A Low-Iron-Available, Polyphenol-Enriched, Carbohydrate-Restricted Diet to Slow Progression of Diabetic Nephropathy

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Diabetic nephropathy has become the leading cause of uremia. Several lines of evidence suggest dietary factors other than protein intake have a substantial role in the progression of diabetic nephropathy to end-stage renal disease. The present investigation was initiated to evaluate whether a carbohydrate-restricted, low-iron-available, polyphenol-enriched (CR-LIPE) diet may delay and improve the outcome of diabetic nephropathy to a greater extent than standard protein restriction. To this aim, 191 diabetic patients, all with type 2 diabetes, were randomized to either CR-LIPE or standard protein restriction and the following outcomes monitored: doubling of serum creatinine, cumulative incidence of end-stage renal disease, and all cause mortality. Over a mean follow-up interval of 3.9 ± 1.8 years, serum creatinine concentration doubled in 19 patients on CR-LIPE (21%) and in 31 control subjects (39%) ($P < 0.01$). Renal replacement therapy or death occurred in 18 patients on CR-LIPE (20%) and in 31 control subjects (39%) ($P < 0.01$). These differences were independent from follow-up interval, sex, mean arterial blood pressure, HbA_{1c}, initial renal dysfunction, and angiotensin system inhibitor use. In conclusion, CR-LIPE was 40–50% more effective than standard protein restriction in improving renal and overall survival rates. *Diabetes* 52: 1204–1209, 2003

Current methods used in clinical practice to slow progression of diabetic nephropathy include angiotensin system inhibition (1–3), blood pressure and glycemic control (4,5), and isocaloric protein restriction (6). Despite these remedies, the incidence of diabetic end-stage renal disease (ESRD) steadily increased over the past three decades, reaching epidemic proportions (7,8). Because little change occurred over the same time-span in average per capita protein intake (9), it seems possible other nutritional factors may be implicated. Several lines of evidence support this view.

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ASI, angiotensin system inhibition; CHO, carbohydrate; CR-LIPE, carbohydrate-restricted, low-iron-available, polyphenol-enriched; ESRD, end-stage renal disease; GFR, glomerular filtration rate; MAP, mean arterial pressure; RRT, renal replacement therapy.

First, although protein restriction delays aging-related glomerulosclerosis (6,10), animal studies showed that limiting calorie intake is, by far, more effective (10–15). Second, not all proteins have the same renal effects. For example, in normal (16), dyslipidemic (17), and diabetic humans (18) ingestion of vegetable protein did not increase glomerular filtration rate (GFR), leading to hyperfiltration. Furthermore, some plant foods have advantageous properties, apparently unrelated to macronutrient composition. For example, polyphenolics extracted from tea inhibited mesangial proliferation (19) and significantly prolonged renal survival in experimental models of glomerulosclerosis (20). Third, iron was an important factor in the progression of experimental nephropathy after the initial offending agent was removed (21). Iron promoted acute tissue damage during ischemia reperfusion (22) and chronic interstitial inflammation and fibrosis in animal models of renal failure associated with chronic proteinuria (23,24). Conversely, iron deficiency or chelation with deferoxamine prevented renal histological and functional deterioration (23,24). Reduction of body iron can be induced by use of a low-iron available diet, i.e., a diet where iron absorption inhibitors (dairies, phytates, and polyphenols) prevail on enhancers (red meat, ascorbate, and citrate) (25).

Because limitation of carbohydrate (CHO) intake was the main component of calorie restriction (26), it was therefore hypothesized that combining high polyphenols intake and low iron availability with CHO restriction may prolong kidney survival and delay the need of renal replacement therapy (RRT) more effectively than protein restriction. To assess this hypothesis, a 50% CHO-restricted, low-iron-available, polyphenol-enriched, diet (CR-LIPE) was recommended to a cohort of diabetic patients with various degrees of renal failure and proteinuria. Outcome development, e.g., doubling of initial serum creatinine, ESRD, and death, were subsequently noted over a mean time course of 3.9 years and compared with that of similar patients treated by standard protein restriction (control subjects).

