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SUSCEPTIBILITY OF NON-HDL FRACTION TO OXIDATION IN EXPERIMENTAL NEPHROTIC SYNDROME

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INDEXING WORDS

nephrotic syndrome; non-HDL fraction; vitamin E

SYNOPSIS

Hyperlipidemia is a striking feature of nephrotic syndrome (NS) and the lipid profile seen in NS is accepted as atherogenic. Both low density lipoprotein (LDL) and very low density lipoprotein (VLDL) are apolipoprotein B-containing lipoproteins which are accepted as atherogenic. Oxidized-LDL (ox-LDL) has been suggested to play a fundamental role in atherogenesis. In this study, male Sprague-Dawley rats were made nephrotic by a single intraperitoneal injection of puromycin aminonucleoside (100 mg/kg body weight). We found significant elevation in serum triglycerides, total cholesterol, malondialdehyde, vitamin E levels and total cholesterol / vitamin E ratio and decrease in total protein and albumin levels in the NS group (n:8) compared with the control group (n:9). High density lipoprotein (HDL) - cholesterol and free fatty acid levels were not significantly different between these two groups. Apolipoprotein B-containing lipoproteins (non-HDL fraction) were separated by precipitation and amount of thiobarbituric acid-reactive substances (TBARS) of non-HDL fraction were measured after 60, 90, 120, 180 minutes of incubation with copper sulphate. TBARS levels of non-HDL fraction were significantly higher in the NS group compared with the control group at all of the time periods above. In nephrotic animals, the increased lipid peroxidation was influenced by serum lipids.

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INTRODUCTION

Nephrotic syndrome (NS) may occur during the course of kidney diseases of varying causes and is characterised by proteinuria, lipiduria, hypoalbuminemia, hyperlipidemia and edema. Hyperlipidemia is frequently seen in nephrotic syndrome, and includes increases in serum concentrations of cholesterol, triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), lipoprotein (a) [Lp(a)], as well as low, normal or elevated levels of high density lipoprotein (HDL).[16,17,25,32,33,36,39] However, cause of hyperlipidemia and mechanism by which it increases the risk of cardiovascular disease (CVD) is still unclear. Causes of hyperlipidemia may be related to increases in lipoprotein synthesis in the liver in response to low serum oncotic pressure and/or abnormalities in the catabolism of lipoproteins. Lipoprotein lipase activity has been found to decrease in NS and a major catabolic defect has been attributed to this decrease. Lecithin:cholesterol acyltransferase, the key enzyme in the reverse cholesterol transport, has also been reported to be less active in the patients with NS.[5,7,30,37]

The incidence of CVD is higher in NS cases than that of normal population. Patients with NS have some risk factors for atherosclerosis other than hyperlipidemia, such as hypertension, hypercoagulability, etc..[3,26] Hyperlipidemia in NS mainly consists of hypertriglyceridemia and hypercholesterolemia, especially with a rise in LDL-cholesterol levels. Elevated LDL-cholesterol level has been associated with increased incidence of CVD. In some experimental studies, it was proposed that hyperlipidemia might cause renal damage by some mechanisms.[19,22] It was demonstrated that the lipid deposits which were composed of cholesterol, triglycerides, phospholipids, apolipoproteins (apo) A, B, E and oxidized-LDL (ox-LDL) particles, accumulated in renal tissues of rats with experimental NS.[14,21,38] In recent years, ox-LDL has been accepted as an important factor in the pathogenesis of atherosclerosis.[34,35] Ox-LDL and ox-VLDL share some common properties: both are chemoattractant for monocytes and T lymphocytes and are cytotoxic and both can be taken up by the scavenger receptors of macrophages and smooth muscle cells.[43] Some investigators suggested that not only ox-LDL but also ox-VLDL could play a role in the development of atherosclerotic lesions.[10]

The lipid abnormalities seen in NS formed by puromycin aminonucleoside (PAN) injection in rats and those seen in nephrotic patients are similar. The NS caused by injection of PAN in rats is an experimental model of minimal change disease seen in humans.[13,20]

In this study, we have formed experimental NS in rats with PAN injection and determined the susceptibility of apo B-containing lipoproteins (non-HDL fraction; LDL,VLDL) to oxidation, plasma malondialdehyde (MDA) levels and interaction of these parameters with serum lipids and vitamin E levels.

