Tissue differences in the response of the pyruvate dehydrogenase complex to a glucose load during the development of obesity in gold–thioglucose-obese mice

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The activity of pyruvate dehydrogenase (PDHC), a key enzyme complex in the oxidative disposal of glucose, was measured after an oral glucose load in the heart, liver, quadriceps muscle, white adipose tissue (WAT) and brown adipose tissue (BAT) of gold–thioglucose (GTG)-obese mice at different stages during the development of obesity and in age-matched controls. Significant responses to the glucose load were seen 30 min post-gavage in heart, WAT and BAT of control mice but no change was observed in quadriceps muscle. The increase in activity of the active form of PDHC (PDHCa) in response to glucose in heart was reduced 2 weeks after the induction of GTG-obesity with no response in 5 or 10 week obese mice. A 2–3-fold increase in the

PDHCa response in both WAT and BAT of 2 week obese mice was absent in 5 and 10 week obese animals. Basal PDHCa activity in quadriceps muscle was increased in 2 week obese mice but subsequently returned to control levels as obesity progressed. The glucose load produced no change in the activity of PDHCa in quadriceps muscle of obese mice. These results demonstrate that changes in the capacity for oxidative glucose disposal in different tissues, as indicated by changes in PDHCa activity, may contribute to glucose-intolerance and insulin-resistance in GTG-obese mice and that the response of the PDHC to insulin during the development of obesity varies in different tissues.

INTRODUCTION

Studies in both animal models of non-insulin dependent diabetes (NIDDM) and in humans with NIDDM have shown that the hyperglycaemia that occurs following a glucose load is due to both increased basal hepatic glucose production and decreased cellular glucose uptake. Reductions occur in both non-oxidative and oxidative glucose disposal [1,2]. In normal subjects given an oral glucose load, both hepatic and peripheral tissues are important in glucose disposal, with forearm balance measurements suggesting that the predominant fate of glucose in skeletal muscle is oxidation [3]. Less is known of the fate of ingested glucose taken up by tissues other than muscle in either normal or NIDDM subjects.

The activity of the pyruvate dehydrogenase complex (PDHC) is a major determinant of the rate of oxidative glucose disposal [4]. The complex has an active and inactive form and its activity changes with the hormonal and nutritional status of the animal [4,5]. The proportion of PDHC in the active form (PDHCa) in a given tissue is often used as an index of glucose oxidation in that tissue [4]. Increased acetyl-CoA concentrations produced by the β oxidation of fats stimulate the activity of a specific PDH kinase, resulting in the conversion of the active dephosphorylated form of PDHC to the inactive phosphorylated form. This ability of fat oxidation to inhibit glucose oxidation by reducing PDHCa activity forms the basis of the glucose/fatty acid cycle, which was first described in heart muscle by Randle et al. [6]. The operation of this cycle is supported by human studies in which decreased whole-body glucose oxidation in NIDDM is accompanied by increased lipid oxidation [7], and by studies in which lipid infusion in normal subjects causes decreased glucose oxidation [8]. Studies of human PDHC activity are few and are restricted to skeletal muscle obtained from muscle biopsies. A recent study has shown that lipid infusion causes a decrease in insulin-

stimulated PDHCa activity in skeletal muscle in normal subjects [9]. Euglycemic clamp studies at physiological insulin levels in NIDDM patients have suggested a major defect in glucose oxidation [10]. However, reports of PDHC activity measurements in NIDDM patients have been inconclusive [11,12] and there is no information available about how the activity of PDHCa might change in different tissues during the disposal of a glucose load.

The gold–thioglucose (GTG)-obese mouse is a model of NIDDM and obesity. A single injection of GTG causes a necrotic lesion of the hypothalamus and results in hyperphagia, increased body weight and the development of hyperglycaemia and hyperinsulinaemia [13,14]. This model has been used to study the metabolic changes that occur during the development of insulin resistance [15–19]. Most of the reported metabolic changes associated with insulin resistance occur within 6 weeks of GTG injection during the period of the most rapid weight gain. Impaired glucose tolerance and increased insulin levels after an intravenous glucose load have been observed as early as 2 weeks post-GTG injection [20]. In previous studies, we have reported decreased PDHCa activity in the heart, quadriceps muscle and adipose tissue of fed obese animals together with increased activity in the liver [5,21], suggesting that in this animal model of NIDDM, glucose oxidation was increased in some tissues but decreased in others. However it is unclear how these changes in PDHCa activity (and presumably glucose oxidation) in the fed state relate to the impaired glucose tolerance observed in these animals.

