



MUSCLE SUPPORT:

GRAMINEX Flower Pollen Extract

Metabolic Adaptation of Muscles to Exercise, Vibration and Raised Temperature under the Influence of Cernitin™™

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Sawicka, T. J., Laszczyca, P., Smylla, B. and Jethon, Z.: Metabolic adaptation of muscles to exercise, vibration and raised temperature under the influence of Cernitin™. *Acta physiol. pol.*, 1984, 35(2), 141-150. Wistar rats were used to study the effects of Cernitin™, i.e. aqueous and oil extracts of pollens, on the metabolic adaptation of the soleus muscle to exercise, vibration and raised ambient temperature. The animals were exposed to selected combinations of these factors for 5 days during 1.5 hour daily. A part of the animals was given orally Cernitin™ in daily doses of 6 mg/mg of body weight for 10 days before the exposure. Among the adaptation changes studied in the soleus muscle, 24 hours after the last exposure, Cernitin™ caused: 1) a reduction of the amount of total protein, 2) an increase in the proportion of soluble proteins in the protein fraction, 3) an increase in the tissue oxygen consumption, 4) an increase of already elevated pyruvate kinase activity, 5) a further rise in ATP level, 6) an increase in lactic dehydrogenase activity, 7) a rise in the activity of cholinesterases. Moreover, it increased significantly the body weight and the weight of the studied soleus muscle. Cernitin™, in combination with certain types of exposure used in this experiment, exerted a catabolic action, increased the rate of anaerobic metabolism and enhanced adaptation to exercise, vibration and temperature. The direction of the adaptation changes depended on the type of exposure to environmental factors.

The adaptation of human organism to the living and working environment becomes often difficult, due to the influence of new noxious environmental factors. This is true particularly of extreme conditions, which exist in certain types of work places. The means used for facilitating the adaptation of man – operator are not always sufficient for reducing the intensity of the harmful effects of the environment, nevertheless they enhance the ability of carrying out work.

One of the factors enhancing adaptation processes are Cernitin™, components of extracts obtained from pollens of flowers. Their favourable effect has been demonstrated on the rate of weight gain [23], course of adaptation to increased ambient temperature [5], resistance to ambient temperature antagonizes in this case the effects of vibration [9, 22].

infections [25], and alleviation of the intensity of inflammatory processes [20, 25]. Moreover Cernitin™ have been shown to exert a favourable effect on the capability of performing exercise [1, 11] and the magnitude of training effect [1]. Cernitin™ influence also favourably the well-being and the ability to carry out intellectual and operational work [5].

Exposure to a combination of high ambient temperature and vibration is not infrequent during occupational work. Physical effort of the worker may increase the harmful effects of these factors leading to a rapid decrease of his ability to work. A synergistic action of vibration and physical activity leading to development of vibration-induced lesions is known [9]. Raised

The aim of the present study was to investigate the possibility of using Cernitin™ as an agent

increasing the adaptation effect, that is maintenance of the ability to work under conditions of high ambient temperature and exposure to vibration. The studied problems were limited to the metabolic effects of Cernitin™ in the light of the data known as yet on the mechanism of action of these substances, especially on protein and steroid metabolism [2, 25].

The effects of Cernitin™ were investigated on certain aspects of the exercise metabolism in rats subjected to swimming exercise, sinusoidal vibration and raised ambient temperature.

Materials and Methods

The experiments were carried out on 48 male Wistar rats weighing 190-240 g. The animals were divided into 8 groups in the following way:

Cernilton® was given for 10 days (the rats in groups KC, PC, PWC, PWTC) orally in daily doses of 2 ml. This dose was equivalent to 6

- K — control, sedentary group,
- KC — sedentary group receiving Cernilton®,
- P — group subjected to swimming until exhaustion,
- PC — group subjected to swimming and receiving Cernilton®,
- PW — group exposed to swimming effort and sinusoidal vibration,
- PWC — group treated as PW and receiving Cernilton®,
- PWT — group exposed to swimming effort, sinusoidal vibration and high ambient temperature of 37°C during exposure to vibration,
- PWTC — group exposed as the PWT group after pretreatment with Cernilton®.

