The effect of body weight and the fatty acid-oxidation inhibitor 2-tetradecylglycidic acid on pyruvate dehydrogenase complex activity in mouse heart

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The proportion of pyruvate dehydrogenase complex in the active, dephosphorylated form was decreased (compared with lean controls) in heart muscle in gold thioglucose-treated obese hyperinsulinaemic mice, and the extent of enzyme inactivation was significantly linearly correlated with both body weight and body fat content. A single oral dose (25 mg/kg body wt.) of the β -oxidation inhibitor 2-tetradecylglycidic acid to obese animals restored pyruvate dehydrogenase complex activity to that of lean controls. It is suggested that increased fatty acid oxidation may be a major factor in mediating the phosphorylation and inactivation of pyruvate dehydrogenase complex in mouse heart muscle in obesity, and this may represent an important mechanism in the development and/or expression of insulin resistance in respect of abnormalities of cellular glucose homeostasis in these animals.

Cellular resistance to the action of insulin occurs in both human and animal obesity syndromes (Assimakopoulos-Jeannet & Jeanrenaud, 1976; Crettaz *et al.*, 1980; Olefsky, 1980) and is characterized by hyperinsulinaemia and a substantial inhibition (compared with lean controls) in the rates of glucose transport and oxidation in skeletal muscle, heart muscle and adipocytes (Le Marchand-Brustel *et al.*, 1978; Olefsky, 1980; Kolterman *et al.*, 1981). Changes at the level of insulin-receptor-complex interaction may contribute to the development and/or expression of insulin resistance in obesity, but may not be the sole determinant of insulin resistance under these conditions, and a defect(s) distal to insulin-receptor interaction may be responsible for the observed changes in glucose metabolism (Olefsky, 1980; Kolterman *et al.*, 1981; Rosen & Reinauer, 1984).

Intramitochondrial pyruvate dehydrogenase complex (EC 1.2.4.1 + EC 2.3.1.12 + EC 1.6.4.3) activity is the major determinant of glucose oxidation in animal cells. It is inactivated by phosphorylation (pyruvate dehydrogenase kinase) and re-activated by dephosphorylation (pyruvate

dehydrogenase phosphate phosphatase) (Linn *et al.*, 1969). The kinase reaction is activated by increasing mitochondrial concentration ratios of NADH/NAD⁺ and acetyl-CoA/CoA (Kerbey *et al.*, 1976, 1977). In the rat, glucose oxidation in a number of tissues, including heart muscle, is less in conditions in which serum insulin is low (starvation and alloxan-diabetes), and this is caused by a decrease in the proportion of pyruvate dehydrogenase complex in the active, dephosphorylated form (Kerbey *et al.*, 1976; Caterson *et al.*, 1982). Increased kinase activity appears to be mainly responsible, and this is due partly to kinase activation by products of fat metabolism (Kerbey *et al.*, 1976; Hutson & Randle, 1978; Kerbey & Randle, 1981; Caterson *et al.*, 1982).

The observation that glucose oxidation in heart muscle, skeletal muscle and adipocytes is markedly impaired in animal models of obesity, (Crettaz *et al.*, 1980; Olefsky, 1980) suggested that inhibition of pyruvate dehydrogenase complex may be a major factor leading to diminished rates of glucose oxidation in these tissues. Furthermore, it seemed that the mechanisms that operate in experimental and possibly human obesity to regulate inter-conversion of pyruvate dehydrogenase complex between the active and inactive forms (and hence rates of glucose oxidation) may be related to those that operate in animal tissues in starvation and

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insulin-dependent diabetes, and in isolated tissue preparations oxidizing fatty acids and ketone bodies (for review, see Randle *et al.*, 1978). In a study with gold thioglucose-treated obese hyperinsulinaemic mice, certain aspects of this hypothesis were realized when we demonstrated *in vivo* a marked decrease (compared with lean control animals) in the proportion of active, dephosphorylated, pyruvate dehydrogenase complex in heart muscle, which persisted during the preparation and extraction of heart mitochondria (Kerbey *et al.*, 1984).

In the present study we have investigated the effect of increasing body weight and body fat content on heart muscle pyruvate dehydrogenase complex activity in gold thioglucose-treated mice. In addition, we have assessed [using 2-tetradecylglycidic acid, an inhibitor of the oxidation of long-chain fatty acids (Tutwiler *et al.*, 1978, 1979; Tutwiler & Dellepigne, 1979; Pearce *et al.*, 1979; Bowden *et al.*, 1981; Caterson *et al.*, 1982)] the contribution of β -oxidation of fatty acids to phosphorylation and inactivation of pyruvate dehydrogenase complex in heart muscle in gold thioglucose-treated obese mice.

