Effects of Induced Fatigue on the Levels of Selected Essential Elements in the Blood and Tissues of Rats*

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ABSTRACT Objective: To identify the changes of some selected elements in blood, skeletal muscle, liver, and brain in a rat model with complex fatigue. Methods: 30 rats in the experiment were randomly divided into three groups: the control group, the food-restricted group, the complex fatigued group. Blood and tissue samples were collected at the end of the 5-day and levels of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and copper (Cu) were measured with atomic absorption spectrophotometer. Results: Compared with that in the control and food-restricted group, the concentrations of potassium in the skeletal muscle, liver, and brain ($P<0.01$), the concentration of iron in the liver ($P<005$), were increased in the complex fatigued group, and the concentration of copper in the blood ($P<0.01$, to the control, $P<0.05$, to the food-restricted) decreased in animals with complex fatigue; compared with that in the control group, the concentration of potassium in the blood was increased in the complex fatigued group ($P<0.05$), the concentrations of magnesium and zinc in the blood ($P<0.05$), the concentrations of calcium, magnesium, and zinc in the skeletal muscle ($P_{Ca}<0.05$, $P_{Mg}<0.05$, $P_{Zn}<0.01$), the concentrations of calcium, and zinc in the liver ($P_{Ca}<0.01$, $P_{Zn}<0.05$), and the concentrations of iron, magnesium, and zinc in the brain ($P_{Fe}<0.05$, $P_{Mg}<0.05$, $P_{Zn}<0.01$) were decreased in animals with complex fatigue. Conclusions: The metabolism of potassium, calcium, magnesium, iron, zinc, and copper in blood, skeletal muscle, liver, and brain changed differently in rats with complex fatigue, which indicated that these minerals may play some important role in the occurrence and relieving of fatigue.

Key words: Fatigue; Minerals; Trace elements; FAAS

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Introduction

Fatigue remains the most common symptom in modern society and it accounts for up to half of the general population affected [1]. Although many hypotheses, such as "failure", and "free radicals", have been proposed, the intrinsic mechanism has not been completely explored. There is a consensus that fatigue is a symptom in a delicate organism with many tissues and bio-chemical reactions involved. Some previous studies have demonstrated that minerals might play a essential role in the occurrence and relieving of fatigue. For example, during strenuous physical activity, the rate of energy turnover in skeletal muscles may increase 10-15-folds and as exercise augments reactive oxygen species (ROS) that can damage cells, the body contains an elaborate antioxidant defense system that depends on the endogenous production of antioxidant compounds and the dietary intake of antioxidant minerals [2,3]. D'Almeida reported that fatigue induced by sleep deprivation could be partly explained by changes of the metabolism of essential minerals [4]. The prolonged forced restriction of muscular activity has been shown to result in a significant increase of the excretion of electrolytes and trace elements [5-8]. Acute and chronic immobilization stress altered some trace element concentrations in the brain of rats [9]. These results suggest that changes of minerals induced by exercise may be associated with deterioration of performance or sensations of fatigue.

Based on the findings mentioned above, we hypothesized that the complex fatigue may be associated with changes of the levels of essential trace elements. Thus the purpose of this study thus was to examine the possible relationship between the levels of selected essential elements in various tissues and fatigue in a rat model with complex fatigue.

1 Materials and methods

1.1 Animals

7-week-old (170-200g) Sprague-Dawley male rats were supplied by the Experimental Animal Center of Fourth Military Medical University (FMMU). The animals were kept in a 12-h light/12h dark cycle (lights on at 8 h) with the room temperature of 23 ± 1℃ (relative humidity 50 ± 5%). All experiments in this study were approved by the animal ethics committee of Fourth Military Medical University and were carried out in accordance with the guidelines laid down by the U.S. National Institutes of Health re-

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garding the care and use of animals for experimental procedures.

A total of 30 rats were randomly divided into three groups: the control group, the food-restricted group, and the complex fatigued group. We first gave rats 8-day burden swimming training, making them adapt to this new swimming style. Then the animal model of complex fatigue was done as described by Masaaki Tanaka et al. in Osaka City University [10] that rats were kept in a cage filled with water to a height of 2.2 cm for 5 d. Food and water were available ad libitum for the control and fatigued group. The food-restricted group was kept in normal cages for 5 d and was restricted to a food intake of 10 g/d. Food and water for all these groups were removed 6 h before the surgical procedure to exclude the influence of food or water on tissue element levels.

