Effects of Streptozocin-Induced Diabetes on Glucose Metabolism and Lactate Release by Isolated Fat Cells From Young Lean and Older, Moderately Obese Rats

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Streptozocin-induced diabetes (STZ-D) was produced in male Wistar rats at two stages of development: young, lean rats, weighing 150–220 g (6–8 wk), and older, moderately obese rats, weighing 450–500 g (6–8 mo). A comparable degree of hyperglycemia (420–500 mg/dl) without ketosis was generated by injection of 50 mg/kg i.v. STZ for young, lean rats and 30 mg/kg i.v. for older, fatter rats. The animals were killed 8–11 days after injection. Insulin binding by the isolated adipocytes of both groups was not significantly altered on a per-cell basis by the presence of diabetes. Total adipocyte glucose metabolism, both basal and insulin stimulated, was reduced (63 and 88%, respectively) by the induction of diabetes in young, lean rats. In contrast, the induction of diabetes in the older, moderately obese rats had no suppressive effect on total glucose metabolism by their fat cells. Diabetes increased the relative conversion of glucose to lactate by fat cells from both groups of rats, but in absolute terms, the fat cells from the obese diabetic rats produced significantly more lactate from glucose than cells from the lean diabetic rats, both in the absence and presence of insulin. Diabetes did not alter the glucose concentration at which peak insulin response occurred in either group. We conclude that STZ-D in rats, at different stages of development and degrees of adiposity, results in quantitatively different alterations of adipocyte metabolism, which appear to be postreceptor in nature and result in an increase in glucose conversion to lactate. *Diabetes* 38:237–43, 1989

Glucose	1 mM = 18 mg/dl	Lactate	1 mM = 1 meq/L
Insulin	1 pM = 0.139 μU/ml		

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Studies in human and animal models have shown that obesity frequently accompanies or precedes the onset of non-insulin-dependent diabetes mellitus (NIDDM) (1–3). As obesity develops, there is an accumulation of excess adipose mass accompanied by hyperinsulinemia and varying degrees of insulin resistance. In an attempt to understand the apparent link between obesity and the development of NIDDM, the metabolic alterations produced by obesity and/or diabetes in adipose tissue from both animals and humans have been extensively examined (2–16). The enlargement of adipocytes during growth and development of obesity is known to be accompanied by a marked shift in the pattern of glucose metabolism and a progressive decline in responsiveness to insulin (5,16,17). Large fat cells from older, fatter rats have a higher basal lipolytic rate and a decrease in rate of glucose conversion to fatty acids and CO_2 compared with small fat cells from lean rats. In addition, they have an increased rate of glucose conversion to glyceride-glycerol, suggesting a greater rate of triglyceride breakdown and resynthesis (5,17).

In a reexamination of the metabolic products of glucose in adipocytes, we and others recently found that enlarged adipocytes from older, moderately obese rats have significantly increased rates of lactate production from glucose, which can account for 40–60% of total glucose metabolism (16,18,19). This finding has also been confirmed in human adipocytes (9,12,20). Moreover, fasting of donor animals markedly increases the rate of lactate production by small fat cells and, to a lesser extent, that of large fat cells, whose rate of lactate production is already elevated (19,21,22).

Because diabetes has been compared with cellular starvation, we postulated that the presence of diabetes might also lead to increased adipocyte lactate production. In this study, we examined insulin binding, insulin response, and the rate and pattern of glucose metabolism and lactate production in adipocytes from streptozocin-induced diabetic

(STZ-D) rats. We have extended these studies to two stages of growth and development (young lean 150- to 220-g rats and older, moderately obese 450- to 500-g rats), because experience with the acute effects of STZ-D is minimal in the older, fatter animal (4,13), which may be a better model of human obesity-related NIDDM (13).

Our results show that experimental diabetes produces greater metabolic alterations in adipocytes from lean animals than in those from older, fatter animals. A shift in the metabolic fate of glucose toward a greater production of lactate was observed in both diabetic groups.

