

PLASMA GROWTH HORMONE, CORTISOL AND TESTOSTERONE RESPONSES TO REPEATED AND INTERMITTENT ERGOMETER EXERCISE AT DIFFERENT PEDALLING RATES

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Abstract. The aim of this work was the evaluation of the hormonal response to the repeated and intermittent exercises at different rates. Nine students of the physical education (age: 24.1 ± 0.39 years; body mass: 81.2 ± 10.17 kg; height: 182.1 ± 7.32 cm; $VO_2\max - 4.121 \pm 0.697$ l O_2/\min) performed in the laboratory conditions series of 5 efforts on the cycle ergometer lasting for 3 min with the loading of 250 W and divided with 2 min intervals. In the first day the pedalling rate amounted to 45 circ/min (W_{45}), in the second day to 80 circ/min (W_{80}). In both cases the work performed (225 kJ), time lasting and power of efforts were the same. The growth hormone (GH), testosterone (T) and cortisol (C) were denoted in the capillary blood taken with the immuno-enzymatic methods from the finger tip. The anabolic – catabolic index (T/C) was also determined. The blood acid – alkali balance parameters were registered using the gas analyser Ciba-Corning 248. The blood was taken: before effort, after third effort (13 min), immediately after fifth effort (23 min) and 30 min after the test. Series of the repeated and intermittent exercises caused the significant ($P < 0.05$) lowering of the capillary blood pH, respectively from: 7.392–7.409 before effort to: 7.316 ± 0.092 (W_{45}) and 7.287 ± 0.068 (W_{80}) after fifth effort. The differences between W_{45} and W_{80} were statistically insignificant. The highest concentrations of GH and T were noticed after all efforts in both series. The T concentration grew significantly, similarly in both series: $W_{45} - 5.8 \pm 1.93$ and $W_{80} - 5.9 \pm 1.59$ ng/ml from the restful level ≈ 4.6 ng/ml. The significant differentiation of the response on different pedalling rates was observed in case of GH. The highest GH concentration amounted to: 7.7 ± 5.43 ng/ml after W_{45} and after W_{80} to: 16.8 ± 6.68 ng/ml. The GH concentration changes presented lower level ($P < 0.05$) during work performed with the smaller rate i.e.: 45 circ/min. As regards cortisol (C), the significant increase until the 30th min after effort occurred after the series with the rate of 80 circ/min (increase from: 158.8 ± 52.55 ng/ml before to 236.1 ± 102.43 ng/ml after) unlike in the series with the rate of 45 circ/min where the concentration of C, 30 min after effort, presented level close to the level reached before the test (181.6 ± 62.50 ng/ml before effort and

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161.5±102.62 ng/ml in 13th min of the rest). The effort test with the higher pedalling rate caused the significant lowering of the T/C index unlike the W_{45} when its growth in the third and thirteenth min of the repose was observed ($P<0.05$). Negative correlations between the highest GH concentration in both series and the decrease of the blood pH ($r=-0.569$; $P<0.01$); a GH peak and the T/C index ($r=-0.819$; $P<0.001$) and the examined VO_2 max and the GH peak ($r=-0.753$; $P<0.05$) were determined. As a conclusion it may be stated that the rate/speed of performed work influences hormonal responses through the increase of cortisol and growth hormone concentrations alongside the similar level of the acid-alkali blood balance disturbances during work with the higher pedalling rate. The anabolic – catabolic balance disturbances (decrease of the T/C index) present strong impulse for the growth hormone secretion.

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Key words: Growth hormone (GH) – Cortisol – Testosterone – Intermittent exercise

Introduction

The physical effort is a strong impulse of the endocrinous secretion hormones with the existence of some stimulation threshold which is determined through the force and time of effort performance. It refers especially to the growth hormone (GH). Growing during effort metabolic acidosis causes increase of the hormones secretion although, it is not the sole factor determining hormones response [2,6,9,18,22]. The form and kind of the physical activity may have an influence on the hormones production as well.

