AUGMENTATION OF DIETARY FAT PREFERENCE BY CHRONIC, BUT NOT ACUTE, HYPERCORTICOSTERONEMIA

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Summary

Numerous studies have documented a role for corticosterone in appetitive behavior, including caloric intake and dietary fat preference. In the present study, we have examined the mechanism(s) underlying modulation of dietary fat preference by corticosterone. The results of these studies show a) an increased fat preference with increased basal urinary output, or decreased stimulation of corticosterone output on fasting, b) elevation of fat preference following chronic, but not acute, hypercorticosteronemia produced by exogenous corticosterone administration, and c) emergence of hypercorticosteronemia prior to the development of increased fat preference in developing rats. These observations have led us to suggest that increased fat preference after chronic hypercorticosteronemia may be secondary to changes in the levels or actions of agents known to affect fat intake.

Key Words: corticosterone, fat preference, carbohydrate preference, hypercorticosteronemia

When given the opportunity to select the proportion of macronutrients (fat, protein and carbohydrate) in their diets, animals generally exhibit a relatively high level of consistency in day-to-day macronutrient selection patterns (2). In addition, results of studies using inbred rats and mice show consistent strain-dependent differences in the pattern of diet selection (2-6), suggesting a role for hereditary factors in nutrient choices. While the precise mechanism(s) underlying regulation of macronutrient preference is not yet understood, involvement of over a half-dozen mediators has been suggested. These include cyclo(His-Pro), galanin, vasopressin, enterostatin and serotonin (1,7-12).

Results of many studies (13-18) clearly suggest a major role for corticosterone, primarily a metabolic hormone, in the regulation of caloric intake. For example, adrenalectomy reduces caloric intake in many rat strains, including Sprague-Dawley and Zucker, and such decrements in appetite are reversed after corticosterone therapy (13-15). Studies on the role of corticosterone in macronutrient preference, however, have yielded controversial data (16-18). Castonguay and his associates (16,17) have suggested that corticosterone is the major regulator of fat intake; in contrast, Leibowitz and her associates have suggested that corticosterone ensures and stabilizes the intake of all three macronutrients, with increased carbohydrate and decreased fat preferences (18). Although the reasons for the above differences are not clear, several procedural

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differences in experimentation have been suggested as possible explanations (17). To resolve this issue and to gain further insight into the role of corticosterone in macronutrient preference, we have examined the relationship between macronutrient preference and adrenal output of corticosterone in a population of outbred male Sprague-Dawley rats whose adrenal function was not altered by surgery or drugs. Results of this study show that chronic, but not acute, hypercorticosteronemia produces an increase in dietary fat preference. Furthermore, the changes in fat preference must be secondary to changes initiated by chronic hypercorticosteronemia.

**Materials and Methods**

**Animals**

Adult (150-250 g) and juvenile outbred male Sprague-Dawley rats were from Holtzman Sprague-Dawley, Inc., Madison, WI. All rats were housed individually in hanging stainless steel wire-mesh cages at an ambient temperature of 22-23°C with a 12 hr reversed light-dark cycle (lights off at 1100 hr).

**Macronutrient Preference Analysis (MP)**

MP analysis was performed using a 24 hour ad lib or 4 hour feeding and 20 hour fast (4/20) paradigm as described in detail elsewhere (2). Briefly, during the feeding period each rat had access to three feeding jars, each containing a diet that derived 90% of its total calories from protein, carbohydrate or fat alone (Table I). MP analyses were performed on 4-5 consecutive days after rats became accustomed to feeding from three separate cups for 72-96 hours. The order in which the three food cups were set in the

