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In vivo turnover study demonstrates diminished clearance of lipoprotein(a) in hemodialysis patients

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Lipoprotein(a) (Lp(a)) consists of a low-density lipoprotein-like particle and a covalently linked highly glycosylated protein, called apolipoprotein(a) (apo(a)). Lp(a) derives from the liver but its catabolism is still poorly understood. Plasma concentrations of this highly atherogenic lipoprotein are elevated in hemodialysis (HD) patients, suggesting the kidney to be involved in Lp(a) catabolism. We therefore compared the *in vivo* turnover rates of both protein components from Lp(a) (i.e. apo(a) and apoB) determined by stable-isotope technology in seven HD patients with those of nine healthy controls. The fractional catabolic rate (FCR) of Lp(a)–apo(a) was significantly lower in HD patients compared with controls (0.164 ± 0.114 vs 0.246 ± 0.067 days^{-1}, $P = 0.042$). The same was true for the FCR of Lp(a)–apoB (0.129 ± 0.097 vs 0.299 ± 0.142 days^{-1}, $P = 0.005$). This resulted in a much longer residence time of 8.9 days for Lp(a)–apo(a) and 12.9 days for Lp(a)–apoB in HD patients compared with controls (4.4 and 3.9 days, respectively). The production rates of apo(a) and apoB from Lp(a) did not differ significantly between patients and controls and were even lower for patients when compared with controls with similar Lp(a) plasma concentrations. This *in vivo* turnover study is a further crucial step in understanding the mechanism of Lp(a) catabolism: the loss of renal function in HD patients causes elevated Lp(a) plasma levels because of decreased clearance but not increased production of Lp(a). The prolonged retention time of Lp(a) in HD patients might importantly contribute to the high risk of atherosclerosis in these patients.

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Cardiovascular morbidity and mortality are markedly elevated in patients with chronic kidney disease.[1] Like other atherogenic lipoproteins (e.g. intermediate and low-density lipoproteins (LDLs)), lipoprotein(a) (Lp(a)) has been suggested to contribute to the high cardiovascular burden.[2–7] Lp(a) derives from the liver but sites and mechanisms of Lp(a) catabolism are still discussed controversially (reviewed by Utermann[8]). Animal models have shown that the kidney may function as a catabolic organ for various lipoproteins including Lp(a)[9] but not for LDL.[10] Most studies in patients with chronic kidney disease treated by peritoneal dialysis or hemodialysis (HD) revealed significantly elevated Lp(a) plasma levels which return to pre-disease values after successful kidney transplantation (reviewed by Kronenberg *et al.*[11]). The elevated Lp(a) levels cannot be explained by differences in apolipoprotein(a) (apo(a)) isoform frequencies and are therefore considered non-genetic and caused by the disease.[6,12,13] These findings led to the assumption that the human kidney might be directly or indirectly involved in Lp(a)/apo(a) removal and degradation from plasma.

So far, no kinetic studies have been performed to examine whether elevated Lp(a) levels in HD patients are caused by increased synthesis and/or decreased catabolism. Decreased catabolism would be in line with the previously suggested metabolic function of the human kidney for Lp(a). We therefore used the technology of stable-isotope-labelled amino acids to investigate the metabolism of apo(a) and apoB of Lp(a) in seven HD patients and nine healthy controls.

RESULTS

General parameters

Biometric data, lipids and lipoprotein profiles of HD patients and healthy controls are summarized in Table 1. In accordance with former studies (see review by Kronenberg[14]), high-density lipoprotein cholesterol levels were lower and triglyceride levels higher in HD patients than in controls, whereas total cholesterol, LDL cholesterol, and apoB concentrations did not differ significantly between the two

Table 1 | Biometric data, proteins, lipids, and lipoproteins in plasma from controls and hemodialysis patients

Subjects	Age (years)	BMI (kg/m^2)	Prot. (g/dl)	Alb (g/dl)	CRP (mg/l)	Chol. (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	ApoB (mg/dl)	Duration HD (months)	Creatinine (mg/dl)	Urea (mg/dl)
Controls													
1	28	21.0	6.4	3.9	2.1	99	31	79	52	43			
2	21	22.0	7.0	4.4	0.1	187	56	90	113	94			
3	50	27.6	5.8	3.6	5.8	204	36	216	125	125			
4	27	22.3	6.5	4.2	1.9	141	34	86	90	66			
5	32	24.2	7.4	4.5	4.0	299	41	91	240	194			
6	23	22.5	6.5	4.1	2.0	150	37	100	93	150			
7	42	24.6	7.0	4.0	0.1	154	52	50	92	97			
8	36	30.6	7.0	4.8	1.0	196	45	124	126	116			
9	43	25.4	6.3	4.0	2.0	170	42	57	117	98			
Patients													
10	33	20.6	6.7	4.2	2.0	126	29	127	72	100	108	13.5	170
11	61	24.5	7.1	3.7	2.0	253	35	250	168	163	34	12.4	160
12	35	23.5	6.6	3.9	5.0	174	25	160	117	97	5	9.3	146
13	67	18.4	6.4	3.6	3.3	262	38	166	191	154	41	9.7	94
14	31	26.5	6.7	3.9	4.7	184	27	140	129	163	75	13.4	193
15	60	21.0	6.6	3.7	8.0	194	45	63	136	102	27	9.0	63
16	61	22.3	6.5	3.6	12.0	168	31	111	115	84	44	13.0	175
Controls *n*=9	34±10	24.5±3.1	6.7±0.5	4.2±0.4	2.1±1.8	178±56	42±8	99±49	116±52	109±45			
HD patients *n*=7	50±16	22.4±2.7	6.7±0.2	3.8±0.2	5.3±3.6	194±48	33±7	145±58	133±39	123±35	48±34	11.5±2.0	143±47
P-value	0.055	0.21	0.75	0.03	0.04	0.61	0.055	0.055	0.21	0.41			

Alb, albumin; BMI, body mass index; Chol., cholesterol; CRP, C-reactive protein; HD, hemodialysis; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Prot., protein; TG, triglycerides.
Values are means±s.d.

groups. Markers for malnutrition and inflammation such as total plasma protein, albumin, and C-reactive protein which are predictors for cardiovascular disease in chronic kidney patients[15] differed slightly between patients and controls but were still within normal range.

