Fatty acid synthase and adipsin mRNA levels in obese and lean JCR:LA-cp rats: effect of diet

G. Shillabeer, J. Hornford, J.M. Forden, N.C.W. Wong, J.C. Russell,* and D.C.W. Lau[1]

Department of Medicine and Julia McFarlane Diabetes Research Centre, The University of Calgary, Calgary, Alberta, and Department of Surgery,* University of Alberta, Edmonton, Alberta, Canada

Abstract In Sprague-Dawley rats, fatty acid synthase (FAS) activity is suppressed by dietary fat. To test the hypothesis that a defect in regulation of de novo fatty acid synthesis exists in massive obesity, we investigated the effect of diet on FAS mRNA levels in genetically obese JCR:LA-corpulent (cp) rats. We also determined levels of mRNA encoding adipsin, a fat cell-derived protein possibly associated with lipid metabolism. Hepatic FAS mRNA levels were elevated five-fold in obese compared to lean cp rats and were unsuppressed by dietary fat. Dietary sucrose increased FAS mRNA levels in lean cp rats, but, in contrast to Sprague-Dawley rats, little deposition of lipid resulted. Adipsin mRNA levels were fivefold lower in obese cp and Sprague-Dawley rats than in lean cp rats and were unaffected by diet. We conclude that exaggerated de novo fatty acid synthesis may play a major role in the pathogenesis of obesity in obese JCR:LA-corpulent rats.—**Shillabeer, G., J. Hornford, J.M. Forden, N.C.W. Wong, J.C. Russell, and D.C.W. Lau.** Fatty acid synthase and adipsin mRNA levels in obese and lean JCR:LA-cp rats: effect of diet. *J. Lipid Res.* 1992. **33:** 31-39.

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A defect in the regulation of fatty acid synthesis can be postulated to be a feature of massive obesity. Preferential shunting of dietary substrate into de novo lipid synthesis, predominantly in the liver (1), has been reported in the obese Zucker (fa/fa) rat despite caloric restriction (2–4). In the short term, acetyl-CoA carboxylase is considered to be the regulatory enzyme that determines the rate of synthesis of fatty acids. However, long term adaption of the rate of de novo lipogenesis to the nutritional and hormonal status of the animal is correlated to the level of fatty acid synthase (EC 2.3.1.85, FAS) and the rate of synthesis of this enzyme (5). FAS is the multiunit enzyme complex involved in the biosynthesis of long chain fatty acids, the activity of which was suppressed by a high fat diet in both the obese Zucker (6) and normal Sprague-Dawley (SD) rat (7, 8). Dietary fats may regulate FAS at the level of gene transcription (9).

We are studying a recently established model of genetic obesity, the JCR:LA-corpulent (cp) rat (10–12). Rats of this strain (formerly designated LA/N-cp) are lean when homozygous normal (+/+) or heterozygous (+/cp), and massively obese when homozygous for the recessive corpulent gene (cp/cp). The cp/cp rats are also characterized by hyperphagia, hyperlipoproteinemia, and hyperinsulinemia (10). We have observed significant body weight gains (11) and 12-fold increases in fat pad weight of cp/cp rats pair-fed a low fat diet compared to their lean partners (12). These observations suggest a possible defect in the regulation of lipogenesis. The hyperphagia manifested by the cp/cp rats suggests that there may also be defect in the feedback regulation of food intake (10).

Adipsin, a differentiation-dependent serine protease, secreted by adipocytes, has been advanced as a marker associated with animal models of metabolic and genetic obesity and as a possible feed-back signal in food intake regulation (2, 13). Adipsin has recently been shown to have activity identical to factor D in regulating the complement pathway thus suggesting a role for this pathway in obesity (14). Because "an increase in adipsin mRNA appears to be part of the catabolic state in normal rodents," adipsin has also been postulated to be a regulator of lipolysis and perhaps lipogenesis (13). Adipsin was suppressed more than 100-fold in genetically obese db/db and ob/ob mice, a suppression that was not reversed by fasting. However, fasting increased adipsin mRNA in normal rats and in streptozotocin-induced diabetes while levels decreased in the hyperglycemic, hyperinsulinemic state (13). Adipsin mRNA was only suppressed two-fold in weanling obese Zucker rats

Abbreviations: FAS, fatty acid synthase; SD, Sprague-Dawley; GPDH, glycerophosphate dehydrogenase; TG, triacylglycerol.

[1]To whom reprint requests should be addressed at: Division of Endocrinology and Metabolism, Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa, ON, K1Y 4E9, Canada.

compared to the lean and this suppression could be prevented by weaning on a high fat diet (15).

