Reduced maximum capacity of glycolysis in brown adipose tissue of genetically obese, diabetic (db/db) mice and its restoration following treatment with a thermogenic β-adrenoceptor agonist

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The maximal activities of the key glycolytic enzymes hexokinase and 6-phosphofructokinase, were reduced in brown adipose tissue in db/db mice compared to their lean littermates. Treatment of db/db mice with the thermogenic β-adrenoceptor agonist, BRL 26830, restored normoglycaemia. The only significant increase in activity of hexokinase and 6-phosphofructokinase in the BRL 26830-treated db/db mice occurred in brown adipose tissue where the total tissue activity increased 10- and 11-fold respectively. These changes together with increased 2-deoxyglucose uptake in vivo suggest that brown adipose tissue can play a quantitatively important role in the removal of glucose from the blood.

Brown adipose tissue *Blood glucose* *Glycolysis* *2-Deoxyglucose* *db/db mouse*

1. INTRODUCTION

The role of brown adipose tissue as a major site of non-shivering thermogenesis in hibernators and other small mammals and in the newborn of larger mammals is now well established [1,2]. It is generally accepted that fatty acids are the oxidative fuel for non-shivering thermogenesis [1,3] but there is no direct evidence that this is always the case [4]. In animals fed on a high carbohydrate diet, blood glucose might be a direct substrate for thermogenesis in brown adipose tissue as well as an indirect substrate via its role as a lipogenic precursor [5,6]. If this is so, brown adipose tissue may play an important quantitative role in the regulation of blood glucose [7]. In support of this argument, it has been shown that the glycolytic capacity of brown adipose tissue as indicated by the maximum activity of glucose phosphorylation (hexokinase) is approximately 25% of that of the liver in normal rats and equal to the liver in cold-acclimated rats [7].

C57Bl/KsJ db/db mice are both obese and diabetic [8]. The development of obesity, which precedes the development of overt diabetes, is attributed to a defective activation of non-shivering thermogenesis [9]. The reasons for the development of diabetes are less well understood and defects in both insulin secretion [10] and insulin action [11] have been demonstrated. Notwithstanding the primary cause of the diabetic condition, if brown adipose tissue is normally a major site of glucose utilisation in small mammals, then defective activation of thermogenesis in db/db mice could contribute to the glucose intolerance of these mice.

Here, the maximum activities of hexokinase, 6-phosphofructokinase and 2-oxoglutarate dehydrogenase have been measured in selected tissues of db/db mice and their lean littermates. These enzymes provide a quantitative index of the maximum capacity of glycolysis from glucose, glycolysis from glycogen, and the tricarboxylic acid cycle activity [7,12] respectively. In addition, measure-

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ments have been made of the relative tissue uptake of 2-deoxyglucose in vivo. Finally, the effect of chronic administration of a thermogenic β-adrenoceptor agonist on these parameters was determined.

2. MATERIALS AND METHODS

Female C57Bl/KsJ db/db mice and their lean littermates were obtained from Jackson Laboratories, Bar Harbour, ME. The mice used came from a stock in which the db gene was in opposition to the coat colour gene misty. The lean littermates were all $db^+/^+m$, the mice were aged 4/5 weeks upon purchase and were maintained at 23 ± 1°C under a 12 h light–12 h dark light cycle. During this time, they were fed on Oxoid rat and mouse breeders diet (H.C. Styles, Bewdley, Worcs.). Chemicals and enzymes were obtained from Boehringer (Lewes, Sussex) or Sigma (Poole, Dorset).

In experiments to study the effect of chronic treatment with a thermogenic β-agonist, BRL 26830 [13], (*R**,*R**)-(±)-methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino]propyl]benzoate, (*E*)-2-butenedioate (2:1) salt, was given as dietary admixture (5 mg/100 g diet) for 10 weeks (enzyme studies) or 8 days (2-deoxyglucose studies). To measure enzyme activity, tissues were removed from the mice and homogenized in 11 volumes of an extraction medium containing (mM) 50 Tris, 1 EDTA, 5 $MgCl_2$ and 0.02 β-mercaptoethanol, at pH 8.2. For the measurement of 2-oxoglutarate dehydrogenase, dithiothreitol (1 mM) replaced mercaptoethanol. Hexokinase, 6-phosphofructokinase and 2-oxoglutarate dehydrogenase were measured spectrophotometrically [14–16]. Since liver possesses both hexokinase and glucokinase activities [17], total glucose phosphorylating activity was determined in the presence of 100 mM glucose. This is not necessary for brown adipose tissue since phosphorylation of glucose is catalyzed by hexokinase [7].

Mitochondrial proteins were separated by discontinuous slab gel electrophoresis and the relative amount of uncoupling protein (M_r 32000) was determined as described previously [18]. Briefly, proteins were located using protein markers (M_r 14000–62000). Only the 32000-Da protein showed changes in height relative to other mitochondrial protein bands and its height was therefore measured relative to an adjacent band (M_r 30000).

