Food Intake Is Inversely Correlated with Central Nervous System Histamine Receptor (H₁) Concentrations in Male Sprague-Dawley Rats Fed Normal, Low Protein, Low Energy or Poor Quality Protein Diets¹,²

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ABSTRACT The reported studies were designed to examine relationships between whole-brain histamine receptors (H₁) and food intake in male Sprague-Dawley rats. Three different experiments were conducted. In each experiment, control rats were fed normal protein (25 g casein/100 g food) and normal metabolizable energy (16.21 kJ/100 g food) diets. Feeding low protein diets (1 g casein/100 g food) elevated central H₁ receptor concentrations (P < 0.0027) and reduced voluntary food intake (P < 0.007) compared with normal diets. Feeding low energy diets lowered H₁ receptor concentrations (P < 0.0089) and increased voluntary food intake (P < 0.0012). Low quality protein diets also affected the central nervous histaminergic system. Whole-brain H₁ receptor concentrations were significantly higher for rats fed low quality protein (25 g gelatin/100 g food) compared with rats fed casein (P < 0.0001). Rats fed medium quality protein (25 g wheat gluten/100 g food) or low quality protein ate significantly less food (P < 0.0001). In all experiments, dietary manipulation affected central histamine receptors. Elevated concentrations of H₁ receptors were associated with a decrease in food intake whereas lowered concentrations of H₁ receptors were associated with an increase in food intake (P < 0.001). The results of these experiments support the hypothesis that central histamine H₁ receptor concentrations in male rats are inversely correlated with voluntary food intake and affected by dietary composition. J. Nutr. 126: 3083–3089, 1996.

INDEXING KEY WORDS:
• histamine receptor • food intake • rats
• low protein • low energy

The regulation of food intake is a complex, hierarchi-
cal phenomenon, influenced by both internal and exter-
nal factors. Among these factors, dietary imbalances (deficiencies or excesses) of protein or amino acids have been shown to cause suppression of voluntary food in-
take [Ashley and Anderson 1975, Mercer et al. 1989a
and 1996, Peters and Harper 1985]. However, the inter-
imal, neurochemical signaling leading to the suppression
of food intake is not fully understood.

Histamine, the decarboxylation product of histidine,
is a neurotransmitter and/or neuromodulator. Hista-
machine concentration in the brain influences various
hypothalamic functions, such as feeding [Sakata et al.
1988], drinking [Leibowitz 1973], neuroendocrine se-
cretion [Schwartz et al. 1991], the sleep/wakefulness cy-
cle [Kiyono et al. 1985], free-running rhythm [Itowi et
al. 1990], locomotor activity [Kalivas 1982], cata-
lepsy [Kamei et al. 1983], neuroendocrine regulation
[Jorgenson et al. 1989] and thermoregulation [Fukagawa
et al. 1989]. An inverse relationship between brain his-
tamine concentration and food intake has also been
reported (Fukagawa et al. 1989).

Cerebroventricular, intraperitoneal, and subcuta-
aneous infusion of histamine receptor (H₁) antagonists,
such as doxepin, cyproheptadine, promethazine, pro-
mazine and chlorpheniramine induce feeding in rats
(Fukagawa et al. 1989, Mercer et al. 1989a, Oishi et
al. 1990, Ookuma et al. 1990, Orthen-Gambill 1988,
Other studies show that only the more potent H₁ recep-

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ressed.
⁴ Abbreviations used: H₁, histamine receptor; HQ, high quality;
LE, low energy; LP, low protein; LQ, low quality; ME, metabolizable
energy; MQ, medium quality; NE, normal energy; NP, normal pro-
tein; VLE, very low energy.
tor antagonists, such as the antidepressant doxepin and the antipsychotic promazine, alter food intake.

The amino acid histidine is elevated in rats fed low protein diets. We have shown that food intake can be predicted as a function of brain histidine concentration [Mercer et al. 1989a and 1989b]. We have also shown that blocking H1 receptors attenuates the weight loss response of rats fed low protein (4 g/100 g diet) diets [Mercer et al. 1994]. Histaminergic blockade with H1-receptor antagonists results in increased food intake or weight gain, whereas increased histamine levels are associated with appetite suppression.

Thus, considerable evidence exists to indicate that histamine/histidine metabolism and histaminergic activity play an important role in the regulation of food intake. This study was designed to examine the role of central histamine H1 receptors and voluntary food intake in rats fed low energy or low protein diets.