MATERIALS AND METHODS

Reagents. Porcine monocomponent insulin (28.5 U/mg) and A14-[^{125}I]monoiodoinsulin (311.04 mCi/mmol) were a gift from Lilly (Indianapolis, IN). Bovine serum albumin (BSA, fraction V) and lactic acid kits (826-B) were purchased from Sigma (St. Louis, MO), collagenase (type I, 190 U/mg) from Cooper (Freehold, NJ), [U-^{14}C]glucose (358.6 mCi/mmol) from New England Nuclear (Boston, MA), and N-Multistix reagents from Ames (Elkhart, IN). STZ was obtained from the National Cancer Institute.

Animals and experimental design. Male Wistar rats, weighing ~150 g (5–6 wk of age) or ~450 g (6–8 mo), were purchased from Charles River (Kingston, NY). Rats weighing 450–500 g were chosen for comparison of the effects of experimental diabetes because these rats are moderately obese (body fat 15–20% of body wt) and have epididymal adipocyte volumes 4–5 times larger (400 vs. 80 pl) than those of the younger 150-g animals (23). The rats were allowed access to Purina Lab Chow and water ad libitum for at least 5 days before the start of the experiments and were housed in individual wire-mesh cages in a temperature-controlled (22–24°C) room with a 12-h light/dark cycle. Diabetes was produced by injecting into the tail vein either 50 mg/kg (young, lean rats) or 30 mg/kg (older, moderately obese rats) STZ dissolved in citrate buffer, pH 4.0. The latter STZ dosage was determined in preliminary experiments in which hyperglycemia without ketonuria, comparable with that produced in the leaner rats, was produced in the older, fatter rats. Controls were injected with an equal volume of citrate buffer. Body weights were measured daily, and blood glucose was monitored every 3rd day by testing a drop of blood from the tail vein with an Accu-Chek blood glucose reflectance meter (Boehringer Mannheim, Indianapolis, IN). Eight to 11 days after STZ injection, the animals were decapitated in the fed state, and the thoracic blood was collected. After allowing the blood to clot at room temperature, serum was separated for determination of glucose and insulin levels.

Serum glucose was measured on the day of the experiment with a Beckman Glucose Analyzer II. Serum insulin was measured (in the laboratory of R. Martin, University of Georgia, Athens) with a Cambridge diagnostic radioimmunoassay kit (24) and rat insulin standard. Urine samples were analyzed on the day of the experiment for the presence of ketone bodies with Ames N-Multistix.

Preparation of isolated adipocytes. In each experiment, the epididymal fat pads were removed from a single large rat or from five or six lean rats. Fat cells were isolated after collagenase digestion by a modification (23) of Rodbell's method (25). Briefly, the pads were rinsed in saline, blotted, minced with scissors, and incubated at 37°C for 30–45 min in Krebs-Ringer bicarbonate (KRB) buffer, 4% BSA (wt/vol, pH 7.4) with collagenase (1 mg/ml). The isolated fat cells were filtered through a 250-μm nylon screen and resuspended in fresh buffer without collagenase. The cells were allowed to float, and the infranatant buffer was aspirated; the cells were washed 3 times in this manner before the final resuspension in fresh medium.

Glucose metabolism. To measure glucose conversion to CO_2 and triglycerides, resuspended fat cells (cell density range 0.144–0.450 × 10^6 cells/ml) were incubated in a final volume of 1 ml KRB-BSA buffer containing 0.25 μCi [U-^{14}C]-glucose and unlabeled D-glucose to a final concentration of 5 mM. Where appropriate, insulin was added to final concentrations of 0, 3, 10, 30, 100, and 1000 μU/ml. Parallel incubations without labeled glucose were used to measure lactate released into the medium. Duplicate or triplicate samples were incubated for 90 min at 37°C in a Dubnoff metabolic shaker at 60–80 cpm. Radioactivity in CO_2 and triglycerides was recovered and measured as previously described (17).

