

# Lipoprotein (a) is associated with endothelial function in healthy postmenopausal women

Hanneke W. Wilmink<sup>a,b</sup>, Miriam J.J. de Kleijn<sup>b</sup>, Michiel L. Bots<sup>b,\*</sup>,  
Annette A.A. Bak<sup>b</sup>, Yvonne T. van der Schouw<sup>b</sup>, Sylvia Engelen<sup>c</sup>, Jose Planellas<sup>c</sup>,  
Jan-Dirk Banga<sup>a</sup>, Diederick E. Grobbee<sup>b</sup>

<sup>a</sup> Division of Internal Medicine, University Medical Center Utrecht (UMC), Utrecht, The Netherlands

<sup>b</sup> Julius Center for Patient Oriented Research, D01.335, Utrecht University Medical Center (UMC), Heidelberglaan 100,  
3584 CX Utrecht, The Netherlands

<sup>c</sup> Clinical Development Department, NV Organon, Oss, The Netherlands

Received 29 August 1999; received in revised form 23 December 1999; accepted 21 January 2000

## Abstract

**Background.** Lipoprotein (a) (Lp(a)) is an independent risk factor for atherosclerotic cardiovascular disease. The atherogenic potential of Lp(a) may be by impairment of endothelial function. **Objectives.** We investigated the relation of Lp(a) plasma levels to endothelium dependent and independent dilatation of the brachial artery in healthy postmenopausal women. **Methods.** One hundred and five healthy postmenopausal women aged 52–67 years were included in the study. Endothelial function was assessed non-invasively by measuring percent lumen diameter change in the brachial artery after reactive hyperemia and sublingual nitroglycerine spray. **Results.** Flow mediated dilatation was inversely related to the plasma log Lp(a) level. Mean change per unit log Lp(a) increase:  $-2.83\%$  (95% CI:  $-5.22 - -0.43$ ). Elevated Lp(a) ( $> 239$  mg/l) (upper quartile) was associated with an impaired flow mediated vasodilatation ( $2.4\% \pm 1.2$ ) compared to Lp(a)  $\leq 239$  mg/l ( $5.2\% \pm 0.7$ ). Adjustment for other cardiovascular risk factors did not change the magnitude of the association. Nitroglycerine-induced vasodilatation was not significantly lower in the high Lp(a) level group, compared to the group with normal levels of Lp(a) ( $\leq 239$  mg/l) ( $8.0 \pm 1.2$  vs  $11.4\% \pm 0.8$ ). **Conclusion.** Elevated lipoprotein (a) levels are associated with an impaired endothelial function in healthy postmenopausal women, independent of conventional risk factors for cardiovascular disease. Since Lp(a) may be pathogenetically important for early vascular damage, elevated Lp(a) levels might contribute to the increased cardiovascular risk seen in postmenopausal women. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Lipoprotein (a); Endothelial function; Postmenopausal women; Nitric oxide

## 1. Introduction

Endothelial function is impaired in the presence of known atherogenic risk factors such as hypercholesterolemia [1,2], hyperlipidemia [3,4], diabetes mellitus [5,6], cigarette smoking [7,8], low plasma estrogen levels [9,10], aging [11] and hypertension [12], even before atherosclerosis becomes clinically manifest. Impaired endothelial function of the brachial artery has also been shown to be present in patients with coronary artery

disease [13–15]. These findings led to the view that endothelial dysfunction may be important in the atherosclerotic process and could be of use as an early marker of cardiovascular risk.

Lipoprotein (a) (Lp(a)) is an independent risk factor for atherosclerotic vascular disease [16–18]. Many prospective studies have shown that excess Lp(a) is associated with premature coronary atherosclerosis and cardiovascular disease risk, in men and women. In particular in subjects with premature coronary atherosclerosis, therapy to lower Lp(a) deserves attention [19]. To our knowledge no results have been published studying the relation between plasma Lp(a) levels and endothelium dependent dilatation of the

\* Corresponding author. Tel.: +31-30-2509352; fax: +31-30-2505485.