Apo(a) phenotype and Lp(a) concentration

Half of the subjects (1, 2, 3, 4, 7, 8, 10, and 11) expressed one apo(a) isoform in plasma, whereas the other half (5, 6, 9, 12, 13, 14, 15, and 16) displayed two different apo(a) isoforms (Table 2). The second, larger isoform, however, was of markedly lower intensity (<10% of total staining) and could not be included for sensitivity reasons in the mass-spectrometric and kinetic analysis.[16] This isoform thus contributes very little to the total Lp(a) plasma concentration and is therefore unlikely to significantly influence apo(a) metabolism in these subjects. The mean Lp(a) plasma concentration was 20% higher in HD patients (50.8±28.0 mg/dl) than in controls (42.3±23.0 mg/dl) (Table 2). When we considered whole blood concentrations, the absolute values of Lp(a) were markedly lower in patients and controls when calculated with the formula [Lp(a)$_{plasma}$ * (1−hematocrit)] (33.9 vs 23.9 mg/dl; Table 2). The relative difference in Lp(a) concentrations between patients and controls became more pronounced, as Lp(a) in HD patients was then distributed in a larger plasma volume. Similar to an earlier study,[17] the relative difference was twice as high (41%) when calculated for whole blood levels (Table 2).

Lp(a) turnover rates

Based on the measurement of isotope enrichment, tracer/tracee ratios were calculated (Figure 1) and converted into final kinetic parameters.

The fractional catabolic rate (FCR) of Lp(a)–apo(a) was significantly lower in HD patients than in controls (0.164 vs 0.246 days^{-1}, $P=0.042$). The same was true for the FCR of Lp(a)–apoB (0.129 vs 0.299 days^{-1}, $P=0.005$) (Table 2 and Figure 2). This resulted in a 2–3 times longer residence time (RT) for Lp(a)–apo(a) (8.85 vs 4.39 days, $P=0.042$) and Lp(a)–apoB (12.86 vs 3.91 days, $P=0.008$) in HD patients compared with controls. Interestingly, the RT of the two proteins was very similar in controls (4.39 vs 3.91 days, $P=0.260$) but significantly different in patients (8.85 vs 12.86 days, $P=0.028$). The production rate (PR) of apo(a) and apoB from Lp(a), which equals the product of FCR, Lp(a) blood concentration, and blood volume, did not differ significantly between controls and HD patients at a first glance (1.161 vs 1.109 nmol/kg for Lp(a)–apo(a) and 1.283 vs 0.832 nmol/kg for Lp(a)–apoB). However, when we performed a paired comparison of the FCR of Lp(a)–apo(a) and Lp(a)–apoB from patients and controls with similar molar Lp(a) blood concentrations, we observed, with one exception, clearly lower FCR levels in patients (Table 3). This consequently resulted in a decreased PR of Lp(a)–apo(a) and Lp(a)–apoB in HD patients compared with controls. As expected, the apo(a) PR significantly correlated with plasma Lp(a) concentrations in controls ($r=0.717$, $P=0.030$),

Table 2 | Lipoprotein(a) and kinetic data in controls and hemodialysis patients

Subjects	Lp(a) plasma (mg/dl)	Apo(a) isoform	Hematocrit	Lp(a) blood (mg/dl)	Lp(a) blood (nM)	Apo(a)-FCR ($days^{-1}$)	ApoB-FCR ($days^{-1}$)	Apo(a)-RT (days)	ApoB-RT (days)	Apo(a)-PR (nmol/kg d)	ApoB-PR (nmol/kg d)
Controls											
1	37.2	13	0.42	21.7	66.21	0.133	0.232	7.52	4.31	0.616	1.075
2	60.7	19	0.45	33.4	99.07	0.278	0.183	3.60	5.47	1.928	1.269
3	49.0	20	0.48	25.5	75.27	0.191	0.205	5.24	4.88	1.006	1.080
4	92.1	20	0.37	58.1	171.68	0.214	0.203	4.67	4.93	2.572	2.440
5	35.2	22/34	0.45	19.4	56.61	0.316	0.367	3.16	2.72	1.252	1.454
6	16.6	24/29	0.45	9.1	26.46	0.229	0.202	4.37	4.95	0.424	0.374
7	19.5	26	0.49	10.0	28.50	0.314	0.568	3.18	1.76	0.626	1.133
8	34.0	30	0.45	18.7	52.74	0.331	0.489	3.02	2.04	1.222	1.805
9	36.2	32/35	0.46	19.6	54.60	0.210	0.240	4.76	4.17	0.803	0.917
Patients											
10	42.9	20	0.33	28.7	84.91	0.095	0.073	10.53	13.70	0.565	0.434
11	48.0	21	0.33	32.2	94.59	0.403	0.316	2.48	3.16	2.668	2.092
12	48.1	22/37	0.39	29.3	85.79	0.045	0.028	22.22	35.71	0.270	0.168
13	111.0	25/34	0.30	77.4	223.28	0.133	0.079	7.52	12.66	2.079	1.235
14	31.1	26/33	0.34	20.4	58.71	0.142	0.080	7.04	12.50	0.584	0.329
15	48.0	28/34	0.34	31.8	90.53	0.155	0.146	6.45	6.85	0.982	0.925
16	26.5	30/33	0.33	17.7	49.92	0.176	0.183	5.68	5.46	0.615	0.639
Controls n=9	42.3±23.0	23.6±5.9	0.45±0.04	23.9±14.8	70.1±44.1	0.246±0.067	0.299±0.142	4.39±1.42	3.91±1.38	1.161±0.696	1.283±0.582
HD patients n=7	50.8±28.0	24.6±3.7	0.34±0.03	33.9±20.0	98.2±57.6	0.164±0.114	0.129±0.097	8.85±6.36	12.86±10.86	1.109±0.904	0.832±0.664
P-value – Controls/HD+	0.61	0.46	<0.001	0.21	0.21	0.042	0.005	0.042	0.008	0.47	0.14
P-value – apo(a)/apoB++				Controls		0.139		0.314		0.260	
				Patients		0.028		0.028		0.028	

Apo(a), apolipoprotein(a); FCR, fractional catabolic rate; HD, hemodialysis; Lp(a), lipoprotein(a); RT, retention time; PR production rate.
Values are means±s.d. +Mann–Whitney U-test for comparison of controls vs patients, ++paired Wilcoxon test for comparison of FCR, RT, and PR of apo(a) and apoB moieties of Lp(a) from a given subject, stratified for patients and controls.

