Starvation-realimentation overshoot in glycerophosphate acyltransferase in adipose tissue and liver of rats is influenced by type of dietary fat

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The purpose of this study was to determine effects of dietary fat source on changes in glycerophosphate acyltransferase that occur with starvation-realimentation. Forty male Sprague-Dawley rats were randomly assigned to dietary treatments at 209 ± 3 g of body weight. Ten rats each were assigned to a 65 g/100 g sucrose diet that contained 5 g/100 g fat from the following sources: stearate (42% stearate, 41% oleate); oleate (high-oleate safflower oil); palm oil; or beef tallow. Five rats received free access to their diet for 4 days and five rats were starved for 2 days and then fed for 2 days. Rats were then killed and liver and epididymal adipose tissue dissected and homogenized for glycerophosphate acyltransferase assay. The starvation-realimentation regimen on the stearate diet stimulated adipose tissue glycerophosphate acyltransferase above non-starved controls. Realimentation on high-oleate and high-palmitate diets stimulated liver glycerophosphate acyltransferase most, as well as produced a starvation-realimentation overshoot in steady-state glycerolipid production. Results indicate that ad libitum consumption of diets containing high proportions of stearate after a period of starvation could overshoot liver glycerophosphate acyltransferase to a lesser extent than if the diet contained low proportions of this fatty acid. Diet fatty acid composition had less impact on adipose tissue than liver glycerophosphate acyltransferase activity in rats subjected to starvation-realimentation. (J. Nutr. Biochem. 5:161–166, 1994.)

Keywords: rats; liver; adipose tissue; glycerophosphate acyltransferase; starvation, realimentation

Introduction

Starvation is associated with decreased rates of lipid synthesis; however, shortly after realimentation rates of fatty acid biosynthesis as well as activities of fatty acid biosynthesis enzymes in liver increase to values above prestarvation levels.[1–3] The starvation-realimentation (SR) effect on lipid biosynthesis enzymes, the "enzyme overshoot," is regulated by hormonal and nutritional factors. Berdanier and coworkers have shown that glucocorticoids and insulin are required for rat liver SR overshoot to occur for glucose-6 phosphate dehydrogenase (EC 1.1.1.49) and malic enzyme (EC 1.1.1.40).[4–7] Also, estrogen treatment of castrated male rats was shown to abolish the enzyme overshoot in liver while estrogen treatment did not exert this effect in ovariectomized rats.[8]

Fatty acid biosynthesis was previously shown to be inhibited by dietary fat.[9] Clarke et al.[10] observed that palmitic and stearic acids were not inhibitory but (n-6,9) linoleic and (n-3,6,9) linolenic acids were. Feeding starved rats diets that contained fat attenuated realimentation hyperlipogenesis,[11] with unsaturated fatty acids being shown to be more effective than saturated fatty acids.[12] When included at 5 g/100 g of diet, corn and coconut oils support the SR enzyme overshoot in rat liver,[2] while the overshoot is much lower with menhaden oil or beef tallow when compared with corn oil.[13] Thus, the type of fat influences fatty acid biosynthesis in both normal rats and rats subjected to SR.

An overshoot in rat adipose tissue glycerolipid biosynthesis in response to SR has been shown.[14] Ide et al.[15] observed

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depressed triacylglycerol (TG) synthesis rates in liver when starved rats were fed soy protein compared with casein or egg proteins. Three days of starvation followed by 2 days of realimentation resulted in an overshoot of glycerophosphate acyltransferase (GPAT, EC 2.3.1.15) activity in rats fed both fat-free and 5 g/100 g fat diets,[16] but only liver microsomes were studied. Feeding rats bread after starvation resulted in an overshoot of GPAT activity in adipose tissue, an effect that was not present when starved rats were subsequently fed a very high (56 g/100 g) corn oil diet.[17] Thus, dietary fat affects GPAT in adipose tissue and liver of rats, but the influence of different sources of dietary fat is unclear. The purpose of the present study was to determine how SR of rats on diets that contained 5 g/ 100 g of either high-stearate fat, high-oleate safflower oil, palm oil, or tallow affects GPAT in homogenates of adipose tissue and liver.

Methods and materials

Reagents

Coenzyme A (CoA, sodium salt), ATP (grade II disodium salt), ethylenediaminetetraacetic acid (EDTA), N-2-hydroxyethylpiperazine-N-2-ethane-sulfonic acid (HEPES), dithiothreitol (DTT), palmitic acid, and glycerol-3-phosphate (disodium salt) were purchased from Sigma Chemical Company, St. Louis, MO USA. ^{14}C-glycerol-3-phosphate (L-(14C(U))-glycerol-3-phosphate, disodium salt) was purchased from New England Nuclear, Boston, MA USA. All other assay reagents were of reagent grade quality. Materials used to prepare diets were purchased from U.S. Biochemical Corp., Cleveland, OH USA.