RESEARCH DESIGN AND METHODS

The following investigation was carried out in accordance with the principles of the Declaration of Helsinki as revised in 1996. A total of 191 consecutive type 2 diabetic patients referred to nephrology clinics for various degrees of renal failure (GFR 15 ÷ 75 ml/min) and otherwise unexplained proteinuria (350 ÷ 12,000 mg/day) were randomized to either CR-LIPE or conventional standard-of-care dietary treatment (control subjects). Nephropathy was attributed to diabetes when it satisfied the following criteria: slowly increasing serum creatinine concentration (e.g., chronic renal failure), negative serolog-

TABLE 1
Macronutrient composition of CR-LIPE and control diets

Variable	CR-LIPE	Control
Carbohydrate	35	65
Fat	30	25
Protein	25–30	10
Ethanol	5–10	0

Data are %.

ical work-up (ANA, RA, HIV, hepatitis C, B, C3, C4, and serum and urine protein electrophoresis), no history of offending drug or toxin exposure, inactive sediment on urinalysis and symmetrical kidneys of normal or increased size on abdominal ultrasonography. When it subsisted a doubt (hematuria, lack of documented retinopathy, or small kidneys on ultrasonography), a renal biopsy was undertaken to confirm the diagnosis. Randomization and concealed patient allocation to either treatment arm was performed by staff personnel blinded to the aim of the study. Sample size calculation was estimated on the basis of former survival analysis from CHO-restricted animal experiments and from iron depletion experiments leading to 50% reduction of insulin resistance. Sample size calculation, according to the method delineated by Lakatos (27) for randomized trials with a survival or binary outcome, yielded a number of 93 subjects per group. Dietary recommendations were given in an intent-to-treat mode, and they complemented, not substituted, angiotensin system inhibition (ASI) and pharmacotherapy for glycemic and blood pressure control.

CR-LIPE main features included the following:

- 50% reduction of CHO (from the previous level of intake)
- substitution of iron-enriched red meats (beef and pork) with iron-poor (28) white meats (poultry and fish) and with protein-enriched food items known to inhibit iron absorption, e.g., dairy, eggs, and soy (25,29)
- elimination of all beverages other than tea, water, and red wine. Milk was recommended for breakfast. Tea was highly recommended. Red wine was not to exceed 150 ml with lunch and 150 ml with dinner. Outside mealtimes, water was the only approved beverage
- exclusive use of polyphenol-enriched extra-virgin olive oil (30,31) for both dressing and frying

Except for limiting CHO intake, CR-LIPE was fed ad-libitum.

The control diet was a standard protein-restricted (0.8 g/kg) diet (32), isocaloric for ideal body weight maintenance where no specific recommendations were given regarding pattern of beverage use (except for avoiding sucrose-containing beverages). The macronutrient composition of the two diets, as suggested to the patients, are illustrated in Table 1. The control diet was estimated (25) to have a four- to fivefold greater iron bioavailability than CR-LIPE.

At presentation and follow-up, weight, automated blood pressure, and serum chemistries were obtained. Twenty-four–hour urinary creatinine and protein excretion rates were determined at baseline. Hemoglobin, glycosylated hemoglobin, serum cholesterol, creatinine, and ferritin concentrations were determined at baseline and follow-up. All assays were performed in the same laboratory by routine techniques.

Outcomes were doubling of serum creatinine, ESRD (as defined by a sustained elevation of serum creatinine concentration to levels ≥530 μmol/l [6.0 mg%], RRT, or transplantation) and all-cause mortality.

Statistical analysis. Results are expressed as means ± SD. Unpaired and paired two-tailed Student's *t* tests were used for intra- and intergroup comparisons. Survival analysis (Kaplan-Meier procedure) and Cox's regression (proportional hazards model) were used to analyze outcome development in a time-independent manner and to adjust for other predictors of diabetic nephropathy progression. Comparison of the survival curves was made by the log-rank test. All calculations were performed with a commercial statistical software (Statsoft, Tulsa, OK) for the MacIntosh (mod ibook; Apple Computers, Cupertino, CA).

