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Circulating Vascular Cell Adhesion Molecules VCAM-1, ICAM-1, and E-Selectin in Dependence on Aging

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Key Words
Cell adhesion molecules · Aging · Atherosclerosis · Cardiovascular risk · VCAM-1 · ICAM-1 · E-selectin

Abstract
Background: Elevated levels of circulating cell adhesion molecules (cCAMs) such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) are found in subjects with vascular diseases and in subjects with several risk factors for atherosclerosis. However, data evaluating cCAMs and biological age are limited. ***Objective:*** The purpose of this study was to assess in subjects with different cardiovascular risk profiles the levels of cVCAM-1, cICAM-1, and cE-selectin in dependence on age. ***Methods:*** The following groups of subjects were included in the study: 282 apparently healthy subjects of the average population aged 18–89 years, 77 vegetarians who are characterized by a favourable global cardiovascular risk profile, 94 patients with coronary heart disease, and 181 patients with peripheral arterial occlusive disease. Blood samples were obtained after an overnight fast for measurement of cCAMs, lipoproteins, and other clinical/biochemical parameters. The cCAM levels were determined by the use of monoclonal antibody based enzyme-linked immunosorbent assays. ***Results:*** Amongst the cCAMs, cVCAM-1 is uniquely elevated in elderly persons with different risks for atherosclerosis, including subjects of the average population, vegetarians with a favourable risk profile, and patients with both coronary heart disease and peripheral arterial occlusive disease. With respect to cICAM-1, an age-dependent elevation was found in the control subjects included in the study. The cE-selectin levels were not correlated with age. Moreover, no associations of cCAMs with serum lipid and lipoprotein levels were found. ***Conclusion:*** The results of the present study indicate that cVCAM-1 is an age-dependent parameter independent of cardiovascular risk.

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Introduction

Aging is associated with several structural and functional modifications of the vascular endothelial wall. These changes occurring with aging may contribute to an increased susceptibility to develop atherosclerotic lesions [1, 2]. However, the mechanisms by which aging acts are still unknown. The predisposition of the old vessels to develop atherosclerotic lesions may be related to an age-dependent increase of the expression of cell adhesion molecules (CAMs) on the surface of vascular endothelial cells [3]. CAMs, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin), are known to modulate cell-endothelium interactions. The expression of these CAMs is suspected of playing in a key role in adhesion and transendothelial migration of monocytes which results in the formation of fatty streaks [4]. Upregulation of CAMs is accompanied by the release of soluble forms of adhesion molecules into the bloodstream. Therefore, increased plasma levels of circulating CAMs (cCAMs) have been suggested as indexes of elevated CAM expression [5–7].

The levels of cCAMs may be useful markers for stratifying cardiovascular disease severity or prognosis [8–11]. Furthermore, the cCAM levels are elevated in subjects with insulin resistance, in type 2 diabetic patients, in hypertensive patients, and in smokers [7, 12, 13]. However, data evaluating cCAMs and biological age are limited [14–16].

The aim of the present study was to assess in subjects with different cardiovascular risk profiles the levels of cVCAM-1, cICAM-1, and cE-selectin in dependence on age. Therefore, both apparently healthy subjects aged 18–89 years and patients with atherosclerotic diseases were included in the study.

Study Design and Methods

Four groups of subjects were included in the study. The first group consisted of apparently healthy subjects of the average population aged 18–89 years, the second group consisted of vegetarians who are characterized by a favourable global cardiovascular risk profile [17], and the third and fourth groups consisted of patients with coronary heart disease (CHD) and patients with peripheral arterial occlusive disease (PAOD), respectively.

Subjects (18–89 Years Old)

Control Subjects. Two hundred and eighty-two male and female control subjects without a personal history or clinical evidence of cardiovascular diseases were selected from the Lipid Study Leipzig (LSL) and the Interdisciplinary Long-Time Study of Health Adult Age (ILSE). These studies were designed to investigate biochemical and physiological parameters on a population basis in dependence on aging.

