The Influence of Sodium Phosphate Supplementation on VO_{2max}, Serum 2,3-diphosphoglycerate Level and Heart Rate in Off-road Cyclists

by

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The main objective of the work was to evaluate the influence of a six day supplementation with sodium phosphate on circulatory and pulmonary variables, the level of 2,3-diphosphoglycerate (2,3-DPG) and the concentration of inorganic phosphates in blood serum of elite off-road cyclists. The research material included 19 cyclists which were randomly divided into a experimental group, supplemented with sodium phosphate and a control group receiving a placebo The subjects in the experimental group ingested sodium diphosphate in a dose of 50mg/kg of fat free mass per day. The supplement was ingested in even doses, four times per day. The control group received 4g of glucose in gelatin capsules (500mg), which were also divided into 4 even portions. During the experiment a significant (p < 0.05) increase in maximal oxygen uptake was observed (VO_{2max}) , maximal minute ventilation (VE_{max}) , as well as oxygen pulse (O_2/HR) . Also a significant decrease in resting and maximal exercise heart rate occurred. This was also true for each exercise load. A significant (p < 0.05) increase in the serum concentration of non-organic phosphates (P) was observed which was accompanied by a decrease in serum calcium (Ca) concentration. The changes in the resting and post exercise concentration of 2,3-DPG were non significant, yet the supplementation procedure showed a tendency for increased level of this variable. **Key words:** sodium phosphate supplementation, 2,3-DPG, VO_{2max} heart

rate, off-road cyclists

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Introduction

Every human has its own, specific genetic endowment of sport capabilities. This potential can be expressed only through long term sports training of specific stimulus, which brings about adaptive changes of particular cells, tissues, organs and the whole body (Williams 1998). The adaptive changes that occur through training may be additionally stimulated by different ergogenic aids. Much of the ergogenic substances available and used by athletes are classified as doping and are forbidden in international competition. Many substances are harmful to the health of athletes and may even cause death if abused. An alternative to doping includes dietary supplementation, which is a natural form of increasing physical work capacity.

Off-road cycling creates great demands for the human organism, especially in regards to the systems that supply oxygen and energy substrates to the working muscles. The greatest demand in cross country cycling is placed on the circulatory and respiratory systems, thus these systems influence sport results in endurance disciplines to the highest degree. Supplementation in of-road cycling is geared at increasing the energetic potential of the athletes and improving the efficiency of the cardio-respiratory system (Stewart et al. 1990).

Phosphate salts are one of those substances that play an extensive role in human exercise metabolism. They are classified as ergogenic nutritional substances and are deemed legal by sports law. Due to various functions in the organism, phosphates are involved in all three energetic systems. Phosphates are a part of the ATP and phosphocreatine (CP), which are the basic substrates for the high energy phosphagen system (ATP-CP). They also play a significant role in anaerobic glycolisis, by buffering lactic acid generated during speed endurance exercise. Phosphates also improve the aerobic energy system by increasing the synthesis of 2, 3-diphosfphoglicerate (Williams 1998). As a result, the hemoglobin dissociation curve moves to the right, thus simplifying the release of oxygen in tissues and increasing the difficulty in assimilating this gas in the lungs. Earlier research results regarding the ergogenic benefits of phosphate salts are not convincing. The literature provides both types of data, supporting and not supporting the ergogenic benefits of these supplements. (Cade et al. 1984, Kreider 1992, Tremblay et al. 1996, Brennan et al. 2001).

The main objective of this paper was to evaluate the ergogenic effects of sodium phosphate supplementation (6 days) on maximal oxygen uptake (VO_{2max}), the level of 2,3-DPG in erythrocytes, and serum concentration of inorganic phosphates as well as heart rate in off road cyclists.

Material and methods

The research material included 19 well trained mountain bike cyclists competing in the cross country and marathon events. The subjects participating in the research were well trained athletes with at least 7 years of sports experience and an oxygen uptake of at least 65 ml/kg/min. All subjects participating in the research project were randomly divided into a supplemented group (n=11), which received sodium phosphate salts over a prescribed period and a control group which was given a placebo (n=8) The data related to age, sports experience, (VO_{2max}), body height and body mass as well as FFM and FAT content are presented in table 1.

