage of the workload gradient, the phosphorus treatment and the placebo with their standard deviation

d. Perception of fatigue:

The Borg scale (graded from 6 "no exertion at all" through 20 "maximal exertion") was used to estimate the perception of fatigue during each of the cycling phases. The participants were asked to rate the effort once at the second minute and once at the 5th minute of each phase. We recorded both but used the second to calculate the rating. No significant difference was detected:

Borg scale	n	Phosphorus	Placebo	*P*-value
Pair 1 at 75 Watts	14	8.92±1.89	8.85±1.87	0.883 (NS)
Pair 2 at 100 Watts	14	10.50±2.34	10.78±2.08	0.629 (NS)
Pair 3 at 125 Watts	14	12.42±2.68	12.78±1.80	0.535 (NS)
Pair 4 at 150 Watts	14	14.92±2.86	14.78±2.66	0.793 (NS)

Table 9: Average perception of fatigue difference between phosphorus and placebo:
Means and means difference significance during cycling phases at different power rates
Shows the average perception of fatigue in 11 pairs at each stage of the workload gradient, the phosphorus treatment and the placebo with their standard deviation

e. Respiratory Quotient (RQ):

A significant difference was detected at rest. No significant difference was detected in RQ following phosphate supplementation during exercise:

Respiratory Quotient (RQ)	n	Phosphorus	Placebo	*P*-value
Pair 1 at rest	11	0.814±0.062	0.873±0.103	0.031
Pair 2 at 75 Watts	12	0.895±0.068	0.925±0.103	0.232 (NS)
Pair 3 at 100 Watts	12	0.912±0.063	0.933±0.075	0.300 (NS)
Pair 4 at 125 Watts	12	0.924±0.061	0.949±0.067	0.215 (NS)
Pair 5 at 150 Watts	12	0.932±0.052	0.957±0.069	0.270 (NS)

Table 10: Respiratory quotient mean after phosphorus intervention and placebo during cycling phases at rest and at different power rates.
Shows the respiratory quotient of n pairs at each stage of the workload gradient, the phosphorus treatment and the placebo with their standard deviation

NS= non-significant difference

f. Breaths per Minutes (BrMn):

No differences in breathing rate were detected at any stage of the protocol between placebo and the Phosphate supplemented participants. Of the 14 participants 3 had to be excluded from this analysis: 1 had a mask malfunction, 1 did not have a resting rate measured and 1 followed two different protocols for placebo and the phosphorus intervention:

Breaths per Minutes	n	Phosphorus	Placebo	*P*-value
Pair 1 at rest	11	15.99±3.70	15.60±4.37	0.745 (NS)
Pair 2 at 75 Watts	12	26.47±6.04	26.17±6.41	0.736 (NS)
Pair 3 at 100 Watts	12	29.26±7.28	28.70±7.47	0.589 (NS)
Pair 4 at 125 Watts	12	35.20±7.48	32.98±8.11	0.157 (NS)
Pair 5 at 150 Watts	12	38.21±7.53	37.57±7.00	0.296 (NS)

Table 12: Breaths per Minutes means during cycling phases at different power rates

Shows the breaths per minute of n pairs at each stage of the workload gradient, the phosphorus treatment and the placebo with their standard deviation

