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Does Lipoprotein(a) Inhibit Elastolysis in Abdominal Aortic Aneurysms?

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Purpose: *to test the hypothesis that there is a negative association between serum levels of lipoprotein(a) (Lp(a)) and elastin-derived peptides (EDP) as well as matrix metalloproteinase (MMP)-9 activation in the aneurysm wall in patients with asymptomatic abdominal aortic aneurysms (AAA).*
Material and Methods: *from 30 patients operated for asymptomatic AAAs, preoperative serum samples and AAA biopsies were collected. Lp(a) (mg/L) and EDP (ng/ml) in serum were measured by enzyme linked immunosorbent assays. MMP-9 activity (arbitrary units) in the AAA wall was measured by gelatin zymography and the ratio: active MMP-9/total MMP-9 were calculated.*
Results: *there was a significant negative correlation (Spearman's rho) between serum levels of Lp(a) and EDP ($r = -0.707$, $p < 0.001$), as well as the share of activated MMP-9 ($r = -0.461$, $p = 0.01$) in the AAA wall.*
Conclusion: *this preliminary study indicate that Lp(a) inhibit elastolysis in asymptomatic AAA.*

Key Words: Abdominal aortic aneurysms; Lipoprotein(a); Elastin; Elastin-derived peptides; Elastolysis; Matrix metalloproteinases.

Introduction

Lipoprotein(a) (Lp(a)) was discovered by Berg in 1963.[1] Several studies have shown that Lp(a) plays a significant role in atherogenesis and has been established as an independent risk marker of atherosclerosis.[2] Serum levels of Lp(a) have been shown to be elevated in patients with abdominal aortic aneurysms (AAA)[3,4] independently of cardiovascular risk factors and the extent of atherosclerosis.[5] Lp(a) is entrapped in the aneurysm wall and thrombus.[4]

An interesting aspect of Lp(a) is that the lipid and protein composition is similar to that of LDL(6) with the exception of an extra glycoprotein, apolipoprotein(a) (apo(a)) linked to apoB-100(7) by a disulfide linkage. Apo(a) has been identified as a member of the plasminogen gene family.[8–10] In apo(a) there are kringle modules structurally related to those of plasminogen, the precursor of plasmin in the fibrinolytic system, endowing Lp(a) with the ability to compete with plasminogen for binding to fibrin[11,12] and cell membranes.[13,14] Thereby plasmin generation is inhibited.[15] Since apo(a) cannot be transformed into a plasmin-like enzyme by tissue-plasminogen activator (t-PA)[16,17] and apo(a) has no protease activity towards substrates for plasmin[18] plasmin-like activity is impaired. Additionally, Lp(a) enhance the expression of plasminogen activator inhibitor-1 (PAI-1)[19] and inhibit endothelial cell synthesis of t-PA[20] thereby further reducing plasmin generation.

In AAA, the fibrinolytic (plasminogen/plasmin) and matrix metalloproteinase (MMP) systems are working in concert, degrading components of the extracellular matrix (ECM) of the aortic wall. Whereas MMPs directly degrade several ECM components, such as elastin and collagen, the plasminogen/plasmin system have a limited direct proteolytic activity against ECM components and acts mainly indirect by activating pro-MMPs (pro-MMP-1 pro-MMP-3, pro-MMP-9, pro-MMP-10, pro-MMP-13)[21] (Fig. 1).

MMP-9 play a significant role in AAA formation and progression towards rupture.[22–24] MMP-9 has significant elastolytic as well as collagenolytic capacity and plasmin directly activates proMMP-9.[25] Impaired generation of plasmin by Lp(a) may therefore decrease elastolysis and the generation of elastin-derived peptides (EDP) through reduced activation of MMP-9.

The aim of this study was to test the hypothesis that there is a negative association between serum levels of Lp(a) and elastolysis in patients with asymptomatic AAA.

