Effect of Adrenalectomy and Glucocorticoid Replacement on Development of Obesity

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Effect of adrenalectomy and glucocorticoid replacement on development of obesity

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FREEDMAN, MARJORIE R., BARBARA A. HORWITZ, AND JUDITH S. STERN. Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am. J. Physiol. 250 (Regulatory Integrative Comp. Physiol. 19): R595-R607, 1986.—Female obese and lean Zucker rats were adrenalectomized (ADX) or sham-operated at 4 wk of age. ADX animals were given daily injections of 0.01, 0.05, 0.50, 1.0, or 2.0 mg hydrocortisone/100 g body wt for 30 days. ADX rats gained less weight than sham-operated controls. Obese ADX rats at the lowest dose (0.01) had a net positive energy gain but lost body fat. As steroid dose increased, obese rats deposited more fat and less protein. Doses of 0.01 and 0.05 mg produced rats that were less fat than sham-operated controls, whereas doses of 0.50, 1.0, and 2.0 mg produced rats of comparable body fat composition. Obese rats were consistently fatter and had a significantly smaller percentage body protein than lean rats at each dose. Body fat elevation was reflected by heavier pancreatic and retroperitoneal fat depots and larger fat cells at all doses except the lowest. Compared with sham-operated controls, lean and obese rats at the two lowest replacement doses (0.01, 0.05) exhibited significantly decreased plasma insulin and triglyceride levels and significantly elevated brown adipose tissue protein content and citrate synthase (CS) activity. Obese rats at these doses had significantly reduced adipose tissue lipoprotein lipase (LPL) activity in the retroperitoneal depot and lower food intake. Furthermore, these obese rats had adipose depot weights, cell sizes, LPL activity, and plasma insulin, glucose, and triglyceride comparable to that of lean sham-operated controls. As steroid dose increased (0.5, 1.0, 2.0), plasma insulin and triglyceride and food intake markedly increased only in obese rats. Adipose tissue LPL activity appeared unaffected by dose. Brown adipose tissue protein content and CS activity significantly decreased as dose increased in both lean and obese rats. At all doses of replacement obese rats were more responsive to steroid than were lean rats. Obese rats receiving 0.01 mg had comparable fat depot weights, cell sizes, and plasma insulin and triglyceride as lean rats receiving 50 times as much steroid per day (0.50 mg). These results suggest glucocorticoids play an important role in the early development of obesity in the Zucker rat and support the hypothesis that obese rats are more responsive to glucocorticoids than are lean rats.

Zucker rat; brown adipose tissue; body fat composition

HORMONAL IMBALANCES, such as high circulating levels of insulin or glucocorticoids, are characteristic of some obesities in humans and experimental animals (13, 33, 35). An animal model often used to investigate hormonal influences on the etiology of obesity is the genetically obese Zucker rat (fa/fa). This animal exhibits hyperinsulinemia (40), normal to slightly elevated blood glucose levels (40), and peripheral tissue insulin resistance both in vivo and in vitro (11, 36). In addition, the obese rat exhibits impaired thermogenic capacity, as indicated by lower rectal temperature (18), and decreased GDP binding to brown adipose tissue mitochondria (21). In a developmental study Zucker and Antoniades (40) found that fatty rats had a greater percentage of body fat than their lean littermates by 2 wk of age and that hyperinsulinemia was not evident until 3 wk of age. However, pancreatic perfusion studies from our laboratory revealed significantly elevated plasma insulin in the obese rat when compared with the homzygous lean (Fa/Fa) rat as early as 2 wk of age (4). The contribution of the increased amounts of circulating insulin to the development of obesity was evaluated using alloxan-diabetic lean and obese Zucker rats comparably treated with insulin (8). Metabolizable energy intake and carcass weight gain were comparable in both genotypes and increased when exogenous insulin was increased. However, fat gain was higher and protein gain was lower in obese rats receiving comparable doses of insulin as lean rats. In addition, adipose tissue lipoprotein lipase activity was markedly elevated in obese compared with lean rats at the same dose of insulin replacement (9). These results suggest that both insulin and some other genetic factor(s) were important in elevating adipose lipoprotein lipase activities and thus lipid deposition in obese Zucker rats.

Increasing evidence points to a role for adrenal glucocorticoid hormones in the expression of obesity in fatty rats (7, 38, 39). To date, adrenalectomy of 10-wk-old Zucker obese rats has been the only procedure to effectively normalize rate and composition of weight gain and food intake to levels comparable to those found in lean rats (7, 38). In addition, adrenalectomy of 10-wk-old obese rats decreased plasma insulin and ameliorated insulin resistance in comparison to sham-operated obese control rats (16, 32). In contrast, when body weight gain of the obese rat is reduced to that of its lean littermate by food restriction, the expression of obesity and hyperinsulinemia is not prevented (10). Adrenalectomy also decreased plasma triglyceride, fat cell size, and lipoprotein lipase activity (32, 38). It also restored GDP binding in brown adipose tissue from obese rats to that of lean control values (21). Despite daily corticosterone and adrenocorticotropin concentrations being indistinguishable from those of lean littermates (19), obese adrenalectomized rats appeared more responsive than lean ad-
renalactomized rats to glucocorticoid replacement, as measured by food intake and body weight gain (15, 39). These results were reported for both 10- to 19-wk-old obese rats exhibiting hyperphagia (39) and 26-wk-old obese rats no longer hyperphagic (15).

