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## Medium chain triglyceride feeding to normal and diabetic rats.

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MEDIUM CHAIN TRIGLYCERIDE FEEDING  
TO NORMAL AND DIABETIC RATS

A Thesis Presented

By

NEILE KAY EDENS

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

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Department of Psychology

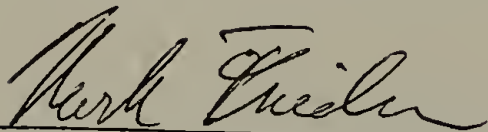
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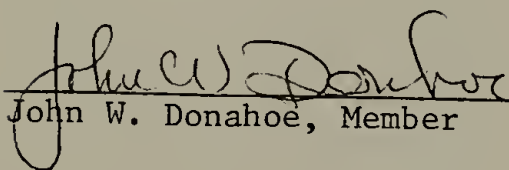
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# ABSTRACT

## Medium Chain Triglyceride Feeding to Normal and Diabetic Rats

February 1982

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Medium chain triglyceride (MCT) feeding to normal male rats has been shown to cause marked alterations in circulating levels of metabolic fuels. Despite this, MCT feeding has not been shown to reliably alter food intake in normal rats. It seemed possible that MCT feeding might affect food intake in diabetic rats, because diabetics have been shown to respond selectively to alterations of the fat content of their diet.

To investigate this possibility, normal and diabetic rats were fed purified diets containing either corn oil (a long chain triglyceride (LCT)) or MCT as the fat component of the diet. After food intake had stabilized, blood was sampled for assay of plasma metabolites, and the concentration of fat in the diet was increased. This procedure was repeated after food intake had stabilized on the higher fat diet.

It was found that diabetic rats fed MCT were able to adjust their caloric intake to the appropriate level more quickly than those fed LCT. Normal rats showed a similar pattern of response, but only at the higher concentrations of dietary fat. The alterations of plasma metabolites caused by MCT feeding were consistent with previous reports in the literature.

Because of the possibility that different concentrations of LCT and MCT diet might differ in palatability as well as metabolic consequences, preference tests between low and high concentration LCT diet or low and high concentration MCT diet were carried out in normal and diabetic rats. The results indicated that both normal and diabetic rats prefer high concentration LCT diet more than they prefer high concentration MCT diet.

Therefore, the more rapid caloric regulation of MCT-fed rats may be explained by their decreased preference for higher concentrations of dietary fat. On the other hand, it seems possible that the metabolic consequences of MCT ingestion may result in rapid feedback about the caloric density of the diet. This can only be directly investigated by using LCT and MCT diets equated for palatability.

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## C H A P T E R I

### GENERAL INTRODUCTION

One approach to the study of food intake involves altering the composition of the diet fed to experimental animals, and correlating subsequent changes in food intake with the metabolic consequences of ingestion of the diet. Several attempts have been made to alter food intake by replacing standard dietary fat, which consists mostly of long chain triglycerides (LCT), with medium chain triglycerides (MCT) in purified diets fed to rats. MCT consist of triacylglycerols that primarily contain acyl groups six to twelve carbons long. They are not found in abundance in ordinary sources of dietary fat, but may be purified from coconut oil.

Because of the relatively short chain length of the acyl groups in MCT, their absorption from the intestine and subsequent metabolism differ in several ways from that of a typical LCT such as corn oil, which primarily contains acyl groups of sixteen or eighteen carbons. MCT, like LCT, are hydrolyzed in the lumen of the intestine, but their hydrolysis occurs more rapidly and more completely, resulting in more rapid absorption from the intestine (Greenberger and Skillman, 1969). The fatty acids and monoglycerides released by hydrolysis of LCT are absorbed by the mucosal cells of the intestine where they are re-esterified to triglycerides. These triglycerides are combined with proteins to form chylomicrons which

enter the lymphatic system and subsequently, the general circulation (Newsholme and Start, 1979). MCT, on the other hand, are completely hydrolyzed to fatty acids and glycerol within the intestinal lumen. These fatty acids are absorbed by the mucosal cells, but they have a low affinity for the esterifying enzymes (Newsholme and Start, 1979), so the fatty acids released by hydrolysis of MCT are transported directly to the liver via the hepatic portal vein as salts or complexed with albumin (Scheig and Klatskin, 1968).

Once LCT enter the circulation, a small proportion may be taken up by the liver (Robinson, 1970). The rest may be hydrolyzed at and absorbed by the adipose tissue, or if not taken up by the adipose tissue, eventually reach the liver. The fact that a given quantity of LCT is partially cleared from the circulation by adipose tissue before it reaches the liver, whereas the same quantity of MCT goes directly and entirely to the liver, in part explains one of the most important metabolic consequences of MCT ingestion, the induction of ketosis.

Ketone body ( $\beta$ -hydroxybutyrate and acetoacetate) production by the liver is controlled by several factors, one of which is the supply of substrate (fatty acids) to liver (McGarry and Foster, 1980). Thus, MCT, partially by virtue of their complete hydrolysis to fatty acids which are delivered directly to the liver, are ketogenic. In addition, the cytoplasmic enzymes in the liver which esterify glycerol with acyl groups have a very low affinity for medium chain acyl groups, so that medium chain fatty acids tend not

to be channelled into triglyceride synthesis, and are available for transport into the mitochondria and subsequent oxidation to ketone bodies.

Finally, the rate of ketone body formation may be controlled by limiting the entrance of fatty acids into the mitochondria where ketogenesis occurs. Before fatty acids can undergo reactions in the cell, they must be activated by linkage to coenzyme A to form fatty acyl CoA. Because the mitochondria membrane is impermeable to fatty acyl CoA, it must be converted to fatty acyl carnitine, a reaction catalysed by carnitine acyl transferase I. Fatty acyl carnitine may then pass through the mitochondrial membrane into the mitochondrial matrix, where it is reconverted to fatty acyl CoA by carnitine acyl transferase II. Fatty acyl CoA is then degraded via  $\beta$ -oxidation into molecules of acetyl CoA, which are the substrate for ketone body formation. The conversion of fatty acyl CoA to fatty acyl carnitine is rate limiting for the entrance of long chain fatty acids into the mitochondria, however medium chain fatty acids may be activated in the mitochondrial matrix, and thus their oxidation is independent of control of carnitine acyl transferase (Bremer et al., 1974).

Because of these properties of MCT -- rapid hydrolysis in the intestine and transport to the liver, low affinity for intestinal and hepatic esterifying enzymes, and independence of carnitine acyl transferase in the hepatocyte -- the metabolic consequences of MCT ingestion are very different from LCT ingestion. A number of

studies have compared the effects on blood levels of metabolites of long term feeding of MCT or LCT (corn oil) to male rats.

Leveille et al. (1967), found decreased plasma lipids and cholesterol in rats fed a diet consisting of 2% corn oil and 12% MCT compared to rats eating a 14% LCT diet for three weeks. Wiley and Leveille (1973) found increased plasma  $\beta$ -hydroxybutyrate and decreased plasma triglycerides in male rats eating similar diets. Interestingly, both Harkins and Sarett (1968) and Bray et al. (1980), although they replicated the results mentioned above in male rats, report that MCT feeding increases, rather than decreases, plasma triglycerides and cholesterol levels in female rats.

In a short term study, Yeh and Zee (1976) found that intubating male rats with 10 ml/kg MCT produced dramatically increased plasma ketone bodies within one half hour. Plasma ketones were elevated to a lesser degree and with a greater delay (2 hours) by LCT intubation. Plasma free fatty acids were increased by both MCT and LCT intubation, although larger increases resulted from LCT intubation. MCT intubation also resulted in an increase in plasma immunoreactive insulin (IRI) by one half hour after intubation, and a concomitant decrease in blood glucose, both of which normalized by four hours after the intubation. The effect of long term MCT ingestion on plasma or serum IRI levels is controversial, however. Both increases (Wiley and Leveille, 1973; Bray et al., 1980) and no difference (Lavau and Hashim, 1978) have been reported.

In view of the different metabolic consequences of MCT and LCT



ingestion, their effects on food intake and body weight regulation are of considerable interest and have been extensively studied. In general, no reliable differences in consumption of MCT and LCT diets have been found. For instance, in an early paper, Kaunitz (1958) reported that rats fed MCT for several weeks did not eat less than lard-fed rats, although they gained significantly less weight. In addition, Kaunitz reported that rats eating a lard diet are able to maintain a constant body weight on a significantly smaller caloric intake than rats eating MCT diet. Other investigators have reported either a decrease (Lavau and Hashim, 1978; Bray et al., 1980) or no difference (Leveille et al., 1967; Harkins and Sarett, 1968; Wiley and Leveille, 1973) in body weight gain in rats after long term ingestion of MCT compared to rats eating corn oil diets.

