Apolipoprotein A-I, B-100, and B-48 Metabolism in Subjects With Chronic Kidney Disease, Obesity, and the Metabolic Syndrome

Marcelo C. Batista, Francine K. Welty, Margaret R. Diffenderfer, Mark J. Sarnak, Ernst J. Schaefer, Stefania Lamon-Fava, Bela F. Asztalos, Gregory G. Dolnikowski, Margaret E. Brousseau, and Julian B. Marsh

The metabolism of apolipoproteins (apo)B-48, B-100, and A-I was studied with a primed constant infusion of deuterium-labeled leucine in the fed state in 3 male individuals with chronic kidney disease (CKD), a glomerular filtration rate (GFR) of 28 to 57 mL/min/1.73 m^2, obesity (body mass index [BMI] 33.1), and the metabolic syndrome. Compared to 5 obese controls (BMI 30.1) and 13 non-obese controls (BMI 25.2), these CKD subjects had high plasma levels of triglycerides (TG) (343 ± 27.5 mg/dL *v* 144 ± 34.4 in the obese controls, $P < .001$) and low apoA-I (86.7 ± 3.9 mg/dL). An abnormal high-density lipoprotein (HDL) particle subpopulation pattern was found, with low levels of pre β-1 and α1. Compared to the obese controls, very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) apoB-100 levels were elevated 2- to 3-fold, while LDL apoB-100 levels were slightly lower (−7 %) and apoB-48 levels were comparable. The high TG levels were not associated with statistically significant changes in VLDL apoB-100 kinetics, although the production rate (PR) was higher and the fractional catabolic rate (FCR) was lower. The slightly lower LDL apoB-100 levels were accompanied by a significant 3-fold increase in the FCR and a 2.7-fold increase in the PR. The lower apoA-I levels were accompanied by a 1.6-fold increase in the FCR. Compared to the non-obese controls, the PR of apoA-I was increased by 61% and 38%, respectively ($P < .001$) in CKD and in obese control subjects. In the control subjects, the PR of apoA-I was significantly correlated with the BMI ($r = 0.81$, $P < .0001$). The kinetic results are consistent with these hypotheses: (1) CKD is associated with decreased clearance of the TG-rich lipoproteins (TRLs) and increased catabolism of LDL; (2) obesity increases apoB-100 and apoA-I production; and (3) in CKD, TG transfer to HDL, making HDL more susceptible to catabolism, accounts for the low apoA-I levels.

IN PATIENTS with chronic kidney disease (CKD), the leading cause of death is atherosclerotic cardiovascular disease, accounting for 45% of all deaths.[1] In atherosclerosis, cholesterol deposition occurs in macrophages and smooth muscle cells in the arterial wall as the result of elevated low-density lipoprotein (LDL), lipoprotein (a), and remnant lipoprotein levels, and decreased high-density lipoprotein (HDL) levels.[2] Alterations in lipid metabolism can be detected in CKD as soon as renal function begins to decline. The typical features of CKD-associated dyslipidemia are increased levels of triglycerides (TG) and decreased levels of HDL-cholesterol, specifically the large HDL_2.[3] Levels of very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), but not necessarily LDL, are frequently increased. The dyslipidemia in CKD subjects is similar to that seen in obesity and the metabolic syndrome.[4]

Few studies of apolipoprotein metabolism, and no studies of apolipoprotein (apo)B-48, in CKD have been reported to date. Our purpose was to define the underlying metabolic cause for the elevated levels of TG-rich lipoproteins (TRLs) and the low levels of HDL in CKD patients, by means of apolipoprotein kinetic studies using deuterium-labeled leucine. The CKD subjects reported here were obese males with an accompanying metabolic syndrome. The present study has revealed a considerable effect of the presence of obesity and the metabolic syndrome on the dyslipidemia associated with CKD.

MATERIALS AND METHODS

Three male patients with glomerular filtration rates (GFRS) less than 60 and greater than 20 mL/min/1.73 m^2 were recruited from the renal clinic at Tufts-New England Medical Center, without regard to body weight, age, or lipid levels, and excluding the presence of disorders with metabolic consequences such as diabetes or acquired immunodeficiency syndrome (AIDS). Their GFRs were estimated by the 4-parameter prediction equation derived from the Modification of Diet in Renal Disease (MDRD) study.[5] None of the subjects had consistent proteinuria exceeding 300 mg/d. Subject 1 had hypertension and had previously undergone a nephrectomy for renal cell carcinoma. Subject no. 2 had polycystic disease, and subject no. 3 had obstructive uropathy. Only one subject (subject no. 2) was taking drugs known to affect lipid metabolism. Atenolol was not taken on the day of the study, and simvastatin was stopped 1 month before the study. These subjects met the criteria for the metabolic syndrome, since all had a body mass index (BMI) greater than 30, with abdominal obesity, associated with high TG and low HDL, and were receiving antihypertensive medications.