Animals and diets

The experimental protocol was approved by the University of Wyoming Animal Care and Use Committee. Forty male Sprague-Dawley rats were randomly assigned to one of four diets and then further divided so that for each diet, five rats were either fed continuously or starved for 2 days and then realimentated for 2 days. Rats weighed 209 ± 3 g when assigned to treatments. Upon arrival all rats were individually housed in a ventilated room with a 12-hr light/dark cycle and given free access to a standard chow diet (Rodent Laboratory Chow 5001, Purina Mills Inc., St. Louis, MO USA) and water for at least 7 days before starting the study. Treatment designations were based on the source of dietary fat: stearate, prepared by interesterification of high-oleate safflower oil and hydrogenated soybean oil (provided by Kraft/General Foods, Glenview, IL USA); oleate, from high-oleate safflower oil; palm oil; and tallow. Diet composition (g/100 g) was sucrose, 65; casein, 20; cellulose, 5; mineral mix, 3.5;[18] vitamin mix, 1.0;[18] D/L methionine, 0.3; and choline bitartrate, 0.2; this composition was based on that used previously.[2] For each treatment, continuous feeding of controls and SR were concurrent, after which rats were killed in ether. This feeding and SR regimen was the same as that used by Berdanier and coworkers.[2,5,6,8,13]

Homogenate preparation

Epididymal fat pads and livers were removed, weighed, and then the entire fat pad (both sides) and 2 g of liver were placed in ice-cold homogenization buffer (0.15 mol/L KCl, 10 mmol/L HEPES, 1 mmol/L EDTA, and 1 mmol/L DTT, pH 7.4). Adipose tissue and liver were homogenized (Tissumizer, Tekmar Company, Cincinnati, OH USA) for 1 min (3 × 20 sec) on ice in 2 mL and 5 mL of buffer per g, respectively. Adipose tissue homogenates were centrifuged at 700*g*, and liver homogenates at 10,000*g*. Thus, GPAT assays for liver did not include mitochondria. Infranatant fractions of centrifuged homogenates were transferred to 7-mL glass vials, snap frozen by immersion in liquid nitrogen for 1 min, and then stored at −70° C for up to 2 months. In previous studies using homogenates of swine[19] and rat[20] adipose tissue, no losses in GPAT activity were observed after snap-freezing in liquid nitrogen and then thawing rapidly up to 2 months later.

Glycerophosphate acyltransferase assay

GPAT was measured by determining the rate of glycerol-3-phosphate incorporation into total glycerolipids (TG, diacylglycerol, and polar glycerolipids). The protocol described for measuring GPAT has been used by others,[14,17] and was conducted as previously described for adipose tissue[20] and liver.[21] Briefly, 0.2 mL of adipose tissue homogenate were combined with 0.2 mL of a buffer (pH 7.4) at a final concentration of 50 mmol/L HEPES, 20 mmol/L K_2HPO_4, 0.16 mmol/L CoA, 12 mmol/L ATP, 12 mmol/L $MgCl_2$, 1 mmol/L DTT, 0.5 mg/mL BSA, 17.5 mmol/L glycerol-3-phosphate, 0.3 μCi of ^{14}C-glycerol-3-phosphate, and 0.6 mmol/L potassium palmitate. For liver 0.1 mL of homogenate was combined with 0.3 mL of the buffer, substrate, and cofactor solution, at the same final concentration as that described above. Substrates and cofactors were at saturating concentrations.

In previous experiments using homogenates of both liver and adipose tissue prepared from rats from the present study (unpublished data), linearity of glycerol-3-phosphate incorporation into total glycerolipids with time was observed for 10 to 20 min. By 60 min, incorporation attained a plateau (steady state), which was maintained for at least 2 hr. In the present study, GPAT was determined after 2 and 4 min, and steady-state glycerolipid production determined after 120 min of incubation to insure incorporation was linear and to determine the magnitude of steady-state incorporation. The rationale for determining steady-state glycerolipid production is that in adipose tissue homogenates from lean and obese swine, steady-state glycerolipid production was greater in the obese pigs than in the lean ones, while linear incorporation of glycerol-3-phosphate was not consistently responsive.[22] Moreover, adipose tissue homogenates from rats and sheep had increased steady-state glycerolipid production when the concentration of ATP was increased, while homogenates from human adipose tissue did not; no response to ATP was observed for linear glycerolipid synthesis rates for the rat and sheep homogenates.[20] Additionally, steady-state glycerolipid synthesis with the rat and sheep adipose tissue homogenates were responsive to fluoride at low ATP concentrations, while homogenates from human adipose tissue were not.[23] These observations indicate that steady-state glycerolipid production in vitro may be an indicator of genetic propensity to deposit fat (or maintain adiposity) and may be influenced by factors such as diet and SR.

