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Decreased low-density lipoprotein receptor function and mRNA levels in lymphocytes from uremic patients

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Decreased low density lipoprotein receptor function and mRNA levels in lymphocytes from uremic patients. The mechanisms by which renal failure causes hyperlipoproteinemia remain unclear. To investigate the potential role of the low-density lipoprotein (LDL) receptor in lipoprotein metabolism in uremia we measured LDL receptor function in peripheral blood mononuclear cells (PBMC) from uremic patients and control subjects using a functional assay in which proliferation of lectin-stimulated PBMC in the presence of lovastatin was dependent upon internalization of exogenous cholesterol via a functional LDL receptor. The amount of LDL required to reverse 50% of lovastatin-induced inhibition of proliferation in PBMC from uremic patients was significantly greater (3.6 ± 1.8 μg/ml, $N = 33$, $P < 0.05$) than controls, (1.99 ± 0.6 μg/ml, $N = 37$). Abnormal LDL receptor function in four uremic patients normalized following renal transplantation. To investigate the molecular basis for LDL receptor dysfunction, we directly quantitated LDL receptor messenger RNA (mRNA) in PBMC from uremic patients and control subjects using a ribonuclease protection assay. LDL receptor mRNA expression in uremic patients was 0.42 ± 0.08 ($N = 10$), significantly lower ($P < 0.015$) than in normal subjects, 0.71 ± 0.08 ($N = 14$). These data suggest that an acquired defect in LDL receptor function in PBMC from uremic patients exists which may be due to decreased LDL receptor expression. These abnormalities, if present in other tissues, could contribute to the aberrant lipoprotein metabolism which is a consistent feature of uremia.

Hyperlipidemia is a common metabolic complication of chronic renal failure. Significant elevations of serum triglycerides [1] and cholesterol [2], as well as the appearance of abnormal lipoproteins such as β-VLDL [3], lipoprotein remnant particles [4], and triglyceride-enriched LDL are frequently observed in these patients and appears to be independent of the length of hemodialysis [1, 5]. Such lipid abnormalities have been identified as significant risk factors for the development of atherosclerosis [6], as well as other adverse clinical sequelae such as hyperlipidemic pancreatitis [7]. The hyperlipoproteinemia associated with chronic renal failure may be the critical risk factor which places these patients at high risk for developing severe generalized atherosclerotic cardiovascular disease [8–11]. In this regard, accelerated atherosclerosis and coronary vascular disease are considered major factors limiting long-term survival in patients with renal failure [6]. Moreover, animal studies suggest that hyperlipidemia may also be a major factor contributing to the progression of renal disease, regardless of the initial insult. Indeed, the end stage lesion, glomerulosclerosis, bears a remarkable resemblance to atherosclerosis [12–14].

While many theories have been postulated to explain the etiology of uremic hyperlipoproteinemia [15], the mechanisms by which renal failure alters lipoprotein metabolism remain unclear. The low density lipoprotein (LDL) receptor is a critical determinant of the regulation of lipid metabolism, and dysfunction of the LDL receptor is the primary cause of several clinically significant dyslipoproteinemias [16]. Recent studies in guinea pigs have suggested that uremia is associated with an abnormality in hepatic LDL receptor function and cholesterol synthesis [17, 18]. To date however, such studies have not been conducted in uremic human subjects. We have therefore investigated LDL receptor function and LDL receptor mRNA levels in peripheral blood mononuclear cells (PBMC) from uremic patients and healthy controls using a recently developed assay of LDL receptor function [19], and a highly sensitive and specific ribonuclease protection assay.

Methods

Subjects

The study was approved by the Committee for the Protection of Human Subjects of the University of Texas Medical School at Houston. Informed consent was obtained from each subject. Thirty-three patients with uremia treated with either conservative management, hemodialysis, or peritoneal dialysis were studied. The clinical characteristics of these patients are listed in Tables 1 and 2. None of the patients was taking any medications known to affect serum lipoprotein levels. Four subjects were studied on two occasions at different levels of renal function or during treatment with different dialysis modalities. Four patients were studied before and at least one month after renal transplantation at the time when maintenance immunosuppression with prednisone and cyclosporin A had been instituted [20]. Thirty-seven healthy subjects, all but two of whom were adults, served as controls. Informed consent was obtained from all subjects.

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Table 1. Clinical characteristics of the uremic patients

Age range	7 to 69 years
Average age	27.2 ± 16 years
Dialysis modality (*N*)	Peritoneal dialysis (11)
	Hemodialysis (19)
	Conservative management (3)
Diagnosis (*N*)	Focal segmental glomerulosclerosis (7)
	Glomerulonephritis (4)
	Diabetes mellitus (3)
	Hypertension (3)
	Familial juvenile nephronophthisis (2)
	Cystinosis (1)
	Obstructive uropathy (1)
	Recurrent pyelonephritis (1)
	Etiology obscure (11)
Mean BUN *mg/dl*	75.6 ± 25 (*N* = 28)
Mean creatinine *mg/dl*	9.35 ± 3.4 (*N* = 31)

Table 2. Biochemical parameters for controls and patients

	Controls	Patients
TC *mg/dl*	189 ± 25.4	210 ± 68
	(*N* = 5)	(*N* = 30)
TG *mg/dl*	94 ± 38	288 ± 166
	(*N* = 4)	(*N* = 30)
LDL *mg/dl*	111 ± 20	106 ± 53
	(*N* = 5)	(*N* = 10)
HDL *mg/dl*	57.8 ± 14.5	36.7 ± 10.7
	(*N* = 5)	(*N* = 8)

Abbreviations are: TC, total cholesterol; TG, total triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein. Each value is presented as the mean ± standard deviation.

The study population for the mRNA assay consisted of a population of 10 of the uremic patients studied above (6 males and 4 females, age 32.2 ± 20.2 years) at various clinical degrees of uremia (mean duration of hemodialysis was 48 ± 39 months), and 14 clinically healthy individuals served as controls (7 males and 7 females, age 28.9 ± 7.3 years).

Blood from the uremic subjects was obtained at the beginning of dialysis, before heparin administration. Serum cholesterol and triglyceride levels, blood urea nitrogen (BUN), and creatinine were determined by standard methods in our clinical chemistry laboratory. LDL levels were calculated by the standard Fredrickson formula [21]. Dialysis efficiency was assessed by pre-dialysis BUN in the hemodialysis patients [22], or by steady state creatinine levels in the peritoneal dialysis patients [23].

