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Suppressed Fat Accumulation in Rats Fed a Histidine-Enriched Diet

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Abstract

The effect on body fat accumulation on male Wistar rats undergoing continuous feeding with a histidine-enriched diet was investigated. Five-week-age rats were assigned to two groups and were fed either the control diet (purified diet AIN-76™) or the histidine-enriched diet containing 3% histidine for 28 days. It was observed that both adipose tissue masses in retroperitoneal and epididymal areas of rats fed histidine-enriched diet significantly decreased ($p<0.05$) compared to those of control rats, while there was no significant difference in the food efficiency ratio between them. The blood levels of histidine derivatives of 3-methylhistidine and carnosine were significantly ($p<0.05$) increased in the rats fed a histidine-enriched diet, whereas there were no significant different between the histidine-enriched diet and control groups in the general amino acid distribution. Our results demonstrate that a histidine-enriched diet suppresses body fat accumulation in rats.

Key words: wistar rat, histidine-enriched diet, adipose tissue mass, fat accumulation, 3-methylhistidine, carnosine

INTRODUCTION

Obesity, a growing epidemic, is an excess of adipose tissue in the body that often causes serious impairment of health (1). The accumulation of visceral adipose tissue increases the risk of developing metabolic syndrome, including cardiovascular disease. In fact, the combination of obesity with the cluster of hypertension, hypertriglyceridemia, and hyperinsulinemia is referred to as the deadly quartet (2,3). It is reported that the prevalence of obesity now involves more than 1 billion individuals worldwide (4), although some evidences are reported for multiple alterations of the endocrine systems, including abnormal blood hormone, growth factors and cytokines (5-7). Most obesity guidelines suggest the need for weight reduction using behavioral change to reduce caloric intake and increasing physical activity (8-10). However, the behavior therapy has not been successful, because management of eating control for a prolonged period has been proven troublesome. In addition, as the maintenance of the reduced weight is generally a laborious task, many subjects experience undesired weight regain (11-13).

Although anorexic drugs or appetite suppressants approved by the FDA may be useful for despairing cases as an adjunct to diet, there was concern that these drugs would affect neurotransmitters in the brain (14,15). Therefore, the development of naturally-occurring supplements has been looked to as an alternative approach. Questionnaire surveys revealed that there was a negative correlation between energy intake and histidine intake in people living in Japan (16,17). Histidine is a precursor for the synthesis of histamine. Reports state that the neurotransmitter histamine in the hypothalamus plays an important role in obese Zucker rats (18,19), and the neuronal system contributes to suppression of fat accumulation by upregulating mRNA expression of uncoupling protein (UCP), which expands energy consumption in rats (20). The blood-brain barrier protects the brain from foreign substances in the blood that may cause brain neuron damage. Since histamine cannot cross the blood-brain barrier (21), oral administration of histamine might not be useful for the prevention and/or treatment of obesity. It is thought that dietary histidine might suppress appetite by activating histamine neurons; therefore, we conducted a study previously that investigated the effects of short-term feeding with a histidine-enriched diet. As the result, a significant weight reduction with decreased fat accumulation was observed in rats (22). The aim of the present study is to clarify if long-term continuous

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feeding with dietary histidine is effective on diminishing body fat accumulation in rats.

MATERIALS AND METHODS

Materials

Casein, methionine, cornstarch, cellulose, mineral mixture, and vitamin mixture for AIN-76^{TM} purified diet were obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan). L-histidine was purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan). Sucrose and corn oil was from Nissin Sugar Manufacturing Co. Ltd. (Tokyo, Japan) and Ajinomoto Co. Ltd. (Tokyo, Japan), respectively. Triglyceride E-test Wako, cholesterol E-test Wako, HDL-cholesterol E-test Wako, NEFA C-test Wako, and glucose CII-test Wako were also from Wako Pure Chemical Industry Ltd. All other chemicals used in this study were of analytical grade.

Test animal and feeding procedure

Five-week-old male Wistar rats were obtained from CLEA Japan (Tokyo, Japan). The rats were kept individually under hygienic conditions with free access to food and water in a stainless steel cage with wire mesh bottoms. The room temperature was maintained at 23 ± 2^{o}C with a 12 hr light/dark cycles. The experimental manipulation was undertaken in accordance with the institutional guideline for the care and use of laboratory animals of Bunkyo University Women's College, Japan. For acclimation to the experimental conditions, rats were given free access to a commercial diet (CE-2, CLEA Japan) for 4 days. They were assigned into two groups at random on the basis of body weight and allowed free access to experimental diets for 28 days. Experimental diets were prepared based on the formula AIN-76^{TM} in the report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies (23), as shown in Table 1. The amount of histidine used in the present study was fixed to 3% as final concentration according to (22). Since 20% casein is required in the diet for the maintenance of normal growth of rats, the added amount was subtracted from the amount of cornstarch. Food intake and body weight were recorded every morning before the food was replenished during 28 days.