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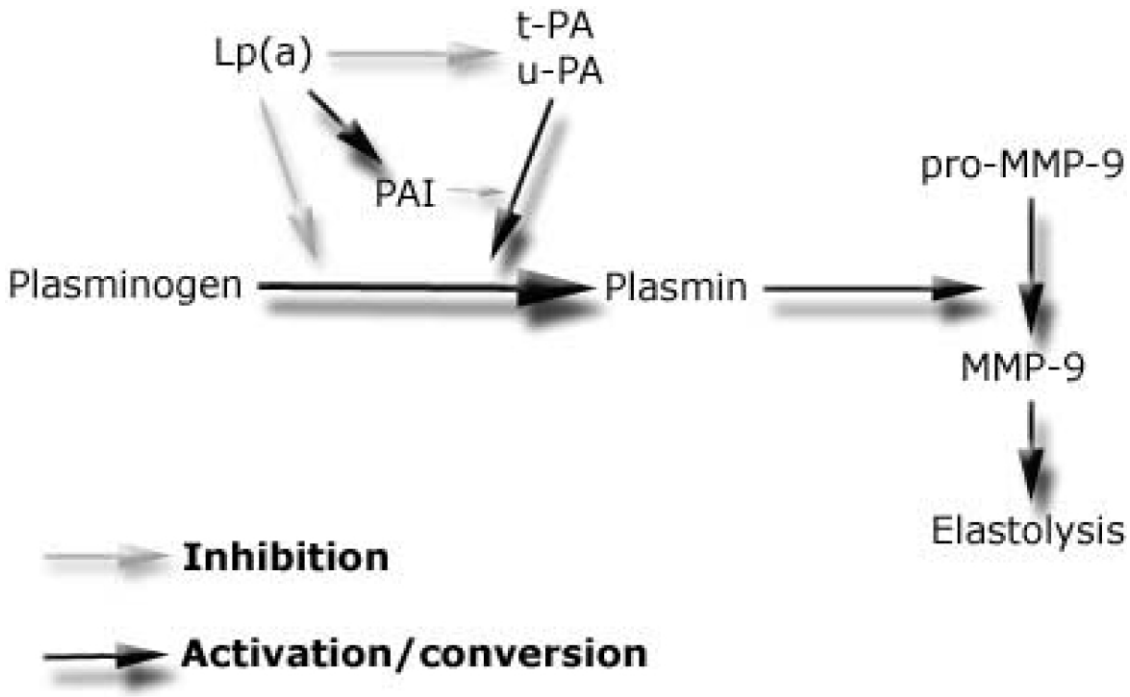

Fig. 1. Schematic presentation of interactions between the plasminogen/plasmin and the MMP systems. The homology between apo(a) and plasminogen implies that Lp(a) competes with plasminogen for binding on fibrin and cell receptors, leading to reduced plasmin generation and MMP-9 activation. Additionally, Lp(a) enhance the expression of PAI-1 and inhibit endothelial cell synthesis of t-PA thereby further reducing plasmin generation and MMP-9 activation. As MMP-9 is a potent elastase, elastolysis is hypothetically inhibitet.

Materials and Methods

Thirty consecutive patients having surgical repair of an asymptomatic infrarenal AAA were included in the study. None of the patients were on anti-lipid therapy. Patient demographics are shown in Table 1. AAA diameter was measured by CT-scan. Routine measurements of serum creatinin (μmol/L) were made.

Serum samples were collected by antecubital venous puncture the day before surgery. Per-operatively a full thickness specimen was collected from the anterior aneurysm wall. The tissues were rinsed for blood, thrombus and connective tissue and stored in −70°C until analysis.

The study was approved by the local scientific ethics committee. All patients gave informed consent.

Table 1. Demographics of the patient group.

	$n = 30$
Age (years) (median, range)	65 (52–84)
Gender (F/M)	5/25
AAA diameter (cm) (median, range)	6 (5–10)
Diabetes mellitus (*n* (%))	3 (10)
Serum creatinine (μmol/L) (median, range)	92 (65–344)
Arterial hypertension (*n* (%))	15 (50)
Smoking (*n* (%))	11 (37)

Measurements

Lp(a): the apo(a) content was measured in serum by a Tint ELISA (Biopool AB, Umeå Sweden) utilizing affinity purified polyclonal goat antibodies raised against apo(a). The results were given as total Lp(a) in mg/L. The detection limit was 0 mg/L and the interassay CV were 7.7% at 100 mg/L and 2.7% at 400 mg/L. The corresponding intraassay CV were 6.6 and 2.3% according to the manufacturer. The analyses were performed at the Department of Clinical Chemistry, Umeå University.

