Effects of adrenalectomy before weaning and short- or long-term glucocorticoid administration on the genetically obese Zucker rat

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Intact obese rats were hyperinsulinaemic, had higher rates of whole-body fatty acid synthesis, higher activities of hepatic acetyl-CoA carboxylase and tyrosine aminotransferase and a higher hepatic glycogen concentration than intact lean animals. Adrenalectomy abolished all these factors of the obese phenotype. Treatment of adrenalectomized rats with corticosterone for 24 h increased the rate of whole-body fatty acid synthesis to the same extent in both phenotypes, but caused a larger increase in glycogen concentration, tyrosine aminotransferase activity and plasma insulin concentration in obese rats.

INTRODUCTION

The genetically obese Zucker rat (fa/fa) shows an abnormal pattern of growth such that by maturity it displays excessive adiposity and a decreased body protein content (Pullar & Webster, 1974). Although the abnormal metabolism of the obese rat has been extensively documented (for review see Bray & York, 1979), the primary lesion responsible for the obese phenotype has not been elucidated.

Further evidence suggests that the glucocorticoid hormones may play an important role in expression of the obese phenotype. Adrenalectomy abolished and corticosterone treatment restored the decreased binding of GDP shown by isolated mitochondria from brown adipose tissue of obese rats (Holt & York, 1982). Interpretation of these findings with regard to the role of glucocorticoid hormones in the obese rat is difficult, however, because adrenalectomy only decreased in severity a number of their other metabolic abnormalities. For example, although the hyperinsulinaemia and elevated rates of fatty acid synthesis of obese rats were decreased by adrenalectomy, plasma insulin concentrations remained 2-fold greater and fatty acid synthesis rates remained 3–4-fold higher compared with adrenalectomized or intact lean (Fa/−) rats (York & Godbole, 1979). Similarly the activity of hepatic tyrosine aminotransferase, a glucocorticoid-inducible enzyme, remained higher in adrenalectomized obese rats compared with adrenalectomized lean rats (Shargill et al., 1983). Short-term treatment of adrenalectomized rats with corticosterone, over a wide range of doses, induced a larger rise in tyrosine aminotransferase activity in lean than in obese rats (Shargill et al., 1983).

The effects of adrenalectomy on obese rats have previously been studied in animals of 5 weeks of age or older, by which time obese rats are grossly different in body composition and metabolism from lean rats (Bray & York, 1979; Pullar & Webster, 1974). In order to study the effects of glucocorticoids on expression of the obese phenotype, the present paper reports the effects of adrenalectomy performed at 18 days of age, 3 days before weaning. The effects of long-term glucocorticoid treatment from weaning to 41–42 days of age or short-term glucocorticoid treatment for 24 h at 41–42 days have also been investigated in adrenalectomized rats.

MATERIALS AND METHODS

Chemicals were purchased either from B.D.H. or from Sigma Chemical Co. (both at Poole, Dorset, U.K.). All radioisotopes were purchased from Amersham International (Amersham, Bucks., U.K.).

Animals

Male lean and obese Zucker rats were bred from heterozygote parents from the colony of the Rowett Research Institute (Pullar & Webster, 1974). Phenotype identification was made at 17 days post partum on the basis of the lower rectal temperature of fa/fa rats (Godbole et al., 1978). Preliminary observations in my laboratory on rats allowed to reach 40 days of age confirmed that this procedure correctly identified 18-day-old obese rats with 100% accuracy.

After weaning rats were housed singly at 22 °C with access to food ad libitum (Oxoid; Herbert C. Styles, Bewdley, Worcs., U.K.). Food intakes were measured during the 48 h before rats were killed at 41–42 days of age.

Experimental

Bilateral adrenalectomies or sham operations were performed under diethyl ether anaesthesia via a single dorsal incision at 18 days. After weaning at 21 days, adrenalectomized rats were given drinking water containing NaCl (9.0 g/l) and glucose (40 g/l). Sham-operated rats received water containing glucose (40 g/l) only. At weaning all rats were injected with 50 µg of dexamethasone in 0.1 ml of sesame oil. Adrenalectomized rats having plasma corticosterone concentrations > 20 µg/l or evidence of surviving adrenal fragments post mortem were excluded from these studies. Similarly adrenalectomized animals treated with corticosterone were discarded if adrenal fragments were found to be present at post mortem.

