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The Effects of Sodium Phosphate Supplementation on the Cardiorespiratory System and Gross Efficiency during Exercise under Hypoxia in Male Cyclists: A Randomized, Placebo-Controlled, Cross-Over Study

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Abstract: The main aim of this study was to evaluate the effects of six days of tri-sodium phosphate (SP) supplementation on the cardiorespiratory system and gross efficiency (GE) during exercise under hypoxia in cyclists. Twenty trained male cyclists received SP (50 mg·kg^{−1} of fat-free mass/day) or placebo for six days in a randomized, cross-over study, with a three-week washout period between supplementation phases. Before and after each supplementation phase, the subjects performed an incremental exercise test to exhaustion under normobaric hypoxia (FiO₂ = 16%, ~2500 m). It was observed that short-term SP supplementation led to a decrease in heart rate, an increase in stroke volume, and an improvement in oxygen pulse (VO₂/HR) during low and moderate-intensity exercise under hypoxia. These changes were accompanied by an increase in the serum inorganic phosphate level by 8.7% ($p < 0.05$). No significant changes were observed in serum calcium levels. GE at a given workload did not change significantly after SP supplementation. These results indicated that SP promotes improvements in the efficiency of the cardiorespiratory system during exercise in a hypoxic environment. Thus, SP supplementation may be beneficial for endurance exercise in hypoxia.

Keywords: phosphate loading; ergogenic aids; hypoxia; altitude; cardiorespiratory system; myocardium; gross efficiency; athletes



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1. Introduction

Over the years, it has been suggested that supplementation with phosphate salts may have a positive effect on athletic performance [1]. In 2021, phosphate was classified by the Australian Institute of Sport as a substance for which the scientific evidence does not support a benefit amongst athletes or no research has been conducted to form an informed opinion about the substance [2]. Undoubtedly, more research is needed to determine the effectiveness of phosphate supplementation in athletes and to establish the best practice protocol in this regard. To date, several studies have supported the exercise performance benefits of phosphate salt supplementation in normoxic conditions. These studies showed an increase in maximal oxygen uptake [3–8] and anaerobic threshold [4,7,9], better time trial performance [10,11], and improvements of the repeated-sprint ability [11–14]. A number of mechanisms have been proposed to explain the ergogenic effects of phosphate loading, including increasing oxidative metabolism and adenosine triphosphate (ATP) synthesis, and also more rapid restoration of ATP and phosphocreatine in the muscles (through greater availability of extracellular and intracellular phosphates), an improvement of oxygen unloading in muscles by reducing Hb-O₂ affinity (via increasing erythrocyte 2,3-diphosphoglycerate concentrations), increasing buffering capacity (via enhancing extracellular hydrogen phosphate concentration), and improving myocardial function [1].

The improvement in myocardial and cardiovascular responses to exercise following phosphate supplementation is explained by increased myocardial contractility as a result

of an increase in the levels of cardiac cell ATP [9]. Myocardial contractility is one of the determinants of heart stroke volume (SV). When the force of myocyte contraction increases, the heart can eject more blood out into the vasculature, thus increasing the SV [15]. Several studies have shown that phosphate loading leads to an increase in SV [9] and a decrease in heart rate (HR) [6,7,16,17] at rest and during exercise in normoxia. These adaptations were accompanied by improved aerobic capacity and time trial performance in athletes [6,7,9]. Keeping in mind that the heart is dependent on aerobic metabolism to sustain contractile function, and that in hypoxic conditions it responds with a compensatory increase in cardiac output (Q) that further increases myocardial O₂ demand [18], it can be presumed that an improvement in myocardial function following phosphate loading may also be beneficial in hypoxic conditions. However, there are currently no studies analyzing the effect of phosphate salts on exercise performance and cardiorespiratory system efficiency under hypoxia.

Hypoxic exposure leads to important changes in the cardiovascular system [19]. During acute hypoxia, resting SV is unchanged, but HR increases through stimulation of the cardiac β -adrenergic receptors and an increase in circulating epinephrine, resulting in higher Q [20]. In contrast, maximal HR and SV are lowered and thus maximal Q declines in hypoxic conditions [21]. During submaximal exercise, at a given exercise intensity (at the same absolute workload), HR is greater and SV is lower in hypoxia compared to normoxia [19,20,22]. For example, Clark et al. [23] found that submaximal HR during 50–250 W workloads was significantly elevated by a mean of 5–21 bpm at both 2200 m and 3200 m compared to sea level. In other words, at the same HR, exercise capacity is lower in hypoxia than in normoxia. Recently, we observed that mean power generated by cyclists during a 30 km time trial in acute hypoxia (FiO₂ = 16.5%, 2000 m) was 9.6% lower with the same average HR as in normoxia [24]. Similarly, Weavil et al. [25] reported a 9% decrease in average power during a five km cycling time trial in hypoxia (FiO₂ = 17%), while HR did not differ from that in normoxia.

Achieving adequate Q during exercise to ensure sufficient oxygen uptake for a given workload is determined by the changes in SV and HR [26]. In terms of myocardial oxygen demand, increasing SV is much more efficient than increasing HR during exercise [27]. Reduction of myocardial oxygen demand should be particularly preferred during exercise in hypoxic conditions when oxygen availability is limited. Therefore, the first aim of this study was to investigate the effect of six-day sodium phosphate (SP) supplementation on cardiorespiratory variables in cyclists during exercise in normobaric hypoxia (FiO₂ = 16.0%; ~2500 m). We hypothesize that phosphate salt loading improves the efficiency of the cardiorespiratory system.

The second purpose of this study was to analyze whether SP supplementation would change gross mechanical efficiency (GE). GE is one of the main factors determining exercise performance [28,29]. Mechanical efficiency refers to the ability to transfer the energy expended into performing external work [30]. The increase in efficiency is associated with the improved conversion of chemical energy from ATP hydrolysis into mechanical energy during muscle contraction [28]. Less efficiency for a given work output is attributed to the higher energy cost of exercise [31,32]. Hypoxic conditions may decrease mechanical efficiency, but the results of previous studies are still inconclusive [23,33,34]. Noordhof et al. [35] proposed that the reduction of GE under hypoxic conditions could be caused by an increased cost of ventilation and HR in combination with a higher respiratory-exchange ratio (RER) during exercise.

It was demonstrated that the intracellular concentration of inorganic phosphate (Pi) is an important determinant of mitochondrial oxidative phosphorylation [36,37], and that a decrease in muscle ATP synthesis may be associated with low blood Pi concentrations [37]. Recently, Marcos et al. [38] reported that sodium phosphate intake improves energy efficiency (reduction of oxygen consumption at the same workload) during exercise at low intensity in normoxia. Thus, we expect that SP supplementation improves GE under hypoxic conditions.

2. Materials and Methods

2.1. Study Participants

Twenty trained male cyclists (aged 34.6 ± 4.3 years; body height 180.5 ± 5.9 cm; body mass 73.2 ± 6.6 kg; fat content (%) 13.9 ± 3.4 %; fat-free mass (FFM) 63.0 ± 5.8 kg) participated in this study. The basic inclusion criteria were: training experience of at least six years and at least a six-month wash-out period without altitude training and sodium phosphate supplementation. Furthermore, before starting the experiment, blood ion concentrations were analyzed in all participants under fasting conditions. All athletes had the correct blood ion levels before the experiment. All athletes had current medical examinations without any contraindications to performing exhaustive exercise in a hypoxic environment. The participants provided their written voluntary informed consent before participation. Additionally, participants declared that for at least one month before testing, they did not take either medications or dietary supplements. Study participants were randomly divided into two equal research groups, G1 and G2, using a computer-generated randomized list [39].

