Insulin Response in Individual Tissues of Control and Gold Thioglucose-Obese Mice In Vivo With [1-^{14}C]2-Deoxyglucose

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SUMMARY

The dose-response characteristics of several glucose-utilizing tissues (brain, heart, white adipose tissue, brown adipose tissue, and quadriceps muscle) to a single injection of insulin have been compared in control mice and mice made obese with a single injection of gold thioglucose (GTG). Tissue content of [1-^{14}C]2-deoxyglucose 6-phosphate and blood disappearance rate of [1-^{14}C]2-deoxyglucose (2-DG) were measured at nine different insulin doses and used to calculate rates of 2-DG uptake and phosphorylation in tissues from control and obese mice. The insulin sensitivity of tissues reflected in the ED_{50} of insulin response varied widely, and brown adipose tissue was the most insulin-sensitive tissue studied. In GTG-obese mice, heart, quadriceps, and brown adipose tissue were insulin resistant (demonstrated by increased ED_{50}), whereas in white adipose tissue, 2-DG phosphorylation was more sensitive to insulin. Brain 2-DG phosphorylation was insulin independent in control and obese animals. The largest decrease in insulin sensitivity in GTG-obese mice was observed in brown adipose tissue. The loss of diet-induced thermogenesis in brown adipose tissue as a result of the hypothalamic lesion in GTG-obese mice could be a major cause of insulin resistance in brown adipose tissue. Because brown adipose tissue can make a major contribution to whole-body glucose utilization, insulin resistance in this tissue may have a significant effect on whole-animal glucose homeostasis in GTG-obese mice. *Diabetes* 36:152–58, 1987

Gold thioglucose (GTG) is transported into the cells of the hypothalamus where it produces necrotic lesions that are responsible for the development of hyperphagia, hyperinsulinemia, hyperglycemia, and obesity (1). Several studies have used this model of obesity to examine the development of insulin resistance in soleus muscle, adipose tissue, and liver (2) and to study postreceptor insulin effects such as changes in the activity of the insulin-sensitive enzyme pyruvate dehydrogenase in heart and liver (3) and defective insulin-receptor tyrosine kinase (4). The acknowledgment of the role of brown adipose tissue (BAT) in energy expenditure in cold- (5) and diet-(6) induced thermogenesis has also led to the study of changes in the metabolism of this tissue in the GTG-obese model (7).

Most of the experiments assessing insulin resistance in tissues of GTG-obese mice have been done with in vitro preparations of the tissues (2,4,7). Recently, however, methods have been developed for examining the insulin response of several tissues from one animal in vivo with the uptake and metabolism of the glucose analogue 2-deoxyglucose (2-DG) as a relative index of glucose metabolism (8,9). This in vivo method under steady-state (10) and non-steady-state (12) conditions has demonstrated the heterogeneity of tissue responses to insulin. Changes in the insulin response during starvation (13) and exercise (14) have also been investigated.

In this study the insulin sensitivity of several glucose-utilizing tissues from control and GTG-obese mice was investigated with the single-injection model of assessing tissue metabolism of [1-^{14}C]2-DG at nine different insulin doses (12). BAT was included because of the postulated involvement of this tissue in obese syndromes (7,15–17) and recent work that showed the insulin-sensitive nature of BAT (18) and suggested a significant role for this tissue in whole-animal glucose utilization (19–21).

MATERIALS AND METHODS

Male mice (6 wk old, 20–25 g) were of the inbred CBA/T6 strain (Blackburn Animal House, Department of Immunology, University of Sydney, NSW, Australia). They were allowed free access to food (Rat and Mouse Kubes, Allied Feeds,

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TABLE 1
Changes in body weight, tissue weight, blood glucose, and plasma insulin in CBA/T6 mice 4 wk after single intraperitoneal injection of gold thioglucose (GTG)

	Control	GTG obese
Body weight (g)	24.57 ± 0.16 (86)	33.70 ± 0.33 (84)*
Epididymal fat pad weight (g)	0.288 ± 0.008 (86)	1.066 ± 0.032 (84)*
Protein content (mg/g)	18.81 ± 1.66	12.32 ± 0.90*
Interscapular BAT weight (g)	0.052 ± 0.001 (86)	0.147 ± 0.004 (84)*
Protein content (mg/g)	142.85 ± 12.15	90.70 ± 11.28*
Initial blood glucose (mM)	8.04 ± 0.12 (86)	11.49 ± 0.16 (84)†
Initial serum insulin (μU/ml)	40.3 ± 2.6 (30)	92.8 ± 8.7 (28)†

Assays for blood glucose and serum insulin were carried out as described in MATERIALS AND METHODS. All values are means ± SE for number of observations in parentheses. Statistical difference was determined by Student's *t* test.
*$P < .005$, †$P < .01$.