Exercises with the various force and duration are used in the sport practice. They cause different hormones responses designated among others by the physical capacity level, training level and the training cycle phase [1,3,4,11,16,21,26]. The references state that the smaller response of the hormonal system is seen during the growth of sport fitness e.g.: stress hormones (cortisol, nor- and adrenaline, growth hormone) reactions are less intense. Hence, the increase of the training capacity (force x time) becomes not very effective training stimulus. On the other hand, the decrease of the energetic and biological costs of performed physical exercises is observed as an after effort effect. This leads to constant increase of training loads – higher intensification. The differentiation of biological effects, with the same work, may be gained using various forms of the physical effort e.g.: intermittent vs.

permanent work, different rate/speed of performed exercises. On the other hand, it is believed that the balance between anabolic and catabolic processes in an organism seems to be necessary in the process of the "optimal" sport training realisation. Hereof, changes of circular concentrations of chosen hormones (in blood) may bring objective information about the physiological effects of applied exercises. The growth of the cortisol concentration under the influence of 30 s effort was observed in examined athletes [16,17]. The cortisol level decreased during the series of 5 exercises lasting for 15 s and after 15 min rest it went back to the initial values [12]. The testosterone level did not change in this time. In case of efforts with the same amount of performed work the permanent (60% VO_2max) and intermittent effort (>80% VO_2max) caused similar GH production [2]. However, in the research of Vanhelder *et al.* [24] the maximal intermittent effort caused higher GH production in comparison to constant effort (with the same work value).

Hormonal responses after sub-maximal and maximal load efforts in permanent and intermittent exercises were described in the references [2,11,14,18]. The level of minute oxygen intake (present in % VO_2max) or the total time of performed work were taken as the criterion of various physical effort forms. It is believed that the hormones secretion increase depends on the effort intensity [9,13,25], effort duration [14,25], body temperature changes [18] and the physical capacity [23]. There is a lack of papers evaluating influence of various work rates, keeping the same kind of work and its power, on hormones responses. The aim of this paper was the evaluation of chosen hormones response to the repeated and intermittent exercises at different rates.

Material and Methods

Nine students of the physical education, practising sports irregularly, took part in the research. The study was approved by The Scientific Research Board of Ethics. Examined students were informed about the aim, methodology of research and the possibility of the immediate resignation. All the participants agreed for the experiment in written. Examined students characteristics were: age 24.1 ± 0.93 years; body height 182.1 ± 7.32 cm; body mass 81.2 ± 10.17 kg and the maximal oxygen intake (VO_2max) 4.121 ± 0.697 l O_2/min .

All examined performed at the beginning the PWC_{170} test according to Sjöstrand [19] as for determination of the physical capacity. This index was calculated basing on the dependence of the effort power and heart systole through the extrapolation to 170 beats/min. The maximal oxygen intake (VO_2max) was

determined through the indirect method of Karpman *et al.* [15]. Next, at the interval of one week, participants were submitted to various effort loads on the cycle-ergometer (Monark E 824 Sweden, connected to the IBM PC Pentium with "MCE v.4.0", "IBA" programme; Zbigniew Staniak, Poland), 5x3 min with the power of 250 W and with 2 min intervals between them. The total test time equalled 23 min and total work – 225 kJ. The MCE programme was used for the control of work time, intervals, amount of performed work and the average power. In the first test (W_{45}) examined students pedalled with the rate equal 45 circ/min and in the second (W_{80}) with 80 circ/min. Both series were conducted before noon (9⁰⁰-11⁰⁰) after light breakfast in the day not preceded by some heavy exercises and after 8 h sleep.

