Glucose Metabolism in Perfused Skeletal Muscle

DEMONSTRATION OF INSULIN RESISTANCE IN THE OBESE ZUCKER RAT

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1. The effect of insulin (0.5, 10 and 50 munits/ml of perfusate) on glucose uptake and disposal in skeletal muscle was studied in the isolated perfused hindquarter of obese (*fa/fa*) and lean (*Fa/Fa*) Zucker rats and Osborne–Mendel rats. 2. A concentration of 0.5 munit of insulin/ml induced a significant increase in glucose uptake (approx. $2.5 \mu\text{mol/min}$ per 30 g of muscle) in lean Zucker rats and in Osborne–Mendel rats, and 10 munits of insulin/ml caused a further increase to approx. $6 \mu\text{mol/min}$ per 30 g of muscle; but 50 munits of insulin/ml had no additional stimulatory effect. In contrast, in obese Zucker rats only 10 and 50 munits of insulin/ml had a stimulatory effect on glucose uptake, the magnitude of which was decreased by 50–70% when compared with either lean control group. Since under no experimental condition tested was an accumulation of free glucose in muscle-cell water observed, the data suggest an impairment of insulin-stimulated glucose transport across the muscle-cell membrane in obese Zucker rats. 3. The intracellular disposal of glucose in skeletal muscle of obese Zucker rats was also insulin-insensitive: even at insulin concentrations that clearly stimulated glucose uptake, no effect of insulin on lactate oxidation (nor an inhibitory effect on alanine release) was observed; [^{14}C]glucose incorporation into skeletal-muscle lipids was stimulated by 50 munits of insulin/ml, but the rate was still only 10% of that observed in lean Zucker rats. 4. The data indicate that the skeletal muscle of obese Zucker rats is insulin-resistant with respect to both glucose-transport mechanisms and intracellular pathways of glucose metabolism, such as lactate oxidation. The excessive degree of insulin-insensitivity in skeletal muscle of obese Zucker rats may represent a causal factor in the development of the glucose intolerance in this species.

Peripheral insulin resistance, which is frequently observed in obese hyperlipaemic patients with maturity-onset diabetes mellitus, is associated with hyperinsulinaemia and impaired glucose tolerance. The cellular basis of this insulin resistance has extensively been investigated *in vitro* in adipose tissue and isolated fat-cells of various animal models as well as of obese man (Salans *et al.*, 1968; Gries, 1970; York & Bray, 1973; Harrison & King-Roach, 1976; Herberg & Coleman, 1977). In contrast with adipose tissue, which plays only a minor role in the disposal of a glucose load, skeletal muscle appears to be an important regulator of glucose homeostasis (Curtis-Prior *et al.*, 1969; Cahill, 1971). Therefore we have investigated the insulin-sensitivity of isolated skeletal muscle *in vitro* with respect to glucose metabolism in a chronic insulin-resistant state. As an animal model of insulin resistance, we used the Zucker rat (*fa/fa*), which exhibits excessive hyperinsulinaemia, obesity

and hyperlipaemia associated with glucose intolerance (Bray, 1977). We first characterized the metabolic status of 15–20-week-old obese Zucker rats and their appropriate controls *in vivo*, and then determined the insulin sensitivity of glucose metabolism of the isolated perfused hindquarter of these animals.

Preliminary reports of this work have been presented at the 2nd International Congress on Obesity, Washington, DC, 1977 (Kemmer *et al.*, 1978a) and at the 13th Annual Meeting of the European Society for Clinical Investigation, Rotterdam (Kemmer *et al.* 1978b).

Experimental

Animals

Three groups of animals were investigated: (1) female Osborne–Mendel rats weighing 180–240 g; (2) female lean Zucker rats (*Fa/Fa*) weighing 190–250 g; (3) female obese Zucker rats (*fa/fa*) 15–20 weeks old weighing 400–600 g. All animals were bred in the animal laboratories of the Diabetes Research Insti-

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tute; they had free access to food and tap water until the beginning of the experiments at 09:00h. The animals were anaesthetized with pentobarbital (40mg/kg body wt.) injected intraperitoneally.

Materials

Bovine serum albumin (Cohn fraction V), obtained from Miles Laboratories, Slough, Bucks., U.K., was dissolved in Krebs–Henseleit (1932) buffer, and dialysed twice against bicarbonate saline (Ruderman, 1971) for 24h. L-[1-¹⁴C]Lactate, D-[U-¹⁴C]glucose and D-[1-³H]sorbitol were obtained from New England Nuclear Chemicals, Dreieichenhain, Germany. All substrates for experiments and metabolic assays were purchased from E. Merck, Darmstadt, Germany, all enzymes for metabolic assays from Boehringer Mannheim Corp., Mannheim, Germany, and crystalline bovine insulin was from Hoechst, Frankfurt (Main), Germany.