Rhodes, NSW, Australia) and water and were kept at 21°C on a 12-h light-dark cycle.

Obesity was induced by a single intraperitoneal injection of GTG (Sigma, St. Louis, MO; 0.5 mg/g body wt). Control mice of the same age and weight received 0.9% NaCl. Mice were used 4 wk after the GTG injection. Food was removed from the cages 3–4 h before the start of each experiment, which was always between 1100 and 1200 h.

Mice were lightly anesthetized with phenobarbitone (50 mg/kg i.p.), and body temperature was maintained at 37°C. Control and GTG-obese animals were injected through the tail vein with [1-^{14}C]2-DG (50 μCi/ml; Amersham, Amersham, UK) at a dose of 150 μCi/kg. The same injection included insulin (Actrapid, Novo, Copenhagen, Denmark) at concentrations of 0.001–10 U/kg with the total injection volume not exceeding 0.2 ml. Samples of blood (10 μl) were taken from the tip of the tail at 1, 5, 10, 20, and 30 min after injection of insulin and radioactivity. These samples were immediately deproteinized by addition of 0.05 ml 6% $HClO_4$, centrifuged, and the supernatant used to determine blood glucose level (22) and blood radioactivity.

The mice were killed 30 min after injection of tracer plus insulin, and brain, heart, epididymal white adipose tissue (WAT), interscapular BAT, and quadriceps were removed and frozen in liquid nitrogen. Tissues were extracted and analyzed for [1-^{14}C]2-DG phosphate as previously described (20). Blood samples taken from the left ventricle of the heart at death were analyzed for immunoreactive insulin by a double-antibody method (Burroughs Wellcome, Sydney, Australia) with human insulin standards.

The rate of tissue metabolism of [1-^{14}C]2-DG (K) was calculated by use of the single-injection model of Hom and Goodner (12) with one modification. [1-^{14}C]2-DG-phosphate concentration in the tissue (C; dpm/g or dpm/g protein for WAT and BAT) was used to calculate the amount of 2-DG metabolized in the tissue in preference to the use of total tissue [1-^{14}C]2-DG to calculate tissue uptake. The initial blood [1-^{14}C]2-DG concentration (C_{p_0}; dpm/ml) and the rate of disappearance of [1-^{14}C]2-DG from the blood (K_p) were derived from a single exponential fit of the [1-^{14}C]2-DG concentration in the blood at the time points (t) studied for each animal. The equation used was

$$K = \frac{C\,K_p}{C_{p_0}(1 - e^{-K_p t})}$$

The insulin dose-response data obtained were fitted to a four-parameter sigmoidal dose-response curve with the computer program ALLFIT (23), which enabled the calcu-

