The Serum Lathosterol to Cholesterol Ratio, an Index of Cholesterol Synthesis, Is Not Elevated in Patients With Glomerular Proteinuria and Is Not Associated With Improvement of Hyperlipidemia in Response to Antiproteinuric Treatment

Robin P.F. Dullaart, Ron T. Gansevoort, Wim J. Sluiter, Dick de Zeeuw, and Paul E. de Jong

The hypothesis that increased cholesterol synthesis provides a mechanism that contributes to nephrotic syndrome–associated hyperlipidemia is mainly based on experimental evidence. The serum level of the cholesterol precursor, lathosterol (expressed per millimole cholesterol), is a reliable marker of whole-body cholesterol synthesis in normocholesterolemia and primary hypercholesterolemia. Serum lathosterol and lipoprotein levels were measured in 11 moderately hyperlipidemic patients with nephrotic-range proteinuria and 22 matched controls. The proteinuric patients were evaluated before and during three antiproteinuric treatment periods with angiotensin-converting enzyme (ACE) inhibition therapy (n = 6) or a low-protein diet (n = 5) alone, in combination, and again as a single treatment. In untreated patients, serum total cholesterol, very–low-density (VLDL) and low-density (LDL) lipoprotein cholesterol, apolipoprotein B (apo B), and lipoprotein (a) [Lp(a)] levels were higher than in controls ($P < .01$ to $P < .001$), but the lathosterol to cholesterol ratio tended to be lower in patients (0.99 ± 0.43 μmol/mmol) as compared with controls (1.29 ± 0.41 μmol/mmol, $P < .10$). During combined antiproteinuric treatment, total and VLDL + LDL cholesterol, apo B, and Lp(a) decreased ($P < .02$ to $P < .01$), but remained higher than levels in controls. Yet the serum lathosterol to cholesterol ratio changed little and was even lower ($P < .05$) in treated patients than in controls. Serum total cholesterol ($r = -.82$, $P < .01$) and apo B ($r = -.84$, $P < .01$) were inversely correlated with serum albumin in untreated patients, whereas the serum lathosterol to cholesterol ratio was not ($r = -.01$, NS). In the patient group, multiple regression analysis showed that changes in the lathosterol to cholesterol ratio during the study were only related to changes in the dietary polyunsaturated to saturated fatty acids ratio (P:S) coinciding with the low-protein diet ($P < .01$). In contrast, the decrease of VLDL + LDL cholesterol, apo B, and Lp(a) was independently related to reduction of proteinuria ($P < .02$ to $P < .001$), but not to changes in the lathosterol to cholesterol ratio. In conclusion, the present data, based on the serum lathosterol to cholesterol ratio, do not support the concept that increased cholesterol synthesis plays an important role in the maintenance of human nephrotic syndrome–associated hypercholesterolemia. Moreover, it appears unlikely that the decrease of apo B–containing lipoproteins with antiproteinuric treatment is attributable to inhibition of cholesterogenesis. These findings warrant further documentation of cholesterol synthesis in human nephrotic syndrome by direct methods.

ELEVATED LEVELS of serum total cholesterol, very–low-density and low-density lipoprotein (VLDL + LDL) cholesterol, and triglycerides, as well as lipoprotein(a) [Lp(a)], are well-established features of the nephrotic syndrome.[1-6] It is generally assumed that these lipoprotein abnormalities contribute to premature development of cardiovascular disease.[5,7] Indeed, a threefold to fivefold increased cardiovascular risk has been recently documented in patients with glomerular proteinuria.[8]

Both increased production of apolipoprotein B (apo B)–containing lipoproteins (VLDL + LDL) and impaired catabolism have been suggested to contribute to the hyperlipidemia.[2,4,5] It is assumed that hepatic lipoprotein synthesis is stimulated in response to hypoalbuminemia, low oncotic pressure, and urinary albumin loss.[1,2,4,5,9] This hypothesis is largely derived from experimental studies in animals showing increased hepatic production of apolipoproteins and lipids,[10-13] but there is little evidence to demonstrate increased cholesterol synthesis in human nephrotic syndrome.[14] The serum level of the cholesterol precursor, lathosterol, is considered a valid index of cholesterol synthesis in man.[15-21] Serum lathosterol, expressed per millimole cholesterol, is positively correlated with whole-body cholesterol synthesis in normolipidemic[16,18] and hypercholesterolemic subjects.[17,21] There is also a close relationship between the level of this cholesterol precursor and hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, the rate-limiting enzyme of cholesterol production.[15,22] Moreover, interventions that cause inhibition of cholesterol synthesis such as HMG-CoA reductase inhibitor treatment result in a decrease in the serum lathosterol to cholesterol ratio in primary hypercholesterolemic patients[16,19,21,23] and nephrotic patients,[24] whereas serum lathosterol increases in parallel with cholesterol synthesis in response to bile acid sequestrants.[20,23]

In the present study, we measured the serum lathosterol to cholesterol ratio as an index of cholesterol synthesis in patients with nephrotic-range proteinuria and matched healthy controls. Recent reports demonstrate that decreasing the proteinuria improves the hyperlipidemia.[25-28] Since it is unknown whether this decrease in lipoprotein cholesterol coincides with changes in cholesterol synthesis, the serum lathosterol to cholesterol ratio and lipoprotein levels were also examined in response to antiproteinuric therapy with an angiotensin-converting enzyme (ACE) inhibitor and a low-protein diet, both as a single treatment and in combination.

SUBJECTS AND METHODS

Patients and Clinical Procedures

All subjects consented to participate in the study, which was approved by the local medical ethics committee. Entry criteria

From the Divisions of Endocrinology and Nephrology, Department of Internal Medicine, State University Hospital, Groningen, The Netherlands.

Submitted March 6, 1995; accepted November 13, 1995.

Address reprint requests to Robin P.F. Dullaart, MD, Department of Internal Medicine, Division of Endocrinology, University Hospital Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.