The purpose of the present report was to determine the susceptibility of FAS in the JCR:LA-corpulent rat to regulation by diets known to suppress or stimulate the activity of lipogenic enzymes (7, 8). Specifically, we have determined the relative abundance of mRNAs coding for FAS and glycerophosphate dehydrogenase (EC 1.1.1.8; GPDH) in obese and lean cp rats. While +/+ rats are the valid control for the cp/cp rats, data obtained in the present and in an earlier study (12) suggest that lean +/+ rats may be "abnormal" in regard to lipogenic activity. Therefore, we have compared data obtained from both lean and obese cp rats to those from the Sprague-Dawley (SD) rat as an example of "normal" lipogenic activity. GPDH holds a central position at the branch point between the glycolytic pathway and triacylglycerol synthesis and is the sole source of glycerol in the adipocyte. We have also examined the effect of diet on the abundance of adipsin mRNA in adipose tissue of the cp rats to assess whether this strain of genetically obese rats conforms to the reported relationship between adipsin and obesity (13, 14, 16).

METHODS

Obese and lean JCR:LA-cp rats were bred at the University of Alberta. This colony was established with stock donated by Dr. C.T. Hansen, Small Animal Section, Division of Research Services, NIH, Bethesda, MD. The lean +/+ rats were bred from demonstrated homozygote normal parents. After transfer to the animal facilities at the University of Calgary, male rats were maintained on standard rat chow (Wayne Rodent Blox, Continental Grain Co., Chicago, IL; 8.6% of calories as fat, 68.6% as carbohydrate, and 22.8% as protein) in wire-bottomed cages at 22°C on a 12 h–12 h light-dark cycle until 10 months of age.

Four-month-old female lean and corpulent rats were maintained for 12 weeks under similar conditions but were fed nutritionally adequate, semi-synthetic diets that varied in the major source of energy. These diets have been described in more detail previously (7). Lean rats were fed standard rat chow, high starch (65% of calories as starch, 10.5% sucrose, 20% protein, and 4.5% fat) or high sucrose (28.5% starch, 47% sucrose, 20% protein, and 4.5% fat) diets. The sucrose diet was supplemented by 20 ml/rat per day of a 20% sucrose solution. Intake of the sucrose solution had to be restricted to 20 ml/day, because the lean rats consumed it preferentially at the expense of the nutritionally complete sucrose diet. A high sucrose diet is known to stimulate lipogenesis (17) and the development of obesity particularly when given in the liquid form (18). Food intake was measured twice weekly and water was freely available. Obese rats were fed rat chow or a high fat diet (35% starch, 20% protein, and 45% fat which was safflower oil).

Male, weanling Sprague-Dawley rats (Charles River) were maintained on chow, or on the same high fat, starch, or sucrose diets as the cp rats until they were 5 months old. At the time of killing, they weighed 503 ± 17, 578 ± 14, 554 ± 17, and 525 ± 15 g, respectively.

Rats were killed in the fed state by exsanguination under light ether anesthesia. Blood was collected for radioimmunoassay of plasma insulin using the double antibody method with rat insulin standards (11). Total plasma triacylglycerols were determined in the diet-fed rats by the L-α-glycerol phosphate oxidase colorimetric method (GPO-pack; Boehringer Mannheim Canada, Dorval, Que.) (19).

Retroperitoneal fat depots and liver were rapidly excised, placed in pre-tared vials containing phosphate-buffered saline (0.01 M K_2HPO_4, 0.01 M KH_2PO_4, 0.154 M NaCL, pH 7.4) at 37°C and weighed. The tissues were then blotted dry, frozen in liquid nitrogen, and stored at –85°C for later analysis.

Quantitation of mRNAs coding for adipsin, GPDH, and FAS

Total RNA was extracted from liver samples using guanidine-HCl (20, 21) and from adipose tissue by centrifugation over a cesium chloride cushion as described by Chirgwin et al. (22). RNA samples were analyzed by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde and MOPS buffer (0.2 M morpholenopropanesulfonic acid, 50 mM sodium acetate, 5 mM EDTA (23)) and separated RNA species were transferred to nitrocellulose membranes by standard blotting techniques. The relative amounts of mRNA coding for GPDH and FAS were determined by Northern blot or by dot blot hybridization (23, 24) with specific ^{32}P-labeled cDNA probes. The probes were labeled by the random primer method (23). The adipsin and GPDH probes used were generously donated by Dr. H. Green of Harvard Medical School (25) and the FAS probe (pFAS-7) used was a gift from the laboratory of Dr. J.W. Porter (26). Autoradiographs of hybridizations were quantitated by a video-assisted densitometer (27). The relative amount of mRNA in each sample was normalized to 28S ribosomal RNA (28) and between-blot variation was corrected using RNA extracted from the liver of 2-month-old Sprague-Dawley rats as an external standard.