This study reports the changes in PDHCa activity in the heart, liver, quadriceps muscle, and brown (BAT) and white (WAT) adipose tissues in response to short-term changes in serum glucose following an oral glucose load and the differences that occur in these responses during the development of obesity and insulin-resistance in the GTG-injected mouse.

Abbreviations used: PDHC, pyruvate dehydrogenase complex; PDHCa, active form of PDHC; WAT, white adipose tissue; BAT, brown adipose tissue; GTG, gold–thioglucose; NIDDM, non-insulin-dependent diabetes; CS, citrate synthase; NEFA, non-esterified free fatty acids.

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MATERIALS AND METHODS

Animals

Male CBA/T6 mice were obtained at 6 weeks of age from the Blackburn Animal House, University of Sydney. Obesity was induced by a single intraperitoneal injection (0.5 g/kg) of GTG (Sigma Chemical Co., St Louis, MO, U.S.A.) [12]. Mice were kept on a 12-h light/dark cycle, with the light cycle between 06:00 and 1800 h, and were allowed free access to food and water. The mice were studied at 2, 5 and 10 weeks after injection of GTG. On each experimental day, a minimum of eight GTG-obese mice from one age group and age-matched controls were used (i.e. one mouse per time-point). After an overnight fast, conscious mice were given an oral gavage of 200 μ l of 50% glucose solution (approx. 3 g/kg). Mice were then anaesthetized with an overdose of Nembutal at 0, 5, 10, 15, 30, 60, 90 and 120 min and tissues were removed and immediately homogenized (see below) for preparation of mitochondria for the assay of PDHCa activity and citrate synthase (CS) activity. PDHCa and CS activities were measured at 0, 15, 30, 60, 90 and 120 min. The tissues collected were the heart, liver, red quadriceps muscle, WAT from the epididymal fat pad and interscapular BAT. Blood was collected from the chest cavity after the heart had been removed and serum was frozen for subsequent assay of insulin, glucose and non-esterified free fatty acids (NEFA).

Mitochondrial preparation

Mitochondria were prepared after immediate homogenization of tissues in ice-cold mitochondrial preparation buffer as previously described [22]. This buffer consisted of 0.25 M sucrose, 0.5 mM Tris/HCl, 2 mM EGTA (pH 7.5) with 50 mM NaF and 10 mM sodium dichloroacetate added to inhibit interconversion of the PDHC and maintain the complex in its state *in vivo*. After the homogenate had been centrifuged at 800 *g* for 10 min, the supernatant was decanted and kept on ice. The pellet was rehomogenized in the preparation buffer and recentrifuged at 800 *g*. The combined supernatants were spun at 10000 *g* and the resulting mitochondrial pellet was transferred in a small amount of buffer to a microfuge tube, repelleted at 10000 *g* and stored in liquid nitrogen.

Enzyme assays

Mitochondria prepared from the heart, liver, quadriceps muscle, BAT and WAT were extracted for enzyme assay as previously described [22] by alternate thawing and refreezing in extraction buffer [50 mM potassium phosphate/10 mM EGTA/2 mM dithiothreitol (pH 7.0)]. PDHCa activity was assayed spectrophotometrically by coupling the production of acetyl-CoA to the acetylation of the dye *p*-(*p*-aminophenylazo)benzenesulphonic acid with the enzyme arylamine acetyltransferase [23]. CS activity was also measured [24] and PDHCa activity was expressed per unit of CS activity to correct for any differences in the recovery or purity of the mitochondrial extracts. CS activity when expressed as units/mg of protein was unchanged by age or obesity (J. M. Bryson and V. R. Wensley, unpublished work). Total PDHC activity was not measured in this study as previous studies have shown it to be unaffected by the age of the animal or the duration of obesity [19].

