Biochem. J. (1987) 243, 549–553 (Printed in Great Britain)

The activity of the pyruvate dehydrogenase complex in heart and liver from mice during the development of obesity and insulin resistance

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The amount of pyruvate dehydrogenase in the active form (PDH_a) was increased 1.7-fold compared with controls in heart muscle of mice 1 week after induction of obesity with a single injection of gold–thioglucose. At 4 weeks post injection, the amount of PDH_a was decreased to 32% of control, a value which was observed in later stages of the obesity syndrome. In contrast, liver PDH_a was increased and remained at an increased activity during the development of obesity. Despite normal post-prandial serum insulin contents, liver membrane insulin-receptor numbers were decreased 1 week after gold–thioglucose injection, and there was no change in receptor affinity. The decrease in heart PDH_a in the obese animals was reversed by a single dose of 2-tetradecylglycidic acid, but this inhibitor of mitochondrial fatty acid oxidation did not affect liver PDH_a in these animals. These early and diverse changes in PDH_a argue for a multifactorial aetiology in the development of the whole-body insulin resistance seen in older gold–thioglucose-treated obese animals.

INTRODUCTION

The proportion of pyruvate dehydrogenase in the active dephosphorylated form (PDH_a) is decreased in heart muscle of the gold–thioglucose-treated obese mouse, despite an increase in the total PDH activity (Kerbey *et al.*, 1984; Caterson *et al.*, 1984) and the degree of enzyme inactivation is linearly correlated with both body weight and body fat content (Caterson *et al.*, 1984). Glucose oxidation is also impaired in heart muscle, skeletal muscle and adipocytes in animal models of obesity (Crettaz *et al.*, 1980; Olefsky, 1981). These observations suggest that inhibition of the PDH complex may be a factor leading to decreased glucose oxidation in these tissues in obesity, since the activity of the PDH complex is a major determinant of glucose oxidation in animal cells.

Inactivation (by phosphorylation) of the PDH enzyme complex requires a specific kinase that is activated by increasing mitochondrial [NADH]/[NAD^+] and [acetyl-CoA]/[CoA] ratios (Linn *et al.*, 1969; Kerbey *et al.*, 1976). The enzyme complex requires dephosphorylation to be re-activated (Linn *et al.*, 1969). In the rat, both starvation and alloxan-diabetes are conditions in which glucose oxidation is decreased, as is the proportion of PDH_a (Kerbey *et al.*, 1976; Caterson *et al.*, 1982). This decrease in activity is brought about in part by the activation of kinase by the products of fatty acid oxidation (Hutson & Randle, 1978; Caterson *et al.*, 1982). Serum insulin is also low in these animals, and the decreased PDH activity may be a result of the hypoinsulinaemia in these conditions.

However, in the obese mouse, despite increased total PDH activity and hyperinsulinaemia, the proportion of PDH_a in heart muscle is decreased (Kerbey *et al.*, 1984). Treatment of these obese animals with 2-tetradecylglycidic acid, an inhibitor of the mitochondrial β-oxidation of long-chain fatty acids, increased the amount of PDH_a (Caterson *et al.*, 1984). Therefore, it is presumed that the decrease in the proportion of PDH_a in heart muscle is a consequence of increased β-oxidation in that tissue. However, to provide the energy and precursors for synthesis of the triacylglycerol that is stored in the gold–thioglucose-induced obesity syndrome, the PDH complex must be activated in some tissues during the development of obesity. The current study establishes that there are diverse changes in the activity of the PDH complex in heart muscle and liver from gold–thioglucose-treated mice during the development of obesity. It also seeks to correlate the observed changes in the proportion of PDH_a with the decrease in insulin-receptor numbers that occurs before the onset of hyperinsulinaemia and insulin resistance in starvation in this animal model of obesity (Assimacopoulos-Jeannet & Jeanrenaud, 1976).

