THE IMPACT OF WINGATE AND PROGRESSIVE TESTS ON HOMOCYSTEINE, VITAMIN B_6, B_{12} AND FOLIC ACID LEVELS IN ATHLETES' BLOOD

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Abstract. Homocysteine is an indirect metabolite of methionine metabolism, as well as of creatinine, and it plays an important role in many biochemical processes. Physical effort modifies homocysteine concentration in the blood, as well as the substances taking part in its metabolism.

The aim of the study was to assess the influence of intensive efforts of diverse energy changes on the concentrations of homocysteine and the vitamins involved in its metabolism – vit. B_6, vit. B_{12} and folic acid.

In the study athletes performed Wingate and progressive test. Before and after tests homocysteine, vitamins B_6, B_{12} and folic acid and creatinine were assayed.

Concentration of homocysteine, vit. B_{12} and creatinine in the blood increased after both tests. Concentration of vit. B_6 decreased and folic acid increased after Wingate test while they did not change after a progressive test. Homocysteine concentration negatively correlated with folic acid but positively with creatinine concentration in the blood, as well as with LBM.

Regardless of its duration and energetic changes, intensive effort leads to an increase in homocysteine concentration. Correlation of homocysteine with creatinine and the LBM suggest that people with bigger muscle mass can have higher homocysteine concentration in the blood.

Key words: homocysteine, vitamin B_6, B_{12}, folic acid, Wingate test, progressive test

Introduction

Homocysteine, a methionine derivative, is an amino acid that takes part in many essential biochemical reactions in the body. It is also a risk factor which can have a significant influence on the development of cardiovascular diseases (Boushey et al. 1995).

An increase in homocysteine level by 5 μmol/l increases the risk of ischaemic heart disease by at least 20%, regardless of traditional risk factors (Humphrey et al. 2008).

Research into homocysteine metabolism regulation indicates that demethylation, remethylation and transsulfuration – its main metabolic pathways, are processes dependent on the amount of methionine supplied in a diet, and therefore on the type of food consumed as well as on other lifestyle factors, including physical activity levels (Joubert et al. 2006; Murawska-Ciałowicz et al. 2008; Selhub 1999).

In homocysteine metabolism the key role is played by the B vitamins – folic acid, vit. B_6 and vit. $B_{12,}$ which take part in remethylation, and vit. B_6 additionally also in transsulfuration. Changes in the balance of metabolic pathways can be caused by shortages of these vitamins in the body. A reduction in the supply of B vitamins in a diet and a reduction of their level in the bloodstream contribute to homocysteine concentration in the blood. Lack of folates is the main factor for elevated homocysteine levels in the blood. A diet rich in folates and folic acid in the form of dietary supplements is thought to lower the levels of this amino acid in the blood (Brouwer et al. 1999).

Vit. B_{12} and, to a lesser extent, vit. B_6 supplementation is also an effective way of lowering homocysteine levels. Thus, one of the main factors for high homocysteine levels in the blood is a diet lacking in the three above-mentioned vitamins (Joubert et al. 2006).

Physical activity and physical effort also influence homocysteine levels, however, different directions in post-exertion changes are observed, which might suggest that the kind of exercise and its intensity are factors determining the observed results. One of the more extensive reports in this field was the research done by Norwegian researchers. It turned out that off-work physical activity was negatively correlated with homocysteine levels in the bloodstream of both men and women. Subjects who most often took up physical activity in their leisure time had the lowest concentrations of homocysteine in the blood (Nygard et al. 1995). Research done by Murawska-Ciałowicz (2012) indicates that obese women have higher homocysteine levels than lean ones, and a 9-month exercise training aimed at body mass reduction and improvement in cardiorespiratory fitness significantly lowers homocysteine concentration in their bloodstreams. Results published by Saw et al. (2001) also indicate that lack or low level of physical activity is connected with higher homocysteine concentration in the blood. Chrysohoou et al. (2004) proved that people who claim to do endurance physical activities have lower homocysteine levels in the blood compared to people with sedentary lifestyles or those who do regular strength exercise. What is more, Dankner et al. (2004) showed that sedentary lifestyle increases homocysteine levels by 7% as compared with active lifestyle. Contrary to the presented findings, De Bree et al. (2001) claim that an increased physical activity contributes to higher homocysteine levels in the blood.

Experimental attempts to analyze the influence of single physical exercise on changes in homocysteine levels do not provide unequivocal conclusions. Looking for such relationships, Herrmann et al. (2003) used 3 different types of intensive endurance efforts. The authors came to the conclusion that an increase in homocysteine concentration due to the efforts was determined by the duration and intensity of exercise. Interesting information was also provided by the research done by König et al. (2003), who analyzed the influence of long-lasting endurance training and intensive exercise on the levels of homocysteine, folic acid and vit. B_{12} in the blood of trained triathletes and proved that an increase in homocysteine levels due to exercise depends on the initial level of folates and training volume but not on the level of vit. B_{12}. According to the authors, intensive training increases homocysteine levels.

Due to the fact that in literature on the influence of maximal effort on homocysteine concentration there is a lot of research whose results are contradictory to one another, our aim was to check in laboratory conditions how homocysteine levels would change in athletes' bloodstreams after maximal exercise training varying in energetic

metabolism and duration. To this end, different athletes performed a Wingate (W) test to assess the peak anaerobic capacity, and a progressive (P) test to assesses peak aerobic capacity.

Materials and Methods

The studied group consisted of 39 active athletes, aged 16 to 24, who do different sports: acrobatic gymnastics (n = 6), judo (n = 8), biathlon (n = 8), rowing (n = 8) and road bicycle racing (n = 12). The athletes were divided into 2 groups. Each group performed a different endurance test of maximal intensity. The first group, consisting of acrobatic gymnasts and judo fighters, performed a Wingate test. The other group, which included biathletes, rowers and road bicycle racers, performed a progressive test up to exhaustion. The choice of test and qualification to one of the groups depended on the type of effort dominant in the athlete's sport. Table 1 shows anthropometric data of both studied groups. The only difference between the groups was age, and the athletes of strength and speed disciplines were older. There were no differences in their body mass or its composition.

