# Influence of the Source of Dietary Fat on Some Metabolic Responses of Meal-Fed Rats (35360)

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Tepperman *et al.* (1) described a greatly increased ability of rats to convert carbohydrate to fat when the animals were meal-fed. Leveille (2) found that high-fat diets markedly depressed this lipogenic response to meal feeding in rat adipose tissue. However, Kritchevsky and Tepper (3) and Leveille *et al.* (4) found that the ingestion of a high-fat diet containing medium-chain triglycerides (MCT), triglycerides of 8- and 10-carbon fatty acids, enhanced lipogenesis in the rat. The portal absorption of medium-chain fatty acids and their rapid hepatic oxidation apparently give MCT some metabolic properties similar to those of carbohydrate. Accordingly, feeding an MCT diet reduced plasma lipid levels in patients with dietary fat-induced hyperlipemia (5), similar to the effect of feeding a high-carbohydrate diet (6)

If MCT differs markedly from LCT in its effect on lipogenesis in the meal-fed rat, the levels of blood glucose, nonesterified fatty acids, triglycerides, and ketones in the blood of meal-fed animals fed MCT or LCT diets also should differ markedly. The present study investigated the effect of MCT and LCT on the blood levels of these metabolites in meal-fed rats.

*Methods and Materials.* Male Wistar rats,[1] weighing 140 to 160 g, were divided randomly into two groups fed either an MCT or an LCT diet. The diets (Table I) supplied about 40% of calories as fat and about 16% of calories as protein. The animals were accustomed to a two-meal-a-day schedule. All rats were housed in individual galvanized screen-bottomed cages in a constant-temperature animal room (25°). Water was available *ad libitum*. The meal periods were from 8 to 9 a.m. and from 4 to 5 p.m.

TABLE I. Composition of the Diets.

| Ingredient | Diet (%) | |
|---|---|---|
| | MCT | LCT |
| Casein | 20.2 | 20.2 |
| Sucrose | 51.3 | 51.3 |
| MCT | 21.1 | — |
| Oleo Oil | — | 10.4 |
| Partially hydrogenated soybean oil | — | 10.4 |
| Safflower oil | 1.0 | 1.3 |
| Salt mixture[a] | 4.0 | 4.0 |
| Fiber | 2.0 | 2.0 |
| Vitamin mixture[b] | 0.35 | 0.35 |
| α-Tocopherol, 33% | 0.015 | 0.015 |
| Oleum percomorphum | 0.015 | 0.015 |

[a] Jones and Foster (15) with 10 ppm F added as NaF.

[b] Sarett and Snipper (16) ascorbic acid omitted.

After 2 weeks of the dietary regime, the animals were treated in the following manner. Five rats from each group were sacrificed at 8 a.m., just before the morning meal hour, five at 9 a.m. and five each at 10 and 11 a.m. and 2 p.m. These rats received pentobarbital (60 mg/kg) intraperitoneally 15 min before they were sacrificed by desanguination (7). Blood was drawn from the heart in heparinized syringes and placed in cold, heparinized centrifuge tubes. A 1-ml sample of blood was immediately removed for ketoacid determination; the remaining blood was centrifuged and the plasma was removed for fatty acid and triglyceride determination.

Two subgroups of 8 rats fed the MCT diet

[1] Obtained from Harlan Industries, Indianapolis, Indiana.

TABLE II. Glucose Tolerance of Rats Bled Once and of Rats Bled Repeatedly from the Orbital Plexus.

| After glucose dosage[a] (hr) | Blood glucose (mg/100 ml) | |
|---|---|---|
| | Single bleeding | Repeated bleeding |
| 0 | 65 ± 5 (4)[b] | |
| 0.5 | 116 ± 13 (4) | 127 ± 15 (4) |
| 1 | 105 ± 13 (5) | 114 ± 19 (4) |
| 2 | 75 ± 6 (5) | 82 ± 10 (4) |
| 3 | 67 ± 11 (5) | 74 ± 9 (4) |

[a] One g of glucose/kg of body weight as a 50% solution.