Experiments in vivo

Animals of all three groups underwent an intravenous glucose-tolerance test. For this 1g of glucose/kg body wt. was injected into the left femoral vein after surgical exposure. Wounds were closed with silk sutures. Immediately before and 5min, 20min, 60min and, except for the Osborne–Mendel rats, 120min after the glucose injection, blood samples were collected from the tip of the tail; portions were used for determination of blood glucose concentrations (Bittner & Manning, 1966), and the remainder of the sample was transferred into chilled, heparin-treated plastic tubes, centrifuged (3000g for 10min), and the plasma was frozen and stored at -18°C for later insulin assay. Blood samples for determination of serum triacylglycerol, serum cholesterol, serum non-esterified fatty acids and plasma glucagon concentrations were taken by puncture of the inferior vena cava during the preparation procedure before the hindquarter perfusion, and transferred into chilled tubes; samples for glucagon determinations (0.9ml) were immediately transferred into chilled tubes containing 0.1ml of EDTA (disodium salt) and Aprotinin. Serum samples were extracted for non-esterified fatty acids by the method of Dole & Meinerts (1960). Plasma and serum samples were stored at -18°C until later determination of glucagon, triacylglycerol and cholesterol.

Perfusion procedures

Details of the perfusion apparatus, the preparation of the hindquarter and the composition of the perfusate were described previously (Ruderman *et al.*, 1971). The operative procedure differed only slightly in that the skin of only one hindlimb was partially removed to allow freeze-clamping of the muscle at the end of each experiment. After being placed in the

perfusion apparatus, the hindquarter was cyclically perfused with 150ml of a standard medium containing 4g of albumin/100ml, aged washed human erythrocytes (7–8g of haemoglobin/100ml), 6.6mm-glucose, 0.15mm-pyruvate, 1.5–2mm-lactate and 5μCi of L-[1-¹⁴C]lactate. The flow rate through the preparation (10ml/min) was monitored with a Gilmont flow meter (A. S. Thomas Corp., Philadelphia, PA, U.S.A.). Throughout the perfusion, pressure in the tubing leading to the aortic catheter was 8–9.5kPa (60–70mmHg). Perfusion was carried out for 10min to allow for equilibration; thereafter, zero samples were collected. The hindquarter was then perfused for 30min without or with addition of 0.5, 10 or 50munits of insulin/ml of perfusate. In some perfusions the medium contained in addition 5μCi of D-[U-¹⁴C]glucose or D-[1-³H]sorbitol instead of labelled lactate.

Composition of the perfused tissue of obese and lean Zucker rats

To delineate the perfused tissue, Evans Blue was injected into the arterial cannula after a routine perfusion procedure. The stained tissue representing the perfused part of the body was then severed from the remainder of the carcass and weighed after the skin and all visible adipose tissue had been removed and weighed. Thereafter, lipids were extracted with acetone in a Soxhlet apparatus. The extracted lipids were weighted after all acetone had been evaporated. The remaining portion of the hindquarter was then dissolved in 50% (w/v) KOH, and the bones were isolated and weighed. The weight of the skeletal-muscle mass was then calculated as (weight of perfused skeletal-muscle mass) = (total hindquarter weight) – (skin weight + adipose-tissue weight + weight of bones). The extracellular space of the muscle tissue of lean and obese Zucker rats was determined by using D-[1-³H]sorbitol as previously described (Berger *et al.*, 1975).

Sampling of perfusate and tissue

Duplicate samples of the perfusate were taken at 0 and 30min and production and utilization rates of lactate, alanine and glucose were calculated from the changes in perfusate concentration. At the end of the perfusion period, a sample of the perfused muscle was freeze-clamped and stored in liquid N₂ for later determination of tissue metabolites and lactate specific radioactivity. ¹⁴CO₂ was collected as described by Ruderman *et al.* (1971). The O₂ content of the whole perfusate was determined in arterial and venous samples at the mid-point of the perfusion period by using a LEX-O₂-CON analyser (Lexington Instruments, Lexington, MA, U.S.A.). In experiments during which D-[U-¹⁴C]glucose was added to the perfusion medium, the entire gastrocnemius muscle

of one hindlimb and the superficial adipose tissue of both hindlimbs were rapidly dissected immediately after the end of the perfusion period. These tissue samples were frozen and stored in liquid N₂ for later determination of glycogen and radioactivity of glucose converted into glycogen or lipids.

