

Effect of dietary linoleic acid on the progression of chronic renal failure in rats

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Abstract

The role of linoleic acid in chronic renal failure (CRF) is controversial. In the present study 21 male Wistar rats submitted to 5/6 renal mass reduction (R) and 16 normal controls (C) were fed a supplement (S) or normal (N) linoleic acid diet for 60 days starting 10 days after CRF. As expected, serum creatinine, cholesterol and triglycerides (mean \pm SEM) were higher in the CRF groups compared to the C groups ($P < 0.05$). The RS group presented lower cholesterol (84 ± 4 vs 126 ± 13 mg%) and triglyceride (88 ± 9 vs 132 ± 19 mg%) levels compared to the RN group. Proteinuria and kidney weight did not differ between CRF groups. Glomerular area increased 78% in RS and 100% in RN compared to control rats. Glomerular sclerosis index tended to be lower in RS (27%) compared to RN (38%), tubulointerstitial damage was similar between CRF groups (RS = 1.91 ± 0.2 and RN = 2.14 ± 0.3), and mesangial fractional volume increased to the same extent in both CRF groups. The data suggest that a linoleic acid-enriched diet did not protect against the progression of CRF after 60 days.

Key words

- Chronic kidney failure
- Diet
- Glomeruli
- Linoleic acid

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Introduction

In the 1980's, the diet started to be considered the factor that would solve almost all problems related to the progression of chronic renal failure (CRF) (1). The protein diet has been extensively studied, together with some other dietary components. Lipids and precursors of some autacoids like prostaglandins and leukotrienes have also been implicated in the progression of CRF (2-4). Dietary prostaglandin precursors are different in different parts of the world. Linoleic acid, common in the western diet, and eicosapentanoic acid, common in the diet of inhabitants of the far north, are examples of this

difference. Many studies have compared these two sources of prostaglandins (those from dienoic series with those of trienoic series) but with inconclusive results (5-7). Studies using different experimental models such as a 5/6 reduction of renal mass, lupus nephritis, and immunologic glomerulonephritis have suggested that increases in vasodilatory prostaglandins could retard the progression of CRF (8-10).

Following experimental reduction in renal mass, the glomerular and urinary excretion of prostaglandins and thromboxane A_2 increases several-fold (11,12). There is evidence that these substances play a role in the progression of CRF (13,14). Prostaglandins

and thromboxanes are cyclic derivatives of polyunsaturated fatty acids (PUFAs) (15-17). Linoleic acid is an essential fatty acid ingested at the rate of approximately 10 g per day, and is the most abundant fatty acid in western diets. It is present in many vegetable oils and is the precursor of arachidonic acid. Some beneficial effects of PUFAs, especially linoleic acid, are related to the reduction of blood pressure (18,19), platelet aggregability and blood viscosity (2,5). Subtotally nephrectomized rats fed a low linoleic acid diet had progressive deterioration of renal function. By contrast, those fed the high linoleic acid diet had better preservation of renal function with less proteinuria and glomerular sclerosis (20,21), and also an increase in the renal content of arachidonic acid (22). Other investigators have shown that rats with CRF fed a linoleic acid-enriched diet had an increase in proteinuria and glomerular sclerosis index (GSI), and also a worsened progression of CRF (23).

The role of linoleic acid in the diet of animals with CRF has not been established regarding the progression of CRF, despite the studies published in the last years (1,9,13,22). Thus, the objective of the present investigation was to study the effects of a high and low linoleic acid content in the diet on the progression of CRF.

Material and Methods

Male Wistar rats weighing 200 to 250 g at the beginning of the study were used. Animals were divided into two groups: controls (C) and animals submitted to 5/6 reduction (R) of total renal mass. Reduction of renal mass was performed by partial left nephrectomy, with an abdominal incision under ether anesthesia. The renal artery was isolated and two or three branches were ligated. Right nephrectomy was then performed immediately through a lumbar incision. This procedure was performed on day 0, and the animals were maintained on a regular diet until

the 10th day after surgery, when they started to receive the experimental diets. Control animals also started to receive the experimental diets at the same time as the CRF groups.