Isolation of peripheral blood mononuclear cells and low density lipoproteins

Ten to fifteen milliliters of heparinized blood were obtained from patients and control subjects. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over a layer of Ficoll-Hypaque, and were thoroughly washed. PBMC were pipetted into plastic microtiter plates at 10^5 cells per well in RPMI 1640 supplemented with L-glutamine, penicillin, streptomycin, and 1% (vol/vol) lipoprotein poor plasma (LPP) obtained from ultracentrifugation of AB human plasma at a density of 1.25 g/ml. The cholesterol content of LPP was <10 mg/dl as determined by a standard colorimetric assay (Sigma Chemical Company, St. Louis, Missouri, USA). LDL was isolated by sequential flotation of plasma at a density of 1.006 to 1.063 g/ml [24], and dialyzed against phosphate-buffered saline. LDL concentration was expressed as its protein content determined by standard protein determinations [25].

Assay of LDL receptor function

Accurate quantitation of LDL receptors on freshly-isolated peripheral mononuclear cells by ligand binding techniques is difficult because of the low level of LDL receptor expression in non-dividing cells [26]. However, when PBMC are stimulated to grow in the presence of lovastatin, an inhibitor of cellular cholesterol synthesis, cellular proliferation becomes totally dependent upon the internalization of LDL by functional LDL receptors; hence LDL receptors are maximally expressed and easily assayed [19].

PBMC were stimulated with phytohemagglutinin (PHA) (Difco) 1/1000, and cultured in triplicate in the presence of 1 to 80 μg/ml LDL. PBMC cultured in the absence of LDL and PHA served as a control for spontaneous blastogenesis. For each concentration of LDL, PBMC were grown in the presence of 0.5 μM lovastatin dissolved in dimethyl sulfoxide (DMSO), or DMSO alone as controls (final DMSO concentration/well was 0.2%). To assess the specificity of the inhibition of cholesterol synthesis produced by lovastatin and the viability of PBMC in culture, additional control and uremic cells were treated with 10 mM mevalonic acid in lieu of LDL [19]. To determine the relative sensitivity of uremic PBMC to lovastatin, dose response studies were performed using lovastatin concentrations of 0.1 to 5.0 μM. Cell viability was determined by trypan blue exclusion. Mevalonic acid was prepared by saponification of mevalonic acid lactone (Sigma Chemicals) with 0.5 N NaOH followed by neutralization with 1 N HCl to a final pH of 7.0.

PBMC were incubated in 5% carbon dioxide and air at 37°C for four days, and were then pulsed with tritiated thymidine (Amersham, 5 Ci/mmol) for 18 hours. After labeling, PBMC were harvested on glass fiber filters and assayed by scintillation counting to measure incorporation of label into newly synthesized DNA. At each LDL concentration, percent inhibition was calculated as the difference in counts-per-minute (CPM) between the means of the DMSO control and the lovastatin-treated PBMC by the formula:

$$[1 - (\text{cpm with lovastatin/cpm with DMSO alone})] \times 100$$

Fresh PBMC from normal subjects were included in each set of incubations as additional controls to monitor the proper functioning of the assay.

Preparation of RNA from HL60 cells and peripheral blood mononuclear cells

A human promyelocytic cell line, HL60, was maintained in RPMI 1640 medium (Hazleton, Lenexa, Kansas, USA) containing penicillin (100 U/ml) and streptomycin (100 μg/ml). In some experiments HL60 cells were treated with 25-hydroxycholesterol (Steraloids, Wilton, New Hampshire, USA) dissolved in ethanol. Approximately 10×10^6 HL60 cells or 20 to 40×10^6 PBMC were extracted with 5 M guanidinium thiocyanate (Fluka Chemical Corp., Ronkonkoma, New York), 25 mM sodium citrate, 100 mM β-mercaptoethanol, 17 mM sodium n-lauryl

sarcosine. Total cellular RNA was isolated by centrifugation at 80,000 × g for 18 hours at 22°C through a 5.7 M cesium chloride cushion (Schwartz/Mann Biotech, Cleveland, Ohio, USA) [27]. Total extracted RNA was quantitated by spectrophotometry at A_{260nm} and stored in water treated with diethylpyrocarbonate (Sigma Chemicals).

Plasmid constructions

The human LDL receptor clone HUMLDL(316) consists of a 316 base-pair (bp) fragment (Pvu II endonuclease digested fragment of pTZ1) from a full length human LDLr cDNA [28], provided by Dr. D.W. Russell (U.T. Southwestern Medical Ctr., Dallas, Texas, USA), and subcloned into the plasmid pGEM-3 (Promega, Madison, Wisconsin, USA). This fragment contains 220 bp of the 5'-end of the LDL receptor beginning with the adenosine in the initiator methionine, and is a highly conserved region that encodes for part of the ligand-binding domain of the LDL receptor [29], with an additional 96 bp of bacterial sequence linked 5' to the LDL receptor sequences.

As an internal standard, a human fibroblast cytoplasmic β-actin clone pHFβA(455) comprised of a 455 bp fragment from a pBR322 plasmid vector (produced by digestion with Eco RI and Rsa I endonucleases of pHFBA-3'UT) containing the last 460 bp of the human fibroblast β-actin [30] was constructed. The insert consists of 140 bp corresponding to nucleotides +3480 to +3620 of the 3'-untranslated region, and is highly conserved and specific for cytoplasmic β-actin and does not cross react with other cellular actins [31, 32].

Ribonuclease protection assay of LDL receptor mRNA

Single-stranded antisense RNA probes complementary to the LDL receptor and β-actin mRNA were obtained by first digesting the LDL receptor and β-actin subclones with Eco RI and Mse I endonucleases, respectively. Labeled RNA probes were synthesized from linearized templates with SP6 polymerase (Boehringer-Mannheim, Indianapolis, Indiana, USA) in the presence of 100 μCi of 32P-UTP (800 Ci/mmol, Amersham, Arlington Heights, Illinois, USA) as described by the manufacturer (Promega, Madison, Wisconsin, USA) with sufficient unlabeled UTP to achieve 11.4 μM final UTP concentration for LDL receptor probe and 21 μM for the β-actin probe. Probe synthesis was carried out at 37°C for 45 minutes. Transcripts were precipitated with 2.7 M ammonium acetate, 48.8 μg/ml glycogen, and 3 × (vol/vol) ethanol and resuspended in depc-H_2O. The LDL receptor probe was 365 bases and β-actin probe was 165 bases in length. In the presence of a complementary RNA these probes yield protected fragments of 220 bases (LDL receptor) and 140 bases (β-actin).