Analytical methods

In blood samples collected *in vivo* the following hormones and metabolites were determined: plasma insulin (Herberg & Kley, 1975), plasma glucagon by using the antiserum 30K (Unger *et al.*, 1970), serum non-esterified fatty acids (Dole & Meinerts, 1960), serum cholesterol (by using the Boehringer test combination by the method of Trinder, 1969), and serum triacylglycerol (Eggstein & Kreutz, 1966). Triacylglycerol and cholesterol were also determined in very-low-density-lipoprotein, low-density-lipoprotein, and high-density-lipoprotein fractions that were isolated by ultracentrifugation (Havel *et al.*, 1955). Perfusate and freeze-clamped tissue specimens (after being ground in a mortar under liquid N₂) were deproteinized with ice-cold 10% (w/v) HClO₄. The extracts were neutralized with KOH, and the precipitate was removed by centrifugation (3000g for 10 min). Metabolites from the perfusate and the tissue were then measured spectrophotometrically by using standard enzymic assays (Ruderman *et al.*, 1971;

was evaporated and the lipids were weighed. The mean specific radioactivity of glucose in the perfusion medium (d.p.m./μmol of glucose) was obtained by forming osazones by addition of phenylhydrazine hydrochloride/sodium acetate to deproteinized and neutralized perfusate samples taken at 0 and 30 min. Measurement from 12 experiments gave 12911 ± 292 (S.E.M.) d.p.m./μmol of glucose at the beginning and 12257 ± 360 (S.E.M.) at the end of the perfusion period, demonstrating a constant glucose specific radioactivity throughout the experiment.

Calculations

Uptake and release of metabolites were determined from concentration changes in the perfusate and expressed as μmol/min per 30 g of muscle (Ruderman *et al.*, 1971). Flux rates through metabolic pathways were estimated as described previously (Berger *et al.*, 1976) based on mean values for glucose uptake, lactate release, lactate oxidation and alanine release. The O₂ consumption was determined from arteriovenous differences in O₂ content and the flow rate, and expressed as μmol/min per 30 g of muscle. Intracellular glucose concentrations were calculated by the method of Berger *et al.* (1975). The rate of glucose conversion into total lipids of skeletal muscle and adipose tissue and into glycogen was calculated for each individual experiment as follows:

Glucose incorporation into total lipids of skeletal muscle or adipose tissue (μmol/min per 30 g of muscle or adipose tissue respectively) =
$$\frac{\text{radioactivity of lipid extract (d.p.m./30g wet wt. of tissue)} \times \text{min}^{-1}}{\text{specific radioactivity of glucose in perfusate water (d.p.m./μmol)}}$$

Glucose incorporation into glycogen of skeletal muscle (μmol/min per 30 g of muscle) =
$$\frac{\text{radioactivity of glycogen (d.p.m./30g wet wt. of muscle)} \times \text{min}^{-1}}{\text{specific radioactivity of glucose in perfusate water (d.p.m./μmol)}}$$

Goodman *et al.*, 1974), and lactate specific radioactivity was determined as described previously (Berger *et al.*, 1976). One portion of the gastrocnemius muscle was taken for determination of glycogen (Good *et al.*, 1933). The glycogen extracts were counted for ¹⁴C radioactivity by using the following scintillator composition: 500 ml of dioxan, 500 ml of toluene, 300 ml of methanol, 104 g of naphthalene, 6.5 g of 2,5-diphenyloxazole, 130 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene. The other portion of the gastrocnemius muscle and the adipose tissue were weighed and lipids were extracted as described above. After acetone had been evaporated, lipids were separated from water-soluble substrates by dissolving in light petroleum (b.p. 40°C). The light-petroleum solution was then transferred to another flask and adjusted with light petroleum to a final volume of 25 ml. Samples of this solution were measured in a liquid-scintillation counter for ¹⁴C radioactivity. The light petroleum of the remainder

The method used allows merely an assessment of the apparent incorporation of [¹⁴C]glucose into glycogen rather than a measurement of net glycogen synthesis; although the perfusion period was only 30 min, the incorporation rates could be affected by differences in the glycogen breakdown between the experimental groups.

Statistical analysis

All results are expressed as means ± S.E.M. Statistical significance was assessed by using Student's *t* test.

Results

Measurements *in vivo* (Table 1; Fig. 1)

Body weight was greatly increased in obese Zucker rats compared with lean rats of either strain. Basal blood glucose concentrations were decreased in lean Zucker rats. An excessive degree of hyperinsulinaemia was present in obese Zucker rats, circulating glucagon

Table 1. *Body weight and concentrations of metabolites and hormones in obese and lean Zucker rats and Osborne-Mendel rats*
 Blood glucose and plasma insulin concentrations were determined from tail blood, and plasma glucagon, serum non-esterified fatty acids, serum cholesterol and serum triacylglycerol from samples collected from the inferior vena cava in anaesthetized rats. Values are means $\pm$ S.E.M. for the numbers of experiments in parentheses.

