Adipsin mRNA amounts are not decreased in the genetically obese Zucker rat

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Adipsin gene expression as assessed by mRNA amounts was examined in adipose tissue of genetically obese rats at the onset (16 days of age) or at later stages (30 and 60 days of age) of obesity. Amounts of mRNA were equivalent in obese and lean rats at 16 days of age. In adult rats, we observed a 2-fold decrease in adipsin mRNA in the obese rats compared with control lean rats, which was abolished by weaning the animals on a high-fat diet. Our data show that, in sharp contrast with genetically obese mice, adipsin mRNA is not suppressed in genetically obese Zucker rats.

INTRODUCTION

It is becoming increasingly clear that genetic influences have an important role in determining human fatness and obesity [1–3]. Therefore, findings in laboratory rodents in which obesity is genetically inherited may help to elucidate the biochemical basis of this disorder in man. Two strains of mice, *db/db* and *ob/ob*, and the Zucker *fa/fa* rat, whose obesity presents many traits in common with the human disease, provide suitable models. In spite of intensive research, the nature of the genetic lesion that causes obesity in these animals remains obscure. Recently, new perspectives have been opened by the report by Flier *et al.* [4] that the expression of the adipsin gene was suppressed in adipose tissue of both *db/db* and *ob/ob* mice. Adipsin, discovered by Spiegelman *et al.* [5], has been shown to be a serine protease homologue [6] which is synthesized mainly by adipose tissue and secreted into the bloodstream [7]. Although the physiological role of this protein is not known, the severity of the defect in adipsin expression in both strains of genetically obese mice (decrease by a factor of 100) is an interesting finding with potentially great implications for the understanding of this disorder [4]. The proposition that adipsin might be a marker of genetic and metabolic obesities prompted us to examine this question in the genetically obese Zucker rat.

MATERIALS AND METHODS

Animals and diets

Obese (*fa/fa*) rats and their lean littermates were bred in our laboratory from pairs originally provided by the Harriet G. Bird Memorial Hospital, Stow, MA, U.S.A. Known heterozygous (*Fa/fa*) lean females and obese *fa/fa* males were mated. From this mating, 50 % of the litter is expected to be obese and 50 % lean of the heterozygous genotype. Rats in the fed state were studied either during suckling (16 days of age) or after weaning (30 and 60 days of age). Pups were routinely separated from their mothers at 28 days of age. Weaning was performed either on to standard rat diet (high-carbohydrate diet) or on to

a high-fat diet. In this case, 2-week-old pups and their mother were shifted to a low-carbohydrate/high-fat diet (30 % lipid, w/w) and studied at 30 days of age. Rats were weighed, killed by decapitation, and subcutaneous inguinal fat-pads were removed under sterile conditions. When 16-day-old pups were used, fat-pads from pups of the same genotype, identified as previously described [8], were pooled within each litter; for older animals, fat-pads were processed individually.

Northern-blot analysis

Total RNA from adipose tissue was extracted as described previously [9], by using the guanidinium isothiocyanate/LiCl procedure [10]. For each RNA preparation, the absence of DNA contamination was checked on agarose gels after ethidium bromide staining. RNA samples were separated by electrophoresis through 1.5 % agarose gels containing 2.2 M-formaldehyde, transferred to nylon membranes (Hybond-N; Amersham, Les Ulis, France) and probed with a 940 bp cDNA coding for adipsin [5], kindly given by Dr. H. Green (Harvard Medical School, Boston, MA, U.S.A.). Radiolabelling of the probe with ³²P was performed by the random-priming method (Multiprime DNA-labelling kit, from Amersham) and gave a specific radioactivity greater than 10⁸ c.p.m./μg. Hybridization and washings were performed as described previously [9]. Comparison of signals between lean and obese rats was assessed by densitometric scanning (Cliniscan) of autoradiograms.

RESULTS AND DISCUSSION

Adipsin gene expression was first assessed in adipose tissue of young (16 days old) pups, at a stage when obesity at the onset is not yet complicated by hyperinsulinaemia [11]. The results (Fig. 1) show that the amounts of adipsin mRNA in adipose tissue were very similar in lean and obese rats, ruling out a fall in adipsin mRNA contents as a primary defect in this obesity. To examine the possibility that the defect in adipsin expression might emerge secondarily to the obese state, the amounts of adipsin mRNA were next determined in

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Fig. 1. Northern-blot analysis of adipsin mRNA in inguinal adipose tissue of 16-day-old suckling lean (l) and obese (o) Zucker rats

Mean fat-pad weights were 220 ± 20 mg and 450 ± 28 mg for 17 lean and 23 obese rats ($P < 0.001$) from four separate litters. No difference between the two genotypes in the intensity of signals could be detected by densitometric scanning of the autoradiograms.

