Metabolic Consequences of Dietary Medium-Chain Triglycerides in the Pig[1] (36158)

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Dietary medium-chain triglyceride (MCT), a fat consisting almost exclusively of caprylic and capric acids has been shown to depress serum cholesterol levels in the rat (1–5), dog (6), rabbit (7), and in man (8, 9). In contrast to these observations, MCT has been reported to be hypercholesterolemic in the chick (3, 10, 11).

The effect of MCT on lipid synthesis has been studied in the rat and chick. Kritchevsky and Tepper (4) reported that the incorporation of acetate into hepatic fatty acids, both *in vivo* and *in vitro*, was enhanced by MCT ingestion compared with animals consuming corn oil or coconut oil. The adipose tissue of rats fed MCT converts acetate to fatty acids at a much faster rate than does adipose tissue from animals fed corn oil (5). Evidence has also been presented (5) to show that the enhanced incorporation of acetate into fatty acids by adipose tissue of rats fed MCT represents *de novo* synthesis of fatty acids and not chain-lengthening activity. *In vitro* fatty acid synthesis was higher in liver slices from chicks fed MCT than in those from chicks fed corn oil (11).

The studies reported herein were conducted to investigate the effect of feeding MCT, lard, corn oil, tallow, or coconut oil on lipid metabolism in the pig.

Materials and Methods. Thirty-six crossbred pigs (5 weeks of age and av 11 kg) were randomly assigned from littermate groups to 6 treatments. The treatments imposed were: control (1% tallow) and 10% dietary fat as tallow, corn oil, lard, coconut oil, or medium-chain triglyceride (MCT)[4]. Food and water were supplied *ad libitum*. Food consumption and body weights were determined at weekly intervals thoughout a 21-day experimental period.

The composition of the control diet was as follows (g/100 g of diet): soybean meal (50% protein), 36.0; DL-methionine, 0.25; cornstarch, 57.35; stabilized tallow, 1.0; mineral mix, 4.0 (12); vitamin mix, 1.0 (12); antibiotic premix, 0.40 (12). Fat was added to the diet at the expense of cornstarch, with sand being used as a nonnutritive diluent to make the diets isocaloric.

Biopsy adipose tissue samples (approx 2 g) were obtained on day 22 of the experiment as previously described (12). Duplicate adipose tissue slices were prepared using a Stadie-Riggs hand microtome. Incubation conditions, extraction, and $^{14}CO_2$ collection procedures, as well as procedures used to prepare homogenates for enzyme assays, have been previously described (12).

Malic enzyme (EC 1.1.1.40) was assayed by the method of Ochoa (13) and citrate cleavage enzyme (EC 4.1.3.8) according to the method of Cottam and Srere (14). The

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[4] The MCT used in these studies was generously supplied by Dr. Herbert P. Sarett of the Mead Johnson Research Center, Evansville, Indiana 47721.

TABLE I. Influence of Source of Dietary Fat on Performance and Plasma Constituents in Young Pigs.[a]

Criteria	Control[c]	Source of dietary fat[b]				
		Tallow	Corn oil	Lard	Coconut oil	MCT
Daily gain (kg)	0.536 ± 0.04	0.568 ± 0.01	0.568 ± 0.02	0.550 ± 0.04	0.573 ± 0.03	0.486 ± 0.02
Gain/feed	0.500	0.561	0.563	0.571	0.607	0.531
Plasma free fatty acids (μEq/liter)	95 ± 5	189 ± 14	207 ± 13	197 ± 12	232 ± 14	148 ± 23
Plasma cholesterol (mg/100 ml)	85 ± 5	100 ± 8	100 ± 6	101 ± 8	112 ± 5	91 ± 6

[a] Each value represents mean ± SEM of 6 pigs with initial weight of 11.0 kg.
[b] Each diet contained 10% fat.
[c] Control diet contained 1% tallow.

protein content of the tissue homogenates used for enzyme assays was determined by the procedure of Lowry *et al.* (15).