Vegetarians. Seventy-seven apparently healthy male and female vegetarians with a favourable cardiovascular risk profile were included in the study. This group consisted of lacto-ovo-vegetarians, lacto-vegetarians, and vegans with vegetarian nutrition and lifestyle of at least 2 years, partly more than 20 years. Mainly members of the German Society of Vegetarians were recruited.

Patients with CHD. Ninety-four male and female patients with angiographically defined CHD (>50% stenosis of one or more major coronary artery, e.g. left main, left anterior descending, left circumflex, or right coronary) who had to undergo coronary bypass surgery (Heart Center, Department of Cardiac Surgery, University of Leipzig) were included in this group. Those patients with myocardial infarction within 2 months, or angioplasty within 3 months, and other significant comorbid illnesses were excluded.

Patients with Peripheral Arterial Occlusive Disease. One hundred and eighty-one male and female PAOD patients, stage IIb–IV according to Fontaine (Clinic of Surgery II, University of Leipzig), were included in the study.

Measurement of cCAMs

Blood samples were obtained after an overnight fast for measurement of cCAMs, lipoproteins, and other clinical/biochemical parameters. Serum was analyzed immediately, or aliquots were stored frozen at –80 °C until analyzed. Serum levels of cVCAM-1, cICAM-1, and cE-selectin were determined by the use of monoclonal antibody based enzyme-linked immunosorbent assays (R&D Systems, Abingdon, UK). All samples were tested twice. Both interassay and intraassay coefficients of variation were < 5%.

Statistics

Baseline values are expressed as mean ± SD as well as median (minimum and maximum) for continuous variables and as counts (percentages) for categorical variables. To assess the association between age and adhesion molecule-1, the Pearson correlation coefficient was calculated. Multiple regression models were built to analyze the association between age and cVCAM-1 adjusted for low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and body mass index. A t test for independent groups for men and women was used. For all statistical tests, differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using SPSS 9.0.0 (SPSS, Chicago, Ill., USA).

Results

cCAMs in Subjects of Different Age Groups

The profiles of all subjects included in the study are presented in table 1. In serum, there was a significant age-dependent increase of cVCAM-1 both in apparently healthy subjects (control subjects and vegetarians) and in patients (CHD and PAOD patients) included in the study (fig. 1). With respect to cICAM-1, an age-dependent ele-