[b] Means given with standard deviation; number of animals is given in parentheses.

and 6 rats fed the LCT diet were selected for blood glucose determination. At each of the sampling times, blood was drawn from the orbital plexus of each rat and analyzed for glucose. Glucose was measured by a glucose oxidase method.[2]

Justification for taking replicate blood samples at hourly intervals from the same rats is provided by the data shown in Table II. There was little difference between the oral glucose tolerance of 4 rats bled repeatedly, 5 times in 4 hours, and that of rats bled only once each.

Plasma nonesterified fatty acid (NEFA) levels were measured by a modification of the colorimetric adaptation by Mosinger (8) of the method of Dole and Meinertz (9). Plasma triglycerides were estimated by the method of Jagannathan (10). Palmitic acid and tripalmitin[3] served as respective standards.

Concentrations of acetoacetate and β-hydroxybutrate in protein-free filtrates of blood were determined using a salicylaldehyde method (11, 12).

*Results.* The most noticeable difference in blood glucose levels between the MCT and LCT groups occurred at 8 a.m,. before the meal (Table III). The fasting blood glucose levels of the MCT-fed rats were only 75% of those of the LCT-fed rats. Although the rats fed the MCT diet showed a greater rise in blood sugar after the meal, the blood sugar levels of the MCT-fed rats were always lower than those of the LCT-fed rats. The blood glucose values within each group at 11 a.m. and 2 p.m. were similar, suggesting that a steady state had been at least temporarily attained.

The fasting NEFA levels of the MCT-fed rats were significantly higher than those of the LCT-fed rats (Table III). After the meal, no significant differences were noted.

Plasma triglyceride levels for both the LCT-fed and MCT-fed rats were similar before the meal (8 a.m.) and shortly after it (9 and 10 a.m.) (Table III). However, the plasma triglyceride level of the MCT-fed rats decreased thereafter, whereas that of the LCT-fed rats remained elevated. These observations are probably related to the more rapid and complete digestion of MCT as well as its portal transport primarily as free fatty acids (13). LCT, on the other hand, is released into the general circulation more slowly and is transported principally through the lymphatic system as triglycerides.

In both groups the blood concentrations of β-hydroxybutyrate were elevated in the fasting state and decreased following the meal (Table III). The blood acetoacetate concentration of fasted MCT-fed rats was significantly higher than that of fasted LCT-fed rats and fell precipitously after the MCT meal (Table III). Only a slight fall in the acetoacetate concentration was observed after the meal in the LCT rats.

The total ketone level (β-hydroxybutyrate + acetoacetate) was higher in the fasting MCT-fed rats, but not significantly so (Table III). Feeding the meal effected a large decline in the blood total ketone levels in the rats of both groups. Large variation within both groups at most time periods may have masked real differences. By 2 p.m., the level of each of these three blood constituents was practically identical for the MCT-fed and LCT-fed rats.

*Discussion.* Muiruri and Leveille (14) found that rats ingesting two 1-hr meals/day consumed as much as control nibbling animals fed *ad libitum*, but gained more weight and exhibited the enhanced rate of lipo-

[2] Glucostat, Worthington Biochemical Corp., Freehold, New Jersey.

[3] Obtained from Hormel Institute, Austin, Minnesota.

TABLE III. Metabolite Levels in the Blood of Meal-Fed Rats Fed MCT or LCT Diets.[a]