Materials and methods

Materials

Male CBA/T6 mice (6 weeks old; 20–25 g) were provided by the Blackburn Animal House, Department of Immunology, University of Sydney, N.S.W., Australia. These animals were allowed free access to food (Rat & Mouse Kubes, Allied Feeds, Rhodes, N.S.W., Australia) and water. Obesity was induced by a single intraperitoneal injection of gold thioglucose (Sigma Chemical Co., St. Louis, MO, U.S.A.; 0.5 mg/g body wt.) as described previously (Kerbey *et al.*, 1984). 2-Tetradecylglycidic acid (McNeil 3802) was generously given by Dr. G. Tutwiler, McNeil Laboratories, Fort Washington, PA, U.S.A. Bovine serum albumin was obtained from Sigma. Anti-insulin antibody (binding reagent) was obtained from Burroughs Wellcome (Aust.) Pty. Ltd., Sydney, N.S.W., Australia. All other chemicals and biochemicals were of the highest grade of purity available.

Animals and enzymes

Mice were injected intraperitoneally with sodium pentobarbitone (60 mg/kg body wt.) to induce anaesthesia, and the heart was removed some 2 min later. Hearts were frozen immediately with a tissue clamp pre-cooled in liquid N₂. Pyruvate dehydrogenase complex and citrate synthase activity were extracted from frozen tissue

as described previously (Kerbey *et al.*, 1976; Caterson *et al.*, 1982).

In some experiments fed mice (both lean control and obese) were given 2-tetradecylglycidic acid as a suspension in 0.5% (w/v) methylcellulose by intragastric tube as described by Tutwiler *et al.* (1979). The dose given was 25 mg/kg body wt. and was given at 74, 50, 26 and 2 h before anaesthesia or just 2 h before anaesthesia as described above. Control mice received methylcellulose alone.

Assays

Pyruvate dehydrogenase complex and citrate synthase. Pyruvate dehydrogenase complex (active form) and citrate synthase in extracts of heart were assayed spectrophotometrically as described by Kerbey *et al.* (1976). One unit of enzyme activity converts 1 μ mol of substrate into product/min at 30°C.

Glucose and insulin. Immediately after removal of the heart, a sample of blood was withdrawn from the chest cavity. Blood samples were centrifuged (10000g, 15 min, 4°C), and serum was used for the determination of glucose (by a glucose oxidase method) and immunoreactive insulin (double-antibody radioimmunoassay using human insulin standards).

Total body fat. This was measured by an adaptation of the method of Bligh & Dyer (1959). All operations were performed at 24°C. A mouse carcass was homogenized in a Sorvall Omnimixer (2 min) in chloroform/methanol (1:2, v/v; 1.0 ml of chloroform/g carcass wt.). After addition of a further 1 vol. of chloroform, the mixture was homogenized for 30 s. This was followed by the addition of 1 vol. of distilled water and a further period of homogenization. The homogenate was filtered in a Buchner funnel through Whatman no. 1 filter paper and washed with 3 vol. more of chloroform. The filtrate was allowed to separate, and the total volume of chloroform was measured. A portion (25 ml) was evaporated to dryness, weighed and redissolved in 10 ml of chloroform. This was chromatographed on a Sephadex G25F column [25 cm $\times$ 1 cm; equilibrated with chloroform/methanol (19:1, v/v, saturated with water)]. After evaporation of the void-volume eluate of this column, the lipid content of the chloroform sample was calculated, together with the total lipid content of the mouse carcass. Total lipid was expressed as a percentage of the carcass weight.

Results and discussion

In this strain of mice, the percentage of body fat increases significantly and linearly as carcass weight increases (results not shown; $r = 0.926$, $P < 0.001$). In both control (lean) and gold thio-

Fig. 1. Relationship between the activity of the pyruvate dehydrogenase complex (PDH_a) and body weight (a) in control mice (■) and (b) in obese (gold thioglucose-injected) mice (●)

Animals were of ages 8–40 weeks (0–32 weeks after gold thioglucose injection). For details of feeding of mice, induction of obesity and assay of pyruvate dehydrogenase complex (active form) see the Materials and methods section. Experiments with both groups of animals were performed in parallel and completed within 1 day: 68 control and 111 gold thioglucose-injected mice were studied. Least-squares linear-regression analysis gave a correlation coefficient of -0.449 ($P < 0.002$) for control animals and of -0.557 ($P < 0.0001$) for gold thioglucose-injected obese animals. (For difference between groups: $F = 0.418$, $P > 0.5$.)