To determine relative tissue uptake of 2-deoxyglucose in vivo, conscious mice were given 2-deoxy-[^{14}C]glucose (0.2 μCi/mouse) in 0.2 ml saline by intravenous injection. After 45 min, the mice were killed and blood was obtained for the measurement of glucose and radiochemical content. The mice were rapidly dissected and samples taken for combustion in a tissue oxidiser. The results are expressed as ^{14}C dpm in tissue/^{14}C dpm in whole animal.

3. RESULTS AND DISCUSSION

The maximum activities of hexokinase, 6-phosphofructokinase and 2-oxoglutarate dehydrogenase in various tissues of C57Bl/KsJ db/db mice and their lean littermates are given in table 1. The activities of 6-phosphofructokinase and 2-oxoglutarate dehydrogenase were increased 5- and 3-fold, respectively, in the livers of the db/db mice. Liver enzyme changes are well documented for obese-diabetic animals and are thought to be associated with but not causal of the obesity [19]. The activities of all three enzymes were decreased in the quadriceps muscle of the db/db mice. This was in part due to a decrease in muscle mass.

In brown adipose tissue of db/db mice, the maximum activities of all three enzymes on a whole tissue basis were significantly reduced relative to the lean littermates (table 1). If the enzyme activities are expressed on a g wet weight of tissue basis, the reductions in enzyme activities in brown adipose tissue were further exaggerated (table 2).

The maximum catalytic activity of hexokinase in muscle provides a quantitative index of the maximal rate of glycolysis from glucose [20]. Table 1 shows that the maximum activity of hexokinase in the interscapular brown adipose tissue of the lean littermates is 50% of the maximum activity of glucose phosphorylation (hexokinase and glucokinase) in liver. However, it is estimated that interscapular brown adipose tissue represents only 25% of the total tissue in the mouse [21] and thus the glycolytic capacity of brown adipose tissue in these lean littermates exceeds that of liver and is 3 times greater than the heart. It is therefore probable that the tissue is of quantitative importance

Table 1

Maximal activities of phosphofructokinase, hexokinase and oxoglutarate dehydrogenase in various tissues of 16-week-old db/db and db/+ mice (μmol/min per tissue)

		Phospho-fructokinase	Hexokinase + glucokinase	Oxoglutarate dehydrogenase
Brown adipose tissue (interscapular)	db/+	4.1 ± 0.7	0.92 ± 0.07	1.66 ± 0.14
	db/db	2.2 ± 0.1*	0.60 ± 0.10*	0.63 ± 0.02***
Liver	db/+	7.5 ± 1.8	1.94 ± 0.36	2.34 ± 0.22
	db/db	37.9 ± 1.5***	1.51 ± 0.23	6.52 ± 0.25***
Heart	db/+	5.5 ± 0.9	0.43 ± 0.08	0.92 ± 0.04
	db/db	4.7 ± 0.2	0.56 ± 0.06	0.75 ± 0.08
Muscle (quadriceps)	db/+	28.0 ± 3.2	0.07 ± 0.02	0.20 ± 0.04
	db/db	6.8 ± 0.5***	not detected	0.05 ± 0.01**

Enzyme activities are presented as the mean ± SE for 5 different mice and statistical significance (Student's t-test) is indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2

Effect of BRL 26830 on the maximum catalytic activities of hexokinase, phosphofructokinase and oxoglutarate dehydrogenase in interscapular brown adipose tissue of db/db mice

	db/+	db/db	db/db + BRL 26830
Interscapular brown adipose tissue wt (mg)	84.8 ± 6.0	295.0 ± 9.5	734.0 ± 34.0
Hexokinase (μmol/min per g tissue)	10.4 ± 0.5***	2.0 ± 0.7	7.5 ± 0.2***
Phosphofructokinase μmol/min per g tissue)	34.9 ± 2.9***	8.1 ± 2.4	29.1 ± 0.4***
Oxoglutarate dehydrogenase (μmol/min per g tissue)	16.7 ± 1.4***	1.40 ± 0.02	4.60 ± 0.80**
Total interscapular BAT mitochondrial protein (mg)	2.56 ± 0.21*	1.86 ± 0.25	8.25 ± 0.83***
Ratio of protein bands (32 kDa/30 kDa)	0.51 ± 0.01***	0.23 ± 0.03	0.57 ± 0.06***

The mice were treated with BRL 26830 for 10–11 weeks as described in section 2. Results are presented as mean ± SE for 5 different mice and statistical significance (Student's t-test) relative to untreated db/db mice is indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

in whole animal glucose utilisation and may play a role in the regulation of blood glucose. If this is the case, any reduction in the maximum glycolytic capacity, as seen in the db/db mice, could contribute to the development of diabetes.

