High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat

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High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1168–E1177, 1997.—High-fat feeding induces insulin resistance and increases the risk for the development of diabetes and coronary artery disease. Glucocorticoids exacerbate this hyperinsulinemic state, rendering an individual at further risk for chronic disease. The present studies were undertaken to determine whether dietary fat-induced increases in corticosterone (B) reflect alterations in the regulatory components of the hypothalamic-pituitary-adrenal (HPA) axis. Adult male rats were maintained on a high-fat (20%) or control (4%) diet for varying periods of time. Marked elevations in light-phase spontaneous basal B levels were evident as early as 7 days after fat diet onset, and B concentrations remained significantly elevated up to 21 days after fat diet onset compared with controls. In contrast, there were no significant effects on any parameters of spontaneous growth hormone secretory profiles, thus providing support for the specificity of the effects on the HPA axis. In a second study, all groups of rats fed the high-fat diet for 1, 9, or 12 wk exhibited significantly elevated levels of plasma adrenocorticotropic hormone, B, fatty acid, and glucose before, during, and/or at 20, 60, and/or 120 min after the termination of a restraint stress. Furthermore, 12-wk fat-fed animals showed a significant resistance to insulin compared with normally fed controls. There were no differences in negative feedback efficacy in high-fat-fed rats vs. controls. Taken together, these results suggest that dietary fat intake acts as a background form of chronic stress, elevating basal B levels and enhancing HPA responses to stress.

glucocorticoids; adrenocorticotropic hormone; corticosterone; fatty acid; glucose

HIGH-FAT DIETS contribute to insulin resistance (37, 42), impaired glucose metabolism (15), type 2 or non-insulin-dependent diabetes mellitus (NIDDM) (2, 19, 24), stroke, and coronary artery disease (28), although the mechanisms underlying these effects are not completely understood. Dietary cholesterol is associated with increased low-density-lipoprotein (LDL) concentrations and the elevated triglyceride levels in very low-density lipoproteins (vLDL). Dietary fat not only lowers glucose uptake but also stimulates inappropriate glucose production (6), resulting in elevations in both circulating insulin and glucose (15, 37). High-fat diets decrease the number of insulin receptors in liver, skeletal muscle, and adipose tissue, decrease glucose uptake into skeletal muscle and adipose tissue, and decrease hepatic glycogenolysis and glycogen synthesis (6). Glycogen accumulation and glucose oxidation are also lower with high-fat diets, and the rate of gluconeogenesis is increased in the liver (3), a common problem for many diabetics. In sum, high-fat diets are associated with a Syndrome X-like state that includes hypertriglyceridemia, decreased high-density lipoproteins, high LDL and vLDL, abnormal glucose production, hyperinsulinemia, and insulin resistance (36).

High-fat diets may also influence hypothalamic-pituitary-adrenal (HPA) activity, elevating adrenal glucocorticoid (GC) production (11, 21, 35). This is of considerable interest here because increased levels of GCs also stimulate secretion of triglycerides from the liver in vLDL, as demonstrated in perfused liver and monolayer cultures of hepatocytes (7, 30). Furthermore, GCs decrease levels of lipoprotein lipase, which controls the hydrolysis of vLDL; this decrease has been shown to exaggerate hypertriglyceridemia (44). Normally, most LDL formed after the degradation of vLDL is removed from the circulation via receptor-mediated endocytosis (16). The binding and degradation of LDL by rat hepatocytes are decreased by dexamethasone, a synthetic GC, which could result in elevations in LDL levels.

A prolonged excess in GC levels leads to various adjustments, altering the balance between insulin and GCs. Elevated GCs antagonize most of insulin’s actions and result in increased basal and glucose-stimulated insulin levels and pancreatic β-cell hyperplasia (27, 31). Insulin inhibits the secretion of triacylglycerol, phospholipid, cholesterol ester, and apolipoproteins B and E associated with vLDL (10). GCs antagonize these effects by increasing the breakdown of protein, glycogen, and triacylglycerol. Amino acids that are released from proteins can be used for gluconeogenesis. Other enzymes that are released from this pathway are increased in activity by GCs. Thus the effects of increased GCs mimic those of a high-fat diet, raising the possibility that some of the effects of high fat might be mediated by increases in circulating GC levels.