	Obese rats	Lean rats	Osborne-Mendel rats
Body wt. (g)	518 $\pm$ 10 (33)	221 $\pm$ 6 (28)†	206 $\pm$ 4 (28)†
Blood glucose (mM)	8.17 $\pm$ 0.50 (8)	6.33 $\pm$ 0.11 (10)†	8.78 $\pm$ 0.17 (8)
Plasma insulin (μ units/ml)	266 $\pm$ 52 (5)	50 $\pm$ 5 (10)†	59 $\pm$ 7 (8)†
Plasma glucagon (pg/ml)	143 $\pm$ 2 (4)	163 $\pm$ 8 (4)*	130 $\pm$ 12 (4)
Serum non-esterified fatty acids (μ mol/l)	1301 $\pm$ 84 (3)	1149 $\pm$ 20 (3)	920 $\pm$ 82 (3)*
Serum cholesterol (mM)	4.19 $\pm$ 2.59 (3)	1.55 $\pm$ 0.16 (3)†	1.37 $\pm$ 0.10 (3)†
Serum triacylglycerol (mM)	6.98 $\pm$ 1.12 (3)	0.71 $\pm$ 0.06 (3)†	0.74 $\pm$ 0.04 (3)†
Cholesterol (mM):			
Very-low-density lipoprotein	0.39 $\pm$ 0.18 (3)	0.03 $\pm$ 0.01 (3)	—
Low-density lipoprotein	0.65 $\pm$ 0.38 (3)	0.16 $\pm$ 0.13 (3)	—
High-density lipoprotein	3.15 $\pm$ 0.62 (3)	1.37 $\pm$ 0.28 (3)*	—
Triacylglycerol (mM):			
Very-low-density lipoprotein	6.84 $\pm$ 1.13 (3)	0.41 $\pm$ 0.05 (3)†	—
Low-density lipoprotein	0.03 $\pm$ 0.01 (3)	0.17 $\pm$ 0.04 (3)	—
High-density lipoprotein	0.10 $\pm$ 0.04 (3)	0.12 $\pm$ 0.03 (3)	—

* Significantly different from corresponding values of obese Zucker rats at $P < 0.05$.

† Significantly different from corresponding values of obese Zucker rats at $P < 0.01$.

Fig. 1. *Blood glucose (a) and plasma insulin (b) concentrations during intravenous glucose-tolerance tests (1g of glucose/kg body wt.) in obese and lean Zucker rats and Osborne-Mendel rats*

Symbols: ●, obese Zucker rats ($n = 5$); ○, lean Zucker rats ($n = 6$); ■, Osborne-Mendel rats ($n = 6$). Values are means $\pm$ S.E.M. (bars). * indicates statistically significant differences between obese Zucker rats and both lean groups of rats at $P < 0.05$.

concentrations were significantly lower in obese than in lean Zucker rats, but no difference was observed between the obese and Osborne-Mendel rats. Serum non-esterified fatty acids were increased in obese Zucker rats when compared with Osborne-Mendel rats. Serum cholesterol in obese Zucker rats was approx. 3-fold and serum triacylglycerol concentrations were more than 10-fold as high in obese rats as in the lean Zucker rats and Osborne-Mendel rats. Further analysis of lipoprotein fractions in Zucker rats demonstrated that most of the triacylglycerols were associated with the very-low-density-lipoprotein fraction and most of the cholesterol was in the high-density-lipoprotein fraction. Glucose tolerance was significantly impaired in obese rats compared with lean Zucker rats and Osborne-Mendel rats (Fig. 1). Basal plasma insulin concentrations in the obese rats were approx. 6 times those in the two lean groups. Although in both groups of lean rats insulin secretion was significantly stimulated after the intravenous glucose load, in obese rats circulating insulin concentrations increased only slightly.

Composition of the perfused tissue

In lean rats, approx. 75% of the hindquarter is represented by skeletal muscle. In the obese Zucker rats the muscle tissue contributed only approx. 50% of the perfused hindlimb mass, whereas the proportion of adipose tissue was increased to 22%, compared with 3% in lean rats (Table 2). As in normal Wistar rats (Ruderman, 1971), in obese and lean Zucker rats the weight of the perfused hindquarter muscle mass is closely correlated with the body weight of the animal ($y = 1.244 + 0.1711x$, $n = 6$, correlation coefficient

Table 2. *Composition of the isolated perfused hindquarter of obese and lean Zucker rats*

Values are expressed as percentage wet wt. of perfused tissue, except for lipid content of muscle and adipose tissue, which is expressed as mg of total lipids/g wet wt. Values are means $\pm$ S.E.M. for the numbers of experiments in parentheses.

