Plasma growth hormone secretion is impaired in obesity-prone rats before onset of diet-induced obesity

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Lauterio, Thomas J., Ariel Barkan, Mark DeAngelo, Roberta Demott-Friberg, and Ray Ramirez. Plasma growth hormone secretion is impaired in obesity-prone rats before onset of diet-induced obesity. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E6–E11, 1998.—Sprague-Dawley rats, which become obese (obesity prone) when fed a moderately high-fat (MHF; 32.5% of kcal as fat) diet, have decreased growth hormone (GH) concentrations compared with obesity-resistant rats fed the same diet. To determine whether plasma GH concentrations are different in obesity-prone rats compared with obesity-resistant rats before diet-induced obesity occurs, total integrated GH concentrations were determined in male Sprague-Dawley rats before exposure to the MHF diet. After initial blood sampling, rats were fed an MHF diet for 15 wk, over which time the animals were separated into two discrete populations based on body weight gain. Analysis of GH in episodic blood samples showed that the obesity-prone group had a GH secretion deficit before the onset of obesity (115.2 ± 12.9 ng·ml⁻¹·200 min⁻¹) compared with obesity-resistant rats (237.2 ± 47.1 ng·ml⁻¹·200 min⁻¹). The GH concentration difference was due to a decrease in mean GH peak height in rats that later became obese (34.8 ng/ml) compared with rats that remained lean (74.2 ng/ml). The results suggest that GH secretion impairment exists before dietary challenge or onset of obesity and may contribute to the susceptibility to obesity observed in these animals.

episodic growth hormone secretion; dietary fat

OBESITY IS one of the most prevalent diseases in the United States, affecting an estimated 25–35% of the population (9, 17). However, factors that contribute to the susceptibility of the disease are only recently coming to light. The task of identifying causative or contributory factors is made more difficult because of the multietiologic nature of obesity and the multiple metabolic perturbations that occur in obese individuals.

Because it is difficult to study susceptibility to obesity in humans, animal models have been utilized to acquire information that would not otherwise be obtainable. These models include genetically obese animals [Zucker rat, ob mouse (34)], surgically induced obese animals [ventromedial hypothalamus-lesioned rat (10, 33)], spontaneously obese animals [rhesus monkey (6)], and diet-induced obese rat and mouse (21, 29, 30, 46). However, perhaps the most relevant of these models with regard to human obesity are those in which obesity develops in response to increased dietary fat. In particular, the dietary model originated by Levin et al. (29), which was later developed into a purified diet (26), is of considerable interest because it allows one to examine resistance and susceptibility to obesity. In this paradigm, rats fed a moderately high-fat (MHF) diet (32.5% of kcal as fat) diverge into distinct populations based on body weight gain. Approximately one-half of the rats fed this diet will gain weight rapidly compared with Chow-fed or control (low fat) rats. The remaining one-half, on the other hand, will gain body weight at a rate that is equivalent to or lower than control fed animals. The former group is referred to as obesity prone (OP), whereas those in the latter group are referred to as obesity resistant (OR). Work in our laboratory has focused on characterizing this model with regard to the metabolic and endocrine status of OP and OR rats (26, 27).

One of the endocrine abnormalities observed in the diet-induced obese model is decreased circulating levels of growth hormone (GH) in the obese compared with the OR rats (27). This reduction in GH concentration is also a consistent finding in obese humans (5, 14, 19, 25). The diet-induced obese rat also has a decreased somatotroph response to growth hormone-releasing hormone (GHRH) (27), which parallels the impaired response to GH secretagogues observed in human obese individuals (13, 16, 24, 25). Because GH stimulates lipolysis (8, 11, 15, 41), it is feasible that lack of GH contributes to increased adipose accumulation in obesity. However, it is not known when GH concentrations change during the onset of obesity and what factors lead to these changes. The animal model described above affords us an opportunity to examine GH levels from the preobese state. Thus the primary objective of the following study was to determine whether plasma GH concentrations differ in OP rats compared with their OR counterparts before dietary changes or onset of obesity. If differences do not exist, subsequent studies could be aimed at elucidating the time point at which dietary or obesity changes affect GH levels. This would then allow studies to identify factors important in this metabolic perturbation. If differences in GH do exist before animals are fed the high-fat diet, further studies to examine the factors underlying these changes could be pursued.