E-mail address: m.l.bots@jc.azu.nl (M.L. Bots).

brachial artery in postmenopausal women. Sørensen et al. [20] showed a positive relation between plasma Lp(a) levels and impairment of endothelium dependent dilatation in the femoral artery of children with familial hypercholesterolemia. In addition there is evidence that Lp(a) is related to coronary artery dysfunction [21].

Since Lp(a) plasma levels in postmenopausal women are elevated compared to premenopausal women [22] and hormone replacement therapy could be associated with a decreased risk of cardiovascular disease [23] and a reduced level of Lp(a) [24], the relation between Lp(a) plasma levels and endothelial function in healthy postmenopausal women is of interest.

To assess whether impairment of endothelial function is related to plasma concentrations of Lp(a), we studied endothelium vasodilator function of the brachial artery in response to increased blood flow.

## 2. Methods

### 2.1. Subjects

The study included 105 healthy postmenopausal women recruited from the Dutch EPIC-cohort (European Prospective Investigation into Cancer and Nutrition) [25], as part of a program to study the effect of hormone replacement therapy on flow mediated vasodilatation of the brachial artery. The study was performed from June 1997 to March 1998. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht and written informed consent was obtained from all participants. Subject selection was based on the following criteria: age between 45 and 65 years; natural menopause and intact uterus and body mass index between 18 and 29 kg/m<sup>2</sup>. Women with disorders related to the genital tract, a history or presence of any malignant disease, a history or presence of cardiovascular or cerebrovascular disease or thromboembolism/thrombosis or diabetes mellitus type 1 or 2 were not eligible for the study. Women with hypertension (systolic blood pressure > 170 mmHg and/or diastolic blood pressure > 105 mmHg); significant hyperlipidaemia (fasting total cholesterol > 8.0 mmol/l and/or fasting triglycerides > 4 mmol/l); history or presence of hepatic (defined by ALAT > 70 IU/L or ASAT > 70 IU/L or gamma GT > 50 IU/L) or renal disorders (creatinine > 150 µmol/l) were excluded. Women who reported use of drugs influencing cardiac and/or vascular dynamics; use of drugs influencing lipids and lipoproteins; use of oral sex-steroids within the last 60 days or transdermal sex-steroids within the last 30 days, conjugated equine estrogens within the last 3 months, hormonal injections or ethinylestradiol within the last 6 months or hormone implants at any time previously were also excluded.

Finally, alcohol and/or drug abuse within the last 12 months and smoking more than 10 cigarettes per day were exclusion criteria.

Weight and height were measured and body mass index calculated. Blood pressure and heart-rate were measured at the right upper arm in sitting position using a Boso oscillomat (BOSCH + SOHM GMBH U). Fasting blood samples were obtained between 08.00 and 12.00 h. Basic haematological lab values were measured with Cell Dyn<sup>®</sup> 4000 (Abbott). Basic biochemical lab values, including cholesterol (cholesterol oxidase) and triglycerides (glycerolkinase, glycerolphosphate oxidase), were measured with a Vitros 950 (Dry chemistry; Johnson and Johnson). HDL-cholesterol was measured on a Synchron CX4 (Beckman), after precipitation with phosphowolfram/phosphotungstic acid and 2 mmol of manganese chloride per litre. The LDL-cholesterol concentration was calculated with the Friedewald formula. The lipoprotein (a) concentration was measured with N Latex Lp(a) Reagent by nephelometry (Behring Diagnostics). The coefficients of variation for the intra-assay precision and the inter-assay reproducibility were 1.5–3.0% and 1.7–3.2%, respectively.

All laboratory parameters were determined by the Central Diagnostics Laboratory in corporation with the Department of Clinical Chemistry, University Medical Center Utrecht.