Wingate test

The Wingate test was performed on a Monark E895 cycle ergometer for 30 sec. Taking a studied athlete's body mass into consideration, the workload value (F) was calculated by the formula: F = 0,075 × body mass. Exercise training was preceded by a 5-minute warm-up on a cycloergometer at a workload of 50W or until an athlete's heart rate (HR) reached 150 bpm. After a 5-minute rest, a test was carried out in which an athlete was expected to achieve a maximal rotation frequency in the shortest time possible and to maintain the frequency for 30 sec. As a result, maximal muscle power of the studied athletes (P_{max}), time in which it was achieved (T_1) and maintained (T_2) were determined. Because of the fact that very intensive exercise can be done for a very short period of time, after achieving P_{max}, muscle power in each second of the test was decreasing until the end of the test. As a result, pedaling rhythm was getting slower and slower. The power achieved at the 30th second was defined as minimal power (P_{min}). The difference between the value of P_{max} and P_{min}, expressed as a percentage, reflects the rate of the increasing fatigue. The difference is called the fatigue index (FI). The test procedure was the same as described by Lovell et al. (2013) for the lower body.

The Wingate test is performed to measure anaerobic capacity. In the test, phosphagen power and volume, as well as glycolytic power and volume, can be determined. Phosphagen power is understood as a maximal power achieved by muscles during dynamic training using the energy from the decomposition of ATP and phosphocreatine (PCr). Phosphagen power is gained in the first few seconds of the exertion. Its value is determined by P_{max} and T_1. Phosphagen volume is dependent on the PCr concentration in the muscles and is determined by T_2. Glycolytic power is measured on the basis of the concentration of lactic acid (LA) in the blood 2 minutes after the test and it reflects the role of anaerobic glycolysis in the resynthesis of ATP. Glycolytic volume is determined by total amount of work (W_{total}) done in the test and the fatigue index.

While interpreting the results, it was assumed that the athlete was characterized by high anaerobic potential when the following were observed: high P_{max}, shorter T_1, longer T_2 and lower value of IF, as well as high value of the performed work, lower value of pH and higher concentration of LA after the test.

Progressive test

The progressive test consisted in a subject performing physical effort of increasing intensity on an Excalibur (LODE) cycloergometer (road bicycle racers), a mechanical treadmill (biathletes) and a Concept 2 Indoor Rower rowing ergometer (rowers). The type of ergometer used in the test was to reflect and recreate the specificity of movements in the subjects' disciplines as faithfully as possible.

This test is used in endurance capacity diagnosis as a direct method of measuring maximal oxygen uptake (VO_2max). The subject's task was to overcome external resistance of the ergometer that increased at regular intervals.

In the case of the cycloergometer the initial workload was 50 W and it was increased by another 50 W every 3 minutes. The test was continued for as long as the athlete was able to overcome the external resistance while maintaining proper work rhythm (80 rpm). On the rowing ergometer the initial workload was 50 W and it was increased by 10 W every 3 minutes. The subject performed the test up to exhaustion. The mechanical treadmill test consisted in an athlete running at the initial speed of 16 km/h, which was increased by 1 km/h every 3 minutes.

During the progressive test all the subjects breathed through a facemask from which the exhaled air was directed to a K4b2 portable gas analysis system (Cosmed, Italy), which measured the real value of breathing parameters while also calculating the oxygen uptake with regard to body mass. Throughout the test the subjects' heart rate (HR) was monitored using a sportester 810i (Polar, Finland), and before the test and right after it, blood pressure (RR) was measured with a Riva Rocci sphygmomanometer.

Anthropmetric measurements

Before the test started, the subjects taking part in the research had their height and body mass measured; their BMI was calculated and body composition was examined by analyzing the absolute content of adipose tissue [FAT (kg)] and its percentage (% FAT), percentage of lean body mass (% LBM) as well as the absolute content of water [H_2O (L)] and its percentage in the body (% H_2O).

The assessment of body composition parameters was done by means of spectrometry in near-infrared. In the applied method, researchers used light with 940–950 nm wavelength, which penetrates into the tissues to the depth of 1 cm. It undergoes absorption, transmission and reflection while getting through the tissues. The more fat there is in the subcutaneous adipose tissue, the greater the absorption of the outgoing ray of light and the smaller absorption of the incoming one, returning to the detector as a refracted ray.

Each subject was informed about test procedures, their aims, unwanted reactions and hazards. All subjects started performing tests after signing a written consent, or in the case of underage subjects, after the consent was signed by their legal guardians.

Biochemical parameters

Biochemical examinations were carried out twice, before exertion and at the 10th minute of restitution. Blood samples were taken from the basilic vein. After removing the serum, concentrations of the chosen biochemical parameters were assayed - homocysteine, vit. B_6, vit. B_{12}, folic acid, creatinine.

Homocysteine was assayed using the FPIA method (Fluorescence Polarization Immunoassay) and a AxSYM Homocysteine kit (Abbott, USA) in a AxSYM analyzer. Vitamin B_6 was assayed using the ELISA method and

a microbiological kit ID-Vit Vitamin B_6 (Immunodiagnostic AG, Germany). Vitamin B_{12} and folic acid were assayed using the MEIA method (Microparticle Enzyme Immunoassay). They were assayed in a AxSYM analyzer, and AxSYM B12 Assay and AxSYM Folate kits (Abbott, USA) were used. The concentration of lactate was assayed using the colorimetric method and a Lactate Cuvette Test kit (Dr. Lange, Germany). The parameters of acid-base homeostasis and concentrations of K^+ and Na^+ were assayed with the Siemens analyzer and kits. Creatinine was estimated using the colorimetric method and a Creatinine Assay Kit (Cell Biolabs, Inc.). Cortisol was assayed using an Architect Cortisol kit (Abbott, USA). The complete blood count was assayed using ABX Micros manufactured by HORIBA ABX Diagnostics.

