



MUSCLE SUPPORT:

GRAMINEX Flower Pollen Extract

Effect of Cernilton on Anaerobic Metabolism

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Introduction

There is evidence that training increases the physical capacity of the muscles by changing the biological adaptation of various types of muscle fibres (6,15,16). It appears from the investigations carried out on various groups of athletes that the effect of training can be enhanced by administration of Cernilton (3). It appears that the effect of Cernilton is increased by its administration in conjunction with hydrolyzed protein (7). So far the investigation has not differentiated between the adaptation of various types of muscle fibres. One might assume that evaluation of the effect of Cernilton on various types of muscle fibres could lead to a better understanding of the nature of its action. The following points were investigated and analyzed in this paper.

1. How Cernilton affects the utilization of energy in the muscle adapted to strength and endurance type of work.
2. How Cernilton affects the anaerobic metabolism of various types of muscle fibres.
3. Effect of Cernilton on metabolic processes in various types of muscle fibres.

The investigations were carried out on 48 male Wistar rats. Their body weight was between 150 and 200 g. The animals were divided into 4 experimental groups, each containing 12 rats:

Group I – trained animals treated as control in relation to Group II.

Similar changes were observed in the creatine level with the exception of FTb type fibres.

Group II – trained animals given Cernilton and hydrolyzed protein.

Group III – trained animals which on the day of experiment were subjected to a continuous physical effort until exhaustion.

This group represents control for Group IV.

Group IV – trained animals given Cernilton and hydrolyzed protein and subjected to continuous physical effort until exhaustion.

The physical effort of animals was produced by forced swimming in water at a temperature of 32°C, with the weight of ca. 10% of body weight attached to the tail. The training lasted 10 minutes and was conducted every day for a period of 3 weeks. Animals in the Groups II and IV were given 0.5 tablets of

Cernilton and 0.2 g hydrolyzed protein (Stark Protein) per 100 g of body weight. One tablet of Cernilton contained 60 mg of Cernilton T60 (soluble in water) and 3 mg Cernilton GBX (soluble in oils).

After 3 weeks of training and one continuous physical effort until exhaustion, the animals were killed by decapitation and muscles, containing mainly fibres type ST and gastrochemius were removed. Gastrochemius was then separated into white and red fibres obtaining thus fibres of FTb and FTa type. The procedure of separating fibres was conducted at a temperature of 0°C.

In the separated muscle fibres the following tests were carried out:

1. Determination of lactic acid by the Barker & Summerson method (1) q modified by Strom (14).
2. Determination of urea
3. Determination of creatine by Ennor and Rosenberg method (5) modified by Drabikowski (4).
4. Determination of glycogen using Doe and Dailey method (13)
5. Determination of ATP using "Eskalab" tests.
6. Determination of total protein and soluble protein using Lowry method (10).

The obtained results were statistically evaluated according to generally accepted methods.

Results

Effect of Cernilton on Metabolism of Muscle Fibres

In the group of animals given Cernilton and hydrolyzed protein the level of glycogen and lactic acid in the fibres of FTb and FTa type did not differ from that of control group I. Only in the fibres of ST type following administration of Cernilton the increase of glycogen by 25.8% and lactic acid by 18.5% ($p < 0.05$) was statistically significant. However, in all three types of muscular fibres the increase in level of ATP was found following the administration of Cernilton and hydrolyzed protein. The level of urea was significantly increased in the FTb and FTa type fibers compared with the values obtained for group I. The increase was accordingly 20.9% and 37.4% and was statistically significant ($p < 0.05$).

Total protein content was lowered significantly only in the FTb and FTa type fibres compared with group I. In the remaining type of fibres following the administration of Cernilton and hydrolyzed protein the changes were insignificant. The content of soluble protein increased significantly in the FTb and FTa by respectively 34.6% and 19.7%.