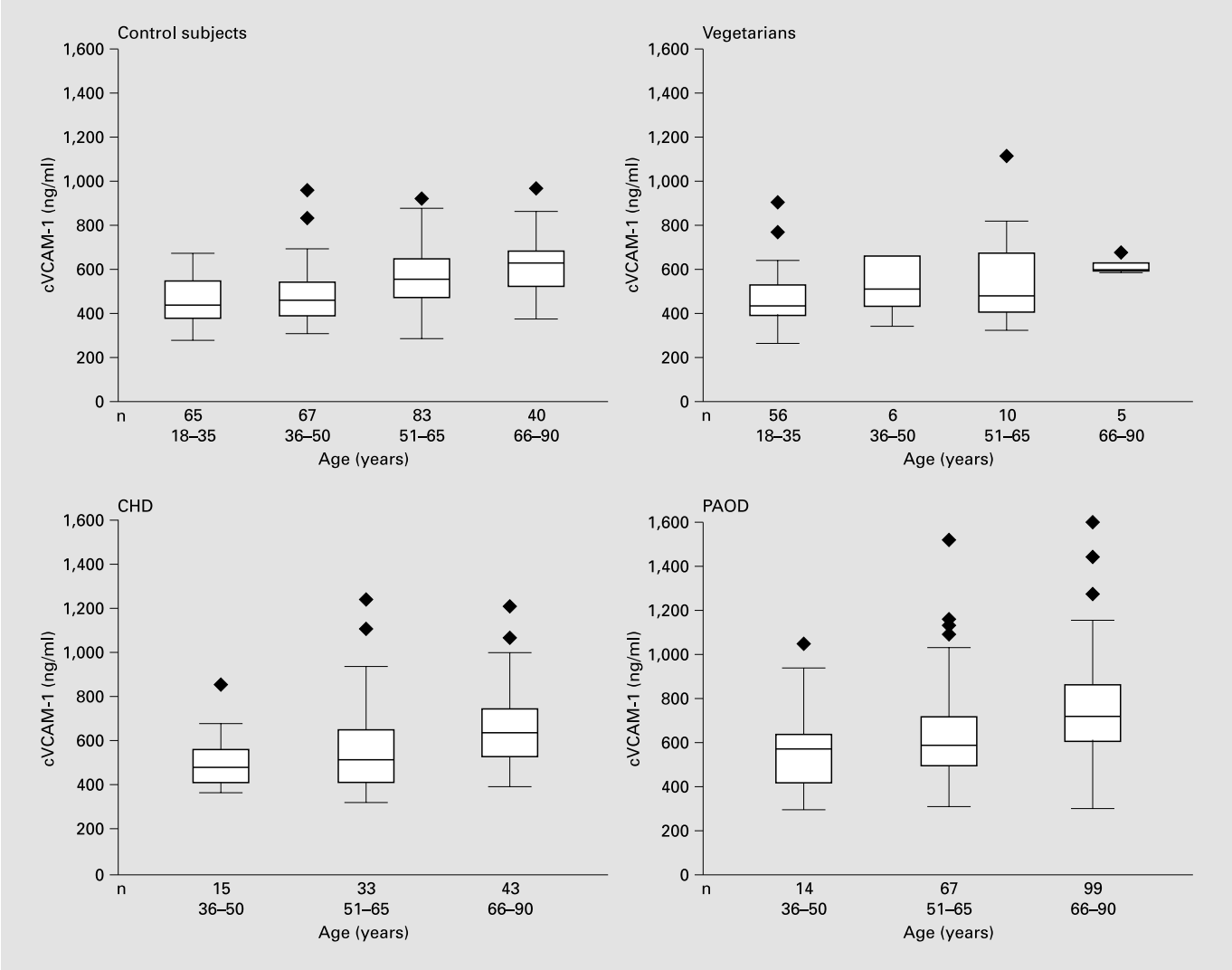


Fig. 1. Levels of cVCAM-1 in serum of healthy control subjects, vegetarians, patients with coronary heart disease (CHD), and patients with peripheral arterial occlusive disease (PAOD) in dependence on age. Box plots with medians, quartiles, minimum and maximum values, as well as outliers; one outlier (1,922 ng/ml) in the age group 66–90 years (PAOD) is not shown.

vation was found in the control subjects included in the study. However, no significant age-dependent changes were found in the other groups (fig. 2). The levels of cE-selectin do not change significantly in dependence on age (fig. 3).

