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Original Scientific Paper

Medium-Chain Triacylglycerols Effect Fatty Acid Modification in Rat's Kidney

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The aim of this investigations was to study the effect of a short-time medium-chain triacylglycerols diet (MCTD) on the lipids (particularly of fatty acids) of the rat kidney. During 14 days, two groups of rats (Fischer strain) were fed a MCTD (18.7% MCTs, 16.5 kJ/g dry matter) or an isoenergetic control diet (standard rat chow). Total lipids were isolated and separated into particular lipid classes by thin-layer chromatography. No essential differences in the distribution of analyzed lipids (cholesterol, cholesterol ester, tracylglycerols, free fatty acids, phosphatidylcholine, phosphatidylethanolamine and sphingomyeline) were noticed after MCTD.

The results showed no changes in the concentration of total neutral lipids, but slight changes appeared in the concentration of particular classes of neutral lipids. Concentrations of cholesterol and cholesterol ester were decreased by approximately 14% by MCTD feeding and, at the same time, the concentration of triacylglycerols, free fatty acids and esters of fatty acids was increased by 4%. The ratio of free cholesterol to total phospholipids (C/P) decreased (17%) after MCTD.

The data indicate a significant increase in fatty acid saturation (expressed as a saturation factor (S_f)) affected by MCTD.

Both of the mentioned data (C/P ratio, S_f), as well as some additional ones reported in this paper, suggest that MCTD can affect the membrane fluidity, although at present it is not possible to judge whether or not such effects could change the physiological function of the kidney.

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INTRODUCTION

Dietary intervention is becoming increasingly popular as an alternative to or as cointervention for the treatment of many diseases.

The dietary medium-chain triacylglycerols in patients with malabsorption syndrome and pancreatic insufficiency provide energy because long chain triacylglycerols cannot be completely absorbed.[1]

Medium-chain fatty acids with 8–12 C atoms were introduced as a well absorbed, energy-rich nutrient used for treating patients having impaired absorption of traditional long-chain triacylglycerols (LCTs) and for other uses, such as appetite control.[2,3] The major advantage of medium-chain triacylglycerols (MCTs) is their faster catabolism. Relative hydrophilia of medium chain fatty acids facilitates their intestinal absorption and directs transport to the liver *via* portal vein.[4] Intracellular transport through the mitochondrial inner membrane is generally independent of carnitine.

MCTs are energetically less dense providing 32 kJ/g of metabolized energy as compared to an average of 35 kJ/g provided by LCTs. In addition, MCTs feeding has been reported to increase thermogenesis to a greater extent than LCTs.[5,6]

The aim of this investigation was to study the effect of a short-time MCTD on rat kidney tissues and rat kidney lipids (particulary of fatty acids).

MATERIALS AND METHODS

Animals and diet

Male Fischer rats weighing 275 g were housed in stainless steel cages. The animals had free access to food and water during 14 days. One group of animals was fed a medium-chain triacylglycerols diet (MCTD), and the other was on control diet (CD) *i.e.* the usual standard rat chow.[7] The two diets were almost isoenergetic, *i.e.* MCTD *vs.* CD, 16.5 *vs.* 15.4 kJ/g.

MCTs obtained from Edelfettwerke, (Hamburg, Germany) contained about 60% caprylic acid and 40% capric acid. Fresh diet was provided daily to each group. After 14 days, the rats were sacrified and their kidneys were removed. The kidneys were washed in saline, dried, weighed, frozen in liquid nitrogen and finally stored at –20 °C until they were used for analyses.

Lipid analysis

Lipids were extracted from kidney homogenates by the method of Folch *et al.*[8] using chloroform/methanol mixtures of increasing polarity. The obtained total lipids (TL) were quantitatively analyzed by preparative TLC on

glass plates coated with Silica gel (Merck Darmstadt). In order to determine neutral lipids, TLC plates were developed using a modified method by Popović *et al.*[11] by a two-step development. In addition, polar lipids were determined by developing TLC in chloroform : methanol : water = 65 : 25 : 4 (*v/v*) as previously described by Wagner *et al.*[10]

Hydrolysis of individual complex lipids was performed alternatively in alkaline and acidic media.[12] Fatty acid derivatization was done with diazomethane.[13] Fatty acid methylesters were analyzed by gas chromatography on a Perkin Elmer 8500 Series, provided with FID, with a SPB-5 capillary column, at a constant temp. of 190 °C. Identification of individual acids was done on the basis of retention times of the authentic standards obtained from Supelco Co., Switzerland.