The blood was taken from the finger tip at ease (before first effort), after third repetition (13 min), immediately after 5th effort (23 min) and after 30 min of passive rest. The acid – alkali blood balance was registered on the on the blood gas analyser – Ciba-Corning 248 (the arterialized blood pH was analysed in the paper). IBL ELISA (Orion Diagnostica, Finland) were used for determination of the cortisol (C) and testosterone (T) concentration and hGH ELISA sets (EUROGENICS, Germany) for the growth hormone. The results reading was done on the Micro-Reader 1 (INTIMEX, England) with the 450 nm wave. The anabolic – catabolic index (T/C) was calculated basing on the designated testosterone and cortisol concentrations:

$$\left[\frac{T \text{ (nmol/l)}}{C \text{ (nmol/l)}} \times 100 \right]$$

The analysis of variance (ANOVA) with repeated measurements was used for the result arrangement. The average values differences were compared with the post hoc test of Scheffé. The AUCs fields values were compared for conditioned trials using the t-Student test. The Pearson's correlation coefficients were used in the evaluation of the dependence between examined variables. Calculations were done with the STATISTICA™ v.5.5 programme (StatSoft, USA).

Results

The intermittent effort (5x3 min x250 W) presenting for examined average load on level – $87.9 \pm 13.8\%$ VO_2 max caused significant changes in the acid – alkali blood balance and in levels of examined hormones. The lactic metabolic acidosis caused by effort effected in crucial decrease of the blood pH – from rest values:

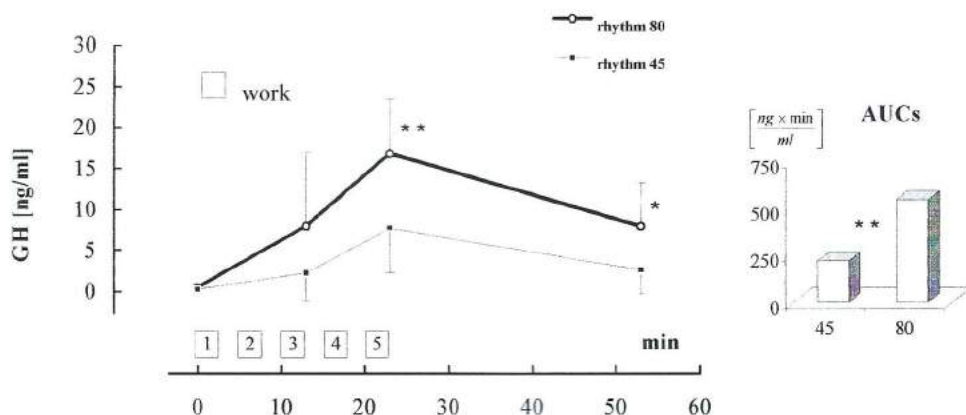


Fig. 1

The average (\pm SE) growth hormone (GH) concentration in blood during the repeated and intermittent efforts ($5 \times 3 \times 250$ W) performed on the cycle ergometer with the 45 and 80 circ/min pedalling rates; AUCs average values ($n=9$) of the field under the curve. (differences significant at the level of $*P<0.05$ and $**P<0.01$ in relation to 45 circ/min rate)

7.392 ± 0.056 and 7.409 ± 0.030 in both trials to: 7.316 ± 0.02 in W_{45} and 7.288 ± 0.069 in W_{80} . The differences between groups are statistically insignificant. After the 30 min rest the blood pH was equal the restful standard values ($7.350 \div 7.450$). The highest growth hormone concentration occurred immediately after performance of the equipollent efforts with various pedalling rates. For W_{45} the GH concentration grew from 0.27 ± 0.151 ng/ml before effort to 7.27 ± 5.434 ng/ml ($P<0.05$) and for W_{80} from 0.45 ± 0.409 to 16.82 ± 6.682 ng/ml ($P<0.05$; Fig. 1). Similarly in the case of testosterone (T) the significant increase of this hormone concentration ($P<0.05$) in blood was observed after finishing the 5 effort: from 4.61 ± 1.013 and 4.67 ± 1.139 ng/ml before effort to: 5.83 ± 1.936 ng/ml in W_{45} and 5.94 ± 1.598 ng/ml in W_{80} (Table 1). The T level went back to the before effort values after the 30 min restitution. The effort performed with the pedalling speed of 45 circ/min caused insignificant decrease of the cortisol (C) concentration – from 181.67 ± 62.500 ng/ml to 161.17 ± 102.622 ng/ml in the 30th min of the rest and in case of 80 circ/min its concentration grew from 158.89 ± 52.546 ng/ml to 236.11 ± 102.432 ng/ml ($P<0.05$) in the 30th min of the rest (Fig. 2). The anabolic – catabolic index

