Diurnal patterns of cardiac and hepatic pyruvate dehydrogenase complex activity in gold–thioglucose-obese mice

Janet M. BRYSON,* Gregory J. COONEY, Victoria R. WENSLEY, Stephen C. BLAIR and Ian D. CATERSON

Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, N.S.W. 2050, Australia

The diurnal pattern of the activity of the pyruvate dehydrogenase complex (PDHC) was studied in the heart and liver of gold–thioglucose (GTG)-obese mice and age-matched controls. The diurnal pattern of lipogenesis was also measured in the liver. Both lean and obese mice had one main eating period, from 20:00 to 24:00 h. Eating produced no change in serum glucose of control mice but there was a significant rise in serum insulin and triacylglycerols. There was also a 3-fold increase in cardiac PDHC activity and a 3-fold increase in hepatic lipogenesis in the control mice, but little change in hepatic PDHC activity. GTG-obese mice were hyperglycaemic, hyperinsulinaemic and hypertriglyceridaemic at all times studied, with significant increases in these parameters being seen in response to eating. Eating produced little change in cardiac PDHC activity, but there was a

5-fold increase in hepatic PDHC activity, paralleled by a 10-fold increase in hepatic lipogenesis. Hepatic PDHC activity was significantly higher in GTG-obese mice at all times except 16:00 h. The simultaneous rise of hepatic PDHC activity, lipogenesis and serum triacylglycerols in GTG-obese mice suggests an increased utilization of glucose for lipogenesis. The lack of change in heart PDHC activity in GTG-obese mice over 24 h suggests that a general decrease in PDHC activity may contribute to the development of the glucose intolerance and insulin resistance of obesity and non-insulin-dependent diabetes. However, it appears that a different level of metabolic control allows hepatic PDHC activity of the same obese animals to increase in response to hyperinsulinaemia and contribute to the higher rates of lipogenesis seen in obese mice.

INTRODUCTION

The gold–thioglucose (GTG)-injected mouse is a chemically induced animal model of obesity. A single injection of GTG causes a necrotic lesion of the hypothalamus, resulting in hyperphagia and the development of hyperglycaemia, hyperinsulinaemia and overt obesity over a period of 6–10 weeks [1]. This model has been used to study metabolic changes that may be involved in the development of obesity and insulin resistance. Changes in glucose uptake [2], insulin-receptor tyrosine kinase activity [3], enzymes involved in glucose disposal [4–7] and lipogenesis [8] have been observed. In general, the results indicate the presence of insulin resistance in heart muscle, skeletal muscle and adipose tissues, whereas the liver appears to remain responsive to the higher circulating insulin levels.

The pyruvate dehydrogenase complex (PDHC) is a key enzyme in the oxidative disposal of glucose because of its role in converting glucose into acetyl-CoA for oxidation to CO₂ or conversion into fatty acids. The proportion of the PDHC in the active form is closely correlated with the availability and oxidation of non-esterified fatty acids. This ability of fat oxidation to inhibit glucose oxidation by inhibiting PDHC activity forms the basis of the glucose/fatty acid cycle first described by Randle and co-workers [9]. In GTG-obese mice in the 'fed' state, PDHC activity is decreased in heart and increased in liver, implying that different mechanisms are involved in the control of the PDHC activity in these two tissues. However, most of the estimations of PDHC activity in the GTG-obese model have been done on 'fed' mice killed between 10:00 h and 12:00 h. Since the nutritional state of the animal is important in determining the PDHC activity [10], it is possible that the reported changes in PDHC activity are due to different eating behaviour in the GTG-obese mice rather than a chronic change in activity. Previous studies of hepatic lipogenesis in GTG-obese mice have only been done in

animals which had been starved overnight and then re-fed [8], and the differences seen may not reflect the normal diurnal pattern of lipid synthesis. Therefore the aim of this study was to investigate the diurnal variation of PDHC activity in heart and liver and the diurnal pattern of hepatic lipogenesis in relation to eating patterns and consequent changes in serum glucose, insulin and triacylglycerols in lean and obese mice.