0026-0495/96/4506-0010$03.00/0

were biopsy-proven glomerulopathy, stable proteinuria greater than 3 g/24 h, and creatinine clearance greater than 40 mL/min. Exclusion criteria were diabetes mellitus, thyroid dysfunction, familial forms of hyperlipidemia (familial hypercholesterolemia, familial combined hyperlipidemia, and known dysbetalipoproteinemia), use of corticosteroids or cytotoxic agents, edema, and renovascular hypertension. None of the patients had xanthomas or palmar streaks. The protocol was designed to evaluate the possible additive effect of ACE inhibition and a low-protein diet on urinary protein excretion in 14 patients, and the results have been reported elsewhere.[29] Of these 14 patients, 11 subjects participated in the present study. Three patients could not be included because serum samples for lipid analysis were not available. Each patient was individually matched with two healthy controls with respect to age, gender, body mass index (calculated as weight in kilograms divided by height in meters squared), smoking status, and oral contraceptive use. All subjects were white. Eight patients used diuretics, which were unchanged during the study.

The healthy control subjects (n = 22) were studied once, while on their habitual diet. The proteinuric patients were investigated during four consecutive study periods, each lasting 2 months. Clinical and laboratory measurements were made at the end of each period. The patients were first studied during a baseline period in which they followed a dietician's advice to consume 1.5 or 1.0 g protein/kg/d (depending on preentry creatinine clearance being > or <70 mL/min). Thereafter, five patients (subgroup A) started a low-protein diet aimed to reduce protein intake by 50% of baseline intake. These five participants continued the low-protein diet throughout the following three study periods. In period 3, an ACE inhibitor (enalapril, 10 mg once daily) was used, which was discontinued in period 4. The six other patients (subgroup B) started treatment with enalapril 10 mg once daily in period 2, which was continued during the following three study periods. In period 3, a low-protein diet was started in these patients, which was replaced by the baseline diet in period 4. If proteinuria exceeded 15 g/24 h, patients were allocated to subgroup B, since it was considered undesirable to withhold ACE inhibition therapy in such patients over a prolonged period. Thus, the patients were studied twice during a single treatment with either the low-protein diet or the ACE inhibitor (periods 2 and 4) and once during combined therapy (period 3).

The diets were isocaloric for each patient, and a sodium restriction of 50 to 100 mmol/d was prescribed throughout the study. The composition of the diets was analyzed with the aid of a computer-assisted Nutrient Data Base.[30] Dietary protein intake was assessed by measurement of urinary urea excretion using the formula proposed by Maroni et al.[31] Assuming nitrogen balance, protein intake (grams per 24 hours) was calculated as 6.25 × (0.0276 × urea excretion [millimoles per 24 hours] + 0.031 × body weight [kilograms]) + proteinuria (grams per 24 hours). Three 24-hour urine collections were obtained at the end of each period for urea measurement, and the results were averaged for analysis. Supine blood pressure was recorded with an automated device (Dinamap, Critikon, FL) in patients and by the auscultatory method in controls. Mean arterial pressure (MAP) was calculated as two thirds of the diastolic pressure plus one third of the systolic pressure. In each participant, venous blood was obtained after a 12-hour fast at 8 AM.

Laboratory Methods

Venous blood for measurement of lathosterol, lipid, apolipoprotein, and Lp(a) levels was collected into vacuum tubes. Serum was separated from erythrocytes by centrifugation at 3,000 rpm for 15 minutes within 1 hour after collection. Samples were kept frozen at −20°C and assayed within 2 months after completion of the study. Serum samples from control subjects were stored under identical conditions for approximately 1 year. Thus, the storage period in untreated patients and controls was similar. The serum level of total (esterified plus free) 7-lathosterol (5α-cholest-7-en-3β-ol) was quantified by gas chromatographic analysis of saponified extracts as described previously.[18] 5β-Cholestan-3α-ol was used as an internal standard. Since noncholesterol sterols like lathosterol are carried on lipoproteins in serum, use of the serum lathosterol to cholesterol ratio as an index of cholesterol synthesis has been advocated to avoid nonspecific effects of differences in serum cholesterol on the lathosterol level.[16-21] In the present study, we expressed serum lathosterol in absolute terms and as per millimole cholesterol and per gram apo B. Cholesterol and triglyceride levels were measured enzymatically using automated methods. Cholesterol was determined in serum and in the high-density lipoprotein (HDL)–containing supernatant fraction after precipitation of apo B–containing lipoproteins with polyethylene glycol 6000.[32] Apos AI and B were assayed by immunoturbidimetry using commercially available kits (Boehringer, Mannheim, Germany; no. 726478 and 726494, respectively). Lp(a) was determined with an enzyme-linked immunosorbent assay (no. 610221, Tint-Elize Lp(a); Biopool, Umea, Sweden).[33] Cross-reactivity of the Lp(a) antibodies with LDL cholesterol and plasminogen is negligible. The detection limit of the assay is 1 mg/L. With this method, a minor decrease in apparent serum Lp(a) concentration has been noted after prolonged freezing.[27] Lathosterol, lipid, apolipoprotein, and Lp(a) measurements for each patient were performed in one run, and all assays were performed on blinded samples. Serum creatinine and albumin and urinary creatinine and urea levels were measured on SMAC and SMA-II autoanalyzers, respectively (Technicon Instruments, Tarrytown, NY). Urinary protein was quantified by the pyrogallol red–molybdate method.[34]

Statistical Analysis

The data are expressed as the mean ± SD, except for Lp(a), which is presented as the geometric mean and range because of its skewed distribution. Parameters from patients and control subjects were compared by the unpaired Wilcoxon test (two data sets) and by Kruskal-Wallis ANOVA (three data sets: controls *v* patients at baseline and during combined treatment). Changes in parameters within the whole patient group and within subgroups were analyzed by the paired Wilcoxon test (combined treatment *v* baseline) and by Friedman's two-way ANOVA (three treatment periods *v* baseline). Duncan's method was applied to correct for multiple comparisons. Bivariate correlation coefficients were calculated by linear regression analysis. Multiple regression analysis was applied to discern the independent contributions of parameters. A two-sided P value less than .05 was taken as significant.

