In Vivo Glucose Utilization by Individual Tissues in Virgin and Pregnant Offspring of Severely Diabetic Rats

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Adult offspring of diabetic rats or SDF rats are characterized by insulin resistance in the liver and extrahepatic tissues; this insulin resistance does not worsen during pregnancy (1,2). In this study, we determined the glucose metabolic index in tissues of anesthetized virgin and pregnant control and SDF rats in basal conditions and during a euglycemic hyperinsulinemic clamp. Tissues comprised insulin-sensitive tissues (five skeletal muscles, diaphragm, and periovarian white adipose tissue) and control tissues (duodenum and cerebrum). In addition, this study measured the GMI of placenta and fetuses. In basal conditions, SDF rats showed a slight decrease (9–29%) in the GMI of skeletal muscles compared with control rats; it was not altered by pregnancy in any of the tissues. During physiological hyperinsulinemia, virgin SDF rats exhibited a 25–70% decrease in the GMI of skeletal muscles compared with control rats; this decrease was not observed in diaphragm, or in adipose tissue in which the GMI was found to be increased. During pregnancy, SDF rats did not show an additional drop in the GMI of skeletal muscles, whereas the GMI of both skeletal muscles and adipose tissue was clearly diminished (25–60%) in control rats. The GMI of skeletal muscles was therefore comparable in pregnant control rats and SDF rats. The placental, but not fetal, GMI was increased by 24% during hyperinsulinemia in control rats; the placental and fetal GMIs, in basal and hyperinsulinemic conditions, were similar in control rats and SDF rats. In conclusion, skeletal muscles, but not white adipose tissue, are involved in the peripheral insulin resistance of the SDF rats. However, pregnancy does not induce a further decrease in glucose utilization by skeletal muscles in SDF rats. ***Diabetes*** **42:530–36, 1993**

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SDF rats, adult offspring of diabetic rats; GMI, glucose metabolic index; STZ, streptozocin; GTT, glucose tolerance test; 2-D-G, 2-deoxy-D-glucose; 2-[^{3}H]D-G, 2-deoxy-[1-^{3}H]-glucose; ANOVA, analysis of variance; NEFA, nonesterified fatty acid.

Hyperglycemia during rat gestation causes perturbations of glucose homeostasis in the offspring (3–7). SDF rats exhibit high insulin levels during a GTT (4) and an increased renal clearance of insulin (8). These SDF rats are markedly resistant to the action of insulin, as revealed by the euglycemic hyperinsulinemic clamp (1). There is a decreased sensitivity and a decreased maximal response to insulin in the liver as well as a decreased sensitivity to insulin but with a normal maximal response in the extrahepatic tissues (1). Gravid SDF rats develop features of gestational diabetes (2,3,9), although the insulin resistance is not significantly aggravated during gestation in these rats in contrast to what is observed during normal gestation (2,10,11). The hyperinsulinemic clamp does not give any information as to the individual response of the insulin-sensitive tissues that contribute to the whole-body glucose utilization. To examine the glucose utilization of the peripheral tissues, we used the euglycemic hyperinsulinemic clamp combined with the use of the radiolabeled nonmetabolizable glucose analogue 2-deoxy-[1-^{3}H]-D-glucose as described by Ferré et al. (12). We thus determined the GMI (13) under basal conditions and during euglycemic hyperinsulinemia in five skeletal muscles (soleus, adductor longus, epitrochlearis, extensor digitorum longus, and tibialis anterior muscle) diaphragm muscle, periovarian white adipose tissue, and two control tissues (cerebrum and duodenum) of virgin and pregnant control rats and SDF rats.