Because fatty acid synthesis rates were to be measured, corticosterone was administered via the gastro-intestinal tract, rather than with an oil-based injection vehicle. To study the short-term effects of glucocorticoid, adrenalectomized rates were given by gastric intubation 2.0 mg of corticosterone 21-acetate suspended in 0.2 ml of water containing gum tragacanth (5%, w/v) 24 h and 12 h before being killed at 41–42 days. For study of long-term glucocorticoid administration, adrenalectomized lean...
(n = 4) and obese (n = 4) rats were fed on powdered chow containing corticosterone 21-acetate (0.1 mg/g of chow) from weaning.

**Fatty acid synthesis**

Whole-body fatty acid synthesis rates were measured by $^3$H incorporation from $^3$H$_2$O (Windmueller & Spaeth, 1966). Rats were injected intraperitoneally with 9.25 MBq of $^3$H$_2$O (37 MBq/ml of H$_2$O) and killed 3 h later (between 12:00 and 13:00 h). A blood sample was taken and, after removal of the stomach, the remaining carcass was rapidly frozen. A portion of the freeze-dried minced carcass was extracted for total lipid (Folch et al., 1957) and then saponified with ethanolic KOH. The saponifiable lipid was extracted from the non-saponifiable fraction by the method of Lough et al. (1966). To remove chemiluminescence it was necessary to treat the saponifiable fraction with activated charcoal. Radioactivity was determined by counting in a Packard Tri-Carb 460 CD liquid-scintillation spectrometer. $^3$H was counted with an efficiency of approx. 30%. Rates of fatty acid synthesis were calculated as $\mu$mol synthesized/3 h by using the $^3$H radioactivity in plasma to estimate tissue water radioactivity, and the factors for conversion of $^3$H incorporation into $\mu$mol of fatty acid synthesized given by Windmueller & Spaeth (1966, 1967).

**Analytical**

Plasma glucose was measured by the glucose oxidase method (Huggett & Nixon, 1957). Plasma insulin was measured by radioimmunoassay, insulin antiserum (batch 65-101-1) was obtained from Miles Scientific (Stoke Court, Stoke Poges, Slough, U.K.), and rat insulin standard was generously given by Eli Lilly (Indianapolis, IN, U.S.A.). Plasma corticosterone was analysed by radioimmunoassay (Gosdow-Cohen et al., 1982) with antiserum (batch CO2) purchased from Miles Scientific.

Immediately after rats were killed, a liver sample (approx. 50 mg) was freeze-clamped in liquid $N_2$ and hepatic glycogen was subsequently assayed (Keppeler & Decker, 1984). A second liver sample was rapidly homogenized in Tris buffer, pH 7.4 (50 mm), containing EDTA (0.1 mm), pyridoxal phosphate (0.05 mm) and dithiothreitol (1.0 mm). The homogenate was centrifuged at 14000 g for 60 min, and tyrosine aminotransferase (EC 2.6.1.5) activity measured in the supernatant by the method of Granner & Tomkins (1970). After gel filtration and activation with citrate (20 mm), Mg$^{2+}$ (10 mm) and fatty-acid-free albumin (2 mg/ml), acetyl-CoA carboxylase (EC 6.4.1.2) was assayed by H$^1$CO$_3$ fixation for 5 min at 37°C as described by Inoue & Lowenstein (1975).

Protein concentration was determined by a modification of the Lowry technique (Hartree, 1972).

The statistical significance of differences between means was assessed by Student’s $t$-test.

**RESULTS**

In a preliminary study it was found that adrenalectomized obese, but not lean, rats died within 72 h of weaning unless treated with glucocorticoid (Fletcher, 1985). In the present study a single injection of dexamethasone (50 $\mu$g) at 21 days was sufficient to ensure > 95% viability of adrenalectomized fa/fa rats maintained at 22°C.

The lipid content of both phenotypes was significantly decreased by adrenalectomy, and obese rats still had slightly more lipid than did lean rats after adrenalectomy (Table 1). Glucocorticoid treatment for 24 h did not significantly affect the content of adrenalectomized obese or lean rats. Glucocorticoid addition to the food of adrenalectomized rats from weaning restored their lipid contents to values not significantly different from those of sham-operated rats, 6.1 ± 0.6 and 28.2 ± 3.7 g of lipid/rat in lean and obese rats respectively (n = 4 for each group).