Table 1

	Group I Supplemented	Group II Control
	n=11	n=8
Age [years]	$25,36 \pm 2,50$	24,5 ± 4,31
Sports experience[years]	$8,64 \pm 1,29$	7,75 ± 1,39
VO _{2max} [ml/kg/min]	$73,53 \pm 4,46$	$73,88 \pm 4,25$
Body height [cm]	$180,5 \pm 5,8$	$173 \pm 3,89$
Body mass [kg]	$70,33 \pm 5,36$	66,43 ± 2,32
FFM [kg]	$65,22 \pm 5,02$	$61,45 \pm 2,60$
FAT content [%]	7,01 ± 1,33	7,28 ± 2,97

Basic characteristics of the tested off-road cyclists

The research project was approved by the Ethics Committee for Scientific Research at the Academy of Physical Education in Katowice. All of the tested subjects possessed current medical examinations, confirming proper health status and the ability to perform exhaustive exercise. Before the start of the experiment all subjects gave their written consent for participation.

In the week prior to the experiment dietary habits of the cyclist were collected and analyzed for the caloric value as well as carbohydrate, protein and fat content. During the experiment training loads were fully individualized, with the two days before testing treated as active rest.

Before the start of the experiment initial values of body mass and composition (BM, BH, FFM, FAT%, TBW) were evaluated with the use of electrical impedance. Also rest blood samples were drawn from the vein and capillaries to determine several biochemical variables. Later the progressive ergocycle tests were carried on until volitional exhaustion to determine maximal oxygen uptake. The second phase of the experiment began with a 6-day supplementation with sodium phosphate for the S group, while a placebo was provided for the C group. The subjects in group S were given sodium phosphate in a dose of 50mg/kg of fat free mass per day, divided into 4 even portions. The control group received a placebo in the form of glucose in gelatin capsules also administered 4 times daily. After the supplementation the exercise tests were repeated. Considering that the concentration of 2,3-DPG is influenced by daily fluctuations, the order and time of testing was similar during the second evaluations.

The atmospheric conditions in regards to air pressure, temperature and humidity were held constant to increase the reliability of measurements (table 2).

Table 2

Phase	Air Temperature (°C)	Atmospheric preassure (hPa)
Ι	18,9±1,04	1037,9± 9,83
II	18,8±0,87	1034,8± 5,17

Air temperature and pressure during both phases of the experiment

Body mass and body composition were evaluated initially and before the second phase of the experiment. Venous and capillary blood samples were drawn each time to determine plasma lactate concentration (LA), hemoglobin concentration (Hb), haematocrit value (Hct), number of erythrocytes (RBC) concentration of non-organic phosphates, serum calcium concentration, the level of 2,3-DPG, acid-base balance variables and oxygen saturation of hemoglobin.

After blood samples were drawn for biochemical evaluations, the exercise test protocol was applied to determine maximal oxygen uptake of the participants. The test was performed on an ergocycle (Jaeger), beginning with a work load of 40W which was increased by that value every 3 min until volitional exhaustion. The cadence was maintained between 60-70 revolutions per minute.

During the exercise the following variables were constantly registered: heart rate (HR), minute ventilation (VE), oxygen uptake (VO₂) and expired carbon dioxide (CO₂). At the end of each load capillary blood samples were drawn to determine lactate concentration. These values allowed to determine the anaerobic thresholds for each athlete. After the cessation of the test, venous blood samples were drawn to determine changes in hemoglobin concentration (Hb), haematocrit value (Hct), number of erythrocytes (RBC), concentration of non-organic phosphates, serum calcium concentration, the level of 2,3-DPG, acid-base balance variables and oxygen saturation of hemoglobin.

The obtained data was analyzed statistically with the use of Statistica 6.0. The results were presented as arithmetic means (x) and standard deviations (SD). To determine the normality of distribution, the Kolmogorov - Smirnov and Liliefors tests were applied. To determine the significance of differences in succeeding series of examinations, the Wilcoxon's test was applied. The relationships between particular variables were determined by calculating the Spearman correlation coefficients. The level of statistical significance was set at p<0,05.

Results

The values of chosen biochemical and physiological variables obtained, as well as the significance of differences between both phases of the experiment are presented in table 3.