The research project was conducted according to the Declaration of Helsinki and was approved (no. 4/2018, approval date: 15 November 2018) by the Ethics Committee for Scientific Research at the Jerzy Kukuczka Academy of Physical Education in Katowice, Poland.

2.2. Study Design

The study was carried out in a cross-over design (Figure 1). The experiment included two six-day supplementation phases with a three-week washout period (for details, see in the “Supplementation with sodium phosphate” section). Before and after each supplementation phase, a test series (S1, S2) was performed. All series were based on the same methodology. The time of day and the order of the participants were maintained in order to ensure similar conditions for the measurements. Each test series began by obtaining venous blood (10 mL) from an antecubital vein under fasting conditions to determine resting levels of the following variables: hemoglobin concentration ([Hb]), hematocrit (Hct), red blood cell count (RBC), and serum levels of inorganic phosphate (Pi) and calcium (Ca). After blood had been obtained, body height, body mass, and body composition were evaluated using the DXA (dual-energy X-ray absorptiometry) method (GE Lunar Prodigy). Hematocrit value and anthropometric variables were used to calculate plasma volume (PV) according to the formula proposed by Nadler et al. [40].

Two hours after a light mixed meal (5 kcal/kg body weight, 50% CHO, 30% Fat, 20% Pro) and after 15 min of passive exposure to normobaric hypoxia ($\text{FiO}_2 = 16\%$; ~2500 m), all participants performed an incremental exercise test under normobaric hypoxia to determine the values of $\text{VO}_{2\text{max}}$ and lactate threshold (LT). The incremental test was performed using the Excalibur Sport cycle ergometer (Lode, Netherlands). Exercise tests were performed in a normobaric hypoxic chamber (AirZone 25, Air Sport, Poland). During all test series, the atmospheric conditions such as temperature (19°C), humidity (50%), the concentration of carbon dioxide (700–800 ppm), and oxygen ($\text{FiO}_2 = 16\%$) were controlled and held constant to increase the reliability of the investigations.

2.3. Incremental Exercise Test

The same test protocol was applied for all exercise tests in each series of testing. The incremental test started with a load of 80 W which was increased by 40 W every three min to exhaustion or until the participant was unable to maintain the minimal cadence of 60 rpm. At rest (three min before the test) and during the exercise, heart rate (HR), minute ventilation (VE), breathing frequency (BF), oxygen uptake (VO_2), expired carbon dioxide (VCO_2) and respiratory exchange ratio (RER) were measured continuously with a fast gas analyzer (MetaLyzer 3B, Cortex) using the breath-by-breath method. At the end of each workload (last 15 s), capillary blood samples were drawn from the fingertips to determine blood lactate levels (SUPER GL2, Dr. Müller Gerätebau GmbH). These data were used to evaluate individual lactate threshold (LT) based on the D_{max} method [41]. Our previous

studies [42,43] demonstrated that LT determined using the D-max method corresponds to the maximal lactate steady state (MLSS).

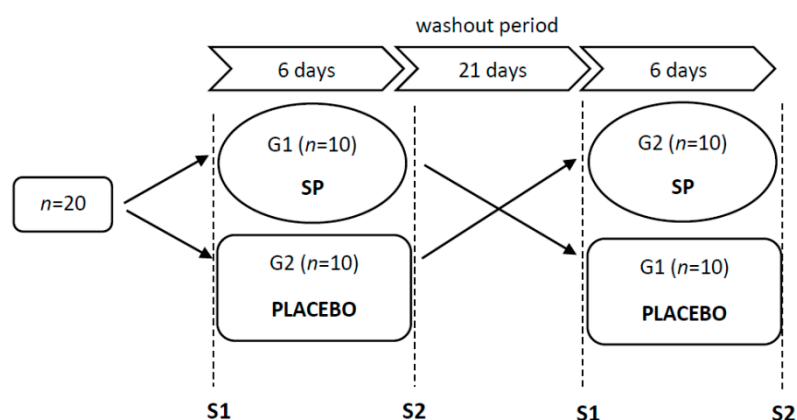


Figure 1. Study design. SP—sodium phosphate supplementation (50 mg/kg FFM per day); S1 and S2—two test series, before and after each supplementation phase.

During the first test of each supplementation phase (S1), HR, VO_2 , oxygen pulse (VO_2/HR), stroke volume (SV), and cardiac output (Q) at LT workload (LT), as well as one and two workloads below and above LT (LT−1, LT−2, LT+1, LT+2) were analyzed. During the next tests (S2), these variables were identified at the same absolute workloads as during the first test. Q was estimated by $\text{VO}_{2\text{max}}$, and was calculated by the following formula: $Q = 100 \times \text{VO}_2 \div \left(5.72 + 10.5a_{\frac{\text{VO}_2}{\text{VO}_{2\text{max}}}} \right)$ used by MetaSoft Studio (Cortex, Germany). SV was calculated as follows: $\text{SV} = Q \div \text{HR}$.

2.4. Supplementation with Sodium Phosphate

During 6 days of the supplementation phase, subjects from group G1 received tri-sodium phosphate at a dose of 50 mg/kg FFM per day. The dose was divided into equal portions and administered four times a day at similar intervals. The participants in group G2 received a placebo in the form of 4 g of cellulose per day, also at a dose divided into four equal portions. Study participants were not informed which substance they were taking. The first six-day supplementation phase was followed by a 21-day substance wash-out period during which participants were not supplemented. Next, the second supplementation phase began. It was identical to the first but the groups were changed so that the six-day supplementation with SP was used in group G2, whereas group G1 received a placebo. Throughout the experiment, all participants received nutritional, training and supplementation recommendations.

SP dose (50 mg/kg FFM per day) and supplementation time (6 days) were selected based on the methodology used in previous studies on ergogenic effects of phosphate salt supplementation in normoxia [7,44–46]. Buck et al. [1] demonstrated that the dose of 3–5 g of SP per day applied over a period of 3–6 days is adequate to achieve sufficient serum phosphate levels and to ensure the expected supplementation benefits. Doses greater than 6 g are usually avoided during supplementation, as they are associated with a reduction in the serum phosphate concentration through regulation of its level through parathormone. Doses below 3 g are generally considered too low to significantly increase serum phosphate levels [1,47,48]. The duration of the washout period (21 days) was selected according to the suggestion that the washout time should be two to three weeks in order to remove any carryover effects from previous phosphate supplementation [3].

2.5. Determination of Gross Efficiency

GE was calculated as the ratio of work accomplished per minute (watts converted to kcal/min) to energy expended per minute (kcal/min). Energy expenditure was calculated from VO_2 and RER using the tables of Lusk [49].

$$\text{GE} = \frac{\text{Work accomplished}}{\text{Energy expended}} \times 100\% \quad (1)$$

During the first test of each supplementation phase (S1) GE at one and two workloads below LT (LT−1, LT−2) was analyzed. During the next tests (S2), GE was identified at the same absolute workloads as during the first test. GE was only calculated when the RER was ≤ 1.0 . The measured RER was converted to the caloric equivalent of oxygen for the non-protein respiratory quotient [49]. During exercise at LT and higher workloads, RER was >1.0 , thus GE was calculated only for workloads below LT.