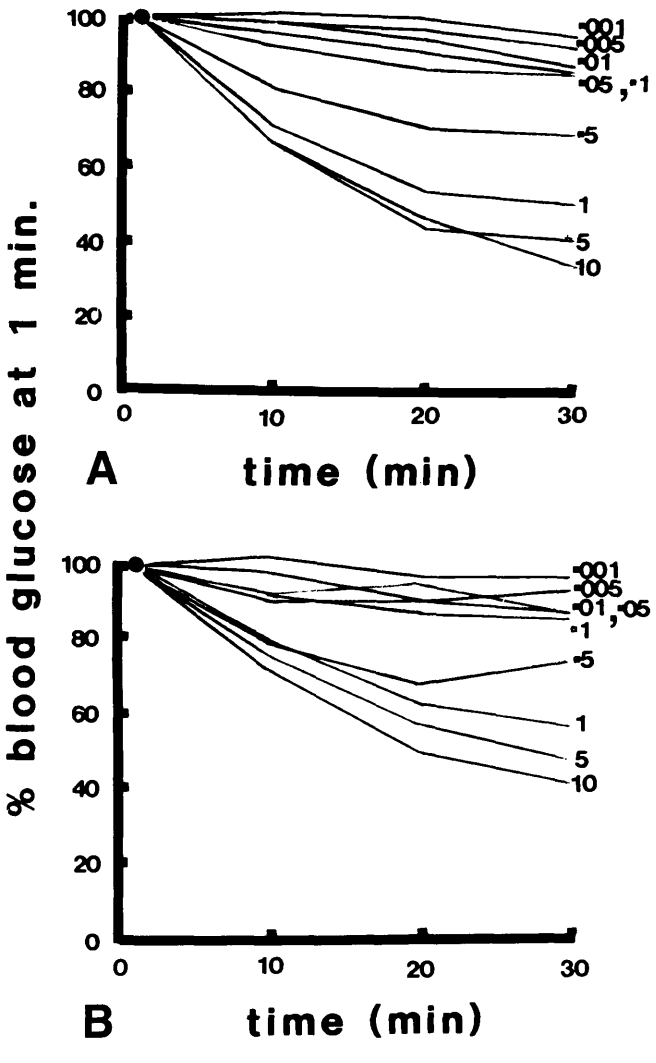

FIG. 1. Response of blood glucose to 9 doses of insulin (U/kg) in control and gold thioglucose-induced (GTG) obese mice. Insulin together with [1-^{14}C]2-deoxyglucose was injected intravenously through tail vein of anesthetized mice. *A*: response to insulin in control mice. *B*: response to insulin in GTG-obese mice. Numbers by each *line* correspond to dose of insulin injected (U/kg).

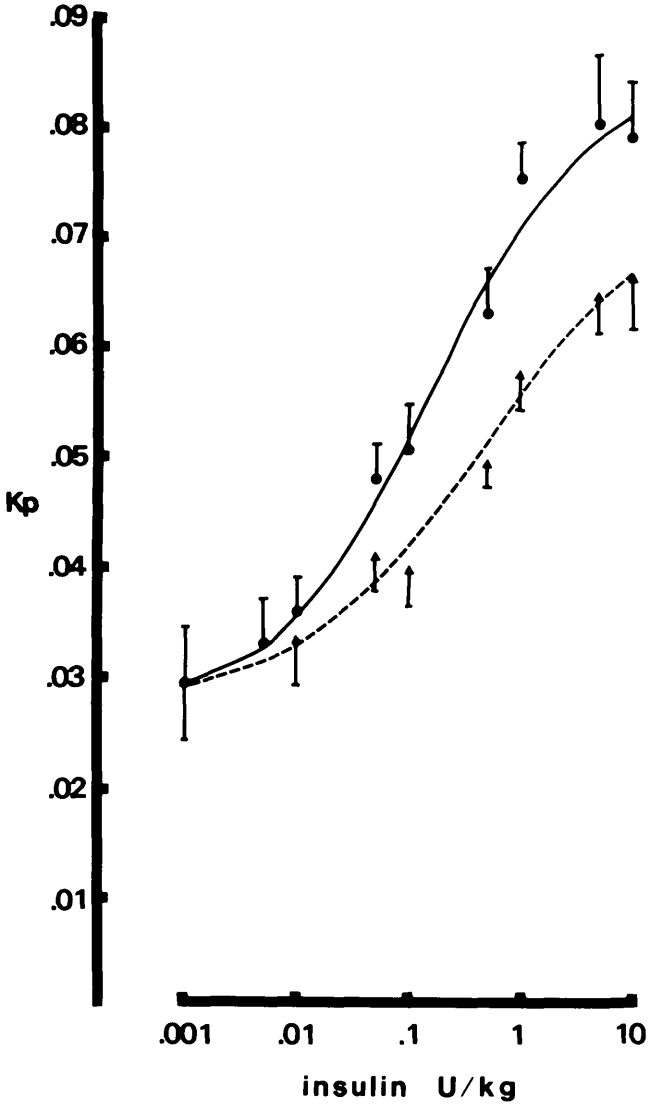

FIG. 2. Insulin dose-response relationship for rate of blood [1-^{14}C]2-deoxyglucose disappearance (K_p) in control and GTG-obese mice. K_p was calculated as described in MATERIALS AND METHODS, and each *point* represents mean ± SE for 5–11 separate animals. ●, Control mice; ▲, GTG-obese mice.

lation of an ED_{50} for insulin response and provided the ability to test the statistical significance of differences between the ED_{50} obtained in each tissue for control and GTG-obese mice.

