Growth Hormone Secretion and Synthesis Are Depressed in Obesity-Susceptible Compared With Obesity-Resistant Rats

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Human obesity is characterized by a low basal growth hormone (GH) concentration and a blunted response to GH secretagogues. The aim of this experiment was to determine whether a perturbation in GH synthesis or secretion occurs in rats that develop obesity only in response to a dietary fat challenge. Female Sprague-Dawley rats were fed a purified 32.5% fat diet ad libitum for 21 weeks. Approximately half of the rats fed this diet developed obesity (obesity-susceptible) while the others remained lean (obesity-resistant) compared with chow-fed (control) animals. Pituitary glands obtained from all three groups were enzymatically dissociated, and somatotrope response to GH secretagogues and inhibitors was determined in vitro. Plasma GH concentrations were decreased in obesity-susceptible rats compared with obesity-resistant rats, and in vitro GH secretory response was blunted in cells obtained from the pituitary glands of obese compared with lean rats. In addition, pituitary GH content was reduced in obese versus lean rats even though the proportion of somatotropes in the two groups did not differ. Since the changes in GH concentration in this dietary obese rat model parallel those found in human obesity, this model may be useful in determining the relationship between GH and obesity.

OBESITY is a complex disease of multiple etiologies. The prevalence of obesity and its associated complications make it one of the costliest diseases in our society in terms of human health.[1] It is estimated that between 25% and 35% of the US population is obese,[2,3] and obese individuals are at greater risk for developing diabetes, atherosclerosis, cardiovascular disease, and stroke. The study of the development of obesity and its relationship to these other disease states is an area of intensive investigation.

One endocrine abnormality often associated with obesity is low circulating growth hormone (GH) concentrations.[4-7] Both basal and stimulated GH concentrations are decreased in obese individuals,[5,8,9] but both GH concentrations and the GH response to secretagogues approach normality when obese patients lose weight.[9-11] However, it is still unknown whether the perturbations in GH secretion are causally related to the obese state or whether they are secondary to the onset of obesity. It is known that GH is lipolytic and that GH administration to GH-deficient or obese patients improves body composition,[12,13] but the obverse (ie, GH secretion deficits result in fat deposition, leading to obesity) has not been proven. To fully evaluate the role of GH in obesity, one would need to assess the GH status in individuals before the onset of obesity to determine whether GH secretory abnormalities exist that predispose individuals to obesity.

One approach to resolving the role of GH in the susceptibility to or development of obesity is to use animal models. Genetically obese Zucker rats have been extensively studied with regard to endocrine abnormalities, and obese Zucker rats have decreased GH and thyroid hormone concentrations compared with their lean littermates.[14-16] Also, similar to humans, GH treatment of obese Zucker rats reduces body fat content.[17] Other animal models of obesity exhibit decreased GH secretion, including ventromedial hypothalamus–lesioned rats and rhesus monkeys.[18-20] However, perhaps the most relevant animal model with regard to human obesity is that which develops obesity in response to dietary changes in fat. One such model, extensively used by Levin et al,[21,22] is of particular interest because rats fed the identical diet diverge into obesity-susceptible and obesity-resistant populations. Often, the obesity-susceptible rats gain body fat without consuming or absorbing more energy than their obesity-resistant counterparts.[21] Our laboratory has recently developed a purified diet that mimics these divergent effects and that, because of its defined nature, can be micromanipulated to further examine the relationship between dietary components and endocrine responses.[23]