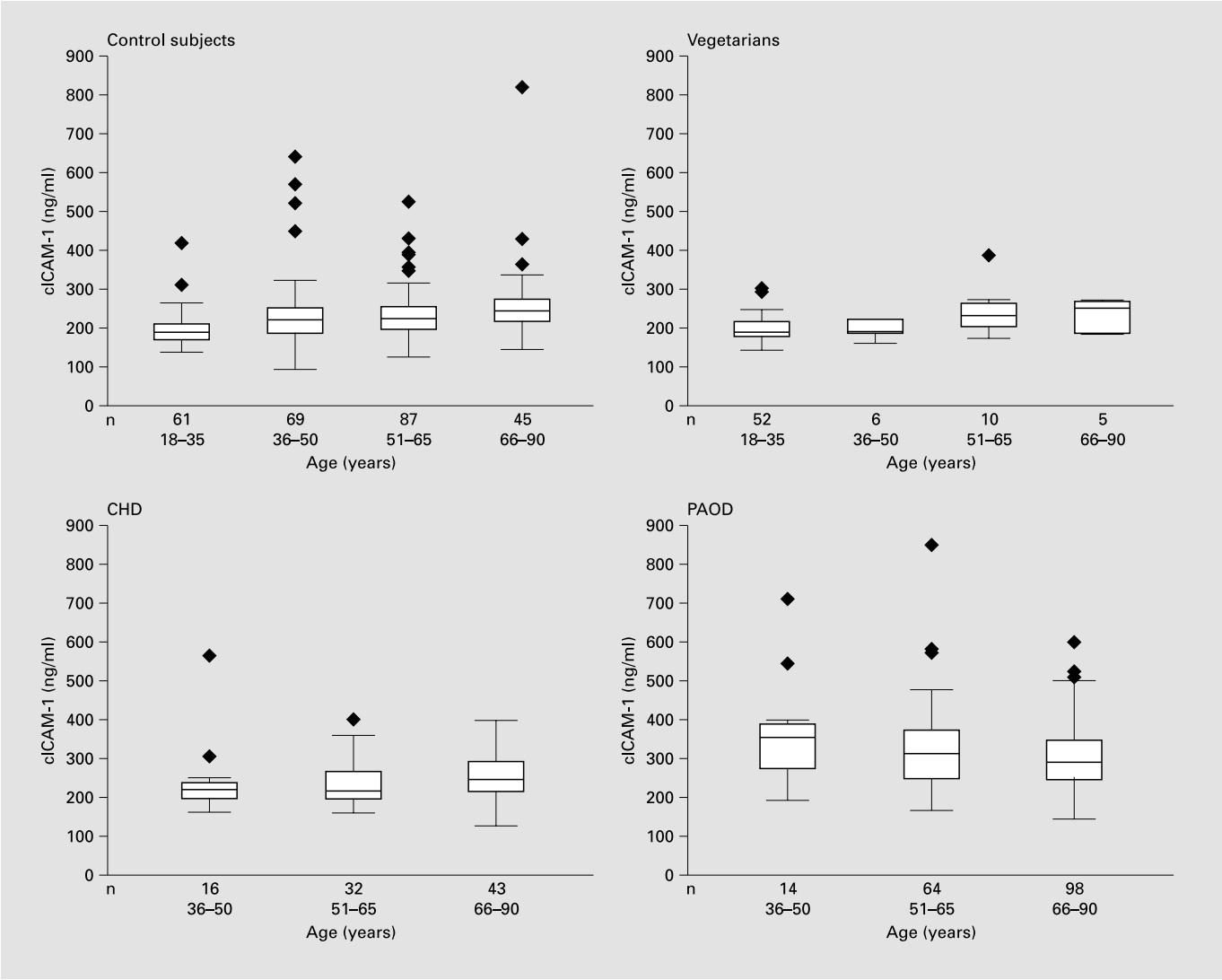
Correlations between Age and cVCAM-1

The levels of serum cVCAM-1 were positively correlated with the age of the subjects included in the study, independent of their cardiovascular risk profile. This positive correlation was seen in apparently healthy control subjects, in vegetarians with a favourable global risk profile, and in patients with atherosclerotic diseases (fig. 4). To analyze the association between cVCAM-1 and age, a multiple regression model, controlled for LDL cholesterol, HDL cholesterol, triglycerides, and body mass index was built. The regression coefficients with the 95% confidence intervals are shown in table 2. There were no significant differences in cVCAM-1 levels between male and female subjects included in the study. Moreover, no associations with serum lipid and lipoprotein levels were found. The results of the multiple regression analysis indicate that age is an independent factor with respect to the increase of the cVCAM-1 concentration.

Table 1. Characteristics of healthy subjects and patients included in the study

	Control subjects	Vegetarians	CHD patients	PAOD patients
Number of subjects	282	77	94	181
Age, years	48 ± 16	32 ± 16	63 ± 11	66 ± 10
Males, %	42	27	76	82
Hypertension, %	22	3	69	75
Diabetes, %	2	1	43	36
Smoking, %	29	20	61	64
Body mass index, kg/m^2	26.2 ± 4.1	22.0 ± 2.4	26.3 ± 3.1	25.8 ± 3.8
Cholesterol, mmol/l	5.8 ± 1.2	5.0 ± 1.1	5.8 ± 1.1	5.9 ± 1.5
LDL cholesterol, mmol/l	3.5 ± 1.2	3.0 ± 1.1	4.2 ± 1.1	4.1 ± 1.4
HDL cholesterol, mmol/l	1.5 ± 0.4	1.5 ± 0.4	1.1 ± 0.3	1.2 ± 0.4
Triglycerides, mmol/l	1.4 ± 0.9	0.9 ± 0.5	2.0 ± 1.4	2.6 ± 2.2

Body mass index and lipid values are given as mean ± SD; data are based on information without missing values.