Considering the evidence that MCT ingestion radically alters the metabolic state of the animal, it is of interest that no consistent effects on food intake and body weight have been found. It would appear that normal animals, within the designs of the experiments cited above, do not respond to the metabolic perturbations caused by MCT feeding by altering their food intake. However, no investigators have reported the short term effect of giving rats MCT diets. It may be that the normal intake of MCT diet occurs only after a period of adaptation to the diet. It would be of interest, therefore, to monitor the daily consumption of MCT and LCT diets immediately after they are presented to the rats. In addition, diabetic animals, because of their inability to utilize the

carbohydrate portion of their diet, rely upon the fat content of their diet and thus may be a more appropriate model for studying the effect of MCT on feeding behavior.

Friedman (1978) has reported that diabetic rats, who are hyperphagic on standard high carbohydrate laboratory chow, will decrease their food intake to near normal levels when the fat content of their diet is increased. In addition, it was found that whereas normal rats will increase their food intake in response to a dilution of either the fat or carbohydrate portion of their diets, diabetics increase their food intake only when the fat content of their diet is decreased. From these data it appears that rats adjust their food intake only in response to changes in the concentration of utilizable components. Thus, replacing standard dietary fat with MCT might be expected to have a larger impact in diabetic than in normal rats.

On the other hand, it should be noted that studies of the control of ketogenesis using the perfused rat liver preparation have shown that livers from diabetic rats do not increase ketone body production when octanoic acid (a medium chain fatty acid) is added to the perfusate (McGarry and Foster, 1971). Diabetes would tend to obscure differences in the ketogenic properties of MCT and LCT because the enzyme that catalyses the rate-limiting step in ketogenesis, carnitine acyl transferase I, is released from inhibition by the absence of insulin. Nevertheless, the fact that MCT are absorbed from the intestine as fatty acids and are delivered

directly to the liver make it possible that MCT feeding to chronic diabetic rats would result in alterations in metabolism which could influence food intake.

To investigate this possibility, experiments were carried out which involved feeding diabetic and normal rats diets containing MCT or LCT in ascending concentrations. Plasma levels of metabolites were measured after adaptation to each concentration of fat in the diet. In addition, because differences in the consumption of MCT and LCT may be ascribed to differences in preference for one or the other rather than their metabolic consequences, experiments were performed which allowed the evaluation of the role of palatability in the effects on food intake observed in the first experiments.



## CHAPTER II

### EXPERIMENTS

#### Experiment Ia

Introduction. The first experiment investigated the effects on food intake and body weight of feeding purified diets containing either MCT or corn oil to normal and diabetic rats. Several concentrations of dietary fat were fed to rats in an ascending order. In order to assess the metabolic impact of MCT feeding, plasma samples were analyzed for glucose, ketone bodies, triglycerides and glycerol after rats had adapted to eating each concentration of fat.

Method. 48 male Sprague-Dawley rats (Charles River) weighing 200-250 grams at the start of the experiment were housed in wire mesh hanging cages in an animal room with a 12:12 light:dark cycle. Rats had free access to Purina laboratory chow and tap water until they were given purified diets as described below. Ten days after arrival in the laboratory, one half of the rats were injected intraperitoneally with 60 mg/kg streptozotocin in citrate buffer. One week after injection, 20  $\mu$ l plasma samples were obtained from the tail of each rat and assayed for glucose content with a Beckman glucose analyzer. Any rats with plasma glucose below the criterion level of 400 mg/dl were re-injected with the same dose of streptozotocin. The re-injected rats had their plasma glucose checked again one week

later, and any rats with plasma glucose still below 400 mg/dl were excluded from the study.

Three weeks after the second injection of streptozotocin, food intake was measured for two consecutive days. On the basis of body weight and the mean food intake for those two days, the normal and diabetic rats were each divided into two matched groups. The rats were then given access to a purified diet containing either 5% corn oil or 3% MCT plus 2% corn oil to supply essential fatty acids. The composition of the diets is presented in Table 1. Food intake was measured daily and body weight was measured weekly.

After the rats had been eating the 5% oil diet for approximately two weeks, blood samples were obtained from the tail of each rat. Because of the large amount of blood needed for the assays, blood samples were taken from each rat two days in a row. Rats were divided into two squads. The first squad had blood taken on the first day one hour after lights on, to be assayed for triglycerides and glycerol, and on the second day three hours after lights on for assay of blood glucose and ketone bodies. The order was reversed for the second squad.

After blood had been collected from all the rats, their diet was changed to contain 15% fat. Food intake was again measured daily. After the rats had been eating the 15% oil diet for approximately two weeks, blood samples were obtained from the tail of each rat as described above. After blood samples were collected the rats were given access to a diet containing 25% fat. Food intake was measured

TABLE 1  
Diet composition

	Fat content				
	5%	10%	15%	25%	30%
Cornstarch	57	54	51	45	42
Casein	24	22	21	19	18
Cellulose	9	9	8	8	7
Minerals	4	4	3	3	3
Vitamins	1	.9	.85	.75	.7
MCT (MCT diet)	3	8	13	23	28
(LCT diet)	0	0	0	0	0
LCT (MCT diet)	2	2	2	2	2
(LCT diet)	5	10	15	25	30

(Values are grams per 100 grams diet)

daily. After the rats had been eating the 25% oil diet for approximately two weeks, blood samples were obtained from the tail of each rat as described above.

The blood that was collected was processed and assayed as follows. Approximately 240  $\mu$ l of blood was collected into a heparinized glass tube, transferred into a chilled microcentrifuge tube, and centrifuged at 12,800  $g$  for one minute. Plasma for triglyceride and glycerol assay was then pipetted into iced glass test tubes and frozen at  $-37^{\circ}\text{C}$  until the day of the assay. Plasma

samples to be used for assay of ketone bodies were deproteinated with barium hydroxide and zinc sulphate at 4°C and centrifuged at 733 g for 10 minutes. The protein-free supernatant was drawn off and stored at -37°C until the day of the assay. Plasma glucose was measured with a Beckman glucose analyzer the same day blood was drawn. Plasma ketones were measured fluorometrically by a modification of the method described by Bates et al. (1968). Plasma samples to be used for the glycerol assay were deproteinated on the day of the assay as described above. Plasma glycerol was measured by the method of Wieland (1957); plasma triglycerides were hydrolyzed to glycerol and free fatty acids by the method of Bucolo (1973) and glycerol measured as above. Triglyceride concentration was estimated by subtraction of free glycerol from total glycerol.

Physiological data were analyzed by means of repeated measures analysis of variance. Differences in body weight gain were evaluated with Student's t-test. The mean change in caloric intake that occurred when the MCT-fed rats were switched to a higher percentage fat diet was compared to the mean change in caloric intake that occurred when LCT rats were switched to a higher percentage fat diet with Student's t-test. A test was considered significant when  $p \leq .05$ .

## Results.

Body weight. Over the course of the experiment there was no difference in body weight gain between the LCT- and MCT-fed normals

(Table 2). On the other hand, the MCT-fed diabetics lost a significant amount of body weight ( $t(14) = 3.14$ ) compared to the LCT-fed diabetics, whose mean body weight remained relatively stable.

---

TABLE 2  
Body Weight Gain in Experiment Ia

	Normals	Diabetics
	(grams)	(grams)
LCT	$82 \pm 10$	$0 \pm 3$
MCT	$71 \pm 10$	$-55 \pm 17$