MATERIALS AND METHODS

Animals

Obesity was induced in male CBA/T6 mice (Blackburn Animal House, University of Sydney, N.S.W., Australia) by a single intraperitoneal injection of gold–thioglucose (0.5 mg/kg body wt.) as described previously (Caterson & Taylor, 1982; Kerbey *et al.*, 1984). Then, 2–3 h after removal from food, mice were injected with sodium pentobarbitone (60 mg/kg body wt., intraperitoneally) to induce anaesthesia, and tissues were removed some 2 min later and immediately frozen with a tissue clamp precooled in liquid N_2.

Some mice (4 weeks after gold–thioglucose injection)

Abbreviations used: PDH, pyruvate dehydrogenase; PDH_a, its active form.

were treated with a single oral dose of 2-tetradecylglycidic acid (25 mg/kg body wt.) as a suspension in 0.5% methylcellulose by intragastric tube 2 h before being killed (Caterson *et al.*, 1984). 2-Tetradecylglycidic acid, an inhibitor of mitochondrial β-oxidation of long-chain fatty acids, was generously given by Dr G. Tutwiler, McNeil Pharmaceuticals, Fort Washington, PA, U.S.A.

Enzyme assays

The PDH complex and citrate synthase activity were extracted from the frozen tissue as described previously (Kerbey *et al.*, 1976; Caterson *et al.*, 1982) and was assayed within 4 h. Total PDH activity was assayed after incubation of tissue extracts with PDH phosphate phosphatase (Whitehouse *et al.*, 1974). One unit of enzyme activity converts 1 μmol of substrate into product per min at 30 °C. These enzyme activities were measured in extracts of heart and liver. The methods used for extraction and assay of PDH, both active (PDH_a) and total forms, have been validated and described previously (Whitehouse *et al.*, 1974; Kerbey *et al.*, 1976). Citrate synthase activity of each extract was used to determine the adequacy of extraction.

Serum assays

Immediately the heart was removed from the animal, a sample of blood (approx. 0.5 ml) was withdrawn from the chest cavity. This sample was centrifuged (14000 ***g***, 15 min, 4 °C), and serum was stored for assay. Serum glucose was measured by a glucose oxidase method. Immunoreactive insulin was measured by a double-antibody radioimmunoassay as in Kerbey *et al.* (1984).

Serum triacylglycerols were measured by a colorimetric determination of glycerol released by the enzymic hydrolysis of triacylglycerols by using esterase, glycerol kinase, glycerolphosphate oxidase and a peroxidase. Reagents were supplied as a kit by Boehringer Mannheim, Sydney, Australia.

Insulin-binding studies

Monocomponent pig insulin (Novo Industries AS, Copenhagen, Denmark) was iodinated by the chloramine-T method to a specific radioactivity of 6.6–7.4 MBq/μg. ^{125}I was obtained from Amersham International, Amersham, Bucks., U.K. A liver membrane fraction was prepared as described for placental membranes by Williams & Turtle (1979). ^{125}I-insulin binding to liver membrane preparations was conducted at 4 °C as described previously (Williams & Turtle, 1979). Insulin-displacement curves were analysed by the method of Scatchard (1949). Association constants for high- and low-affinity binding sites (K_{a1}, K_{a2}) and the concentrations of high- and low-affinity receptors (N_1, N_2) were calculated by using the non-linear least-squares curve-fitting computer program 'Ligand' (Munson & Rodbard, 1980).

Source of all chemicals and reagents were otherwise as described in Kerbey *et al.* (1984).

RESULTS

After a single intraperitoneal injection of gold–thioglucose, CBA/T6 mice gained weight more rapidly than did control mice. This weight increase is statistically significant after 3 weeks (Table 1). There was no consistent difference in serum glucose concentrations

Table 1. Changes in weight, glucose, insulin and triacylglycerols in CAB/T6 mice with time after a single intraperitoneal injection of gold–thioglucose (0.5 mg/g body wt.)