Thus the purpose of this study was to investigate the effect of glucocorticoids on earlier stages of development of obesity in the Zucker rat and to further test the hypothesis that obese rats are more responsive to glucocorticoids than are lean rats. We first adrenalectomized 30- to 32-day-old lean and obese rats. A two-way design comprised of two genotypes (lean and obese) and five different doses of replacement for 30 days allowed the evaluation of the effects of the obese gene, glucocorticoids, and their interactions on deposition of fat and protein, food intake, white adipose tissue cellularity, plasma insulin, white adipose tissue lipoprotein lipase activity, brown adipose tissue protein content, and brown adipose tissue enzymatic activity. Thus the contribution of circulating glucocorticoids to the development of obesity was evaluated.

METHODS

Animals. At 30-32 days of age female obese (fa/fa, body wt 75–90 g) and lean (fa/-, body wt 70–80 g) Zucker rats were randomly assigned to one of four different groups: obese adrenalectomized (ADX), obese sham-operated control, lean ADX, and lean sham-operated control. Bilateral adrenalectomies were performed from the dorsal approach under methoxyflurane anesthesia (Metrofan, Pitman-Moore, Washington Crossing, NJ). Sham-operated controls were exposed to the same surgical procedures excluding removal of adrenal glands. ADX rats were maintained on 1.0% (wt/vol) NaCl in drinking water. Control rats received tap water. All animals were fed stock diet (Purina rat chow) ad libitum. Rats were doubly housed in a temperature-controlled room (23 ± 1°C) for 15 days after surgery to prevent death from hypothermia, a problem previously noted in young ADX rats. After 15 days and for the remaining 2 wk, rats were single housed and daily food intakes were recorded, with careful deductions for spillage. A 12-h light-dark cycle was maintained, with lights off at 1700 h.

After surgery and for the subsequent 2 days, ADX rats were injected subcutaneously with 0.1 mg hydrocortisone 21-acetate and 0.05 mg deoxyxycorticosterone acetate (Sigma Chemical, St. Louis, MO). Between 0900 and 1100 h on the third day postsurgery, rats were killed to provide base-line data. That afternoon steroid replacement began. ADX rats were randomly assigned to one of five replacement dose groups: 0.01, 0.05, 0.5, 1.0, and 2.0 mg hydrocortisone/100 g body wt (Cortef, Upjohn, Kalamazoo, MI). Between 1600 and 1700 h each ADX rat was weighed and injected subcutaneously with its assigned dose for a total of 30 days. Sham-operated controls were also weighed daily but received no injection.

Thirty to thirty-one days postsurgery, between 0900 and 1200 h, all animals were killed by decapitation. Blood was collected in heparinized tubes for analysis of plasma insulin, glucose, triglyceride (TG), and corticosterone. ADX animals with corticosterone levels >0.05 mg/dl were not used. Carcasses were eviscerated for analysis of body composition. Left and right parametrial and retroperitoneal fat depots were removed, weighed, and sampled for fat cell number and size determinations. Adipose tissue homogenates were prepared for lipoprotein lipase (LPL) activity in ice-cold 0.25 M sucrose-1 mM EDTA buffer (1:4, wt/vol; pH 7.4) from both retroperitoneal fat depots by use of ground glass-on-glass homogenizing equipment. All homogenates were centrifuged at 12,000 g for 15 min at 4°C, and the postmitochondrial supernatant was aspirated and stored at −70°C. Scapular and cervical brown fat depots were dissected, weighed, and then immediately frozen at −70°C for subsequent determination of maximal citrate synthase (CS) (EC 4.1.3.7) and β-hydroxyacyl-CoA dehydrogenase (HOAD) (EC 1.1.1.35) activities and for protein content. CS activity was taken as a measure of potential flux through the Krebs (tricarboxylic acid) cycle, and HOAD was used as an indicator of potential flux through the fatty acid β-oxidation pathway.

Analyses. Total carcass water, fat, protein, and ash were determined by freeze-drying, ether-acetone extraction, and ashing in a muffle oven as previously described (3). Insulin was measured by radioimmunoassay using a rat insulin standard (20.7 U/mg; Novo, Bagsvaerd, Denmark) and [125I]insulin (Cambridge Nuclear, Cambridge, MA) (8). Plasma glucose was determined using the glucose oxidase method with a Beckman Glucose Analyzer 2. TG was assayed by measuring free glycerol (Boehringer Mannheim, Indianapolis, IN), and corticosterone was measured by radioimmunoassay (Radiosys Systems, Carson, CA). Adipose cell number of fat depots was measured for individual rats using electronic counting of osmium-fixed cells as described by Hirsch and Gallian (20). Percent lipid for each depot was determined using the chloroform:methanol extraction (2:1) technique of Folch et al. (14). Fat cell size (μg TG/cell) was calculated by dividing the fat depot cell number into total pad lipid. Adipose tissue LPL activity of the retroperitoneal depot was assayed as described by Chan et al. (9). The substrate was [14C]triolein, and lysolecithin was added as an emulsifier. LPL activity is defined as that activated by fasted human serum and inhibited by 1 M NaCl. For brown adipose tissue enzyme analyses, weighed tissue samples were thawed and homogenized in phosphate buffer (100 mM phosphate, 2 mM EDTA, pH 7.3). Homogenates were then refrozen at −70°C until assayed. On the day of the assay the frozen homogenates were thawed and sonicated (at 2–4°C) for two 8-s intervals. For CS [method of Bass et al. (1) and Srere (30)] the reaction medium contained (in mM) 10 tris(hydroxymethyl)aminoethane-HCl, 2.5 EDTA, 0.1 5,5′-dithiobis(2-nitrobenzoic acid), 0.2 acetyl-CoA, and 0.5 oxaloacetate. For HOAD [method of Bass et al. (1)] the reaction medium consisted of (in mM) 100 triethanolamine-HCl, 5 EDTA, 0.225 NADH, and 0.1 acetoacetyl-CoA, pH 7.0. Enzyme activities were measured as the change in absorbance at 412 (CS) or 340 (HOAD) at 25°C. Tissue homogenates were assayed for protein by the method of Lowry et al. (24), with bovine serum albumin as the
standard. To avoid interference from fat present in the sample, all assay tubes were filtered (0.45 μm, Millipore, Bedford, MA) after the Lowry reagents were added. Samples were then assayed spectrophotometrically.