The food intake over the 48 h period from 38 days of age was higher in intact obese rats than in intact lean animals, 47.7 ± 1.9 and 33.9 ± 1.6 g respectively (P < 0.001). Adrenalectomized rats of both phenotypes ate less than intact rats, 26.6 ± 1.9 and 28.8 ± 1.4 g in obese and lean rats respectively (not significantly different). Addition of glucocorticoid to the diet from weaning restored the hyperphagia of obese rats, 46.1 ± 1.7 and 33.8 ± 1.4 g in obese and lean rats respectively (P < 0.001).

<table>
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<tr>
<th>Table 1. Effects of pre-weaning adrenalectomy and glucocorticoid treatment at 40–41 days on body weight, body lipid content and rates of whole-body fatty acid synthesis of lean and obese Zucker rats</th>
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<tbody>
<tr>
<td><strong>Lean (Fa/−)</strong></td>
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<td><strong>SHAM (6)</strong></td>
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<tr>
<td>Body wt. (g)</td>
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<tr>
<td>Body lipid (g)</td>
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<tr>
<td>Fatty acid synthesis ($\mu$mol/3 h per rat)</td>
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</table>

Values are the means ± 1 S.E.M. for the numbers of animals in parentheses. Statistical significance of phenotypic differences within the same treatment: †P < 0.01, ††P < 0.001. Statistical significance of differences from the SHAM group within the same phenotype: †P < 0.05, ††P < 0.01, †††P < 0.001.
Pre-weaning adrenalectomy of the Zucker rat

Table 2. Effects of pre-weaning adrenalectomy and glucocorticoid treatment at 40–41 days on hepatic glycogen content and hepatic activities of tyrosine aminotransferase and acetyl-CoA carboxylase of lean and obese Zucker rats

Rats were adrenalectomized (ADX) or sham-operated (SHAM) at 18 days of age, and at 40–41 days one group of adrenalectomized rats (ADX CST) were given by gavage corticosterone 21-acetate (2.0 mg) 24 and 12 h before being killed. A sample of liver was rapidly freeze-clamped for assay of glycogen, and a second liver sample was homogenized in Tris buffer, pH 7.4 (50 mm), containing EDTA (0.1 mm), pyridoxal phosphate (0.05 mm) and dithiothreitol (1.0 mm). After centrifugation, enzyme activities were determined in the supernatant as described in the Materials and methods section. Values are the means ± 1 S.E.M. for the numbers of animals in parentheses. Statistical significance of phenotypic differences within the same treatment: * P < 0.05, ** P < 0.01, *** P < 0.001. Statistical significance of differences from the SHAM group within the same phenotype: †† P < 0.01, ††† P < 0.001.

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<th>Lean (Fa/−)</th>
<th>Obese (fa/fa)</th>
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<tr>
<td></td>
<td>SHAM (6)</td>
<td>ADX (7)</td>
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</tr>
<tr>
<td>Hepatic glycogen (µmol of glucose/g of liver)</td>
<td>240 ± 20</td>
<td>193 ± 27</td>
</tr>
<tr>
<td>Tyrosine aminotransferase (nmol/min per mg of protein)</td>
<td>41 ± 3.9</td>
<td>21 ± 3.1†††</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase (nmol/min per mg of protein)</td>
<td>5.5 ± 0.9</td>
<td>5.9 ± 1.2</td>
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<td>326 ± 34*</td>
<td>172 ± 29††</td>
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<td></td>
<td>60 ± 4.3**</td>
<td>24 ± 2.5†††</td>
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<td>36.2 ± 5.3***</td>
<td>5.1 ± 0.7†††</td>
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Rates of fatty acid synthesis were approx. 4-fold higher in intact obese rats than in lean animals, and adrenalectomy decreased these rates in both phenotypes and abolished the phenotypic differences (Table 1). Glucocorticoid treatment for 24 h significantly increased the rates of fatty acid synthesis in both lean and obese rats and, although the rate was higher in obese rats, this difference did not reach statistical significance. Fatty acid synthesis rates were not measured in adrenalectomized rats after long-term glucocorticoid treatment.

