Changes in the lipogenic response to feeding of liver, white adipose tissue and brown adipose tissue during the development of obesity in the gold–thioglucose-injected mouse

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Lipogenic response to feeding was measured *in vivo* in liver, epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT), during the development of obesity in gold–thioglucose (GTG)-injected mice. The fatty acid synthesis after a meal was higher in all tissues of GTG-treated mice on a total-tissue basis, but the magnitude of this increase varied, depending on the tissue and the time after the initiation of obesity. Lipogenesis in BAT from GTG mice was double that of control mice for the first 2 weeks, but subsequently decreased to near control values. In WAT, lipogenesis after feeding was highest 2–4 weeks after GTG injection, and in liver, lipid synthesis in fed obese mice was greatest at 7–12 weeks after the induction of obesity. The post-prandial insulin concentration was increased after 2 weeks of obesity, and serum glucose concentration was higher in fed obese mice after 4 weeks. These results indicate that increased lipogenesis in GTG-injected mice may be due to an increase in insulin concentration after feeding and that insulin resistance (assessed by lipogenic response to insulin release) is apparent in BAT before WAT and liver.

INTRODUCTION

The major characteristic of obesity in humans and animals is excessive synthesis and deposition of triacylglycerol. This overproduction of fat may be due to increased food intake (hyperphagia), but there is also a significant amount of evidence from animal studies (Rothwell & Stock, 1984) and human experiments (Ravussin *et al.*, 1988) suggesting that energy expenditure is an important factor in determining the proportion of energy intake that is diverted to fat synthesis. In other words, decreased energy expenditure may lead to the development of obesity in animals and man, even though energy intake is not increased. Other factors, such as hyperinsulinaemia and hyperglycaemia, have also been suggested as important stimuli for the increased fat synthesis of obesity, and obesity is often associated with the development of non-insulin-dependent diabetes in animals and humans.

Since increased lipid deposition is an obvious metabolic abnormality of obesity, several studies have been performed comparing lipogenesis in liver, white adipose tissue (WAT) and brown adipose tissue (BAT) of control and obese rats and mice. However, in most of these studies different animal models of obesity were used, and the duration of obesity at the time of study was variable. This makes comparison of the lipogenic role of individual tissues at different stages of obesity difficult. For example, in *ob/ob* obese mice Hems *et al.* (1975) reported that lipogenesis in liver, WAT and BAT was higher in 3-month-old mice throughout the 24 h cycle. Mercer & Trayhurn (1983) studied lipogenesis in BAT during the development of obesity in *ob/ob* mice, and showed a transitory hyperlipogenesis in BAT of obese mice at

weaning, which then decreased to the level of lipid synthesis in BAT of lean mice. However, lipid synthesis in liver and WAT remained increased in *ob/ob* mice throughout the development of obesity. Godbole & York (1978) showed an increase in lipogenesis in liver and WAT of genetically obese *fa/fa* rats at 5 weeks of age, but, although fatty acid synthesis in WAT from obese rats at 13 weeks of age was still higher than in lean controls, it had fallen dramatically in comparison with the value obtained for 5-week-old obese rats. In contrast, the lipogenic rate of liver increased further in 13-week-old obese rats compared with 5-week-old obese rats, and was 20-fold higher than lipogenesis in lean rats of the same age. Lavau *et al.* (1982) presented evidence for a higher rate of fatty acid synthesis in BAT of *fa/fa* obese rats at 30 days of age, whereas, in the ventromedial hypothalamic-lesioned obese rat at 8–10 weeks after lesion, lipogenesis is increased in liver and WAT but decreased in BAT (Saito & Shimazu, 1984). In all these studies it appears that the amount of lipid synthesis in different tissues varies at different stages of the development of obesity. This variability may be due to the nutritional state of the animal at the time of study (i.e. the time since the last meal), or to the existence of a defined pattern for the appearance of increased lipid synthesis in the major lipogenic tissues as obesity develops.

