Development of fatty acid-synthetic capacity in interscapular brown adipose tissue during suckling in genetically obese Zucker rats

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(Received 10 June 1983/Accepted 2 August 1983)

The development of the lipogenic capacity in brown adipose tissue was studied in suckling lean (*Fa/fa*) and obese (*fa/fa*) Zucker pups aged from 7 to 22 days. In both lean and obese pups, activities of the two key lipogenic enzymes, fatty acid synthetase and acetyl-CoA carboxylase, and of citrate cleavage enzyme rose from the early to the late suckling period. Compared with lean pups, 7-day-old *fa/fa* pups showed a 35% increase in fat accumulation in interscapular brown adipose tissue and a 25% increase in fatty acid synthetase activity. By 10 days of age, fat deposition, lipogenesis *in vivo* (assessed by the incorporation of ^3H from $^3\text{H}_2\text{O}$ into fatty acids) and fatty acid synthetase activity were 1.5–2-fold higher in pre-obese than in lean pups. Compared with lean pups, the increased lipogenesis *in vivo* observed in brown adipose tissue of 10-day-old pre-obese pups could not entirely account for the difference in fat deposition observed in this tissue, suggesting that additional mechanisms are at play to explain the increased fat content of this tissue.

We have shown that the interscapular brown adipose tissue of 30-day-old genetically obese rats displayed a dramatically increased lipid-synthesis activity as compared with their lean littermates (Lavau *et al.*, 1982). This metabolic abnormality could be causally related to the marked hyperinsulinaemia (Zucker & Antoniades, 1972) and hyperphagia (Dilettuso & Wangsness, 1977; Stern & Johnson, 1977) shown by the obese animals at that age, since both these states have been demonstrated to trigger lipogenesis in rat brown adipose tissue (Agius & Williamson, 1980). Alternatively, the capacity for hyperlipogenesis could be an early expression of the *fa/fa* genotype emerging in brown adipose tissue during the suckling period before hyperinsulinaemia, as this has been shown to be the case in white adipose tissue (Bazin & Lavau, 1982). If so, brown adipose tissue, which is not a negligible portion of body weight in suckling pups, would play a significant role in the onset of the obesity syndrome in the Zucker rat. The aim of this work was therefore to document the developmental changes in lipogenic enzyme activities that occur during suckling in brown adipose tissue in obese and lean Zucker rats.

Materials and methods

Animals and diets

All rats used in this study were bred in our laboratory from pairs originally provided by the Harriet G. Bird Memorial Laboratory, Stowe, MA, U.S.A. They were fed on stock diet (UAR, 91360 Epinay sur Orge, France) *ad libitum*. Known heterozygous (*Fa/fa*) lean females and obese males (*fa/fa*) were mated. From this mating, 50% of the litter is expected to be obese and 50% to be lean and of the heterozygous (*Fa/fa*) genotype. We selected litters numbering between 9 and 11 pups of both sexes. Pups may begin to eat the mother's stock diet as early as 13 days of age (Redman & Sweney, 1976). To avoid a switch from a high-fat diet (mother's milk) to a low-fat diet (stock diet) by the pups during the time period studied, the dams were fed on a high-fat diet when the pups were 10 days old. The high-fat diet, as described previously (Lavau *et al.*, 1979), contained 70% of its energy as fat and 10% as carbohydrate, representing approximately the composition of Zucker-rat milk at 10 days of lactation (Godbole *et al.*, 1981). The rats were maintained at 22–23°C on a fixed 12h-light

(07:00–19:00h)/12h-dark cycle. All experiments were started between 09:00 and 10:00h. Since the genotype of pups younger than 16 days of age (7, 10 and 14 days old) could not be diagnosed, we performed surgical biopsies (approx. 30mg) of interscapular brown adipose tissue under light diethyl ether anaesthesia, in order to keep the pups alive for later (7 weeks of age) genotype diagnosis. At 16 and 22 days of age the interscapular brown adipose tissue was totally dissected out under the same anaesthesia conditions, an inguinal fat-pad was removed for the identification of genotypes as previously described (Lavau & Bazin, 1982), and the pups were killed by decapitation. Some representative plots of the weight of inguinal fat-pad versus body weight, used in this study for the identification of genotypes in litters of 16-day-old pups, are illustrated in Fig. 1. It shows that lean (*Fa/fa*) and obese (*fa/fa*) genotypes could be assigned without any ambiguity.