glucose-induced obese animals there was a gradual decrease in the amount of pyruvate dehydrogenase in the active form as weight increased (see Figs. 1a and 1b). Least-squares linear-regression analysis of variance gave a good correlation ($r = -0.449$; $P < 0.002$) for the control animals (pyruvate dehydrogenase complex activity versus weight)

and for gold thioglucose-injected obese animals the correlation was -0.557 ($P < 0.0001$). The slope of the regression line was -0.040 for control animals and -0.035 for the gold thioglucose-injected animals. For any given weight above 28 g, the lean control animals are older, and hence in these animals an effect of age on the activity of the pyruvate dehydrogenase complex cannot be eliminated. The values for active pyruvate dehydrogenase complex obtained with lean animals less than 16 weeks of age (i.e. within 8 weeks of injection) were qualitatively similar to the values obtained in hearts from lean rats (Kerbey *et al.*, 1976; Caterson *et al.*, 1982). The mean value obtained in gold thioglucose-injected obese animals was 33.7% of that of lean controls (1.55 ± 0.33 and 4.59 ± 0.62 units/g dry wt., with 14 and 5 observations respectively).

A single dose of 2-tetradecylglycidic acid (25 mg/kg) given by intragastric tube to obese mice 2 h before death increased the activity of the dehydrogenase complex (Table 1) to a value higher than, but not significantly different from, that of treated lean control mice. There was no significant change in the dehydrogenase complex activity in control mice. The carrier (methylcellulose) for this drug had little if any effect on the complex activity in control and obese mice. The mice used in this experiment were older (26 weeks after gold thioglucose injection, 34 weeks old), and this age difference may account for the fact that serum glucose was slightly increased in these animals. This effect of age and stress in gold thioglucose-induced obesity has been recognized previously (Katsuki *et al.*, 1962), and these animals are not diabetic under normal conditions. The mice with increased serum glucose were not necessarily the most obese. As well as age, starvation and stress have been reported to affect serum glucose concentrations in *ob/ob* mice (Dubuc *et al.*, 1983). An acute dose of 2-tetradecylglycidic acid had no significant effect on serum insulin concentrations in control mice, but it did significantly decrease the raised serum insulin in gold thioglucose-injected obese mice. The insulin concentration in treated obese mice remained extremely high, indicating that a degree of cellular insulin resistance still existed.

In a group of control mice (34 weeks old), 2-tetradecylglycidic acid (25 mg/kg) given in four doses between 74 and 2 h before death raised the activity of the dehydrogenase complex from 1.91 ± 0.26 (11) to 3.30 ± 0.52 (13) units/g dry wt. ($P < 0.05$). The lower dehydrogenase complex activity in these control mice may have been due to the effect of weight or perhaps of age (see Fig. 1a). In a group of obese mice, dehydrogenase complex activity was also increased from 0.61 ± 0.09 (12) to 1.98 ± 0.33 (13) units/g dry wt. ($P < 0.001$) by 2-

Table 1. *Effects of 2-tetradecylglycidic acid on heart muscle pyruvate dehydrogenase complex activity and on serum glucose and insulin concentrations in lean control and gold thioglucose-treated obese mice*

Mice (lean and obese; 34 weeks of age) were given 2-tetradecylglycidic acid (TDG; 25 mg/kg body wt.) as a suspension in 0.5% (w/v) methylcellulose by intragastric tube (Tutwiler *et al.*, 1979) 2 h before being killed. Control animals received methylcellulose alone. For details of feeding of mice, induction of obesity, extraction and assay of pyruvate dehydrogenase complex (active form) and citrate synthase, and assay of serum glucose and insulin, see the Materials and methods section. Experiments with all four groups of animals were performed in parallel and were completed in 1 day. Results are given as means $\pm$ S.E.M. for the numbers of animals shown in parentheses. For statistical significance of differences: * $P < 0.05$, ** $P < 0.01$ against lean controls in the same treatment group; † $P < 0.01$, †† $P < 0.001$ against obese animals treated with methylcellulose. Other differences are not significant.