	Obese rats	Lean rats	Wistar rats*
Muscle tissue (%)	50.4 $\pm$ 1.2 (6)	71.8 $\pm$ 0.7 (8)	77.7
Total lipids in muscle (mg/g wet wt.)	33 $\pm$ 4.3 (8)	12 $\pm$ 0.6 (8)	—
Adipose tissue (%)	22.4 $\pm$ 1.2 (6)	3.2 $\pm$ 0.7 (8)	0
Total lipids in adipose tissue (mg/g wet wt.)	799 $\pm$ 22 (8)	548 $\pm$ 29 (8)	—
Bone (%)	6.9 $\pm$ 0.4 (6)	9.4 $\pm$ 0.1 (8)	10.0
Skin (%)	21.3 $\pm$ 1.5 (6)	15.6 $\pm$ 0.7 (8)	12.3

* Values taken from Ruderman (1971).

$r = 0.95$, where y is the weight of perfused muscle and x is the body weight of lean Zucker rats; and $y = 10.03 + 0.0551x$, $n = 17$, correlation coefficient $r = 0.79$, where y is the weight of perfused muscle and x is the body weight of obese Zucker rats). Therefore in subsequent experiments the weight of the perfused muscle mass was calculated from the rats' total body weight. The extracellular space of skeletal muscle was $227 \pm 12 \mu\text{l/g}$ wet wt. (mean $\pm$ S.E.M., $n = 4$) in obese and $201 \pm 5 \mu\text{l/g}$ wet wt. (mean $\pm$ S.E.M., $n = 3$) in lean Zucker rats; thus it was not different from that in Sprague-Dawley rats (Berger *et al.*, 1975).

Perfusion experiments

In none of the groups examined was a significant rate of glucose uptake observed in the absence of added insulin (Table 3). The stimulation of glucose uptake was identical in both groups of lean rats: 0.5 munit of insulin/ml of perfusate increased the glucose uptake to approx. $2.5 \mu\text{mol/min}$ per 30g of muscle, and 10 munits of insulin/ml caused an increase to approx. $6 \mu\text{mol/min}$ per 30g of muscle. However, 50 munits of insulin/ml induced no further stimulation of glucose uptake. By contrast, in obese rats 0.5 munits of insulin/ml had no stimulatory effect on glucose uptake. At 10 and 50 munits of insulin/ml, glucose uptake was significantly increased in obese Zucker rats, but it was still decreased by 50–70% compared with either lean control group.

A dose-dependent inhibition of alanine release by insulin was observed in Osborne-Mendel rats (Table 3). In lean Zucker rats, only at 50 munits of insulin/ml was alanine release inhibited (to approx. 65% of the basal value). In obese Zucker rats alanine release remained unaffected by insulin. Lactate release in obese Zucker rats was approximately twice that in rats of both lean groups; it remained unaffected by insulin in all experimental groups. Lactate oxidation was stimulated in Osborne-Mendel rats at 10 and 50 munits of insulin/ml (Table 3). In lean Zucker rats basal lactate oxidation was lower than

in Osborne-Mendel rats and a stimulatory effect of insulin could only be observed at 0.5 munit of insulin/ml. In obese Zucker rats, no stimulation of lactate oxidation was observed at any insulin concentration used.

The calculated glycolytic flux rate varied considerably between the groups examined (Table 3). In Osborne-Mendel rats it increased when higher insulin concentrations were present; in lean Zucker rats it was lower than in both other groups and remained unaffected by insulin; and in obese Zucker rats insulin had no stimulatory effect. In all three experimental groups, calculations indicated a net glycogenolysis in the basal state (Table 3). In Osborne-Mendel rats and lean Zucker rats addition of insulin to the perfusate appeared to induce a net glycogen synthesis. In the absence of added insulin, the calculated net glycogenolysis in obese Zucker rats was approx. 2.5 times as high as in the lean groups; the apparent rate of glycogen breakdown decreased when insulin was added and a slight net glycogen synthesis was estimated in the presence of 50 munits of insulin/ml. Rates of O_2 consumption (Table 3) did not differ between the experimental groups and remained unaffected by insulin. In lean Zucker rats glucose conversion into glycogen was 1.23 ± 0.43 ($n = 4$) and increased to $4.75 \pm 0.93 \mu\text{mol/min}$ per 30g of muscle ($n = 4$) ($P < 0.05$) when 50 munits of insulin/ml were present. In obese Zucker rats insulin did not stimulate glucose conversion into glycogen ($1.50 \pm 0.43 \mu\text{mol/min}$ per 30g of muscle without addition of insulin, $n = 4$; $1.40 \pm 0.59 \mu\text{mol/min}$ per 30g of muscle at 50 munits of insulin/ml, $n = 4$). Glucose incorporation into adipose-tissue lipids proceeded at a very low rate and was not stimulated by insulin in either group (Table 4). From these data and from the lipid content of adipose tissue (Table 2) it can be calculated that in obese Zucker rats only approx. $0.01 \mu\text{mol}$ of glucose was incorporated into lipids/min per g of adipose tissue. In both lean and obese Zucker rats a significant stimulatory effect of insulin on glucose conversion

Table 3. *Glucose uptake and disposition in perfused hindquarters of obese and lean Zucker rats and Osborne-Mendel rats*
 Results are means $\pm$ s.e.m. for the numbers of experiments in parentheses. Different insulin concentrations were added to initial perfusate as indicated. Negative values for glycogen synthesis indicate net glycogenolysis. Values significantly different from corresponding values obtained from lean Zucker rats are indicated by * (at $P < 0.05$) and † (at $P < 0.01$). Values significantly different from controls obtained in perfusions without added insulin are indicated by ‡ (at $P < 0.05$) and § (at $P < 0.01$).