All results are expressed as means ± S.E.M., with numbers of animals shown in parentheses. Statistical significance of differences (compared with corresponding lean control group): $*P < 0.05$; $**P < 0.01$; $***P < 0.005$. For details of feeding of mice, induction of obesity and assay of serum glucose and insulin see the Materials and methods section.

Time after injection (weeks)	Controls				Gold–thioglucose-injected			
	Body wt. (g)	Serum glucose (mM)	Serum insulin (μunits/ml)	Serum triacylglycerol (mM)	Body wt. (g)	Serum glucose (mM)	Serum insulin (μunits/ml)	Serum triacylglycerol (mM)
0	23.7 ± 0.8 (10)	11.5 ± 1.6 (10)	23 ± 5 (5)					
1	23.1 ± 0.6 (9)	14.9 ± 0.8 (9)	50 ± 8 (9)		23.0 ± 0.8 (9)	14.2 ± 0.5 (9)	56 ± 4 (7)	
2	24.9 ± 0.2 (8)	12.4 ± 2.4 (7)	70 ± 6 (6)		25.8 ± 0.6 (16)	17.0 ± 0.8 (16)	58 ± 11 (16)	
3	24.8 ± 0.3 (13)	15.6 ± 0.7 (10)	50 ± 12 (12)		29.0 ± 0.7 (17)***	16.4 ± 0.6 (11)	47 ± 6 (15)	
4	24.7 ± 0.6 (18)	15.7 ± 0.6 (18)	66 ± 9 (15)		30.2 ± 0.8 (17)***	17.6 ± 0.6 (17)*	65 ± 6 (16)	
6	26.2 ± 0.6 (21)	15.1 ± 0.6 (7)	55 ± 9 (21)		32.8 ± 0.8 (27)***	15.9 ± 0.5 (13)	80 ± 13 (23)	
8	25.9 ± 0.3 (48)	13.6 ± 0.4 (43)	65 ± 7 (46)	3.12 ± 0.27 (37)	38.3 ± 0.4 (40)***	20.4 ± 0.9 (38)**	266 ± 37 (37)***	3.98 ± 0.29 (33)*

until 4–6 weeks after gold–thioglucose injection, and post-prandial serum insulin concentrations in injected (obese) animals became significantly elevated at 8 weeks (Table 1).

PDH activity in heart and liver

The proportion of PDH_a in control mice was approx. 20%, as previously reported (Kerbey *et al.*, 1984) (Table 2). In the gold–thioglucose-injected animal, the proportion of heart enzyme as PDH_a was initially increased to 34.4% (a 73% increase) 1 week after injection. At week 4 after injection this proportion fell to 6.8% of total activity (a 68% decrease), and it remained at this low value. Total PDH activity was similar in hearts from control and injected mice, but at weeks 6 and 8 there was an increase in total PDH in the obese animals (as previously reported; Kerbey *et al.*, 1984).

In contrast, the proportion of PDH_a in liver from gold–thioglucose-treated animals was significantly increased from the first week after injection (Table 2). Total PDH activity was similar in both control and injected mice.

Insulin-receptor analysis

The results of Scatchard analysis of displacement curves for insulin binding to liver membranes are shown in Table 3 for both lean control and gold–thioglucose-treated animals over the first 8 weeks of the experiment. There was no difference in the affinity of the binding sites (analysed as a two-site model) at any stage, but there was a progressive decrease in the number of insulin-binding sites (N_1, N_2). The decrease in receptors was significant at 4 weeks after injection of gold–thioglucose, but a definite trend to a decrease in receptor numbers was evident from the first week after injection. This decrease in insulin-receptor numbers occurred despite the absence of a change in post-prandial serum insulin concentrations.

