Evaluation of the Protein Quality of Diets Containing Medium- and Long-Chain Triglyceride in Healthy Rats[1]

PEI-RA LING,[2] KARIM J. HAMAWY, LYLE L. MOLDAWER, NAWFAL ISTFAN, BRUCE R. BISTRIAN AND GEORGE L. BLACKBURN

The Nutrition/Metabolism Laboratory, Cancer Research Institute, New England Deaconess Hospital, Harvard Medical School, 194 Pilgrim Rd., Boston, MA 02215

ABSTRACT In this study, protein efficiency ratio and net protein utilization together with the kinetic estimates of protein turnover were used to compare the effect of different protein and fat sources in healthy rats. Male Sprague-Dawley CD rats were pair-fed different diets for 14 d. All diets were isonitrogenous and isocaloric, containing 10.4% protein, 10.9–11.4% fat, 31.9–32.8% carbohydrate and 43.5–44.5% moisture (wt/wt). After 14 d of feeding, protein efficiency ratio, net protein utilization, weight gain, intake, fat and protein content in the whole-body and fractional synthetic rates in various tissues were determined. Animals given diets containing medium-chain triglycerides (MCT) demonstrated decreased weight gain and fat content compared to the pair-fed controls receiving long-chain triglycerides (LCT). No difference was seen in protein content, net protein utilization and fractional synthetic rates in the liver and whole body of these MCT-fed rats when compared to those given LCT. Protein efficiency ratios in both of the MCT groups fed MCT + casein and MCT + soy protein were lower than those in the groups given LCT + casein. Although this study did not include a group for LCT and soy protein, these results suggest that MCT reduces the fat deposition without affecting the whole-body protein content. This may have implications for the treatment of obesity. Secondly, the protein efficiency ratio may not be a useful indicator of dietary protein quality when the fat source is MCT. J. Nutr. *116*: 343–349, 1986.

INDEXING KEY WORDS casein • hydrolyzed soy protein • long-chain triglycerides • medium-chain triglycerides • protein kinetics

The metabolism of triglycerides composed of medium-chain fatty acids (MCT) differs substantially from that of long-chain triglycerides (LCT) (1). Differences in the mechanism of gastrointestinal absorption, as well as in the utilization by tissues for energy production have been described by several investigators (2). These differences are in part related to the fact that MCT are transported in the portal circulation at a faster rate than LCT, which are transported as chylomicrons in the lymphatic channels. Furthermore, the transport of MCT into the mitochondria for β-oxidation is carnitine independent. More recently, it has been shown that MCT have a thermogenic effect, which results in less weight gain in overfed rats (3–4).

These properties of MCT, i.e., the rapidity of absorption and oxidation and the increased thermogenesis, have led to their consideration

 Received for publication: 31 May 1985. Accepted for publication: 19 November 1985.

[1]Reprint requests should be directed to: George L. Blackburn, M.D., Ph.D., Cancer Research Institute, 194 Pilgrim Rd., Boston, MA 02215.
[2]Dr. Ling is a visiting research fellow from the Peking Union Hospital, Peking Union Medical College, Beijing, China.

for use as energy substrates in the nutritional support of critically ill patients (5) as well as in the management of obesity (6). However, before MCT-based nutritional formulas can be widely advocated for these purposes, their effect on protein nutrition and dietary protein utilization should be thoroughly evaluated. This is especially important in view of the close relationship between energy and protein metabolism.

Assessment of protein quality in experimental animals has traditionally been dependent on the measurement of body weight changes and nitrogen retention (7). However, these techniques do not easily differentiate between body compartments and they fail to provide information regarding the loss or accretion of protein by individual tissues. New methods have been developed that reflect the changes in host protein synthesis and degradation rates. These methods (8), which are based on the infusion of radioactive tracers of amino acids, provide a more detailed characterization of the dynamic nature of body protein in the different tissues and can be used to supplement classical methods.

The present study was undertaken to examine the effect of varying the protein (casein and soy protein) and nonprotein (LCT and MCT) energy sources of enteral diets on host protein metabolism in healthy rats. The kinetic parameters of protein turnover were used as an adjunct to explain the mechanisms underlying the potential changes in dietary protein efficiency ratio and net protein utilization ratio.