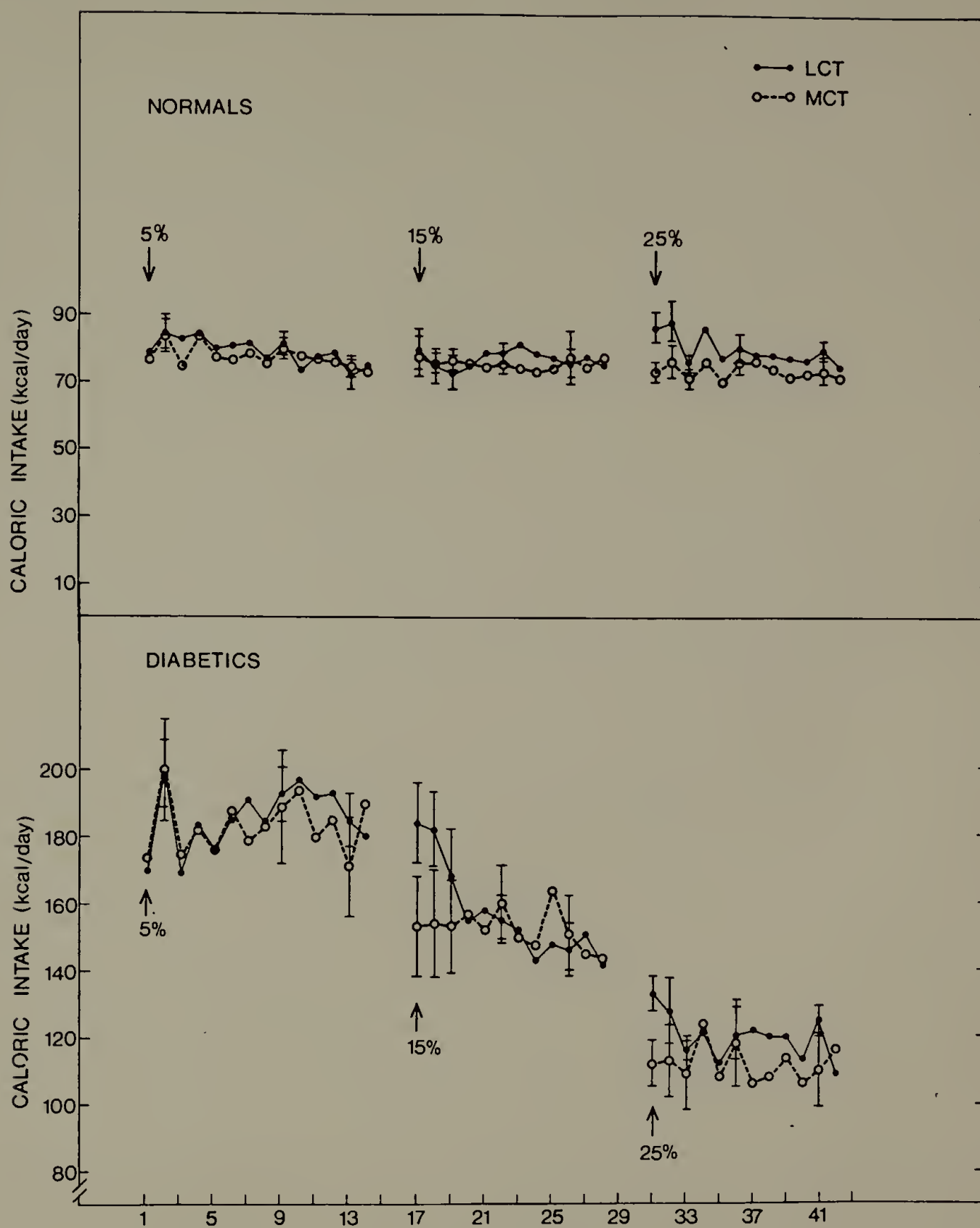
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(Values are mean  $\pm$  s.e.m.)

Food intake. MCT feeding had a more striking effect on the caloric intake of diabetic than normal rats (Fig. 1, bottom). Although MCT feeding had no effect on the mean daily caloric intake of 5% fat diet, when diabetic rats were switched to 15% fat diet, the MCT-fed rats significantly decreased their caloric intake on the first day compared to the LCT-fed diabetics ( $t(14) = 2.86$ ). The caloric intake of the LCT-fed rats gradually declined to the same level, and both groups maintained their mean daily caloric intake at approximately the same level while they were fed the 15% fat diet.

When diabetic rats were switched from 15 to 25% fat diet, a similar pattern of response was evident (Fig. 1, bottom). On the

Figure 1. Caloric intakes of normal and diabetic rats eating diets given in ascending order of concentration of dietary fat. Points shown are mean  $\pm$  s.e.m.





first day after the diets were switched, MCT-fed rats decreased their caloric intake relative to LCT-fed rats. This difference in the magnitude of the decrease is not statistically significant ( $t(14) = 1.88$ ) unless the datum from one rat in the MCT-fed group, which increased, rather than decreased its caloric intake on the first day, is deleted: ( $t(13) = 2.79$ ). Again the LCT-fed diabetics gradually decreased their caloric intake to the same level as the MCT-fed diabetics, and both groups maintained their mean daily caloric intake at approximately the same level for the rest of the time they were fed the 25% fat diet.

In contrast, when normal rats were switched from 5 to 15% fat diet, there was no difference between the LCT-fed and MCT-fed rats in their caloric intake (Fig. 1, top). Both groups maintained their caloric intake at the same (pre-switch) level. When normal rats were switched from 15 to 25% fat diet however, LCT-fed rats significantly increased their caloric intake on the first day relative to MCT-fed rats, who maintained their caloric intake at the pre-switch level ( $t(20) = 2.32$ ). This increase in caloric intake by the LCT-fed rats was transient; by the third day of access to the 25% fat diet the caloric intake of the LCT-fed group was the same as that of the MCT-fed group.

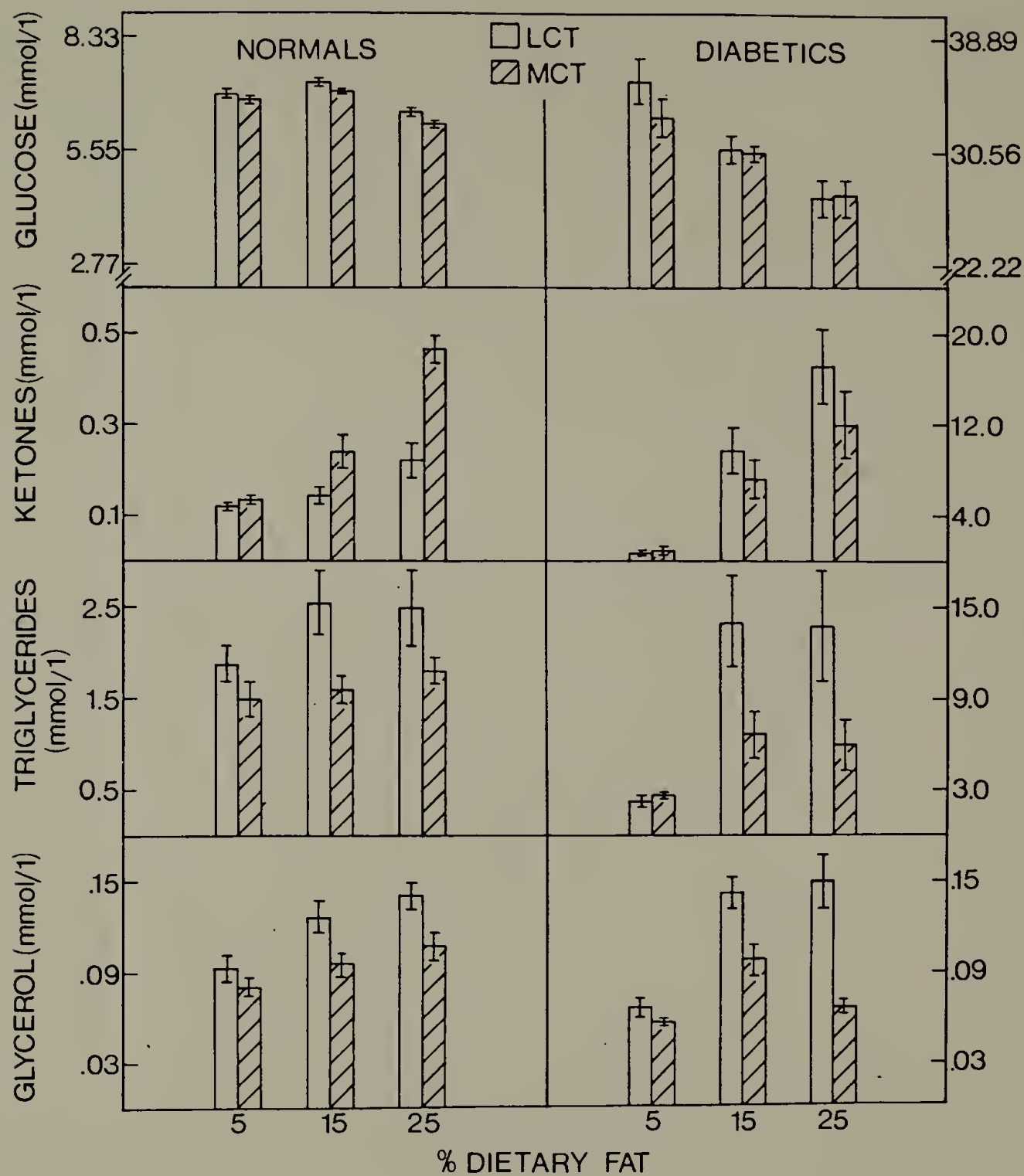
### Physiology.

#### Normals

MCT feeding had no effect on plasma glucose levels (Fig. 2).



Figure 2. Plasma levels of metabolites of normal and diabetic rats eating either LCT or MCT diets given in ascending order of concentration of dietary fat.



Although there was a significant effect of dietary fat content ( $F(2,40) = 5.46$ ), this appeared to be attributable to a slight elevation in plasma glucose when the rats were eating 15% fat diet and a slight decrease when they were eating 25% fat diet, rather than any consistent effect of dietary fat.

