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Effects of streptozotocin diabetes and insulin treatment on adipose tissue glucose utilization in the rat

BY

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(2 figures)

[U-^{14}C] glucose utilization by pieces of epididymal fat-pad was studied in streptozotocin-diabetic rats, food-restricted animals, and normal controls. Both CO_2 and fatty acids formation were greatly reduced in tissues from the diabetic animals treated or not with insulin and this change was milder in tissues from food-restricted rats. A positive correlation was found between [^{14}C] glucose utilization *in vitro* and plasma RIA-insulin levels when values from all animals were pooled. The percentual response to high doses of insulin was small in adipose tissue from streptozotocin-diabetic animals for CO_2 and total lipids formation, but it was high for fatty acids synthesis; the response in food-restricted animals was high and was greatly augmented in tissues from the streptozotocin-diabetic rats treated with insulin. Results indicate that in adipose tissue from streptozotocin-diabetic animals the alteration of insulin responsiveness occurs distal to the receptor events and that impaired basal glucose metabolism is a direct consequence of their hypoinsulinemia.

Introduction

Basal and insulin-stimulated glucose utilization is reduced in adipose tissue from alloxan and streptozotocin-diabetic rats (S-DR) (WINEGRAD & RENOLD, 1958; DENTON *et al.*, 1966; SCHOENLE *et al.*, 1977; KASUGA *et al.*, 1978; WIERINGA & KRANS, 1978; KOBAYASHI & OLEFSKY, 1979). The mechanism for this insulin resistance is not completely understood because insulin binding to adipocyte receptors from diabetic animals is known to be increased (OLEFSKY, 1976; SCHOENLE *et al.*, 1977; KASUGA *et al.*, 1978; WIERINGA & KRANS, 1978; KOBAYASHI & OLEFSKY, 1979). A similar situation occurs in fasting where insulin binding increases but adipose tissue insulin response is impaired (OLEFSKY, 1976; KASUGA *et al.*, 1977; WIERINGA & KRANS, 1978) and it has been proposed that these mechanisms of insulin resistance are not related to insulin receptors, and that the rate limiting process occurs after insulin binding (GLIEMANN *et al.*, 1980). Although an impairment in the adipocyte glucose transport system could limit both basal and insulin-mediated glucose

utilization in diabetes, it has been suggested that other intracellular enzyme steps may also be affected (WIERINGA & KRANS, 1978) and enhanced glycerolipid formation has been even detected in adipose tissue from streptozotocin-treated rats (JAMDAR, 1981).

In this work the intracellular glucose metabolism *in vitro* was studied in basal and insulin-stimulated adipose tissue from S-DR. To disassociate possible effects produced by the catabolic state of diabetes and to determine recuperation effects produced by insulin treatment, the investigation was extended to food-restricted rats whose weight change approximated that of streptozotocin-treated animals but whose insulin affinity in adipocytes was unchanged (WIRTH *et al.*, 1980), and also to insulin-treated S-DR.

Materials and Methods

Animals

Male Wistar rats divided into four groups of similar body weight were maintained in collective cages in a temperature (22 ± 1 °C) and light (from 7.00 to 19.00 h) controlled room and fed purina rat chow. One group was made diabetic by a single intraperitoneal injection of streptozotocin (The *Upjohn Co.*, Michigan, USA) (55 mg/kg) freshly dissolved in citrate buffer pH 4.8, after a 24-h fast. Another group of rats received the same treatment until the seventh day following the streptozotocin injection after which they were subcutaneously injected daily with monocomponent porcine insulin (Actrapid, from *Novo Industri A/S,* Copenhagen, Denmark) (2 IU/100 g). A third group of animals received no treatment and was used as normal and a fourth untreated group was food-restricted. These animals were weighed every three days and the food-pellets were conveniently administered to obtain a mean daily body weight change similar to that of the diabetics. All rats were killed by decapitation without anaesthesia 22 days after onset of the experiment. Blood was collected into heparinized, chilled tubes and plasma samples were stored at —20 °C until processing.

