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Influence of Dietary Safflower Oil and Tallow on Growth, Plasma Lipids and Lipogenesis in Rats, Pigs and Chicks[1] (39033)

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It is well established that dietary fat influences lipogenesis in rats, chicks and pigs (1). Few investigators have examined the influence of source of dietary fat on lipogenesis in species other than the rat and in this species the vast majority of studies have dealt with the influence of various fats on lipogenesis in the liver (2–8), but not on adipose tissue. It is now clear that there are tissue-specific responses to various dietary factors. Dietary fructose increases fatty acid synthesis in rat liver, but depresses the rate of fatty acid synthesis in rat adipose tissue (9) whereas dietary butanediol depresses the rate of hepatic fatty acid synthesis without influencing fatty acid synthesis in rat adipose tissue (10).

The purpose of this investigation was to study the influence of dietary safflower oil and tallow on lipogenesis in three species known to differ with regard to major site of fatty acid synthesis. In the rat, adipose tissue accounts for over 50% of total fatty acid synthesis. Adipose tissue is responsible for virtually all fatty acids synthesized in the pig whereas quantitatively the liver is the most important site of fatty acid synthesis in the chick (11). Additionally, the influence of dietary safflower oil and tallow on plasma lipid levels was investigated.

Materials and methods. Experimental animals and diets. Male Sprague–Dawley[3] rats were used. Seven and 10 rats per treatment, in two separate experiments, were individually housed in stainless steel cages having raised wire floors. Male crossbred chicks[4] were employed for these experiments 8–10 days after hatching. Chicks were housed, six birds per pen, in electrically heated pens with raised wire floors. Two separate experiments were conducted. Castrated male Yorkshire–Hampshire crossbred pigs were weaned at 3 weeks of age and provided a commercial diet for 1 wk. At this time the 16 pigs were assigned to one of two experimental diets. The pigs were housed in groups of eight in pens with aluminum-slat floors. Diets and water were provided *ad libitum* in all experiments. Rats, chicks, and pigs were allowed to consume the diets for 22, 22, and 28 days, respectively. Individual body weights were recorded weekly. Individual food consumption was recorded for the rat experiments, while group food intakes were recorded in the chick and pig experiments. The composition of the diets fed is presented in Table I. Approximately 4% safflower oil was added to the rat and chick diets to provide a source of essential fatty acids. The tallow containing diet fed to pigs did not contain safflower oil; however, the pigs did not exhibit visual symptoms of essential fatty acid deficiency during the study.

Blood sampling and analyses. Blood was collected in heparinized beakers following decapitation of the rats, by heart puncture of the chicks, and by anterior vena cava puncture of the pigs. Plasma triglycerides were analyzed by the method of Van Handel (16), plasma cholesterol by the method of Babson *et al.* (17), and plasma free fatty acids by the method of Duncombe (18).

In vitro lipogenesis. Distal portions of rat

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TABLE I. COMPOSITION OF DIETS.[a]

	Species		
	Rat	Chick	Pig
Casein	24.6	—	—
Isolated soy protein	—	28.0	31.4
Methionine	0.4	0.5	0.3
Mineral mix[b]	4.9	6.8	3.0
Vitamin mix[c]	0.5	0.7	0.6
Choline chloride	0.2	0.4	—
Fiber	4.9	6.1	3.6
Glucose	42.3	34.5	37.2
Safflower oil	3.7	3.6	—
Safflower oil or tallow	18.5	19.4	23.9
Total	100.00	100.00	100.00

[a] The isolated soy protein, tallow and safflower oil were generously supplied by Central Soya, Decatur, Indiana; Carolina By-Products, Greensboro, North Carolina and Oscar Mayer, Madison, Wisconsin; and by Pacific Vegetable Oil Corporation, Richmond, California, respectively.

[b] The composition of the rat and chick mineral mix has been presented by Leveille and O'Hea (12) and by Velu *et al.* (13), respectively. The mineral mix fed to the pigs contained 20% sodium chloride, 40% dicalcium phosphate and 40% limestone.