The data for 16 of the 18 control subjects have been published previously.[6,7] Each subject underwent a medical history and physical examination. They had no evidence of any chronic illness, including endocrine, hepatic, thyroid, or cardiac dysfunction. All control subjects had normal kidney function. They did not smoke and were not taking any medications known to affect lipid levels. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University.

From the Lipid Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University; and the Renal Division, Department of Medicine, New England Medical Center, Boston, MA.

Submitted June 19, 2003; accepted May 6, 2004.

Supported by a General Clinical Research Center grant from the National Institutes of Health (5M01RR00054), a contract from the US Department of Agriculture Research Service (53-3k06-5-10), and by a grant from the National Heart, Lung, and Blood Institute of the National Institutes of Health (HL-64738). Support for M.C.B. was provided by the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (Brazil).

Address reprint requests to Julian B. Marsh, MD, Atherosclerosis Research Laboratory, Lipid Division, Box 216 New England Medical Center, 750 Washington St, Boston, MA 02111.

0026-0495/04/5310-0003$30.00/0

doi:10.1016/j.metabol.2004.05.001

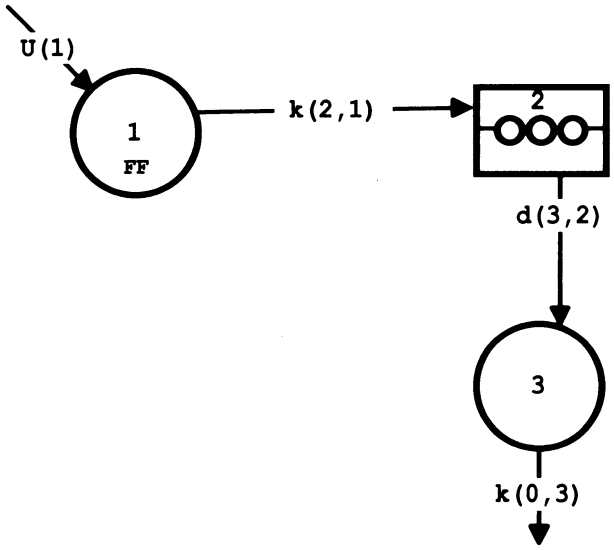

Fig 1. Single-pool model of apoA-I and of apoB-48 metabolism. Compartment 1 represents the forcing function—the plasma leucine enrichment during the constant infusion of deuteroleucine—reflecting the intracellular precursor enrichment. Compartment 2 represents a delay, accounting for the time required for the synthesis and secretion of the apolipoprotein. Compartment 3 represents the plasma pool of the apoprotein, with the rate constant k(0,3) equal to the FCR.

Experimental Protocol for Stable Isotope Kinetics

To determine the kinetics of TRL apoB-48 and apoB-100 in VLDL, IDL, LDL, and apoA-I in HDL, after an overnight fast the subjects underwent a primed constant infusion of deuterated leucine while they were in the fed state as previously described.[6,7] Starting at 6 AM, the subjects received 20 identical small hourly meals, each equivalent to 1/20th of their daily food intake, with 15% of calories as protein, 49% carbohydrate, 36% fat (15% saturated, 15% monounsaturated, 6% polyunsaturated), and 180 mg cholesterol/1,000 kcal. At 11 AM, with 2 intravenous lines in place, one for the infusate and one for blood sampling, (5,5,5-$^{2}H_{3}$)-leucine (10 μmol/kg body weight) was injected as an intravenous bolus over 1 minute and then by continuous infusion (10 μmol/kg body weight^{-1}/h^{-1}) over a 15-hour period. Blood samples (20 mL) were collected at hours 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 15.

Plasma Lipid and Lipoprotein Characterization

The blood was collected in sterile tubes containing EDTA (0.1% final concentration). Plasma was separated from red blood cells in a refrigerated centrifuge at 3,000 rpm for 30 minutes at 4°C. Plasma and lipoprotein fractions were assayed for total cholesterol and TG with an Abbott Spectrum analyzer with enzymatic reagents. HDL cholesterol was measured as previously described.[6] Lipid assays were standardized through the Centers for Disease Control Lipid Standardization Program.

The VLDL (d = 1.006 g/mL), IDL (d = 1.006 to 1.019 g/mL), LDL (d = 1.019 to 1.063 g/mL), and HDL (d = 1.063 to 1.21 g/mL) fractions were isolated from fresh plasma by sequential ultracentrifugation. ApoB was assayed in plasma and lipoprotein fractions with a noncompetitive enzyme-linked immunosorbent assay (ELISA) using immunopurified polyclonal antibodies.[6] ApoA-I was measured in whole plasma by an immunoturbidimetric assay as previously described.[7] The coefficient of variation for the assays was less than 5% within runs and less than 10% between runs.