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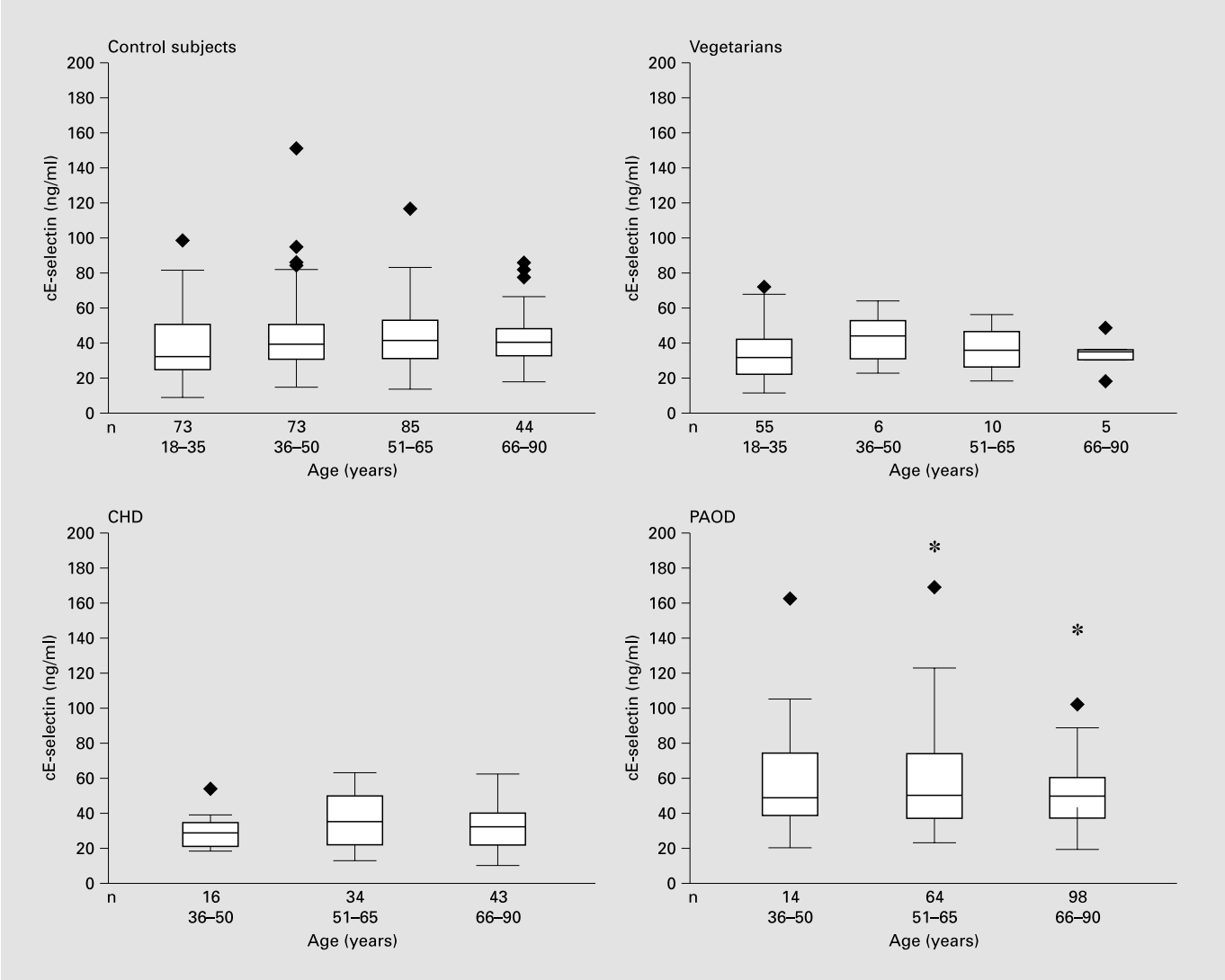

Fig. 3. Levels of cE-selectin in serum of healthy control subjects, vegetarians, patients with CHD, and patients with PAOD in dependence on age. Box plots with medians, quartiles, minimum and maximum values, as well as outliers; one outlier (258 ng/ml) in the age group 18–35 years (vegetarians) is not shown.