Serum analyses

Serum glucose was measured by a glucose oxidase/peroxidase method with 4-amino-antipyrine as the dye, serum insulin by a double-antibody radioimmunoassay using rat insulin standards

and anti-rat insulin first antibody (Linco Research, St Louis, MO, USA), and serum NEFA by a commercial enzymic colorimetric method *in vitro* (Wako Chemical Co., Osaka, Japan).

Statistics

Results are expressed as the mean $\pm$ S.E.M. Data were analysed by two-way analysis of variance (ANOVA) with or without repeated measurements, followed by Scheffe's *S post hoc* comparisons (SuperANOVA; Abacus Concepts, Berkeley, CA, U.S.A.). Differences between individual points and group means were determined using Student's *t*-test.

RESULTS

Serum analyses

There was no difference in the fasting level of serum glucose or glucose tolerance in the three age-groups of lean control-mice (Figure 1), with the serum glucose level peaking in all groups 10–15 min after the oral glucose load. Fasting serum glucose was elevated in the 5 and 10 week GTG-injected mice (Figure 1b and 1c) and all three GTG-injected groups exhibited impaired glucose

Figure 1 Serum glucose concentrations following an oral glucose load (3 g/kg) in control (○) and GTG-injected (●) mice at 2 weeks (a), 5 weeks (b) and 10 weeks (c) post-GTG injection

Values are mean $\pm$ S.E.M. ($n = 4-8$). Fasting glucose was greater than control levels in 5 week (control versus GTG: $P < 0.05$) and 10 week (control versus GTG: $P < 0.001$) GTG-injected animals. Significant increases in the overall response to glucose were seen at 2 weeks ($P < 0.05$), 5 weeks ($P < 0.001$) and 10 weeks ($P < 0.01$) post-injection.

Figure 2 Serum insulin concentrations following an oral glucose load (3 g/kg) in control (○) and GTG-injected (●) mice at 2 weeks (a), 5 weeks (b) and 10 weeks (c) post-GTG injection

Values are mean $\pm$ S.E.M. ($n = 4-8$). Significant increases in the overall response to glucose were seen in the obese mice at 5 weeks ($P < 0.01$) and 10 weeks ($P < 0.01$) post-injection.

disposal which worsened with the duration of obesity. Fasting insulin levels (Figure 2) were the same in all control groups with the peaks in serum insulin levels coinciding with the peaks in serum glucose. There was no fasting hyperinsulinaemia in any GTG-injected group. A hyperinsulinaemic response to the glucose load was seen after 2 weeks in the GTG mice when compared with age-matched controls (Figure 2a) but only reached significance in the 5 and 10 week CTG-injected groups (Figure 2b and 2c).

Fasting NEFA levels were similar in all control groups, with a small but not significant decrease in NEFA levels occurring 15 min after the glucose gavage. Fasting NEFA levels in GTG-obese mice were only slightly higher than controls at 5 weeks (controls: 2.77 ± 0.19 ; GTG: 3.50 ± 0.21 mequiv. per litre, $P < 0.05$) and the levels in all GTG-injected groups also fell slightly as the serum insulin levels rose.

PDHCa activity

The PDHCa activity in the fasting state, the change in PDHCa activity in response to a glucose load and the effect of GTG injection on the activity of PDHCa varied in each tissue studied. In the heart, fasting PDHCa activity was the same in all control groups (Figure 3). There was a 5-fold increase in heart PDHCa activity in all control groups 30 min after the glucose gavage, which was 15 min later than the observed peak in serum glucose

Figure 3 Heart PDHCa activity following an oral glucose load (3 g/kg) in control (○) and GTG-injected (●) mice at 2 weeks (a), 5 weeks (b) and 10 weeks (c) post-GTG injection

Values are mean $\pm$ S.E.M. ($n = 4-8$). A significant response to glucose was seen at 30 min. in all control groups (0 min versus 30 min: $P < 0.001$) and in the 2 weeks GTG-injected group (0 min versus 30 min: $P < 0.05$).

Table 1 Basal (0 min) PDHCa activity levels in quadriceps muscle of both control and GTG-obese mice during the development of obesity

Values are mean $\pm$ S.E.M. with n in parentheses. * $P < 0.01$ for differences between GTG-obese and control mice, ** $P < 0.001$ for differences from value at 2 weeks.