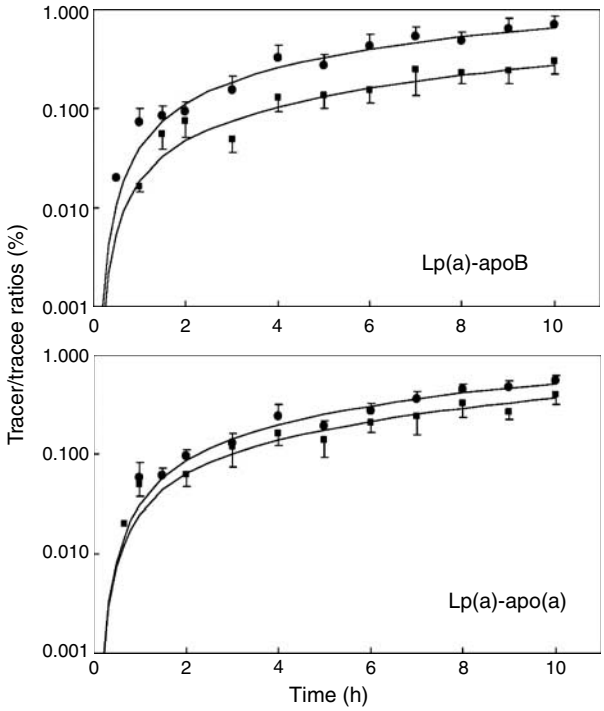

Figure 1 | Tracer/tracee ratio curves for Lp(a)–apoB (upper panel) and Lp(a)–apo(a) (lower panel) in seven HD patients (squares) and nine control subjects (circles). Plasma concentrations of Lp(a) did not change at any time point during the study, indicating steady-state conditions. Data are given as mean±s.e. and were fitted by the multicompartmental model using SAAMII.

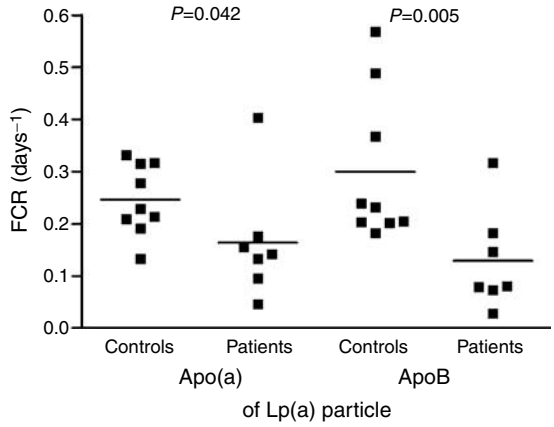

Figure 2 | Significantly decreased FCR of Lp(a)–apo(a) and Lp(a)–apoB in seven HD patients compared with nine healthy controls.

whereas it did not reach statistical significance in HD patients ($r=0.607$, $P=0.148$).

Table 4 shows the rate constants for each individual pathway depicted in the multicompartmental kinetic Lp(a) model (Figure 3). Comparing the rate constants for the hepatic and LDL-derived apoB moiety of Lp(a) (d(5,2) and k(5,4)) revealed that Lp(a) is almost exclusively (92% in controls, 99% in HD patients) synthesized directly from the liver. The remaining 8% and 1%, respectively, are derived from plasma LDL. The fractional standard deviation values

for $k(0,4)$, $k(0,5)$, and $k(0,7)$ were 6.7 ± 4.7, 16.0 ± 17.7, and $6.7 \pm 3.3\%$, respectively. The fractional standard deviation for $k(5,4)$ could not be adequately calculated because of extremely low rate constants for this pathway.

DISCUSSION

In this study, we investigated the turnover of Lp(a) in seven HD patients and nine healthy controls. This endogenously labelled stable-isotope amino-acid technology allows simultaneous analysis of the two protein components of Lp(a), namely apo(a) and apoB.[18]

The observed significantly decreased FCR of Lp(a)–apo(a) (−33%) and Lp(a)–apoB (−57%) together with a 41% higher whole blood Lp(a) concentration in HD patients compared with controls argues for a catabolic block caused by kidney dysfunction. Our turnover study is one further important step in understanding Lp(a) catabolism and in line

Table 3 | Comparison of apo(a)-FCR and apoB-FCR between all possible pairs of controls and patients with a difference in Lp(a) blood concentration of less than ± 15%

Lp(a) blood (nM)		Apo(a)-FCR (days^{-1})			ApoB-FCR (days^{-1})		
Control	Patient	Control	Patient	Change	Control	Patient	Change
52.74	49.92	0.331	0.176	⇓	0.489	0.183	⇓
	58.71	0.331	0.142	⇓	0.489	0.080	⇓
54.60	49.92	0.210	0.176	⇓	0.240	0.183	⇓
	58.71	0.210	0.142	⇓	0.240	0.080	⇓
56.61	49.92	0.316	0.176	⇓	0.367	0.183	⇓
	58.71	0.316	0.142	⇓	0.367	0.080	⇓
66.21	58.71	0.133	0.142	⇒	0.232	0.080	⇓
75.27	84.91	0.191	0.095	⇓	0.205	0.073	⇓
	85.79	0.191	0.045	⇓	0.205	0.028	⇓
99.07	84.91	0.278	0.095	⇓	0.183	0.073	⇓
	85.79	0.278	0.045	⇓	0.183	0.028	⇓
	90.53	0.278	0.155	⇓	0.183	0.146	⇓
	94.59	0.278	0.403	⇑	0.183	0.316	⇑

Apo(a), apolipoprotein(a); FCR, fractional catabolic rate.
The arrow indicates the deviation of the FCR in a given patient when compared with a respective matched control.

