Influence of a High Ambient Temperature and Administration of Clenbuterol on Body Composition in Rats

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Summary This study was conducted to determine the effects of the oral administration of the beta agonist clenbuterol on body composition in growing rats reared under a high ambient temperature. Forty-three male Wistar-strain rats of 5 weeks of age were divided into 6 groups: 2 levels of ambient temperature (26 and 33°C) and 3 dose levels of clenbuterol (0, 50, and 100 µg/kg diet) under each ambient temperature. All rats were raised for 7 weeks. From the 3rd week, rats in the clenbuterol-treated groups were fed a diet containing clenbuterol. Both the lipid and cholesterol content in the rat liver, and the epididymal adipose tissue weight were significantly higher in the hot environment than in the temperature environment. Body fat component was significantly higher in rats in the 33°C groups in comparison with that in rats in the 26°C groups. On the other hand, body protein component was significantly lower in the 33°C groups than in the 26°C groups. Although the administration of clenbuterol significantly decreased fat and increased protein in the 26°C groups, no particular influence of clenbuterol administration on body composition was observed in the 33°C groups.

Key Words rats, beta agonist, clenbuterol, body composition, high ambient temperature, liver, epididymal adipose tissue

Promoted body fat deposition was observed in animals raised in a high ambient temperature compared with animals receiving isoenergy under room or low temperature (1–5). This could be related to the reduced basal metabolic rate of animals in a hot environment (6, 7). The reduction in basal metabolic rate under a high ambient temperature might be attributed to simultaneous decrease of blood thyroid hormone concentration, hypothyroidism, under a high ambient temperature (8, 9). In a previous study, a physiological dose of thyroid hormone, thyroxine, to growing
rats reared under a high ambient temperature predominantly suppressed body fat deposition and consequently the percentage of protein became similar to that in rats raised under room temperature (10). Hence it can be considered that thyroid hormone status might contribute to the change of body composition—in particular, promotion of body fat deposition—in animals reared under a high ambient temperature.

Recently a number of researchers have investigated actions of beta agonists, the structure of which is similar to cathecolamines, as nutrient repartitioning agents that alter carcass composition by partitioning energy away from fat deposition towards protein accretion in several animal species such as sheep (11), cattle (12), pigs (13, 14), broilers (15), and rats (16). Beta agonists exert their actions through specific cell-membrane receptors, beta-receptors, and activate adenylate cyclase, which promotes intracellular accumulation of cAMP. In the case of adipose tissue, cAMP accumulation leads to activation of hormone-sensitive lipase and then results in lipolysis. However, it was reported that lipolysis induced by cathecolamines was suppressed in hypothyroidism (17–21).

This study was conducted to determine the effect of oral administration of the beta agonist clenbuterol on body composition in growing rats reared under a high ambient temperature, and to compare the result with the effect of thyroid hormone administration observed in our previous experiment.

MATERIALS AND METHODS

Forty-three male Wistar rats (Slc-Wistar-KY strain purchased from Shimizu Laboratory Supplies, Kyoto) aged 5 weeks were divided into 6 group: 26°C, control group (this group is abbreviated as L, n = 7); 26°C, lower-dose clenbuterol group (L50, n = 7); 26°C, higher-dose clenbuterol group (L100, n = 7); 33°C, control group (H, n = 6); 33°C, lower-dose clenbuterol group (H50, n = 7); 33°C, higher-dose clenbuterol group (H100, n = 9). The average initial body weight of the rats was 135 g. All rats were raised for 7 weeks. Rats in groups L and H received a commercial diet, which was a basal diet, throughout the experimental period. Rats in L50, L100, H50, and H100 groups were fed a basal diet without clenbuterol for the first 3 weeks and then fed a diet containing clenbuterol (Spiropent; Teijin) at a level of 50, 100, 50, and 100 µg/kg diet, respectively. These dose levels of clenbuterol were referred to the dose level of a previous work by Abe and Saitoh (22). Rats were fed ad libitum in the 33°C groups while rats in the 26°C groups were fed according to the average levels of voluntary intake of the 33°C groups. Drinking water was always available, and light condition was 12 h light/dark cycle.

At the end of the experimental period, blood samples were collected from the abdominal aorta under ether anesthesia and serum was separated from blood by standing at 4°C for 2 h. Immediately after blood collection, epididymal adipose tissue and liver were separated and weighed. Liver samples were subjected to the determination of chemical analysis, protein, total lipid, and cholesterol. After

washing with a saline solution to remove the alimentary digesta, the gastrointestinal tracts and the rest of the carcasses were analyzed for carcass composition. All samples were stored at −20°C until analysis.

Serum concentrations of triglyceride were determined by the modified Fletcher method (23), total cholesterol concentrations by the Zurkowski method (24) and blood urea nitrogen concentrations by BUN test (Wako Pure Chemical, Osaka).