MATERIALS AND METHODS

Animals

Experiments were performed on male Sprague-Dawley rats, weighing 325-480 g and maintained ad libitum on a regular laboratory chow and tap water. In order to form NS, 8 rats were injected intraperitoneally 100 mg/kg body weight 3% PAN (Sigma, St. Louis, MO) that was dissolved in 0.15 mol/L NaCl. Control rats (n:9) were also injected with the same volume of 0.15 M NaCl without PAN. 15 days after the injection, blood was taken from both of the groups by cardiac puncture under mild diethyl ether anaesthesia. Some of the blood was transferred to tubes containing EDTA (1.5 g / L blood) and then centrifuged at 1000 g to obtain plasma for measuring the susceptibility of non-HDL fraction to oxidation. The liver and kidneys were quickly removed, blotted on a filter paper to remove blood and weighted.

Methods

Serum total cholesterol (TC), TG and free fatty acid (FFA) levels were determined with enzymatic spectrophotometric methods (Randox, UK). HDL-cholesterol was quantified by the same enzymatic method after precipitation of VLDL and LDL with dextran sulphate-magnesium chloride.[6] Biuret and brom crezol methods were performed to measure total protein (TP) and albumin levels, respectively.[8,9] Plasma MDA levels were determined spectrophotometrically.[15]

Precipitation of the non-HDL fraction by dextran sulphate-magnesium, which is followed by the removal of EDTA, was carried out as described by Zhang et al .[43] Cholesterol content of EDTA-free non-HDL fraction was adjusted to 200 μg/ml for measurement of thiobarbituric acid-reactive substances (TBARS). Five tubes were prepared for each case. Tubes containing 0.5 ml sample (200 μg cholesterol/ml) were added 50 μl 0.5 mM $CuSO_4$ and incubated at 37°C except the first tube. Other tubes were removed one by one from the incubator at 60, 90, 120 and 180th minutes. The concentration of TBARS in the sample was estimated by comparison with a standard curve prepared from 1,1',3,3' tetraethoxypropane. The final results were expressed as nmol MDA equivalents/mg non-HDL cholesterol.

Statistical analysis

Data were expressed as mean ± SD. Differences between the groups were considered significant at $p<0.05$ by nonparametric methods (Mann-Whitney U-test). Correlation were determined by calculation of Pearson correlation coefficient.

RESULTS

The characteristics of the nephrotic and control rats were summarized in Table I.

Table I. Body, liver and kidney weights in all rats.

Rats	Control	Nephrotic Syndrome
n	9	8
Body weight, g		
initial	414 ± 32	385 ± 45
final	435 ± 78	324 ± 42[a]
Liver weight, g/100 g	2.50 ± 0.39	4.10 ± 0.27[b]
Kidney weight, g/100 g	0.79 ± 0.10	1.10 ± 0.03[b]

Values are expressed as mean ± SD. [a] $p<0.01$ vs. controls [b] $p<0.001$ vs. controls (Mann Whitney U-test)

Table II. Serum triglycerides, total cholesterol, HDL-cholesterol, total protein, albumin, free fatty acids and vitamin E levels of the groups.

Parameters	Control (n:9)	Nephrotic Syndrome (n:8)	p
TG (mg/dl)	30 ± 8	180 ± 88	<0.001
TC (mg/dl)	57 ± 11	260 ± 98	<0.001
HDL-C (mg/dl)	42 ± 6	70 ± 31	>0.05
TP (g/dl)	7.6 ± 0.2	7.2 ± 0.4	<0.01
Albumin (g/dl)	4.4 ± 0.2	2.0 ± 0.4	<0.001
FFA (mEq/L)	0.51 ± 0.09	0.45 ± 0.07	>0.05
Vitamin E (mg/dl)	0.62 ± 0.04	1.13 ± 0.19	<0.001
TC/Vitamin E (mmol/μmol)	10.2 ± 1.8	26.2 ± 11.3	<0.001

Data are expressed as mean ± SD
p: Statistical significance of the differences, compared with Mann-Whitney U-Test
TG: Triglycerides, TC: Total cholesterol, HDL-C: High density lipoprotein-cholesterol, TP: Total protein, FFA: Free fatty acid

TG, TC, MDA, vitamin E levels and the TC/vitamin E ratio were significantly higher ($p<0.001$ for each) while TP and albumin levels were

significantly lower ($p<0.01$ and 0.001, respectively) in the NS group than those in the control group. There were no significant differences between HDL-cholesterol and FFA levels between the groups (Table II, III).At the beginning (unincubated tube) there was no significant difference between the MDA levels of non-HDL fraction among the groups. However, the amounts of MDA measured at 60, 90, 120 and 180th minutes were significantly higher in the NS group than those in the control group ($p<0.01$ for each) (Figure).