The RNAse protection assay was a modification of an earlier published procedure [33]. Total RNA (10 μg) was co-precipitated with both ^{32}P-labeled antisense LDL receptor and β-actin probes (2.5 to 5.0 × 10^5 cpm) in the presence of 3 × (vol/vol) ethanol, 4 μg glycogen, and 0.2 M NaCl. The precipitate was resuspended in a hybridization buffer (80% deionized formamide, 620 mM NaCl, 3 mM PIPES, and 0.3 mM EDTA), denatured at 95°C for five minutes, and then incubated overnight at 55°C. RNAse A digestion of the unprotected RNA was performed by adding 37.8 μg/ml RNAse A (Boehringer Mannheim, 50 U/mg) in buffer (10 mM Tris HCl, pH 8.0; 5 mM EDTA; 200 mM NaCl; and 100 mM LiCl) to the hybridization solution,

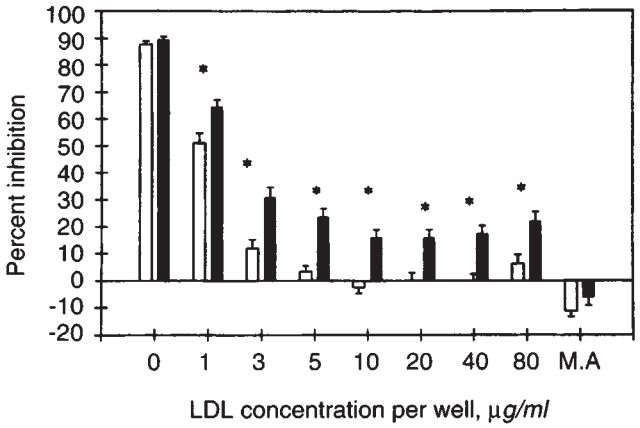

Fig. 1. *LDL receptor function assay.* The percent inhibition of PBMC proliferation by lovastatin was determined at increasing concentrations of LDL. The control subjects' mean values ± SEM (N = 37) are represented at each concentration by the open bars, and the uremic patients' (N = 33) by the solid bars. The solid horizontal line represents zero inhibition, that is, the point where the lovastatin inhibition has been completely reversed by LDL. (*$P < 0.001$)

and incubating at 33°C for 60 minutes. The digestion was terminated by adding SDS (0.35%), 86.7 μg/ml proteinase K (Boehringer Mannheim, 20 U/mg), and incubating at 37°C for 15 minutes. The samples were then phenol:chloroform-extracted, ethanol-precipitated in the presence of 20 μg of glycogen, and fractionated by electrophoresis on 8 M urea, 5% denaturing polyacrylamide gels. After electrophoresis, gels were dried and exposed to Kodak XAR film at room temperature with intensifying screens. Autoradiographs were analyzed by laser densitometry and bands corresponding to LDL receptor and β-actin mRNAs were quantitated. Results presented are expressed as the ratio of LDL receptor mRNA/β-actin mRNA to normalize for any differences in sample loading or hybridization efficiency.

Statistical analysis

The Student's t-test with Bonferroni correction was used to compare percent inhibition between groups; $P < 0.005$ was considered statistically significant [34]. Differences in LDL concentrations, BUN, creatinine, and lipoprotein levels between groups were measured by a two-tailed Student's t-test; $P < 0.05$ was considered statistically significant. Correlation between clinical parameters with assay results was measured by logistic regression analysis. Levels of cellular LDL receptor mRNA were reported as the mean ± SEM, and the significance of differences in levels was determined by unpaired t-test, with significance defined as $P < 0.05$. Correlation between mRNA levels and biochemical parameters from uremic patients was calculated by Pearson product moment correlation.

Results

LDL receptor function assay in control and hypercholesterolemic subjects

Addition of LDL to peripheral blood mononuclear cells from the control subjects reversed the lovastatin-mediated inhibition of proliferation at concentrations less than 10 μg/ml (Fig. 1). The slight inhibition of cellular proliferation noted at LDL

Table 3. Sensitivity of patient and control cells to inhibition by lovastatin

Lovastatin μM	% Inhibition Uremic N = 2	Normal N = 2
0.1	59.4	58.3
0.5	78.6	75.1
1.0	76.5	74.5
2.0	85.3	92.4
5.0	89.1	90.0

50,000 cells/well were cultured in complete media with 1% LPP, PHA 1/1000 in the presence of increasing concentrations of lovastatin, and % inhibition calculated as described in the text.

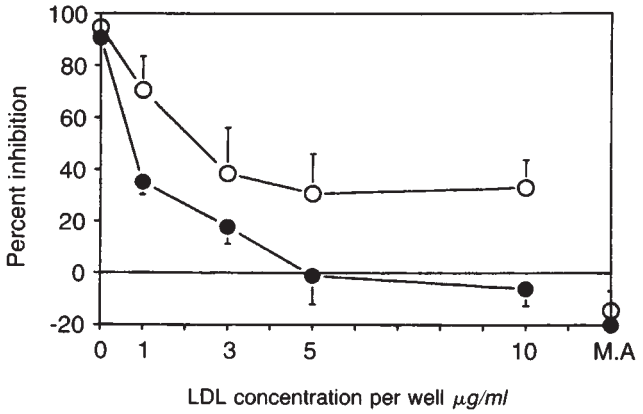

Fig. 2. *LDL receptor function assay ion four patients beforeand after renal transplantation.* The percent inhibition of PBMC proliferation by lovastatin was determined at increasing concentrations of LDL. Results are expressed as mean ± SEM pre-transplantation (open circles) and post-transplantation (closed circles).

concentrations of 80 μg/ml in both patient and control cells was likely due to the direct cellular toxicity of high LDL concentrations [35]. In the control subjects the mean LDL_{50}, that is, the concentration of LDL required to reverse 50% of the lovastatin inhibition, was 1.99 ± 0.6 μg/ml. In initial experiments PBMC from three patients with documented heterozygous familial hypercholesterolemia, the LDL_{50} was greater than 3.00 μg/ml; in PBMC from a patient with homozygous familial hypercholesterolemia even the highest concentration of LDL could not reverse lovastatin-mediated inhibition (data not shown). These results are in accord with previously published values [36], and demonstrate that the assay accurately detects differences in PBMC LDL receptor function.