Effect of 2-tetradecylglycidic acid

At 4 weeks after the induction of obesity, the amount of PDH_a was 3.74 ± 0.77 (12) and 0.99 ± 0.28 (12) units/g dry wt. in hearts from control and obese mice respectively (body wts. 24.8 ± 0.4 and 32.0 ± 1.4 g). After a single dose of 2-tetradecylglycidic acid (25 mg/kg) given to lean control and obese mice by intragastric tube, PDH_a was increased to 4.75 ± 0.75 (12) and 4.78 ± 0.51 (12) units/g dry wt. of heart respectively (body wts. 25.1 ± 0.4 and 31.9 ± 0.5 g respectively).

In liver from control mice, PDH_a was increased from 1.39 ± 0.27 (8) to 1.98 ± 0.27 (8) units/g dry wt. by administration of 2-tetradecylglycidic acid, an increase of 42.5%. In contrast, in 'obese' animals, 2-tetradecylglycidic acid had no effect on PDH_a in liver [1.83 ± 0.32 (10) and 1.89 ± 0.17 (10) units/g dry wt., for obese and obese + 2-tetradecylglycidic acid respectively]. 2-Tetradecylglycidic acid had no effect on the number of liver membrane insulin receptors or receptor affinity.

DISCUSSION

As reported previously (Caterson & Taylor, 1982; Kerbey *et al.*, 1984), the weight of male CBA/T6 mice increased rapidly after a single intraperitoneal injection of gold–thioglucose. At 6 weeks after injection mice were 25% heavier than controls, and at 8 weeks they were

Table 2. Time course of effects of induction of obesity on activity of PDH complex in heart muscle and liver from gold–thioglucose-treated and lean control mice

For details of extraction and assay of PDH_a, see the Materials and methods section. Experiments with both groups of animals for each time point were performed in parallel and completed in 1 day. Results are given as means ± S.E.M. for the numbers of animals shown in parentheses. Statistical significance of differences compared with matched lean control: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$.

Tissue	Time after injection (weeks)	Control			Gold–thioglucose-treated		
		PDH_a (units/g dry wt.)	% Activity (PDH_a/total PDH)	Total PDH (units/g dry wt.)	PDH_a (units/g dry wt.)	% Activity (PDH_a/total PDH)	Total PDH (units/g dry wt.)
Heart muscle	0	4.21 ± 0.40 (9)	27.84 ± 2.66	15.14 ± 1.48			
	1	4.25 ± 0.73 (7)	19.89 ± 3.41	21.38 ± 0.73	6.31 ± 0.63 (8)**	34.39 ± 3.45**	18.35 ± 1.26
	3	4.53 ± 0.76 (11)	21.39 ± 3.56	21.18 ± 0.94	5.28 ± 0.68 (10)	25.00 ± 3.24	21.10 ± 1.62
	4	4.56 ± 0.50 (18)	21.08 ± 2.44	21.61 ± 0.92	1.34 ± 0.27 (16)***	6.81 ± 1.35***	19.93 ± 1.13
	6	4.99 ± 0.83 (11)	22.18 ± 2.97	21.54 ± 0.90	2.29 ± 0.29 (18)***	7.83 ± 1.01***	31.54 ± 1.16
	8	3.89 ± 0.35 (21)	22.42 ± 2.02	17.36 ± 0.66	1.28 ± 0.30 (15)***	5.98 ± 1.38***	21.36 ± 0.47
Liver	0	0.81 ± 0.14 (5)	19.78 ± 2.45	4.55 ± 0.50			
	1	1.34 ± 0.32 (10)	31.81 ± 7.63	4.20 ± 0.37	2.18 ± 0.24 (15)**	49.27 ± 5.45**	4.43 ± 0.21
	3	1.15 ± 0.16 (16)	30.15 ± 4.25	3.82 ± 0.36	2.27 ± 0.28 (20)**	49.36 ± 6.17**	4.59 ± 0.23
	4	1.02 ± 0.22 (14)	23.76 ± 5.17	4.32 ± 0.39	1.98 ± 0.17 (17)**	44.78 ± 3.77**	4.42 ± 0.27
	6	0.91 ± 0.11 (21)	23.63 ± 2.77	4.01 ± 0.38	2.19 ± 0.31 (20)**	40.64 ± 4.12**	5.40 ± 0.22
	8	0.87 ± 0.11 (26)	15.32 ± 1.94	5.79 ± 0.32	1.81 ± 0.13 (38)**	38.91 ± 2.87**	4.65 ± 0.21