Table 1

Average (\pm SD) values of the arterialized blood pH, growth hormone (GH), testosterone (T), cortisol (C) concentrations, anabolic – catabolic (T/C) index during the repeated work (5x3 x 250 W) performed with 45 and 80 circ/min pedalling rates on the cycle ergometer: 0 – restful value, 13°, 23°, 53° – measurements in 13 (after 3 effort), 23 (after 5 effort) and 53 (after 30 min of rest) minute of the examination

	45 circ/min pedalling rates					80 circ/min pedalling rates				
	0°	13°	23°	53°	0°	13°	23°	53°		
PH	x	7.392	7.330*	7.316*	7.408	7.409	7.294*	7.288*	7.410	
	SD	\pm 0.056	\pm 0.079	\pm 0.092	\pm 0.021	\pm 0.030	\pm 0.056	\pm 0.069	\pm 0.013	
GH (ng/ml)	x	0.27	2.24	7.72*	2.62	0.45	7.97*	16.82*	7.94*	
	SD	\pm 0.151	\pm 3.426	\pm 5.434	\pm 2.896	\pm 0.409	\pm 8.997	\pm 6.682	\pm 5.342	
T (ng/ml)	x	4.61	5.42	5.83*	4.92	4.67	5.86*	5.94*	4.74	
	SD	\pm 1.013	\pm 1.414	\pm 1.936	\pm 1.264	\pm 1.139	\pm 1.638	\pm 1.598	\pm 1.129	
C (ng/ml)	x	181.67	176.67	185.00	161.17	158.89	166.11	215.00	236.11*	
	SD	\pm 62.500	\pm 80.739	\pm 110.510	\pm 102.622	\pm 52.546	\pm 72.877	\pm 75.952	\pm 102.432	
T/C	x	3.48	4.37	4.65*	4.66*	4.00	4.80	3.70	2.99*	
	SD	\pm 1.134	\pm 1.552	\pm 1.715	\pm 1.742	\pm 1.551	\pm 1.443	\pm 1.436	\pm 1.475	