MATERIALS AND METHODS

Diets. Three diets were evaluated (table 1). The AIN-76 diet is a purified diet that includes vitamin-free casein (C) and corn oil (an LCT). Corn oil was added to the AIN-76 (C + LCT) diet to make it equivalent in total fat content to the other two test diets. The other two purified diets contained either casein or partially hydrolyzed soy protein (S) as the protein source and MCT as the principal lipid source. The hydrolyzed soy protein and MCT diet (S + MCT) was produced by NOVO Industries (DK-2880, Bagsvaerd, Denmark). The casein and MCT diet (C + MCT) was obtained from Pfrimmer Company (Erlangen, West Germany). Electrolytes, trace minerals, vitamins and essential nutrients, including choline, were added to the three diets in accordance with the AIN-76 standard diet composition. All diets were then mixed with the appropriate quantities of 2% agar solution. The final

TABLE 1

Composition of the diets (wt/wt)[1]

Ingredient	C + LCT		S + MCT		C + MCT	
	% wt	% kcal	% wt	% kcal	% wt	% kcal
LCT (corn oil)	10.4	35.6	—	—	—	—
MCT	—	—	11.4	36.7	10.9	35.2
Casein	10.4	15.7	—	—	10.4	15.6
Soy hydrolysate protein	—	—	10.4	15.6	—	—
Sucrose	32.4	48.7	31.9	47.7	32.8	49.2
Salt mix (AIN-76)[2]	1.8		1.8		1.8	
Vitamin mix (AIN-76)[3]	0.5		0.5		0.5	
Choline chloride	—		0.01		0.01	
Moisture[4]	44.5		44.0		43.5	

[1]Values are means. C, casein; LCT, long-chain triglycerides: 9.1 kcal/g; S, soy protein; MCT, medium-chain triglycerides: 8.6 kcal/g. [2]Vitamin mix supplied in milligrams/kilogram diet (except as noted): retinyl palmitate, 4000 IU; D-L-tocopheryl acetate, 50 IU; cholecalciferol, 1000 IU; menadione sodium bisulfite, 0.8; cyanocobalamin (vitamin B-12), 10; biotin, 0.2; folic acid, 2; calcium pantothenate, 16; niacin, 30; pyridoxine · HCl, 7; riboflavin, 6; thiamin · HCl, 6. (Ref. 8a.) [3]Salt mix supplied in grams/kilogram diet: calcium, 5200; phosphorus, 4000; potassium, 3600; sodium, 1020; chloride, 1560; sulfur, 337; magnesium, 507; iron, 35; copper, 6.0; manganese, 35.0; zinc, 30.0; chromium, 2.0; iodine, 0.2; selenium, 0.10. (Ref. 8a.) [4]Moisture includes 2% agar and 98% water (wt/vol).

ratios (wt/wt) were: protein 10.4%, fat 10.4–11.4%, carbohydrate 31.9–32.8% and moisture 43.5–44.5%. All diets were isocaloric (caloric density = 265 kcal/100 g of wet weight) and the mixtures were stored at 4°C to be used within 48 h.

Animals. Fifty male Sprague-Dawley CD rats (Taconic Farms, Germantown, NY) weighing 100–135 g were allowed to consume the three diets for 14 d. All animals were individually housed in stainless-steel suspension cages in a light-controlled room (12 h on/12 h off) at an ambient temperature (21–23°C). Animals were randomly divided into five groups. Animals consuming the two test diets (group 2, S + MCT, group 4, C + MCT) were allowed to eat ad libitum and control rats (group 1, group 3) were pair-fed equivalent quantities of the AIN-76 (C + LCT) diet. Ten additional animals (group 5) were allowed to eat the AIN-76 (C + LCT) diet ad libitum.

The study was conducted in two phases: during the first phase, the effects of these three diets on weight gain and protein efficiency ratios were measured. During the 14 d of feeding, food intake was recorded every day, and the animals were weighed every 3 d. All rats were maintained in a light- and temperature-controlled room and were given tap water ad libitum. At the end of 2 wk of feeding, animals were fasted for 24 h and subsequently prepared for phase 2.

