

The effects of dietary fat content on the growth and body composition of lean and genetically obese Zucker rats adrenalectomized before weaning

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1. Lean (*Fa/–*) and obese (*fa/fa*) Zucker rats were adrenalectomized or sham-operated at 18 d of age (3 d before weaning). After weaning the rats were fed *ad lib.* on semi-synthetic diets containing either a low (8 g/kg) or a high (178 g/kg) proportion of fat. Other groups of sham-operated rats were given the same amount eaten by adrenalectomized animals (restricted intake). Rats were killed at 40 d of age.

2. Adrenalectomy reduced the body lipid content of lean and obese rats compared with intact animals fed *ad lib.* or given a restricted intake. Adrenalectomized obese rats contained more body lipid than intact or adrenalectomized lean rats.

3. Sham-operated obese rats given a restricted intake had less body protein than similarly treated lean animals and this phenotypic difference was abolished by adrenalectomy.

4. There were no effects of diet on growth or body composition of intact or adrenalectomized rats.

5. It is concluded that preweaning adrenalectomy prevented development of the obese phenotype when rats were fed on either diet. Comparison of these results with a previous study, in which adrenalectomized Zucker rats were fed on a stock diet (Fletcher, 1986*b*), showed, however, that feeding either of the semi-synthetic diets caused greater deposition of body lipid in obese rats.

The underlying defect responsible for development of genetic obesity in the rat (*fa/fa*) or mouse (*ob/ob*) is not understood. The abnormal body composition, hyperphagia, hyperinsulinaemia and insulin resistance of the obese rat and mouse can, however, be prevented by adrenalectomy (Yukimura *et al.* 1978; Saito & Bray, 1984; Fletcher, 1986*a*). The effects of adrenalectomy are thought to be a consequence of glucocorticoid deprivation as treatment of adrenalectomized obese animals with natural or synthetic glucocorticoids restored the obese phenotype (Saito & Bray, 1984; Freedman *et al.* 1986; Fletcher, 1986*a*).

After weaning, laboratory rats are customarily fed on a diet containing a high proportion of carbohydrate and the excessive adipose tissue deposition shown by obese rats is largely due to *de novo* lipogenesis (Haggarty *et al.* 1987). Glucocorticoids are important factors in the control of lipogenesis in normal animals. For example, intact rats refed on a high-carbohydrate diet after a period of starvation increase their rate of hepatic lipogenesis several-fold compared with normally fed animals (Kaul & Berdanier, 1975; Berdanier *et al.* 1976). Adrenalectomized rats are unable to make this adaptation and glucocorticoid replacement restores this capacity (Bouillon & Berdanier, 1980). It is therefore possible that adrenalectomy prevents development of obesity in Zucker rats by inhibition of lipogenesis from dietary carbohydrate. If this hypothesis is correct, adrenalectomized obese rats would still be able to accumulate excess adipose tissue from diets containing a high proportion of dietary fat. It has been reported that giving a diet containing a high proportion of fat to adrenalectomized obese mice allowed development of the obese phenotype (Smith & Romsos, 1985).

In the present study the effects of preweaning adrenalectomy on the growth and body composition of lean (*Fa/–*) and obese (*fa/fa*) Zucker rats have been investigated when rats

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Table 1. *Composition of low- and high-fat diets (g/kg)*

	Low-fat	High-fat
Casein	200	223
Flour	287	322
Sucrose	432	76
Maize oil	8	178
Vitamin and mineral mix*	73	83
Cellulose	0	118

* As described by Miller & Bender (1955).

were fed on semi-purified diets containing either a low or a high proportion of fat from weaning to 40 d of age. Because adrenalectomy is associated with a reduced food intake of both lean and obese rats, the effects of these diets have also been investigated in intact rats fed to the intake of adrenalectomized animals.