EDP: serum levels of EDP were measured by a competitive ELISA as described elsewhere.[26]

MMP activity: gelatine zymography was used to estimate MMP-9 activity in the AAA wall as previously described.[23] The gelatinolytic activities of pro-MMP-9 (92 kDa) and active MMP-9 (84 kDa) were quantified by densitometry using 2020 Ultrascan Laser Densitometer (LKB, Sweden). The intra-assay CV of gelatine zymography was 10.9% and the inter-assay CV 10.8%. For each of the patients and MMPs the ratio between active MMP and total MMP activity was calculated.

Statistics

The statistical calculations were performed using SPSS 11.0. Spearman's rank correlation analysis was used for univariate analyses. After log transformation of Lp(a) a multiple linear regression analysis using Lp(a) as the dependent variable was performed to adjust for potential confounding factors. However, Lp(a) failed even after transformation to be reasonably normally distributed.

Statistical significance was set at 95% ($p < 0.05$). Assuming a correlation ($r = 0.5$) exist between the variables serum Lp(a) and serum EDP or active MMP-9/total MMP-9, a number of 30 patients would allow a 86% chance of detecting a correlation this large at the chosen level of statistical significance (Sample Power 1.0).

Results

The distribution of serum Lp(a) in the AAA patients was skewed (Fig. 2).

Serum Lp(a) was negatively correlated (Spearman's rho) to serum EDP ($r = -0.707$, $p < 0.001$) (Fig. 3) and the share of active MMP-9 ($r = -0.461$, $p = 0.01$) in the AAA wall (Fig. 4).

Both serum EDP and the share of activated MMP-9 in the AAA wall were still significantly correlated to serum Lp(a) after adjustment for age, gender, diabetes mellitus, serum creatinin, AAA diameter, hypertension and smoking. Among these variables serum creatinin (μmol/L) was positively correlated with Lp(a) ($r = 0.373$, $p < 0.05$).

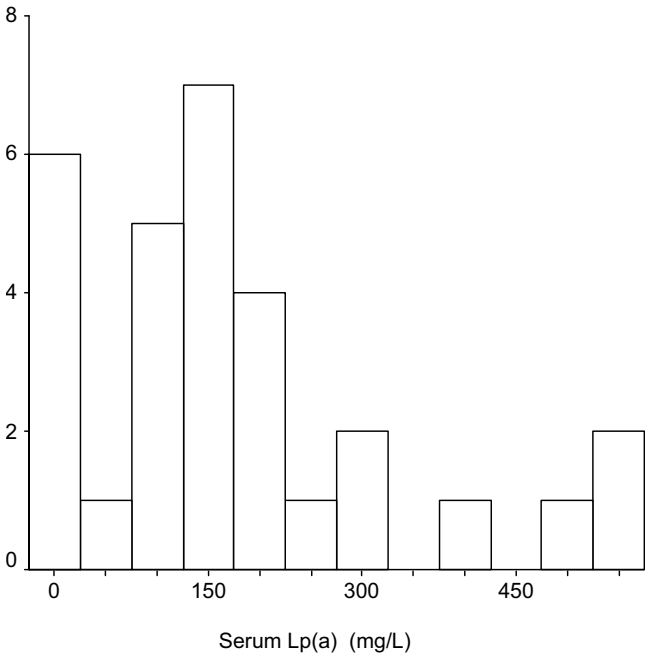

Fig. 2. Distribution of Lp(a) in the patient group.

Discussion

We found a significant inverse association between serum levels of Lp(a) and EDP as well as the share of activated MMP-9 in the AAA wall of patients with asymptomatic AAA. As serum EDP seems to reflect elastin turnover in the presence of a pathological process,[27,28] our results therefore indicate that Lp(a) may have a significant influence on the elastolytic process in AAA disease, through inhibition of plasmin generation and decreased activation of MMP-9 (Fig. 1).