Plasma ketone bodies were significantly elevated by both MCT ( $F(1,20) = 30.18$ ) and increasing dietary fat content ( $F(2,40) = 39.74$ ), but they were elevated significantly more by increasing dietary MCT content ( $F(2,40) = 11.02$ ).

Plasma triglyceride concentration was significantly decreased by MCT feeding ( $F(1,20) = 5.51$ ). The elevation of plasma triglycerides seen with increasing dietary fat content was not statistically significant.

Finally, plasma glycerol concentration was significantly decreased by MCT feeding ( $F(1,20) = 7.66$ ) but was elevated by increasing dietary fat content in both the LCT- and MCT-fed rats ( $F(2,40) = 17.62$ ).

#### Diabetics

MCT feeding did not have any effect on plasma glucose levels; however, increasing concentration of dietary fat significantly decreased plasma glucose in a monotonic fashion ( $F(2,28) = 25.13$ ). (Fig. 2).

MCT feeding also had no significant effect on plasma ketone body concentration. Increasing dietary fat significantly elevated

plasma ketones in both LCT- and MCT-fed rats ( $F(2,28) = 34.78$ ).

Although MCT feeding had no significant effect on plasma triglyceride levels, increasing dietary fat content caused a significant elevation ( $F(2,28) = 16.69$ ) and this elevation was significantly larger in LCT-fed than MCT-fed rats ( $F(2,28) = 4.66$ ).

Plasma glycerol concentration was significantly depressed by MCT feeding ( $F(1,14) = 23.46$ ). Although plasma glycerol was elevated by increasing dietary fat content ( $F(2,28) = 23.22$ ), this elevation was significantly larger, and more consistent for LCT-fed rats ( $F(2,28) = 8.01$ ).

### Experiment Ib

Introduction. To determine whether long term adaptation to LCT or MCT can influence consumption of a novel fat, at the end of experiment Ia, all rats were switched from their accustomed diet (MCT or LCT) to a diet containing the alternative oil in the same concentration.

Method. Daily food intake of the 25% diet was measured for three days after the end of experiment Ia. At that time rats formerly fed the MCT diet were given a diet containing 25% corn oil, and vice versa. Food intake was measured daily for six days. Food intake data obtained after the diets were switched was analyzed with repeated measures analysis of variance. An effect was considered significant if  $p \leq .05$ .

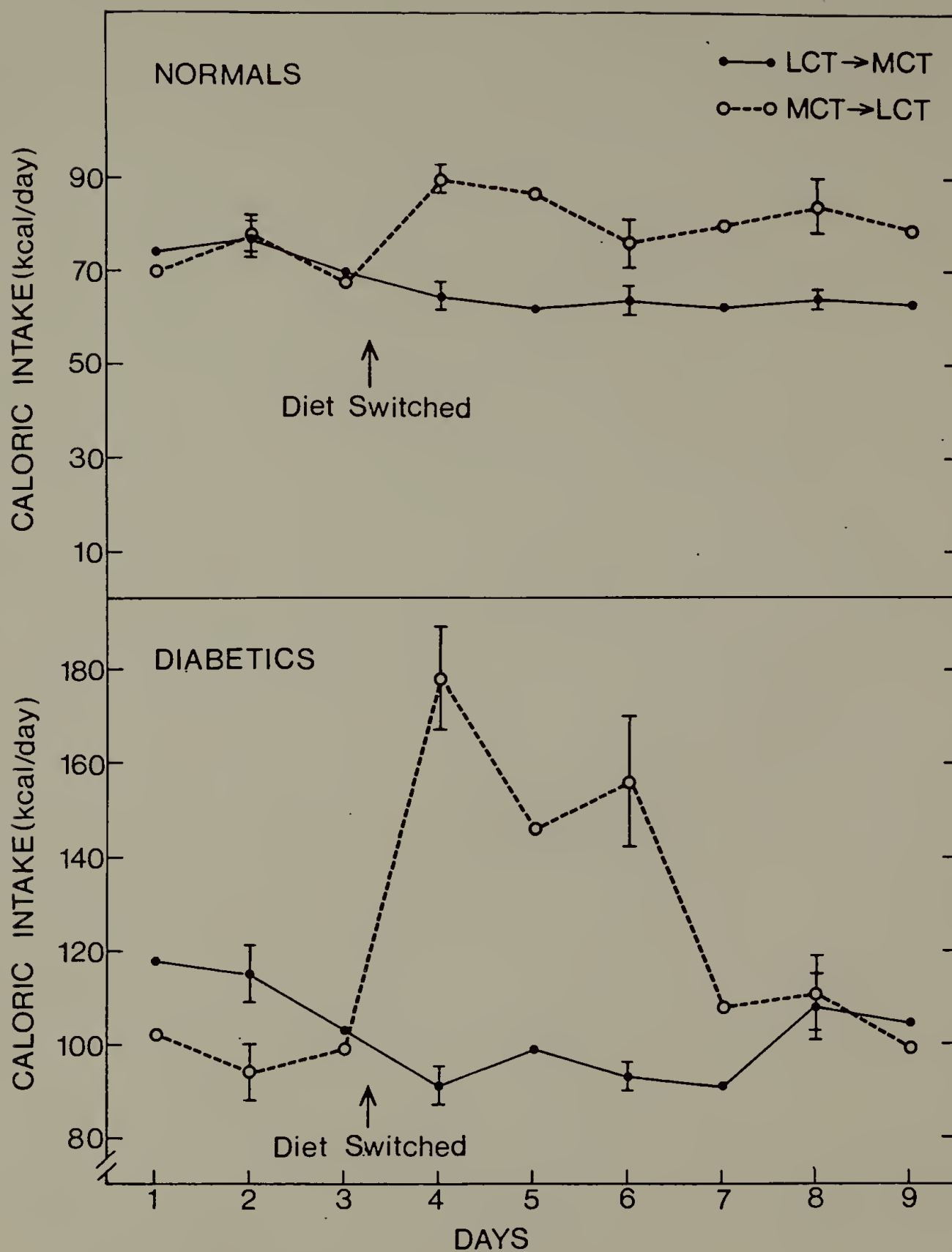
Results. At the end of two weeks of 25% fat diet feeding, when diabetic rats previously fed LCT were switched to MCT ( $L \rightarrow M$ ) and vice versa ( $M \rightarrow L$ ), there was an immediate and dramatic increase in the caloric intake of the  $M \rightarrow L$  rats which persisted for three days and then disappeared (Fig. 3, bottom).  $L \rightarrow M$  diabetics showed, if anything, a slight depression in caloric intake. There was a significant effect of diet ( $F(1,12) = 16.73$ ), days ( $F(5,60) = 10.51$ ), and a diet by days interaction ( $F(5,60) = 17.70$ ). This interaction is due to the relative constancy of caloric intake of the  $L \rightarrow M$  group compared to the large, transient change seen in the  $M \rightarrow L$  group.

When normal rats were switched from 25% LCT diet to 25% MCT diet or vice versa, there was an enduring difference in the caloric intake of the two groups ( $F(1,19) = 21.34$ ) (Fig. 3, top). This difference appears to be attributable to a slight increase in intake by the  $M \rightarrow L$  group and a slight decrease by the  $L \rightarrow M$  group.

### Experiment IIa

Introduction. Any differences in intake of diets containing MCT and LCT in experiment Ia may be attributed to a difference in preference for the taste of the two diets rather than the metabolic consequences of ingestion of the diets. This series of preference tests was undertaken in the expectation that it would allow conclusions to be drawn about the role of palatability in differential consumption of MCT and LCT diets.

Figure 3. Caloric intake of normal and diabetic rats eating 25% fat diet after the type of oil in the diet was switched. Points shown are mean  $\pm$  s.e.m.



Method. 18 normal and 18 diabetic male Sprague-Dawley rats were maintained as described above. Normal and diabetic rats were each divided into matched groups on the basis of baseline food intake and body weight. One group was fed the 5% corn oil diet, the other group was fed the diet containing 3% MCT plus 2% corn oil for two weeks. The diet was presented in glass food jars attached to the front of the cage with a spring. Food intake was measured daily. Ten days after the rats were given access to the 5% diet, an extra, empty food jar was attached to the front of the cage with a spring to accustom the rats to its presence. The side of the cage on which the empty food jar was presented was counterbalanced for each diet group. Two weeks after the rats were given access to the 5% diet, the second food jar was filled with the 15% oil diet appropriate for each group (MCT for the group eating 3% MCT, corn oil for the group eating 5% corn oil). Food intake was measured at 24 and 48 hours. The jar containing the 5% oil diet was then removed from the cage and daily intake of the 15% oil diet was measured for two weeks. Ten days after the 5% oil diet was removed from the cage, an extra, empty food jar was placed in the cage as described above. Two weeks after the 5% diet was removed from the cage, the extra food jar was filled with the appropriate 25% diet for each group. Food intake was measured at 24 and 48 hours.