Cholesterol and cholesterolesters were determined chemically by a method described by Le Grimellec *et al.*[14] The phosphorus content of phospholipid classes was determined according to Broekhuyse.[15]

Protein determination

Kidney tissue was dried in a vacuum desiccator to a constant weight, and ground finely to a powder form. Protein content was determined by the method of Lowry *et al.*[16] using bovine serum albumin as a standard.

Statistical analysis

Dunett's multiple comparison test[17,18] was used to evaluate the significance of the differences between population means. Namely, $P > 0.05$ was not taken to be significant.

RESULTS

Food consumption and growth

Even though the animals were overfed with two different diets that were energetically equivalent (MCTD 16.5, CD 15.4 kJ/g), there were no significant differences in the food consumption by rats kept on diets containing MCTs.

Organ and body weights

No differences were observed in the body weight and the weight of particular organs during either of the diet periods (Table I).

TABLE I

Mean body weight and absolute and relative (g/100 g body weight) organ weights

	CD		MCTD	
	abs. (g)	rel. (g/100g)	abs. (g)	rel. (g/100g)
Body weight	275		272	
Kidneys	1.57		1.52	
		0.57		0.56

Lipid composition

Under such conditions, no significant changes occured in the amount of total lipids. In Table II, the neutral lipid composition of kidney tissues as mol % of total lipids is shown. The data obtained from the two groups of rats were compared. The results showed no changes in the concentration of total neutral lipids, but slight changes appeared in the concentration of particular classes of neutral lipids. Concentrations of cholesterol and cholesterolesters were decreased by approximately 14% by MCTD feeding. At the same time, the concentration of triacylglycerols, free fatty acids and esters of fatty acids was increased by 4% by the MCT diet (Table II).

TABLE II

Neutral lipid composition of kidney tissues (mol % of the total)

Neutral lipids	CD	MCTD
Cholesterol	22.4 ± 0.1*	19.0 ± 0.4*
Cholesterol-ester	4.3 ± 0.8*	3.9 ± 0.1*
Triacylglycerols	41.6 ± 0.3*	42.3 ± 0.9*
Free fatty acids	29.3 ± 0.4*	31.4 ± 0.1*
Esters of Fatty acids	3.4 ± 0.1*	3.6 ± 0.6*

Data are means ± S.E., *P (8 samples)

Table III shows the phospholipid composition. These results showed no changes of the content of total phospholipids but, in particular phospholipid classes (sphingomyelin, phosphatidylserine and phosphatidylinositol), slight changes appeared after MCTD application. The content of phosphatidylethanolamine and phosphatidylcholine decreased after MCTD in comparison with CD.

TABLE III

Phospholipid composition of kidney tissues (mol % of the total)

Phospholipids	CD	MCTD
Sphingomyeline	34.6 ± 0.8*	35.5 ± 0.2*
Phosphatidyletanolamine	24.5 ± 0.7*	21.2 ± 1.0*
Phosphatidylcholine	20.3 ± 0.6*	20.4 ± 0.1*
Phosphatidylserine + Phosphatidylinositol	20.3 ± 0.8*	21.0 ± 0.9*

Data are means ± S.E.,* P (8 samples)

The ratio of free cholesterol to total phospholipids

Table IV shows the effect of the diet on the cholesterol, cholesterol ester and phospholipid contents of the kidney, as well as on the ratio of free to esterified cholesterol and on the cholesterol/phospholipid ratio. The results showed a decrease in the ratio of cholesterol to cholesterolester, after MCTD vs CD. The content of phospholipids in both cases remained unchanged.