Anatomical experiments

At the end of the feeding period, rats were anesthetized with 0.2 mL of pentobarbital (Kyoritsu Seiyaku, Tokyo, Japan) between 1:00 and 4:00 PM, according to the procedure approved by the animal use committee of Bunkyo University Women's College, Japan. Blood was collected through a heart puncture with a syringe, and serum was separated from the blood and used for the measurement of lipid components and glucose. In parallel with the collection of blood sample, major organs were removed and measured their weights. In addition, retroperitoneal and epididymal fat pads were also harvested to determine the accumulated adipose tissue mass.

Table 1. Composition of histidine-enriched diet (g/kg)

	Control diet	Histidine-enriched diet
Casein	200	200
Methionine	3	3
Histidine	0	30
Cornstarch	427	397
Sucrose	210	210
Corn oil	100	100
Mineral mixture[1)]	40	40
Vitamin mixture[1)]	10	10
Cellulose	10	10

[1)]Both mixtures were prepared based on American Institute of Nutrition's dietary allowance for rats (AIN-76^{TM} purified diet).

Biochemical analyses

Triglyceride, total cholesterol, high density lipid (HDL)-cholesterol, free fatty acids and glucose levels in serum were calorimetrically measured by using commercially available assay kits, triglyceride E-test Wako, cholesterol E-test Wako, HDL-cholesterol E-test Wako, NEFA C-test Wako and glucose CII-test Wako. Distribution of free amino acids and their derivatives in the serum was determined using an amino acid analyzer L-8500 (Hitachi High-Technologies Co., Tokyo, Japan).

Statistical analysis

Significant differences were evaluated by Student's *t*-test. Paired test was conducted to assess the differences in each group between control diet and histidine-enriched diet in all experimental data. The effects of treatment were considered statistically significant at $p<0.05$. All data are expressed as means $\pm$ SD.

RESULTS

Body weight gain and food efficiency ratio

After acclimation with normal feed for 4 days, rats were fed with control diet and a histidine-enriched diet containing 3% histidine for 28 days. Measurement of body weight of test rats was done every day, and the amount of food intake was recorded before replenishing diets. Food efficiency was expressed as the ratio of body weight gain to the total food intake in 28-day feeding period. A tendency to suppress body weight gain and food intake was only observed for histidine-enriched diet. The food-intakes of rats fed the control diet and

histidine-enriched diet were 547±20 g (n=5) and 530±29 g (n=5), respectively. The gains in weight of rats fed the control diet and histidine-enriched diet were 182±12 g (n=5) and 175±28 g (n=5), respectively. The food efficiency ratio of rats fed histidine-enriched diet slightly but not significantly ($p<0.05$) decreased compared with that of rats fed the control diet (Table 2).

Body fat accumulation

All rats used for 28-day feeding were anesthetized, and weights of major organs including liver and brain were measured after isolation each other. As the result, it was found that there was no significant difference in all organs isolated between rats fed the control diet and histidine-enriched diet. As shown in Fig. 1, adipose tissue masses harvested from retroperitoneal and epididymal areas in rats fed the control diet were respectively 1.99±0.21 and 1.82±0.15 g·100 g-body weight^{-1} (n=5), whereas those in rats fed histidine-enriched diet were respectively 1.54±0.32 and 1.32±0.19 g (n=5) g·100 g-body weight^{-1}. Thus, compared with the control group, it is revealed that the body fat accumulation was significantly ($p<0.05$) lower in the dietary histidine group.

Table 2. Changes of body weight rat fed with histidine-enriched diet

	Control diet	Histidine-enriched diet
Initial body weight (g)	145±5	145±5
Final body weight (g)	327±16	320±37
Total food intake (g/28 days)	547±20	530±29
Fist week (g/7 days)	121±5	117±5
Second week (g/7 days)	144±7	136±7
Third week (g/7 days)	134±6	127±10
Fourth week (g/7 days)	148±9	150±9
Food efficiency ratio[1)]	0.334±0.016	0.329±0.017

Shown values are mean±SD (n=5).
[1)]Food efficiency ratio indicates the ratio between the body weight gain (final body weight−initial body weight) and total food intake.