Discussion

Although age is a known risk factor for the development of atherosclerosis, it is unclear whether age may directly influence processes of the formation of atherosclerotic plaques. Adhesion and transendothelial migration of monocytes play an important role in atherogenesis, and these processes are mediated, among others, by CAMs on the surface of vascular endothelial cells [4]. CAMs are also found in soluble forms in the circulation, and several data indicate that the levels of circulating CAMs may serve as molecular markers for atherosclerosis [10].

Fig. 2. Levels of cICAM-1 in serum of healthy control subjects, vegetarians, patients with CHD, and patients with PAOD in dependence on age. Box plots with medians, quartiles, minimum and maximum values, as well as outliers.

The serum level of cVCAM-1 of control subjects included in the present study show an age-dependent increase. This finding may imply age-dependent changes in VCAM-1 expression and shedding from cell surfaces. The results of the present study may be related to an effect

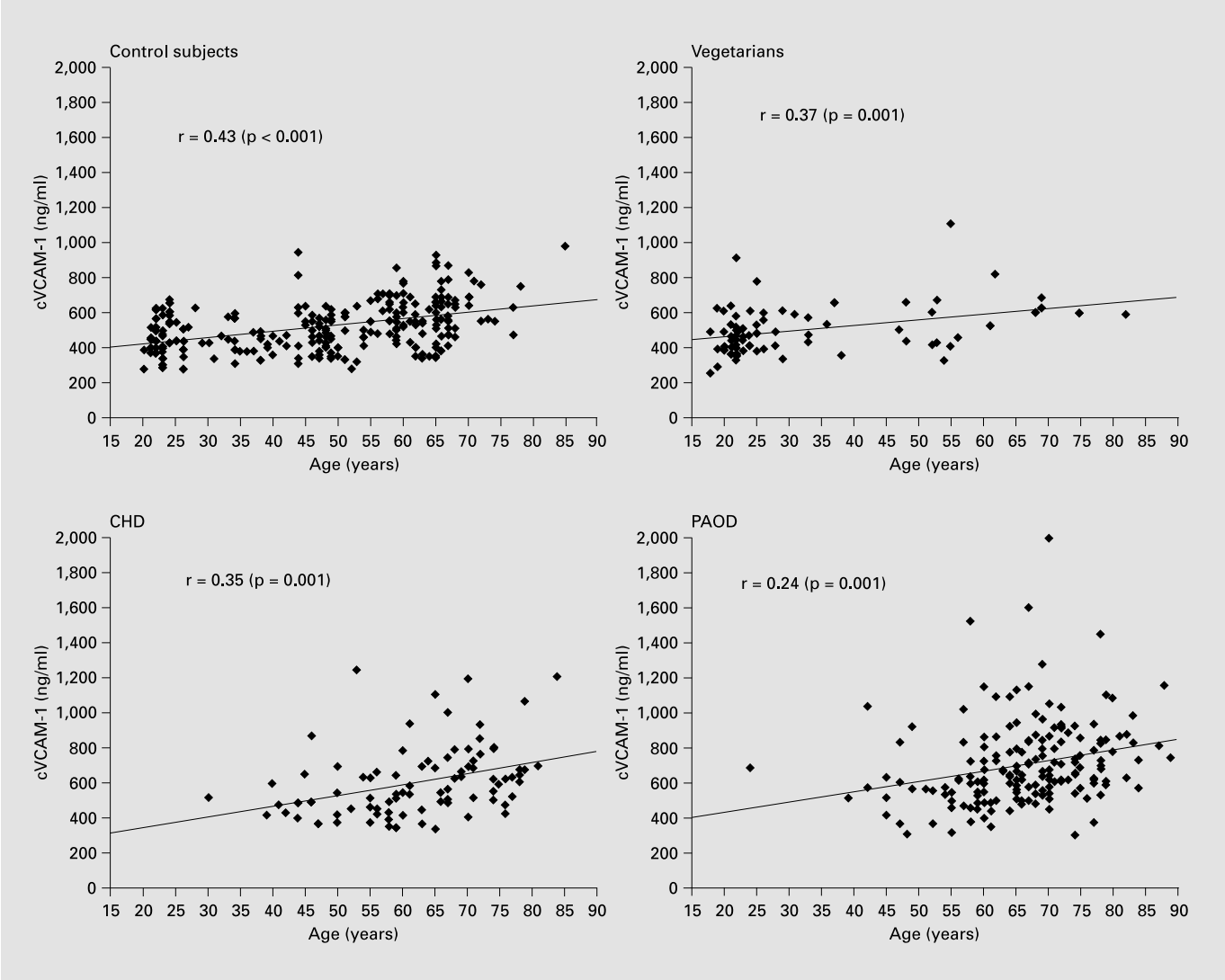

Fig. 4. Correlations (r) between serum cVCAM-1 and age in the four groups of subjects included in the study.

Table 2. Regression analysis regarding age and cVCAM-1 of control subjects, vegetarians, and patients with CHD and PAOD

Group	Regression coefficient	95% confidence interval	p
Controls	4.2	2.0–6.3	<0.001
Vegetarians	3.8	0.9–6.8	0.013
CHD patients	5.2	1.0–9.4	0.017
PAOD patients	5.5	2.0–9.0	0.002

of aging on aortic expression of VCAM-1 and atherosclerosis as described by Merat et al. [3] in murine models of atherosclerosis. These authors reported that old mice had a significantly higher VCAM-1 expression in the aortic arch in comparison with young mice. However, no atherosclerotic lesions were observed in the aortic origin of both young and old animals which have low plasma cholesterol levels. Moreover, they observed that both VCAM-1 expression and the extent of atherosclerosis were significantly greater in old low-density-receptor-deficient (LDL R–/–) mice than in young LDL R–/–. The data demonstrate that in the presence of elevated plasma cholesterol levels, an age-dependent increase of VCAM-1 expression