The purpose of this study was to determine whether the dietary obesity observed in this animal model is also accompanied by decreased GH concentrations. The divergent body weight response of these animals allows a determination of whether the effects observed are due to obesity per se or rather to the high-fat diet, since both obesity-susceptible and obesity-resistant animals are fed the same diet. In vitro experiments were conducted to determine the contribution of the pituitary versus the hypothalamus to this GH deficit. A secondary objective was to ascertain whether this divergent body weight response is observed in slower-growing female rats. All previous experiments using this model have been conducted exclusively in male rats.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing approximately 300 g were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were individually housed in a temperature- and light-controlled room (21° ± 1°C; lights on 7 AM to 7 PM) in hanging stainless steel cages with food and water available ad libitum. One third of the rats were then fed a control diet (AIN76A, Diet #D10001) and the remainder were fed a purified moderately high-fat (MHF) diet (Diet #D12266; Research Diets, New Brunswick, NJ) for 21 weeks. The MHF diet, the

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composition of which has been described in detail elsewhere,[23] contains 32.5 kcal% fat, 15.1 kcal% protein, and 52.4 kcal% carbohydrate. Food intake and body weight were measured on a weekly basis with corrections for food spillage. Over the course of the MHF dietary regimen, rats diverged into two groups based on body weight gain. Approximately half of the rats fed the MHF diet gained weight at a rate that was similar to the rate of those fed the AIN76A control diet, while the rest gained weight at an accelerated rate. Frequency distribution analysis of body weight gain demonstrated the existence of two distinct populations by chi-square test and Student's *t*-test.[24] Rats that gained weight at an accelerated rate were referred to as gainers, while those remaining lean on the MHF diet were referred to as resisters. Seven animals from each of the three experimental groups were used in the following studies. These rats were killed by decapitation following a 4-hour fast, and blood was obtained for plasma in a K^+/EDTA-coated tube (Sarstedt, Princeton, NJ). Rats were killed in sets of three (one from each group) to avoid temporal differences in hormone or metabolite profiles. An aliquot of plasma was refrigerated at 4°C for glucose analysis by a YSI biochemical analyzer (Yellow Springs Instruments, Yellow Springs, OH), and the remainder was frozen (−20°C) until assayed for hormones. Pituitary glands were quickly excised after decapitation for use in the studies described later, and abdominal fat pads (retroperitoneal and epididymal) were removed and weighed. This study was conducted in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and with the oversight of the Eastern Virginia Medical School veterinarian and Animal Care and Use Committee.

Materials

Eagle's minimum essential medium α-modification (αMEM), MEM containing spinner culture salts (sMEM), penicillin, fetal bovine serum, normal rabbit serum, normal goat serum, and streptomycin were purchased from GIBCO (Grand Island, NY). Trypsin (crude, 1:250) was obtained from Difco Laboratories (Detroit, MI). Bovine serum albumin ([BSA] fraction V), HEPES, $NaHCO_3$, deoxyribonuclease (DNase I), diaminobenzidine, somatostatin (SRIF), and gentamicin sulfate were obtained from Sigma Chemical (St Louis, MO). Insulin-like growth factor-I (IGF-I) was purchased from Upstate Biotechnology (Lake Placid, NY), and rat GH-releasing factor was obtained from Bachem (Torrance, CA).

Enzymatic Treatment of Tissue

Sterile conditions were maintained throughout this procedure. Each adenohypophysis was placed in sMEM (pH 7.35) supplemented with 0.3% BSA, 25 mmol/L HEPES, 0.2% $NaHCO_3$, 25 μg/mL gentamicin sulfate, 100 U/mL penicillin, and 100 μg/mL streptomycin (sMEM/BSA). Individual cells from intact tissue were obtained using a modified method of Wilfinger et al.[25] Each gland was cut into small blocks that were placed in sMEM/BSA containing trypsin (0.3%) and DNase I (20 μg/mL). Tissue blocks were incubated (75 minutes) with constant gyratory shaking (60 rpm) in a humidified chamber equilibrated with 5.0% CO_2 and air. After incubation, tissues were gently triturated with a flame-tapered Pasteur pipette. Dispersed cells were washed once with sMEM before counting. Mean values for cell yield were determined by hemacytometry. Phase-contrast microscopy was used to assess cell viability, which was consistently greater than 90%.