It is recognized that MMP-9 plays a critical role in aneurysm formation[22] and progression towards rupture.[23,24] In a guinea pig-to-rat aortic xenograft model, seeded with syngeneic rat smooth muscle cells retrovirally transduced with rat PAI-1 cDNA, over expression of PAI-1 resulted in inhibition of t-PA and lower levels of MMP-9 activity as well as prevention of aneurysm formation and arterial rupture.[29] Accordingly, the enhancement of PAI-1 expression[19] and inhibition of endothelial cell t-PA synthesis[20] executed by Lp(a) may thereby theoretically and to some extent inhibit AAA formation and progression towards rupture. It has previously been reported that elevated plasma levels of Lp(a) were not a risk marker for AAA rupture.[30] However, our results may indicate that low levels of Lp(a) may be a possible marker of AAA progression towards rupture.

The elastolytic activity of MMP-9 in the AAA wall is dependent of the total amount of activated MMP-9 present. The ratio active MMP-9/total MMP-9 calculated in this study reflects only the magnitude of proMMP-9 activation in which plasmin is participating. The physiologic role of Lp(a) has not been entirely determined yet. Elevated levels of Lp(a) is believed cause endothelial damage and thus may increase the susceptibility for intimal injury and initiation of aneurysm formation. It has also been suggested to play a role in thrombus formation and reinforcement of the aortic wall in AAA disease. AAA concentrations of Lp(a) is correlated to Lp(a) plasma levels and is 5 times higher in the thrombus than in the aneurysm wall.[4] Lp(a) is thus present in AAA and may influence proteolysis and thrombus formation. However, previous findings that rapid increase of thrombus area is a predictor of AAA rupture[31] do not correspond with our results. Increased concentrations of Lp(a) may promote further thrombus formation because of its

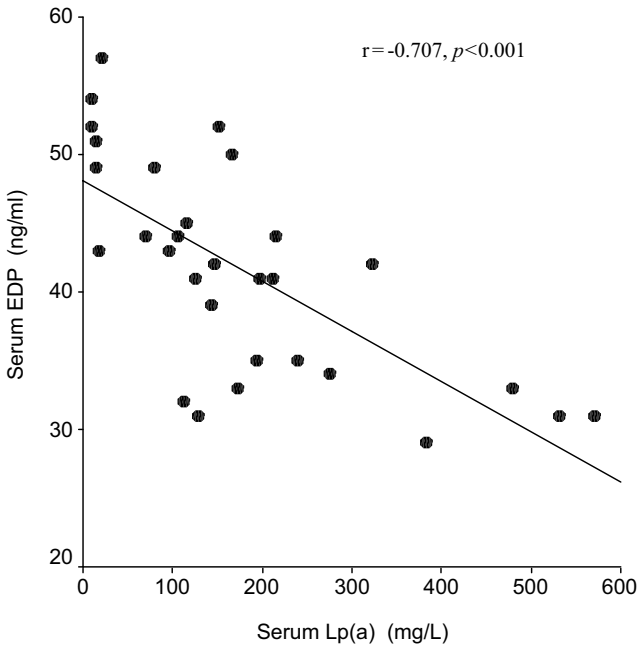

Fig. 3. Association between serum levels of Lp(a) and EDP (Spearmen's rho).

thrombogenic effect, whereas proteolysis may be inhibited, theoretically decreasing progression towards rupture.

Hypertriglyceridemia has been shown to be associated with low Lp(a) levels[32] and AAA rupture.[30] These observations are in accordance with our results as Lp(a) may be a common link, between triglycerides and ECM degradation in the aortic wall.

Lp(a) has been found not to be correlated to AAA expansion rate.[33] Increased MMP-2 but not MMP-9 levels are associated with large size AAA.[24] As proMMP-2 activation is a two-step process,[25] resulting in an intermediate 64 kDa form generated by MMP-14 and a mature 62 kDa form generated by plasmin, the indirect influence of Lp(a) on MMP-2 activation and possibly AAA expansion is dependent of step one. We were not able to identify the intermediate (64 kDa) MMP-2 on the zymograms and therefore the indirect influence of Lp(a) on proMMP-2 activation could not be evaluated.

Among Caucasians the distribution of Lp(a) levels are highly skewed,[34] a distribution also found in the present study (Fig. 2). We found no correlation between age and Lp(a) levels, an association otherwise earlier described.[35] One of the main sites of catabolism of Lp(a) is the kidney. Renal dysfunction may lead to accumulation of Lp(a) and to increased serum levels.[36] We found an sleigh positive correlation between serum creatinin and Lp(a) in our patients. It may be speculated that renal dysfunction theoretically may exert some inhibitory influence on AAA disease.