Preference scores were obtained by calculating the number of



grams eaten of the higher fat diet divided by the total grams eaten in twenty-four hours (e.g., "% grams from 15% cup"). Preference scores were compared by means of Student's t-test. A comparison was considered significant when  $p \leq .05$ .

Results. When normal rats were given a choice between 5 and 15% fat diets, LCT-fed rats had a significantly greater preference for 15% diet than MCT-fed rats ( $t(16) = 8.31$ ) (Fig. 4). The same pattern of results was apparent when normal rats were given a choice between 15 and 25% fat diet for 24 hours ( $t(16) = 5.82$ ). In neither case did the MCT-fed rats show an aversion to the higher percentage fat diet; if anything, they showed a slight preference for it. The results from the second day of the preference test did not differ substantially from those on the first day (Table 3).

When diabetic rats were given a choice between 5 and 15% fat diets for 24 hours, LCT-fed rats had a significantly greater preference for 15% diet than the MCT-fed rats ( $t(16) = 2.51$ ) (Fig. 4). Similar to normal rats, MCT-fed diabetics showed no aversion to the 15% fat diet. On the other hand, when diabetic rats were given a choice between 15 and 25% diet, not only did LCT-fed rats have a significantly greater preference for 25% fat diet than MCT-fed rats ( $t(16) = 4.88$ ), but the MCT-fed rats appeared to have an aversion to the 25% fat diet. The difference between LCT-fed and MCT-fed rats was attenuated on the second day of the test, when the LCT-fed group no longer showed a strong preference for 25% fat diet.

Figure 4. Percentage of 24 hour gram intake eaten from the higher fat cup by normal and diabetic rats eating either LCT or MCT diet.

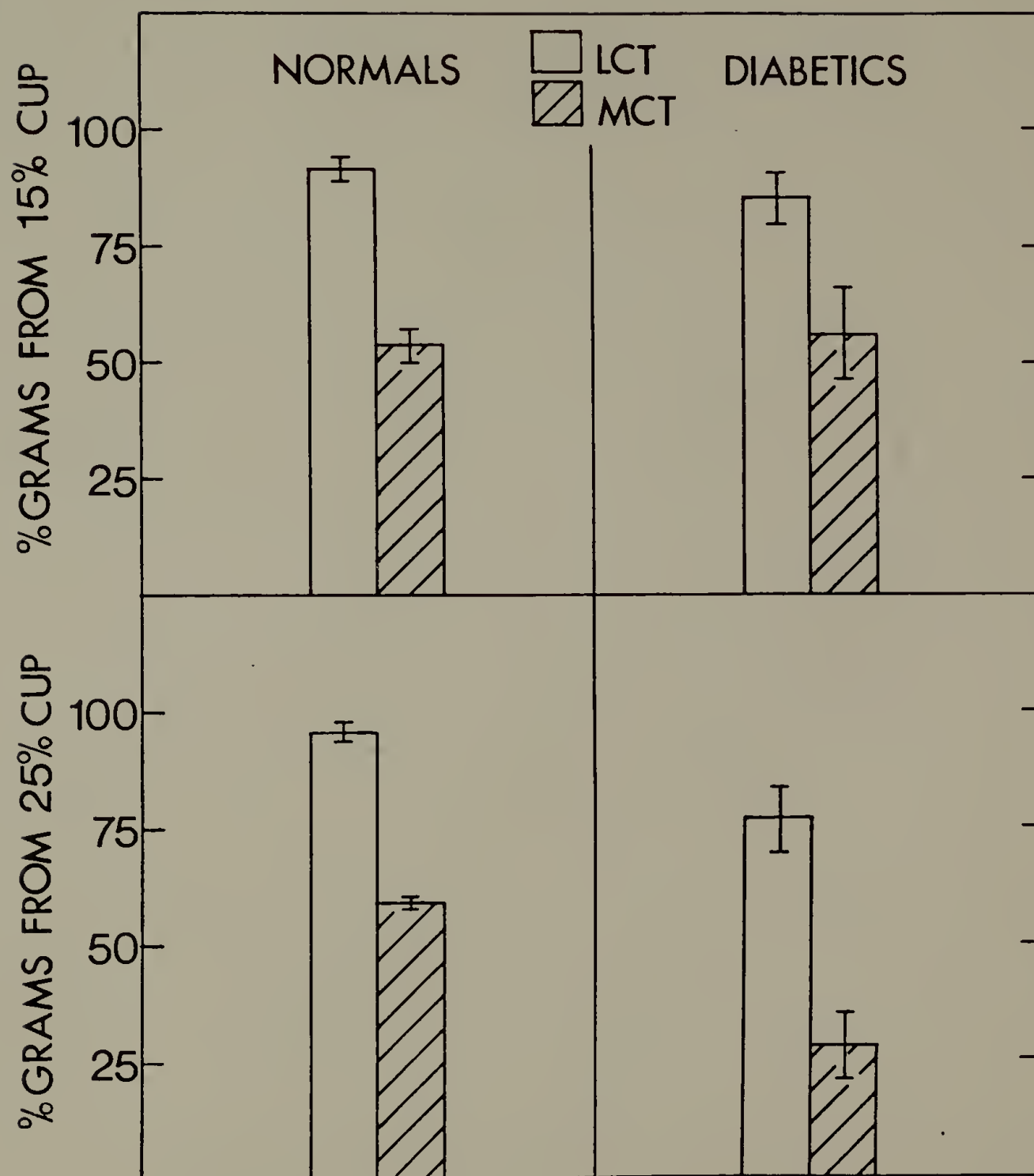


TABLE 3

Preference test summary-Experiments IIa and IIb

	Normals		Diabetics	
	LCT	MCT	LCT	MCT
% from 15% cup				
Day 1	92.9 $\pm$ 2.6	54.5 $\pm$ 6.0	87.4 $\pm$ 6.0	56.8 $\pm$ 11.5
Day 2	90.1 $\pm$ 2.1	65.9 $\pm$ 6.3	82.6 $\pm$ 8.9	46.9 $\pm$ 12.4
% from 25% cup				
Day 1	95.1 $\pm$ 1.7	60.2 $\pm$ 6.3	76.9 $\pm$ 7.6	27.6 $\pm$ 7.6
Day 2	89.4 $\pm$ 7.0	52.3 $\pm$ 8.4	57.1 $\pm$ 11.7	19.1 $\pm$ 7.6
% from 30% cup				
Day 1	80.8 $\pm$ 3.0	52.0 $\pm$ 6.9		
Day 2	93.6 $\pm$ 2.7	54.4 $\pm$ 10.3		

(Values are mean  $\pm$  s.e.m.)Experiment IIb

Introduction. Experiment IIa was designed to yield information about the concentration of dietary fat preferred by normal and diabetic rats

eating either LCT or MCT. To address the question of whether normal and diabetic rats naive to purified diets prefer the taste of MCT or corn oil, a preference test between the two using a high concentration (25%) of oil was performed. The high concentration was used to maximize the saliency of the taste of the oil.

Method. 10 normal and 10 diabetic male Sprague-Dawley rats were maintained as described above. Three weeks after the induction of diabetes in the diabetic group, all rats were given access to two food cups. One food cup contained the 25% corn oil diet, the other contained the 23% MCT plus 2% corn oil diet. Food intake was measured at 24 and 48 hours after the presentation of the food cups.

Preference for the LCT diet was evaluated by subtracting grams of MCT diet eaten from grams of LCT diet eaten for each rat, and comparing the mean difference to zero with Student's t-test. The comparison was considered significant if  $p \leq .05$ .

Results. Both normal and diabetic rats ate significantly more LCT diet in the first 24 hours after presentation of the food cups when given a choice between LCT and MCT diet ( $t(9) = 3.98$  for diabetics and  $t(9) = 5.29$  for normals) (Fig. 5). The results from the second day of the preference test are not substantially different from those of the first day in either normal or diabetic rats (Table 4).

### Experiment IIIa

Introduction. In experiment Ia, a difference in caloric intake be-

Figure 5. Twenty-four hour intake of 25% LCT or 25% MCT diet by normal and diabetic rats given a choice between the two.