1.2 Weight-loaded forced swimming test

The weight-loaded forced swimming test was used to evaluate the extent of fatigue in all experimental groups. The animals in each group were forced to swim at 8:00 am with a load of lead rings that weighed approximately 8% of their body weight and were attached to their tails roots 2 cm from the hip. The time from entry into the water until the point at which rats could not return to the water surface for 10 s after the tip of the nose submerged was measured. Then they were helped out and returned to their own cages for recovery. The swimming pool was a plastic cylinder (30-cm inside diameter, 60-cm height) filled with water to a height of 40 cm, and the temperature of the water in the pool was maintained at 30 ± 1℃ with a thermostat.

1.3 Biochemical analyses

Whole blood samples were obtained from the ventriculus dexter, and the tissues, including brain, liver, and quadriceps muscle, were removed after the rats were anaesthetized with diethyl ether in a way, which avoided any damage to the tissues. Each segment was rapidly flushed with deionized water for removing the residual blood. The tissue samples were digested by conventional wet acid digestion (CAD) method and then converted into acidic digest solutions for analysis by Hitachi Z-2000 flame atomic absorption spectrometry (FAAS). Briefly, 0.1g of samples were weighed and placed in metal-free glass tubes (washed with hydrochloric acid) added with 0.3ml perchloric acid and 0.1ml hydrogen nitrate. Then they were heated on a 130℃ oven until digestion was completed.

Concerned with the function of some elements demonstrated and the possible mechanisms of fatigue, the concentrations of potassium, calcium, magnesium, iron, zinc and copper in the whole blood, skeletal muscle, liver, and brain were measured by a FAAS and blank digestions were also carried out. Wavelengths of hollow cathode lamps were 766.5, 422.7, 285.2, 248.3, 213.9, 324.8 and 279.5 nm for potassium, calcium, magnesium, iron, zinc, and copper, respectively. For all the measurements of these elements, air-acetylene fuel mixture was used. Results were calculated as micrograms per microliter of blood (μg/μl) and micrograms per gram wet weight of tissues. (μg/g)

1.4 Statistical Evaluations

Data was analyzed by one-way classification ANOVA. Student-Newman-Keuls test was used to evaluate significant differences between groups. All results were presented as means ± SD. All P values were two-tailed, and P<0.05 was considered statiscally significant.

2 Results

2.1 The body weights of rats in each group

The average of body weights of rats in each group at the beginning and at the end of the experiment were shown in Table 1. At the beginning of the experiment, body weights of rats were not statistically significant among groups (P>0.05) (Fig.1). Compared with the control group, rats in fatigued group and food-restricted group were thinner at the end of the experiment (Fig.1). But there was no significant difference between the food-restricted and the fatigued group (P>0.05) (Fig.2).

2.2 The swimming time of rats in each group

The swimming time of rats among 3 groups was not significantly different during the adaptation stage (P>0.05) (Fig.3). During the experiment, fatigued rats showed a significant decrease in swimming time compared with rats in the control (P<001) and food-restricted (P<005) group. And the swimming time of rats in fatigued group decreased more radically (Fig. 4).

2.3 The concentration of essential elements in blood and tissues

In the blood, the concentration of potassium (approximately 1.2-fold of control level, P<0.05) in the fatigued group was significantly higher than those in the control group (Table 2). But the concentration of potassium in the food-restricted group was also lower than that in the control group (P<0.05), which indicated that changes in potassium level of blood in the fatigued group may include the effects of restriction of food intake. In contrast to the other two groups, the concentration of zinc (approximately 64% of control level, P<0.01; approximately 77% of food-restricted level, P<0.05) in the fatigued group was significantly lower. The concentrations of magnesium and copper in fatigued group were also lower than that in the control group (P<0.05). There were not any significant differences in concentrations of iron and calcium among groups.

