

Hepatic and adipose tissue lipogenic enzyme mRNA levels are suppressed by high fat diets in the rat

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Abstract Small changes in lipogenic enzyme activity induced by dietary fats of different composition may, over the long term, have significant impact on the development of obesity. We have investigated the effect of high fat diets (45% of calories as fat) on abundance of mRNA encoding fatty acid synthetase (FAS) and glycerophosphate dehydrogenase (GPDH) in male Sprague-Dawley rats. When caloric intake was equal, the relative amount of hepatic FAS mRNA was greater in rats fed a saturated compared to a polyunsaturated fat diet. This difference could not be attributed to diet-induced changes in plasma insulin concentration. However, both fat diets suppressed hepatic FAS mRNA, compared to a sucrose diet. Close correlation between FAS specific activity and the relative amount of mRNA suggested that regulation was mainly at a pre-translational level. Adipose tissue FAS mRNA was suppressed by the two fat diets equally while GPDH mRNA was unaffected by dietary composition. Retroperitoneal fat pads were significantly larger in rats fed saturated compared to those fed polyunsaturated fat for 26 weeks. ■ We concluded that dietary saturated fats fail to suppress hepatic de novo lipogenesis as effectively as polyunsaturated fats, which may have implications for the prevention of obesity in humans. — Shillabeer, G., J. Hornford, J.M. Forden, N. C. W. Wong, and D. C. W. Lau. Hepatic and adipose tissue lipogenic enzyme mRNA levels are suppressed by high fat diets in the rat. *J. Lipid Res.* 1990. 31: 623–631.

Supplementary key words fatty acid synthetase • glycerophosphate dehydrogenase • obesity

In the typical North American diet, approximately 45% of calories are derived from fats (1). Current evidence suggests that the metabolic consequences of ingesting this high-fat diet may, in part, be dependent on the type of fat ingested (2). The degree of saturation of dietary fat has been shown to alter cell membrane enzyme function, receptor-mediated responses, and lipogenic enzyme activity (2–4). In the liver, de novo lipogenesis was reduced by polyunsaturated fats (1–25% by weight of diet) but not saturated fats (5, 6). Data on the effect of dietary fat on adipose tissue lipogenesis are conflicting. De novo lipogenesis was variously not suppressed (7, 8), was more sup-

pressed (9, 10), or was less suppressed (11, 12) by a saturated fat than a polyunsaturated fat diet.

Most reports are based on the results of relatively short-term (48 h to 2 weeks) studies. The observed small differences in lipogenic enzyme activity induced by changing the fatty acid composition of dietary fat may, over the long term (3 months or more), lead to significant changes in total lipid accumulated in adipose tissue. Expansion of adipose tissue mass leads to the development of obesity, a prevalent nutritional disorder associated with increased risk of heart disease, hypertension, and diabetes mellitus (13, 14). Thus, elucidation of the long-term effects of dietary fats on endogenous synthesis of lipids is essential for development of appropriate measures for the prevention of obesity and its associated metabolic abnormalities.

Inhibition of lipogenesis in both liver and adipose tissue has been shown to be dependent on the amount of fat intake (2) and to be a specific effect independent of the concomitant decrease in carbohydrate intake (5). The efficacy of a polyunsaturated fat diet was attributed mainly to its linoleic acid content (7). A diet high in saturated fat (40% by weight) containing adequate essential fatty acid depressed lipogenesis in young rats, but with decreasing efficiency as the rats aged, although a polyunsaturated fat diet remained effective for 12–18 months (15). Long-term feeding of the high polyunsaturated fat

Abbreviations: FAS, fatty acid synthetase; GPDH, glycerophosphate dehydrogenase; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; HFS, high saturated fat; HFP, high polyunsaturated fat; HSU, high sucrose; HST, high starch; HSA, high saturated fat diet fed ad libitum; HPA, high polyunsaturated fat diet fed ad libitum.

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diet induced a reduction in synthesis of hepatic lipogenic enzymes by an as yet unidentified mechanism (2). However, the long-term effect of feeding saturated fats has not been clarified.

We hypothesized that the constituents of dietary fat, possibly by divergent effects on *de novo* lipogenesis, in the long term, may have significant influence on the development of obesity and its associated metabolic complications. To test this postulate, we have studied the long-term effects of feeding diets, high in saturated or polyunsaturated fats, on the mRNA levels coding for various lipogenic enzymes in adipose tissue and liver of rats. Specifically, we have studied fatty acid synthetase (FAS; EC 2.3.1.85), the enzyme complex involved in the *de novo* synthesis of long chain fatty acids, and glycerophosphate dehydrogenase (GPDH; EC 1.1.1.8), which is a key intermediate at the branch point between the glycolytic pathway and triacylglycerol synthesis. In adipose tissue, GPDH is solely responsible for the supply of glycerol. We have quantitated the mRNAs coding for FAS and GPDH by Northern and dot blot (16, 17) analyses using specific rat cDNA probes. The results of these studies were correlated with the specific activity of the enzymes. To assess diet-induced changes in adipose tissue lipid uptake and release, we have also determined the activity of lipoprotein lipase (LPL; EC 3.1.1.34) and hormone-sensitive lipase (HSL; EC 3.1.1.3).