To investigate the possibility that changes in lipid synthesis of tissues may reflect their relative importance in the increased lipogenesis characteristic of obesity, we have measured the lipogenic response to feeding in liver, WAT and BAT during the development of obesity in gold–thioglucose (GTG)-injected mice. This model of obesity exhibits hyperphagia, hyperinsulinaemia and

Abbreviations used: WAT, white adipose tissue; BAT, brown adipose tissue; GTG, gold–thioglucose.

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hyperglycaemia, and we have previously demonstrated tissue-specific differences in pyruvate dehydrogenase activity (Caterson *et al.*, 1987) and insulin-sensitivity (Cooney *et al.*, 1987) in this animal model of obesity. The results show that lipogenesis is higher in liver, WAT and BAT in obese mice throughout the development of obesity. However, the magnitude of the lipogenic response to feeding follows a separate pattern in each tissue, and suggests that the importance of individual tissues in whole-animal lipogenesis changes during the development of obesity.

MATERIALS AND METHODS

Animals

Male CBA/T6 mice were obtained from the Blackburn Animal House, Department of Pathology, University of Sydney, at 6 weeks of age. 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 over 90 % of these become obese. Injected animals and age-matched controls were allowed free access to 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 (by wt.): crude protein 23 %, crude fat 5 %, crude fibre 6 % and carbohydrate 65 %. Access to food was restricted to between 09:00 and 16:00 h (during the light cycle) to make sure that at 09:00 h on the day of experimentation, when food was presented, the mice would commence eating immediately. Mice that did not receive food are defined as overnight-starved. To confirm that the mice had eaten during this time, each mouse was checked for a full stomach at the time of dissection.

Measurement of lipogenic response to feeding

Overnight-starved control and GTG-injected mice of the required age were divided into four groups. One group of control and one group of GTG-injected mice received an intraperitoneal injection (0.5 mCi) of $^3\text{H}_2\text{O}$ (Amersham International, Amersham, Bucks., U.K.) while in the overnight starved state, and one group of control and one of GTG-injected mice were presented with food for 2 h from 09:00 h and were injected with $^3\text{H}_2\text{O}$ for the second hour of feeding. Exactly 1 h after injection of $^3\text{H}_2\text{O}$, mice were killed by cervical dislocation, and the liver, epididymal white fat-pads and interscapular brown adipose tissue were rapidly excised and frozen in liquid N_2 . A blood sample was taken from the heart at the time of killing for the determination of serum glucose, serum insulin and specific radioactivity of $^3\text{H}_2\text{O}$ in serum. Fatty acid synthesis was measured by the method of Stansbie *et al.* (1976). Tissues were 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 on a Packard Tri-Carb 300 scintillation counter with external quench correction. Serum glucose was measured by a hexokinase/glucose-6-phosphate dehydrogenase assay (Bergmeyer *et al.*, 1974) with reagents purchased from Boehringer Mannheim Aust. Pty. Ltd. Serum insulin was measured with a double-antibody radioimmunoassay (Burroughs Wellcome Aust. Pty. Ltd.), with human insulin as the standard.

RESULTS

Body and tissue weights

The changes in body and tissue weights during the development of obesity in GTG-injected mice are shown in Fig. 1. The body weight of the GTG-injected mice is significantly higher than that of control mice from 3 weeks after injection ($P < 0.005$), and by 12 weeks after injection the GTG-injected mice were more than 10 g heavier than age-matched control mice (a 38 % increase in body weight) (Fig. 1a). The weight of the liver appears slightly greater (not statistically significant) in GTG-injected mice from 2 to 5 weeks after GTG injection, but at 7 and 12 weeks after induction of obesity the wet weight of the liver increases, to be double the weight of age-matched controls at 12 weeks ($P < 0.001$) (Fig. 1b). The weight of the epididymal fat-pads and interscapular brown fat-pads was significantly increased ($P < 0.01$) in GTG mice from 2 weeks after injection (Figs. 1c and 1d).