Although this study is small and preliminary it generates some interesting speculations and questions. What impact may antilipid drug therapy have on aneurysm formation and progression? It may be speculated whether efforts to decrease atherogenesis and the risk of thrombogenic and embolic complications by lowering LDL, including Lp(a), may imply that increased risk of AAA formation and complications come without fail. Recently, it has been shown that antilipid treatment with niacin or statins effectively lowers Lp(a) levels.[37–39] Additionally there are evidence that aspirin also decrease serum Lp(a) concentrations.[40] The observations in recent years that death from coronary heart disease and cerebrovascular disease is decreasing[41] whereas death from AAA disease is not[42] may theoretically and to some extent be explained by the use of aspirin and antilipid drug therapy.

The increased presence of the obligate intracellular bacterium *Chlamydia pneumoniae* in AAA tissue compared to non-aneurysmal aortic tissue[43] and increased Lp(a) levels in AAA patients[3–5] may start speculations on a possible influence of circulating immune complexes containing Lp(a) and *C. pneumoniae* specific IgG antibodies on AAA formation and progression. A hypothesis linking immunological mechanisms, high Lp(a) levels, *C. pneumoniae* and atherosclerosis was proposed by Dahlèn.[44] As it has been proposed that

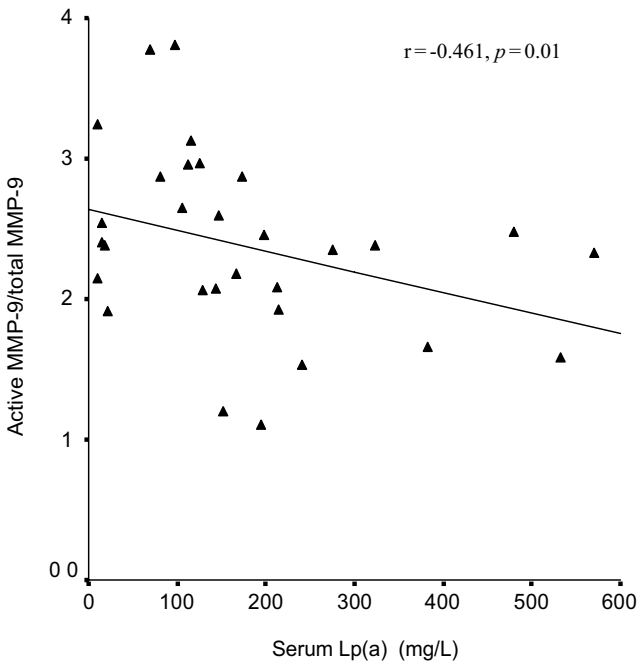

Fig. 4. Association between serum levels of Lp(a) and the share of activated MMP-9 to total MMP-9 in the AAA wall (Spearmen's rho).

aneurysmal disease partially represents an immune-mediated disease,[45] studies on these circulating immune complexes could be interesting.

In conclusion, this small and preliminary study suggest an inverse association between serum Lp(a) and elastolysis in patients with asymptomatic AAA. Further studies on the influence of Lp(a) on AAA disease is warranted.