Incubations

Four pieces of epididymal fat-pad (47-73 mg) from each rat were placed in 20-ml vials containing 2 ml of Krebs-Ringer bicarbonate buffer, pH 7.4 (UMBREIT *et al.*, 1964), supplemented with bovine serum albumin (10 mg/ml) purified by the method of CHEN (1967), and [U-^{14}C] glucose (5 mμ, 0.5 mCi/ml). In some vials the medium was also supplemented with porcine monocomponent insulin (Actrapid, from *Novo Industri A/S,* Copenhagen, Denmark) (1 mU/ml). Incubations were performed for 1 h as described elsewhere (HERRERA & AYANZ, 1972) in a O_2-CO_2 (95-5 %) atmosphere and they were ended by the addition of 0.5 ml 20 % $HClO_4$ to the medium. CO_2 was trapped in Hyamine 10-X hydroxyde. Incubated tissues were rinsed with saline and lipids were extracted (FOLCH *et al.*, 1957), purified and fractionated (DOMINGUEZ & HERRERA, 1976). In the fraction containing glycerides, phospholipids were discarded by treatment with activated silicic acid in a chloroform medium, so that values of glycerides glycerol corresponded to neutral lipids. Aliquots of all lipidic fractions were used for counting their radioactivity in a PPO/POPOP xylene/Triton X-100 based scintillation cocktail. Radioactivity values were adjusted to 1×10^6 cpm for the ^{14}C-label contained in each vial before incubation. Proteins were measured (WANG & SMITH, 1975) in aliquots of the tissue preparations. Plasma aliquots were deproteinized (SOMOGYI, 1945) for glucose (HUGGET & NIXON, 1957)

and β-hydroxybutyrate (WILLIAMSON & MELLANBY, 1974) which were evaluated by enzymatic procedures. Insulin was assayed (HEDING, 1972) in other aliquots of plasma with a rat insulin radioimmunoassay kit for rat, generously provided by *Novo Industri A/S* (Copenhagen, Denmark). Statistical analysis of the data was performed by using the Student's *t* test, *t* paired test, and linear regressions with estimation of the error and comparison of the lines according to the *F* values (SNEDECOR, 1956; SOKAL & ROHLF, 1969).

Results

Animals studied

Table I shows some characteristics of the animals studied. Initial body weight was similar for the different groups, however in the following 22 days normal animals gained weight while S-DR showed a reduction which was not counteracted by insulin treatment. Food-restricted animals showed no change in body weight and with values similar to those of S-DR treated or not with insulin. Epididymal fat-pad protein content was lower in normal animals than in the other groups, indicating a proportionally greater fat content in the normals. Plasma glucose level was markedly augmented in diabetic rats and also in diabetic rats receiving insulin until 24 h prior to sacrifice, while in food-restricted animals glycemia was slightly but not significantly reduced as compared with normals. Plasma β-hydroxybutyrate concentration was significantly lower in normal rats than in the other groups and the level attained in S-DR treated with insulin was even greater than in either the streptozotocin or food-restricted rats. Plasma RIA-insulin concentration was greatly reduced in diabetic animals while this decrease was milder in both diabetics treated with insulin and in food-restricted rats as compared with normals, although these differences remained statistically significant.

TABLE I. *Characteristics of the animals studied.*

Body weight (g)	Normal (n = 28)	Diabetic (n = 31)	Diabetic + Insulin (n = 10)	Food-restricted (n = 6)
Initial (day 0)	174 ± 5^a	165 ± 4^a	176 ± 5^a	160 ± 10^a
Final (day 22)	240 ± 8^a	159 ± 3^b	172 ± 4^b	160 ± 9^b
Change	65 ± 7^a	−6 ± 4^b	−4 ± 5^b	0 ± 3^b
Epididymal fat-pad protein (mg/100 mg)	1.50 ± 0.06^a	2.84 ± 0.25^b	4.15 ± 0.53^c	1.98 ± 0.16^b
Plasma glucose (mg/100 ml)	121 ± 4^a	671 ± 40^b	674 ± 50^b	109 ± 10^a
Plasma β-hydroxybutyrate (μmol/litre)	90 ± 9^a	244 ± 21^b	499 ± 66^c	313 ± 33^b
Plasma insulin (μU/ml)	43 ± 4^a	4 ± 1^b	10 ± 3^c	20 ± 4^c

All animals were sacrificed on day 22 (day 0 ± day prior streptozotocin or saline treatment). Diabetic + insulin rats received a daily s.c. injection of 2 IU/100 g monocomponent porcine insulin from day 7 and were sacrificed 24 h after the last injection. Values are mean ± SEM. For each line, values bearing different superscripts differ significantly ($P < 0.05$, or less).