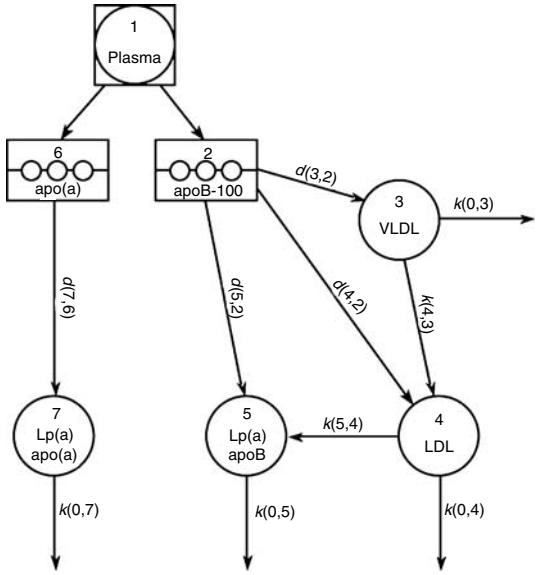

Figure 3 | Multicompartmental model for apoB-100 and apo(a) metabolism. The model consists of a plasma leucine pool (compartment 1), used as a forcing function, and delay compartments that account for assembly and subsequent secretion of apoB-100 (compartment 2) and apo(a) (compartment 6), respectively. ApoB in VLDL, LDL, Lp(a), and apo(a) in Lp(a) consist all of single compartments. The input of apoB in Lp(a) is twofold: one via *de novo* synthesis from the liver and one from LDL-apoB. $d(i,j)$ denotes the distribution of transfer from the delay compartment j to compartment i and $k(i,j)$ represents the rate constant from compartment j to compartment i. In this model, tracer/tracee data for VLDL, LDL, Lp(a)–apoB, and Lp(a)–apo(a) as well as leucine masses (nmol/l) in these compartments were fitted simultaneously. The FCR of Lp(a)–apo(a) and Lp(a)–apoB was equal to $k(0,7)$ and $k(0,5)$, respectively.

Table 4 | Rate constants (pools/h) for individual pathways shown in Figure 3 in controls and hemodialysis patients

Subjects	$d(3,2)$	$d(4,2)$	$d(5,2)$	$k(4,3)$	$k(5,4)$	$k(0,3)$	$k(0,4)$	$k(0,5)$	$k(0,7)$
Controls									
1	0.0440	0.8631	0.0929	0.109	0.00027	0.0000	0.0140	0.0097	0.0056
2	0.6809	0.2919	0.0272	0.093	0.00000	1.3285	0.0124	0.0076	0.0116
3	0.4076	0.5778	0.0146	0.103	0.00015	0.2085	0.0249	0.0085	0.0079
4	0.7528	0.1855	0.0617	0.044	0.00025	0.3643	0.0128	0.0084	0.0089
5	0.6450	0.3177	0.0373	0.962	0.00000	0.0000	0.0127	0.0153	0.0132
6	0.9962	0.0000	0.0039	1.749	0.00002	0.0000	0.0380	0.0084	0.0095
7	0.0496	0.9213	0.0291	0.148	0.00009	0.1311	0.0223	0.0237	0.0131
8	0.3059	0.6159	0.0782	0.330	0.00000	0.0000	0.0112	0.0204	0.0138
9	1.0000	0.0000	0.0000	0.054	0.00000	0.5325	0.0093	0.0100	0.0088
Patients									
10	0.9642	0.0000	0.0358	0.751	0.00000	0.0000	0.0067	0.0030	0.0040
11	0.9235	0.0546	0.0219	0.007	0.00003	0.4154	0.0018	0.0132	0.0168
12	0.9221	0.0702	0.0077	0.031	0.00000	0.1375	0.0028	0.0012	0.0019
13	0.0818	0.8728	0.0454	0.126	0.00000	0.2322	0.0088	0.0033	0.0056
14	0.9971	0.0000	0.0029	0.086	0.00000	0.2259	0.0087	0.0033	0.0059
15	0.3985	0.5546	0.0469	1.353	0.00002	0.5209	0.0081	0.0061	0.0065
16	0.9858	0.0000	0.0142	0.094	0.00000	0.1984	0.0072	0.0076	0.0073
Controls n=9	0.542 ± 0.363	0.419 ± 0.344	0.038 ± 0.033	0.399 ± 0.583	0.00009 ± 0.00011	0.28498 ± 0.43456	0.01751 ± 0.00928	0.01245 ± 0.00593	0.01026 ± 0.00280
Patients n=7	0.753 ± 0.363	0.222 ± 0.350	0.025 ± 0.018	0.350 ± 0.512	0.00001 ± 0.00001	0.24718 ± 0.17292	0.00631 ± 0.00286	0.00538 ± 0.00404	0.00684 ± 0.00475
P-value$^+$	0.351	0.252	0.536	0.536	0.210	0.536	<0.001	0.005	0.042

$k(i,j)$ describes the transfer of leucine from compartment j to compartment i; d(i,2) describes the distribution of material leaving the delay compartment 2 and entering compartments 3, 4, 5; $d(3,2)+d(4,2)+d(5,2)=1$.
Values are means ± s.d.; $^+$Mann–Whitney U-test.

with previous studies suggesting the kidney to be involved in the metabolism of Lp(a). Kronenberg *et al.*[19] measured Lp(a) levels in the ascending aorta and renal vein in 100 patients with normal kidney function. Lp(a) levels differed significantly between the two blood vessels with lower values in the venous samples.[19] The authors suggested a removal of Lp(a) from the renal circulation and assigned the kidney a potentially active role in the catabolism of Lp(a). Oida *et al.*[20] showed that urinary Lp(a) excretion correlates positively with kidney function, although the mechanisms involved were unclear. Previous findings of proteolytic apo(a) fragments up to a size of 215 kDa in human urine suggest an active transport mechanism rather than simple glomerular filtration.[21,22] An active and direct role of the kidney in catabolizing Lp(a) was supported by intra-glomerular localization of apo(a)/apoB and localization of apo(a) in tubular cells in a heterologous rat model after injection of human Lp(a).[9]

In this study, there was a mean age difference of 16 years between patients and controls which could have influenced the kinetic data of this study as shown earlier for LDL–apoB.[23] However, a subanalysis of an age-matched sample of six controls (subjects 1, 2, 4, 5, 6, and 8, mean age 28 years) and three patients (subjects 10, 12, and 14, mean age 33 years) revealed similar results with a significantly decreased FCR of Lp(a)–apo(a) (0.094 ± 0.049 vs $0.250 + 0.074\,\text{days}^{-1}$, $P = 0.048$) and Lp(a)–apoB (0.060 ± 0.028 vs $0.279 \pm 0.123\,\text{days}^{-1}$, $P = 0.024$) in patients compared with controls. Therefore, the observed kinetic results cannot be explained by age differences between patients and controls.