Statistical methods

The computer program Statistica PL Stat Soft version 9.0 was used for statistical analysis. In all applied statistical tests the level of $p \leq 0.05$ was considered statistically significant. The following tests were used for the data analysis. The normality of the distribution was measured with the Shapiro-Wilk test. If a variable was normally distributed, further calculations were done with the T test for independent trials to assess the differences in the analyzed variable between the groups, and the T test for dependent trials to assess the differences in a given variable before and after exertion. To measure the correlation between the variables, Spearman's rank correlation coefficient was calculated. The correlation between the variables was considered at the level of $p \leq 0.05$.

Results

Table 1 shows the results of anthropological, physiological and biochemical results for all the athletes as well as for groups performing the Wingate test and the progressive test separately.

Table 1. Mean values and standard deviation of the measured anthropological, physiological and biochemical parameters and significances before and after exercise and before Wingate and progressive tests

Parameters	All athletes		Wingate group		Progressive group	
	before test	after test	before test	after test	before test	after test
1	2	3	4	5	6	7
Age [years]	18.32 ±2.75	–	20.33 ±2.81	–	17.62 ±2.32^{m}	–
Height [cm]	177.37 ±6.83	–	173.71 ±9.21	–	178.15 ±6.12	–
Body mass [kg]	69.56 ±8.45	–	68.70 ±9.71	–	69.84 ±8.10	–
BMI [kg/m^2]	22.17 ±1.74	–	22.55 ±1.90	–	22.03 ±1.69	–
FAT [kg]	9.90 ±3.30	–	9.67 ±3.47	–	10.09 ±3.27	–
%FAT	14.38 ±5.46	–	14.37 ±5.45	–	14.40 ±5.66	–
LBM [%]	60.40 ±10.37	–	58.46 ±9.80	–	61.96 ±10.88	–
H_2O [L]	44.65 ±7.38	–	43.24 ±6.99	–	45.78 ±7.72	–
% H_2O	63.34 ±3.40	–	63.36 ±3.38	–	63.32 ±3.53	–
Homocysteine [µmol/L]	9.43 ±1.71	10.70 ±2.04*	9.25 ±1.48	10.18 ±2.21^{a}	9.58 ±1.92	10.88 ±1.98^{c}
B_6 [µg/L]	11.05 ±4.87	10.58 ±4.80	11.50 ±4.24	10.02 ±4.16^{b}	10.85 ±4.08	11.06 ±3.21
B_{12} [pmol/L]	278.31 ±83.76	316.29 ±94.88^{b}	248.37 ±59.82	285.67 ±61.97^{a,m}	287.68 ±88.77^{m}	328.25 ±77.67^{b,m}
Folic acid [ng/ml]	10.52 ±3.59	10.79 ±2.67	12.93 ±2.93	13.67 ±2.85^{b}	9.57 ±2.41^{m}	9.76 ±3.78^{m}
Lactic acid [mmol/L]	0.70 ±0.29	10.42 ±2.82$^{\#}$	0.85 ±0.24	10.89 ±2.15$^{\#}$	0.58 ±0.28^{m}	10.11 ±3.24$^{\#}$
pH	7.42 ±0.02	7.22 ±0.07$^{\#}$	7.41 ±0.01	7.23 ±0.04$^{\#}$	7.42 ±0.02	7.21 ±0.08$^{\#}$
BE [mmol/L]	0.35 ±2.19	–13.64 ±3.05$^{\#}$	–0.96 ±2.49	–13.93 ±1.65$^{\#}$	1.40 ±1.17	–13.43 ±3.81$^{\#}$

1	2	3	4	5	6	7
pO_2 [mmHg]	70.72 ±7.83	95.55 ±7.07$^{\#}$	70.66 ±7.07	99.51 ±7.07$^{\#}$	70.77 ±9.36	92.65 ±5.68$^{\#}$
pCO_2 [mmHg]	39.30 ±4.21	32.05 ±3.94$^{\#}$	37.56 ±5.04	29.47 ±4.62$^{\#}$	40.69 ±2.87	33.94 ±1.84$^{m,\#}$
$RR_{systole}$ [mmHg]	121.11 ±7.64	182.11 ±18.45$^{\#}$	120.42 ±8.11	176.82 ±14.01$^{\#}$	121.67 ±7.48	186.00 ±20.72$^{\#,m}$
$RR_{diastole}$ [mmHg]	76.11 ±5.43	56.53 ±19.17*	76.25 ±6.44	71.89 ±9.82	76.00 ±4.71	45.33 ±16.42 $^{\#,m}$
K^+ [mmol/L]	4.62 ±0.61	4.40 ±0.69	4.59 ±0.64	4.83 ±0.71	4.65 ±0.60	4.08 ±0.49^{c}
Na^+ [mmol/L]	142.74 ±5.95	140.75 ±27.70	143.58 ±8.59	134.44 ±41.53	142.07 ±2.60	145.80 ±2.96^{a}
Creatinine [mg/dL]	0.98 ±0.18	1.12 ±0.21*	1.02 ±0.18	1.15 ±0.17*	0.96 ±0.17	1.13 ±0.23^{c}

$^{\#}p \leq 0.000001$; $^{*}p \leq 0.00001$; $^{a}p \leq 0.005$; $^{b}p \leq 0.05$; $^{c}p \leq 0.01$ as compared with before test value; $^{m}p \leq 0.05$ – as compared with the related Wingate test group.

