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**Summary**

Lean (Fa/f) and obese (fa/fa) Zucker rats were adrenalectomized (ADX) in order to assess the contribution of adrenal hormones to insulin resistance of the obese Zucker rat. Glucose utilization was measured using an insulin suppression test.

Sham-operated obese rats gained almost twice as much weight as sham-operated lean littermates. However, body weight gain of ADX animals was comparable in both genotypes. It was significantly less than that of the respective sham-operated controls. Body weight differences can be accounted for almost entirely by a marked loss of adipose tissue.

Although insulin resistance may be attributable to obesity in part, steroid hormones are thought to be directly antagonistic to insulin for glucose metabolism. Adrenalectomy resulted in a decrease in serum glucose concentrations for both lean and obese Zucker rats compared with their respective sham-operated groups. Serum insulin concentration of lean ADX rats was 23% of sham-operated controls; in obese ADX rats, it was 9% of controls. Elevated levels of steady state serum glucose (SSSG) levels in sham-operated obese rats demonstrate a marked resistance to insulin induced glucose uptake compared with sham-operated lean animals. Adrenalectomy caused a marked improvement in insulin sensitivity of obese rats. The hyperglycemic SSG levels of the obese rats were reduced 2.5 times by ADX.

These results indicate that insulin resistance of Zucker obese rats can be ameliorated by ADX, suggesting adrenal hormones contribute to insulin resistance in these animals.

**Key-Words:** Adrenalectomy — Glucose Metabolism — Insulin Resistant Zucker Obese Rat

**Introduction**

The genetically obese Zucker rat is characterized by hyperinsulinemia, mild hyperglycemia and peripheral tissue insulin resistance both *in vivo* and *in vitro* (Triscari, Stern, Johnson and Sullivan 1979; Czech, Richardson, Beckner, Walters, Gitomar and Heinrich 1978). When body weight gain of the obese rat is reduced as during pair-feeding to its lean littermate or during food restriction, the expression of obesity and hyperinsulinemia is not prevented (Cleary, Vasselli and Greenwood 1980). However, adrenalectomy results in dramatic reductions of body weight gain, fat deposition, fat cell size and plasma immunoreactive insulin (IRI) in these obese rats (Yukimura and Bray 1978). Thus, it appears that adrenal hormones participate either directly or indirectly in the establishment and maintenance of the obese and insulin resistant state in the Zucker rat. The present study was initiated to address this point of view, and involved the use of a specific *in vivo* estimate of insulin stimulated glucose disposal to study the effect of adrenalectomy on insulin action in lean and obese Zucker rats.

**Material and Methods**

**Animals**

Male obese (fa/fa) and lean (Fa/f) Zucker rats were adrenalectomized or sham-operated at 10 to 11 weeks of age. After surgery, adrenalectomized rats were provided with saline (1.0 g NaCl/l) in drinking water and sham-operated rats with tap water. All animals were fed standard laboratory chow *ad libitum* and maintained in a constant temperature (23°C ± 1) animal quarter with fixed artificial 12-h light/dark cycle. After 9 weeks, blood taken from the tail vein was assayed for plasma corticosterone to confirm the success of the adrenalectomy (Radioassays Systems). After ten weeks, food was removed at 8:30 a.m. and insulin sensitivity determined 5 h later.

**Insulin Sensitivity Test**

Sensitivity to the ability of insulin to stimulate glucose uptake was evaluated using the insulin suppression test (Wright, Hansen, Mondorn and Reaven 1983). Rats were anesthetized with an intraperitoneal injection of sodium thioumyl (6 mg/100 g body weight). Subsequently, an initial blood sample was obtained from the tip of the tail for assessment of basal glucose and insulin levels. Immediately thereafter, the right internal jugular vein was cannulated for administration of a continuous infusion of glucose, insulin, epinephrine and propranolol for 160 min. With this technique, endogenous secretion of insulin is suppressed by the addition of epinephrine and propranolol and steady-state serum glucose and insulin levels are attained during the last-hour of infusion. Thus, by comparing the steady-state serum glucose (SSSG) concentrations during the third hour, a direct assessment of the ability of a fixed concentration of insulin to stimulate glucose uptake by the various groups can be made. Due to the large preponderance of adipose tissue in obese (fa/fa) rats, it was deemed necessary to infuse the various constituents of the infusion solution on the basis of lean body weight, rather than gross body weight, to prevent overloading of the more obese animals. Previous studies from our laboratory have indicated that fat comprises 7.9% of total body weight of lean sham-operated Zucker rats, 5.0% of lean adrenalectomized, 37.7% of obese sham and 20% of obese adrenalectomized animals. Thus, body weight was corrected for lean body mass and epinephrine was infused at rates of 0.08 μg/kg/min, propranolol at 1.7 μg/kg/min and insulin at 2.5 mU/kg/min. Glucose, on the other hand, is usually infused at 8 mg/kg/min (Wright et al. 1983), and was found to yield severe hypoglycemic serum glucose concentrations (< 25 mg/dl) in pet-
Adrenalectomy and Glucose Metabolism in Zucker Rats

Summary studies on adrenalectomized lean rats. Therefore, glucose infusion was provided at increased rates (9.6 mg/kg lean body weight/min) for all experimental groups. SSSG and SSSI values were calculated from the mean of tail blood samples taken at 10-minute intervals between 120 and 160 minutes of the infusion. Glucose concentrations were determined by the glucose oxidase methods and insulin levels by radioimmunoassay (Wright et al. 1983).