In the present study, we tested the hypothesis that elevations in HPA activity could modulate some of the effects of high-fat feeding. To accomplish this, we exami-
ined both basal and stress-induced alterations in HPA axis functioning as well as carbohydrate and fatty acid metabolism after short- and long-term exposure to a high-fat diet. We also monitored spontaneous growth hormone (GH) secretory profiles, to assess the specificity of the response, and assessed dietary-induced alterations in exogenous corticosterone (B) negative feedback efficacy.

**METHODS**

Animals. Because of the multicenter nature of this study, different rat strains were used. Adult male Long-Evans hooded rats (Charles River Canada, St. Constant, QC, Canada) were used in most experiments. In both the negative feedback experiment [M. F. Dallman Lab, University of California at San Francisco (UCSF)] and the experiment on the effects of high-fat diets on spontaneous basal B and GH levels (G. S. Tannenbaum Lab, McGill University), adult male Sprague-Dawley rats (Harlan-Holtzman, Madison, WI and Charles River, Canada) were employed. Long-Evans rats were housed on a 12:12-h light-dark cycle (lights on at 0800) and were group housed until catheterization. In the negative feedback and basal B/GH studies, the rats were individually housed on arrival and kept on a 12:12-h light-dark cycle (lights on at 0600) in a humidity- and temperature-controlled environment. In the basal study, body weight was monitored daily, and 24-h food intake was assessed daily over several days. The rats used in these experiments weighed 260–320 g (basal and feedback experiments) and 175–225 g (stress experiments) and were assigned randomly to groups fed either the control or high-fat diet for 5 days or 1, 3, 9, or 12 wk. A subset of these rats was implanted with indwelling jugular catheters; those selected for negative feedback and glucocorticoid receptor binding were not catheterized.

Diet. Both the control (4% fat) and high-fat (20% fat) diets were obtained from ICN Biomedical (Mississauga, ON, Canada). The same source of diet was used in both the McGill and UCSF studies. The diets had been formulated in a previous study (11) and were modified slightly for the present studies (Table 1). Diets were balanced for protein as a percentage of energy intake and for essential vitamins and minerals. The fat source in both the control and high-fat diets was corn oil. The high-fat diet contained 4.8 kcal/g and the 4% fat control diet, 4.0 kcal/g. In place of fat (corn oil), the 4% fat control diet each day, and their intake was measured the following day by subtracting uneaten food plus spillage from total food given; spillage was collected on a diaper under the rat cages. In this experiment, rats were fed the control or high-fat diet for either 1 or 3 wk before testing.

On the day of testing, food was removed 1.5–2 h before the start of sampling and was returned at the end. Blood samples (0.4 ml) were withdrawn every 15 min for periods of 6 h (1000–1600). All blood samples were centrifuged immediately, and the plasma was separated and stored at −20°C for subsequent measurement of B and GH. Red blood cells were resuspended in isotonic saline and returned to the animal after removal of the next blood sample to prevent hemodynamic instability.

Restraint stress. After 1, 9, or 12 wk on the diets and 3 days before testing, another set of animals was anesthetized under methoxyflurane (Metofane; MTC Pharmaceuticals, Mississauga, ON, Canada) and implanted with indwelling Silastic jugular catheters (Dow Corning), which were led subcutaneously and externalized to the nape of the neck. The catheter was filled with heparinized (100 U/ml) isotonic saline and closed off with a stainless steel obturator. Animals were housed singly for the remaining 3 days of the study (while being maintained on the high-fat or control diets).

Restraint stress was performed between 1000 and 1300 with the use of tubular, plastic restrainers lined with foam rubber. This period was chosen to avoid the elevated basal B and peak HPA responses to stress associated with the dark phase of the cycle (8). A blood sample (0.15 ml) was taken immediately before the rat was placed in the restrainer and within 10 s after removal from the home cage. The animals were restrained for 20 min, and blood samples were taken at both 5 and 10 min after the onset of restraint. Additional blood samples were obtained at the termination of restraint and at 20, 60, and 120 min thereafter. Blood samples for B, fatty acid (FA), and glucose measurement were collected into tubes coated with EDTA, placed on ice, and then centrifuged and stored at −20°C until assayed. Blood samples for adrenocorticotropic hormone (ACTH) assays were collected in tubes containing EDTA and aprotinin (Trasylol), centrifuged, and stored at −20°C as well.