NS= non-significant difference

CHAPTER V

DISCUSSION

Phosphorus is a widely used sport supplement. Most athletes who use it follow a phosphorus loading approach which consists of a weeklong phosphorus intake of 3-4 gr per day for optimal effect. Several factors could explain the ergogenic benefits, like higher ATP availability for energy expenditure. The body reserve of ATP is derived from the oxidation of blood glucose and muscle glycogen stores. As a consequence enhanced glycogenesis, which correlates to muscle signaling, may be an explanation of phosphate's ergogenic advantage. The aim of this experiment is to investigate whether acute phosphate supplementation, in smaller amounts that do not exceed usual meal content, is responsible for the performance enhancement. The research team led by Dr. Omar Obeid at the Nutrition Department has been exploring the various effects of phosphorus supplementation on metabolism and energy expenditure. Investigating phosphate supplementation in the athletic context allows for a gain in Sports Nutrition Literature, and to an exploration of mild phosphate supplementation. The glycogen replenishment or supercompensation determines the intensity of the following bout of exercise. The trial may therefore allow us to detect the possibility of inducing optimal metabolic reserve through phosphate supplementation. Another gain is the discovery of the threshold for phosphate supplementation, in order to avoid unwarranted excess. Previous trials used 4 g of phosphate for up to six days while we are using a onetime 400 mg dose. One of the trial's hypotheses is that 1 mg phosphate per Kcal is sufficient to elicit an effect, be it ergogenic, thermic, or immunotropic. Acute Phosphorus supplementation did not increase exercise efficiency in water polo players. The perception of fatigue did not change, and neither did the breathing rate during exercise nor

the respiratory quotient. The only significant difference we were able to detect was an increase in heartrate (HR) during exercise (6.75 ±2.62 bpm, table 7), and specifically after the 100 Watts resistance (Table 9A). These results lead us to conclude that acute phosphorus supplementation does not seem to increase glycogen synthesis in muscle. The heart rate increase during exercise lead us to think that core temperature was elevated (Buller, Tharion, Duhamel, & Yokota, 2015; Mark et al., 2013; Rubin, 1987). Our results show that HR only increased during exercise and not during rest after the phosphorus ingestion, leading us to conclude that the effect of supplementation was not on the cardiac rhythm. Increased myocardial efficiency was suggested as an explanation of a significant increase in $VO_{2\ max}$ along with a decrease in maximal heart rate and no change in lactate concentration or 2,3-DPG, after 6 days supplementation of 25 mg of sodium phosphate per kg of fat free mass (FFM) per day (Czuba et al., 2009). Our trial showed that increased heartrate did not have an effect on efficiency. A curious effect since an increased heartrate would normally lead to a decrease in efficiency and increased fatigue (Nelesen, Dar, Thomas, & Dimsdale, 2008). One investigation, which found no effect on athletic performance upon acute phosphate supplementation, was done using calcium phosphate (Galloway et al., 1996). Their trial was done following an overnight fast, and the phosphorus was administered without a meal, which may have eliminated the possible glycogen contribution. The existing link between serum phosphate, calcium and PTH may have led to their conclusion (Deroisy et al., 1997; Silverberg et al., 1986). Recent work on the acute effect of phosphorus on higher blood glucose clearance (Khattab et al., 2015) are indications that its acute metabolic effects are complex and warrant further investigation. Recent phosphorus research showed increased protein synthesis when an incomplete protein and its complementing amino acid were jointly administered with phosphorus (Ragi, American University of Beirut. Faculty of, Food

Sciences.Department of, & Food); gluten fed rats that co-ingested lysine alone with it, did not increase in weight, while the addition of phosphorus led to significant protein synthesis and weight gain. When amino acids were administered intravenously to surgery patients, anesthesia induced hypothermia was mitigated (Yamaoka, 2008). Phosphorus on the other hand seems to induce an increase in heat shock proteins (HCPS), which protect eukaryotic cells against temperature elevation, and phosphorus deficient animals were unable to produce the required homeostatic compensation during heat stress (Edens, Hill, & Wang, 1992; Mahmoud, Edens, Eisen, & Havenstein, 2004; Pespeni, Hodnett, & Pittet, 2005; Staib, Tümer, & Powers, 2009). This narrative leads us to conclude that our next focus should be on the nature of protein synthesis accompanying phosphorus supplementation.