Table III. Plasma MDA levels in the control and nephrotic syndrome groups.

Parameter	Control (n:9)	Nephrotic Syndrome (n:8)	p
MDA (nmol/ml)	9.1 ± 1.6	13.4 ± 0.7	<0.001

Data are expressed as mean ± SD
MDA: Malondialdehyde

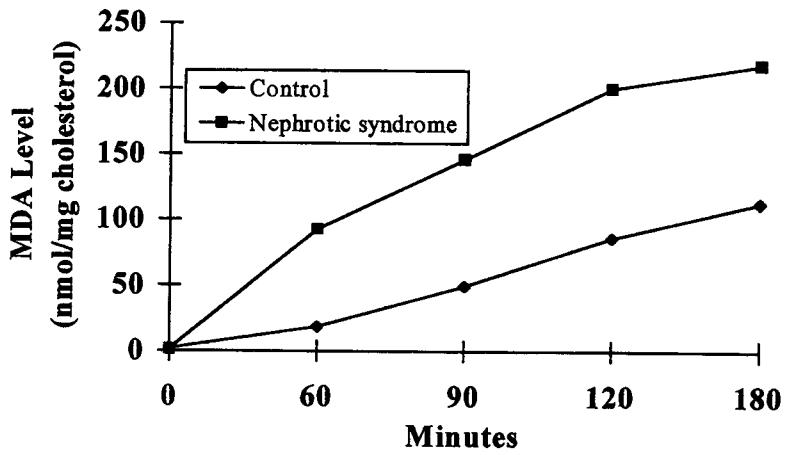

Figure. MDA levels of the non-HDL fraction at the prior and after 60, 90, 120 and 180 minutes of incubation with copper sulphate.

Correlations between TC, TG levels, TC/vitamin E ratios and the MDA levels of non-HDL fractions measured at 0, 60, 90, 120 and 180th minutes were shown in Table IV. In the control group there were no correlation between these parameters; but in the NS group there were significantly positive correlations between TC, TG levels, TC/vitamin E ratio and the MDA levels of non-HDL fraction at 60, 90, 120 and 180th minutes.

Table IV. Correlation coefficients between MDA levels of non-HDL fraction and serum total cholesterol, triglycerides and total cholesterol/vitamin E ratio

	CONTROL GROUP			NEPHROTIC SYNDROME GROUP		
MDA levels measured at the time intervals	TC	TG	TC/ Vit E	TC	TG	TC/ Vit E
Beginning	-0.568	0.017	-0.505	0.407	0.057	0.032
60th minute	0.093	-0.136	-0.064	0.869[a]	0.865[a]	0.742[a]
90th minute	0.078	0.152	0.060	0.874[a]	0.793[a]	0.684
120th minute	-0.039	0.348	0.020	0.841[a]	0.848[a]	0.715[a]
180th minute	-0.094	0.531	0.114	0.870[a]	0.835[a]	0.716[a]

[a]$p<0.05$
See Table II for abbreviations.

DISCUSSION

TC and TG levels were significantly higher, TP and albumin levels were significantly lower in the PAN-injected group compared to the NaCl-injected (control) group. These data were accepted as the evident of formation of NS with PAN injection.