Carcass samples were minced by a meat chopper (GM. CHOPPER) and then percentages of moisture, protein, fat, and ash were determined. Moisture was determined by drying for 24 h at 100°C and ash was determined by the usual method with these dried samples. Protein and fat were determined by the Kjeldahl method and the ethyl ether extraction method, respectively.

The content of liver protein was determined by the Kjeldahl method and total lipid and cholesterol contents in liver samples were determined by chloroform/methanol extraction and Abell-Kendall method (25), respectively.

Differences among least squares means and analysis of variance were tested using the General Linear Models procedure of SAS.

RESULTS

Table 1 shows that the final body weights of rats in the temperate environment groups (L, L50, and L100) were significantly higher than those of rats in the hot environment groups (H, H50, and H100). Average daily feed intakes of rats during the experimental period were 12.1 g for H and L groups, 12.7 g for H50 and L50 groups, and 12.6 g for H100 and L100 groups. The liver weight of rats in the hot environment groups was significantly lower than that of rats in the temperate environment groups and, in particular, the liver weight of rats in the H100 group was the lowest (Table 1). The epididymal adipose tissue weight, which can become an indicator of body fat content in rats, was significantly higher in the hot environment groups than in the temperate environment groups (Table 1).

Table 2 shows the contents of cholesterol, lipid, and protein in the liver of rats. The contents of both cholesterol and lipid in the liver of rats in the hot environment groups were significantly higher than those of rats in the temperate environment groups, while the content of protein was lower in the hot environment groups. As shown in Table 1, the liver weight of rats in the hot environment groups was lower than that of rats in the temperate environment groups. Although the reason why the liver weight was reduced in a hot environment is not known exactly, there may be a possibility that a high ambient temperature suppresses differentiation of hepatic cells in rats.

A significant influence of ambient temperature on body composition in rats was observed (Table 3). The percentages of moisture, protein, and ash were significantly lower in rats in the hot environment groups; however, the fat percentage in rats in the hot environment groups was significantly higher than that of
Table 1. Effects of a high ambient temperature and clenbuterol administration on final body weight, liver weight, and epididymal adipose tissue weight of rats.

<table>
<thead>
<tr>
<th></th>
<th>Final body weight (g)</th>
<th>Liver weight (g/100 g body weight)</th>
<th>Epididymal adipose tissue weight (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>202 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.73 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>H50</td>
<td>201 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.13 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>H100</td>
<td>204 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.52 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.08 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L</td>
<td>219 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.94 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L50</td>
<td>223 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.89 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.76 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>L100</td>
<td>227 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.79 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.68 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Temperature **  **  **
Clenbuterol  N.S.  **  N.S.
Interaction  N.S.  N.S.  N.S.

All values are presented as least squares means ± SE. Values in the same column with different superscript letters differ significantly (p<0.05). **, p<0.01; N.S., not significant.

Table 2. Effects of a high ambient temperature and clenbuterol administration on hepatic lipid, cholesterol, and protein content in rats.

<table>
<thead>
<tr>
<th></th>
<th>Lipid (g/100 g wet liver tissue)</th>
<th>Cholesterol (mg/g wet liver tissue)</th>
<th>Protein (g/100 g wet liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>6.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.1 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>H50</td>
<td>7.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.1 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>H100</td>
<td>7.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.1 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L</td>
<td>4.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L50</td>
<td>5.0 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.8 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L100</td>
<td>4.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Temperature **  **  **
Clenbuterol  N.S.  N.S.  N.S.
Interaction  N.S.  N.S.  N.S.

All values are presented as least squares means ± SE. Values in the same column with different superscript letters differ significantly (p<0.50). **, p<0.01; N.S., not significant.

Rats in the temperate environment groups. These results indicate that lean tissue content in the body of rats was higher in the 26°C groups and that of adipose tissue content was higher in the 33°C groups. Therefore the higher content of moisture observed in rats in the 26°C groups, in particular the highest value of the L100 group, might reflect higher lean tissue content in rats in the 26°C groups, since moisture content of lean tissue is considered to be higher than adipose tissue.

Table 4 represents concentrations of serum triglyceride, total cholesterol and
Table 3. Effects of a high ambient temperature and clenbuterol administration on body composition in rats (%).