Incubations were conducted in 16 × 125 mm test tubes at 37° C, and were terminated by addition of 3.0 mL of 1:2 chloroform:methanol (vol/vol). Triacylglycerol production was calculated from the percentage TG in extracts of homogenates from steady-state incubations that were subjected to thin layer chromatography. In a previous study,[24] long incubation times were required for maximal TG production in adipose tissue homogenates; therefore, steady-state incubation extracts were used. Extraction of total lipids as well as separation and quantitation of glycerolipids after thin layer chromatography are described elsewhere.[24]

Total homogenate protein was determined by using the Biuret procedure.[25] Fatty acid methyl esters of each dietary lipid were prepared[26] and analyzed by gas-liquid chromatography. Activity of glucose-6-phosphate dehydrogenase (G6PD) was determined in liver[27] to compare responsiveness of SR in the present study with responses observed by others.[13] Data were analyzed by analysis of

variance for the complete randomized design,[28] and differences between means were determined by Duncans's New Multiple range test[28] when significant F values were encountered. Differences were considered significant at $P < 0.05$; significance at $P < 0.01$ also is indicated.

Results

Weight percents of dietary fatty acids are presented in *Table 1*. Proportions of individual fatty acids as well as combinations of fatty acids (for example saturated, monounsaturated, and polyunsaturated) were diverse across treatments. Fatty acids of stearate and palm diets had similar ratios of total saturated to total unsaturated fatty acids (about 1:1), with the difference in composition largely from 18:0 and 16:0. The oleate diet contained primarily 18:1 (n-9), but also a substantial proportion of 18:2 (n-6,9). Tallow consisted of over half unsaturated fatty acids, most of which was from 18:1 (n-9).

Effects of SR on body and organ weights are shown in *Table 2*. For all treatments except tallow, rats subjected to SR were lighter than their fed controls ($P < 0.01$ for stearate; $P < 0.05$ for oleate and palm diets). Total fat pad weights also tended to be lighter after SR ($P < 0.05$ for oleate and tallow diets) when expressed either in g or as a percentage of body weight. When expressed in g, no differences in liver weight were observed. As a percentage of body weight, however, liver weights were greater after SR of rats with stearate, oleate ($P < 0.01$), and palm ($P < 0.05$) diets. Thus, in contrast to decreased adipose tissue weights after SR, liver weights increased after SR. Total assayable protein concentration in adipose tissue homogenates was greater after SR with rats fed the stearate diet ($P < 0.05$). For liver, protein concentration was greater in homogenates of rats after SR than in fed controls ($P < 0.01$ for stearate, oleate, and palm diets; $P < 0.05$ for the tallow diet).

Effect of dietary fat source and SR on liver G6PD activity are shown in *Figure 1*. Regardless of dietary fat source, SR resulted in significant ($P < 0.01$) enzyme activity overshoot. Control rats fed stearate or oleate diets had greater ($P = 0.03$) G6PD activities than those fed the palmitate diets, whereas G6PD activity in rats fed tallow were intermediate. On the other hand, no effects of fat source on liver G6PD activity in rats subjected to SR were observed. Results clearly indicate that a G6PD overshoot occurred in response to SR, which is consistent with results of others.[2,5–8,11,13] Values shown in *Figure 1* for SR G6PD were similar in magnitude to values reported by Johnson and Berdanier,[13] especially for the tallow diet. Values for fed controls, however, were greater in the present study than in the report of Johnson and Berdanier,[13] which resulted in lower changes in G6PD overshoot after SR. The reason for higher values for control rats in the present study is not clear.

Effects of SR on GPAT activity are shown in *Figure 2*. GPAT activity in adipose tissue homogenates was significantly influenced in the stearate-fed rats. Controls fed stearate had lower GPAT than their SR counterparts, but it also was lower than in fed controls in all other treatments ($P < 0.01$). Adipose tissue GPAT activity was similar for all rats after SR. GPAT activity in liver *(Figure 2)* was greatest after

Table 1 Weight percents of dietary fatty acids

Fatty acid	Diet			
	Stearate[a]	Oleate[b]	Palm	Tallow
14:0	0.2	0.2	1.3	3.6
16:0	8.6	5.5	45.6	26.7
16:1	0.2	0.1	0.1	4.4
18:0	42.0	2.5	4.8	13.1
18:1	41.2	76.6	38.9	49.7
18:2	7.6	14.8	9.0	1.6
18:3	0.2	0.3	0.2	1.0

[a]Developed by interesterification of high-oleate safflower oil and hydrogenated soybean oil.
[b]High-oleate safflower oil.

