Phosphorus supplementation raised the heart rate of male water polo players during a randomised graded dryland exercise test

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ABSTRACT

Objective The impact of phosphorus supplementation on athletic performance is unclear. Ingestion of phosphorus for several days has been reported to increase cardiac capacity, improve oxygen muscle kinetics and enhance lactate buffering capacity. Recent studies have shown that phosphorus ingestion with a meal increases postprandial glucose uptake and thermogenesis. The present study aimed to assess the effect of acute phosphorus ingestion with a meal on specific workload parameters.

Methods A double-blind, crossover trial of 12 male water polo players between 18 and 22 years old was conducted. Overnight fasted subjects were asked to cycle for 20 min before ingesting 100 g of glucose with phosphorus or placebo (400 mg). Three hours later, they were asked to perform a graded cycling exercise for 25 min.

Results Expenditure, respiratory quotient, perception of fatigue and exercise efficiency were similar between treatments. However, heart rate was significantly higher in the phosphorus group (142±10 beats/min) compared with placebo (135±10 beats/min).

Conclusion Exercise performance 3 hours after the coingestion of glucose with phosphorus did not affect substrate use, while heart rate was increased. The heart rate increase could be attributed to a rise in core body temperature.

Trial registration number NCT03101215.

What are the new findings?

► Phosphorus supplementation increased heart rate during exercise performed 3 hours later.
► Acute phosphorus supplementation has a significant metabolic effect.
► Phosphorus supplementation does not affect the breathing rate, perception of fatigue or exercise efficiency.

How might it impact on clinical practice in the near future?

► Use of phosphorus supplementation in competitions taking place in cold water as possible protection from hypothermia.

BACKGROUND/RATIONALE

The ergogenic benefits of phosphorus supplementation have been explored since the beginning of the 20th century, but the results are often ambivalent.1 2 In most studies, the effect of chronic phosphorus loading, 2–3 g/day, was studied.3 The impact of phosphorus was thought to be its capacity to increase plasma 2,3diphosphoglycrate (2,3 DPG), which reduces O2 affinity to haemoglobin. We recently found that phosphorus coingestion with carbohydrates led to an increase in postprandial intracellular glucose uptake.4 Phosphorus is also known to enhance glycogenolysis and glycogenesis, since it is needed for glycogen phosphofructokinase activity, and ATP production.5–7 In most studies, phosphorus was not ingested with the meal, which may have reduced its impact.5–8 In one study, acute supplementation using calcium phosphate, but administered without a meal, failed to detect an effect on athletic performance.9 However, the impact of acute phosphorus ingestion with a meal on athletic performance remains to be elucidated especially since phosphorus coingestion with a meal is known to affect glycogen and ATP statuses. The acute dose also helps in isolating its metabolic effect on glycogen alone rather than 2,3 DPG known to be affected by chronic intake, making it a confounding variable.9–11

Objective The objective of this study was to investigate the ergogenic effect of acute phosphorus (400 mg) ingestion with 100 g glucose using male water polo players.

Methods Experimental protocol A crossover double-blinded study was conducted on male water polo players. Neither the participants nor the person in charge of the protocol knew which substance was ingested.

Experimental design A randomised controlled crossover trial of 12 male water polo players was conducted. Over a period of 6 weeks, subjects ingested a meal consisting of 400 mg of phosphorus or placebo (100 g glucose) before ingesting 400 mg of phosphorus or placebo (100 g glucose, two times). Randomisation was performed using a random number generator (one of 12 subjects was assigned to each trial). Subjects were tested after an overnight fast on a cycle ergometer. The exercise consisted of a 20 min warm-up at 50 W, followed by a 25 min graded exercise protocol. A cold water trial was also performed in order to test the effect of phosphorus ingestion on hypothermia.

Conclusions The present study showed that phosphorus ingestion with a meal increased the heart rate 3 hours after the coingestion of glucose and did not affect substrate use.

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Conflict of interest None.

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The training routine starts with warm-up dives for 10 min that include 10 s rest intervals between bouts, and reaching an estimated 95% of maximum heart rate capacity. Another 5 min are spent doing water treading, which consists of holding both arms at shoulder level while doing eggbeaters. Eggbeater is a specific water polo leg movement technique consisting of alternate breaststroke kicks. This strenuous routine done two times a week makes the sample relatively similar in exercise tolerance and perception of fatigue.

The study consisted of two visits to the metabolic lab at the Department of Nutrition and Food Sciences, where all data were collected; in each visit, the subject received the placebo or phosphorus treatment in a random order. Only the lab assistant in charge of conducting the test knew which treatment sequence was being administered. The training routine starts with warm-up dives for 10 min that include 10 s rest intervals between bouts, and reaching an estimated 95% of maximum heart rate capacity. Another 5 min are spent doing water treading, which consists of holding both arms at shoulder level while doing eggbeaters. Eggbeater is a specific water polo leg movement technique consisting of alternate breaststroke kicks. This strenuous routine done two times a week makes the sample relatively similar in exercise tolerance and perception of fatigue.