Serum glucose and insulin concentrations

The concentration of glucose in the serum of overnight-starved control mice was reasonably constant at 8.5–9.5 mM throughout the study (Table 1). At 5, 7 and 12 weeks after injection the serum glucose concentration in obese mice after overnight starvation was significantly higher than in age-matched controls, and was higher than values for fed control mice at 7 and 12 weeks after induction of obesity (Table 1). The serum glucose concentrations of obese mice after 2 h of feeding was significantly higher than in fed controls at 4, 5, 7 and 12 weeks after injection, indicating a lack of glycaemic control in obese mice by 4–5 weeks after induction of obesity.

The serum insulin concentration of overnight-starved GTG-injected mice was not different from that of age-matched controls for the first 4 weeks of obesity, but at 5, 7 and 12 weeks after induction of obesity the insulin concentration was significantly increased in overnight-starved obese mice (Table 1). The serum insulin concentration approximately doubled in control mice after 2 h of feeding over the 12 weeks of the study, but the increase in serum insulin in response to feeding was significantly higher in the obese mice compared with controls from 2 weeks after the induction of obesity (Table 1).

Lipogenesis in liver

The rate of lipogenesis in liver of overnight-starved GTG-injected mice was similar to that of overnight-starved control mice for 1 and 2 weeks after injection, whether expressed per g fresh wt. or per total liver (Table 2). From 3 to 12 weeks after injection there was a significantly higher lipogenic rate in the liver of obese overnight-starved mice. The lipogenic rate in liver after feeding was significantly increased in GTG-injected mice 1 week after injection as compared with control mice, and this increase was of a similar magnitude on a 'per g fresh wt.' basis at all stages of obesity studied (Table 2). On a 'whole-liver' basis there was a significant increase in lipogenesis at 7 and 12 weeks in liver after induction of obesity (Table 2), owing to the marked increase in the size of the liver that occurred at this stage (cf. Fig. 1b).

Fig. 1. Changes in body weight (a), liver weight (b), epididymal white-fat-pad weight (c) and interscapular brown-fat-pad weight (d) during the development of obesity in GTG-injected mice

Mice were injected with GTG (0.5 g/kg) or 0.9% NaCl as described in the Materials and methods section. Each point represents the mean $\pm$ S.E.M. for 15–20 separate animals. The broken line represents GTG-injected mice and the continuous line represents age-matched control mice.

Table 1. Serum glucose and insulin concentrations during the development of obesity in GTG-injected mice

Glucose and insulin concentrations in serum from GTG-injected mice and age-matched controls were assayed before and after feeding as described in the Materials and methods section. Each result represents the mean $\pm$ S.E.M. for 10–15 separate animals. Statistical analyses were performed by Student's *t* test: * $P \leq 0.01$, ** $P \leq 0.001$ for difference between GTG-injected mice and controls of the same age and nutritional state; † $P \leq 0.01$, †† $P \leq 0.001$ for the difference from week 1 for mice with the same experimental treatment.

Time after injection (weeks)	Nutritional state	Mice ...	Plasma glucose (mM)		Plasma insulin (μ units/ml)	
			Control	GTG-injected	Control	GTG-injected
1	Starved		8.4 $\pm$ 0.6	9.5 $\pm$ 0.3	37.5 $\pm$ 2.3	32.1 $\pm$ 0.6
	Fed		14.2 $\pm$ 0.5	13.3 $\pm$ 0.5	69.3 $\pm$ 5.9	64.5 $\pm$ 3.2
2	Starved		9.0 $\pm$ 0.4	8.5 $\pm$ 0.5	31.0 $\pm$ 1.5	31.5 $\pm$ 1.6
	Fed		15.2 $\pm$ 0.7	15.5 $\pm$ 0.4††	53.1 $\pm$ 2.3	80.9 $\pm$ 5.1**†
3	Starved		8.9 $\pm$ 0.4	8.1 $\pm$ 0.3	34.6 $\pm$ 2.5	37.1 $\pm$ 1.5†
	Fed		13.9 $\pm$ 0.2	14.6 $\pm$ 0.3	66.7 $\pm$ 4.5	89.3 $\pm$ 6.3*†
4	Starved		8.4 $\pm$ 0.3	8.9 $\pm$ 0.4	28.9 $\pm$ 1.5	39.5 $\pm$ 2.1**†
	Fed		14.5 $\pm$ 0.4	16.1 $\pm$ 0.4†††	60.0 $\pm$ 3.6	70.4 $\pm$ 5.1
5	Starved		9.0 $\pm$ 0.4	10.2 $\pm$ 0.4	31.6 $\pm$ 1.1	52.0 $\pm$ 4.1***†
	Fed		13.6 $\pm$ 0.3	14.6 $\pm$ 0.5	68.5 $\pm$ 4.3	92.0 $\pm$ 7.3*†
7	Starved		9.6 $\pm$ 0.1	16.1 $\pm$ 0.8***†	31.6 $\pm$ 2.3	49.9 $\pm$ 6.8*†
	Fed		13.3 $\pm$ 0.2	16.2 $\pm$ 0.4***†	62.0 $\pm$ 3.6	106.4 $\pm$ 9.3***†
12	Starved		9.8 $\pm$ 0.4	16.9 $\pm$ 0.9***†	34.6 $\pm$ 1.9	58.1 $\pm$ 4.7***†
	Fed		14.1 $\pm$ 0.5	18.3 $\pm$ 0.8***†	59.4 $\pm$ 5.8	204.7 $\pm$ 22.9***†