**MATERIALS AND METHODS**

**Animals.** The subjects in all of these experiments were male Sprague-Dawley rats [Harlan Sprague Dawley, Indianapolis, IN]. All animals were housed in an environmentally controlled room with a 12-h light: dark cycle (0600–1800). Rats were housed and fed in the animal care facility of the Division of Laboratory Animal Resources, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. The temperature was maintained at 23–25°C and 55–60% relative humidity. On arrival, all rats were maintained on free-access feeding schedules, and a nonpurified diet [Wayne Laboratory Animal Diets, Denver, CO] was fed for 4 d to acclimate the rats. Water was freely available after being purified by deionization and reverse osmosis. On the fifth day, all rats were housed individually in standard stainless steel wire-bottom cages and fed normal protein (25 g casein/100 g food) powder diet (Table 1) for 3 d to acclimate to the experimental regimen. The diet was placed in shallow glass food cups with stainless steel follow-through disks to reduce food spills. All diets were stored at 4°C in plastic containers and handled with gloves and appropriate utensils to avoid contamination.

Rats and food jars were weighed daily at 1030 h on a time-integrated Sartorius balance [Brinkmann Instruments, Westbury, NY] interfaced to a computer. Data were transmitted from balance to computer directly into a Lotus 1-2-3 [Lotus Development, Cambridge, MA] spreadsheet to facilitate analysis and to minimize time and transcription errors.

**Daily weight gain and daily food intake analysis.** Average daily weight gain rates (dW/dt) and food intake rates (dF/dt) were calculated for the studies according to the following equations: 

\[ W = (dW/dt)t + W_0 \]

and 

\[ F = (dF/dt)t + F_0 \]

where \( W \) is live weight, \( F \) is cumulative food intake at time \( t \) and \( W_0 \) and \( F_0 \) are initial conditions. We chose average daily intakes [the slope of the cumulative food intake curve] to minimize the effects of bioperiodicity in daily food intakes which we have demonstrated [Mercer et al. 1993].

**Tissue collection and preparation.** At the end of the experiment, killing of rats was started at 1030 h. Food was left in each rat's cage until the time of killing. Rats were individually removed from the animal quarters to a nearby preparation room where they were anesthetized with metofane and decapitated. Immediately after decapitation, brains were removed, frozen on dry ice and stored at –80°C until homogenized.

Whole rat brains were homogenized in 5.5 volumes ice-cold sodium-potassium phosphate buffer (50 mmol/L, pH 7.5) with a Polytron homogenizer [Ultra-Turrax T25, Tekmar, Cincinnati, OH] for 3–10 s periods at 10-s intervals. The homogenates were centrifuged at 25,000 × g for 20 min at 4°C. Pellets were then resuspended in the same volume of buffer. Pellets were again centrifuged at 25,000 × g for 20 min at 4°C and resuspended in original volume of same buffer (5.5 mL buffer/g wet tissue). The homogenate was frozen at –80°C until analyses could be performed.

**Protein analysis.** Protein analysis was done by utilizing the Bio-Rad Protein Assay Kit [Bio-Rad Laboratories, Melville, NY]. The instructions of the manufacturer were followed for the assay. All samples and standards were analyzed in duplicate.

**H1-Receptor assay.** The assay was based on the specific binding of tritiated mepyramine ([3H]-mepyramine, an H1 receptor antagonist) to H1 receptors in the rat brain [Liu et al. 1993, Nakai et al. 1991]. All samples were analyzed in triplicate.

To determine total mepyramine binding, whole brain tissue preparations (total protein of ~0.7 mg) were incubated with 2 nmol/L [3H]-mepyramine (NET 594) [918 GBq/mmol, New England Nuclear, Boston, MA] and with Na/K phosphate buffer (50 mmol/L, pH 7.5) to a total volume of 1.5 mL. To determine nonspecific binding, tissue preparations (total protein ~0.7 mg) were incubated with 2 µmol/L tripropidine, 2 nmol/L [3H]-mepyramine and Na/K phosphate buffer to a total volume of 1.5 mL. Incubation was carried out for 60 min at 30°C. Following incubation, the mixture was filtered onto Whatman glass-fiber filters (GF/B), presoaked in 3% (v/v) polyethyleneimine [P-3143] (Sigma Chemical, St. Louis, MO) positioned over a vacuum. Filters were washed three times with 4 mL ice-cold Na/K phosphate buffer. Filters were soaked for 12–18 h in 10 mL scintillation mixture [111152] [Research Product International, Mt. Prospect, IL]. [3H]-Mepyramine concentration was then determined by scintillation counting. Specific radioactivity was equal to total bound radioactivity minus nonspecific binding determined in the presence of 2 µmol/L tripropidine.
Table 1
Composition of diets