TABLE IV

Cholesterol and phospholipid contents of kidney tissues

Phospholipids	CD	MCTD
Cholesterol (μmol/mg protein)	0.24 ± 0.04	0.20 ± 0.03
Cholesterolesters (μmol/mg protein)	0.03 ± 0.01*	0.04 ± 0.01*
Cholesterol/Cholesterol-esters (mol/mol)	8.01 ± 0.01	5.11 ± 0.07
Phospholipids (μmol/mg protein)	0.44 ± 0.03*	0.44 ± 0.06*
Cholesterol/Phospholipids (mol/mol)	0.54 ± 0.01*	0.45 ± 0.03*

Values are means ± S.E., * P = 0.05 (8 samples)

The ratio of free cholesterol to total phospholipids (C/P) using MCTs decreased significantly decreased (17%) after MCTD.

Fatty acid composition of kidney lipids

The fatty acid composition of the main lipid classes is compiled in Figure 1, and in Table VI. The results are expressed for particular lipid classes, important for the structure and the function of the kidney.

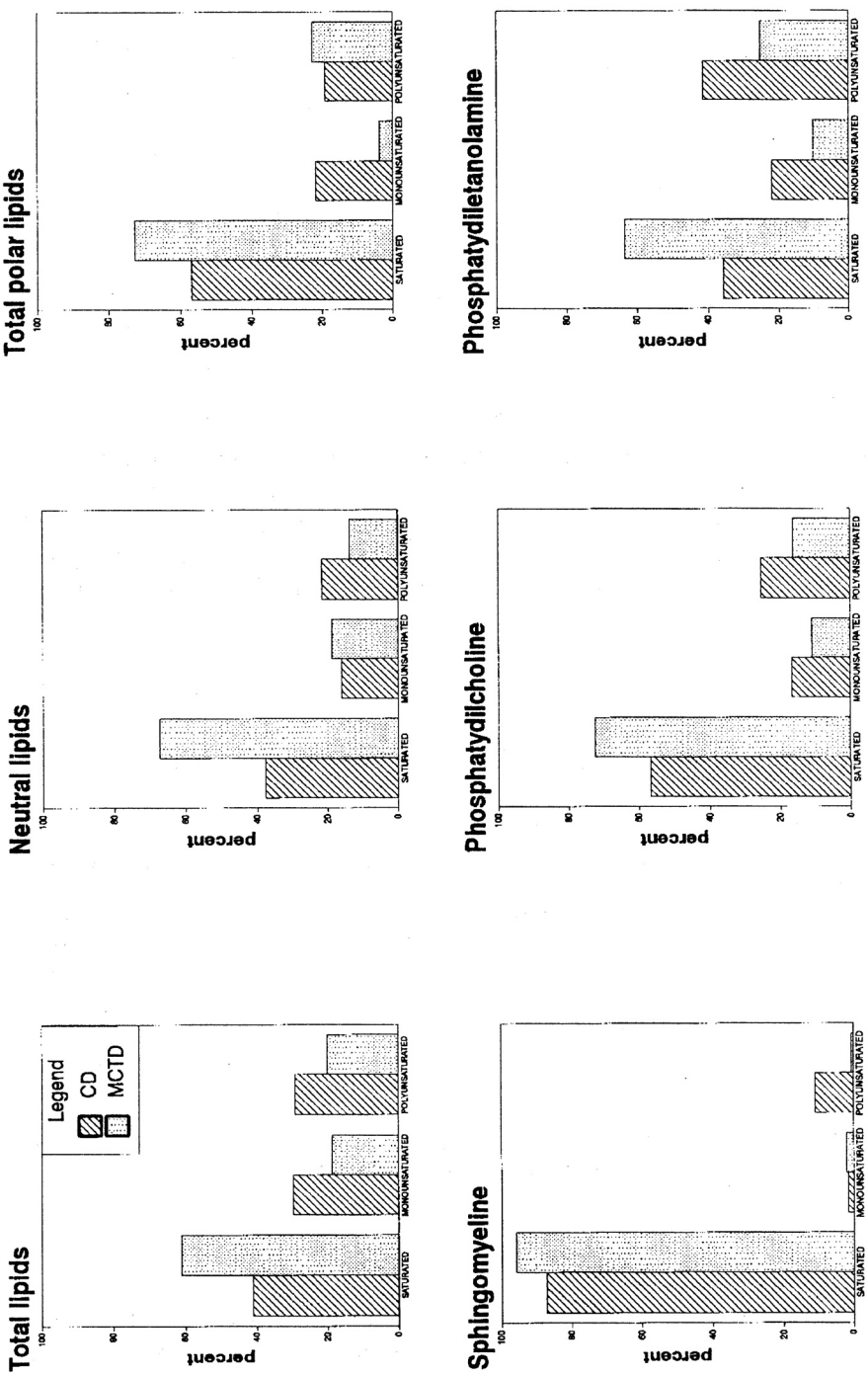

Figure 1. Fatty acid composition of kidney lipids.

TABLE V

Fatty acids saturation factors S_f (saturated fatty acids/unsaturated fatty acids)

	CD	MCTD	*
Total lipids	0.70	1.58	125
Neutral lipids	0.61	2.07	239
Total polar lipids	1.39	2.71	95
Sphingomyeline	7.02	34.21	387
Phosphatidylcheoline	1.33	2.64	98
Phosphatidylethanolamine	0.57	1.79	70

* Percent of the increase of Saturation factor (S_f) after MCTD

TABLE VI

Kidney fatty acids composition (in % of total fatty acids)

Fatty acids	Total lipids		Neutral lipids		Sph		PC		PE	
	CD	MCTD	CD	MCTD	CD	MCTD	CD	MCTD	CD	MCTD
C 14 : 0	0.6	0.5	1.5	2.6	5.8	3.7	–	–	–	–
C 16 : 0	28.4	30.4	29.7	38.4	29.9	34.0	39.3	42.3	16.4	33.0
C 16 : 1	5.1	3.4	7.8	0.4	0.8	–	2.0	0.9	1.2	–
