Rapid Publication

Amelioration of High-Fat Feeding–Induced Insulin Resistance in Skeletal Muscle With the Antiglucocorticoid RU486

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Fat feeding produces whole-body insulin resistance and decreased glucose uptake in muscle tissue of rats. To examine the effect of glucocorticoid blockade on the insulin resistance caused by high-fat feeding, four groups of rats were fed diets high in starch (70% of calories) or fat (59% of calories) for 4 weeks with or without the antiglucocorticoid RU486 (69.8 μmol · kg^{-1} · day^{-1}) in the food. Whole-body insulin action was assessed by the euglycemic clamp technique at an upper physiological insulin level with bolus 2-[^{3}H]deoxyglucose to determine individual tissue insulin-stimulated glucose uptake. Whole-body glucose utilization (clamp glucose infusion rate [GIR]) was decreased by high-fat feeding (GIR 68.3 ± 12.2 vs. 182.6 ± 12.8 μmol · kg^{-1} · min^{-1} for the starch-fed group; $P < 0.001$). Addition of RU486 to the diet significantly improved (GIR 133.9 ± 12.8 μmol · kg^{-1} · min^{-1}; $P < 0.01$), but did not fully reverse, the insulin resistance caused by fat feeding. RU486 was without effect in the starch-fed rats. In skeletal muscles, RU486 ameliorated 62 and 68% of the insulin resistance produced by fat feeding in red quadriceps and extensor digitorum longus hindlimb muscles, respectively, but had no effect in heart or white adipose tissue. These results suggest that glucocorticoids play, in a tissue-specific manner, a role in the maintenance and/or production of insulin resistance produced by high-fat feeding. *Diabetes* 44:718–720, 1995

Acute and chronic alterations in glucocorticoid levels strongly influence insulin action with deficiency linked to improved insulin sensitivity (1,2) and excess glucocorticoids associated with insulin resistance (3,4). Hyperreactivity of the hypothalamic-pituitary-adrenal axis has been demonstrated in a number of animal models with a genetic predisposition to insulin resistance, diabetes, and obesity, including the sand rat, *ob/ob* mouse, and Zucker fatty rat (5–8). Similar results have also been reported for the high-fat–fed model of diet-induced insulin resistance (9).

Adrenalectomy ameliorates or reverses many of the metabolic abnormalities of the genetic and lesion-induced rodent models of obesity and insulin resistance (10–13). In addition, while not an entirely consistent finding (14), it has been reported that blockade of the glucocorticoid II receptor with RU486 ameliorates the obesity of the Zucker *fa/fa* fatty rat (15) and reduces the hyperphagia and weight gain of high-fat–fed rats (16).

There are no reports of the effect of glucocorticoid II receptor blockade on insulin action in any model of insulin resistance. Thus, the aim of this study was to investigate the effect of chronic administration of RU486 on insulin action in the high-fat–fed rat model of insulin resistance.

RESEARCH DESIGN AND METHODS

All experiments were approved by the institution ethics committee and comply with the National Health and Medical Research Council (Australia) guidelines for the care and use of animals for research purposes.

Adult male Wistar rats (bred at the Garvan Institute, Sydney, Australia) aged between 54 and 60 days and weighing ~270 g at the beginning of the study were housed individually in wire cages in a temperature-controlled room (22 ± 1°C) on a 12/12 light/dark cycle (lights on at 0600).

Four groups of rats were fed diets high in starch (starch group; 70% of calories) or fat (fat group; 59% of calories as safflower oil) with or without RU486 (Roussel-Uclaf, Romainville, France) in the food at a dosage of 69.8 μmol · kg^{-1} · day^{-1} (30 mg · kg^{-1} · day^{-1}). Diets were matched in terms of protein, fiber, vitamins, and minerals as previously described (17). Each rat was given 310 kJ/day, which was approximately equal to their ad libitum intake of laboratory diet. Rats were fed using small metal dishes introduced into the cage just before the beginning of the dark cycle. A pair-feeding regimen was used to avoid differences in caloric intake. Any spillage was collected and additional diet of equal weight was added to the following day's intake. Diets were freshly made every 3–4 days and stored at 4°C.

Euglycemic clamp studies. After 25 ± 1 days on their respective diets, rats were anesthetized with sodium pentobarbital (Nembutal, Abbott, Sydney, Australia; 263 μmol/kg [55 mg/kg] i.p.) and atropine sulfate (Astra, Sydney, Australia; 0.43 μmol/kg [0.3 mg/kg], i.m.) and fitted with chronic carotid and jugular cannulas exteriorized in the intrascapular area and capped. Studies were conducted 6–7 days after surgery in unrestrained conscious rats housed in 25 × 25 cm wire cages. This postoperative period was sufficient time for the rats to be eating their entire ration of food for at least 4 days before the study and to have regained preoperative weight. The studies were carried out during the light period when the rats were observed to be generally inactive. Euglycemic hyperinsulinemic clamps were performed as described previously (18). Briefly, a continuous infusion of porcine insulin (Actrapid, Novo, Copenhagen, Denmark) was given at a dose of 29.4 pmol · kg^{-1} · min^{-1} to achieve plasma insulin concentrations in the mid-to-upper physiological range. This infusion was maintained for ~2

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GIR, glucose infusion rate.