Cell Culture and Secretagogue Studies

Cells (2.5×10^5) contained in sMEM were allowed to attach to a plastic surface (24-well tissue culture plate). After 1 hour, the medium was replaced with αMEM supplemented with 10% fetal bovine serum and the cells were cultured for 48 hours. Following culture, cells were exposed (2 hours) to fresh αMEM containing the test agents; spent medium was centrifuged, and supernatant solutions were stored at −70°C. To determine the amount of GH contained in cells obtained from rats in each of the treatment groups, cells attached to culture wells were frozen, thawed, and sonicated briefly in 0.01 mol/L $NaHCO_3$. Extracts were centrifuged ($10,000 \times g$ for 15 minutes), and supernatant solutions were stored for assay.

GH Immunocytochemistry

GH cells were identified according to a modified method of Denef et al.[26] Cells (2.0×10^5) suspended in sMEM (50 [67Ud] mL) were allowed to attach to glass cover slips for 1 hour at 37°C. After this step, the cells were fixed (1 hour at 22°C) with 4% Formalin in phosphate-buffered saline ([PBS] pH 7.3), washed three times, and then stored at 5°C. Samples from all three groups (controls, gainers, and resisters) were processed simultaneously. Cells were initially incubated (1 hour) in PBS containing 5% normal goat serum and then exposed (5°C) to anti–rat GH serum (1:5,000; NIH) prepared in PBS containing 0.07% BSA and 0.1% Triton X-100. The control consisted of replacing the primary antiserum with normal rabbit serum (1:5,000). On the following day, cells were exposed (1 hour at 22°C) to goat anti–rabbit horseradish peroxidase (1:500). Reaction product was developed (10 minutes) with 10 mg diaminobenzidine in Tris-NaCl containing 0.006% H_2O_2. Cover slips were mounted on microscope slides before viewing. A minimum of 500 cells were counted under oil immersion and bright-field illumination to determine the frequency of somatotropes.

Assays

Primary antiserum for GH radioimmunoassay and somatotrope labeling (described earlier) was obtained through the National Hormone and Pituitary Program (NHPP) of the NIH. Standards for the rat GH assay were dilutions of the NHPP reference preparation (RP-2). Iodinated GH for the assay was obtained from Amersham Life Sciences (Arlington Heights, IL). All other components for the assay were purchased from Linco (St Louis, MO), and the assay was conducted according to the procedure of Kacsoh et al.[27,28] Plasma insulin level was measured using a homologous radioimmunoassay with components and ^{125}I-labeled human insulin obtained from Linco according to their enclosed protocol.

Statistics

The data are presented as the mean ± SE in all cases. Student's *t* test was used to confirm that separate populations existed for gainers and resisters.[24] Bartlett's test of homogeneity of variances was calculated for each comparison to ensure that variances of the treatment groups did not differ before calculation of ANOVA. The three experimental groups ($n = 7$ per group) were then compared using ANOVA and Tukey's multiple comparison test at each time point or within each treatment as appropriate.[24]

RESULTS

Food Intake, Body Weight, and Efficiency Data

Initial and final body weights are presented for all three groups in Table 1. Although initial body weights for the groups did not vary, the mean final body weight of gainers was 15% greater than that of controls and 22% greater than that of resisters ($P < .001$). Food intake measured in grams was similar for gainers and controls, but intake was greater in these groups compared with resisters ($P < .001$ and $P < .01$, respectively). However, because the MHF diet has a higher energy density per gram than the AIN76A diet, the mean energy intake of resisters and controls was not different. The energy intake of gainers (cumulative kilojoules) was 11.6% greater than that of

Table 1. Food Intake and Body Weight Data of Gainers, Resisters, and Controls

Parameter	Controls	Gainers	Resisters
Cumulative food intake			
g	2,105 ± 28	2,075 ± 32	1,875 ± 40*†
kJ	34,943 ± 466	39,010 ± 602*‡	35,250 ± 752
Body weight (g)			
Initial	305 ± 3	303 ± 4	302 ± 4
Final	375 ± 9	433 ± 8§	356 ± 8

NOTE. Data are the mean ± SE.
*$P < .001$ *v* controls.
†$P < .01$ *v* gainers.
‡$P < .01$ *v* resisters.
§$P < .001$ *v* controls and resisters.

animals fed the control diet ($P < .01$) and 10.6% greater than that of the resisters that were also fed the MHF diet.