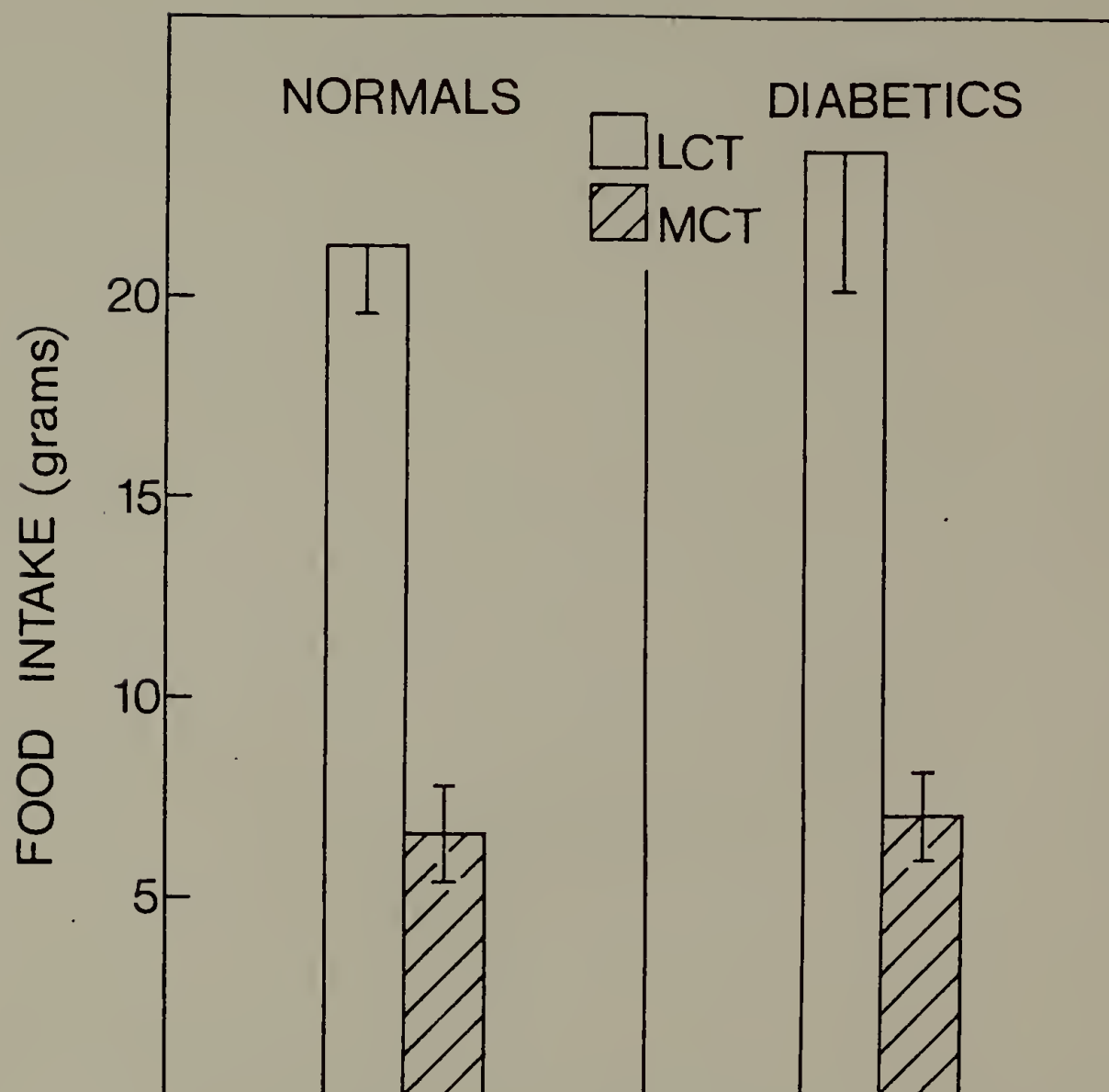


TABLE 4

LCT vs. MCT Preference Test-Experiment IIb

	Normals		Diabetics	
	LCT	MCT	LCT	MCT
Day 1	21.3 $\pm$ 1.7	6.6 $\pm$ 1.2	23.7 $\pm$ 3.5	7.0 $\pm$ 1.1
Day 2	22.6 $\pm$ 1.3	3.9 $\pm$ 1.7	22.4 $\pm$ 4.9	5.8 $\pm$ 2.2

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(Values are mean gram intake  $\pm$  s.e.m.)

tween LCT and MCT diet by normal rats was seen only at the highest concentration of dietary fat. Therefore, the experiment was repeated in normal rats using higher concentrations of fat in the diet to make it a more salient component.

Method. 30 male Sprague-Dawley rats were maintained as described above. Rats were fed a 10% oil diet for two weeks. Blood was sampled as described above for measurement of plasma triglycerides, glycerol, glucose and ketone bodies. Following blood sampling, the diets were changed to contain 30% fat. Rats were allowed to eat the 30% oil diet for two weeks, when blood was sampled again. Further increases in the fat content of the diet were not possible because oil separates from the other components of the diet at higher concentrations.



Results were analyzed as described for experiment Ia.

### Results.

Body weight. Body weight gain over the course of the experiment was slightly, but not significantly decreased by MCT feeding (Table 5).

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TABLE 5

Body Weight Gain in Experiment IIIa

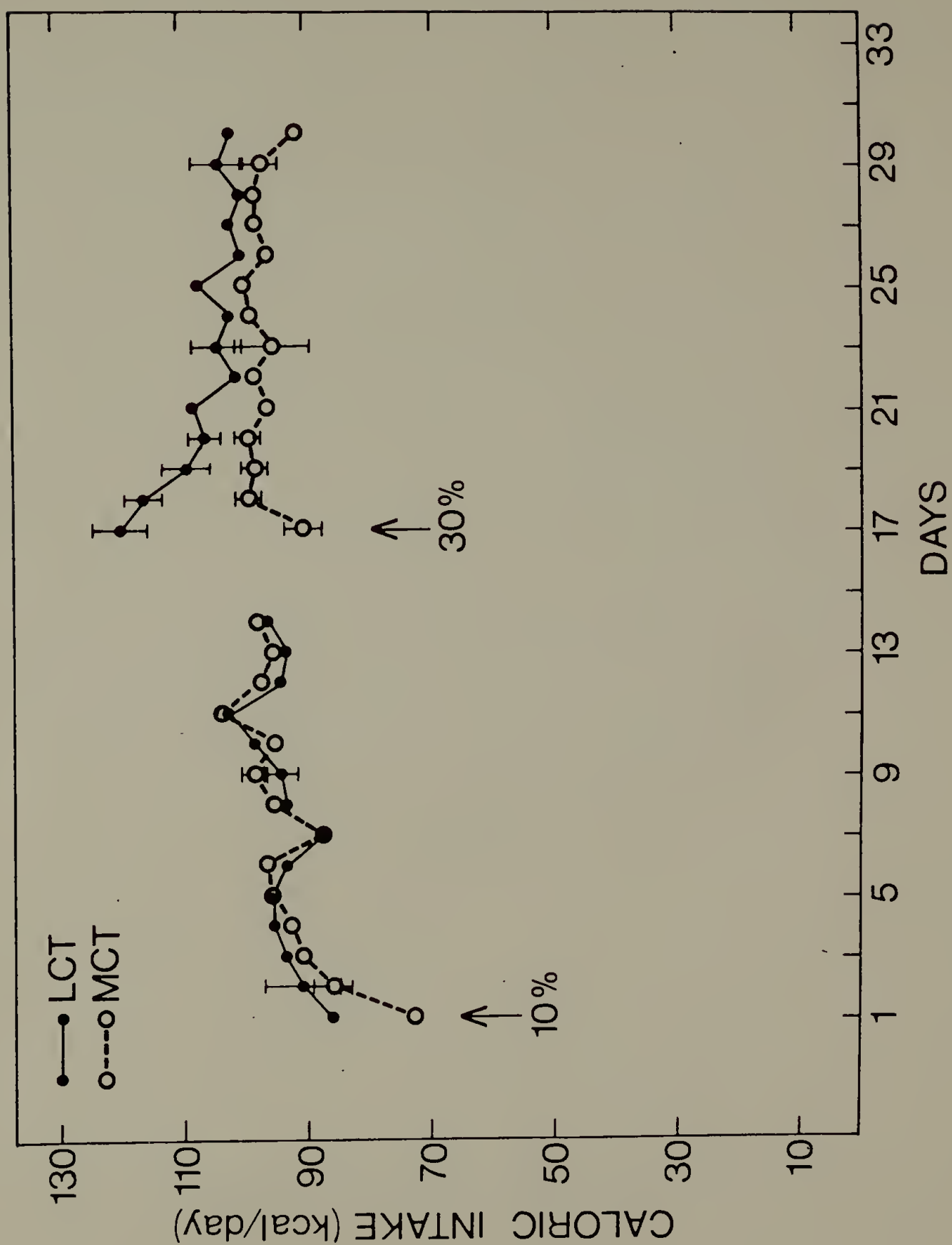
	(grams)
LCT	197 $\pm$ 8
MCT	177 $\pm$ 6

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(Values are mean  $\pm$  s.e.m.)

Food intake. The effect of MCT feeding on caloric intake was similar to that seen in normal rats in experiment Ia (Fig. 6). Mean daily caloric intake was not different in the LCT-fed and MCT-fed while they ate 10% fat diet, however on the first day the diet was switched to 30% fat, LCT-fed rats increased their caloric intake significantly more than the MCT-fed rats, who actually decreased their intake slightly ( $t(28) = 12.27$ ). The LCT-fed rats gradually decreased their caloric intake to a level only slightly higher than that of MCT-fed rats and maintained their consumption at this somewhat elevated level for the rest of the experiment.