METHODS

Animals. All procedures involving animals were approved by the Animal Care and Use Committee of Eastern Virginia Medical School. Twenty male Sprague-Dawley rats weighing between 300 and 350 g (Charles River Laboratories, Wilmington, MA) were individually housed in hanging stainless steel cages. Room temperature was controlled (22 ± 2°C), and lighting was on a 12:12-h light-dark cycle. Food and water
were provided ad libitum throughout the experiment. Initially, rats were maintained on standard laboratory chow (Purina Mills, St. Louis, MO) and were not switched to the experimental diet until after blood sampling occurred. Rats were cannulated in the jugular vein and allowed 1 wk of recovery before sampling. However, cannulas were aspirated, flushed with heparinized saline (50 IU/ml of heparin; Sigma, St. Louis, MO), and heplocked with 0.1 ml of heparin (500 IU/ml) daily to maintain patency.

On the day of sampling, extension cannulas were connected to the exteriorized jugular catheter to allow remote blood sampling. Blood samples (100 µl) were drawn in 1-ml heparinized syringes at 20-min intervals for a period of 200 min. All animals were sampled at the same time of day, between 0900 and 1300, to reduce influence of diurnal rhythms on GH secretion. A total of 11 samples was obtained from each animal. These samples were immediately centrifuged, and the plasma was stored at -20°C until assay for GH.

One week after the blood samples for GH analysis were obtained, the rats were fed a purified MHF diet (32.5% of kcal from fat; D12266, Research Diets, New Brunswick, NJ), described previously (26), ad libitum. Food intake and body weights were monitored weekly, with intake corrected for spillage. After divergence of rats into obese and OR populations, animals were killed by decapitation 15 wk after the MHF diet was initiated. Trunk blood was collected in K+-EDTA tubes, and plasma was stored at -20°C until analysis. Abdominal fat pads (perirenal, epididymal, and mesenteric) were excised, weighed, and frozen. Carcass weights were also obtained minus fat pads for calculation of the index of adiposity. Assays and calculations. Plasma GH levels were measured in duplicate by RIA, using the reference standard rat GH-RP2, and materials were obtained from National Institute of Diabetes and Digestive and Kidney Diseases as previously described (7). Radiolabeled GH was iodinated with 125I obtained from Amersham Life Sciences (Arlington Heights, IL). The mean detection limit was 1.3 µg/l, and the mean intra-assay covariance for GH concentrations was 20 µg/l. Samples with GH concentrations >20 µg/l were diluted and reassayed. Episodic GH concentrations were integrated over the 200-min sampling period, and cluster analysis in a 1 × 1 matrix was conducted to determine discrete parameters of GH pulsatility (22). Dietary obese and OR populations were determined by subjecting frequency plot of body weight gain to chi-square analysis as described previously (26). The seven animals with the lowest body weight gain were defined as resistant (OR), whereas the seven rats demonstrating the greatest weight gain were labeled OP. The index of adiposity was calculated as the sum of the fat pad weights (in g) divided by the carcass weight of the animal minus fat depots (in kg). Other data values, represented as means ± SE, were compared between the two groups by Student’s t-test (42).

RESULTS

Food intake, body weight, and composition data. Body weights of Sprague-Dawley rats fed the MHF diet are presented in Fig. 1. Initial body weights of the OP rats (385.5 ± 17.3 g) did not differ from those of the OR subgroup (387 ± 8.4 g), but after 4 wk of the MHF diet, differences in body weight became apparent. At that point, body weights of OP rats were 13% greater than those of the OR group (P = 0.025). Body weights continued to diverge over the 15-wk period, and final OP body weights were 35.5% greater than those of the OR group (P < 0.0001).