*significantly different values in relation to the rest (0°) for $P < 0.05$

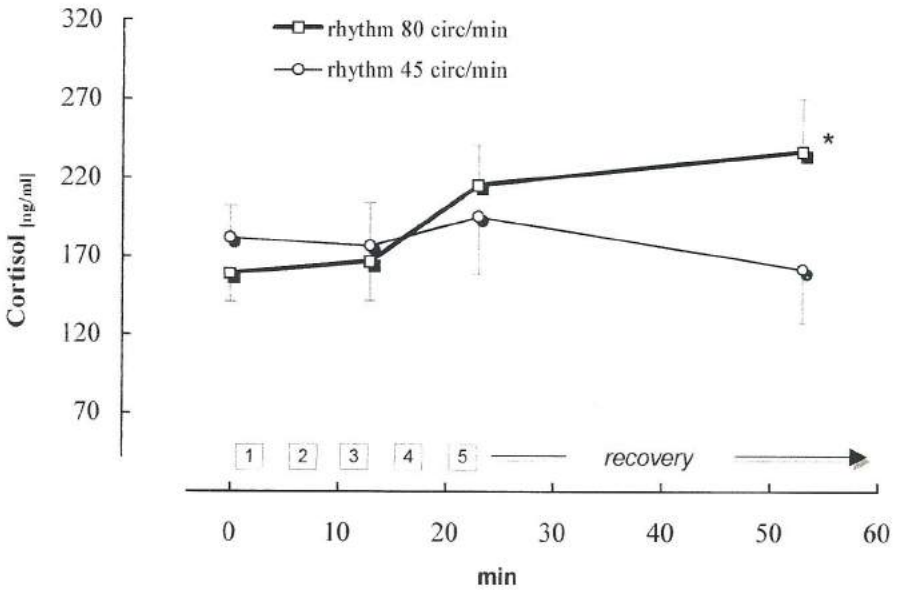


Fig. 2

Average (\pm SE) values of the cortisol concentration in blood during the repeated and intermittent efforts (5x3x250 W) performed on the cycle ergometer with the 45 and 80 circ/min pedalling rates (rate x time, inter., $F(3,48) = 4.92$, $P < 0.01$), (*difference significant at the level of $P < 0.05$ in relation to 45 circ/min rate)

(T/C) calculated basing on the testosterone and cortisol concentration displayed inconstancy in time and according to the work rhythm. The significant T/C growth ($P < 0.05$) after work and in the 30th min of the restitution was observed during the effort with pedalling rate of 45 circ/min (in rest: 3.48 1.134 and after the test respectively: 4.65 1.715 and 4.66 1.742). During the effort with 80 circ/min the T/C index grew initially (after 3rd effort) and next significantly lowered itself ($P < 0.05$): in rest – 4.00 1.551; after 3rd effort – 4.80 1.443; after 5th effort – 3.70 \pm 1.436 and in 30th min of the passive restitution – 2.99 \pm 1.475 ($P < 0.05$); (Fig. 3).

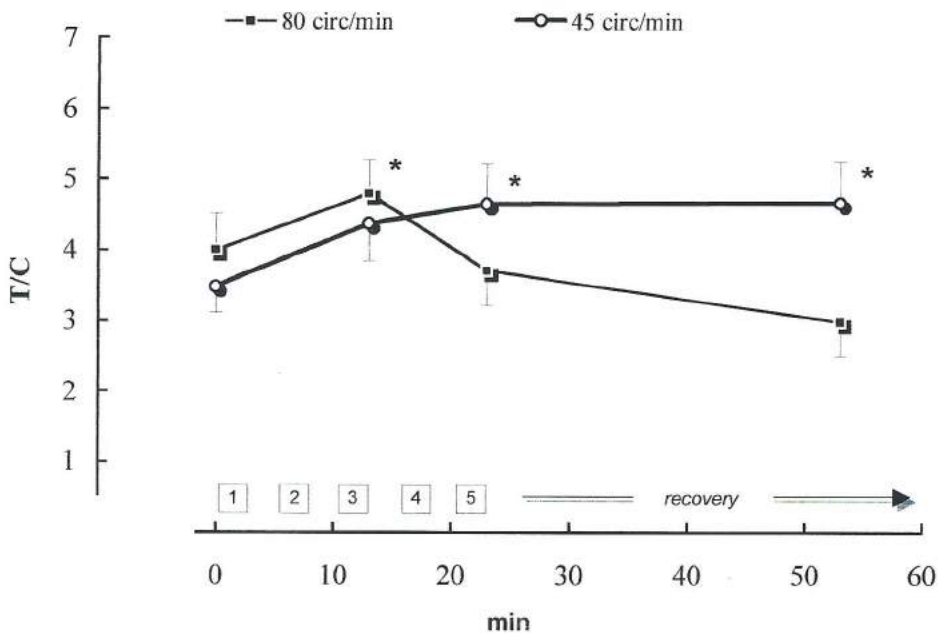


Fig. 3

Changes of the T/C index (average values \pm SE) during the repeated and intermittent efforts ($5 \times 3 \times 250$ W) performed on the cycle ergometer with 45 and 80 circ/min pedalling rates (rate \times time, inter., $F(3.48)=5.34$; $P<0.003$), (*differences significant at the level of $P<0.05$ in relation to 30th min of the rest in series with 45 circ/min)