Figure 6. Caloric intake of normal rats eating LCT or MCT diet given in ascending order of concentration of dietary fat. Points shown are mean  $\pm$  s.e.m.



Physiology. Plasma glucose concentration was unaffected by MCT feeding, but significantly elevated by increasing dietary fat content ( $F(1,28) = 195.20$ ) (Fig. 7).

Plasma ketone body concentration was significantly elevated by MCT feeding ( $F(1,28) = 39.30$ ), and by increasing dietary fat content ( $F(1,28) = 50.57$ ). Plasma ketones were elevated significantly more by increasing dietary MCT than by increasing dietary LCT content ( $F(1,28) = 12.71$ ).

Plasma triglycerides were significantly decreased by MCT feeding ( $F(1,28) = 4.52$ ), but significantly elevated by increasing dietary fat content ( $F(1,28) = 12.71$ ).

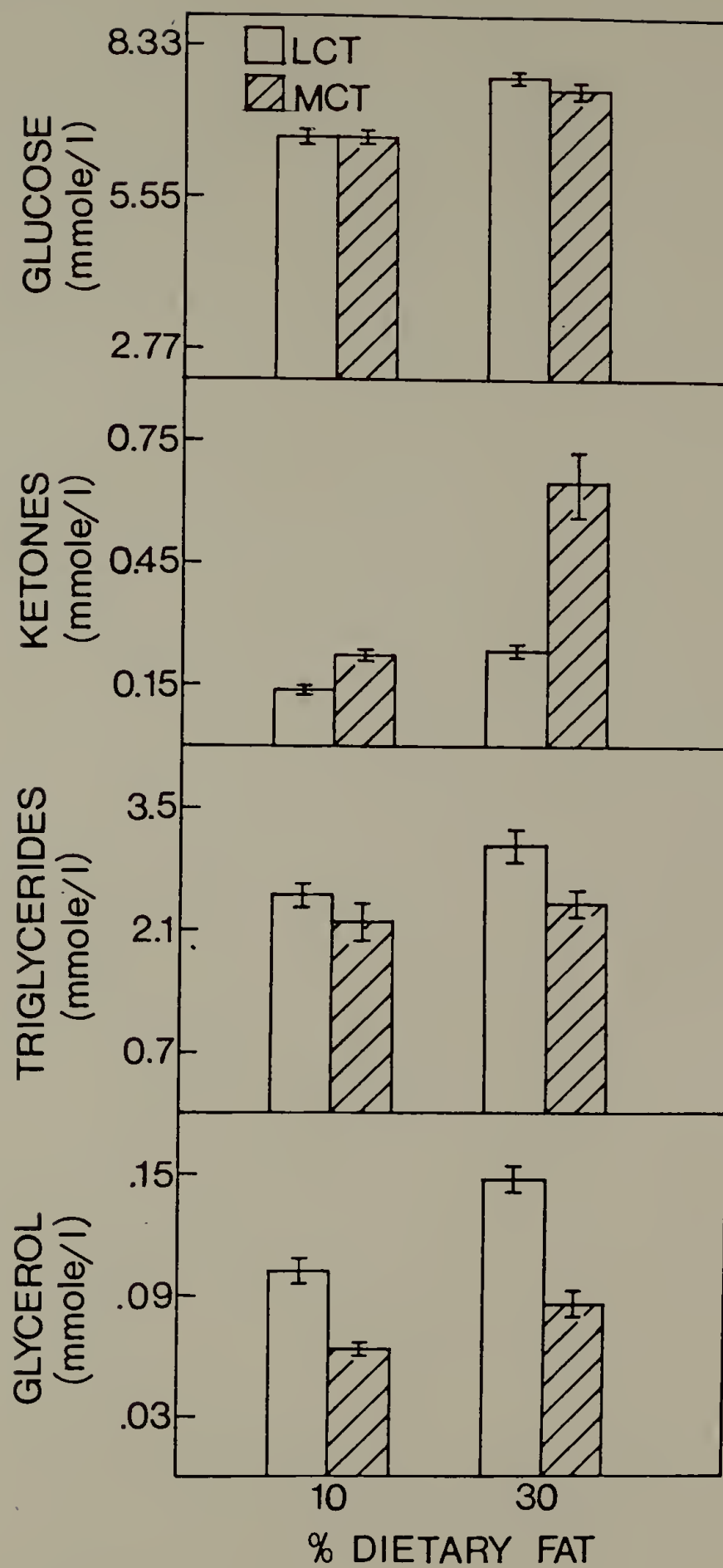
MCT feeding also significantly decreased plasma glycerol concentration ( $F(1,28) = 70.15$ ). Plasma glycerol was significantly elevated by increasing dietary fat content ( $F(1,28) = 39.92$ ), but this effect was significantly more pronounced for LCT-fed rats than MCT-fed rats ( $F(1,28) = 5.70$ ).

### Experiment IIIb

Introduction. This experiment investigated the role of palatability in the results obtained in experiment IIIa. Essentially it was a replication of experiment IIa, except that only normal rats were used, and the concentration of fat in the diet was increased to make it a more salient component of the diet.

Method. 18 male Sprague-Dawley rats were maintained as described

Figure 7. Plasma levels of metabolites of normal rats eating either LCT or MCT diet given in ascending order of concentration of dietary fat.

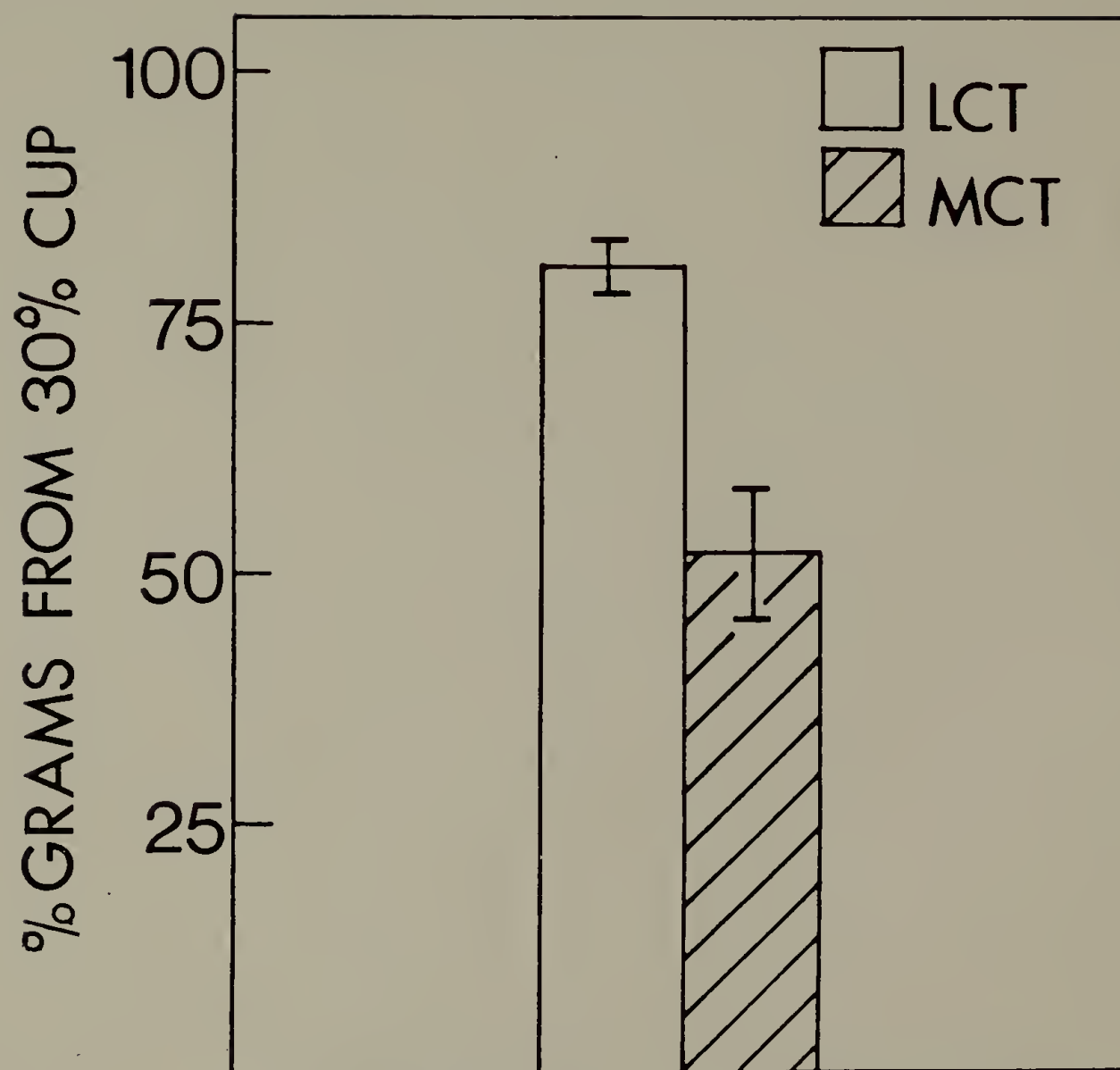


above. The procedure of experiment IIa was replicated except that the diets contained 10 or 30% oil. Preference was evaluated in the same way as experiment IIa.