RESEARCH DESIGN AND METHODS

The animals were adult offspring (second generation) of control and diabetic Wistar rats. Diabetes in the maternal

rat (first generation) was induced experimentally by a single intravenous injection of STZ (76.5 μmol/kg body wt; Upjohn, Puurs, Belgium) on day 1 of pregnancy (i.e., the day of copulation plug). Severe hyperglycemia was confirmed on days 6 and 20 of gestation. Only the offspring of rats that had given birth to ≥8 pups were included; after weaning only the female offspring were kept. All animals had free access to water and a standard rat laboratory chow. At 100–110 days of age, part of the rats were mated overnight. Experiments were performed on day 20 of gestation and in appropriately age-matched virgin rats. Pregnant animals with <8 fetuses were discarded. After a 3-h fast, the animals were anesthetized with pentobarbitone (0.24 mmol/kg, i.p.), the right carotid artery was catheterized for blood sampling, and a tracheotomy was performed. Body temperature was maintained at 37–38°C.

Euglycemic hyperinsulinemic clamp. The clamp studies were performed as described previously (1). Based on previous data (1,2), different doses of insulin (porcine monocomponent insulin, Novo Industri, Bagsvaerd, Denmark) were infused in a saphenous vein at a constant rate (20 μl/min) in virgin control rats (0.06 mmol · kg^{-1} · min^{-1}), virgin and pregnant SDF rats, and pregnant control rats (0.04 mmol · kg^{-1} · min^{-1}) to obtain steady-state plasma insulin concentration of ~1.7 nM in all animals.

Measurement of GMI in individual tissue. The method used is based on the biochemical properties of 2-D-G, which is phosphorylated by hexokinase intracellularly. 2-Deoxyglucose-6-phosphate accumulates if the tissue glucose-6-phosphatase activity is very low; hydrolysis of glucose-6-phosphate is therefore negligible (14). Glucose-6-phosphatase activity is very low in the maternal tissues used in this study (12) as well as in the fetal liver and kidney tissue (15) and the placenta (16, 17). The GMI was determined as follows (14): the accumulation of 2-deoxy-[1-^{3}H]-D-glucose-6-phosphate in the tissues was determined after a bolus injection of (2-[^{3}H]D-G), as previously described in anesthetized rat (12). In the basal state, 30 μCi of 2-[^{3}H]D-G (Amersham Laboratories, Buckinghamshire, UK) dissolved in 200 μl of 0.9% NaCl was given as a bolus through a saphenous vein 30 min after surgery. Arterial blood (50 μl) was sampled at 1, 3, 5, 10, 20, 45, and 60 min. The clamp studies were started 30 min after surgery. When steady state was reached (after 40 min), 30 μCi of 2-[^{3}H]D-G in 200 μl of 0.9% NaCl was given as a bolus through the saphenous vein. Arterial blood (50 μl) was again sampled at 1, 3, 5, 10, 20, 30, and 45 min. Blood samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$, and centrifuged at 10,000 rpm. An aliquot was used for the determination of blood glucose concentration by the D-glucose-oxidase D-peroxidase method (GOD-PAP, Boehringer Mannheim, Mannheim, Germany). Another aliquot was used to count the 2-[^{3}H]D-G in a liquid scintillation counter (Packard, Canberra, Australia). A larger blood sample was taken at the start and at the end of each experiment for determination of plasma insulin by radioimmunoassay using rat and porcine insulin standards, respectively (Novo Industri, Bagsvaerd, Denmark) (1).

After the last blood sampling, rats were killed by cervical dislocation, and skeletal muscles (soleus, adductor longus, epitrochlearis, extensor digitorum longus, tibialis anterior, and diaphragm), pieces of periovarian white adipose tissue, the cerebral hemispheres, and pieces of duodenum were rapidly removed within 5 min. The tissues were placed in 0.5 ml of 1M NaOH. The content of 2-[^{3}H]D-G was determined after digestion (45 min at 60°C) and neutralization with 1 M HCl. Neutralized solution (200 μl) was added to 1 ml of 6% $HClO_4$, and 200 μl was added to 1 ml of $Ba(OH)_2$-$ZnSO_4$. After centrifugation, samples of both supernatants were counted in a Packard liquid scintillation counter to determine the amount of 2-[^{3}H]D-G plus 2-deoxy-[1-^{3}H]glucose-6-phosphate in the $HClO_4$ supernatant and the amount of 2-[^{3}H]D-G in the $Ba(OH)_2$-$ZnSO_4$ supernatant.