This second phase was designed to study the effect of the diets on dynamic measures of protein metabolism in the postabsorptive state. The total nitrogen and fat contents were also measured. Forty-two rats were studied in this phase. After fasting for 24 h, rates of tissue protein synthesis were measured with a flooding dose of radiolabeled amino acid, as described by McNurlan et al. (9). Briefly, on the day of study, the rats were gently restrained in cloth and were given a dose of [^{3}H]leucine through a lateral tail vein (50 μCi [^{3}H]leucine and 100 μmol of unlabeled leucine/100 g body weight). At the end of 10 min, all animals were killed by decapitation. Blood and tissue samples were collected and quickly frozen in liquid nitrogen (10). The protein fractional synthetic rates of liver, muscle and whole-body tissues were determined by using the equation $K_s = 100\ [S_b/(S_i \cdot t)]$ (8), where K_s is the fractional synthetic rate, S_b is the specific activity of leucine in the protein bound fraction and S_i is the specific activity in the acid soluble fraction. The actual time (t) of incorporation included the time required to remove and freeze the tissue samples in liquid nitrogen. The entire procedure required about 2 min.

The contribution of leucine in various tissues studied was determined by acid hydrolysis of 200 mg of tissue (wet wt) in 12 N sulfuric acid. This was then heated for 3 h at 120°C until the samples were hydrolyzed. The pH of each sample was adjusted to approximately 1.8–2.5 by addition of 2 N HCl. Each sample was diluted 1:2 with lithium citrate buffer. The leucine concentration of the hydrolysate was determined by a Dionex D-400 cation–exchange amino acid analyzer with the use of ninhydrin and photometric determination (Dionex Corp., Sunnyvale, CA). Integration was performed by a Spectra Physics SP-4100 Integrator (Spectra Physics Co., Piscataway, NJ).

Nitrogen content in tissues, carcass and food was determined using a micro-Kjeldahl digestion method as previously described, assuming that protein is comprised of 16% nitrogen (11). The frozen carcass was wrapped in a clean cloth and broken into small pieces with a mallet, which were subsequently pulverized in a Waring Blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, CT) with dry ice. Samples (1–3 g) were placed in 5 ml saline for analysis of total nitrogen in the carcass.

Fat content of carcass was measured by the method of Bligh and Dyer (12). Briefly, the sample was homogenized for 3 min with chloroform, methanol and water (3:2:1). The homogenate was then filtered through Whatman No. 1 paper in a Buchner funnel under constant suction. Methanol was added to ensure greater yield of the sample. The solution was well mixed and allowed to separate into two phases. Twenty-five milliliters of the clear chloroform layer was then collected. The chloroform was evaporated under a constant flow of nitrogen gas, and the weight of extracted liquid was determined. Total fat content was calculated as percentage of fat per gram of tissue.

Calculation and statistics. Protein efficiency ratio (7) was calculated as follows:

PER = gain in body weight after 14 d of feedings divided by the amount of protein consumed during the study period. Net protein utilization (7) was calculated from the equation: NPU = nitrogen retained in carcass divided by total nitrogen in food consumed during the 14-d period. This value represents the percent nitrogen retained in carcass. Fractional rates of protein synthesis (percent/day, ref. 9) were calculated from the equation: $K_s = [S_b/(S_i \cdot t)]$ 100, where S_b is the specific radioactivity of leucine in bound protein, S_i is the mean specific radioactivity of leucine in the intracellular pool, and t is the time expressed in days.

Statistical analysis of the data for the different groups was made with one-way analysis of variance by using a statistical software program (BMDP Statistical Software Package, BMDP Software, Los Angeles, CA). Whenever applicable, intergroup comparison was made with the Bonferroni test for multiple comparison of means. The relative contribution of food intakes versus the type of fat in the diet was further analyzed with analysis of covariance by using the same software package.

RESULTS

Feeding studies. The daily intake and weight gain during the 14-d feeding periods are shown in table 2. The dietary intake for both pair-fed groups did not significantly differ. Animals allowed to consume the modified AIN-76 diet ad libitum ate more than the pair-fed groups. Although intake did not vary among the pair-fed groups, there were significantly different weight gains. Analysis of covariance revealed that these differences were related to the type of diet and were independent of the actual amount of food consumed by the rats. As shown in table 2, feeding a diet comprised of MCT resulted in a significant reduction in weight gain. Group 2 and group 4 had a 35.0% ($P < 0.01$) and a 10.0% ($P < 0.01$) reduction in weight gain, respectively, when compared to their pair-fed groups fed AIN-76 (group 1 and 3, respectively). The C + MCT diet differed predominantly from C + LCT diet in the type of fat it contained. The S + MCT diet contained both a different fat and a different protein source compared to the C + LCT diet. When comparing PER (Fig. 1), the lowest value, 2.00, was attained in the group 2 ($P < 0.01$ vs. group 1, 3 and group 5). The PER value for group 4 was 2.56. There was no significant difference in the three groups given C + LCT diet, and an average value of 3.10 was attained.