Enzyme-activity assays

At least three litters were used for the determination of lipogenic enzyme activities at each of the following ages: 7, 10, 14, 16 and 22 days. The tissues were processed as described previously

(Lavau *et al.*, 1982). Briefly, tissues carefully trimmed free of white adipose tissue were homogenized (all-glass homogenizer) in 0.5 or 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), 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. Three additional litters of rats were used for the determination of acetyl-CoA carboxylase (EC 6.4.1.2) activity as described previously (Lavau *et al.*, 1982). Protein was determined in the infranatants by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Fig. 1. Determination of genotype by using inguinal fat-pad weights (mg) plotted versus body weights (g) by the method of Lavau & Bazin (1982), in 16-day-old lean and obese Zucker littermates

●, Lean pups; ○, obese pups. Regression lines were fitted by the method of least squares. Data from four litters are represented.

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.

Lipogenesis in vivo

The rate of lipogenesis *in vivo* was determined in three litters of 10-day-old pups by measuring the incorporation of ^3H from $^3\text{H}_2\text{O}$ into tissue fatty acids, essentially as described previously (Lavau *et al.*, 1982). The pups were injected intraperitoneally with 1 mCi of $^3\text{H}_2\text{O}$ in 0.1 ml of 0.9% NaCl and returned to their cages with their mothers. Then 1 h later, interscapular brown adipose tissue was surgically removed and blood samples were collected for the determination of the specific radioactivity of plasma water. Brown adipose tissue was carefully trimmed free of white adipose tissue, added to 3 ml of 30% (w/v) KOH and processed as described by Stansbie *et al.* (1976). Rates of fatty acid synthesis were calculated as μg -atoms of H incorporated/h from the radioactivity in the fatty acid fraction and the specific radioactivity of plasma water. No correction was made for isotope effects of ^3H versus H.

Lipid content of interscapular brown adipose tissue

At least one additional litter was used at each age. Surgical samples of interscapular brown adipose tissue were extracted with chloroform/methanol (2:1, v/v) during 24 h and lipids were determined gravimetrically.

Statistical analysis

Group comparisons were made by Student's *t* test.

Results

Fig. 2 shows the developmental changes in the content of lipid in brown adipose tissue of lean and obese Zucker pups during suckling. In lean pups, the fat content of the tissue increased from 10% at 7 days of age to more than 40% by 22 days of age. The fat-accretion process was particularly active during the second week of life, when brown-adipose-tissue lipid content increased by 200%. Thereafter, the rate of lipid deposition slowed down. The pattern of development of brown-adipose-tissue lipid content in obese Zucker rats was similar to that observed in the tissue of lean heterozygous pups in terms of developmental changes. However, the brown-adipose-tissue lipid content was significantly higher in pre-obese pups than in their lean littermates in the first week of life, as interscapular brown adipose tissue of the obese pups already contained 30% more fat than did that of lean pups. Beyond this age the differences in fat content between the two groups of

Fig. 2. Development of lipid content of interscapular brown adipose tissue of Zucker rats

For experimental details see the text. The values are means $\pm$ S.E.M.: * $P < 0.05$ and ** $P < 0.01$ compared with values for lean pups at the same age. The numbers of lean pups (●) were 8, 7, 4, 32 and 9, and of obese pups (○), 10, 9, 5, 28 and 11, at 7, 10, 14, 16 and 22 days respectively.