Table 2. Lipogenesis in liver during the development of obesity in GTG-injected mice

Fatty acid synthesis was measured *in vivo* in liver from overnight-starved and fed GTG-injected mice and age-matched controls as described in the Materials and methods section. Lipogenesis is expressed as μg -atoms of ^3H incorporated into fatty acid/h per g of liver and as μg -atoms of ^3H incorporated into fatty acid/h per total liver. Each result represents the mean $\pm$ S.E.M. for 10–15 separate animals. Statistical analyses were performed by Student's *t* test; symbols as in Table 1.

Time after injection (weeks)	Nutritional state	Mice ...	Lipogenesis			
			μg -atoms of ^3H /h per g fresh wt.		μg -atoms of ^3H /h per liver	
			Control	GTG-injected	Control	GTG-injected
1	Starved		11.3 $\pm$ 0.9	10.6 $\pm$ 1.4	12.5 $\pm$ 1.0	11.2 $\pm$ 1.5
	Fed		77.4 $\pm$ 7.5	123.3 $\pm$ 6.8**	86.0 $\pm$ 8.3	131.2 $\pm$ 7.2**
2	Starved		7.6 $\pm$ 0.4	12.9 $\pm$ 1.7*	7.5 $\pm$ 0.4	14.7 $\pm$ 2.0*
	Fed		86.8 $\pm$ 6.6	139.1 $\pm$ 10.4**	86.9 $\pm$ 7.7	162.2 $\pm$ 12.1**
3	Starved		8.9 $\pm$ 0.7	19.2 $\pm$ 1.6***†	9.9 $\pm$ 0.8	23.8 $\pm$ 2.0***†
	Fed		77.2 $\pm$ 4.6	115.0 $\pm$ 8.6**	85.8 $\pm$ 5.1	142.7 $\pm$ 10.7
4	Starved		11.4 $\pm$ 1.0	18.1 $\pm$ 1.8*†	13.1 $\pm$ 1.1	22.6 $\pm$ 2.3*†
	Fed		93.9 $\pm$ 5.4	106.6 $\pm$ 9.8	107.2 $\pm$ 6.2	133.3 $\pm$ 12.2
5	Starved		8.0 $\pm$ 0.6	19.7 $\pm$ 2.5***†	9.2 $\pm$ 0.7	25.5 $\pm$ 3.3***†
	Fed		90.1 $\pm$ 4.7	122.2 $\pm$ 8.4*	104.0 $\pm$ 5.4	157.7 $\pm$ 10.9**
7	Starved		7.0 $\pm$ 0.3	19.2 $\pm$ 1.6***†	7.9 $\pm$ 0.3	31.7 $\pm$ 2.7***†
	Fed		96.1 $\pm$ 8.7	121.5 $\pm$ 8.1*	107.6 $\pm$ 9.7	200.2 $\pm$ 13.4***†
12	Starved		9.0 $\pm$ 0.6	29.6 $\pm$ 3.7***†	11.2 $\pm$ 0.7	61.0 $\pm$ 7.6***†
	Fed		86.9 $\pm$ 7.0	117.2 $\pm$ 9.1*	108.5 $\pm$ 8.8	241.7 $\pm$ 18.8***†