Calculations. The GMI (13) for each tissue was calculated by dividing the amount of 2-deoxy-[1-^{3}H]glucose-6-phosphate (dpm in the $HClO_4$ supernatant minus dpm in the $Ba(OH)_2$-$ZnSO_4$ supernatant) in the tissue by the calculated integral of the ratio of arterial blood 2-[^{3}H]D-G to glucose concentration. The lumped constant was not determined in this study, nor was a lumped constant that was determined elsewhere used for the calculation of the GMI for the various tissues. Based on data from previous studies (13,18), we assumed that the relative changes in GMI are sufficient to reflect the effect of maternal diabetes on the adult offspring. However, in the DISCUSSION section, we used a previously determined lumped constant (11) for placenta (0.72) and fetuses (0.85) to give an approximation of the contribution of the rat conceptus to the maternal glucose utilization in basal postabsorptive conditions (2).

Statistical analysis. Data are presented as means ± SE in SI units. Statistical analysis was performed with a computer software program (Minitab Statistics, Pennsylvania State University, State College, PA). Intergroup differences were first analyzed by one-way ANOVA. When significant ($P < 0.05$), an unpaired Student's t test was used to compare pairs of means.

RESULTS

All pregnant rats of the first generation that were injected with STZ were severely hyperglycemic, were hypoinsulinemic, and had a decreased body weight (Table 1). Their second generation offspring (SDF rats) had a decreased body weight at 100 days of age, increased nonfasting insulin levels, but normal glucose levels. The pregnancy-induced fall in glucose levels and rise in insulin levels, as observed in control rats, was blunted in SDF rats.

GMI of individual tissues of virgin and pregnant control and SDF rats.

Virgin SDF rats versus virgin control rats. Under basal conditions the GMI of the epitrochlearis muscle was lower in SDF rats than in control rats ($P < 0.05$; Fig. 1). The GMI of all other tissues was not significantly altered by maternal diabetes (Figs. 1 and 2).

To obtain a similar hyperinsulinemic plateau (± 1.85 nM) in control rats and SDF rats during the clamps,

TABLE 1
Body weight, nonfasting plasma glucose, and plasma insulin concentrations of first and second generation control rats and diabetic rats

	Control rats			Diabetic rats		
	Body weight (g)	Glucose (mM)	Insulin (nM)	Body weight (g)	Glucose (mM)	Insulin (nM)
First generation						
Pregnant	297 ± 2	4.22 ± 0.17	0.39 ± 0.33	276 ± 5*	20.10 ± 0.83†	0.12 ± 0.02‡
n	7	7	7	6	6	6
Second generation						
Virgin	206 ± 2	5.22 ± 0.22	0.18 ± 0.01	162 ± 3	5.11 ± 0.17	0.24 ± 0.02*
n	21	21	21	21	19	16
Pregnant	292 ± 3	4.31 ± 0.17‖	0.37 ± 0.04§	274 ± 8†§	4.82 ± 0.27*	0.25 ± 0.03*
n	12	12	11	12	12	12

Data are means ± SE. Measurements were made at 100 days of age in virgin animals and on day 20 of gestation in pregnant animals.
*$P < 0.05$ vs. values in control rats.
†$P < 0.001$ vs. values in control rats.
‡$P < 0.01$ vs. values in control rats.
§$P < 0.001$ vs. values in virgin rats.
‖$P < 0.01$ vs. values in virgin rats.

different doses of insulin were used to obtain a constant infusion rate (20 μl/min); the insulin infusion rate was 0.06 mmol · kg^{-1} · min^{-1} in control rats, and 0.04 mmol · kg^{-1} · min^{-1} in SDF rats (Table 2). The glucose infusion rates necessary to maintain euglycemia were significantly lower in SDF rats compared with control rats ($P < 0.001$). Hyperinsulinemia increased the GMI of the skeletal muscles, diaphragm, and white adipose tissue in both control and SDF virgin rats (Figs. 1 and 2) but not the GMI of brain and duodenum (Fig. 2).