Hepatic glycogen concentrations were higher in intact obese rats than in intact lean animals, and this difference was abolished by adrenalectomy and restored by short-term glucocorticoid treatment (Table 2). Long-term glucocorticoid treatment also caused a larger increase in hepatic glycogen concentration in obese than in lean rats, 452 ± 33 and 274 ± 31 µmol of glucose/g of liver respectively (P < 0.01). The activity of hepatic tyrosine aminotransferase was higher in intact obese rats than in intact lean rats; this difference was abolished by adrenalectomy and restored by short-term (Table 2) and long-term glucocorticoid treatment (55.4 ± 2.6 and 26.2 ± 2.9 nmol/min per mg of protein in obese and lean rats respectively; P < 0.001).

Adrenalectomy lowered the activity of hepatic acetyl-CoA carboxylase in obese rats, but had no effect in lean animals (Table 2). Glucocorticoid treatment for 24 h had no effect on acetyl-CoA carboxylase activity in either phenotype, but long-term treatment restored the phenotypic difference, 32.2 ± 4.7 and 8.5 ± 3.8 nmol/min per mg of protein in obese and lean rats respectively (P < 0.01). Adrenalectomy lowered the plasma insulin concentration of obese rats to that of lean animals, and glucocorticoid treatment for 24 h was sufficient to restore the hyperinsulinaemia of obese animals (Table 3). Long-term glucocorticoid replacement also restored the hyperinsulinaemia of adrenalectomized Fa/Fa rats, 13.4 ± 3.0 and 2.1 ± 0.8 ng/ml in obese and lean rats respectively (P < 0.001), but there was no phenotypic difference in plasma glucose concentration (results not shown).

Concentrations of corticosterone in the plasma of

Table 3. Effects of pre-weaning adrenalectomy and glucocorticoid treatment at 40–41 days on plasma insulin and glucose concentrations of lean and obese Zucker rats

Rats were adrenalectomized (ADX) or sham-operated (SHAM) at 18 days of age, and at 40–41 days one group of adrenalectomized rats (ADX CST) were given by gavage corticosterone 21-acetate (2.0 mg) 24 and 12 h before being killed. A blood sample was taken when rats were killed, and glucose and insulin were measured in plasma as described in the Materials and methods section. Values are the means ± 1 S.E.M. for the numbers of animals in parentheses. Statistical significance of phenotypic differences within the same treatment: ** P < 0.01, *** P < 0.001. Statistical significance of differences from the SHAM group within the same phenotype: †† P < 0.01, ††† P < 0.001. Plasma insulin concentrations of two adrenalectomized lean rats and two adrenalectomized obese rats were below the detection level of the assay.

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<th>Lean (Fa/−)</th>
<th>Obese (fa/fa)</th>
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<tr>
<td></td>
<td>SHAM (6)</td>
<td>ADX (7)</td>
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</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>7.8 ± 0.3</td>
<td>7.2 ± 0.4</td>
</tr>
<tr>
<td>Plasma insulin (ng/ml)</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.2</td>
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<td></td>
<td>7.9 ± 0.2</td>
<td>7.0 ± 0.2††</td>
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<td>10.4 ± 2.1***</td>
<td>1.0 ± 0.2†††</td>
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adrenalectomized rats did not significantly differ between phenotypes after short-term glucocorticoid treatment (0.28 ± 0.09 and 0.24 ± 0.06 μmol/l in obese and lean rats respectively) or long-term glucocorticoid treatment (0.18 ± 0.04 and 0.22 ± 0.03 μmol/l in obese and lean rats respectively).

DISCUSSION

An objective of the present study was to examine the effects of adrenalectomy performed before the full development of the obese phenotype. At 18 days of age, when adrenalectomies were performed in the present study, obese rats are not hyperphagic (Bell & Stern, 1977) or hyperinsulinaemic (Turkenkopf et al., 1982) compared with Fa/− animals, and there is only a slight phenotypic difference in body lipid content (Bell & Stern, 1977). Compared with sham-operated animals, adrenalectomy caused a small decrease in body weight and lipid content of lean rats, but a large decrease in lipid content of obese rats at 41-42 days. The body lipid content of adrenalectomized obese rats, however, remained slightly but significantly elevated compared with adrenalectomized lean animals. This residual obesity may represent the small degree of phenotypic difference in lipid content already present at 18 days, or the effects of glucocorticoid replacement over the weaning period. The effects of adrenalectomy on the obesity and other aspects of the obese phenotype cannot be attributed to the prevention of their hyperphagia. Intact obese rats pair-fed on the intake of lean animals remain obese (Bray et al., 1973; Cleary et al., 1980), hyperinsulinaemic (Cleary et al., 1980), and have higher rates of hepatic fatty acid synthesis (Martin, 1974). Pre-weaning adrenalectomy successfully normalized each feature of the obese phenotype examined in the present study with respect to the condition of adrenalectomized lean rats, and daily replacement of glucocorticoid, by addition of corticosterone to the food of adrenalectomized rats, restored the obese phenotype. This finding strongly suggests that the effects of adrenalectomy were a consequence of glucocorticoid deprivation rather than lack of mineral corticoid hormones or lack of adrenal-medullary catecholamines. A similar conclusion can be drawn from studies of the adrenalectomized obese mouse (ob/ob) and the effects of glucocorticoid replacement (Saito & Bray, 1984).