Table 2 Body and organ weights and assay protein content of tissue of fed and starved-realimentated (SR) rats

Diet	Treatment	Initial body weight g	Final body weight g	Fat pad weight g	Fat pad weight %[a]	Liver weight g	Liver weight %[a]	Assay Protein Fat mg/mL	Assay Protein Liver mg/mL
Stearate	Fed	213	222	1.4	0.6	12.8	5.8	2.8	7.8
	SR[d]	207	198	1.0	0.5	13.6	6.9	3.8	16.3
	SEM[e]	4	5[b]	0.1	0.04	0.4	0.2[b]	0.3[b]	1.3[b]
Oleate	Fed	207	217	1.5	0.7	11.6	5.4	3.0	7.5
	SR[d]	210	200	1.1	0.6	13.5	6.8	3.8	17.5
	SEM	3	5[c]	0.1[c]	0.01[b]	0.4	0.2[b]	0.3	1.0[b]
Palm	Fed	211	219	1.3	0.6	12.4	5.6	3.5	7.0
	SR[d]	214	199	1.1	0.5	13.4	6.7	3.5	12.0
	SEM	2	7[c]	0.1	0.03	1.0	0.3[c]	0.3	1.0[b]
Tallow	Fed	206	223	1.4	0.6	14.2	6.3	3.3	6.3
	SR[d]	207	210	1.0	0.5	14.1	6.7	3.5	9.8
	SEM	4	6	0.1[b]	0.03[c]	0.8	0.2	0.3	1.0[c]

[a]Percentage of body weight.
[b]For individual diets fed was different from SR, $P < 0.01$.
[c]For individual diets fed was different from SR, $P < 0.05$.
[d]SR were starved for 48 hr and then fed for 48 hr.
[e]Pooled standard error of the mean.

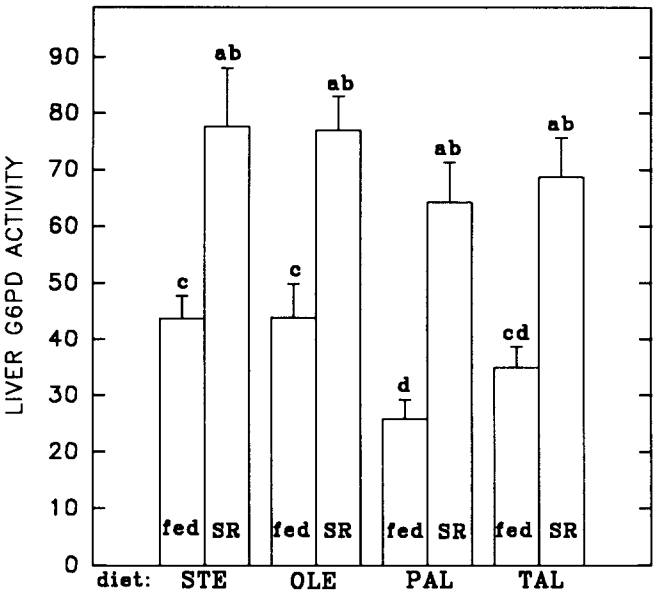

Figure 1 Glucose-6 phosphate dehydrogenase (G6PD) activity in livers of control (fed) and starved-realimentated (SR) rats fed 5 g/100 g of dietary fat as either high-stearate (STE), high-oleate safflower oil (OLE), palm oil (PAL), or beef tallow (TAL). G6PD activity is expressed as units per 100 g of body weight where one unit of enzyme activity is the amount of enzyme that will produce 1 μmole of product per min. [a]SR greater than fed ($P < 0.01$). [b]Between dietary treatments of SR rats no differences in G6PD activity were observed. [cd]For fed control rats only, different superscripts indicate a dietary effect on G6PD activity ($P = 0.03$). Data are means ± standard error of the mean.

SR when rats were fed oleate ($P < 0.01$) or palm ($P < 0.05$) diets, but no differences in liver GPAT were observed after SR when rats were fed the stearate or tallow diets.