The statistical analysis indicates that the supplementation with sodium phosphate accompanied by training caused a significant increase (p<0.05) in maximal oxygen uptake (VO_{2max}), as well as in the oxygen uptake measured at the ventilation threshold (VO_{2VAT}).

A significant decrease (p<0,05) in rest (HR_{min}) and maximal heart rate (HR_{max}) occurred in the supplemented group. A significant decrease (p<0.05) in maximal heart rate (HR_{max}) and a significant increase (<0.05) in maximal oxygen uptake VO_{2max}) in group S caused a significant increase p<0.05) in oxygen pulse (O₂/HR).

During the experiment also a significant improvement <0.05) in maximal minute ventilation (VE_{max}) was observed in the supplemented group. Such changes were not observed in the control group receiving a placebo (table 3). Additionally significant (p<0,05) changes were registered in the concentration of inorganic phosphates (P) in the S group, while rest and post exercise concentration of 2,3-DPG increased, yet these changes were insignificant.

Table 3

Average values of chosen biochemical and physiological variables in the supplemented	
and control groups during both phases of the experiment; ***- p<0,05	

Variable	Phase 1		Phase 2	
	Gr. S	Gr. C	Gr. S	Gr. C
VO_{1} (m1/min)	554,55	561,5	646,09	556,5
VO _{2sp} (ml/min)	± 81,33	± 131,85	± 175,98	± 120,78
VO _{2sp} (ml/kg/min)	7,91	8,43	8,98	8,35
	±1,29	± 1,92	± 2,17	±1,71
VO _{2max} (ml/min)	5164,73	4906,75	5438,5 ***	4910,75
	± 319,17	± 328,17	± 346,28	± 328,17
VO_{1} (m1/leg/min)	73,53	73,88	77,54 ***	73,89
VO _{2max} (ml/kg/min)	± 4,46	± 4,25	± 5,98	± 4,36
VO _{2VAT} (ml/min)	3981,5	3893,8	4315,3***	3908,2
v O 2VAT (IIII/IIIIII)	± 367,82	$\pm 290,5$	± 289,68	$\pm 263,45$
VE _{max} (l/min)	183,27	169,7	192,73***	171
V Emax (1/IIIIII)	± 22,56	± 9,57	± 21,66	± 8,98
IID (hts/min)	68	68	62 ***	69
HR _{rest} (bts/min)	± 6,7	± 3,78	± 6,11	± 3,48
HR _{max} (bts/min)	190	195	185 ***	195
	± 6,66	± 4,31	± 6,45	± 3,66
O(IIII)(-1/1/c)	27,71	25,38	29,29 ***	25,2
O ₂ /HR (ml/bts)	± 1,84	± 1,98	± 1,6	± 1,93
P _{max} (W)	403,64	390	414,55	390
P max (VV)	$\pm 28,03$	± 18,52	± 26,97	± 18,52
D (147)	280,4	273,13	295 ***	274,38
$\mathbf{P}_{\mathrm{VAT}}(\mathbf{W})$	± 26,22	± 11,32	± 19,36	$\pm 10,84$
2,3-DPGrest (mmol/l	3,88	3,88	4,00	3,83
er)	±0,65	$\pm 0,43$	$\pm 0,58$	$\pm 0,4$
2,3-DPG _{exer} (mmol/l	3,64	3,54	3,48	3,62
er)	$\pm 0,57$	$\pm 0,18$	± 0,65	$\pm 0,48$
$\mathbf{P}(\mathbf{mmo}^{1/1})$	0,8	0,95	1,00 ***	0,94
P (mmol/l)	± 0,16	$\pm 0,09$	± 0,22	$\pm 0,05$
	7,408	7,416	7,401	7,411
pH _{rest}	$\pm 0,02$	$\pm 0,02$	$\pm 0,02$	± 0,02
	7,253	7,254	7,236	7,255
pHexer	± 0,05	± 0,04	± 0,03	$\pm 0,03$

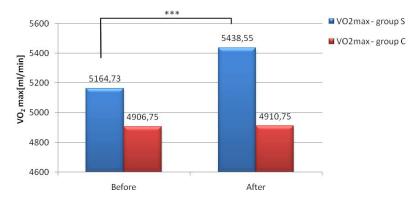


Fig. 1

Changes in absolute values of maximal oxygen uptake (VO_{2max}) *in the supplemented and control groups in successive phases of the experiment; ***- p<0,05.*