pups were highly significant, with a 50% increase in fat content in tissue of obese pups as compared with lean pups by 10 days of age. In 14-day-old pups, the brown-adipose-tissue fat contents clustered in two widely separated groups, with values ranging from 25.4 to 31.1% in lean pups (four rats) and from 40.2 to 46.6% in obese pups (five rats). In 16-day-old pups a clear bimodal distribution of brown-adipose-tissue fat content was found within each of the six litters investigated. The same observation was made in litters of pups aged 22 days. The question of whether the excess accumulation of lipid resulted in an increase in total tissue weight could not be answered in pups younger than 16 days of age, since the total tissue weight was not available. In 16-day-old pups, where the whole pad of interscapular brown fat could be dissected out, since the survival of the pups was not required for the identification of genotypes, a very significant tissue hypertrophy was observed in obese pups as compared with their lean littermates (112.1 ± 2.9 mg and 87.9 ± 2.54 mg respectively; $P < 0.01$). This increase in tissue weight strictly equalized the excess lipid store (47.5 mg in *fa/fa* and 27.3 mg in *Fa/fa* rats). At 22 days of age the increase in brown-adipose-tissue weight (from 143 mg in lean to 174 mg in obese rats) was also entirely accounted for by the increase in fat content (from 60 mg in lean to 90 mg in obese rats). Figs. 3 and 4 depict the developmental changes in lipogenic enzyme activities in brown adipose tissue of pre-obese *fa/fa* pups, as compared with heterozygous (*Fa/fa*) lean pups, during

Fig. 3. Development of fatty acid synthetase (a) and acetyl-CoA carboxylase (b) activities, in interscapular brown adipose tissue of lean (*Fa/fa*; ●) and obese (*fa/fa*; ○) pups

A unit is defined as the amount of enzyme needed to catalyse the formation of 1 nmol of product/min at 37°C. Results are expressed as means $\pm$ s.e.m. (a) The numbers of lean pups were 14, 12, 18, 12 and 11, and of obese pups 14, 14, 15, 13 and 11, at 7, 10, 14, 16 and 22 days respectively. (b) The numbers of lean pups were 15, 10, 14 and 7, and of obese pups 11, 10, 7 and 12, at 10, 14, 16 and 22 days respectively. * $P < 0.05$ and ** $P < 0.01$ as compared with lean pups of the same age.

Fig. 4. Development of citrate cleavage enzyme (a), malic enzyme (b), glucose 6-phosphate dehydrogenase (c) and 6-phosphogluconate dehydrogenase (d) in interscapular brown adipose tissue of lean (*Fa/fa*) and obese (*fa/fa*) pups. Results (units/mg of protein) are expressed as means $\pm$ s.e.m. The numbers of lean pups were 14, 12, 18, 12 and 11, and obese pups 14, 14, 15, 13 and 11, at 7, 10, 14, and 16 and 22 days respectively. Symbols are as in Fig. 3.

Table 1. *Lipogenesis in interscapular brown adipose tissue of lean (Fa/fa) and obese (fa/fa) Zucker rats (10 days old)*For experimental details see the text. The values are means $\pm$ S.E.M. for the numbers of rats shown in parentheses.** $P < 0.01$ versus values for lean rats.

	Fat (%)	Fatty acid synthetase (units/mg of protein)	Lipogenesis <i>in vivo</i> (rate of fatty acid synthesis) (μ g-atoms of H incorporated/h)	
			(per g wet wt. of tissue)	(per g fat-free wt. of tissue)
Lean	18.6 $\pm$ 1.88 (7)	4.0 $\pm$ 0.51 (12)	5.2 $\pm$ 0.29 (13)	6.5 $\pm$ 0.39 (13)
Obese	29.2 $\pm$ 1.26 (9)**	7.7 $\pm$ 0.65 (14)**	6.9 $\pm$ 0.36 (17)**	10.2 $\pm$ 0.55 (17)**