**Table I**

<table>
<thead>
<tr>
<th>Ration</th>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>48.4</td>
<td>86.7</td>
<td>834.4</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>417.9</td>
<td>34.7</td>
<td>19.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>417.9</td>
<td>34.7</td>
<td>19.1</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>21.4</td>
<td>38.5</td>
<td>21.2</td>
</tr>
<tr>
<td>Crisco</td>
<td>----</td>
<td>635.9</td>
<td>----</td>
</tr>
<tr>
<td>Cellulose</td>
<td>48.3</td>
<td>86.7</td>
<td>48.3</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>33.8</td>
<td>60.7</td>
<td>33.8</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>9.7</td>
<td>17.3</td>
<td>9.7</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.7</td>
<td>1.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>1.9</td>
<td>3.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Total (g)</td>
<td>1,000</td>
<td>1,000</td>
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Macronutrient Kcal%

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>90</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
</tr>
<tr>
<td>Protein</td>
<td>5</td>
</tr>
</tbody>
</table>

Caloric density

<table>
<thead>
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<th>Kcal/Kg</th>
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<tr>
<td>3,768</td>
</tr>
<tr>
<td>6,762</td>
</tr>
<tr>
<td>3,724</td>
</tr>
</tbody>
</table>

a ICN Pharmaceuticals, Cleveland, OH  
b Proctor and Gamble, Cincinnati, OH  
c Alphacel non-nutritive bulk (ICN Pharmaceuticals)  
d Briggs salt mixture (ICN Pharmaceuticals)  
e Vitamin fortification mixture (ICN Pharmaceuticals)
cage was changed daily and randomly to eliminate the development of positional preference over time. The mean daily MP for each macronutrient was calculated as the % of total calories consumed. Under the aforementioned conditions for evaluating MP, 90% or more rats exhibited a mean daily MP with a coefficient of variation (CV) of 20% or less; rats with a CV of >20% were excluded from the study.

Urine Collection

All urine collections were made prior to MP analyses. Rats were kept in individual stainless-steel metabolic cages and 24 hour urine was collected on two consecutive days. On day 1 (basal) of urine collection, rats had access to both food (Purina Rat Chow) and water, whereas on day 2 (fasting), they had only water; the day 1 of the urine collection was the first exposure of the rat to the metabolic cage. Fasting, which serves as a mild form of stressor, was used to evaluate the activation of hypothalamo-pituitary-adrenal axis. The urine collection tube fitted at the bottom of the metabolic cage was packed with glasswool to prevent entry of feces into the urine. The urine was centrifuged to clarify and the clear urine was stored frozen at -40°C.

Corticosterone and Creatinine Assay

Corticosterone was assayed by radioimmunoassay using a kit purchased from ICN Biomedicals, Costa Mesa, CA. Urinary creatinine was measured colorimetrically using a kit obtained from Sigma Chemical Co., St. Louis, MO. The urinary corticosterone levels were expressed as ng corticosterone/mg creatinine.

Prior to RIA, urine samples were extracted as follows. A 0.15 ml urine sample was mixed with 0.75 ml methylene chloride by vortexing for 30 sec in a 1.5 ml microfuge tube, followed by centrifugation at 12,000 xg for 15 min. The top aqueous phase was aspirated and discarded. To the bottom methylene chloride (organic) phase, 0.15 ml of 0.1M NaOH was added followed by vortexing and centrifugation; the top aqueous phase was then eliminated as above. The alkali-washed organic phase was further washed with 0.1M acetic acid and water sequentially. The organic phase was dried overnight at room temperature, stored at -80°C and reconstituted in 125 µl steroid diluent (phosphosaline gelatin buffer containing rabbit gamma-globulin, pH 7.0) and 25 µl absolute ethanol just before RIA.

Statistical Analysis

The data are presented as mean ± sem and were considered to be statistically different when p<0.05. Data were analyzed by Students’ t-test, regression analysis and Kruskal-Wallis ANOVA followed by Mann-Whitney U-test.

Results

A correlation between urinary corticosterone output and dietary fat preference

One hundred and forty outbred Holtzman male Sprague-Dawley rats were used for this study. Since the number of the animals used was large, the study was conducted on three separate occasions using 40-50 animals each time. The housing and experimental conditions in different studies (see Methods) were kept as similar as possible. Twenty-four hour basal and fasting (from Purina Rat Chow but not water) urine was collected for corticosterone and creatinine measurements. Immediately following the last urine collection, rats were prepared for MP analyses as described under Methods.