may be connected with the formation of atherosclerotic lesions [3].

No significant differences were noted regarding the cCAM levels in the comparison of male and female subjects in the present study. Moreover, there are no correlations between cCAMs and serum total cholesterol and LDL cholesterol levels. This observation is in agreement with findings that changes in LDL cholesterol are not associated with changes in cCAMs [18]. Interestingly, the data of the present study including subjects with different cardiovascular risk profiles show that the age-dependent increase of cVCAM-1 levels does not appear to be related to the lipid or the global cardiovascular risk profile. The increase of serum cVCAM-1 levels in dependence on age is seen in subjects of the average population, in vegetarians with a favourable cardiovascular risk profile, and in patients with different atherosclerotic manifestations. Low cE-selectin levels of vegetarians may reflect the favourable cardiovascular risk profile of this group [19]. Both CHD and PAOD patients show the age-dependent increase of cVCAM-1 levels. The results of the present study with respect to control subjects and patients with coronary heart disease are in agreement with those of Morisaki et al. [14] regarding a positive correlation between cVCAM-1 and age.

The plasma concentrations of cCAMs may be higher in patients with atherosclerosis. Several data indicate that they may serve as molecular markers for atherosclerosis [10]. The data of the present study support the concept that cVCAM-1 could be used as a marker for biological aging and further strengthen the link between inflammatory responses in atherosclerosis and an age-dependent increased susceptibility to develop atherosclerotic lesions [1, 2]. The results of the present study are in agreement with observations regarding significant positive linear correlations between age and levels of both cVCAM-1 and cICAM-1 in healthy subjects [15]. However, the present data indicate that only cVCAM-1 is an age-dependent parameter in healthy subjects and in patients with atherosclerotic disease as well.