To measure lactate released into the incubation medium, the cell suspension was transferred after 90 min to plastic 1.5-ml microcentrifuge tubes, and the cells were allowed to float. The infranatant was aspirated, added immediately to an equal volume of ice-cold perchloric acid (PCA, 8% wt/vol), vortexed, and centrifuged at 2000 × *g* for 20 min. The clear supernatant was transferred to polyethylene vials and stored at −4°C until it was assayed enzymatically for lactate within 7 days (26).

Total glucose metabolized was calculated as the sum of glucose converted to CO_2, triglycerides, and lactate. It was expressed as micromoles of glucose metabolized by 10^7 fat cells during 90 min of incubation.

To determine if the hyperglycemia of diabetes affected the glucose concentration at which the maximal insulin response was observed, the effect of glucose concentration on basal glucose oxidation and the response of glucose oxidation to insulin were studied as parts of the same experiments. Cells were incubated with [U-^{14}C]glucose and unlabeled glucose to final glucose concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10, and 20 mM in the absence or presence of 1 mU/ml insulin. Fat cell size and number were determined by standard methods (23).

Insulin binding. Binding studies were carried out by previously described techniques (27). Each flask contained, in a 1.5-ml volume, 1 mM glucose; 0.1 μCi A14-[^{125}I]monoiodoinsulin; unlabeled insulin to the desired insulin concentrations of 10, 30, 100, 300, and 1000 μU/ml; and 1 ml fat cell suspension. After a 45-min incubation at 24°C, 300-μl aliquots were removed from each flask and placed in microfuge tubes containing 110 μl dinonyl phthalate. After centrifugation in a Beckman microfuge for 15 s, the tubes were cut across the oil layer, and the fat cell layer was recovered and counted in a γ-scintillation counter (ISO-DATA 20/20).

Hormone concentration and binding were corrected for the volume occupied by the fat cells. Insulin degradation was monitored by 5% trichloroacetic acid precipitation. Specific binding of ^{125}I-labeled insulin was calculated by subtracting the nonspecific binding (^{125}I-insulin bound in the presence of 100 mU/ml unlabeled insulin) from the total

TABLE 1
Animal characteristics at death

Rat group	Body weight (g)	Fat cell volume (pl)	Serum glucose (mg/dl)	Serum insulin* (μU/ml)
Lean control	224 ± 5	137.8 ± 15.4	140 ± 1	16.2 ± 1.0
Lean diabetic	206 ± 5†	62.4 ± 3.5†	466 ± 20†	12.0 ± 1.2
Obese control	499 ± 1	362.1 ± 55.2	122 ± 6	20.8 ± 1.2
Obese diabetic	467 ± 6†	283.8 ± 35.9†	450 ± 15†	12.6 ± 1.0†

Values are means ± SE of 6–7 experimental observations in each group.
*n = 12 for lean rats and 7 for older, fatter rats.
†Significantly different from respective control group ($P < .05$).

insulin bound to the cells. Results were expressed as nanograms of insulin specifically bound by 10^6 fat cells.

Data analysis. Results were expressed as means ± SE. All dependent metabolic variables (glucose metabolized to specific products and insulin binding) were analyzed by an analysis of variance (ANOVA) followed by post hoc t tests where appropriate. Serum glucose and insulin and animals' weights were analyzed by group comparison with Student's t test. Differences were considered significant at $P \leq .05$.

RESULTS

Animal characteristics. Characteristics of the control and diabetic animals are presented in Table 1. After a mild initial weight loss (~15 g), the lean diabetic animals had a growth rate that paralleled that of the lean controls. In contrast, the older, fatter diabetic animals had an initial weight loss and then plateaued at a level ~30 g lower than the controls. Both diabetic groups weighed ~8% less than their respective control group on the day of the experiment. Fat cell volume decreased by 75 pl (−54%) in lean diabetic rats and by 78 pl (−22%) in older, fatter diabetic rats compared with their respective controls. This result indicates a similar absolute lipid loss per cell (Table 1).