Group of rats	Insulin (munits/ml)	Metabolism (μ mol/min per 30 g of muscle)					
		Glucose uptake	Alanine release	Lactate release	Lactate oxidation	Calculated:	
						Rate of glycolysis	Net glycogen synthesis
Obese Zucker	0	-0.03 ± 0.46 (11)	0.56 ± 0.05 (10)	2.70 ± 0.45 (11)*	0.93 ± 0.16 (7)	2.10	-2.13
	0.5	0.40 ± 0.61 (7)*	0.54 ± 0.06 (7)	2.37 ± 0.75 (7)*	0.82 ± 0.07 (7)	1.87	-1.47
	10	1.75 ± 0.63 (7)†	0.61 ± 0.09 (7)*	2.05 ± 0.49 (7)*	0.86 ± 0.17 (8)	1.76	-0.01
	50	2.81 ± 0.19 (8)†§	0.50 ± 0.03 (8)†	2.53 ± 0.34 (8)†	0.42 ± 0.04 (4)*	1.73	1.08
Lean Zucker	0	0.22 ± 0.49 (9)	0.41 ± 0.03 (6)	1.28 ± 0.34 (9)	0.55 ± 0.14 (5)	1.12	-0.84
	0.5	2.53 ± 0.45 (5)†	0.42 ± 0.05 (5)	0.72 ± 0.38 (5)	1.09 ± 0.16 (5)§	1.12	1.41
	10	5.29 ± 0.65 (5)§	0.36 ± 0.06 (5)	1.08 ± 0.27 (5)	1.84 ± 0.07 (5)	1.14	4.15
	50	6.37 ± 0.42 (9)§	0.27 ± 0.02 (9)§	1.10 ± 0.35 (9)	1.68 ± 0.13 (5)	1.03	5.34
Osborne-Mendel	0	0.52 ± 0.54 (12)	0.52 ± 0.04 (12)	1.10 ± 0.17 (12)	1.09 ± 0.14 (10)*	1.36	-0.84
	0.5	2.09 ± 0.47 (7)†	0.37 ± 0.04 (7)†	1.40 ± 0.28 (7)	1.03 ± 0.14 (7)	1.40	0.69
	10	6.07 ± 0.95 (5)§	0.32 ± 0.03 (7)§	1.02 ± 0.38 (5)†	2.02 ± 0.38 (5)†	1.68	4.39
	50	7.15 ± 0.74 (4)§	0.23 ± 0.02 (4)§	2.12 ± 0.25 (4)	1.59 ± 0.18 (4)†	1.97	5.18
							11.3 ± 1.2 (9)
							10.9 ± 0.4 (6)
							12.5 ± 0.7 (8)
							11.5 ± 0.7 (8)
							9.5 ± 0.5 (9)
							10.3 ± 0.5 (4)
							10.9 ± 0.7 (5)
							10.5 ± 1.0 (9)
							13.4 ± 1.3 (12)
							10.1 ± 1.0 (7)
							11.0 ± 0.5 (5)
							12.5 ± 1.1 (4)

Table 4. *Effect of 50 munits of insulin/ml of perfusate on incorporation of glucose into total lipids of adipose tissue and skeletal muscle of obese and lean Zucker rats*
Values are means $\pm$ S.E.M. for four experiments.

	Addition of insulin	Glucose incorporation into total lipids (μ mol/min per 30 g of adipose tissue or skeletal muscle)
Adipose tissue		
Obese	—	$0.30 \pm 0.03^*$
Obese	+	$0.49 \pm 0.09^*$
Lean	—	0.80 ± 0.04
Lean	+	0.75 ± 0.06
Skeletal muscle		
Obese	—	$0.16 \pm 0.03^*$
Obese	+	$0.37 \pm 0.09^{*\dagger}$
Lean	—	0.16 ± 0.03
Lean	+	$1.49 \pm 0.03^\dagger$

* Value significantly different from corresponding value obtained from lean Zucker rats at $P < 0.01$.