RESULTS

The animals in this study were used 4 wk after injection with GTG (10 wk of age). This is the "dynamic" phase of obesity (7) in which the animals are hyperphagic and gain weight. Table 1 shows that at this stage the GTG-injected mice were significantly heavier than control mice and were hyperglycemic and hyperinsulinemic. Of the tissues analyzed in this study, the epididymal fat pad and intercapsular BAT were significantly larger in the GTG-obese animals. The decrease in protein content (mg/g) of WAT and BAT of GTG-obese mice indicated the accumulation of lipid in these tissues.

Because blood samples taken from the tail were not large enough to enable plasma insulin determination, the effectiveness of each insulin dose was monitored by measuring blood glucose levels during the course of experiments (Fig. 1). For both control and GTG-obese mice, increased insulin concentration resulted in an increased hypoglycemic effect. However, there was no significant difference in the hypoglycemic effect of the same insulin dose in control and GTG-obese mice. This lack of difference could be due to differences in hepatic glucose production, which was not measured in this study.

The effect of insulin on the [1-^{14}C]2-DG K_p is shown in Fig. 2. The relationship between K_p and insulin dose fitted a sigmoidal dose-response curve for both control and GTG-obese mice. The increased ED_{50} of the insulin dose-response curve for obese mice (0.43 ± 0.26 U/kg) was not significantly different from controls (0.16 ± 0.04 U/kg); $P = .092$). However, the apparent lower clearance of [1-^{14}C]2-DG from blood in obese mice cannot be taken as an absolute measure of whole-body insulin action, because 2-DG is cleared via the kidneys into urine, and differences in renal threshold for glucose and 2-DG in obese mice could influence K_p values.

Figure 3 shows the effect of insulin on [1-^{14}C]2-DG metabolism in brain. As has been previously reported, brain appeared to be insulin independent for glucose uptake and metabolism, and no significant difference in the rate of uptake and phosphorylation of [1-^{14}C]2-DG between control and GTG-obese mice was found at any of the insulin doses studied (8,9).

Analysis of the dose-response curves for [1-^{14}C]2-DG metabolism in the heart from control and GTG-obese mice (Fig. 4) showed a significant insulin resistance in the obese mice

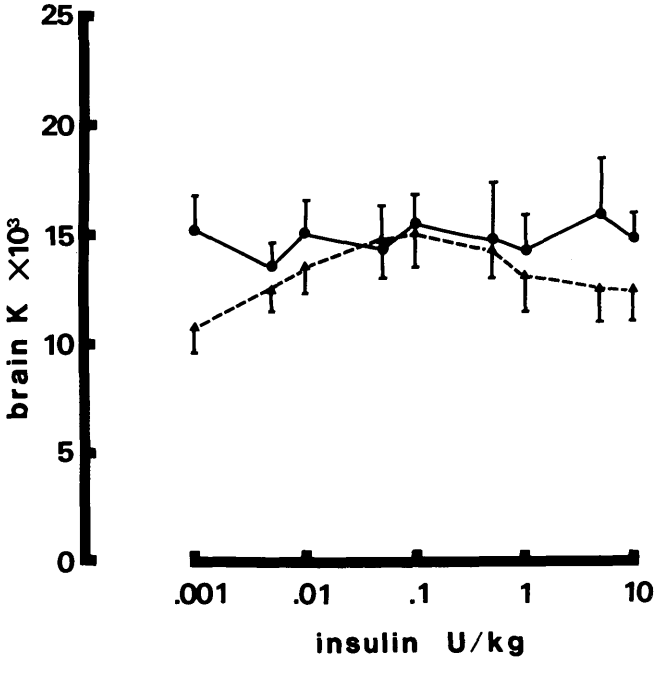

FIG. 3. Insulin dose-response curve for net tissue uptake and metabolism (K) of [1-^{14}C]2-deoxyglucose in brain from control and GTG-obese mice. K was calculated as described in MATERIALS AND METHODS, and each *point* represents mean ± SE for 5–10 separate animals. ●, Control mice; ▲, GTG-obese mice.