### 2.2. Endothelial function

Ultrasound measurements of the brachial artery were performed in supine position at the elbow of the right arm using a vessel wall-movement detection system (Wall Track System, Pie Medical, Maastricht), which consists of an ultrasound imager with a 7.5 MHz linear array transducer connected to a data acquisition system and a personal computer.

In short, an optimal two dimensional B-mode image of the artery was obtained. An M-line perpendicular to the vessel was selected. Next the ultrasound system was switched to M-mode, after which storage of data started. The vessel wall-movement detector system repeatedly registered end-diastolic vessel diameter during a period of five to six cardiac cycles. This procedure was performed three times. The measurements of the lumen diameter were averaged to obtain a baseline (before ischemia) lumen diameter estimate.

By inflation of a blood pressure cuff for 4 min at a pressure of 100 mmHg above the systolic blood pressure, ischemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 3 min after cuff release with measurements at 30 s intervals. The lumen diameter measurement showing the largest lumen diameter was taken as 'maximal lumen diameter after ischemia'. After 10 min of rest,

allowing the artery to return to its baseline diameter, sublingual nitroglycerine spray was administered as an endothelium independent vasodilator. Measurements were obtained for another 5 min, with 1 min intervals. All measurements were done by two technicians.

Endothelium dependent and independent dilatation were expressed as a percentage change relative to the baseline diameter ((maximal diameter – baseline diameter)/baseline diameter) × 100%.

A reproducibility study in which 35 subjects were measured twice within a three month interval showed significant Spearman correlation coefficients for repeat baseline and maximal lumen diameter measurements of 0.73 and 0.74, respectively. The coefficients of variation were 6.2 and 7.4%, respectively.

Table 1  
General characteristics of the studied 105 postmenopausal women<sup>a</sup>

		Range
Age (years)	58.9 (3.6)	(51–65)
Body mass index (kg/m <sup>2</sup> )	24.3 (2.3)	(20–29)
Smoking (%)	11	
Systolic blood pressure (mmHg)	134.7 (16.8)	(98–176)
Diastolic blood pressure (mmHg)	85.7 (9.2)	(63–105)
Total cholesterol (mmol/l)	6.2 (0.94)	(3.1–8.2)
LDL cholesterol (mmol/l)	4.0 (0.86)	(1.6–5.8)
HDL cholesterol (mmol/l)	1.62 (0.41)	(0.71–2.87)
Lipoprotein (a) (mg/l)	197.2 (257.5)	(21–1634)
	35, 105 and 239	
	(25, 50, 75 percentile)	
Triglycerides (mmol/l)	1.23 (0.5)	(0.35–2.91)
Vessel size (mm)	4.3 (0.6)	(2.81–5.95)
Flow mediated vasodilatation, %	4.5 (6.3)	(–10.4–23.0)
Nitroglycerine-induced vasodilatation (%)	10.5 (6.7)	(–8.9–25.8)

<sup>a</sup> Values are percentages or means with standard deviation in parentheses

Table 2  
Association of Lp(a) with flow mediated vasodilatation and nitroglycerine-induced vasodilatation<sup>a</sup>

	Quartile 1 (<35 mg/l)	Quartile 2 (35–105 mg/l)	Quartile 3 (105–239 mg/l)	Quartile 4 (>239 mg/l)
FMD	6.6 (1.3)	4.4 (1.3)	4.5 (1.0)	2.4 (1.2)*
NTG	11.2 (1.2)	11.8 (1.5)	11.2 (1.2)	8.0 (1.2)**

<sup>a</sup> Values are percentages with standard errors given in parentheses.

\* *P*-value for trend: 0.02.

\*\* *P*-value for trend: 0.09.

### 2.3. Data analysis

Linear regression analysis was used to assess the association of cardiovascular risk factors with endothelium dependent and endothelium independent vasodilatation. Distribution of Lp(a) was skewed. Analyses on Lp(a) were conducted on log-transformed Lp(a) data.