Enzyme assays

The liver was homogenized with a tissue homogenizer (Kinematica, Lucerne, Switzerland) in phosphate-carbonate buffer (70 mM $NaHCO_3$, 85 mM K_2HPO_4, 9 mM KH_2PO_4, 1 mM EDTA, 1 mM dithiothreitol, pH 8.0) and the homogenate was centrifuged at 20,000 *g* for 10 min. The supernatant was centrifuged again at 100,000 *g* for 1 h at 4°C and aliquots of this supernatant were taken and frozen at –85°C until used for GPDH and FAS assay.

Frozen fat tissue was homogenized at 37°C in a 10 mM HEPES buffer containing 0.25 M sucrose, 1 mM EDTA, and 1 mM dithiothreitol. The homogenate was centrifuged at 100,000 *g* at 0–4°C for 20 min. The floating fat layer was removed and aliquots of the fluid infranatant fraction were taken for determination of FAS and GPDH activity.

Soluble protein was determined by the Coomassie blue method using a Bio-Rad assay kit (29). All enzyme assays were performed under optimal substrate and linear kinetic conditions.

FAS activity was determined in liver and adipose tissue by the method of Nepokroeff, Lakshmanan, and Porter (30). Aliquots of the tissue extracts were incubated at 37°C for 15 min with phosphate buffer (1 mM potassium phosphate, 0.2 mM EDTA, and 1 M dithiothreitol, pH 7.0). After the addition of NADPH (100 μM final concentration), the reaction was initiated by the addition of acetyl- and malonyl-CoA (35 μM and 100 μM final concentration, respectively). The change in absorbance at 340 nm due to oxidation of NADPH was followed with a Gilford response spectrophotometer at 30°C. A unit of FAS activity is defined as 1 nmol of NADPH oxidized/min.

GPDH activity was determined in both adipose tissue and liver under zero order kinetics by the method of Kozak and Jensen (31). The substrate, dihydroxyacetone phosphate (Sigma), was added to aliquots of the appropriate fractions (see above) of the tissue homogenates in the presence of NADH (0.12 mM; Sigma). The change in absorbance at 340 nm due to NADH oxidation was followed spectrophotometrically at 23°C. One unit of enzyme activity corresponded to 1 nmol of NADH oxidized/min.

TABLE 1. Total body and fat pad weights of age-matched male lean (+/+) and obese (cp/cp) JCR:LA-corpulent rats.

	Obese cp/cp (n = 4)	Lean +/+ (n = 4)
	g	
Body weight	801 ± 9.8*	416 ± 3.2
Epididymal pad	13.7 ± 0.1*	1.1 ± 0.1
Retroperitoneal pad	38.2 ± 1.9*	1.9 ± 0.2
Inguinal pad	66.3 ± 1.0*	3.5 ± 0.2
Subscapular pad	29.7 ± 10.7*	0.4 ± 0.1

Rats were fed standard rat chow until 10 months of age. Values shown are the means ± SEM of n rats per group. *Values are significantly different by Student's paired *t*-test from those of the lean (+/+) rats ($P < 0.01$).

Data analysis

Data were analyzed by one-way ANOVA and Tukey's method for multiple comparisons or, where appropriate, by Student's t-test for paired or independent data.

RESULTS

Total body and adipose tissue weights of male rats

Ten-month-old male cp/cp rats fed a low fat chow diet were two-fold heavier than lean +/+ rats fed the same diet (**Table 1**). Regional fat depots of obese rats were 12- to 70-fold larger than those of their age-matched lean counterparts ($P < 0.01$). Plasma insulin concentrations were 268.0 ± 62.7 mU/l in the obese and 17.0 ± 3.3 mU/l in the lean rats ($P < 0.03$).

Hepatic and adipose tissue FAS of male rats

Representative dot blot (FAS) or Northern blot analyses (GPDH, adipsin, and 28S) of hepatic and adipose tissue mRNAs are show in **Figs. 1A and 1B**, respectively. We have demonstrated previously that the FAS probe hybridized only with FAS mRNA (8) and, thus, just the dot blot is shown here. The combined data from four rats per group are presented graphically (mean ± SEM) (see Figs. 2–6). Hepatic FAS mRNA was significantly elevated in the cp/cp rats compared to the lean +/+ while the specific activity of hepatic FAS was increased compared to both lean and normal SD rats (**Fig. 2A and B**). The amount of FAS mRNA and the specific activity in the +/+ rats were, respectively, 60% and 73% lower than those in SD rats but the differences were not significant.

In adipose tissue, the specific activity of FAS was significantly increased in the lean +/+ compared to SD rats but was not different from the cp/cp rats. There were no differences in the mRNA level between these study groups (Fig. 2C and D).