The pair-wise comparison of kinetic parameters from patients and controls with similar Lp(a) blood levels provided an interesting detail (Table 3): HD patients had lower FCR and consequently lower PR values compared with controls. This finding could suggest an inhibitory feedback mechanism on Lp(a) synthesis in response to impaired clearance and subsequently increased plasma concentrations in HD patients.

The FCR and hence RT values of Lp(a)–apo(a) and Lp(a)–apoB were very similar in controls suggesting the clearance of Lp(a) as an integral particle from the circulation. These findings are consistent with previous studies using stable isotopes[24,25] but inconsistent with a recent kinetic Lp(a) study in non-fasting probands.[26] In this latter study, Lp(a) was purified from plasma by lectin-mediated affinity chromatography which could have resulted in co-purification of other, minor apo(a)-containing plasma fractions that might have influenced the kinetic analysis. In our study, the mean RT of Lp(a)–apoB was significantly prolonged compared with Lp(a)–apo(a) in HD patients (12.86 vs 8.85 days, $P = 0.028$). Whether these differences indicate a preferential clearing of Lp(a)–apo(a) compared with the apoB moiety of Lp(a) in HD patients or simply reflect technically caused variation in a relatively small sample remains presently unclear. Metabolic studies in larger patient groups are therefore needed to clarify whether these differences do reflect different metabolic fates for both Lp(a) protein components.

PR values of apo(a) correlated significantly with plasma Lp(a) levels in controls which has been shown in previous kinetic studies[27] indicating regulation of Lp(a) levels predominantly by synthesis and not by degradation. Such a significant correlation was not observed in the HD group underlining the non-genetic and disease-induced origin of elevated Lp(a) levels in these patients.[12,13]

Calculation of individual rate constants for each pathway (Table 4, Figure 3) revealed a further important detail: Lp(a) was almost exclusively (92% in controls, 99% in HD patients) derived from the liver and only to a very small extent from the circulating LDL plasma compartment indicating a very similar Lp(a) assembly pathway in healthy controls and HD patients. This finding is compatible with the formation of an Lp(a) particle within the hepatocyte or on its surface in a compartment protected from plasma LDL as suggested by Su *et al.*,[25] but in contrast to several *in vitro* studies assuming extracellular or plasma membrane-associated Lp(a) assembly from circulating LDL (reviewed by White and Lanford[28]).

Our turnover study and one describing patients with nephrotic syndrome[29] point, however, to fundamental differences in the metabolism of Lp(a) and other proteins between these two patient groups. Patients with nephrotic syndrome showed no differences in the FCR of Lp(a) compared with controls but an increased synthesis rate of Lp(a). It is well known that nephrotic patients show a generally increased synthesis of lipoproteins.[29,30] Decreased clearance of Lp(a) in these patients might not necessarily be expected, as renal function is still relatively preserved. Metabolic differences between nephrotic and dialysis patients are not only evident for Lp(a) but also for albumin. Whereas FCR of albumin in HD patients is similar or even reduced compared with controls, FCR in patients with nephrotic syndrome is increased.[31,32]

The above-mentioned observation of markedly decreased FCR of apo(a) and apoB in Lp(a) causes a prolonged RT of Lp(a) in plasma of HD patients. This is reminiscent of a recently described disturbed plasma turnover of IDL and LDL in HD patients: the observed decreased FCR and fractional synthesis rate of apoB of both IDL and LDL led to the paradox of a prolonged RT of LDL despite normal circulating plasma levels.[33] Owing to the long retention period, 'aged' lipoprotein complexes are thus more susceptible for alterations such as oxidation damage, which was shown to be associated with accelerated atherogenesis in HD patients.[34] A previous kinetic study investigated the turnover of the two LDL subclasses, 'buoyant' LDL_1 and the smaller cholesterol-poor 'dense' LDL_2, in subjects with familial defective apoB-100. The authors found a more than four-fold longer RT for small dense LDL_2 in those patients as compared to normolipidemic controls.[35] It was therefore suggested that oxidative damage of an 'aged' LDL_2, which is present in large concentrations in both blood and the subendothelial space, may be an important mechanism for the development of

premature atherosclerosis in patients with familial defective apoB-100. Considering that the LDL particle from Lp(a) is compositionally similar to LDL_2,[24] it is tempting to speculate that the increased RT of circulating Lp(a) might pose an additional risk factor for the increased incidence of cardiovascular disease in HD patients.

Limitations of the study

Similar to other kinetic studies,[29,31,32] this study is limited by its small number of subjects. However, owing to the labor- and cost-intensive nature of these experiments, a high-throughput investigation is far from being feasible.

We had to exclude subjects with plasma Lp(a) concentrations <10 mg/dl for technical reasons, as this would not allow to purify Lp(a) in sufficient quantities. This resulted together with the relatively small sample size in a non-significant 41% difference in Lp(a) blood levels between patients and controls. On the other hand, this allowed us in a subanalysis to match patients and controls with similar Lp(a) concentrations, which might be the most appropriate procedure to investigate differences in the metabolism, which are not simply caused by Lp(a) plasma concentration differences between patients and controls.

In summary, this *in vivo* turnover study supports the role of the kidney in the clearance of Lp(a)/apo(a) without providing direct evidence for renal Lp(a) uptake. The lack of renal function in HD patients might explain the elevated circulatory levels of Lp(a) because of decreased clearance and not increased production of Lp(a). The prolonged RT of Lp(a) in HD patients compared with controls might pose a further strong contribution to the high risk of atherosclerosis in these patients.