2.6. Statistical Analysis

The results of the study were analyzed using StatSoft Statistica 13.0 software. The results were presented as mean \pm SEM. The statistical significance level was set at $p < 0.05$. Prior to all statistical analyses, normality of the distribution of variables was checked using the Shapiro-Wilk test. The analysis of variance (ANOVA) for repeated measures (intervention [SP, placebo] \times time [S1, S2]) was used to determine differences in each of the dependent variables. When significant differences were found, the post hoc Tukey test was used. Effect sizes (ESs) were calculated from standardized differences (Cohen's d units). Threshold values for Cohen ES statistics were considered to be small (0.20–0.60), moderate (0.60–1.20), large (1.20–2.0), very large (2.0–4.0), or extremely large (>4.0) [50].

3. Results

3.1. Exercise Intensity

The LT workload (LT), as well as one and two workloads below and above LT (LT−2, LT−1, LT+1, LT+2), corresponds to absolute workload determined during the initial exercise test (S1). The exercise intensity reported as a % of actual $\text{VO}_{2\text{max}}$ measured during S1 and S2 is presented in Table 1.

ANOVA with repeated measures showed a significant interaction (intervention \times time) for % of actual $\text{VO}_{2\text{max}}$ at two workloads above LT ($F = 6.64$, $p < 0.05$). The post-hoc Tukey's test revealed a decrease in % of actual $\text{VO}_{2\text{max}}$ at LT+2 workload ($p < 0.05$) following SP supplementation (Table 1). No significant changes were observed after placebo ingestion.

Table 1. Exercise intensity presented as a percentage of actual maximal oxygen uptake.

Workload	Variable	Sodium Phosphate		Placebo	
		S1	S2	S1	S2
LT−2	% of actual $\text{VO}_{2\text{max}}$	61.5 \pm 1.3	59.9 \pm 1.2	62.0 \pm 1.3	62.7 \pm 1.3
LT−1	% of actual $\text{VO}_{2\text{max}}$	72.7 \pm 1.1	72.0 \pm 1.2	74.1 \pm 1.2	74.7 \pm 1.3
LT	% of actual $\text{VO}_{2\text{max}}$	83.9 \pm 0.9	82.3 \pm 1.0	84.5 \pm 0.9	85.6 \pm 1.1
LT+1	% of actual $\text{VO}_{2\text{max}}$	93.7 \pm 0.7	92.0 \pm 0.9	94.4 \pm 0.8	94.9 \pm 1.0
LT+2	% of actual $\text{VO}_{2\text{max}}$	99.3 \pm 0.5	96.6 * \pm 0.8	97.3 \pm 0.6	98.6 \pm 1.0

LT−2, LT−1, LT, LT+1, LT+2—absolute workload corresponding to workload at lactate threshold (LT) as well as one and two workloads below and above LT which were determined during the initial exercise test (S1); S1—before supplementation phase, S2—after supplementation phase; * $p < 0.05$ S1 vs. S2.

3.2. Cardiorespiratory Variables

HR, SV, Q, VO_2 and VO_2/HR were analyzed at workloads (LT−2, LT−1, LT, LT+1, LT+2) determined during the initial exercise test (S1). During the next test (S2), these variables were identified at the same absolute workloads as during the S1.

ANOVA with repeated measures showed a significant interaction (intervention \times time) for HR at workloads below LT (HR_{LT-2} : $F = 3.83$, $p < 0.05$; HR_{LT-1} : $F = 5.97$, $p < 0.05$) and for HR at LT (HR_{LT} : $F = 4.90$, $p < 0.05$). Statistically significant changes were also found for SV at LT and at one workload below LT (SV_{LT-1} : $F = 7.72$, $p < 0.01$; SV_{LT} : $F = 5.98$, $p < 0.05$). Furthermore, changes at the limit of the adopted significance level were observed for oxygen pulse at one workload below LT (VO_2/HR_{LT-1} ; $F = 3.64$, $p < 0.07$).

The post-hoc Tukey test showed that HR_{LT-2} and HR_{LT-1} significantly ($p < 0.01$) decreased due to SP supplementation (Figure 2A) by 3.9% ($d = 0.36$) and 3.4% ($d = 0.38$), respectively. Additionally, HR_{LT} dropped by 1.9% following SP, although not significantly ($p < 0.06$, $d = 0.27$). Similar changes were not observed after placebo ingestion (Figure 2B). Analysis also revealed a significant increase in SV_{LT-1} by 4.1% ($p < 0.01$, $d = 0.47$) and in SV_{LT} by 2.5% ($p < 0.05$, $d = 0.30$). SV_{LT-2} increased by 3.7%, but these changes did not reach statistical significance ($p < 0.08$, $d = 0.44$) (Table 2). Furthermore, SP supplementation improved VO_2/HR_{LT-1} by 5.2% ($p < 0.05$, $d = 0.45$) (Figure 3A). At workloads above LT, there were no significant changes in HR, SV and VO_2/HR . VO_2 at the given workloads also did not change significantly after the interventions (Table 2).

There were no statistically significant changes in cardiac output (Q) at the given workloads (Table 2). Q_{max} and SV_{max} were unchanged after SP supplementation (S1 vs. S2: SP 19.13 ± 0.29 vs. 19.01 ± 0.30 L·min⁻¹; PI 19.23 ± 0.31 vs. 19.23 ± 0.32 L·min⁻¹ and SP 107.2 ± 2.2 vs. 107.0 ± 2.4 mL; PI 106.2 ± 2.3 vs. 108.4 ± 2.5 mL, respectively). Furthermore, VO_{2max} and HR_{max} also did not change significantly following the interventions (S1 vs. S2: SP 3.45 ± 0.06 vs. 3.51 ± 0.06 L·min⁻¹; PI 3.47 ± 0.06 vs. 3.44 ± 0.06 L·min⁻¹ and SP 178.7 ± 2.4 vs. 178.1 ± 2.5 bpm; PI 179.6 ± 2.6 vs. 179.4 ± 2.6 bpm, respectively).

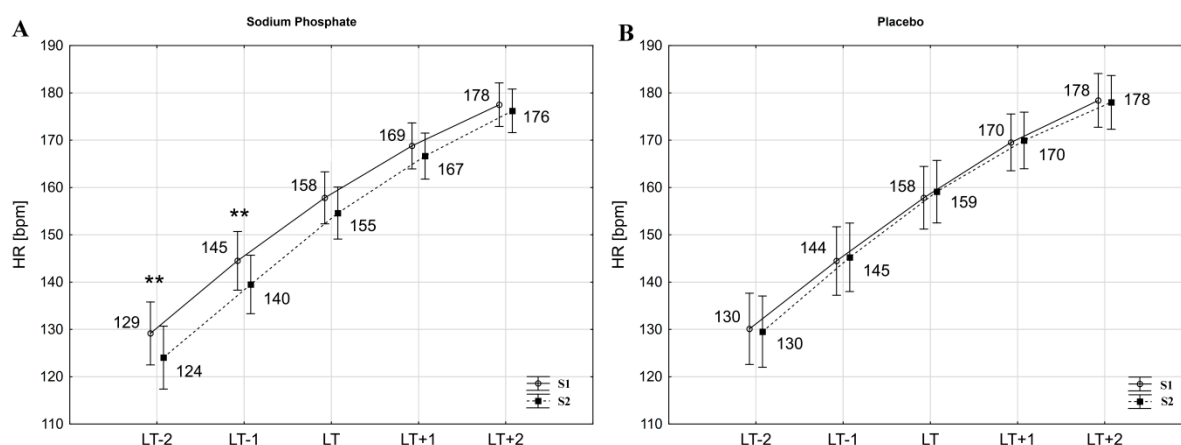


Figure 2. Heart rate (HR) during exercise in hypoxia before (S1) and after (S2) sodium phosphate (A) and placebo (B) ingestion. ** $p < 0.01$ S1 vs. S2. LT-2, LT-1, LT, LT+1, LT+2—absolute workload corresponding to workload at lactate threshold (LT) as well as one and two workloads below and above LT which were determined during the initial exercise test (S1).