Hepatic and adipose tissue GPDH of male rats

Both the relative amount of hepatic mRNA for GPDH and the specific activity of the enzyme were elevated in the obese rats compared to the lean +/+ rat but not compared to SD rats (**Fig. 3A and B**). Hepatic GPDH mRNA was also significantly elevated in the SD rats compared to the lean, although the difference in the specific activity failed to reach significance. In adipose tissue, the specific activity of GPDH was elevated in both SD and cp/cp rats rela-

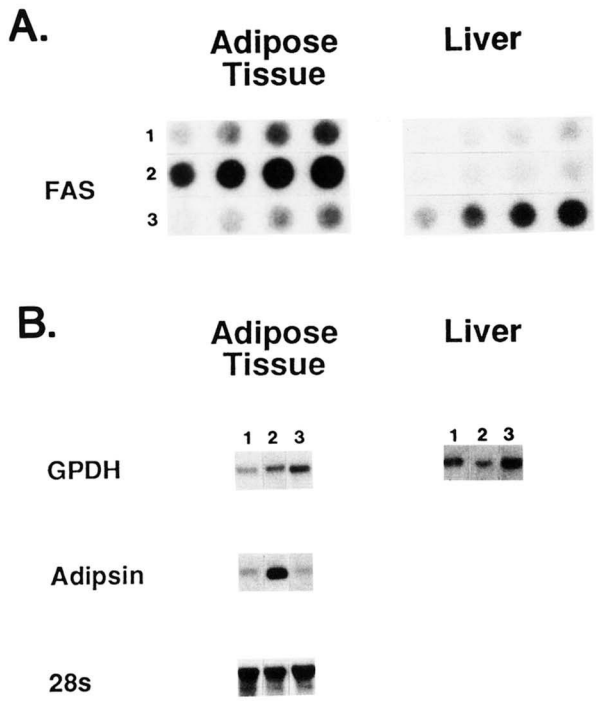

Fig. 1. A: Representative dot blot analysis of FAS mRNAs extracted from liver and adipose tissues of obese (cp/cp) and lean (+/+) JCR:LA-corpulent and Sprague-Dawley (SD) rats. Total RNA was loaded at four concentrations (1, 2, 3, and 4 μg RNA/dot) and hybridized with ^{32}P-labeled cDNA probes as described in Methods. Lane 1: SD; lane 2: +/+; lane 3: cp/cp. B: Representative Northern blot analyses of hepatic and adipose GPDH mRNAs and adipose tissue adipsin mRNAs extracted from the same rats as in Fig. 1A. The latter blot was rehybridized for 28S ribosomal RNA for normalization of the data. Lane 1: SD; lane 2: +/+; lane 3: cp/cp.

tive to the lean +/+, but levels of mRNA did not vary between groups (Fig. 3C and D).

Adipsin mRNA levels in adipose tissue of male rats

The relative level of adipsin mRNA was not suppressed in the obese cp/cp compared to SD rats but was elevated fivefold in the lean +/+ rats compared to both cp/cp and SD rats ($P < 0.05$) (**Fig. 4**).

Body and fat pad weights of diet-fed female rats

Over the 12-week feeding period, high starch and high sucrose diets failed to induce a gain in body weight relative to standard chow (**Table 2**) and, in fact, starch-fed rats weighed significantly less than chow-fed rats. Although the parametrial fat pads of sucrose-fed rats were significantly larger than those of both chow- and starch-fed rats, the growth was small (1.3-fold). The same diet (without the liquid supplement) fed to male SD rats induced 2.5-fold increases in fat pad size relative to those fed chow (retroperitoneal fat pads: 19.7 ± 1.0 g in sucrose-fed rats compared to 7.5 ± 0.9 g in chow-fed rats). Total plasma triacylglycerols were 1.05 ± 0.31 and 1.18 ± 0.23 mmol/l in chow- and sucrose-fed rats, respectively. Total caloric intake was not different between diets (Table 2).

The obese rats fed the high fat diet for 12 weeks weighed significantly more than chow-fed rats (596 ± 10 and 564 ± 9 g, respectively, $P < 0.05$) although there was only a small increase in the combined weight of the parametrial, retroperitoneal, and inguinal fat pads (79.6 ± 3.9 vs. 75.4 ± 1.5 g). Total plasma triacylglycerols were similar in chow- and fat-fed obese rats (12.67 ± 2.84 and 15.03 ± 2.33 mmol/l in chow- and fat-fed rats, respectively) and both values were significantly elevated compared to those of the lean rats ($P < 0.01$).

Hepatic and adipose tissue FAS mRNA in female rats

The high sucrose diet induced an increase in the relative amount of hepatic FAS mRNA compared to the chow diet in the SD rats (n = 4) and a similar trend in the +/+ rats (n = 3) (**Fig. 5**). In the adipose tissue of the +/+ rats, there was little change in FAS mRNA level in response to diet (0.70 ± 0.10, 0.84 ± 0.15 and 0.84 ± 0.14 units in chow-, sucrose-, and starch-fed rats, respectively).