LDL receptor function assay in uremic patients

Cells from uremic patients demonstrated significant inhibition of proliferation in the assay (Fig. 1). The mean LDL_{50} in the uremic subjects was 3.6 ± 1.7 μg/ml, which was significantly higher than the controls ($P < 0.00001$). Moreover, the percent inhibition in the uremic patients was statistically different from control subjects at each concentration of LDL. Cells from both normal and uremic patients proliferated equally well in the presence of mevalonic acid (Fig. 1); hence, the abnormal proliferation of the uremic cells was not a consequence of an intrinsic cellular defect. Furthermore, uremic and control PBMC were identical in their sensitivity to different concentrations of lovastatin (Table 3). These findings suggest that the LDL receptor does not function normally in PBMC from uremic patients.

Normalization of LDL receptor function following renal transplantation

LDL receptor function was studied in four uremic patients who underwent renal transplantation. The pre-transplantation mean LDL_{50} of these patients was 5.77 ± 3.7 μg/ml. However, when re-studied at least one month after transplantation, the mean LDL_{50} of the group had fallen to 1.98 ± 0.42 μg/ml, and relatively low LDL concentrations were able to completely abolish lovastatin inhibition of PBMC proliferation (Fig. 2). These observations suggest that LDL receptor dysfunction associated with renal failure is reversible, and may rapidly improve with resolution of the uremic state. One patient with refractory nephrotic syndrome due to focal segmental glomerulosclerosis had an LDL_{50} of 2.7 μg/ml. After nephrectomy and treatment with peritoneal dialysis, the LDL_{50} increased to 4.8 μg/ml, and even the highest concentrations of LDL were unable to abolish lovastatin inhibition of PBMC proliferation, as previously observed with the other uremic patients. This observation suggests that deterioration of renal function may be associated with worsening of LDL receptor function.

Validation of RNA probes and the RNAase protection assay

A dose-response analysis of the protection of total cellular RNA against RNAse digestion by LDL receptor and β-actin RNA probes was performed to establish the linearity of the assay. Increasing amounts of RNA extracted from HL60 cells were hybridized with LDL receptor and β-actin RNA probes, digested with RNAse A, and analyzed by electrophoresis and autoradiography. With increasing amounts of RNA, a progressive increase in the LDL receptor and β-actin hybridization signals was observed on the autoradiograph (Fig. 3). Laser densitometry of the autoradiograph established that a linear response of the hybridization signal was obtained with both probes upon increasing RNA mass. The correlation coefficient was greater than 0.99 for the LDL signal and 0.98 for the β-actin signal (Fig. 4). Even with a total RNA mass of 30 μg (more than 3 times the amount used in standard RNase protection assays), the response was still linear; hence under these assay conditions there is probe excess even at 30 μg of total RNA. Standard assay conditions therefore employed 10 μg of total RNA per sample.

To establish that the assay was sensitive to changes in cellular LDL receptor mRNA, LDL receptor mRNA was measured in HL60 cells treated with 25-hydroxycholesterol. The addition of 25-hydroxycholesterol (24 hrs treatment with 0.0625 μg/ml) to HL60 cells produced a 47% decrease in LDL receptor mRNA relative to untreated cells (data not shown), in agreement with the known down-regulation of the LDL receptor and its mRNA by this oxysterol [37].

RNAase protection assay of LDL receptor mRNA in PBMC from uremic patients and controls

To investigate the molecular basis for the LDL receptor dysfunction observed in the uremic subjects, we quantitated

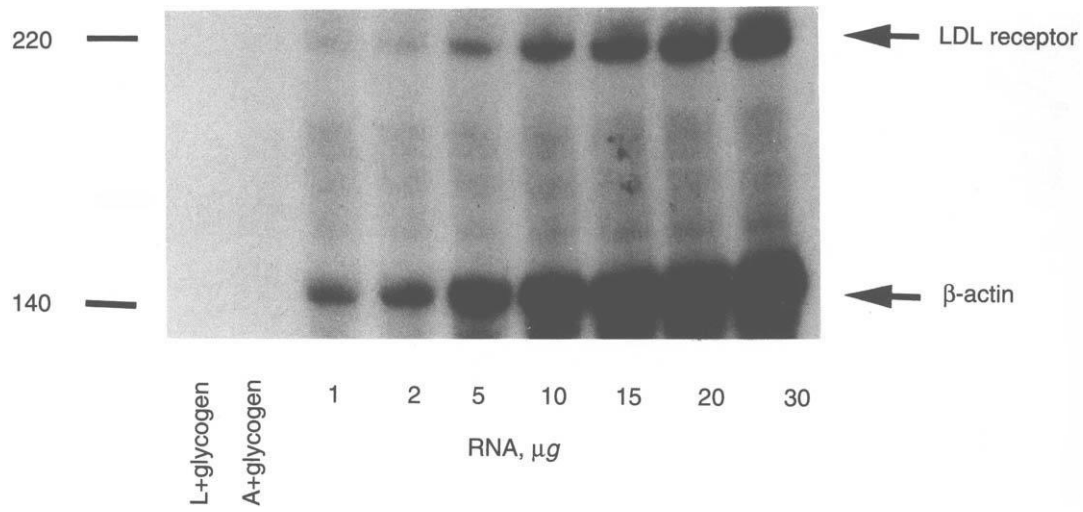

Fig. 3. *Dose-response analysis of HL60 total cellular RNA with LDL receptor and β-actin RNA antisense probes in a ribonuclease protection assay.* Increasing amounts of HL60 total cellular RNA was RNase protected with LDL receptor and β-actin RNA probes as described in **Methods**. The figure indicates the position of the protected mRNA fragments (220 bases for LDL receptor mRNA; 140 bases for β-actin RNA) relative to radiolabeled Hae III endonuclease digested φX-174 DNA used as size markers.