RESULTS

Renal biopsy showed glomerulosclerosis in five patients, membranous glomerulopathy in three, membranoproliferative glomerulonephritis in two, and IgA nephropathy in one. In the patient group, proteinuria ranged from 3.3 to 21.2 g/24 h. Patients were closely matched to controls with respect to age (43 ± 17 *v* 41 ± 13 years, NS), body mass index (24.2 ± 3.1 *v* 24.5 ± 3.1 kg/m^2, NS), and gender (patients, three women and eight men; controls, six women and 16 men). In patients, baseline serum creatinine (124 ± 39 μmol/L; range, 66 to 206) was higher than in controls (91 ± 11 μmol/L, $P < .01$) and serum albumin was lower (26.6 ± 5.6 g/L) than in controls (91 ± 11 μmol/L, $P < .01$) and serum albumin was lower (26.6 ± 5.6

g/L) than in controls (47.4 ± 2.2 g/L, $P < .0001$). MAP was not significantly different in patients (99 ± 13 mm Hg) and controls (94 ± 9 mm Hg, NS). One male patient and two male controls smoked cigarettes, and one female patient and two female controls used oral contraceptives (Table 1).

Since the two subgroups were studied during a different sequence of antiproteinuric treatment regimens, clinical, dietary and lipid parameters are given both for the whole patient group (baseline and combined antiproteinuric treatment) and for the separate subgroups that started with either a low-protein diet (subgroup A, n = 5) or with ACE inhibition therapy (subgroup B, n = 6). Table 1 shows clinical and dietary parameters during the antiproteinuric treatment periods. Urinary protein excretion was lowest during combined treatment. The decrease in proteinuria was accompanied by a modest increase in serum albumin. Serum creatinine also increased during the treatment periods. ACE inhibition treatment elicited the expected decrease in MAP, but the low-protein diet did not influence blood pressure. Dietary protein intake was clearly lower during the low-protein diet periods, although the prescribed 50% reduction was not fully achieved (Table 1). Concomitant with the advice to decrease protein intake, dietary fat and cholesterol intake was reduced but the polyunsaturated to saturated fatty acids ratio (P:S) increased (Table 1).

Table 2 shows that serum levels of total cholesterol, triglycerides, VLDL + LDL cholesterol, apo B, and Lp(a) were higher in untreated patients than in controls. In contrast, HDL cholesterol was lower but serum apo AI was not different in patients compared with controls. With antiproteinuric treatment, serum total cholesterol, triglycerides, VLDL + LDL cholesterol, apo B, and Lp(a) decreased (Table 2). The lowest levels of apo B–containing lipoproteins were attained during combined antiproteinuric therapy. Of importance, all these lipid parameters remained significantly higher in patients compared with controls. HDL cholesterol did not change during treatment. Apo AI decreased, particularly in the low-protein diet group, but the apo AI/B ratio improved during combined treatment.

Table 3 shows the absolute serum lathosterol level, the lathosterol to cholesterol ratio, and the lathosterol to apo B ratio in nephrotic patients and controls. In contrast to the clearly elevated levels of apo B–containing lipoproteins, the

Table 1. Clinical Parameters and Dietary Protein and Fat Intake in Proteinuric Patients During Four Study Periods

Parameter	Baseline	Period 2 (single treatment)	Period 3 (combined treatment)	Period 4 (single treatment)
Proteinuria (g/24 h)				
All patients (n = 11)	11.5 ± 5.8		5.4 ± 4.1‡	
Subgroup A (n = 5)	7.9 ± 3.0	6.3 ± 2.8‡	3.6 ± 2.2‡§	5.2 ± 3.1‡
Subgroup B (n = 6)	14.4 ± 6.6	9.4 ± 6.1	6.5 ± 5.2‡	8.8 ± 4.9*
Serum albumin (g/L)				
All patients (n = 11)	26.6 ± 5.6		31.4 ± 5.2†	
Subgroup A (n = 5)	28.6 ± 4.3	29.2 ± 4.1	34.8 ± 4.0†	33.0 ± 5.3
Subgroup B (n = 6)	25.0 ± 26.8	29.0 ± 5.6	28.5 ± 5.0	28.2 ± 5.8
Serum creatinine (μmol/L)				
All patients (n = 11)	124 ± 39		144 ± 51†	
Subgroup A (n = 5)	107 ± 19	120 ± 36	124 ± 48	111 ± 33
Subgroup B (n = 6)	138 ± 48	155 ± 56*	162 ± 51‡	178 ± 75‡
MAP (mm Hg)				
All patients (n = 11)	99 ± 13		87 ± 13‡	
Subgroup A (n = 5)	96 ± 10	95 ± 9	82 ± 7‡§	94 ± 17
Subgroup B (n = 6)	101 ± 16	88 ± 13‡	92 ± 17*	92 ± 11*
Protein intake (g/kg/24 h)				
All patients (n = 11)	1.26 ± 0.30		0.82 ± 0.09‡	
Subgroup A (n = 5)	1.39 ± 0.40	0.79 ± 0.08‡	0.81 ± 0.09‡	0.83 ± 0.06‡
Subgroup B (n = 6)	1.16 ± 0.21	1.22 ± 0.23	0.83 ± 0.09*§	1.14 ± 0.24
Total fat (energy %)				
All patients (n = 11)	36.6 ± 5.4		28.5 ± 4.4‡	
Subgroup A (n = 5)	37.4 ± 4.1	25.9 ± 2.7		
Subgroup B (n = 6)	35.9 ± 6.6		30.7 ± 4.5*	
P:S ratio				
All patients (n = 11)	0.42 ± 0.20		0.60 ± 0.28*	
Subgroup A (n = 5)	0.53 ± 0.23	0.74 ± 0.37		
Subgroup B (n = 6)	0.33 ± 0.12		0.48 ± 0.13*	
Cholesterol (mg/24 h)				
All patients (n = 11)	289 ± 97		175 ± 65‡	
Subgroup A (n = 5)	303 ± 114	167 ± 66		
Subgroup B (n = 6)	278 ± 89		182 ± 70*	

NOTE. Data are the mean ± SD.

*$P < .05$, †$P < .02$, ‡$P < .01$: *v* baseline by paired Wilcoxon test or Friedman's 2-way ANOVA (subgroup A and B).

§$P < .05$ *v* periods 2 and 4 by paired Wilcoxon test.