References

1 Berg K. A new serum type system in man—The Lp system. *Pathol Microbiol Scand* 1963; **59**: 369–382.
2 Morrisett JD. The role of lipoprotein[a] in atherosclerosis. *Curr Atheroscler Rep* 2000; **2**: 243–250.
3 Norrgard O, Angquist KA, Dahlen G. High concentrations of Lp(a) lipoprotein in serum are common among patients with abdominal aortic aneurysms. *Int Angiol* 1988; **7**: 46–49.
4 Papagrigorakis E, Iliopoulos D, Asimacopoulos PJ *et al.* Lipoprotein(a) in plasma, arterial wall, and thrombus from patients with aortic aneurysm. *Clin Genet* 1997; **52**: 262–271.
5 Schillinger M, Domanovits H, Ignatescu M *et al.* Lipoprotein (a) in patients with aortic aneurysmal disease. *J Vasc Surg* 2002; **36**: 25–30.
6 van Den EA, van Der Hoek YY, Kastelein JJ, Koschinsky ML, Labeur C, Rosseneu M. Lipoprotein [a]. *Adv Clin Chem* 1996; **32**: 73–134.
7 Albers JJ, Kennedy H, Marcovina SM. Evidence that Lp[a] contains one molecule of apo[a] and one molecule of apoB: evaluation of amino acid analysis data. *J Lipid Res* 1996; **37**: 192–196.
8 McLean JW, Tomlinson JE, Kuang WJ *et al.* cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 1987; **330**: 132–137.
9 Eaton DL, Fless GM, Kohr WJ *et al.* Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. *Proc Natl Acad Sci USA* 1987; **84**: 3224–3228.
10 Ichinose A. Multiple members of the plasminogen-apolipoprotein(a) gene family associated with thrombosis. *Biochemistry* 1992; **31**: 3113–3118.
11 Harpel PC, Gordon BR, Parker TS. Plasmin catalyzes binding of lipoprotein (a) to immobilized fibrinogen and fibrin. *Proc Natl Acad Sci USA* 1989; **86**: 3847–3851.
12 Beisiegel U, Niendorf A, Wolf K, Reblin T, Rath M. Lipoprotein(a) in the arterial wall. *Eur Heart J* 1990; **11**(Suppl. E): 174–183.
13 Hajjar KA, Gavish D, Breslow JL, Nachman RL. Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. *Nature* 1989; **339**: 303–305.
14 Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF. A potential basis for the thrombotic risks associated with lipoprotein(a). *Nature* 1989; **339**: 301–303.
15 Soulat T, Loyau S, Baudouin V *et al.* Evidence that modifications of Lp(a) *in vivo* inhibit plasmin formation on fibrin—a study with individual plasmas presenting natural variations of Lp(a). *Thromb Haemost* 1999; **82**: 121–127.
16 Sangrar W, Bajzar L, Nesheim ME, Koschinsky ML. Antifibrinolytic effect of recombinant apolipoprotein(a) *in vitro* is primarily due to attenuation of tPA-mediated Glu-plasminogen activation. *Biochemistry* 1995; **34**: 5151–5157.
17 Palabrica TM, Liu AC, Aronovitz MJ, Furie B, Lawn RM, Furie BC. Antifibrinolytic activity of apolipoprotein(a) *in vivo*: human apolipoprotein(a) transgenic mice a resistant to tissue plasminogen activator-mediated thrombolysis. *Nat Med* 1995; **1**: 256–259.
18 Gabel BR, Koschinsky MI. Analysis of the proteolytic activity of a recombinant form of apolipoprotein(a). *Biochemistry* 1995; **34**: 15777–15784.
19 Poon M, Zhang X, Dunsky K, Taubman MB, Harpel PC. Apolipoprotein(a) is a human vascular endothelial cell agonist: studies on the induction in endothelial cells of monocyte chemotactic factor activity. *Clin Genet* 1997; **52**: 308–313.
20 Levin EG, Miles LA, Fless GM *et al.* Lipoproteins inhibit the secretion of tissue plasminogen activator from human endothelial cells. *Arterioscler Thromb* 1994; **14**: 438–442.
21 Carmeliet P, Moons L, Lijnen R *et al.* Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nat Genet* 1997; **17**: 439–444.
22 Pyo R, Lee JK, Shipley JM *et al.* Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J Clin Invest* 2000; **105**: 1641–1649.
23 Petersen E, Gineitis A, Wagberg F, Angquist KA. Activity of matrix metalloproteinase-2 and -9 in abdominal aortic aneurysms. Relation to size and rupture. *Eur J Vasc Endovasc Surg* 2000; **20**: 457–461.
24 Petersen E, Wagberg F, Angquist KA. Proteolysis of the abdominal aortic aneurysm wall and the association with rupture. *Eur J Vasc Endovasc Surg* 2002; **23**: 153–157.
25 Baramova EN, Bajou K, Remacle A *et al.* Involvement of PA/plasmin system in the processing of pro-MMP-9 and in the second step of pro-MMP-2 activation. *FEBS Lett* 1997; **405**: 157–162.
26 Petersen E, Gineitis A, Wagberg F, Angquist KA. Serum levels of elastin-derived peptides in patients with ruptured and asymptomatic abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2001; **22**: 48–52.
27 Kucich U, Christner P, Lippmann M *et al.* Immunologic measurement of elastin-derived peptides in human serum. *Am Rev Respir Dis* 1983; **127**: S28–S30.
28 Kucich U, Christner P, Lippmann M *et al.* Utilization of a peroxidase antiperoxidase complex in an enzyme-linked immunosorbent assay of elastin-derived peptides in human plasma. *Am Rev Respir Dis* 1985; **131**: 709–713.
29 Allaire E, Hasenstab D, Kenagy RD, Starcher B, Clowes MM, Clowes AW. Prevention of aneurysm development and rupture by local overexpression of plasminogen activator inhibitor-1. *Circulation* 1998; **98**: 249–255.
30 Watt HC, Law MR, Wald NJ, Craig WY, Ledue TB, Haddow JE. Serum triglyceride: a possible risk factor for ruptured abdominal aortic aneurysm. *Int J Epidemiol* 1998; **27**: 949–952.
31 Stenbaek J, Kalin B, Swedenborg J. Growth of thrombus may be a better predictor of rupture than diameter in patients with abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2000; **20**: 466–469.
32 Bartens W, Rader DJ, Talley G, Brewer Jr HB. Decreased plasma levels of lipoprotein(a) in patients with hypertriglyceridemia. *Atherosclerosis* 1994; **108**: 147–149.
33 Lindholt JS, Heegaard NH, Vammen S, Fasting H, Henneberg EW, Heickendorff L. Smoking, but not lipids, lipoprotein(a) and antibodies against oxidised LDL, is correlated to the expansion of abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2001; **21**: 51–56.
34 Guyton JR, Dahlen GH, Patsch W, Kautz JA, Gotto Jr AM. Relationship of plasma lipoprotein Lp(a) levels to race and to apolipoprotein B. *Arteriosclerosis* 1985; **5**: 265–272.
35 Akita H, Matsubara M, Shibuya H, Fuda H, Chiba H. Effect of ageing on plasma lipoprotein(a) levels. *Ann Clin Biochem* 2002; **39**: 237–240.
36 Kostner KM, Clodi M, Bodlaj G *et al.* Decreased urinary apolipoprotein (a) excretion in patients with impaired renal function. *Eur J Clin Invest* 1998; **28**: 447–452.
37 Pan J, Lin M, Kesala RL, Van J, Charles MA. Niacin treatment of the atherogenic lipid profile and Lp(a) in diabetes. *Diabetes Obes Metab* 2002; **4**: 255–261.
38 Gonbert S, Malinsky S, Sposito A *et al.* Atorvastatin lowers lipoprotein(a) but not apolipoprotein(a) fragment levels in