MATERIALS AND METHODS

Study subjects

Seven HD patients and nine healthy controls – all male Caucasians from Tyrol, Austria – were enrolled in the study. None of the subjects showed any evidence of illness affecting lipid metabolism at the time of the study. The HD patients took vitamins, erythropoietin, and bicarbonate but no medication known to affect Lp(a) metabolism. For methodological reasons, we enrolled only subjects who had Lp(a) plasma concentrations >10 mg/dl and who expressed either a single apo(a) band or a second larger apo(a) band with minor intensity ($<10\%$ of total intensity). For 3 days preceding the study, all subjects received a standardized isocaloric diet composed of 30 kcal/kgBW, 50% carbohydrates, 30% fat, and 20% protein with a maximum of 300 mg cholesterol per day. The primary reasons for the development of end-stage renal disease in our patients were pyelonephritis (subjects 10 and 11), small kidney (subjects 13 and 16), Goodpasture syndrome (subject 14), renal arterial stenosis (subject 15), and IgA nephritis (subject 12). They had been dialysed for 4 h three times weekly for 48 ± 31 months on average and showed no residual urinary output.

Study protocol

The previously described study protocol[36] was approved by the Internal Review Boards of the Universities of Marburg and Innsbruck. Written informed consent was obtained from all participants. The study started after a 10-h overnight fasting period (in all HD patients 1 day after dialysis), whereby all study subjects maintained their fasting state during the first 12 h of the study. The day after dialysis was chosen to ensure that no blood volume shifts occur within the study period, which would not allow steady-state conditions. After a starting bolus of 1.34 mg trideuterated L-leucine/kgBW (95% pure, Cambridge Isotopes Laboratories Inc., Andover, MA, USA) to an antecubital vein, a continuous infusion of 1.34 mg d_3-leucine/kgBW/h followed. Before bolus injection and during constant infusion for 10 h, 9 ml ethylenediaminetetraacetic acid blood samples were taken from the opposite antecubital vein after 10, 20, 30, 40, 60, 90, 120 min followed by hourly blood withdrawal until 10 h after start of infusion.

Lp(a) preparation

After ethylenediaminetetraacetic acid plasma was obtained by low-speed centrifugation, Lp(a) was isolated as follows: after removal of very low-density lipoprotein (VLDL) ($d<1.006$ mg/ml) and LDL ($1.019<d<1.063$ mg/ml) by sequential ultracentrifugation, Lp(a) was isolated from the remaining plasma fraction by immunomagnetic separation with antibody-bound magnetic beads (Magna-Bind™ carboxylated derivatized beads, Pierce, Rockford, IL, USA), according to the manufacturer's recommendations. Monoclonal anti-apo(a) antibody 1A2, which recognizes the repetitive kringle IV type 2 of apo(a),[37] was mixed with magnetic beads at a weight ratio of 1:50 (wt/wt) and incubated for 45 min at room temperature on a rotator in a final volume of 1 ml phosphate-buffered saline. After removal of all unbound antibody by means of an external magnetic field, VLDL/LDL-depleted plasma (0.1–0.6 ml, depending on Lp(a) plasma concentration) was incubated overnight with 1.7 mg antibody-bound beads at 4°C. Afterwards the beads/antibody/Lp(a) complex was washed in phosphate-buffered saline. With this purification protocol, on average 60% of Lp(a) could be recovered which was comparable to previous work[38] and which was substantially higher than achieved with conventional sequential ultracentrifugation (approximately 15%, according to previous observations in our laboratory). To assess for purity, Lp(a) was subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis under non-reducing conditions followed by silver staining. Lp(a) was found to be pure, not degraded/fragmented and, in particular, not contaminated with LDL. Likewise, purified VLDL and LDL did not contain Lp(a)/apo(a) (Figure 4). To separate the two protein components of Lp(a), Lp(a)–apo(a), and Lp(a)–apoB, a sample buffer (ratio 1:1.5:15 β-mercaptoethanol, 0.5% bromphenol blue (wt/vol) in 5% glycerol (vol/vol), 10% SDS (wt/vol)) was added and the solution boiled in a water bath for 10 min. To extract and prepare both proteins for preparative SDS-polyacrylamide gel electrophoresis (8%) under reduced conditions, an external magnetic field was again used.

Mass-spectrometric and kinetic analysis

Both protein bands, corresponding to Lp(a)–apo(a) and Lp(a)–apoB, were excised from the gel after SDS-polyacrylamide gel electrophoresis, dried overnight at 60°C, hydrolyzed in 6 M HCl at 110°C for 24 h under nitrogen and afterwards lyophilized. In case of subjects with two visible apo(a) isoforms, only the major apo(a) band was excised and analyzed. Free amino acids were analyzed by gas chromatography as described by Schweer *et al.*[16] Briefly, free amino acids were prepared from plasma by cation exchange chromatography, derivatized, and analyzed by gas chromatography/triple-stage quadrupole mass spectrometry. Isotopic enrichment

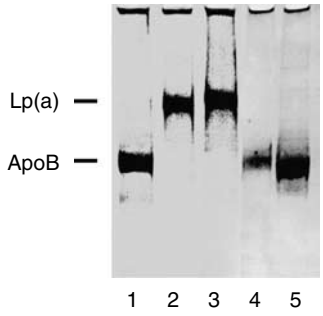

Figure 4 | Purified Lp(a) is not contaminated with LDL and purified VLDL and LDL are not contaminated with apo(a)/Lp(a). Lp(a) was purified from VLDL/LDL-depleted plasma by immunoprecipitation with magnetic beads bound to monoclonal antibody 1A2 and by conventional stepwise ultracentrifugation as described in 'Materials and Methods'. Purified VLDL, LDL, and Lp(a) were subjected to 3–8% SDS-polyacrylamide gel electrophoresis under non-reducing conditions; 0.5 μg protein each were applied to the gel. Proteins were identified by silver staining. Lane 1, LDL–apoB served as standard; Lane 2, Lp(a), purified by sequential ultracentrifugation as described;[47] Lane 3, Lp(a), purified by magnetic bead separation; Lane 4, VLDL–apoB; Lane 5, LDL–apoB. VLDL and LDL were obtained by sequential ultracentrifugation as described in 'Material and Methods'.

was converted to tracer/tracee ratios as described previously.[39] A multicompartmental model (SAAMII, Washington University, Seattle, WA, USA) was developed[40] to define the kinetics of Lp(a)–apo(a) and Lp(a)–apoB, as shown in Figure 3. Based on previous kinetic studies, plasma Lp(a) was assumed to be assembled both from circulating and *de novo* secreted LDL.[24]

Plasma concentrations of Lp(a), apoB, triglycerides, total and high-density lipoprotein cholesterol did not change at any time point during the study (data not shown), indicating steady-state conditions. Therefore, the fractional synthesis rate was assumed to be equal to the FCR. RT equals 1/FCR.