Food intake data are presented as total cumulative energy intake as well as relative food consumption (Table 1). OP rats consumed 32% more food over the course of the 15-wk dietary regimen compared with OR animals (P = 0.001). However, relative food consumption, calculated as food consumed per kilogram of body weight, was not different between groups at week 15. Consumption expressed in this manner also did not differ for weeks 1–14 (data not presented). Efficiency of weight gain, calculated as body weight gain (in g) divided by kilojoules of energy consumed times 10³, was 74% greater for OP (P < 0.0001) compared with OR rats for the entire study period.

Fat pad weights obtained from OP and OR rats at the end of the study are presented in Fig. 2. Every fat pad depot from OP rats weighed significantly more than the respective OR depot. The greatest difference was observed in the mesenteric depot, which was 2.6-fold heavier in the OP than in the OR animals (P < 0.0001). Perirenal fat pads differed by only twofold (P = 0.0014) and were the least-different depot in terms of weight. The epididymal depot and total fat depot weights were similarly 2.2-fold greater in the OR group (P < 0.0001).

Table 1. Food intake, relative food consumption, efficiency of weight gain, and thyroid hormone concentrations in rats fed a moderately high-fat diet for 15 wk

![Body weight](https://via.placeholder.com/150)

Fig. 1. Body weights of male Sprague-Dawley rats fed a moderately high-fat (MHF) diet for 15 wk. Data are means ± SE; n = 7 for both groups. Obesity-susceptible rats are labeled as obesity prone (OP), whereas those resistant to obesity are referred to as obesity resistant (OR). *Significantly different from OP body weight means at equivalent week (P < 0.05 or less) by Student’s t-test.

![Food consumption](https://via.placeholder.com/150)

Food consumed, g/kg body wt (week 15) 226.8 ± 5.7 218 ± 6.8
Food consumed × 10³ 1,573 ± 35,405 35,405 ± 542
Efficiency of wt gain, g body wt/kj food consumed 4.30 ± 0.25 4.89 ± 0.19
Total T₄, µg/dl 4.89 ± 0.19 3.88 ± 0.30
Free T₃, pg/ml 3.42 ± 0.17 2.94 ± 0.4

Data are means ± SE; n = 7. Relative food consumption, calculated for week 15, and free triiodothyronine (T₃) concentrations did not differ between obesity-prone and obesity-resistant groups. T₄, thyroxine. Significantly different from obesity-prone rats: *P = 0.001, †P = 0.0001, ‡P = 0.0145.
for both). The index of adiposity reflected the increased body fat content of the OP animals and was 67% higher in the OP compared with the OR rats \((P < 0.0003, \text{Fig. 3})\).

GH secretion data. Total integrated plasma GH values are presented in Fig. 4 for the 200-min blood-sampling period. For the equivalent time, GH released was 2.1-fold greater in the OR rats than in the OP group \((P = 0.037)\). The GH peak height (Fig. 5) was determined also to be 2.1-fold greater in the OR group \((P = 0.025)\). There were no differences in GH peak frequency, peak duration, or mean nadir level between groups. Representative GH secretion profiles for both OP and OR groups are shown in Fig. 6.

**DISCUSSION**

This study further characterizes the diet-induced obese rat model with regard to GH deficits. As in previous experiments, Sprague-Dawley rats fed the MHF diet (26) diverged into OP and OR populations. The data reported here suggest that these groups differ metabolically even before MHF challenge, which accounts for their end-point differences in body weight and fat content. Earlier data (27) have demonstrated lower GH levels in the obese subpopulation. These data led to the question of how much of that difference is induced by the dietary fat vs. being due to a different endocrine or metabolic status before dietary fat challenge. In other words, does the dietary fat somehow differentially trigger endocrine responses in the OP rats that are different from those of OR animals, or does the endocrine status of the preobese rats determine how dietary fat will be utilized? The data obtained in this study would suggest that the latter situation occurs.