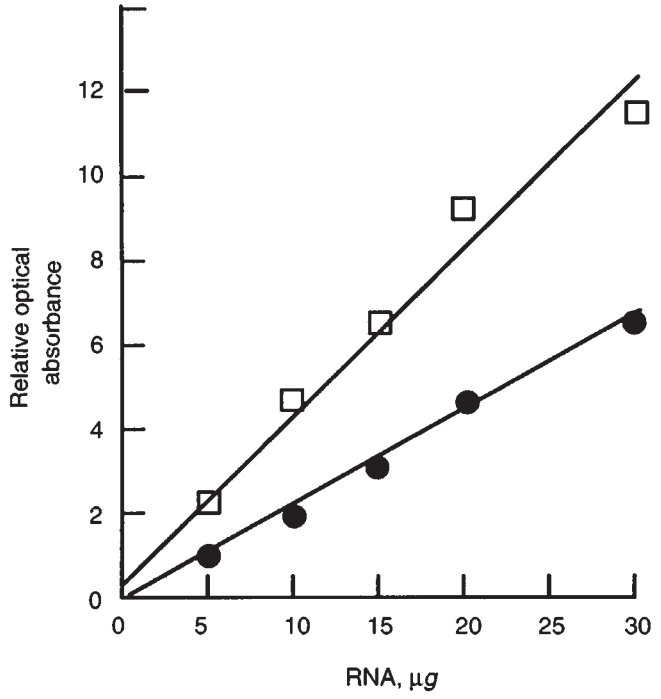

Fig. 4. *Demonstration of dose-response and linearity of the RNase protection assay system used in experiments.* Laser densitometry was performed on autoradiograph from the HL60 RNA dose-response experiment. Relative optical absorbance area-under-the-curve for the LDL receptor and β-actin RNA probes are plotted as a function of increasing HL60 total cellular RNA mass. Symbols are: (□) β-actin, r > 0.98; (●) < DLr, r > 0.99.

LDL receptor mRNA in freshly collected PBMC from uremic patients and control subjects with the RNAase protection assay. As indicated by the LDL receptor hybridization signal at 220 bases and the β-actin signal at 140 bases, a marked decrease in LDL receptor mRNA was observed in uremic patients relative to the controls (Fig. 5). The LDL receptor mRNA signal, normalized to the β-actin mRNA signal, was 0.42 ± 0.08 (N = 10) for the uremic patients, versus 0.71 ± 0.09 (N = 14) for the controls (Fig. 6). This represents a 40% relative decrease in LDL receptor mRNA, which was highly significant at $P < 0.015$. No alteration in β-actin mRNA level was observed in the uremic patients compared to controls. These data suggest that a significant reduction in LDL receptor mRNA was present in PBMC from uremic patients.

Associations between assay results and clinical parameters

The mean serum cholesterol level in the uremic subjects was 210 ± 68 mg/dl (mean ± SD); 72% of the patients had elevated levels for their age [38] (Table 2). The mean serum triglyceride levels in uremic subjects was 288 ± 166 mg/dl (Table 2), and 75% of the patients had levels which were elevated for their age [38]. Regression analysis revealed no correlation between the LDL_{50} or LDL receptor mRNA levels and measures of renal function (that is, blood urea nitrogen or serum creatinine) or dialysis efficiency (that is, pre-dialysis BUN in the patients receiving hemodialysis or steady state creatinine in patients treated with peritoneal dialysis). Nor was there a significant correlation between these tests and serum total cholesterol, triglycerides, LDL or HDL concentrations. This was not unexpected as previously noted [19, 39]. Examination of different dialysis modalities revealed that LDL_{50} and serum cholesterol levels were significantly higher in patients treated by peritoneal dialysis than by hemodialysis.

Discussion

The mechanisms which cause altered lipoprotein metabolism in renal disease have not been well delineated. Although abnormalities of lipoprotein lipase [40, 41], hepatic lipase [42] and lecithin-cholesterol acyltransferase [43] in uremia have been described, few studies have addressed the function of the LDL receptor. Our data establish that LDL receptor function as measured by this assay in PBMC from uremic patients is

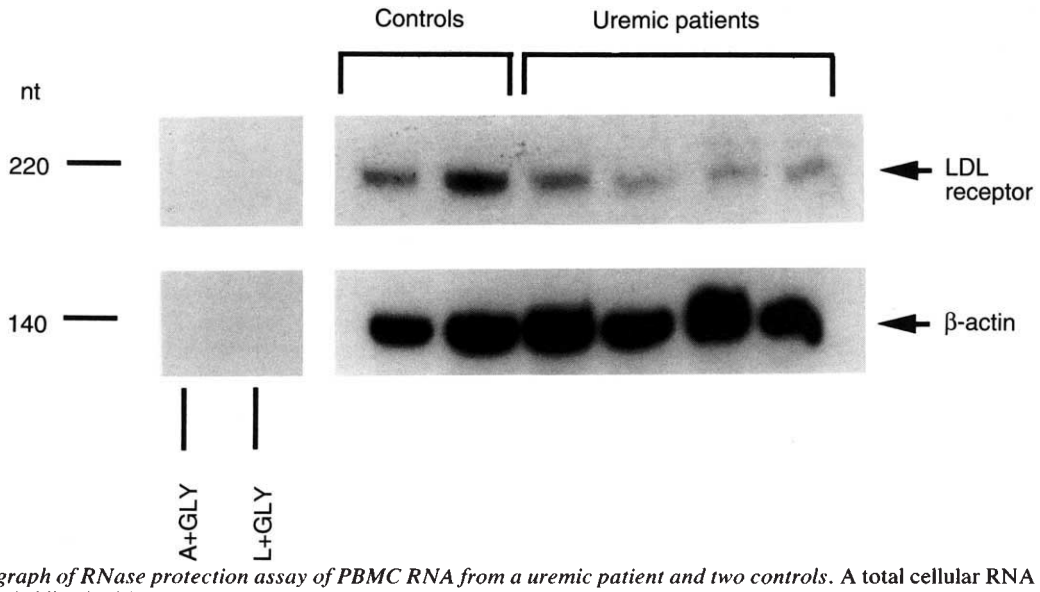

Fig. 5. *Autoradiograph of RNase protection assay of PBMC RNA from a uremic patient and two controls.* A total cellular RNA mass of 10 g from each sample was hybridized with the LDL receptor and β-actin antisense RNA probes and RNase A protected fragments were subjected to 8 M urea, 5% polyacrylamide gel electrophoresis. The LDL receptor protected band = 220 bases and the β-actin protected band = 140 bases.