METHODS

Animals and diets

Male Sprague-Dawley rats (Charles River) were housed individually in wire-bottomed cages and maintained on a 12:12-h light-dark cycle at 22°C. The rats were fed nutritionally complete diets, differing only in their major source of energy (Table 1): high saturated fat (HFS) consisting of beef tallow (Teklad Test Diets, Madison, WI); high polyunsaturated fat (HFP) consisting of safflower oil high sucrose (HSU) or high starch (HST) (ICN Canada, Montreal, PQ). All diets contained adequate essential fatty acid. Sucrose and safflower oil were obtained from the local supermarket. The high sucrose group was included as a control for stimulating lipogenesis (2). To eliminate effects due to a difference in intake of calories by the two high fat groups, intake of the high fat diets was calorically pair-matched to the intake of the high starch-fed group. Pair-fed rats were given the same amount of food, corrected for the different caloric density that was consumed by the HST rats on the previous day. A further two groups of rats were allowed to consume the high saturated and polyunsaturated fat diets *ad libitum* (HSA and HPA, respectively). *Ad libitum* diet-fed groups were compared to control rats fed standard chow (4% fat, 72% carbohydrate, 24% protein).

TABLE 1. Nutrient composition of test diets

Nutrients	Diets			
	HST	HSU	HFS	HFP
	% of calories			
Casein	20.0	20.0	20.0	20.0
Safflower oil	4.5	4.5	4.5	45.0
Beef tallow			40.5	
Starch	65.0	28.5	35.0	35.0
Sucrose	10.5	47.0		

HST: high starch; HSU: high sucrose; HFS: high saturated fat; HFP: high polyunsaturated fat. All diets were supplemented with vitamin mix including choline chloride (1.2%), salt mixture (3.8%), and Alphacel, a nonnutritive fiber (1.6%) (ICN Canada) and DL-methionine (0.02%; Gibco, NY).

After 15 or 26 weeks on the diets, the rats were killed in the fed state by exsanguination under light halothane anesthesia. Blood was collected for radioimmunoassay of plasma insulin using anti-rat antiserum (Muttart Diabetes Unit, University of Alberta, Edmonton, AB). 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 , 0.15 M NaCl, pH 7.4) at 37°C and weighed. The tissues were then frozen in liquid nitrogen and stored at -85°C for subsequent analysis.

Quantitation of mRNAs coding for GPDH and FAS

Total RNA was extracted from liver samples by the guanidine-HCl method (18, 19) and from adipose tissue by centrifugation over a cesium chloride cushion as described by Chirgwin et al. (20). Total RNA was analyzed by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde and MOPS buffer (0.2 M morpholinopropanesulfonic acid, 50 mM sodium acetate, 5 mM EDTA) (16) and resolved RNA was 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 (16, 17) with specific ^{32}P -labeled cDNA probes. The probes were labeled by the random primer method (16). The GPDH probe used was donated by Dr. H. Green of Harvard Medical School (21) and the FAS probe used was a gift from the laboratory of Dr. J. W. Porter (pFAS-7) (22). Autoradiographs of hybridizations were quantitated by a video-assisted densitometer. The amount of mRNA in each sample was normalized to 28S ribosomal RNA (23) and between-blot variation was corrected using RNA extracted from the liver of 2-month-old rats as an external standard.

Enzyme assays

The right lobe of 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, pH 8.0, con-

taining 1 mM dithiothreitol). 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 assays.

Part of the 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 infranant fraction were assayed for FAS, GPDH, LPL, and HSL activity.

Soluble protein was determined by the Coomassie blue method (24). All enzyme assays were performed under optimal substrate and zero order kinetic conditions.

FAS activity was determined in liver and adipose tissue as described by Nepokroeff, Lakshmanan, and Porter (25). Briefly, 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 μ M final concentration), the reaction was initiated by the addition of acetyl- and malonyl-CoA (35 μ M and 100 μ M 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 by the method of Kozak and Jensen (26). The substrate, dihydroxyacetone phosphate (Sigma), was added to aliquots of the appropriate fractions (see above) of the tissue homogenates in the presence of NADH (Sigma). The change in absorbance at 340 nm due to oxidation of NADH was followed at 23°C. One unit of enzyme activity corresponded to the oxidation of 1 nmol of NADH/min.

LPL activity was determined by the method of Ramirez et al. (27). Briefly, an homogeneous suspension of the substrate, triolein (>99% pure; Supelco) and [3 H]triolein (Amersham) was formed by sonication (3 \times 15 sec pulses at 75 W, Braun Sonicator 1510) and added to aliquots of the infranant fluid fraction of the fat tissue homogenate immediately prior to incubation. A pooled sample of rat serum was used to activate LPL; this activity could be inhibited by the addition of 1 M NaCl, thereby distinguishing between LPL and hormone-sensitive lipase activity. The [3 H]oleic acid released by lipase activity was extracted with a solution containing methanol-chloroform-heptane 1.41:1.25:1. An aliquot of the upper aqueous layer containing the released fatty acid was quantitated in ACS scintillant (Amersham) using a LKB Rackbeta liquid scintillation counter.