Weeks post-GTG injection	PDHCa (m-units/unit CS)	
	Control	GTG
2	25.52 ± 2.62 (6)	37.29 ± 1.73 (8)*
5	24.53 ± 2.18 (5)	31.86 ± 3.0 (6)
10	26.40 ± 2.33 (5)	25.08 ± 2.86 (6)**

and insulin. In all groups of GTG-injected mice, there was no difference in the fasting PDHCa activity, but 2 weeks after GTG injection the increase in PDHCa activity in heart in response to the glucose load was reduced (30% of the increase in controls) (Figure 3a). There was no change in heart PDHCa activity

Figure 4 BAT PDHC activity following an oral glucose load (3 g/kg) in control (○) and GTG-injected (●) mice at 2 weeks (a), 5 weeks (b) and 10 weeks (c) post-GTG injection

Values are mean $\pm$ S.E.M. ($n = 4-8$). Fasting PDHCa was significantly elevated in all GTG-injected groups (control versus GTG: $P < 0.05$). Response to glucose was significant in control mice at 2, 5 and 10 weeks ($P < 0.01$) and in GTG-injected mice at 2 weeks ($P < 0.001$).

observed in either 5 or 10 week GTG-obese animals after the oral glucose load (Figure 3b and 3c).

Basal activity of PDHCa in quadriceps muscle of control mice (Table 1) was much higher than in other tissues and was significantly increased 2 weeks post-GTG injection. This difference had decreased by 5 weeks post-GTG injection and at 10 weeks there was no difference between PDHCa activity in quadriceps muscle of control and GTG-injected mice. Neither the PDHCa in quadriceps muscle of control or of GTG-obese mice at any stage of obesity showed any increased activity in response to the glucose load.

Fasting PDHCa activity in BAT of control mice was unchanged with age (Figure 4). There was a significant, 1.5–2-fold increase in PDHCa activity in response to the glucose load in controls which became less with increasing age. In all GTG-injected animals, fasting PDHCa activity was higher throughout the glucose-tolerance test when compared with controls. An enhanced (2.5-fold) increase of BAT PDHCa activity was seen after the glucose load in the 2 weeks GTG-injected group (Figure 4a), but this increased response was lost by 5 weeks post-GTG injection (Figure 4b). In fact, there was no significant change in PDHCa activity in BAT in both 5 and 10 week GTG-injected animals after the glucose load.

Figure 5 WAT PDHC activity following an oral glucose load (3 g/kg) in control (○) and GTG-injected (●) mice at 2 weeks (a), 5 weeks (b) and 10 weeks (c) after GTG injection

Values are mean $\pm$ S.E.M. ($n = 4-8$). Significant age-related decreases in fasting PDHCa were seen in both control and GTG-injected mice (2 weeks versus 10 weeks: $P < 0.05$). GTG injection also caused a significant decrease in fasting PDHCa (control versus GTG: $P < 0.01$). Significant overall responses to glucose were seen in the 2 and 10 week control groups ($P < 0.05$) and in the 2 week GTG-injected mice ($P < 0.01$).

In WAT there was a small increase in PDHCa activity after the glucose load in control mice which decreased with age (Figure 5). In WAT of GTG-injected mice there was a decreased fasting level of PDHCa activity from 2 weeks post-GTG injection, but an increased response of PDHCa activity to the glucose load at 2 weeks post-GTG injection (Figure 5a) and no response of PDHCa activity to the glucose load in the more obese animals (Figures 5b and 5c).

Overnight fasting resulted in very low activities of liver PDHCa in lean mice of every age. In the control animals, increased PDHCa activity was seen in all age-groups at 30 min post gavage, but this response only reached significance in the 10 week group (0 min: 1.59 ± 0.44 ; 30 min: 5.57 ± 1.27 munits/unit CS, $P < 0.05$). Hepatic PDHCa of GTG mice showed only a slight response to the glucose load at all ages, with no difference between control and GTG-injected mice at any age.

DISCUSSION

The activity of PDHC has often been used as an indicator of glucose oxidation in a particular tissue, with the amount of this enzyme complex in the active form representing the maximal possible flux of glucose to carbon dioxide. Therefore, after a glucose load, an increase in PDHCa would be expected in tissues

important in oxidative glucose disposal. The results of this study show that tissue-specific patterns of PDHCa activity are obtained in response to an oral glucose challenge and that these responses are altered during the development of obesity in the GTG-injected mouse.