Statistical procedures. Data were analyzed by two-way analysis of variance (ANOVA) for the effects of genotype, dose, and their interaction. When the analysis gave a significant F value, differences between means were evaluated using the Newman-Keuls test, and multiple t tests with the Bonferroni adjustment were used when variances were unequal. Data for lean and obese rats were analyzed separately (one-way ANOVA) to assess effect of dose. Differences between sham-operated lean and obese rats were tested by Student's t. A P < 0.05 was taken as significant.

RESULTS

The effects of adrenalectomy and steroid replacement on body weight gain, food intake, body composition, adipose cellularity, plasma insulin, glucose, and TG, adipose tissue LPL activity, and brown adipose tissue mass, protein content, and enzymatic activities are reported below. Doses of 0.01, 0.05, 0.50, 1.0, and 2.0 mg hydrocortisone/100 g body wt are referred to as doses I, II, III, IV, and V, respectively. Comparisons are first presented

FIG. 1. A: body weight (grams) of sham-operated and adrenalectomized 4-wk-old lean Zucker rats from beginning of steroid replacement (day 1) to death. Results are mean values. B: body weight (grams) of sham-operated and adrenalectomized 4-wk-old obese Zucker rats from beginning of steroid replacement (day 1) to death. Results are mean values.
between sham-operated lean and obese rats, next between sham-operated and ADX-steroid-replaced rats within each genotype, and then between ADX-steroid-replaced rats of both genotypes. Last, comparisons between obese ADX rats at low replacement doses (doses I and II) and lean sham controls were made to assess the effects of ADX and low doses of steroid replacement on the development of obesity.

**Body weight gain.** Growth of lean and obese rats during the course of this experiment is illustrated in Fig. 1. At the start of the experiment the initial weight of lean and obese sham control rats was not significantly different (76.0 ± 1.3 vs. 79.8 ± 1.7 g, lean vs. obese, P > 0.05). However, daily body weight gain (g/day) was significantly greater in obese compared with lean sham controls (P < 0.01; Table 1). The initial weight of rats at the time of adrenalectomy averaged 76.77 ± 0.5 and 84.10 ± 1.1 g (P < 0.05) for lean and obese rats, respectively. Lean ADX rats at all doses of replacement gained less weight than their sham controls (Table 1). Weight gain of these replaced rats increased as dose increased from dose I to II. Thereafter, weight gain significantly decreased as dose increased (doses III, IV, and V). Obese ADX rats at all replacement doses also gained less weight than their sham controls. Daily weight gain increased as dose increased from dose I to III, although there was no significant difference in weight gain at doses II, III, and IV. Weight gain of obese rats decreased at dose V. From these data the optimal dose of steroid for body weight gain was taken to be dose II in lean and dose III in obese rats. Obese rats receiving doses IV and V gained significantly more weight than did lean rats at these doses (P < 0.01). At all doses of replacement obese ADX rats gained less weight than did lean sham controls.

**Food intake.** Daily food intake, measured over the last 2 wk of the experiment, was significantly higher in obese control compared with lean control rats (P < 0.001; Table 1). Lean ADX rats at the three lowest doses (doses I, II, and III) had comparable food intakes to control rats; those at the highest doses (doses IV and V) had decreased food intakes compared with controls. All obese ADX-replaced rats, however, ate less food per day than did sham controls. There was a tendency toward increased food intake as steroid dose increased from dose I to III, although this was not significant. Contrary to what was seen in lean rats, obese rats did not decrease food intake at higher replacement doses. Daily food intake was significantly higher in obese compared with lean rats receiving doses III, IV, and V. Obese rats at the lowest dose of steroid consumed significantly less food per day than did lean sham controls (P < 0.05).

When food intake data were expressed per metabolic size (kcal/BW<sup>0.75</sup>), where BW is body weight, obese control rats still ate significantly more than did the lean sham controls (P < 0.001; Table 1). Although lean rats tended to decrease food intake per BW<sup>0.75</sup> as dose increased, no significant differences between lean rats at any dose or between lean ADX and sham-operated controls were noted. In contrast, obese rats tended to increase food intake per BW<sup>0.75</sup> as dose increased. However, this was not significant nor different from sham-operated controls. Food intake per BW<sup>0.75</sup> of lean and obese rats differed only at dose V. Because body compositions differed, we expressed food intake per gram metabolic fat-free weight (kcal/g FFW). Data expressed this way still showed significant differences between lean and obese control rats (Fig. 2). No difference in energy intake per gram FFW between lean rats and their sham controls or between lean rats at different doses was noted. Interestingly, obese rats at the two lowest doses now showed a significant decrease in food intake per FFW compared with their sham controls. As dose increased, kilocalories per FFW also significantly increased to levels greater than (but not significantly different from) sham controls. Obese rats had significantly higher energy intakes per gram FFW than lean rats at doses III, IV, and V. Last, obese rats at dose I consumed significantly more kilocalories per gram FFW than did lean controls.

**Body energy gain.** Carcass fat and protein gain over 30 days was calculated by the method of comparative slaughter. The fat and protein contents of both ADX and sham-operated rats killed 3 days after surgery were 5.09 ± 0.3 and 11.82 ± 0.2 g for the lean rats and 18.45 ± 0.4 and 10.25 ± 0.2 g for the obese rats, respectively. In terms of percent body fat and protein these values represent 8.14 ± 0.3 and 18.94 ± 0.2% for the lean rats.