[c] The composition of the rat and chick vitamin mix (14) and the pig vitamin mix (15) has been presented previously.

epididymal adipose tissue, pieces of chick abdominal adipose tissue, slices of pig subcutaneous adipose tissue and rat and chick liver slices were incubated for 2 hr at 37° in 3 ml Krebs–Ringer bicarbonate buffer. The tissue slices were prepared with a Stadie–Riggs hand microtome. The tissue preparations weighed approximately 150 mg. The incubation buffer contained per ml: 0.1 units porcine insulin,[5] 5 μmoles glucose, 10 μmoles acetate and 0.1 μCi ^{14}C-acetate. This system does not estimate the rate of fatty acid synthesis from endogenous substrates; however, the contribution of endogenous substrates should be small relative to the large (10 m*M*) exogenous pool of acetate. The incubation procedures and methods for isolating and counting the radioactive fatty acids have been described (19).

Enzyme analyses. About 1 g liver or adipose tissue was weighed, homogenized in 9 ml 0.15 *M* KCl, 4 m*M* $MgCl_2$, 4 m*M* *N*-acetylcysteine, pH 7.0 and centrifuged at 100,000*g* at 4° for 30 min. The resulting supernatant was used to determine the activities of malic enzyme (E.C. 1.1.1.40) (20), acetyl CoA carboxylase (E.C. 6.4.1.2) (21) and fatty acid synthetase (22). Protein content of the supernatant was determined by the method of Lowry *et al.* (23). Enzyme activities were expressed as units where a unit is the amount of enzyme which will catalyze the utilization of one nanomole of substrate per minute.

Results. Results are presented in Tables II and III. Body weight gain and food intake were not influenced by the source of dietary fat in rats, chicks or pigs. Plasma triglyceride levels were depressed in rats, unchanged in chicks and increased in pigs fed diets containing safflower oil rather than tallow. Plasma cholesterol and free fatty acid levels were not influenced by the source of dietary fat in rats, chicks or pigs.

Hepatic lipogenic enzyme activities and in vitro rates of fatty acid synthesis were determined in rats and chicks fed safflower oil or tallow containing diets (Table II). Liver weight was increased and hepatic soluble protein concentration decreased in rats fed safflower oil rather than tallow. Hepatic lipogenic enzyme activities in rats and chicks tended to be depressed to a greater extent when safflower oil, rather than tallow, was added to the diet. *In vitro* rates of fatty acid synthesis in rats and fatty acid synthetase activities in chicks were significantly lower when safflower oil, rather than tallow, was fed. These results are in general agreement with a number of studies suggesting that dietary fats containing polyunsaturated fatty acids depress hepatic lipogenesis in the rat and mouse to a greater extent than do fats containing saturated fatty acids (2–8, 24–26).

Adipose tissue quantitatively is an important site of fatty acid synthesis in the rat and pig (11); consequently the influence of safflower oil and tallow on adipose tissue lipogenesis was investigated. In contrast to the influence of these dietary fats on hepatic lipogenesis, dietary tallow depressed adipose

[5] Generously supplied by Eli Lilly, Indianapolis, Indiana.

TABLE II. EFFECTS OF DIETARY FAT SOURCE ON BODY WEIGHT GAIN, PLASMA LIPIDS, AND HEPATIC AND ADIPOSE TISSUE LIPOGENESIS IN RATS AND CHICKS[a]