Mean serum glucose concentrations were comparable in both diabetic groups (466 and 450 mg/dl in the younger and older rats, respectively). Mean serum insulin levels were also similar in the two diabetic groups (12.0 vs. 12.6 μU/ml). When compared with mean control levels of 16.2 and 20.8 μU/ml, respectively, insulin values were reduced 26% in the lean diabetic animals and 39% in the older, fatter diabetic animals.

Insulin binding. Experimentally induced diabetes had no significant effect on insulin binding by adipocytes from either the young, lean or the older, moderately obese rats, (Fig.1). At the highest concentration of insulin (1 mU/ml), there was a trend toward reduced binding to the fat cells for the lean diabetic rats (Fig. 1*A*) and toward increased binding by fat cells for the moderately obese diabetic rats (Fig. 1*B*), but these differences did not reach statistical significance. Insulin binding per cell was similar for fat cells from the lean and the older, fatter controls, except at very low insulin concentrations (<0.10 mU/ml), where binding to cells from older, fatter rats was slightly higher ($P < .05$), confirming previous findings from our laboratory (27). Insulin degradation was <15% of total during the 45-min incubation.

Total glucose metabolism. Table 2 and Fig. 2 show the effects of diabetes on total glucose metabolism. When diabetes was induced in lean rats, their epididymal adipocytes, under basal conditions, metabolized 63% less glucose than adipocytes from lean controls (0.96 vs. 2.6 μmol per 10^7 fat cells in 90 min, respectively; Fig. 2*A*). When incubated with insulin, their fat cells metabolized a maximum of 2.2 μmol glucose per 10^7 fat cells in 90 min compared to 17.9 μmol per 10^7 fat cells in 90 min for cells from lean controls, a decrease of 88%. In contrast, the induction of diabetes in older, moderately obese rats did not change the total amount of glucose metabolized by their large fat cells in the absence of insulin (Fig. 2*B*). When incubated with insulin, large fat cells from obese diabetic rats metabolized slightly more glucose than cells from obese control rats.

Glucose metabolism to lactate. Table 2 shows glucose metabolism to the individual products, i.e., CO_2, triglycerides, and lactate, by fat cells from control and diabetic lean and moderately obese rats. Figure 3 illustrates the amount of glucose metabolized to lactate by fat cells from the four

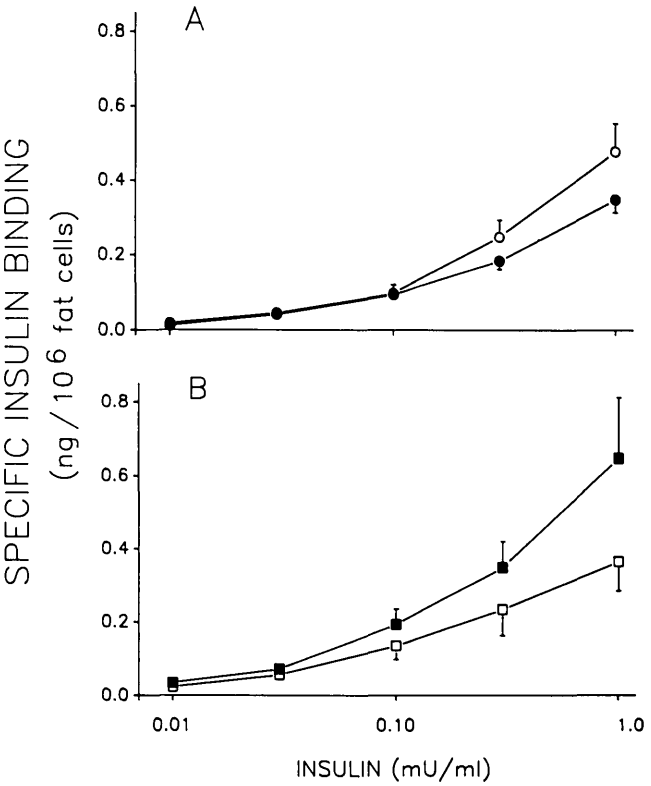

FIG. 1. Specific insulin binding by small (*A*) and large (*B*) adipocytes from lean control (○), lean diabetic (●), obese control (□), and obese diabetic (■) rats. Specific binding by 10^6 fat cells is plotted versus log insulin concentration (mU/ml). Values are means ± SE of 5–7 experiments. When not shown, SE is smaller than symbol size.