TABLE 2
Baseline characteristics of the two groups

Variables	CR-LIPE group (n = 100)	Control group (n = 91)	P
Age (years)	59 ± 10	60 ± 12	NS
Sex (M/F)	53/47	48/43	NS
BMI (kg/m^2)	28 ± 5	28 ± 5	NS
Diabetes duration (years)	9 ± 4	10 ± 5	NS
SBP (mmHg)	156 ± 22	157 ± 25	NS
DBP (mmHg)	87 ± 8	89 ± 9	NS
MAP (mmHg)	107 ± 16	108 ± 17	NS
HbA_{1c} (%)	7.6 ± 1.6	7.7 ± 1.6	NS
Creatinine (μmol/l)	159 ± 53	168 ± 62	NS
GFR (ml/min)	64 ± 28	62 ± 32	NS
Proteinuria (mg/day)	2,411 ± 2,371	2,533 ± 2,488	NS

Data are means ± SD. DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure.

TABLE 3
Use of conventional medications in the two study groups

Drug	Baseline	2 years	4 years
Aspirin			
CR-LIPE	57 (57)	49 (56)	43 (59)
Control	59 (54)	45 (60)	29 (60)
Statins			
CR-LIPE	9 (9)	8 (9)	7 (10)
Control	7 (8)	8 (11)	6 (12)
ASI			
CR-LIPE	78 (78)	67 (76)	53 (72)
Control	69 (76)	56 (75)	34 (70)
Ca^{2+} antagonist			
CR-LIPE	26 (26)	24 (27)	21 (29)
Control	23 (25)	19 (25)	14 (29)
Central adrenergic blocker			
CR-LIPE	13 (13)	15 (17)	13 (18)
Control	13 (14)	12 (16)	10 (21)
β-blocker			
CR-LIPE	15 (15)	17 (19)	15 (20)
Control	16 (18)	13 (17)	9 (19)
α-blocker			
CR-LIPE	11 (11)	12 (14)	11 (15)
Control	12 (13)	10 (13)	9 (19)
Diuretic			
CR-LIPE	62 (62)	55 (63)	48 (66)
Control	55 (60)	48 (64)	31 (65)
Insulin			
CR-LIPE	49 (49)	41 (47)	34 (47)
Control	46 (51)	38 (51)	26 (54)
Metformin			
CR-LIPE	6 (6)	5 (6)	5 (7)
Control	5 (5)	5 (7)	4 (8)
Sulfonylurea			
CR-LIPE	23 (23)	18 (20)	14 (19)
Control	24 (26)	19 (25)	10 (21)

Data are n (%)

RESULTS

A total of 21 patients were lost to follow-up, 9 in CR-LIPE and 12 in the control group, due to either loss of insurance or moving out of town. The demographic, clinical, and metabolic characteristics of these patients were similar to the ones who returned for follow-up.

There were 170 patients who met the last follow-up assessment or in whom an outcome developed: 91 in the CR-LIPE and 79 in the control groups. The two groups had comparable age and BMI (Table 2). Also, Tables 2 and 3 show that initial serum creatinine and 24-h proteinuria, blood pressure, glycosylated hemoglobin, and proportion of patients using ASI or other medications were similar between the two groups. Furthermore, follow-up blood