HDL subpopulations in the CKD subjects were determined by 2-dimensional gel electrophoresis as described by Asztalos et al.[8] The control subjects used for comparisons were from the Framingham Offspring Study.[9]

Isolation of Apolipoproteins

ApoB-48, apoB-100, and apoA-I were isolated from lipoproteins by preparative sodium dodecyl sulfate–polyacrylamide gel electropheresis (SDS-PAGE) using a Tris-glycine buffer system as previously described.[6,7] Individual apoB species were assessed by scanning each gel by laser densitometry. We scanned lipoprotein fractions from each time point and averaged all values to calculate ratios and from this we estimated concentrations from the total apoB as determined by ELISA.

Isotopic Enrichment Determinations

ApoB-48, apoB-100, and apoA-I bands were excised from the polyacrylamide gels and hydrolyzed in 12N HC1 at 100°C for 24 hours. The free amino acids were isolated from plasma by Dowex AG-50W-X8 100- to 200-mesh cation exchange chromatography (Dow Chemical Corp, MI). The free amino acids from apoB-48 and apoB-100 were initially isolated with the Dowex columns and subsequently with centrifugation at 2,000 × g for 5 minutes as previously described.[6,7] The amino acids were converted to the n-propyl ester N-heptafluorobutyramide derivatives before analysis on an Agilent Technologies 5973N gas chromatograph/mass spectrometer.

Isotope enrichments (%) and tracer/tracee ratios (%) were calculated from the observed ion current ratios by the method of Cobelli et al.[10]

Table 1. Characteristics of Study Subjects

	Age (yr)	Weight (kg)	BMI (kg/m^2)	Creatinine (mg/dL)	GFR* (mL/min/1.73 m^2)	Albumin (g/dL)
CKD subjects						
CKD 1	55	95.0	31.3	1.6	57	4.2
CKD 2	47	109.5	36.7	1.9	46	4.2
CKD 3	66	99.5	31.5	2.7	28	4.1
Mean ± SD	56.0 ± 10	101 ± 7.4†	33.1† ± 3.1	2.1 ± 0.57	44 ± 15	4.2 ± 0.06
Obese controls (5)						
Mean ± SD	51.0 ± 8.9	94.7 ± 10.0	30.1 ± 1.3			
Non-obese controls (13)						
Mean ± SD	57.7 ± 13	73.8 ± 6.94	25.2 ± 1.8			

*GFR was estimated from the creatinine level by the equation of Levy et al.[5] The control subjects had normal values for serum creatinine, blood urea nitrogen, and albumin.

†Significantly different from the mean of the non-obese controls, $P < .001$, but not significantly different from the mean of the obese subjects ($P = .39$).

Table 2. Plasma Lipid Levels

	TG (mg/dL)	Total Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)
Subject no.				
1	371	239	134	30.9
2	316	196	100	24.8
3	342	189	87	26.8
Mean ± SD	343 ± 27.5*	202 ± 25.5	107 ± 24.3	27.5 ± 3.11†
Obese controls (5)				
Mean ± SD	144 ± 34.5	210 ± 48.0	143 ± 35.7	33.0 ± 3.39‡
Non-obese controls (13)				
Mean ± SD	115 ± 34.2	223 ± 41.7	149 ± 33.3	43.8 ± 8.26

*Significantly different from the mean of the obese or non-obese controls, $P < .001$.
†Significantly different from the mean of the non-obese controls, $P < .005$.
‡Significantly different from the mean of the non-obese controls, $P = .013$.

Data in this format are analogous to specific radioactivity in radiotracer experiments.

Kinetic Analysis

The kinetics of apoB-100 in the VLDL, IDL, and LDL fractions were determined after the primed constant infusion of $^2H_3$-leucine, using the multicompartmental model previously described.[6] The SAAM II program was used to fit the model to the observed tracer data by a weighted least-squares approach to find the best fit. In the control subjects, the forcing function for the estimation of the intracellular precursor pool was the plateau value for the isotopic enrichment of VLDL apoB-100. Because in the CKD subjects, no plateau was reached in 15 hours, due to the hypertriglyceridemia, a value of 71% of the plasma leucine enrichment was used as the forcing function. This value was based on the relationship between the plasma leucine enrichment compared to the plateau for VLDL apoB-100 in the control subjects.[6] A similar, though somewhat higher, factor was found by Batal et al[11] in subjects with hyperlipidemia. We have made the assumption that a similar correction factor would be applicable to subjects with CKD. Support for this assumption was obtained in a kinetic study (data not shown here) of an overweight subject with severe CKD (GFR, 23 mL/min) with a TG level of 88 mg/dL. The plasma leucine enrichment was 71% of that of the VLDL apoB-100 plateau.

