Thermogenic Responses to High-Energy Phosphate Contents and/or Hindlimb Suspension in Rats

T. Wakatsuki¹, F. Hirata*, H. Ohno†, M. Yamamoto‡, Y. Sato§, and Y. Ohira

Department of Physiology and Biomechanics and *Department of Sports Recreation, National Institute of Fitness and Sports, Kanoya, 891-23 Japan; †Department of Hygiene and ‡Department of Biochemistry II, National Defense Medical College, Tokorozawa, 359 Japan; and §Research Center of Health, Physical Fitness and Sports, Nagoya University, Nagoya, 464-01 Japan

Summary: Effects of chronic depletion of high-energy phosphate compounds by feeding β-guanidinopropionic acid (β-GPA) with or without hindlimb suspension (HS) on body temperature were studied in rats. Lower rectal and skin temperatures were observed in rats after 10 d of HS. Suspension-related enlargement of the interscapular brown adipose tissue (BAT), associated with adrenal hypertrophy, was seen. Feeding β-GPA also caused a hypothermia and BAT enlargement. It is suggested that the hypothermic response to HS may be due to decreased contractile activity and metabolic rate in skeletal muscles, associated with stress. It is also speculated that the changes in the thermogenesis in rats fed β-GPA might be related to a stimulated ATP synthesis with sacrificed heat production, but not associated with stress. [Japanese Journal of Physiology, 46, 171–175, 1996]

Key words: thermogenesis, high-energy phosphates, hindlimb suspension.

The metabolic rate has a close association with body temperature because heat is produced as a by-product of metabolic reaction. Decreased body temperature in restraint animals has been reported [1, 2]. One of the causes for such hypothermia may be reduced muscular activity [3]. However, the hypothermia may not necessarily be related to the metabolic rate, because the level of oxygen consumption is even increased in the restraint animals [4]. Nagasaka et al. [2] reported that a thermal equilibrium was reached during restraint. However, the mechanism responsible for the restraint-induced hypothermia is still unclear.

Hindlimb suspension causes a decrease in the turnover rate of adenosine triphosphate (ATP) [5], as well as the activities of mitochondrial enzymes measured in homogenates of whole muscle [6], suggesting a reduction of the metabolic rate. However, obvious stress responses, such as adrenal hypertrophy, thymus atrophy, and increased urinary excretion of stress hormones, are observed in response to hindlimb suspension [7] and such phenomena may affect the metabolic rate.

Chronic depletion of creatine, which then lowers the contents of high-energy phosphates, by feeding of the creatine analogue β-guanidinopropionic acid (β-GPA) [5, 8] causes an elevation of resting oxygen consumption in rats [9]. It is suggested that the turnover rate of ATP, estimated by the method of Chance et al. [10] as 1/(1+0.6×Pcr/Pi) using the peak heights of ³¹P-nuclear magnetic resonance spectroscopy, is increased in skeletal muscles treated with β-GPA [5]. Such a phenomenon was associated with stimulated mitochondrial enzyme activities [11], even though the daily physical activity level of rats was subnormal [9].

Energy expenditure for thermogenesis in brown adipose tissue (BAT) serves to maintain body temperature by non-shivering thermogenesis [12]. The BAT mass is enlarged in response to several factors, such as cold exposure, insulin, norepinephrine, or stress [13, 14] which may affect the metabolic rate. However, it is not clear how thermogenesis and/or BAT mass are affected by an increased metabolic rate without enhanced muscular activity or reduced muscular

Received on December 15, 1995; accepted on February 19, 1996
Correspondence should be addressed to: Y. Ohira, Department of Physiology and Biomechanics, National Institute of Fitness and Sports, Kanoya, 891-23 Japan. Tel and Fax: +81-994-46-4031
¹Present address: Department of Biochemistry II, National Defense Medical College, Tokorozawa, 359 Japan
activity caused by gravitational unloading. Therefore, the current study was carried out to investigate the thermogenic responses to chronic depletion of high-energy phosphates and/or hindlimb suspension in rats.

**Methods**

The animal care and treatment were performed following the institutional guidelines for experimental animals. Twenty newly weaned male Wistar rats (Kyudo, Kumamoto) were randomly divided into two groups \( n = 10 \) in each group. Rats in the control group were fed powdered diet (CE-2, Nihon Clea, Tokyo). The same diet, but containing \( \beta \)-GPA (1% w/w), was pair-fed for the experimental group. The \( \beta \)-GPA was synthesized in our laboratory following the methods shown previously [15, 16]. The daily food supply was gradually increased following growth. From week 3 to the end of experiment, each rat was fed 20 g of diet daily. Water was supplied ad libitum. Temperature and humidity in the animal room with a 12:12 h of light:dark cycle were maintained at approximately 23°C and 55% R.H., respectively. After approximately 9 weeks, five rats from each control and \( \beta \)-GPA group were hindlimb-suspended for 10 d by a method described previously [17]. The remaining rats served as the cage-controls.