HSL activity in adipose tissue was quantitated by the method of Khoo and Steinberg (28). The incubation mixture was prepared by sonication of the substrate, triolein,

and [3 H]triolein, and fatty acid-free bovine serum albumin (Fraction V, Sigma) in aliquots of the infranant fluid fraction of the adipose tissue homogenate. HSL activity was stimulated by the addition of an activation mix containing 0.5 mM ATP, 5 mM Mg²⁺, 0.01 mM cyclic AMP, and 1 mM theophylline (Sigma). To inhibit LPL activity, 1 M NaCl was added to the reaction mixture. The [3 H]oleic acid released by lipase activity was extracted in the manner described above and its radioactivity was quantitated using a LKB scintillation counter.

Data analysis

Unpaired data were analyzed by one-way ANOVA and where significant intergroup differences were indicated, the data were further analyzed by the least significant difference method for multiple comparisons. Intake-matched groups were compared by Student's *t*-test for paired data.

RESULTS

Body weight gain and growth of abdominal fat tissue

The retroperitoneal fat pads of rats fed saturated fats were significantly larger than those of rats fed a calorically equivalent amount of starch after 15 weeks ($P < 0.02$) or of polyunsaturated fats after 26 weeks on the diets ($P < 0.05$) although their total body weights were significantly less than those of the HFP group at 26 weeks ($P < 0.001$) (Table 2).

While total body weights of the ad libitum-fed diet groups were not different at 26 weeks, the epididymal, retroperitoneal, and inguinal fat pads of the saturated fat-fed rats (HSA) were significantly larger than those of the chow-fed controls (Fig. 1). Furthermore, the retroperitoneal pads of rats fed both high fat diets were significantly larger than those from rats fed starch or sucrose diet.

Plasma insulin

Plasma insulin concentrations in rats after 26 weeks on the diets were (mU/l): control 85 ± 31 ($n = 3$); HST 84 ± 20 ($n = 3$); HSU 111 ± 8 ($n = 3$); HFS + HSA 65 ± 7 ($n = 6$); HFP + HPA 60 ± 13 ($n = 7$) (data for the equicaloric and ad libitum-fed high fat groups were combined since the data for the two groups were very similar). The mean plasma insulin level for the high sucrose group was significantly different from both high fat-fed groups, $P < 0.01$.

Hepatic FAS

Northern blot analysis for hepatic mRNA for one rat from each diet group is shown in Fig. 2, panel A. The combined data from four rats per diet group are presented graphically (mean \pm SEM) in Fig. 2, panel C.

TABLE 2. Effect of dietary energy source on body weight gain and fat pad growth with constant caloric intake

	Time on Diet	Weight on Different Diets		
		HST	HFS	HFP
	wk		g	
Initial body weight	15	59.8 ± 2.3 (9)	59.6 ± 2.3 (8)	57.4 ± 1.6 (8)
	26	84.5 ± 1.1 (4)	84.6 ± 0.9 (4)	83.0 ± 1.7 (4)
Final body weight	15	553.7 ± 17.4 (9)	542.9 ± 15.7 (8)	533.4 ± 10.7 (8)
	26	666.2 ± 32.7 (4)	641.8 ± 8.0 ^b (4)	681.6 ± 8.6 ^b (4)
Epididymal	15	10.3 ± 1.0 (9)	11.0 ± 1.7 (8)	10.2 ± 1.2 (8)
	26	13.7 ± 0.7 (4)	15.5 ± 0.2 (4)	16.9 ± 2.8 (4)
Retroperitoneal	15	18.9 ± 1.7 ^a (9)	25.6 ± 2.2 ^a (8)	22.2 ± 1.8 (8)
	26	25.4 ± 4.0 (4)	36.4 ± 3.1 ^a (4)	29.5 ± 2.5 ^a (4)
Inguinal	15	15.9 ± 2.0 (9)	18.2 ± 2.3 (8)	18.3 ± 2.0 (8)
	26	30.7 ± 3.6 (4)	26.2 ± 1.9 (4)	25.7 ± 1.8 (4)
Liver	26	22.0 ± 2.3 (4)	18.9 ± 0.6 (4)	17.6 ± 1.1 (4)

Abbreviations: HST, high starch diet; HFS, high saturated fat diet with food intake pair-matched to HST; HFP, high polyunsaturated fat diet with food intake pair-matched to HST. Values shown are the means ± SEM of four to nine animals (exact number in parentheses). Rats fed the diets for 26 weeks were approximately 1 week older at the start of the study than those fed the diets for 15 weeks.

Values within lines with the same superscript are significantly different: ^a, $P < 0.05$; ^b, $P < 0.001$.