Basal plasma glucose and FA levels. To assess basal plasma glucose and FA levels, separate sets of animals (noncatheterized) were fed the high-fat diets for 1, 9, or 12 wk. After termination of the dietary periods, the animals were killed rapidly (i.e., <10 s) by decapitation after removal from the home cage between 1000 and 1300. Trunk blood was collected and stored at −20°C until assayed. Plasma FAs were determined with a nonesterified fatty acid (NEFA-C) test kit (Wako, Richmond, VA). The assay protocol followed the manufacturer's instructions but was miniaturized, and absorbancies were measured with a microtitration test reader. Plasma glucose concentrations were measured by an automated glucose oxidase method with a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA).

Adrenalectomies, brain dissections, and GR receptor binding. At the end of 1, 9, and 12 wk of exposure to the diet, two additional groups of control and high-fat-fed rats (noncatheter-
ized) were bilaterally adrenalectomized (ADX). These animals were killed by rapid decapitation 12–14 h after ADX, a time period that allows for clearance of the endogenous steroid (32). The brain was removed quickly and placed on ice, and the hippocampus, frontal cortex, hypothalamus, and pituitary were dissected, frozen on dry ice, and stored at −80°C. On the day of the GC receptor (GR) binding assay, brain tissue was homogenized in 30 mM tris(hydroxymethyl)aminomethane, 1 mM EDTA, 1 mM dithiothreitol, 10% (vol/vol) glycerol, and 10 mM sodium molybdate (TEDGM; pH adjusted to 7.4), and the homogenates were centrifuged at 4°C for 45 min at 105,000 g. Binding in all tissues was measured by single point assays in which aliquots (225 µl) of the soluble fraction from a single animal were incubated for 18–24 h [a time that has been shown to be sufficient for maximal exchange to occur and during which binding is stable (22)] with 150 µl of TEDGM containing a saturating, 10 nM concentration of [3H]dexamethasone (88.7 Ci/mmol; Amersham, Oakville, ON, Canada). Nonspecific binding was determined in parallel incubations containing a 500-fold excess of unlabeled RU-28362. RU-28362 binds selectively to the GR, with very little affinity for the mineralocorticoid receptor (39).

Separation of bound from unbound steroid was achieved using Sephadex LH-20 (Pharmacia Fine Chemicals, Dorval, QC, Canada) columns of 10 × 1 cm made with disposable pipette tips filled with one 3-mm glass bead and equilibrated with TEDGM. After incubation, 100 µl of the samples were washed onto the columns with 100 µl TEDGM. The columns were eluted 30 min later with 500 µl of TEGM into mini-vials, which were then filled with 4.5 ml of Liquiscint (National Diagnostics, Sommerville, NJ). Radioactivity was determined in a Packard scintillation counter at 56% efficiency. Protein content was determined by the method of Bradford (9), and the results were expressed as fentomoles of specific binding per milligram protein. Protein concentrations ranged from 300 to 500 µg/ml for hippocampus and frontal cortex and from 150 to 250 µg/ml for hypothalamus and pituitary.

Insulin sensitivity tests. At the end of 12 wk of exposure to either the high-fat or control diet, animals were food deprived for 12 h overnight, and blood was collected via the tail vein at 0800 the following day for measurement of basal plasma glucose levels. Two hours later, animals were injected with 0.125 U/kg ip of insulin (Humulin R, Eli Lilly, Indianapolis, IN). Samples were then taken at 15, 30, 60, 120, and 180 min after injection. All samples were collected in tubes containing EDTA, centrifuged, and stored at −20°C until assayed for plasma glucose.

Test of feedback with exogenous B. In this study, rats were bilaterally ADX and implanted with 0, 25, or 50% B pellets. The pellets remained implanted for 5 days while the rats were exposed to either the high fat or the control diet. At the end of 5 days, and within 2 h of lights on, rats were exposed to 30 min of restraint stress. Blood was collected immediately before (0 min) and at 15 min into restraint. At the termination of restraint (30 min), animals were rapidly decapitated, and trunk blood was collected at this time point as well. Trunk blood was collected into plastic tubes, and the plasma was separated and stored at −20°C for subsequent assay of ACTH and B.