	Rat Fat source		Chick Fat source	
	Safflower oil	Tallow	Safflower oil	Tallow
Initial weight (g)	117 ± 2	115 ± 3	154 ± 6	157 ± 6
Final weight (g)	293 ± 7	282 ± 6	1020 ± 33	1014 ± 34
Daily food intake (g)	17.8 ± 0.3	18.3 ± 0.4	49.5	50.1
Plasma triglycerides, (mg/100 ml)	136 ± 13	243 ± 28[c]	63 ± 8	73 ± 6
Plasma cholesterol, (mg/100 ml)	191 ± 12	174 ± 7	183 ± 7	192 ± 11
Plasma free fatty acids, (mg/100 ml)	5.9 ± 0.38	5.5 ± 0.48	7.7 ± 0.55	8.4 ± 0.43
Liver weight (g)	13.1 ± 0.35	12.1 ± 0.31[c]	21.8 ± 0.66	21.6 ± 0.39
Liver soluble protein (mg/g)	109 ± 2.6	117 ± 2.1[c]	120 ± 3.3	120 ± 2.1
Hepatic fatty acid synthetase[b]	4.2 ± 0.3	4.5 ± 0.37	9.8 ± 0.61	12.7 ± 0.7[c]
Hepatic acetyl CoA carboxylase[b]	2.65 ± 0.17	2.45 ± 0.27	20.4 ± 2.90	22.3 ± 3.00
Hepatic fatty acid synthesis[b]	621 ± 56	828 ± 61[c]	4255 ± 321	3972 ± 558
Epididymal fat pad weight (g)	4.21 ± 0.23	4.40 ± 0.19	—	—
Adipose soluble protein (mg/g)	6.64 ± 0.33	6.84 ± 0.43	4.89 ± 0.16	4.92 ± 0.20
Adipose fatty acid synthetase[b]	30.6 ± 2.10	18.4 ± 1.78[c]	2.80 ± 0.28	2.50 ± 0.30
Adipose acetyl CoA carboxylase[b]	23.8 ± 3.03	10.8 ± 1.81[c]	2.20 ± 0.38	2.04 ± 0.36
Adipose fatty acid synthesis[b]	2027 ± 90	1617 ± 102[c]	103 ± 18	82 ± 8

[a] Values represent Mean ± SEM; rat $n = 17$, chick $n = 18$.

[b] Enzyme activity expressed as nanomoles substrate converted to product per mg protein per minute at 37° and fatty acid synthesis expressed as nanomoles ^{14}C-acetate incorporated into fatty acids per gram tissue per hour.

[c] Safflower oil versus tallow, significantly different, $P < 0.05$ level.

TABLE III. EFFECTS OF DIETARY FAT SOURCE ON BODY WEIGHT GAIN, ADIPOSE TISSUE LIPOGENESIS, AND PLASMA COMPONENTS IN PIGS.[a]

	Dietary fat source: Safflower oil		Tallow	
	3 weeks	4 weeks	3 weeks	4 weeks
Daily body weight gain (g)		510 ± 30		480 ± 20
Daily food intake (g)		760		750
Plasma, (mg/100 ml)				
Triglycerides		56 ± 6		37 ± 6[c]
Cholesterol		116 ± 6		125 ± 4
Free fatty acids		4.7 ± 0.6		4.0 ± 0.7
Adipose tissue				
Soluble protein (mg/g)	7.60 ± 0.30	6.31 ± 0.24	7.85 ± 0.43	6.55 ± 0.28
Fatty acid synthetase[b]	7.10 ± 0.60	8.50 ± 0.70	4.50 ± 0.60[c]	6.50 ± 0.70[c]
Acetyl CoA carboxylase[b]	2.61 ± 0.33	3.39 ± 0.53	2.00 ± 1.31	2.89 ± 0.47
Malic enzyme[b]	145 ± 17	145 ± 17	99 ± 13[c]	101 ± 12[c]
Fatty acid synthesis[b]	4390 ± 591	4512 ± 378	2889 ± 195[c]	3313 ± 406[c]

[a] Values represent Mean ± SEM; $n = 8$. The initial weight of the pigs was 10.4 ± 0.45 kg. Biopsy adipose tissue samples and blood samples were obtained after the pigs had been fed the respective diets for 3 or 4 weeks, as indicated.

[b] See Table II.