In the skeletal muscle, the concentration of potassium (approximately 3-fold of control level, P<0.01; 3.4-fold of food-restricted level, P<0.01) in the fatigued group was significantly higher than those in the control and food-restricted group (Table 3). By contrast, the concentration of zinc (approximately 68% of control level, P<0.01; approximately 30% of food-restricted level, P<0.01) in the fatigued group was obviously lower compared with those in other two groups. Muscular concentrations of calcium, and magnesium, in fatigued group were also lower than that in the control group (P<0.05). However, the concentration of magnesium was evidently different between the control and food-restricted group (P<0.05), which indicated that changes in magnesium levels in the fatigued group include the effects of restriction of food intake. There were not any distinct differences in concentrations of iron and copper among groups.

In the liver, concentrations of potassium (approximately 3.7-fold of control level, P<0.01; 3.3-fold of food-restricted level, P<0.01), and iron (approximately 1.6-fold of control level, P<0.05; 1.6-fold of food-restricted level, P<0.05) in the fatigued group were significantly higher than those in the control and food-restricted group (Table 4). Comparatively, hepatic concentrations of calcium, and zinc in the fatigued rats were significantly lower compared with values in the control groups (PCa<0.01, PZn<0.05). There were no significant differences between the control and food-restricted groups in hepatic concentrations of these 7 elements.

In the brain, the concentration of potassium (approximately 3.6-fold of control level, P<0.01; 3.1-fold of food-restricted level, P<0.01) in the fatigued group was significantly higher than those in the control and food-restricted group (Table 5). In contrast, concentrations of zinc (approximately 21% of control level, P<0.01; approximately 31.2% of food-restricted level, P<0.01) in the fatigued group were significantly lower compared with the control and food-restricted groups. The concentration of zinc in food-restricted group was also significantly lower than that in the control group, indicating that changes in zinc levels in the fatigued group may include the effects of restriction of food intake. Brain concentrations of iron and magnesium in the fatigued rats were significantly lower than these in the control group, but not the food-restricted rats. There were no significant differences in levels of potassium, calcium, magnesium, iron, and copper, between the control group and food-restricted groups.

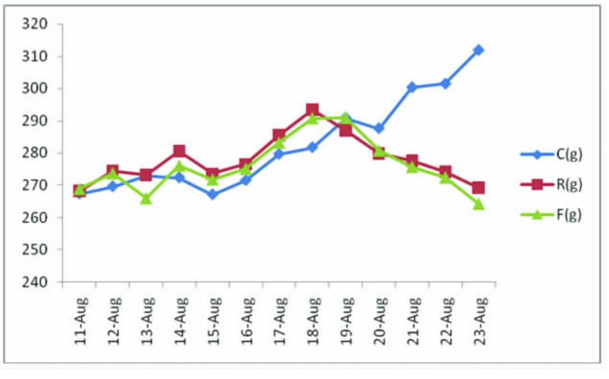

Fig. 1 The body-weight of the rats in each group during the experiment. The body-weight between groups before experiment started is not significantly different, C, control group; R, food-restricted group; F, fatigued group

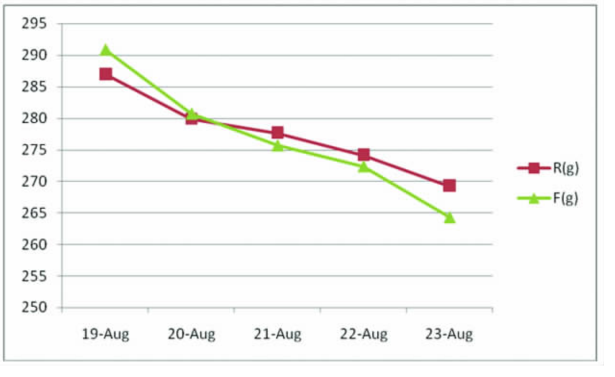

Fig. 2 The weight-loss of the rats in food-restricted and fatigued groups during the experiment. The weight-loss between the food-restrict and fatigued groups is not significantly different, R, food-restricted group; F, fatigued group