Radioimmunoassays. Plasma B was measured by the radioimmunoassay (RIA) of Krey et al. (25), with a highly specific B antiserum (B3–163, Endocrine Sciences, Tarzana, CA), [3H]B (101 Ci/mmol; NEN, Boston, MA) as tracer, and 10 µl of plasma. The minimum level of detection of the assay was 10 pg/ml. The antiserum cross-reacts slightly with deoxycorticosterone (4%) but not with cortisol (<1%). Separation of bound from unbound hormone was achieved using dextran-coated charcoal. Samples were then decanted into miniscintillation vials filled with 4.5 ml of Liquiscint (National Diagnostics, Sommerville, NJ), and radioactivity was determined in a Packard scintillation counter at 56% efficiency. The intra- and interassay coefficients of variation were 3.2 and 3.9%, respectively.

Plasma ACTH was measured by the RIA described by Walker et al. (47) with an ACTH antiserum (immunoglobulin G, Nashville, TN) and 125I-labeled ACTH (Institut, Stillwater, MN) as tracer. The ACTH antibody cross-reacts 100% with ACTH-(1–39), ACTH-(1–18), and ACTH-(1–24) but not with ACTH-(1–16), β-endorphin, α- and β-melanocyte-stimulating hormone, and α- and β-lipotropin (<1%). Plasma (25 µl) was incubated for 48 h at 4°C with antiserum and tracer, after which precipitation serum (Peninsula Laboratories, Belmont, CA) was added and incubated overnight. Bound peptide was obtained by centrifugation at 5,000 g for 45 min. The minimum level of detection of the assay was 10 pg/ml; inter- and intra-assay variabilities were 4.0 and 2.8%, respectively.

Plasma GH levels were determined in duplicate by double-antibody RIA with materials supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD). The averaged plasma GH values are reported in terms of the rat GH reference preparation, rGH RP-2. The standard curve was linear between 0.62 and 320 ng/ml. The intra- and interassay coefficients of variation were 8.1 and 8.7%, respectively, for duplicate samples of pooled plasma containing a mean GH concentration of 11.5 ng/ml.

Statistical analyses. The results were analyzed by repeated-measures and factorial analyses of variance and by paired and unpaired Student’s t-tests. Scheffé post hoc tests were performed when appropriate. Integrated hormone levels were determined with the trapezoidal rule, and the data were expressed over time of sampling. P < 0.05 was considered significant.

RESULTS

Effects of high-fat diet on spontaneous plasma B and GH profiles. Plasma B levels measured every 15 min over 6 h and averaged over 2-h blocks (1000–1200, 1200–1400, and 1400–1600) are shown in Fig. 1. All rats displayed the typical circadian elevation in basal

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**Fig. 1.** Two-hour plasma corticosterone (B) profiles (means ± SE) in freely moving rats exposed to a high-fat diet for 7 (n = 8) or 21 (n = 6) days vs. controls (n = 6) across the day. *Seven-day-fat-fed rats differ from controls at P < 0.05; †21-day-fat-fed vs. 7-day-fat-fed rats differ at P < 0.05; ‡21-day-fat-fed rats differ from controls at P < 0.05.
plasma B, with increasing levels observed during the latter hours of the day; spontaneous basal plasma B values obtained in the 1000–1200 time period were significantly (P < 0.05) lower in all groups compared with their respective profiles from either the 1200–1400 or 1400–1600 period. However, compared with normal-fed controls, mean 2-h plasma B levels were markedly elevated in both groups of high-fat-fed rats (Fig. 1). Rats fed a high-fat diet for 1 wk showed two- to threefold increases in plasma B levels throughout the sampling period. After 3 wk of exposure to high fat, significant elevations in B were observed in the 1200–1400 and 1400–1600 sampling periods compared with those of normal-fed controls. Interestingly, mean plasma B profiles obtained from rats fed the high-fat diet for 7 days were significantly (P < 0.05) higher at both the 1000–1200 and 1200–1400 phases than the profiles obtained from rats fed the high-fat diet for 21 days.

In contrast to the effects observed on plasma B, exposure to a high-fat diet for either 7 or 21 days failed to significantly alter the spontaneous GH profiles. As shown in Table 2, there were no significant differences in GH peak amplitude, GH trough value, or mean 6-h plasma GH levels among the three groups. Daily body weight gain and food intake were also similar across the three groups (Table 2). These latter data essentially reflect what was found in all other studies (stress response and negative feedback).