The efficiency of weight gain is an index of how efficient an animal is at depositing nutritional substrates as body mass. It can be calculated as body weight gain in grams divided by food intake in grams or body weight gain divided by food intake in kilojoules. Both of these calculations are listed in Table 2. Efficiency of weight gain was approximately twofold greater in gainers compared with either controls or resisters when calculated on a gram/gram basis ($P < .001$). Efficiency expressed as grams of body weight divided by kilojoules of energy consumed was still greater in gainers compared with controls ($P < .01$) or resisters ($P < .001$); however, the increases calculated in this manner were 70% and 123%, respectively. Both measures indicate that gainers were different at using the dietary substrates available to them over the experimental period. Abdominal adipose tissue weights expressed per kilogram body weight were also greater in gainers than in controls (26%, $P < .05$) or resisters (23%, $P < .05$). This measure indicates that the body weight increases realized by the gainers were not solely due to increases in nonadipose (muscle, bone, etc.) tissues and that the animals differed in the degree of fatness.

Hormone and Metabolite Data

To assess metabolic responses between animals fed control and MHF diets and between gainers and resisters, plasma concentrations of insulin, glucose, and GH were measured (Table 3). Although gainers achieved numerically higher insulin

Table 2. Efficiency of Weight Gain and Abdominal Adipose Tissue Weight of Gainers, Resisters, and Controls

Parameter	Controls	Gainers	Resisters
Efficiency of gain			
g/g × 10^3	32.8 ± 1.4	63.2 ± 5.6*	28.4 ± 2.2
g/kJ × 10^3	1.98 ± 0.23	3.36 ± 0.3†‡	1.51 ± 0.12
Adipose tissue weight (g)/body weight (kg)	20.5 ± 1.2	25.8 ± 1.4§	21.0 ± 0.8

NOTE. Data are the mean ± SE.
*$P < .001$ *v* controls and resisters.
†$P < .01$ *v* controls.
‡$P < .001$ *v* resisters.
§$P < .05$ *v* controls and resisters.

Table 3. Terminal Plasma Insulin and Glucose Concentrations of Gainers, Resisters, and Controls

	Controls	Gainers	Resisters
Insulin (ng/mL)	4.2 ± 0.5	4.6 ± 0.5	3.7 ± 0.4
Glucose (mg/dL)	110 ± 3	121 ± 4*	118 ± 3

NOTE. Data are the mean ± SE.
*$P < .05$ *v* controls.

levels than the other two groups, the mean insulin concentration did not differ significantly across treatment groups. Glucose concentrations of gainers did not differ from those of resisters, but were significantly increased over control levels ($P < .05$). Plasma glucose concentrations of resisters were intermediate and did not differ from control levels.

Terminal plasma GH concentrations (Fig 1) were 60% lower in gainers compared with resisters ($P < .01$) and 54% lower in gainers compared with controls ($P < .05$). However, plasma GH concentrations of resisters and controls did not differ.

Cell Culture Data

A dose-response curve for pituitary cells obtained from all three treatment groups was generated using three concentrations of GH-releasing hormone ([GHRH] 0.5, 5, and 50 ng/mL). MEM without GHRH was used to determine basal GH release for the 2-hour incubation period. For all three treatment groups, GH secretion from dispersed pituitary cells increased with increasing secretagogue concentration (Fig 2). The GH secretory response of pituitary cells obtained from gainers was significantly less than that of resister cells under basal and all stimulated conditions. This decrease in GH secretion ranged from 38% ($P < .05$ at 0.5 ng/mL GHRH) to 56% (for basal release using MEM media only, $P < .01$). GHRH-stimulated or basal GH secretion from control animal pituitary cells was intermediate and did not differ from that for gainers or resisters.