Two responses observed in the OP group may be the result of GH deficiency rather than the cause of suppressed GH secretion. These are the increased food efficiency and increased adiposity observed in the OP rats. Both of these responses have been shown in many species, including humans, to be regulated by GH concentrations. For example, porcine somatotropin administration to swine increases feed efficiency while dramatically decreasing carcass fat deposited com-

**Fig. 2.** Total and individual (mesenteric, perirenal, and epididymal) fat pad weights of male Sprague-Dawley rats fed an MHF diet for 15 wk. Data are means ± SE; \(n = 7\) for both groups. *Significantly different from mean of equivalent OP group fat depot \((P < 0.05\) or less) by Student’s t-test.

**Fig. 3.** Index of adiposity \((g\text{ total fat/kg carcass wt} - \text{fat})\) calculated from rats fed an MHF diet for 15 wk. Data are means ± SE; \(n = 7\) for both groups. *Significantly different compared with OP group \((P = 0.0003)\) by Student’s t-test.

**Fig. 4.** Total integrated growth hormone (GH) secretion in obesity-susceptible (gainers) and OR (resisters) Sprague-Dawley rats over a 200-min sampling period before placement of animals on an MHF diet. Data are means ± SE; \(n = 7\) for both groups. *Significantly different compared with gainers \((P = 0.037)\) by Student’s t-test.

**Fig. 5.** Mean peak GH concentrations in obesity-susceptible and OR Sprague-Dawley rats during a 200-min blood-sampling period before placement of animals on an MHF diet. Data are means ± SE; \(n = 7\) for both groups. *Significantly different compared with gainers \((P = 0.025)\) by Student’s t-test.
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GH SECRETION IS INHERENTLY IMPAIRED IN OBESITY-PRONE RATS

In this experiment, as calculated, the fat gain appears to be greater than suggested by the index of adiposity. This may be due, in part, to increased lean body mass on the part of the OP rats, but body composition was not determined in this study. Plasma leptin concentrations also did not differ initially between preobese and resistant, although they were different at the termination of the experiments after divergence of body weight (unpublished data).

Although it is still not established that GH is a causative factor in the increased fat deposition, differences in GH before the initiation of the diet strengthen the possibility for direct GH involvement. Our laboratory and others have demonstrated that plasma insulin levels often increase along with circulating glucose concentrations over the course of the dietary treatment (27, 28, 31). However, we observed neither hyperglycemia nor hyperinsulinemia in OP rats before feeding the Sprague-Dawley rats the MHF diet. Thus, although it is possible that hyperinsulinemia leads to greater adiposity, it is more likely that decreased GH secretion initiates divergent body weight response to the dietary fat. Moreover, GH decreases the effect of insulin on glucose oxidation in adipose tissue in vitro (37). In the animal model characterized here, the GH deficit in preobese rats may lead to an enhanced response of fat cells to insulin. Because caloric density is increased along with fat content in the MHF diet, increased insulin effectiveness along with increased substrates could initiate fat cell size increases in obesity-susceptible rats.

In addition to the possible enhancement of the effect of insulin, a hypothesis that needs to be examined more closely is that lack of GH in the OP rats reduces the lipolytic capacity or response of the adipose tissue. If the presence of adequate GH concentrations is necessary for normal levels of lipolysis, a reduction of GH levels may lead to decreased ability of the OP rats to utilize fat stores compared with those that remain lean. If this is true, regardless of whether there is an enhancement of insulin’s action, the fat that is stored would be less likely to be drawn on to meet energy needs in OP compared with OR rats. GH is a potent stimulator of lipolysis, and it is capable of mobilizing fat stores for energy instead of glucose (8, 15, 38–41). This makes GH an effective agent for improving body composition in humans. However, most studies have focused on effects of administration of GH to the already obese individual rather than determining its role in the etiology of obesity. The diet-induced obese rat model provides an opportunity to examine that question in a prospective manner.