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>66.6±0.5b</td>
<td>20.0±0.3c</td>
<td>10.5±0.6a</td>
<td>3.5±0.1ab</td>
</tr>
<tr>
<td>H50</td>
<td>66.8±0.4b</td>
<td>20.0±0.2c</td>
<td>10.0±0.6a</td>
<td>3.4±0.1b</td>
</tr>
<tr>
<td>H100</td>
<td>66.9±0.4b</td>
<td>20.0±0.2c</td>
<td>9.6±0.5a</td>
<td>3.4±0.1b</td>
</tr>
<tr>
<td>L</td>
<td>67.4±0.4c</td>
<td>21.2±0.3b</td>
<td>7.7±0.5b</td>
<td>3.7±0.1a</td>
</tr>
<tr>
<td>L50</td>
<td>67.7±0.4c</td>
<td>21.1±0.3b</td>
<td>7.5±0.5b</td>
<td>3.5±0.1ab</td>
</tr>
<tr>
<td>L100</td>
<td>69.3±0.1a</td>
<td>22.3±0.3a</td>
<td>5.4±0.5c</td>
<td>3.6±0.1ab</td>
</tr>
</tbody>
</table>

Table 4. Effects of a high ambient temperature and clenbuterol administration on serum concentrations of triglyceride, total cholesterol, and urea nitrogen in rats (mg/100ml).

<table>
<thead>
<tr>
<th></th>
<th>Triglyceride</th>
<th>Total cholesterol</th>
<th>Urea nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>28.2±3.7</td>
<td>61.4±5.9b</td>
<td>19.1±1.3a</td>
</tr>
<tr>
<td>H50</td>
<td>27.5±3.1</td>
<td>69.9±5.4b</td>
<td>18.9±1.2ab</td>
</tr>
<tr>
<td>H100</td>
<td>22.7±2.9</td>
<td>57.9±4.7b</td>
<td>20.5±1.1a</td>
</tr>
<tr>
<td>L</td>
<td>34.0±3.4</td>
<td>108.2±5.4a</td>
<td>17.4±1.2ab</td>
</tr>
<tr>
<td>L50</td>
<td>28.5±3.4</td>
<td>123.3±5.9a</td>
<td>14.1±1.2b</td>
</tr>
<tr>
<td>L100</td>
<td>30.0±3.1</td>
<td>113.5±5.0a</td>
<td>17.6±1.1a</td>
</tr>
</tbody>
</table>

All values are presented as least squares means±SE. Values in the same column with different superscript letters differ significantly (p<0.05). *, p<0.05; **, p<0.01; N.S., not significant.

urea nitrogen in rats. The data show that the high ambient temperature significantly increased serum urea nitrogen concentrations and decreased serum total cholesterol concentration in rats.

The epididymal adipose tissue weight of rats in the L100 group was significantly lower than that in the L group; however, clenbuterol administration did not affect the epididymal adipose tissue weight of rats in the hot environment groups (Table 1).

Although there was no significant difference in body composition between the L and L50 groups, a higher dose (100 μg/kg diet) of clenbuterol in the L100 group significantly increased body protein content and decreased body fat in rats (Table
3). On the other hand, the clenbuterol administration at both levels showed no effects on body composition in rats reared under a high ambient temperature. These results with respect to body fat, the amount of which was higher and not influenced by clenbuterol administration under a high ambient temperature, were also obtained in epididymal adipose tissue weight (Table 1).

**DISCUSSION**

It has been reported in previous studies, that body weight gain of rats reared under a high ambient temperature, 33°C, was higher than that of rats reared under room temperature, 24°C, when rats were given the same amount of feed (9,10). They suggested that it might be due to a suppressed basal metabolic rate of rats associated with a reduced blood thyroid hormone concentration under a high ambient temperature (9). The data about final body weight shown in Table 1 contradicts the results of previous studies described above (9, 10). Although the exact reason for this contradiction is not known, a possible explanation might be the difference of the rat strain used in two studies, i.e., Jcl-Wistar rats and Slc-Wistar-KY rats. The data about body weight gain for those two strains suggested that the growth rate, in particular before 12 weeks old, of Slc-Wistar-KY rats was higher than that of Jcl-Wistar rats. In addition, it seems that the physiological sensitivity of the two strains to ambient temperature may be different.

Effects of beta agonists on feed efficiency varied among several studies. Moser et al. (13) reported that the administration of cimaterol, a beta agonist, did not change feed efficiency in pigs. However, some researchers reported that the administration of beta agonists improved weight gain/feed ratio in pigs and chicks (14,15) and others reported that gain/feed ratio was reduced by the administration of beta agonists in cattle, mice, and sheep respectively (12,26,27). The different results might be attributed to different concentrations of beta agonists in feedstuff and animal species. In the present study, no particular influence of clenbuterol administration on final body weight was observed in rats (Table 1), and no significant influence of clenbuterol administration on feed efficiency of rats was observed in the present study.

According to Akiba et al. (28) and Raheja and Linscheer (29), lipid deposition in chick liver seems to be promoted by both heat exposure and administration of antithyroid agent such as thiouracil and propylthiouracil. And Hsu et al. (30) suggested that the increase in hepatic lipid deposition in laying hens reared under a hot environment temperature was owing to a decrease in blood thyroid hormone concentrations. As for the present study, the high ambient temperature significantly increased the contents of lipid and cholesterol in the liver of rats (Table 2). Based on the general understanding that blood thyroid hormone concentration is reduced in a hot environment, the results obtained in the present study were in agreement with previous ones in chicks described above.