Table 2. Serum Lipids, Apolipoproteins, and Lp(a) in Control Subjects and Proteinuric Patients During Four Study Periods

		Proteinuric Patients (n = 11)			
	Control Subjects (n = 22)	Baseline	Period 2 (single treatment)	Period 3 (combined treatment)	Period 4 (single treatment)
Serum total cholesterol (mmol/L)	5.25 ± 1.01	8.87 ± 2.88§		7.26 ± 2.04‡¶	
Subgroup A (n = 5)		8.05 ± 2.35	7.13 ± 2.28‖	6.51 ± 2.21#	6.81 ± 2.59#
Subgroup B (n = 6)		9.56 ± 3.56	8.38 ± 2.18	7.89 ± 2.04	8.45 ± 2.05
Serum triglycerides (mmol/L)	1.45 ± 0.50	3.08 ± 1.22§		2.38 ± 0.89‡‖	
Subgroup A (n = 5)		2.71 ± 1.51	2.16 ± 0.39	2.46 ± 1.27	2.58 ± 1.45
Subgroup B (n = 6)		3.39 ± 1.10	2.85 ± 0.76	2.32 ± 0.66‖	2.95 ± 0.91
HDL cholesterol (mmol/L)	1.16 ± 0.26	0.94 ± 0.34*		0.99 ± 0.45*	
Subgroup A (n = 5)		1.05 ± 0.46	1.00 ± 0.37	1.01 ± 0.65	0.97 ± 0.49
Subgroup B (n = 6)		0.85 ± 0.24	0.91 ± 0.27	0.96 ± 0.31	0.93 ± 0.22
VLDL + LDL cholesterol (mmol/L)	4.08 ± 1.05	7.93 ± 3.17§		6.27 ± 2.29‡¶	
Subgroup A (n = 5)		6.99 ± 2.52	6.13 ± 2.37¶	5.50 ± 2.53#	5.84 ± 2.85#
Subgroup B (n = 6)		8.71 ± 3.66	7.47 ± 2.12	6.92 ± 2.06	7.52 ± 2.07
Apo AI (g/L)	1.47 ± 0.15	1.58 ± 0.44		1.45 ± 0.34	
Subgroup A (n = 5)		1.48 ± 0.25	1.36 ± 0.28¶	1.32 ± 0.32¶	1.28 ± 0.23¶
Subgroup B (n = 6)		1.67 ± 0.60	1.55 ± 0.26	1.56 ± 0.37	1.55 ± 0.26
Apo B (g/L)	0.76 ± 0.17	1.49 ± 0.50§		1.13 ± 0.30‡¶	
Subgroup A (n = 5)		1.36 ± 0.37	1.08 ± 0.26‖	1.00 ± 0.28#	1.06 ± 0.38#
Subgroup B (n = 6)		1.59 ± 0.65	1.23 ± 0.32	1.23 ± 0.32	1.36 ± 0.37
Apo AI/B ratio	2.02 ± 0.48	1.21 ± 0.60‡		1.40 ± 0.61†‖	
Subgroup A (n = 5)		1.16 ± 0.40	1.31 ± 0.43	1.46 ± 0.79	1.36 ± 0.65
Subgroup B (n = 6)		1.25 ± 0.77	1.31 ± 0.40	1.35 ± 0.47	1.22 ± 0.38
Lp(a) (mg/L)	22 (1-243)	336 (10-1,878)§		245 (9-1,303)‡#	
Subgroup A (n = 5)		363 (28-939)	213 (19-924)#	269 (11-926)¶	240 (7-908)#
Subgroup B (n = 6)		315 (10-1,878)	244 (9-1,272)¶	224 (9-1,303)‖	259 (11-1,969)

NOTE. Data are the mean ± SD, except for Lp(a), which is reported as the geometric mean (range).

*$P < .05$, †$P < .02$, ‡$P < .01$, §$P < .001$: *v* control subjects by Kruskal-Wallis ANOVA.

‖$P < .05$, ¶$P < .02$, #$P < .01$: *v* baseline by paired Wilcoxon test (whole group) or Friedman's 2-way ANOVA (subgroups A and B).

absolute lathosterol level was not significantly higher in patients than in controls ($P > .20$). With combined antiproteinuric therapy, serum lathosterol decreased to a mean level that was similar in patients and controls. Importantly, in the combined untreated nephrotic patients and controls, the absolute concentration of lathosterol was positively related to total cholesterol and apo B ($r = .61$, $P < .001$ and $r = .65$, $P < .001$, respectively; data not shown), indicating a relationship between lathosterol level and the concentration and number of its carrying lipoproteins in serum. In addition, changes in serum lathosterol during combined treatment were correlated with changes in serum cholesterol ($r = .76$, $P < .01$; not shown). When lathosterol was therefore corrected for differences in cholesterol and apo B, it was found that the lathosterol to cholesterol ratio tended to be lower ($P < .10$; type II error, $\beta < .05$) and that the lathosterol to apo B ratio was significantly lower ($P < .05$) in untreated patients compared with controls. With antiproteinuric treatment, modest and variable changes were noted in these ratios. During combined treatment,

Table 3. Serum Lathosterol, Lathosterol to Cholesterol Ratio, and Lathosterol to Apo B Ratio in Control Subjects and Proteinuric Patients During Four Study Periods

		Proteinuric Patients (n = 11)			
Parameter	Control Subjects (n = 22)	Baseline	Period 2 (single treatment)	Period 3 (combined treatment)	Period 4 (single treatment)
Lathosterol (μmol/L)	6.63 ± 2.02	8.80 ± 4.87		5.79 ± 2.12§	
Subgroup A (n = 5)		9.14 ± 2.38	7.0 ± 2.79	5.48 ± 1.52‖	5.72 ± 1.02‖
Subgroup B (n = 6)		8.52 ± 6.54	5.97 ± 3.17‡	6.05 ± 2.81‡	6.55 ± 2.92
Lathosterol to cholesterol ratio (μmol/mmol)	1.29 ± 0.41	0.99 ± 0.43		0.85 ± 0.36*	
Subgroup A (n = 5)		1.19 ± 0.41	1.01 ± 0.36	0.91 ± 0.35	0.89 ± 0.20
Subgroup B (n = 6)		0.83 ± 0.41	0.71 ± 0.36	0.80 ± 0.40	0.79 ± 0.32
Lathosterol to apo B ratio (μmol/g)	8.75 ± 2.25	5.96 ± 2.70*		5.60 ± 2.99†	
Subgroup A (n = 5)		7.14 ± 2.98	6.45 ± 1.74	6.01 ± 3.10	5.87 ± 2.10
Subgroup B (n = 6)		4.98 ± 2.20	4.77 ± 2.29	5.26 ± 3.15	5.06 ± 2.34

NOTE. Data are the mean ± SD.