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**Table 1. Body weight gain and food intake**

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<th>sham Control</th>
<th>dose 1, 0.05 mg</th>
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<td><strong>Lean rats</strong></td>
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<td>BW gain, g/day</td>
<td>3.2±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0±0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2±0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.4±0.1&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Food intake, g/day</td>
<td>15.6±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.9±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.9±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.8±0.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.8±0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.9±0.5&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Food intake/BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td>0.58±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.64±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.55±0.02&lt;sup&gt;f&lt;/sup&gt;</td>
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<td><strong>Obese rats</strong></td>
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<td>BW gain, g/day</td>
<td>5.0±0.3&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.8±0.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.1±0.03&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.4±0.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.2±0.3&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1.2±0.1&lt;sup&gt;***&lt;/sup&gt;</td>
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<td>Food intake, g/day</td>
<td>26.2±0.3&lt;sup&gt;**&lt;/sup&gt;</td>
<td>14.4±0.5&lt;sup&gt;**&lt;/sup&gt;</td>
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<td>17.6±0.9&lt;sup&gt;**&lt;/sup&gt;</td>
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<td>Food intake/BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
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<td>0.77±0.01&lt;sup&gt;**&lt;/sup&gt;</td>
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Values are means ± SE. Food intake measurements were made daily for last 2 wk of experiment. Food intake/BW<sup>0.75</sup> represents daily food intake and corresponding body weight (raised to two-thirds power) of individual rats within each group over last 2 wk of experiment. Values that do not share common superscript are significantly different from those of other groups of rats within same genotype (P < 0.05). ** *** Obese rats that significantly different from lean, P < 0.05, P < 0.01, and P < 0.001, respectively.
FIG. 2. Food intake per fat free weight (kcal/g FFW) of lean and obese sham-operated and adrenalectomized (ADX) rats. Values are means ± SE. ADX rats received steroid replacement at 5 doses. These values are plotted on semilog scale. ***Between lines: obese ADX rats and lean ADX rats at same replacement dose significantly different, P < 0.01 and P < 0.001, respectively. *Above lines: obese ADX rats significantly different from obese sham-operated rats, P < 0.05. **Obese and lean sham-operated rats significantly different, P < 0.01.

FIG. 3. Body energy gain of lean (L) and obese (O) sham-operated and adrenalectomized rats, in fat and protein. Kilocalorie values were calculated by multiplying average grams of fat gain by 9.3 kcal/g and average grams of protein gain by 5.25 kcal/g. **Significance of bars: significant differences in either protein or fat gain between lean and obese rats at same replacement dose, P < 0.05 and P < 0.01, respectively. ***Above bars: significant differences in total body energy gain, P < 0.05 and P < 0.01, respectively.

and 27.06 ± 0.7 and 15.15 ± 0.2% for the obese rats, respectively. In obese sham control rats elevated body energy gain was attributed to the tremendous increase in fat gain (60.1 ± 11.4 vs. 61.9 ± 8.4 kcal, fat vs. protein gain; Fig. 3). In contrast, body energy gain of lean control rats was evenly distributed between fat and protein gain (90.40 ± 8.5 and 83.34 ± 3.8 kcal, respectively). Thus lean control rats deposited significantly more protein and less fat than did obese controls (P < 0.05). In the lean genotype body fat gain was greatest in the sham-operated group (P < 0.05) and least at doses I and V. Body protein gain was also greatest in sham controls, followed by doses II and I. Thereafter, protein gain significantly decreased at each increasing dose. In obese rats body fat gain was greatest in sham controls (P < 0.05). Obese rats maintained on the lowest dose of steroid showed a loss of body fat over the experimental period. As the amount of steroid injected was increased from dose I to IV, carcass fat gain increased. However, fat gain decreased at dose V. Body protein gain was greatest in obese sham controls and rats at doses I and II (P > 0.05). Thereafter, protein gain significantly decreased as dose increased. Between genotypes fat gain was significantly higher in obese rats at doses III, IV, and V, whereas protein gain was significantly lower in obese rats only at dose III. Obese rats at the lowest dose of replacement deposited significantly less protein and less fat than did lean sham controls.

Total body energy gain was calculated by summing body protein gain (5.25 kcal/g) and body fat gain (9.3 kcal/g). When expressed by total kilocalories, body energy gain was greatest in obese sham control rats (663 ± 29 vs. ± 173 ± 8 kcal, obese vs. lean). In both lean and obese ADX-replaced rats total body energy gain paralleled fat gain. Interestingly, both lean and obese rats at the two lowest doses retained more of their body energy as protein and less as fat. Lean rats at dose III (like lean sham controls) deposited equal amounts of fat and protein. Therefore, decreased body energy gain of lean rats was reflected by decreased protein and fat deposition, although rats at these doses (doses IV and V) deposited more kilocalories as protein than fat. In contrast, obese rats at doses III and IV dramatically increased fat and decreased protein deposition. Even at the highest dose (dose V), where energy gain was less than at doses III and IV, fat gain far outweighed protein gain (158 ± 13 vs. 4.0 ± 4.3 kcal, fat vs. protein).

Body composition. Obese sham-operated control rats had a significantly greater percentage of body fat than did lean controls (47.8 ± 4.1 vs. 11.4 ± 0.8%, respectively; Fig. 4). When lean ADX rats were compared with lean controls, doses I, II, and V produced rats that were less fat; doses III and IV produced rats of similar body fat composition (Table 2). In obese ADX rats doses I and II produced rats that were less fat then obese sham-operated controls; doses III, IV, and V produced rats of
comparable body fat composition to controls. Obese rats were consistently fatter than lean rats at each dose of replacement (P < 0.01), although replacement of obese rats at the lowest dose resulted in percent body fat comparable to lean sham controls.