Table 3. Insulin binding to liver membrane from gold–thioglucose-injected obese mice and lean control mice

For details of membrane preparation, insulin binding and analysis of data see the Materials and methods section. K_{a1} and K_{a2} are the affinity constants for the high- and low-affinity components (respectively) of the insulin receptor (as analysed), and N_1 and N_2 are the corresponding receptor-component concentrations. All results are expressed as means ± S.D. and are obtained from membranes prepared from five animals. Statistical significance of differences compared with lean control: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$.

	Controls				Gold–thioglucose-treated			
Time after induction of obesity (weeks)	$10^9 \times K_{a1}$ (M^{-1})	$10^7 \times K_{a2}$ (M^{-1})	$10^{11} \times N_1$ (M)	$10^{11} \times N_2$ (M)	$10^9 \times K_{a1}$ (M^{-1})	$10^7 \times K_{a2}$ (M^{-1})	$10^{11} \times N_1$ (M)	$10^{11} \times N_2$ (M)
1	0.7±0.2	1.0±0.85	24.8±10.6	154±73.3	0.67±0.07	0.8±1.2	12.0±1.2*	95.1±13.8
2	0.5±0.4	0.8±0.4	28.5±7.5	197±62	0.4±0.08	0.8±0.75	23.1±4.1	68.5±41.6
3	0.86±0.38	1.6±1.4	15.6±3.0	142.1±73.3	0.5±0.2	0.8±1.5	20.8±7.6	103.6±69
4	0.6±0.1	1.0±1.3	26.8±4.3	152±30.5	0.85±0.23	0.8±1.5	5.3±1.7***	94.6±22.1
8	0.7±0.06	1.0±0.8	24.6±2.7	147±20.4	0.5±0.2	0.73±0.88	11.5±3.7**	109±57

almost 50% heavier. Though it is generally accepted that mice made obese with gold–thioglucose do not become diabetic, except under conditions of stress (Katsuki *et al.*, 1962; Caterson & Taylor, 1982), the serum glucose concentration of mice at 4 and 8 weeks after injection was elevated compared with controls (Table 1).

Post-prandial serum insulin concentrations did not rise in these obese animals until 8 weeks after injection, at which stage the amount of PDH_a had decreased in heart muscle (Table 2). This increase in serum insulin occurred together with and despite an increase in serum glucose, and these two changes imply that a state of insulin resistance had developed in the obese mice. Indeed it has been shown *in vivo* that tissues of gold–thioglucose-treated mice are insulin-resistant (Cooney *et al.*, 1987).

The serum insulin concentrations were post-prandial in that they were measured in the morning after the mouse had fed overnight and then been removed from food for 3 h. Though it has been suggested that an effect of the hypothalamic infarct produced by gold–thioglucose is an immediate increase in serum insulin (Bray & York, 1979), no such early change in post-prandial serum insulin was detected. Measurement of insulin response to food or of mean 24 h insulin release might give a more accurate picture of the role of insulin in the development of obesity in this animal model.

The number of insulin receptors was decreased progressively over time (Table 3). This decrease was evident in the first week after gold–thioglucose injection, but it was not until 4 weeks after injection that both high- and low-affinity receptor numbers were significantly decreased. These receptor-number alterations preceded by several weeks the occurrence of overt hyperinsulinaemia and hyperglycaemia. The decrease in receptor numbers may be due to the hyperphagia induced by gold–thioglucose causing higher than normal 24 h mean insulin concentrations or higher insulin secretion in response to food. It should be realized, however, that attempting to measure serum glucose and insulin frequently is difficult in mice, owing to their small blood volume. Also, with repeated sampling from the same animal stress is likely to have an influence on the results.