The kinetics of apoB-48 in the TRL fraction and the kinetics of apoA-I in the HDL fraction were described by a single-pool multicompartmental model, shown in Fig 1. In kinetic studies of this kind, it is assumed that each subject remains in a steady state with respect to apolipoprotein metabolism during the study. As in normal individuals, in the CKD subjects there was no significant change in the concentration of any of the apolipoproteins at any of the four time points chosen for measurement (data not shown). In the steady state, the fractional catabolic rate (FCR) is equivalent to the fractional synthetic rate. ApoB and apoA-I production rates (PRs) were determined by the formula PR $(mg/kg^{-1}/d^{-1})$ = FCR (pools/d) multiplied by the apoB pool size/body weight in kg. The pool size was calculated as the plasma concentration (mg/L) multiplied by the plasma volume, assumed as 4.5% of the body weight (kg).

Statistical Analysis

Data for the subjects were analyzed with the Instat2 program (GraphPad Software, San Diego, CA), and presented as means ± SD. Unpaired standard *t* tests were performed. Where the variances differed, Welch's approximate *t* test was used. Probability values ≤ .05 were considered significant.

RESULTS

Characteristics of the Subjects

For comparative purposes, all individuals in the control group with a BMI greater than 28 were isolated as a subset of obese controls. Table 1 shows the characteristics of the 3 groups of subjects: CKD, obese controls, and non-obese con-

Table 3. Plasma Apolipoprotein Levels

	B-48	VLDL B-100 (mg/dL)	IDL B-100 (mg/dL)	LDL B-100 (mg/dL)	Apo A-I (mg/dL)
Subject no.					
1	0.471	17.7	3.37	91.5	91.0
2	0.625	13.3	4.23	91.1	83.5
3	0.401	18.1	5.21	61.8	85.6
Mean ± SD	0.499 ± 0.115	16.4 ± 2.66*	4.27 ± 0.92†	81.5 ± 17.0	86.7 ± 3.87‡
Obese controls (5)					
Mean ± SD	0.366 ± 0.129	6.82 ± 3.94	1.37 ± 0.68	87.7 ± 14.1	110 ± 4.32§
Non-obese controls (14)					
Mean ± SD	0.500 ± 0.339	7.81 ± 5.06	1.72 ± 0.92	104 ± 24.4	124 ± 11.9

*Significantly different from the mean of the obese and non-obese controls, $P < .014$.
†Significantly different from the mean of the obese or non-obese controls, $P < .007$.
‡Significantly different from the mean of the obese or non-obese controls, $P < .001$.
§Significantly different from the mean of the non-obese controls, $P = .022$.

Table 4. Plasma Concentration of HDL Particles (mg/dL)

Subjects	ApoA-I	Pre β-1	Pre β-2	α-1	α-2	α-3	Pre α-1	Pre α-2	Pre α-3
CKD (4)									
Mean	86.9	5.3	1.4	4.5	28.2	38.5	1.0	3.3	3.3
SD	3.75	0.7	0.2	0.7	4.7	1.9	0.2	0.4	0.9
N-TG (25)									
Mean	126	11.2	2.2	18.7	39.7	35.9	6.3	7.1	4.6
SD	15.4	4.3	1.1	8.1	10.1	10.7	3.6	2.2	2.9
H-TG (25)									
Mean	117	13.1	2.2	8.9	33.7	45.3	3.1	4.8	5.8
SD	23.2	5.5	1.1	3.5	9.4	10.0	1.6	1.4	2.0
P v N-TG	<.001	.012	.16	.0019	.036	.64	.007	.002	.39
P v H-TG	.017	.0095	.16	.02	.27	.19	0.016	.045	.02

NOTE. The control subjects were from the Framingham Offspring Study[9] and were males averaging 56.9 and 58.6 years for the normal TG (N-TG) and high TG (H-TG) groups, respectively. The N-TG group averaged 89 ± 8.7 mg/dL of TG, while the H-TG averaged 303 ± 14.9 mg/dL. The high-TG group did not differ significantly from the CKD group with respect to TC, HDL-C, or LDL-C levels, which averaged 210, 117, and 34.1 mg/dL, respectively.

trols. CKD patients did not differ significantly in average age as compared to the controls. The CKD subjects had a 10 % higher BMI than the obese controls ($P = .10$).

Table 2 indicates the lipid levels of the 3 groups. The HDL-C levels in the CKD subjects were significantly decreased by 38% compared to the non-obese and were 25% lower than in the obese controls, although the latter difference was not significant. In agreement with studies in the literature,[3] both total cholesterol and LDL cholesterol levels were lower, but the difference was not statistically significant.