As regards GH the significant changes between W_{45} and W_{80} were stated immediately after the effort and in the 30th min of the restitution. The field (AUCs) calculated under the curve of GH changes in time demonstrated significant differences between both tests: 221 ± 188.41 and 550 ± 261.70 ng \cdot min \cdot ml $^{-1}$ ($P<0.01$). Significant differences in the testosterone concentrations between efforts performed with speeds of 45 and 80 circ/min were not stated. As considering cortisol and the T/C index the significant differences between equipollent efforts occurred not before 30 min after the test.

Calculated correlation coefficients showed negative co-dependence between the GH peak and T/C index ($r = -0.819$; $P<0.001$) and between the lowest pH concentration in the arterialized blood and GH ($r = -0.569$; $P<0.01$; Fig. 4). The

correlation coefficient equalled: $r = -0.774$ ($P < 0.01$) in the case of the dependence between the blood pH and the highest cortisol concentration. Correlations between the C and GH peak ($r = 0.593$; $P < 0.05$) and between C and T/C ($r = -0.835$; $P < 0.01$) were significant as well. The smaller concentration of GH and C, smaller blood pH decrease and higher T/C value accompanied the higher physical capacity of examined. The simple correlation coefficient between $VO_2\max$ and the GH peak equalled: $r = -0.753$ ($P < 0.05$); C $r = -0.734$ ($P < 0.05$); pH, $r = 0.594$; T/C, $r = 0.844$ ($P < 0.05$).

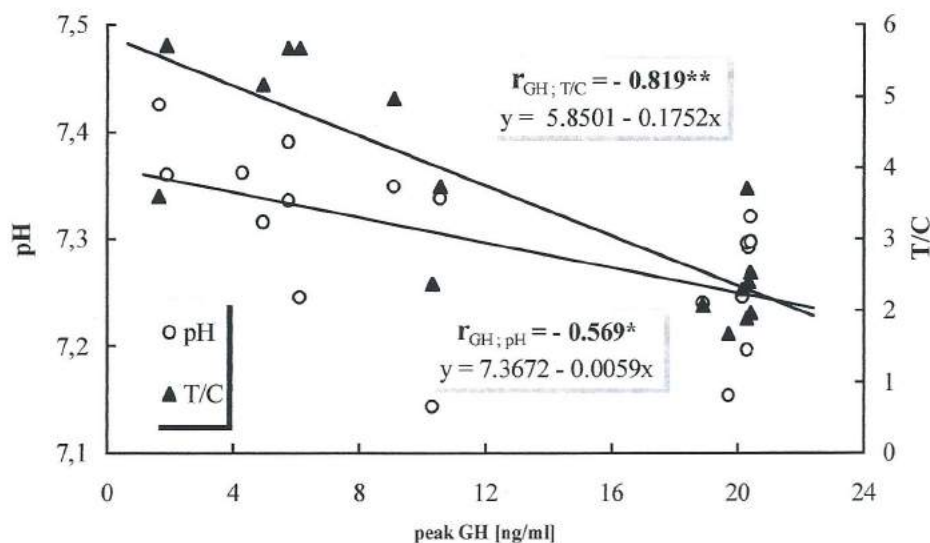


Fig. 4

Relations between the growth hormone concentration (GH peak) and the after effort arterialized blood pH and the anabolic – catabolic index (T/C) for $n=18$, in both trials (* $P < 0.01$; ** $P < 0.001$)