Obese control rats had a significantly lower percentage of body protein than did lean control rats (Fig. 4). At all doses of replacement except dose III, lean rats deposited significantly more protein (as a percentage of carcass weight) than did their sham controls. Obese rats at the two lowest doses (doses I and II) deposited significantly more protein than all other obese rats, including sham controls (Table 2). At all doses obese rats had a significantly smaller percentage body protein than lean rats (P < 0.01). Contrary to what was seen with body fat, replacement of obese ADX rats at the lowest dose did not normalize body protein to levels comparable to lean sham control rats.

**Fat depot weights.** Obese control rats had significantly heavier parametrical and retroperitoneal fat depots than did lean control rats (P < 0.01; Figs. 5A and 6A). Both lean and obese control rats had significantly larger depots than respective ADX-replaced rat at all doses (P < 0.05). Depot weights increased as dose increased from dose I to III in lean rats and from dose I to IV in obese rats. Thereafter, depot weights decreased. At each dose of steroid except the lowest, obese rats had heavier fat depots than lean rats. Replacement of obese rats at dose I resulted in normalization of both parametrical and retroperitoneal fat depot weights to levels comparable to lean sham controls.

**Adipose cellularity.** Sham-operated obese control rats had significantly increased fat cell size, but not fat cell number, compared with lean sham controls, findings consistent with other studies (8). In lean rats adenectomy and steroid replacement affected cell number and size. Lean control rats had the largest cells (P < 0.05). Cell size increased in both depots as steroid dose increased from dose I to III; thereafter cell size decreased (Figs. 5B and 6B). In the parametrical depot only rats at doses II and III had comparable cell number to sham-operated controls; rats at doses I, IV, and V had fewer cells (Fig. 5C). In the retroperitoneal depot only rats at dose II had comparable cell number to controls; all other rats had fewer cells (Fig. 6C).

In obese rats cell size was affected by adenectomy and steroid replacement. In the parametrical depot obese control rats had the largest cells. In obese ADX rats cell size increased as dose increased from dose I to IV and then decreased at dose V (Fig. 5C). In the retroperitoneal depot cell sizes of control rats were not different from those of rats at doses III and IV; sizes were smaller in depot of rats from all other doses (Fig. 5C). Between genotypes, at the same dose of steroid (except for dose I in the retroperitoneal depot), fat cell size was significantly larger in obese rats compared with lean rats. Fat cell number was consistently, although not significantly higher, in obese compared with lean rats. Again, steroid replacement of obese rats at the lowest dose resulted in normalization of fat cell size and fat cell number to levels comparable to lean sham controls.

When the number of cells in both depots was combined (Fig. 7), there was no difference in total cell number between control rats of either genotype (P > 0.2). In lean rats the same pattern as seen in the retroperitoneal depot was noted. Lean ADX rats at dose II had comparable total cell number to controls, and lean ADX rats at all other doses had significantly fewer cells. In obese rats there was no difference in total cell number between sham controls and rats in all other groups. Only obese rats at the highest dose had significantly more cells than lean rats at that dose. Total cell number of obese rats at dose I was comparable to that of lean sham controls.

**Insulin.** Plasma insulin levels of obese control rats were almost 200-fold higher than those of lean controls (Fig. 8). Compared with lean sham controls, only lean rats at dose IV had significantly elevated plasma insulin. In the obese genotype, however, replacement at doses III and V resulted in insulin values comparable to obese sham controls, whereas replacement at dose IV resulted
in significant elevation of plasma insulin compared with obese controls. Rats in these groups were severely hyperinsulinemic. Obese rats at doses IV and V had significantly higher insulin levels than lean rats at these doses (P < 0.01). There was no difference in plasma insulin between obese rats at dose I and lean sham controls.

Glucose. Obese sham controls had significantly elevated blood glucose compared with lean controls (Fig. 9). In lean rats plasma glucose was unaffected by dose except
at the highest dose, where a significant decrease in plasma glucose was noted. In obese rats there was no effect of steroid on plasma glucose except at dose V, where one case of steroid-induced diabetes (blood glucose 318 mg/dl) and another of hypoglycemia (82 mg/dl) were observed. There was no significant difference in blood glucose between lean and obese rats at any dose. Plasma glucose was comparable between obese rats at dose I and lean sham controls.

Plasma TG. Obese control rats also had significantly higher TG values than did lean control rats (Fig. 10). Plasma TG was comparable in lean control rats and lean ADX rats receiving doses III, IV, and V. Only lean rats at doses I and II had plasma TG significantly lower than sham controls and rats at dose III, although these levels were not significantly different from rats at doses IV and V. In obese rats plasma TG was lowest in rats at doses I and II. These values were significantly different from that of obese sham controls. Thereafter, as dose increased, plasma TG increased to levels significantly greater than sham controls (dose V). The trend toward significantly higher plasma TG in obese compared with lean rats began at dose III but reached significance at doses IV and V. Adrenalectomy and steroid replacement of obese rats at the two lowest doses resulted in normalization of plasma TG levels.

LPL activity. LPL activity expressed per retroperitoneal depot or per cell ($\times 10^6$) was highest in obese controls compared with lean controls (Fig. 11). Because obese rats had significantly larger cells and higher insulin values...
than did lean rats, separate analyses of covariance using cell size and insulin as independent variables were done. These analyses did not change results. In the lean genotype no LPL values for rats at doses I and V were reported due to inadequate tissue sizes for sampling. Activity per depot was greatest in lean sham control rats compared with lean ADX rats at doses II, III, and IV. However, there was no difference in activity in lean ADX rats at these doses. Activity per cell was again significantly greater in control rats compared with that of rats at doses III and IV but not dose II. In obese rats activity per depot and per cell was greatest in control rats compared with rats in all other groups (P < 0.05). Obese rats at doses IV and V had significantly lower LPL activities per cell than obese rats at doses I, II, and III. When cell size was used as a covariate, these differences were no longer significant. Activity per depot appeared to be greatest in obese rats at doses III; however, this difference was not significant. Although enzyme activities tended to be higher in obese compared with lean rats at doses II, III, and IV, significant differences were noted only at dose III. Using cell size as a covariate did not change these results. However, when plasma insulin was used as the covariate, differences were no longer seen. Adrenalectomy and replacement of obese rats at the lowest doses (doses I and II) decreased LPL activity of obese rats to levels comparable to lean sham controls.

