Evidence for a high fatty acid synthesis activity in interscapular brown adipose tissue of genetically obese Zucker rats

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Obese (*fa/fa*) rats (30 days old) exhibited a 50% increase in the weight of interscapular brown adipose tissue compared with their lean (*Fa/fa*) littermates. The tissue weight increase was accounted for by an increased fat content. Lipogenesis *in vivo*, as assessed by the incorporation of ^3H from $^3\text{H}_2\text{O}$ into lipid, was increased 5-fold in brown adipose tissue of obese as compared with lean rats. Accordingly, acetyl-CoA carboxylase, fatty acid synthetase, citrate-cleavage enzyme and malic enzyme in this tissue were 4–8 times more active in obese than in lean rats.

Subsequent studies have substantiated the initial observation by McCormack & Denton (1977) in cold-acclimated rats that interscapular brown adipose tissue is an important site of fatty acid synthesis. Thus, in female rats kept at normal environmental temperature, lipogenesis in interscapular brown adipose tissue has been shown to be 3–4 times that in white (parametrial) adipose tissue and liver per g fat-free weight of tissue (Agius & Williamson, 1980). This observation was extended to mice housed at 22°C, where the rates of fatty acid synthesis in interscapular brown adipose tissue were about 6–10 times those in white adipose tissues (Rath *et al.*, 1979; Trayhurn, 1981). The regulation of the function of lipogenesis in brown adipose tissue has not yet been extensively investigated, but the few relevant studies indicate that lipogenesis in rat or mouse brown adipose tissue responds dramatically to the known regulatory factors of this pathway in the other lipogenic tissues. So acute elevation of insulinaemia in female rats increased the rate of lipogenesis in brown and white adipose tissues by 800% and 160% respectively (Agius & Williamson, 1980). In keeping with this result, it was observed that pregnancy, which is associated with both hyperinsulinaemia and hyperphagia, induced a 5-fold increase in lipogenesis in interscapular brown adipose tissue (Agius & Williamson, 1980). Feeding mice on diets rich in long-chain fatty acid inhibited lipogenesis in interscapular brown adipose tissue more than in other lipogenic tissues (Van den Brandt & Trayhurn, 1981). This particular sensitivity of brown adipose tissue to dietary lipid might explain the very low fatty acid synthesis observed in this tissue in suckling mice (Trayhurn, 1981). Acute

(Rath *et al.*, 1979) or chronic (Trayhurn, 1979, 1981) exposure of rats or mice to cold (4°C) induced a marked activation of fatty acid synthesis in both white and brown adipose tissues.

The above studies point out the extreme sensitivity of brown-adipose-tissue lipogenesis to environmental, dietary and hormonal conditions. This led us to hypothesize that brown adipose tissue in obese Zucker rats, which are hyperphagic and hyperinsulinaemic, might well be a very active site of fatty acid synthesis and contribute extensively to the hyperlipogenesis that characterizes this obesity. However, the obese Zucker rat is characterized also by a subnormal metabolic rate (Kaplan, 1979) and lower body temperature (Godbole *et al.*, 1978), which strongly suggest the presence of some abnormalities in the heat-production function of brown adipose tissue, possibly including an impairment in the fatty acid synthesis and breakdown cycle. The present work was therefore undertaken to clarify whether interscapular brown adipose tissue of genetically obese rats displayed an altered lipogenic capacity compared with their lean controls.

Materials and methods

Zucker rats are bred in our laboratory from pairs originally provided by the Harriet G. Bird Memorial Laboratory, Stowe, MA, U.S.A. The four litters of rats used in this study were obtained from lean (*Fa/fa*) female and obese (*fa/fa*) male breeding pairs. The pups were weaned at 22 days of age and fed on stock diet (U.A.R., 91360 Epinay sur Orge, France) *ad libitum*. They were maintained at 22–23°C on a fixed 12h-light (07:00–19:00h)/

12h-dark cycle, and used at 30 days of age in the fed state. All experiments were started between 09:00 and 10:00h.