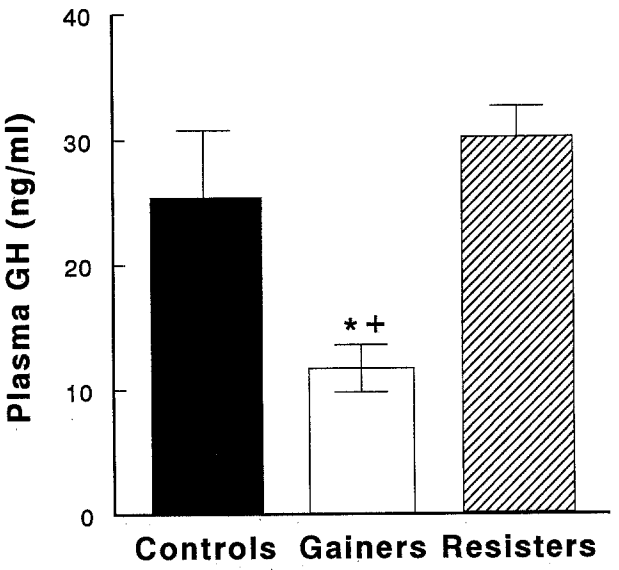

Fig 1. Plasma GH concentrations in rats fed a MHF or control (AIN76A) diet for 21 weeks. Plasma samples were obtained from a terminal trunk bleed, and all were assayed in a single radioimmunoassay. *$P < .05$ *v* controls; †$P < .01$ *v* resisters. Data are the mean ± SEM (n = 7) for each treatment.

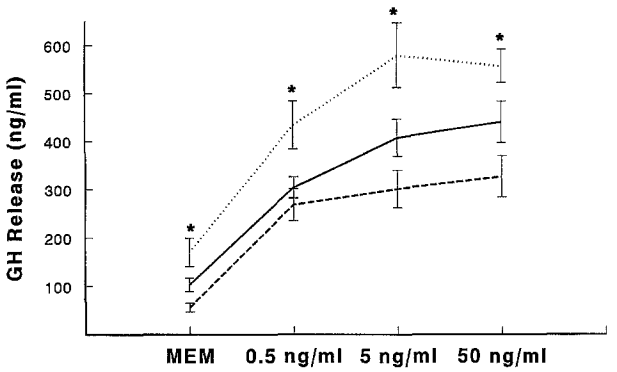

Fig 2. GH concentrations in media collected from pituitary cell cultures in the presence of varying concentrations of GHRH. Pituitaries were collected from rats fed MHF or control diets for 21 weeks. Data are the mean ± SEM (n = 7) for each treatment. (—) Controls; (---) gainers; (....) resisters. *$P < .05$ v gainers at the same GHRH dose by ANOVA and Tukey's multiple comparison test.

To determine whether inhibitors of GH secretion would exert a differential effect on somatotropes obtained from the treatment groups, dispersed cells were incubated in the presence of IGF-I, SRIF, or both, with and/or without GHRH (Fig 3). The greatest GH secretory response was observed for cells exposed to GHRH and GHRH + IGF-I regardless of the group from which the cells were obtained. SRIF effectively blunted GHRH-stimulated GH secretion whenever the two agents were combined. Perhaps most important, for each secretagogue/inhibitor treatment group, GH secretion was lower for somatotropes obtained from gainers than for those obtained from resisters. This decrease was most pronounced in the GHRH + SRIF treatment, where GH secretion from gainer somatotropes was 68% lower than GH secretion from resister somatotropes ($P < .001$). However, in this treatment, GH secretion from gainer somatotropes did not differ from that of control somatotropes. GH released in response to GHRH + IGF-I or in the presence of SRIF alone was also lower in gainers than in resisters by 37% ($P < .05$) and 62% ($P < .01$), respectively.