The relative abundance of hepatic FAS mRNA in the female obese rats was similar to that in male cp/cp rats (0.70 ± 0.13 units in females vs. 0.77 ± 0.17 units in males). The 45% fat diet did not suppress the high level of mRNA observed in chow-fed cp/cp rats (Fig. 5).

Adipsin mRNA in adipose tissue of diet-fed female rats

The difference in adipsin mRNA level between female lean and obese cp rats was similar to that between male lean and obese rats (Fig. 4 and **Fig. 6**). Diet did not affect the relative abundance of adipsin mRNA in adipose tissue of lean or obese cp rats (Fig. 6).

DISCUSSION

The data reported here are consistent with a major role for exaggerated de novo fatty acid synthesis in the pathogenesis of obesity in the obese JCR:LA-corpulent rat. The relative hepatic abundance of mRNA encoding FAS was elevated 5-fold and the specific activity by more than 20-fold in the obese compared to the lean cp rat. This is compatible with earlier findings by Russell et al. (32) that hepatic secretion of triacylglycerols was elevated 5- to 9-fold in obese compared to lean rats. Furthermore, there was no adaptive change in FAS levels in response to dietary

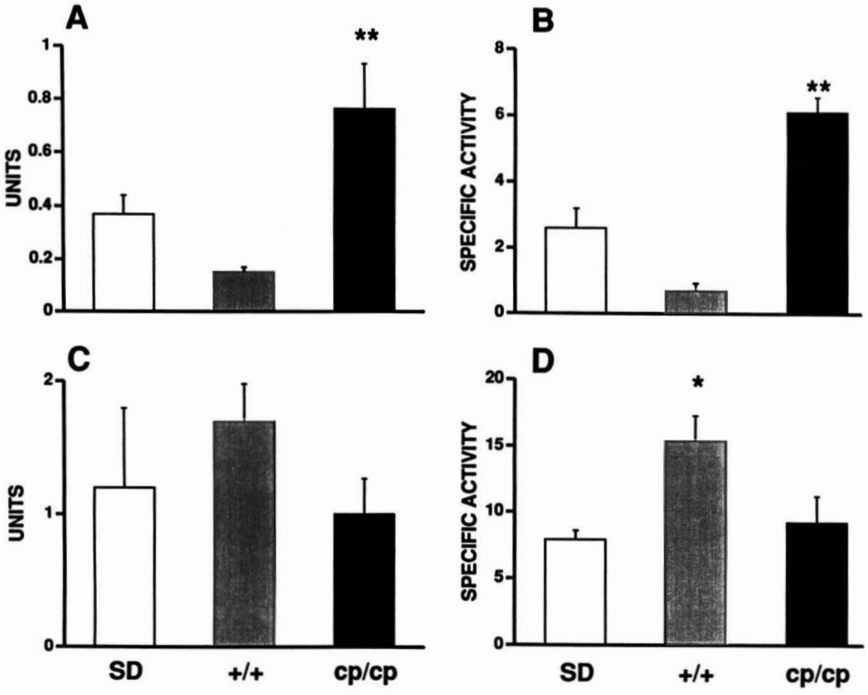

Fig. 2. The relative abundance of hepatic (panels A and B) and adipose tissue (panels C and D) FAS mRNA and specific activity in Sprague-Dawley (SD), and male lean (+/+) and obese (cp/cp) rats. The data shown are the means ± SEM of four rats per group. mRNA relative units were derived from computer-assisted laser densitometry of autoradiographs of Northern or dot-blots prepared as described in Fig. 1 and Methods. Data were normalized to 28S ribosomal RNA as internal standard. Between-blot variation was corrected using RNA extracted from the liver of a 2-month-old Sprague-Dawley rat as external standard. The specific activity of FAS, determined by the method of Nepokroeff et al. (30) as described in Methods, is expressed as nmol NADPH utilized/min per mg protein. Values significantly different by Tukey's test for multiple comparisons: Hepatic FAS mRNA, panel A: ** different from +/+, $P < 0.01$; Hepatic FAS specific activity, panel B: ** different from SD and +/+ ($P < 0.01$). Adipose FAS mRNA panel C: no significant differences; Adipose FAS specific activity, panel D: * different from SD ($P < 0.05$).

fat in obese rats. In contrast, FAS activity was suppressed by dietary fat (6) although not by fasting (3) in obese Zucker rats.

The close correlation between mRNA levels and specific activity of the enzyme in both hepatic and adipose tissue suggests that regulation of FAS was principally at a pre-translational level, which is in keeping with earlier studies in differentiating mouse preadipocytes (33) and in avian liver (34). Little change in turnover rate of hepatic FAS mRNA was detected in response to feeding and fasting (35) and, by comparison of changes in in vitro transcription rate with changes in mRNA level, it was demonstrated that FAS synthesis was regulated mainly at the level of transcription (34). Moreover, Blake and Clarke (9) have recently shown that dietary polyunsaturated fats inhibit the transcription of FAS. Thus, the lack of response to dietary fat in the female cp/cp rats of our study is probably due to changes in regulation at the level of FAS transcription.