When caloric intake of the two high fat diets was equal, the amount of mRNA coding for hepatic FAS was significantly elevated in the saturated fat-fed rats compared to the polyunsaturated fat-fed rats ($P < 0.01$). The HFS diet failed to suppress FAS mRNA compared to the high starch diet whereas there was a trend suggesting suppression in the HFP-fed rats. Furthermore, there was a decrease in FAS mRNA when the rats were fed the saturated diet ad libitum (HSA) relative to the intake-matched HFS group ($P < 0.02$), even though there was only a small increase in the total calories consumed during the 15 weeks measured (8320 ± 319 vs 7845 ± 241 kcal for HSA and HFS groups, respectively). No additional suppression of FAS mRNA was obtained when the high polyunsaturated diet was fed ad libitum (HPA) compared to the intake-matched group (HFP), which was consistent with the negligible difference in intake observed.

The diet-induced changes in the level of mRNA for FAS were in parallel with changes in the specific activity of FAS. There was threefold greater FAS activity in the saturated fat-fed animals than in those fed the polyunsaturated fat diet ($P < 0.02$) (Fig. 2, panel B). FAS activity was increased almost tenfold in both high starch- and high sucrose-fed groups compared to control and HFP groups, but only 2- to 3-fold compared to the HFS group.

Adipose tissue FAS

As in the liver, retroperitoneal fat pad FAS mRNA was elevated in the high sucrose-fed rats compared to all other groups except HST (Fig. 3, panels A and C). There were no differences between fat-fed groups, in which FAS mRNA was only slightly suppressed below the level in the chow-fed control rats. Similarly, the specific activity of

FAS in adipose tissue was not different between fat-fed groups of rats, while activity in the HST- and HSU-fed rats was significantly elevated (Fig. 3, panel B). The small increase in caloric intake when the fat diets were fed ad libitum in the HSA and HPA groups (not shown) did not change the enzyme activity or the quantity of mRNA coding for FAS relative to the intake-matched groups.

Hepatic GPDH

The hepatic levels of mRNA coding for GPDH were elevated in the HSU-fed group compared to those in both starch- and chow-fed rats ($P < 0.01$). There was no difference between the fat-fed groups although the specific mRNA was increased in the HFS group compared to con-

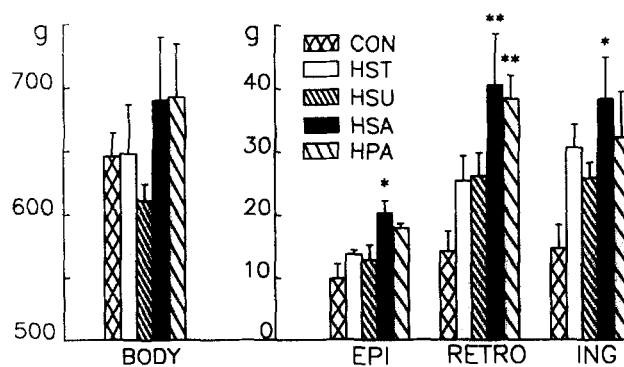


Fig. 1. Total body and fat pad weights of 7-month-old male Sprague-Dawley rats. Three-month-old rats were fed one of the following diets (described in detail under Methods) ad libitum for 26 weeks: standard rat chow (CON); high starch (HST); high sucrose (HSU); high saturated (HSA) or polyunsaturated (HPA) fats. There were four rats in each diet group. The fat pads examined were the epididymal (EPI), retroperitoneal (RETRO), and inguinal (ING). Values shown are means ± SEM and are significantly different from control (CON) when denoted by * and from control, HST, and HSU when denoted by **, $P < 0.05$.