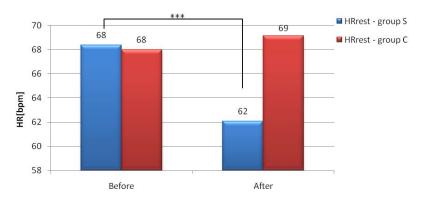
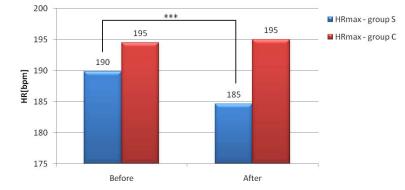


Fig. 2

*Changes in resting heart rate (HR_{rest}) in the supplemented and control groups in successive phases of the experiment ***- p<0,05.*

*The Influence of Sodium Phosphate Supplementation on VO*_{2max} ...





*Changes in maximal heart rate (HR_{exer}) in the supplemented and control groups in successive phases of the experiment; ***- p<0,05.*

To determine the relationships between all of the analyzed variables and the serum level of inorganic phosphates as well as the concentration of 2,3-DPG, correlation coefficients were calculated. Significant correlation coefficients are presented in table 4.

Table 4

Correlation coefficients between analyzed variables and the level of inorganic phosphates (P) as well as the erythrocyte concentration of 2,3-DPG in the tested off-road cyclists.

Paired Variables	R	t(N-2)	р
VO _{2max} & P	0,501693	3,17658	0,003440
VE _{max} & P	0,518859	2,71438	0,013351
O2/HR & P	0,590016	3,26809	0,003848
2,3-DPGrest & P	0,444570	2,21958	0,038172
VO2vat & P	0,568144	3,08753	0,005807
Pvat & P	0,566907	3,07762	0,005939
VO2max & 2,3-DPGrest	0,636158	3,687324	0,001460
VEmax & 2,3-DPGrest	0,621996	3,552463	0,001997
O2/HR & 2,3-DPGrest	0,602941	3,379900	0,002976
Pmax & 2,3-DPGrest	0,515310	2,689061	0,014112
PVAT & 2,3-DPGrest	0,533767	2,822834	0,010512

Discussion

Most of the recent papers dealing with sodium phosphate salts are based on the results of Cade et al. (1984) which showed a significant effect (p<0.05) of this

supplement on the erythrocyte concentration of 2,3-DPG (13,00 vs. 13,92 mg/g Hb). Additionally the supplement caused a 6-12% increase in VO2max.

In a similar experiment Stewart et al. (1990) evaluated the effects of sodium phosphate intake on VO_{2max}, time to exhaustion, blood concentration of 2,3-DPG and blood inorganic phosphate concentration in 8 well trained cyclists. The results indicated insignificant changes in resting blood concentrations of 2,3-DPG, while the post exercise values were significantly (p<0,05) higher in the group supplemented with sodium phosphate. No changes in VO_{2max} were registered in the control and placebo groups while cyclists that ingested sodium phosphate salts increased their maximal oxygen uptake by 11%. Research conducted by Kreider et al. (1990) indicated a significant influence of sodium phosphate salts on VO_{2max}, (9%) and VO_{2VAT}, (12%) as well as on the time of the 5 mile run. The concentration of 2,3-DPG was not registered in this project.

These results are conclusive with those presented in this paper. The six day supplementation with 50mg/kgFFM/d of sodium diphosphate caused a significant (p<0.05) increase in absolute (5164,73±319,17 vs. 5438,55±346,28 ml/min) and relative (73,54 vs. 77,54ml/kg/min) values of VO_{2max}.

The supplemented group also showed a significant (p<0.05) increase in oxygen uptake at the ventilation threshold (VO_{2VAT}). These changes are in accordance with those observed by Kreider et al. (1990), who observed increases in (VO_{2VAT}) close to 12% following a 6 days of sodium phosphate supplementation. The margin of improvement in this variable was much smaller in this research (5%) yet the sports level of the tested subjects was much higher. An improvement in VO_{2VAT} in the supplemented group, allowed for a significant shift of the VAT in the direction of greater loads. During the second test trial a 5.4% increase in VAT was observed (280.4 vs. 295W).