Contrary to the above interpretation of our data, it is possible that up-regulation of FAS in the obese rat masked or negated the response to dietary fat. A normal down-regulatory response to dietary fat might be revealed if the fat diet was compared to a sucrose diet, since the difference in FAS levels induced by these two diets is more pronounced than that between fat and starch in mice (36). But, it is also feasible that FAS activity was maximal in both chow- and fat-fed cp/cp rats and that a sucrose diet would not have increased the activity further.

The high, and apparently unsuppressible, abundance of hepatic FAS mRNA may be associated with the markedly elevated plasma insulin concentration in the cp/cp rats. Insulin and triiodothyronine (T_3) have been shown to increase both the activity and the rate of synthesis of FAS in avian hepatocytes in vitro (35). Insulin may also increase the efficiency of translation of FAS mRNA (37). T_3 accelerated the synthesis of FAS in preadipocytes in culture, acting at a pre-translational level (38), whereas FAS activity was depressed in hypothyroid rats (39). While we did not measure T_3 levels of the rats in our study, serum T_3 levels were reduced in obese compared to those in lean LA/N-cp rats from which the JCR:LA-cp rats are derived (40, and P. Dolphin, personal communication).

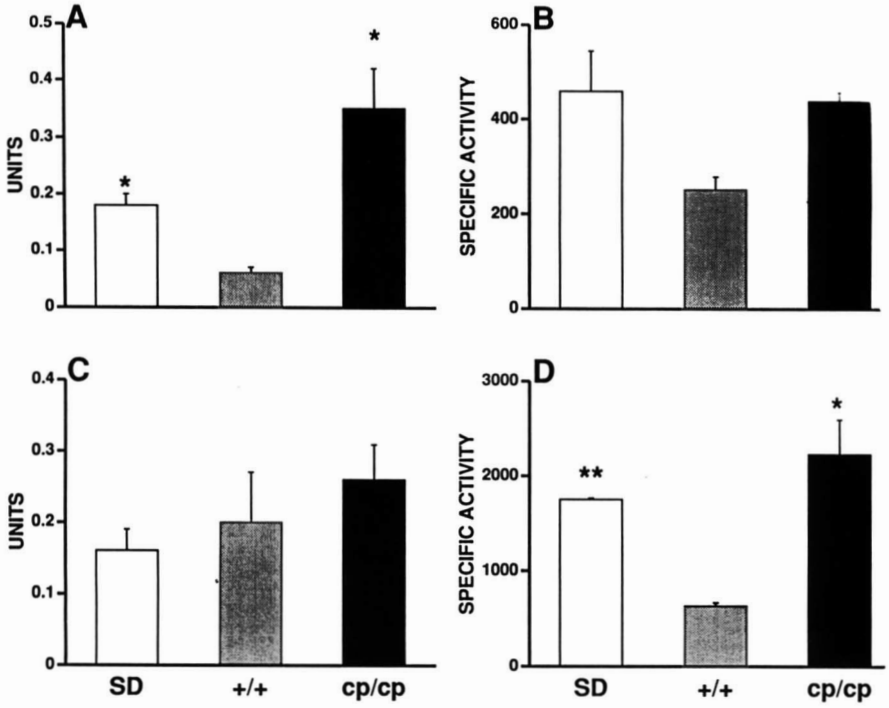

Fig. 3 The relative abundance of hepatic (panels A and B) and adipose tissue (panels C and D) GPDH mRNA and specific activity in Sprague-Dawley (SD), and male lean (+/+), and obese (cp/cp) rats. RNA extraction and hybridization, mRNA units, and number of rats per group are the same as in Fig. 2. The specific activity of GPDH, determined by the method of Kozak and Jensen (31) as described in Methods, is expressed as nmol NADH utilized / min per mg protein. Values significantly different by Tukey's test for multiple comparisons: Hepatic GPDH mRNA, panel A: * different from +/+, $P < 0.05$); Hepatic GPDH specific activity, panel B: ** from +/+, ($P < 0.01$); Adipose GPDH mRNA, panel C: no significant differences; Adipose GPDH specific activity, panel D: * from +/+, $P < 0.05$ and ** from +/+, $P < 0.01$.