suckling. In lean pups, two different developmental profiles of enzyme activities were observed: one group of enzymes, including fatty acid synthetase and acetyl-CoA carboxylase, the two key lipogenic enzymes, and citrate cleavage enzyme, an enzyme providing the cytoplasm with acetyl units, increased steadily from day 7 to day 22. The rises in enzyme activities were very substantial, 6-fold for fatty acid synthetase and 3-fold for citrate cleavage enzyme over that time period; acetyl-CoA carboxylase, whose activity was only studied from day 10 onwards, showed a 2-fold increase. In contrast, NADPH-producing enzymes (malic enzyme, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) underwent no significant changes over the whole suckling period. The developmental patterns exhibited by lipogenic enzymes in adipose tissue of obese pups were very similar to those observed in brown adipose tissue of their lean littermates in that fatty acid synthetase, acetyl-CoA carboxylase and citrate cleavage enzyme increased severalfold during suckling, whereas NADPH-producing enzymes remained approximately at the same activity. However, several differences were observed between the two genotypes. Some of the enzymes appeared to have lower activities in obese pups than in their lean littermates, such as glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which were decreased by 20–30% in the obese rats during the second and third weeks of life. In contrast, fatty acid synthetase activity was significantly increased in obese pups in the first week of life, and by 10 days of age this key lipogenic enzyme was 92% higher in activity in the obese rats than in the normal rats; the increase in fatty acid synthetase was significant throughout the suckling period. In addition, during the late suckling period, from day 16 to day 22, the developmental patterns of fatty acid synthetase, acetyl-CoA carboxylase, citrate cleavage enzyme and malic enzyme activities in brown adipose tissue of obese pups differed from those observed in lean pups in that they rose more sharply, leading to very significant differences between the two groups of rats by 22 days of age. That suggests the presence of a triggering factor in the mutant rat.

A candidate for this effect could be the increase in insulinaemia that has been reported to occur in obese pups from 17 days of age (Bazin & Lavau, 1982). The hyperphagia that develops in obese pups at that age is also likely to play a role in this phenomenon (Stern & Johnson, 1977).

The fact that fatty acid synthetase was the only lipogenic enzyme to show a marked increase in brown adipose tissue of pre-obese pups within the first 2 weeks of life raised questions of the physiological significance of this genotype effect for the regulation of brown-adipose-tissue lipogenesis in the whole animal. Therefore the rate of brown-adipose-tissue fatty acid synthesis was determined *in vivo* in lean and obese pups at 10 days of age. In good agreement with the enzyme data, the results shown in Table 1 clearly indicate that the rate of fatty acid synthesis *in vivo* in brown adipose tissue was higher in obese pups than in lean pups.

Discussion

Previous studies by Pillay & Bailey (1982a,b) have documented the changes in lipogenic enzyme activities and lipogenesis that occur in brown adipose tissue during the perinatal period and at the suckling-weaning transition. However, nothing was known about the developmental changes in the fatty acid-synthesis capacity of brown adipose tissue during suckling. Our data for the lean control rats showed that activities of citrate cleavage enzyme and the key lipogenic enzymes, fatty acid synthetase and acetyl-CoA carboxylase, rose steadily in this tissue from early to late suckling, leading to a severalfold increase during this period. Therefore these developmental profiles were very different from the steady low values exhibited by those enzymes in liver and adipose tissue throughout suckling, in Wistar rats (Ballard & Hanson, 1967; Taylor *et al.*, 1967) and in lean Zucker rats (Bazin & Lavau, 1982). The tissue-specificity of the rise in lipogenic enzyme activities during suckling might be related to the gradual decrease in the environmental temperature to which the pups are exposed as they switch from nest (33°C) to cage (22°C). Indeed, cold has been shown to elicit increases in the

capacity for fatty acid synthesis much greater in brown adipose tissue than in liver and white adipose tissue (Trayhurn, 1979).