Table 3 lists the apolipoprotein levels of the 3 groups. In the TRL density class containing apoB-48, there were no significant differences. The CKD subjects had levels of VLDL and IDL apoB-100 that were more than twice as high as those in obese or non-obese controls. LDL apoB-100 levels were 21% lower than in the non-obese subjects, although the difference did not reach statistical significance. With respect to apoA-I, the CKD subjects had lower levels than the obese (−21 %, $P <$ .001), as well as lower levels than the non-obese subjects (−30 %, $P < 0.0001$).

Table 4 shows that the large apoA-I HDL α-I subspecies in CKD subjects had a plasma concentration of only 4.5 mg/dL, compared with 18.7 mg/dL in a control population of subjects with TG levels in the normal range. Moreover, this concentration was also lower than the 8.9 mg/dL seen in a subset of controls with high TG levels. The pre α-1, -2, and -3 particles and especially the pre β-1 subgroup were also significantly lower than in the high-TG controls. Similar results were observed when the percent distribution of particles was estimated, independent of the concentration (data not shown). Therefore, the pattern seen in individuals with normal renal function and high TG was exacerbated in our CKD subjects.

Kinetic Analysis

The fit of the data to the multicompartmental models for apoB-100 is shown for a hypertriglyceridemic CKD subject (subject no. 3) in Fig 2. Figure 3 shows the fit of the curve to the data points for apoB-48, and Fig 4, for apoA-I.

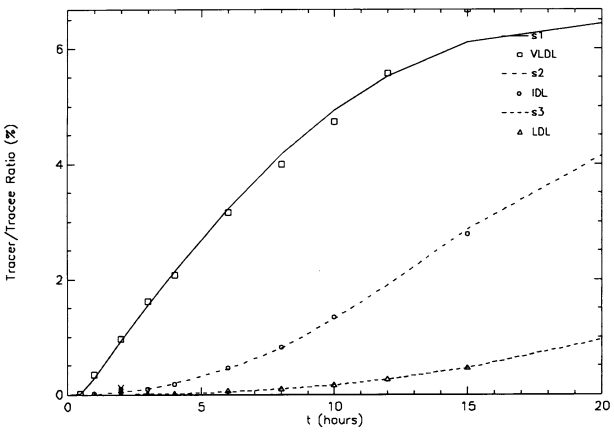

Fig 2. Fit of the isotopic enrichment data for apoB-100 to the curves (s1, s2, s3) calculated from the SAAMII program for subject no. 3. The ordinate numbers in this and succeeding figures are the % tracer/tracee ratios.

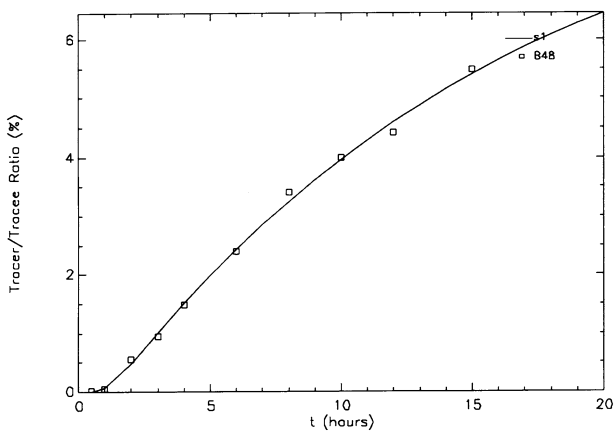

Fig 3. Ft of the isotopic enrichment data for apo B-48 to the curve calculated from the SAAMII program for subject no. 3.

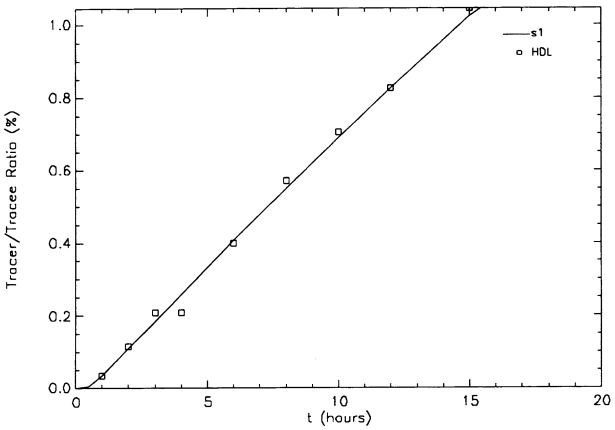

Fig 4. Fit of the isotopic enrichment data for apoA-I in subject no. 3.

The estimated FCRs for apoB-48 and apoB-100 are listed in Table 5.

For apoB-48, the FCR was 62% lower in CKD than in the non-obese controls ($P = .01$). For VLDL and IDL, the apoB-100 FCRs were not significantly different, although they were 37% lower in CKD compared to the obese controls. For LDL, the FCRs were 2-fold higher than in the obese controls ($P = <.003$).