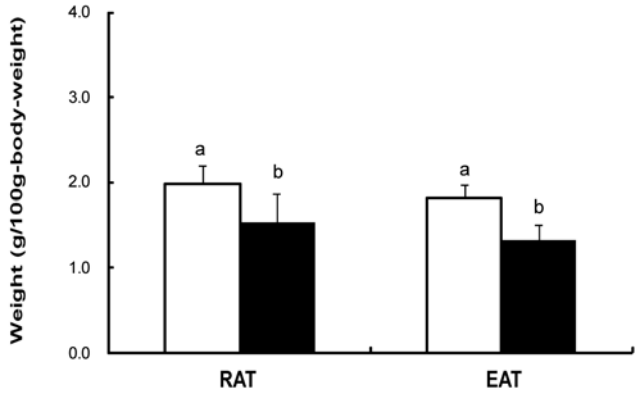

Fig. 1. Aetroperitoneal and epididymal tissue masses of rats fed with histidine-enriched diet. Error bars represent means ±SD (n=5). Means labeled with a different symbol between two different feeding systems are significantly different ($p<0.05$). RAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue. □, control diet; ■, histidine-enriched diet for 28 days.

Blood biochemical findings including amino acid distribution

Serum separated from the withdrawn blood was used for the determination of lipid components' concentration, glucose concentration, and amino acid. As shown in Table 3, it was observed that both groups fed control diet and histidine-enriched diet had almost the same level in total cholesterol and HDL-cholesterol. Concentrations of triglyceride and glucose for the rats fed the control diet were 41.7±27.7 and 127±23.0 mg·dL^{-1}, respectively, whereas those from the histidine-enriched diet were 37.8±17.0 and 111±8.0 mg·dL^{-1}, respectively. The levels of triglycerides and glucose in the histidine-enriched group tended to be lower than those in the control group. It was shown that there was no significant difference in the profile of general amino acid

Table 3. Changes of serum lipids and glucose in rats fed with histidine-enriched diet

	Control diet	Histidine-enriched diet
Total cholesterol (mg/dL)	77.0±6.8	77.4±11.3
HDL-cholesterol (mg/dL)	51.4±3.4	51.6±5.6
Triglyceride (mg/dL)	41.7±27.7	37.8±17.0
Glucose (mg/dL)	127±23.0	111±8.0

Shown values are mean±SD (n=5).

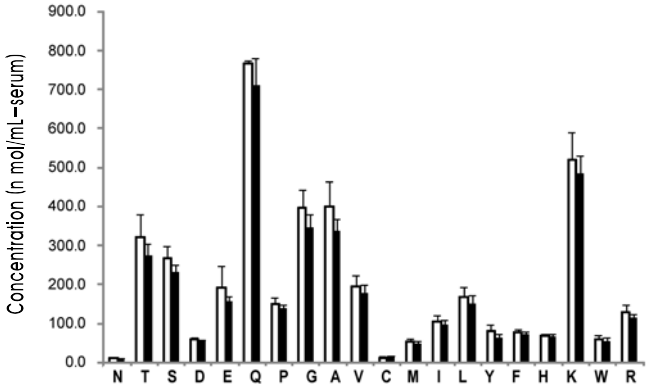

Fig. 2. Profiles of amino acid distribution in serum of rats. Error bars represent means±SD (n=5). No significant difference ($p<0.05$) was observed between two different feeding systems for all amino acids. N, asparagine; T, threonine; S, serine; D, asparatic acid; E, glutamic acid; Q, glutamine; P, proline; G, glycine; A, alanine; V, valine; C, cystine; M, methionine; I, isoleucine; L, leucine; Y, tyrosine; F, phenylalanine; H, histidine; K, lysine; W, tryptophan; R, arginine. □, control diet; ■, histidine-enriched diet for 28 days.

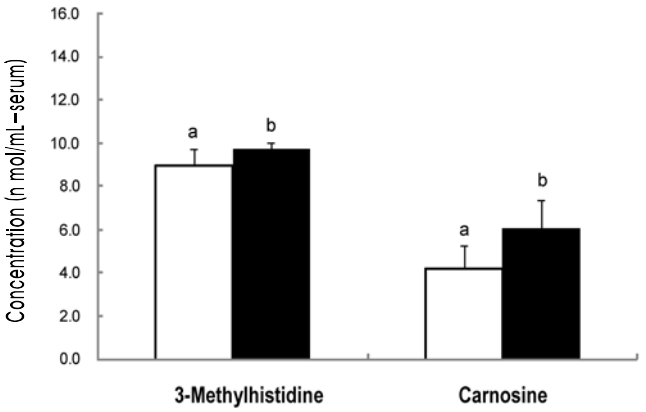

Fig. 3. Histidine derivatives in serum of rats. Error bars represent means±SD (n=5). Means labeled with different letters within individual feeding systems are significantly different ($p<0.05$). □, control diet; ■, histidine-enriched diet for 28 days.