TABLE 2

Food intake, weight gain and protein content in carcass[1,2]

Group	Intake (avg daily)	Wt gain (during 14 d)	Protein (in carcass)
	g	g	g
1	27.21 ± 2.21	120.64 ± 16.57	33.47 ± 6.82
2	27.99 ± 1.71	81.78 ± 7.36*	34.82 ± 4.02
3	22.55 ± 1.60	100.30 ± 13.53	32.92 ± 8.34
4	23.13 ± 1.67	89.60 ± 16.39†	31.63 ± 4.77
5	29.59 ± 1.96	140.10 ± 15.09	36.94 ± 6.08

[1]Data are means ± SD; n = 10. Groups: 1. Control 1, C + LCT diet. 2. Test, S + MCT diet. 3. Control 2, C + LCT diet. 4. Test, C + MCT diet. 5. Fed ad libitum, C + LCT.
[2]Significant differences: *compared to group 1 and 5, $P < 0.01$; †compared to group 3 and 5, $P < 0.05$.

Significant differences were also observed in the total fat content in the carcass (fig. 1). Both MCT-containing diets produced less fat deposition in the tissues of the whole body when compared to animals fed LCT diets. The total fat content averaged 19.0% of body weight in the control groups, whereas only 14.3% ($P < 0.01$) and 12.6% ($P < 0.01$) fat was found in group 2 and group 4, respectively (fig. 1). It should be noted here that these changes in fat content of the carcass do not totally account for the differences observed in weight. In view of the nitrogen analysis (table 2), this finding indicates that either the water content of the carcass did not comprise the same percentage of body weight in the different groups of rats (and this water accounts for the remainder of the weight difference); or that the methodology used for fat analysis did not adequately account for differences in the fat content of tissues outside lipid depots. To better characterize the effect of MCT on body composition in a more quantitative way, analysis of the total-body water would also be needed.

Despite differences in weight gain, fat content of the carcass and protein efficiency ratios, there was no difference in net protein utilization (fig. 1) and in protein content of the carcass (table 2) between the control and test groups.

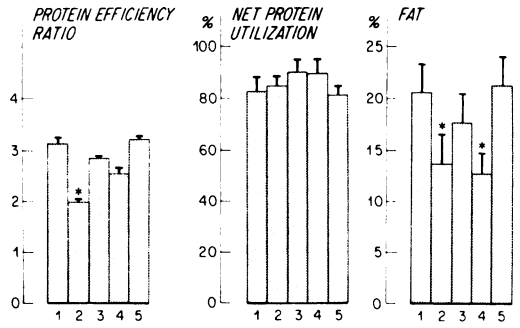

Fig. 1 Protein efficiency ratio (PER), net protein utilization (NPU) and fat content in the carcass. All data were compiled after 14 d of feeding and are means ± SEM. Two control groups were pair-fed to test diet groups. These control groups (group 1 and 3) and group 5, fed ad libitum, received diets comprised of casein and long-chain triglyceride. The other two groups received diets consisting of casein and medium-chain triglyceride (MCT) or soy protein and MCT, groups 2 and 4, respectively. Results suggest that MCT reduces total-body fat and PER, while not affecting NPU. *Significantly different from groups 1, 3 and 5 at $P < 0.01$.

Protein turnover studies. A comparison of the protein fractional synthetic rate in tissues and whole body is summarized in table 3. There were no significant differences in the fractional synthetic rate of liver and whole body when compared to the pair-fed groups. The protein fractional synthetic rate of muscle was significantly higher in group 3 ($P < 0.05$ vs. group 1, 2, 4 and 5). There were no differences among the other groups.

DISCUSSION

The protein efficiency ratio has been used extensively as a sensitive indicator of protein quality. Munro and Allison (7) have recommended that protein efficiency ratio testing be conducted at a level of protein intake equivalent to 10% of the diet by weight. In weanling rats receiving restricted protein intakes, the ratio is considered the ratio of weight gain over a 28-d period to the quantity of protein consumed. Another frequently used technique to assess the protein quality is net protein utilization. The protein is given as the sole nitrogen source in quantities less than required for maximal growth. The net protein utilization is subsequently represented as the fraction of ingested nitrogen retained in the body. Furthermore, the functions of body growth and nitrogen balance are used to evaluate the quality of dietary protein.