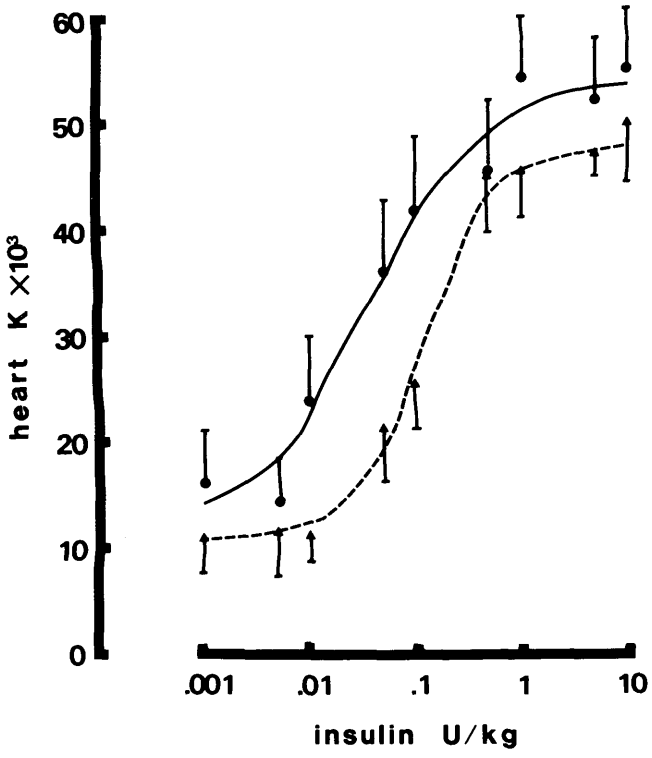

FIG. 4. Insulin dose-response curve for net tissue uptake and metabolism (*K*) of [1-^{14}C]2-deoxyglucose in heart from control and GTG-obese mice. *K* was calculated as described in MATERIALS AND METHODS, and each *point* represents mean ± SE for 5–10 separate animals. ●, Control mice; ▲, GTG-obese mice.

(control ED_{50} = 0.040 ± 0.014 U/kg; GTG obese = 0.117 ± 0.024 U/kg; P = .019). The basal and maximal responses to insulin were unaltered in heart from control or GTG-obese mice.

The epididymal WAT of GTG-obese mice appeared more sensitive to insulin than that of control mice (Fig. 5). These results were calculated with disintegrations per minute per gram protein to compensate for the difference in fat content of WAT in control and obese mice. The ED_{50} for insulin response in control mice was 0.245 ± 0.022 U/kg, whereas the ED_{50} in GTG-obese mice for insulin response in WAT was 0.159 ± 0.024 U/kg (P = .042). There was also a significantly higher basal (P = .001) and maximal (P = .001) response to insulin in GTG-obese WAT. A more valid comparison of total [1-^{14}C]2-DG metabolism in control and GTG-obese adipose tissue could be based on cell number or DNA content, but this would not alter the ED_{50} values; calculation of ED_{50} is based on the relationship between basal and maximal response to insulin, and expressing the results per cell number would not alter this relationship.

In quadriceps muscle the sensitivity of [1-^{14}C]2-DG metabolism was decreased by approximately twofold (Fig. 6). The ED_{50} of [1-^{14}C]2-DG metabolism for control muscle was 0.434 ± 0.085 U/kg and for obese muscle was 1.00 ± 0.261 U/kg (P = .002). There was no significant difference between basal (P = .78) and maximal (P = .38) response to insulin in quadriceps muscle from control and GTG-obese mice.

The ED_{50} of [1-^{14}C]2-DG metabolism for interscapsular BAT was the lowest of all the tissues studied (0.026 ± 0.004 U/kg; Fig. 7). This tissue also showed the greatest change in ED_{50} in GTG-obese mice (0.282 ± 0.14 U/kg; P = .014), indicating that BAT became very insulin resistant in hypothalamic obesity. Because of the differences in fat content of BAT from control and GTG-obese mice, the *K* values for BAT were calculated with disintegrations per minute per gram protein. The maximal response in BAT of obese mice was only 49% (P = .001) of that of BAT from control animals, which is consistent with a postreceptor defect causing insulin resistance in BAT.

