

## Glucogenic and Ketogenic Capacities of Lard, Safflower Oil, and Triundecanoin in Fasting Rats

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**ABSTRACT** The glucogenic and ketogenic capacities of lard, safflower oil, and triundecanoin were compared. Rats were fed diets containing 30% of either lard (a ketogenic fat), triundecanoin (a glucogenic fat), or safflower oil (a fat high in linoleic acid). After 61 days, the rats were fasted for 72 hours. Plasma glucose and ketone body concentrations and carcass fatty acid loss were measured during fasting. The lard-fed animals, which lost mostly saturated even-chain length fatty acids during fasting, did not maintain their prefasting plasma glucose levels and became ketotic. The animals that had been fed triundecanoin (which mobilized considerable odd-chain fatty acid) maintained their prefasting plasma glucose levels and did not become ketotic. The animals fed safflower oil (which mobilized massive amounts of linoleic acid) showed even lower levels of plasma glucose and higher levels of ketone bodies than did the animals fed lard. This failure of safflower oil to avert fasting hypoglycemia suggests that linoleic acid is oxidized in a manner more like the saturated fatty acid of lard than like the glucogenic odd-chain fatty acid (undecanoic). *J. Nutr.* 105: 185-189, 1975.

**INDEXING KEY WORDS** fatty acid oxidation · glucogenesis · plasma glucose levels during fasting · plasma ketone bodies during fasting

In an attempt to explain why linoleic acid (18:2) is oxidized more rapidly than other fatty acids both in vivo and in vitro, DuPont and Mathias (1) have suggested that the  $\gamma$  oxidation of linoleate to yield propionate as hypothesized by Sinclair (2) is responsible. In this proposed pathway, three acetyl CoA units are cleaved from 18:2 via  $\beta$  oxidation, and then the  $\Delta^3,6$ -dodecadienoyl CoA is cleaved at the 3-position via  $\gamma$  oxidation producing propionyl CoA and  $\Delta^3$ -nonenoyl CoA. Propionyl CoA can then be converted to succinate (3) and provide intermediates for acetyl CoA oxidation by the tricarboxylic acid cycle.

Studies on 18:2 oxidation both in vivo (4, 5) and in vitro with liver mitochondria (4, 6) and heart mitochondria (7) have yielded results consistent with complete degradation of 18:2 via  $\beta$  oxidation. In addition, Stoffel et al. (8) and Stoffel and Caesar (9) have shown that liver mitochondria contain enzymes that can accom-

plish the complete oxidation of 18:2 via  $\beta$  oxidation.

Recently, several investigators<sup>1</sup> (10, 11) have reported that odd-chain fatty acid oxidation allows fasting animals to maintain nonfasting plasma glucose levels. The glucogenic capacity of odd-chain fatty acids relies upon  $\beta$  oxidation yielding propionyl CoA from the terminal three carbons and conversion of this propionate to succinate (10).

We have compared the glucogenic and ketogenic capacities of an 18:2 triglyceride (safflower) with that of a known propionate precursor (triundecanoin) and a saturated fat (lard) in fasting animals in an attempt to assess the extent to which 18:2 is catabolized by the proposed  $\gamma$  oxidation pathway (1).

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<sup>1</sup>Guy, D. G. & Theurer, R. C. (1972) The effect of feeding various levels of triundecanoin on some blood and tissue metabolites. *Federation Proc.* 31, 674. (Abstr.)

TABLE 1  
*Experimental diets*

Component	Diet no.		
	720	721	722
		%	
Casein	20	20	20
Sucrose	36	36	36
Bernhart and Tomarelli salt mix <sup>1</sup>	5	5	5
Cellulose <sup>2</sup>	3	3	3
Water-soluble vitamins <sup>3</sup>	5	5	5
Fat-soluble vitamins <sup>4</sup>	1	1	1
Triundecanoin	29	—	—
Lard	—	29	—
Safflower oil	1	1	30

<sup>1</sup> Nutritional Biochemicals, Inc., Cleveland, Ohio (12).  
<sup>2</sup> Cellu flour, Chicago Dietetic Supply House, Chicago, Ill.  
<sup>3</sup> Contains (in mg): menadione, 0.3; thiamin-HCl, 0.4; ribo-  
 flavin, 0.7; niacin, 2.0; Ca pantothenate, 0.2; folic acid, 0.002;  
 pyridoxine, 0.4; vitamin B-12, 0.015; choline chloride, 300;  
 inositol, 200; ascorbic acid, 10; biotin, 0.015; and *p*-amino-  
 benzoic acid, 10. <sup>4</sup> Prepared using the appropriate fat for the  
 diet and containing 1,250 U.S.P. units of retinyl acetate, 1,250  
 U.S.P. units of ergocalciferol, and 10 U.S.P. units of *d*- $\alpha$ -  
 tocopherol.