Table 3. Lipogenesis in epididymal WAT during the development of obesity in GTG-injected mice

Fatty acid synthesis was measured *in vivo* in epididymal WAT from overnight-starved and fed GTG-injected mice and age-matched controls as described in the Materials and methods section. Lipogenesis is expressed as μg -atoms of ^3H incorporated into fatty acid/h per g of epididymal WAT and as μg -atoms of ^3H incorporated into fatty acid/h per total epididymal WAT. Each result represents the mean $\pm$ S.E.M. for 10–15 separate animals. Statistical analyses were performed by Student's *t* test; symbols as in Table 1.

Time after injection (weeks)	Nutritional state	Mice ...	Lipogenesis			
			μg -atoms of ^3H /h per g fresh wt.		μg -atoms of ^3H /h per total fat-pads	
			Control	GTG-injected	Control	GTG-injected
1	Starved		7.5 $\pm$ 0.6	7.4 $\pm$ 0.6	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1
	Fed		39.3 $\pm$ 4.1	102.4 $\pm$ 9.5**	6.1 $\pm$ 0.6	12.2 $\pm$ 1.0**
2	Starved		6.8 $\pm$ 0.5	8.7 $\pm$ 0.5	1.0 $\pm$ 0.1	2.0 $\pm$ 0.2***†
	Fed		40.0 $\pm$ 6.6	126.4 $\pm$ 14.0***†	5.9 $\pm$ 0.7	31.0 $\pm$ 2.1***†
3	Starved		7.0 $\pm$ 0.7	7.0 $\pm$ 0.8	1.3 $\pm$ 0.1	2.6 $\pm$ 0.3***†
	Fed		25.3 $\pm$ 2.2†	57.5 $\pm$ 10.3*†	4.8 $\pm$ 0.4	20.9 $\pm$ 3.2***†
4	Starved		6.8 $\pm$ 0.4	6.1 $\pm$ 0.9	1.7 $\pm$ 0.1	5.0 $\pm$ 0.7***†
	Fed		22.2 $\pm$ 5.5†	33.2 $\pm$ 3.9†	5.1 $\pm$ 0.4	23.8 $\pm$ 2.5***†
5	Starved		5.8 $\pm$ 0.2†	6.1 $\pm$ 0.6	1.7 $\pm$ 0.1	5.9 $\pm$ 0.6***†
	Fed		24.5 $\pm$ 2.7†	20.1 $\pm$ 3.0†	6.6 $\pm$ 0.6	17.0 $\pm$ 1.8***†
7	Starved		3.0 $\pm$ 0.3†	3.4 $\pm$ 0.4†	1.3 $\pm$ 0.2	4.6 $\pm$ 0.6***†
	Fed		24.2 $\pm$ 4.9†	6.8 $\pm$ 0.3***†	6.7 $\pm$ 1.6	10.1 $\pm$ 0.6*
12	Starved		5.0 $\pm$ 0.5†	3.6 $\pm$ 0.2†	1.4 $\pm$ 0.2	5.3 $\pm$ 0.5***†
	Fed		20.3 $\pm$ 1.9†	7.1 $\pm$ 0.4***†	6.0 $\pm$ 0.4	10.6 $\pm$ 0.8**

Lipogenesis in epididymal WAT

In epididymal WAT the changes in lipogenesis during the development of obesity were considerably different from those observed in liver. On a g-fresh-wt. basis

(Table 3) the lipogenic response to feeding in WAT was approx. 3-fold higher in GTG-injected mice than in control mice 1 week after injection, but by 3 weeks after injection the lipogenic rate of WAT had decreased to near control values, and was below control values for 5,

Table 4. Lipogenesis in interscapular BAT during the development of obesity in GTG-injected mice

Fatty acid synthesis was measured *in vivo* in interscapular BAT from overnight-starved fed GTG-injected mice and age-matched controls as described in the Materials and methods section. Lipogenesis is expressed as μg -atoms of ^3H incorporated into fatty acid/h per g of interscapular BAT and as μg -atoms of ^3H incorporated into fatty acid/h per total interscapular BAT. Each result represents the mean $\pm$ S.E.M. for 10–15 separate animals. Statistical analyses were performed by student's *t* test; symbols as in Table 1.