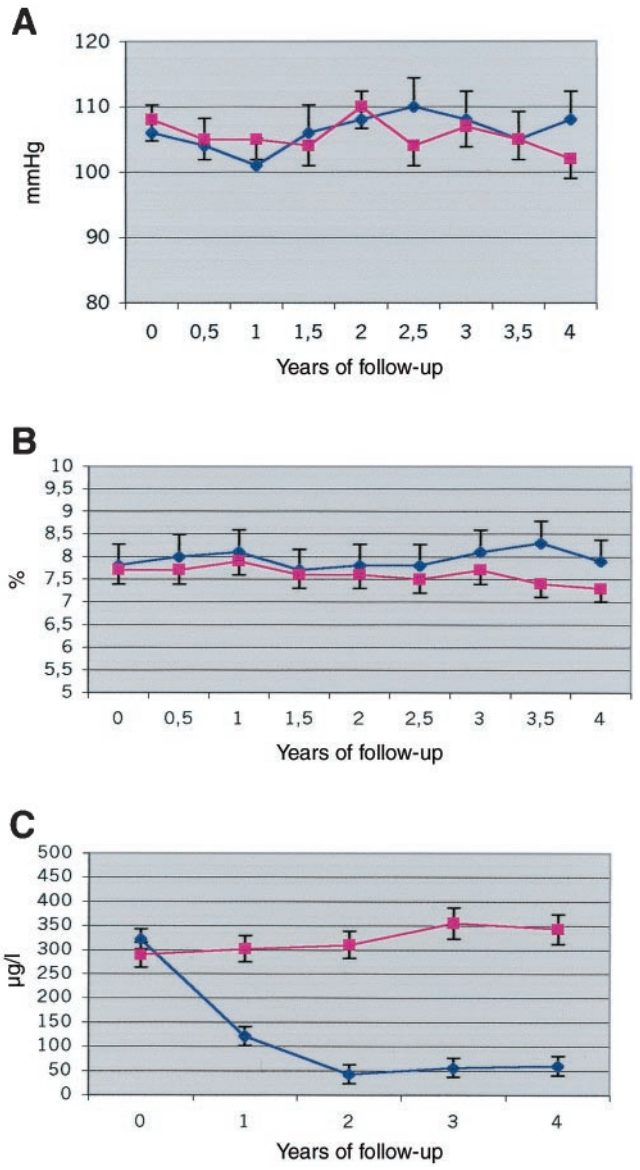

FIG. 1. Longitudinal changes of mean arterial pressure (*A*), glycosylated hemoglobin (*B*), and serum ferritin (*C*) concentrations in CR-LIPE (◆) and control (■) subjects.

pressure, HbA_{1c}, and ASI use were comparable between the two groups (Fig. 1*A* and *B* and Table 3). Body iron stores were assessed by serum ferritin. In patients with chronic renal failure not on RRT, serum ferritin is a fairly reliable estimate of iron stores (33). In CR-LIPE patients, serum ferritin concentration decreased from 301 ± 162 to 36 ± 31 µg/l ($P < 0.001$), while it was unchanged in control subjects (Fig. 1*C*).

Over a mean follow-up interval of 3.9 ± 1.8 years (range 0.7–5.3), serum creatinine concentration doubled in 19 patients on CR-LIPE (21%) and in 31 control subjects (39%) ($P < 0.01$) (Fig. 2*A*). RRT or death occurred in 18 patients on CR-LIPE (20%) and in 31 control subjects (39%) ($P < 0.01$) (Fig. 2*B*). RRT and death occurred in 10 and 8 patients on CR-LIPE and in 17 and 14 control subjects, respectively. Renal and general survival are graphically illustrated in Fig. 3.

The different rate of outcome development among CR-LIPE and control subjects was independent of length of time from entry to the last follow-up (or until an outcome occurred), sex, MAP, HbA_{1c}, ASI use, and baseline proteinuria (Table 4). Because differences in outcome occurrence might also relate to severity of the initial renal dysfunction, group subanalysis was performed on the basis of whether initial serum creatinine was ≤132.6 µmol/l (1.5 mg%) or >132.6 µmol/l. In the CR-LIPE group there were 45 of 91 individuals with an initial serum creatinine ≤132.6 µmol/l. Serum creatinine doubled in 29% of these patients and in 13% of those with values >132.6 µmol/l ($P < 0.02$). In the control group, there were 38 of 79 individuals with a serum creatinine >132.6 µmol/l. Among control subjects, the proportion of patients with doubling of serum creatinine was similar (37 and 41%, respectively) regardless of initial serum creatinine concentration.