#### MATERIALS AND METHODS

Weanling, male, Sprague-Dawley rats were fed semipurified diets (table 1) containing 30% fat for 61 days. The dietary fats were: (1) 30% safflower oil (72% linoleic acid); (2) 29% lard and 1% safflower oil; and (3) 29% triundecanoin<sup>2</sup> and 1% safflower oil. Animals were fed a daily 2-hour meal (8–10 AM) during which time they were allowed to eat ad libitum. After consuming the diets for 8 weeks, the animals were placed in metabolism cages that allowed for urine collection. After an acclimation period of 4 days, a control 24-hour urine sample was collected. The animals were then fed a final meal, and five animals per dietary fat were killed at 4, 24, 48, and 72 hours after the final meal. During the fasting period, daily weights were

recorded, and 24-hour urine volumes were measured.

Blood was collected in heparinized syringes via heart puncture after anesthesia with ether (4-hour samples) or with sodium pentobarbital (50 mg/kg body weight). Plasma was separated by centrifugation, frozen, and stored at  $-20^{\circ}$  until analyzed. The entire carcasses were frozen and ground in a Wiley mill in the presence of dry ice. The ground carcasses were freeze-dried, and their dry weights were determined.

Plasma glucose was measured enzymatically (13), and plasma and urine ketone body concentrations were determined chemically (14). Carcass total lipids were measured (15).

Carcass total fatty acid compositions were determined by gas-liquid chromatography (GLC) after methyl ester formation (16). The mass of individual fatty acids present in the carcass was calculated by total lipid weight (grams per animal)  $\times$  percentage of the individual acid as determined by GLC.

Statistical analyses were conducted by analysis of variance and comparison among means by the method of Tukey as outlined by Snedecor (17).

#### RESULTS

Weight gains and feed efficiencies (g of gain/100 g of diet ingested) during weeks 5–8 of the study are presented in table 2. Animals fed lard and safflower oil gained more weight than animals fed triundecanoin. The poorer gain observed with triundecanoin was associated with reduced

<sup>2</sup> Triundecanoin kindly supplied by H. D. Hamilton, Drew Chemical Corp., Boonton, N.J. 07005.

TABLE 2  
*Effects of dietary oils on weight gain, feed efficiency, and weight loss during fasting*

Dietary oil	Final body wt <sup>1</sup>	Weeks 5–8 of feeding <sup>2</sup>		Loss during fast	
		Gain <sup>3</sup>	Efficiency <sup>3,4</sup>	Live wt <sup>4</sup>	Dry wt <sup>4,5</sup>
Triundecanoin	141 $\pm$ 4 <sup>a</sup>	46 $\pm$ 2 <sup>a</sup>	32.8 $\pm$ 1.0 <sup>a</sup>	23.8 $\pm$ 1.2 <sup>a</sup>	6.7 $\pm$ 0.9 <sup>a</sup>
Safflower oil	195 $\pm$ 4 <sup>b</sup>	77 $\pm$ 3 <sup>b</sup>	40.2 $\pm$ 1.4 <sup>b</sup>	26.6 $\pm$ 0.7 <sup>a</sup>	15.6 $\pm$ 0.4 <sup>b</sup>
Lard	222 $\pm$ 5 <sup>c</sup>	81 $\pm$ 3 <sup>b</sup>	41.5 $\pm$ 1.4 <sup>b</sup>	24.2 $\pm$ 0.8 <sup>a</sup>	13.0 $\pm$ 0.8 <sup>b</sup>

Values with different superscripts are significantly different from each other at  $P < 0.05$ . <sup>1</sup> Weight at time of initiation of fast. Mean  $\pm$  SEM for 20 animals per diet. Initial body weight was 70  $\pm$  1 g at start of the experimental diet feeding. <sup>2</sup> Mean  $\pm$  SEM for 20 animals per dietary treatment. <sup>3</sup> Grams of gain/100 g of diet consumed. <sup>4</sup> Mean  $\pm$  SEM for five animals per treatment. <sup>5</sup> Difference in dry mass observed at 72 hours and values calculated for the same animals at 4 hours using percentage dry weight obtained for animals fed the same diet but killed at 4 hours.