Hepatic FAS mRNA levels and activity of the enzyme were two- to fourfold lower in male lean +/+ rats than in SD rats although this difference did not reach significance (Fig. 2). Very low FAS levels are appropriate to the extreme leanness of the +/+ rats which have less than 2% body weight as fat at 7 months of age (an approximation based on total resected fat weight). However, when stimulated by a high sucrose diet, the response in the female, lean +/+ rats paralleled the changes induced in SD rats (Fig. 5). No accompanying rise in serum triacylglycerols (TG) was observed, due perhaps to a greater rate of clearance. An increase in plasma insulin concentration, possibly induced by the sucrose diet, would stimulate lipoprotein lipase activity. The slight, albeit significant, gain in fat pad weight (Table 2) may have been the result of increased lipid uptake or of increased FAS activity in adipose tissue. However, there was so little fat deposition in lean +/+ compared to SD rats fed the same sucrose diet, an increase in β-oxidation of fatty acids could be postulated. Alternatively, there may have been no increase in TG secretion by the liver, despite the elevated FAS levels. In vivo studies of hepatic fatty acid synthesis and oxidation will be necessary to ascertain these possible interpretations. It could be argued that, since total caloric intake was not augmented by the high sucrose diet, compared to the chow or starch diets (Table 2), no substantial gain in lipid accumulation could be expected. The concomitant decrease in fat and protein intake in the sucrose fed rats might be expected to have increased caloric intake. Never-

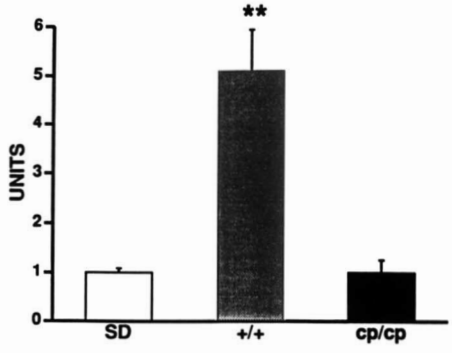

Fig. 4. The relative abundance of adipose tissue adipsin mRNA in Sprague-Dawley (SD, n = 2), and male lean (+/+, n = 3) and obese (cp/cp, n=4) rats. Methods and mRNA units are the same as in Fig. 2. **, Value significantly different from cp/cp and SD ($P < 0.01$).

TABLE 2. Total body and fat pad weights of female lean JCR:LA-corpulent rats at 7 months of age

	Chow (n = 4)	Sucrose (n = 3)	Starch (n = 4)
Body weight, g	233 ± 3.0[a]	226 ± 3.2	220 ± 4.1[a]
Parametrial pad, g	1.7 ± 0.1[a]	2.3 ± 0.1[ab]	1.6 ± 0.2[b]
Retroperitoneal pad, g	1.3 ± 0.2	2.4 ± 0.1[a]	1.1 ± 0.1[a]
Inguinal pad, g	1.8 ± 0.1	2.2 ± 0.2	1.8 ± 0.1
Liver weight, g	8.5 ± 0.3[a]	7.8 ± 0.2	6.7 ± 0.6[a]
Calories ingested, kcal	4751 ± 91	4614 ± 186	4348 ± 51

Lean rats were fed for 3 months on standard rat chow (Chow), high sucrose supplemented with 20% sucrose solution (Sucrose), or high starch (Starch) diets. Values shown are the means ± SEM of n rats per group. Values for each pad/organ sharing the same superscript are significantly different by Tukey's test for multiple comparisons ($P < 0.05$).

theless, the lean +/+ rats appear to have inordinately efficient control of food intake. We propose that these lean rats may not be "normal" but represent an opposite extreme to the cp/cp rats.

Adipose tissue FAS mRNA levels were almost the reciprocal of hepatic levels (Fig. 2). This finding suggests that regulation of FAS mRNA levels in adipose tissue may be dependent on substrate availability rather than on plasma hormonal concentrations, and thus, may differ from hepatic regulation. The elevation of FAS levels in the fat pads of lean +/+ rats implies that adipose tissue is not a passive storage depot but actively involved in lipogenesis despite very low plasma lipid and insulin concentrations. Another interpretation of the data might be that adipose FAS levels are constitutively high unless suppressed by exogenous substrate availability.

The GPDH mRNA levels and the specific activity of the enzyme in both liver and adipose tissue were consistent with the production of glycerol appropriate to

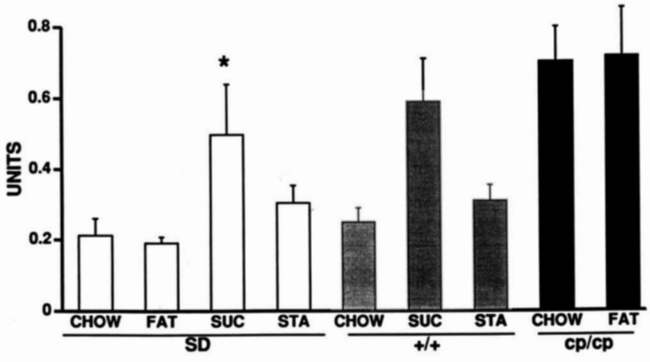

Fig. 5. The effect of diet on the relative levels of hepatic FAS mRNA in Sprague-Dawley (SD), and female lean (+/+) and obese (cp/cp) rats. The number of rats in each diet group was four with the exception of the sucrose-fed lean +/+ group in which there were three rats. Methods and mRNA units are the same as in Fig. 2. The diets, described in Methods, were standard rat chow (CHOW), high fat (FAT), high sucrose (SUC) or high starch (STA). *, Value significantly different from SD rats fed starch or fat ($P < 0.05$).