Steady-state glycerolipid production tended to be greater in adipose tissue homogenates after SR than with continuous feeding, but differences were not significant ($P > 0.1$; data not shown). With liver homogenates, however, an SR overshoot in steady-state glycerolipid production was observed ($P < 0.01$ with stearate and oleate diets; $P < 0.05$ with palm and tallow diets; *Figure 3*). Steady-state TG production also was significantly greater after SR than with continuous feeding ($P < 0.01$ with oleate and tallow diets: $P < 0.05$ with stearate and palmitate diets).

Discussion

In the present study, final body weights were lower for rats after SR than their respective fed controls, which has been observed previously.[2,4–8,13] Fat pad weights decreased after SR, but differences were significant only for rats fed oleate or tallow diets. Because of relatively high variation in fat pad weights, no statistical differences were observed for the other treatments. Bouillon and Berdanier[7] and Jamdar and Osborne[14] also observed decreased fat pad weights in rats after SR. Apparently, despite the SR-enzyme overshoot commonly observed in lipogenic enzymes in adipose tissue, not enough time had elapsed to produce and deposit at least similar amounts of fat as fed controls.

For the most part, liver weights tended to increase after SR, and when expressed as a percentage of body weight, SR resulted in significantly greater liver weights in rats fed stearate, oleate, or palm diets. This observation was consistent with those of others.[2,4–6,16,29] Johnson and Berdanier[13] observed increased relative liver weights after SR in rats fed corn oil but not in rats fed menhaden oil.

Assayable protein concentrations in adipose tissue homogenates were similar for all treatments except stearate, in which SR resulted in a greater concentration. Bouillon and Berdanier[7] did not find an SR effect on fat pad soluble protein concentration; however, in the present study, protein concentration for liver of all treatments nearly doubled. Soluble protein concentrations in liver homogenates has also

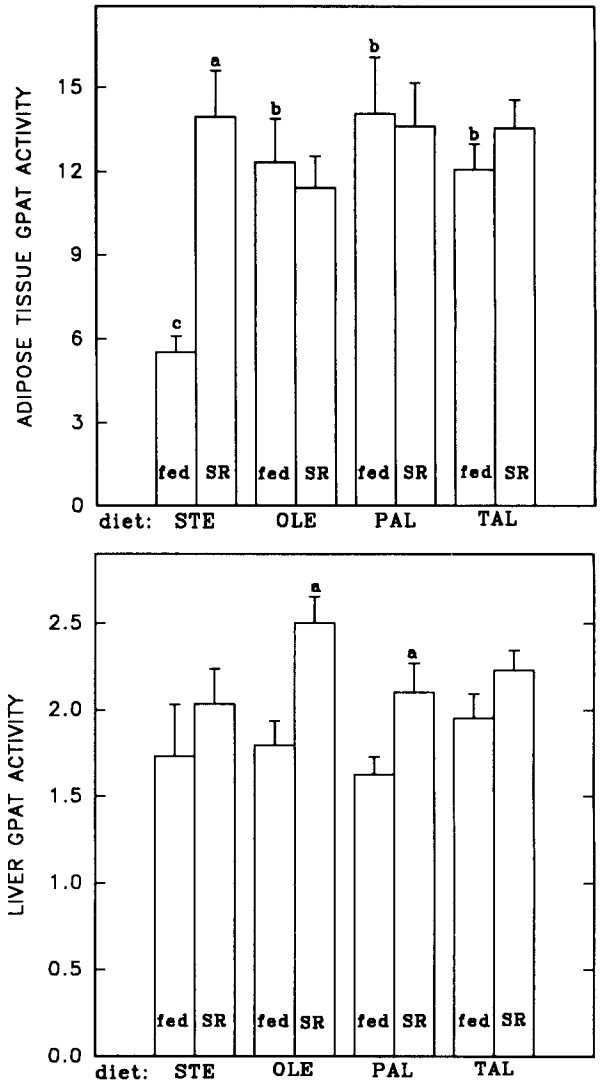

Figure 2 Glycerophosphate acyltransferase (GPAT) activity in adipose tissue and liver homogenates of control (fed) and starved-realimentated (SR) rats fed 5 g/100 g of dietary fat as either high-stearate (STE), high-oleate safflower oil (OLE), palm oil (PAL), or beef tallow (TAL). Enzyme activity is expressed as nmol glycerol-3-phosphate → total glycerolipids/min/100 g of body weight. [a]For both adipose tissue and liver, GPAT activity in SR rats was greater than that in fed rats ($P < 0.01$). [bc]Different superscripts indicate a diet effect on GPAT activity within fed controls for adipose tissue only. Data are means ± standard error of the mean.