The large increase in cardiac PDHCa activity in control mice in response to glucose is consistent with the concept of increased glucose availability and circulating insulin levels leading to decreased fatty acid oxidation and increased glucose oxidation (the glucose/fatty acid cycle). However, no significant change in the activity of PDHCa was observed in quadriceps muscle, the skeletal muscle examined in this study. As skeletal muscle is considered to be the major site of glucose uptake following glucose ingestion, with oxidation being the major pathway for disposal [3], a substantial change in quadriceps muscle PDHCa activity was expected. The proportion of PDHC activity in the active form as a percentage of total PDHC activity is higher in quadriceps muscle than in other tissues [21]. The lack of a glucose effect in this experiment suggests that either the high basal PDHCa in quadriceps muscle is not rate-limiting for glucose oxidation or that glucose oxidation in quadriceps muscle is not increased after a glucose load. It may be that other skeletal muscles are more important in oxidative glucose disposal. Determination of PDHCa in rat muscles [25] and in human muscle biopsy samples following hyperinsulinaemic clamps have also shown little or no change in PDHCa [11,12], indicating that the capacity for glucose oxidation does not alter greatly in this tissue. Thus the responses of the PDHC in tissues other than quadriceps muscle may also be important in whole-body oxidative glucose disposal.

Overnight fasting reduced liver PDHCa activity to almost undetectable levels and the increase in serum insulin after the glucose load was not sufficient to significantly reactivate PDHC in this tissue. This is consistent with results of refeeding studies in starved rats, in which levels of PDHCa activity remained low for 4–8 h while glucose was directed towards glycogen synthesis [26]. In no tissue in the present study did the increase in PDHCa activity in response to the glucose load reach the level of activity seen in the fed state [5,21], indicating that a glucose load cannot reverse the effects of overnight starvation.

The induction of obesity in these mice was accompanied by tissue-specific PDHCa changes that were consistent with a period of enhanced insulin responsiveness preceding the development of insulin resistance. Thus, increased basal quadriceps muscle PDHCa and increased responses to glucose in BAT and WAT were seen in the 2 weeks post-injection group. This is in agreement with reports of increased insulin sensitivity in the early phases of the development of insulin-resistance in other models [27–34]. In heart muscle, the response of PDHCa activity was significantly reduced by 2 weeks post-GTG. Earlier studies showed increased heart PDHCa in fed animals 1 week post-GTG [19] and it is possible that a period of enhanced heart PDHCa activity has already been missed in the 2 weeks post-GTG animals. Thus, the hyperinsulinaemia demonstrated in these mice during intravenous glucose-tolerance tests at 2 weeks post-injection [20] initially increases the activity of processes sensitive to insulin, with insulin resistance occurring later as a result of prolonged periods of hyperinsulinaemia.

The decreased activity of PDHCa in the heart of fed GTG-obese mice and in adipocytes from high-fat fed diabetic mice can be restored by inhibitors of fatty acid oxidation [14,35], suggesting that the lack of a PDHC response in tissues of obese mice might be due to increased availability and oxidation of fatty acids. Hyperinsulinaemic/euglycaemic clamp studies in rats have shown a close correlation between insulin stimulation of PDHCa activity

and insulin suppression of serum fatty acid levels [25]. The differential changes in PDHCa activity in tissues of the same obese mice suggest that intracellular availability of fatty acids for oxidation, as well as circulating fatty acids, may contribute to the increased fatty acid oxidation which inhibits PDHC activity. Differences in the rate of accumulation of triacylglycerol stores in different tissues during the development of obesity could therefore explain the different PDHCa activities seen in this study.

Impaired glucose tolerance in humans can lead to many of the complications of NIDDM. This study demonstrates that the impaired glucose tolerance that develops in GTG-obese mice is associated with reduced PDHCa response to a glucose load in some tissues but not in skeletal muscle. It is possible that increasing PDHCa activity, perhaps by modification of fatty acid metabolism during the ingestion of glucose might help normalize glucose tolerance.

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