adipose tissue of 30- and 60-day-old obese rats, when hyperphagia and hyperinsulinaemia had fully developed. Adipose tissue from 60-day-old *db/db* mice was also included in our study. The results show that, in sharp contrast with adipose tissue of adult *db/db* mice, where adipsin mRNA was virtually absent (as previously reported by Flier *et al.* [4]), adipsin mRNA was readily detected in adipose tissue of adult obese Zucker rats at both 30 and 60 days of age (Figs. 2 and 3). However, an approx. 2-fold decrease in adipsin mRNA per unit of total RNA could be observed in the obese rats as compared with lean rats during the post-weaning period. At this time the genotype-induced hyperphagia had developed, resulting in an over-intake of carbohydrate in obese rats when standard rat chow was used. This could be responsible for the difference between the two genotypes, since glucose infusion has been shown to decrease (by 65%) adipsin mRNA in the normal rat [4]. Therefore we have examined adipsin mRNA amounts in Zucker rats weaned on to a high-fat diet. As shown in Fig. 3 (inset), there was no longer any difference between lean and obese rats, although the high-fat diet had markedly aggravated obesity.

Altogether, our data show that adipsin mRNA is not suppressed during either the early onset or the development of obesity in the fatty rat. Although the possibility that the amount of adipsin protein might be different between lean and obese rats cannot be discounted at the

Fig. 2. Adipsin mRNA contents in (a) inguinal adipose tissue of 2-month-old Zucker rats and (b) perigonadal adipose tissue of *db/db* mice and age-matched congenic C57/BL controls

(a) Northern-blot analysis was performed, as described in the Materials and methods section, on RNA preparations obtained from individual rats whose fat-pad weights were 1776 ± 430 mg and 15390 ± 1330 mg (mean values for five lean and six obese rats respectively; $P < 0.001$). Each lane shows the signal obtained for one rat. Results are representative of two separate experiments. Compared with lean rats, adipsin mRNA is decreased 1.7-fold in obese rats, as estimated by densitometric scanning of the autoradiograms. (b) Obese and control female mice were obtained from Jackson Laboratory and studied at 2 months of age in the fed state. Adipose-tissue weights were 245 ± 34 mg and 3460 ± 135 mg (mean values for five control and two obese *db/db* mice respectively). RNA preparations were obtained from two individual obese mice and five lean mice pooled in two batches. The results shown are from one experiment. Amounts of total RNA per lane are shown.

Fig. 3. Adipsin mRNA contents in inguinal adipose tissue of 30-day-old lean and obese Zucker rats weaned on either a standard diet or a high-fat diet (inset)

Inguinal fat-pad weights were 669 ± 66 mg and 2156 ± 148 mg for seven lean and ten obese rats respectively ($P < 0.001$). Adipose-tissue total RNA contents, as measured by A_{260} , were 292 ± 61 and 430 ± 78 μ g/fat-pad in lean and obese rats respectively ($P < 0.05$). Signals obtained after hybridization with the adipsin cDNA probe are shown for four lean and six obese rats from the same litter. The same results were obtained in two separate litters. Densitometric scanning of the autoradiograms revealed a 2-fold decrease in the intensity of the signals of obese rats as compared with lean. Inset: Northern-blot analysis of adipsin mRNA in lean and obese Zucker rats weaned on a high-fat diet. In these experiments, rats from two separate litters (ten rats for each genotype), whose inguinal fat-pads weighed 1.39 ± 0.09 g and 3.17 ± 0.14 g for lean and obese rats respectively, were studied. Results representative of three separate experiments are shown. Amounts of total RNA per lane are shown.

present time, it is unlikely, in view of observations that adipsin mRNA and protein amounts are modulated in a consistent manner [4]. Our data rather suggest the presence of species-specific difference in the genetic lesion causing obesity. However, a question of major importance that remains to be answered is whether the impairment of adipsin gene expression is also present in genetically obese mice in the pre-obese state. Alternatively the differences in amounts of adipsin mRNA between adult obese Zucker rats and adult obese mice might reflect the differences in hormonal status between these rodents. Insulin is unlikely to play a role, since

these animal models are all characterized by a frank hyperinsulinaemia. However, it is associated with a severe hyperglycaemia in obese mice, whereas obese Zucker rats are normoglycaemic [12]. The diabetic insulin-resistant state of the mouse models might play a role in the early defect of adipsin mRNA expression in these animals. Another candidate would be the serum corticosterone concentration, markedly elevated in both *ob/ob* and *db/db* mice [13,14] and unchanged in Zucker rats [15–17], since this hormone has very recently been demonstrated to suppress markedly adipose-tissue adipsin mRNA [18].

The present work demonstrates that suppression of adipsin mRNA is not a general feature of inherited obesities in rodents, and adipsin is therefore not suitable as a marker of genetic obesities. The full significance of adipsin deficiency in the pathophysiology of some obesities will require further investigation, and especially the elucidation of the physiological function of this protein.

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