The amount of PDH_a and the influence of obesity differed in the tissues studied. Both heart muscle and liver showed an increase in PDH_a at 1 week after injection. At 4 weeks after injection, before the animals were grossly obese, PDH_a in heart muscle decreased to 29% of control values, and remained low as obesity developed (Kerbey *et al.*, 1984). As in older mice (Caterson *et al.*, 1984), the decrease in PDH_a in heart 4 weeks after gold–thioglucose injection may represent increased β-oxidation of fatty acids in the tissue, since 2-tetradecylglycidic acid reversed the decrease in PDH_a. The change in PDH_a was unlikely to be due to altered insulin binding, as there was no change in liver insulin receptor number or affinity in the animals treated with 2-tetradecylglycidic acid, nor was there any difference in post-prandial serum insulin concentrations. An alteration in fuel availability (by inhibiting β-oxidation with 2-tetradecylglycidic acid) increased heart PDH_a in obese animals, with no effect on PDH_a in liver from these animals. The oxidation of fatty acids in heart muscle must therefore play a part in the decreased activity of the PDH complex in this model of obesity. This may occur via alterations in [NADH]/[NAD^+] and [acetyl-CoA]/[CoA] ratios. In addition, if fatty acids are available to heart muscle, e.g. in starvation (Palmer & Kane, 1983) or obesity (Golay *et al.*, 1986), they will be preferentially oxidized, despite insulin concentrations which normally cause an increase in PDH_a. This metabolic change in heart muscle and other tissues may be an early post-binding change in the development of insulin resistance. The decrease in PDH_a may therefore indicate tissue insulin resistance.

Total PDH activity in heart muscle from animals was greater than that reported previously (Kerbey *et al.*, 1984), but there was no difference between control and gold–thioglucose-injected animals except when the animals were obese, and then total PDH was increased in heart muscle. The lower values reported previously may be due to the age of the animals in that study, as there was a tendency for the total PDH activity to fall in control animals over 8 weeks. The animals studied in previous experiments were older (16 weeks after injection of gold–thioglucose) (Kerbey *et al.*, 1984).

In liver, PDH_a was increased during the development of obesity, and this was consistent with increased lipogenesis in this tissue. In the liver, increased glucose oxidation would contribute an energy source and also provide the precursors for the role of the liver in synthesizing fatty acids and triacylglycerols.

In this model of obesity, the stimulation of liver PDH_a

occurred early in the development of the obesity syndrome. In heart muscle there was an initial increase in PDH_a (at week 1), but a decrease in heart PDH_a occurred suddenly at 4 weeks after injection, before a rise in post-prandial insulin concentrations. These changes in PDH_a may reflect differences in tissue function and fuel utilization in the onset of obesity. Increased PDH_a in liver is paralleled by increased triacylglycerol synthesis by the liver (G. J. Cooney & I. D. Caterson, unpublished work), and the increased availability of lipid may result in suppressed PDH_a in heart muscle, as this tissue metabolizes the products of liver lipogenesis in preference to other fuels (as demonstrated by the effect of 2-tetradecylglycidic acid on PDH_a). The observations that insulin receptors in obesity are generally decreased (as in this study) and the observations that the PDH complex is both activated and inhibited in different tissues in obesity support the contention that the aetiology of the insulin resistance in gold–thioglucose-induced obesity is multi-factorial (Le Marchand *et al.*, 1978; Caterson *et al.*, 1984).

We thank Joanne Marsh and Jenny Nicks for technical assistance, and Toni Meucci for typing the manuscript. We also thank Dr Alan Kerbey for helpful discussion. This study was supported by the National Health and Medical Research Council of Australia.

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Received 30 July 1986/24 November 1986; accepted 30 December 1986