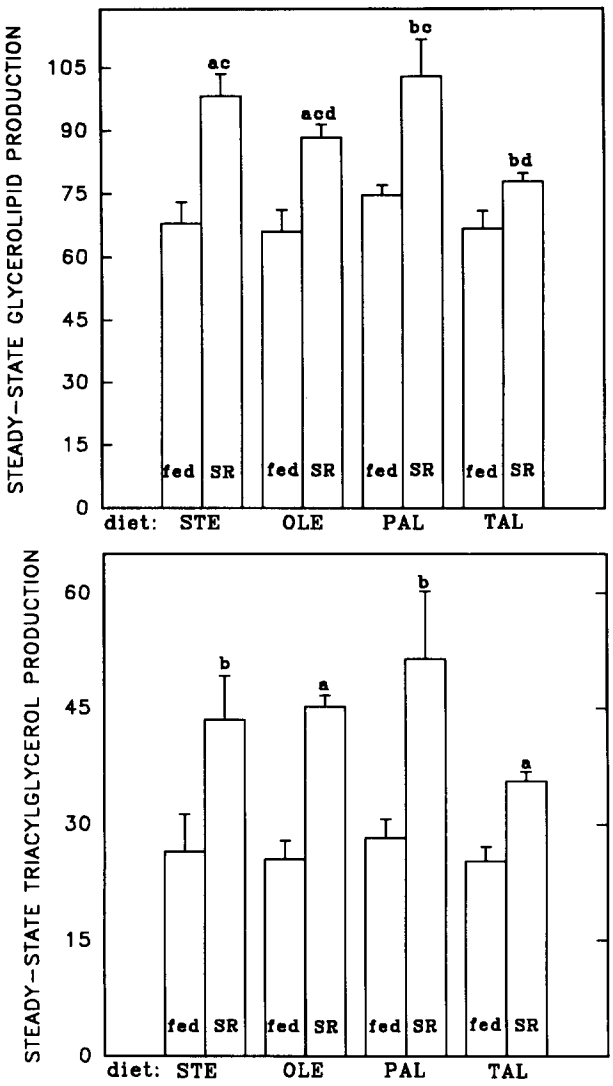

Figure 3 Steady-state glycerolipid and triacylglycerol production in liver homogenates of control (fed) and starved-realimentated (SR) rats. Data represent nmol glycerol-3-phosphate → total glycerolipids or triacylglycerol/2 hr/100 g of body weight. [a]SR values are greater than fed ($P < 0.01$). [b]SR values are greater than fed ($P < 0.05$). [cd]For steady-state glycerolipid production different superscripts indicate diet effects with SR rats ($P = 0.02$). Data are means + standard of the mean.

been shown to increase after SR of rats fed either corn oil or coconut oil.[2] In the present study, food intake was not reported because of losses of food out of feeders, especially during realimentation. Others, however, have shown that food intake of rats subjected to the same SR regimen was not different than fed controls.[4,5,8]

Feed efficiency of rats subjected to SR was greater than in rats fed ad libitum when all animals were fed a commercially prepared chow diet.[8] Bartley and Abraham[12] reported 5 days were required for rats to regain weight lost after 48-hr starvation. The similar body weights of rats subjected to SR in the present study indicates that realimentation on diets composed of different lipid sources probably did not influence feed efficiency beyond that expected during realimentation.

The greater liver homogenate protein of rats subjected to SR was likely related to the SR effect on GPAT activity and steady-state glycerolipid production. In adipose tissue the greatest effect of SR on GPAT was observed for rats fed stearate who also had significantly greater assayable protein concentrations than their controls, possibly contributing a greater concentration of GPAT and, thus, greater GPAT activity.[20] In fed controls, those that received stearate had the lowest adipose tissue GPAT activity. In bovine adipose tissue, greater in vitro rates of fatty acid esterification were observed with palmitate than with stearate as the substrate.[30] Thus, high dietary stearate depressed GPAT, but 48 hr of realimentation with a high-stearate fat did not. Results indicate that dietary fatty acid composition did not affect adipose tissue GPAT after SR.

With liver homogenates, rats subjected to SR with diets highest in 18:0 (stearate and tallow) were influenced least in the SR overshoot in GPAT. The two diets that supported the greatest SR GPAT overshoot in liver homogenates (oleate and palm) also had the highest and second highest (respectively) proportion of 18:2 (n-6,9). This result is consistent with that of Johnson and Berdanier,[13] who observed G6PD activity to be greatest in livers of starved rats refed a corn oil diet compared with beef tallow. On the other hand, Weigand et al.[16] observed an increase in GPAT activity in rats refed for 2 days after a 3-day fast on a diet that contained 5 g/100 g fat from either cocoa butter or safflower oil when data were expressed per mg of microsomal protein. This contrasts with results of the present study; however, the present study used a 10,000*g* supernatant fraction and not reconstituted microsomes.