Results are presented as 'beta coefficient' of the linear regression model with corresponding 95% CI. Multivariate linear regression was used to assess the association between Lp(a) and flow mediated dilatation (FMD) adjusted for other cardiovascular risk factors. To evaluate whether the association between Lp(a) and FMD differed across smoking status and blood pressure levels, multiplicative interaction terms (Lp(a) × smoking; Lp(a) × systolic blood pressure) were constructed and evaluated with a linear regression model.

### 3. Results

The general characteristics of the study population are given in Table 1.

Associations between risk factors and endothelial function are shown in Table 2. Log(Lp(a)) and smoking were inversely related to flow mediated dilatation. FMD gradually decreased with increasing Lp(a) (Table 2). Mean change in FMD per unit log(Lp(a)) increase was –2.83% (95% CI: –5.22– –0.43). Elevated Lp(a) (239 mg/l) (upper quartile) was associated with an impaired flow mediated vasodilatation (2.4% ± 1.2) compared to Lp(a) ≤ 239 mg/l (5.2% ± 0.7) (*P*-value: 0.02). The association between Lp(a) and FMD remained after adjustment for other cardiovascular risk factors, notably age, diastolic and systolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, smoking and body mass index (Table 3). The association between Lp(a) and FMD did not differ across smoking status and blood pressure levels (*p* values of the multiplicative interaction terms > 0.15) (data not shown).

The associations between cardiovascular risk factors and nitroglycerine-induced vasodilatation are given in Table 4. When multivariate analyses were performed with nitroglycerine response as the dependent variable, only body mass index and smoking were significantly (inversely) related to endothelium independent vasodilatation (*P* values < 0.05) (Table 4). Elevated Lp(a) (> 239 mg/l), upper quartile, was associated with an impaired nitroglycerine-induced vasodilatation, compared to Lp(a) levels ≤ 239 mg/l: 8.0 and 11.4%, respectively. This difference was not statistically significant (Table 2).

Table 3  
Association of cardiovascular risk factors with flow mediated vasodilatation

	Univariate			Multivariate <sup>b</sup>		
	Beta <sup>a</sup>	95% CI		Beta <sup>a</sup>	95% CI	
		Lower	Upper		Lower	Upper
Age, year	0.12	−0.24	0.48			
Diastolic blood pressure, mmHg	0.001	−0.14	0.14			
Systolic blood pressure, mmHg	0.07	−0.004	0.15			
Total cholesterol, mmol/l	−0.17	−1.58	1.23			
LDL cholesterol, mmol/l	0.31	−1.21	1.83			
HDL cholesterol, mmol/l	−1.28	−4.29	1.72			
Log(Lp(a)), mg/l	−2.93	−5.36	−0.50	−2.81	−5.47	−0.15
Triglycerides, mmol/l	−1.1	−3.66	1.45			
Smoking, yes/no	−5.95	−9.79	−2.10	−5.31	−9.36	−1.25
Body mass index, kg/m <sup>2</sup>	0.23	−0.32	0.77			

<sup>a</sup> Beta coefficients reflect the mean change in FMD with the change in characteristic by one 'unit': e.g. in smokers the mean FMD was 5.95% lower than in non-smokers.

<sup>b</sup> All variables were included in the model, only log (Lp(a)) and smoking were significant independent predictors.