*$P < .05$, †$P < .01$: *v* control subjects by Kruskal-Wallis ANOVA.

‡$P < .05$, §$P < .02$, ‖$P < .01$: *v* baseline by paired Wilcoxon test (whole group) or Friedman's 2-way ANOVA (subgroups A and B).

both ratios were lower in patients than in controls. Figure 1 shows the serum lathosterol to cholesterol ratio in the study subjects.

In untreated nephrotic patients, inverse correlations of serum albumin with serum total cholesterol ($r = -.82$, $P < .01$; Fig 2A), VLDL + LDL cholesterol ($r = -.81$, $P < .01$; not shown), and apo B ($r = -.84$, $P < .01$; Fig 2B) were observed, whereas these lipid parameters were positively correlated with the degree of proteinuria ($r = .74$, $P < .01$, Fig 2D; $r = .74$, $P < .01$, not shown; and $r = .73$, $P < .01$, Fig 2E, respectively). In contrast, no significant correlations could be demonstrated between either serum albumin or proteinuria and the absolute serum lathosterol level ($r = -.53$, NS, and $r = -.48$, NS; not shown) and the lathosterol to cholesterol ratio ($r = -.01$, NS, Fig 2C; and $r = .01$, NS, Fig 2F).

Multiple regression analysis was performed for the patient group to establish which parameters independently contributed to changes in VLDL + LDL cholesterol, apo B, and Lp(a) during treatment. In this analysis, the serum lathosterol to cholesterol ratio was used as an index of cholesterol synthesis. Since they were studied on four occasions, each patient was included in the analysis as a separate categorical variable. Group assignment (as a categorical covariate), serum albumin, proteinuria, and dietary parameters (total energy from fat, cholesterol intake, and the P:S ratio) were included as possible independent factors. Patient category was an independent variable ($P < .001$ in all models), indicating that the serum lathosterol to cholesterol ratio, as well as lipoprotein levels, were individually determined. Group assignment was not a significant covariate ($P > .10$ in all models). The serum lathosterol to cholesterol ratio was only related to the P:S ratio (the higher this ratio, the lower the latherosterol to cholesterol ratio, $P < .01$), the relationships with lipid parameters, serum albumin, proteinuria, and dietary fat and cholesterol intake were not significant (all $P > .10$). VLDL + LDL cholesterol, in contrast, was independently related to proteinuria ($P < .001$), whereas the relationships with serum albumin and dietary factors were not significant (all $P > .10$). Apo B was related to both protein-

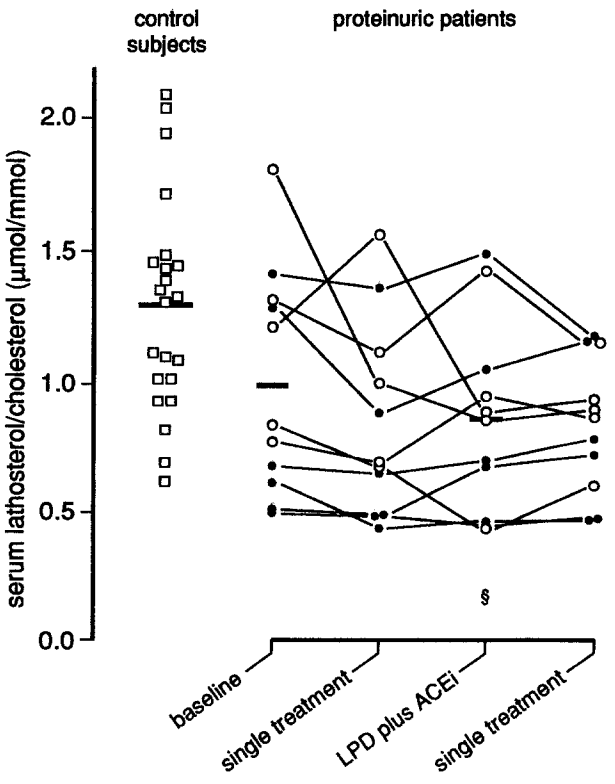

Fig 1. Serum lathosterol to cholesterol ratio in control subjects (□) and proteinuric patients during 4 consecutive study periods. Patients were assigned to either a low-protein diet (LPD, ○) or ACE inhibition (ACEi, ●) in periods 2, 3, and 4. Bars indicate mean values in controls and proteinuric patients at baseline and during combined antiproteinuric treatment. §$P < .05$ *v* control subjects by Kruskal-Wallis ANOVA.