Diets

Each group was divided into two groups fed the experimental diet containing linoleic acid as 1.5% (normal, N) or 21% (supplement, S) of the total caloric intake for 61 days. Throughout the study, the animals were pair-fed (CN *vs* CS and RN *vs* RS). At the beginning of the study, animals were paired according to body weight and food consumption. Body weight and food consumption were determined daily throughout the study. Diets were elaborated in the Experimental Laboratory of the Faculty of Nutrition, Federal Fluminense University, Rio de Janeiro, RJ, Brazil, using an adaptation of the model of Tobian et al. (24). The diet consisted of 80% of standard Purina® rodent chow (Campinas, SP, Brazil), which was ground to a powder, and 20% soy oil or butter, with water added to form a paste. The diet was homogenized with an industrial mixer (Hobart), transformed into pellets and dried in an oven (Fabber Primar). After drying, the diets were identified and stored under refrigeration. All ingredients were weighed on a digital electronic scale.

The diet called "N", in which butter was the main source of lipids, contained approximately 0.67 g linoleic acid/100 g chow, which corresponded to 1.5% of the total energy, i.e., a normal linoleic acid content. The "S" diet, which contained soy oil, had approximately 9.60 g of linoleic acid/100 g chow, which corresponded to 21% of the total energy, corresponding to a linoleic acid-rich diet. Both diets offered about 400 kcal/100 g and 19% protein. The estimated centesimal chemistry composition (Table 1) of the experimental diets followed established criteria (25).

A sample of each diet was analyzed for linoleic acid content by the Brazilian Company of Agriculture and Stock Raising Research (EMBRAPA). The result showed that the "S" diet had nine times more linoleic acid than the "N" diet. The basic composition of the standard chow from Labina/Purina®, according to the manufacturer, contains corn, wheat bran, soy bran, meat meal, raw rice meal, calcium carbonate, bicalcium phosphate, and salt.

Groups

The following groups were studied: control group fed the diet with normal linoleic acid content (CN, N = 9), CRF group fed the diet with normal linoleic acid content (RN, N = 10), control group fed the linoleic acid-rich diet (CS, N = 7), and CRF group fed the linoleic acid-rich diet (RS, N = 11). On days 21, 41 and 61 after starting the specific diets, 24-h proteinuria was determined. Urine was collected in metabolic cages, where the animals were kept only with water *ad libitum*. Blood was collected at the end of the study to measure serum creatinine, cholesterol and triglycerides.

Morphology

At the end of the study, the kidneys were perfused with 1.25% glutaraldehyde in sodium phosphate buffer, pH 7.4. Kidney fragments were fixed, and 3-4- μ m sections were stained with periodic acid-Schiff. Glomerular area was determined after measuring the glomerular diameter with a caliper mounted on the eyepiece. The reported glomerular area is the mean of 25 measurements. The GSI was determined by measuring the frequency of sclerosis in 100 glomeruli from each animal as described by Raij et al. (26), and the result is reported as the median. A score of 0 to 4+ was assigned to each glomerulus according to the extent of sclerosis: normal glomerulus, 0; up to 25% involve-

ment, 1+; up to 50% involvement, 2+; up to 75% involvement, 3+; more than 75% involvement, 4+. The GSI score for each animal was the sum of individual glomerular scores multiplied by the percent of glomeruli with the same score. Tubulointerstitial damage was evaluated as described by Shih et al. (27). Briefly, cortical involvement was examined and graded according to a 0 to 4 scale (0 = normal, 0.5 = small focal areas of cellular infiltration and tubular damage, 1 = involvement of less than 10% of the cortex, 2 = involvement up to 25% of the cortex, 3 = involvement up to 50 to 75% of the cortex, and 4 = involvement greater than 75%). Additionally, mesangial fractional volume (Vv) was measured in ten glomeruli from each animal using the M42 test-system grid; Vv was estimated by counting the number of points falling on mesangium (pMes), including its matrix and cellular components, using $Vv = pMes \times 100/42$ (28).

Analytical procedures

Serum creatinine, cholesterol and triglycerides were determined with an autoanalyzer in the Central Laboratory of the Pedro Ernesto Hospital. Proteinuria was determined using the sulfosalicylic acid method.

Statistical analysis

Data are reported as means \pm SEM, and medians were compared by analysis of variance. The difference between groups was determined by the Duncan test. The Mann-

Table 1. Estimated centesimal chemistry of the diets.