mg/kg of body weight of Cernitin™ T60 (the water-soluble fraction) and 0.3 mg/kg of body weight of Cernitin™ GBX (the lipid-soluble fraction). In accordance with the declaration of the producer this dose corresponded after calculation for 1 kg of body weight daily to: 0.36-0.55 mg of free amino acids, about 0.2 mg of a

mixture of various lipids, among them sterols 0.030 to 0.048 mg. The amount of vitamins received by the animals with Cernilton® was of the order of 10⁸ ng for thiamin, riboflavin, pyridoxamine, pantothenic acid, folic acid and inositol, 10⁸ ng for niacin, 10⁴ ng for ascorbic acid, 10⁰ ng for tocopherol, 10⁰ mIU for calciferol. Besides that, this dose contained carotens, 10¹ ng, xantophils 10⁸ ng, and mineral components such as Ca, K, P, Cl, Na, Mg, Al, Fe, Si, Zn, Mn, Cu in amounts found in vegetable tissues ranging from 50 ng to 60 ug. The preparation used was Cernilton® produced by AB Cernelle, Vegeholm 6250, S 2620 Engelholm, Sweden. The rats in groups K, P, PW, PWT received during the same time 2 ml of a 0.9% NaCl solution orally, as placebo.

On the first day after completion of Cernilton® or placebo administration the animals were subjected to swimming effort, vibration and raised ambient temperature for 5 days.

The animals swam without load until exhaustion in water at 32°C, in tanks making impossible passive floating on water surface. Sinusoidal vibration was applied during 1.5 hour daily using a vibration table steered by a vibration generator, at a mean acceleration of 1.11 m/sec² and 4 Hz frequency [8].

During the exposure to vibration the animals in groups PW and PWC remained in an ambient temperature of 19-20°C, and the animals in groups PWT and PWTC were exposed to a temperature of 37°C. Twenty-four hours after the last exposure the animals were killed by decapitation. The soleus muscle was taken from the hindpaws. Muscle fragments were homogenized at 0°C in a proportion of 40 mg of muscle tissue for 1 ml of 0.9% NaCl solution. The following determinations were carried out in the homogenate:

- 1) concentrations of total protein and protein soluble in isotonic saline by the method of Lowry [21],
- 2) ATP content in the muscle using Eskalab test kit, (in HClO₄ homogenate),

3) pyruvate kinase (PK) activity using Eskalab test kit,

4) lactic dehydrogenase activity (LDH) using Eskalab LDH-UV kit,

5) muscle acetylcholinesterase activity (AChE) by Hestrin's method using acetylcholine as substrate [10].

A part of the obtained muscle mass was homogenized in Tyrode's solution until a final proportion of 100 mg of tissue per 1 ml of the solution for determining the intensity of tissue respiration by Warburg's method.

The results were subjected to statistical analysis by the generally accepted methods. The calculated results included the arithmetical mean and standard deviation. The differences were accepted as significant at $p < 0.05$.

Results

The rate of weight gain in the rats not receiving Cernilton® was higher than in the controls (group K) during exercise and exercise combined with vibration (groups P and PW) by 19.2% and 9.3% respectively, while combined exposure to exercise, vibration and high ambient temperature decreased the rate of weight gain by 14.2%. The ratio of the soleus muscle weight to the total body weight decreased significantly as compared with controls: by 9.2% in the group subjected to exercise (P) and by 4.2% in the group subjected to exercise and vibration (PW). The effect of combined exercise, vibration and high ambient temperature appeared as a decrease of the content of total protein in the soleus muscle which was significant only in group P decreasing by about 17% in relation to controls. The concentration of soluble protein in the soleus muscle decreased significantly in all groups in relation to controls: P – 17.9%, PW – 25.0%, PWT – 23.1%. The ratio of the content of soluble protein to that of total protein decreased significantly by 17.5% only in group PW.

Table 1. Metabolic adaptation of soleus muscle to exercise, vibration and high ambient temperature after treatment with Cernitin™.