Utilization of [^{14}C] glucose

Pieces of epididymal fat-pads were incubated in basal conditions for 1 h and the conversion of [U-^{14}C] glucose into $^{14}CO_2$ and [^{14}C] lipids was measured. Observed values are summarized in Table II demonstrating that tissues from S-DR show a marked reduction in the formation of both $^{14}CO_2$ and [^{14}C] lipids. Lipidic fractionation denotes great differences in the comparative effects on fatty acids and glyceride glycerol formation, fatty acid synthesis being almost negligible while the formation of [^{14}C] glyceride glycerol was reduced in tissues from diabetic animals to only 60 % of values found in normals. These changes were not improved when the diabetic animals were treated with insulin, with the single exception of a slight but significant increase in the formation of [^{14}C] fatty acids as compared with values in diabetic animals not treated with insulin. In food-restricted rats, utilization of [^{14}C] glucose in epididymal fat pads was also impaired as compared with normals, but observed changes were smaller than those in diabetic rats for all parameters studied (Table II).

TABLE II. *Utilization of [U-^{14}C] glucose by epididymal fat-pads incubated* in vitro.

	Normal (n = 23)	Diabetic (n = 27)	Diabetic + Insulin (n = 10)	Food-restricted (n = 6)
$^{14}CO_2$	10 478 ± 1 212^a	1 573 ± 136^b	1 569 ± 445^b	3 041 ± 457^c
[^{14}C] Total lipids	11 916 ± 1 261^a	2 160 ± 147^b	1 996 ± 536^b	4 347 ± 449^c
[^{14}C] Fatty acids	8 481 ± 1 071^a	100 ± 16^b	316 ± 128^c	1 780 ± 389^d
[^{14}C] Glyceride glycerol	3 436 ± 331^a	2 052 ± 143^b	1 679 ± 424^b	2 567 ± 288^{a,b}

Incubations were carried out for the 1 h in the presence of bovine serum albumin (10 mg/ml) and [U-^{14}C] glucose (5 mM, 0.5 μCi/ml). Values are expressed as cpm/mg of tissue protein, adjusted to 1×10^6 cpm for the ^{14}C-label contained in each vial before incubation. For each line, values bearing different superscript differ significantly ($P < 0.05$, or less).

Relationship between utilization of [^{14}C] glucose by adipose tissue in vitro *and circulating RIA-insulin levels*

In an attempt to determine whether a direct relation existed between basal utilization of [^{14}C] glucose *in vitro* and actual plasma RIA-insulin levels, firts-order regressions were calculated for all individual values. As shown in Figure 1, correlations appeared positive and highly significant : ($P < 0.001$) for CO_2, fatty acids and glyceride glycerol formation as compared with plasma RIA-insulin concentrations. Statistical comparison (F values) between the lines showed no significance between CO_2 and fatty acids while comparison between glyceride glycerol and either CO_2 or fatty acids was highly significant ($P < 0.001$) indicating that the former parameter change was less affected by the plasma insulin levels than the two others.

Response to insulin in vitro

When incubations were performed in the presence of high doses of insulin (1 mU/ml), [^{14}C] glucose utilization was enhanced by the fat-pad pieces from all the groups, but great differences were observed among them. Observed values expressed as percent of insulin in response *vs.* basal values (those shown in Table II)