	Proteins	Glucides	Lipids	Energy		Linoleic acid
				kcal	g/100	%/energy
N	19.78	35.87	19.1	394.5	0.67	1.53
S	19.78	38.35	20.0	412.5	9.60	21.0

N, S = normal and supplemented linoleic acid diets, respectively.

Whitney test was used to compare GSI data. The differences were considered to be statistically significant if $P \leq 0.05$.

Results

Body weights after surgery on the day the animals began the specific diet were: CS =

Figure 1. Effect of linoleic acid dietary supplement on serum cholesterol (Chol) and triglycerides (Tri) 61 days after renal mass reduction. For group abbreviations, see legend to Table 2. * $P < 0.05$ compared to CS; * $P < 0.05$ compared to CN (Duncan test).

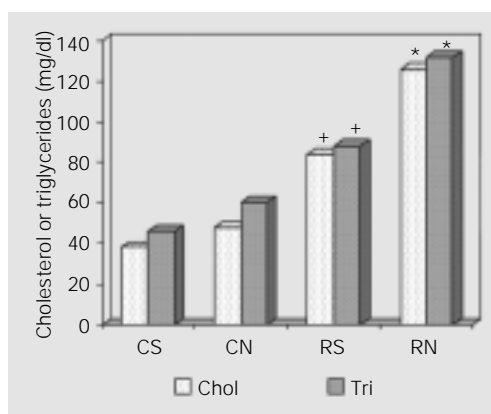


Figure 2. Effect of linoleic acid dietary supplement on proteinuria (PTN) 21, 41 and 61 days after renal mass reduction. For group abbreviations see legend to Table 2. * $P < 0.05$ compared to CS; * $P < 0.05$ compared to CN (Duncan test).

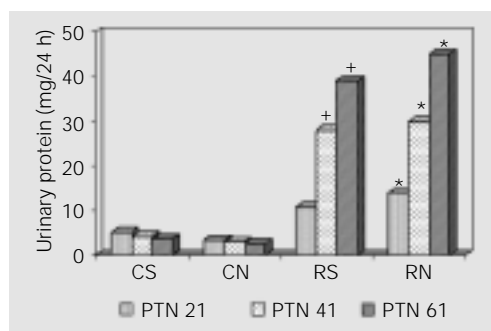


Table 2. Effect of linoleic acid dietary supplement on kidney morphology after mass reduction.

	CS	CN	RS	RN
	N = 7	N = 7	N = 6	N = 7
GA ($\mu\text{m}^2 \times 10^3$)	11 \pm 0.8	9.6 \pm 0.4	19 \pm 1.1 ⁺	20 \pm 0.4*
GSI	0	0	36 (3-112)	22 (21-103)
TID	0	0	1.9 \pm 0.2	2.1 \pm 0.3
Vv (%)	62 \pm 2.6	64 \pm 1.7	62 \pm 0.7	63 \pm 2.1

GA = glomerular area, GSI = glomerular sclerosis index, TID = tubulointerstitial damage, Vv = mesangial fractional volume, C = control, R = 5/6 renal mass reduction, S = linoleic acid-rich diet, N = normal linoleic acid diet. Data are reported as means \pm SEM or as medians. * $P < 0.02$ compared to CS; * $P < 0.02$ compared to CN (Duncan test for all parameters and Mann-Whitney for GSI).

323 \pm 8, CN = 317 \pm 6, RS = 258 \pm 12, and RN = 231 \pm 11 g, and at the end of the study were CS = 387 \pm 10, CN = 367 \pm 7, RS = 317 \pm 15, and RN = 282 \pm 14 g. C groups were different from the respective R groups ($P < 0.001$). Mean serum creatinine values were: CS = 0.65 \pm 0.06, CN = 0.69 \pm 0.03, RS = 1.44 \pm 0.12, and RN = 1.80 \pm 0.42 mg%. The CRF groups presented higher creatinine values ($P < 0.05$), and RN tended to be higher than RS. Cholesterol values were higher in the CRF groups compared to their respective controls after 61 days (Figure 1). The RN group showed higher serum lipid levels compared to RS ($P < 0.05$). Values for proteinuria were higher in the CRF groups after 21, 41 and 61 days ($P < 0.05$) except for RS compared to CS after 21 days (Figure 2). Values for kidney weight: CS = 1.41 \pm 0.09, CN = 1.19 \pm 0.06, RS = 1.44 \pm 0.08, and RN = 1.13 \pm 0.06 g, were not statistically different among groups. Values for glomerular area, GSI, and tubulointerstitial damage were higher in CRF groups compared to the respective controls, but were not significantly different among them. Mesangial fractional volume was not different among the groups studied (Table 2).