A. Limitations

The use of a standard phosphorus dose for all participants didn't allow us to test for correlation of phosphorus and workload. The dose of 400mg was chosen based on 1 mg/Kcal estimation, which was noted to be the sufficient rate to detect an effect in previous landmark phosphorus supplementation studies (Ayoub, Samra, Hlais, Bassil, & Obeid, 2015; Hazim et al., 2014; Khattab et al., 2015; Obeid, Dimachkie, & Hlais, 2010). The acute setting in our experiment allows for the testing of the glycogenesis rate improvement hypothesis which suggests that the ability to exert effort at 75% of $VO2_{max}$ is related to pre-exercise level of glycogen in the muscle (Ivy, 1991). Another limitation in this study was the lack of precise determination of $VO2_{max,}$ HR_{max}, and their relationship; an often daunting task due to the inaccurate determination of their relationship (Lounana et al., 2007; Poole, Wilkerson, & Jones, 2008). Choosing the intensity of 72-86% of maximal heart rate during exercise seemed to fall between 60% and 80% of $VO2_{max}$ (Lounana et al., 2007; Pollock et al., 1998) and thus

comprising over 70% glycogen as energy source being used aerobically for optimal calorie production in a period of less than an hour (Holloszy & Kohrt, 1996; Romijn et al., 1993). The intensity level is also below the first ventilatory threshold value observed for water polo players (Galy et al., 2014), it was chosen to avoid the lactate phase and any confounding with the role that phosphorus can play in relation to the Cori cycle. The aim is to detect the effect of acute phosphorus supplementation on workload, and the dose given hypothetically enhances the glycogen production and aerobic expenditure. However, the phosphorus supplementation may have an effect on muscle signaling regardless of glycogen content, due to its influence on circadian rhythm (Kawai, Kinoshita, Shimba, Ozono, & Michigami, 2014), and which may be conceived as a limitation due to the inability to control for the phosphorus level in the participants, this was mitigated by requesting the trials be done after an overnight fast, and at the same time for all participants, besides controlling for the amount of phosphorus in the carbohydrate source. Conversely, the benefit of phosphorus supplementation may be the result of higher glycogen stores but not myoplasmic energy (ATP and Phosphocreatine) level (Joachim Nielsen et al., 2011). Glycogen was found to conform to several structures depending on its location in the cell inside the myofibril (intramyofibrillar), between them (intermyofibrillar) or under the cell membrane (subsarcolemmal), where it accordingly undergoes interactions with scaffolding proteins, phosphorylase kinase, glycogen phosphorylase, and phosphatase among others (Marchand et al., 2002; Roach et al., 1998; Wanson & Drochmans, 1968). Phosphorus supplementation can have a particular effect on any of these interactions (Pugazhenthi & Khandelwal, 1995; Torabi, Moemeni, Ahmadiafshar, & Mazloomzadeh, 2014). Further work is therefore warranted to elucidate the mechanism behind any detectable effect, and take into considerations these potential confounders.

B. Conclusion

Potassium phosphate supplementation along 400 Kcal Glucose solution after an overnight fast and 20 minutes cycling bout did not seem to significantly affect the cardiac rhythm at rest, when measured for 5 minutes before the start of the exercise protocol, but it did lead to a higher HR once the participants used the ergometer at 85 RPM and above a 75 Watts resistance. The effect of phosphate supplementation on glucose uptake and on lipemia has been reported in previous research (Hazim et al., 2014; Khattab et al., 2015). We therefore opted to record the effect of phosphate on the respiratory quotient (RQ). The room temperature was always kept at 21-23^{O}C and all participants had both their tests done at the same temperature. This leads us to suspect that acute phosphate supplementation, while being protective against hyperthermia, may be raising core temperature, as deduced from increased heartrate. Enhanced protein synthesis may be one venue of interpreting the increase in core temperature. If phosphorus is associated to this increase, without being an agent of hyperthermia or tachycardia then it is likely through the increased metabolism demands related to non-fat non-glycogen processes. Further research on the nature of these processes need to be undertaken to specify the type of proteins if any (Ohdachi et al., 1999), and their physiologic context. Recent research shows an effect of fibroblast growth factor 23 (FGF23) on macrophage number (Kovesdy & Quarles, 2016; Masuda et al., 2015). It may therefore be suggested that phosphorus regulation through FGF23 (Bergwitz & Juppner, 2010) could also be a factor therein. This only goes to show the importance of further research on the topic.

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