		K	KC	P	PC	PW	PWC	PWT	PWTC
AM	g/24h	1.40	2.36	1.67	1.68	1.53	1.52	1.20	1.80
M _m	mg/g b.m.	0.418 0.024	0.375 0.017	0.380 0.021	0.397 0.007	0.400 0.012	0.393 0.025	0.441 0.019	0.401 0.027
PrT	mg/g t.	101.0 11.7	73.6 14.6	83.6 10.5	72.3 14.2	91.9 15.4	56.8 4.1	79.3 34.3	69.0 12.0
PrS	mg/g t.	15.6 2.4	15.2 1.4	12.8 0.9	14.3 2.2	11.7 2.0	13.8 2.2	12.0 1.7	24.2 4.8
VO ₂	Nmol O ₂	19.7	34.2	40.2	33.2	25.6	64.1	54.6	41.8
	min mg prot.	5.9	12.3	9.3	20.9	10.1	12.1	25.5	15.4
ATP	mg/g prot.	16.8 3.3	39.4 7.2	17.3 4.3	22.7 14.6	45.9 30.6	47.4 6.9	32.7 25.4	35.2 10.4
LDH	IU/g prot.	438 78	777 125	444 49	743 156	437 74	1346 146	496 235	1005 162
PK	IU/ g Prot.	6.6 0.6	13.8 0.8	16.8 0.4	14.2 0.6	9.6 0.4	20.7 0.3	10.1 0.5	15.7 0.5
ChE	IU/ mg prot.	21.53 1.69	23.54 2.24	21.03 2.06	23.52 1.99	20.84 2.44	25.03 1.42	19.65 1.90	24.93 2.65

Designations: K – control group, P – swimming, T – high temperature, C – Cernitin™ administration, AM – weight gain, m_m – proportion of muscle mass to body mass, PrT and PrS – total and soluble protein levels in the muscle, ATP – ATP level, LDH, PK, ChE – activity of: lactic dehydrogenase, pyruvate kinase and cholinesterase

The activity of tissue metabolism expressed as oxygen consumption by tissue homogenate showed an increasing tendency during the exposure to these external factors by 104.5% in group P and 179.0% in group PWT ($p < 0.01$). The increase of kinase activity in the studied muscle was significant in all groups in relation to controls and it was 154.5% in group P, 45.5% in group PW and 53.1% in group PWT. Attention is called to the agreement between the directions of changes in tissue respiratory activity and pyruvate kinase activity. The ATP content of the muscle calculated for one unit of total protein was 173% above the control level in group PW ($p < 0.05$). In the remaining groups the rise was statistically not significant. The activity of LDH and ChE showed no significant changes in the groups of rats not subjected to treatment with Cernitin™, independently of the action of other factors.

A particularly evident effect of Cernitin™ on the weight gain rate was observed in the group of sedentary rats (KC) as compared with group K (a 68.2% increase) and in group PWTC as compared with PWT group (a 50.0% increase). In the remaining groups changes in relation to the control group and between the corresponding groups were below 20%. The ratio of the soleus muscle weight to the total body weight was not significantly changed. The only exceptions were: a decrease of this index by 9.4% in group KC as compared with group K, and a decrease by 9.1% in group PWTC as compared with PWT.

The concentration of total protein in the soleus muscle was decreased by Cernitin™ in all groups amounting in the case of group pairs to the following values: KC/K – 27.2%, PC/P – 13.5%, PWC/PW – 38.2% and PWTC/PWT – 13%.

A reverse relationship is observed in the concentration of soluble protein since Cernitin™ raised this concentration. However, only a rise by 100% between PWTC/PWT groups was significant. This increased the proportion of enzymatic soluble proteins in the total protein

pool by 34.3% for KC/K, 29.4% for PC/P, 91% for PWC/PW and 131.0% for PWTC/PWT.

The oxygen consumption by the homogenates of muscles from the animals treated with Cernitin™ was always higher than in controls independently of the exposure, and in the rats in groups KC and PWC Cernitin™ caused a significant increase in tissue respiration by 73.7% for KC/K and 150.0% for PWC/PW. The remaining changes were not significant.

Significant differences in pyruvate kinase activity were observed only between groups KC and K, and PWC and PW. In both these cases this increase was 115.5% and 115.4% respectively.

The ATP content showed an increasing tendency during treatment with Cernitin™, but this increase was significant only for KC/K, being 111.0%.

LDH activity increased in all groups treated with Cernitin™: 77.5% for KC/K, 67.3% for PC/P, 207.2% for PWC/PW, 102.5% for PWTC/PWT.

ChE activity increased significantly in all groups treated with Cernitin™ with the exception of the sedentary group: 9.2% for PC/P, 11.8% for PWC/PW, and 27.0% for PWTC/PWT.