Blood was obtained from the vena cava using a 10-ml heparinized syringe on day 19 of the experiment. Plasma was collected after centrifugation and was frozen at —20° until analyzed. Plasma free fatty acids were determined by the method of Ko and Royer (16). Total plasma cholesterol was determined by a modification (17) of the method of Searcy and Bergquist (18). Plasma glucose was determined with a commercial glucose oxidase reagent. Plasma β-hydroxybutyrate and acetoacetate levels were determined by the method of Williamson *et al.* (19). The data were analyzed statistically by means of analysis of variance.

Results. The influence of source of dietary fat on weight gain and plasma constitutents is presented in Table I. Feeding 10% fat as corn oil, lard, tallow, or coconut oil increased rate and efficiency of gain, although this increase was not statistically significant. The pigs fed MCT gained at the same rate and had a similar feed efficiency as the pigs consuming the control diet. Plasma cholesterol levels were significantly ($p < .05$) increased by the ingestion of 10% dietary fat as corn oil, lard, tallow, or coconut oil. There was no significant difference in plasma cholesterol between the pigs consuming the control, low-fat diet and those consuming the MCT diet. Pigs consuming coconut oil had slightly higher plasma cholesterol values than animals consuming tallow, corn oil, or lard; and pigs fed corn oil, lard, tallow, or coconut oil had significantly higher ($p < .01$) plasma free fatty acid levels than the animals consuming the control or the MCT-supplemented diets. But the group receiving MCT had a significantly ($p < .05$) higher level of plasma free fatty acids than the group fed the control diet.

The effect of source of dietary fat on lipogenesis and enzymatic activity is shown in Table II. The ingestion of diets containing tallow, lard, corn oil, or coconut oil resulted in approximately a 50% depression in the capacity for fatty acid synthesis in adipose tissue as measured by the incorporation of glucose-U-^{14}C into fatty acids. The ingestion of MCT resulted in a significantly ($p < .05$) lower rate of fatty acid synthesis than that observed in adipose tissue from the pigs fed the control diet, but MCT was less inhibitory than the other sources of fat. Similar effects were noted for the oxidation of glucose-U-^{14}C to $^{14}CO_2$

The activity of malic enzyme was significantly ($p < .01$) reduced by all fat sources except MCT. Similarly, the activity of citrate cleavage enzyme was reduced as a result of adding 10% fat from all fat sources except

TABLE II. Effect of Source of Dietary Fat on Lipogenesis and Enzymatic Activity in Pig Adipose Tissue.[a]

Criteria	Control[c]	Source of dietary fat[b]				
		Tallow	Corn oil	Lard	Coconut oil	MCT
Fatty acid synthesis[d]	659 ± 46	312 ± 30	348 ± 53	419 ± 44	317 ± 39	526 ± 28
$^{14}CO_2$ production[d]	466 ± 35	271 ± 21	294 ± 24	354 ± 29	289 ± 35	418 ± 10
Malic enzyme[e]	113 ± 7	77 ± 7	71 ± 7	80 ± 5	70 ± 5	125 ± 10
Citrate cleavage enzyme[e]	44 ± 4	30 ± 3	35 ± 4	40 ± 2	33 ± 4	45 ± 4

[a] Each value represents mean ± SEM of 6 pigs with initial weight of 11.0 kg.

[b] Each diet contained 10% fat.

[c] Control diet contained 1% tallow.

[d] Results expressed as nanomoles of glucose-U-^{14}C converted to product indicated per 100 mg of tissue/2 hr.

[e] Activity expressed as nanomoles of substrates converted per minute per milligram of protein at 25°.

MCT. The activities of both enzymes were similar in adipose tissue of MCT-fed pigs and of those consuming the low-fat control diet.

In Table III, data are presented on the effect of long-chain or medium-chain triglyceride ingestion on plasma glucose and ketone body levels. The ingestion of 10% MCT resulted in a slight decrease in plasma glucose but increased plasma ketone levels 5-fold. There was no difference in plasma glucose or ketone body levels between animals fed 10% corn oil and those consuming the low-fat control diet.