TABLE 3  
Effect of dietary oil on mass of individual fatty acids lost from the carcass during a 72-hour fast<sup>1</sup>

Fatty acid	Dietary oil <sup>1</sup>		
	Triunde- canoin	Safflower oil	Lard
	g		
Total acids	4.2±0.2 <sup>2</sup>	10.3±0.7	3.9±1.2
Saturates			
Odd-chain	3.0±0.1		
Even-chain	0.9±0.1	1.7±0.1	1.6±0.4
Monounsaturates	0.8±0.03	2.0±0.1	1.8±0.6
Linoleic	0.1±0.03	6.7±0.4	0.5±0.2

<sup>1</sup> Five rats in each dietary group were killed after a 4-hour fast and five were killed after a 72-hour fast. The former rats provided the base-line data corrected for lipid mass differences.

<sup>2</sup> Mean ± SEM for five animals.

feed efficiency and is consistent with results reported by other workers (11).

The effects of fasting on live and dry weights are shown in table 2. All three groups of animals showed similar weight losses during fasting, and the percentage of weight lost during fasting was inversely proportional to the live weight at initiation of the fast (table 2). The amount of dry weight lost during fasting (table 2) was not merely a function of the original dry mass of the animal but was also influenced by the dietary oil the animals had consumed prior to fasting. For example, the animals fed safflower oil and the originally heavier lard-fed animals lost similar amounts of dry mass, and the lightest triundecanoin-fed animals lost the least amount of dry mass (6.7 g). When the dry weight loss data are expressed as percentages of the original dry weight, safflower oil-fed animals lost a greater percentage of their dry mass during the 72-hour fast (24%) than either the lard-fed animals (16%) or the triundecanoin-fed animals (18%).

The greater amount of dry weight lost by the safflower oil-fed animals was accounted for by total carcass lipid loss, as shown in table 3. That is, the safflower oil-fed animals lost approximately twice as much lipid during fasting (10.3 g) as the animals fed either lard (3.9 g) or triundecanoin (4.2 g).

The mass of individual fatty acids lost from the carcass during fasting are presented in table 3. The triundecanoin-fed

animals lost mostly odd-carbon chain acids during fasting. The animals fed lard and safflower oil mobilized similar amounts of saturated and monounsaturated acids, but the safflower oil-fed animals mobilized 13 times as much linoleic acid as that of the lard-fed animals.

Plots of the plasma concentrations of glucose and ketone bodies as a function of fasting time are shown in figure 1A and B, respectively. Plasma glucose concentrations 4 hours after the final meal were highest in the animals fed safflower oil (173 mg/100 ml) and lowest in animals fed lard (146 mg/100 ml). All the animals had similar plasma glucose concentrations 24 hours after the final meal. These 4- and 24-hour values should reflect the diurnal variations in plasma glucose levels in these animals, because they are approximately the time of maximal absorption and the between-meal time, respectively.

During the second 24 hours of fasting (24-48 hours), the plasma glucose concentrations in both the lard-fed and safflower oil-fed groups dropped rapidly while that

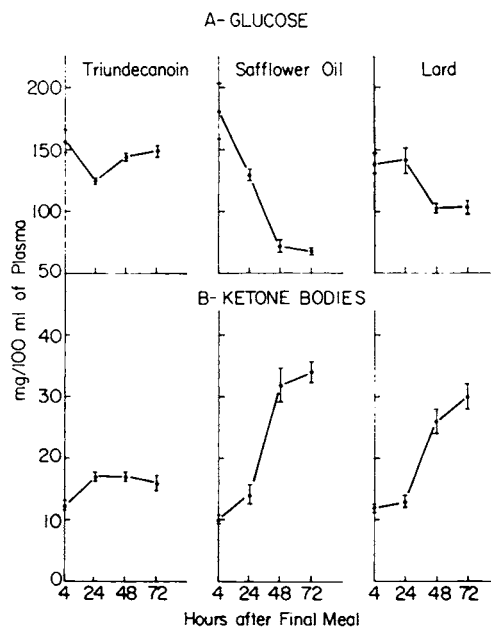


Fig. 1. Effect of length of fasting period on plasma glucose and ketone body concentrations in animals fed the fats indicated prior to fasting. Each point is the mean value for five animals, and the bars are SEM.

of the triundecanoin-fed group showed a recovery to the 4-hour postprandial level. During the final 24-hour fast, none of the groups showed appreciable change in plasma glucose concentration.