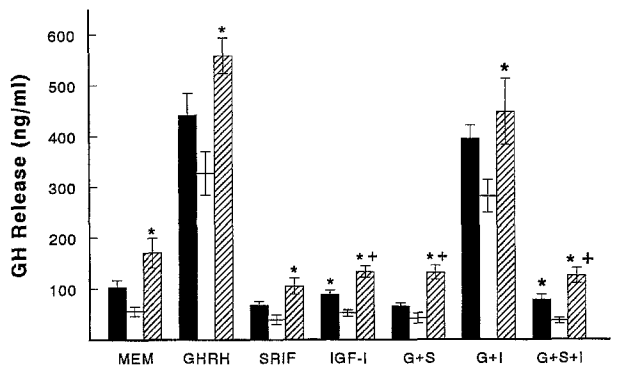

Fig 3. GH concentrations in media collected from pituitary cell cultures in the presence of medium only (MEM), GH-releasing factor (GHRH or G, 50 ng/mL), IGF-I (IGF-I or I, 50 ng/mL), SRIF (SRIF or S, 50 ng/mL), or combinations of these agents. Pituitaries were collected from rats fed MHF or control diets for 21 weeks. Data are the mean ± SEM (n = 7) for each treatment. (■) Controls; (□) gainers; (▨) resisters. *$P \leq .05$ v gainers by ANOVA and Tukey's multiple comparison test. †$P \leq .05$ v controls.

But in these treatments, the amount of GH released from control somatotropes was intermediate to that of both gainer and resister somatotropes and did not differ significantly from either group. Finally, for both GHRH + IGF-I + SRIF and IGF-I alone, three different levels of GH secretion were observed for the three different animal groups. Somatotropes obtained from gainers released the lowest amount of GH in response to these secretagogues/inhibitors, whereas somatotropes obtained from controls and resisters exhibited greater GH secretion to the same agents. In the case of GHRH + IGF-I + SRIF treatment, GH secretion of control somatotropes was 2.1-fold greater than that of gainer somatotropes ($P < .05$), whereas GH secretion was 3.4-fold greater in resister pituitary cells than in those of gainers ($P < .001$). Additionally, GH secretion for resister somatotropes was 59% greater than that observed for control cells ($P < .05$). For IGF-I alone, the amount of GH released from resister somatotropes was 2.6-fold greater than the amount of GH released from gainer somatotropes ($P < .001$). Similar to the GRF/SRIF/IGF-I treatment, the amount of GH secreted from control somatotropes was intermediate to that of the other groups, being 69% greater than the amount released from gainer cells ($P < .01$) but still 34% less than that secreted from resister cells ($P < .05$).

The cell yield and percent somatotrope data presented in Table 4 show that the number of cells obtained from gainer pituitaries was greater ($P < .001$) than that obtained from either resister or control pituitaries. However, the proportion of cells identified as somatotropes within the pituitary was similar for all three animal groups. When cells were assayed for GH content, resisters were found to have a 3.4-fold greater amount ($P < .01$) than gainers. Control somatotrope GH content was intermediate to the amount found in resister or gainer cells and differed from neither.