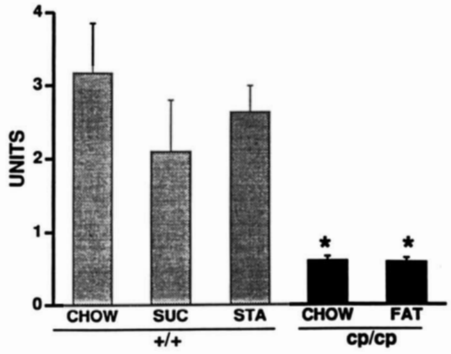

Fig. 6. The effect of diet on the relative abundance of adipose tissue adipsin mRNA in female lean (+/+) and obese (cp/cp) rats. Methods and mRNA units are the same as in Fig. 2. Diets are as described in Fig. 5. The number of rats in each diet group was four with the exception of the sucrose-fed lean +/+ group in which there were three rats. *, Values significantly different from lean +/+ rats of all diet groups ($P < 0.05$).

the amount of lipid deposited in the adipose tissue. The discrepancy observed between the mRNA level and the enzyme activity in the fat tissue of the lean rats may be the result of post-translational modifications.

Spiegelman et al. (41) has proposed that defective hormonal regulation of the adipsin gene expression occurs in obese states. In our study, adipsin mRNA levels in adipose tissue of cp/cp rats were similar to those in normal SD rats, but were increased fivefold in lean +/+ rats. Obese and lean cp rats were age-matched (9–10 months) whereas the SD rats were younger (5–7 months). While age-related changes in adipsin message cannot be excluded, our data should perhaps be interpreted as an elevation of the mRNA in the lean rat rather than as suppression in the obese rat, in contrast to interpretation of earlier data from obese mice (13). An increased total mRNA content of the expanded adipose tissue mass in the obese animals will, in part, compensate for the reduced relative amount of adipsin mRNA. This may account for the smaller difference in plasma adipsin protein concentration observed between lean and obese mice (13, 42). The absence of a significant effect of diet on adipsin mRNA levels in the lean +/+ rats suggests that adipsin may not play a role in the regulation of lipogenesis/lipolysis. Adipsin gene expression has been shown to be responsive to changes in plasma glucocorticoid concentration in obese mice (41). Elevated plasma corticosterone concentrations are unlikely to have been a factor since adipsin mRNA levels were similar in the obese cp/cp rats to those in the normal SD rats and, indeed, this similarity implies that adipsin does not play a role in obesity.

The smaller difference in adipsin mRNA levels observed between obese and lean JCR:LA-cp rats compared to differences reported between lean and obese

mice (13) may be partly a function of the use of 28S ribosomal RNA as internal standard (28). Previous studies have used actin mRNA to normalize their data (13, 16, 41). Another explanation of this discrepancy may be that obese db/db and ob/ob mice demonstrate overt diabetes (13) whereas cp/cp rats show only transient hyperglycemia (11). Zucker obese rats are normoglycemic and have adipsin mRNA levels only twofold lower than those of the lean rats (15). Adipsin may have a function more related to glucose metabolism than to obesity per se.

The obese phenotype of cp/cp rats was apparent by 21 days of age (11), implying that lipid deposition and, thus, perhaps de novo fatty acid synthesis had already been induced. Plasma insulin levels were slightly, but not significantly, elevated at 1 month (11). To further define the role of excessive fatty acid synthesis in genetic obesity, it will be necessary to establish the time course of the development of FAS activity and to identify the hormone(s) or other factors responsible for its induction in the obese cp/cp rat.

In summary, we have demonstrated in the obese JCR:LA-corpulent rat that the relative abundance of hepatic mRNA coding for fatty acid synthase and the activity of the enzyme are excessively elevated and that they are not responsive to suppression by dietary fats. We concluded that exaggerated de novo fatty acid synthesis may play a major role in the pathogenesis of obesity in the cp rat. We have also found that, while hepatic FAS mRNA was responsive to dietary stimulation, the lean +/+ rats demonstrate an exceptional ability to resist lipid accumulation and weight gain. Consistent with other models of obesity (12, 14), there was a fivefold difference in abundance of adipose tissue adipsin mRNA between lean and obese cp rats, the significance of which has yet to be determined.

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