MATERIALS AND METHODS

Lean (*Fa/-*) and obese (*fa/fa*) Zucker rats were bred from heterozygote parents from a colony at the Rowett Research Institute. Phenotype identification was made at 18 d of age on the basis of the lower rectal temperature of obese animals (Godbole *et al.* 1978) as described previously (Fletcher 1986*a*). After weaning, rats were housed singly at 22°. Because there were no significant differences in body composition between male and female rats at 40 d of age, both sexes have been used in the present study. Each experimental group, however, consisted of an equal number of male and female rats. The food intakes of adrenalectomized animals were measured every 2–3 d and intact pair-fed lean and obese rats were provided daily with their appropriate diet and amount of food. The composition of the diets are given in Table 1.

Bilateral adrenalectomies or sham operations were performed under diethyl ether anaesthesia using a single dorsal incision at 18 d of age. After weaning at 21 d, adrenalectomized rats were given drinking water containing sodium chloride (9 g/l) and glucose (40 g/l). Sham-operated rats received water containing glucose (40 g/l) only. To ensure survival of adrenalectomized obese rats, at weaning all rats were injected with 50 µg Dexamethasone (Sigma Chemical Co., Poole, Dorset) in 0.1 ml sesame oil (Fletcher, 1986*a*). Adrenalectomized rats having plasma corticosterone concentrations greater than 20 µg/l, or evidence of surviving adrenal fragments post-mortem, were excluded from these studies.

Rats were killed and blood samples taken between 09.00 and 10.00 hours at 40 d of age. Stomachs were removed and the remaining carcasses freeze-dried, minced and analysed for total lipid by the method of Atkinson *et al.* (1972) and nitrogen by the Kjeldahl method (crude protein was calculated at $N \times 6.25$). Ash was determined as the residue after combustion.

Plasma glucose was analysed by the glucose oxidase (*EC* 1.1.3.4) method (Hugget & Nixon, 1957). Plasma insulin and corticosterone were measured by radioimmunoassay. Iodinated insulin and tritiated corticosterone were purchased from Amersham International Plc, Bucks. Insulin and corticosterone anti-sera were purchased from Miles Scientific Plc, Stoke Poges, Slough. Rat insulin standard (lot 615-063-12-3) was obtained from Eli Lilly and corticosterone standard was purchased from Sigma Chemical Co.

Table 2. Body-weights, food intakes and ash contents of sham-operated (sham ADX), adrenalectomized (ADX) and sham-operated rats fed to the same intake as adrenalectomized animals (sham ADX and restricted intake). Rats were fed on a diet containing a low or high proportion of fat

(Mean values with their standard errors for six to eight rats for each group except for sham ADX and restricted-intake obese rats where four rats were used)

Diet†...	Sham ADX				ADX				Sham ADX and re- stricted intake				
	Low-fat		High-fat		Low-fat		High-fat		Low-fat		High-fat		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Body-weight (g):													
Lean (Fa/ -)	124	4.6	122*	7.6	110	6.0	103	4.7	112	112	113	3.5	3.6
Obese (fa/fa)	138**	9.7	134**	9.3	110	5.4	113	5.0	102	102	107	3.5	2.3
Food intake (g):													
Lean (Fa/ -)	236*	20	209*	10	184	17	173	8	—	—	—	—	—
Obese (fa/fa)	289**	13	259***	12	192	6	185	9	—	—	—	—	—
Body ash (g)													
Lean (Fa/ -)	4.2*	0.31	4.0*	0.28	3.3	0.23	3.1	0.21	3.4	3.4	3.7	0.12	0.4
Obese (fa/fa)	3.4	0.33	3.3	0.25	3.5	0.22	3.4	0.12	3.0	3.0	2.8	0.17	0.37

Mean values were significantly different from those of ADX rats of the same genotype fed on the same diet: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
 † For details, see Table 1 and p. 564.