The data summarized in Table 2 show the MP and corticosterone output profile of outbred male Sprague-Dawley rats. Both MP and corticosterone output varied widely from animal to animal. However, the median values for all measurements were closer to
the mean value. The frequency analysis for the protein and carbohydrate preference patterns suggested almost symmetrical distribution, with the highest frequency between 20-30% (33% animals) for protein and 30-40% (23.5% animals) for carbohydrate. In contrast, the frequency curve for fat preference was multi-modal, with 15.2%, 13.6% and 19.7% animals with preferences between 0-10%, 30-40% and 50-60% fat, respectively. Similarly, 24-hour urinary corticosterone output exhibited a wide animal-to-animal variation.

The relationships between MP and corticosterone output were analyzed by correlation analysis and the data are summarized in Table 3. Fat preference exhibited a significant positive correlation with basal corticosterone output ($r=0.22$, $n=125$, $p=0.0137$) and negative correlation with fasting corticosterone calculated as the % of basal ($r=-0.256$, $n=123$, $p=0.0042$). The relationship between corticosterone and protein preference was opposite to that with fat preference. In contrast, however, carbohydrate preference did not exhibit any correlation with corticosterone output. Considering a very wide variation in 24-hour urinary CORT-output and MP by outbred male Holzman S-D rats (Table 2), it is not surprising that the analysis of relationships between dietary fat preference and basal or increase in urinary corticosterone output on fasting (stress) revealed a significant but weak association (Table 3). To further analyze the relationship between corticosterone output and MP, rats were divided into two groups on the basis of their basal corticosterone output or stimulation of corticosterone output on fasting. The two groups were low (<half of mean value, 540±46, $n=47$) and high (> twice the mean value, 9412±1490, $n=8$) basal secretors or low (<half of mean value, 41±3, $n=42$) and high (>twice the mean value, 567±66, $n=13$) stimulators. Rats with high basal CORT-output ($p=0.0004$) or low stimulation of CORT-output ($p=0.0051$) had significantly higher fat preference than those with low basal CORT-output or high stimulation (Figure 1). These data suggest that animals with high dietary fat preference have generally a higher basal urinary corticosterone output, with attenuated stimulation of urinary corticosterone output on fasting (a mild stressor). In addition, animals with low dietary fat preference exhibit corticosterone output patterns opposite to those that show a high fat preference.

**Acute corticosterone does not alter dietary fat preference**

Thirty male Sprague-Dawley rats were acclimated to 4/20 feeding paradigm on a 3-cup MP diet as described in Methods and were divided in three equal groups. On day 1, all groups were treated subcutaneously with the vehicle (sesame oil, 1 ml/Kg) 30 min prior to the 4 hour feeding. Both the total caloric intake and the MP for day 1 (pretreatment) were calculated. The following day rats in the three groups were administered with the vehicle, 2 mg/kg and 20 mg/kg corticosterone (1 ml/Kg), respectively. Thirty minutes later all rats were allowed access to MP diets. One hour after feeding, 5 rats from each group were killed by decapitation and the trunk blood
Table III

Coefficient of Linear Correlation Between Macronutrient Preference and Urinary Output of Corticosterone under Basal and Fasting Conditions.

<table>
<thead>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. % Protein preference</td>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. % Carbohydrate preference</td>
<td>(C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. % Fat preference</td>
<td>(F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Basal Corticosterone output, ng/mg creatinine</td>
<td>(Cb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Fasting Corticosterone output, ng/mg creatinine</td>
<td>(Cf)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Fasting Corticosterone (% of basal)</td>
<td>C%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-value equal (number of pairs are shown in parantheses): $a=0.7990(132)$, $b=0.0001(132)$, $c=0.0126(125)$, $d=0.6343(128)$, $e=0.002(123)$, $f=0.0001(132)$, $g=0.1694(125)$, $h=0.6541(128)$, $i=0.2063(123)$, $j=0.0137(125)$, $k=0.4713(126)$, $l=0.0042(123)$, $m=0.0125(123)$, $n=0.0015(123)$ and $o=0.0001(123)$.