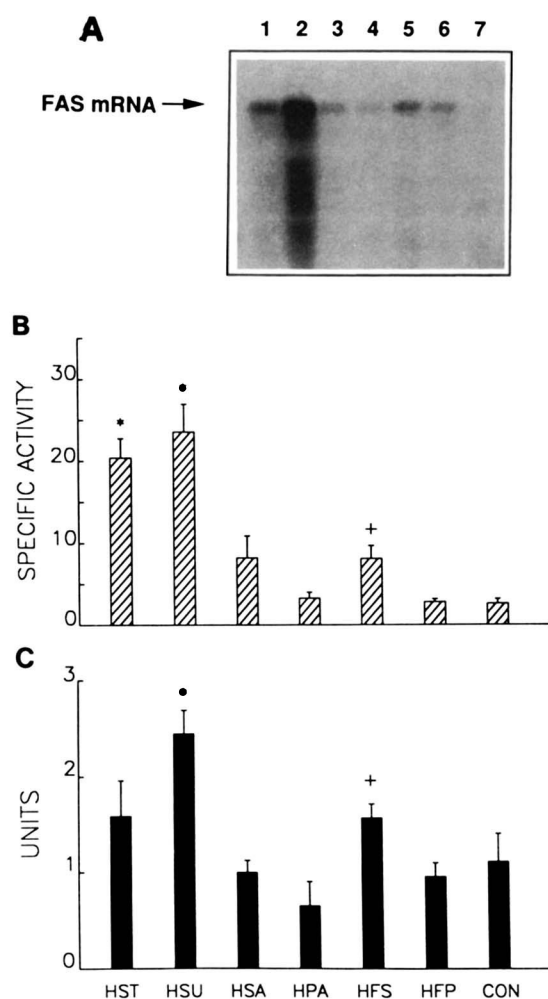


Fig. 2. Effect of dietary manipulation on hepatic FAS mRNA and enzyme specific activity. Panel A: a representative Northern blot analysis of hepatic FAS mRNA extracted from diet-fed rats. Total RNA was hybridized with the 32 P-labeled cDNA probe for FAS (9.4 kb) as described in Methods. The diets, as described in Fig. 1 and shown in the same sequence as in the graphs below, were: HST (lane 1); HSU (lane 2); HSA (lane 3); HPA (lane 4); control (lane 7). Two additional diet groups in which intake of the high fat diets was pair-matched to the intake of rats of the HST group are also shown: high saturated fat (HFS) (lane 5); high polyunsaturated fat (HFP) (lane 6). Panel B: the enzyme specific activity of FAS expressed as nmoles NADPH utilized/min per mg protein. * denotes values that are significantly different from control, HSA, HPA, HFS, and HFP; + significant difference from HFP, $P < 0.03$ (both by Tukey's test for multiple comparisons). Panel C: the relative amount of mRNA expressed in units, as quantified by densitometric scanning, normalized to 28S ribosomal mRNA, and corrected for between blot variation using an external standard. * Value is significantly different from HPA, control and HFP, $P < 0.01$. • Value is significantly different from HFP, $P < 0.03$.

trol ($P < 0.05$) (Fig. 4, panels A and C). Diet had little effect on the specific activity of GPDH in the liver (Fig. 4, panel B).

Adipose tissue GPDH

The amount of mRNA coding for GPDH in the retroperitoneal fat pad of the HSU ($P < 0.04$) and HFS ($P < 0.02$) groups was increased compared to the starch-

fed group (Fig. 5, panels A and C). In addition, the HFS group was increased relative to the HFP group ($P < 0.03$). The specific activity of GPDH was elevated in parallel to the mRNA levels in both the high fat- and sucrose-fed groups ($P < 0.05$) compared to control and there was a similar trend in the HST group ($P < 0.06$) (Fig. 5, panel B).

Specific activities of LPL and HSL in adipose tissue

LPL and HSL activities were mainly unaffected by the source of dietary energy (Table 3).

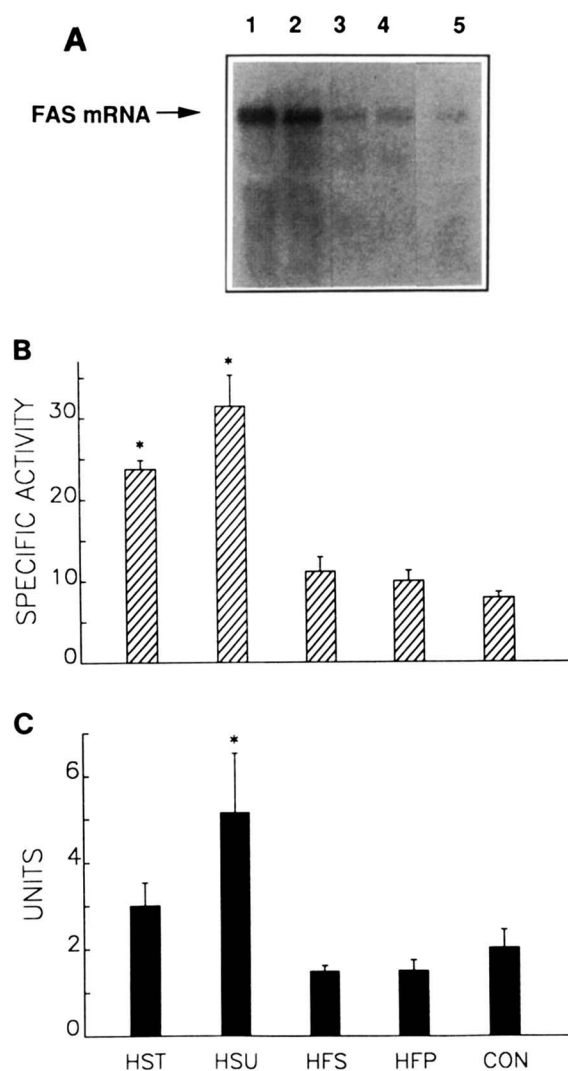


Fig. 3. Effect of the major source of dietary energy on adipose tissue FAS mRNA and specific activity. The procedure, diets, and units were the same as in the Figs. 1 and 2. Panel A: a representative Northern blot analysis of adipose tissue FAS mRNA in response to dietary manipulation. The diets were: HST (lane 1); HSU (lane 2); HFS (lane 3); HFP (lane 4); control (lane 5). Panel B: enzyme specific activity. Panel C: relative abundance of FAS mRNA. * Values are significantly different from control, HFS, and HFP, $P < 0.01$.

