Preference for Safflower Oil in Rats Exposed to a Cold Environment under Free-Feeding Conditions

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ABSTRACT There are several benefits to a high-fat diet for animals exposed to cold, including improved tolerance to severe cold conditions and increased survival rates in cold environments. It is therefore of interest to examine whether animals exposed to cold will selectively consume lipids. We examined the intake of safflower oil (SO) by rats exposed to cold (4 ± 2°C) under a feeding condition in which the rats were given free access to SO. Rats exposed to cold consumed more SO than those housed at 25 ± 2°C. This finding suggests that rats prefer SO in a cold environment. There was no significant difference in the ratio of calories of SO ingested to that of matter (standard laboratory chow plus SO) ingested between rats exposed to cold and those at 25 ± 2°C. The high SO intake also affected cold tolerance and metabolite kinetics in the rats. Factors that affected the SO intake of rats exposed to cold are also discussed.

KEY WORDS: cold exposure, cold tolerance, interscapular adipose tissue, rats, safflower oil.


There are several benefits to a high-fat diet for animals exposed to cold, including improved tolerance to severe cold conditions and increased survival rates in cold environments [5, 15, 18]. Previous studies have examined diets that included a higher portion of lipid than standard diets. Thus, animals were forced to eat a high-fat diet to satisfy their energy needs and to obtain essential nutrients. However, whether animals prefer lipids in a cold environment has not been studied. It is therefore of interest to examine whether animals exposed to cold will selectively consume lipids. Safflower oil (SO) has often been used as a lipid in experiments related to diet [8, 20–22, 26, 27]. Thus, in the present study we examined the SO intake of rats exposed to cold (4 ± 2°C) under a free-feeding condition in which both SO and a standard laboratory chow were available ad libitum. We also measured rectal temperature upon acute cold exposure (ACE: −20°C in a cold room, 60 min) to evaluate the effect of ingestion of SO on cold tolerance of rats compared with rats not given SO [15]. Changes in the blood levels of glucose, nonesterified fatty acids (NEFA), and β-hydroxybutyrate caused by ACE were also measured to evaluate changes in the use of these substances by cold exposure [15]. The interscapular brown adipose tissue (IBAT) was weighed to assess its generation of heat [10, 15].

MATERIALS AND METHODS

Male SLC: Wistar rats 9 week of age (Japan SLC, Shizuoka, Japan) were divided into 4 groups with 2 rats per cage (Fig. 1). Two groups were housed at room temperature (25 ± 2°C) for 4 week and then exposed to cold (4 ± 2°C, using a cold room) for the following 3 week period. The 1st group served as controls exposed to the cold environment (CECNT), and the 2nd group had access to SO (CESO). The other 2 groups were fed at room temperature for 7 week. One group served as controls housed at room temperature (RTCNT), and the other group had access to SO (RTSO). All of the animals had free access to a standard laboratory chow (CLEA Rodent Diet CE-2, CLEA Japan Inc., Tokyo).

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Fig. 1. Temperature and diet conditions for the four groups of rats. For each pair of bars, the upper bar represents a temperature condition and the lower bar a diet condition. The bottom line shows the age of the rats. Dashed line A indicates the start of offer of safflower oil (SO). Dashed line B indicates the start of exposure to cold (4 ± 2°C). DW: deionized water. RTCNT: rats kept at room temperature (25 ± 2°C) and given standard laboratory chow and DW. RTSO: rats kept at room temperature and given SO in addition to standard laboratory chow and DW. CECNT: rats exposed to cold and given the same diet as RTCNT. CESO: rats exposed to cold and given the same diet as RTSO. See MATERIALS and METHODS for details.
Japan; fat content = 4.6 g/100 g chow), and were subjected to a 12 hr dark/12 hr light cycle, lights on at 7:00 a.m. RTCNT and CECNT were given deionized water (DW) ad libitum with 2 drinking bottles attached to each cage throughout the experimental period (7 week). RTSO and CESCO were given 2 bottles of DW for the first 2 week of the experimental period and then DW and SO (100%, Sigma Chemical Co., St. Louis, MO) for the remainder by filling one of the 2 drinking bottles with SO. The positions of the two bottles were altered daily to avoid a position preference. Body weight and intake of standard laboratory chow, DW and SO were monitored daily. Each of the 4 groups of rats was divided into 2 experimental groups at the end of the experimental period. With one experimental group, rectal temperature upon ACE (∼20°C in a freezing room, 60 min) was measured, and then a blood sample was collected from the jugular veins. With the other experimental group, a blood sample was obtained, and then the weight of the interscapular brown adipose tissue (IBAT) was measured. This experimental group did not undergo ACE. The blood samples were utilized for measurement of blood levels of glucose, NEFA, and β-hydroxybutyrate. All of the tested animals were fasted for 18 hr before the above-mentioned measurements to attenuate effects of ingestion of food on the concentrations of glucose, NEFA, and β-hydroxybutyrate in the blood, and to stabilize the concentrations. The rats in the cold room were transferred to room temperature 18 hr before the measurements. Blood glucose, NEFA, and β-hydroxybutyrate were measured using the glucose oxidase-color, enzyme color, and enzyme UV methods, respectively [10, 17, 23]. IBAT samples were dissected from the interscapular subcutaneous region and freed from the connective tissue. All procedures for the care and use of experimental animals were approved by the Animal Research Committee of Obihiro University, and were conducted under both the Guidelines for Animal Experiments at Obihiro University and the Guiding Principles in the Use of Animals in Toxicology that were adopted by the Society of Toxicology in 1989.

The data were expressed as mean ± SD. Statistical analysis was performed using the Student’s t-test and Bonferroni’s test for multiple comparisons. A p value of less than 0.05 was considered to be significant.

RESULTS

The body weights of the four groups were similar on the first day of the experiment period (Fig. 2, 9 week of age). At 13 week of age, RTSO and CESCO ingested SO for 2 week (Fig. 1). However, ingestion of SO did not affect body weight when compared with rats not given SO (Fig. 2, 13 week). Cold exposure (4 ± 2°C) for 3 week significantly decreased body weight, but SO intake for the same period had no influence on body weight in either temperature condition (Fig. 2, 16 week).

Daily intake of standard laboratory chow for the last week of the 7 week experimental period was significantly reduced by the ingestion of SO, both in the rats housed at room temperature and in those exposed to cold (Table 1). Cold exposure significantly increased chow intake, both in the rats given SO and in those not given it.

Ingestion of SO decreased daily DW intake in both temperature conditions for the last week of the experimental period (Table 1). CESCO also drank less DW than RTSO, but not significantly.

The rats in the cold environment ingested more SO than those at room temperature for the last week of the experimental period (Table 1, p<0.05).

Daily lipid intake, i.e., the sum of daily fat intake from the laboratory chow and SO, was significantly higher in the rats given SO than those not given it, both at room temperature and in the cold environment for the last week of the experimental period (Table 1). Cold exposure significantly increased lipid intake only in rats given SO.

There was no significant difference between RTSO and RTSO, or between CESCO and CESCO.

The relative weight of IBAT to body weight was significantly higher in CESCO than in either RTSO or CECNT.

Fig. 2. Effects of safflower oil ingestion and cold exposure on the body weight of rats. RTCNT: rats kept at room temperature (25 ± 2°C) and given standard laboratory chow and deionized water (DW). RTSO: rats kept at room temperature and given safflower oil in addition to standard laboratory chow and DW. CECNT: rats exposed to cold (4 ± 2°C) and given the same diet as RTCNT. CESCO: rats exposed to cold and given the same diet as RTSO. See MATERIALS and METHODS and Fig.1 for details of temperature and diet conditions. The number of rats tested was 20 from each group. Vertical bars indicate SD of means. *p<0.05 vs. corresponding group at room temperature.
SAFFLOWER OIL PREFERENCE IN RATS IN COLD

Table 1. Daily intake of standard laboratory chow, deionized water (DW), safflower oil (SO), lipids and total calories for the last week of the 7 week experiment period, and the ratio of calories

<table>
<thead>
<tr>
<th></th>
<th>Laboratory chow (g)</th>
<th>DW (g)</th>
<th>SO (g)</th>
<th>Total calories (cal)</th>
<th>Lipid (g)</th>
<th>Ratio of calories (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTCNT</td>
<td>39.8 ± 2.2</td>
<td>95.6 ± 15.6</td>
<td>-</td>
<td>137.2 ± 7.5</td>
<td>1.8 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>RTSO</td>
<td>18.6 ± 3.7*</td>
<td>57.9 ± 8.9 (9)</td>
<td>6.8 ± 1.1</td>
<td>125.0 ± 8.6</td>
<td>7.6 ± 1.0 (9)</td>
<td>49.0 ± 8.3</td>
</tr>
<tr>
<td>CECNT</td>
<td>62.4 ± 2.3*</td>
<td>94.5 ± 8.1</td>
<td>-</td>
<td>214.9 ± 8.0</td>
<td>2.9 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>CESO</td>
<td>30.3 ± 5.9 (9)</td>
<td>49.4 ± 10.5 (9)</td>
<td>11.3 ± 2.4*</td>
<td>206.0 ± 15.6 (9)</td>
<td>12.7 ± 2.3 (9b)</td>
<td>49.6 ± 10.0</td>
</tr>
</tbody>
</table>

See Fig. 2 for the abbreviations used in this table, and also MATERIALS and METHODS and Fig.1 for temperature and diet conditions. Daily intake of lipid is the sum of daily fat intake from standard laboratory chow and daily SO intake. Daily intake of total calories is the sum of daily caloric intake from standard laboratory chow and SO. Ratio of calories is defined as the ratio of calories of SO ingested to that of matter (standard laboratory chow plus SO) ingested for the last 8 days of the experimental period. The number of rats tested was 20 from each group. Values are means ± SD. * p<0.05 vs. RTSO. * p<0.05 vs. corresponding group at room temperature. * p<0.05, RTCNT vs. RTSO, or CECNT vs. CESO.

(Table 2). The relative weight in RTCNT was not significantly different from CECNT.

Cold exposure for 3 week significantly decreased the drop in rectal temperature upon ACE (−20°C, 60 min), both in rats given SO and in those not given it (Table 2). Ingestion of SO significantly attenuated the rectal temperature decrease due to ACE in CESO compared with CECNT.

The glucose concentration in CESO significantly increased following ACE (Table 3).

ACE significantly increased NEFA in RTCNT, RTSO, and CESO (Table 3). There was a significant difference between RTCNT and CECNT for after-ACE.

Cold exposure for 3 wk significantly decreased β-hydroxybutyrate concentrations, both in rats given SO and in those not given it for before-ACE (Table 3). Ingestion of SO significantly increased β-hydroxybutyrate levels, both in rats housed at room temperature and in those exposed to cold for before-ACE. ACE significantly increased β-hydroxybutyrate concentrations in CECNT. ACE did not affect the metabolite concentration of CESO.

Table 2. Relative weight of interscapular brown adipose tissue (IBAT) to body weight (BW) and drop in rectal temperature due to acute cold exposure (−20°C, 60 min)

<table>
<thead>
<tr>
<th></th>
<th>IBAT weight / BW (%)</th>
<th>Fall in rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTCNT</td>
<td>0.097 ± 0.019</td>
<td>2.81 ± 0.69</td>
</tr>
<tr>
<td>RTSO</td>
<td>0.084 ± 0.017</td>
<td>2.42 ± 0.41</td>
</tr>
<tr>
<td>CECNT</td>
<td>0.098 ± 0.014</td>
<td>1.26 ± 0.96</td>
</tr>
<tr>
<td>CESO</td>
<td>0.123 ± 0.011 (9a)</td>
<td>0.45 ± 0.82 (9a)</td>
</tr>
</tbody>
</table>

See Fig. 2 for the abbreviations used in this table, and MATERIALS and METHODS and Fig.1 for temperature and diet conditions. The number of rats tested was 10 from each group. Values are means ± SD. a) p<0.05 vs. corresponding group at room temperature. b) p<0.05, CECNT vs. CESO.

DISCUSSION

High-fat diet feeding is beneficial to animals exposed to cold [5, 15, 18]. We examined the SO intake of rats exposed to cold in a free-feeding condition.