Resting rectal temperature was measured using a thermister (MGA 3-219, Nihon Kohden, Tokyo) in unanesthetized rats. The determination in the suspended rats was performed when rats were still hindlimb-suspended. Determination of skin temperature (calf, foot, and tail) was performed using a thermography system (TVM-2000, Nippon Avionics, Tokyo) after shaving the right hindlimb of rats lightly anesthetized by ether inhalation. Each rat was placed on a styrene foam plate in a prone position (Fig. 1). Reading of skin temperature was performed by moving the cursor to the specific portion of the body in the thermograph. All measurements were performed between 8 and 10 a.m. in the temperature- and humidity-regulated room at approximately 23°C and 55% R.H.

After the measurement of body temperature, the interscapular BAT and adrenals were dissected out under deep anesthesia with an additional ether inhalation. All tissues were weighed immediately and stored at \(-80\)°C for further analyses. All data are presented as means±SEM. Statistical significance was examined by analysis of variance and Student’s t-test. Differences were considered significant at the 0.05 level of confidence.

**Results and Discussion**

Rectal and skin temperatures are shown in Table 1. Rats fed the diet containing \( \beta \)-GPA had a lower rectal temperature than controls \((p<0.05)\). Tail skin temperature of \( \beta \)-GPA group was also lower than controls \((p<0.001)\), but that in the calf and foot was not significantly different from controls. Rectal and all skin temperatures tended to decrease by hindlimb suspension. In the control-diet group, all of the values decreased significantly following hindlimb suspension. A significant suspension-related decrease in the \( \beta \)-GPA group was seen only in rectal and calf temperatures \((p<0.001)\).

The body weight of the \( \beta \)-GPA group was significantly less than the control group (Table 2, \( p<0.05 \)), but the absolute weight of interscapular BAT in \( \beta \)-GPA group was greater than in controls \((p<0.001)\). The BAT weight relative to body weight was also increased by \( \beta \)-GPA feeding \((p<0.001)\). Following the

---

**Fig. 1.** Thermography showing temperature distribution of the rat body. Normal-diet cage control (left) and \( \beta \)-GPA fed cage control (right) rats were lightly anesthetized with ether inhalation. After shaving, the tail, calf, and foot temperatures were measured. The overall skin temperature was decreased following creatine depletion.
Phosphorus Compound Effect on Thermogenesis

Table 1. Rectal and skin temperature (°C) in resting rats.

<table>
<thead>
<tr>
<th></th>
<th>Control-diet</th>
<th></th>
<th>β-GPA-diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cage-control</td>
<td>Suspended</td>
<td>Cage-control</td>
<td>Suspended</td>
</tr>
<tr>
<td>Rectal</td>
<td>37.3±0.2</td>
<td>36.1±0.1T</td>
<td>36.5±0.1*</td>
<td>35.8±0.1TTT</td>
</tr>
<tr>
<td>Tail</td>
<td>31.9±0.6</td>
<td>25.8±1.0TTT</td>
<td>27.3±0.4***</td>
<td>27.3±0.1</td>
</tr>
<tr>
<td>Calf</td>
<td>32.9±0.3</td>
<td>29.2±0.3TTT</td>
<td>32.7±0.2</td>
<td>29.4±0.3TTT</td>
</tr>
<tr>
<td>Foot</td>
<td>32.4±0.4</td>
<td>27.9±1.3*</td>
<td>30.9±0.7</td>
<td>27.9±1.2</td>
</tr>
</tbody>
</table>

Values are means±SEM. n=5 in each group. *p<0.05 and ***p<0.001 vs control-diet group and †p<0.05, ‡p<0.01, and †††p<0.001 vs cage-control group.

Table 2. Body and tissue weight.

<table>
<thead>
<tr>
<th></th>
<th>Control-diet</th>
<th></th>
<th>β-GPA-diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cage-control</td>
<td>Suspended</td>
<td>Cage-control</td>
<td>Suspended</td>
</tr>
<tr>
<td>BW (g)</td>
<td>338±11</td>
<td>299±16†</td>
<td>309±5*</td>
<td>237±14†</td>
</tr>
<tr>
<td>BAT (mg)</td>
<td>420±25</td>
<td>485±20</td>
<td>620±30***</td>
<td>463±50†</td>
</tr>
<tr>
<td>(% BW×10⁻³)</td>
<td>124±8</td>
<td>167±9TT</td>
<td>207±11***</td>
<td>190±21</td>
</tr>
<tr>
<td>Adrenal (mg)</td>
<td>59.2±4.6</td>
<td>62.3±2.5</td>
<td>54.6±2.9</td>
<td>61.7±3.5</td>
</tr>
<tr>
<td>(% BW×10⁻³)</td>
<td>16.8±1.5</td>
<td>21.4±1.1†</td>
<td>19.0±1.0</td>
<td>24.2±1.2TT</td>
</tr>
</tbody>
</table>

Values are means±SEM. n=5 in each group. *p<0.05 and ***p<0.001 vs control-diet group and †p<0.05 and ‡p<0.01 vs cage-control group. BW, body weight; BAT, interscapular brown adipose tissue.