Table 2 shows a few selected results of the Wingate test – the value of peak power generated in the test duration (P_{max}), time needed to reach peak power (T_1) and time of peak power maintenance (T_2), fatigue index (FI), and a total amount of work completed in the test duration (W_{total}).

Table 2. Mean values and standard deviation of selected parameters in the Wingate test

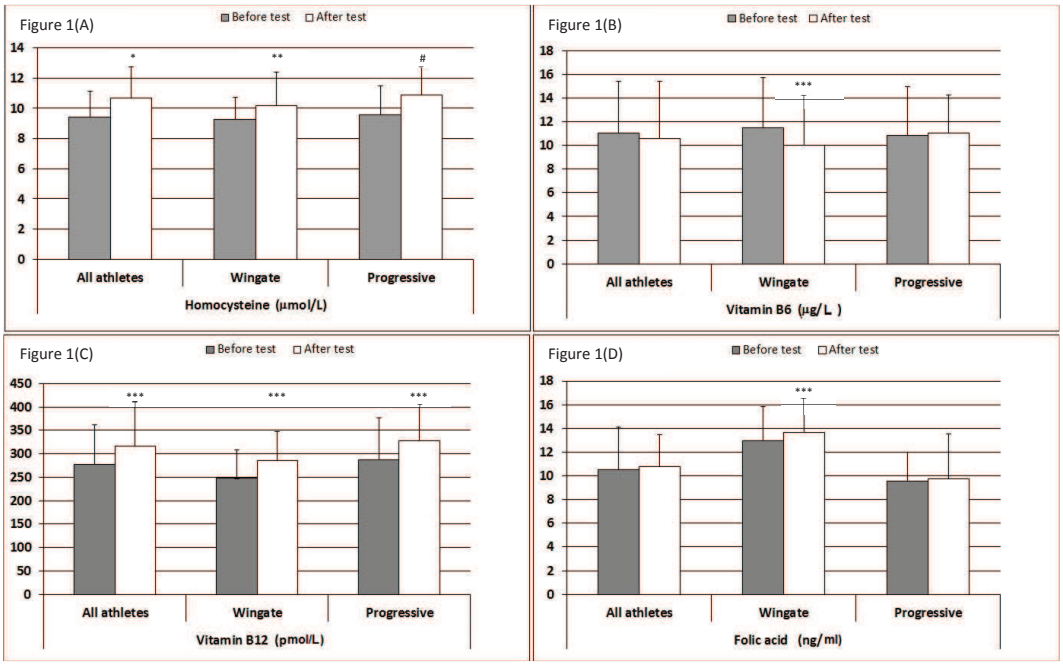
P_{max} [W]	P_{max} [W · kg^{-1}]	T_1 [s]	T_2 [s]	FI [%]	W_{total} [kJ]	W_{total} [J · kg^{-1}]
700.18 ±155.87	10.12 ±1.17	5.8 ±1.13	3.89 ±0.86	22.09 ±4.74	243.36 ±157.23	16.82 ±3.61

Table 3 shows the results of mean values of maximal oxygen uptake (VO_2max), maximum heart rate (HR_{max}) recorded in the subjects, and maximal value of minute ventilation of the lungs (VE_{max}) as well as the amount of work completed in the test duration. Following the formula for HR_{max} as 220-age, the subjects' HR should range from 188 to 200 bpm. Thus, after comparing theoretical values with the directly measured ones, it turns out that the intensity of the subjects' effort was really maximal.

Table 3. Mean values and standard deviation of selected parameters in the progressive test

VO_2max [L · min^{-1}]	VO_2max [ml · kg^{-1} · min^{-1}]	HR_{max} [b · min^{-1}]	VE_{max} [L · min^{-1}]	W_{total} [kJ]
4.4 ±0.80	64.20 ±7.35	193.27 ±5.46	148.53 ±28.51	232.28 ±54.74

The resting concentration of homocysteine in the blood of all the subjects measured after exertion (Figure 1A) increased by 13.5%. In both separate groups there was an increase in the concentration of homocysteine in comparison to the resting values, by 10% in the W group and by 11.36% in the P group.



* p = 0.001; ** p = 0.005; *** p < 0.05; # p = 0.01 as compared with the values before test.

Figure 1. Concentration of homocysteine (1A), vitamin B_6 (1B), vitamin B_{12} (1C) and folic acid (1D) before and after Wingate and progressive test, in groups and in all athletes

After exertion the concentration of vit. B_6 (Figure 1B) in the whole group as well as in the group performing the progressive test was not changed, but there was a decrease by 12.9% in the group performing the Wingate test.

The concentration of vit. B_{12} (Figure 1C) after exertion increased by 13.65% in the whole group of athletes. Separately, in the Wingate test group by 15%, and in the progressive test group by 14.1%. Higher resting values of this parameter were characteristic of athletes performing aerobic endurance disciplines.

After physical exertion the concentration of folic acid in the whole group and in the progressive test group was not changed (Figure 1D). However, in the Wingate test group it increased by 5.7%. What is more, in this group both resting concentration of the parameter and its value after exertion were higher in comparison with the progressive test group. In the resting state the concentration of homocysteine correlated positively with the number of erythrocytes ($r = 0.49$, $p = 0.021$), HGB concentration in the blood ($r = 0.51$, $p = 0.015$) and hematocrit ($r = 0.61$, $p = 0.003$). In the resting state a positive correlation with the percentage of lean body mass was also noted ($r = 0.45$, $p = 0.034$). Before and after exertion homocysteine concentration correlated with the concentration of creatinine in the blood (respectively $r = 0.31$, $p = 0.042$ and $r = 0.48$, $p = 0.025$). A negative correlation with the concentration of folic acid was observed both in the resting state ($r = –0.48$, $p = 0.023$) and after exertion ($r = –0.66$, $p = 0.001$).

Discussion

Physical effort disturbs the organism's homeostasis. The extent of biochemical and physiological changes is dependent on the type, intensity and duration of exertion and also on the level of the organism's physiological adaptation.

From the presented findings, it can be inferred that standard efforts measuring maximal power of anaerobic and aerobic processes cause changes in homocysteine metabolism and they also modify its concentration in the blood.

An increase in homocysteine concentration in the blood, which is evident in our presented research results, can be caused by a few mechanisms.

Physical effort can change the amount of homocysteine produced by an organism through its influence on the increase in protein turnover. Increased consumption of proteins during exertion accelerates methionine catabolism, which in turn might foster an increase in the level of homocysteine in the blood, as well as a decrease in the availability of the B vitamins (Forslund et al. 2000).

High-intensity physical exertion also increases the turnover of the methyl groups, which might also lead to an increase in homocysteine concentration in the blood (Joubert et al. 2006). In this process methionine is initially converted into s-adenosyl methionine – the most important donor of methyl groups in people. It transfers the methyl group to guanidinoacetate methyltransferase (GAMT). As a result of this reaction, creatinine and S-adenosyl homocysteine are created (Brosnan et al. 2011). The very last substance is then hydrolized to form adenosine and homocysteine. Creatine synthesis constitutes ca. 40% of all methyl groups supplied by S-adenosyl methionine (SAM). Creatine synthesis in the liver constitutes ca. 75% of the daily production of homocysteine (Steenge et al. 2001). According to Stead et al. (Stead et al. 2006) ca. 70% of S-adenosyl methionine produced in the metabolism of methionine, and in this way of homocysteine too, is used in creatine synthesis. During massive accumulation of ADP followed by ATP hydrolysis, creatine kinase (CK) catalyses a reversible reaction of phosphocreatine (PCr) with ADP to regenerate ATP and to stabilize the intracellular ATP pool.

Phosphocreatine and creatine kinase form the CK/PCr system, that is sensitive to energy fluctuations and plays the role of an immediately available energy buffer and metabolic regulator. In anaerobic high-intensity efforts, phosphocreatine is an energy carrier. The creatine produced in this process together with ATP takes part in phosphocreatine resynthesis. As a result of intensive physical effort, creatine from phosphocreatine is transformed to creatinine, whose concentration in the blood increases (Joubert et al. 2006). Its higher concentration is then also observed in the urine (Stead et al. 2006). In our tests the concentration of creatinine in the blood of the athletes after exertion increased in both groups of the studied subjects. In the research done by Bakońska-Pacoń (2006), a significant increase in the concentration of creatinine in the blood was also observed. At the same time, she noted creatinine clearance in athletes performing the progressive test even though the work done by the athletes was much smaller (175.69 ±8.54 kJ), as compared to our athletes performing the progressive test (232.28 ±54.74 kJ).

Moreover, in our research the concentration of homocysteine in the blood after exertion correlated highly with the concentration of creatinine. It can be thus inferred that high intensity and long duration efforts, which cause an increase in creatine concentration necessary for phosphocreatine resynthesis, lead to an increase in homocysteine levels (Joubert et al. 2006). It might be assumed that an increase in homocysteine level after intensive efforts is provoked by increased methionine metabolism. Methyl derivatives, such as creatine or acetylcholine, play an important role during physical effort. Due to effort-induced metabolic demand, the turnover of the substances is

greater and it is possible that their regeneration is accompanied by an increased methionine metabolism, which, in turn, can disturb the equilibrium between production and degradation (through remethylation and transsulfuration) of homocysteine, and as a result, lead to its increase. Herrman et al. (2003) and Joubert et al. (2006) are of a similar opinion.

Another probable mechanism responsible for an increase in homocysteine level in the blood immediately after exertion might be connected to vit. B_6 availability. Intensive effort disturbs glycogen reserves and in this way leads to vit. B_6 consumption (Okada et al. 2001). The most biologically active form of vit. B_6 – pyridoxal phosphate (PLP) is a coenzyme for transaminases, decarboxylases, and other enzymes used in metabolic pathways as well as other nitrogen compounds. PLP presence is also required by glycogen phosphorylase – the key enzyme in the process of degradation of glycogen present in the muscles and liver (Howlett et al. 1998; Parolin et al. 1999). While increasing muscles' demand for vit. B_6, physical effort (Choi and Cho 2008) can cause the shifting of the substance's pool towards energetic processes, and thus decrease its use in methionine remethylation processes. It might result in higher concentration of homocysteine in the blood.

König et al. (2003) suppose that only very intensive and exhausting efforts based on anaerobic processes lead to a significant increase in homocysteine concentration because, at that time, increased creatine and protein turnover takes place. Their hypothesis is based on research done by De Cree et al. (1999), who noted a high increase in homocysteine level in the blood of women performing a progressive test. Yet, they did not observe homocysteine increase in the group who performed submaximal effort at 60% VO_2max.

Efficient renal filtration seems to be an important factor in proper functioning of the organism and in the removal of homocysteine from the organism after exertion (Friedman et al. 2001; Patel et al. 2005; Wollesen et al. 1999). Brattstrom et al. (1992) stated that in patients with a pulmonary embolism resulting from deep vein thrombosis, and with diabetic nephropathy, homocysteine concentration depends on plasma creatinine level. Gelecek et al. (2007), who studied the influence of single and cyclic aerobic exercise on homocysteine level, are of the same opinion. Their results are similar to the results presented in our paper. In their studied group of students after a 30-minute submaximal aerobic effort at 70–80% HR_{max}, the concentration of homocysteine in the blood increased significantly immediately after exertion.