NS is characterised with abnormalities of lipoprotein metabolism which lead to increase in the risk of CVD. On the other hand, the patients with NS have also other risk factors than hiperlipidemia, such as hypertension or hypercoagulability. There are some studies reporting that the risk of CVD in NS increases 5.5 to 85 fold than the normal population.[2,24] However, there has been no consensus on this subject. Moreover, there are some other studies indicating no increase in the risk.[40] Glomerular and tubulointerstisial damage have also been known as other harmful effects of hyperlipidemia in NS.[19,22]

The plasma concentration of MDA was significantly higher in the NS group. Shishkin et al. also reported MDA levels to be elevated in NS.[31] Serum vitamin E levels were higher in the NS group than those in the control group, whereas lipid-standardized vitamin E (TC/vitamin E ratio) was significantly higher in the NS group than that in the control group. The latter could be an explanation why the MDA levels are higher in the NS group. It has been accepted that low serum vitamin E levels or inadequate intake of vitamin E is associated with increased risk of CVD. Mydelik et al. found that vitamin E and A levels were elevated in 33 NS patients and proposed that it was related to the increase in lipoprotein synthesis.[23]

Increased susceptibility of LDL to oxidation in *in vitro* conditions is accepted as an indicator for its atherogenic potential. In this purpose, the most commonly performed method is the incubation of LDL with Cu^{2+} and then measuring the lipid peroxidation products.[4,11,27] In our study, we separated LDL and the other apo B-containing lipoprotein (VLDL) and then incubated this fraction with Cu^{2+} for 60, 90, 120 and 180 minutes. After the incubation, we measured MDA levels. MDA levels were significantly higher in the NS group at all of the periods. In the control group, there were no significant correlations between the MDA levels of non-HDL fraction and serum TG, TC levels or TC/vitamin E ratio; but in the NS group there were significantly positive correlations between these parameters. Apanay et al. reported that there were no correlations between serum LDL oxidation and serum cholesterol or TG levels.[1] The conflict between our results and the latter could be the result of the difference between the methods that had been performed. We determined not only the susceptibility of LDL but VLDL as well.

In the glomeruli there is a homeostasis in LDL entrance, usage and removal in normolipidemic conditions. In these conditions LDL contains enough antioxidants to protect itself from oxidation and is not subject to oxidative modification until there is an oxidative stress. However, when in the case of excessive LDL in the mesengial tissue or in the case of an oxidative stress, LDL loses its antioxidants and is faced to oxidative modification.[28]

Ox-LDL is cytotoxic and induces the formation and secretion of cytoxins and also induces monocytes to migrate into the arterial wall. Ox-LDL is also taken up by mesangial cells via scavenger receptors and the mesangial cells are then transformed

into foam cells. Mediators released from macrophages and ox-LDL induce formation of eicosanoids and modify mesangial cells' functions by affecting matrix components.[29,42] The endothelial cell damage caused by ox-LDL leads to platelet aggregation.[12] All these changes result in the disruption of glomerular hemodynamic function and vessel permeability.[41] In a study, performed by Kaplan et al., healthy rats were fed in cholesterol rich diet and the observed vasoconstriction was proposed to be associated with the increase in ox-LDL level.[18] Elevation of ox-LDL in NS may cause both renal damage and acceleration in atherogenesis.

The increased amount of vitamin E was probably a manifestation of enhanced lipoprotein synthesis in the liver in response to protein depletion. However, when the results were standardized for cholesterol content, TC/vitamin E ratio was higher in the nephrotic rats. Thus, in nephrotic syndrome a higher cholesterol to vitamin E ratio could contribute to the enhanced oxidizability of apo-B containing lipoprotein particles. In conclusion, high serum cholesterol, triglycerides levels and cholesterol/vitamin E ratio seem to represent very important factors in nephrotic animals.

REFERENCES

1. Apanay, D.C., Neylan, J.F., Ragab, M.S., and Sgoutas, D.S.: Transplantation. 1994. 58(6). 663/669. Cyclosporine increases the oxidizability of low density lipoproteins in renal transplant recipients.
2. Berlyne, G.M. and Mallick, N.P.: Lancet. 1969. 2. 399/400. Ischemic heart disease as a complication of nephrotic syndrome.
3. Bernard, D.B.: Kidney. Int. 1988. 33.1184/1202. Extrarenal complications of the nephrotic syndrome.
4. Bowie, A., Owens, D., Collins, P., Jonshon, A., and Tomkin, G.H.: Atherosclerosis. 1993. 102. 63/67. Glycosylated low density lipoprotein is more sensitive to oxidation:implications for the diabetic patient?
5. Cohen, S.L., Cramp, D.G., Lewis, A.D., and Tickner, T.R.: Clin. Chim. Acta. 1980. 104. 393/397.Serum LCAT activity in nephrotic syndrome.
6. Demacker, P.N.M., Vos-Janssen, H.E., Hijmans, A.G.M., Van't Laar, A., and Jansen, A.P.: Clin. Chem. 1980. 26(13). 1780/1786. Measurement of high-density lipoprotein cholesterol in serum: Comparison of six isolation methods combined with enzymic cholesterol analysis.
7. Diamant, S. and Shafrir, E.: Biochim. Biophys. Acta.1974.360.241/251. Lipogenesis in aminonucleoside-induced nephrotic syndrome.
8. Doumas, B.T., Bayse, D., Borner, K., Carter, R.J., Peters, T., and Schaffer, R.: Clin. Chem. 1981. 27.1642/1647. A candidate reference method for determination of total protein in serum: I. Development and validation.