TABLE 1
Body weights at the time of the euglycemic clamp and basal and clamp steady-state plasma insulin and glucose levels

	Group			
	Starch	Starch + RU	Fat	Fat + RU
Body wt (g)	348 ± 11	339 ± 16	366 ± 9	358 ± 12
Basal glucose (mmol/l)	7.5 ± 0.2	7.3 ± 0.1	7.6 ± 0.2	7.4 ± 0.2
Basal insulin (pmol/l)	272 ± 44	289 ± 40	311 ± 50	298 ± 36
Clamp glucose (mmol/l)	7.0 ± 0.1	6.9 ± 0.2	7.0 ± 0.1	6.8 ± 0.2
Clamp insulin (pmol/l)	890 ± 84	939 ± 77	969 ± 63	907 ± 70

Data are means ± SD.

h. The arterial blood glucose concentration was clamped at the basal fasting level using a variable-rate glucose infusion.

Insulin sensitivity of individual tissues in vivo was studied as described previously (5–9). The nonmetabolizable glucose analogue 2-deoxy-D-[2,6-^{3}H]glucose (2.96 MBq; 2-[^{3}H]DG) was administered as an intravenous bolus ~80–90 min after the start of the insulin infusion. Blood samples for determination of blood and plasma glucose concentrations and plasma tracer concentrations were obtained at 2, 5, 10, 15, 20, 30, and 45 min after bolus administration. At the completion of the clamp, rats were instantly anesthetized (sodium pentobarbital, 526 μmol/kg [110 mg/kg] i.v.) and the following hindquarter muscles were rapidly removed and frozen for subsequent analysis: deep-red part of the quadriceps (containing mainly fast-twitch oxidative-glycolytic fibers) and extensor digitorum longus (containing a mixture of fast-twitch oxidative-glycolytic and glycolytic fibers) (19). In addition, heart and inguinal white adipose tissue samples were taken. The estimate of tissue glucose metabolic rate (the glucose metabolic index [Rg']) was calculated as described previously (20,21). Blood samples for plasma insulin determinations (0.4 ml) were taken at the beginning of the clamp, before tracer administration, and at the time the rats were killed. The red blood cells of the first two samples were reconstituted in 0.4 ml of saline and returned to the animal. The sampling cannula was kept patent with lightly heparinized saline, and <1 U of heparin was infused with the return of the red blood cells.

Analytical methods. Plasma samples for determination of tracer concentration were deproteinized immediately in 5.5% $ZnSO_4$ and saturated $Ba(OH)_2$. ^{3}H in an aliquot of the supernatant was determined by scintillation spectrometry (Beckman, Palo Alto, CA) using a quench-corrected (external standard) counting program. Blood and plasma glucose concentrations were measured using a glucose analyzer (YSI 23 AM, Yellow Springs, OH). Plasma samples for insulin determination were stored at −20°C before analysis.

Statistical analysis. Statistical comparisons were made using the Statview 512+ statistical package (Abacus Concepts, Berkeley, CA). Unless noted, group statistical comparisons were by one-way analysis of variance and individual comparisons by Fisher post hoc tests with $P < 0.05$ being considered as significant.

RESULTS

Table 1 shows the body weights of the 4 groups along with the basal and clamp plasma glucose and insulin levels. There were no differences between groups in food intake or rate of body weight gain either pre- or postoperatively (data not shown).

Figure 1 shows the glucose infusion rate (GIR) during the hyperinsulinemic euglycemic clamp as a measure of whole-body insulin action. The addition of RU486 to the diet of starch-diet rats (starch+RU group) had no effect on whole-body insulin-stimulated glucose metabolism. Fat feeding caused a profound insulin resistance (to a GIR of ~30% of that of starch-fed rats). Addition of RU486 to the diets of fat-fed rats (fat+RU group) significantly improved insulin action ($P < 0.01$ vs. fat feeding alone), although not to the level of the starch groups ($P < 0.01$, fat+RU group vs. both starch-fed groups).

The results in the skeletal muscles paralleled the whole-body results (Table 2). High-fat feeding impaired insulin action in both the red quadriceps and the extensor digitorum longus ($P < 0.01$, starch vs. fat). Treatment with RU486 had no effect in the starch-fed group but partially ameliorated the insulin resistance of the fat-fed group ($P < 0.05$, fat vs. fat+RU). In contrast to the skeletal muscles, there were no differences between any of the groups in insulin-stimulated glucose metabolism in the heart. In the inguinal adipose tissue depot, fat feeding impaired insulin action ($P < 0.05$; starch vs. fat) but RU486 had no effect on the insulin-stimulated glucose uptake of the starch-fed or fat-fed groups.