TABLE 2
Rate and pattern of glucose metabolism to products by fat cells from lean and moderately obese control and diabetic rats

	CO_2		Triglyceride		Lactate		
Rat group	Amount metabolized	%	Amount metabolized	%	Amount metabolized	%	Total
Lean control							
−	0.94 ± 0.22*	33	1.66 ± 0.22*	67	ND	<1	2.60 ± 0.43*
+	4.48 ± 1.06†	26	8.02 ± 0.86†	52	3.35 ± 0.49*	21	15.85 ± 2.32†
Lean diabetic							
−	0.13 ± 0.02‡	12	0.75 ± 0.07‡	78	0.08 ± 0.04†	8	0.96 ± 0.08‡
+	0.26 ± 0.04§	11	1.01 ± 0.09§	46	0.91 ± 0.07‡	42	2.18 ± 0.18§
Obese control							
−	0.45 ± 0.13*	12	2.32 ± 0.22‖	67	0.84 ± 0.21‡	22	3.61 ± 0.48*
+	0.78 ± 0.21*	13	2.89 ± 0.34‖	51	2.18 ± 0.44§	36	5.85 ± 0.79‖
Obese diabetic							
−	0.32 ± 0.08‖	9	1.90 ± 0.15¶	56	1.39 ± 0.34‡	34	3.62 ± 0.47¶
+	0.50 ± 0.09*	7	2.88 ± 0.19‖	42	3.49 ± 0.45*	50	6.87 ± 0.59‖

Values are means ± SE of duplicate or triplicate determinations in 5–7 experiments for each experimental condition shown and are expressed as micromoles glucose metabolized per 10^7 fat cells during 90 min incubation. Within each column, values without superscripts in common differ significantly ($P < .05$). −, Without insulin; +, with 1 mU/ml insulin; %, percentage of total glucose metabolized to each product; ND, not detectable (<0.05 μmol/10^7 fat cells).

groups of rats when incubated with increasing amounts of insulin. In the absence of insulin, cells from lean diabetic rats converted slightly more glucose to lactate than cells from lean controls (Fig. 3*A*); however, when incubated with 1 mU/ml insulin, fat cells from lean diabetic rats converted significantly less glucose to lactate than did cells from lean control rats (0.91 vs. 3.35 μmol per 10^7 fat cells in 90 min, respectively). Although the presence of diabetes decreased the absolute amount of glucose metabolized by fat cells from lean rats, the relative conversion of glucose to lactate was significantly increased (8 vs. <1% without insulin and 42 vs. 21% with insulin, $P < .001$ for both). Whereas cells from lean diabetic rats had decreased absolute lactate production compared with lean controls, the large fat cells from moderately obese diabetic rats had a 60–66% higher rate of lactate production than cells from obese controls (Fig. 3*B*). The presence of diabetes in the older, moderately obese rats also resulted in an increased relative conversion of glucose to lactate by the large fat cells when compared with controls (34 vs. 22% without insulin, 50 vs. 36% with insulin). Thus, experimental diabetes altered the pattern of glucose metabolism to promote lactate production in fat cells from both lean and obese rats.

Glucose concentration at which peak insulin response occurs. All the above experiments were performed at 5 mM glucose, a concentration similar to that seen in normal, but not in diabetic, serum. Because the prolonged exposure of the cells to hyperglycemia could have shifted the glucose concentration at which maximal adipocyte insulin responsiveness occurred, we incubated fat cells from the four groups of animals at concentrations of glucose ranging from 0.01 to 20 mM without insulin and with insulin at 1 mU/ml. The effect of glucose concentration on insulin's ability to stimulate glucose oxidation is shown in Fig. 4. The induction of diabetes did not appreciably shift the glucose concentration (0.5 mM) at which peak insulin stimulation occurred in the adipocytes, regardless of the size of the animal studied. Insulin stimulated relative glucose oxidation by 1200% in fat cells from lean control rats and by 670% in cells from lean diabetic rats. In fat cells from obese rats, the presence of diabetes did not change the relative insulin response, which was 270% for cells from controls and 220% for cells from diabetic rats.