A pronounced decrease (25–70%) was observed in the effect of hyperinsulinemia on the glucose utilization by skeletal muscles in SDF rats compared with control rats (Fig. 1); in contrast, the GMI of diaphragm was similar in both groups of rats (Fig. 2). An increased effect of hyperinsulinemia was observed in the periovarian white adipose tissue of SDF rats compared with control rats ($P < 0.05$).

Pregnant versus virgin control rats. The basal GMI of the soleus, adductor longus, and tibialis anterior muscles; the diaphragm; white adipose tissue; brain; and duodenum were not altered by pregnancy (Fig. 1). In contrast, the GMI of the extensor digitorum longus and epitrochlearis muscles was lower ($P < 0.05$) in pregnant than in virgin control rats (Fig. 1).

During the hyperinsulinemic clamp, the glucose infu-

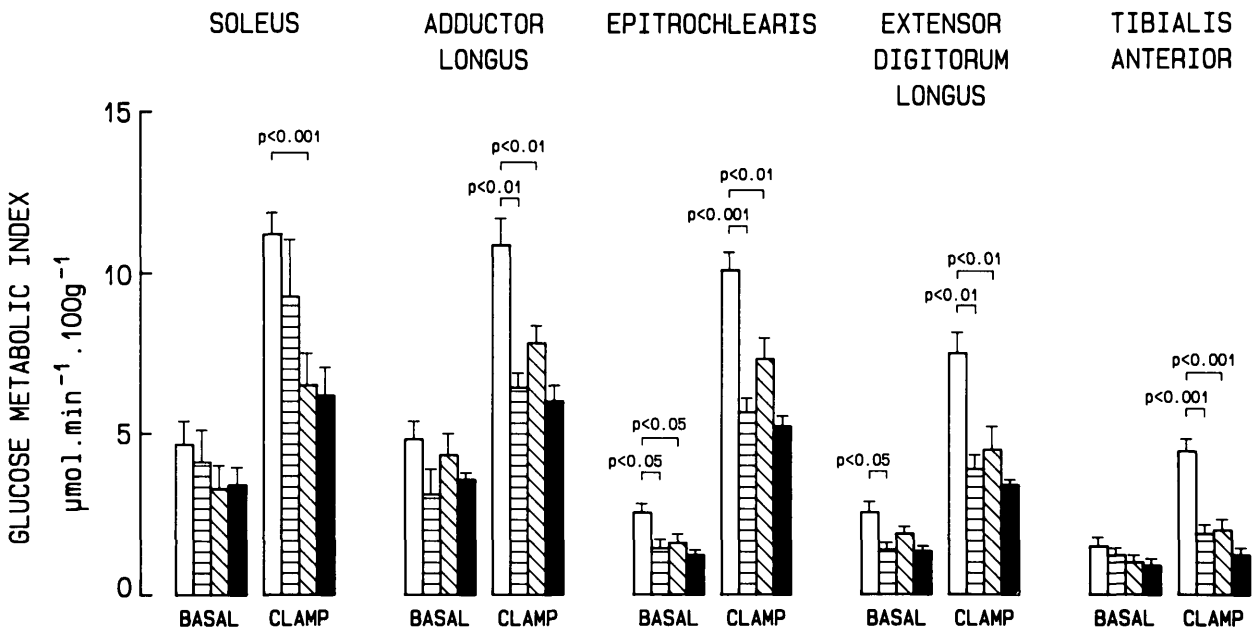

FIG. 1. In vivo GMI of soleus, adductor longus, epitrochlearis, extensor digitorum longus, and tibialis anterior muscles in virgin (▨) and pregnant (▤) control rats and in virgin (▧) and pregnant (■) offspring of rats made diabetic with STZ under basal conditions and at physiological hyperinsulinemia. Data are means ± SE of 5–10 experiments.