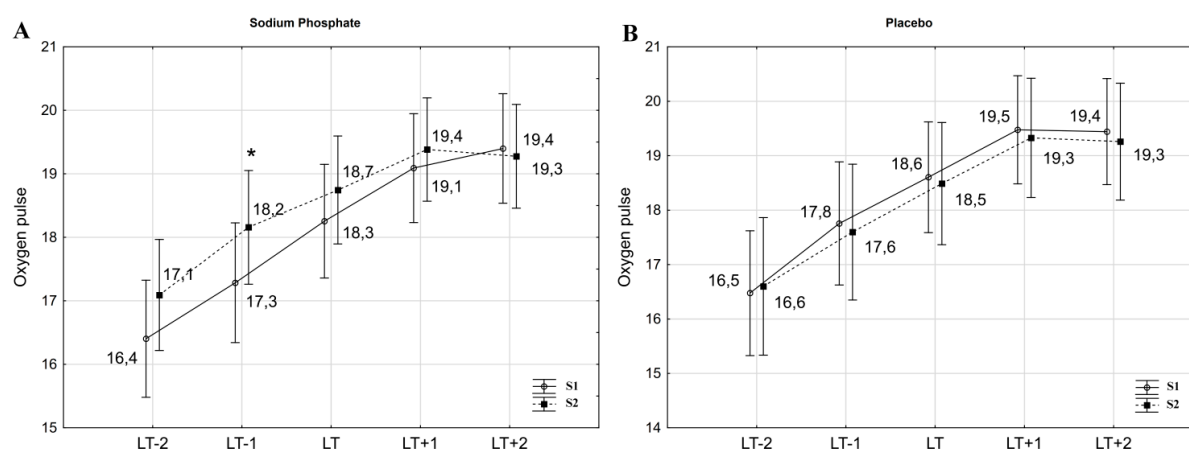


Figure 3. Oxygen pulse (VO_2/HR) during exercise in hypoxia before (S1) and after (S2) sodium phosphate (A) and placebo (B) ingestion. * $p < 0.05$ S1 vs. S2. LT−2, LT−1, LT, LT+1, LT+2—absolute workload corresponding to workload at lactate threshold (LT) as well as one and two workloads below and above LT which were determined during the initial exercise test (S1).

Table 2. Stroke volume (SV), cardiac output (Q) and oxygen uptake (VO_2) during exercise in hypoxia before (S1) and after (S2) sodium phosphate and placebo supplementation.

Workload	Variables	Sodium Phosphate		Placebo	
		S1	S2	S1	S2
LT−2	SV (mL)	123.6 ± 2.9	128.2 ± 3.6	120.8 ± 3.0	121.2 ± 3.7
	Q ($\text{L} \cdot \text{min}^{-1}$)	15.95 ± 0.27	15.85 ± 0.32	16.15 ± 0.28	15.88 ± 0.38
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	2.12 ± 0.06	2.10 ± 0.06	2.17 ± 0.06	2.18 ± 0.06
LT−1	SV (mL)	117.9 ± 2.7	122.7 ** ± 3.1	117.4 ± 2.8	116.9 ± 3.2
	Q ($\text{L} \cdot \text{min}^{-1}$)	17.10 ± 0.27	17.08 ± 0.32	17.36 ± 0.28	17.17 ± 0.32
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	2.51 ± 0.06	2.53 ± 0.07	2.59 ± 0.06	2.59 ± 0.07
LT	SV (mL)	113.8 ± 2.6	116.6 * ± 2.5	113.6 ± 2.6	113.3 ± 2.6
	Q ($\text{L} \cdot \text{min}^{-1}$)	18.05 ± 0.27	17.97 ± 0.28	18.22 ± 0.28	18.19 ± 0.29
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	2.90 ± 0.06	2.89 ± 0.06	2.95 ± 0.06	2.97 ± 0.06
LT+1	SV (mL)	110.8 ± 2.4	112.3 ± 2.3	110.2 ± 2.5	110.6 ± 2.4
	Q ($\text{L} \cdot \text{min}^{-1}$)	18.75 ± 0.29	18.69 ± 0.28	18.94 ± 0.30	18.93 ± 0.29
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	3.23 ± 0.06	3.22 ± 0.06	3.32 ± 0.07	3.30 ± 0.06
LT+2	SV (mL)	107.6 ± 3.0	109.2 ± 3.2	109.4 ± 3.5	109.4 ± 3.7
	Q ($\text{L} \cdot \text{min}^{-1}$)	19.17 ± 0.40	19.10 ± 0.44	19.50 ± 0.47	19.48 ± 0.51
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	3.44 ± 0.06	3.38 ± 0.06	3.46 ± 0.07	3.43 ± 0.07

LT−2, LT−1, LT, LT+1, LT+2—absolute workload corresponding to workload at lactate threshold (LT) as well as one and two workloads below and above LT which was determined during the initial exercise test (S1). * $p < 0.05$, ** $p < 0.01$ S1 vs. S2.

3.3. Gross Efficiency

Gross efficiency (GE) and RER during S1 and S2 were analyzed at the same absolute workloads. There were no statistically significant changes in GE and RER at the given workloads after SP supplementation or placebo ingestion (Table 3).

3.4. Serum Phosphate and Calcium Concentrations

ANOVA with repeated measures showed a significant interaction (intervention × time) for serum Pi level ($F = 4.16$, $p < 0.05$). The post-hoc Tukey test revealed that Pi concentration increased by 8.7% ($p < 0.05$, $d = 0.72$) following the SP supplementation (S1 vs. S2: SP 3.11 ± 0.08 vs. 3.38 ± 0.09 mg/dL; PI 3.32 ± 0.08 vs. 3.33 ± 0.09 mg/dL). No significant changes were observed in serum Ca level (S1 vs. S2: SP 9.55 ± 0.06 vs. 9.51 ± 0.06 mg/dL; PI 9.53 ± 0.06 vs. 9.47 ± 0.06 mg/dL).

Table 3. Gross efficiency (GE) and respiratory exchange ratio (RER) during exercise in hypoxia before (S1) and after (S2) sodium phosphate and placebo supplementation.

Workload	Variables	Sodium Phosphate		Placebo	
		S1	S2	S1	S2
LT−2	GE (%)	18.73 ± 0.47	18.96 ± 0.45	19.06 ± 0.50	19.00 ± 0.48
	RER	0.94 ± 0.01	0.93 ± 0.01	0.94 ± 0.01	0.93 ± 0.01
LT−1	GE (%)	20.20 ± 0.43	20.05 ± 0.41	20.54 ± 0.45	20.39 ± 0.43
	RER	0.97 ± 0.01	0.97 ± 0.01	0.98 ± 0.01	0.97 ± 0.01

LT−2, LT−1—absolute workload corresponding to one and two workloads below lactate threshold (LT) which was determined during the initial exercise test (S1).