On the other hand, Loireau et al. (31) and Mathe and Chevaller (32) reported...
that thyroidectomy increased total blood cholesterol concentrations in rats, and the administration of thyroid hormone to thyroidectomized rats restored the concentration to a normal level. In the present study, however, total blood cholesterol concentrations in rats in the hot environment groups were significantly lower than those in the temperate environment groups (Table 4), and in addition, we also observed both lower blood thyroxine and lower blood cholesterol concentrations in rats reared under a high ambient temperature compared with those in temperate environment in our previous study. In that study, serum thyroxine concentration was significantly reduced by heat exposure—from 4.7 in the 26°C group to 3.9 (μg/100 ml) in the 33°C group—while serum total cholesterol concentration in the 33°C group, 60 mg/100 ml, was significantly lower than in the 26°C group, 77 mg/100 ml, (unpublished data). These results may suggest that blood cholesterol concentration is not always increased by hypothyroidism and that it is affected by other factors than thyroid hormone in a hot environment. For instance, Christie (33) and Bell (34) mentioned in their reviews that blood lipid concentrations of cattle, in particular total and free cholesterol, were decreased by heat exposure or during the summer season. The authors attributed these observations to a reduction in the activity of the enzyme lecithin-cholesterol acyltransferase in a hot environment. And the result in the present study that hepatic cholesterol contents were significantly higher in rats reared under the high ambient temperature (Table 2) suggests that the environment temperature affected hepatic cholesterol metabolism. However, the specific influence of an ambient temperature on hepatic cholesterol metabolism, such as the activity of rate-limiting enzyme of cholesterol synthesis in liver, hydroxymethylglutaryl-CoA reductase, has not been determined, so that further investigations are necessary to elucidate the mechanism by which a high ambient temperature reduces blood cholesterol concentration and enhances hepatic content of cholesterol.

It was confirmed in the present study that a high ambient temperature significantly increased body fat percentage and epididymal adipose tissue weight in rats (Tables 3 and 1) as observed in several previous studies (1-5). We also observed a significant lower content of body protein in rats reared under a high ambient temperature. On the other hand, blood urea nitrogen concentrations in rats reared under the high ambient temperature were significantly higher (Table 4) in the present study. It may be attributed to higher turnover rate of nitrogen or a lower glomerular filtration of urea nitrogen in a hot environment, or both. Holmes (35) reported that urinary nitrogen concentrations of pigs in a hot environment were higher than those under a temperate environment, and simultaneously lower deposition of whole body protein was observed in pigs reared under a hot environment. Hence higher blood urea concentration in rats in the 33°C groups might be due to a higher turnover rate of nitrogen, and it may be related to the lower content of body protein in the 33°C groups in the present study.

In agreement with several previous studies, repartitioning effects of the beta agonist clenbuterol, such as altering body composition by partitioning energy away
from fat deposition towards protein accumulation, were observed in rats reared under room temperature, 26°C, in the present study. However, the administration of clenbuterol did not affect body composition and epididymal adipose tissue weight in rats reared under the high ambient temperature (Tables 3 and 1). One of the main mechanisms by which beta agonists reduce body fat mass has been understood to be that the agent acts on beta receptors on adipocytes and then stimulates a series of reactions such as activation of adenylate cyclase, accumulation of cyclic AMP and activation of hormone-sensitive lipase, resulting in lipolysis (36). Malbon et al. (17), Goswami and Rosenberg (18), Fain (19), Stiles et al. (20), and Coorreze et al. (21) suggested that lipolytic activity of adipocytes, mainly stimulated by catecholamines in those previous studies, was reduced by hypothyroidism. In addition, Loireau et al. (31) suggested that altered lipid metabolism in genetically obese Zucker rats was partly due to its hypothyroidism. Moreover, in our latest study, we observed that clenbuterol did not decrease epididymal fat-pad weight on the basis of both absolute weight and per 100 g of body weight in hypothyroid rat induced by oral administration of propylthiouracil (unpublished data). Based on these observations, it might be considered that both the higher body fat content and the phenomenon that clenbuterol administration did not decrease body fat in rats in a hot environment in the present study may relate to thyroid hormone status, which was reduced under a high ambient temperature in previous works (8, 9) and that the action of clenbuterol might be dependent on the physiological level of blood thyroid hormone. And it might be a reason why thyroid hormone administration reduced body fat in rats under a high ambient temperature in our previous work (10), but clenbuterol administration in the present work did not. However, there remains the possibility that the temperature, 33°C, which was used as a high ambient temperature in the present study, might not be an appropriate pharmacological condition for the action of clenbuterol.

REFERENCES