C 18 : 0	11.1	20.6	6.4	16.6	20.4	22.1	17.1	21.2	19.3	21.6
C 18 : 1	24.1	15.3	32.1	18.4	0.8	0.1	14.9	10.4	20.0	10.3
C 18 : 2	17.3	5.5	19.9	11.3	–	–	14.3	6.3	9.7	5.1
C 18 : 3	0.2	–	–	–	–	–	0.8	–	1.9	–
C 20 : 0	0.8	5.3	–	6.3	12.4	15.4	–	5.4	–	4.8
C 20 : 4	12.1	13.4	1.7	2.4	0.1	0.6	8.6	8.7	26.0	17.6
C 22 : 0	0.2	1.6	–	–	10.4	11.2	0.4	0.1	–	–
C 22 : 5	–	–	–	–	–	–	1.3	1.3	2.6	2.5
C 22 : 6	0.1	1.0	–	–	–	–	–	–	0.6	0.1
C 24 : 0	–	3.1	–	3.4	8.2	9.4	–	3.6	–	4.2
C 24 : 1	–	–	–	–	10.8	2.1	–	–	–	–

DISCUSSION

Lipid abnormalities are common in patients with chronic progressive renal disease.[19,20] This was shown by a number of investigations carried out on animal models of immune and nonimmune mediated renal diseases, where dietary and pharmacologic modification of lipid metabolism was predominantly affected.[21,22] Lipid profiles can be changed and to some extent

controlled, in different types of lipid diets.[23] Even fat-free or low-fat diets will essentially affect the fatty acid composition in most lipid classes of different tissues. We have recently reported our investigations on this subject.[7,24]

MCTs diet has been introduced in the clinical treatment for years. MCTs can be administrated in all of the three possible ways *i.e.* orally, enterally and parenterally. In hepatocytes they will be predominantly catabolized and a remarkable part of the generated energy will be transformed into heat. Consequently, in no accumulation of adipose fat can be expected MCTD.[1,25] Our data have proved this assumption (Table I).