3.5. Hematocrit and Plasma Volume

There were no statistically significant changes in Hct (S1 vs. S2: SP 43.6 ± 0.6 vs. $43.1 \pm 0.5\%$; Pl 44.1 ± 0.5 vs. $43.3 \pm 0.5\%$) and PV (S1 vs. S2: SP 2888 ± 58 vs. 2909 ± 58 mL; Pl 2831 ± 57 vs. 2872 ± 56 mL) after SP supplementation or placebo ingestion.

4. Discussion

According to the authors' knowledge, this is the first study to analyze the ergogenic effects of phosphate salt supplementation on exercise performance under hypoxic conditions. Our study revealed that short-term SP supplementation leads to a decrease in HR, an increase in SV, and an improvement in oxygen pulse (VO_2/HR) during exercise at low to moderate intensity ($\leq \text{LT}$) under hypoxia (2500 m). These results indicated that SP promotes improvements in the efficiency of the myocardial and cardiorespiratory systems during exercise in a hypoxic environment. Thus, SP supplementation may be beneficial for endurance exercise performance in hypoxia. Lowering HR and increasing SV while maintaining the same ability to perform work during exercise reduces myocardial oxygen demand and may improve exercise tolerance.

Our findings are in line with the results of several previous studies conducted in normoxic conditions. It has been reported that phosphate loading caused a decrease in HR [6,7,16,17] and an increase in end-diastolic volume, SV and Q [9] during endurance exercise. In our previous studies [6,7] we found that after using the same supplementation protocol as in this study, the resting HR and HR at LT dropped by 9.6% and 1.7%, respectively, and maximal VO_2/HR increased by 5.8% in elite mountain bike cyclists during an incremental exercise test in normoxia.

Notably, in our study a significant decrease in HR (by 5 bpm) and an improvement in VO_2/HR (by 5.2%) following SP supplementation occurred only at workloads below the LT, where the exercise intensity was $<75\% \text{VO}_{2\text{max}}$. At LT workload, the reduction in HR was less pronounced (decrease by 3 bpm), and at higher intensities, no statistically significant changes in HR were found. Perhaps, as the exercise intensity increased above the anaerobic threshold, the stimulation of the sympathetic nervous system activity and an overproportional increase in release of catecholamines occurred [51,52]. Consequently, stimulation of the myocardium by the sympathetic nervous system resulted in an increase in HR [53,54]. We suggest that above LT, this mechanism was dominant in relation to the reduction in HR caused by the phosphate salts.

The basis for the assumption that ingestion of phosphate salts can contribute to improvement in myocardial efficiency are the observations that hypophosphatemia causes impaired contractility of the myocardium and reduced SV, while reversal of hypophosphatemic states has been reported to significantly improve the cardiac muscle work [55–57]. O'Connor et al. [55] reported that after intravenous infusion of potassium phosphate solution in patients with severe hypophosphatemia there was a significant increase in SV. The increase in SV was explained by an improvement in myocardial contractility. The authors suggested that the increase in myocardial contractility is most likely the result of an increase in intracellular ATP concentration, the level of which is low during hypophosphatemia.

Recently, Pesta et al. [37] reported that ATP synthesis in muscle cells is markedly decreased in hypophosphatemic mice, suggesting that intramyocellular phosphate regulates ATP synthesis. Additionally, Brautbar and Altura [58] demonstrated that reduction in the myocardial concentrations of creatine phosphate and Pi is associated with a decline in the activity of mitochondrial and myofibrillar creatine phosphokinase, which plays a crucial role in skeletal and cardiac muscle contractility.

Davis et al. [59] reported that an improvement of ventricular function after phosphate supplementation is mainly observed in patients with severe hypophosphatemia (0.9 ± 0.15 mg/dL), but not in patients with mild hypophosphatemia (1.4 ± 0.11 mg/dL) or in the normal range. In contrast to these data, we noted an improvement in cardiorespiratory efficiency during exercise following SP supplementation in athletes within the physiological reference range of serum Pi levels (2.7–4.5 mg/dL). Similar results were also recorded in earlier studies in normoxia [7,9], which indicate that phosphate salts may be beneficial not only in subjects with low Pi serum level.

In our study, we recorded a significant (8.7%) increase in serum Pi concentrations after six-day SP supplementation. Similar results were reported by other authors [3,4,7,9,60,61], who observed an increase in blood Pi levels in the range of 5–39% after short term phosphate salt intake. There is evidence of a correlation between serum Pi concentration and Pi level in the myocardium [58]. Thus, it is likely that the administration of phosphate salts led to an increase in extracellular phosphate availability, promoting an increase in the intracellular phosphate level, thus enhancing ATP synthesis in the myocardium and improving myocardial contractility and SV in athletes.

Since the SV is also largely determined by blood volume [27,62], it is possible that an increase in SV after SP supplementation results from an increase in PV caused by an increase in serum sodium concentration [63,64]. During our experiment, blood sodium concentration was not determined. However, we did not observe changes in Hct and PV in cyclists after administration of phosphate salts. This is partially consistent with the results of previous study conducted by Marcos et al. [38], who reported no changes in Hct. Thus, it may be concluded that the increase in PV was not the mechanism responsible for the improvement in cardiovascular efficiency after SP supplementation in our study.

It is worth noting that the short-term SP supplementation did not change blood Ca levels, which is consistent with previous reports [7,60]. A decrease in blood Ca concentration was registered after prolonged (four weeks) SP intake [7]. Calcium plays an essential role in several metabolic processes in the organism: it is a bone component and is also necessary for muscle contraction, nerve conduction, and blood coagulation [65]. Furthermore, intracellular calcium ions (Ca^{2+}) are central regulators of cardiomyocyte contraction [66,67]. It was recognized that low blood Ca levels (hypocalcemia) are associated with neuromuscular irritability, muscle cramps, bone fragility, cognitive impairment, and cardiac symptoms, including prolonged QT intervals and cardiac arrhythmias [68]. Thus, the absence of disturbance in serum Ca levels after phosphate loading is favorable for athlete health and performance.

The second purpose of the present study was to investigate the effect of SP supplementation on mechanical efficiency in cyclists. We found that GE during exercise at the same absolute power output did not change after phosphate salt loading. When GE is considered for the same absolute power output, its improvement is strictly dependent on a reduction in VO_2 and/or RER at this workload. In our study, we did not observe any significant changes in VO_2 and RER during exercise at a given workload. Similarly, no significant decrease in submaximal RER or VO_2 after phosphate loading was reported in studies conducted under normoxic conditions [10,38,44,69]. Our findings suggest that phosphate salts did not shift the use of metabolic substrates towards fat utilization and did not reduce the energy cost of exercise at an intensity $> 60\%$ of $\text{VO}_{2\text{max}}$ in hypoxic conditions. Interestingly, Marcos et al. [38] found that SP ingested for seven days at a dose of 50 mg/kg lean body mass improved energy efficiency at low exercise intensity, where the major metabolic substrate for energy production is fat oxidation. It should be noted that efficiency in their study was calculated for a lower exercise intensity than in our experiment, which

may be reflected by the differences in RER (0.82–0.86 vs. 0.93–0.98). These discrepancies may have contributed to the differences in the occurrence of efficiency improvement. The above observation suggests that SP supplementation may offer more ergogenic benefits for low-intensity exercise. Since hypoxia impairs aerobic metabolism [70], it is also possible that the energy cost of exercise is reduced following intake of phosphate salts when exercise is conducted under normoxic [38] but not hypoxic conditions. However, further investigation is needed to clarify this issue.