DISCUSSION

The single-injection model was used in this study for three reasons. First, for ease of experimentation, because the catheterization and infusions needed to produce a euglycemic clamp in the mouse, as distinct from the rat, would be technically difficult. Second, because the 40- to 75-min period of insulin and glucose infusion required to stabilize the euglycemic clamp before 2-DG injection might seriously reduce the uptake of labeled 2-DG by tissues that are not actively oxidizing glucose under the conditions of the experiment. For example, in WAT, BAT, and nonoxidative fast-twitch muscle, the uptake and utilization of glucose during the plateau of the euglycemic clamp may be limited by the amount of glycogen and/or lipid accumulated during the stabilization period before the injection of labeled 2-DG.

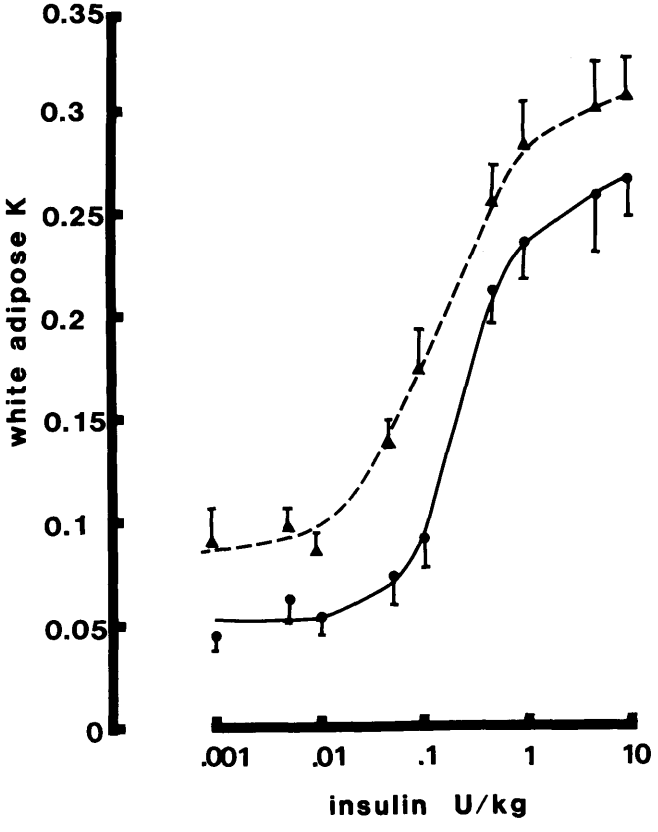

FIG. 5. Insulin dose-response curve for net tissue uptake and metabolism (*K*) of [1-^{14}C]2-deoxyglucose in white adipose tissue from control and GTG-obese mice. *K* was calculated as described in MATERIALS AND METHODS, and each *point* represents mean ± SE for 5–10 separate animals. ●, Control mice; ▲, GTG-obese mice.

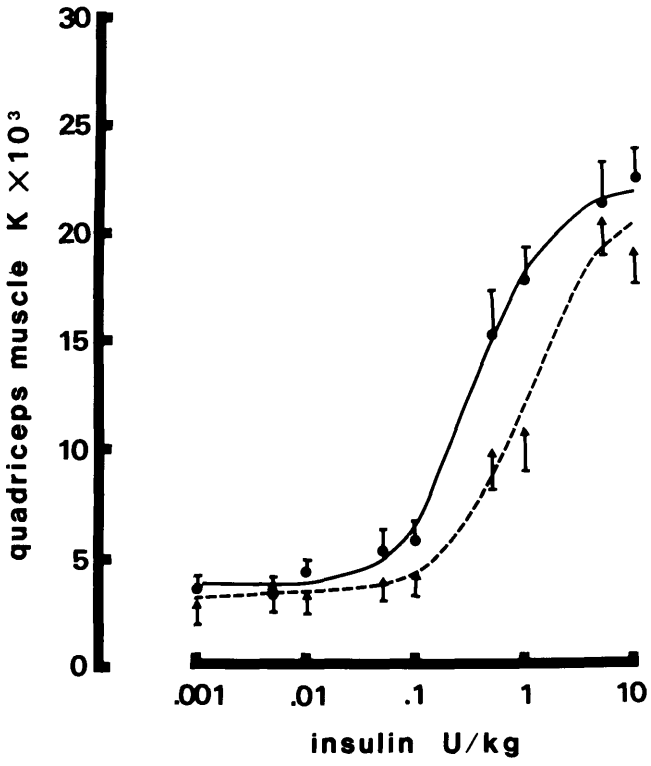

FIG. 6. Insulin dose-response curve for net tissue uptake and metabolism (*K*) of [1-^{14}C]2-deoxyglucose in quadriceps muscle from control and GTG-obese mice. *K* was calculated as described in MATERIALS AND METHODS, and each *point* represents mean ± SE for 5–10 separate animals. ●, Control mice; ▲, GTG-obese mice.