Discussion

The diets were isocaloric and all groups showed the same percentage of increase in body weight, suggesting that the diet was adequate.

Animals fed a normal linoleic acid diet showed a worse lipid profile when compared to those fed a high linoleic acid diet. Disturbance of lipid metabolism, a characteristic of CRF (29,30), has been associated with the development of glomerular sclerosis, and consequently with the progression of CRF (31,32). The mechanisms whereby lipids contribute to renal injury are incompletely understood. The PUFAs of the n-6 and n-3 series have beneficial effects on plasma lipids. Those of the n-6 series are

more effective in reducing cholesterol (23) whereas those of the n-3 series exert their main effect on triglycerides and their effect on lipoproteins has been questioned (33). It has also been suggested that PUFAs improve renal lesions not by reducing the serum levels of lipoproteins, but by modifying lipoprotein composition, their deposition within the glomeruli or the cholesterol or ester composition in the tissues (20,34-36).

A marked increase in proteinuria was observed in both CRF groups, suggesting that glomerular selectivity was compromised to the same extent in both groups. Some studies have shown that dietary supplementation with PUFAs reduces proteinuria and prevents functional deterioration in immune complex glomerulonephritis, and in genetic systemic lupus erythematosus (2,3,8). Dietary supplementation by linoleic acid prevented functional deterioration after subtotal nephrectomy in rats (37). Heifets et al. (22) showed that a linoleic acid-enriched diet fed to female rats with CRF led to an increase in glomerular filtration rate when compared with rats fed a low linoleic acid diet. Additionally, they showed better levels of arterial pressure and proteinuria. It has been suggested that PUFAs of the n-6 series may reduce the deterioration of renal function and the glomerular sclerosis of rats with CRF (20,21,30). Conversely, Brown et al. (38) found that in dogs with CRF, supplementation with omega 6 PUFA enhanced renal injury, and supplementation with omega 3 was renoprotective. In a study evaluating partially nephrectomized rats fed a high linoleic acid diet (21), protection of renal function was demonstrated, but both groups developed proteinuria. However, proteinuria occurred later and was significantly less severe in rats fed the high linoleic acid diet. Serum cholesterol and triglycerides were not different between groups. Protection of the renal architecture was also demonstrated in CRF rats fed the high linoleic acid diet. The authors suggested that evolution of CRF could

be prevented by increasing the linoleic acid content of the diet, and showed that this protection was associated with increased cortical production of PGE₂. These data show that there is no consensus about this issue and they should be interpreted carefully, firstly because they are not homogenous and secondly because different models of progressive renal disease were used as well as different strains of rats.

Animals submitted to 75% reduction of total renal mass showed kidney weights that did not differ from control. These data suggest the occurrence of hypertrophy of the residual kidney (1), and show that this process was independent of the dietary lipid content. The glomerular area was larger in the CRF groups, increasing 78% in the RS group and 100% in the RN group. GSI and tubulointerstitial damage increased to the same extent in the CRF groups, while V_v did not change, suggesting that diet did not affect these parameters. These results were expected since proteinuria, that is considered to be the main mediator of mesangial expansion and tubulointerstitial damage (39), was similar between CRF groups.

Sometimes the extent of glomerular lesion is correlated with serum cholesterol (40). Endogenous hyperlipidemia in obese Zucker rats is well correlated with the development of albuminuria and glomerular lesion. When the animals are treated with lipid-lowering agents, albuminuria decreases, and glomerular lesions (40) as well as mesangial matrix expansion are reduced (33). Thus, in some forms of glomerular lesion, serum lipid reduction is associated with the reduction of the structural lesions of the kidney.

In the present study we found that animals with CRF fed a linoleic acid-enriched diet had a better serum lipid profile; however, other indicators of renal function and architecture were not different from controls. These data suggest that lowering serum lipids is not the main mechanism for

protecting renal function in this model.

Questions related to the source of lipids of the diet, playing a role in the progression

of CRF, remain unanswered, and longer studies will be necessary to answer them.

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