As hematocrit levels are remarkably lower in HD patients than in controls, lipoproteins including Lp(a) distribute in a larger plasma volume in these patients than in controls.[17] Therefore, lipoprotein levels measured in HD patients are generally too low when the lower hematocrit is not considered. To account for the significantly decreased hematocrit in HD patient, we calculated concentrations of Lp(a) in whole blood from both groups with the formula $[\text{Lp(a)}_{\text{plasma}} \star (1-\text{hematocrit})]$[17] to evaluate PR. The resulting PR equals the product of FCR $\star$ Lp(a) blood concentration $\star$ blood volume (assumed to be 7% of body weight) divided by body weight as described previously[41] with modifications for blood concentration and blood volume[42] instead of plasma concentration and plasma volume.

Laboratory procedures

Triglycerides, total and high-density lipoprotein cholesterol were determined with kits from Roche Diagnostics GmbH (Mannheim, Germany). LDL-cholesterol was calculated with the Friedewald formula. Urea, creatinine, total protein, albumin, and C-reactive protein were determined using standard assays on a COBAS INTEGRA® analyzer (Roche Diagnostics, Mannheim, Germany). Plasma concentrations of Lp(a) and apoB were measured by double-antibody enzyme-linked immunosorbent assay as described previously.[43] In short, an affinity-purified polyclonal apo(a) coating antibody and the horseradish peroxidase-conjugated monoclonal detection antibody 1A2[37] was used for Lp(a) determination. To measure apoB levels, an affinity-purified rabbit polyclonal antibody against apoB was used for coating and the same antibody, labelled with horseradish peroxidase, was used for detection. Apo(a) phenotyping was performed by SDS agarose gel electrophoresis under reducing conditions.[3] The molecular weight of Lp(a) to calculate PR was equal to the molecular weight of the LDL moiety of Lp(a) + mass of apo(a) isoform, which was derived from the amino-acid composition of the protein multiplied by the carbohydrate moiety (30%) of apo(a).[44–46] The PR was indicated in nmol/kg day in order to distinguish between PRs of apo B and apo(a) (of different size isoforms) from Lp(a).

Statistical analysis

Data are presented as mean $\pm$ s.d., except when noted. For statistical analysis, we used the non-parametric Mann–Whitney *U*-test and the paired Wilcoxon test. Correlation was analyzed by Spearman-Rho test. A $P<0.05$ was considered significant.

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REFERENCES

1. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998; **32**: S112–119.
2. Cressman MD, Heyka RJ, Paganini EP *et al.* Lipoprotein(a) is an independent risk factor for cardiovascular disease in hemodialysis patients. *Circulation* 1992; **86**: 475–482.
3. Kronenberg F, Neyer U, Lhotta K *et al.* The low molecular weight apo(a) phenotype is an independent predictor for coronary artery disease in hemodialysis patients: a prospective follow-up. *J Am Soc Nephrol* 1999; **10**: 1027–1036.
4. Longenecker JC, Klag MJ, Marcovina SM *et al.* Small apolipoprotein(a) size predicts mortality in end-stage renal disease: The CHOICE study. *Circulation* 2002; **106**: 2812–2818.
5. Kronenberg F, Kathrein H, König P *et al.* Apolipoprotein(a) phenotypes predict the risk for carotid atherosclerosis in patients with end-stage renal disease. *Arterioscler Thromb* 1994; **14**: 1405–1411.
6. Zimmermann J, Herrlinger S, Pruy A *et al.* Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 1999; **55**: 648–658.
7. Longenecker JC, Klag MJ, Marcovina SM *et al.* High lipoprotein(a) levels and small apolipoprotein(a) size prospectively predict cardiovascular events in dialysis patients. *J Am Soc Nephrol* 2005; **16**: 1794–1802.
8. Utermann G. Lipoprotein(a). In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic Bases of Inherited Disease*. 8th edn. McGraw Hill Inc.: New York, 2001, pp 2753–2787.
9. Reblin T, Donarski N, Fineder L *et al.* Renal handling of human apolipoprotein(a) and its fragments in the rat. *Am J Kidney Dis* 2001; **38**: 619–630.
10. Pittman RC, Steinberg D. Sites and mechanisms of uptake and degradation of high density and low density lipoproteins. *J Lipid Res* 1984; **25**: 1577–1585.
11. Kronenberg F, Utermann G, Dieplinger H. Lipoprotein(a) in renal disease. *Am J Kidney Dis* 1996; **27**: 1–25.
12. Dieplinger H, Lackner C, Kronenberg F *et al.* Elevated plasma concentrations of lipoprotein(a) in patients with end-stage renal disease are not related to the size polymorphism of apolipoprotein(a). *J Clin Invest* 1993; **91**: 397–401.
13. Kronenberg F, König P, Neyer U *et al.* Multicenter study of lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease treated by hemodialysis or continuous ambulatory peritoneal dialysis. *J Am Soc Nephrol* 1995; **6**: 110–120.
14. Kronenberg F. Lipoprotein abnormalities in end-stage renal failure. In: Loscalzo J, London GM (eds). *Cardiovascular Disease in End-stage Renal Failure*. Oxford University Press: Oxford, 2000, pp 175–208.