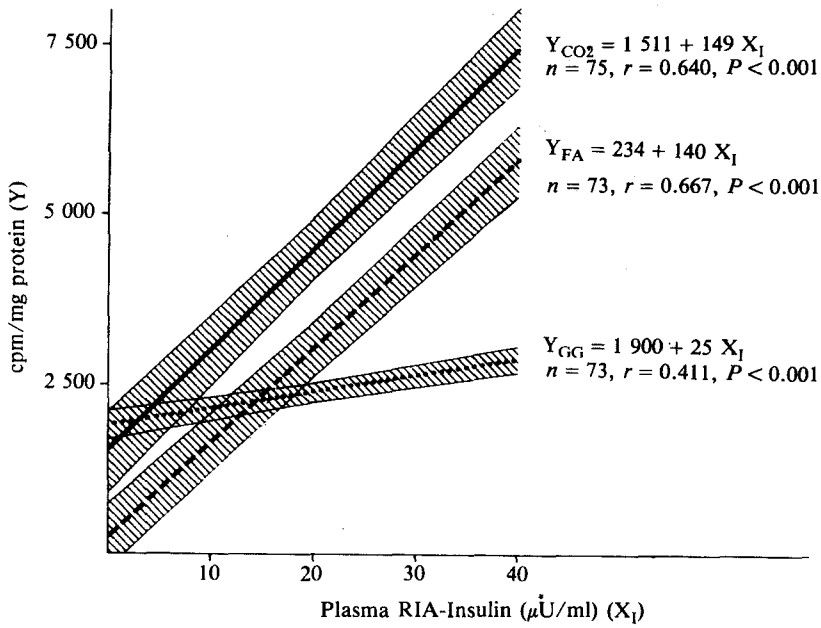

FIG. 1. *Regression lines and standard error of the estimation of Y in the correlations between basal utilization* in vitro *of [^{14}C] glucose by pieces of epididymal fat-pads from all animals used in the present study for the formation of $^{14}CO_2$, [^{14}C] fatty acids* (FA) *or* [^{14}C] *glyceride glycerol* (GG) *and plasma levels of RIA-insulin.*

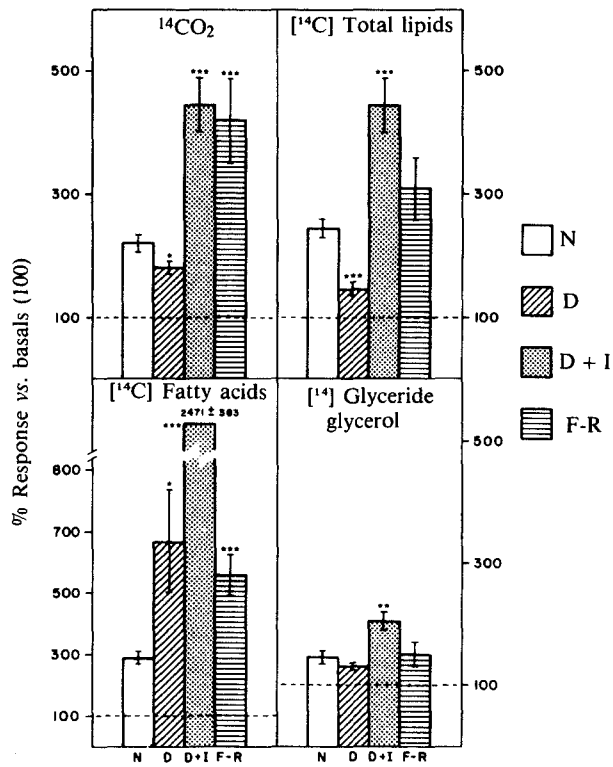

FIG. 2. *Percentual response to 1 mU/ml of porcine monocomponent insulin in the formation* in vitro *of $^{14}CO_2$ and ^{14}C-lipids from [U-^{14}C] glucose by pieces of epididymal fat-pads from normal* (N), *streptozotocin-diabetic* (D), *streptozotocin-diabetic treated with insulin* (D + I), *and food-restricted* (F-R) *rats. Comparison vs.* values in N are shown by asterisks : * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

are shown in Figure 2. Insulin enhanced the conversion of [^{14}C] glucose into $^{14}CO_2$ and this effect was smaller in tissues from S-D animals and greater in those from both diabetic insulin-treated and food-restricted animals than in normal. A similar change was found for the formation of [^{14}C] total lipids although in this case the insulin effect in tissues from food-restricted rats did not differ from that in normals. When lipids were fractionated it was seen that insulin effect was much greater in all the groups for the formation of [^{14}C] fatty acids than for [^{14}C] glyceride glycerol (Fig. 2). For fatty-acids formation, the insulin effect was much greater in tissues from diabetic insulin-treated animals than in the other groups, and both diabetics and food-restricted animals showed a significantly higher effect than normals. The insulin effect was significantly higher in diabetic insulin-treated rats than in normals for the formation of [^{14}C] glyceride glycerol while differences were not observed among the other groups (Fig. 2).