Table 6 lists the PRs for the apoB-containing lipoproteins. For apoB-48, the PR was lower in the CKD subjects and in 2 obese controls than in the non-obese controls, but the considerable variability in the data rendered these changes not significant. In the CKD group, the PR of VLDL apoB-100 tended to be higher (especially in subject no. 1) in comparison to either group of controls.

The PR of apoB-100 in IDL tended to be increased compared to the controls, but this was not significant. However, in LDL, the PR of apoB-100 was increased 2-fold in CKD compared to the obese and non-obese controls ($P < .01$).

HDL-associated apoA-I kinetics are shown in Table 7. The FCR in the CKD subjects was 1.6 times higher than in the obese controls ($P < .0001$). The values in obese controls were 1.5 times higher than in the non-obese controls ($P = .014$). The PR of apoA-I was increased in the CKD subjects by 61% ($P < .001$) and in the obese controls by 38% ($P < .01$), compared to the non-obese controls.

DISCUSSION

Our 3 subjects, with a combination of obesity and CKD, all had the metabolic syndrome, as judged by medication for hypertension, a BMI greater than 30, a low HDL concentration, and a very high TG concentration. However, the underlying renal pathology differed in each case. Since we have not studied non-obese CKD subjects, the present finding cannot be extrapolated to the general population of CKD patients. An additional caveat is that in comparing the apolipoprotein kinetic parameters in the CKD subjects with the obese controls having normal renal function, our obese controls had a lesser degree of obesity, with a 10% lower average BMI of 30.1.

The TG levels in our CKD subjects averaged 343 mg/dL, considerably higher than in our obese subjects at 144 mg/dL and higher than the 170 mg/dL reported in 35 obese males with the metabolic syndrome and an average BMI of 34.[12] Therefore, much of the higher TG could be attributed to the presence of CKD. Taken together, the 66% increase in the PR of VLDL apoB-100 combined with a 41% decrease in the FCR can partially account for the high TG level. Overproduction of TG due to obesity could have further increased the TG. VLDL-TG production has been shown to be increased in obese males.[12-14] One explanation for this is that in the metabolic syndrome associated with insulin resistance, there is greater flux of fatty acids from visceral adipose tissue to the liver and increased VLDL secretion.[12]

The average FCR of apoB-48 in chylomicrons from the CKD subjects was lower than in the non-obese controls, which is in accord with a decreased lipoprotein lipase activity, which has been reported in CKD.[15] To our knowledge, there have been no previous studies of apoB-48 metabolism with stable isotopes in obesity. However, Watts et al[16] have reported decreased chylomicron-remnant catabolism in abdominally obese subjects, using a stable isotope breath test. Since the PR of apoB-48 was also lower in our CKD subjects, it offset the decreased FCR, resulting in a normal plasma level of apoB-48.

In the case of apoB-100 in IDL, the high plasma level was associated with a trend toward a lower FCR and a higher PR of apoB-100 in the CKD subjects compared to the obese controls.

Table 5. FCRs of apoB-48 and apoB-100 (pools per day)

	apoB-48	VLDL apoB-100	IDL apoB-100	LDL apoB-100
CKD subjects				
1	3.30	5.13	6.91	0.669
2	1.04	3.23	3.87	0.530
3	1.51	2.26	3.26	0.681
Mean ± SD	1.95 ± 1.19*	3.54 ± 1.46	4.68 ± 1.96	0.627 ± 0.0834†
Obese controls (5)				
Mean ± SD	(1.68, n = 2)	5.95 ± 2.74	7.42 ± 4.80	0.294 ± .095
Non-obese controls (13)				
Mean ± SD	5.11 ± 1.74	6.26 ± 4.00	10.8 ± 8.77	0.240 ± 0.0741

*Significantly different from the mean of the non-obese controls, $P = .01$

†Significantly different from the mean of the obese and the non-obese controls, $P < .003$.

Table 6. PRs of apoB-48 and apoB-100 (mg/kg/d)

	apoB-48	VLDL apoB-100	IDL apoB-100	LDL apoB-100
CKD subjects				
1	0.705	41.6	10.8	27.6
2	0.292	19.4	7.36	21.8
3	0.273	18.4	7.65	18.9
Mean ± SD	0.423 ± 0.244	26.5 ± 13.1	8.50 ± 1.72	22.8 ± 4.40*
Obese controls (5)				
Mean ± SD	(0.202, n = 2)	16.0 ± 10.2	4.56 ± 3.54	11.0 ± 3.52
Non-obese controls (13)				
Mean ± SD	1.25 ± 1.00	17 ± 7.65	6.41 ± 4.17	10.5 ± 2.78

*Significantly different from the mean of the obese or non-obese controls, $P < .01$.