Table 4  
Association of cardiovascular risk factors with nitroglycerine-induced vasodilatation

	Univariate			Multivariate <sup>b</sup>		
	Beta <sup>a</sup>	95% CI		Beta <sup>a</sup>	95% CI	
		Upper	Lower		Upper	Lower
Age, year	−0.1	−0.47	0.28			
Diastolic blood pressure, mm Hg	−0.06	−0.19	0.09			
Systolic blood pressure, mm Hg	−0.01	−0.09	0.07			
Total cholesterol, mmol/l	0.17	−1.23	1.58			
LDL cholesterol, mmol/l	0.10	−1.43	1.62			
HDL cholesterol, mmol/l	2.13	−1.02	5.28			
Log(Lp(a)), mg/l	−2.0	−4.53	0.63	−2.33	−5.06	0.4
Triglycerides, mmol/l	−2.44	−5.02	0.15			
Smoking, yes/no	−4.10	−8.27	0.09	−3.51	−7.95	0.93
Body mass index, kg/m <sup>2</sup>	−0.76	−1.31	−0.22	−0.65	−1.3	−0.005

<sup>a</sup> Beta coefficients reflect the mean change in NTG-induced vasodilatation with the change in characteristic by one 'unit': e.g. in smokers the mean NTG-induced vasodilatation was 4.10% lower than in non-smokers.

<sup>b</sup> All variables were included in the model, only body mass index was a significant independent predictor.

#### 4. Discussion

In the present population-based study among healthy postmenopausal women we provide evidence that elevated Lp(a) levels and smoking are associated with endothelial dysfunction, independent of other cardiovascular risk factors.

These findings raise some issues that need to be discussed. The size of the present study limits the power to detect small associations. In addition, our participants reflect a healthy sample. All subjects with classical cardiovascular risk factors, except for Lp(a) and smoking, were excluded. Finally this is a cross-sectional study, so no statement on cause or consequence can be made.

There is ample data to indicate that abnormal endothelial physiology plays an important role in both early atherogenesis and subsequent progression of the disease by controlling the dynamic plaque behavior [26]. The biologic link between endothelial damage and atherosclerosis is probably formed by decreased arterial bio-availability of nitric oxide (NO) [27]. NO is a signaling molecule in blood vessels, which acts on the underlying smooth muscle to maintain vasodilatation and nutritious blood flow [28]. Arterial endothelium and smooth muscle function can be studied non-invasively by examining brachial artery responses to endothelium dependent and endothelium independent stimuli [29]. An endothelium dependent stimulus is increased blood flow, which leads to endothelial NO

production and measurable vasodilatation. This vasoactivity of the arterial wall, flow mediated vasodilatation (FMD), has been shown in both experimental and clinical studies to depend on endothelial nitric oxide release [30].

Lp(a) was found to be an independent risk factor for cardiovascular disease [31]. The mechanism of Lp(a) atherogenicity has not been elucidated yet, but the unique features of Lp(a) suggest that this lipoprotein has both thrombogenic and atherogenic potential [32]. It interacts with the fibrinolytic system due to its homology to plasminogen. Lp(a) retards fibrinolysis by inhibiting plasmin generation. It competes with plasmin on the endothelial surface and activates plasminogen activator inhibitor (PAI-1) [32]. Since plasmin is an activator of transforming growth factor beta (TGF- $\beta$ ), which is a key inhibitor of atherosclerosis by stimulating NO-synthase gene expression, Lp(a) may directly interact with the process of atherosclerosis.

Moreover, Lp(a) has similar effects to LDL cholesterol [32]. Lp(a) inactivates NO through superoxide anion production and interferes with NO-synthase stimulation [21]. The findings in our study provide support for this view. We have shown that FMD, which depends on endothelial NO release, gradually decreases with increasing Lp(a) levels.

Non-invasive measurement of flow mediated dilatation in superficial arteries provides insight into the etiology of functional vascular abnormalities in many clinical settings. In the presence of known atherogenic risk factors for cardiovascular disease, FMD will be impaired [33]. Smoking is one of those risk factors that correlate with endothelial dysfunction [7], an association detected in our study as well.