Research done by Whrite et al. (1998) confirmed that homocysteine concentration increases after a 30-minute effort at 70% HR_{max}. However, in the researchers' opinion, hemoconcentration increase accounts for the elevated homocysteine concentration, and not for any biochemical mechanism. They did not observe any correlation between homocysteine level and VO_2max, either. As a consequence, the researches came to the conclusion that intensive exercise does not affect homocysteine concentration in any significant way. An increase in hemoconcentration during exertion is caused by an increase of hydrostatic pressure in the capillaries, which moves part of the water to extravascular compartments (Austin et al. 2011).

In our research we also observed an increase in hemoconcentration after exertion. As far as the value of hematocrit is concerned, it increased by 5.3% in all athletes, while in the W group it increased by 3.6%, and in the P group by 6.7%. However, an increase in homocysteine concentration was twice as big – in the whole group the concentration increased by 13%, while in the W and P groups by 10% and 11.36%, respectively. Thus, how can a decrease in vit. B_6 concentration, which we observed in our research, be explained? In our opinion, the assumption that hemoconcentration is the only reason for the observed increase in the concentration of homocysteine in the blood after exertion is too big a simplification.

Research papers often emphasize the existence of a strong correlation of folic acid and vitamin B_{12} with the level of homocysteine in the blood. In the present study this correlation was examined in the context of physical effort. The subjects were divided into 2 groups. It was noted that folic acid concentration in the W group was significantly higher both before and after exertion in comparison to the P group subjects, and post-exertion increase was only observed in the W group. On the other hand, vit. B_{12} concentration before exertion was significantly higher in the progressive test group.

The B vitamins play an essential role in homocysteine metabolism, both in remethylation and transsulfuration. Folic acid and vit. B_{12} as coenzymes of methionine synthase take part in remethylation, which is a conversion of homocysteine into methionine. However, vit. B_6 as a coenzyme of cystathionine-ß-synthase and cystathionine-δ-lyase participates in the transsulfuration pathway, during which homocysteine is converted into cysteine.

In our research in the whole group of athletes we noted a negative correlation between folic acid concentration and homocysteine before and after exertion. A similar correlation was observed by König et al. (2003). In their opinion, a lower increase in homocysteine concentration after exertion might be caused by a higher resting concentration of folic acid. At the same time, the authors cannot explain why higher concentration of folic acid was observed in subjects whose training volume was bigger. However, in our studies we noticed that higher levels of folic acid were characteristic of speed and strength discipline athletes in contrast to endurance ones.

In the present study no correlation between homocysteine and vit. B_{12} was observed. However, on the basis of research publications it could be concluded that such a correlation should have taken place. König et al. (2003) did not notice such a correlation in their research either, not only in the case of subjects performing an intensive single effort, but also in the subjects whose measurements were taken after a whole training unit and after a whole training cycle, regardless of training volume. However, research done by Murawska-Ciałowicz (2012) showed that such a correlation was observed in women participating in a 9-month exercise training.

A high positive correlation between homocysteine and LBM, which was observed in the presented research, also seems to be very interesting. A similar correlation was noticed by Battezzati et al. (2007). They noted a higher homocysteine level in men as compared to women. Their explanation for the difference is higher LBM in men, whose percentage has a profound effect on a higher concentration of plasma homocysteine. Ebenbichler et al. (2001) also observed higher concentrations of this amino acid in body builders in the period of muscle mass building.

Correlations between homocysteine and body mass can be corroborated by a positive correlation between concentrations of homocysteine and creatinine, which was observed in the present study. During intensive exercise, when ATP is resynthesised thanks to a transfer of a phosphate group from phosphocreatine, creatine is created and converted into creatinine. On exertion, an excess of creatine is removed from the muscles in the form of creatinine, that is why, after exertion it is possible to observe a lack in creatine, which is essential for phosphocreatine resynthesis. It might lead to an increase in the synthesis of disposable creatine and to an increase in homocysteine concentration.

The present research confirmed that a single, intensive exercise leads to an increase in homocysteine concentration; however, regular physical effort might contribute to a decrease in plasma homocysteine levels. The above-mentioned research done by König et al. (2003) seems to be a good example. The researchers achieved a significant increase in homocysteine levels immediately after exertion; yet, after a 30-day training cycle they noticed a decrease in the concentration of this amino acid, especially in subjects who train more regularly. Similar research done by Murawska-Ciałowicz (2012) in women of different body mass also indicated that a 9-month

exercise training significantly reduced homocysteine concentration, especially in obese women, even though after single exercises a significant increase in homocysteine concentration was observed, especially in obese and overweight women.

Gaume et al. (2005) claim that a combination of training and a diet rich in folic acid and vit.B_{12} is a better method for lowering homocysteine in the blood, which might be extremely useful in disease prevention. They came to such conclusions after examining middle-aged men, who were divided into 2 groups of training and non-physically active subjects. Homocysteine concentration was negatively correlated with the levels of vit. B_{12} and folic acid. Additionally, homocysteine concentration was significantly lower in training subjects. Interesting findings were also provided by Czajkowska et al. (2008) on the assessment of the influence of energy expenditure connected with physical activity on plasma homocysteine concentrations in young men. The researchers noticed that in subjects whose energy expenditure caused by physical activity amounted to 1284 kcal/day, homocysteine level was significantly lower than in subjects whose energy expenditure totaled 316.4 kcal/day, which is only a little more than the recommended 2100 kcal/week. Therefore, in order to lower homocysteine level in the blood one should take up physical activity whose expenditure exceeds 2100 kcal/week.

As it seems obvious from the presented material, there are a lot of contradictory reports on the real influence of physical activity on homocysteine levels. Thorough knowledge of the mechanisms connected with the changes of this amino acid in the blood that happen as a result of different forms of physical activity can be used in the prophylaxis of cardiovascular diseases, which are, among others, diseases correlated with an elevated homocysteine concentration.