The study consisted of two visits to the metabolic lab at the Department of Nutrition and Food Sciences, where all data were collected; in each visit, the subject received the placebo or phosphorus treatment in a random manner. The protocol of each visit was as follows:

1. Overnight fasted participants were received at the testing facility (Department of Nutrition and Food Sciences) in the morning; they underwent a body composition analysis using bioelectrical impedance analysis (InBody brand 770 model) and were asked to rest for 30 min before wearing the heart rate monitor and gas exchange mask using the indirect calorimeter (Quark, CPET, Cosmed, Rome, Italy) in order to determine baseline energy expenditure and heart rates.

2. After baseline measurements, participants were asked to cycle on the ergometer for 20 min at an average of 70% of the maximal heart rate while wearing the mouthpiece, to be familiar with the process, and the Borg scale was introduced.

3. After baseline measurements, participants were asked to cycle on the ergometer for 20 min at an average of 70% of the maximal heart rate while wearing the mouthpiece, to be familiar with the process, and the Borg scale was introduced.

4. After baseline measurements, participants were asked to cycle on the ergometer for 20 min at an average of 70% of the maximal heart rate while wearing the mouthpiece, to be familiar with the process, and the Borg scale was introduced.

5. Cycling started at 75 W and an average of 85 revolutions per minute (RPM) was maintained throughout the test. The load was increased by 25 W every 5 min. In case the participants were unable to reach 150 W, an increment of 10 W was used instead. The perception of fatigue was recorded at the last minute of each 5 min stage using the 6–20 Borg scale.

During the workload test, several parameters were tested, including heart rate, energy expenditure, exercise efficiency, respiratory quotient, breathing rate and perception of fatigue.

### Statistical design

#### Sample size

Sample size was estimated based on previous studies, varying from 6 to 11 participants. 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% ($Z_a=0.84$), and a 10% probability of false positive ($Z_a=1.64$). Sample size calculation for two unpaired samples with an estimated 10 beats/min difference, requires a sample of 10 (using the following link: https://select-statistics.co.uk/calculators/sample-size-calculator-two-means/).

#### Statistical methods

The unpaired t-test for two means was used based on the recommendation of Wellek and Blettner, since it is a crossover sample with randomised AB-BA assignment of treatment (online supplementary files 1 and 2). Carry-over effect was estimated using the aforementioned method (T score=$0.397$, p value=$0.7006$). A column indicating carry over T-score and p value were added to the tables.

### RESULTS

The workload test showed that energy expenditure increase by about 10 kcal/min over the test period (from a rest value of about 2 kcal/min until more than 12 kcal/min), and this increase was similar between the two treatments and thus not affected by phosphorus addition to the glucose load (table 1). Moreover, exercise efficiency during the workload test of the phosphorus treatment (21.0±2.2) was similar to that of the placebo (21.2±2.5).

The average heart rate in beats per minute at rest or before the start of the workload test was similar between the two treatments, while the heart rate of the phosphorus

### Table 1: Energy expenditure (kcal/min) of subjects after the four stages of workload

| Workload | n | Phosphorus       | Placebo         | P value | Carry over T score | Carry over P value |
|----------|---|------------------|-----------------|---------|--------------------|--------------------|
| Rest     | 11 | 2.06±0.316       | 1.96±0.219      | 0.146   | 1.093              | 0.302              |
| At 75 W  | 12 | 8.17±1.165       | 7.91±1.085      | 0.128   | 0.702              | 0.498              |
| At 100 W | 12 | 9.58±1.032       | 9.54±0.993      | 0.788   | 0.659              | 0.524              |
| At 125 W | 12 | 11.18±1.018      | 11.13±1.126     | 0.798   | 0.240              | 0.815              |
| At 150 W | 12 | 12.95±1.138      | 12.73±1.205     | 0.078   | -0.0585            | 0.954              |
treated group maintained a significantly higher value throughout the testing period. During the workload test, the heart rate of the phosphorus treatment averaged 142±9 compared with 135±9 for the placebo treatment (P value<0.001) (table 2).