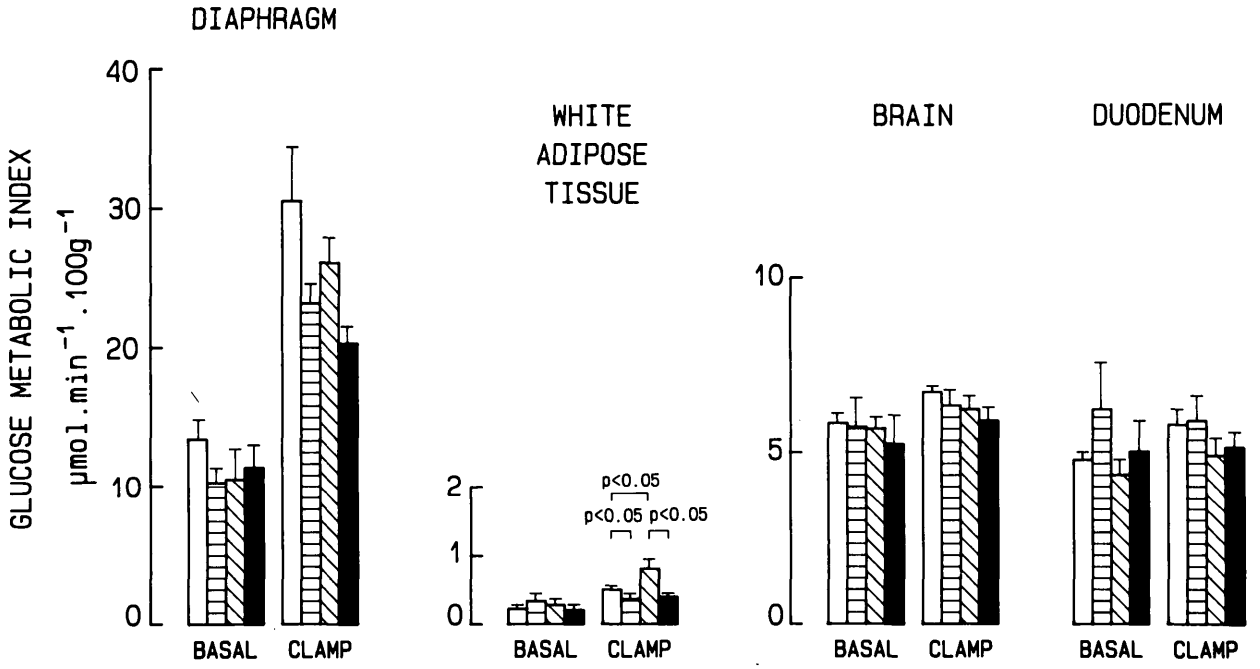

FIG. 2. In vivo GMI of diaphragm, periovarian white adipose tissue, cerebrum, and duodenum in virgin (▨) and pregnant (▤) control rats and virgin (▧) and pregnant (■) offspring of rats made diabetic with STZ under basal conditions and at physiological hyperinsulinemia. Data are means ± SE of 5–10 experiments.

sion rate necessary to maintain euglycemia was lower in pregnant than in virgin rats, but steady-state insulin levels were not different in both groups (Table 2). The GMI of brain and duodenum were not altered by hyperinsulinemia, whereas the GMI of all five skeletal muscles, the diaphragm, and white adipose tissue were stimulated by hyperinsulinemia. However, the GMI was significantly decreased in the adductor longus, extensor digitorum longus, tibialis anterior, and epitrochlearis muscles (40–60% decrease; Fig. 1) and in white adipose tissue (25% decrease; Fig. 2) of pregnant rats compared with those of virgin rats; the GMI of the soleus muscle and diaphragm was not different.