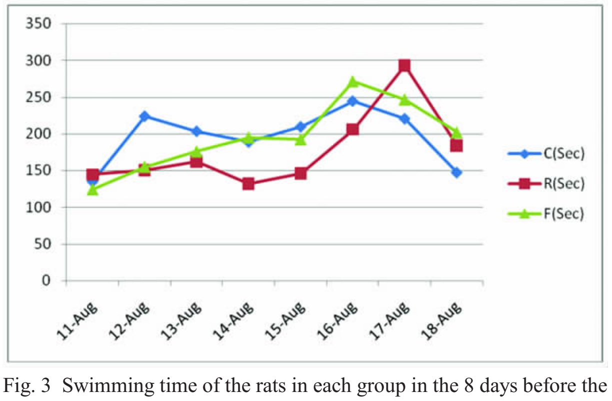

Fig. 3 Swimming time of the rats in each group in the 8 days before the experiment started. C, control group; R, food-restricted group; F, fatigued group

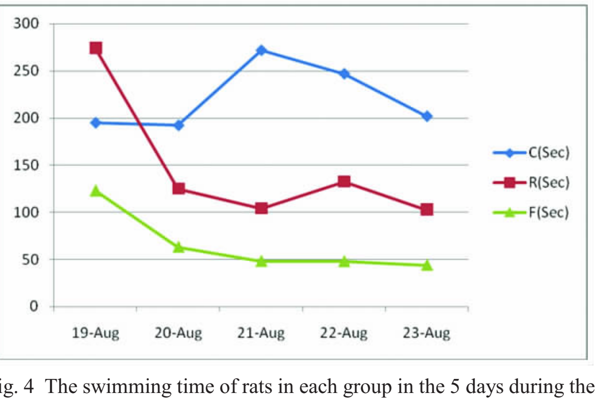

Fig. 4 The swimming time of rats in each group in the 5 days during the experiment. C, control group; R, food-restricted group; F, fatigued group

Table 1 Body weight changes (mean ± SD)

Date	Control (g)	Food-restricted (g)	Fatigued (g)
18-Aug	281.68± 18.02	293.51± 26.91	290.66± 23.61
23-Aug	311.84± 19.81	269.26± 28.65	264.27± 23.47

Table 2 Element levels in the blood of fatigued group (mean ± SD)

Elements (μg/μl)	Control	Food-restricted	Fatigued
Potassium	27.12± 2.45	32.05± 3.58$^{\#}$	32.84± 5.72$^{\#*}$
Calcium	0.59± 0.18	0.58± 0.23	0.54± 0.23
Magnesium	1.25± 0.15	1.13± 0.16	1.08± 0.11$^{\#}$
Iron	2.19± 1.46	1.89± 1.08	1.35± 0.29
Zinc	0.14± 0.06	0.12± 0.01	0.10± 0.02$^{\#}$
Copper	0.011± 0.002	0.009± 0.002	0.007± 0.003$^{\#*}$

Note: # P<0.05, significantly different from the control group, ## P<0.01, significantly different from the control group
*P<0.05, significantly different from the food restrict group, **P<0.01, significantly different from the food restrict group

Table 3 Element levels in the skeletal muscle of fatigued group (mean ± SD)

Elements (μg/g)	Control	Food-restricted	Fatigued
Potassium	564.2± 103.5	507.2± 183.6	1703.3± 238.4$^{\#\#,**}$
Calcium	153.3± 71.6	121.0± 47.9	83.7± 35.9$^{\#}$
Magnesium	395.3± 69.7	340.9± 65.4$^{\#}$	335.5± 38.5$^{\#}$
Iron	28.3± 14.7	23.3± 7.4	19.2± 4.3
Zinc	74.3± 19.7	63.5± 13.3	19.2± 10.1$^{\#\#,**}$
Copper	1.33± 0.27	1.26± 0.27	1.19± 0.38

Note: # P<0.05, significantly different from the control group, ## P<0.01, significantly different from the control group
* P<0.05, significantly different from the food restrict group, ** P<0.01, significantly different from the food restrict group

Table 4 Element levels in the liver of fatigued group (mean ± SD)

Elements (μg/g)	Control	Food-restricted	Fatigued
Potassium	461.2± 171.6	514.8± 147.6	1707.0± 325.7$^{\#\#,**}$
Calcium	158.7± 49.0	85.0± 23.1	64.9± 16.0$^{\#\#}$
Magnesium	375.6± 142.6	306.1± 48.4	302.4± 53.7
Iron	111.5± 27.3	114.5± 27.1	182.7± 64.1$^{\#,*}$
Zinc	86.6± 39.1	66.3± 11.9	47.7± 17.8$^{\#}$
Copper	3.77± 0.66	5.00± 2.16	5.13± 1.03