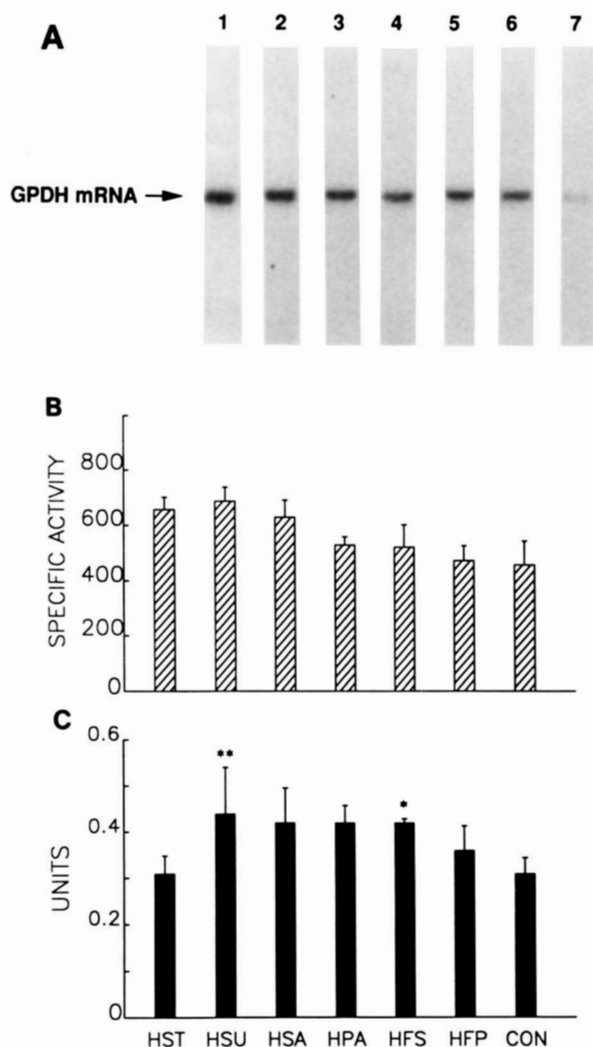


Fig. 4. Effect of dietary manipulation on hepatic GPDH mRNA and specific activity. The procedure, mRNA units and diets are the same as in Fig. 2. Panel A: a representative Northern blot analysis of hepatic GPDH mRNA. The diets were: HST (lane 1); HSU (lane 2); HFS (lane 3); HFP (lane 4); control (lane 5). The amount of GPDH mRNA in lane 7 appears fivefold lower than in other lanes. However, after normalization to 28S mRNA and combining the data from four rats per group, most of this difference was eliminated as shown in panel C. Panel B: the enzyme specific activity is expressed as nmol NADH utilized/min per mg protein. Panel C: the abundance of GPDH mRNA. * Values are significantly different from control; ** values are significantly different from control and HST, $P < 0.01$.

DISCUSSION

In this study, we have demonstrated by enzymatic assays and determination of mRNA levels for two lipogenic enzymes that dietary saturated fats over the long term fail to suppress hepatic de novo lipogenesis as efficiently as a polyunsaturated fat diet. When total caloric and total fat intake are kept constant, a diet that induces endogenous triacylglycerol synthesis can be postulated to promote a greater increase in body fat content compared to a diet

that suppresses lipogenesis. In support of this tenet we found that rats fed the saturated fat diet had significantly larger retroperitoneal fat pads than rats fed equicaloric amounts of diets high in polyunsaturated fats (Table 2).

We examined the retroperitoneal fat pad since its growth appears to be unlimited compared to that of the epididymal fat pad (Fig. 1), which has been used in all previous reports on the effect of dietary fat on de novo lipogenesis (2). Neither of the high fat diets was effective in suppressing retroperitoneal fat pad FAS mRNA or specific activity below levels measured in the chow-fed control rats (Fig. 5) despite an increase in fat intake from