Cold exposure (4 ± 2°C) increased standard laboratory chow and caloric intake in rats given only chow and DW. On the other hand, cold exposure decreased body weight

Table 3. Changes in the concentrations of glucose, nonesterified fatty acids (NEFA), and β-hydroxybutyrate in the blood by acute cold exposure (ACE: −20°C, 60 min)

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mg/dl)</th>
<th>NEFA (mEq/l)</th>
<th>β-hydroxybutyrate (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before-ACE</td>
<td>After-ACE</td>
<td>Before-ACE</td>
</tr>
<tr>
<td>RTCNT</td>
<td>132.7 ± 183.3</td>
<td>127.1 ± 175</td>
<td>1.03 ± 0.18</td>
</tr>
<tr>
<td>RTSO</td>
<td>124.6 ± 247.5</td>
<td>139.8 ± 30</td>
<td>1.042 ± 0.12</td>
</tr>
<tr>
<td>CECNT</td>
<td>136.6 ± 221.1</td>
<td>125.0 ± 30</td>
<td>0.843 ± 0.17</td>
</tr>
<tr>
<td>CESO</td>
<td>126.3 ± 21.3</td>
<td>160.5 ± 30</td>
<td>0.827 ± 0.30</td>
</tr>
</tbody>
</table>

See Fig. 2 for the abbreviations used in this table, and MATERIALS and METHODS and Fig.1 for temperature and diet conditions. The number of rats tested was 10 from each group. Values are means ± SD. * p<0.05, vs. before-ACE within a group of rats. a) p<0.05 vs. corresponding group at room temperature for before-ACE. b) p<0.05 vs. corresponding group at room temperature for after-ACE. c) p<0.05, RTCNT vs. RTSO, or CECNT vs. CESO for before-ACE. d) p<0.05, RTCNT vs. RTSO, or CECNT vs. CESO for after-ACE.
and the drop in rectal temperature following ACE (~20°C for 60 min). Lipid intake and the relative weight of IBAT to body weight were not affected by cold exposure in rats given only the chow and DW. The increase in chow, or caloric intake, by cold exposure is consistent with previous studies [9, 15], and is sufficient to satisfy the increased energy needs of the animals. The decrease in body weight was due to an initial decrease in body weight for approximately 1 week after the start of cold exposure (data not shown). The initial decrease suggests a preferential utilization of energy previously stored in the body mainly for the production of heat [9]. Cold exposure for 3 week attenuated the decrease in rectal temperature following ACE. This result is consistent with previous studies [4, 13, 15]. The relative weight of IBAT to body weight was not increased by cold exposure for 3 week in the present study. The recruitment of brown adipose tissue is a well-known phenomenon of animals exposed to cold [10, 15]. This discrepancy cannot be explained from the results of this experiment. However, the color of the IBAT of CECNT was obviously different from that of RTCNT; that is, the IBAT of CECNT was dark brown, and that of RTCNT was pale and almost indistinguishable from the surrounding white adipose tissue. This finding suggests that cold exposure for 3 week in the present study caused at least qualitative changes of IBAT in rats, and those changes might be involved in the increased cold tolerance of the rats.

Ingestion of SO induced a variety of effects. SO intake significantly reduced chow intake both in rats housed at room temperature and in those exposed to cold, and thus induced no changes in caloric intake compared with rats given only standard laboratory chow and DW. The mechanism that maintains a constant caloric intake in rats is not well understood. Studies of relations between the regulation of body temperature in animals and their feeding behavior are needed [12]. Ingestion of SO resulted in a higher lipid intake in RTSO than in RTCNT. This finding suggests that rats in the high-lipid feeding condition with access to SO were utilizing an efficient strategy for consuming the required calories because SO is higher in calories per weight than the laboratory chow. The cold exposure used in the present study significantly increased SO intake, suggesting that rats prefer SO in a cold environment. However, further experiments with other lipids are needed to reveal whether rats prefer lipids in cold environments. The lipid intake of CESCO was the highest of the four groups of rats. High-fat diets are beneficial to animals exposed to cold [5, 15, 18]. That is, high-fat diets improve cold tolerance, increase the survival rate in cold environments, and enhance the function of brown adipose tissue mitochondria. In the present study, a high-lipid diet feeding resulting from the ingestion of SO was also beneficial to rats exposed to cold. Specifically SO intake increased cold tolerance and IBAT/body weight in CESCO. Because brown adipose tissue grows and the non-shivering thermogenesis of this tissue increases in cold environments [6, 7, 10, 15], an increase in IBAT/body weight of CESCO might contribute to their increased cold tolerance.