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.) [11]. Mice were used 10 weeks after injection, when obesity was fully developed and GTG-obese mice were more than 30% heavier than lean controls. GTG-obese animals and age-matched controls were placed 4–5 to a cage and allowed free access to water and food (Rat and Mouse Cubes; Young Stockfeed, Young, N.S.W., Australia). The composition of the diet was protein (23%), fat (4%), carbohydrate (56%). A light/dark cycle was maintained with the dark period from 18:00 h to 06:00 h. The food in each cage was weighed every 4 h over a 24 h period, and the food intake per mouse was calculated.

Mitochondrial preparation and enzyme assays

At the end of each 4 h period, one cage of lean and one cage of obese mice were killed by cervical dislocation. Heart and liver were rapidly excised, the liver was weighed and the whole heart and approx. 200 mg of liver were homogenized immediately in ice-cold homogenization buffer for preparation of mitochondria for PDHC and citrate synthase (CS) assay as previously described

Abbreviations used: GTG, gold–thioglucose; PDHC, pyruvate dehydrogenase complex; CS, citrate synthase; ACC, acetyl-CoA carboxylase.

* To whom correspondence should be addressed.

[11–15]. The homogenization buffer contained 0.25 M sucrose/0.5 mM Tris/HCl/2 mM EGTA, pH 7.5, with 50 mM NaF and 10 mM sodium dichloroacetate. We have previously demonstrated the efficacy of sodium dichloroacetate and NaF in preventing interconversion of PDHC and maintaining the complex in its activation state *in vivo* [13].

Mitochondrial extracts were prepared from the mitochondrial pellets by alternate thawing and refreezing in extraction buffer (50 mM potassium phosphate/10 mM EGTA/2 mM dithiothreitol, pH 7.0) [12–15]. PDHC 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 [11]. CS activity was also measured, and PDHC activity was expressed per unit of CS activity to correct for any differences in the recovery or purity of the mitochondrial extracts [14]. Total PDHC activity was not measured in this study, as previous studies have shown it to be unaffected by the nutritional state of the animal [12]. Although similar results may be obtained by the simpler procedure of freeze-clamping tissue and measuring PDHC in tissue homogenates, in our hands we find that measuring PDHC activity in mitochondrial extracts gives more consistent rate changes in the assay and more reproducible values for PDHC activity [13,14].

Lipogenesis

At 1 h before the end of each 4 h period, the mice to be killed were each given an intraperitoneal injection (0.5 mCi) of $^3\text{H}_2\text{O}$ (Amersham International, Amersham, Bucks., U.K.). At the time of death, a piece of liver was weighed and immediately saponified in ethanolic KOH, acidified with H_2SO_4 , and fatty acids were extracted in light petroleum (b.p. 60–80 °C). Organic extracts were evaporated to dryness, and the ^3H content of fatty acids was determined by liquid-scintillation counting in a Packard Tri-Carb 300 scintillation counter with external quench correction [8].

Serum analyses

Blood was collected from the chest cavity after the heart had been removed, centrifuged for 2 min at 10000 *g* and serum frozen for subsequent assay of insulin, glucose and triacylglycerols.

Figure 1 Diurnal patterns of food intake in lean and obese mice

Food intake of lean (○) and GTG-obese mice (●) over 24 h. Mice were housed 4–5 to a cage, food was weighed every 4 h and the amount eaten/4 h per mouse was calculated. The number of cages at each time interval varied between 3 and 12. Statistical differences were determined by Student's *t* test: ***P* < 0.001 for differences between control and GTG-obese groups. Error bars in all Figures represent S.E.M.

Serum glucose was measured by a glucose oxidase/peroxidase method with 4-amino-antipyrine as the dye, and serum insulin by a double-antibody radioimmunoassay using rat insulin standards (Linco Research, St. Louis, MO, U.S.A.). Serum triacylglycerols were measured by an enzymic colorimetric assay (Triglycerides GPO-PAP from Boehringer Mannheim).

RESULTS

Food intake and serum analyses

Both control and GTG-obese mice commenced eating during the 4 h period 16:00–20:00 h, with the highest food intake occurring

Figure 2 Diurnal patterns of serum glucose, insulin and triacylglycerols

Diurnal patterns of serum glucose (a), insulin (b) and triacylglycerols (c) in control (○) and GTG-obese (●) mice. Statistical differences were determined by Student's *t* test: **P* < 0.01, ***P* < 0.001 for differences between controls and GTG-obese at corresponding times (*n* varied between 9 and 18).