The lower FCR may be related to decreased lipolysis associated with CKD.

For LDL, plasma levels of apoB-100 in CKD were only slightly lower (−7 %) than in the obese controls. However, this was associated with a 2-fold increase in both the FCR and the PR. These findings contrast with the decreased FCR and PR of LDL apoB-100 reported in obese males by Chan et al,[12] suggesting that the combination of CKD and obesity was the dominant factor in our 3 subjects. A possible explanation for the increased FCR could be an increased appearance of LDL in the glomerular filtrate, with subsequent renal tubular catabolism, since all subjects did have some degree of proteinuria.

The low apoA-I levels in our CKD subjects were associated with an increased FCR. With hypertriglyceridemia, one would anticipate TG enrichment of HDL via the neutral lipid transfer protein (CETP), which has been reported to be more active in subjects with obesity,[17] as well as in end-stage renal disease.[18] TG-rich HDL is known to be associated with an increased FCR.[19] In an earlier report in CKD subjects using radioisotope methodology,[20] an impaired apoA-I clearance and a decreased PR were found, contrasting with the present study. However, their CKD subjects were not obese.

The obese controls, compared to the non-obese, had a significant 46% increase in apoA-I production. This has not been well documented in the literature to date, although it was mentioned by Barrett and Watts.[4] Pont et al[21] noted a 41% increase in their obese subjects, which they stated did not reach statistical significance. However, a simple *t* test applied to their data does indicate significance. A calculation of the correlation between the BMI and the PR of apoA-I in the control subjects used in the present study[7] yielded an *r* value of 0.81 ($P < .0001$). It is possible that an increased production of apoA-I in obesity may be of intestinal rather than hepatic origin, although at present we do not have reliable methods to estimate hepatic versus intestinal production of apoA-I.

Since there was an increase in the FCR in our CKD subjects, it was of interest to examine the HDL subpopulation distribution. The changes were similar to those seen with hypertriglyceridemia in a population without renal disease, except that the effects were more pronounced, and they also occurred in another CKD subject who was not hypertriglyceridemic. Much less of the apoA-I was in the larger α-1 and α-2 particles, in agreement with data showing lower HDL_2.[3] The α-1 HDL particles have been associated with efficient reverse cholesterol transport.[22] Moreover, the pre $\beta-1$ particles were very low, which would imply a diminished ability to remove cholesterol and phospholipid from cell membranes in the process of becoming mature HDL.[23] Sasahara et al[24] found low levels of α-1 HDL particles in obesity, but higher levels of pre β-1 particles. Since these pre β-1 particles are quite small, increased glomerular filtration and subsequent renal tubular catabolism may have occurred in our CKD subjects, offsetting any increase due to obesity. The proximal renal tubules contain the cubilin receptor, which recognizes HDL and apoA-I.[25]

A methodological consideration affecting the interpretation of the present kinetic observations is the fact that the high TG level necessitated using a corrected value of the plasma leucine enrichment as the forcing function in these subjects rather than the VLDL apoB-100 plateau that was used for the controls. The correction factor was derived from the ratio of the VLDL apoB-100 enrichment at plateau to plasma enrichment determined previously for 18 control subjects.[6] In support of the use of this correction factor, the ratio of the VLDL apoB-100 plateau to plasma enrichment in a non-obese CKD subject, who had a normal TG and thus did reach plateau, was identical to that determined for the control subjects.

The present study indicates that in individuals with impaired renal function, obesity and the metabolic syndrome exacerbate the dyslipidemia. Further studies, especially in non-obese CKD subjects, appear warranted.

Table 7. HDL apoA-I Kinetics

	FCR (pools/d)	PR (mg/kg/d)
CKD subjects		
1	0.319	13.1
2	0.323	12.2
3	0.273	10.6
Mean ± SD	0.305 ± 0.0278*	12.0 ± 1.27†
Obese controls (5)		
Mean ± SD	0.228 ± .042	10.9 ± 1.83‡
Non-obese controls (11)		
Mean ± SD	0.133 ± 0.0274	7.45 ± 1.58

*Significantly different from the mean of the obese controls, $P = .033$, and the non-obese controls, $P \leq .0001$.

†Significantly different from the mean of the non-obese controls, $P = .0007$.

‡Significantly different from the mean of the non-obese controls, $P = .0017$.

ACKNOWLEDGMENT

The authors wish to thank the nursing and administrative staff at the GCRC and the nutrition staff at the USDA-HNRC, for their expert care of the patients and control subjects of this study. Two obese control subjects were made available to us by Dr Daniel Rader. We thank the faculty of the renal division of the Department of Medicine for their help in recruiting subjects with renal disease. We particularly thank the subjects for their wholehearted participation.