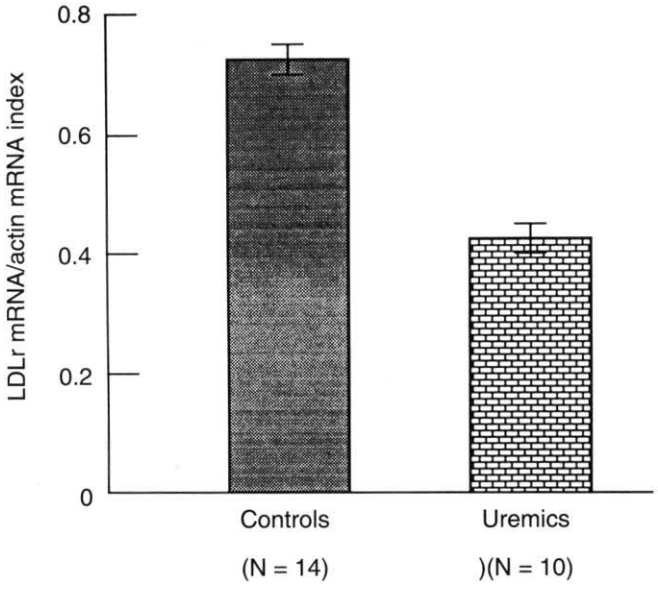

Fig. 6. *LDL receptor mRNA:β-actin mRNA index for a larger study of uremic patients and healthy controls.* RNase protection assays were performed on 10 μg of PBMC RNA from uremic patients (N = 10) and controls (N = 14). LDL receptor and β-actin mRNA levels were quantitated from autoradiographs by laser densitometry. LDL receptor mRNA level was normalized with β-actin to correct for any differences in RNA mass from sample to sample, and an L:A index is shown for uremic patients and controls.

depressed. The level of dysfunction in this assay is similar to that seen in patients with familial hypercholesterolemia and other lipoprotein disorders associated with decreased lipoprotein clearance but by different mechanisms. Moreover, the LDL receptor dysfunction appears to be reversible, as it is reversed by renal transplantation, which suggests a transient down-regulation by physiological factors.

The receptor dysfunction observed in uremic patients was associated with a significant reduction of PBMC LDL receptor mRNA when compared to healthy controls. This decrease could be due to any number of factors, such as uremic factors that either specifically inhibit transcription of LDL receptor mRNA, or modulate the amount of activity of critical factors required for LDL receptor gene transcription. Uremia could also reduce LDL receptor mRNA stability and/or accelerate degradation, thereby leading to decreased steady-state LDL receptor mRNA levels. Another possible mechanism for uremic-induced alteration in LDL receptor gene expression is a uremic effect on mitogenic stimulation of LDL receptor gene expression. It has been shown that mitogens can increase LDL receptor mRNA levels in human lymphocytes [44]. Uremic conditions may somehow interfere with normal mitogenic signaling in these lymphocytes. Interestingly, Cuthbert and Lipsky found that an oxygenated sterol could down-regulate LDL receptor gene expression equally in *both* mitogenic stimulated and control lymphocytes [44].

While it has proved difficult to directly measure LDL receptor protein on PBMC from uremic subjects, all studies to date suggest that reduced levels of LDL receptor mRNA should result in diminished synthesis and cellular expression of LDL receptor protein.

Several other candidate mechanisms for the observed receptor dysfunction must also be considered. It is possible that the depressed receptor function is a consequence of a global alteration in PBMC cholesterol synthesis secondary to nonspecific toxic effects of uremia. This is unlikely, however, as the addition of mevalonic acid completely restored the proliferative response in the uremic PBMCs. Structural alteration of the LDL molecule or the LDL receptor in the uremic environment could alter the affinity of their interaction, or disrupt the process of LDL internalization and digestion [45]. However, in our studies we utilized normal LDL isolated from healthy donors, and the incubation conditions for the PBMC used in our assay

is sufficiently long that newly synthesized LDL receptors would likely have been cycled to the PBMC surface. One could also speculate that a mechanism unrelated to LDL receptor activity could account for the observed functional defect. If this were the case, however, mevalonate should not have been able to restore normal proliferation to the uremic cells.

It is possible that differences in the lymphocyte/monocyte ratio of the cells isolated from the patients and controls could influence the response to lovastatin or phytohemagglutinin. However, in the assay of LDL receptor function, each patient serves as his own control, that is, inhibition of cellular proliferation is calculated relative to PBMC grown in optimal conditions. Thus the effect of differences in mononuclear cell subpopulation distribution upon the specificity of the assay for inhibition of LDL receptor function should be minimized. The possibility that uremic PBMC are more sensitive to lovastatin was made less plausible by the identical behavior of uremic and control PBMC in the dose-response studies.

A final consideration is that our findings may be a consequence of cellular toxicity of chemically modified forms of cholesterol. The decreased reductive capacity of uremic serum [46] may generate oxidatively modified sterols, such as 25-hydroxycholesterol, 7-ketocholesterol or 4-hydroxynonenal, some of which have been identified in LDL isolated from uremic patients [47]. Some of these oxysterols are 100 times more potent than LDL in inhibiting LDL receptor biosynthesis [37, 48], and prolonged exposure of PBMC to oxysterols in vivo could account for the decreased LDL receptor function and receptor mRNA levels observed in this study. However, since uremic PBMC grown with LDL in the absence of lovastatin proliferated normally, it seems unlikely that uremic PBMC directly generated oxysterols in vitro.

The elevations in serum triglycerides and moderate elevations in cholesterol in renal failure patients are directly associated with the degree of uremia [49], yet we observed no relationship between PBMC LDL receptor function or mRNA and serum lipids, lipoprotein levels, or crude measures of uremic severity in our patients. Significant correlations could have been obscured by the fact that the receptor function assay measures the maximum expression of PBMC LDL receptors, and not their receptor status immediately upon removal from the uremic milieu, or by the insensitivity of pre-dialysis BUN and steady-state creatinine levels to the clinical severity of uremia. Further, the LDL levels in these patients were not directly measured and the hypertriglyceridemia can lead to an overestimation of VLDL cholesterol by this formula and subsequently, a falsely low LDL fraction [21]. Uremia is associated with an increase in abnormal lipoproteins including particles which are β-VLDL-like in that they contain both apolipoproteins B and E and are typically triglyceride rich with some cholesterol [4]. The uremic LDL particle itself is triglyceride enriched. These particles are cleared, in part by the LDL (B/E) receptor. Thus a decrease in the LDL receptor funciton would be expected to produce an increase in both triglycerides and to a lesser extent cholesterol. There was also great variability in cholesterol and triglyceride levels in uremic patients based on variable nutritional status of some of the patients as evidenced by the large standard deviations of these values (Table 2). Nonetheless, improvement in patients' clinical status post transplantation was associated with a decrease in LDL_{50}, and deterioration after nephrectomy was associated with increased LDL_{50}.