Discussion

Physical exercise, vibration and high ambient temperature produced disturbances in the protein metabolism in the soleus muscle manifesting themselves as a decrease in the amounts of total and soluble proteins and of the ratio of the soleus weight to the total body weight. These changes were associated with increased intensity of tissue respiration and pyruvate kinase activity. The rate of weight gain decreased or increased in different groups without an unequivocal correlation with the metabolism of the studied muscle. This absence of correlation might be explained as due to brief exposure time or to considerable metabolic, functional differences between various muscles and tissues. The muscle fibres of the ST type prevailing in the soleus respond differently than

the FT muscle fibres prevailing in other muscles. Moreover, these responses are specific with respect to the stimulus and hormonal regulation [23, 29]. Metabolic changes observed by other authors induced by exercise [15, 26, 30], raised ambient temperature [28] and vibration [9, 22] were similar to those observed by us. The character of these changes resembled the metabolic changes observed during stress reaction. Less data are found on the combined effect of several stress-inducing factors on the organism. The effect of exercise combined with vibration and the effect of vibration and high temperature studied by us suggested that under these conditions the reaction of the organism was changed. The most characteristic finding was the change in the equilibrium of the anabolic and catabolic processes. The change was manifested as a decrease in protein concentration and an increase of tissue respiration, as well as an increase in ATP level, which was particularly evident after exposure to vibration (PW group). Raised ambient temperature seemed to exert a protective effect on the equilibrium between catabolism and anabolism. The damaging effect of low ambient temperature on humans exposed to vibration has been reported in the literature [9, 22].

The observed absence of changes in LDH activity after the exposures used in this experiment indicates that their intensity was too small to cause mobilization of anaerobic metabolism [31, 34].

In our experiment Cernitin™ were given before exposing the rats to exercise, vibration and high ambient temperature. Thus the effects observed after Cernitin™ administration were due either to metabolic changes caused by them prior to exposure or to the action of tissue deposits of Cernitin™ or their derivatives mobilized by exposure to stress-inducing factors. The rise in the requirements for amino acids, vitamins, steroids, trace elements, and energy in animals subjected to stress-inducing exposure is known [12, 13, 14, 18, 32]. The five-day exposure to stress-inducing factors in this experiment failed probably to exhaust the stores of these

substances in the organism, since the work of Karvonen [14] shows that they can cover much longer time periods.

Administration of Cernitin™ to rats caused in all groups an increase in the proportion of the soluble protein fraction and in the activity of catabolic enzymes (PK, LDH, ChE) in the soleus muscle. Cernitin™ potentated also tissue respiration during exercise, vibration and high ambient temperature. Similarly also, a further rise of ATP was observed in the muscle. The increase of the catabolic activity induced with administration of Cernitin™ manifesting itself as a better utilization of the energy of food components [1, 6], enhanced training effect and effort tolerance has been already described in man and rats [1, 25]. Cernitin™ raise also intestinal absorption of food components [33] and in this field their action is contrary to that of vibration which decreases absorption [27]. Increased LDH activity and reduction of post-exercise blood lactate concentration following intake of Cernitin™ have been described by Jethon [13] and Dabrowski [5]. This effect was observed also in our experiment.

The effect of Cernitin™ on protein metabolism in the soleus muscle manifested itself as an increase in the proportion of the soluble fraction of cell proteins with a decrease in the total protein concentration. The suggested intensification of protein catabolism at the expense of protein anabolism, and intensification by Cernitin™ of changes induced by stressors. These results disagree with those obtained by other authors who found decreased catabolism of amino acids and proteins in humans receiving Cernitin™, with decreased loss of nitrogen in the form of urea [11, 13]. The above discussed differences in the metabolic characteristics of muscles [23, 29] and acceleration, or at least stabilization, by Cernitin™ of the weight gain of rats suggest that in muscles belonging to other metabolic type than the soleus muscle, or in other tissues Cernitin™ stimulated the anabolic processes. This supposition is supported by observations of other authors that Cernitin™ enhanced protein

synthesis during healing of wounds or fractures [20, 25] and increased the rate of weight gain [6, 25, 29].