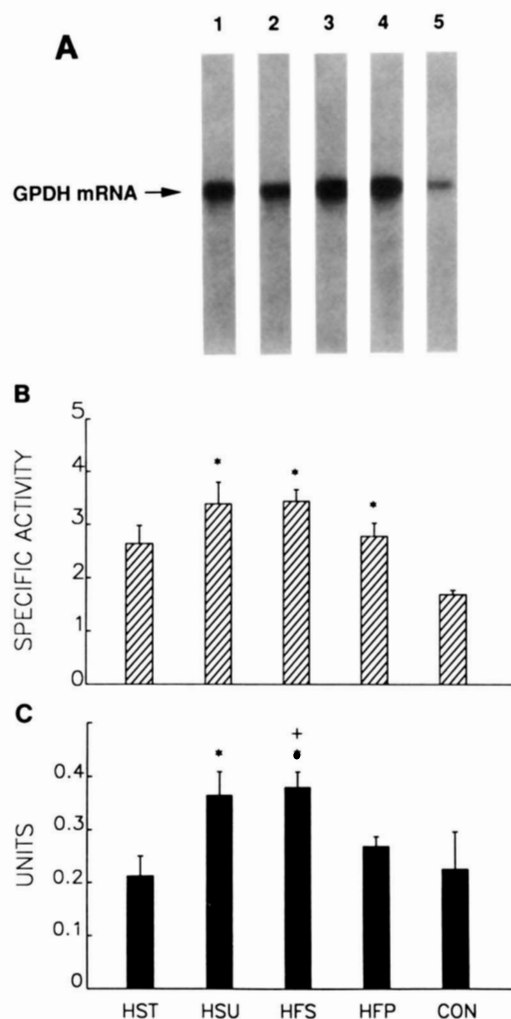


Fig. 5. Effect of changing the source of dietary energy on adipose tissue GPDH mRNA and specific activity. Procedure, diets, and mRNA units are as in Figs. 1 and 2. Units of GPDH specific activity are as in Fig. 4. Panel A: a representative Northern blot analysis of adipose tissue GPDH mRNA extracted from diet-fed rats. The diets were: HST (lane 1); HSU (lane 2); HSA (lane 3); HPA (lane 4); HFS (lane 5); HFP (lane 6); control (lane 7). As in Fig. 4, the apparent difference between lane 7 and the other lanes was mainly eliminated by normalization to 28S mRNA. Panel B: GPDH specific activity. * Values are significantly different from control, $P < 0.02$. Panel C: relative abundance of GPDH mRNA. * Values are significantly different from HST, $P < 0.05$, and • from HFP, $P < 0.03$.

TABLE 3. Lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activity in the retroperitoneal fat pad

Diet	LPL		HSL	
	Specific	Total	Specific	Total
Control	173.3 ± 16.1	4991 ± 407	36.6 ± 7.0	985 ± 59
HST	131.4 ± 19.3	5059 ± 410	37.4 ± 5.4	1514 ± 205
HSU	158.1 ± 7.4	4585 ± 888	39.7 ± 4.6	1362 ± 291
HFS	111.2 ± 12.4	5687 ± 658	34.8 ± 3.6	1672 ± 168 ^a
HFP	139.0 ± 23.1	5458 ± 759	30.4 ± 3.7	1225 ± 59
HSA	101.2 ± 12.6	6263 ± 1037	25.9 ± 2.5	1338 ± 139
HPA	125.8 ± 12.7	6401 ± 513	21.7 ± 1.8	1111 ± 80

Values are means ± SEM. The diets are: chow (Control); high starch (HST); high sucrose (HSU); high saturated fat (HFS) or high polyunsaturated fat (HFP), intake matched to the starch diet; high saturated (HSA) or polyunsaturated fat (HPA) fed ad libitum. LPL and HSL activity is expressed as nmol oleate/h. Specific activity is in units/mg protein and total activity is in units/total fat pad.

^aDenotes value significantly different from control by Tukey's method for multiple comparisons ($P < 0.05$).

12% of calories in control rats to 45% of calories in fat-fed rats. However, both fat diets significantly reduced the specific activity of FAS in the adipose tissue to the same extent as equicaloric intake of the starch diet, in contrast to earlier reports of short-term studies using the epididymal pad (7–12). These data suggest that the similar degree of FAS activity in high fat and control groups was due to a disparity in caloric intake. De novo lipogenesis in the epididymal fat pad was previously reported to be decreased in a dose-dependent manner by dietary fat, but to a lesser extent than in the liver (2). Using the incorporation of tritiated water to measure fatty acid synthesis in rats fed low-fat diets, Gandemer, Durand, and Pascal (29) demonstrated that adipose tissue produced 27% of total body fatty acids. However, the synthesis of fatty acids in adipose tissue in animals fed a high fat diet probably accounts for only a small percentage of the total lipid accretion (30).

There was generally a close correlation between levels of FAS mRNA and the specific activities of the enzyme in both liver and adipose tissue (Figs. 2 and 3). This observation was consistent with earlier reports demonstrating that the relative mRNA level and the rate of synthesis of FAS increase in parallel in differentiating mouse preadipocytes (31) and upon feeding in avian liver (32). These data suggested that regulation by nutritional factors was mainly pre-translational (33). Back and colleagues (32) demonstrated that feeding and fasting regulated FAS synthesis by acting predominantly at the level of transcription. These extremes of nutritional status had little effect on the half-life of FAS mRNA (32) which implies that a change in the rate of degradation of the mRNA is unlikely to be the basis of the differences in mRNA abundance induced by modification of dietary composition in our study.