At this time we can only speculate upon the relationship between LDL receptor dysfunction and the genesis of hyperlipidemia in renal failure. If other cells, such as endothelial cells, hepatocytes, and macrophages, manifested LDL receptor abnormalities similar to those seen in the PBMC, then intravascular LDL clearance would be decreased and hypercholesterolemia would ensue. Delayed clearance of LDL would also increase its exposure time to uremic plasma, and encourage the formation of oxidized species of LDL which have been implicated in foam cell formation [50]. Since the LDL receptor, or B/E receptor, also recognizes very low density lipoproteins (VLDL), reduced hepatic LDL receptor function could lead to the accumulation of VLDL, and VLDL remnants which, as they contain a high percentage of triglycerides, would lead to hypertriglyceridemia. Finally, reduced hepatic uptake of VLDL, VLDL remnants, and LDL would result in the up-regulation of hepatic cholesterol biosynthesis, as has been documented by Shapiro [18].

The increasing evidence that uremic hyperlipidemia constitutes a significant risk factor for accelerated atherosclerosis and progression of renal failure mandates an aggressive approach to the management of this condition. Nonetheless, therapy for these patients, especially in the pediatric population where early intervention may be most productive, is problematic. Dietary modification of cholesterol intake is extremely difficult because of other dietary restrictions imposed on these patients and, by itself, is rarely sufficient to control serum lipid levels. Although fibric acids are effective [51, 52], their renal excretion and the risk of serious myopathy limit their use. Recently it was demonstrated that depressed LDL receptor function in the lymphocytes of heterozygous FH patients was normalized by treatment with hypolipidemic drugs [36]. Pharmacotherapy with an HMG-CoA reductase inhibitor which enhances LDL receptor function may be useful in uremic patients. Probucol, which has been shown to decrease oxysterol production [53], could potentially be effective in treating uremic hyperlipidemia by preventing LDL receptor mRNA down-regulation.

In summary, we have demonstrated abnormal LDL receptor function in PBMC's in uremic patients, which is reversed by renal transplantation. We have further documented a concomitant reduction in lymphocyte LDL receptor mRNA levels in uremic patients compared to controls, suggesting that down-regulation of the transcription of LDL receptor mRNA or accelerated mRNA degradation could be the cause of this phenomena. Further investigation of LDL receptor function in renal disease will contribute to the elucidation of the causes of uremic hyperlipidemia and may hasten the development of effective therapy to retard the progression of renal failure and atherosclerosis in uremia.