Lipogenesis in vivo

The rats of two litters were injected intraperitoneally with 1 mCi of $^3\text{H}_2\text{O}$ in 0.2 ml of 0.9% NaCl and returned to their cages. Then 60 min later interscapular brown adipose tissue was surgically removed under light diethyl ether anaesthesia, and blood samples were collected for the determination of the specific radioactivity of plasma water. Surgery was performed in order to keep the animals alive for later (7 weeks of age) genotype diagnosis. Brown adipose tissue was carefully trimmed free of white adipose tissue and divided into two pieces. One piece (80–120 mg) was added to 3 ml of 30% (w/v) KOH and processed as described by Stansbie *et al.* (1976). The lipid content of the tissue was estimated from the weight of the residue multiplied by a factor of 1.06 (to compensate for the loss of glycerol). Rates of fatty acid synthesis were calculated as μg -atoms of H incorporated per h from the radioactivity in the fatty acid fraction and the specific radioactivity of plasma water (no correction being made for isotope effects of ^3H versus H).

Enzyme-activity assays

The rest of the tissue was homogenized (all-glass homogenizer) in 1 ml of ice-cold 0.25 M-sucrose containing 1 mM-dithiothreitol and 1 mM-EDTA, pH 7.4. Homogenates were centrifuged at 105 000 g at 0°C for 60 min. The clear infranatants below the fat-cake were either used immediately for assays of fatty acid synthetase (Halestrap & Denton, 1973) and citrate cleavage enzyme (EC 4.1.3.8) (Cottam & Srere, 1969) or frozen at -25°C and used on the following day for assays of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) (Glock & McLean, 1953), and malic enzyme (EC 1.1.1.40), which was measured by the method of Ochoa (1955), but with 10 mM-malate. All the enzyme

assays were run at 37°C in duplicate, with a double-beam spectrophotometer (Perkin-Elmer model 555) equipped with a recorder; they were linear with respect to time and to sample concentration. Two additional litters of rats were used for the determination of acetyl-CoA carboxylase (EC 6.4.1.2) activity. Interscapular brown adipose tissue was removed and processed as described above for the other enzymes. Acetyl-CoA carboxylase was assayed immediately by the method described by Martin & Vagelos (1962), but with Tris/acetate buffer as recommended by Allred & Roehrig (1980). Supernatant samples (0.1 or 0.2 ml) representing 8–15 mg of tissue were incubated at 37°C for 20 min in the presence of potassium citrate (20 mM), dithiothreitol (3 mM), EDTA (0.5 mM), magnesium acetate (10 mM), defatted bovine serum albumin (10 mg/ml) and Tris/acetate (60 mM), pH 7.8, in a final volume of 0.7 ml, in a glass scintillation vial sealed with a rubber stopper. Then 0.1 ml of 100 mM- $\text{KH}^{14}\text{CO}_3$ (sp. radioactivity 0.5 Ci/mol) was delivered through the stopper and the reaction was started by addition of 0.2 ml of a solution of 25 mM-ATP and 1 mM-acetyl-CoA, pH 7.8. Assays were terminated at 1, 2 and 3 min by addition of ice-cold 2 M-HCl (0.2 ml). Last traces of $^{14}\text{CO}_2$ were removed either by gassing the vials with CO_2 for 45 min or by evaporation to dryness at 80°C and the subsequent addition of 1 ml of water. Then 10 ml of scintillation fluid, appropriate for aqueous solutions, was added. All radioactivity counts were corrected for blanks run in the absence of ATP and acetyl-CoA. Protein was determined in the supernatants by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. All enzyme data are expressed as units of activity: 1 unit is defined as the amount of enzyme needed to catalyse the formation of 1 nmol of product/min at 37°C.

Results and discussion

As shown in Table 1, the increase in body weight of the *fa/fa* rats as compared with lean hetero-

Table 1. *Rates of fatty acid synthesis in vivo in interscapular brown adipose tissue of lean (Fa/fa) and obese (fa/fa) Zucker rats (30 days old)*

For experimental details see the text. The values are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. ** $P < 0.001$ versus values for lean rats.