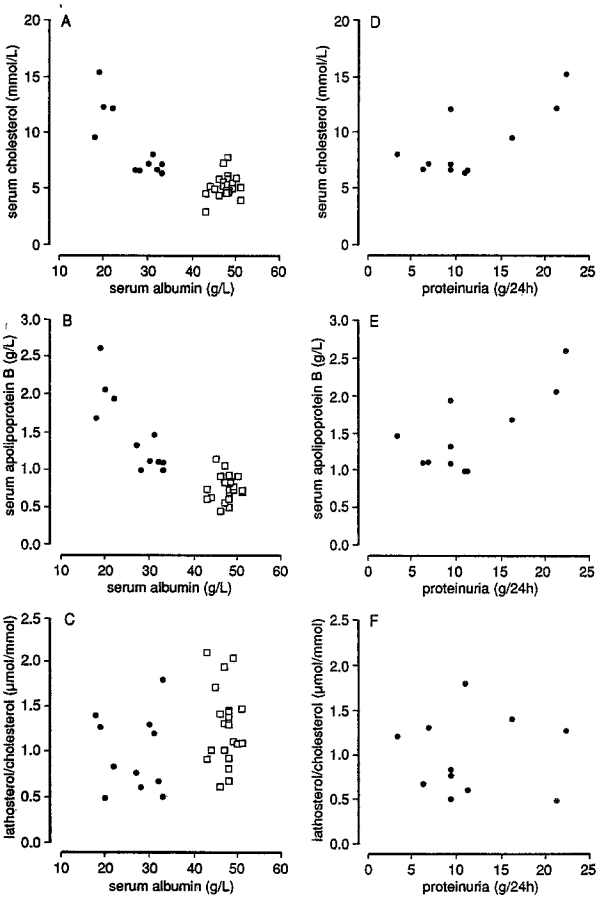

Fig 2. Relationships of serum total cholesterol, apo B, and the lathosterol to cholesterol ratio with serum albumin and with urinary protein excretion in 11 untreated proteinuric patients (●) and 22 control subjects (□). (A) Serum total cholesterol and serum albumin. Nephrotic patients, $r = -.82$, $P < .01$; control subjects, $r = .25$, NS. (B) Serum apo B and serum albumin. Nephrotic patients, $r = -.84$, $P < .01$; control subjects, $r = .07$, NS. (C) Serum lathosterol to cholesterol ratio and serum albumin. Nephrotic patients, $r = -.01$, NS; control subjects, $r = .01$, NS. (D) Serum total cholesterol and proteinuria. Nephrotic patients, $r = .74$, $P < .01$. (E) Serum apo B and proteinuria. Nephrotic patients, $r = .73$, $P < .01$. (F) Serum lathosterol to cholesterol ratio and proteinuria. Nephrotic patients, $r = .01$, NS.

uria ($P = .002$) and serum albumin ($P = .008$), but not to dietary parameters (all $P > .10$). Lp(a) was only related to proteinuria ($P < .02$). Neither VLDL + LDL cholesterol nor apo B and Lp(a) were independently related to the lathosterol to cholesterol ratio (all $P > .50$), indicating that the effect of antiproteinuric therapy to decrease these (apo)lipoproteins was not related to an effect on this ratio.

DISCUSSION

This study demonstrates that the lathosterol to cholesterol ratio tends to be lower in moderately hyperlipidemic nephrotic patients compared with healthy controls. Symptomatic antiproteinuric treatment improved but did not normalize the elevated levels of apo B–containing lipoproteins and Lp(a) in patients. Yet the serum lathosterol to cholesterol ratio changed little and was even lower in patients during combined antiproteinuric treatment than in controls. These findings cast doubt on the hypothesis that increased cholesterol synthesis provides an important mechanism responsible for the maintenance of hypercholesterolemia associated with human nephrotic syndrome. Obviously, the patients studied had established nephrotic syndrome, and it cannot be excluded that cholesterol synthesis was elevated at the initial phase of their renal disease.

The serum lathosterol to cholesterol ratio is the primary measure of whole-body cholesterol synthesis in the present study, and the validity of its use is crucial to our conclusions. Lathosterol is produced along the major route of cholesterol synthesis.[15] It is assumed that this cholesterol precursor is released into the serum compartment at a rate proportional to that of cholesterogenesis.[16] Serum lathosterol is bound to lipoproteins, and its absolute concentration is positively correlated with total cholesterol and with apo B[16,18] (and the present study). Thus, in hypercholesterolemia an elevated lathosterol level can be nonspecific and does not necessarily imply enhanced cholesterol synthesis.[16-21] Indeed, in familial hypercholesterolemia, serum lathosterol is elevated[16,23] although cholesterol is synthesized at a normal rate.[35] In this situation, cholesterol synthesis is correctly estimated by the lathosterol to cholesterol ratio, which is within normal limits.[16,23] Hence, it seems of particular relevance to correct the serum lathosterol level for an increased concentration and number of its carrying lipoproteins when comparing hypercholesterolemic with normolipidemic subjects, and so we followed this practice. In our study, the group-based coefficient of variation for the serum lathosterol to cholesterol ratio was high in patients and controls, in keeping with other data.[16,20] Despite this high variability, post hoc type II error analysis excluded a higher ratio in patients at baseline compared with controls ($\beta < .05$). We also calculated the lathosterol to apo B ratio, which was significantly lower in untreated patients. This observation is clearly inconsistent with an increased hepatic output of lathosterol into the serum pool of apo B–containing lipoproteins. Moreover, the absolute lathosterol concentration was not significantly higher in nephrotic patients at baseline. During combined treatment, serum lathosterol was similar in patients and controls, although the hypercholesterolemia was incompletely corrected. Therefore, the interpretation of our data did not essentially change when using the lathosterol concentration per se. Since the lathosterol to cholesterol ratio is a reliable index of whole-body cholesterol synthesis in both normocholesterolemia and hypercholesterolemia,[16-18,21] it is likely that this ratio is also a valid measure of cholesterogenesis in human nephrotic syndrome. However, the use of this index has limitations, and it should be emphasized that the presently suggested lack of an increase in cholesterol synthesis needs to be confirmed by direct methods in future studies. Furthermore, extrahepatic cholesterol synthesis may largely contribute to whole-body cholesterol synthesis.[36,37] It is therefore uncertain whether the lathosterol to cholesterol ratio accurately reflects hepatic cholesterol synthesis under all circumstances. On the other hand, the lathosterol to cholesterol ratio parallels changes in hepatic cholesterol synthesis induced by medical interventions[16,19-21,23,24] and is well related to hepatic HMG-CoA reductase activity.[15,22] The current results may thus support the possibility that hepatic cholesterol synthesis is unaltered in human nephrotic syndrome.

It is generally accepted that hepatic synthesis of lipids and apolipoproteins is increased in rats with experimentally induced nephrotic syndrome.[10-13] Hepatic ^3H_2O incorporation into newly synthesized cholesterol is enhanced, and an increased proportion of mevalonic acid, the product of HMG-CoA reductase, is converted to cholesterol.[11,13] In addition, the secretion of apolipoproteins by the liver and the hepatic mRNA content of various apolipoproteins, including apo B, is increased in nephrotic animals.[10,38,39] In human nephrotic syndrome, McKenzie and Nestel[14] showed increased turnover of esterified cholesterol. These early results suggesting increased cholesterol synthesis appear to be discrepant with the present data. Since the patients in the earlier study had extreme hyperlipidemia, it is possible that this discrepancy is related to the degree of hyperlipidemia. Several kinetic studies have demonstrated that LDL production is increased.[3,40-42] Normally, LDL is to a large extent, if not exclusively, derived from VLDL, which is directly excreted by the liver.[43] Therefore, enhanced apo B input into the LDL pool does not unequivocally establish increased hepatic apo B synthesis. VLDL apo B production, in turn, has been found to be either unchanged[40] or increased.[42] Thus, the issue of increased direct hepatic apo B production in human nephrotic syndrome is still unsettled. Moreover, it remains possible that hepatic synthesis of lipids other than cholesterol is enhanced.