Another effect of GH is to regulate metabolic rate (20, 36). Whereas increased insulin effectiveness or decreased lipolysis are specific changes in nutrient utilization, the metabolic effect of GH is a general one. Decreased GH leads to a reduction in energy expenditure, which, in turn, would also help increase efficiency of fat deposition. With the assumption of equal caloric

pared with noninjected littermates (43). Porcine somatotropin injections also increase lean muscle mass in swine, conserving protein while utilizing fat for energy needs (23). This is also confirmed by the decreased urinary nitrogen excretion observed in swine administered porcine somatotropin (23). Thus fat is preferentially used as fuel, whereas protein is stored to account for increased efficiency. However, in the case of the OP rats, it would appear that increased food efficiency is tied to decreased rather than increased GH concentrations. This might be explained by the nature of the weight gain in the obese rats vs. the GH-injected swine. In the former case, increased body weight gain appears to be in the form of fat, especially the abdominal depot (Fig. 2). Regardless of whether the fat is expressed as absolute weight or relative to body size, the fat depots of the OP rats significantly exceed those of the resistant rats. The index of adiposity data further confirms the body fat accumulation in obese animals (Fig. 3). This index has been shown to correlate extremely well with body composition analysis data in mice (46), although we and others have successfully utilized this index for other species (28). One possible explanation for the difference in efficiency of weight gain may be that the adipose cells of the OP rats more easily take up circulating lipids than those from resistant rats. Because the MHF diet differs from chow or control diets in the percentage of fat employed, both prone and resistant rats would have had exposure to greater-than-normal levels of circulating lipids in this study than with less calorically dense diets. A greater proportion of these circulating fats was deposited in the depots of the OP animals compared with those that remained lean.

Fig. 6. Plasma GH concentrations over sampling period are presented for an OR (animal no. 517; A) and an OP (animal no. 510; B) rat. Samples were obtained before placing rats on MHF diet.
intake in OP and OR animals per gram of body weight, decreased energy expenditure of the OP group would provide more energy substrates for storage as fat. It would be important to examine whether prone and resistant animals differ in energy expenditure, as measured by indirect calorimetry, before exposing the animals to the MHF diet.

There are other possible relationships between GH and lipolysis that have not been explored here. Aside from altering lipid metabolism, GH also changes the fat cell response to epinephrine, which may in turn affect adipose cell size and lipolytic sensitivity (2). The effect of GH on hormone-sensitive lipase has also been well characterized (15), and the differential regulation of this enzyme in the two groups may explain, at least in part, the resistance to obesity observed in some of the Sprague-Dawley rats fed the MHF diet. Other studies have shown a role for insulin-like growth factor I, a GH-dependent hormone, in body fat and lipid mobilization (4, 12). However, it is not possible to determine to what degree each of these factors plays a role in the diet-induced obese model at this time. Future studies should surely explore these mechanisms, especially considering these data.

Finally, the reason for the decreased GH secretion in preobese rats is unknown. Because frequency and duration of GH pulses did not differ between the two groups, the deficit is likely to involve mechanisms for GH storage and/or secretion rather than one regulating pulsatility. Potential mechanisms would include inducers or inhibitors of GH synthesis and storage, or composition and amount of releasing or inhibiting factors. GHRH is one peptide that affects both synthesis and secretion (1, 18, 44, 45). Thus GHRH or a similar factor would be a good candidate on which to focus. The attenuated GH pulse amplitude in rats destined to become obese may also suggest higher somatostatinergic tone. Conversely, there are a number of hypothalamic factors, including neuropeptide Y and galanin, that alter GH secretion as well as regulate metabolism and food consumption. Finally, it is possible that a neuroendocrine abnormality exists that causes the predisposition to body weight gain and concurrently decreases GH secretion. Thus lower GH may be just a marker rather than the cause of increased obesity. If this is the case, GH levels would still be useful as a marker to help determine the events initiating the diet-induced obesity in response to elevated dietary fat. The model of diet-induced obesity described here allows for a systematic and prospective analysis of the discrete neuroendocrinological mechanisms leading to obesity.

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