The present study was designed in growing rats that were fed diets containing 10.4% protein for 14 d, which is sufficient to meet the basal growth requirements of these animals. The sole nitrogen source was given in quantities less than that required for maximal growth. Under these conditions, the protein efficiency ratio and net protein utilization should be reliable indicators of the protein quality. However, the protein efficiency ratio calculated for group 2 and group 4 did not concur with the results of net protein utilization and estimates of protein synthesis in the liver, muscle and whole body as measured by radioactive tracer labeling.

Generally, the availability of amino acids in many foods must be reasonably complete, since the biologic and chemical scores of protein quality correlate very well (7). In this study, the diets containing MCT as the predominant fat source produced a significantly slower weight gain in the animals after 14 d of feeding. This decrement in weight was due, to a large extent, to a reduction in total-body fat. The lower PER in group 2 (S + MCT) is probably due to an effect of soy protein, which is well described in the literature (7), in addition to a possible effect of MCT on weight gain. Although we have not included a control group for LCT and soy, the results of the PER and fat content measurements in group 4 (C + MCT) allow us to conclude that there is significant effect of MCT on weight gain and body fat

TABLE 3

Fractional synthetic rate[1,2]

Group	Fractional synthetic rate in		
	Liver	Muscle	Whole body
	%/d	%/d	%/d
1 (6)	182.48 ± 32.57	8.84 ± 0.31	25.38 ± 2.88
2 (9)	179.83 ± 26.56	7.52 ± 0.60	26.37 ± 5.12
3 (9)	133.66 ± 10.77	14.05 ± 1.13*	18.11 ± 1.31
4 (9)	142.30 ± 18.02	10.42 ± 1.19	23.30 ± 1.31
5 (9)	179.24 ± 19.00	10.09 ± 0.46	26.13 ± 3.14

[1]Data are means ± SEM for the number of rats in parentheses. Groups: 1. Control 1, C + LCT diet. 2. Test, S + MCT diet. 3. Control 2, C + LCT diet. 4. Test, C + MCT diet. 5. Fed ad libitum, C + LCT. [2]Significant differences: *compared to groups 1, 2, 4 and 5, $P < 0.05$.

content, separate from that of soy. However, the experimental design of this study does not resolve the relative contribution of soy protein and MCT to this observation.

The phenomenon of decrease in weight gain and fat content did not have an effect on the protein content of the carcass or overall protein kinetics in the various tissues examined. The results from the net protein utilization support this conclusion. Therefore, changes in body weight gain that can occur as a result of MCT consumption do not adequately reflect the protein retained in body but are reflective of the change in fat content of body. These findings further suggest that the protein efficiency ratio may not be a sensitive indicator of dietary protein quality under the conditions of our study.

Studies in experimental animals have shown that the protein nutritional value of soy protein is significantly lower than that from animal sources (13), and a number of studies with specific soy protein products indicate similar findings in human subjects (14). Recent studies have shown that improvements in techniques for processing of soybeans have increased their protein nutritional values (15–17). In the present study, the diet containing a soy protein hydrolysate did not result in a change in protein synthetic rate in tissues and the animals eating it maintained the same protein content in the carcass when compared to the animals fed diets containing casein. The enzymatic treatment of the soy protein may have improved the bioavailability to a level similar to that of casein.

It is well established that MCT is absorbed from the intestine more rapidly than LCT. The medium-chain fatty acids are quickly oxidized into CO_2, water and energy (18). Furthermore, feeding with MCT is associated with a greater oxygen consumption than that achieved by LCT feeding (19) and thus, there is an increased heat production after ingestion of MCT. If more MCT than LCT is converted into heat, less would be available for storage as fat. In this study, food intakes were controlled by using the pair-feeding technique. Thus, it was possible to compare the effect of LCT and MCT on weight gain and body composition. In normal healthy rats, enteral feeding with MCT resulted in lower weight gain and body fat as compared to an isocaloric diet containing only LCT. Although it was unclear on this basis whether MCT-containing diets are inferior or superior to diets containing LCT, the results presented here show that MCT in the diets does not adversely affect protein synthesis while at the same time reducing fat deposition. These findings suggest that a diet with MCT may have implications for the treatment of obesity.