Rats with High Basal Corticosterone Output or with Low Stimulation of Corticosterone Output on Fasting Exhibit Increased Preference for Fat. Rats (n=125) were screened for corticosterone output and divided into high and low subgroups as described under results. The data are presented as mean ± sem. The group differences were analyzed statistically using two-tailed unpaired t-test.
collected for corticosterone, whereas the remainder of the animals was allowed to feed for 4 full hours. Since animals vary widely in their total caloric intake as well as in their MP, to evaluate the effect of corticosterone on feeding, both total caloric intake and MP for day 2 were calculated as the % of day 1 (pretreatment).

The data presented in Figure 2 (bottom panel) show that there was a dose-dependent increase in serum levels of corticosterone (vehicle=250±9, 2 mg=870±126, and 20 mg=9347±185 ng/ml, mean ± sem, n=5) after acute corticosterone administration. Total caloric intake increased with an increasing serum corticosterone level (Fig 2, top panel). However, the increase in the total caloric intake was not due to a selective change in the preference for protein (p=0.275), carbohydrate (p=0.651) or

![Figure 2](image)

**A**

- **TREATMENT**
  - VEHICLE (n=5)
  - CORT, 2mg / Kg
  - CORT, 20mg / Kg

- **% OF PRETREATMENT**
  - Total Cal Intake
  - Prot Preference
  - CHO Preference
  - Fat Preference

- **p-values**
  - 0.024
  - 0.275
  - 0.651
  - 0.827

**B**

- **Corticosterone (ng/ml)**
  - VEHICLE
  - CORT, 2mg / Kg
  - CORT, 20mg / Kg

**Fig. 2**

Acute Hypercorticosteronemia Does Not Alter Dietary Fat Preference. Thirty rats, prescreened for MP using 20-hr fast/4-hr feeding paradigm, were administered (IP) with vehicle (sesame oil) 30 min prior to 4-hr feeding. Next day, rats were divided into three groups (n=10) and administered 30-min prior to food presentation, with vehicle, 2 mg/Kg or 20 mg/Kg corticosterone, respectively. Both the total caloric intake and MP were determined for each of the two days of feeding. A separate group of rats (n=5/treatment) were decapitated, one hour into feeding period, and trunk blood collected for the measurement of serum corticosterone levels by RIA. Both total caloric intake and MP for the second day was calculated as the % of the first day and the data are presented as mean ± sem. The group differences were analyzed statistically using one-way ANOVA.
fat (p=0.827) (Fig 2, top panel). In conclusion, these data suggest that the relationship between corticosterone output and fat preference shown in Fig. 1 may be due to changes in feeding control mechanisms secondary to chronic hypercorticosteronemia. If this hypothesis is correct, chronic hypercorticosteronemia should convert low-fat-preferring rats to high-fat-preferring animals.

Chronic hypercorticosteronemia augments dietary fat preference

Of twenty-five male Sprague-Dawley rats screened for MP, eleven with low fat preference (24.2±4.2%, mean±SEM) were selected for this study. Rats were divided into two groups: control (n=5) and treatment (n=6). The treatment group received 5 mg% corticosterone water as the sole drinking fluid for three weeks whereas the control group remained on tap water containing 0.1% ethanol, solvent used to dissolve corticosterone. During treatment, both groups received Purina Rat Chow ad lib. Both the initial and the final body weights of rats in the two groups were similar (Initial: control, 184±6; cort, 171±12 and Final: control, 319±6; cort, 319±10, p>0.3). The MP for each rat in the control and corticosterone-treated groups was measured before the start of treatment (pretreatment) and 3 weeks after treatment. The post-treatment MP was calculated as the % of pretreatment MP and the data are presented in Figure 3. These data show that while there was a significant increase in fat preference (p=0.048) after chronic corticosterone treatment, there was a significant decrease (p=0.003) in the preference for carbohydrate, with no change in protein preference (p=0.861). In conclusion, these
data confirm our hypothesis that increased fat preference may be secondary to chronic hypercorticosteronemia. Therefore, one must assume that in the sequential progression of events leading to increased fat preference, hypercorticosteronemia appears early on. In other words, hypercorticosteronemia must precede the emergence of increased fat preference. The next series of experiments was conducted to test this hypothesis.