$\dagger$ Value significantly different from perfusion without added insulin at $P < 0.01$.

into lipids of skeletal muscle was observed (Table 4), but the rate of insulin-stimulated glucose conversion into lipids of skeletal muscle in obese Zucker rats was decreased by 90% in obese rats when compared with that of lean Zucker rats. Independently of the presence of added insulin, tissue glucose concentrations in all groups of rats (Table 5) indicated a glucose distribution well below that of the extracellular space; thus an accumulation of free glucose in muscle cell water could be excluded (Berger *et al.* 1975). In obese rats the ATP and phosphocreatine concentrations were significantly lower than in both lean groups. No differences were observed in lactate and pyruvate concentrations and in the lactate/pyruvate ratio (Table 5).

Discussion

In accordance with earlier observations, the obese Zucker rats used in this study showed substantial hormonal and metabolic abnormalities compared with their lean homozygous controls: hyperinsulinaemia (York *et al.*, 1972; Zucker & Antoniadis,

Table 5. *Tissue metabolites of isolated perfused muscle of obese and lean Zucker rats and Osborne-Mendel rats at the end of the perfusion period in the absence and presence of different insulin concentrations*

Values are means $\pm$ S.E.M.

Group of rats	No. of expts.	Insulin added to the perfusate (munits/ml)	Intra-cellular glucose (mM)	Content (μ mol/g wet wt.)					Lactate/pyruvate ratio
				Glucose	Lactate	Pyruvate	Phospho-creatine	ATP	
Obese Zucker	11	0	0	0.63	1.82	0.12	13.84	4.78	14.4
				± 0.09	± 0.21	± 0.01	± 0.58	± 0.28	± 1.85
	7	0.5	0	1.05	1.82	0.14	12.83	3.89	13.8
				± 0.14	± 0.20	± 0.01	± 0.42	± 0.32	± 2.14
	8	10	0	0.98	1.69	0.13	14.96	3.92	14.8
				± 0.12	± 0.29	± 0.01	± 1.23	± 0.28	± 3.40
	8	50	0	0.44	1.19	0.13	15.58	4.10	9.3
				± 0.11	± 0.11	± 0.01	± 0.80	± 0.37	± 0.84
Lean Zucker	8	0	0	0.73	0.87	0.10	18.48*	6.41*	9.1
				± 0.05	± 0.10	± 0.01	± 1.13	± 0.27	± 1.35
	5	0.5	0	0.67	1.49	0.10	20.12	6.02*	15.0
				± 0.07	± 0.28	± 0.01	$\pm 0.78^*$	± 0.34	± 2.40
	5	10	0	0.54	1.12	0.13	18.76*	5.44*	9.4
				± 0.09	± 0.09	± 0.02	± 0.67	± 0.26	± 1.37
	9	50	0	0.78	1.26	0.11	17.78*	5.49*	12.3
				± 0.08	± 0.27	± 0.02	± 0.53	± 0.23	± 2.91
Osborne-Mendel	11	0	0	1.27	1.27	0.14	18.82*	5.58*	9.6
				± 0.14	± 0.18	± 0.02	± 0.90	± 0.30	± 1.41
	7	0.5	0	1.20	1.07	0.08	18.38*	5.12*	15.0
				± 0.18	± 0.22	± 0.03	± 0.91	± 0.14	± 1.88
	5	10	0	0.60	1.60	0.09	18.32*	5.27*	17.3
				± 0.12	± 0.23	± 0.01	± 0.78	± 0.26	± 2.70
	4	50	0	0.70	0.94	0.11	17.19*	4.68	12.9
				± 0.14	± 0.34	± 0.01	± 1.31	± 0.39	± 4.38

*Significantly different from corresponding value obtained in obese Zucker rats at $P < 0.05$.

1972), impaired glucose tolerance (Zucker & Antoniadis, 1972), decreased glucagon concentrations in the circulation (Eaton *et al.*, 1976), increased serum non-esterified fatty acids (Zucker, 1965; Malewiak *et al.*, 1977) and hyperlipaemia with increased triacylglycerol and cholesterol concentrations (Zucker, 1965; Barry & Bray, 1969; Schonfeld *et al.*, 1974). Thus the obese Zucker rat presents a high degree of chronic insulin resistance. In various other states of peripheral insulin resistance, skeletal muscle has been found to exhibit a decrease in insulin sensitivity (Fraser *et al.*, 1962; Rabinowitz & Zierler, 1962; Stauffacher *et al.*, 1965; Butterfield *et al.*, 1965; Rabinowitz, 1968; Lemonnier *et al.*, 1974; Stern *et al.*, 1975; Czech *et al.*, 1977; Le Marchand-Brustel *et al.*, 1978). The present study demonstrates that the perfused hindquarter of the obese Zucker rat is excessively insulin-resistant. In normal Wistar rats the hindquarter's glucose metabolism is almost exclusively due to skeletal muscle (Ruderman, 1971). The present study demonstrates that the composition of the perfused tissue in the hindquarter of lean Zucker rats does not differ from that of normal rats. In hindquarters of obese Zucker rats, however, the skeletal-muscle mass is decreased in favour of adipose tissue, which contributes approx. 22% of the perfused hindquarter mass. Nevertheless, the contribution of the adipose-tissue metabolism to glucose utilization by the hindquarter can be neglected, because in subcutaneous fat of obese Zucker rats glucose-incorporation rates into lipids were found to be relatively trivial (Table 4). Similar observations have already been described in obese Zucker rats (Bray, 1968; York & Bray, 1973), in Wistar rats (Curtis-Prior *et al.*, 1969) and in humans (Björntorp *et al.*, 1971). Therefore, as in lean rats (Ruderman, 1971), in obese Zucker rats glucose metabolism of the hindquarter can be assumed to reflect the metabolism of skeletal muscle.