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References

1. BAGDADE JD, PORTE D, BIERMAN EL: Hypertriglyceridemia, a metabolic consequence of chronic renal failure. *N Engl J Med* 279:181–185, 1968
2. ATGER V, DUVAL F, FROMMHERZ K: Anomalies in composition of uremic lipoproteins isolated by gradient ultracentrifugation. *Atherosclerosis* 74:75–83, 1988
3. MUJAIS SK, SABATINI S, KURTZMAN NA: Pathophysiology of the Uremic Syndrome, in *The Kidney* (3rd ed), edited by BRENNER BM, RECTOR FC, Philadelphia, W.B. Saunders, 1986, pp 1593–1595
4. NESTEL PJ, FIDGE NH, TAN MH: Increased lipoprotein-remnant formation in chronic renal failure. *N Engl J Med* 307:329–333, 1982
5. HAAS LB, WAHL PW, SHERRARD DJ: A longitudinal study of lipid abnormalities in renal failure. *Nephron* 33:145–149, 1983
6. LINDNER A, CHARRA B, SHERRARD DJ, SCRIBNER BH: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290:697–701, 1974
7. RUTSKY EA, ROBARDS M, VAN DYKE JA, ROSTAND SG: Acute pancreatitis in patients with end stage renal disease without transplantation. *Arch Int Med* 146:1741–1745, 1986
8. BURTON BT, KRUEGER KK, BRYAN FA: National registry of long-term dialysis patients. *J Am Med Assoc* 218:718–722, 1971
9. BONOMINI CF, SCOLARI MP, STEFONI S, VANGELISTA A: Atherosclerosis in uremia: A longitudinal study. *Am J Clin Nutr* 33:1493–1500, 1980
10. ROSTAND SG, KIRK KA, RUTSKY EA: Relationship of coronary risk factors to hemodialysis-associated ischemic heart disease. *Kidney Int* 22:304–308, 1982
11. PORTMAN RJ, VERANI R, HAWKINS E: Premature atherosclerosis in pediatric renal patients. Report of the Southwest Pediatric Nephrology Study Group. (abstract) *Pediatr Res* 29:349A, 1991
12. SCHMITZ PG, KASISKE BL, O'DONNELL MP, KEANE WF: Lipids and progressive renal injury. *Semin Nephrol* 9:354–369, 1989
13. DIAMOND JR, KARNOVSKY MJ: Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int* 33:917–924, 1988
14. PENNISI AJ, HEUSER EJ, MICKEY MR, LIPSEY A, MALEKZADEH MH, FINE RN: Hyperlipidemia in pediatric hemodialysis and renal transplant patients: Associated with coronary artery disease. *Am J Dis Child* 130:957–961, 1976
15. MANSKE CL: Lipid abnormalities and renal disease. *The Kidney* 20:25–30, 1988
16. BROWN MS, GOLDSTEIN JL: A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47, 1986
17. SHAPIRO RJ: Cholestyramine normalizes LDL catabolism in uremic guinea pigs. (abstract) *Kidney Int* 33:384, 1988
18. SHAPIRO RJ: Cholesterol biosynthesis is altered in uremic guinea pigs. (abstract) *Kidney Int* 35:437, 1989
19. CUTHBERT JA, EAST CA, BILHEIMER DW, LIPSKY PE: Detection of familial hypercholesterolemia by assaying functional low-density-lipoprotein receptors on lymphocytes. *N Engl J Med* 314:879–883, 1986
20. CONLEY SB, PORTMAN RJ, LEMIRE JM, VAN BUREN CT, LEWIS R, KAHAN BD: Five years experience with cyclosporine in children. *Trans Proc* 20(Suppl 3):280–4, 1988
21. FRIEDEWALD WT, LEVY RI, FREDRICKSON DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentifuge. *Clin Chem* 18:499–502, 1972
22. SERGENT JA, GOTCH FA: Mathematical modeling of dialysis therapy. *Kidney Int* (Suppl 10):S2, 1980
23. BALFE JW, STEFANIDES CJ, STEELE BJ, HEWITT IK: Continuous ambulatory peritoneal dialysis:Clinical aspects, in *End Stage Renal Disease in Children*, edited by FINE RN, GRUSKIN AB, Philadelphia, WB Saunders, 1984, pp 138–141
24. OVERTURF ML, SMITH SA, HEWETT-EMMETT D, LOOSE-MITCHELL DS, SOMA MR, GOTTO AM, MORRISETT JD: Development and partial characterization of a dietary cholesterol-resistant colony of rabbits. *J Lipid Res* 30:263–273, 1989
25. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin Phenal Reagent. *J Biol Chem* 193:266–275, 1951
26. HO YK, BROWN MS, BILHEIMER DW, GOLDSTEIN JL: Regulation of low density lipoprotein receptor activity in freshly isolated human lymphocytes. *J Clin Invest* 58:1465–1474, 1976
27. CHIRGWIN JM, PRZYBYLA AE, MACDONALD RJ, RUTTER WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294–5299, 1979
28. YAMAMOTO T, DAVIS CG, BROWN MS, SCHNEIDER WJ, CASEY ML, GOLDSTEIN JL, RUSSELL DW: The human LDL receptor: A cysteine-rich protein with multiple Alu sequences in its mRNA. *Cell* 39:27–38, 1984
29. SUDHOF TC, GOLDSTEIN JL, BROWN MS, RUSSELL DW: The LDL receptor gene: A mosaic of exons shared with different proteins. *Science* 228:815–822, 1985
30. PONTE P, NG S-Y, ENGEL J, GUNNING P, KEDES L: Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. *Nucleic Acids Res* 12:1687–1696, 1984
31. PONTE P, GUNNING P, BLAU H, KEDES L: Human actin genes are single copy for alpha-skeletal and alpha-cardiac actin but multicopy for beta- and gamma- cytoskeletal genes: 3′ untranslated regions are isotype specific but are conserved in evolution. *Molec Cell Biol* 3:1783–1791, 1983
32. HANUKOGLU I, TANESE N, FUCHS E: Complementary DNA sequence of a human cytoplasmic actin. *J Mol Biol* 163:673–678, 1983
33. MELTON DA, KRIEG PA, REBAGLIATI MR, MANIATIS T, ZINN K, GREEN MR: Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res* 12:7035–7056, 1984
34. POCOCK SJ, GELLER NL, TSIATIS AA: The analysis of multiple end points in clinical trials. *Biometrics* 43:487–498, 1987
35. MORSE JH, WITTE LD, GOODMAN DS: Inhibition of lymphocyte proliferation stimulated by lectins and allogeneic cells by normal plasma lipoproteins. *J Exp Med* 146:1791–1803, 1977
36. CUTHBERT JA, EAST CA, LIPSKY PE: Normalization of LDL receptor function by lymphocytes of patients with heterozygous familial hypercholesterolemia after treatment with plasma cholesterol lowering agents. *Am J Med Sci* 298:152–160, 1989
37. BROWN MS, GOLDSTEIN JL: Regulation of the activity of the low density lipoprotein receptor in human fibroblasts. *Cell* 6:307–316, 1975
38. BERENSON GS: *Cardiovascular Risk Factors in Children*. New York, Oxford University Press, 1980, pp 151–211
39. CORTESE C, TURNER PR, MARENAH CB, SULE U, PRICE S, MILLER NE, LEWIS B: Lack of correlation between the maximum low-density lipoprotein receptor activity of blood lymphocytes and plasma concentration in normal men. *Clin Sci* 65:95–98, 1983
40. ROULLET JB, LACOUR B, YVERT JP, PRAT JJ, TILMAN D: Factors of increase in serum triglyceride-rich lipoproteins in uremic rats. *Kidney Int* 27:420–5, 1985
41. BAGDADE JD, YEE E, WILSON DE, SHAFRIR E: Hyperlipidemia in renal failure: Studies of plasma lipoproteins, hepatic triglyceride production, and tissue lipoprotein lipase in a chronically uremic rat model. *J Lab Clin Med* 91:176–186, 1978
42. MORDASINI R, FREY F, FLURY W, KLOSE G, GRETEN H: Selective deficiency of hepatic triglyceride lipase in uremic patients. *N Engl J Med* 297:1362–1366, 1977
43. MCLEOD R, REEVE CE, FROHLICH J: Plasma lipoproteins and lecithin cholesterol acyltransferase distribution in patients on dialysis. *Kidney Int* 25:683–688, 1984
44. CUTHBERT JA, LIPSKY PE: Mitogenic stimulation alters the regulation of LDL receptor gene expression in human lymphocytes. *J Lipid Res* 31:2067–2078, 1990
45. SHAPIRO RJ: Mechanisms for hyperlipidemia in chronic renal failure. *Kidney Int* 29:326, 1986
46. KURODA M, ASAKA S, TAKEDA R: Serum antioxidant activity in uremic patients. *Nephron* 41:293–298, 1985
47. CHISOLM G: The role of lipid oxidation in endothelial cell toxicity

and altered endothelial cell function. (abstract) *Am J Kid Dis* 14:427, 1989

48. BROWN MS, GOLDSTEIN JL: Suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. *J Biol Chem* 249:7306–7314, 1974
49. GRUETZMACHER P, MAERZ W, PESCHKE B, GROSS W, SCHOEPPE: Lipoproteins and apolipoproteins during the progression of chronic renal failure. *Nephron* 50:103–111, 1988
50. STEINBERG D, PARATHASWARATHY S, CAREW TE, KHOO JC, WITZTUM JL: Beyond cholesterol. Modifications of low density lipoprotein that increase its atherogenicity. *N Engl J Med* 320:915–924, 1989
51. PASTERNACK A, VANTTINEN T, SOLAKIVI T, KUUSI T, KORTE T: Normalization of lipoprotein lipase and hepatic lipase by gemfibrozil results in correction of lipoprotein abnormalities in chronic renal failure. *Clin Nephrol* 27:163–168, 1987
52. GRUNDY SM: Management of hyperlipidemia of kidney disease. *Kidney Int* 37:847–853, 1990
53. PARTHASARATHY S, YOUNG SG, WITZTUM JL, PITTMAN RC, STEINBERG D: Probucol inhibits oxidative modification of low density lipoprotein. *J Clin Invest* 77:641–644, 1986