LITERATURE CITED

1. Bach, A. C. & Babayan, V. K. (1982) Medium-chain triglycerides: an update. Am. J. Clin. Nutr. *36*, 950–962.
2. Baba, N., Bracco, E. F. & Hashim, S. A. (1982) Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. Am. J. Clin. Nutr. *35*, 678–682.
3. Gelieber, A., Torbay, N., Bracco, E. F., Hashim, S. A. & van Itallie, T. B. (1983) Overfeeding with medium-chain triglyceride diet results in diminished deposition of fat. Am. J. Clin. Nutr. *37*, 1–4.
4. Lavar, M. M. & Hashim, S. A. (1978) Effect of medium chain triglyceride on lipogenesis and body fat in the rat. J. Nutr. *108*, 613–620.
5. Babayan, V. K. (1981) Medium chain length fatty acids esters and their medical and nutritional applications. J. Am. Oil Chem. Soc. *58*, 49A–51A.
6. Hashim, S. A. (1967) Medium chain triglycerides—clinical and metabolic aspects. J. Am. Diet. Assoc. *51*, 221–227.
7. Munro, H. N. & Allison, J. B. (1964) An introduction to nutritional aspects of protein metabolism. In: Mammalian Protein Metabolism II, pp. 10–41, Academic Press, New York.
8. Waterlow, J. C. & Stephen, J. M. L. (1967) The measurement of total lysine turnover in the rat by intravenous infusion of L-[U-^{14}C]lysine. Clin. Sci. *33*, 489.

8a. American Institute of Nutrition (1977) Report of the AIN Ad Hoc Committee on standards for nutritional studies. J. Nutr. *107*, 1340–1348.

9. McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979) The effect of starvation on the rate of protein synthesis in rat liver and small intestine. Biochem. J. *178*, 373–379.
10. Pomposelli, J. J., Palombo, J. D., Yamazaki, K., Hamawy, K. J., Bistrian, B. R., Blackburn, G. L. & Moldawer, L. L. (1985) Comparison of different techniques for estimating rates of protein synthesis in vivo in healthy and bacteremic rats. Biochem. J. *226*, 37–42.
11. Moldawer, L. L., O'Keefe, S. J. D., Bothe, A. J., Bistrian, B. R. & Blackburn, G. L. (1980) In vivo demonstration of the nitrogen sparing mechanism for glucose and amino acids in the injured rat. Metabolism *29*, 173–180.
12. Bligh, E. G. & Dyer, W. J. (1959) A rapid

method of total lipid extraction and purification. Can. J. Biochem. Physiol. *37*, 911.
13. Liener, I. E. (1977) Nutritional aspects of soy protein products. J. Am. Oil. Chem. Soc. *54*, 454A (abs.).
14. Kies, C. & Fox, H. M. (1971) Comparison of the protein nutritional value of TVP, methionine enriched TVP and beef at two levels of intake for human adults. J. Food Sci. *36*, 841–845.
15. Torun, B., Viteri, F. E. & Young, V. R. (1981) Nutritional role of soya protein for humans. J. Am. Oil. Chem. Soc. *137*, 400–405.
16. Wayler, A., Queiroz, E., Scrimshaw, N. S., Steinke, F. H., Rand, W. M. & Young, V. R. (1983) Nitrogen balance studies in young men to assess the protein quality of an isolated soy protein in relation to meat proteins. J. Nutr. *113*, 2485–2491.
17. Scrimshaw, N. S., Wayler, A. H., Murray, E., Steinke, F. H., Rand, W. M. & Young, V. R. (1983) Nitrogen balance response in young men given one of two isolated soy proteins or milk protein. J. Nutr. *113*, 2492–2497.
18. Lossow, W. J. & Chaikoff, I. L. (1955) Carbohydrate sparing fatty acid oxidation: 1. The relation of fatty acid chain length to the degree of sparing. 2. The mechanism by which carbohydrate spares the oxidation of palmitic acids. Arch. Biochem. Biophys. *57*, 23–40.
19. Travis, D., Minenna, A. & Frier, H. (1979) Effects of medium chain triglyceride on energy metabolism and body composition in the rat. Fed. Proc. *38*, 561 (abs.).