The intake of sodium phosphate delayed the drastic increase of carbon dioxide pressure in the blood (pCO₂) which stimulates ventilation. This on the other hand delays hyperventilation what suggests better saturation of oxygen in the working muscles.

The resting blood concentration of 2,3-DPG showed a increasing tendency yet these changes were statistically insignificant. The calculation of correlation coefficients showed a significant relationship between the resting concentration of 2,3-DPG and VO_{2max} (r=0,63; p=0,001), as well as between (2,3-DPG_{rest}) a O₂/HR (r=0,6; p=0,003). There was a small, insignificant decrease in the post exercise concentration of 2,3-DPG in the supplemented group, what could have been caused by an increase (2.7%) of maximal power (403,64±28,03 vs. 414,55±26,97 W).

The increase in P_{max} caused a post exercise decrease in blood pH (before – 7,353±0,05; after – 7,236±0,03) and the blood concentration of 2,3-DPG. A decrease of blood pH by 0.01 units causes a drop in 2,3-DPG concentration of about 4% (Bard et al. 1979). Another factor that could be responsible for the decrease of 2,3-DPG in the erythrocytes includes the increase of 2,3-DPG phosphate observed during exercise. During exercise of progressive intensity a concomitant acidosis of the erythrocyte occurs. An increase in the concentration of H ions causes an inhibition of glycolysis and stimulates the activity of 2,3-DPG phosphatase (Brennan K. M., Connolly D. A. (2001).

On the other hand research conducted by Brendle et al. (1988) with calcium phosphate (5,7g/d) over a period of 4 days showed no significant changes in 2,3-DPG and VO_{2max}.

Brennan et al. (2001) supplemented a group of cyclists (n=12) (VO_{2max} = 60,6 \pm 4,4 ml/kg/min) with sodium diphosphate (4g/d) over 4 days and reached no significant changes in the above considered variables. An additional factor that may regulate the erythrocyte concentration of 2,3-DPG includes the concentration of serum inorganic phosphates (P). This may be confirmed by a low correlation between inorganic phosphate and resting level of 2,3-DPG obtained in this research (r=0,44; p=0,038). In general when the concentration of phosphates decreases a concomitant drop in 2,3-DPG occurs while the opposite is true when the serum level of (P) rises (Lichtman et al. 1971, Card et al. 1973). Not all research confirms this tendency. Cadet et al. (1984) after supplementing athletes with sodium phosphate for 3 days registered a significant (p<0.05) rise in resting serum phosphate concentration (1,17 vs. 1,22 mmol/L), as well as in the concentration of erythrocyte 2,3-DPG (13,0 vs. 13,92 mg/Hb).

In a similar experiment Kreider et al. (1992) also showed a significant (17%) increase in serum phosphate concentration following supplementation and 9% higher values of VO_{2max}, yet they did not evaluate 2,3-DPG concentration.

Bradle et al. (1988) despite a significant (p<0,05) rise in the concentration of phosphates in the blood (35%) following 4 day of calcium phosphate intake, registered no changes in 2,3-DPG, P₅₀, pH and VO_{2max}. Further research by Mannix et al. (1990) conducted with a single intake of calcium phosphate showed a significant blood increase in (P) (13%) and 2,3-DPG (11%), with no changes in VO_{2max}, and in the contractility of the heart muscles.

In a recent experiment with sodium phosphate salts Brennan et al. (2001) showed a significant relationship between the blood serum concentration of phosphates and phosphate content in the erythrocyte (r=0,51; p=0,009), as well as between erythrocyte concentration of phosphates and the level of 2,3-DPG (r=0,69; p<0,001). No significant relationships were detected between the blood

serum phosphate concentration and the erythrocyte level of 2,3-DPG. The 7 day supplementation procedure cause a 30 % increase in serum phosphate concentration (p=0,03) and a 25% rise in erythrocyte 2,3-DPG (p=0.03). The authors suggested that the increase in 2,3-DPG was caused by higher concentration of phosphates in the erythrocytes.