Effects of high-fat diet on plasma ACTH and B responses to restraint. Plasma ACTH and B responses immediately before, during, and up to 2 h after the termination of restraint are shown in Figs. 2 and 3. Compared with animals fed the control diet, rats fed the high-fat diet for 1 wk showed elevated levels of ACTH throughout the sampling period that reached significance 60 min after termination of stress (P < 0.05; see Fig. 2A). Overall, the integrated levels of plasma ACTH were significantly (P < 0.05) increased in animals fed the high-fat diet for 1 wk compared with controls (Fig. 3A). One-week high-fat-fed rats also showed a significant elevation in plasma B at 60 min after the termination of restraint (Fig. 2B) in addition to significantly (P < 0.05) higher integrated levels of B compared with controls (Fig. 3B).

After 9 wk on the high-fat diet, a significant (P < 0.05) elevation in plasma ACTH was observed at 20 and 120 min after the termination of stress (Fig. 2C), and integrated ACTH levels were significantly (P < 0.05) elevated compared with controls (Fig. 3C). High-fat fed rats also showed significant (P < 0.05) elevations in plasma B at 60 and 120 min poststress, as shown in Fig. 2D, as well as significantly (P < 0.05) higher integrated levels of ACTH compared with control-fed animals (Fig. 3D).

Finally, rats fed for 12 wk on the high-fat diet did not exhibit a significant increase in plasma ACTH compared with control rats (Figs. 2E and 3E) but did show significant (P < 0.05) elevations in plasma B at both 60 and 120 min posttermination of stress (Fig. 2F) and integrated levels of B (Fig. 3F). Plasma B levels in high-fat-fed rats were also elevated 20 min after the termination of restraint, although this difference failed to reach significance (0.1 < P < 0.05).

Basal and stress-induced plasma FA and blood glucose concentrations. Figure 4A illustrates basal FA levels in 1-, 9-, and 12-wk high-fat-fed and control animals. Basal FA levels were significantly (P < 0.03) elevated in 1- and 9-wk high-fat-fed animals compared with controls, but no significant differences were found at 12 wk. Plasma FA responses before and after exposure to a 20-min period of restraint are shown in Fig. 4B. High-fat-fed animals maintained on the diet for 1 wk showed significant (P < 0.03) elevations in FA immediately before and at 5 and 10 min after the onset of restraint compared with controls. On termination of restraint, rats fed the high-fat diet maintained significantly (P < 0.03) elevated FA levels compared with controls. Integrated FA levels were augmented twofold (P < 0.05) in fat-fed rats (1,110 ± 112 µeq·l⁻¹·min⁻¹) vs. controls (585 ± 50 µeq·l⁻¹·min⁻¹).

Basal plasma glucose levels obtained at the time of killing were similar in fat-fed vs. control-fed rats at all time points (Fig. 5A). Whereas high-fat-fed rats showed a significant (P < 0.05) elevation in plasma glucose 5 min into the restraint (Fig. 5B), there were no significant differences in integrated plasma glucose levels compared with controls (110 ± 5 mg·dl⁻¹·min⁻¹ for high-fat-fed rats and 103 ± 4 mg·dl⁻¹·min⁻¹ for controls).

Similar FA and glucose responses to stress were found in rats fed for 9 wk with the high-fat diet; integrated FA levels were 1,170 ± 66 µeq·l⁻¹·min⁻¹ and 871 ± 66 µeq·l⁻¹·min⁻¹ in rats fed the high-fat and control diets, respectively (P < 0.05), whereas the integrated level of plasma glucose in high-fat-fed rats was 103 ± 3 mg·dl⁻¹·min⁻¹ compared with 92 ± 3 mg·dl⁻¹·min⁻¹ in controls.

Rats exposed to the high-fat diet for 12 wk exhibited no significant differences in stress-induced FA levels compared with controls (integrated FA levels: 1,091 ± 131 µeq·l⁻¹·min⁻¹ vs. 947 ± 49 µeq·l⁻¹·min⁻¹). Integrated plasma glucose levels were also similar in the two groups: 101 ± 3 mg·dl⁻¹·min⁻¹ vs. 107 ± 2 mg·dl⁻¹·min⁻¹ for controls.