Increased activity of muscle cholinesterases observed after administration of Cernitin™ may be due to the action of these substances on the nervous system. Cernitin™ are known to increase the psychotechnical performance and intellectual ability as well as the sense of well-being [5, 7, 20, 25]. The possibility of intensification of the tropic action of the nerves on the muscles is not ruled out and the activity of acetylcholinesterase (AChE) depends on this action [3, 4, 35]. Another possibility is increased production or change of the turnover half time of enzymatic proteins similar to the hepatic fraction of secretory enzymes, such as pseudocholinesterase. This supposition is confirmed by the parallelism between increasing ChE activity and the proportion of soluble protein fraction.

The mechanism of the action of Cernitin™ on the effects of the tested exposures depends, probably on a synergistic action of the various components of this mixture of substances [2]. This mechanism may be connected with increased availability (through better absorption) of vitamins as precursors of coenzymes and trace elements as activators. Among the literature reports attention is called to the fact that Cernitin™ raise the amount of 17-ketosteroids and 17-hydroxysteroids excreted with urine [7, 25] and cause vacuolization of the fascicular zone of the adrenal cortex [25], without concomitant signs of adrenal cortical hypertrophy [2, 6]. The possibility cannot be ruled out that 27-29-carbon steroids of Cernitin™ undergo metabolic changes in the organism. The possibility of increased production of 21-carbon corticosteroids is suggested by the investigations described by Oudot [25], and mobilization of the production of 18 and 19-carbox sex steroids is suggested by the results of the investigations of Diczfalusy [6]. Chemical analysis carried out by Kvanta [17, 19] showed a similarity between Cernitin™ steroids and certain oestrogens. Thus the changes

observed in our experiment may be due to “facilitation” of the hormonal regulation considering the interaction between steroids, and differences in the sensitivity of the metabolism of various types of muscles and tissues to their action [16, 23, 29].

In summary, the administration of Cernitin™ during exposure of the organism to combined exercise-vibration-thermal stress produces multi-directional changes not always favorable for such metabolic indices as: muscle mass, level of muscle protein or weight gain. Administration of Cernitin™ seems to be advantageous in individuals not exposed to physical work (KC rats) and subjected to thermal stress (PWTC rats) in view of the necessity of maintenance of homeostasis in protein metabolism and growth processes.

However, during exercise and vibration stress Cernitin™ increases the metabolic fitness (PC and PWC rats) which is unquestionably and advantageous effect enhancing adaptation to working environment.

The always-present Cernitin™ effect increasing LDH activity with increased aerobic metabolism suggests a greater anaerobic potential and tolerance.

Conclusions

1. Cernilton® shows an action stimulating the cellular metabolism and when administered to rats exposed to stress it increases the intensity of catabolic processes.
2. Significantly raised LDH activity after Cernilton® suggests intensification of aerobic metabolism, and increased potential and tolerance of anaerobic metabolism.
3. Cernilton® increases also the energy stores of the phosphagen pool of the muscles at rest during exercise stress.
4. The anabolism of proteins in muscles with prevalence of ST fibers (soleus muscle) in animals exposed to stress was increased much less than the overall catabolism of protein. Metabolic changes induced by Cernilton®, particularly those of protein

anabolism, are probably, quite different, in various tissues, and probably the ST muscles respond to this agent differently than FT muscles and various non-muscular tissues.

5. Cernilton® effect differed significantly during various types of stress to which the rats were exposed:
 - during vibration stress the drug caused mobilization of catabolism at the expense of anabolism,
 - during exercise stress it caused no drastic changes in the anabolism-catabolism equilibrium,
 - the exposure to high ambient temperature and other stress types applied in this experiment increased anabolism above the level observed at room temperature, although catabolism activation by Cernilton® was still significant.
6. Changes in the activity of enzymes and weight gain in the rats exposed to thermal stress point to a favorable effect of Cernilton® on thermal adaptation.
7. Changes in cholinesterase activity in the soleus muscle caused by Cernilton® indicate that its effects either the production of proteins similar to hepatic secretory fraction or the tropic action of motor neurons on the muscle and on the metabolism of muscle proteins.
8. In the potential mechanism of Cernilton®, besides the importance of vitamin and amino acid supplements, the effect of this drug should be analyzed on steroid metabolism and the related effects on hormonal regulation which are probably of essential importance in the development of the observed changes.

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