Note: # P<0.05, significantly different from the control group, ## P<0.01, significantly different from the control group
* P<0.05, significantly different from the food restrict group, ** P<0.01, significantly different from the food restrict group

Table 5 Element levels in the brain of fatigued group (mean ± SD)

Elements (μg/g)	Control	Food-restricted	Fatigued
Potassium	531.2± 140.9	618.8± 92.1	1922.4± 169.4$^{\#\#,**}$
Calcium	114.7± 75.6	102.2± 46.5	63.5± 33.5
Magnesium	271.7± 34.4	235.2± 53.0	195.5± 10.7$^{\#}$
Iron	40.0± 23.7	28.2± 4.4	20.2± 2.3$^{\#}$
Zinc	97.3± 34.1	65.7± 13.4$^{\#\#}$	20.5± 4.9$^{\#\#,**}$
Copper	2.26± 0.17	2.22± 0.29	2.13± 0.41

Note: # P<0.05, significantly different from the control group, ## P<0.01, significantly different from the control group
* P<0.05, significantly different from the food restrict group, ** P<0.01, significantly different from the food restrict group

3 Discussion

This study made a modified rat model with complex fatigue. In this model, rats were forced to endure a continuous physical load and psychological stress, which both made them fatigued. After 5 d of water immersion, rats showed shorter swimming times compared with that in the control or food restricted animals, which suggested that water-immersed animals were fatigued. After that, the concentration of selected minerals in complex fatigued rats was analyzed, including potassium, calcium, magnesium, iron, zinc, and copper, in the blood, skeletal muscle, liver, and brain. The changes of exercise-induced trace elements in plasma were widely investigated. However, erythrocyte plays a vital role in trace element metabolism, therefore, the blood was used instead.

Minerals are essential for metabolic and physiologic processes in the human body. It has been reviewed that minerals are involved in muscle contraction, nerve impulse conduction, oxygen transport, oxidative phosphorylation, enzyme activation, antioxidant activity, and acid base balance of the blood[11]. Most of the elements have an impact on the physiological events in the organisms of fatigue[12].

Potassium, calcium, and magnesium, were involved in the propagation of nerve impulses and in muscle contraction. It was reported that plasma potassium obviously increased and magnesium significantly decreased after a long-distance running in high heat and humidity [13]. Dressendorfer and other researchers examined the effects of 10-week intense endurance training on serum and urinary mineral levels [14]. And they found that calcium excretion might increase and serum calcium decrease below the clinical norm with high-intensity training. Some earlier studies have shown that magnesium supplementation improved strength and cardiorespiratory function in healthy persons and athletes [15, 16]. And Vedat Cinar demonstrated that training athletes while receiving magnesium supplements might cause increased magnesium loss in the body [17]. This study found that the concentration of potasium in blood, skeletal muscle, liver, and brain showed a notable increase, the concentration of calcium in the skeletal muscle, and liver, and the concentration of magnesium in the blood, skeletal muscle and brain showed a notable decrease in complex fatigued rats. As for reasons mentioned, the increase of potassium in tissues may be attributed to the decrease of urine. Several longitudinal studies on the effect of exercise training have revealed that exercise training is related to rising sweat rate for evaporative cooling during the exercise. So as body fluid was mostly excreted out of the body in the form of sweat during exercise to lower the body temperature, which make the amount of urine, the major way to excrete potassium, reduce and the concentration of potassium in body increase. Another result of the sweat increase is the decrease of calcium and magnesium. Maughan RJ indicated that strenuous exercise might promote calcium loss, especially in women [18]. And Pohl considered that sweat loss were responsible for a substantial fall of magnesium in football players after a match[19].

Iron, a component of oxygen-binding molecules (hemoglobin and myoglobin) in cytochromes and many enzyme cofactors (heme and Fe-S clusters), can accept and donate electrons, interconverting between ferric and ferrous forms [20]. The use and metabolism of iron increased with exercises[21]. Abdulkerim Kasim Baltaci et al. found an increase in iron level just after an acute exercise when compared with the control group [2]. And Abdullah Sivrikaya et al. discovered high iron leval in exercising diabetic rats [22]. According to the present study, levels of iron significantly increased in the liver and decreased in the brain ($P<0.05$). And the increase of iron in liver may be the result of the continuous contraction of muscle, which makes more myoglobin damaged, and is converted to the liver to resynthesize hemoglobin and myoglobin. The need of iron increases in tissues having a high metabolic rate during exercise, such as liver, heart, and muscle, and iron moves to these organs which contribute to the decrease of iron in brain [23]. However, the iron in the skeletal muscle did not change visibly in our study. Further study should be done on this problem.