Finally, the effects of CR-LIPE on some nutritional parameters, including serum cholesterol, are illustrated in Table 5. Fasting lipid data were available in 53 of 91 patients on CR-LIPE and in 48 of 79 control subjects. In the CR-LIPE group there were statistically significant changes of HDL cholesterol and of the ratio among total and HDL cholesterol, as expected on the basis of a reduction of CHO intake.

DISCUSSION

Diabetic renal disease is one of the most disabling conditions known, with a generally ominous prognosis. The aim of the current study was to identify a dietary pattern capable of slowing the progression of diabetic nephropathy more effectively than a low-protein diet. Because CHO restriction, polyphenol intake, and iron lowering delayed ESRD in animal models of chronic renal failure (10,11,20,23,24), all were combined and simultaneously

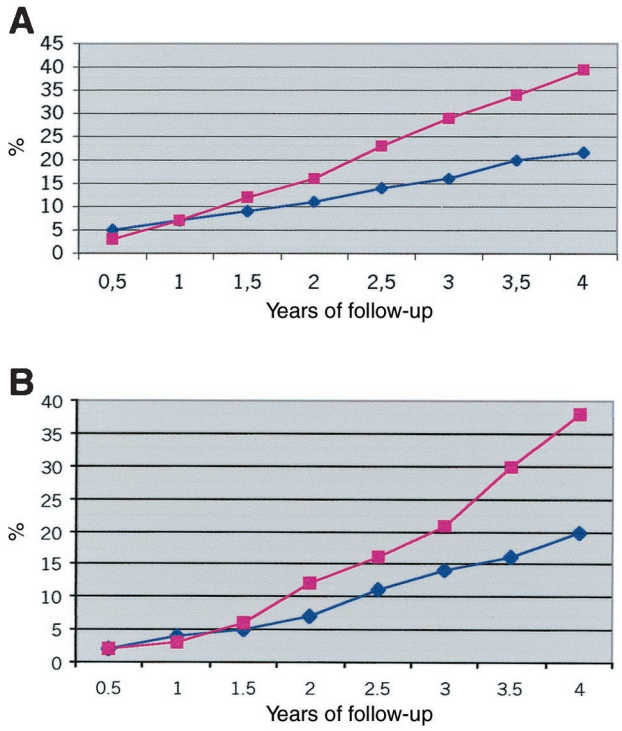

FIG. 2. Cumulative percentage of individuals in whom serum creatinine concentration doubled from baseline (*A*) and of those who either died or reached ESRD (*B*) ◆, CRP-LIPE; ■, control.

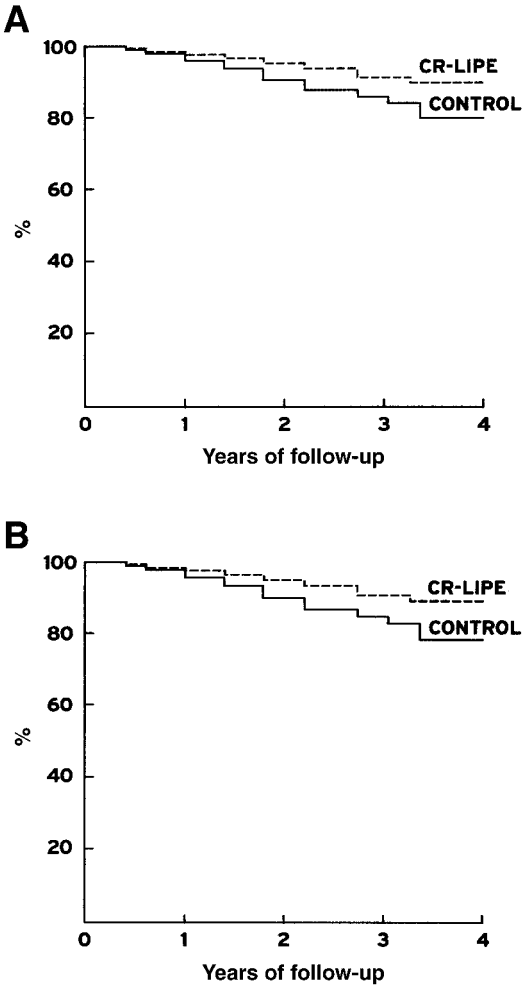

FIG. 3. Kaplan-Meier survival curves with death (*A*) and ESRD (*B*) as nonsurvival criteria.