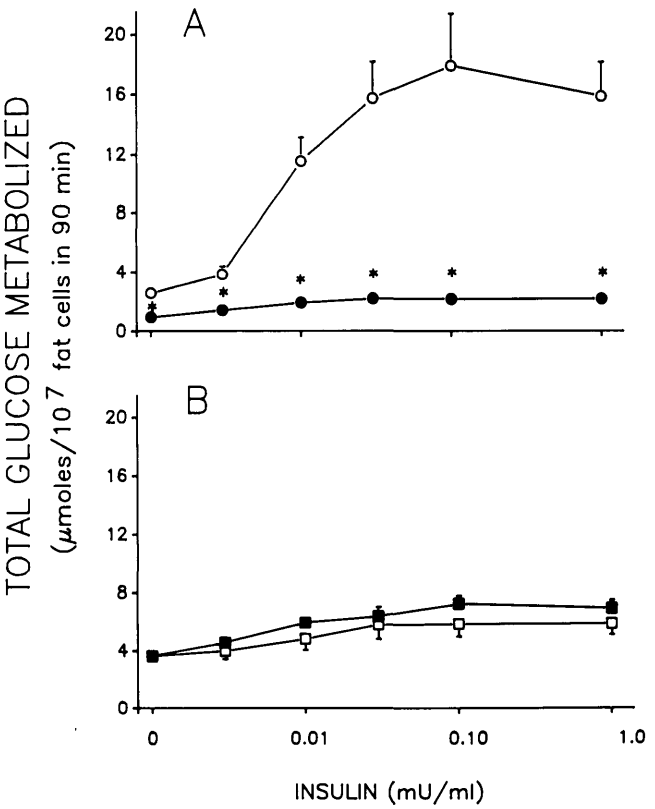

FIG. 2. Insulin-dose response for total glucose metabolism by small (*A*) and large (*B*) adipocytes from lean control (○), lean diabetic (●), obese control (□), and obese diabetic (■) rats. Total glucose metabolized is calculated as sum of micromoles of glucose converted to CO_2, triglycerides, and lactate. Values are means ± SE of duplicate or triplicate incubations in 5–7 experiments. Note lack of effect of diabetes on total glucose metabolism by large fat cells of obese rats. When not shown, SE is smaller than symbol size. *Difference ($P \leq .05$) between indicated value and analogous value of control group.