The aim of the present work was to monitor any changes in lipogenic enzyme activities in brown adipose tissue of *fa/fa* pups during the earliest stages of development of obesity. Previous studies have established that lipogenic enzyme activities were not enhanced in the liver of suckling obese pups as compared with their lean littermates (Godbole *et al.*, 1978; Turkenkopf *et al.*, 1980; Bazin & Lavau, 1982). In contrast, in inguinal adipose tissue fatty acid synthetase activity was 50% higher in the obese rats than in the lean rats by 9 days of age (Bazin & Lavau, 1982). Several lines of evidence indicate that brown adipose tissue was also a very early site of expression of the *fa/fa* genotype.

By 7 days of age the fat content of the tissue was already increased by as much as 30% in obese pups as compared with their lean littermates. These data, in good agreement with the increase in interscapular brown-adipose-tissue weight observed in 7-day-old *fa/fa* pups by Boulangé *et al.* (1981), were at variance with those reported on brown adipose tissue of genetically obese (*ob/ob*) mice, where no major excess accumulation of lipids was detected as late as 14 days of age (Goodbody & Trayhurn, 1982). Several altered metabolic fluxes could contribute to the increased fat accretion observed in brown adipose tissue of obese Zucker pups: an increased uptake of circulating triacylglycerols through the action of lipoprotein lipase, a decreased lipid mobilization and oxidation, an enhanced fatty acid synthesis activity. The last hypothesis was substantiated by the finding that both fatty acid synthetase activity and fatty acid synthesis *in vivo* were markedly increased in brown adipose tissue of obese pups within the first days of life. This increased fatty acid-synthetic capacity could be secondary to small periodic increases in insulin secretion, since York *et al.* (1981), in 20-day-old *fa/fa* pups, and Rohner-Jeanrenaud *et al.* (1983), in 17-day-old *fa/fa* pups, have shown that pre-obese rats have an increased capacity for insulin secretion in response to a glucose challenge before any increase in basal insulin concentration. However, there is no data in the literature suggesting that such an increased capacity for insulin secretion could occur in the early suckling period when pups are restricted to high-fat maternal milk.

The increment in the rate of fatty acid synthesis above the values for lean rats found in the tissue of obese pups at 10 days of age would approximately account for 1–2 mg of lipid/day per g wet wt. of tissue (by using the factor of 13.3 for the number of ³H atoms incorporated for each molecule of fatty acid synthesized; Windmueller & Spaeth, 1966) when the excess lipid accumulation per g of tissue

amounted to more than 10 mg/day. This result indicates that additional mechanisms were at play in obese rats. As lipoprotein lipase has been shown to be substantially decreased in brown adipose tissue of 2-week-old obese pups as compared with lean pups (Boulangé *et al.*, 1981), the uptake of circulating triacylglycerols in brown adipose tissue cannot be proposed to explain the altered fat content of the tissue. Therefore, taken together these results strongly suggest the presence of a low rate of fatty acid oxidation in brown adipose tissue of obese pups. This conclusion, supported by current observations that brown-adipose-tissue thermogenic capacity, as assessed by GDP binding to mitochondria, is decreased in obese rats in the very first days of life (R. Bazin, D. Eteve & M. Lavau, unpublished work), is in keeping with the decrease in energy expenditure observed in obese pups by 7 days of age (Planche *et al.*, 1983). Therefore in brown adipose tissue of pre-obese pups a high fatty acid synthesis activity would be associated with a low rate of thermogenesis, similarly to what has been reported in older obese Zucker rats, where both a high fatty acid synthesis (Lavau *et al.*, 1982) and a low GDP binding (Holt & York, 1982) have been demonstrated. Thus the hyperlipogenesis triggered by the *fa/fa* genotype in brown adipose tissue differs from the cold- or hyperphagia-induced hyperlipogenesis (McCormack & Denton, 1977; Trayhurn, 1979; Agius & Williamson, 1980), which are always associated with an increased rate of thermogenesis (Rothwell & Stock, 1980).