implemented in a cohort of patients affected by diabetic nephropathy. When compared with standard protein restriction, ad-libitum intake of CR-LIPE significantly reduced all-cause mortality and rate of renal function loss and delayed ESRD and RRT. These effects were independent from other predictors of progression and more apparent in those individuals with an initial serum creatinine >132.6 μmol/l. Although no proven explanation exists for this latter finding, better compliance was possibly a factor, especially among symptomatic patients. Conversely, control subjects showed the expected steady rate of renal function loss, independent of initial serum creatinine and quite similar to that recently reported in patients with nephropathy due to type 2 diabetes (3). In the CR-LIPE group, the combination of high polyphenols, dairy, and no red meat also lead to a marked reduction of serum ferritin concentration. Polyphenols inhibit the digestion and absorption of protein, energy, and iron, particularly those contained in red wine and tea (35). For example, as little as 200–300 mg of red wine tannins (contained, on average, in 200 ml of red wine) diminished greater than threefold iron absorption from either 7% ethanol in water or from a bread-based meal (36).

TABLE 4
Cox regression analysis between predictor and outcome variables

	ESRD		Death	
	HRR	(95% CI)	HRR	(95% CI)
Age	1.05*	(1.01–1.1)	1.08*	(1.03–1.11)
Sex	0.98	(0.88–1.1)	0.97	(0.89–1.13)
MAP	1.02	(0.81–1.16)	1.03	(0.87–1.18)
HbA_{1c}	1.03	(0.9–1.19)	0.99	(0.75–1.26)
Creatinine	1.15†	(0.95–1.23)	1.11	(0.89–1.33)
Proteinuria	1.09	(0.92–1.3)	1.13	(0.69–1.7)
Not on ASI	1.36*	(1.11–1.56)	1.19	(0.88–1.34)
Not on CR-LIPE	1.32†	(1.1–1.44)	1.44*	(1.13–1.68)

*$P < 0.02$; †$P < 0.05$. HRR, hazard risk ratio.

TABLE 5
Effect of CR-LIPE and control diets on body weight, hemoglobin, serum albumin, and plasma cholesterol

Variable	Baseline	Follow-up
Weight (kg)		
CR-LIPE	78 ± 15	76 ± 14
Control	79 ± 16	78 ± 14
Albumin (g/l)		
CR-LIPE	42 ± 5	41 ± 6
Control	41 ± 6	41 ± 7
Hgb (g/l)		
CR-LIPE	141 ± 21	140 ± 20
Control	144 ± 22	140 ± 26
TC (mmol/l)		
CR-LIPE	5.5 ± 1.3	5.8 ± 1.4
Control	5.7 ± 1.3	5.5 ± 1.5
LDLC (mmol/l)		
CR-LIPE	3.61 ± 0.97	3.68 ± 1.01
Control	3.59 ± 1.10	3.47 ± 1.99
HDLC (mmol/l)		
CR-LIPE	0.99 ± 0.39	1.22 ± 0.50*
Control	0.97 ± 0.35	0.92 ± 0.41
TC/HDLC		
CR-L	5.4 ± 0.9	4.7 ± 0.7*
Control	5.5 ± 1.0	5.8 ± 1.1

Data are means ± SD. HDLC, HDL cholesterol; LDLC, LDL cholesterol; TC, total cholesterol. *$P < 0.05$.