Several studies have postulated that an increase in cold tolerance of animals ingesting a high-fat diet in a cold environment might be induced by metabolic changes that improve gluconeogenesis and lipid metabolism [15, 16]. The metabolic response to ACE in the present study lends support to this idea, although the exact mechanism is unclear. There was a significant increase in glucose concentration following ACE in CESCO, which might indicate an increase in gluconeogenesis. A high-fat diet and cold exposure both elevate gluconeogenesis [1, 24, 25]. Therefore, the two factors might synergistically affect the metabolism related to gluconeogenesis in CESCO. There was no ACE-induced change in the β-hydroxybutyrate concentration of CESCO, and the concentration of the metabolite was lower in CESCO than in RTSO. In addition, ACE increased the NEFA concentration of CESCO. The β-hydroxybutyrate and NEFA levels in CESCO suggest a combined effect of SO intake and cold exposure on lipid oxidation and mobilization [2, 3, 19, 24]. However, we cannot determine whether the rats in the cold environment increased their intakes of SO to obtain the benefits induced by a high-lipid diet feeding. Studies of relations between feeding behavior of animals, their heat generation, and metabolic changes caused by high-fat diets are needed.

The increase in SO intake in CESCO might also be explained by other aspects of feeding in cold environments in terms of the animals’ energy balance. That is, feeding is related to energy intake, but feeding in a cold environment also entails increased heat loss because animals are exposed to cold. Johnson and Cabanac [14] reported that rats in a cold environment decreased meal duration and increased the speed of eating. These results suggest that rats efficiently ingest food and decrease the time spent exposed to a cold environment to decrease heat loss and preserve a net energy gain. The results of the present study also suggest that CESCO preferentially ingested SO to more efficiently consume energy so that heat loss was further decreased.

Ingestion of SO lowered daily DW intake in both temperature conditions for the last week of the experimental period. CESCO also drank less DW than RTSO. The results may be related to the water obtained by metabolism of SO in the body. The decrease in DW intake by ingestion of SO might be also make heat loss of the rats given SO in the cold environment lower than those not given this oil because a decrease in DW intake diminishes the time spent exposed to the cold environment. Intake of SO might decrease heat loss of rats in a cold environment when feeding and drinking DW. Further investigation for behaviors of rats in cold environments, including feeding and drinking behavior, are required to examine the speculations concerning heat loss of rats.

There was no significant difference in the ratio of calories of SO ingested to that of matter (standard laboratory chow plus SO) ingested between RTSO and CESCO. This result, combined with the results of SO intake, suggests that rats exposed to cold prefer SO while keeping the ratio of calories approximately constant. Additionally, the findings for the
ratio of calories imply that the feeding behavior of rats is also affected by factors other than SO. These factors might be related to essential nutrients, for examples, vitamins, that can be obtained from the standard laboratory chow used in this study. However, the results for the ratio of calories also suggest the opposite of the preference of rats for SO; that is, the result might indicate that rats do not prefer SO in a cold environment. That the ratio for calories was almost constant regardless of environmental temperature might indicate that the feeding behavior of rats exposed to cold was no different from that of rats at room temperature, and that the increase in SO intake for rats exposed to cold was a result caused by satisfying an increased energy demand in the cold environment. The discrepancy between the two suggestions can not be solved by the results of the present study. Further experiments are required. For example, examinations of SO intake while varying the content of essential nutrients in the laboratory chow are needed.

In conclusion, the cold exposure used in the present study significantly increased SO intake, suggesting that rats prefer SO in a cold environment.

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REFERENCE


Safflower oil preference in rats in cold

Japanese Society of Veterinary Science

ABBREVIATIONS

ACE, acute cold exposure; BW, body weight; CECNT, rats exposed to cold and given the same diet as RTCNT; CESO, rats exposed to cold and given the same diet as RTSO; DW, deionized water; IBAT, interscapular brown adipose tissue; NEFA, nonesterified fatty acids; RTCNT, rats kept at room temperature and given standard laboratory chow and deionized water as a control diet; RTSO, rats kept at room temperature and given safflower oil in addition to standard laboratory chow and deionized water; SO, safflower oil.