Another aspect of our study is the relationship between lipid levels, serum albumin, and the degree of proteinuria. Although serum VLDL + LDL cholesterol and apo B are inversely correlated with serum albumin in nephrotic patients[1,3,9] (and the present study), the decrease in apo B–containing lipoproteins and Lp(a) during antiproteinuric therapy was more closely related to the decrease in proteinuria than to changes in serum albumin. These observations confirm and extend recent findings.[27] Kaysen et al[9,44]

originally reported that enhanced renal albumin clearance is a more important determinant of hyperlipidemia than hepatic albumin synthesis in experimental and in human nephrotic syndrome. The present study documents that there is no relationship between either the proteinuria or serum albumin level and the cholesterol to lathosterol ratio at baseline and during treatment. In addition, the decrease in apo B–containing lipoproteins during treatment was not related to changes in the lathosterol to cholesterol ratio. Thus, it is improbable that improvement of the lipoprotein profile following antiproteinuric therapy is associated with inhibition of cholesterol synthesis.

If human nephrotic syndrome–associated hypercholesterolemia is unlikely related to enhanced cholesterogenesis and if the decrease in cholesterol following antiproteinuric treatment is not related to inhibition of cholesterol synthesis, which other mechanisms could be responsible? In nephrotic patients, impaired catabolism of VLDL[40,42] and possibly also of LDL[41] cause accumulation of these lipoproteins in the circulation. Such an impaired lipoprotein catabolism might be related to a defect in the metabolism of a lipid-regulatory factor, concomitant with urinary protein loss.[2,5] A prolonged residence of apo B–containing lipoproteins can lead to further enrichment of these lipoproteins with esterified cholesterol, as a result of sustained esterified cholesterol transfer from HDL to VLDL and LDL.[45] This process is mediated by the cholesteryl ester transfer protein, and it has been shown that this factor is elevated in the plasma of nephrotic patients.[25,46]

Cholesterol synthesis is known to be influenced by dietary factors. A low-fat, low-cholesterol diet enhances whole-body and hepatic cholesterol synthesis.[37,47] Conversely, cholesterol synthesis is inhibited by increasing the dietary P:S ratio.[48] In the untreated nephrotic patients, dietary fat intake closely resembled the fat composition of the average Dutch diet.[49] It is therefore most unlikely that the absence of an elevated serum lathosterol to cholesterol ratio in nephrotic patients compared with controls was attributable to differences in dietary fat intake. With the low-protein diet, dietary fat intake and fat composition were altered, and a slight decrease in the lathosterol to cholesterol ratio was observed during combined antiproteinuric treatment. This suggests some inhibition of cholesterol synthesis, despite a decrease in fat and cholesterol intake, coinciding with the low-protein diet. In these nephrotic patients, the P:S ratio in the individual diet was the only significant determinant of the serum lathosterol to cholesterol ratio.

The present results, based on the serum lathosterol to cholesterol ratio, suggest that elevated cholesterogenesis is not important in the hypercholesterolemia associated with established human nephrotic syndrome. Furthermore, it appears unlikely that the decrease of apo B–containing lipoproteins is attributable to inhibition of cholesterol synthesis. Further evaluation of cholesterol synthesis in human nephrotic syndrome should be made by direct methods.

ACKNOWLEDGMENT

The technical assistance of Dr B.G. Wolthers, J. van der Molen, and H. Breukelman is appreciated. B.J. Beusekamp provided dietary advice.

REFERENCES

1. Appel GB, Blum CB, Chien S, et al: The hyperlipidemia of the nephrotic syndrome. Relation to plasma albumin concentration, oncotic pressure, and viscosity. N Engl J Med 312:1544-1548, 1985

2. Kaysen GA, Myers BD, Couser WG, et al: Mechanisms and consequences of proteinuria. Lab Invest 54:479-498, 1986

3. Joven J, Villabona C, Villella E, et al: Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome. N Engl J Med 323:579-584, 1990

4. Keane WF, Kasiske BL: Hyperlipidemia in the nephrotic syndrome. N Engl J Med 323:603-604, 1990

5. Short CD, Durrington PN: Hyperlipidaemia and renal disease. Balliere's Clin Endocrinol Metab 4:777-806, 1990

6. Wanner C, Rader D, Bartens W, et al: Elevated plasma lipoprotein (a) in patients with the nephrotic syndrome. Ann Intern Med 119:263-269, 1993

7. Wanner C, Bartens W: Lipoprotein (a) in renal patients: Is it a key factor in the high cardiovascular mortality? Nephrol Dial Transplant 9:1066-1068, 1994

8. Ordonez JD, Hiatt RA, Killebrew EJ, et al: The increased risk of coronary heart disease associated with nephrotic syndrome. Kidney Int 44:638-642, 1993

9. Kaysen GA, Gambertoglio J, Felts J, et al: Albumin synthesis, albuminuria and hyperlipemia in nephrotic patients. Kidney Int 31:1368-1376, 1987

10. Marsh JB, Sparks CE: Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. J Clin Invest 64:1229-1237, 1979

11. Shafrir E, Brenner T: Lipoprotein lipid and protein synthesis in experimental nephrosis and plasmapheresis. I. Studies in rat in vivo. Lipids 14:695-702, 1979

12. Marsh JB: Lipoprotein metabolism in experimental nephrosis. J Lipid Res 25:1619-1623, 1984

13. Golper TA, Feingold KR, Fulford MH, et al: The role of circulating mevalonate in nephrotic hypercholesterolemia in the rat. J Lipid Res 27:1044-1051, 1986

14. McKenzie IFC, Nestel PJ: Studies on the turnover of triglycerides and esterified cholesterol in subjects with the nephrotic syndrome. J Clin Invest 47:1685-1695, 1968

15. Björkhem I, Miettinen T, Reihnér E, et al: Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver. J Lipid Res 28:1137-1143, 1987

16. Van Kempen HJM, Glatz JFC, Gevers Leuven JA, et al: Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. J Lipid Res 29:1149-1155, 1988

17. Gylling H, Kuusi T, Vanhanen H, et al: Apolipoprotein E phenotype and cholesterol in familial hypercholesterolemia. Atherosclerosis 80:27-32, 1989

18. Miettinen TA, Tilvis RS, Kesäniemi YA: Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. Am J Epidemiol 131:20-31, 1990