The fetal (10.4 μmol · min^{-1} · 100 g^{-1}) and placental (11.4 μmol · min^{-1} · 100 g^{-1}) GMIs were relatively high under basal conditions and comparable to the GMI we measured in the maternal diaphragm (10.5 μmol · min^{-1} · 100 g^{-1}). The fetal GMI was not altered during hyperinsulinemia, but the placental GMI was increased by 24% during hyperinsulinemia (Fig. 3).

Pregnant versus virgin SDF rats. The basal GMI of the various tissues studied were not altered by pregnancy in SDF rats (Figs. 1 and 2).

During the clamp, the steady-state glucose infusion rates needed to maintain euglycemia were not different in pregnant and virgin SDF rats and steady-state insulin levels were similar (Table 2). The GMI of the skeletal muscles and the diaphragm were stimulated by hyperinsulinemia the same as in virgin SDF rats, so that no difference was observed between the GMI of the skeletal muscles in pregnant and virgin SDF rats; however, the increase in the GMI of white adipose tissue during hyperinsulinemia was blunted, and the GMI of adipose tissue was lower in pregnant than in virgin SDF rats.

Fetal (9.2 μmol · min^{-1} · 100 g^{-1}) and placental (10.8 μmol · min^{-1} · 100 g^{-1}) GMIs were relatively high when

TABLE 2
Plasma insulin, blood glucose, and steady-state glucose infusion rate in virgin and pregnant control rats and offspring of diabetic rats under basal conditions and during euglycemic hyperinsulinemia

		Plasma insulin (nM)		Blood glucose (mM)		Glucose infusion rate
	n	Basal	Clamp	Basal	Clamp	(μmol · min^{-1} · kg^{-1})
Control rats						
Virgin	9	0.25 ± 0.02	1.92 ± 0.27	5.0 ± 0.1	4.8 ± 0.1	104 ± 3
Pregnant	5	0.32 ± 0.07	1.60 ± 0.11	3.8 ± 0.05*	3.9 ± 0.2	68 ± 3*
SDF rats						
Virgin	10	0.30 ± 0.04	1.79 ± 0.36	5.3 ± 0.05	5.1 ± 0.1	72 ± 2†
Pregnant	5	0.32 ± 0.03	1.64 ± 0.05	3.9 ± 0.3*	3.9 ± 0.1	72 ± 3

Data are means ± SE of 5–10 experiments.
*P < 0.001 vs. values in virgin rats.
†P < 0.001 vs. values in control rats.

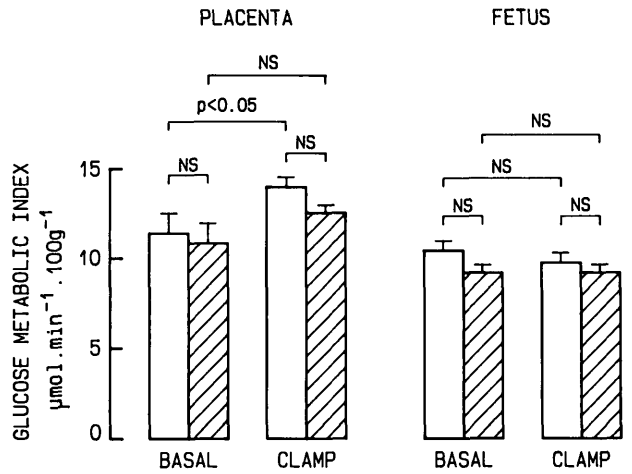

FIG. 3. In vivo GMI in fetus and placenta of 20-day pregnant control rats (□) and offspring of rats made diabetic with STZ (▨) under basal conditions and during euglycemic hyperinsulinemic clamp. Data are means ± SE of 5 experiments.

compared with the GMI of several maternal tissues (e.g., 5.3 $\mu mol \cdot min^{-1} \cdot 100\ g^{-1}$ in maternal brain), and the values were comparable with the GMI in maternal diaphragm (11.3 $\mu mol \cdot min^{-1} \cdot 100\ g^{-1}$). The GMI of fetuses and placenta were similar in basal conditions and during hyperinsulinemia (Figs. 2 and 3).