Figure 3 Diurnal patterns of cardiac and hepatic PDHC activity

PDHC activity of mitochondrial extracts of heart muscle (a) and liver (b) taken from GTG-obese (●) and age-matched control (○) mice; $n = 4$ for each point. Statistical differences were determined by Student's t test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for differences between control and GTG-obese at corresponding times.

Figure 4 Diurnal pattern of lipogenesis in lean and obese mice

Diurnal pattern of lipogenesis in the liver of control (○) and GTG-obese (●) mice. Lipogenesis is expressed as μg-atoms of ^3H incorporated into fatty acid/h per total liver. Statistical differences were determined by Student's t test: * $P < 0.01$, ** $P < 0.001$ for differences between control and GTG-obese groups at corresponding times (n varied between 9 and 18).

between 20:00 h and 24:00 h (Figure 1). The eating pattern was the same in both groups. Differences between food intake over each 4 h period were slight but the total food intake over 24 h was significantly higher in GTG-obese mice (control, 4.66 ± 0.25 g/24 h per mouse; GTG-obese, 6.08 ± 0.34 g/24 h per mouse; $P < 0.05$). GTG-obese mice were hyperglycaemic, hyperinsulinaemic and hypertriglyceridaemic at all times throughout the 24 h of study (Figure 2). Serum glucose in control mice did not change (Figure 2a), but there was a significant rise in insulin between 16:00 h and 24:00 h ($P < 0.05$) (Figure 2b). Serum triacylglycerols rose in lean mice (Figure 2c) to double the basal level ($P < 0.001$). Much greater diurnal variations were seen in the GTG-obese mouse, with significant rises occurring in serum glucose, insulin and triacylglycerols during the feeding period.

PDHC activity in heart and liver

Cardiac PDHC activity in control mice increased 3-fold after the commencement of feeding ($P < 0.001$) and remained significantly higher than the preprandial level for the rest of the 24 h study period (Figure 3a). PDHC activity in the heart of GTG-obese mice was always significantly less than controls ($P < 0.05$ to $P < 0.001$), except at 16:00 h, when PDHC activity was lowest in both groups. There was no significant increase in heart PDHC activity in the GTG-obese mice in response to feeding.

In contrast, hepatic PDHC activity was higher in GTG-obese mice at all time points except 16:00 h ($P < 0.01$ to $P < 0.001$) (Figure 3b). There was a 5-fold increase in hepatic PDHC activity in obese animals by 24:00 h, which was maintained for the following 12 h. In the lean mice, there were no significant diurnal changes in liver PDHC activity. Liver lipogenesis increased in both control and GTG-obese mice with a similar diurnal pattern to that for feeding behaviour, serum insulin and circulating triacylglycerols (Figure 4). However, lipogenesis was 4–5 times higher in GTG-obese mice for the full 24 h period.

DISCUSSION

The GTG-injected mouse is characterized by increased body weight, hyperglycaemia and hyperinsulinaemia [5]. This obesity is accompanied by several manifestations of insulin resistance. There is decreased glucose uptake *in vivo* in heart, quadriceps muscle and brown adipose tissue at 4 weeks after the GTG injection [2] and decreased PDHC activity in fed animals in heart, quadriceps muscle and white adipose tissue [5–7]. Lipogenesis is initially increased in both brown and white adipose tissue, but, by 10 weeks after injection, lipid synthesis in adipose tissues is resistant to insulin stimulation [8] and muscle glycogen metabolism appears unaffected by the hyperinsulinaemia [16]. By contrast, the liver appears to remain sensitive to these high circulating insulin levels, with increases in lipogenesis [8], PDHC activity in the fed state [5] and glycogen storage [16]. What is not clear from these previous studies is whether the apparent obesity-related differences that have been found in either the fed or the starved state reflect consistent changes in the diurnal patterns of food intake, insulin action, enzyme activity and lipid synthesis that would be necessary for the accumulation of lipid that occurs as the mice become obese.