hypercholesterolemic subjects at high cardiovascular risk. *Atherosclerosis* 2002; **164**: 305.
39 Duriez P, Dallongeville J, Fruchart JC. Lipoprotein(a) as a marker for coronary heart disease. *Br J Clin Pract Suppl* 1996; **77A**: 54–61.
40 Akaike M, Azuma H, Kagawa A *et al.* Effect of aspirin treatment on serum concentrations of lipoprotein(a) in patients with atherosclerotic diseases. *Clin Chem* 2002; **48**: 1454–1459.
41 Stern MP. The recent decline in ischemic heart disease mortality. *Ann Intern Med* 1979; **91**: 630–640.
42 Lilienfeld DE, Gunderson PD, Sprafka JM, Vargas C. Epidemiology of aortic aneurysms: I. Mortality trends in the United States, 1951 to 1981. *Arteriosclerosis* 1987; **7**: 637–643.
43 Petersen E, Boman J, Persson K *et al.* Chlamydia pneumoniae in human abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1998; **15**: 138–142.
44 Dahlen GH. Lp(a) lipoprotein in cardiovascular disease. *Atherosclerosis* 1994; **108**: 111–126.
45 Wills A, Thompson MM, Crowther M, Sayers RD, Bell PR. Pathogenesis of abdominal aortic aneurysms—cellular and biochemical mechanisms. *Eur J Vasc Endovasc Surg* 1996; **12**: 391–400.

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