5. Practical Applications

It is considered that for ultraendurance events (i.e., >4 h), an optimal performance intensity exists below the anaerobic threshold [71,72]. Relative exercise intensity observed during ultraendurance competitions is around 60–75% of $\text{VO}_{2\text{max}}$ in 4–12 h events [73–75], and decreases gradually during longer races [76,77]. In our study, we observed an ergogenic effect of SP on cardiovascular efficiency at workloads below LT, where the exercise intensity was <75% $\text{VO}_{2\text{max}}$. Thus, we suggest that phosphate salt supplementation may be particularly beneficial during prolonged endurance exercise at altitude, for example during ultra-distance cycling, triathlon, running, and cross country skiing races. Future research should examine the effect of phosphate salt supplementation on exercise performance and physiological and biochemical responses during prolonged exercise protocols lasting several hours to determine phosphate loading suitability for ultraendurance athletes.

6. Study Limitations

To the best of our knowledge, our study is the first in which the effect of SP supplementation was assessed in normobaric hypoxia. Our experiment, however, is not without certain limitations. Firstly, during the experiment, subjects did not share the same accommodations and did not follow the same training schedule. However, they received nutritional, training, and supplementation recommendations that were constantly monitored. Secondly, SV and Q were estimated from ergospirometric measurements, which could have influenced the results. Future research should use more accurate methods to measure SV [78] and other indicators of myocardial efficiency.

7. Conclusions

Short-term sodium phosphate supplementation promotes improvements in the efficiency of the cardiorespiratory system during low- and moderate-intensity exercise in a hypoxic environment without calcium-phosphate imbalance. Gross efficiency in hypoxia did not change following supplementation. We suggest that sodium phosphate should be considered as an ergogenic aid for endurance athletes, especially for ultra-race competitors.

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References

- Buck, C.L.; Wallman, K.E.; Dawson, B.; Guelfi, K.J. Sodium phosphate as an ergogenic aid. *Sports Med.* **2013**, *43*, 425–435. [CrossRef]
- The AIS Sports Supplement Framework. Available online: <https://www.ais.gov.au/nutrition/supplements> (accessed on 29 May 2021).
- Cade, R.; Conte, M.; Zauner, C.; Mars, D.; Peterson, J.; Lunne, D.; Hommen, N.; Packer, D. Effects of phosphate loading on 2,3-diphosphoglycerate and maximal oxygen uptake. *Med. Sci. Sports Exerc.* **1984**, *16*, 263–268. [CrossRef]
- Kreider, R.; Miller, G.W.; Williams, M.H.; Somma, C.T.; A Nasser, T. Effects of phosphate loading on oxygen uptake, ventilatory anaerobic threshold, and run performance. *Med. Sci. Sports Exerc.* **1990**, *22*, 250–256.
- Stewart, I.; McNaughton, L.; Davies, P.; Tristram, S. Phosphate loading and the effects on VO₂max in trained cyclists. *Res. Q. Exerc. Sport* **1990**, *61*, 80–84. [CrossRef]
- Czuba, M.; Zajac, A.; Poprzącki, S.; Cholewa, J. The influence of sodium phosphate supplementation on VO₂max, Serum 2,3-diphosphoglycerate level and heart rate in off-road cyclists. *J. Hum. Kinet.* **2008**, *19*, 149–164. [CrossRef]
- Czuba, M.; Zajac, A.; Poprzącki, S.; Cholewa, J.; Woska, S. Effects of sodium phosphate loading on aerobic power and capacity in off road cyclists. *J. Sports Sci. Med.* **2009**, *8*, 591–599.
- Brewer, C.P.; Dawson, B.; Wallman, K.E.; Guelfi, K.J. Effect of repeated sodium phosphate loading on cycling time-trial performance and VO₂peak. *Int. J. Sport Nutr. Exerc. Metab.* **2013**, *23*, 187–194. [CrossRef]
- Kreider, R.; Miller, G.W.; Schenck, D.; Cortes, C.W.; Miriel, V.; Somma, C.T.; Rowland, P.; Turner, C.; Hill, D. Effects of phosphate loading on metabolic and myocardial responses to maximal and endurance exercise. *Int. J. Sport Nutr.* **1992**, *2*, 20–47. [CrossRef]
- Folland, J.P.; Stern, R.; Brickley, G. Sodium phosphate loading improves laboratory cycling time-trial performance in trained cyclists. *J. Sci. Med. Sport* **2008**, *11*, 464–468. [CrossRef]
- Brewer, C.P.; Dawson, B.; Wallman, K.E.; Guelfi, K.J. Effect of sodium phosphate supplementation on repeated high-intensity cycling efforts. *J. Sports Sci.* **2014**, *33*, 1109–1116. [CrossRef]
- Buck, C.L.; Henry, T.; Guelfi, K.; Dawson, B.; McNaughton, L.R.; Wallman, K. Effects of sodium phosphate and beetroot juice supplementation on repeated-sprint ability in females. *Eur. J. Appl. Physiol.* **2015**, *115*, 2205–2213. [CrossRef]
- Buck, C.; Guelfi, K.; Dawson, B.; McNaughton, L.; Wallman, K. Effects of sodium phosphate and caffeine loading on repeated-sprint ability. *J. Sports Sci.* **2015**, *33*, 1971–1979. [CrossRef]
- Kopec, B.J.; Dawson, B.T.; Buck, C.; Wallman, K.E. Effects of sodium phosphate and caffeine ingestion on repeated-sprint ability in male athletes. *J. Sci. Med. Sport* **2016**, *19*, 272–276. [CrossRef]
- King, J.; Lowery, D.R. *Physiology, Cardiac Output*; StatPearls Publishing: Treasure Island, FL, USA, 2021.
- Farber, O.M.; Sullivan, T.Y.; Fineberg, N.; Carlone, S.; Manfredi, F. Effect of decreased O₂ affinity of hemoglobin on work performance during exercise in healthy humans. *J. Lab. Clin. Med.* **1984**, *104*, 166–175.
- Lunne, D.; Zauner, C.; Cade, R.; Wright, T.; Conte, M. Effect of phosphate loading on RBC 2–3 DPG, cardiac-output, and oxygen utilization at rest and during vigorous exercise. *Clin. Res.* **1990**, *28*, 810.
- Siebenmann, C.; Lundby, C. Regulation of cardiac output in hypoxia. *Scand. J. Med. Sci. Sports* **2015**, *25*, 53–59. [CrossRef]
- West, J.B.; Schoene, R.B.; Luks, A.M.; Milledge, J.S. *High Altitude Medicine and Physiology*, 5th ed.; CRC Press Taylor & Francis: Boca Raton, FL, USA, 2013; pp. 101–120.
- Mazzeo, R.S. Physiological responses to exercise at altitude: An update. *Sports Med.* **2008**, *38*, 1–8. [CrossRef]
- Mourot, L. Limitation of maximal heart rate in hypoxia: Mechanisms and clinical importance. *Front. Physiol.* **2018**, *9*, 972. [CrossRef]
- Mallet, R.T.; Burtcher, J.; Richalet, J.-P.; Millet, G.P.; Burtcher, M. Impact of high altitude on cardiovascular health: Current perspectives. *Vasc. Health Risk Manag.* **2021**, *17*, 317–335. [CrossRef]
- Clark, S.A.; Bourdon, P.C.; Schmidt, W.; Singh, B.; Cable, G.; Onus, K.J.; Woolford, S.M.; Stanef, T.; Gore, C.J.; Aughey, R. The effect of acute simulated moderate altitude on power, performance and pacing strategies in well-trained cyclists. *Eur. J. Appl. Physiol.* **2007**, *102*, 45–55. [CrossRef]
- Płoszczyca, K.; Czuba, M.; Chalimoniuk, M.; Gajda, R.; Baranowski, M. Red blood cell 2,3-diphosphoglycerate decreases in response to a 30 km time trial under hypoxia in cyclists. *Front. Physiol.* **2021**, *12*, 670977. [CrossRef]
- Weavil, J.C.; Duke, J.W.; Stickford, J.L.; Stager, J.M.; Chapman, R.F.; Mickleborough, T.D. Endurance exercise performance in acute hypoxia is influenced by expiratory flow limitation. *Eur. J. Appl. Physiol.* **2015**, *115*, 1653–1663. [CrossRef]
- Warburton, D.E.R.; Haykowsky, M.J.; Quinney, H.A.; Blackmore, D.; Teo, K.K.; Humen, D.P. Myocardial response to incremental exercise in endurance-trained athletes: Influence of Heart rate, contractility and the frank-starling effect. *Exp. Physiol.* **2002**, *87*, 613–622. [CrossRef]
- Vella, C.A.; Robergs, R.A. A review of the stroke volume response to upright exercise in healthy subjects. *Br. J. Sports Med.* **2005**, *39*, 190–195. [CrossRef]
- Joyner, M.J.; Coyle, E.F. Endurance exercise performance: The physiology of champions. *J. Physiol.* **2008**, *586*, 35–44. [CrossRef]
- Płoszczyca, K.; Foltyn, J.; Goliniewski, J.; Krężelok, J.; Poprzącki, S.; Ozimek, M.; Czuba, M. Seasonal changes in gross efficiency and aerobic capacity in well-trained road cyclists. *Isokinet. Exerc. Sci.* **2019**, *27*, 193–202. [CrossRef]
- Faria, E.W.; Parker, D.L.; Faria, I.E. The science of cycling: Factors affecting performance-Part 2. *Sports Med.* **2005**, *35*, 313–337. [CrossRef]