The influence of the lipid composition on the membrane permeability has been proven in numerous experiments. Recently, it has been shown that, like in the intestine,[26] the lipid asymmetry of kidney cell membranes also reflects their physical state. Data concerning the latter case are still limited and to some extent contradictory.[27] This was one of the reasons that triggered us to reinvestigate the effect of orally administered MCTs on the lipid profile changes of rat kidneys. On the basis of the data represented in Table II, some of the neutral lipids (triacylglycerols, free fatty acids, esters of fatty acids) were increased. This class of neutral lipids are not essential for the composition of membrane lipids. This is certainly not true of cholesterol and cholesterol ester, which were decrease. The decrease of cholesterol was found to be 15% (Table II). Since no changes of total phospholipid quantities were found, the decrease of the cholesterol/phospholipid (C/P) ratio of 16.7% (Table IV) is caused only by the cholesterol decrease. According to Carmel *et al.*,[28] the C/P ratio correlates very well with the physical properties of membranes and it can be a very reliable chemical parameter in estimating the changes of both physical and physiological properties of kidney membranes. Based on this fact, the data presented in this paper suggest a certain restriction in membrane fluidity, which does not necessarily influence the physiological functions of rat kidneys.

The other basic parameter for the determination of membrane fluidity is, as it is well known, the saturation factor (S_f), which is in this paper calculated as the molar ratio of saturated *vs.* unsaturated fatty acids. Taking into account only the lipid classes most important for the membrane construction, *i.e.* total polar lipids and sphingomyelin (Sph) (Table V), one can notice a 387% increase of S_f for sphingomyeline by 387% and 95% for total polar lipids. Sphingomyeline is the major phospholipid of kidney tissue[29,30] in relation to other most common phospholipids; the ratio being: Sph : PC : PS : PI as 13 : 5 : 4 : 3 : 1 of total lipids.[28] The data for sphingomyelin and total polar lipids show a significant increase in fatty acid saturation, as affected by MCTD. This fact is in very good correlation with the above mentioned C/P ratio and is an additional indication that MCTD could, at least to a certain extent, limit the membrane fluidity.

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SAŽETAK

Utjecaj dijete sa srednjelančastim triacilglicerolima na lipide bubrega štakora

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Promatran je utjecaj dijete s kratkolančastim triacilglicerolima (MCTD) na lipide bubrega, posebice promjene masnih kiselina u pojedinim lipidnim klasama bubrega štakora. Dvije skupine štakora soja Fischer hranjene su 14 dana sa dvije različite dijete, koje su bile izoenergijske (MCTD 32 kJ/g, CD 35 kJ/g).

Ukupni lipidi izolirani su iz tkiva bubrega, te razdvojeni na pojedine lipidne frakcije tankoslojnom kromatografijom.

Ustanovljeno je da nema značajnijih promjena u raspodjeli lipidnih klasa (kolesterol, kolesterolni esteri, triacilgliceroli, slobodne masne kiseline, fosfatidilkolin, fosfatidiletanolamin i sfingomielini) u ukupnim lipidima tkiva bubrega primjenom MCTD.

Dobiveni rezultati pokazuju da nema promjena u koncentraciji neutralnih lipida, ali su primijećene blage promjene koncentracija pojedinih klasa neutralnih lipida. Koncentracija kolesterola i kolesterolnih estera opada za 14% primjenom MCTD, a u isto se vrijeme koncentracija triacilglicerola i slobodnih masnih kiselina povećava za 4%. Primjenom MCTD omjer kolesterol/fosfolipidi opada (17%). Dobiveni rezultati su pokazali su da primjenom MCTD dolazi do zasićenja u masnim kiselinama svih promatranih lipidnih klasa (u ovom radu izraženo kao faktor zasićenja, S_f). Faktor zasićenja u sfingomijelinskoj frakciji, kao najviše zastupljenoj frakciji u tkivu bubrega, povećan je primjenom MCTD za 385%. Dobiveni rezultati pokazuju da MCTD ima utjecaj na propusnost membrane bubrega pa bi bilo potrebno proučiti promjene fiziološke funkcije bubrega kod primjene MCTD.