**DISCUSSION**

The ability of phosphorus to stimulate postprandial glycogen synthesis as well as glycogenolysis (through stimulation of glycogen phosphorylase) incited us to investigate the impact of phosphorus on workload, known to be affected by glycogen status. The dose of 400mg was chosen to remain within one-third of phosphorus recommended dietary allowance (RDA) for males aged 18–50 years old and minimise the carryover effect, also minimised by adopting a week-long washout period. This was verified statistically using the methodology described by Wellek and Blettner. Period effect was minimised by scheduling treatment sequence (AB-BA) at the same time of day, and no more than 2 weeks apart, to account for the circadian phosphorus level. The bolus dosing regime was adopted to detect the effect of supplementing 1 mg phosphorus per kcal, noted to be the sufficient rate to detect an effect in previous phosphorus supplementation studies. The population is composed of team members with similar training routine, which leads to a relatively similar response to prolonged exercise and intensity of workload. Water polo is known for its harsh training conditioning, as described in the methodology, making our population likely to have a lower score on the perception of fatigue scale. The other distinctive feature is the ability to perform intensive exercise in water, compared with leisurely swimming, and the likelihood to practice in a lake or non-heated pool ahead of ideal water temperature season.

Phosphorus addition to food did not affect energy expenditure of the workload test, although it had been reported to raise postprandial energy expenditure (0–4 hours after meal) probably to enhance the postprandial anabolic processes. The heart rate increase during exercise points towards a rise in core temperature.

The similarity in heart rate between the treatment groups before the workload test implies that phosphorus ingestion did not affect the cardiac rhythm. The ability of an acute phosphorus dose to increase heart rate during a workload test may be related to a potential increase in core temperature. The increase in core temperature may be related to the capacity of phosphorus to raise postprandial thermogenesis. Furthermore, the observed increase in heart rate may have been an adaptive mechanism to compensate for a decrease in exercise efficiency. Phosphorus, on the other hand, seems to induce an increase in heat shock protein synthesis, which protects eukaryotic cells against temperature elevation, since phosphorus-deficient animals were found unable to produce the required homeostatic compensation during heat stress. This study showed that increased heart rate did not have an effect on exercise efficiency (table 3). A curious finding since an increased heart rate normally leads to a decrease in exercise efficiency and to increased fatigue. A possible direction of our research in sports nutrition can focus on the effect of acute phosphorus supplementation on efficiency in water polo players swimming in non-heated pools or in open water, in line with researching the effect of new practices in competitive sports. Since many teams start their open water training when the water temperature is relatively lower than traditional pools where temperature is set to 26°C, the increase in core temperature could provide an ergogenic advantage.

**CONCLUSION**

In water polo players, the addition of phosphorus to a glucose load failed to affect subsequent energy expenditure during a workload test performed 3 hours later. At the same time, exercise efficiency was not affected despite a significant increase in heart rate.

Out of 14 participants, 12 had their energy expenditure per minute (EEm) measured following the four-increment protocol; 1 participant followed two different protocols; and

| Table 2 | Heart rate (beats/min) of subjects after the four stages of workload |
|---------|-----------------------------|
| **Workload** | **n** | **Phosphorus** | **Placebo** | **P value** | **Carry over T score** | **Carry over P value** |
| At rest (fasted) | 11 | 74±5 | 73±5 | 0.688 | 0.367 | 0.722 |
| At rest | 10 | 82±8 | 78±6 | 0.105 | 1.493 | 0.170 |
| At 75 W | 11 | 119±9 | 111±8 | 0.0036 | 0.786 | 0.452 |
| At 100 W | 11 | 134±9 | 127±8 | 0.00086 | -0.428 | 0.678 |
| At 125 W | 11 | 149±10 | 142±10 | 0.00047 | -0.793 | 0.448 |
| At 150 W | 11 | 166±12 | 160±13 | 0.00047 | -0.470 | 0.649 |
| Average heart rate | 11 | 142±9 | 135±9 | 0.000017 | -0.397 | 0.7006 |

| Table 3 | Exercise efficiency (%) of subjects after the four stages of workload |
|---------|-----------------------------|
| **Exercise efficiency** | **n** | **Phosphorus** | **Placebo** | **P value** | **Carry over T score** | **Carry over P value** |
| 13 | 21.0±2.2 | 21.2±2.5 | 0.767 | 1.672 | 0.123 |
1 participant had a mask malfunction, requiring us to omit his EEm value. One participant did not have a resting EEm measurement; therefore, the energy expenditure at rest was calculated in 11 participants. There was no significant increase in energy expenditure at any workload and no significant increase throughout all stages of the trial.

Overnight fasted subjects were asked to cycle for 20 min before being given 100 g of glucose with or without phosphorus (400 mg). Three hours later, they were asked to perform a graded cycling exercise for 25 min, and both measures energy expenditure and heart rate were monitored.

Eleven participants’ data for heart rate were analysed. Two subjects’ HR measurements during the rest period on the bike before the start of pedalling were not registered.

Participants (n=13) had their exercise efficiency calculated from four different points of the EEm curve; each phase of the four time trial stages consisted of pedalling for 5 min at a constant speed and a constant load. We measured the EEm after the third 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.

Correction notice The article has been corrected since it was published online.

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