DISCUSSION

These results demonstrate a significant amelioration by the antiglucocorticoid RU486 of the whole-body and skeletal muscle insulin resistance induced by high-fat feeding. As such, the results are consistent with the hypothesis that hyperreactivity of the hypothalamopituitary adrenal stress axis may be involved in the development of insulin resistance in general and its development in fat-fed rats in particular.

The mechanisms underlying the insulin resistance of high-fat feeding have not been fully elucidated. However, it has been shown that increased lipid storage in muscle is closely associated with impaired insulin-stimulated glucose uptake (22,23). A possible explanation for this observation is provided by the glucose–fatty acid cycle hypothesis first proposed by Randle et al. (24) in 1963 and subsequently developed (25). According to this hypothesis, increased lipid availability and utilization will result in increased intramitochondrial concentrations of acetyl-CoA and subsequent inhi-

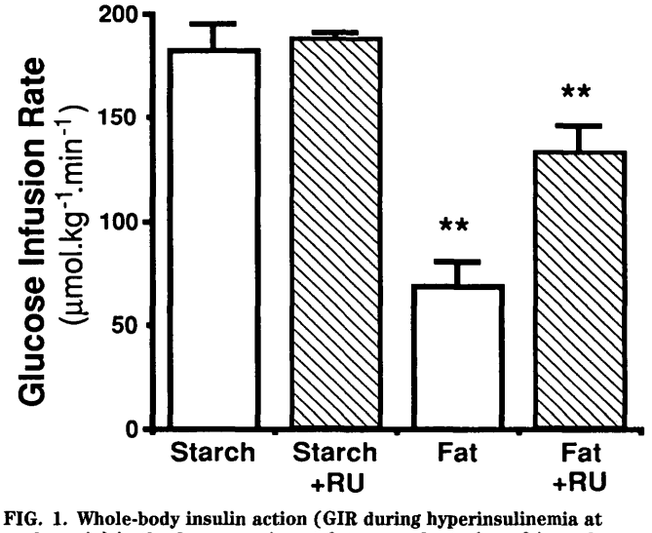

FIG. 1. Whole-body insulin action (GIR during hyperinsulinemia at euglycemia) in the four experimental groups of rats (n = 6 in each group). The fat and fat+RU groups differ, **$P < 0.01$, from each other and from both starch groups.

TABLE 2
Tissue-specific insulin-stimulated glucose metabolism (Rg′) during the hyperinsulinemic euglycemic clamp in two hindlimb muscles, heart, and the inguinal white adipose depot

	Group			
	Starch	Starch + RU	Fat	Fat + RU
Red quadriceps	19.1 ± 2.3	18.2 ± 2.9	10.9 ± 2.2*	16.0 ± 2.5†
Extensor digitorum longus	17.4 ± 1.3	18.1 ± 2.2	11.0 ± 0.9*	15.3 ± 2.6†
Heart	36.9 ± 4.5	40.8 ± 6.3	33.7 ± 5.2	31.8 ± 6.6
Inguinal	4.4 ± 0.3	4.2 ± 0.6	2.3 ± 0.5‡	2.8 ± 0.4

Data are means ± SD. *$P < 0.01$ Starch and Starch + RU vs. Fat; †$P < 0.05$ Fat + RU vs. Fat; ‡$P < 0.05$ Starch vs. Fat.

bition of the pyruvate dehydrogenase complex. This enzyme is essential for the complete oxidation of glucose to CO_2, and decreased activity of this enzyme has been associated with decreased uptake of glucose in several experimental models (26–28). Further, similar inhibition of glucose storage by increased lipid availability has been demonstrated (29). As Standl et al. (30) have shown, increased muscle triglyceride stores will result in increased rates of lipolysis for a given stimulus. This latter observation is particularly germane to the present study, as it has been shown that hyperinsulinemia during a euglycemic clamp increases sympathetic nervous activity (31). Finally, increased stress responsivity has been shown to be predictive of high weight gain on a high-fat/high-sucrose diet (32), and in turn, fat feeding itself results in an increased responsivity to stress (9).

Increased muscle intracellular lipid stores, increased sympathetic nervous activity to elevated insulin levels, and increased responsivity to stress are mechanisms that together or independently could lead to increased lipolysis and therefore decreased glucose uptake in high-fat–fed rats—effects that are ameliorated by RU486. Tissue differences in glucocorticoid type II receptor numbers and/or glucocorticoid responsiveness may then account for differences in the effect of glucocorticoids on insulin action in specific tissues.

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