	Interscapular brown adipose tissue					
	Body weight (g)	Weight (mg)	Fat (%)	Rate of fatty acid synthesis (μg -atoms of H incorporated/h)		
				Per g wet wt. of tissue	Per total tissue	Per g fat-free wt. of tissue
Lean (17)	56.6 $\pm$ 1.74	129.3 $\pm$ 5.62	47.6 $\pm$ 1.30	23.1 $\pm$ 3.88	2.85 $\pm$ 0.410	43.3 $\pm$ 6.95
Obese (6)	61.4 $\pm$ 3.19	198.3** $\pm$ 7.88	62.8** $\pm$ 1.76	71.7** $\pm$ 10.90	14.2** $\pm$ 2.11	190.3** $\pm$ 24.46

zygous rats is not yet significant at 30 days of age. In contrast, there was a very significant 50% increase in the weight of interscapular brown adipose tissue in obese as compared with lean rats. The percentages of fat in interscapular brown adipose tissue were also very different between the two genotypes, with values clustering in two widely separated groups. In fact, the tissue enlargement observed in the obese rats (+69 mg) was nearly entirely accounted for by the increase in lipid content of the tissue (+63 mg). There was no significant difference in fat-free tissue weight between the two genotypes. These results indicate that the hypertrophy of interscapular brown adipose tissue in genetically obese rats occurred primarily by a cell size increase. Similar findings have been reported in two models of obese mice, the *ob/ob* (Himms-Hagen & Desautels, 1978) and the *db/db* mice (Goodbody & Trayhurn, 1981), where the hypertrophy of interscapular brown adipose tissue was shown to be the result of an increased amount of triacylglycerol with no change in DNA or protein content. That is at variance with the morphological characteristics exhibited by interscapular brown adipose tissue in obese cafeteria-fed rats, where the tissue growth was explained by an increase in cell number (Tulp *et al.*, 1980; Himms-Hagen *et al.*, 1981).

The rates of fatty acid synthesis were assessed in an attempt to elucidate the mechanism of the increased triacylglycerol content observed in brown adipose tissue of obese rats. The data obtained in lean Zucker rats (Table 1) compare very well with those reported by Agius & Williamson (1980) in interscapular brown adipose tissue of Wistar rats. To our knowledge, the fatty acid synthesis capacity in brown adipose tissue of genetically obese rodents has never been investigated. The present work shows (Table 1) that the rate of fatty acid synthesis per g of tissue in obese *fa/fa* rats was 3 times that in their

lean (*Fa/fa*) littermates. When the results were normalized for active tissue (g fat-free wt.), the differences between the two groups of rats were further increased. The rate of fatty acid synthesis in obese rats was 5 times that of lean rats, on a total-tissue basis.

However, these data did not demonstrate unequivocally an increased fatty acid synthesis *in situ*, since the excess of ^3H -labelled fatty acids present in interscapular brown adipose tissue of obese rats could result from the increased hepatic production of labelled triacylglycerol and their subsequent uptake through the action of brown-adipose-tissue lipoprotein lipase. We therefore investigated the activity of lipogenic enzymes in interscapular brown adipose tissue. The activities in lean Zucker rats presented in Table 2 were very substantial if we consider those displayed by the two major lipogenic organs, liver and white adipose tissue, in the same rats; thus brown-adipose-tissue fatty acid synthetase activity, per mg of protein, was nearly as high as that in inguinal adipose tissue, and 3 times that in liver (R. Bazin & M. Lavau, unpublished work). Table 2 shows that the lipogenic enzyme activities were all dramatically enhanced by the *fa/fa* genotype, the most altered enzyme being malic enzyme, which was 8-fold higher in obese than in lean heterozygous rats. The nearly 4-fold increase in fatty acid synthetase activity per mg of protein demonstrated here in interscapular brown adipose tissue of obese *fa/fa* rats is quite remarkable in view of the fact that, in the same animals, this enzyme activity was only increased 2-fold in liver and 3-fold in white (inguinal) adipose tissue (R. Bazin & M. Lavau, unpublished work). From Tables 1 and 2 it was evident that the increases in the activities per total tissue of the two key lipogenic enzymes, acetyl-CoA carboxylase (4.2-fold) and fatty acid synthetase (4.5-fold), were very close to the increase

Table 2. Lipogenic enzyme activities in interscapular brown adipose tissue of lean (*Fa/fa*) and obese (*fa/fa*) Zucker rats (30 days old)

For experimental details see the text. The values are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. * $P < 0.005$, ** $P < 0.001$ versus values for lean rats. One unit of enzyme is defined as the amount that will catalyse the formation of 1 nmol of product/min at 37°C.