Effect of Cernilton on Biochemical Parameters in Post-Exercise Period

In animals which were given Cernilton and hydrolyzed protein and which were submitted to a simple exhaustive physical effort on the day of the experiment (Group IV), the glycogen content in all types of muscle fibres was similar as compared to group III. However, there was a significant increase in the level of lactic acid in the ST and FTa type fibers. Similar changes were found in the level of ATP. In the FTb type fibres the content of lactic acid and ATP remained the same as in the control group III.

The level of creatinine and urea increased significantly in the FTa type fibres (creatinine by 33.3% and urea by 39.0%) while remaining without change in the FTb and St type fibres. Also observed was a significant decrease in the total protein level in the ST type fibres. The decrease was 26.4% and was statistically significant at ($p < 0.05$). The fibre types FTb and FTa did not show any change in total protein level. However, there was an increase in the level of soluble protein in these fibres by 40.5% and 17.0% respectively.

Table I – Effect of Cernilton on the Level of Glycogen in Various Types of Muscular Fibres

| GLYCOGEN | | | | |
|----------|-----------|-----------|-----------|-----------|
| | I | II | III | IV |
| FTb | 8.10±0.77 | 7.80±0.82 | 7.50±0.63 | 7.20±0.43 |
| FTa | 9.50±0.92 | 9.80±0.46 | 6.10±0.72 | 5.80±0.66 |
| ST | 6.60±0.67 | 8.30±0.70 | 3.60±0.43 | 3.80±0.51 |

Group I – trained animals used as control for group II

Group II – trained animals given Cernilton and hydrolyzed protein.

Group III – trained animals, submitted to a continuous physical effort until exhaustion on the day of experiment. This group was used as a control group for group IV

Group IV – trained animals given Cernilton and hydrolyzed protein and on the day of the experiment subjected to a continuous physical effort

Table II – Effect of Cernilton on the Level of Lactic Acid in Various Types of Muscle Fibres

| LACTIC ACID (umol/g) | | | | |
|----------------------|-----------|-----------|------------|-------------|
| | I | II | III | IV |
| FTb | 1.63±0.62 | 1.80±0.38 | 10.90±2.43 | 12.30±2.90 |
| FTa | 2.10±0.80 | 2.30±0.51 | 21.40±3.81 | 28.40±4.19 |
| ST | 3.24±0.75 | 3.80±0.66 | 18.92±3.13 | 23.10±03.45 |

*Headings as in table I

Table III – Effect of Cernilton on the Creatine Level in Various Types of Muscle Fibres

| CREATINE mg/g | | | | |
|---------------|-----------|-----------|-----------|-----------|
| | I | II | III | IV |
| FTb | 4.80±0.89 | 5.20±0.67 | 6.80±1.24 | 7.50±1.01 |
| FTa | 3.50±0.47 | 4.90±0.59 | 4.80±0.95 | 6.40±0.89 |
| ST | 2.10±0.38 | 3.00±0.45 | 3.30±0.63 | 3.80±0.57 |

*Headings as in table I

Table IV – Effect of Cernilton on the ATP level in Various Types of Muscle Fibres

| ATP mg/g | | | | |
|----------|-----------|-----------|-----------|-----------|
| | I | II | III | IV |
| FTb | 4.10±0.45 | 4.80±0.57 | 4.40±0.92 | 4.70±0.88 |
| FTa | 3.50±0.62 | 4.60±1.08 | 3.60±0.85 | 4.50±0.90 |
| ST | 1.40±0.27 | 2.90±0.65 | 1.20±0.35 | 3.10±0.76 |

*Headings as in Table I

Table V – Effect of Cernilton on Urea Level in Various Types of Muscle Fibres

| UREA (umol/g) | | | | |
|---------------|------------|------------|------------|------------|
| | I | II | III | IV |
| FTb | 32.10±3.84 | 38.80±3.14 | 48.60±5.22 | 52.30±8.62 |
| FTa | 21.40±3.03 | 29.40±3.62 | 24.10±4.12 | 33.50±4.24 |
| ST | 15.80±2.94 | 17.20±2.11 | 19.10±3.80 | 20.70±2.28 |