**Fig. 4**

**Hypercorticosteronemia Precedes the Emergence of Increased Dietary Fat Preference.** Twenty-three juvenile rats were screened for corticosterone output and MP using 24 hour ad lib feeding at the ages of 28 and 35 days, respectively. Rats were put on Purina Rat Chow and tap water and were allowed to grow to an age of 3.5 months before a second screening for MP. The relationship between fat intake and corticosterone output was analyzed statistically using Regression Analysis.
screening, rats were put on Purina Rat Chow and tap water and allowed to grow to an age of 3.5 months before a second screening for MP. The data presented in Figure 4 analyze the relationship between changes in the corticosterone output on fasting (presented as the % of basal output) as juvenile and fat preference as juveniles and adults. While the fat preference of juveniles exhibited no significant (p>0.30) relationship with the change in fasting corticosterone as juveniles, it had a significant (p<0.005) negative correlation with adult fat preference. In conclusion, these data support the hypothesis that hypercorticosteronemia appears first and when it persists for a long period, the increase in fat preference appears.

**Discussion**

The MP profile of self-selecting rodents varies widely (1,2,19,20). We have taken advantage of this variation in MP to examine the role of corticosterone in caloric intake and macronutrient preference in outbred male Sprague-Dawley rats. The major findings are 1) an increase in fat preference with an increase in basal urinary corticosterone output or decrease in stress-mediated rise in corticosterone; 2) an increase in caloric intake after acute hypercorticosteronemia, with no change in macronutrient preference; 3) augmentation of fat preference after chronic hypercorticosteronemia; and 4) the emergence of hypercorticosteronemia before increased fat preference. The results of our study confirm the concept that corticosterone plays an important role in the regulation of appetitive behavior. Such a concept is the

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Modulation of Dietary Fat Preference by Chronic Hypercorticosteronemia: A Working Model.

result of many studies, including attenuation of genetic- and lesion-induced obesity after adrenalectomy (13-15,21) and an apparent correlation between fat preference
and circulating corticosterone level (16). Furthermore, corticosterone has been shown to facilitate appetite stimulation by adrenergic activation (22).

The mechanism by which corticosterone stimulates fat appetite is not clear. Since it is the chronic, but not acute, hypercorticosteronemia that leads to enhanced fat preference, it is very likely that the increase in fat intake may be secondary to neurochemical changes brought about by chronic hypercorticosteronemia. Today there are at least four known endogenous substances in the brain that inhibit (1,9-12) [e.g. enterostatin, arginine-vasopressin, cyclo(His-Pro), and corticotropin-releasing hormone] and one (galanin) that stimulates (8) fat intake. The illustration in Figure 5 shows a hypothetical model in which corticosterone may decrease inhibitory input and/or increase stimulatory input to elevate fat preference. Consistent with such a hypothesis is decrease in hypothalamic content of CRH, an agent known to cause a decrement in the intake of all macronutrients with fat being more sensitive to CRH inhibition (23 and unpublished observations), as well as the expression of CRH-mRNA after chronic hypercorticosteronemia (24-25).

It must be pointed out, however, that the relationship between corticosterone and fat preference is more complex than it appears. For example, only about one-half of the rats with basal corticosterone output above the median also have fat preference above the median; the same thing is true for the rats with low corticosterone output. This suggests the complexity of the phenomenon and, therefore, it is more likely that both corticosterone-responsive and corticosterone-nonresponsive mechanisms work together to modulate fat preference.

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References