Discussion. The present study was conducted to determine the metabolic consequences of feeding MCT to young pigs. The results obtained with the pig are in general agreement with those previously reported for humans and rats (20).

Plasma cholesterol levels were increased by feeding 10% dietary corn oil, lard, tallow, or coconut oil compared with the pigs fed the low-fat control diet. Pigs ingesting MCT-supplemented diets and plasma cholesterol levels similar to those of pigs consuming the low-fat diet. MCT, when substituted in diets for glycerides containing fatty acids of longer-chain length has been shown to depress serum cholesterol levels in the rat (1–5), dog (6), rabbit (7), and in man (8, 9). Beveridge *et al.* (8) reported that serum cholesterol values in human subjects were the same with MCT-supplemented as with the fat-free diets, rose slightly during coconut oil ingestion, and markedly increased with the ingestion of butter.

TABLE III. Effect of Ingestion of Long- and Medium-Chain Triglycerides on Plasma Glucose and Ketone Levels.[a]

Criteria	Control[c]	Source of dietary fat[b]	
		Corn oil	MCT
β-Hydroxybutyrate[d]	0.317 ± 0.33	1.443 ± 0.74	7.679 ± 1.36
Acetoacetate[d]	2.058 ± 0.27	1.204 ± 0.09	4.901 ± 0.44
Total[d]	2.375 ± 0.12	2.647 ± 0.74	12.580 ± 1.70
Glucose (mg/100 ml)	113 ± 5	117 ± 4	102 ± 4

[a] Each value represents mean ± SEM of 3 pigs after 19 days on the diet.

[b] Each diet contained 10% fat.

[c] Control diet contained 1% tallow.

[d] Micrograms per milliliter.

The effects of saturated and unsaturated fats on plasma cholesterol and cholesterogenesis have been reported by several groups who have used as subjects both experimental animals and man. When consumed by the pig, fats containing long-chain triglycerides usually result in increased plasma cholesterol levels, saturated fats producing higher plasma cholesterol levels than unsaturated fats (21–23). The data reported herein are in agreement with this previous work since the feeding of coconut oil resulted in higher plasma cholesterol levels than observed with the highly unsaturated fat (corn oil).

It has been well established that dietary fat depresses fatty acid synthesis in the rat (24–29), pig (12, 30–33), and chicken (34–36). However, the effect of source of dietary fat on the capacity for fatty acid synthesis has not been extensively studied. In a previous report (33), we demonstrated that corn oil, lard, coconut oil, and tallow were equally effective in suppressing fatty acid synthesis in pig adipose tissue. The present data confirm our previous finding and illustrate that MCT is less effective in depressing fatty acid synthesis in pig adipose tissue than are the other sources of fat tested. Similar results have been reported in the rat. Kritchevsky and Tepper (4) found that MCT ingestion results in an increased rate of hepatic lipogenesis compared with animals fed corn oil or coconut oil, and the rate of fat synthesis in rats consuming MCT was similar to that of animals consuming a low-fat diet. Similarly, Leveille *et al.* (5) have shown that MCT-fed rats convert acetate to fatty acids at a much faster rate than rats fed corn oil. In the chick, hepatic lipogenesis is higher when MCT is fed than when corn oil is fed [Leveille *et al.* (11)].

The activity of malic enzyme and citrate cleavage enzyme in pig adipose tissue is known to be related to lipogenic capacity (12). The role of these enzymes in fatty acid synthesis has been discussed (37, 38). Pigs consuming 10% of their diet as corn oil, lard, tallow, or coconut oil had a lower activity of malic enzyme and citrate cleavage enzyme in their adipose tissue than did pigs consuming the MCT or low-fat diets. Relative to the control (low-fat), the ingestion of MCT neither elevated nor depressed the activity of citrate cleavage and malic enzyme. The enzymatic data support the *in vitro* lipogenesis data in that MCT is less effective in inhibiting fatty acid synthesis than the other sources of fat employed.