<table>
<thead>
<tr>
<th>Component</th>
<th>NP, NE and HQ</th>
<th>LP3</th>
<th>LE4</th>
<th>VLE5</th>
<th>MQ6</th>
<th>LQ7</th>
</tr>
</thead>
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<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AIN mineral mixture 76</td>
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<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
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<td>3.50</td>
</tr>
<tr>
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<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
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<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
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<tr>
<td>Cornstarch</td>
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<td>84.30</td>
<td>36.14</td>
<td>12.15</td>
<td>60.27</td>
<td>60.27</td>
</tr>
<tr>
<td>Cellulose</td>
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<td>5.00</td>
<td>29.13</td>
<td>53.15</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>t-Methionine</td>
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<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Casein</td>
<td>25.00</td>
<td>1.00</td>
<td>25.00</td>
<td>25.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>—</td>
<td>—</td>
<td>25.00</td>
<td>25.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gelatin</td>
<td>—</td>
<td>—</td>
<td>25.00</td>
<td>25.00</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 All dietary ingredients purchased from ICN Nutritional Biochemicals, Cleveland, OH.
2 NP, NE and HQ = normal protein [25 g casein/100 g food] and normal metabolizable energy (16.21 kJ/g food).
3 LP = low protein (1 g casein/100 g food) and normal metabolizable energy (16.21 kJ/g food).
4 LE = normal protein (25 g casein/100 g food) and 25% less metabolizable energy (12.15 kJ/g food).
5 VLE = normal protein (25 g casein/100 g food) and 50% less metabolizable energy (8.10 kJ/g food).
6 MQ = medium quality protein (25 g gelatin/100 g food) and normal metabolizable energy (16.21 kJ/g food).
7 LQ = low quality protein (25 g gelatin/100 g food) and normal metabolizable energy (16.21 kJ/g food).

Experiment 1. The goal of this experiment was to determine the effects of dietary protein deficiency on central H1 receptor binding in male rats. Twelve rats were randomly assigned by weight to two groups of six rats each and fed treatment diets (Table 1) for 7 d. Normal protein [NP] and low protein [LP] groups were fed protein-adequate (25 g casein/100 g food) and low protein (1 g casein/100 g food) diets, respectively. Rats were weighed every day for 7 d. Food intakes were also measured daily. Seven days after initiation of experimental feeding, rats were individually killed and whole brains were collected.

Experiment 2. The goal of this experiment was to determine the effects of dietary energy deficiency on H1 receptor binding in male rats. Eighteen rats were randomly assigned by weight to three treatment groups of six rats each and fed three different powdered diets (Table 1) differing in energy contents. Rats were fed the following diets: normal energy (NE), i.e., normal metabolizable energy (ME) (16.21 kJ/g of food); low energy (LE) which contained 25% less ME than NE; and very low energy (VLE) which contained 50% less ME than NE. The diets were made by adjusting the cellulose and cornstarch contents, assuming that the cellulose provides no metabolizable energy. Thus, the diets were isonitrogenous, each providing ~25 g casein/100 g food. Rats were weighed every day for 7 d. Food intakes were also measured daily. Seven days after initiation of experimental feeding, rats were individually killed and whole brains were collected.

Experiment 3. In this experiment the effect of protein quality on histamine H1 receptor binding in rat brain was studied. Eighteen rats were randomly assigned by weight to three groups of six rats each. The three groups were fed high quality (HQ) protein (25 g casein/100 g food), medium quality (MQ) protein (25 g gelatin/100 g food) and low quality (LQ) protein (25 g gelatin/100 g food) diets (Table 1), respectively. Rats were weighed every day for 4 d. Food intakes were also measured daily. Four days after initiation of experimental feeding, rats were individually killed and whole brains were collected.

Statistical analysis. The data were analyzed by ANOVA in a one- and/or two-way classification using the computer program SYSTAT (SYSTAT, Evanston, IL). Post-hoc comparisons were made using the Tukey Honestly Significant Differences test (Dowdy and Wearden, 1983). Results were considered significant at P < 0.05. Graphics in this study were performed using the PC-based graphics program SigmaPlot (Jandel Corporation, San Rafael, CA).

Results

Experiment 1. Rats fed the LP diet lost weight compared with rats fed the NP diet (P < 0.0001) [Fig. 1A]. Diets also altered food intake: the LP rats ate significantly less food than the NP rats (P < 0.0007) [Fig. 1B]. As can be seen from Figure 4A, the LP rats had significantly greater specific [3H]-mepyramine binding to H1 receptors compared with the NP rats (P < 0.0027).