19. Wolthers BG, Waldrecht HT, Van der Molen JL, et al: Use of determinations of 7-lathosterol (5α-cholest-7-en-3β-ol) and other cholesterol precursors in serum in the study and treatment of disturbances of sterol metabolism, particularly cerebrotendinous xanthomatosis. J Lipid Res 32:603-612, 1991

20. Uusitupa MIJ, Miettinen TA, Happonen P, et al: Lathosterol and other noncholesterol sterols during treatment of hypercholesterolemia with lovastatin alone and with cholestyramine or guar gum. Arterioscler Thromb 12:807-813, 1992

21. Vanhanen H, Kesäniemi YA, Miettinen TA: Pravastatin lowers serum cholesterol, cholesterol-precursor sterols, fecal steroids, and cholesterol absorption in man. Metabolism 41:588-595, 1992

22. Lund E, Sisfontes L, Reihner E, et al: Determination of serum levels of unesterified lathosterol by isotope dilution–mass spectrometry. Scand J Clin Lab Invest 49:165-171, 1989

23. Elmberger PG, Kalén A, Lund E, et al: Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia. J Lipid Res 32:935-940, 1991

24. Warwick GL, Packard CJ, Murray L, et al: Effect of simvastatin on plasma lipid and lipoprotein concentrations and low-density lipoprotein metabolism in the nephrotic syndrome. Clin Sci 82:701-708, 1992

25. Dullaart RPF, Gansevoort RT, Dikkeschei LD, et al: Role of elevated lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein activities in abnormal lipoproteins from proteinuric patients. Kidney Int 44:91-97, 1993

26. Keilani T, Schlueter WA, Levin ML, et al: Improvement of lipid abnormalities associated with proteinuria using fosinopril, an angiotensin-converting enzyme inhibitor. Ann Intern Med 118:246-254, 1993

27. Gansevoort RT, Heeg JE, Dikkeschei LD, et al: Symptomatic antiproteinuric treatment decreases serum lipoprotein (a) concentration in patients with glomerular proteinuria. Nephrol Dial Transplant 9:244-250, 1994

28. De Zeeuw D, Gansevoort RT, Dullaart RPF, et al: Angiotensin II antagonism improves the lipoprotein profile in patients with nephrotic syndrome. J Hypertens 13:S53-S58, 1995 (suppl 1)

29. Gansevoort RT, De Zeeuw D, De Jong PE: Additive antiproteinuric effect of ACE inhibition and a low protein diet in human renal disease. Nephrol Dial Transplant 10:497-504, 1995

30. Stichting Nederlands voedingsstoffen bestand (NEVO) Centraal Onstituut voedingsonderzoeh Institute Toegepast Natuurwetenschappelŷk Onderzoeh. NEVO Table Zeist. Voorlichtingsbureau voor de voeding, 1987

31. Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. Kidney Int 27:58-65, 1985

32. Demacker PNM, Hijmans AOM, Vos-Janssen HF, et al: A study of the use of polyethylene glycol on estimating cholesterol in high density lipoprotein. Clin Chem 26:1775-1779, 1980

33. Dagen MM, Packard CJ, Shepherd J: A comparison of commercial kits for the measurement of lipoprotein (a). Ann Clin Biochem 20:359-364, 1991

34. Watanabe N, Kamei S, Ohkubo A, et al: Urinary protein as measured with pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem 32:1551-1554, 1986

35. Bilheimer DW, Stone NJ, Grundy SM: Metabolic studies in familial hypercholesterolemia. Evidence for a gene-dosage effect in vivo. J Clin Invest 64:524-533, 1979

36. Turley SD, Spady DK, Dietschy JM: Role of liver in the synthesis of cholesterol and the clearance of low density lipoproteins in the cynomolgus monkey. J Lipid Res 36:67-79, 1995

37. Dietschy JM, Turley SD, Spady DK: Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 34:1637-1659, 1993

38. Tarugi P, Nicolini S, Albertazzi L, et al: Dyslipoproteinemia in an inbred strain with spontaneous chronic progressive nephrotic syndrome. J Lipid Res 32:1675-1687, 1991

39. Sun X, Jones H, Joles JA, et al: Apolipoprotein gene expression in analbuminemic rats and in rats with Heymann nephritis. Am J Physiol 262:F755-F761, 1992

40. Vega GL, Grundy SM: Lovastatin therapy in nephrotic hyperlipidemia: Effects on lipoprotein metabolism. Kidney Int 33:1160-1168, 1988

41. Warwick GL, Caslake MJ, Boulton-Jones JM, et al: Low-density lipoprotein metabolism in the nephrotic syndrome. Metabolism 39:187-192, 1990

42. Warwick GL, Packard CJ, Demant T, et al: Metabolism of apolipoprotein B–containing lipoproteins in subjects with nephrotic-range proteinuria. Kidney Int 40:129-138, 1991

43. Babiak J, Rudel L: Lipoproteins and atherosclerosis. Baillière's Clin Endocrinol Metab 1:515-550, 1987

44. Davies WR, Staprans I, Hutchison FN, et al: Proteinuria, not altered albumin metabolism, affects hyperlipidemia in the nephrotic rat. J Clin Invest 86:500-505, 1990

45. Dullaart RPF, Groener JEM, Erkelens DW: Cholesteryl ester transfer between lipoproteins. Diabetes Nutr Metab 4:329-343, 1991

46. Moulin P, Appel GB, Ginsberg HN, et al: Increased concentration of plasma cholesteryl ester transfer protein in nephrotic syndrome: Role in dyslipidemia. J Lipid Res 33:1817-1822, 1992

47. Miettinen TA, Gylling H, Vanhanen H, et al: Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apolipoprotein E phenotypes. Arterioscler Thromb 12:1044-1052, 1992

48. Glatz JFC, Katan MB: Dietary saturated fatty acids increase cholesterol synthesis and faecal steroid excretion in healthy men and women. Eur J Clin Invest 23:648-655, 1993

49. Zo eet Nederland, 1992. Resultaten van de voedselconsumptiepeiling 1992. Ministerie van Welzijn, Volksgezondheid en Cultuur, Ministerie van Landbouw, Natuurbeheer en Visserij. Den Haag. Voorlichtingsbureau voor de voeding, 1993