9. Doumas, B.T., Watson, W.A., and Biggs, H.G.: Clin. Chim. Acta. 1971. 31. 87/96. Albumin standards and the measurement of serum albumin with bromcresol green.
10. Esterbauer, H., Gebicki, J., Puhl, H., and Jurgens, G.: Free. Rad. Biol. Med. 1992. 13. 341/390. The role of lipid peroxidation and antioxidants in oxidative modification of LDL
11. Esterbauer, H., Striegel, G., Puhl, H., and Rotheneder, M.: Free. Rad. Res. Comm. 1989. 6. 67/75.Continuous monitoring of in vitro oxidation of human low density lipoprotein.
12. Evensen, S.A., Galdal, K.S., and Nilsen, E.: Atherosclerosis. 1983. 49. 23/30. LDL-induced cytotoxicity and its inhibition by antioxidant treatment in cultured human endothelial cells and fibroblasts.
13. Frukawa, S., Hirano, T., Mamo John, C.L., Nagano, S., and Takahashi, T.: Metabolism. 1990. 39. 101/107.Catabolic defect of triglyceride is associated with abnormal very-low density lipoprotein experimental nephrosis.
14. Grond, J., van Goor, H., Erkelens, D.W., and Elema, J.D.: Virchows. Arch (B).1986. 51.521/534.Glomerular sclerotic lesions in the rat. Histochemical analysis of their macromolecular and cellular composition.
15. Janero, D.R.: Free. Radic. Biol. Med. 1990. 9. 515/540. Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury.
16. Joven, J., Rubies-Prat, J., Espinel, E., Ras, M.R., and Piera, L.: Nephrol. Dial.Transplant.1987. 2. 149/153.High-density lipoproteins in untreated idiopathic nephrotic syndrome without renal failure.
17. Joven, J., Villabona, C., Viella, E., Masana, l., Albuminerti, R., and Valles, M.: N. Engl. J. Med. 1990. 323. 579/584. Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome.
18. Kaplan, R., Aynedjian, H.S., Schlondorff, D., and Bank, N.: J. Clin. Invest. 1990. 86: 1707-1714. Renal vasoconstriction caused by short term cholesterol feeding is corrected by thromboxane antagonist or probucol.
19. Keane, W.F., Kasiske, B.L., O'Donnell, M.P., and Kim, Y.: Am. J. Kidney. Dis.1991.17. 38/42.The role of altered lipid metabolism in the progression of renal disease: Experimental evidence.
20. Levy, E., Ziv, E., Bar-On, H., and Shafrir, E.: Biochim. Biophys. Acta. 1990.1043. 259/266. Experimental nephrotic syndrome: Removal and tissue distribution of chylomicrons and very-low density lipoproteins of normal and nephrotic origin.
21. Magil, A.B., Frohlich, J.J., Innis, S.M., and Steinbrecher, U.P.: Kidney. Int. 1993. 43.1245/1250. Oxidised low-density lipoprotein in experimental focal glomerulosclerosis.