In liver, a general increase in steady-state TG production occurred with SR, suggesting that phosphatidate phosphohydrolase (EC 3.1.3.4) activity was increased by SR. The large influx of fatty acids expected after SR also would be expected to stimulate phosphatidate phosphohydrolase activity because fatty acids have been shown to stimulate translocation of the soluble form of the enzyme to the membrane of the endoplasmic reticulum, resulting in greater activity in hepatocytes.[31,32]

Variations in dietary fat source were not reflected in liver G6PD activity, indicating that the rapid influx of lipogenic substrates during realimentation was responsible for the SR effects, and that the lipids fed did not accelerate or attenuate these effects. Liver GPAT activity, however, was greater in SR rats fed either the oleate or palm oil diets, indicating that substrate type influenced liver TG production. Steady-state production of glycerolipids and TG in vitro indicated that, as was the case with G6PD, influx of substrate in SR rats controlled overall synthesis more so than the individual lipid sources. Adipose tissue GPAT activity in SR rats was responsive to only stearate, suggesting that energy supply was the major driving force behind adipose GPAT regulation in SR. With continuous feeding, substrate supply from stearate was more important because GPAT activity was much lower when fed controls consumed the high-stearate diet. With the high-stearate diet substrate supply was less a factor in liver than in adipose tissue because no differences in GPAT activity were observed in liver between fed and SR rats with this dietary fat. On the other hand, substrate supply from the oleate and palm oil diets played a role in regulation of liver GPAT, indicating that these two organs respond

to variations in substrate supply patterns differently under different dietary regimens.

In conclusion, 2 days of starvation followed by 2 days of realimentation on a diet high in stearate stimulated adipose tissue GPAT to activities above non-starved controls. Realimentation on high-oleate and high-palmitate diets stimulated liver GPAT most. The SR overshoot in steady-state glycerolipid and TG production was accompanied by large increases in liver homogenate protein concentrations. Results indicate that ad libitum consumption of diets containing high proportions of stearate after a period of starvation could overshoot liver GPAT to a lesser extent than if the diet contained low proportions of this fatty acid. Diet fatty acid composition had little impact on adipose tissue GPAT activity in rats subjected to SR. In comparison with other studies, the most marked effects of feed intake variation and composition of realimentation diets appear to be on enzymes of fatty acid synthesis and less on TG synthesis.

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References

1 Allman, D. W., Hubbard, D. D., and Gibson, D. M. (1965). Fatty acid synthesis during fat-free refeeding of starved rats. *J. Lipid Res.* **6,** 63–74

2 Baltzell, J. K. and Berdanier, C. D. (1985). Effect of the interaction of dietary carbohydrate and fat on the responses of rats to starvation-refeeding. *J. Nutr.* **115,** 104–110

3 Moir, A. M. B. and Zammit, V. A. (1990). Changes in the properties of cytosolic acetyl-CoA carboxylase studied in cold-clamped liver samples from fed, starved and starved-refed rats. *Biochem. J.* **272,** 511–517

4 Berdanier, C. D., Wurdeman, R., and Tobin, R. B. (1976). Further studies on the role of the adrenal hormones in the responses of rats to meal-feeding. *J. Nutr.* **106,** 1791–1800

5 Wurdeman, R., Berdanier, C. D., and Tobin, R. B. (1978). Enzyme overshoot in starved-refed rats: Role of the adrenal glucocorticoid. *J. Nutr.* **108,** 1457–1461

6 Berdanier, C. D. and Shubeck, D. (1979). Interaction of glucocorticoid and insulin in the responses of rats to starvation-refeeding. *J. Nutr.* **109,** 1766–1771

7 Bouillon, D. J. and Berdanier, C. D. (1980). Role of glucocorticoid in adaptive hyperlipogenesis in the rat. *J. Nutr.* **110,** 286–297

8 Berdanier, C. D. (1981). Effects of estrogen on the responses of male and female rats to starvation-refeeding. *J. Nutr.* **111,** 1425–1429

9 Bottino, N. R., Anderson, R. E., and Reiser, R. (1965). Dietary fatty acids: Their metabolic fate and influence on fatty acid biosynthesis. *J. Amer. Oil Chem. Soc.* **42,** 1124–1129

10 Clarke, S. D., Romsos, D. R., and Leveille, G. A. (1977). Differential effects of dietary methyl esters of long-chain saturated and polyunsaturated fatty acids on rat liver and adipose tissue lipogenesis. *J. Nutr.* **107,** 1170–1181