Previously, the thermogenic β-adrenoceptor agonist, BRL 26830, has been shown to have anti-obesity activity in a number of animal models [13] and produce hypertrophy of brown adipose tissue [22]. In addition, it produces an improvement in

glucose tolerance in obese animals [23]. In the current studies, when given to C57Bl/KsJ db/db mice as a dietary admixture, BRL 26830 produced a reduction in the blood glucose concentration from 30.6 ± 1.1 to 11.5 ± 1.1 mM and virtually eliminated glycosuria. This was accompanied by a significant increase in the concentration of plasma insulin from 116 ± 15 μU/ml to 279 ± 57 μU/ml (mean ± SE on 10–14 animals). Treatment of db/db mice with BRL 26830 led to a significant increase in the maximal activities of both glycolytic and tricarboxylate cyclic enzyme activities in brown adipose tissue (table 2). This effect of BRL 26830 was not seen in the lean littermates (not shown). Indeed, since there was also brown adipose tissue hypertrophy including increased mitochondrial protein and increased proportion of the 32-kDa protein, the maximum activities of hexokinase, 6-phosphofructokinase and 2-oxoglutarate dehydrogenase in total interscapular brown adipose tissue of BRL 26830-treated db/db mice were increased 5-, 5- and 2-fold respectively relative to the lean littermates and 10-, 11- and 6-fold relative to the untreated db/db mice. BRL 26830 did not significantly affect the maximum activities of these enzymes in liver, heart or quadriceps muscle in either the db/db mice or their lean littermates.

It is not possible to equate directly increases in the maximum capacity of the glycolytic pathway with increases in the actual flux through that pathway. Therefore, to provide some indication of relative glucose flux into various tissues, the uptake of 2-deoxy[^{14}C]glucose in vivo has been measured (table 3). This glucose analogue is transported across membranes by the same carrier as glucose, is phosphorylated by hexokinase but is not metabolized further [24]. Net uptake of 2-deoxyglucose therefore reflects glucose consumed by a tissue. Two tissues which prove the exception to this are the liver and kidney, which have significant glucose 6-phosphatase activity [25]. In these tissues trapped 2-deoxyglucose 6-phosphate can be dephosphorylated and returned to the blood. Preliminary experiments carried out in these laboratories showed that whole animal recovery of radio label in normoglycaemic lean mice was constant. However, with an increasing, circulating blood glucose concentration, there was a progressive, but linear ($r = -0.83$) decrease in the overall recovery. This related to an increase in the urinary losses of 2-deoxy[^{14}C]glucose.

Since treatment of the db/db mice with BRL 26830 led to a substantial fall in the blood glucose concentration within 3 days of treatment,

Table 3

Effect of BRL 26830 on the relative tissue uptake of 2-deoxyglucose glucose uptake in vivo in db/db mice

	2-Deoxyglucose uptake (% whole animal)	
	db/db	db/db + BRL 26830
Interscapular brown adipose tissue	0.40 ± 0.05	1.85 ± 0.27***
Liver	11.53 ± 0.89	9.03 ± 0.56
Heart	1.38 ± 0.23	4.10 ± 0.39***
Brain	1.56 ± 0.40	1.01 ± 0.09
Kidneys	5.26 ± 0.55	2.46 ± 0.29***
Quadriceps	0.46 ± 0.06	0.57 ± 0.07
White adipose tissue (per g)	0.71 ± 0.08	0.56 ± 0.04

db/db mice were treated with BRL 26830 for 8 days. As a result, blood glucose concentration fell from 30.6 ± 1.1 to 11.6 ± 1.1 mM. Results are expressed as ^{14}C in total tissue (except white adipose tissue)/^{14}C recovered in animal (see text). Each value is the mean ± SE of 9 mice.
*** $P < 0.001$

2-deoxyglucose uptake was measured after a shorter period (8 days) than those of enzyme measurements. The 2-deoxyglucose was introduced into different sized glucose pools in the two sets of mice. Thus, it is not possible to make direct comparisons between the two groups of mice with respect to 2-deoxyglucose uptake into individual tissues. However, it is possible to make comparisons between different tissues within each group of mice. In the control db/db mice, uptake of 2-deoxyglucose into total brown adipose tissue was similar to that of the brain and heart (assuming that interscapular brown adipose tissue comprises 25% of the total tissue). Following treatment of the db/db mice with BRL 26830, there was a selective increase in the uptake of 2-deoxyglucose by brown adipose tissue so that the interscapular brown adipose tissue alone now consumed more 2-deoxyglucose than brain. These findings, taken together with the measurement of maximum enzyme activities of hexokinase and 6-phosphofructokinase, suggest that brown adipose tissue in the diabetic mouse has a low capacity for glucose utilisation and this plays an important role in the development of diabetes in this animal.

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