From the research presented in our study, it can be concluded that a single exercise causes an increase in homocysteine concentration in the blood, whereas from the research done by other authors it can be learned that regular physical activity leads to a decrease in concentrations of this amino acid in the blood.

It can be caused, most of all, by body mass reduction, fat percentage in the body and an improvement in adipose tissue metabolism. A positive correlation between body mass, fat content and homocysteine concentration in women's blood was noticed. What is more, a correlation was observed between homocysteine and hormones secreted by adipose tissue (adipokines) (Murawska-Ciałowicz 2012). Adipokines participate in the regulation of many significant physiological processes. They affect, among others, the endothelium of blood vessels, and also the processes of coagulation and fibrinolysis.

Training-related fat reduction in an organism can decrease lipid peroxidation and limit oxidative stress through an increase in anti-oxidant enzymes' activity (catalase, glutathione peroxidase) (Murawska-Ciałowicz et al. 2006). It was shown that in patients suffering from type 2 diabetes and from insulin resistance homocysteine blood clearance is lower than in healthy patients. There is a lot of research that proves an improvement in cell sensitivity to insulin after regular exercise. It might be expected that an improvement in insulin sensitivity fosters an increase in homocysteine blood clearance and therefore a decrease in homocysteine concentration in the blood (Tessari et al. 2005). Ravaglia et al. (2004) observed a strong relationship between homocysteine and C-reactive protein in patients with cardiovascular diseases. Regular physical activity limits oxidative stress and reduces the inflammatory process that is conducive to the development of obesity and cardiovascular diseases. From the research by Hübner-Woźniak and Ochocki (2009) and by other authors, it can be concluded that there is a significant reduction in CPR (main marker of the inflammatory process) due to physical training.

Summing up, it can be stated that after intense exercise, concentrations of homocysteine, vit. B_{12} and folic acid in the blood increase as a result of many passing adaptive reactions that are to provide an organism with energy and metabolic substances. A decrease in vit. B6 concentration after anaerobic exercise is probably caused by a greater use of its resources by glycogen phosphorylase of an enzyme indispensible in the process of glycogen degradation and production of glucose 6-phosphate.

References

Austin A.W., Patterson S.M., von Känel R. Hemoconcentration and hemostasis during acute stress: interacting and independent effects. Ann. Behav. Med. 2011; 42: 153–173.

Bakońska-Pacoń E. Creatinine clearance and 24-hour creatinine excretion profile in the urine of people after physical exercises. Biol. Sport. 2006; 23: 157–170.

Battezzati A., Bertoli S., San Romerio A., Testolin G. Body composition: An important determinant of homocysteine and methionine concentrations in healthy individuals. Nutr. Metabol. Cardiovas. Dis. 2007; 17: 525–534.

Boushey C.J., Beresford S.A., Omenn G.S., Motulsky A.G. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA. 1995; 274: 1049–1057.

Brattstrom L., Lindgren A., Israelsson B., Malinow M.R., Norrving B., Upson B., Hamfelt A. Hyperhomocysteinaemia in stroke: prevalence, cause, and relationships to type of stroke and stroke risk factors. Eur. J. Clin. Invest. 1992; 22: 214–221.

Brosnan JT., Da Silva R.P., Brosnan M. The metabolic burden of creatine synthesis. Amino Acids. 2011; 40: 1325–1331.

Brouwer I.A., Van Dusseldorp M., Thomas C.M., Duran M., Hautvast J.G., Eskes T.K., Steegers-Theunissen R.P. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. Am.J. Clin. Nutr. 1999; 69: 99–104.

Choi E.Y., Cho Y.O. Vitamin B6 deficiency can reduce fuel storage and utilization in physically trained rats. In. J. Vitam. Nutr. Res. 2008; 78: 64–69.

Chrysohoou C., Panagiotakos D.B., Pitsavos C., Zeimbekis A., Zampelas A., Papademetriou L., Masoura C., Stefanadis C. The associations between smoking, physical activity, dietary habits and plasma homocysteine levels in cardiovascular disease-free people: the "ATTICA" study. Vasc. Med. 2004; 9: 117–123.

Czajkowska A., Lutosławska G., Mazurek K., Ambroszkiewicz J., Żmijewski P. Correlation between physical activity and plasma homocysteine concentration in young men. Pediatr. Endocrinol. Diab. Metab. 2008; 14: 177–180.

Dankner R., Chetrit A., Lubin F., Sela B.A. Life-style habits and homocysteine levels in an elderly population. Aging Clin. Exp. Res. 2004; 16: 437–442.

De Bree A., Verschuren V.M., Blom H.J., Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. Am. J. Epidemiol. 2001; 154: 150–154.

De Cree C., Malinow M.R., Van Kranenburg G.P., Geurten P.G., Longford N.T., Keizer H.A. Influence of exercise and menstrual cycle phase on plasma homocysteine levels in young women – a prospective study. Scand. J. Med. Sci. Sports. 1999; 9: 272–278.

Ebenbichler C.F., Bodner K.J., Gander R., Lechleitner M., Herold M., Patsch J.R. Hyperhomocysteinemia in bodybuilders taking anabolic steroids. Eur. J. Int. Med. 2001; 12: 43–47.

Forslund A.H., Hambraeus L., Van Beurden H., Holmback U., El-Khoury A.E., Hjorth G., Olsson R., Stridsberg M., Wide L., Akerfeldt T., Regan M., Young V. R. Inverse relationship between protein intake and plasma free amino acids in healthy men at physical exercise. Am.J. Physiol. Endocrinol. Metab. 2000; 278: E857–E867.

Friedman A.N., Bostom A.G., Selhub J., Levey A.S., Rosenberg I.H. The kidney and homocysteine metabolism. J. Am. Soc. Nephrol. 2001; 12: 2181–2189.