Discussion

Many authors [2,3,6,9,12,17,21,26] claim that the intensity and time of the effort have the vital significance in the after effort hormonal response framing. Presented above observations show that the set of intermittent efforts is the strong stimulus of the growth hormone, cortisol and testosterone secretion. Moreover, the differentiation of the pedalling rate during exercises on the cycle ergometer but

with the same amount of work and duration of effort caused various response of the hormonal system. The GH and cortisol concentration was significantly higher ($P<0.05$) during the higher pedalling rate exercise (80 circ/min) than during effort with lower pedalling rate (45 circ/min). The change of the work rate did not effect in significant differences as considering the testosterone concentration in blood. The after effort analysis of cortisol concentration in blood, conducted by various authors [3,9], points out that the increase of this hormone concentration occurs while efforts with high intensity and high energetic expense. The higher pedalling rate caused significant increase of the cortisol concentration with similar testosterone concentration in both trials. Those results negate surmises that the cortisol concentration increase sets back the testosterone synthesis in Leidig's cells through the suppression of the luteinizing hormone [7].

Changes of the blood pH were used in the analysis of the acid – alkali blood parameters in this work. The effort, in both cases, caused changes of the blood acid – alkali balance pointing at important participation of the anaerobic – lactic metabolism in the energetic security of the effort series: 5 x 3 min. Gained results are coherent with results of Buśko *et al.* [5] which state that changes of the acid – alkali balance are growing along with the speed during singular, equipollent sub-maximal efforts performed with different pedalling rates. The relation between the intensifying metabolic acidosis and GH secretion was displayed in the research of Gordon *et al.* [10], Chwalbińska-Moneta *et al.* [6] and Opaszowski [18]. It presents significant dependence between pH decrease (H^+ concentration growth) and GH concentration in blood. This relation was significant in this research and formulated the correlation coefficient $r = -0.569$ ($P < 0.01$). Differences between the blood pH decrease while efforts with higher and lower pedalling rate were statistically insignificant. The GH response differentiation in both series was caused by the pedalling rate change. This result shows that the stimulation of GH production through H^+ concentration growth is not the only element stimulating its secretion. In research conducted by Sutton [22] the significant growth of cortisol and GH concentration occurred in examined subjects with low VO_2max (2.3 l/min) after the sub-maximal effort (750 kpm/min) lasting for 20 min. In subjects with $VO_2max = 4.55$ l/min the GH concentration did not change and the cortisol level decreased. Differences between examined subjects maintained in the 50th min of rest. Other research data [9,17,18] show that in sub-maximal efforts during which the anaerobic glycolytic – lactic metabolism growth is not observed the GH concentration increase depends on the growth of the internal body temperature. Hartley *et al.* [11] state that high levels of cortisol during intensive efforts may slow the excessive GH secretion. Bloom *et al.* [3] stated that high concentration of

GH accompanied the high level of the after effort cortisolemia. In this work, the high concentration of cortisol was accompanied by higher GH concentration in the W_{80} test. Our research show that the metabolic changes in muscles and changes in blood may determine the growth hormone concentration in blood. The size of the stimulation flowing from the *motor centres of the cerebral cortex* (via under-protuberance – hypophysis) or the afferent impulsion running through the sympathetic fibre from “the metabolic receptors” of the fast twitching (FT) fibres involved in notable degree in work performed with high rate may decide about the GH secretion during the physical effort with high pedalling rate (sprint like) [9,18,20]. Stokes *et al.* [20] state that the ammonium (growth of the NH_4 concentration), accompanying the sprint like effort and modulating work of the neurotransmitters OUN such as glutamine and GABA which have the control impact on the proprioception mechanisms taking part in regulation and control of the GH production by the pituitary body during the sprint like effort, may have the influence on the GH response on different pedalling rate. The stronger production of ammonia especially in FT fibres accompanies the short, intensive efforts. Particularly high changes of the NH_4 concentration occur in people with high content of the fast twitching fibres (FT) in muscles. The ammonia concentration in blood is tightly correlated with the concentration of lactate and the de-amination of AMP in muscle cells is the cause of its increase [9,18,20]. Changes of the cortisol concentration in blood confirm this hypothesis. Its concentration during effort performed with 45 circ/min was close to the rest value (before test) and had the tendency to decrease in the 30th restitution minute. The effort load 5 x 3 min realized in 45 circ/min rate did not influence the cortisol production despite of performing effort at average level of 88% VO_{2max} . However, the total test time (work + intervals = 23 min) could be too short for the intensified secretion of cortisol. Introduction of the higher rate (80 circ/min) elicited significant changes in cortisol concentration. In the light of this data it may be stated that the growth of the effort intensity is the factor reinforcing the stress reaction on the same kind of the physical work. Cortisol intensifies the gluconeogenesis thereby prevents or slows the hypoglycemia. It has the physiological meaning in this way. The effort performed with the higher pedalling rate activated in higher degree the *under-protuberance – hypophysis – adrenal gland pivot* what effected in bigger changes of the cortisol concentration in time in relation to the test with smaller work rate.