DISCUSSION

In this series of experiments, we have further characterized a model of dietary obesity with regard to the GH axis. Obesity-susceptible rats fed the chow-based high-fat diet of Levin et al[21,22] or the purified diet used here[23] have been shown previously to develop hyperglycemia and hyperinsulinemia over the course of the dietary regimen, whereas obesity-resistant rats have values similar to those of controls fed low-fat diets. The GH abnormalities reported here for obesity-susceptible rats further enhance the value of this model and increase its relevancy for human disease. All of these endocrine perturbations are common in human obesity[5] and coexist in a condition frequently referred to as the metabolic syndrome.[29] In addition, this study examines the response of female Sprague-

Table 4. Cell Yield and Proportion of Somatotropes From Pituitaries of Gainers, Resisters, and Controls

Parameter	Controls	Gainers	Resisters
Cell yield (×10⁶)	7.3 ± 0.6	11.2 ± 0.7*	5.5 ± 0.5
Somatotropes (%)	32.2 ± 1.7	28.8 ± 1.8	28.5 ± 1.3
GH storage (ng/5,000 cells)	63.8 ± 8.2	31.0 ± 8.9†	104.9 ± 23.2

NOTE. Data are the mean ± SE.
*$P < .001$ v controls and resisters.
†$P < .01$ v resisters.

Dawley rats to the MHF diet. Because the growth rate of male rats is greater than that of females of the same strain, it is important to establish whether the divergent response observed in males (gainers *v* resisters) can be duplicated in females. These results confirm that the body weight and fat gain in response to the diet is not limited to the faster-growing sex. It is also not likely that the response is linked to genes on the male chromosome. One difference that does appear to be sex-related is the amount of time necessary to see a complete divergence of populations based on body weight gain. In previous experiments, populations of male Sprague-Dawley rats with similar body weight completely diverge into separate populations between 12 and 15 weeks.[21,23] In the current study, female rats achieved a bimodal distribution of body weight between 18 and 21 weeks. In addition, the degree of separation is greater in males.[23] However, all other energy-balance data parallel those of males. Gainers are far more efficient at depositing energy consumed as body weight, and proportionately more of the weight is deposited as fat (Table 2). Differences in insulin and glucose concentrations are less profound between female gainers and resisters than between those two male populations, but the inclination to develop diabetes also appears to be present in females.

The changes in body composition observed in the animal model described here are similar to those observed for GH-deficient humans.[7,30] Lean body mass was not measured directly in this study, but increases in the adipose tissue depot were associated with decreased levels of GH. The low plasma concentrations of GH observed in the gainers combined with the in vitro somatotrope challenge experiment suggest that there would be a blunted response to GH-stimulating secretagogues in vivo, as well. Again, this is analogous to human obesity,[31,32] in which obese individuals have a decreased response to a variety of secretagogues compared with age-matched lean controls.

There are also several advantages of the dietary obese model used in these studies compared with other animal models of obesity. First, a diet-based model may be more relevant to the study of human obesity than genetic models. Dietary fat intake has been associated with obesity in a number of human studies.[33,34] It is easier to sort out the individual gene defect in the genetic model, but the relationship of the gene identified to the prevalence of obesity in human populations remains unclear. Zucker rats are also GH-deficient,[35] but they exhibit hyperinsulinemia and have abnormalities in corticosteroids and thyroid hormone metabolism, as well as decreased GHRH synthesis.[36] It has been suggested that the Zucker rat has a central nervous system (CNS) defect[14,15,37] that leads to general pituitary dysfunction. This may or may not be the case with the diet-induced obese rat, but it is unlikely that a CNS defect is the precipitating factor in most human obesity. Another difference is that Zucker fatty rats are hyperphagic compared with their lean counterparts, which is not the case with this dietary model. Although slightly increased food consumption is observed in the obesity-susceptible rat, this increased adiposity is not always accompanied by changes in energy intake or energy absorption.[23] Further, the increased food intake by obese rats cannot solely account for the increased body weight gain of this group. A metabolic adaptation such as decreased energy expenditure would also need to occur for the changes in weight gain to be increased significantly in obese versus lean rats.