In attempting to explain the mechanisms by which dietary fat depresses fatty acid synthesis, one must consider the inverse relationship between fatty acid synthesis and plasma free fatty acid levels. Pigs consuming 10% of the diet as corn oil, tallow, coconut oil, or lard had a decreased rate of fatty acid synthesis and an increased level of plasma free fatty acids compared with the pigs consuming MCT or low-fat diets. Free fatty acids or their CoA derivatives have been reported (39–41) to inhibit acetyl CoA carboxylase, which may be the rate-limiting enzyme for cytoplasmic fatty acid synthesis (42). More recent data (43, 44), however, argue against ascribing a regulatory role to long-chain acyl derivatives on the basis of their inhibitory effect on a wide range of enzyme systems.

In attempting to explain why MCT feeding does not depress fatty acid synthesis to the same degree as other fat sources, the following hypothesis has been proposed by this laboratory (45) to explain the metabolic consequences of MCT ingestion: (i) MCT fatty acids are absorbed into the portal vein and transported to the liver; (ii) in the liver, MCT fatty acids are rapidly oxidized to CO_2, acetate and ketones or they may be elongated to long-chain fatty acids; (iii) increased blood ketone levels stimulate insulin release from the pancreatic β-cells; (iv) ketone bodies are preferentially utilized as an oxidative fuel by muscle, thereby sparing glucose; and (v) glucose is therefore utilized by adipose tissue as a substrate for fatty acid synthesis.

It has been adequately demonstrated (46–48) that after intestinal absorption, fatty acid molecules with chain lengths of 10 carbons or less are transported primarily in the portal blood, whereas fatty acids with chain length of greater than 16 carbons are transported predominantly in the lymph. More

recent studies have confirmed that medium-chain fatty acids are transported primarily as fatty acid in the portal blood (20). Data have been presented (20) suggesting that most of the medium-chain fatty acids are converted by the liver to CO_2, ketones, and acetate.

Studies with humans and dogs (20) have demonstrated that a MCT preparation containing C_8 to C_{12} fatty acids when given orally or infused into the liver will increase plasma ketone levels. When a similar quantity of long-chain triglycerides was given, however, the increase in ketones was negligible. In the present study, pigs fed diets containing 10% MCT exhibited a 5-fold increase in ketones compared with pigs fed the low-fat or 10% corn oil diet.

MCT ingestion in man has been reported by Pi-Sunyer (49) to result in a slight decrease in serum glucose and an increase in circulating insulin levels, perhaps because MCT stimulates the islets of the β-cells [Horino *et al.* (50)]. Pigs fed MCT in the present study tended to have lower plasma glucose levels than those fed low-fat or corn oil diets, which may be attributed to increased release of insulin as a result of high ketone levels.

Randle *et al.* (51) have demonstrated that ketones are used preferentially over glucose as an oxidative fuel by muscle. Therefore, since MCT ingestion resulted in a 5-fold increase in ketones in our pigs, these ketones were probably used preferentially as an oxidative fuel, thus, in effect, sparing glucose for fatty acid biosynthesis.

Summary. Lipid metabolism was studied in pigs fed diets containing corn oil, lard, tallow, coconut oil, or medium-chain triglyceride (MCT). The ingestion of diets containing tallow, lard, corn oil, or coconut oil resulted in approximately a 50% depression in the capacity for fatty acid synthesis in adipose tissue as measured by the incorporation of glucose-U-^{14}C into fatty acids. The ingestion of MCT resulted in a significantly lower rate of fatty acid synthesis than observed in adipose tissue from the pigs fed the control diet, but MCT was less inhibitory than the other sources of fat. The activities of malic enzyme and citrate cleavage enzyme were significantly reduced by all fat sources except MCT. Plasma cholesterol levels were increased by feeding 10% dietary corn oil, tallow, lard, or coconut oil as compared with the pigs fed low-fat control diets. Pigs ingesting MCT-supplemented diets had plasma cholesterol levels similar to those of pigs consuming the low-fat diet. The ingestion of 10% MCT resulted in a 5-fold increase in plasma ketone levels. A hypothesis is discussed to explain the metabolic consequences of MCT ingestion in the pig.

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