Third, it was thought that a single intravenous injection of insulin rather than constant infusion would more closely mimic the normal release and clearance of insulin.

The major problem with interpreting the results obtained in this study is the different initial glycemia of the two groups of mice studied. The equation used to calculate rates of 2-DG uptake does not take into account the blood glucose concentration, but the uptake of [1-^{14}C]2-DG will be affected by the size of the glucose pool available for uptake. In essence, a larger glucose pool would lead to a decrease in [1-^{14}C]2-DG uptake, but the actual rate of glucose uptake might be the same as in an animal with a lower level of glycemia. In our study the changes in insulin sensitivity of heart and muscle of GTG-obese mice might be partly explained by the higher glycemia in the obese mice; however, the reduced insulin sensitivity observed in these tissues is supported by other studies with GTG-obese mice (2,3). In WAT the increased insulin sensitivity observed could not be explained by the increased glycemia in GTG-obese mice, and the magnitude of the decrease in insulin sensitivity in BAT of obese mice and decrease in maximal response to insulin would also suggest that a change in the glucose pool could not account for these differences. Finally, despite the lack of any observed insulin effect in brain, the fact that there was no significant difference in [1-^{14}C]2-DG uptake between control or GTG-obese mice at any dose of insulin implies that the difference in glycemia between the two groups of animals studied does not affect the conclusions about relative glucose uptake made on the basis of rates of [1-^{14}C]2-DG uptake and phosphorylation.

The results show that the expected dose-response relationship between insulin dosage and [1-^{14}C]2-DG uptake was obtained for heart, WAT, BAT, and muscle in control mice. The rate of disappearance of [1-^{14}C]2-DG from the blood (K_p) also fitted a sigmoidal dose-response curve, and the increase in ED_{50} for K_p in GTG-obese mice may reflect the whole-animal insulin resistance shown by the hyperglycemia and hyperinsulinemia of the obese mice.

The decrease in insulin sensitivity (increased ED_{50}) seen in the heart from GTG-obese mice substantiates previous work from our laboratory that reported a decreased activity of the insulin-sensitive pyruvate dehydrogenase complex despite hyperinsulinemia in these animals (24). A reduction in pyruvate dehydrogenase activity (possibly due to increased fatty acid oxidation) could feed back through increased intracellular metabolites to decrease glucose uptake manifested in this study by the decreased response of [1-^{14}C]2-DG metabolism to insulin.

The decreased sensitivity to insulin in vivo observed in quadriceps muscle agrees with previous in vitro findings for isolated soleus muscle, even though the results shown here