Plasma ketone body concentrations as a function of dietary oil and fasting time are recorded in figure 1B and are the mirror images of the plasma glucose concentrations shown in figure 1A. For example, both the plasma glucose and ketone body levels showed only small changes during fasting in the animals fed triundecanoin, while the animals fed safflower oil and lard both showed large decreases in plasma glucose and concomitant increases in plasma ketone bodies. The animals fed safflower oil showed the most pronounced drop in plasma glucose (to 68 mg/100 ml) and the highest plasma ketone body concentrations (34 mg/100 ml).

#### DISCUSSION

The work described in this report was designed to assess the ketogenic and glucogenic potentials of a high linoleic-containing dietary oil (safflower oil) compared with that of a known ketogenic fat (lard) and a known glucogenic fat (triundecanoin). The assessment of the ketogenic and glucogenic capacities of linoleic acid is based upon measures of carcass fatty acid losses and plasma glucose and ketone body concentrations at 4, 24, 48, and 72 hours after a final meal.

The results obtained in this study show that animals fed triundecanoin were able to maintain nonfasting blood glucose levels during prolonged fast, whereas the animals fed lard were not. We therefore have a valid base upon which to assess the effects of safflower oil feeding.

The animals fed safflower oil prior to fasting were not able to maintain prefasting plasma glucose levels like those of the animals fed triundecanoin (fig. 1A) and had even lower blood glucose levels during fasting than animals fed lard prior to fasting (fig. 1A).

Plasma ketone body results (fig. 1B) show that safflower oil was more ketotic than lard. These results were unexpected, because it has been reported that polyunsaturated fatty acids are less ketogenic than their saturated analogs (18, 19).

There are several differences in experimental design in the present work and those reported by others (18, 19) that could account for this difference in results.

The glucogenicity of odd-carbon chain fatty acids has been attributed to the fact that  $\beta$  oxidation of such acids would yield propionyl CoA (10). Assuming this to be true, we have calculated the propionic acid pool available to the animals fed triundecanoin (table 3). Assuming 1 mole of odd-chain acid oxidized would yield 1 mole of propionyl CoA, we have calculated that the animals fed triundecanoin produced  $\sim 16$  mmoles of propionic acid during the fasting (3.0 g of odd-chain acids lost/186 mg/mmole of undecanoic acid = 16.1 mmoles of undecanoic acid oxidized). This yield of propionic acid allowed the animals fed triundecanoin to maintain prefasting glucose levels during 72 hours of fasting and also prevented ketosis during the fasting period (fig. 1).

DuPont and Mathias (1) have proposed that linoleic acid may be oxidized via a combination of  $\beta$  oxidation and  $\gamma$  oxidation to acetyl CoA and propionyl CoA. If this oxidative pathway is employed and yields 1 mole of propionic acid/mole of linoleic acid oxidized, the safflower oil-fed animals should have produced  $\sim 24$  mmoles of propionic acid (6.7 g of linoleic acid lost/280 mg/mmole of linoleic acid = 23.9 mmoles 18:2 oxidized). In contrast to the glucogenic response of propionic acid from odd-chain catabolism by fasting rats, linoleic catabolism did not prevent fasting hypoglycemia. In fact, animals catabolizing only one-tenth as much linoleic acid (lard-fed animals) as that of the safflower oil-fed animals had higher fasting plasma glucose concentrations.

Not only did linoleic acid catabolism not avert fasting hypoglycemia, but it also produced a more severe fasting ketosis than that observed in triundecanoin- and lard-fed animals (fig. 1B). Further, urine analysis showed that the safflower oil-fed animals excreted  $\sim 8$  mg of  $\beta$ -hydroxybutyric acid in their urine during the 48-72 hour fasting period while the lard-fed animals showed no ketonuria. It has been demonstrated that ketosis is a function of overproduction (beyond the capacity of the oxidative systems) of acetyl CoA from fatty

acid  $\beta$  oxidation (20). Thus, the data obtained in animals catabolizing mostly linoleic acid (safflower oil fed) during fasting are consistent with the oxidation of linoleic acid by  $\beta$  oxidation to acetyl CoA and inconsistent with  $\gamma$  oxidation of linoleic acid to propionyl CoA to any appreciable extent.

If linoleic acid is oxidized by  $\gamma$  oxidation, the propionic acid produced does not serve as a precursor of plasma glucose as does propionic acid from odd-chain fatty acid oxidation nor does it diminish the conversion of acetyl CoA from linoleic acid  $\beta$  oxidation to plasma ketone bodies in fasting animals.

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