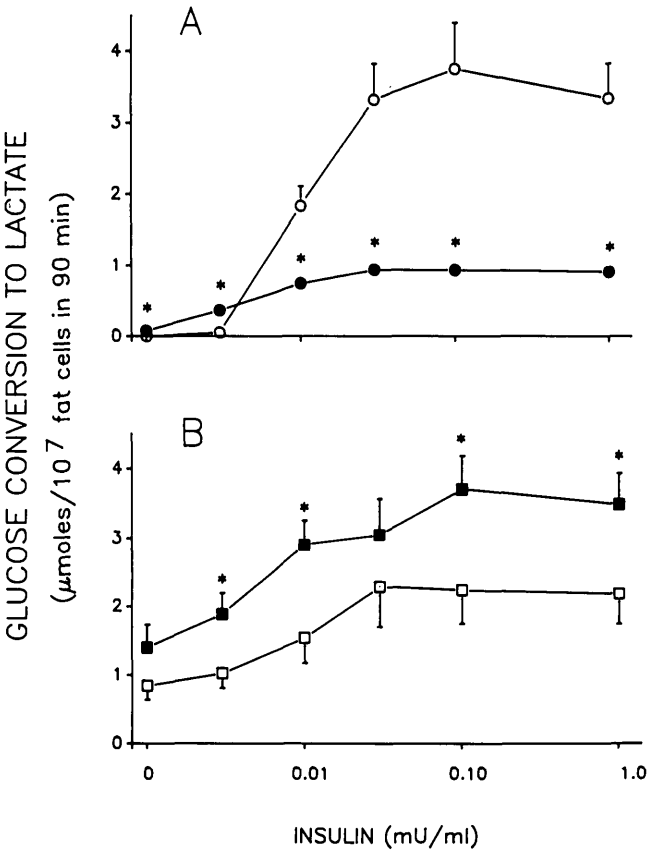

FIG. 3. Insulin-dose response for glucose conversion to lactate by small (*A*) and large (*B*) adipocytes of lean control (○), lean diabetic (●), obese control (□), and obese diabetic (■) rats. Values are means ± SE of duplicate or triplicate incubations in 5–7 experiments. When not shown, SE is smaller than symbol size. *Difference ($P \leq .05$) between indicated value and analogous value of control group.

DISCUSSION

The results of this study demonstrate that the induction of experimental diabetes in rats at different stages of development and with different degrees of adiposity produces marked differences in adipocyte glucose metabolism, even though levels of hyperglycemia and reduced circulating insulin levels were comparable.

In young, lean rats, diabetes caused a severe reduction in the absolute amount of glucose metabolized by adipocytes in both the absence and presence of insulin, confirming previous reports by others (3,7,10,11,15). Our findings of unaltered insulin binding per cell in adipocytes of diabetic animals and reports by others that diabetes increases binding by adipocytes suggest that the loss of insulin response seen in adipocytes from young, lean diabetic rats is not due to diminution of hormone binding but rather to postreceptor abnormalities (3,7,10,11). Studies by other investigators have shown that this absolute decrease in glucose utilization is due, at least in part, to depletion of intracellular glucose transporters (6–8).

With regard to the pattern of glucose metabolism, diabetes produced in adipocytes from young lean animals a significant increase in the relative conversion of glucose to lactate from <1% of total to 8% in the absence of insulin and from 21 to 42% in the presence of 1 mU/ml insulin. Because of the marked reduction in total glucose metabolism produced by diabetes, the absolute amount of lactate produced was decreased compared with results from cells from control rats.

In contrast to the findings with lean rats, the induction of diabetes in older, moderately obese rats caused no reduction in total adipocyte glucose metabolism and no change in response to insulin. With regard to the pattern of glucose metabolism, we observed a decrease in glucose oxidation similar to that reported by Dall'Aglio et al. (4); however, the decrease in glucose oxidation (basal and with insulin) and triglyceride synthesis (basal) was accompanied by a simultaneous increase in lactate production. Thus, when the results from all products measured were added together, there was no change in total glucose metabolism in the adipocytes from the obese diabetic rats; diabetes in these obese animals did result in a significant increase in both absolute and relative conversion of glucose to lactate by adipocytes. The observation that diabetes in older, moderately obese animals had little effect on total glucose metabolism and insulin response by their large fat cells may, at least in part, be due to the already-altered metabolic pattern resembling that produced by diabetes in fat cells from lean animals, i.e., reduced glucose oxidation and insulin responsiveness and increased relative conversion of glucose to lactate (16,18,19,22).