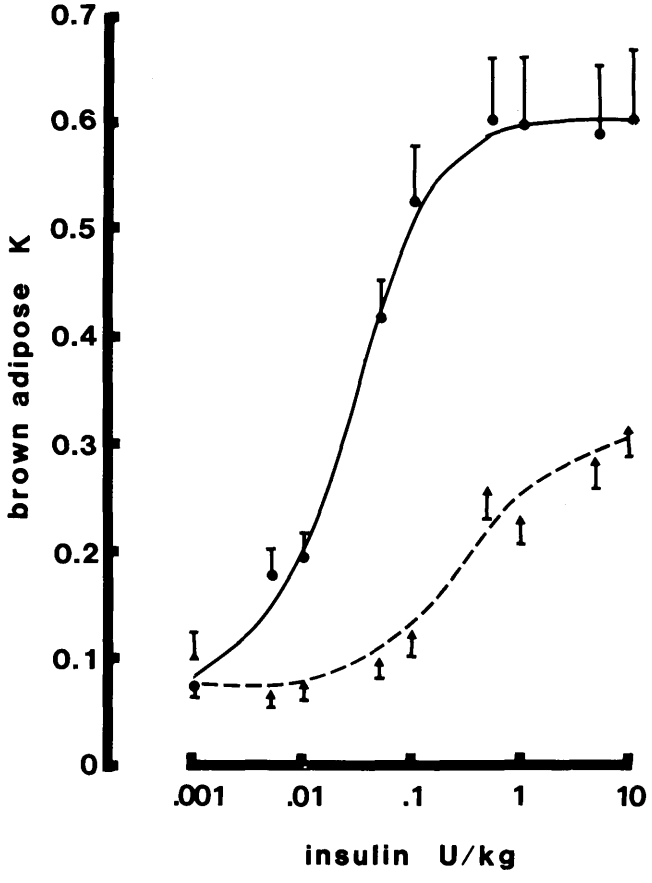

FIG. 7. Insulin dose-response curve for net tissue uptake and metabolism (*K*) of [1-^{14}C]2-deoxyglucose in brown adipose tissue from control and GTG-obese mice. *K* was calculated as described in MATERIALS AND METHODS, and each *point* represents mean ± SE for 5–10 separate animals. ●, Control mice; ▲, GTG-obese mice.

were obtained at an earlier stage of obesity than those reported for soleus (2). Despite the documented heterogeneity of muscle response to insulin (27), the GTG obesity probably results in a general reduction of insulin sensitivity in muscle tissue. Whether the change is a primary cause of obesity or a result of the development of obesity is not clear from this study.

In vitro studies have shown evidence for both increased and decreased insulin sensitivity in WAT from obese mice (25,26). Although it is possible that in the latter stages of GTG obesity in mice the white adipose tissue does become insulin resistant, the in vivo results obtained in this study show that 4 wk after the induction of obesity by injection with GTG, the epididymal fat pad is more insulin sensitive in the GTG-obese mice. This could explain the increased ability of the tissue to convert circulating glucose to triglyceride for storage in GTG-obese mice. Note, however, that all depots of WAT do not exhibit the same insulin sensitivity (12), and therefore the changes observed in our study for epididymal WAT may not occur in other WAT depots.

Analysis of the insulin dose-response curves for interscapular BAT in control and GTG-obese mice showed that BAT was the most insulin-sensitive tissue studied in control mice and that the greatest decrease in insulin sensitivity in obese mice (a 10-fold increase in ED_{50}) also occurred in BAT. Although BAT represents only 1–2% of the total body weight, the importance of this tissue in whole-body glucose disposal has been postulated by several groups (18–21,33). BAT has a large capacity for lipogenesis from glucose and has high activities of the enzymes involved in glucose conversion to lipid (28,29). The role of BAT in diet-induced thermogenesis is well established, and impairment of the thermogenic activity of BAT has been postulated as a major cause of obesity in several genetic models (15,30,31). Also, one report of the use of a specific β-agonist to stimulate BAT in genetic diabetic (*db/db*) mice showed that it led to a reduction in the hyperglycemia (32). In the GTG model of obesity it has been suggested that the inability of the lesioned hypothalamus to activate diet-induced thermogenesis in BAT in response to hyperphagia is the major cause of the increased metabolic efficiency that leads to obesity (7). If the lipid synthesized and stored in BAT under the influence of insulin was not oxidized because of the defective control of the thermogenesis, the accumulation of intracellular lipid could feed back on glucose uptake and lead to the lack of insulin response in BAT seen in our study. Other tissues would then become more important in dealing with the excess energy intake caused by hyperphagia in GTG-obese mice. The increased insulin sensitivity of WAT reported here and increased lipogenesis in the liver (2) are consistent with this hypothesis.

The results presented here show that in obesity some tissues may remain insulin sensitive while others exhibit a varying degree of insulin resistance. It is not clear from these experiments when tissues become insulin resistant after injection with GTG, but the magnitude of the resistance observed in BAT 4 wk after GTG injection suggests that the development of decreased insulin response in this tissue may occur early in the onset of obesity. In view of the recent findings that BAT is responsible for 8–12% of the whole-body glucose turnover after insulin stimulation in rats (33), it is tempting to speculate that the loss of control of diet-induced thermogenesis and the development of insulin resistance in BAT may be initial events in the development of obesity in GTG-injected mice.

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