Mice	Treatment	Body wt. (g)	Citrate synthase (units/g dry wt.)	Active pyruvate dehydrogenase complex		Serum glucose (mmol/l)	Serum insulin (munits/l)
				(units/g dry wt.)	(ratio munits/ unit of citrate synthase)		
Lean control	Control	35.0 $\pm$ 0.8 (9)	506.7 $\pm$ 31.0 (9)	3.82 $\pm$ 0.77 (9)	8.2 $\pm$ 1.9 (9)	13.4 $\pm$ 0.6 (9)	51 $\pm$ 8 (9)
	TDG	32.6 $\pm$ 0.4 (9)	466.5 $\pm$ 30.4 (9)	4.80 $\pm$ 1.08 (9)	11.6 $\pm$ 3.1 (9)	14.4 $\pm$ 0.5 (9)	34 $\pm$ 7 (9)
Obese	Control	45.0 $\pm$ 0.8** (15)	508.2 $\pm$ 23.1 (14)	1.18 $\pm$ 0.19** (12)	2.5 $\pm$ 0.5** (12)	15.6 $\pm$ 0.8* (14)	489 $\pm$ 145** (15)
	TDG	43.4 $\pm$ 1.0** (16)	481.4 $\pm$ 27.3 (16)	7.54 $\pm$ 1.13†† (14)	18.6 $\pm$ 4.7† (14)	16.3 $\pm$ 0.5* (16)	298 $\pm$ 86**† (15)

tetradecylglycidic acid. The initial dehydrogenase complex activity in these obese mice was 35% of that of control mice of the same age. 2-Tetradecylglycidic acid had no effect on serum glucose or insulin concentrations in these mice, serum insulin being increased in the obese animals. In obese animals given methylcellulose, serum insulin was 83 $\pm$ 13 (12) compared with 49 $\pm$ 2 (11) munits/l in control animals ($P < 0.02$). In animals given four doses of 2-tetradecylglycidic acid, the corresponding values were 94 $\pm$ 14 (13) and 48 $\pm$ 3 (12) munits/l ($P < 0.01$).

General discussion and conclusions

In gold thioglucose-induced obesity in mice, there is a decrease in the amount of the pyruvate dehydrogenase complex in the active (dephosphorylated) form in heart. It has also been shown that there is an increase in the total amount of pyruvate dehydrogenase complex in these animals (Kerbey *et al.*, 1984). A decrease in activity of the complex with increasing body weight also occurs in control (non-injected) animals. This decrease in activity may be due to increasing age of the animal. With age and with gold thioglucose-induced obesity there is an increase in the proportion of the body which is adipose tissue (James, 1983), and this increase in adipose tissue may perhaps predispose to a greater availability of fatty acids for oxidation.

In starvation and diabetes, β -oxidation of fatty acids may activate the kinase reaction and facilitate phosphorylation and inactivation of the pyruvate dehydrogenase complex (Kerbey *et al.*,

1976; Kerbey & Randle, 1979). The present study has shown that, in obese mice, a single oral dose of the β -oxidation inhibitor 2-tetradecylglycidic acid is sufficient to increase the activity of heart pyruvate dehydrogenase complex. The activation (dephosphorylation) of the dehydrogenase complex is not due to depletion of ATP, since an identical dose (25 mg/kg) given intravenously to alloxan-diabetic rats does not increase the activity of the pyruvate dehydrogenase complex (Caterson *et al.*, 1982), and rats treated for 3 weeks with this drug show no ill effects (Tutwiler *et al.*, 1979). Treatment with 2-tetradecylglycidic acid only decreased serum insulin concentrations slightly, and it is therefore unlikely that this inhibitor is working through altered insulin concentrations. These results are in agreement with those obtained by Rosen & Reinauer (1984), who studied the effects of the carnitine palmitoyltransferase 1 inhibitor phenylalkyloxiranecarboxylic acid in isolated perfused hearts of streptozotocin-diabetic rats. These investigators found that this inhibitor decreased myocardial lipolysis, accelerated the rate of pyruvate and lactate outflow and increased pyruvate oxidation. They concluded that the inhibition of glucose oxidation in heart muscle in streptozotocin-diabetes is mediated by excessive metabolism of endogenous triacylglycerol.

In obesity, increased oxidation of fatty acids in the heart may be a consequence of relative insulin deficiency and/or of increased fatty acid availability owing to an increased adipose-tissue mass.

Restoration of the activity of the pyruvate

dehydrogenase complex to normal by 2-tetradecylglycidic acid in obesity, as in states of insulin deficiency in rats (Caterson *et al.*, 1982), is consistent with the concept of obesity as a state of relative insulin deficiency and implicates increased β -oxidation of fatty acids as a primary mediator in the inhibition of the activity of the enzyme complex.

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