Statistical Analysis
The results of the treatment programs on serum concentrations are expressed as mean ± SE, and statistical comparisons were made with the Student two-tailed t-test.

Results
Adrenalectomy of extended duration had a profound effect on body weight gain of lean and obese Zucker rats. To illustrate this point, the weight gain of adrenalectomized rats following surgery was compared with sham-operated animals. These results are itemized in Table 1 and show that sham-operated obese (fa/fa) rats gain almost twice as much weight as sham-operated lean (Fa?) animals following surgery. Body weight gain of adrenalectomized rats was comparable in both lean and obese groups and was significantly less (P < .001) than that of the respective sham-operated littermates.

Basal glucose and insulin concentrations at the start of infusion are listed in Table 2. The insulin-resistant state of obese Zucker rats is well established as reflected by the slight increase in serum glucose and marked elevation in serum insulin concentration of sham-operated obese rats compared with sham-operated lean animals. Adrenalectomy resulted in a modest decrease (P < .05) in serum glucose concentration for both lean and obese Zucker rats compared with their respective sham groups. The fall in serum insulin concentration was even more dramatic with adrenalectomized rats showing insulin levels 4-fold lower in the lean group and over 10-fold lower in the obese group.

Mean (± SEM) SSSG concentrations during the insulin sensitivity test are seen in the left panel of Fig. 1, and indicate that they were twice as high in sham-operated obese (fa/fa) as compared to sham-operated lean (Fa?) rats. Since SSSI levels (right panel) were also higher in the obese sham-operated rats, these results emphasize the marked resistance of obese as compared to lean Zucker rats. The data in this figure also indicate that SSSG concentrations were much lower in obese rats following adrenalectomy. Since SSSI levels were also lower in adrenalectomized obese rats, it is clear that resistance to insulin-stimulated glucose uptake improved in these rats in association with adrenalectomy.

SSSG levels also fell following adrenalectomy in lean Zucker rats, but in this case we noted an increase in SSSI concentrations. Consequently the improvement in glucose disposal in these animals cannot be entirely attributed to enhanced insulin action.

Discussion
The results of the current study provide direct evidence that adrenalectomy greatly alters the resistance to insulin-stimulated glucose uptake in obese Zucker rats. As such, they complement previous observations which demonstrate that adrenalectomy effectively reduced weight gain, adiposity and normalized plasma insulin and fat cell size in this animal (Yukimura and Bray 1978). Since neither pair feeding nor food restriction of these animals prevents development of the obese, hyperinsulinemic state (Cleary et al.}
1980), it is tempting to speculate that the ability of adrenalectomy to restore so many of the metabolic defects present in the obese Zucker rat towards normal is due, in part, to the involvement of glucocorticoids in the genesis of these abnormalities. In this regard, it is well established that glucocorticoid-induced abnormalities at the level of the insulin receptor may lead to insulin insensitivity (Kahn, Goldfine, Neville and De Meyts 1978). In addition, glucocorticoids inhibit glucose transport and metabolism in both muscle and adipose tissue (Munck 1971). Finally, several studies have noted decreased basal glucose uptake, glucose transport, and decreased sensitivity to insulin-induced glycogen synthesis in muscle from obese rats (Cuendet, Loten, Jeanrenaud and Renold 1976; Le Marchand-Brustel, Jeanrenaud and Freychet 1978; Crettaz, Prentki, Zaninetti and Jeanrenaud 1980). Thus, decreased glucose uptake and insulin resistance in skeletal muscle from obese rats may be due to the presence of glucocorticoids and, with their removal, insulin sensitivity is normalized. This view is supported by recent studies from Oshishima, Sharfigill, Chen and Bray (1984) who demonstrated that basal glucose uptake and insulin-induced glucose transport in perfused hindlimbs from ob/ob mice was restored to normal after adrenalectomy. On the other hand, the fact that adrenalectomy can ameliorate insulin resistance in obese Zucker rats does not prove that the adrenals are responsible for the development of insulin resistance in these rats. Indeed, it is certainly possible that we are dealing with two entirely unrelated processes (i.e., genetic insulin resistance and adrenocorticism), which coincidentally have equal and opposite effects upon in vivo insulin action.

Although not the major goal of the study, some attention should be focussed on the SSSI levels noted during the insulin sensitivity test. Specifically, the data in Fig. 1 suggest that adrenalectomy, in addition to ameliorating the resistance to insulin-induced glucose uptake in Zucker obese rats, also corrected the impairment in insulin removal as reflected by the elevated SSSI levels in obese rats. The most likely explanation for this is that hepatic degradation of insulin was restored to normal following weight reduction. Previous studies have shown that perfused livers from obese fa/fa rats (Karakash and Jeanrenaud 1983), as well as obese hyperglycemic (ob/ob) mice (Karakash, Assimacopoulos-Jeannet and Jeanrenaud 1976) and spontaneously obese rats (Weiland, and Reaven 1979) exhibit decreased rates of insulin removal. The fact that this effect was not seen in adrenalectomized lean rats also supports the view that the change was related to the obese state.

In conclusion, these findings demonstrate that insulin resistance in obese Zucker rats is corrected following adrenalectomy. Further studies are necessary to clarify the specific mechanisms responsible for decreased insulin sensitivity in these animals, and the manner in which adrenalectomy leads to increased sensitivity.

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References


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