It must be emphasized that repeated blood sampling is the best method for obtaining a true indication of circulating GH concentrations, but the single-point determinations reported here were significantly different for gainer and resisters. The advantage of multiple sampling is that it often illustrates differences that might be obscured by a single-point determination. However, since differences in GH concentration were found in this study by single-point measurements, these differences are likely to reflect lower episodic secretion of GH in obese rats compared with resisters. Otherwise, the natural episodic variation in GH secretion between resisters and gainers would hide the real mean differences. Due to the fact that somatotropes were to be analyzed at the end of this experiment, we used the terminal bleed for GH analysis, and are currently conducting another experiment that assessess integrated GH concentrations before dietary treatment begins. The decrease in circulating GH observed in obesity-susceptible rats appears to be mediated by decreased synthesis, since GH content of the somatotropes was lower in these animals than in resisters. However, mRNA analysis for pituitary GH content needs to be conducted to show that transcription is decreased in addition to the protein levels. A decreased number or proportion of somatotropes in gainers versus resisters is also ruled out as a possible explanation for decreased GH secretion based on our data (Table 4). Decreased GH synthesis would be consistent with data obtained in experiments in Zucker rats in which pituitary GH mRNA content was shown to be lower in fatty rats than in lean rats.[38] However, body composition (ie, body fat stores) and not body weight is likely to be the common denominator for these two models, since selection for rapid growth and body weight alone results in greater GH secretion, not GH deficiency.[39]

The question as to why GH synthesis is decreased is unanswered. Ghigo et al[40] hypothesize that there is a somatotropic defect in obesity. This group demonstrated that repetitive GHRH injections failed to increase GH secretion in obese individuals, in contrast to lean controls.[40] It has also been proposed that SRIF tone plays a role in the blunted GH response observed in obese patients.[41] It is possible that endogenous SRIF release or concentration is higher in the obese than in leaner individuals. However, other studies show that GH-releasing peptide-6 can elicit robust GH release regardless of whether SRIF levels are depressed by pyridostigmine.[42] The experiments presented here also suggest that SRIF levels are not by themselves primary modulators of the diminished GH response of somatotropes obtained from obese rats. If they were, in vitro release of GH should be normalized in the absence of SRIF. Further, the data reported by Cordido et al[42] raise the possibility that the primary defect may not be somatotrophic, since combined secretagogues do effectively stimulate the release of GH in obese individuals. However, the dietary obese model can be used to further explore these questions.

Finally, the diet-induced obese rat model described here has great potential for examining the modulation of endocrine status

by dietary components such as fat. Without the dietary fat challenge, the Sprague-Dawley populations used in these experiments would not demonstrate a divergent GH response. Thus, the underlying mechanisms for the development of obesity and GH deficiency may be studied in animals before and during exposure to the MHF diet. One could evaluate pre-obese animals and follow changes induced by the diet leading to obesity. These experiments would be difficult, if not impossible, to perform in the human population. Also, since resisters consume the same diet as gainers, dietary composition is controlled for in this paradigm. Other dietary obesity models compare animals that consume high levels of dietary fat with animals fed low-fat diets.[43] Although these regimens have utility, they add variables to the study and make the interpretation of results more difficult. In addition, the purified nature of this diet makes it amenable to experimental manipulation. One could, for example, alter the degree or type of fat saturation in the diet and determine whether the GH effect or other effects on insulin resistance still occur.

The differences observed in body fat accretion between gainers and resisters must ultimately be related to energy expenditure, since the energy intake of these groups cannot account for the fat gains observed.[23] But the mechanism whereby these changes occur and the factors that precipitate such changes remain unelucidated. GH has been shown to stimulate energy expenditure in GH-deficient patients,[44] but it is not known whether GH deficiencies existed in the gainers before dietary fat exposure or only afterward. Studies to answer this question are currently being conducted by our laboratory. In addition, it is possible that target-tissue sensitivity differs between animals predisposed to obesity and those that are resistant to obesity. Other factors such as insulin secretion are also being considered, since dietary fat has been shown to alter insulin binding,[45] and insulin's effects on GH secretion and synthesis have been demonstrated previously.[46]

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