profile in serum of rats fed histidine-enriched diet and that of rats fed the control diet (Fig. 2). An analysis of amino acid-associated substances was performed for taurine, hydroxyproline, citrulline, α-amino-n-butyric acid, cystathionine, monoethanolamine, ornithine, 1-methylhistidine, 3-methylhistidine, carnosine. The results revealed that histidine derivatives in serum of rats fed the histidine-enriched diet were significantly ($p<0.05$) higher than those of the control rats, as shown in Fig. 3. Concentrations of 3-methylhistidine and carnosine of the histidine-enriched group were respectively 9.8±0.5 and 6.1±1.3 nmol·mL^{-1}, while those of the control group were respectively 8.9±0.8 and 4.2±1.0 nmol·mL^{-1}.

DISCUSSION

Male Wistar rats were allowed free access to diets with and without histidine for 28 day. During the experimental period, there was no significant difference in food efficiency ratio between the control and experimental rats with 3% dietary histidine. On the other hand, it was demonstrated that fat accumulation in the retroperitoneal and epididymal adipose tissue areas was significantly lower ($p<0.05$) in rats fed histidine-enriched diet than in rats fed the control diet. According to countrywide questionnaire surveys for dietary habit in Japan, it was revealed that there exits a significant correlation ($p<0.05$) between energy intake and histidine intake (16,17). There is a rationale for efficacy of dietary histidine as a therapeutic tool for weight reduction. Histamine in the brain play a crucial role in maintaining homeostatic energy balance, because activation of histaminergic nerve systems in the hypothalamus resulted in enhancement of satiation (19). Additionally, several studies also demonstrate that peripheral loading of histidine, a precursor of histamine, exerts some influences for physiological function in rat brain. Histidine decarboxylase, an enzyme that converts histidine to histamine, is found in the hypothalamus (24). Peripheral administration of histidine increased histamic concentration in the hypothalamus (24-27).

In addition to the body measurement, blood characteristics were investigated for the 28-day feeding rats. The results indicated no significant differences in lipid components and distribution of general free amino acids between two groups fed the control diet or the histidine-enriched diet. On the other hand, concentrations of 3-methylhistidine and carnosine in the serum collected from the histidine-enriched group were significantly ($p<0.05$) higher than those in the control group. It is assumed that histidine has a stimulating effect on upregulation of muscle activity, and the amount of 3-methylhistidine in blood may be reflected in the level of catabolic activity of myoproteins. Recently, we demonstrated that weight gain in musculatures and weight loss in adipose tissues were simultaneously observed in rats fed dried bonito extract containing 9.6% histidine (28). Thus, a successive feeding with histidine-enriched diet for 28 days may accelerate the turnover of muscle protein in rats. The histidine derivate biosynthesized in muscle is assumed to be released into blood systems by the exercise induction (29). L-carnosine, a bioactive dipeptide of aniline and histidine present in mammalian tissues, is involved in the regulation of energy homeostasis through the autonomic nervous system, although its biosynthesis and degradation have not yet been fully elucidated (30). However, it was suggested that L-carnosine released from muscles due to exercise functions could reduce the blood glucose level through the regulation of the autonomic nerves (29-31). Therefore, an elevation of the concentrations of 3-methylhistidine and carnosine in the serum implies that long-term feeding with a histidine-enriched diet could induce increased muscular activity, and therefore result in diminished body fat accumulation in the test animals.

The prevalence of obesity is increasing dramatically around the world. Since obesity is associated with increased risk of cardiovascular morbidity and mortality, increase of an obese population has become a serious problem not only in the developed countries but also in emerging countries (32,33). Guidelines for overcoming obesity suggest the need for weight reduction using behavioral change to reduce caloric intake and increasing physical activity (8-10). Lifestyle modification

may remain the cornerstone for the maintenance of body weight reduction; however, patients frequently face difficulties and often experience a weight gain rebound. Nonetheless, use of anti-obesity medications is not generally recommended due to risks for individuals with co-morbid disease, such as anorexia and appetite disturbance. Interestingly, it has been reported that administration of yeast hydrolysates reduced the level of plasma lipids and the weight of body fat not only in rats but also in college students (34,35). Although there are still concerns to be worked out regarding safety issues, these products may have the potential for use as a functional food or food-based medicine designed for weight reduction. The studies discussed in this report also indicate that histidine-fortification could be useful for an anti-obesity functional food material.

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