*Brown adipose tissue.* When compared with lean sham-operated rats, obese sham-operated rats had significantly heavier brown adipose scapular and cervical depots containing less protein (mg/depot; Table 3). Total activity of CS and HOAD, calculated by expressing activity per depot (μmol/min) and combining values for scapular and cervical depots, was significantly higher in lean compared with obese control rats (P < 0.001) (Fig. 12).

In lean rats scapular and cervical depot weights of steroid-replaced rats were not different from that of sham-operated controls except at the highest dose (dose V) (Table 3). Brown fat protein content (mg/depot) was significantly elevated in rats at low replacement doses (doses I and II). Thereafter protein content tended to decrease, and in the cervical depot, rats receiving the highest replacement dose (dose V) had significantly reduced protein content compared with that of lean controls. In the scapular depot the maximal specific activities (μmol·min⁻¹·mg protein⁻¹) of both CS and HOAD were comparable in lean rats at all replacement doses to those in their sham-operated controls. In the cervical depot CS specific activity was significantly reduced only in rats at the highest dose (dose V), and HOAD specific activity significantly reduced only in rats at dose III. When activity was expressed per depot and values for scapular and cervical depots combined, total maximal CS and HOAD activity (μmol/min) was greatest in lean rats at the lowest dose of replacement (Fig. 12). Thereafter, total activity significantly decreased as dosage increased. At doses IV and V for CS and doses III, IV, and V for HOAD, total activity was comparable to that of lean sham-operated controls.

In obese rats receiving the lowest doses of replacement, scapular and cervical depots weighed significantly less than those of obese sham-operated controls (Table 3). Thereafter, as steroid dose increased, depot weights increased to values no longer significantly different from sham controls. Protein content (mg/depot) of brown adipose tissue was significantly elevated in obese rats at the two lowest doses of replacement. However, as steroid dose increased, protein content decreased to levels comparable to that of obese sham controls. In the scapular depot specific activity (μmol·min⁻¹·mg protein⁻¹) of both CS and HOAD was comparable in obese rats at all doses of replacement and their sham-operated controls. In the cervical depot CS specific activity was greatest in obese sham-operated rats, but there was no consistent change in CS specific activity among obese rats receiving steroid replacement. In this depot only obese rats at dose IV exhibited significantly decreased HOAD activity compared with all other groups of rats. When activities were expressed per depot and values for scapular and cervical depots combined, total activity of CS and HOAD (μmol/min) was greatest in obese rats at the lowest dose of replacement compared with all other groups of rats (Fig. 12). Thereafter, as dose increased, total activity of CS and HOAD decreased to levels no longer significantly different from that of obese sham-operated controls.

Obese rats receiving doses III, IV, and V had significantly heavier scapular and cervical depots than did lean
TABLE 3. Brown adipose tissue mass, protein content, and CS and HOAD specific activity in scapular and cervical depots

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
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<th>Dose I, 0.01 mg</th>
<th>Dose II, 0.06 mg</th>
<th>Dose III, 0.5 mg</th>
<th>Dose IV, 1.0 mg</th>
<th>Dose V, 2.0 mg</th>
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<tr>
<td></td>
<td>n</td>
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<td>6</td>
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<td>90±32</td>
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<td>1.58±0.03</td>
<td>1.85±0.07</td>
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<td>HOAD activity, U/mg protein</td>
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<td>1.26±0.12</td>
<td>1.03±0.07</td>
<td>0.88±0.06</td>
<td>0.98±0.18</td>
<td>1.15±0.12</td>
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<td>390±75**</td>
<td>542±50***</td>
<td>703±52***</td>
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<td>Protein content, mg/depot</td>
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<td>493±44**</td>
<td>287±35**</td>
<td>147±45</td>
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<td>CS activity, U/mg protein</td>
<td>2.57±0.29*</td>
<td>1.92±0.09*</td>
<td>1.83±0.09*</td>
<td>1.94±0.19*</td>
<td>1.55±0.45*</td>
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<td>HOAD activity, U/mg protein</td>
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<td>1.22±0.17**</td>
<td>1.46±0.17***</td>
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<td>43±3*</td>
<td>46±3*</td>
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<td>71±6**</td>
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<td>1.58±0.08*</td>
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<td>HOAD activity, U/mg protein</td>
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<td>0.78±0.07**</td>
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<td>0.88±0.07</td>
<td>0.91±0.09*</td>
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<tr>
<td>Cervical depot in lean rats</td>
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<td>53±4*</td>
<td>59±10*</td>
<td>94±6****</td>
<td>132±17***</td>
<td>138±15***</td>
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<tr>
<td>Protein content, mg/depot</td>
<td>35±3**</td>
<td>154±20*</td>
<td>113±24**</td>
<td>43±8***</td>
<td>37±2***</td>
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<tr>
<td>CS activity, U/mg protein</td>
<td>2.28±0.20**</td>
<td>1.54±0.24*</td>
<td>1.79±0.11**</td>
<td>1.89±0.17*</td>
<td>1.21±0.09*</td>
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<tr>
<td>HOAD activity U/mg protein</td>
<td>0.70±0.13**</td>
<td>0.99±0.14*</td>
<td>0.91±0.06*</td>
<td>0.78±0.13*</td>
<td>0.44±0.06*</td>
<td>0.95±0.36*</td>
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</table>

Values are means ± SE. One unit enzyme activity equals 1 pmol substrate converted to product per minute. BW, body weight; CS, citrate synthase; HOAD, β-hydroxylacyl-CoA dehydrogenase. Values that do not share common superscript are significantly different from those of other groups of rats within same genotype (P < 0.05). *** Significant difference from lean, P < 0.05, P < 0.01, and P < 0.001, respectively.