*Headings as in Table I

Table VI – Effect of Cernilton on the level of Total Protein and Soluble Protein in Various Types of Muscle Fibre

| | Total Protein (mg/g) | | | | Soluble Protein (mg/g) | | | |
|-----|----------------------|-------------|--------------|--------------|------------------------|------------|------------|------------|
| | I | II | III | IV | I | II | III | IV |
| FTb | 138.20±11.80 | 146.50±8.60 | 141.50±9.80 | 148.30±11.80 | 35.30±2.57 | 47.50±1.95 | 34.50±2.82 | 47.90±4.07 |
| FTa | 133.60±10.60 | 140.90±9.70 | 138.00±12.20 | 142.70±13.20 | 38.60±1.82 | 46.20±1.68 | 38.20±2.70 | 44.70±3.85 |
| ST | 108.00±11.70 | 96.00±14.20 | 112.30±15.50 | 82.70±12.80 | 17.40±2.80 | 18.50±2.80 | 18.20±2.46 | 17.90±2.38 |

*Headings as in Table I

Discussion

Analyzing the effect of Cernilton (administered jointly with hydrolyzed protein) on the level of glycogen and lactic acid in various types of muscle fibres, it was evident that there was no change in these parameters in the FTb and FTa types. However, in the case of ST type fibres a significant increase in glycogen was found. Some authors express the view that the main sources of energy in “slow twitch” muscle are fatty acids. Glycogen is recognised as an “energy reserve” which is used in situations when a progressive state of anaerobic conditions occurs or sudden overload takes place (12). The obtained results might also indicate an additional effect of Cernilton, such as limiting the utilisation of glycogen by slow twitch muscle fibres and increased use of other sources of energy. e.g. fatty acids. The increase in the level of lactic acid in these fibres indicates increase in the rate of glycolysis that is utilisation of glucose. At the same time in the ST and FTa type fibres following the administration of Cernilton an increase in the level of creatinine was found. Such an increase was not found in FTb type fibres. Similar tendencies, i.e. increase in lactic acid level in ST and FTa type fibres and creatinine in FTa type fibres were shown in animals of Group IV as compared with Group III where animals were submitted to additional, single effort.

In the light of the results of our investigation it appears that the effect of Cernilton depends on increasing the ability of red fibres (ST and FTa) to utilize the energy produced in anaerobic conditions. A similar suggestion was put forward by Dabrowski (3) who examined the effect of Cernitins on physical capacity in humans. The mechanism of this process, however, is difficult to explain due to the lack of data on Cernilton effect on enzymes activity in particular metabolic pathways.

Also difficult to interpret at the moment is the effect of Cernilton on protein content in various types of fibres. There is some evidence that Cernitins increase the rate of protein synthesis (2). In our work similar results were obtained only in cases of soluble protein in FTb and FTa type muscle fibres. In group II and I, as well as in group IV compared to group III there was a significant decrease of total protein w in ST type fibres while no change was observed in the level of soluble protein.

Kawka-Serweciska (8) suggests that slow-twitch muscles are unable to utilise lactates for synthesis of glucose and in these types of muscles gluconeogenesis is based mainly on alanine cycle. The presence of this cycle as well as closely linked ornithine cycle protects the red ST type fibres from excessive overloading with lactic acid during the intensive glycolytic activity.