Results. LCT-fed rats given a choice between 10 and 30% fat diet had a significantly greater preference for 30% diet than MCT-fed rats given the same choice ( $t(16) = 4.06$ ) (Fig. 8). The MCT-fed rats showed no aversion to the 30% diet, since it constituted approximately half their total intake. The results from the second day of the preference test did not differ substantially from those of the first day (Table 3).



Figure 8. Percentage of 24 hour gram intake eaten from the higher fat cup by normal rats eating either MCT or LCT diet.



## C H A P T E R   I I I

### DISCUSSION

Physiology. The effects of MCT feeding on plasma levels of metabolites in the normal rats of experiments Ia and IIIa were similar to those reported by other investigators. Because the MCFA derived from MCT are more easily oxidized by the liver than re-esterified into triglyceride, MCT feeding in normal rats leads to an increase in plasma ketone body concentration, and a decrease in plasma triglyceride concentration. When dietary fat content was increased, although ketone body concentration increased in both diet groups, ketones were increased more in MCT-fed rats, reflecting the different affinities of MCFA and LCFA for the oxidation and re-esterification pathways.

In diabetic rats, increasing dietary fat content resulted in decreased plasma glucose levels, presumably as a result of decreased carbohydrate intake. Plasma ketone body concentration was also affected only by dietary fat content, and not the type of fat in the diet. This is consistent with the fact that the liver of a chronic diabetic rat is already maximally ketotic, probably because the low insulin level found in diabetic rats has removed inhibition of the rate limiting step in ketogenesis, the enzyme carnitine acyl transferase I. When the activity of this enzyme is increased, LCFA are able to enter the mitochondria and be oxidized at approximately the

same rate as MCFA (McGarry and Foster, 1980). Since plasma ketone body levels are similar in MCT-fed and LCT-fed diabetic rats regardless of dietary fat content, it is of interest that plasma triglycerides do not increase with increasing dietary fat content as much as MCT-fed rats as they do in LCT-fed rats. It may be the case that MCFA, which are unlikely to be re-esterified in either the liver or adipose tissue of diabetic rats, are present in the plasma as free fatty acids, rather than triglyceride fatty acids.

It should be noted that these experiments do not furnish complete information about the metabolic state of diabetic and normal LCT and MCT-fed rats, since blood was sampled at only one time of day. Plasma metabolites have been shown to have a marked diurnal rhythm (Bruckdorfer, 1974), and thus, if blood had been sampled at some other time of day, the results would have been somewhat different. The degree to which the differences between LCT-fed and MCT-fed rats would vary with time of day is unknown. The results of these experiments are of interest in that they demonstrate that the MCT diet used affected plasma metabolites in a fashion similar to that reported by other investigators, and in that they give some indication of the metabolic effects of MCT feeding in diabetics.

Body weight. In normal rats, MCT feeding produced only non-significant decreases in body weight gain. This is somewhat surprising, in that MCFA tend to be oxidized rather than converted to lipid by a variety of tissues, including liver and adipose tissue (Schieg, 1968). It may be the case that body weight was not a reliable

indicator of the degree of adiposity in the rats in these studies, and that MCT-fed rats actually had reduced stores of body fat. On the other hand, lipid synthesis in the adipose tissue of rats fed a high MCT diet has been shown to be less depressed than in the adipose tissue of rats fed high LCT diet (Leveille et al., 1967; Wiley and Leveille, 1973; Lavau and Hashim, 1978). It is possible that MCT-fed rats were partially able to offset their reduced ability to store the fat portion of their diet. The fact that diabetic rats fed MCT showed a significant reduction in body weight may support this possibility, since their capacity for fatty acid synthesis would be decreased (Newsholme and Start, 1979).

Food intake. MCT feeding appeared to facilitate rapid caloric regulation when the fat content and therefore the caloric density of the diet fed to rats was increased. When the fat content of the diet was changed from either 15 to 25% (Expt Ia) or from 10 to 30% (Expt IIIa), MCT-fed normal rats never overate on the higher fat diets. On the other hand, LCT-fed normal rats markedly overate for several days when they were given a higher fat diet.

Diabetic rats, who have previously been shown to decrease their caloric intake when the concentration of utilizable fuels (fat) in their diet is increased, were able to adjust their caloric intake to the appropriate level within twenty-four hours after they were given a higher fat diet if they were fed MCT. This adjustment took several days in LCT-fed diabetics.

There are several possible explanations for this rapid regulatory ability in MCT-fed rats. One of them is provided by the results of the preference tests. Whereas both normal and diabetic rats fed LCT show an unmistakable preference for diets with a higher fat concentration, MCT-fed rats exhibit no clear preference, except diabetics given a choice between 15 and 25% diet, and they prefer the lower fat diet. It may be the case that when LCT-fed rats are switched to a higher fat diet, they initially overeat because the new diet is more palatable than their old diet. This gradual adjustment to an increase in caloric density of the diet has been observed by other investigators (Kanarek, 1976; Gil, personal communication). On the other hand, when MCT-fed rats are switched to a higher fat diet, since they find it no more palatable than their previous diet, they respond appropriately to the metabolic feedback they obtain from the new, more calorically dense diet.

The fact that both LCT- and MCT-fed rats readily ate the new, higher fat diet in the first twenty-four hours of the preference test raises the possibility that the rats were able to derive metabolic feedback from the new diet. Thus, the differences in consumption of the two diets during the preference test might be attributable to the metabolic consequences of the diets, rather than differing palatability.

Decreased intake of higher fat MCT diet relative to higher fat LCT diet could be caused by two types of feedback: the higher fat MCT diet could be just as palatable (relative to the lower fat

diet) as the higher fat LCT diet, but more "satiating", or the higher fat MCT diet could cause malaise. The fact that lower fat diet comprised half the intake of MCT-fed rats during the preference test makes it unlikely that higher fat MCT diet is just as palatable as higher fat LCT diet but more "satiating". If it were, one would expect MCT-fed rats to eat less higher fat diet than LCT-fed rats and little or no lower fat diet.

It also seems doubtful that decreased consumption of higher fat MCT diet in the first twenty-four hours of the preference test was caused by malaise, since MCT-fed rats continued to eat the higher fat diet on the second day of the preference test. One would expect the rats to attribute their illness to the novel diet, and subsequently to avoid it. Thus, the results of the preference tests are best explained by differences in palatability of the higher fat LCT diets relative to their lower fat counterparts.

The foregoing explanation for the rapid caloric regulation seen in MCT-fed rats assumes that both LCT- and MCT-fed rats receive identical feedback about the caloric density of their diet, and that LCT-fed rats temporarily ignore this feedback because of the increased palatability of the new diet. It seems plausible, however, that due to the different metabolic consequences of LCT and MCT ingestion, the feedback obtained from them might be quite different. By virtue of their rapid absorption and delivery (as fatty acids) directly to the liver, MCT seem likely to have a more rapid metabolic impact than LCT. In addition, intubations of MCT have been shown to slow



gastric emptying compared to LCT (Pirk and Skala, 1970) although the interpretation of these data is complicated by the fact that intubations of MCFA have been shown to slow gastric emptying less than intubations of LCFA (Hunt and Knox, 1968).

At any rate, the studies presented above cannot distinguish between the possibilities that LCT-fed rats calorically regulate more gradually than MCT-fed rats (1) because of the increased palatability of higher fat LCT diets, or (2) because they do not obtain relevant metabolic feedback from their diet as quickly as MCT-fed rats. Similarly, the role played by the different routes of absorption and metabolism of MCT and LCT in the changes in food intake seen when normal and diabetic rats are switched from 25% LCT to 25% MCT and vice versa is confounded by the fact that the LCT diet is preferred to the MCT diet. This difference in preference for LCT and MCT diets may also explain the small but enduring differences in intake of 30% LCT and MCT diet, although metabolic factors cannot be excluded.

These experiments illustrate the importance of evaluating the palatability of diet components presumed to affect food intake because of their metabolic consequences. It may be the case that when similar experiments are carried out using MCT and LCT diets equated for palatability, the results will be similar to those described above. If this is the case, the elucidation of the mechanism by which MCT facilitates rapid caloric regulation will be a problem of considerable interest.

## FOOTNOTES

1. Experiments were conducted with the technical support of Patricia Smallman.
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