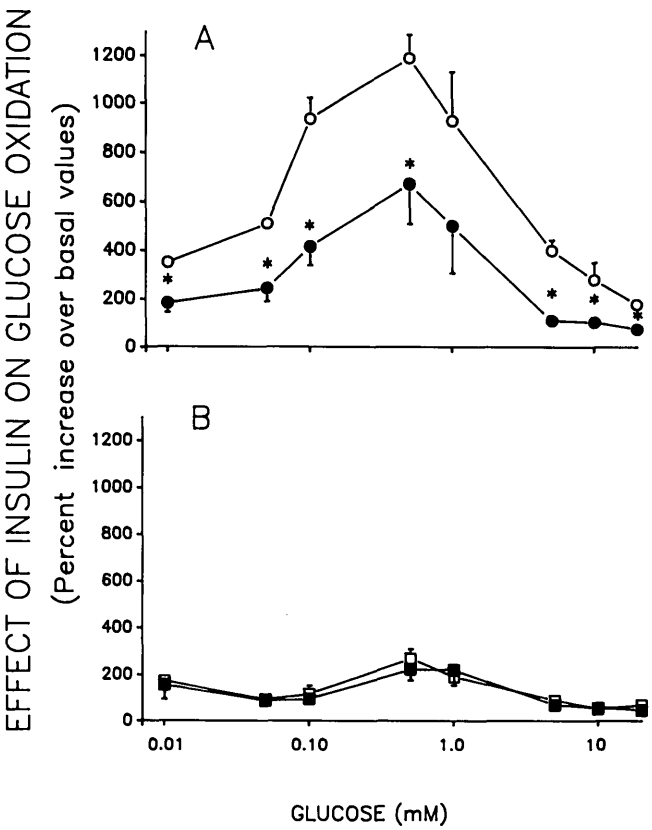

FIG. 4. Effect of experimental diabetes on glucose concentration at which peak insulin stimulation of glucose oxidation occurs in small (*A*) and large (*B*) adipocytes from lean control (○), lean diabetic (●), obese control (□), and obese diabetic (■) rats. Values are means ± SE of duplicate or triplicate observations of 5–7 experiments. When not shown, SE is smaller than symbol size. Note that maximal insulin effect occurred at similar glucose concentration (0.5 mM) in all 4 groups. *Difference ($P \leq .05$) between indicated value and analogous value of control group.

We have previously shown that the relative glucose conversion to lactate by fat cells increases with fat cell enlargement (18,19,22) and with fasting or food restriction of the donor animals (19,22). The data presented here indicate that the induction of diabetes in either lean or moderately obese rats also increases the relative amount of glucose converted to lactate by isolated adipocytes. It is possible, in all these experimental conditions (fat cell enlargement, fasting, and diabetes), that fat cells may contribute significant quantities of lactate to be used for hepatic gluconeogenesis and glycogenesis (28). This contribution could be particularly significant in the moderately obese diabetic rats, because when diabetes was superimposed on obesity, fat cells increased both the relative amount of glucose metabolized to lactate and the absolute amount of lactate released. The quantitative significance of adipose tissue contribution to lactate production and utilization remains to be established. However, because obesity is associated with an increase in both fat cell size and number, the enlarged adipose mass could generate increased amounts of lactate. Marin et al. (12) recently suggested that in obesity, the adipose organ can metabolize up to 15–30% (and possibly more) of an oral glucose load. Because ~50–65% of the glucose metabolized by adipocytes is converted to lactate (9,12,16,18, 19,21,22), adipose tissue in obesity and/or diabetes may contribute a significant quantity of lactate to the total body pool for use as a metabolic substrate. The increased rate of lactate production shown here by adipocytes from moderately obese diabetic rats may provide additional insight for the observations of Consoli et al. (29) that gluconeogenesis from lactate is increased in NIDDM patients and that the increased availability of lactate comes from tissues other than muscle.

In summary, STZ was used to induce a comparable level of diabetes in rats at different stages of development and degrees of adiposity. In young lean rats, this resulted in a large reduction in adipocyte total glucose metabolism. In older, moderately obese rats, the induction of diabetes had no significant effect on fat-cell total glucose metabolism but resulted in a significant increase in both absolute and relative amounts of adipocyte glucose conversion to lactate. No significant changes in either insulin binding or the glucose concentration at which peak insulin stimulation occurred were seen. We conclude that adipose tissue may be an important site of glucose conversion to lactate, particularly in the obese and diabetic states.

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This work was completed by F.D.N. in partial fulfillment of the requirements for a PhD. Portions of this study have been presented elsewhere (30,31).

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