Pregnant SDF versus pregnant control rats. In the basal postabsorptive state, the GMI of the various tissues studied were comparable in pregnant control and SDF rats (Figs. 1 and 2).

During the clamp (0.04 $mmol \cdot kg^{-1} \cdot min^{-1}$), the steady-state glucose infusion rate and the steady-state insulin levels were similar in both pregnant groups of rats (Table 2). Hyperinsulinemia stimulated the GMI to the same extent in the skeletal muscles and in white adipose tissue of both pregnant control and SDF rats (Figs. 1 and 2). During the clamp, the GMI of the various tissues was not different between both these groups.

The fetal GMI was comparable between control and SDF rats in either the basal state or during hyperinsulinemia, whereas the placental GMI was slightly higher in control rats than in SDF rats during hyperinsulinemia (Fig. 3).

DISCUSSION

We previously have demonstrated that the liver and the extrahepatic tissues of the SDF rats are markedly resistant to the action of insulin (1). Somewhat unexpectedly, we also found that no significant deterioration of this insulin resistance occurs during gestation in SDF rats, although some features of gestational diabetes are present. Pregnant SDF rats have higher glucose and NEFA levels than normal pregnant rats, their plasma insulin levels are decreased and the number of granulated β-cells in their endocrine pancreas does not increase as in normal gestation (2,3,9). In this study, we examined further the glucose utilization in several insulin-sensitive and nonsensitive peripheral tissues in both virgin and pregnant SDF rats. Thus we were able to delineate the effect of several factors on peripheral glucose utilization, alone and in combination: *1*) hyperinsulinemia, *2*) pregnancy, and *3*) the effect of maternal diabetes during pregnancy and lactation on their adult offspring.

Muscles represent the main reservoir of insulin-sensitive tissues within the mammalian body, representing ~40% of the body weight (19); the contribution of the muscle mass to the whole-body glucose turnover rate is ~36% in postabsorptive anesthetized Wistar rats (12). We sampled five skeletal muscles and the diaphragm muscle. These skeletal muscles are representative of the several subtypes in fiber composition: slow-twitch oxidative (soleus and adductor longus), fast-twitch oxidative glycolytic (tibialis anterior and extensor digitorum longus), and fast-twitch glycolytic (epitrochlearis) (20–23). In the basal postabsorptive state, the GMI was about twofold higher in the muscles of the slow-twitch type (soleus and adductor longus muscles) than in the other skeletal muscles, as was reported previously (24). The basal GMI was severalfold higher in the diaphragm than in the skeletal muscles (13), which can be explained by the fact that it is the only muscle working continuously during the experiment (10,25).

The physiological hyperinsulinemia obtained during the hyperinsulinemic clamp was associated with an increased glucose utilization by all muscles studied (skeletal muscles and diaphragm) and in white adipose tissue, but not by the two tissues representative of tissues not sensitive to insulin, duodenum, and brain.

Normal late pregnancy is associated with a severe physiological insulin resistance in rats (10,11) as in women (26). In this study, we have confirmed earlier data (10) that the white adipose tissue and the skeletal muscles, but not the diaphragm muscle, are involved in the insulin resistance of late pregnancy. This cannot be explained by differences in glucose levels between virgin and pregnant rats (10). Because the insulin binding to those tissues is unchanged during pregnancy (27), a postreceptor defect has been proposed (28) but is as yet incompletely understood.

The adult offspring of diabetic rats are characterized by normal basal glucose levels and normal (1) or increased (2; Table 1) basal insulin levels. The small increase in basal insulin concentrations, a consequence of the insulin resistance that characterizes SDF rats (1), may explain the mild decrease (9–29%) in the basal GMI in SDF rats compared with control rats. During the hyperinsulinemic clamp, the whole-body glucose turnover rate, as reflected by the glucose infusion rate, was 30% lower in SDF rats than in control rats, confirming previous results on whole-body insulin sensitivity (1). Clear evidence indicated a 25–70% decrease in glucose utilization by the skeletal muscles, but not by the diaphragm, of SDF rats. In contrast, no decrease occurred in the GMI of white adipose tissue in SDF rats. This may seem somewhat surprising; however, one has to bear in mind that the rat is born at an immature stage, with only 1–2% adipose tissue. White adipose tissue develops after birth, and should therefore not be affected by the altered milieu caused by maternal diabetes during early development that affects skeletal muscle glucose metab-

olism so much (29). Diversity between tissues is not exceptional and has also been reported in humans (30).