Gaume V., Mougin F., Figard H., Simon-Rigaud M.L., N'Guyen U.N., Callier J., Kantelip J.P., Berthelot A. Physical training decreases total plasma homocysteine and cysteine in middle-aged subjects. Ann. Nutr. Metab. 2005; 49: 125–131.

Gelecek N., Teoman N., Ozdirenc M., Pinar L., Akan P., Bediz C., Kozan O. Influences of acute and chronic aerobic exercise on the plasma homocysteine level. Ann. Nutr. Metab. 2007; 51: 53–58.

Herrmann M., Schorr H., Obeid R., Scharhag J., Urhausen A., Kindermann W., Herrmann W. Homocysteine increases during endurance exercise. Clin. Chem. Lab. Med. 2003; 41: 1518–1524.

Herrmann M., Wilkinson J., Schorr H., Obeid R., Georg T., Urhausen A., Scharhag J., Kindermann W., Herrmann W. Comparison of the influence of volume-oriented training and high-intensity interval training on serum homocysteine and its cofactors in young, healthy swimmers. Clin. Chem. Lab. Med. 2003; 11: 1525–1531.

Howlett R.A., Parolin M.L., Dyck D.J., Hultman E., Jones N.L., Heigenhauser G.J.F. Spriet L.L. Regulation of skeletal muscle glycogen phosphorylase and PDH at varying exercise power output. Am. J. Physiol. 1998; 275: R418–R425.

Hübner-Woźniak E., Ochocki P. Effects of training on resting plasma levels of homocysteine and C-reactive protein in competitive male and female wrestlers. Biomed. Hum. Kinet. 2009; 1: 42–46.

Humphrey L.L., Fu R., Rogers K., Freeman M., Helfand M. Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis. Mayo Clin. Proc. 2008; 83: 1203–1212.

Joubert L.M., Manore J., Manore M.M. Exercise nutrition and homocysteine. Int. J. Sport. Nutr. Exer. Metab. 2006; 16: 341–361.

König D., Bisse E., Deibert P., Muller H.M., Wieland H., Berg A. Influence of training volume and acute physical exercise on the homocysteine levels in endurance trained men: interactions with plasma folate and vitamin B-12. Ann. Nutr. Metab. 2003; 47: 114–118.

Lovell D.I., Bousson M., McLellan C. The use of performance tests for the physiological monitoring of training in combat sports: A case study of a world ranked mixed martial arts fighter. J. Athl. Enhance. 2013; 2: 1–6.

Murawska-Ciałowicz E. Influence of health training on chosen adipose tissue hormones and homocysteine level in women with different body mass. Studies and Monographs 104. Wroclaw Academy of Physical Education Press. Wroclaw 2012.

Murawska-Ciałowicz E., Januszewska L., Zuwała-Jagiełło J., Milczarska J., Zawadzki M., Paprocka-Borowicz M., Wierzbicka--Damska I. Melatonin decreases homocysteine level in blood of rats. J. Physiol. Pharmacol. 2008; 59: 717–729.

Murawska-Ciałowicz E., Wierzbicka-Damska I, Zawadzki M., Januszewska L., Jastrzębska A., Ochmann B., Paprocka-Borowicz M. Correlation of oxidative stress with physical performance in young cyclists. Pol. J. Environ. Stud. 2006; 15: 276–280.

Nygard O., Vollset S.E., Refsum H., Stensvold I., Tverdal A., Nordrehaug J.E., Ueland M., Kvale G. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA. 1995; 274: 1526–1533.

Okada M., Goda H., Kondo Y., Murakami Y., Shibuya M. Effect of exercise on the metabolism of vitamin B6 and some PLP-dependent enzymes in young rats fed a restricted vitamin B6 diet. J. Nurt. Sci. Vitaminol. (Tokyo) 2001; 47: 116–121.

Parolin M.L., Chesley A., Matsos M.P., Spriet L.L., Jones N.L., Heigenhauser G.J.F. Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. Am. J. Physiol. 1999; 277: E890–E900.

Patel D.R., Torres A.D., Greydanus D.E. Kidney and sports. Adolesc. Med. 2005; 16: 111–119.

Ravaglia G., Forti P., Maioli F., Servadei L., Martelli M., Arnone G., Talerico T., Zoli M., Mariani E. Plasma homocysteine and inflammation in elderly patients with cardiovascular disease and dementia. Exp. Gerontol. 2004; 39: 443–450.

Saw S.M., Yuan J.M., Ong C.N., Arakawa K., Lee H.P., Coetzee G.A., Yu M.C. Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. Am. J. Clin. Nutr. 2001; 73: 232–239.

Selhub J. Homocysteine metabolism Annu. Rev. Nutr. 1999; 19: 217–246.

Stead L.M., Brosnan J.T., Brosnan M.E., Vance D.E., Jacobs R.L. Is it time to reevaluate methyl balance in humans? Am. J. Clin.Nutr. 2006; 83: 5–10.

Steenge G.R., Verhoef P., Greenhaff P.L. The effect of creatine and resistance training on plasma homocysteine concentration in healthy volunteers Arch. Intern. Med. 2001; 161: 1455–1457.

Tessari P., Coracina A., Kiwanuka E., Vedovato M., Vettore M., Valerio A., Zaramella M., Garibotto G. Efects of insulin on methionine and homocysteine kinetics in type 2 diabetes with nephropathy. Diabetes 2005; 54: 2968–2976.

Whrite M., Francis K., Cornwell P. Effect of acute exercise on plasma homocysteine. J. Sports Med. Phys. Fitness 1998; 38: 262–265.

Wollesen F., Brattström L., Refsum H., Ueland P.M., Berglund L., Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus Kidney Int. 1999; 55: 1028–1035.

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