Iron absorption is further blunted by calcium-enriched products, such as dairy and milk (37), as well as by eggs (38), and all of these expedients in conjunction with the elimination of red meat lead to a marked reduction in dietary iron availability and in serum ferritin, e.g., to values commonly seen in lacto-ovo vegetarians (39). Lacto-ovo vegetarians do not eat meat, have low iron stores, are less insulin resistant (39), and have a 40–50% decreased risk of death (40,41), similar in entity to the risk reduction found in the present trial. Despite lower ferritin values and a marginal iron status, lacto-ovo vegetarians do not develop iron deficiency anemia to a greater extent than meat-eaters, and, accordingly, anemia was not more prevalent in patients on CR-LIPE. However, it is possible that with erythropoietin replacement, increasing iron needs may render CR-LIPE less beneficial or even hazardous. In the present trial an insufficient number of patients was on erythropoietin; therefore, further studies will be necessary to clarify this issue. The other main component of CR-LIPE was CHO restriction. Rodents fed sucrose develop a chronic nephropathy characterized by glomerulosclerosis, thickening of basement membranes and kidney enlarge-

ment, five times more frequently than sucrose-restricted controls (11). This effect is not sucrose specific, as it was reproduced with corresponding variations of dietary dextrin, glucose, or cornstarch (10,12,13). Level of animal protein intake, including nonmeat protein (casein), can also delay renal failure (6,10–12). As meat increases iron status, casein-based studies better estimated the effect of protein, independent of iron, on renal survival. The conclusion of such studies was that casein restriction prolonged renal survival but only during ad-lib intake of a 60% CHO diet. However, a 40% calorie restriction was not only more beneficial (than protein restriction) but also abolished the renoprotective effect of casein restriction (10–12), indicating that casein becomes detrimental only above a threshold of CHO intake. The notion that protein intake is harmful for the kidney above a threshold of CHO intake is substantiated by the simultaneous surge of CHO consumption, obesity, diabetes, and renal failure that has happened in countries such as the U.S. over the past three decades. Between 1970 and 1997, in fact, U.S. per capita intake of grains and sweeteners doubled, while that of total CHO increased nearly 50% (9). Conversely, protein per capita intake increased only marginally (~12%) and fat remained the same (9). These trends are consistent with the notion that excess CHO intake is one key factor in raising incidence of diabetic renal failure. Although CHO restriction was presumably important in the better outcome of patients on CR-LIPE, it is not possible at this time to rank which factor was more or less effective. This is one limitation of the present study. However, the aim of this investigation was to delay renal failure and mortality more effectively than by means of protein restriction. Distinguishing the relative importance of each component of CR-LIPE or dissecting mechanistic pathways were not our objectives. On the other hand, there is evidence all three components of CR-LIPE lead to downregulation of insulin signaling and oxidative stress pathways. Oxidative stress and free-radical generation were implicated in the genesis and progression of diabetic nephropathy (42). The insulin-sensitizing effect of iron depletion is known (39,43,44), and glycemia and insulinemia can be further lowered by both CHO restriction (45,46) and polyphenol intake (47,48). Because iron, hyperinsulinemia, and hyperglycemia act in concert to upregulate free-radical reactions (rev. in 26), it is possible, although yet to be demonstrated, that CR-LIPE slowed progression of diabetic nephropathy by downregulating oxidative stress pathways of tissue damage.

Compliance and nutrient and energy intake were not estimated, another limitation of the present study. However, the substantial decrease and subsequent stability of serum ferritin concentration suggests fair adherence to dietary guidelines. Furthermore, patients on CR-LIPE maintained their body weight, indicating that, although the two diets presumably differed in ingested calories, available energy was similar. Given that both greater polyphenol intake and iron depletion augment weight-maintenance energy needs (34,35,49), this finding was not unexpected. Moreover, lack of dietary and compliance information seems an insufficient ground to dismiss the major findings of the present study, such as that CR-LIPE caused a greater than fivefold reduction of serum ferritin and nearly doubled renal and overall survival rates. If confirmed, these preliminary findings should encourage a changing dietary prescription in patients with diabetic nephropathy. In addition, since calorie excess, iron, and pro-oxidants likely hasten progression of other age-related nephropathies, further research should establish whether CR-LIPE may be useful in these circumstances as well.

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