Time after injection (weeks)	Nutritional state	Mice...	Lipogenesis			
			μg -atoms of ^3H /h per g fresh wt.		μg -atoms of ^3H /h per total fat-pad	
			Control	GTG-injected	Control	GTG-injected
1	Starved		16.1 $\pm$ 0.8	16.0 $\pm$ 1.2	1.0 $\pm$ 0.1	0.8 $\pm$ 0.1
	Fed		110.2 $\pm$ 9.1	210.6 $\pm$ 18.8**	6.7 $\pm$ 0.6	11.5 $\pm$ 1.3*
2	Starved		16.2 $\pm$ 0.4	14.6 $\pm$ 0.5	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1
	Fed		106.9 $\pm$ 11.1	133.7 $\pm$ 9.1††	5.8 $\pm$ 0.5	11.5 $\pm$ 0.8**
3	Starved		13.4 $\pm$ 1.0	13.4 $\pm$ 0.8	0.8 $\pm$ 0.1	1.4 $\pm$ 0.1***†
	Fed		118.6 $\pm$ 10.4	70.8 $\pm$ 10.8***†	6.0 $\pm$ 0.5	7.3 $\pm$ 0.9†
4	Starved		18.3 $\pm$ 0.9	11.9 $\pm$ 0.4***†	1.0 $\pm$ 0.1	1.5 $\pm$ 0.1***†
	Fed		95.3 $\pm$ 7.9	40.3 $\pm$ 3.1***†	5.0 $\pm$ 0.5	5.6 $\pm$ 0.6††
5	Starved		15.8 $\pm$ 1.1	14.1 $\pm$ 0.8	1.0 $\pm$ 0.1	2.2 $\pm$ 0.1***†
	Fed		83.2 $\pm$ 3.6	40.1 $\pm$ 3.5***†	4.6 $\pm$ 0.2†	6.0 $\pm$ 0.6††
7	Starved		11.7 $\pm$ 2.3	7.9 $\pm$ 2.2†	0.7 $\pm$ 0.1	1.3 $\pm$ 0.3
	Fed		96.6 $\pm$ 7.9	34.7 $\pm$ 2.4***†	6.0 $\pm$ 0.6	8.2 $\pm$ 0.4†
12	Starved		18.1 $\pm$ 1.1	9.4 $\pm$ 0.8***†	1.4 $\pm$ 0.1†	2.2 $\pm$ 0.2***†
	Fed		81.5 $\pm$ 11.1	16.5 $\pm$ 1.6***†	6.5 $\pm$ 0.5	4.5 $\pm$ 0.4***†

7 and 12 weeks of obesity. These changes at the later stages of obesity could have been due to the accumulation of fat in the tissue, but expression of results on a total-tissue basis revealed a similar pattern (Table 3). The lipid synthesis after feeding in control mice was approx. 5 times that of overnight-starved mice at all time points of the study. In the GTG-injected mice the lipogenic rate after feeding was twice that of control mice after 1 week, and 4–6 fold higher at weeks 2–4 of obesity. It then decreased to near the control response at weeks 7–12 after induction of obesity.

Lipogenesis in interscapular BAT

During the 12 weeks of this study the lipogenic rate in BAT from fed control mice was always 5–6-fold higher than lipogenesis in BAT from overnight-starved control mice (Table 4). At 1 week after the induction of obesity, the lipogenic rate in BAT from fed GTG mice was twice that of fed control mice. From 2 to 12 weeks of obesity the lipogenic rate per g of BAT after feeding decreased to be less than the response in control mice, and was only 10% of the rate per g in control mice at 12 weeks after induction of obesity. If the results are presented on the basis of ' ^3H incorporated/total fat-pad' to account for the accumulation of lipid in BAT during the development of obesity, the results differ slightly. At 1 and 2 weeks after injection of GTG the rate of lipogenesis after feeding was double that of control mice (171% and 198% increase respectively). After this early phase of obesity the lipogenic response to feeding in BAT of obese mice was slightly higher, but not significantly different from the lipogenic response to feeding in control mice (Table 4).