During pregnancy, SDF rats exhibit no further demonstrable decrease in the GMI of skeletal muscles. This agrees with our previous data (2) that the insulin sensitivity and responsiveness of the peripheral tissues during the hyperinsulinemic clamp are unaltered during pregnancy in SDF rats.

Further research should concentrate on the mechanism of insulin resistance in SDF rats. NEFAs, which are known to inhibit insulin-stimulated glucose utilization (31), are almost certainly not involved in the peripheral insulin resistance of SDF rats, because plasma NEFA levels are lower in virgin SDF rats than in virgin control rats (1). A prereceptor defect though can as yet not be excluded; a decreased ability of insulin to stimulate skeletal muscle blood flow as demonstrated in obesity (32), or increased levels of circulating insulin counteragents such as cortisol (33) or epinephrine (18) may also induce a decreased glucose uptake. Both receptor and postreceptor defects (34,35) can reduce glucose utilization by skeletal muscles. Impaired binding of insulin to its receptor seems not to be the cause as ^{125}I-labeled insulin binding to isolated liver plasma membranes and isolated adipocytes of SDF rats is respectively unchanged and enhanced (K.H., R.V.B., L.A., F.A.V.A.; unpublished observations). These data show that the effect of pregnancy and the effect of a diabetic milieu during early development on the glucose utilization by the skeletal muscles of adult rats are roughly comparable and are not additive. We therefore speculate that a similar (postreceptor?) defect may be present in both situations.

Using a previously published (10) lumped constant for placenta (0.72) and fetuses (0.85), we calculated the rate of glucose utilization by the rat conceptus (10 fetuses and 10 placentas) as being 21% of the maternal glucose utilization (61 μmol $\cdot$ min^{-1} $\cdot$ kg^{-1} [2]) in the control group in basal postabsorptive conditions. This value compares with values obtained in previous studies in the rat (10). The glucose utilization by the conceptus was 17% of the maternal glucose utilization rate in SDF rats (72 μmol $\cdot$ min^{-1} $\cdot$ kg^{-1} [2]), which is lower than in the control rats. This may be explained by the lack of pregnancy-induced insulin resistance in SDF rats.

Some caution is needed to extrapolate results obtained in anesthetized animals to the situation in conscious animals. Pentobarbital decreases the whole-body glucose utilization rate in the rat by 30%, primarily because of a drop in glucose utilization of postural muscles, whereas the glucose utilization of nonpostural muscles and other tissues is minimally affected (36). However, the effect of insulin on glucose utilization is very similar in anesthetized (11) and conscious (37) rats.

From these and previous studies, it is clear that the maternal hyperglycemia interferes with fetal development (5,9) and has consequences persisting into adulthood (1–9,38,39). Studies in Pima Indians have shown that, in addition to the genetic transmission of diabetes, a diabetic intrauterine milieu can induce a diabetogenic tendency in the offspring; impaired glucose tolerance is much more frequent in children of mothers who had diabetes during pregnancy than in children of mothers who developed diabetes after pregnancy (38). Also, Martin et al. (39) have shown that women born to diabetic mothers have an increased risk of developing gestational diabetes.

In conclusion, we have shown that the skeletal muscles are primarily responsible for the peripheral insulin resistance in the offspring of diabetic rats. Second, the pregnancy-induced decrease in GMI of skeletal muscles is not present in adult offspring of severely hyperglycemic rats; the GMI of skeletal muscles of normal pregnant rats and pregnant offspring of diabetic rats is therefore comparable.

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