In conclusion, the present work establishes that in the pre-obese Zucker rat the interscapular brown adipose tissue is a very early site of hyperlipogenesis. As has been shown for inguinal white adipose tissue, the hyperlipogenesis occurs in *fa/fa* pups before hyperinsulinaemia and hyperphagia, and suggests that hyperlipogenesis could be a primary factor in the development of obesity in the Zucker rat.

References

- Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480
- Ballard, F. J. & Hanson, R. W. (1967) *Biochem. J.* **102**, 952–958
- Bazin, R. & Lavau, M. (1982) *J. Lipid Res.* **23**, 839–849
- Boulangé, A., Planche, E. & de Gasquet, P. (1981) *Metab. Clin. Exp.* **30**, 1045–1052
- Cottam, G. L. & Srere, P. A. (1969) *Arch. Biochem. Biophys.* **130**, 304–311
- Dilettuso, B. A. & Wangsness, P. J. (1977) *Proc. Soc. Exp. Biol. Med.* **154**, 1–5
- Glock, C. E. & McLean, P. (1953) *Biochem. J.* **55**, 400–408
- Godbole, V. Y., York, D. A. & Bloxham, D. P. (1978) *Diabetologia* **16**, 41–44

- Godbole, V. Y., Grundleger, M. L., Pasquine, T. A. & Thenen, S. W. (1981) *J. Nutr.* **111**, 480–487
- Goodbody, A. E. & Trayhurn, P. (1982) *Biochim. Biophys. Acta* **680**, 119–126
- Halestrap, A. P. & Denton, R. M. (1973) *Biochem. J.* **132**, 509–513
- Holt, S. & York, D. A. (1982) *Biochem. J.* **208**, 819–822
- Lavau, M. & Bazin, R. (1982) *J. Lipid Res.* **23**, 941–943
- Lavau, M., Fried, S. K., Susini, C. & Freychet, P. (1979) *J. Lipid Res.* **20**, 8–16
- Lavau, M., Bazin, R., Karaoghlanian, Z. & Guichard, C. (1982) *Biochem. J.* **204**, 503–507
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- McCormack, J. G. & Denton, R. M. (1977) *Biochem. J.* **166**, 627–630
- Ochoa, S. (1955) *Methods Enzymol.* **1**, 739–753
- Pillay, D. & Bailey, E. (1982a) *Biochim. Biophys. Acta* **713**, 663–669
- Pillay, D. & Bailey, E. (1982b) *Int. J. Biochem.* **14**, 511–517
- Planche, E., Joliff, M., de Gasquet, P. & Lelievre, X. (1983) *Am. J. Physiol.* **245**, E107–E113
- Redman, R. S. & Sweney, L. R. (1976) *J. Nutr.* **106**, 615–626
- Rohner-Jeanrenaud, F., Hochstrasser, A. C. & Jeanrenaud, B. (1983) *Am. J. Physiol.* **244**, E317–E322
- Rothwell, N. J. & Stock, M. J. (1980) *Can. J. Physiol. Pharmacol.* **58**, 842–848
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Stern, J. S. & Johnson, P. R. (1977) *Metab. Clin. Exp.* **26**, 371–380
- Taylor, C. B., Bailey, E. & Bartley, W. (1967) *Biochem. J.* **105**, 717–722
- Trayhurn, P. (1979) *FEBS Lett.* **104**, 13–16
- Turkenkopf, I. J., Olsen, J. L., Moray, L., Greenwood, M. R. C. & Johnson, P. R. (1980) *Proc. Soc. Exp. Biol. Med.* **164**, 530–533
- Windmueller, H. G. & Spaeth, A. E. (1966) *J. Biol. Chem.* **241**, 2891–2899
- York, D. A., Shargill, N. S. & Godbole, V. Y. (1981) *Diabetologia* **21**, 143–148
- Zucker, L. M. & Antoniades, H. N. (1972) *Endocrinology* **90**, 1320–1330