11 Williams, B. H. and Berdanier, C. D. (1982). Effects of diet composition and adrenalectomy on the lipogenic responses of rats to starvation-refeeding. *J. Nutr.* **112,** 534–541

12 Bartley, J. C. and Abraham, S. (1972). Hepatic lipogenesis in fasted, re-fed rats and mice: Response to dietary fats of differing fatty acid composition. *Biochim. Biophys. Acta* **280,** 258–266

13 Johnson, B. and Berdanier, C. D. (1987). Effect of menhaden oil on the responses of rats to starvation-refeeding. *Nutr. Rep. Int.* **36,** 809–817

14 Jamdar, S. C. and Osborne, L. J. (1982). Glycerolipid biosynthesis in rat adipose tissue. 10. Changes during a starvation and re-feeding cycle. *Biochim. Biophys. Acta* **713,** 647–656

15 Ide, T., Murata, M., and Sunada, Y. (1992). Soybean protein-dependent changes in triacylglycerol synthesis and concentration of diacylglycerol in the liver microsomes of fasted-refed rats. *Ann. Nutr. Metab.* **36,** 87–96

16 Wiegand, R. D., Rao, G. A., and Reiser, R. (1973). Dietary regulation of fatty acid synthetase and microsomal glycerophosphate acyltransferase activities in rat liver. *J. Nutr.* **103,** 1414–1424

17 Saggerson, E. D. and Greenbaum, A. L. (1970). The regulation of triglyceride synthesis and fatty acid synthesis in rat epididymal adipose tissue. Effects of altered dietary and hormonal conditions. *Biochem. J.* **119,** 221–242

18 AIN-76. (1977). Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. *J. Nutr.* **107,** 1340–1348

19 Rule, D. C., Smith, S. B., and Mersmann, H. J. (1988). Glycerolipid biosynthesis in porcine adipose tissue in vitro. II. Synthesis by various types of cellular preparations. *J. Anim. Sci.* **66,** 1665–1675

20 Rule, D. C. (1992). Comparison of glycerolipid biosynthesis in homogenates from human, ovine, bovine and rat adipose tissue in vitro. *Comp. Biochem. Physiol.* **103B,** 93–100

21 Steffen, D. G., Phinney, G., Brown, L. J., and Mersmann, H. J. (1979). Ontogeny of glycerolipid biosynthetic enzymes in swine liver and adipose tissue. *J. Lipid Res.* **29,** 246–253

22 Rule, D. C., Smith, S. B., and Mersmann, H. J. (1989). Glycerolipid biosynthesis in porcine adipose tissue in vitro: Effect of adiposity and depot site. *J. Anim. Sci.* **67,** 364–373

23 Rule, D. C. (1993). Effect of fluoride on the ATP requirement for glycerolipid biosynthesis in adipose tissue of four mammalian species. *Comp. Biochem. Physiol.* **104B,** 469–473

24 Rule, D. C., Smith, S. B., and Mersmann, H. J. (1988). Glycerolipid biosynthesis in porcine adipose tissue in vitro. I. Assay conditions for homogenates. *J. Anim. Sci.* **66,** 1656–1664

25 Gornall, A. G., Bardawill, C. J., and David, M. M. (1949). Determinations of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177,** 751–755

26 Morrison, W. R. and Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **5,** 600–608

27 Freedland, R. A. (1967). Effect of progressive starvation on rat liver enzyme activities. *J. Nutr.* **91,** 489–495

28 Snedecor, G. W. and Cochran, W. G. (1967). One-way classifications. Analysis of variance., and Two-way classifications. In *Statistical methods,* (G. W. Snedcor and W. G. Cochran, eds.), p. 258–298 and p. 299–338, Iowa State University Press, Ames, IA USA

29 McDonald, B. E. and Johnson, B. C. (1965). Metabolic response to realimentation following chronic starvation in the adult male rat. *J. Nutr.* **87,** 161–165

30 Lin, K. C., Cross, H. R., and Smith, S. B. (1992). Esterification of fatty acids by bovine intramuscular and subcutaneous adipose tissues. *Lipids* **27,** 111–116

31 Martin-Sanz, P., Hopewell, R., and Brindley, D. B. (1984). Long-chain fatty acids and their acyl-CoA esters cause the translocation of phosphatidate phosphohydrolase from the cytosolic to the microsomal fraction of rat liver. *FEBS Lett.* **175,** 284–288

32 Pittner, R. A., Fears, R., and Brindley, D. N. (1985). Interactions of insulin, glucagon and dexamethasone in controlling the activity of glycerol phosphate acyltransferase and the activity and subcellular distribution of phosphatidate phosphohydrolase in cultured rat hepatocytes. *Biochem. J.* **230,** 525–534