| Metabolite | Dietary fat | a.m. 8 | a.m. 9 | a.m. 10 | a.m. 11 | 2 p.m. |
|---|---|---|---|---|---|---|
| Blood glucose (mg/100 ml) | MCT | 56 ± 6[f] | 83 ± 10 | 81 ± 9 | 89 ± 12 | 91 ± 11 |
| | LCT | 75 ± 10 | 86 ± 8 | 90 ± 13 | 104 ± 14 | 104 ± 15 |
| | *p* value[b] | <0.005 | ns | ns | ns | <0.05 |
| Plasma NEFA[c] (meq/liter) | MCT | 1.33 ± 0.12 | 0.83 ± 0.18 | 0.60 ± 0.20 | 0.74 ± 0.26 | 0.69 ± 0.22 |
| | LCT | 0.79 ± 0.23 | 1.05 ± 0.34 | 0.79 ± 0.20 | 0.87 ± 0.46 | 0.64 ± 0.19 |
| | *p* value | <0.005 | ns | ns | ns | ns |
| Plasma triglycerides (mg/100 ml) | MCT | 62 ± 42 | 42 ± 23 | 133 ± 39 | 62 ± 28 | 45 ± 13 |
| | LCT | 52 ± 35 | 46 ± 36 | 113 ± 35 | 89 ± 25 | 103 ± 37 |
| | *p* value | ns | ns | ns | ns | <0.01 |
| Blood ketoacids | | | | | | |
| β-HBA[d] (μmoles /ml) | MCT | 0.65 ± 0.43 | 0.34 ± 0.24 | 0.21 ± 0.09 | 0.20 ± 0.09 | 0.14 ± 0.13 |
| | LCT | 0.42 ± 0.25 | 0.11 ± 0.04 | 0.13 ± 0.03 | 0.13 ± 0.02 | 0.14 ± 0.12 |
| | *p* value | ns | ns | ns | ns | ns |
| AA[e] (μmoles/ml) | MCT | 0.29 ± 0.14 | 0.14 ± 0.08 | 0.07 ± 0.03 | 0.08 ± 0.02 | 0.07 ± 0.02 |
| | LCT | 0.08 ± 0.04 | 0.05 ± 0.02 | 0.04 ± 0.03 | 0.03 ± 0.02 | 0.06 ± 0.02 |
| | *p* value | <0.025 | ns | ns | <0.005 | ns |
| Total (μmoles/ ml) | MCT | 0.94 ± 0.55 | 0.49 ± 0.31 | 0.28 ± 0.12 | 0.29 ± 0.08 | 0.21 ± 0.16 |
| | LCT | 0.50 ± 0.28 | 0.17 ± 0.04 | 0.17 ± 0.03 | 0.16 ± 0.03 | 0.20 ± 0.12 |
| | *p* value | ns | ns | ns | <0.025 | ns |

[a] Meals were fed from 8 to 9 a.m. and from 4 to 5 p.m. daily.
[b] Statistical analysis by Student's *t* test; ns = not significant.
[c] Plasma nonesterified fatty acids.
[d] β-Hydroxybutyric acid.
[e] Acetoacetic acid.
[f] Mean given with standard deviation.

genesis typical of animals meal-fed a low-fat diet. Thus, the feeding regimen used here is valid.

The significance of the difference between the effects of MCT and LCT diets on lipogenesis in meal-fed rats (3, 4) extends to the effects of MCT and LCT on the indicators of intermediary metabolism studied here. The higher ketoacid and NEFA levels and lower glucose levels of the fasted MCT-fed rats (Table III) suggest that before the meal the MCT-fed rats were in the postabsorptive state longer than the LCT-fed rats and that fatty acid oxidation provided a greater proportion of the fasting energy requirements of the MCT-fed rats than of the LCT-fed rats. Moreover, plasma triglyceride levels indicate more rapid clearing of triglyceride after an MCT meal and a faster return to the postabsorptive state.

The MCT-fed rats showed a rapid decline in NEFA levels and a greater relative and absolute rise in blood glucose levels over the meal hour. These results suggest a sharper transition from a fasting to fed energy economy in the MCT-fed animals.

*Summary.* Male rats were accustomed to a two-meal-a-day feeding pattern of diets containing either medium-chain triglycerides (MCT) or long-chain triglycerides (LCT). Fat comprised 40% of calories in these diets. Before the morning meal, rats fed the MCT diet had higher blood ketone and plasma nonesterified fatty acid levels and lower blood glucose and plasma triglyceride levels than the LCT-fed rats, suggesting that the MCT-fed rats may have been in the postabsorptive state for a longer time.

After the rats received the morning feeding, the MCT-fed rats showed a rapid decline

in plasma NEFA levels and a greater rise in blood glucose levels than LCT-fed rats. The high fasting blood ketone levels decreased in both groups, but the MCT-fed rats maintained a higher level than the LCT-fed rats even after the meals.

Postprandial hypertriglyceridemia was observed in both groups. However, in the MCT-fed rats the triglyceride level returned to the fasting level by 2 hr after the meal; whereas the hypertriglyceridemia was sustained for at least 5 hr after the meal in the LCT-fed rats. This finding probably relates to the more rapid and complete digestion of MCT compared to LCT.

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