Decreased vasodilatation has also been associated with aging. In women this decline in endothelial function begins after menopause, significantly later than in male subjects [11], suggesting a beneficial effect of endogenous estrogens and progestins on vasomotor function [34]. In addition, improvement of endothelial function has been shown after hormone replacement therapy, both intravenously and orally, in postmenopausal women [9,35]. Until now, there is no data showing a relation between plasma Lp(a) levels and endothelial function of the brachial artery in postmenopausal women.

Lp(a) plasma levels in postmenopausal women are elevated compared to premenopausal women [22]. Since cross-sectional studies and clinical trials have shown an association with elevated circulating estrogens and use of combined HRT and decreased levels of Lp(a) [36], one could assume that one of the mechanisms by which HRT reduces the risk of cardiovascular disease [23,37] might be through a reduction in plasma Lp(a), subsequently leading to improved endothelial function.

In conclusion, the results of the present study suggest that increased Lp(a) is an independent risk factor for impaired endothelial function in healthy postmenopausal women.

### Acknowledgements

The study was supported by a grant from N.V. Organon, Oss, The Netherlands. We are grateful to the participants for their essential contribution to this study. We thank L. Middelinck and C. van Geet for their assistance during the study. The ultrasound technicians, Marian Boer and Gea Boschker are gratefully acknowledged. We acknowledge M. Heijmering for his technical support. The study has been supported by N.V. Organon Oss, The Netherlands. The authors have no financial relationship with the sponsors. N.V. Organon did not control or influence the decision to submit the final manuscript for publication. N.V. Organon was given the final draft of the manuscript to become familiar with its content and for discussion upon the interpretation of the data.

### References

- [1] Stroes ES, Koomans HA, deBruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet* 1995;346:467–71.
- [2] Steinberg HO, Bayazeed B, Hook G, Johnson A, Cronin J, Baron AD. Endothelial dysfunction is associated with cholesterol levels in the high normal range in humans. *Circulation* 1997;96:3287–93.
- [3] Chowienczyk PJ, Watts G, Wierzbicki A, Cockcroft J, Brett S, Ritter JM. Preserved endothelial function in patients with severe hypertriglyceridemia and low functional lipoprotein lipase activity. *J Am Coll Cardiol* 1997;29:964–8.
- [4] Lundman P, Eriksson M, Schenck-Gustafsson K, Karpe F, Tornvall P. Transient triglyceridemia decreases vascular reactivity in young, healthy men without risk factors for coronary heart disease. *Circulation* 1997;96:3266–8.
- [5] Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilatation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993;88:2510–6.
- [6] Lambert J, Aarsen M, Donker AJ, Stehouwer CD. Endothelium-dependent and -independent vasodilatation of large arteries in normoalbuminuric insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1996;16:705–11.
- [7] Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993;88:2149–55.
- [8] Woo KS, Robinson JT, Chook P, et al. Differences in the effect of cigarette smoking on endothelial function in chinese and white adults. *Ann Intern Med* 1997;127:372–5.
- [9] Lieberman EH, Gerhard MD, Uehata A. Estrogen improves endothelium-dependent, flow-mediated vasodilatation in postmenopausal women. *Ann Intern Med* 1994;121:936–41.