REFERENCES

1. US Renal Data System: Death rates by primary cause of death: 2000 Annual Data Report, Reference Tables, Table H 18. Bethesda, MD, NIH, NIDDK, 2000, p 659

2. Schaefer EJ: Lipoproteins, nutrition, and heart disease. Am J Clin Nutr 75:191-212, 2002

3. Attman PO, Samuelsson O, Alaupovic P: Lipoprotein metabolism in renal failure. Am J Kidney Dis 21:573-592, 1993

4. Barrett PHR, Watts GF: Kinetic studies of lipoprotein metabolism in the metabolic syndrome including effects of nutritional interventions. Curr Opin Lipidol 14:61-68, 2003

5. Levey AS, Bosch JP, Lewis JB, et al: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Ann Intern Med 130:461-470, 1999

6. Welty FK, Lichtenstein AH, Barrett PHR, et al: Human apolipoprotein (apo) B-48 and apo B-100 kinetics with stable isotopes. Arterioscler Thromb Vasc Biol 19:2966-2974, 1999

7. Velez-Carrasco W, Lichtenstein AH, Li Z, et al: Apolipoprotein A-I and A-II kinetic parameters as assessed by endogenous labeling with [$^2H_3$]leucine in middle-aged and elderly men and women. Arterioscler Thromb Vasc Biol 20:801-806, 2000

8. Asztalos BF, Roheim PS, Milani RL, et al: Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 20:2670-2676, 2000

9. Lamon-Fava S, Wilson PWF, Schaefer EJ: Impact of body mass index on coronary heart disease risk factors in men and women—The Framingham Offspring Study. Arterioscler Thromb Vasc Biol 16:1509-1515, 1996

10. Cobelli C, Toffolo G, Bier DM, et al: Models to interpret kinetic data in stable isotope tracer studies. Am J Physiol 253:E551-E564, 1987

11. Batal R, Tremblay M, Barrett PHR, et al: Plasma kinetics of apoC-III and apoE in normolipidemic and hyperlipidemic subjects. J Lipid Res 41:706-718, 2000

12. Chan DC, Watts GF, Barrett PHR, et al: Plasma markers of cholesterol homeostasis and apolipoprotein B-100 kinetics in the metabolic syndrome. Obesity Res 11:591-596, 2003

13. Cummings MH, Watts GF, Pal C, et al: Increased hepatic secretion of very-low density lipoprotein apolipoprotein B-100 in obesity. A stable isotope study. Clin Sci 88:225-233, 1995

14. Chan DC, Watts GF, Redgrave TG, et al: Apolipoprotein B-100 kinetics in visceral obesity: Associations with plasma apolipoprotein C-III concentration. Metabolism 51:1041-1046, 2002

15. Lee DM, Knight-Gibson C, Samuelsson O, et al: Lipoprotein particle abnormalities and impaired lipolysis in renal insufficiency. Kidney Int 61:209-218, 2002

16. Watts GF, Chan DC, Barrett PHR, et al: Preliminary experience with a new stable isotope breath test for chylomicron remnant metabolism: A study in central obesity. Clin Sci 101:683-690, 2001

17. Arai T, Yamashita AT, Hirano K, et al: Increased plasma cholesteryl ester transfer protein in obese subjects. A possible mechanism for the reduction of serum HD cholesterol levels in obesity. Arterioscler Thromb 14:1129-1136, 1994

18. Asayama K, Hayashibe H, Mishiku Y et al: Increased activity of plasma cholesteryl ester transfer protein in children with end-stage renal disease receiving continuous ambulatory peritoneal dialysis. Nephron 72231-236, 1996

19. Lamarche B, Uffelman KD, Carpentier A, et al: Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. J Clin Invest 106:191-1199, 1999

20. Fuh MM, Lee C, Jeng C, et al: Effect of chronic renal failure on high-density lipoprotein kinetics. Kidney Int 37:1295-1300, 1990

21. Pont F, Duvillard PF, Florentin E, et al: High-density apolipoprotein A-I kinetics in obese insulin resistant patients. An in vivo stable isotope study. Int J Obes Relat Metab Disord 26:1151-1158, 2002

22. Bruce C, Chouinard RA Jr, Tall AR: Plasma lipid transfer protein, high-density lipoprotein, and reverse cholesterol transport. Annu Rev Nutr 18:297-330, 1998

23. Fielding PE, Nagao K, Hakamata H, et al: A two-step mechanism for free cholesterol and phospholipid efflux from human vascular cells to apolipoprotein A-I. Biochemistry 46:14113-14120, 2000

24. Sasahara T, Yamashita T, Sviridov D, et al: Altered properties of high density. subfractions in obese subjects. J Lipid Res 38:600-611, 1997

25. Christensen EI, Bim H: Megalin and cubilin, synergistic endocytic receptors in renal proximal tubule. Am J Physiol Renal Physiol 4:F562-F573, 2001