Table 2. Effects of high-fat diet on GH secretory dynamics, body weight gain, and food intake

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>GH Peak Amplitude, ng/ml</th>
<th>GH Trough Level, ng/ml</th>
<th>Mean 6-h Plasma GH Level, ng/ml</th>
<th>Daily Body Weight Gain, g</th>
<th>Daily Food Intake, g</th>
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<tbody>
<tr>
<td>Control diet, 7–21 days</td>
<td>6</td>
<td>180.5 ± 31.0</td>
<td>1.3 ± 0.1</td>
<td>39.0 ± 4.9</td>
<td>6.1 ± 1</td>
<td>23.9 ± 1.2</td>
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<tr>
<td>High-fat diet</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Days</td>
<td>10</td>
<td>165.5 ± 28.3</td>
<td>1.4 ± 0.2</td>
<td>42.6 ± 8.0</td>
<td>5.8 ± 0.8</td>
<td>24.7 ± 0.7</td>
</tr>
<tr>
<td>21 Days</td>
<td>6</td>
<td>147.1 ± 42.3</td>
<td>1.3 ± 0.1</td>
<td>34.3 ± 9.0</td>
<td>5.5 ± 0.3</td>
<td>20.3 ± 1.4</td>
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Values are means ± SE; n, no. of rats/group. GH, growth hormone.
Insulin sensitivity test. Figure 6 shows plasma glucose levels before and after an injection of 0.125 U/kg of insulin in rats exposed to the high-fat or control diets for 12 wk. No significant differences in plasma glucose concentrations were found before insulin administration. Control-fed rats demonstrated a significant decrease in blood glucose in response to insulin administration. In contrast, fat-fed rats failed to respond to insulin at 10 min, and this resulted in plasma glucose levels being significantly (P < 0.02) higher at 15 and 30 min after injection in high-fat-fed animals compared with controls. High-fat-fed animals showed significantly (P < 0.05) elevated integrated plasma glucose levels (95 ± 4 mg·dl⁻¹·min⁻¹) compared with controls (75 ± 6 mg·dl⁻¹·min⁻¹).

GR binding densities. Examination of GR binding in brain regions known to be implicated in GC negative feedback regulation of HPA activity revealed no significant differences in GR binding densities in either hippocampus, frontal cortex, or pituitary after 1 wk on the diet, although GR binding in the hypothalamus was significantly lower (P < 0.05) in high-fat-fed animals on the diet compared with controls (Fig. 7A). After 9 (Fig. 7B) and 12 (Fig. 7C) wk of exposure to the high-fat or control diet, GR binding densities were similar in all brain areas studied.

Feedback of exogenous B. Table 3 shows both basal plasma ACTH and B concentrations, as well as stress-induced ACTH levels, in rats that were ADX and replaced with 0, 25, or 50% B pellets and exposed to both diets. Replacement with B pellets effectively decreased plasma ACTH levels after ADX. However, there were no significant differences in either basal ACTH or B levels, or stress-induced ACTH, between ADX rats fed the high-fat diet and control-fed animals.
DISCUSSION

These results demonstrate that both basal and stress-induced HPA activity was altered by both short- and long-term exposure to a high-fat diet. Free-moving high-fat-fed animals maintained on the diet for 1 and 3 wk and sampled throughout the day showed two- to threefold increases in plasma B concentrations between 1000 and 1600 compared with controls. Rats fed the high-fat diet for 1, 9, and 12 wk also showed significant elevations in plasma ACTH, B, and FA levels at 20, 60, and 120 min after the termination of restraint and significant augmentation in the overall integrated ACTH, B, and FA responses to restraint stress, compared with normally fed controls. In contrast, there were no significant alterations in any parameters of the spontaneous GH secretory profiles after 1 and 3 wk on the high-fat diet, providing support for the specificity of the effects on the HPA axis.

Interestingly, the pattern of effects of the high-fat diet is similar to that observed after chronic stress. Basal HPA activity is increased in rat models of chronic stress, such as continuous cold exposure or streptozotocin-induced diabetes, and this effect is most apparent in the light phase of the cycle, i.e., the nadir in HPA activity (41). Moreover, chronic stress facilitates HPA responses to subsequent acute stressors (1). High-fat diets clearly augmented both ACTH and B responses to acute stress. The effects of chronic stress (enhanced ACTH and B responses to stress) are not associated with alterations in delayed GC negative-feedback sensitivity. Accordingly, high-fat-fed rats of the present study also did not differ from controls in delayed feedback sensitivity. Thus, in terms of HPA activity, sustained periods of increased fat consumption appear to function as a chronic stressor. The lack of effect of increased fat consumption on GH secretory profiles is consistent with earlier reports demonstrating that plasma GH concentrations are not altered in conditions of chronic stress (18, 26).