Experiment 2. There was significantly lower weight gain in the rats fed a diet containing 50% less ME (VLE diet) than in rats fed the diet containing 25% less ME (LE diet) or the normal ME diet (NE diet) (P < 0.019) [Fig. 2A]. The rats fed diets containing 50 or 25% less ME ate significantly more food than the rats fed the diet containing normal ME levels (P < 0.0012) [Fig.
DISCUSSION

The histaminergic system in brain functions as a neuroregulator and has been implicated in control of food intake. Histaminergic cell bodies project from the tuberomammillary nuclei of the posterior hypothalamus to all regions of the brain (Watanabe and Wada 1991). Histamine H₁, H₂ and H₃ receptors are located in the hypothalamus, the brain region which is intimately involved in fuel substrate homeostasis, hypophagia and hyperphagia (Richelson 1992).

Inverse relationships between brain histamine (or histidine) and food intake have been reported. Histamine is a metabolite of histidine. When histidine is increased, histamine will also tend to increase due to increased rates of decarboxylation. Intracerebral infusion of histamine or histidine, feeding histidine, and intraperitoneal injection of histidine all elevate brain

**FIGURE 1** Effect of protein quantity on cumulative weight gain [panel A] and cumulative food intake [panel B] of rats [Experiment 1]. NP = normal protein and normal metabolizable energy. LP = low protein and normal metabolizable energy. Each point is the mean ± SEM, n = 6. Values on the same day not sharing a letter are significantly different, P < 0.05.

2B). The diet containing 50% less ME significantly lowered the specific [³H]-mepyramine binding to H₁ receptors compared with the diet containing normal ME [P < 0.0089][Fig. 4B].

**Experiment 3.** The dietary treatments had a significant effect on weight gain, food intake and H₁ receptors. Rats fed the HQ diet ate more food than rats fed MQ and LQ diets [P < 0.0001][Fig. 3A] even though protein concentration was the same in each diet. Rats fed the HQ gained more weight than rats fed the MQ and LQ diets [P < 0.0001][Fig. 3B]. Whole-brain H₁ receptor concentrations were significantly higher for rats fed LQ protein or MQ protein than rats fed the HQ diet [P < 0.0001][Fig. 4C].

Converting food intake data from all three experiment data to grams intake per day [to compare experiments of different length] and graphing the results as daily food intake vs. brain H₁ concentration demonstrated a significant inverse relationship [P < 0.001] [Fig. 5].

**FIGURE 2** Effects of different levels of metabolizable energy on cumulative weight gain [panel A] and cumulative food intake [panel B] of rats [Experiment 2]. NE = normal protein and normal metabolizable energy. LE = normal protein and 25% less metabolizable energy. VLE = normal protein and 50% less metabolizable energy. Each point is the mean ± SEM, n = 6. Values on the same day not sharing a letter are significantly different, P < 0.05.
histamine and depress food intake in rodents [Fukagawa et al. 1989, Itowii et al. 1988, Sheiner et al. 1985]. Cerebroventricular infusion of a histamine H₁ receptor antagonist (resulting in increased synthesis and release of brain histamine) depresses feeding in rats [Machidori et al. 1992]. On the other hand, reduction of neuronal histamine by blocking histidine decarboxylase with the enzyme inhibitor α-fluoromethylhistidine elicits feeding in rats [Watanabe and Wada 1991]. An inverse correlation was also reported by Mercer et al. (1989b) who demonstrated that food intake could be predicted as a function of whole-brain histidine concentration.

Protein-deficient diets increase serum histidine, brain histidine and brain histamine which correlates with decreased food intake and weight gain [Lunn and Austin 1983a, Mercer et al. 1989b]. Other studies with protein-deficient animals report elevated brain histidine or histamine with unaltered serum histidine [Enwonwu and Okadigbo 1983, Enwonwu and Worthing-

The previous studies indicate a reciprocal effect: brain histamine concentrations can affect food intake, and dietary patterns can affect brain histamine levels. Our studies were designed to extend the investigation to central nervous system H₁ receptors, dietary effects and their relationship to food intake.

In the first experiment, a low protein diet elevated H₁ receptor concentration. It is already known that low protein diets result in increased brain histidine and histamine concentrations, which, in turn, are associated with decreased food intake and weight gain [Lunn and Austin 1983b, Mercer et al. 1989a]. Increased systemic

![Graph A](image1.png)

**FIGURE 3** Effects of protein quality on cumulative weight gain (*panel A*) and cumulative food intake (*panel B*) of rats (Experiment 3). HQ = normal protein and normal metabolizable energy. LQ = low quality protein and normal metabolizable energy. MQ = medium quality protein and normal metabolizable energy. Each point is the mean ± SEM, n = 6. Values on the same day not sharing a letter are significantly different, *P* < 0.05.