DISCUSSION

A single injection of GTG in mice produces a necrotic lesion of the hypothalamus that is responsible for the development of hyperphagia, hyperinsulinaemia, hyperglycaemia and obesity (Marshall *et al.*, 1955). However, whether these abnormalities develop together, sequentially or as a consequence of each other has not been fully investigated. The aim of the current study was to determine the lipogenic rate in three tissues important in the disposal of excess energy intake during the initial stages of the development of obesity in GTG-injected mice, and to interpret these results in terms of the prevailing glycaemia and insulinaemia, since these factors may be important stimuli for lipogenesis in obesity (Clark *et al.*, 1983). Rather than measure the lipogenic rate of tissues from mice in the post-prandial state, as has been done in previous studies (Mercer & Trayhurn, 1983; Lavau *et al.*, 1982), it was decided to measure lipogenesis before and after feeding. Any observed differences in blood glucose, insulin concentrations and rates of lipogenesis might otherwise be due to the time since the last meal rather than an inherent difference in the metabolism of the GTG-injected mice. This experimental design also provided pre- and post-prandial serum glucose and insulin concentrations, and therefore gave an indication of when hyperglycaemia and hyperinsulinaemia occurred during the development of obesity.

The results obtained in this study show that a significant increase in the weight of the epididymal fat-pads and the interscapular brown fat-pads can be observed as soon as 2 weeks after injection of GTG. On the basis of previous experiments carried out at later stages of obesity in GTG-injected mice (LeMarchand

et al., 1978; Hogan & Himms-Hagen, 1983), this increase is due to the accumulation of lipid in these tissues rather than an increase in cell number. The weight of the liver increases dramatically in the obese mice after 7 weeks, but this hypertrophy of the liver is probably due to the accumulation of lipid rather than an increase in cell number (LeMarchand *et al.*, 1978).

Hyperglycaemia and hyperinsulinaemia in the overnight-starved state were not apparent in GTG-injected mice until 5–12 weeks after injection (Table 1). However, it has been proposed that there may be a greater insulin release in response to food in rats with ventromedial hypothalamic lesions, which produces a transient hyperinsulinaemia responsible for the development of the metabolic abnormalities of obesity (Berthoud & Jeanrenaud, 1979). In the experiments reported here, the serum insulin concentration after 2 h of feeding was significantly higher in GTG-injected mice 2 weeks after injection and remained higher at subsequent time points except at 4 weeks after injection. This post-prandial hyperinsulinaemia substantiates the view that there is an increased insulin response in obese mice, but it is also possible that this hyperinsulinaemia is due to the hyperphagia of the obese mice, as no attempt was made to control the amount of food intake during the 2 h feeding period. As insulin concentration in the serum was only measured at a single time point, there may be differences in the timing of insulin secretion owing to the hyperphagia of the obese mice, which could account for the increased insulin concentration observed.

The expression of the results for lipogenesis as μg -atoms of ^3H incorporated into fatty acids/h per total tissue depot as well as μg -atoms of ^3H incorporated into fatty acids/h per g of tissue enables the total lipogenesis of each tissue to be assessed and also corrects for the accumulation of lipid in the two adipose tissues studied. The lipogenic rate of liver is increased in GTG-injected mice from week 1 after injection, and this increase is maintained throughout the period of study. The increase in lipogenesis per total liver after 7 and 12 weeks is due to the increase in the size of the liver and coincides with a decreased rate of lipogenesis in the epididymal WAT and BAT.