As in other chronic stress models, there appeared to be a small amount of adaptation to high-fat feeding. After 12 wk of exposure to high fat, animals failed to show a significantly elevated ACTH response to stress despite a significantly augmented B response to the restraint. Animals fed the high-fat diet for 12 wk also did not differ in basal FA levels compared with controls.
However, although it is possible that these particular responses adapted after longer exposures to fat feeding, adrenal GC responses remained elevated. Moreover, these animals were clearly nonresponsive to insulin challenge; rats tested after 12 wk of exposure to high-fat diet demonstrated a significant resistance to insulin, as evidenced by a lower decline in blood glucose levels after the administration of insulin. This finding supports the severity of the effects of long-term (12-wk) fat feeding on insulin and glucose dynamics and underscores the functional significance of this high-fat model in rodents. Although high-fat diet-induced insulin insensitivity has been shown in models in which exposure to the diet has been more prolonged, our results suggest that relatively short exposures to high-fat diets can induce severe changes in insulin dynamics without any major change in circulating basal blood glucose levels, a phenomenon often seen in the clinical setting (36).

High-fat diets have been reported to increase serum FA concentrations, which in turn may act to antagonize the action of insulin (38). Feeding experimental ani-

![Figure 4](image1.png)

**Fig. 4.** Basal (means ± SE, A) and stress-induced (means ± SE, B) plasma fatty acid (FA) levels in rats fed either high-fat (n = 10) or CTL (n = 10) diet. A: basal FA levels were obtained from trunk blood at time animals were killed. B: FA response to a 20-min period of restraint (solid black line) in animals fed the high-fat (n = 9) or CTL (n = 10) diet for 1 wk. *Significantly different at P < 0.03.

![Figure 5](image2.png)

**Fig. 5.** Basal (means ± SE, A) and stress-induced (means ± SE, B) plasma glucose concentrations in rats fed either high-fat (n = 10) or CTL (n = 10) diet. A: basal glucose levels obtained from trunk blood at time animals were killed. B: plasma glucose response to a 20-min period of restraint (solid black line) in animals fed high-fat (n = 9) or CTL (n = 10) diet for 1 wk. *Significantly different at P < 0.05.

![Figure 6](image3.png)

**Fig. 6.** Blood glucose levels (means ± SE) before and up to 3 h after a 0.125 U/kg ip injection (arrow) of insulin in control (n = 8) and fat-fed (n = 9) rats. *Significantly different at P < 0.02.
mals with high-fat diets induces insulin resistance and impairs intracellular glucose metabolism by a variety of mechanisms. The binding of insulin to its receptor initiates glucose transport into fat and muscle cells. Insulin stimulates glycolysis, glycogen synthesis, and glucose oxidation; high-fat diets impair all of the intracellular routes of glucose disposal (14, 20). High FA may act directly to reduce the number of insulin receptors in certain tissues (4). High-fat diets also decrease the activities of the key enzymes involved in glycolysis (5) while at the same time stimulating gluconeogenesis (38). The present findings show that high-fat-fed rats exhibit elevations in both basal and stress-induced FA. FA levels were elevated before, during, and immediately after the termination of restraint in the high-fat-fed animals; this elevation in FAs may mediate changes in both insulin sensitivity and HPA function. The high FA levels appeared to coincide with higher basal and stress-induced ACTH. Because ACTH has been shown to be a stimulus for lipolysis, the ACTH response to stress may best predict the FA levels.

Widmaier et al. (49) showed that elevations in FA, achieved by infusions of intralipid, raise plasma levels of ACTH and B. Fatty acids have direct electrophysiological effects on cells of the central nervous system and are taken up by cells in the brain (29, 34). Oomura (34) demonstrated that electroapplication of FA into the ventromedial hypothalamus (VMH) inhibited neuronal firing rates in that area, and Dallman (13) showed that the cells of the VMH exert an inhibitory control on the HPA. Thus FA may act to alter hypothalamic regulation of the HPA axis. We propose that high fat-induced elevations in FA may be partly responsible for the elevation in basal B, as well as the increased stress-induced hypersecretion of both B and ACTH. Furthermore, because GCs tend to stimulate lipolysis, elevations in B may further stimulate the production of FA, which are already elevated because of the fat content of the diet. This may render the animal even more resistant to insulin, because elevations in FA tend to reduce the number of insulin receptors on various tissues, thereby increasing the dependence on GCs for homeostasis and stimulating gluconeogenesis. Taken together, these findings suggest a feed-forward cascade involving FA, GC, and insulin dynamics.