15. Menon V, Greene T, Wang X *et al.* C-reactive protein and albumin as predictors of all-cause and cardiovascular mortality in chronic kidney disease. *Kidney Int* 2005; **68**: 766–772.
16. Schweer H, Watzer B, Seyberth HW *et al.* Determination of isotopic ratios of L-leucine and L-phenylalanine and their stable isotope labeled analogues in biological samples by gas chromatography/triple-stage quadrupole mass spectrometry. *J Mass Spectrom* 1996; **31**: 727–734.
17. Kronenberg F, Trenkwalder E, Kronenberg MF *et al.* Influence of hematocrit on the measurement of lipoproteins demonstrated by the example of lipoprotein(a). *Kidney Int* 1998; **54**: 1385–1389.
18. Marsh JB, Welty FK, Lichtenstein AH *et al.* Apolipoprotein B metabolism in humans: studies with stable isotope-labeled amino acid precursors. *Atherosclerosis* 2002; **162**: 227–244.
19. Kronenberg F, Trenkwalder E, Lingenhel A *et al.* Renovascular arteriovenous differences in Lp[a] plasma concentrations suggest removal of Lp[a] from the renal circulation. *J Lipid Res* 1997; **38**: 1755–1763.
20. Oida K, Takai H, Maeda H *et al.* Apolipoprotein(a) is present in urine and its excretion is decreased in patients with renal failure. *Clin Chem* 1992; **38**: 2244–2248.
21. Mooser V, Marcovina S, White A *et al.* Kringle-containing fragments of apolipoprotein(a) circulate in human plasma and are excreted into the urine. *J Clin Invest* 1996; **98**: 2414–2424.
22. Kostner KM, Maurer G, Huber K *et al.* Urinary excretion of apo(a) fragments. Role in apo(a) catabolism. *Arterioscler Thromb Vasc Biol* 1996; **16**: 905–911.
23. Ericsson S, Eriksson M, Vitols S *et al.* Influence of age on the metabolism of plasma low density lipoproteins in healthy males. *J Clin Invest* 1991; **87**: 591–596.
24. Demant T, Seeberg K, Bedynek A *et al.* The metabolism of lipoprotein(a) and other apolipoprotein B-containing lipoproteins: a kinetic study in humans. *Atherosclerosis* 2001; **157**: 325–339.
25. Su W, Campos H, Judge H *et al.* Metabolism of Apo(a) and ApoB100 of lipoprotein(a) in women: effect of postmenopausal estrogen replacement. *J Clin Endocrinol Metab* 1998; **83**: 3267–3276.
26. Jenner JL, Seman LJ, Millar JS *et al.* The metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein (a) in human beings. *Metabolism* 2005; **54**: 361–369.
27. Rader DJ, Cain W, Ikewaki K *et al.* The inverse association of plasma lipoprotein(a) concentrations with apolipoprotein(a) isoform size is not due to differences in Lp(a) catabolism but to differences in production rate. *J Clin Invest* 1994; **93**: 2758–2763.
28. White AL, Lanford RE. Biosynthesis and metabolism of lipoprotein(a). *Curr Opin Lipidol* 1995; **6**: 75–80.
29. de Sain-van der Velden MG, Reijngoud DJ, Kaysen GA *et al.* Evidence for increased synthesis of lipoprotein(a) in the nephrotic syndrome. *J Am Soc Nephrol* 1998; **9**: 1474–1481.
30. Kaysen GA, De Sain-van der Velden MG. New insights into lipid metabolism in the nephrotic syndrome. *Kidney Int Suppl* 1999; **71**: S18–S21.
31. Giordano M, De Feo P, Lucidi P *et al.* Increased albumin and fibrinogen synthesis in hemodialysis patients with normal nutritional status. *J Am Soc Nephrol* 2001; **12**: 349–354.
32. Kaysen GA. Albumin turnover in renal disease. *Miner Electrolyte Metab* 1998; **24**: 55–63.
33. Ikewaki K, Schaefer JR, Frischmann ME *et al.* Delayed *in vivo* catabolism of intermediate-density lipoprotein and low-density lipoprotein in hemodialysis patients as potential cause of premature atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005; **25**: 2615–2622.
34. Shoji T, Fukumoto M, Kimoto E *et al.* Antibody to oxidized low-density lipoprotein and cardiovascular mortality in end-stage renal disease. *Kidney Int* 2002; **62**: 2230–2237.
35. Pietzsch J, Lattke P, Julius U. Oxidation of apolipoprotein B-100 in circulating LDL is related to LDL residence time. *In vivo* insights from stable-isotope studies. *Arterioscler Thromb Vasc Biol* 2000; **20**: E63–E67.
36. Ikewaki K, Rader DJ, Schaefer JR *et al.* Evaluation of apoA-I kinetics in humans using simultaneous endogenous stable isotope and exogenous radiotracer methods. *J Lipid Res* 1993; **34**: 2207–2215.
37. Dieplinger H, Gruber G, Krasznai K *et al.* Kringle 4 of human apolipoprotein[a] shares a linear antigenic site with human catalase. *J Lipid Res* 1995; **36**: 813–822.
38. Seman LJ, Jenner JL, McNamara JR *et al.* Quantification of lipoprotein(a) in plasma by assaying cholesterol in lectin-bound plasma fraction. *Clin Chem* 1994; **40**: 400–403.
39. Cobelli C, Toffolo G, Foster DM. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am J Physiol* 1992; **262**: E968–E975.
40. Barrett PH, Bell BM, Cobelli C *et al.* SAAM II: simulation, analysis, and modeling software for tracer and pharmacokinetic studies. *Metabolism* 1998; **47**: 484–492.
41. Cryer DR, Matsushima T, Marsh JB *et al.* Direct measurement of apolipoprotein synthesis in human very low density lipoprotein using stable isotopes and mass spectrometry. *J Lipid Res* 1986; **27**: 508–516.
42. Greger R, Windhorst U. *Comprehensive Human Physiology. From Cellular Mechanisms to Integration*. 1st edn. Springer Verlag: Berlin-Heidelberg, 1996.
43. Kronenberg F, Lobentanz E-M, König P *et al.* Effect of sample storage on the measurement of lipoprotein[a], apolipoproteins B and A-IV, total and high density lipoprotein cholesterol and triglycerides. *J Lipid Res* 1994; **35**: 1318–1328.
44. Kostner GM, Ibovnik A, Holzer H *et al.* Preparation of a stable fresh frozen primary lipoprotein[a] (Lp[a]) standard. *J Lipid Res* 1999; **40**: 2255–2263.
45. McLean JW, Tomlinson JE, Kuang W-J *et al.* cDNA sequence of human apolipoprotein (a) is homolgous to plasminogen. *Nature* 1987; **300**: 132–137.
46. Marcovina SM, Albers JJ, Gabel B *et al.* Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). *Clin Chem* 1995; **41**: 246–255.
47. Menzel H-J, Dieplinger H, Lackner C *et al.* Abetalipoproteinemia with an ApoB-100-lipoprotein(a) glycoprotein complex in plasma. Indication for an assembly defect. *J Biol Chem* 1990; **265**: 981–986.