We observed that a twofold increase in hepatic FAS mRNA levels in sucrose-fed rats was accompanied by a ninefold difference in the specific activity of the enzyme. In addition, similar levels of FAS mRNA (1.4-fold relative

to control) in starch- and saturated fat-fed rats were associated with 8- and 3-fold changes in enzyme activity, respectively. These divergent results suggested that regulation at a post-transcriptional level may also be occurring in response to dietary composition.

Post-transcriptional regulation of FAS synthesis may be the consequence of diet-induced variations in hormonal concentrations. We found that plasma insulin concentrations were significantly elevated in rats fed the high sucrose diet compared to those in fat-fed rats. These data are compatible with an insulin-stimulated effect on FAS synthesis. Goodridge and colleagues (33) demonstrated that insulin and triiodothyronine, which are elevated in the fed state, increased both the activity and rate of synthesis of FAS in avian hepatocytes in vitro. Their data suggested that insulin increased the efficiency of translation of FAS mRNA. Since plasma insulin concentrations in rats of the two fat diet groups in our study were not different, changes in FAS activity cannot be explained in this manner.

When rats were allowed to eat the high saturated fat diet ad libitum (HSA), there was a significant decrease in FAS message compared to the group in which intake of the diet was pair-matched to the starch group ($P < 0.02$). This decrease in FAS mRNA, which may perhaps be attributed to a very slight increase in fat consumption (approximately 6%), suggests a very sensitive regulatory mechanism. The concomitant increase in caloric intake would tend to counteract the effect of the greater fat intake.

It has been shown that high fat diets deficient in linoleate stimulate hepatic lipogenesis (2, 5). Since the saturated fat diet used in our study contained adequate essential fatty acids (approximately 4% of calories as linoleate) elevated levels of FAS mRNA and specific activity cannot be attributed to such a deficiency. On the other hand, fatty acid synthesis was shown to be inversely related to the dietary concentration of polyunsaturated fat but was unchanged by saturated fat (5, 6). The difference

that we observed may thus reflect the greater amount of polyunsaturated fat in the HFP diet. But, even at 45% of calories, the fat content of the HFP diet was insufficient to totally suppress fatty acid synthesis, an observation consistent with earlier data (2).

Both the high fat and high sucrose diets induced increases in the mRNA for GPDH in the liver (Fig. 4) compared to control, which is consonant with a greater flow of substrate through the glycolytic pathway. The activity of the enzyme was mainly unaffected by diet since caloric intake between diet groups was comparable (Fig. 4). Although we did not measure chow intake by the control group, the body weight gain of all diet-fed rats relative to control rats suggests a lower caloric intake by the latter group.

Insulin promoted the adipose conversion of 3T3 cells and the associated 17-fold increase in GPDH mRNA (34). However, it is unlikely that the greater abundance of GPDH mRNA observed in adipose tissue from HFS-fed rats could be attributed to stimulation by insulin since plasma insulin values tended to be lower than those from controls. However, given the same intake of dietary fat and total energy, increased de novo fatty acid synthesis would result in the delivery of more lipid to adipose tissue with a concomitant increase in GPDH activity and glycerol production. An increase in LPL, the enzyme that regulates triacylglycerol uptake by adipose tissue, might also be predicted. Nevertheless, in our study, total LPL activity in the retroperitoneal fat pad was scarcely greater in fat-fed than in control rats (Table 2). Elevated plasma corticosteroids, observed following fat ingestion, increased adipose LPL activity (35) and decreased FAS activity in the retroperitoneal pad of rats (36). LPL activity also responded positively to plasma triacylglycerol concentration (37). Conversely, insulin resistance, previously observed in fat-fed rats (38), might act to reduce LPL activation. Decreased release of stored lipid was unlikely to be a factor in the observed excess adipose tissue growth since total HSL activity was elevated in HFS-fed rats compared to control (Table 2). However, small changes in hormonal response to dietary composition, which are below detection limits, may summate over the long term and lead to the excess growth of adipose tissue mass observed in the saturated fat-fed rats.

Visceral accumulation of fat in humans has been correlated with greater impairment of glucose and lipid metabolism than subcutaneous accumulation (39). Similarly, the intra-abdominal fat pads in rats have been demonstrated to be more responsive to metabolic stimuli than subcutaneous fat (40). While the intrinsic synthesis of fatty acids may be negligible, the greater accretion of lipid in the retroperitoneal fat pad in animals fed saturated fat, whether resulting from increased storage of lipid of dietary origin or from de novo hepatic lipogenesis,

may have implications for the prevention of obesity in humans.

In conclusion, we have demonstrated that over the long term dietary saturated fats fail to suppress de novo hepatic fatty acid synthesis as effectively as a polyunsaturated fat diet. We also found that feeding a diet high in saturated fats led to a significantly greater expansion of the retroperitoneal fat pad than a diet high in polyunsaturated fats. Differences in the quantity of FAS mRNA and in specific activity of the enzyme, induced by modification of the composition of dietary fat, are compatible with regulation of FAS at both pre- and post-translational levels. Further studies are required to determine the mechanism by which saturated and polyunsaturated fats diverge in their effect on FAS regulation. ■

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