Table 3. *Body lipid content, protein content and plasma insulin concentrations of sham-operated (sham ADX), adrenalectomized (ADX), and sham-operated rats fed to the same intake as adrenalectomized animals (sham ADX and restricted intake). Rats were fed on a diet containing a low or high proportion of fat*

(Mean values with their standard errors for six to eight rats for each group except for Sham ADX and restricted intake obese rats where four rats were used)

Diet† ...	Sham ADX			ADX			Sham ADX and re- stricted intake					
	Low-fat		High-fat	Low-fat		High-fat	Low-fat		High-fat			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Body lipid (g):												
Lean (Fa/-)	8.8***	0.50	7.2***	0.36	5.4	0.59	4.4	0.42	6.8*	0.40	8.0***	0.54
Obese (fa/fa)	36.8***	2.66	35.2***	3.73	11.9	1.57	11.8	1.44	24.5***	2.41	28.6***	1.18
Body protein (g):												
Lean (Fa/-)	24.5	1.52	23.4	1.39	22.6	1.04	21.9	1.22	22.3	1.12	22.4	0.75
Obese (fa/fa)	18.6	1.46	17.8	1.18	19.9	0.86	20.6	0.61	14.3**	0.33	15.0**	1.04
Plasma insulin (ng/ml):												
Lean (Fa/-)	1.7	0.23	1.0	0.16	1.6	0.55	0.8	0.13	1.5	0.3	0.8	0.10
Obese (fa/fa)	9.3***	0.97	3.2**	0.50	1.8	0.44	1.0	0.17	2.3	0.67	1.0	0.08

Mean values were significantly different from those of ADX rats of the same genotypes fed on the same diet: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details, see Table 1 and p. 564.

Results were analysed by a $2 \times 2 \times 3$ factorial design analysis of variance and the significances of differences between mean values were established by Student's *t* test. Mean values with their standard errors are given.

RESULTS

Intact obese rats ate more than lean animals when fed on either diet (Table 2). Adrenalectomy reduced the intake of both obese and lean rats and prevented the hyperphagia of obese animals.

Tables 2 and 3 show that adrenalectomy reduced body lipid and ash contents of lean rats, and also body-weight in lean rats fed on the high-fat diet.

Adrenalectomy reduced the body-weight and lipid content of obese rats but had no significant effect on body protein and ash contents (Tables 2 and 3). Intact obese rats, pair-fed to the intake of adrenalectomized animals, had more lipid ($P < 0.01$), less protein ($P < 0.01$) and less ash ($P < 0.1$) than adrenalectomized obese rats.

Despite the reduction in lipid content of obese rats caused by adrenalectomy, they still contained more lipid than adrenalectomized lean rats ($P < 0.001$). Body protein and ash contents did not significantly differ between lean and obese rats after adrenalectomy. There were no effects of dietary type on body-weight or composition in either phenotype after adrenalectomy or when intact rats were fed to the intake of adrenalectomized animals.

Plasma insulin concentrations were higher in intact obese rats than in intact lean animals when fed on either diet ($P < 0.001$) and adrenalectomy abolished this hyperinsulinaemia (Table 3). There were no significant differences in glycaemia between obese and lean rats or between diets (values not shown).

DISCUSSION

Adrenalectomy was effective in abolishing the hyperphagia and hyperinsulinaemia of obese rats. Although adrenalectomized obese rats still contained more lipid than adrenalectomized or intact lean rats, they only accumulated approximately one-third of the lipid content of intact obese animals. These effects on the growth and insulinaemia of obese rats were not simply a consequence of reduced food intake, as shown by comparison with the effects of pair-feeding on intact obese rats. In particular, the body protein content of adrenalectomized obese rats was greater than that of pair-fed intact obese rats. The ability of intact obese rats preferentially to deposit lipid at the expense of body protein, when expression of their hyperphagia is prevented, has been well documented (Bray *et al.* 1973; Cleary *et al.* 1980) and increased secretion of glucocorticoids may be instrumental in achieving this abnormal pattern of growth (Fletcher, 1986*c*).