Rats receiving comparable doses at all doses obese rats tended to have significantly less brown adipose tissue protein per depot than did lean rats. With regards to CS and HOAD maximal specific activities there were no consistent differences between lean and obese steroid-replaced rats. However, when activity was expressed per depot and values of scapular and cervical depots combined, total maximal activity of CS and HOAD (μmol/min) tended to be lower in obese compared with lean rats at all doses of replacement. Two-way ANOVA testing the effects of genotype and dose revealed a significant effect of genotype for CS activity [F(1,4), 834, P < 0.01] and a significant effect of dose for both CS [F(1,4), 22.40, P < 0.001] and HOAD [F(1,4), 21.67, P < 0.001].

Compared with lean sham-operated controls, obese rats at the lowest doses of replacement had significantly elevated total activity of CS and HOAD (μmol/min; Fig. 12).

DISCUSSION

The present experiment demonstrates that adrenalectomy and glucocorticoid replacement profoundly affect development of obesity in the Zucker fatty rat. Furthermore, it indicates obese rats are more responsive to glucocorticoid replacement than are lean rats. These results confirm reports implicating glucocorticoids as fundamental to the full expression of the fat gene in 10-wk-old rats (5, 32, 38, 39) and extend implications to weanling rats. Previously, it was reported that adrenalectomy of fatty rats at 10 wk decreased body weight gain and food intake to levels comparable to lean controls (32, 39). The present study indicates that adrenalectomy at 4 wk decreases body weight gain and food intake (g/day) to levels significantly lower than lean sham controls. Similarly, it was reported that adrenalectomy of older fatty rats resulted in lower percent body fat, fat cell size, and plasma insulin and triglyceride compared with obese sham controls (32, 38). Although fat cell size and plasma triglyceride of the obese adrenalectomized rat were comparable to lean controls, percent body fat and plasma insulin were still greater (32, 38). The present study is the first to report that adrenalectomy of weanling obese rats normalizes fat deposition, fat depot weights, adipose cell size, and plasma insulin, triglyceride, and glucose to levels not significantly different from or lower than that of lean controls.

In this and other studies (7, 32, 38, 39) the effects of adrenalectomy were not limited to the obese genotype. After adrenalectomy lean rats also decreased body weight gain, body fat, and fat cell size. However, obese and lean rats’ responsiveness to glucocorticoid replacement was markedly different. In lean rats replacement at doses II and III resulted in comparable body composition and body weight gain to lean sham controls. In obese rats replacement at doses III, IV, and V resulted in comparable body fat content despite a significant reduction in body weight gain compared with sham controls. Furthermore, at each replacement dose obese rats were significantly fatter than lean rats, even when body weight gain was not significantly different (doses I, II, and III). Generally, obese rats responded to steroid by greatly increasing fat deposition, whereas lean rats did not.
Increased responsiveness to glucocorticoids by obese rats was also demonstrated by significant elevations of plasma insulin and triglyceride as dose increased. In some cases levels were significantly greater than those of obese sham controls. No such elevations were seen in lean rats. These results invite speculation as to the role of adrenal hormones in the development and maintenance of body weight and adiposity in the fatty rat. Although plasma corticosterone concentrations over a 24-h period are not different in lean and obese rats (17), obese rats lack diurnal periodicity of glucocorticoid secretion and food intake (15, 17). Perhaps this tonic rather than phasic secretion of steroid results in enhanced responsiveness, which in turn may account for the obesity that occurs (12). When excess glucocorticoids are administered it is believed that the primary action of the hormone remains the same, but the added amount influences secretion rates of other hormones or substrates, which in turn may be responsible for the different effects (31). There is evidence that glucocorticoids administered in excess elevate the rate of insulin secretion in several species including sheep (2), Chinese hamsters (6), mice (28), and rats (25). Conversely, it has been reported that adrenalectomized rats secrete less insulin in response to increased blood glucose concentrations and that their fasting immunoreactive insulin levels are depressed (34). In adult Zucker rats adrenalectomy markedly decreased plasma insulin in both lean and obese animals, whereas glucocorticoid replacement normalized plasma insulin in the lean genotype but significantly elevated it in the obese genotype (T. W. Castonguay, unpublished observations). As indicated in this study, when adrenalectomized weanling rats received glucocorticoid replacement, plasma insulin of lean rats significantly increased only at dose IV, although these rats could hardly be considered hyperinsulinemic at 77 μU/ml. In contrast, plasma insulin of obese rats significantly decreased at low doses of replacement (to levels comparable to lean sham controls) and significantly increased at high doses (to hyperinsulinemic levels not significantly different or significantly greater than obese controls). This is not surprising considering obese rats are insulin resistant, and adrenalectomy ameliorates their insulin resistance (16). Thus it appears that glucocorticoid-induced hyperinsulinemia may be of primary importance to full expression of the fa gene.