![Graph B](image2.png)

**FIGURE 4** Central nervous histamine receptor [H₁] concentrations of rats fed different quantities of protein (*panel A*), different levels of energy (*panel B*) and different quality proteins (*panel C*). NP, NE and HQ = normal protein, normal metabolizable energy and high quality protein. LP = low protein and normal metabolizable energy. LE = normal protein and 25% less metabolizable energy. VLE = normal protein and 50% less metabolizable energy. LQ = low quality protein and normal metabolizable energy. MQ = medium quality protein and normal metabolizable energy. Each point is the mean ± SEM, n = 6. Bars not sharing a letter are significantly different, *P* < 0.05.
histidine due to low protein intake occurs via several possible mechanisms: the increased catabolism of protein, the decreased catabolism of histidine in the liver, the catabolism of carcinosine, and/or a decrease in the 1-carbon metabolic pathway (Mercer et al. 1990). However, the histidine/histamine system is regulated in the liver by catabolism of histidine, which reduces central histamine synthesis. Central \( H_1 \) receptors have another, as yet unknown regulatory system. This experiment indicates that in male rats fed low protein diets, \( H_1 \) receptors are elevated concurrently with histidine and histamine, producing the likelihood of greatly increased histaminergic activity.

The results of the second experiment indicated that feeding low energy diets to the rats decreased \( H_1 \) receptor binding while increasing their voluntary food intake. At this time, the mechanism by which low energy diets cause a decrease in the histaminergic receptors is uncertain. However, we have shown that rats fed restricted diets have decreased numbers of \( H_1 \) receptors (Mercer et al., in press). Also, Hwang and Guntz (1996) showed decreased mRNA for corticotropin-releasing factor in food-deprived rats, indicating decreased histaminergic activity. These observations are consistent with our hypothesis.

The feeding behavior of the rats in the third experiment was similar to that of rats fed the low protein diet. Low quality protein diets formulated from wheat gluten or gelatin, both of which have poor biological value, increased whole-brain \( H_1 \) binding, indicating the probable importance of a single amino acid or group of amino acids in the regulatory mechanism of \( H_1 \) trafficking. We have shown that brain histidine increases rapidly in rats fed diets containing \(< 12 \text{ g} / 100 \text{ g casein} \) or diets containing low levels of essential amino acids (Mercer et al. 1989a). This means that not only the protein quantity but also protein quality increase both central nervous system histidine and \( H_1 \) receptor concentrations and decrease voluntary food intake.

Comparing and graphing the results of all three experiments shows a linear, inverse relationship \( (P < 0.001) \) between central nervous system \( H_1 \) receptor concentrations and food intake in rats over a wide range of dietary treatments (Fig. 5). We have shown that both \( H_1 \) receptor concentrations and food intake patterns are modified after rats are fed experimental diets for 1 d (Bundrant et al. 1996). These experiments indicate that \( H_1 \) modifications are specific, low energy diets decrease receptors and increase food intake, whereas low or poor quality protein diets have the opposite effect and have the same time frame. Also, dietary composition has an impact on whole-brain histamine \( H_1 \) receptor binding, correlating with normal eating patterns, hypophagia and hyperphagia.

We have discussed dysregulation of the histaminergic system and its possible involvement in the etiology of eating disorders (Mercer et al. 1996). Histaminergic responses are gender-specific (Mercer et al., in press), with females having higher "normal" \( H_1 \) receptor concentrations than males and reacting differently to dietary manipulation. Subjects with anorexia have at least ten physiologic symptoms which indicate abnormal histaminergic activity, and over 90% of eating disorders are found in females (Mercer et al. 1996). While many factors impinge upon eating behavior, central histaminergic activity seems to be directly involved in food intake patterns in rats. Low protein diets produce anorexia and activate the histaminergic system, whereas low energy diets produce hyperphagia and depress the histaminergic system.

**FIGURE 5** Food intake as a function of central nervous system histamine receptor \( H_1 \) concentration in rats fed low protein, low quality protein and low energy diets vs. normal diet. LP = low protein and normal metabolizable energy. NP, NE and HQ = normal protein, normal metabolizable energy and high quality protein. LP = low protein and normal metabolizable energy. LE = normal protein and 25% less metabolizable energy. VLE = normal protein and 50% less metabolizable energy. LQ = low quality protein and normal metabolizable energy. MQ = medium quality protein and normal metabolizable energy. Each point is the mean ± SEM, \( n = 6 \).

**LITERATURE CITED**


lately feeding behavior through H₁-receptor in rat hypothalamus. Am. Physiol. Soc. 89: R605-R611.