[c] See Table II.

tissue lipogenesis in rats and pigs to a greater extent than did dietary safflower oil (Tables II and III). Adipose tissue fatty acid synthetase activities and *in vitro* rates of fatty acid synthesis were significantly depressed in both rats and pigs when tallow replaced safflower oil in the diet. Acetyl CoA carboxylase activities and malic enzyme activities were also significantly depressed in rats and pigs, respectively, fed the tallow containing diets. Adipose tissue soluble protein concentration was not influenced by the source of dietary fat (Tables II and III). Adipose tissue is not considered an important site of fatty acid synthesis in the chick (11). In this study lipogenic activity in chick adipose tissue was much lower than values observed in rat or pig adipose tissue and was not influenced significantly ($P > 0.05$) by the source of fat provided in the diet.

Discussion. The results of this investigation indicate that the influence of dietary factors on metabolism depends, in part, on the species, as well as the tissue, studied. Plasma triglyceride response to the fat sources appeared to be species-specific. Plasma triglyceride levels were lower when safflower oil, rather than tallow, was fed to rats (Table II). This effect may be explained, in part, by the elevation of lipoprotein lipase activity observed when safflower oil was fed to rats (27) thereby allowing for a greater removal of triglycerides from the circulation. However in the pig, plasma triglyceride levels were lower when tallow was fed. Whether the changes in circulating triglyceride levels in these species result from changes in triglyceride inflow and/or removal from the circulation remains to be established. These three species, the rat, chick and pig, would appear to represent useful models in the investigation of dietary influences on plasma triglyceride kinetics.

In the present study dietary safflower oil significantly altered only one of the three measured indicies of fatty acid synthesis in rat liver; however, the results observed in rat adipose tissue were more pronounced. While in some situations the source of dietary fat influences hepatic lipogenesis in the rat, the mechanism of action is not clear. In an earlier study (28) the lipogenic rate in chick liver was depressed to a greater extent by the addition of safflower oil to the diet than by addition of tripalmitin primarily because tripalmitin was poorly absorbed. Based on the body weight gain and food intake data, safflower oil and tallow were equally well utilized in this study. This suggests that dietary tallow was well absorbed and that other mechanisms are involved in the distinct influences observed.

The lipogenic response of rat and pig adipose tissue to the dietary fat sources was opposite of the response observed in rat and chick liver (Tables II and III). In a previous experiment, rates of fatty acid synthesis also were lower in adipose tissue slices obtained from pigs fed tallow, rather than corn oil; however, in the second experiment of that study no differences were observed (29). Others have also obtained data which suggest tissue-specific responses to dietary fat sources. Lipogenic enzyme activities in adipose tissue preparations have been reported to be higher in rats fed safflower oil than in animals fed lard (30) and acetyl CoA carboxylase activities were higher in adipose tissue preparations from rats fed linoleic acid rather than oleic acid (31). Launay and Raulin (32) have also observed that the rate of incorporation of ^{14}C-orotic acid into adipose tissue ribosomal RNA is greater in rats fed safflower oil than observed in rats fed lard containing diets.

Whether the metabolic influences of safflower oil and tallow can be wholly contributed to the differences in degree of saturation of the fatty acids contained therein or not remains to be established. It is possible that other factors relating to the composition of these fats might be involved in the responses observed. An understanding of the tissue and species-specific responses observed should contribute to our understanding of the regulation of lipid metabolism in man.

Summary. Rats, chicks and pigs were fed diets containing safflower oil or tallow. Plasma triglyceride levels were elevated when tallow, rather than safflower oil was added to the diet of rats, unchanged in chicks and lowered when tallow, rather than safflower

oil was fed to pigs. The rate of fatty acid synthesis in rat and chick liver was higher, whereas the rate of lipogenesis in adipose tissue preparations from rats and pigs was lower when tallow, rather than safflower oil was fed. These results indicate that there are species-specific, as well as organ-specific, metabolic responses to various dietary fats.

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