Copper is a critical nutrient involved in many aspects of energy metabolism and an essential component for the synthesis of hemoglobin, myoglobin, cytochromes and some peptide hormones [24]. It is needed for proper utilization of Fe and protection against oxidative damage to cells [25, 26]. The previous studies on the effects of exercise on copper are totally different. Olha found a noticeable increasing in plasma copper concentrations after an exhausting cycloergometer test [27]. Bordin explored that post exercise copper concentrations were approx 50% lower than rest values [28]. And Anderson did not find any change in a similar experiment [29]. But copper's playing an important role in exercise and fatigue is widely recognized. In this study, compared with the control and food-restricted group, the level of copper in blood in the fatigued group showed an obvious decrease, but it showed a bit increase in the liver, and a decrease in the skeletal muscle and brain without significance. As reported by Vedat Cinar et al. that magnesium supplement significantly increased copper level in training subjects, and without supplement, copper might be used for the antioxidant system during exercise [17], resulting in a higher metabolic rate and redistribution of copper.

Zinc is a component of over 200 enzymes, involved in functions important to physical performance, such as muscle energy production and protein synthesis. In previous studies, it has been determined that a decrease in plasma zinc levels after exercise[30-32]. In accordance of the results, levels of zinc decreased distinctly in the blood, skeletal muscles, liver, and brain. It has been reported that intense exercise might lead to excessive body zinc loss through the excretion of the sweat and urine[33]. Cordova A and Alvarez-Mon M demonstrated that these post-exercise differences might result from the exchange of elements between the extracellular fluid and tissues [34]. And zinc supplement could elevate serum levels of potassium, magnesium, iron and copper [35], indicating that the decrease of zinc may contribute to the changes of other elements.

In conclusion, up to the functions of these minerals mentioned and many of these processes being accelerated during fatigue, we believe that a vital change in the amount and/or redistribution of minerals will inevitably play an important role in fatigue. But our research is just a preliminary work. The specific mechanism of each mineral in the occurrence and relieving of the fatigue still need some further studies.

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复合疲劳对大鼠不同组织矿物质代谢影响研究 *

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摘要 目的：研究复合疲劳大鼠血液、肌肉、肝脏和脑中矿物元素代谢变化的影响。方法：将 30 只大鼠随机分为正常对照组、食物限制组和复合疲劳组。经过 5 天的实验干预后，提取动物的血液、腓肠肌、肝脏和脑，并利用原子吸收分光光度法测量各组织中的钾(K)、钙(Ca)、镁(Mg)、铁(Fe)、锌(Zn)和铜(Cu)。结果：相对正常对照组和食物限制组，复合疲劳大鼠的肌肉、肝脏和脑中的 K ($P<0.01$)和肝脏中的 Fe($P<005$)明显升高，血液中的 Cu(与正常对照组比较 $P<0.01$,与食物限制组比较 $P<0.05$)明显下降；与对照组相比，复合疲劳大鼠的血液中的 K 明显升高($P<0.05$)，血液中的 Mg 和 Zn ($P<0.05$)，肌肉中的 Ca、Mg 和 Zn ($P_{Ca}<0.05$, $P_{Mg}<0.05$, $P_{Zn}<0.01$),肝脏中的 Ca 和 Zn($P_{Ca}<0.01$, $P_{Zn}<0.05$),以及脑中的 Fe、Mg 和 Zn($P_{Fe}<0.05$, $P_{Mg}<0.05$, $P_{Zn}<0.01$)明显降低。结论：在复合疲劳状态下，大鼠血液、肌肉、肝脏和脑中的 K、Ca、Mg、Fe、Zn 和 Cu 代谢发生变化，可能在疲劳的发生与缓解中发挥作用。

关键词：疲劳；矿物质；微量元素；原子吸收分光光度法

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