31. Layec, G.; Haseler, L.J.; Hoff, J.; Richardson, R.S. Evidence that a higher ATP cost of muscular contraction contributes to the lower mechanical efficiency associated with COPD: Preliminary findings. *Am. J. Physiol. Integr. Comp. Physiol.* **2011**, *300*, R1142–R1147. [CrossRef]
32. Jabbour, G.; Iancu, H.-D. Mechanical efficiency improvement in relation to metabolic changes in sedentary obese adults. *BMJ Open Sport Exerc. Med.* **2015**, *1*, e000044. [CrossRef]
33. Schuler, B.; Thomsen, J.J.; Gassmann, M.; Lundby, C. Timing the arrival at 2340 m altitude for aerobic performance. *Scand. J. Med. Sci. Sports* **2007**, *17*, 588–594. [CrossRef]
34. Van Erck, D.; Wenker, E.J.; Levels, K.; Foster, C.; De Koning, J.J.; Noordhof, D.A. Cycling at altitude: Lower absolute power output as the main cause of lower gross efficiency. *Int. J. Sports Physiol. Perform.* **2019**, *14*, 1117–1123. [CrossRef]
35. Noordhof, D.A.; Schoots, T.; Hoekert, D.H.; De Koning, J.J. Is gross efficiency lower at acute simulated altitude than at sea level? *Int. J. Sports Physiol. Perform.* **2013**, *8*, 319–322. [CrossRef]
36. Lardy, H.A.; Wellman, H. Oxidative phosphorylations: Rôle of inorganic phosphate and acceptor systems in control of metabolic rates. *J. Biol. Chem.* **1952**, *195*, 215–224. [CrossRef]
37. Pesta, D.H.; Tsirigotis, D.N.; Befroy, D.E.; Caballero, D.; Jurczak, M.J.; Rahimi, Y.; Cline, G.W.; Dufour, S.; Birkenfeld, A.L.; Rothman, D.L.; et al. Hypophosphatemia promotes lower rates of muscle ATP synthesis. *FASEB J.* **2016**, *30*, 3378–3387. [CrossRef] [PubMed]
38. Marcos, M.; Olcina, G.; Timón, R.; Sánchez, F.; Maynar, M. Efecto ergogénico de la ingesta de fosfato sódico en sujetos físicamente activos/Ergogenic effect of sodium phosphate intake in physically active subjects. *Rev. Int. Med. Cienc. Act. Física Deporte* **2016**, *16*, 33–44. [CrossRef]
39. Urbaniak, G.C.; Plous, S. Research Randomizer (Version 4.0) [Computer Software]. Available online: <http://www.randomizer.org/> (accessed on 14 September 2020).
40. Nadler, S.B.; Hidalgo, J.H.; Bloch, T. Prediction of blood volume in normal human adults. *Surgery* **1962**, *51*, 224–232.
41. Cheng, B.; Kuipers, H.; Snyder, A.C.; Keizer, H.A.; Jeukendrup, A.; Hesselink, M. A New Approach for the determination of ventilatory and lactate thresholds. *Int. J. Sports Med.* **1992**, *13*, 518–522. [CrossRef]
42. Czuba, M.; Zajac, A.; Cholewa, J.; Poprzęcki, S.; Waśkiewicz, Z.; Mikołajec, K. Lactate threshold (D-Max Method) and maximal lactate steady state in cyclists. *J. Hum. Kinet.* **2009**, *21*, 49. [CrossRef]
43. Płoszczyca, K.; Jazic, D.; Piotrowicz, Z.; Chalimoniuk, M.; Langfort, J.; Czuba, M. Comparison of maximal lactate steady state with anaerobic threshold determined by various methods based on graded exercise test with 3-minute stages in elite cyclists. *BMC Sports Sci. Med. Rehabil.* **2020**, *12*, 70. [CrossRef]
44. West, J.S.; Ayton, T.; Wallman, K.E.; Guelfi, K. The effect of 6 days of sodium phosphate supplementation on appetite, energy intake, and aerobic capacity in trained men and women. *Int. J. Sport Nutr. Exerc. Metab.* **2012**, *22*, 422–429. [CrossRef]
45. Brewer, C.P.; Dawson, B.; Wallman, K.E.; Guelfi, K. Effect of Sodium phosphate supplementation on cycling time trial performance and VO₂ 1 and 8 days post loading. *J. Sports Sci. Med.* **2014**, *13*, 529–534. [PubMed]
46. Buck, C.L.; Dawson, B.; Guelfi, K.; McNaughton, L.; Wallman, K.E. Sodium phosphate supplementation and time trial performance in female cyclists. *J. Sports Sci. Med.* **2014**, *13*, 469–475. [PubMed]
47. Tremblay, M.S.; Galloway, S.D.; Sexsmith, J.R. Ergogenic effects of phosphate loading: Physiological fact or methodological fiction? *Can. J. Appl. Physiol.* **1994**, *19*, 1–11. [CrossRef]
48. Galloway, S.D.R.; Tremblay, M.S.; Sexsmith, J.R.; Roberts, C.J. The effects of acute phosphate supplementation in subjects of different aerobic fitness levels. *Eur. J. Appl. Physiol. Occup. Physiol.* **1996**, *72*, 224–230. [CrossRef] [PubMed]
49. Lusk, G. Animal calorimetry twenty-fourth paper. Analysis of the oxidation of 423 mixtures of carbohydrate and fat. *J. Biol. Chem.* **1924**, *59*, 41–42. [CrossRef]
50. Hopkins, W.; Marshall, S.; Batterham, A.; Hanin, J. Progressive statistics for studies in sports medicine and exercise science. *Med. Sci. Sports Exerc.* **2009**, *41*, 3–12. [CrossRef] [PubMed]
51. Urhausen, A.; Weiler, B.; Coen, B.; Kindermann, W. Plasma catecholamines during endurance exercise of different intensities as related to the individual anaerobic threshold. *Eur. J. Appl. Physiol. Occup. Physiol.* **1994**, *69*, 16–20. [CrossRef] [PubMed]
52. Chmura, J.; Nazar, K.; Kaciuba-Uścilko, H. Choice Reaction time during graded exercise in relation to blood lactate and plasma catecholamine thresholds. *Int. J. Sports Med.* **1994**, *15*, 172–176. [CrossRef]
53. Gordan, R.; Gwathmey, J.K.; Xie, L.-H. Autonomic and endocrine control of cardiovascular function. *World J. Cardiol.* **2015**, *7*, 204–214. [CrossRef]
54. Macdonald, E.A.; Rose, R.A.; Quinn, T.A. Neurohumoral control of sinoatrial node activity and heart rate: Insight from experimental models and findings from humans. *Front. Physiol.* **2020**, *11*, 170. [CrossRef]
55. O'Connor, L.R.; Wheeler, W.S.; Bethune, J.E. Effect of hypophosphatemia on myocardial performance in man. *N. Engl. J. Med.* **1977**, *297*, 901–903. [CrossRef] [PubMed]
56. Stoff, J.S. Phosphate homeostasis and hypophosphatemia. *Am. J. Med.* **1982**, *72*, 489–495. [CrossRef]
57. Rubin, M.F.; Narins, R.G. Hypophosphatemia: Pathophysiological and practical aspects of its therapy. *Semin. Nephrol.* **1990**, *10*, 536–545.
58. Brautbar, N.; Altura, B.M. Hypophosphatemia and hypomagnesemia result in cardiovascular dysfunction: Theoretical basis for alcohol-induced cellular injury. *Alcohol. Clin. Exp. Res.* **1987**, *11*, 118–126. [CrossRef]