22. Moorhead, J.F., Wheeler, D.C., and Varghese, Z.: Am. J. Med.1989. 87. 5N/12N.Glomerular structures and lipids in progressive renal disease.
23. Mydlik, M., Derzsiova, K., Bratova, M., and Havris, S.: Int. Urol. Nephrol. 1991. 23(4). 399/405. Serum vitamin A, retinyl esters and vitamin E in nephrotic syndrome.
24. Ordonez, J.D., Hiatt, R.A., Killebrew, E.J., and Fireman, B.H.: Kidney. Int. 1992. 44. 638/642.The increased risk of coronary heart disease associated with nephrotic syndrome.
25. Querfeld, U., Gnasso, A., Haberbosch, W., Augustin, J., and Scharer, K.: Eur. J. Pediatr. 1988.147. 233/238. Lipoprotein profiles at different stages of the nephrotic syndrome.
26. Radhakrishnan, J., Appel, A.S., Valeri, A., and Appel, G.B.: Am. J. Kidney. Dis. 1993. 22.135/142.The nephrotic syndrome, lipids and risk factors for cardiovascular disease.
27. Regnstrom, J., Nilsson, J., Tornvall, P., Landou, C., and Hamsten, A.: Lancet. 1992. 339. 1183/1186. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man.
28. Schlondorff, D.: Am. J. Kidney. Dis. 1993. 22(1). 72/82. Cellular mechanisms of lipid injury in the glomerulus.
29. Schwartz, C.J., Valente, A.J., and Sprague, E.A.: Am. J. Kardiol. 1993. 71. 9B/14B. A modern view of atherogenesis.
30. Sestak, T.L., Alavi, N., and Subbaiah, P.V.: Kidney. Inter. 1989. 36. 240/248. Plasma lipids and acyltransferase activities in experimental nephrotic syndrome.
31. Shiskin, A.N., Kolmakova, E.V., and Ashirova, K.V.: Urol. Nefrol. 1994. 3. 39/41. Lipid metabolic disorders and lipid peroxidation in patients with the nephrotic syndrome.
32. Short, C.D., Durrington, P.N., Mallick, N.P., Bhatnagar, D., Hunt, L.P., and Mbewu, A.: Nephrol. Dial. Transplant.1992. (suppl 1) 7. 109/113. Serum lipoprotein (a) levels in men with nephrotic syndrome due to idiopathic membranous nephrophathy.
33. Short, C.D., Durrington, P.N., Mallick, N.P., Hunt, L.P., Tetlow, L., and Ishola, M.: Kidney. Int. 1986.29.1224/1228. Serum and urinary high density lipoproteins in glomerular disease with proteinuria.
34. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L.: N. Eng. J. Med. 1989. 320. 915/924. Beyond cholesterol, modification of low-density lipoprotein that increases its atherogenecity.
35. Steinbrecher, U.P., Zhang, H., and Lougheed, M.: Free. Radic. Biol. Med. 1990. 9.155/168. Role of oxidatively modified LDL in atherosclerosis.

36. Thomas, M.E., Freestone, A.L., Varghese, Z., Persaud, J.W., and Mooehead, J.F.: Nephrol. Dial. Transplant .1992. 7.597/601.Lipoprotein (a) in patients with proteinuria.
37. Valeri, A., Gelfand, J., Blum, J., and Appel, G.B.: Am. J. Kidney. Dis. 1986. 8. 388/396.Treatment of hyperlipidemia of the nephrotic syndrome: A controlled trial.
38. van Goor, H., van der Horst, M.L.C., Atmosoerodjo, J., Joles, J.A., van Tol, J., and Grond, J.: Am. J. Pathol. 1993. 142. 1804/1812. Renal apolipoproteins in nephrotic rats.
39. Warwick, G.L., Caslake, M.J., Boulton-Jones, J.M., Dagen, M., Packard, C.J., and Shepherd, J.: Metabolism. 1990. 39.187/192. Low-density lipoprotein metabolism in the nephrotic syndrome.
40. Wass, V. and Cameron, C.S.: Nephron. 1981. 27. 58/61.Cardiovascular disease and the nephrotic syndrome: The other side of the coin.
41. Wheeler, D.C. and Chana, R.S.: Miner. Electrolyte. Metab. 1993. 19.149/164. Interactions between lipoproteins, glomerular cells and matrix.
42. Witztum, J.L. and Steinberg, D.: J. Clin. Invest. 1991. 88. 1785/1792. Role of oxidised low density lipoprotein in atherogenesis.
43. Zhang, A., Vertommen, J., Van Gaal, L., and De Leeuw, I.: Clin. Chim. Acta. 1994. 227.159/173. A rapid and simple method for measuring the susceptibility of low-density-lipoprotein and very-low-density-lipoprotein to copper-catalysed oxidation.