In epididymal WAT the lipogenic rate is also markedly elevated by 1 week after GTG injection, and the rate increases further over the next 2 weeks before decreasing to values similar to those in age-matched control mice. The decrease in the lipogenic response to feeding in WAT indicates the development of insulin resistance in this tissue, because the post-prandial insulin concentrations in obese mice increase as lipogenesis in WAT decreases. This early insulin sensitivity followed by insulin resistance in WAT is in agreement with results published by LeMarchand *et al.* (1978), although the time points for investigation in that earlier study were 6 and 16 weeks after GTG injection, and lipogenesis in epididymal WAT was measured *in vitro*.

During times of thermogenic stress, such as cold exposure, lipogenesis in BAT is greatly increased to provide fatty acids to support thermogenesis (Trayhurn, 1979). BAT is also thought to be important in diet-induced thermogenesis, where excess energy intake is dissipated as heat via the unique uncoupling mechanism present in BAT mitochondria (Rothwell & Stock, 1983). In all animal models of obesity studied so far, this thermogenic mechanism in BAT has been found to be

defective (Ashwell *et al.*, 1985; Hogan & Himms-Hagen, 1983; Goodbody & Trayhurn, 1981), but whether changes in BAT metabolism are the major cause of the development of obesity in rodents is still a matter of conjecture. In the current study the size of the interscapular BAT is increased significantly 2 weeks after GTG injection, owing to the increased accumulation of lipid (Hogan & Himms-Hagen, 1983). On a total-fat-pad basis there is hyperlipogenesis in interscapular BAT for 2 weeks after induction of obesity, but after this time the lipogenic response to feeding returns to that of age-matched control mice. The decrease in lipogenic response to feeding in BAT despite continued high concentrations of serum insulin and glucose is indicative of insulin resistance in BAT at a stage in the development of obesity where the lipogenic response to feeding in liver and WAT of obese mice is still higher than in age-matched control mice. This suggests that liver and WAT remain insulin sensitive at early stages of obesity.

Decreased diet-induced thermogenesis in BAT has been proposed as a major cause of the increased metabolic inefficiency observed in animal models of obesity, because energy intake not used as a thermogenic substrate in BAT is converted into triacylglycerol and stored (Himms-Hagen, 1984). In the GTG-injected mice this occurs because the lesion of the ventromedial hypothalamus prevents stimulation of thermogenesis in BAT. The accumulation of lipid in BAT of GTG-induced obese mice and obese *fa/fa* rats is paralleled by a decrease in insulin-stimulated glucose uptake (Cooney *et al.*, 1987; Ferré *et al.*, 1986) and decreased lipid synthesis in BAT. Together, these results indicate a progressive decrease in the conversion of glucose into lipid for diet-induced thermogenesis in BAT. The increased lipogenesis observed in liver and WAT of obese mice at a stage in the development of obesity when lipogenesis in BAT is decreasing may, at least in part, be a consequence of the inability of BAT to utilize excess energy intake for thermogenesis.

Changes in BAT metabolism are unlikely to be entirely responsible for the development of hyperlipogenesis in liver and WAT, since BAT can only account for 8–12% of insulin-stimulated glucose turnover in normal rats (Ferré *et al.*, 1986) and approx. 10% of total body lipogenesis in normal mice (Trayhurn, 1981). However, the early development of insulin resistance in BAT with regard to glucose uptake and lipid synthesis in GTG-induced obese mice, coupled with the knowledge that thermogenesis in BAT is closely linked to the area of the brain lesioned by GTG (Shimazu & Takahashi, 1980), suggest that initial changes in BAT metabolism may perturb whole-animal glucose and lipid homeostasis enough to accelerate the appearance of abnormalities of glucose and lipid metabolism in other tissues. Because of the differences in lipid and glucose metabolism in tissues at different stages of obesity (Lavau *et al.*, 1982; Saito & Shimazu, 1984; Ferré *et al.*, 1986; Cooney *et al.*, 1987; Penicaud *et al.*, 1987), it seems important that future studies focus on the time course for the appearance of metabolic abnormalities of obesity to help clarify the role of individual tissues in the development of obesity.

This work was supported by a project grant from the National Health and Medical Research Council of Australia and the Kellion Diabetes Research Foundation.

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Received 10 June 1988/9 December 1988; accepted 29 December 1988