In the study reported here, both lean and 10-week obese mice had the same pattern of food intake, although obese mice ate slightly more food than control mice at all time periods studied, which resulted in a significantly higher food intake for the 24 h period. There was no significant change in serum glucose level over the 24 h of study in the control mice, but a marked hyperglycaemia developed in the obese mice with the onset of feeding. This did not appear to be due to a lack of insulin release,

as obese mice were hyperinsulinaemic throughout the 24 h period compared with control mice, and this hyperinsulinaemia was greatly exacerbated by the commencement of feeding.

Cardiac PDHC activity rose dramatically in control animals on the commencement of feeding and remained elevated until the mice stopped eating. This increase coincided with an increase in insulin and suggests a switch from fatty acid to carbohydrate as the energy source for this tissue [10]. Recent studies of the insulin-sensitivity of PDHC in various rat tissues have shown a very tight relationship between insulin suppression of plasma non-esterified fatty acids and stimulation of PDHC activity [15]. The fall in cardiac PDHC activity at 04:00 h occurred as feeding activity decreased and serum triacylglycerol levels were rising, suggesting a switch back to using lipid for energy. The absence of any eating-stimulated rise in cardiac PDHC activity in GTG-obese mice shows that the decreased PDHC activity previously reported between 10:00 h and 12:00 h persists throughout the normal 24 h cycle in these mice.

Unlike cardiac PDHC activity, variations in hepatic PDHC activity and lipogenesis in obese mice closely paralleled the increases in serum glucose, insulin and triacylglycerols. This suggests that increasing amounts of glucose are being used for fatty acid synthesis and are contributing to the increased serum triacylglycerols. In the control mice, the significant rise in hepatic lipogenesis was not accompanied by a parallel increase in hepatic PDHC activity. These results are similar to those found in control rats [17] and imply that the activity of PDHC in liver in the normal situation is sufficient to support lipogenesis that uses glucose as substrate. However, when hyperglycaemia and hyperinsulinaemia are present, PDHC activity is markedly increased to provide substrate to support the observed increased rates of lipogenesis.

There is no clear explanation for the increased PDHC activity in liver and decreased PDHC activity in heart in the same obese mice. It is possible that the difference is due to tissue differences in the activity of acetyl-CoA carboxylase (ACC), an insulin-sensitive enzyme which is thought to be rate-limiting in fatty acid synthesis. We have previously shown that hepatic ACC activity is considerably higher than cardiac activity in control animals and that obesity further increases hepatic, but not cardiac, ACC [6]. Increased hepatic ACC in obese mice may lead to increased malonyl-CoA concentrations, which would inhibit fatty acid oxidation [18] and lead to PDHC activation as well as to increased fatty acid synthesis. In heart, where ACC activity is low and unchanged by obesity, decreased PDHC activity is likely to reflect increased fatty acid availability and oxidation. The fact that a single dose of a pharmacological fatty acid oxidation inhibitor can restore the lower PDHC activity of obese-mouse heart to control values [19] reinforces the idea that fatty oxidation rates are central to the control of PDHC activity.

One important observation from this study is that the most rapid change in all the parameters measured occurs with the onset of the feeding period (16:00–20:00 h) and between 08:00 and 12:00 h in the morning. The results presented here suggest

that, although this period is considered to be a post-prandial steady state, there is the capacity for a wide variety of results to be obtained from the same group of animals. Comparison of results obtained during the middle of the feeding phase (approx. 24:00 h in these experiments) and at 16:00 h may provide more consistent and meaningful data about the changes occurring in animals during a 24 h cycle.

The diurnal variations in PDHC activity reported here confirm that the previously described changes in PDHC activity between 10:00 and 12:00 h reflect the pattern of the whole 24 h period. The lack of change in heart PDHC activity in GTG-obese mice over 24 h suggests that a general decrease in activity of this enzyme complex may contribute to the development of the glucose intolerance and insulin resistance of obesity and non-insulin-dependent diabetes. However, it seems that a different level of metabolic control allows increased hepatic PDHC activity to contribute to the higher rates of lipogenesis in liver of obese mice.

This work was supported by a project grant from the National Health & Medical Research Council of Australia.

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