Exposure to high-fat diets did not alter B negative feedback sensitivity. We did find differences in hypothalamic GR levels after 1 wk, but not after 9 or 12 wk, of high-fat feeding. The absence of differences at 9 and 12 wk of feeding might be attributable to the poor resolution of our technique used for the binding assays. We cannot, therefore, preclude the possibility of differences in other forms of feedback, such as fast feedback. Fast

Table 3. Effects of high-fat diet on basal and stress-induced plasma ACTH and B levels in B pellet-replaced rats

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>ACTH, pg/ml</th>
<th>Basal B, µg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet (ADX &lt; 0%)</td>
<td>6</td>
<td>490 ± 107</td>
<td>1,711 ± 152</td>
</tr>
<tr>
<td>Control (ADX &lt; 25%)</td>
<td>6</td>
<td>152 ± 73</td>
<td>1,312 ± 110</td>
</tr>
<tr>
<td>Control (ADX &lt; 50%)</td>
<td>6</td>
<td>72 ± 21</td>
<td>154 ± 15</td>
</tr>
<tr>
<td>High fat (ADX &lt; 0%)</td>
<td>7</td>
<td>550 ± 97</td>
<td>2,100 ± 154</td>
</tr>
<tr>
<td>High fat (ADX &lt; 25%)</td>
<td>7</td>
<td>110 ± 47</td>
<td>1,100 ± 55</td>
</tr>
<tr>
<td>High fat (ADX &lt; 50%)</td>
<td>7</td>
<td>75 ± 21</td>
<td>201 ± 27</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats/group. ACTH, adrenocorticotropic hormone; B, corticosterone; ADX, adrenalectomy.
feedback represents a process whereby rapidly increasing levels of B dampen ACTH release (23). FA may play a role here. The binding of dexamethasone (DEX) to the rat cytosolic GR can be modified by FA; for example, FAs inhibit the binding of DEX to GR in the liver as a function of increasing dose (45). Because GCs stimulate lipolysis, FA could exert a feedback control on GC by modulating binding of the hormone to the receptor. Also, recent work shows that the binding of GC in vivo to GR is reduced in immature rats after plasma FA is increased by stimulating lipase activity (17). The elevations in FA levels occur under more dynamic conditions of stress, and these conditions would be expected to alter fast, but probably not delayed, forms of feedback. Although this idea clearly remains to be tested, it does provide another potential mechanism whereby high-fat diets could modulate HPA responses to stress.

GCs can antagonize the effects of insulin, produce insulin insensitivity, and decrease glucose uptake in tissues. However, the combination of elevated GCs with concurrent increases in insulin further enhances energy deposition through FA and glycogen synthesis and the activity of lipoprotein lipase in adipose tissue (12). An increased GC control of metabolism is characteristic of many of the risk factors for premature atherosclerosis. The GC-insulin antagonism stimulates the secretion of VLDL and decreases hepatic uptake of LDL (48). High-fat-fed rats are insulin resistant and therefore may be at greater risk for the development of Syndrome X and NIDDM.

A strong correlation exists between consumption of a diet high in fat and many cancers, such as breast, colorectal, pancreatic, prostatic, and uterine cancer (40). Munck et al. (33) demonstrated that GCs inhibit production of interferon, which augments natural killer (NK) cell activity and activates macrophages for clearance of bacterial pathogens and antibody tagged host cells. Thus, in addition to obvious implications for heart disease, increased GC production may mediate some of the effects of high-fat diets on tumor development in addition to the onset and progression of related pathologies. If one considers that high-fat feeding increases GCs and FAs and decreases insulin sensitivity in an atherosclerosis-prone animal (e.g., a human), then these stress-induced changes should aggravate the atherosclerosis.

In summary, the results of the present study demonstrate that high-fat feeding augments both the ACTH and B responses to acute stress, as well as increases in basal B secretion, without any significant alterations in B negative feedback efficacy. Furthermore, we have shown that high-fat feeding results in insulin resistance and elevations in both basal and stress-induced FA and blood glucose concentrations. Taken together, these findings provide initial support for the view that enhanced exposure to counterregulatory hormones can mediate the effects of high-fat diets. These findings may be of considerable clinical importance, because stressful events not only stimulate HPA activity but may also increase fat consumption, leading to a potentially dangerous metabolic cascade.

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