Although it is probable that the liver is the major site of lipogenesis in the obese rat (Godbole et al., 1978), both the white (Kannen et al., 1980) and brown adipose tissue (Bazin & Lavau, 1982) of obese rats have higher rates of fatty acid synthesis than are found in lean animals. Accordingly, whole-body fatty acid synthesis rates were measured in the present study and, as expected, these were higher in intact obese rats than in lean animals. The activity of acetyl-CoA carboxylase is probably an important regulator of fatty acid synthesis (Volpe & Vagelos, 1976), and hepatic activity of this enzyme was higher in sham-operated obese rats than in lean animals. Adrenalectomy was effective in lowering the rate of fatty acid synthesis, the activity of hepatic acetyl-CoA carboxylase and the plasma insulin concentration of obese rats to values not different from those of adrenalectomized lean animals. This contrasts with the effects of adrenalectomy performed at 5-6 weeks of age (Shargill et al., 1983; York & Godbole, 1979).

In agreement with the present study, adrenalectomy of non-obese strains of rat was not associated with any decrease in activity of hepatic acetyl-CoA carboxylase (Volpe & Marasa, 1975). Short-term glucocorticoid treatment did not affect the activity of this enzyme, despite increasing whole-body fatty acid synthesis rates in both phenotypes. This may be due to the increased lipogenesis occurring in the periphery rather than in the liver, or alternatively activation of this enzyme before assay may have masked any allosteric activation occurring in vivo. Stimulation of hepatic acetyl-CoA carboxylase activity by glucocorticoids in non-obese strains of rat showed only a small increase after 24 h, and at least 48 h treatment was required for full activation (Diamont & Shafir, 1975).

The half-life of exogenous corticosterone does not differ between phenotypes (Yukimura et al., 1978), and in the present study the different effects of long- and short-term glucocorticoid treatment were obtained at the same administered dose and corticosterone concentra-
tions in plasma did not differ between phenotypes. It has been suggested that the tissues of the obese rat may be more sensitive to the action of glucocorticoids (Yukimura et al., 1978); alternatively, glucocorticoids may play a merely permissive role in allowing expression of the obese genotype. A striking phenotypic difference in plasma insulin concentration emerged after short-term glucocorticoid treatment. This points to an important effect of glucocorticoids on the obese rat being exerted via increased insulin secretion. The mechanism(s) by which glucocorticoids induce a relative hyperinsulinaemia in obese rats after only 24 h of treatment is not clear. High-dose glucocorticoid treatment of non-obese strains of rat causes hyperinsulinaemia (Diamont & Shafir, 1975), but this is thought to be a consequence of concomitant hyperglycaemia. The hyperinsulinaemia of the intact or glucocorticoid-treated adrenalectomized obese rat was not accompanied by differences in plasma glucose concentration compared with lean animals.

Short-term glucocorticoid treatment caused a larger increase in glycogen deposition and tyrosine aminotransferase activity in the liver of obese rats than in lean animals. These findings may appear to suggest an increased sensitivity of the actions of glucocorticoids in the obese rat. These phenotypic differences cannot be ascribed to the influence of glucocorticoids alone, however, as insulin stimulates glycogen deposition (Whitton & Hems, 1976) and tyrosine aminotransferase activity (Kenney, 1970). As noted above, glucocorticoid treatment of obese rats was associated with a relative hyperinsulinaemia, compared with lean animals.

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Pre-weaning adrenalectomy of the Zucker rat


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