Results of own experiment were similar, with inorganic phosphates rising significantly (25%, p=0.05) in the group supplemented with sodium phosphate. It must be stated that the initial concentration of blood serum phosphates in the experimental group equaled 0.81 mmol/l what indicates a state of hypophosphatemia. The causes of hypophosphatemia are numerous, yet in competitive cyclists, the most probable factors include post exercise trauma and inadequate phosphate content in the diet (Schrier 1997). The presented research also showed significant relationships between blood serum concentration of inorganic phosphate and VO_{2max} (r=0,5; p=0,003), as well as VO_{2VAT} (r=0,56; p=0,006).

Several research papers indicate that the intake of sodium phosphate salts may improve the functional abilities of the hear muscle at rest as well as during exercise. The basis for such a hypothesis comes from hypophosphatemia, during which the contractile properties of the heart are diminished and thus stroke volume decreases. Rubin et al. 1990, Fuller et al. 1978 and O'Connor et al. 1977 all showed in their research that an abnormally low concentration of inorganic phosphates in the blood decreases contractile properties of the heart muscle. O'Connor et al. (1977) in a experiment with patients suffering from hypophosphatemia injected them with potassium phosphate salts and registered a significant increase in the left ventricle stroke volume. The mechanism responsible for such changes is most likely related to a significant rise in cardiac cell ATP increase which is very low during hypophosphatemia. There are also experimental results indicating that hyperphosphtemia improves the contractile properties of the cardiac muscle (Darsee et al. 1978, Stoff 1982).

Several other research projects which implemented sodium phosphate or calcium phosphate supplementation showed a decrease in stroke volume and cardiac output during exercise of low to moderate intensity (Farber et al. 1984, Lunne et al. 1990, Moore et al. 1981), and a significant increase in these variables during maximal endurance exercise (Kreider 1992). Bredle et al. (1988), supplemented a group of men with calcium phosphate for 4 days in a dose of 176 mmol/d and registered a significant (p<0.05) increase in blood concentration of inorganic phosphates and improved cardiac output. There were no changes in 2,3-DPG and VO_{2max} values, yet the increased arteriovenous difference in O₂ and CO₂ concentration suggests a better supply of oxygen to the skeletal and cardiac tissues.

The analysis of resting and exercise heart rate in own research indicates significant effects of sodium phosphate supplementation on these variables. In the group of cyclists that ingested sodium phosphate a significant decrease in resting and maximal heart rate occurred (0.05), while no changes were observed in the control group receiving a placebo.

Of great importance is the fact that despite a decrease in maximal heart rate a increase in peak power occurred in cyclists that ingested sodium phosphate (403,64±28,03 vs. 414,55±26,97 W), what suggests an even greater load on the cardiovascular system in the experimental group.

Further analysis of results; also indicate a significant improvement in oxygen pulse, which is often treated as a noninvasive index of cardiopulmonary fitness. This variable can be successfully used as a marker of training effectiveness in endurance sport disciplines. In this experiment a significant rise in VO_{2max} and a drop in HR_{max} in the supplemented group also caused a significant (p=0.05) improvement of oxygen pulse (O₂/HR). The six day supplementation with so-dium phosphate (50mg/kgFFM/d) caused a 5.8% increase in O₂/HR (27,71±1,84 vs. 29,29±1,6 bts/min).

The last significant finding of this experiment includes the detection of a high relationship between O_2/HR and serum inorganic phosphate concentration (P) (r=0,59; p=0,003), as well as between O_2/HR and rest values of 2,3-DPG (r=0,6; p=0,003).

Conclusions

- The intake if sodium phosphate in well trained endurance athletes allows to increase maximal oxygen uptake (VO_{2max}), oxygen uptake at the ventilation threshold (VO_{2VAT}) and maximal minute ventilation (VE_{max}).
- 2. Sodium phosphate supplementation decreases heart rate both at rest and during maximal exercise what indicates improved contractile properties of the heart muscle.
- 3. Sodium phosphate intake increases oxygen pulse values (O₂/HR), what improves the economy of the cardiopulmonary system.
- 4. Peak power (P_{max}) during endurance exercise and ventilation threshold should improve following sodium phosphate intake.
- 5. Sodium phosphate may increase the erythrocyte concentration of 2,3-DPG, yet the margin of changes are influenced by several factors of which the inorganic serum phosphate concentration and the level of fitness may dominant.

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