The insulin-resistance of skeletal muscle demonstrated in the present study for obese Zucker rats appears to be effective at different levels of the muscle cell's glucose metabolism. Thus glucose uptake by skeletal muscle shows a markedly decreased insulin sensitivity. This is probably due to an impairment of glucose transport processes across the muscle cell membrane, since the inhibition of glucose uptake is not associated with an intracellular glucose accumulation. The disturbance of glucose transport might be linked to an impairment of insulin binding to muscle cell receptors, as has been demonstrated in lymphocytes and adipocytes from obese species (Bar & Roth, 1977; Olefsky, 1976a), in skeletal muscle of genetically obese (*ob/ob*) mice (Le Marchand-Brustel *et al.*, 1978), of aged obese Sprague-Dawley rats (Olefsky *et al.*, 1976) and of obese Zucker rats (Crettaz *et al.*, 1978). It is noteworthy that the decreased insulin sensitivity of skeletal-muscle glucose uptake

could not be overcome by increasing insulin perfusate concentrations up to 50 munits/ml. Furthermore, in accordance with observations on large adipocytes (Czech, 1976; Olefsky, 1976b) the present study seems to indicate intracellular defects of insulin-stimulated glucose metabolism in skeletal muscle of obese Zucker rats: independently of the decreased glucose-uptake rates (Table 3) we have observed an impairment of lactate oxidation, which is probably due to an inhibition of pyruvate dehydrogenase activity (Hagg *et al.*, 1976). In addition, insulin did not inhibit alanine release from skeletal muscle, nor did it stimulate the [^{14}C]glucose incorporation into glycogen, and the insulin stimulation of glucose conversion into lipids of skeletal muscle was markedly decreased.

These data suggest that the impairment of glucose metabolism observed in skeletal muscle of obese Zucker rats might involve at least two different mechanisms. Thus there appears to be a defect in the insulin-sensitivity of glucose transport that might, at least in part, be due to a disturbed insulin-receptor interaction. Secondly, independent of the rate of glucose uptake, there are intracellular defects, namely the inhibition of lactate oxidation. As a possible mechanism for this observation, a glucose fatty-acid-cycle-type (Randle *et al.*, 1966) inhibition of pyruvate oxidation by the permanently increased circulating non-esterified fatty acids in obese Zucker rats has to be considered. Such a hypothesis would be in keeping with findings reported by Crettaz *et al.* (1978) showing an increase in skeletal-muscle concentrations of glucose 6-phosphate, citrate and glycogen in obese Zucker rats. In our perfusion studies, however, calculated rates of glycolysis were not inhibited, but rather enhanced, in obese Zucker rats (Table 3). Furthermore, in earlier experiments using the hindquarter perfusion technique on Sprague-Dawley rats we could not show an inhibition of lactate oxidation by non-esterified fatty acids (Berger *et al.*, 1976). In addition, non-esterified fatty acid concentrations in the perfusate during all our perfusion experiments were less than 500 $\mu\text{mol/l}$ (Berger *et al.*, 1976) and, finally, the basal lactate oxidation in obese Zucker rats was not inhibited [in contrast with diabetes and starvation (Berger *et al.*, 1976)].

On the other hand, it might be speculated that if intracellular effects of insulin were induced via direct interaction of the hormone with intracellular insulin receptors (Goldfine *et al.*, 1977), such receptors could also be down-regulated by chronic hyperinsulinaemia. Further investigation is needed to clarify the basis for the diminished insulin sensitivity of intracellular pathways of glucose metabolism in skeletal muscle of obese Zucker rats.

The present paper demonstrates a generalized insulin resistance of skeletal-muscle glucose metabolism in obese Zucker rats. Since skeletal muscle

appears to be important as a regulator of carbohydrate homeostasis, it is suggested that its insulin-insensitivity should be at least a contributory factor in the development of the glucose intolerance in this species.

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