- [10] McCrohon JA, Adams MR, McCredie RJ, et al. Hormone replacement therapy is associated with improved arterial physiology in healthy post-menopausal women. *Clin Endocrinol Oxf* 1996;45:435–41.
- [11] Woo KS, McCrohon JA, Chook P, et al. Chinese adults are less susceptible than whites to age-related endothelial dysfunction. *J Am Coll Cardiol* 1997;30:113–8.
- [12] Iiyama K, Nagano M, Yo Y, et al. Impaired endothelial function with essential hypertension assessed by ultrasonography. *Am Heart J* 1996;132:779–82.
- [13] Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995;26:1235–41.
- [14] Motoyama T, Kawano H, Kugiyama K, et al. Flow-mediated, endothelium-dependent dilatation of the brachial arteries is impaired in patients with coronary spastic angina. *Am Heart J* 1997;133:263–7.
- [15] Kugiyama K, Ohgushi M, Motoyama T, et al. Nitric oxide-mediated flow-dependent dilation is impaired in coronary arteries in patients with coronary spastic angina. *J Am Coll Cardiol* 1997;30:920–6.
- [16] Scanu AM. Lipoprotein(a). A genetic risk factor for premature coronary heart disease. *J Am Med Assoc* 1992;267:3326–9.
- [17] Maher VMG, Brown BG. Lipoprotein(a) and coronary heart disease. *Curr Opin Lipidol* 1995;6:229–35.
- [18] Mijatovic V, vanderMooren MJ, Stehouwer CDA, Netelenbos JC, Kenemans P. Postmenopausal hormone replacement, risk estimators for coronary artery disease and cardiovascular protection. *Gynecol Endocrinol* 1999;13:130–44.
- [19] Skin JH, Rosenson RS. Lipoprotein Lp(a) excess and coronary heart disease. *Arch Intern Med* 1997;157:1170–6.
- [20] Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. *J Clin Invest* 1994;93:50–5.
- [21] Schachinger V, Halle M, Minners J, Berg A, Zeiher AM. Lipoprotein(a) selectively impairs receptor-mediated endothelial vasodilator function of the human coronary circulation. *J Am Coll Cardiol* 1997;30:927–34.
- [22] Jenner JL, Ordovas J, Lamon-Fava S, et al. Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study. *Circulation* 1993;87:1135–41.
- [23] Stampfer MJ, Colditz GA, Willett WC, et al. Postmenopausal estrogen therapy and cardiovascular disease: 10-year follow-up from the nurses' health study. *N Engl J Med* 1991;325:756–62.
- [24] Espeland MA, Marcovina SM, Miller V. et al., Effect of postmenopausal hormone therapy on lipoprotein(a) concentration. *Circulation* 1998;97:979–86.
- [25] Peeters PH, Beckers C, Hogervorst J, Collette HJ. Effect on breast cancer screening response in The Netherlands of inviting women for an additional scientific investigation. *J Epidemiol Comm Health* 1994;48:175–7.
- [26] Vogel RA. Coronary risk factors, endothelial function, and atherosclerosis: a review. *Clin Cardiol* 1997;20:426–32.
- [27] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;43:109–42.
- [28] Anggard E. Nitric oxide: mediator, murderer, and medicine. *Lancet* 1994;343:1199–206.
- [29] Leeson P, Thorne S, Donald A, Mullen M, Clarkson P, Deanfield J. Non-invasive measurement of endothelial function: effect on brachial artery dilatation of graded endothelial dependent and independent stimuli. *Heart* 1997;78:22–7.
- [30] Fleming I, Busse R. Control and consequences of endothelial nitric oxide formation. *Adv Pharmacol* 1995;34:187–206.
- [31] Sandholzer C, Boerwinkle E, Saha N, Tong M, Utermann G. Apolipoprotein(a) phenotypes, Lp(a) concentration and plasma lipid levels in relation to coronary heart disease in a Chinese population: evidence for the role of the apo(a) gene in coronary heart disease. *J Clin Invest* 1992;89:1040–6.
- [32] Scanu AM, Scandiani L. Lipoprotein(a): structure, biology, and clinical relevance. *Adv Intern Med* 1991;36:249–70.
- [33] Busse R, Fleming I. Endothelial dysfunction in atherosclerosis. *J Vasc Res* 1996;33:181–94.
- [34] Celermajer DS, Sorensen K, Spiegelhalter D, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 1994;24:471–6.
- [35] Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO. Acute vascular effects of estrogen in postmenopausal women. *Circulation* 1994;90:786–91.
- [36] Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnkar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991;325:1196–204.
- [37] Chae CU, Ridker P, Manson JE. Postmenopausal hormone replacement therapy and cardiovascular disease. *Thromb Haemost* 1997;78:770–80.