	Fatty acid synthetase	Citrate cleavage enzyme	Malic enzyme	Glucose 6-phosphate dehydrogenase	6-Phosphogluconate dehydrogenase	Acetyl-CoA carboxylase†
Activity (units/mg of protein)						
Lean (17)	42.1 $\pm$ 5.73	58.6 $\pm$ 6.10	113 $\pm$ 12.2	191 $\pm$ 13	139 $\pm$ 12	4.4 $\pm$ 0.85 (11)
Obese (6)	154.2** $\pm$ 16.60	362.6** $\pm$ 46.0	878** $\pm$ 88.8	577** $\pm$ 61	231* $\pm$ 24	17.1** $\pm$ 3.31 (8)
Activity (units/total tissue)						
Lean (17)	157 $\pm$ 18.9	219 $\pm$ 18.0	432 $\pm$ 39	741 $\pm$ 48	541 $\pm$ 46	15.1 $\pm$ 3.14 (11)
Obese (6)	702** $\pm$ 92.8	1665** $\pm$ 276	4000** $\pm$ 479	2633** $\pm$ 238	1062** $\pm$ 110	63.9** $\pm$ 12.45 (8)

† Two additional litters were used for this assay.

in fatty acid synthesis (5-fold). This strongly suggests that the increase in labelled fatty acids detected in interscapular brown adipose tissue of obese rats represented true fatty acid synthesis *de novo* and was not the result of an increased uptake of exogenous ^3H -labelled fatty acids. Such a conclusion is supported by the observation of Boulangé *et al.* (1981) that brown-adipose-tissue lipoprotein lipase was decreased in obese Zucker rats as compared with lean rats at 4 weeks of age. When the rates of fatty acid labelling *in vivo* were plotted against the activity of fatty acid synthetase (Fig. 1), all the values from both obese and lean rats approximated to a single line going through the origin. This substantiates the conclusion that ^3H -labelled fatty acid present in interscapular brown adipose tissue reflected synthesis *in situ*, irrespective of the genotype of rats.

Fig. 1. Relationship between fatty acid synthetase activity and fatty acid synthesis *in vivo* in interscapular brown adipose tissue

For experimental details see the text. The regression-line equations calculated by the least-squares method were:

obese, ○; $y = (18.3 \pm 76.4) + (1.11 \pm 0.48)x$;
 $r = 0.76, n = 6$;

lean, ●; $y = (-1.83 \pm 6.99) + (1.07 \pm 0.15)x$;
 $r = 0.88, n = 17$;

both groups: $y = (-8.18 \pm 7.77) + (1.26 \pm 0.09)x$;
 $r = 0.95, n = 23$.

It is noteworthy that the increased lipogenesis in interscapular brown adipose tissue reported here occurs in obese rats characterized by a decreased metabolic rate (Godbole *et al.*, 1978; Kaplan, 1979). This is in marked contrast with other conditions, namely cold and hyperphagia, where increased brown-adipose-tissue lipogenesis (McCormack & Denton, 1977; Trayhurn, 1979; Agius & Williamson, 1980) paralleled increased metabolic rates (Rothwell & Stock, 1980). That raises the question of the fate of the excess of fatty acids synthesized in interscapular brown adipose tissue of obese rats. Oxidation *in situ* is likely to be decreased in this model; moreover, the hypertrophy of interscapular brown adipose tissue observed in obese rats was smaller than one would expect from the increment in fatty acid synthesis rates; it could therefore be speculated that some fatty acids are exported from interscapular brown adipose tissue and contribute to the hypertrophy of the surrounding white adipose tissues.

This work establishes that brown adipose tissue is a site of expression of the *fa/fa* genotype. At present it is not clear whether the hyperlipogenesis demonstrated here in interscapular brown adipose tissue of obese rats aged 30 days is secondary to the hyperinsulinaemia of these rats, or whether, as in white adipose tissue (Lavau & Bazin, 1981), the capacity for hyperlipogenesis is present in suckling *fa/fa* pups before hyperinsulinaemia. In contrast with white adipose tissue, brown adipose tissue amounts to a very large portion of the total body weight in suckling rats. Therefore, if present early in life, the hyperlipogenesis capacity described here might permit brown adipose tissue to play a role of paramount importance in the channeling of metabolic fuels into lipid synthesis and the development of obesity.

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