It is recognized that amino acids like isoleucine, leucine and valine act as donors of NH₂ groups used for synthesis of alanine de novo from pyruvic acid. It is therefore probable that the decrease in the level of total protein in ST type fibres is due to its degradation and consequent utilisation of resulting amino acids for the process of gluconeogenesis. This assumption is supported by data indicating that Cernitins increase the utilization of cortisone reserves from the adrenal glands (2). Cortisone among other effects is responsible for an increased rate of gluconeogenesis in the liver by increasing transport of glucogenic amino acids into this organ. It also increases the pool of proteolytic enzymes and therefore the pool of available free amino acids. However, cortisone decreases the rate of transport of glucose through the cell membrane and therefore reduces transport of glucose from blood into the muscle cells. As a result, the supply of glucose into the muscle fibres might be insufficient and the only solution to this problem could be through increasing the rate of gluconeogenesis through the alanine cycle in the muscle fibres themselves. In the "fast twitch" muscle fibres similar processes possibly based on the lactate might take place. It was shown that glucose synthesis from lactate is typical for this type of fibre (11).

In FT type fibres Cernilton also caused an increase in the urea level. This fact could be due to an increase in the rate of protein metabolism linked with increase in synthesis of soluble protein. In ST type fibres, in spite of a fall in the level of total protein, no changes in urea level were found.

Many of the above suppositions due to the lack of more detailed investigation must remain only as hypothesis. Further investigations into this subject seem to be well worth while.

Conclusions

1. Effect of Cernilton on biochemical parameters in the muscle adapted to straight effort character of work differs in various types of fibre.
2. Cernilton does not affect the creation of anaerobic components in metabolic adaptation in exercise in fibres type FTb.
3. Administration of Cernilton affects the ST type fibres and to a lesser degree FTa fibres by changing the character of metabolism in these fibres into one normally found in FTb type fibres, that is processes of anaerobic metabolism.

References

1. Barker S.B., Summerson W.H., The colorimetric determination of lactic acid in biological materials. *J.Biol.Chem.* 1941, 138, 535.
2. Dabrowski I., Effect of Cernilton and hydrolyzed protein on the ability to adapt to physical work in a sub-tropical climate, AWF Warsaw 1980.
3. Drabikowski W. Modification of the method of estimating creatine and phosphocreatine using di aceto. *Acta Biochem.* 1957, 4, 41.
4. Ennor A.H., Rosenberg H. the determination and distribution of phosphocreatine in animal tissues. *Biochem.J.* 1952, 51, 606.
5. Gollnick R.B., Armstrong R.B., Saltin S. Saubert C.W., Sembrowich W.L., Shepherol R.E. Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J.Appl.Physio.* 1973, 34, 1, 107.
6. Jethon Z. Der Einfluss von Stark Protein auf die physische und psychische Arbetiskapzitat im subtropischen Klima. Symposium – Aminosauern sportliche Hochleistung. Homburg 1978.
7. Kawka – Serwecinska E. Changes in the level of amino acid in rats undergoing physical training (swimming). *Acta Biologica.* Katwoice 1983 (in print).
8. Kokof F. Laboratory methods used in clinical practice. PZWL Warsaw 1969.
9. Lowry O.H., Rosenbrought H.J., Farr A.L. Protein measurement with Folin phenol reagent. *J.Biol.Chem.* 1951, 193, 265.
10. McLane J., Holloszy J. Glycogen synthesis from lactate in the three types of skeletal muscle. *J.Biol.Chem.* 1979, 254, 6548.
11. Moruzzi E.V., Bermiini E., Bergamini Z.G. Glycogen metabolism and the function of fast and slow muscle of the rat. *Oflugers Arch.* 1981, 391, 338.
12. Roe J.H., Dailey R.K. Determination of glycogen with anthrone reagent. *Analyt.Biochem* 1966, 15, 245.
13. Thorstensson A., Hulten B. von Dobeln W., Karlsson J. Effect of strength training on enzyme activities and fiber characteristics in human skeletal muscle. *Acta Physiol Scand.* 1976, 96, 392.
14. Strom G. the influence of anoxia on lactate utilization in man after prolonged muscular work. *Acta Physiol Scand.* 1949, 17, 440.
15. Thorstensson A., Sjodin B., Tesch P., Karlsson J. Actomyosin ATPase, *Acta Physiol Scand.* 1977, 99, 225.