Suspension, the BAT weight in the control-diet group tended to increase (p>0.05). The BAT weight normalized by the body weight was significantly elevated (p<0.01). The absolute weight in the β-GPA group, however, decreased by hindlimb suspension (p<0.05).

The absolute weight of the adrenal tended to increase following hindlimb suspension insignificantly (Table 2), but the percent adrenal weight relative to body weight was increased significantly by hindlimb suspension in both the control (p<0.05) and β-GPA-fed rats (p<0.01). The adrenal weight was not influenced by β-GPA feeding alone.

The BAT volume is increased by exposure to cold and non-shivering thermogenesis is stimulated [13]. It is also suggested that hindlimb suspension causes increased thermogenic activity in the BAT mitochondria of rats [18]. Kuroshima et al. [14] reported that a repetitive immobilization stress (3 h/d, 6 d/week for 1–8 weeks) caused an increased BAT mass, although their results showed no significant fall in the resting colonic temperature of rats even in the cold at -5°C. However, a significant reduction of body temperature was observed in response to continuous 10-d hindlimb suspension in our study. The adrenal hypertrophy in the current study and others [7] suggest that the hindlimb suspension of rats is also stressful.

Nagasaki et al. [2] suggested that increased catecholamine levels might be responsible for the restraint-hypermetabolism in rats. The rate of heat production increased sharply in rats in response to physical restraint, but the rate of heat loss increased progressively in these animals. Thus, the rate of heat storage was positive during the first 40 min of restraint and colonic temperature was increased, but the rate of heat storage decreased toward zero later during 2.5 h of restraint. Since the hindlimb-suspended rats in our study may be under stress continuously for 10 d, heat loss may be greater than heat production. Furthermore, a change in the set-point for body temperature could be induced in these rats (Dr. T. Ogawa, personal communication).

One of the causes of lower body temperature in the suspended rats might be reduced contractile activity of skeletal muscle as this is the major site for heat production. Although the electromyogram activity disappears immediately following hindlimb suspension, it is gradually normalized during suspension [19, 20]. However, tension development is inhibited especially in ankle extensors [20]. The decrease in cutaneous fat, as indicated by lesser white adipose tissue mass (data not shown), may be another factor for the lower body temperature in the suspended rats.

A significant reduction of temperature, especially in the rectum and tail, was also observed in rats fed β-
GPA. A greater enlargement of BAT than in suspended group was noted in these animals, as was reported in our previous study [21]. However, the adrenal mass remained normal. Therefore, the chronic depletion of creatine or high-energy phosphates may not be stressful. The whole-body fat content of rats fed β-GPA was significantly less than controls [9], suggesting that the slower gain of body weight in creatine-depleted rats is partly caused by the reduced accumulation of fat. Subnormal fat content could be one of the detrimental factors for the regulation of body temperature.

Stimulation of ATP synthesis in muscles with depleted high-energy phosphates was suggested [5, 8]. The mitochondrial enzymes are also activated in these muscles [11]. Such phenomena, associated with an increased resting oxygen consumption [9], may indicate a stimulated mitochondrial energy metabolism. However, our results showed that the metabolic rate and the body temperature were negatively correlated in rats fed β-GPA, although the volume of BAT and the oxygen consumption level are generally the measures of heat production. These results may indicate that uncoupling or heat production might be inhibited in order to increase ATP synthesis.

The rats in the current study did not perform any exercise training. The daily voluntary activity level in rats fed β-GPA was less than normal [9]. It is clear that the increase in mitochondrial enzyme activities after β-GPA feeding was not stimulated by increased contractile activity of muscles.

On the contrary, an inhibition of mitochondrial biogenesis in response to gravitational unloading by, for example, hindlimb suspension of rats was suggested. The rate of ATP synthesis in suspended ankle extensors was less than in cage-control rats [5, 8]. Further, the specific activities of mitochondrial enzymes in these muscles are less than normal [6]. The density of β-adrenoceptors, which is closely associated with mitochondrial enzyme activity, was also decreased in unloaded soleus [8]. Therefore, the mitochondrial biogenesis or metabolic rate might be decreased and may cause the reduction of heat production.

The increase in the BAT volume of β-GPA rats may be related to both lowered body temperature and increased mitochondrial biogenesis. However, that of hindlimb-suspended rats may not be stimulated directly by the mitochondrial biogenesis, although the lower body temperature may be one of the major factors.

This study was supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES