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Pyruvate dehydrogenase-complex activity in brown adipose tissue of gold thioglucose-obese mice

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The activity of pyruvate dehydrogenase (PDH) complex and PDH kinase were measured in brown adipose tissue (BAT) of 4-week-gold thioglucose (GTG)-obese mice. The proportion of PDH complex in the active dephosphorylated form was 2-fold higher in BAT of post-absorptive obese mice compared with lean controls. This result was consistent with the higher circulating insulin concentration observed in GTG-obese mice. In both obese and lean mice the PDH-complex activity in BAT decreased after 24 h starvation and increased in response to supraphysiological insulin injection, indicating that the PDH complex is insulin-responsive in BAT of GTG-obese mice. There was no difference in the PDH kinase activity of BAT in post-absorptive or insulin-injected lean and obese mice, suggesting that the higher PDH-complex activity in obese mice was not due to decreased PDH kinase activity. There is no evidence for a decreased activity of PDH complex contributing to insulin resistance in BAT of 4-week-GTG-obese mice.

INTRODUCTION

The peripheral insulin resistance observed in non-insulin-dependent diabetes mellitus (NIDDM) and obesity is thought to be due to several factors, including changes in the effect of insulin at the receptor and/or changes in the effect of insulin on intracellular processes [1]. One possible site of intracellular insulin resistance is the pyruvate dehydrogenase (PDH) complex, since this enzyme is important in determining both the amount of glucose oxidized to CO_2 and the amount of glucose converted into fatty acids [2]. PDH exists in two forms: a phosphorylated inactive form and a dephosphorylated active form. The interconversion of these forms is controlled by the activity of specific kinase and phosphatase enzymes [3]. In normal animals PDH-complex activity in adipose tissues is sensitive to the circulating insulin concentration, with the amount of complex in the active form increasing when insulin levels are high (after feeding) and decreasing when the insulin level is low, e.g. starvation and type I diabetes [2,3].

In animals with genetic (*fa/fa* rat) and chemically induced (gold thioglucose, GTG) obesity and NIDDM, an important contributory factor to hyperlipogenesis is the lack of diet-induced thermogenesis in brown adipose tissue (BAT) [4]. Obese animals are more efficient in converting nutrient intake into stored triacylglycerols, because the normal stimulation of the thermogenic mechanisms of BAT after feeding are defective [5,6]. The reduced thermogenesis in BAT of obese animals is accompanied by impaired insulin-sensitivity of deoxy[1-^{14}C]glucose uptake [7,8], and it has been postulated that changes in glucose uptake by BAT may contribute significantly to changes in whole-animal glucose utilization [9]. In long-term obesity in GTG-injected mice, reduced numbers of insulin receptors in BAT may be the cause of the decreased effect of insulin on glucose uptake [10]. However, the status of insulin-sensitive enzymes such as PDH complex in BAT of GTG-obese mice has not been determined, despite the fact that the insulin-responsiveness of this complex and its importance in fatty acid synthesis for thermogenesis has been investigated in normal animals [11,12].

To determine if the insulin resistance in BAT of GTG obese mice is accompanied by a failure of insulin to activate the PDH complex, we have measured the PDH-complex activity in BAT mitochondria isolated from control and GTG-obese mice in the starved and fed state, and after stimulation with a supra-physiological dose of insulin. The activity of PDH kinase was also measured to ascertain if changes in PDH-complex activity correlated with the activity of PDH kinase.

MATERIALS AND METHODS

Animals

Male CBA/T6 mice were obtained at 6 weeks of age from the Blackburn Animal House, Department of Pathology, 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.). At this dose of GTG, more than 95% of injected animals survive, and more than 90% of these become obese. Injected animals and age-matched controls were allowed free access to food and water and were kept at 21 °C on a 12 h-light/12 h-dark cycle. The composition of the diet (Rat and Mouse Kubes; Allied Feeds, Rhodes, N.S.W., Australia) was: crude protein, 23%; crude fat, 5%; crude fibre, 6%; and carbohydrate 65%.

Experimental procedure

Mice were studied 4 weeks after injection with GTG or 0.9% NaCl (controls). Obese and control mice were killed after starvation for 24 h, in the post-absorptive state, or in the post-absorptive state 30 min after an 0.3 ml intraperitoneal injection containing 1 unit of insulin (Actrapid; Novo, Copenhagen, Denmark)/kg and 1 g of glucose/kg (to prevent hypoglycaemia)

Abbreviations used: PDH, pyruvate dehydrogenase; BAT, brown adipose tissue; GTG, gold thioglucose (aurothioglucose); NIDDM, non-insulin-dependent diabetes mellitus; KAP, kinase activator protein.

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[11]. Interscapular BAT was rapidly excised and homogenized immediately in ice-cold mitochondrial preparation buffer.

Preparation of mitochondria

Interscapular BAT from four animals was used to prepare mitochondria in a buffer, containing interconversion inhibitors (10 mM-dichloroacetate and 50 mM-NaF), which maintains the PDH complex in the '*in vivo*' state of activation as previously described [13]. For estimation of total activity, the PDH complex was fully re-activated by incubation of mitochondria prepared in the absence of interconversion inhibitors in KCl medium (15 min, 30 °C) containing 10 μM-carbonyl cyanide *m*-chlorophenylhydrazone to deplete intramitochondrial ATP and inactivate the PDH kinase [14].

Enzyme assays

PDH-complex activity in extracts of BAT mitochondria 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 [13,14]. PDH kinase activity was assayed by measuring the ATP-dependent inactivation of PDH complex [13]. PDH kinase activity is expressed as the apparent first-order rate constant (K, min^{-1}) calculated from least-squares linear-regression analysis of ln(% active PDH complex) as a function of time.

Serum insulin assay

A blood sample was taken from the heart at the time the animal was killed, centrifuged at 10000 *g* (4 °C) for 5 min and the serum used for the assay of insulin by a double-antibody radioimmunoassay using rat insulin standards.

Protein assay

Protein content of mitochondrial preparations was measured by using a Bio-Rad kit, with ovalbumin as the standard.

RESULTS AND DISCUSSION

After 4 weeks, mice injected with GTG were approx. 10 g (25%) heavier than age-matched saline-injected control mice (33.5±1.4 versus 24.4±0.7 g respectively). At this stage in the development of obesity the GTG-injected mice have significantly higher serum insulin concentrations in both the fed and 24 h-starved state when compared with age-matched controls (Table 1).

In the post-absorptive state, PDH-complex activity in BAT of GTG-obese mice was approx. 2-fold higher than that of fed controls. This was paralleled by the higher insulin concentration observed in these animals, implying that the PDH complex in BAT of 4-week-GTG-obese mice is still sensitive to insulin (Table 1). However, there was no significant difference in the PDH-complex activity in BAT of control and obese mice after 24 h starvation, even though the insulin level in obese mice was still significantly higher. If post-absorptive control and GTG-obese mice were injected intraperitoneally with insulin plus glucose, PDH-complex activity in BAT was increased to the same extent, suggesting that insulin-responsiveness is still present in BAT of obese mice (Table 1). The decreased insulin-sensitivity reported for BAT from 4-week-GTG-obese mice with respect to 2-deoxyglucose uptake [8] and lipid synthesis [15] does not therefore appear to be the result of a decreased sensitivity of the PDH complex to insulin. In the experiments reported here, PDH-complex activity in control and GTG-obese mice seem to be directly related to the circulating insulin level of the animals, although it is not possible to tell from these experiments whether the ED_{50} (dose producing 50% of maximum effect) for insulin activation of PDH complex in BAT is altered in GTG-obese mice.

The control of PDH complex by insulin is partially mediated by the activity of the enzyme PDH kinase. In starvation, or when insulin levels are low, the activity of PDH kinase is elevated, owing to the increased activity of a specific protein activator of the kinase (KAP, kinase activator protein) [16]. We have previously shown that the decrease in PDH-complex activity in heart of GTG-obese mice is not due to KAP-induced changes in PDH kinase activity [13], and so it was of interest to determine whether the differences in PDH-complex activity in BAT of control and GTG-obese mice were also independent of changes in PDH kinase activity. There was no difference in the PDH kinase activity of BAT in control and 4-week-GTG-obese mice in the post-absorptive state or after the injection of insulin and glucose (Table 1). However, there was a significant increase in the PDH kinase activity of control- and obese-mouse BAT after 24 h starvation. Although the PDH kinase activity in BAT from both groups of animals increased (the normal response to

Table 1. Activities of PDH complex and PDH kinase in extracts of BAT mitochondria from control and obese mice after starvation and injection with insulin and glucose

Mice were studied at 4 weeks after the injection of NaCl (lean control) or gold thioglucose (obese). Control and obese mice were used in the fed state, after starvation for 24 h or in the fed state after injection with 1 unit of insulin/kg plus 1 g of glucose/kg intraperitoneally. Preparation, incubation and extraction of mitochondria, and assay of PDH complex and PDH kinase were carried out as described in the Materials and methods section. The results show the mean±S.E.M. for a minimum of nine separate preparations of mitochondria. The total activity of PDH complex in mitochondria from control and obese mice was 143.3±5.5 munits/mg of mitochondrial protein and 156.7±8.3 munits/mg of mitochondrial protein respectively (not significantly different). Significance: $^{**}P < 0.01$; $^{***}P < 0.005$ for the difference between control and obese mice under the same experimental conditions; $\#\#P < 0.01$; $\#\#\#P < 0.005$ for the difference from the post-absorptive state within the lean or obese group.

Treatment	Mice	PDH complex (active form) (munits/mg of mitochondrial protein)	PDH complex (active form) (% of total)	PDH kinase activity (K, min^{-1})	Serum insulin (μunits/ml)
Post-absorptive	Lean	8.7±1.1	6.2±0.6	0.45±0.06	18.9±3.9
	Obese	20.8±1.8***	14.1±1.4***	0.53±0.06	80.1±15.5***
Starved	Lean	5.5±1.7	3.2±0.7##	1.25±0.12###	3.5±0.4###
	Obese	6.4±2.0###	3.4±0.6###	0.72±0.05**###	20.7±2.8***###
Insulin+glucose	Lean	47.2±4.8###	29.4±3.3###	0.40±0.03	> 2000###
	Obese	45.2±4.6###	28.8±2.6###	0.50±0.05	> 2000###

starvation), this increase was not as marked in GTG-obese mice (Table 1). KAP-induced increases in PDH kinase activity are thought to be long-term effects mediated by changes in insulin concentration [17,18], and the results reported here support the insulin-sensitive nature of this control mechanism for PDH complex. In BAT of 24 h-starved obese mice the PDH kinase activity increased by only 50 % compared with the 120 % increase in BAT from 24 h-starved lean mice. This may be related to the fact that 24 h starvation in GTG-obese mice only decreases the serum insulin level to 20.8 ± 2.8 μunits/ml compared with 3.5 ± 0.4 μunits/ml in 24 h-starved lean mice.

The role of the PDH complex in BAT is to provide acetyl-CoA units for the synthesis of fatty acids or to allow some of the glucose taken up by this tissue to be oxidized to CO_2 directly [11,12]. There is conflicting evidence for the role of glucose as an oxidative substrate for thermogenesis in BAT. It is widely held that the purpose of glycolysis in BAT is to supply oxaloacetate for the maintenance of tricarboxylic-acid-cycle activity during thermogenesis, or that glycolytically derived ATP is needed to support cellular functions while BAT mitochondria are respiring in the uncoupled state [19]. There is also evidence that, in situations of prolonged thermogenesis, such as cold acclimation, there is a considerable amount of glucose taken up into BAT that is not accounted for by lipid synthesis or lactate production and is therefore being used directly as a thermogenic substrate [20]. Whether glucose is used as an oxidative substrate directly or indirectly via synthesis of fatty acids and subsequent β-oxidation, PDH-complex activity is required to convert pyruvate into acetyl-CoA. The results presented here suggest that the reduced lipid synthesis and glucose uptake in BAT of 4-week-GTG-obese mice is not the result of a decrease in the activity of PDH complex or the inability of PDH complex to respond to an increase in the circulating insulin concentration. It is possible that the activity of another insulin-sensitive enzyme involved in *de novo* fatty acid synthesis, namely acetyl-CoA carboxylase, is altered in the obese mice [11] or that the insulin-resistance observed in BAT with respect to glucose transport and lipogenesis is not due to the inability of insulin to activate key intracellular enzymes.

The control of PDH-complex activity in BAT appears to be similar to that of other tissues. In starvation, PDH-complex activity is decreased and the activity of PDH kinase is increased, owing to increased activity of KAP [16]. As previously reported, PDH-complex activity in BAT is increased by insulin [11], and this insulin-responsiveness does not appear to be affected by GTG-induced obesity. In heart muscle of GTG-obese mice the activity of the PDH complex is decreased compared with lean fed controls [21]. This is thought to be the result of increased fatty acid oxidation, which increases acetyl-CoA levels and stimulates PDH kinase [22]. This in turn, leads to reduced PDH-complex activity, despite high circulating insulin levels. However, in BAT of obese mice, where there is a lack of sympathetic stimulation of thermogenesis [6], β-oxidation does not occur to a great extent, despite increased fatty acid availability. Therefore acetyl-CoA concentrations would be lower and PDH kinase would not be activated, resulting in a PDH-complex activity higher than that of control mice.

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REFERENCES

1. Olefsky, J. M. (1981) Am. J. Med. **70**, 151–168
2. Wieland, O. H. (1983) Rev. Physiol. Pharmacol. **154**, 327–348
3. Randle, P. J. (1986) Biochem. Soc. Trans. **14**, 799–806
4. Rothwell, N. J. & Stock, M. J. (1988) Diabetes/Metab. Rev. **4**, 595–601
5. Ashwell, M., Holt, S., Jennings, G., Stirling, D. M., Trayhurn, P. & York, D. A. (1985) FEBS Lett. **179**, 233–237
6. Hogan, S. & Himms-Hagen, J. (1983) Am. J. Physiol. **244**, E581–E588
7. Ferre, P., Burnol, A.-F., Letturque, A., Terretaz, J., Penicaud, L., Jeanrenaud, B. & Girard, J. (1986) Biochem. J. **233**, 249–252
8. Cooney, G. J., Astbury, L. D., Williams, P. F. & Caterson, I. D. (1987) Diabetes **36**, 152–158
9. Cooney, G. J. & Newsholme, E. A. (1984) Trends Biochem. Sci. **9**, 303–305
10. Tanti, J. F., Gremeaux, T., Brandenburg, D., Van Obberghen, E. & Le Marchand-Brustel, Y. (1986) Diabetes **35**, 1243–1248
11. McCormack, J. G. & Denton, R. M. (1977) Biochem. J. **166**, 627–630
12. McCormack, J. G. (1982) Prog. Lipid Res. **21**, 195–223
13. Caterson, I. D., Kerbey, A. L., Cooney, G. J., Frankland, R. L., Denyer, G. S., Nicks, J. L. & Williams, P. F. (1988) Biochem. J. **253**, 291–294
14. Denyer, G. S., Lam, D., Cooney, G. J. & Caterson, I. D. (1989) FEBS Lett. **250**, 464–468
15. Cooney, G. J., Vanner, M. A., Nicks, J. L., Williams, P. F. & Caterson, I. D. (1989) Biochem. J. **259**, 651–657
16. Denyer, G. S., Kerbey, A. L. & Randle, P. J. (1986) Biochem. J. **239**, 347–354
17. Fatania, H. R., Vary, T. C. & Randle, P. J. (1986) Biochem. J. **234**, 233–236
18. Marchington, D. R., Kerbey, A. L., Girardina, M. G., Jones, A. E. & Randle, P. J. (1989) Biochem. J. **257**, 487–491
19. Ma. S. W. Y. & Foster, D. O. (1986) Can. J. Physiol. Pharmacol. **64**, 609–614
20. Lopez-Soriano, F. J., Fernandez-Lopez, J. A., Mampel, T., Villarroya, F., Iglesias, R. & Alemany, M. (1988) Biochem. J. **252**, 843–849
21. Caterosn, I. D., Astbury, L. D., Williams, P. F., Vanner, M. A., Cooney, G. J. & Turtle, J. R. (1987) Biochem. J. **243**, 549–553
22. Caterson, I. D., Williams, P. F., Kerbey, A. L., Astbury, L. D., Plehwe, W. E. & Turtle, J. R. (1984) Biochem. J. **224**, 787–791

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