In obese rats at low replacement doses, body fat gain significantly decreased, and body protein gain significantly increased. In fact, obese rats at dose I lost 3.3 g of fat while gaining 9.4 g of protein over the course of the experiment. This indicates mobilization of fat energy to promote lean growth, results identical to Chan et al. (8), who found a gain in lean energy and a loss in fat energy in alloxan-diabetic obese rats at a low insulin dose. In addition, Chan et al. (8) reported that the low-insulin group was the only group of obese rats that did not exhibit impaired protein utilization relative to the lean rats. They concluded that a certain level of insulin was necessary for manifestation of the obese gene (8). Present results seem to substantiate this hypothesis; i.e., full expression of obesity may be dependent on a critical level of insulin that may be influenced or mediated in part by glucocorticoids. Glucocorticoids are catabolic hormones known to increase protein breakdown and gluconeogenesis from amino acids. Insulin, on the other hand, is an anabolic hormone known to increase fat deposition and protein synthesis. If obese rats are more responsive to glucocorticoids, hyperinsulinemia may be protective to them. The resulting ratio of insulin to glucocorticoids may help to explain the increased carcass fat but decreased carcass protein often seen in the Zucker fatty rat (8, 27) and seen in the obese but not lean rat receiving higher doses of glucocorticoid replacement.

As reported in this study and previously (38), adipocyte cell size after adrenalectomy was smaller than at the time of surgery, although cell number continued to increase. This implies hyperplasia of fat cells is not dependent on the presence of enlarging fat cells or glucocorticoids. Also, we report no change in cell number with glucocorticoid replacement, even though cell size and carcass fat deposition increased. Similar results were reported in alloxan-diabetic insulin-replaced rats (9) whose capacity to increase adipocyte number appeared to be dissociated from adipocyte hypertrophy and increased carcass lipid deposition. Results are in agreement.
with those of Salans et al. (29), who reported long-term insulin replacement resulted in increased fat deposition and fat cell size, but not cell number, and Krotkiewski and Björntorp (23), who noted increased fat cell size but no change in number due to daily insulin administration over 4 wk. It appears that the adrenalectomized animal (receiving no or a low dose of steroid replacement) is similar to the alloxan-diabetic animal rat at low dose of insulin replacement in that enlargement of existing adipocytes is impaired while adipocyte proliferation is not.

Greenwood et al. (19) have proposed a role for adipose tissue lipoprotein lipase in development and maintenance of obesity. However, our results do not support this proposal. No significant difference in lipoprotein lipase activity was seen at any replacement dose within either the lean or obese genotype, despite significant differences in body fat deposition and fat cell size. Only at dose III was there a significant difference in activity per depot between lean and obese rats. However, when plasma insulin was used as a covariate, no difference was seen. Results are in contrast to those of Chan et al. (9), who reported a marked elevation of adipose tissue lipoprotein lipase activity in obese rats when circulating insulin was made comparable to that of lean rats. We have no explanation for these differences.

The suggestions that adrenalectomy may enhance thermogenesis in order to prevent obesity and that thermogenic capacity of brown adipose tissue is suppressed by adrenal steroids in the obese rat (21) were further evaluated in this experiment. In agreement with Holt and York (21) we found no difference in wet weight of brown adipose tissue of lean rats after adrenalectomy and replacement (except at dose V). Although Holt and York (21) reported no change in brown adipose tissue protein content in lean rats 7 days postadrenalectomy, we observed significant elevations in protein content in lean rats receiving low doses of glucocorticoid replacement for 30 days. It is possible that this additional time was necessary to manifest changes in brown adipose tissue protein content. In obese rats, in agreement with Holt and York (21), we found adrenalectomy and low doses of glucocorticoid replacement significantly decreased brown adipose tissue weight toward values seen in lean rats, despite a significant increase in protein content. These changes probably reflect a loss of tissue lipid stores, substantiated by comparative slaughter analysis illustrating loss of body fat at low replacement doses (Fig. 3).

Holt and York (21) reported that, after adrenalectomy, specific binding of [3H]GDP to brown adipose tissue mitochondria was increased by over 60% in obese rats, indicating enhanced thermogenic capacity. They reported no differences in GDP binding in lean rats after adrenalectomy. It has also been reported that adrenalectomy increases norepinephrine turnover in brown adipose tissue of Ob/ob mice (37), reflecting elevated sympathetic nervous system activity. Our results, reporting significant increases in maximal activity of citrate synthase and β-hydroxylacyl-CoA dehydrogenase (μmol/min) of obese rats after adrenalectomy and low doses of steroid replacement, appear to be in agreement. However, we noted significant increases in enzymatic activity in lean rats at low replacement doses as well. Even though GDP binding appears to be more directly related to the thermogenic capacity of brown adipose tissue than are the maximal activities of citrate synthase and β-hydroxylacyl-CoA dehydrogenase, our findings in lean animals cannot be disregarded. Although maximal depot citrate synthase activity (μmol/min) of lean and obese rats at comparable doses of steroid replacement were not significantly different, this activity was consistently higher in lean compared with obese rats at all doses, resulting in a significant genotypic effect. These results are consistent with the view that differences in thermogenesis play a contributory role in the development of obesity in the Zucker fatty rat. However, because both lean and obese rats exhibit increased maximal citrate synthase activity (μmol/min) after removal of glucocorticoids (to values greater than sham controls) and depressed citrate synthase activity on glucocorticoid replacement (to values comparable to sham controls), the contribution of glucocorticoids to thermogenesis in both lean and obese animals must be further evaluated before the link between thermogenesis and development of obesity is fully understood.

Finally, when food intake data were expressed in grams per day, obese rats at the three highest replacement doses consumed a similar quantity of food, which was significantly greater than that of lean rats at each of these doses. However, when food intake was expressed per fat free weight, it was significantly greater in obese compared with lean rats at these highest doses. A significant elevation in food intake was seen only in the obese genotype as steroid dose increased. This marked increase in food intake per fat free weight helps to substantiate the hypothesis that enhanced responsiveness to glucocorticoids plays an important role in the development and maintenance of obesity of the Zucker fatty rat.

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