Discussion

These studies demonstrate that insulin-deficient diabetes produced by streptozotocin treatment in the rat led to marked impairment in intracellular glucose utilization in adipose tissue. This alterations did not correspond in the same proportion to the overall glucose metabolism as glyceride glycerol formation was much less affected than the formation of either CO_2 or fatty acids. If one considers that glucose transport is decreased in adipocytes from diabetic animals (SCHOENLE *et al.*, 1977; KASUGA *et al.*, 1978; WIERINGA & KRANS, 1978; KOBAYASHI & OLEFSKY, 1979), the resulting synthesis of glyceride glycerol from available glucose is not modified or even augmented in these animals. This conclusion is in agreement with the increased rate of glycerolipid formation in adipose tissue from S-DR found by JAMDAR (1981). Fatty acid synthesis from glucose was practically insignificant in adipose tissue from diabetic animals. This was probably due more to their hypoinsulinemia than to their negative caloric balance as lipogenesis reduction was much less in tissues from food-restricted animals which had a similar negative caloric balance according to their body weight change and their circulating β-hydroxybutyrate concentration. Exogenous insulin treatment of diabetic animals had very little effect on the basal adipose tissue metabolism, perhaps because these animals were studied 24 h after the last insulin injection when circulating RIA-insulin levels were already lower than in normals and plasma glucose and β-hydroxybutyrate levels were greatly augmented. The positive and highly significant correlation found in all animals studied when [^{14}C] glucose conversion into CO_2, fatty acids, and glyceride glycerol by adipose tissue was plotted against circulating insulin levels suggests that both adipose tissue metabolic activity and plasma insulin levels are directly correlated with independence of the condition of the studied subject. This correlation was smaller for glyceride glycerol formation than for either CO_2 or fatty acids, showing that the former parameter is less sensitive to endogenous insulin changes. Present results are in agreement with other reports (SCHOENLE *et al.*, 1977; KASUGA *et al.*, 1978; WIERINGA & KRANS, 1978; KOBAYASHI & OLEFSKY, 1979) describing impaired glucose utilization in adipose tissue from diabetic animals which was apparently directly mediated through their hypoinsulinemia.

Insulin-stimulated glucose oxidation in adipose tissue from diabetic animals decreased with hormone doses much higher than those required to give half-maximal stimulation (THOMAS *et al.*, 1979; PALACIN & HERRERA, 1982) in normal conditions. These results correspond to the decreased glucose transport known to occur in adipocytes from diabetic animals in basal and insulin-stimulated

conditions (SCHOENLE *et al.*, 1977; KASUGA *et al.*, 1978; WIERINGA & KRANS, 1978; KOBAYASHI & OLEFSKY, 1979). This insulin resistance has no relation with the enhanced insulin binding occurring in adipocytes from diabetic animals (SCHOENLE *et al.*, 1977; KASUGA *et al.*, 1978; WIERINGA & KRANS, 1978; KOBAYASHI & OLEFSKY, 1979) and it was not seen here for fatty acid synthesis. It is known that increased lipogenesis by insulin is independent of its action on glucose transport (STEINER & MURTHY, 1971) and that activity of lipogenic enzymes in adipose tissue is very sensitive to enhancement by insulin in conditions *in vitro* (SICA & CUATRECASAS, 1973; BELSHAM *et al.*, 1980). The selective effects on certain metabolic pathways in adipose tissue from S-DR indicate that alterations of insulin responsiveness occurs distal to the receptor events (KAHN, 1978).

The marked response to insulin in adipose tissue from insulin-treated diabetic animals deserves attention as in this group, basal metabolism was similar to that of diabetics while its response to insulin *in vitro* was much greater. Insulin treatment was interrupted 24 h prior to sacrifice. During this last day, circulating insulin reached its maximum level a few h after the injection to decline later, as shown by MORISHIGE *et al.* (1977). In an experimental design similar to ours, these authors obtained values far below those of normal animals, which produced a diabetogenic situation (hyperglycemia and hyperketonemia) at the time of sacrifice. Insulin treatment of S-DR is known to restore the activity of key glucose utilization enzymes in adipose tissue such as hexokinase (BERNSTEIN *et al.*, 1977), and this effect lasts for at least 16 h (BERNSTEIN *et al.*, 1977). In these animals, adipocyte insulin receptors must also augment prior to sacrifice as it is known that although insulin treatment decreases insulin receptors, they are greatly augmented about 6 h after cessation of treatment (KOBAYASHI & OLEFSKY, 1978). Thus increased insulin responsiveness in adipose tissue from insulin-treated diabetic animals must be a consequence of both proper enzymatic machinery of intracellular glucose utilization and augmented insulin affinity. Neither of these changes was effective when tissues were studied in basal conditions, due to their low circulating insulin levels as suggested by the positive correlation between glucose utilization in basal conditions *in vitro* and plasma insulin levels.

In summary, present results show that impaired basal glucose metabolism in adipose tissue from S-DR is a direct consequence of their hypoinsulinism and that reduced insulin responsiveness *in vitro* is due to an intracellular defect distal to the receptor events.

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