59. Davis, S.V.; Olichwier, K.K.; Chakko, S.C. Reversible depression of myocardial performance in hypophosphatemia. *Am. J. Med. Sci.* **1988**, *295*, 183–187. [[CrossRef](#)]
60. Bredle, D.L.; Stager, J.M.; Brechue, W.F.; Farber, M.O. Phosphate supplementation, cardiovascular function, and exercise performance in humans. *J. Appl. Physiol.* **1988**, *65*, 1821–1826. [[CrossRef](#)]
61. Mannix, E.T.; Stager, J.M.; Harris, A.; O Farber, M. Oxygen delivery and cardiac output during exercise following oral phosphate-glucose. *Med. Sci. Sports Exerc.* **1990**, *22*, 341–347. [[CrossRef](#)] [[PubMed](#)]
62. Schierbauer, J.; Hoffmeister, T.; Treff, G.; Wachsmuth, N.B.; Schmidt, W.F.J. Effect of exercise-induced reductions in blood volume on cardiac output and oxygen transport capacity. *Front. Physiol.* **2021**, *12*, 679232. [[CrossRef](#)]
63. Sawka, M.N.; Coyle, E.F. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exerc. Sport Sci. Rev.* **1999**, *27*, 167–218. [[PubMed](#)]
64. Hamouti, N.; Fernández-Elías, V.E.; Ortega, J.F.; Mora-Rodriguez, R. Ingestion of sodium plus water improves cardiovascular function and performance during dehydrating cycling in the heat. *Scand. J. Med. Sci. Sports* **2012**, *24*, 507–518. [[CrossRef](#)]
65. Clarkson, P.M.; Haymes, E.M. Exercise and mineral status of athletes: Calcium, magnesium, phosphorus, and iron. *Med. Sci. Sports Exerc.* **1995**, *27*, 831–843. [[CrossRef](#)] [[PubMed](#)]
66. Bers, D.M. Calcium fluxes involved in control of cardiac myocyte contraction. *Circ. Res.* **2000**, *87*, 275–281. [[CrossRef](#)] [[PubMed](#)]
67. Baird, G.S. Ionized calcium. *Clin. Chim. Acta* **2011**, *412*, 696–701. [[CrossRef](#)] [[PubMed](#)]
68. Murphy, E.; Williams, G.R. Hypocalcaemia. *Medicine* **2009**, *37*, 465–468. [[CrossRef](#)]
69. Brown, J.A.; Glaister, M. The Effects of sodium phosphate supplementation on physiological responses to submaximal exercise and 20 km cycling time-trial performance. *J. Diet. Suppl.* **2019**, *16*, 564–575. [[CrossRef](#)]
70. Cerretelli, P.; Gelfi, C. Energy metabolism in hypoxia: Reinterpreting some features of muscle physiology on molecular grounds. *Eur. J. Appl. Physiol.* **2010**, *111*, 421–432. [[CrossRef](#)]
71. Laursen, P.B.; Rhodes, E.C. Factors affecting performance in an ultraendurance triathlon. *Sports Med.* **2001**, *31*, 195–209. [[CrossRef](#)]
72. Laursen, P.B.; Rhodes, E.C.; Langill, R.H.; McKenzie, D.C.; Taunton, J.E. Relationship of exercise test variables to cycling performance in an Ironman triathlon. *Eur. J. Appl. Physiol.* **2002**, *87*, 433–440. [[CrossRef](#)]
73. Davies, C.T.; Thompson, M.W. Physiological responses to prolonged exercise in ultramarathon athletes. *J. Appl. Physiol.* **1986**, *61*, 611–617. [[CrossRef](#)]
74. Barrero, A.; Chaverri, D.; Erola, P.; Iglesias, X.; Rodríguez, F.A. Intensity profile during an ultra-endurance triathlon in relation to testing and performance. *Int. J. Sports Med.* **2014**, *35*, 1170–1178. [[CrossRef](#)]
75. Fornasiero, A.; Savoldelli, A.; Fruet, D.; Boccia, G.; Pellegrini, B.; Schena, F. Physiological intensity profile, exercise load and performance predictors of a 65-km mountain ultra-marathon. *J. Sports Sci.* **2017**, *36*, 1287–1295. [[CrossRef](#)] [[PubMed](#)]
76. Neumayr, G.; Pfister, R.; Mitterbauer, G.; Gaenzer, H.; Sturm, W.; Hoertnagl, H. Heart rate response to ultraendurance cycling. *Br. J. Sports Med.* **2003**, *37*, 89–90. [[CrossRef](#)] [[PubMed](#)]
77. Mattsson, C.M.; Ståhlberg, M.; Larsen, F.J.; Braunschweig, F.; Ekblom, B. Late Cardiovascular drift observable during ultraendurance exercise. *Med. Sci. Sports Exerc.* **2011**, *43*, 1162–1168. [[CrossRef](#)] [[PubMed](#)]
78. Phillips, R.A.; Smith, B.E.; Madigan, V.M. Stroke volume monitoring: Novel continuous wave doppler parameters, algorithms and advanced noninvasive haemodynamic concepts. *Curr. Anesthesiol. Rep.* **2017**, *7*, 387–398. [[CrossRef](#)]