Many authors' data [1,7,9,12] confirm that the short physiological effort elevates the testosterone concentration in blood. The results were convergent in our work. The higher concentration of testosterone was observed after the end of both series (5 x 3 min). Differences between concentrations of this hormone were not

stated in both series. The testosterone works among others in an anabolic way what effects the muscles mass growth. It is also a counter-regulator to the active muscles tissue in relation to cortisol which has the catabolic feature (influences the muscle protein degradation). Relations of those 2 hormones decide about the metabolic balance. Hence, they become the basis for the calculation of the anabolic – catabolic index which is used in sport for the evaluation of biological effects of sport training [1]. The balance between anabolic and catabolic processes seems to be the basis of the “optimal” sport training. The T/C index decrease below 3.5 may point at the catabolic processes enforcement (to high training loads) and at the lack of full after effort restitution [1,8]. In our research the sprint like effort (80 circ/min) caused significant ($P<0.05$) decrease of this index. It is still seen in the 30th min of the passive rest after the series of 5 intermittent efforts unlike during the 45 circ/min pedalling rate where the significant increase of this index was observed. Hence, the same mechanical, external work performed with different rates points at the distinct biological result of exercises done in the same time unit. The effort performed with smaller rate intensified the anabolic effects (testosterone concentration growth alongside the lack of cortisol changes) and the effort with bigger rate strengthened catabolic effects (the cortisolemia enforcement in relation to the testosterone concentration changes).

The results gained from the above research present a *novum* against the background of the references data. Interesting co-dependence raised (Fig. 4) between the T/C index and the GH concentration. The correlation coefficient for dependence between the T/C and GH peak equalled $r = -0.819$; $P < 0.001$. It enhances the enforcement of production and circulation of GH in organism in the situation of significant anabolic – catabolic balance transgression caused by physical effort/training. The GH with its lipolytic work keeps among others the amount of glucose in blood through the restriction of its transport to muscles during the effort. Hence, the strong metabolic signal from working muscles may be one of the main factors intensifying the GH strain secretion. The enlargement of the work load resulting from the higher activation of FT muscle fibres through the quicker pedalling rate confirms the hypothesis of the increase of GH secretion during the same work loads.

In the conclusion it may be stated that the rate/speed of performed work influences the hormonal response through the increase of cortisol and growth hormone concentrations alongside the similar level of the acid – alkali blood balance disturbances during work with higher pedalling rate. The smaller hormonal response on standard loads (constant power and time of the effort) is conditioned by the high physical capacity. Smaller disturbances of the acid – base blood

balance correspond with the smaller response of stress hormones which in our research were: the growth hormone and cortisol. The direction and size of hormonal changes may present the criterion of better adaptation to sport effort/training and evaluation of the growth of so called "sport condition".

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