There was no effect of dietary type on growth or body composition of intact or adrenalectomized obese rats. It is unlikely, therefore, that a reduced capacity for *de novo* lipogenesis in adrenalectomized rats is responsible for their reduced fatness. In contrast, Smith & Romsos (1985) reported that giving a semi-synthetic diet containing a high proportion of fat to adrenalectomized obese mice restored their body lipid content to that of intact obese mice, and restored all other features of the intact obese phenotype. These conflicting results may represent a fundamental difference between the obese mouse and rat; alternatively they may be attributed to the amount of dietary fat or type of fat. In the study of Smith & Romsos (1985) the high-fat diet contained 320 g fat/kg compared with 178 g/kg in the present study. Whereas beef tallow was a component of the fat in the diet of Smith & Romsos (1985), only maize oil was used in the present study. Tallow and maize oil differ in fatty acid composition and probably in other minor components. In particular,

tallow, being derived from animal adipose tissue, may contain dissolved steroid hormones (Zomzely *et al.* 1959) and only small amounts of exogenous glucocorticoids are required to restore the complete obese phenotype in adrenalectomized obese animals (Saito & Bray, 1984; Freedman *et al.* 1986; Fletcher, 1986*b*).

Although giving a high- or low-fat semi-synthetic diet did not restore the obese phenotype, comparison of the present findings with those of a previous study carried out in this laboratory (Fletcher 1986*b*) does reveal an effect of diet on the ability of adrenalectomized obese rats to deposit body lipid. Lean and obese rats were adrenalectomized and maintained under identical conditions to those described here, except that rats were fed on a commercially available pelleted stock diet from weaning (Fletcher, 1986*b*). At 40 d of age the body lipid contents of adrenalectomized lean and obese rats were 3.6 (SE 0.3) g and 6.4 (SE 0.8) g respectively (Fletcher, 1986*b*). The value for the obese but not the lean rats fed on stock diet was significantly lower ($P < 0.001$) than that found in the present study for adrenalectomized obese rats fed on either low- or high-fat semi-synthetic diets. The stock diet used in the previous study (Fletcher, 1986*b*) had a fat content of 24 g/kg and the fat contents of the semi-synthetic diets used in the present study were chosen so as to provide values above and below that of the stock diet, 8 and 178 g/kg in the low- and high-fat diets respectively. The differential ability of stock and semi-synthetic diets to ameliorate the effects of adrenalectomy on the body lipid content of obese rats, is, therefore, not related to dietary fat content. An alternative explanation may lie in the semi-synthetic diets having a greater palatability compared with stock diet, leading to a larger energy intake.

Intact obese rats retain dietary energy more efficiently than lean animals (Pullar & Webster, 1974) and adrenalectomy has been reported to abolish this phenotypic difference (Marchington *et al.* 1983). In the present study adrenalectomized obese rats did not consume significantly more food than adrenalectomized lean animals and had the same lean body mass, but they did, however, deposit more body lipid. Studies with stock-fed adrenalectomized obese rats (see above) have shown that only a small proportion of this excess lipid deposition may be attributable to the degree of obesity present before adrenalectomy and the effects of glucocorticoid replacement at weaning (Fletcher, 1986*b*). Rats were also given drinking water containing glucose, and water intakes were not measured. The amount of glucose consumed was, however, likely to have been small, and water intake is very closely related to solid food intake. The magnitude of the difference in lipid content between adrenalectomized lean and obese rats therefore suggests that obese rats were still energetically more efficient than lean animals.

Intact lean rats, fed to the same intake as adrenalectomized animals, had the same lean body mass but also accumulated more body lipid than adrenalectomized rats. This finding suggests a greater energetic efficiency in intact than in adrenalectomized lean rats, although the reservations regarding access to drinking water containing glucose, noted previously, are also applicable. Further studies are clearly required to ascertain the energetic efficiency of adrenalectomized lean and obese rats fed on semi-synthetic diets.

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