The surface expression of CAMs appears to be a common response to a variety of stimuli, among others cytokines. Therefore, the age dependence of VCAM-1 may be related to altered cytokine production and cytokine plasma levels in the elderly. Leucocyte/monocyte function seems to be increased age dependently. Leucocytes of elderly subjects produce higher amounts of the cytokines interleukins (ILs) 1 and 6 and tumour necrosis factor alpha (TNF-α) after stimulation with lipopolysaccharide than leucocytes from young human subjects [20]. Furthermore, an age-related increase in IL-6 production by stimulated leucocytes of rhesus monkeys was found [21]. An elevation of constitutive production of various cytokines by unstimulated monocytes from elderly in contrast to young subjects is also indicative of activation [22]. Therefore, aging seems to be associated with an increased inflammatory activity in the blood. In elderly humans increased circulating levels of TNF-α were found, and high TNF-α levels correlated with the clinical diagnosis of atherosclerosis [23]. In this context, the observed increase in cVCAM-1 levels might reflect inflammatory responses to infections which increase with age.

Both age-related changes in functional characteristics of circulating monocytes and increased plasma concentrations of cytokines may contribute to an age dependence of CAM expression and to altered levels of cCAMs. Adhesion of monocytes to endothelial cells induces expression and release of IL-1β by monocytes which, in turn, stimulates release of IL-6 by endothelial cells and upregulation of VCAM-1 on the endothelial surface [24]. Such a positive feedback system could possibly play an important role not only in inflammatory reactions in atherosclerosis, but also in processes of aging. Interestingly, in aged rabbits an increase of monocytes adhering to endothelial cells was found [2].

The nuclear transcription factor NF-kappa B has a central role in gene control associated with inflammatory reactions. This protein is regulated by reactive oxygen intermediates under a great variety of conditions [25]. Several radical-generating systems are involved in mediating NF-kappa B activation and induction of CAMs by cytokines. Interestingly, antioxidants inhibit monocyte adhesion by suppressing NF-kappa B and induction of VCAM-1 in endothelial cells. ICAM-1 expression is only slightly affected, indicating distinct mechanisms for VCAM-1 versus ICAM-1 induction [26]. Aging is believed to be associated with the time-dependent shift in the antioxidant/pro-oxidant balance in favour of oxidative stress [27]. Endothelial VCAM-1, but no ICAM-1, seems to play a critical role in monocyte entry into the subendothelial space in early atherosclerosis [28, 29]. Thus, NF-kappa B activation resulting in increased VCAM-1 induction and elevated cVCAM-1 levels may be related to an age-dependent increased susceptibility to develop atherosclerotic lesions.

In summary, amongst the soluble adhesion molecules, cVCAM-1 is uniquely elevated in elderly persons with different risks for atherosclerosis, including subjects of the average population, vegetarians with a favourable risk profile, and patients with both coronary heart disease and

peripheral arterial occlusive disease. Therefore, the results of the present study indicate that cVCAM-1 is an age-dependent parameter independent of cardiovascular risk. However, further studies are needed to investigate possible explanatory factors with respect to the mechanisms of the age-dependent increase of VCAM-1 and various changes of endothelial functions.

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References

1 Bilato C, Crow MT: Atherosclerosis and the vascular biology of aging. Aging (Milano) 1996; 8:221–234.
2 Orlandi A, Marcellini M, Spagnoli LG: Aging influences development and progression of early aortic atherosclerotic lesions in cholesterol-fed rabbits. Arterioscler Thromb Vasc Biol 2000;20:1123–1136.
3 Merat S, Fruebis J, Sutphin M, Silvestre M, Reaven PD: Effect of aging on aortic expression of the vascular cell adhesion molecule-1 and atherosclerosis in murine models of atherosclerosis. J Gerontol A Biol Sci Med Sci 2000;55:B85–B94.
4 Price DT, Loscalo J: Cellular adhesion molecules and atherogenesis. Am J Med 1999;107: 85–97.
5 Nakai K, Itoh C, Kawazoe Y, Miura H, Sotoyanagi K, Hotta T, Itoh J, Kamata K, Hiramori K: Concentration of soluble vascular cell adhesion molecule-1 (VCAM-1) correlated with expression of VCAM-1 mRNA in the human atherosclerotic aorta. Coron Artery Dis 1995;6: 497–502.
6 Pastore L, Tessitore A, Martinotti S, Toniato E, Alesse E, Bravi MC, Ferri MD, Desideri G, Gulino A, Santucci A: Angiotensin II stimulates intercellular adhesion molecule-1 (ICAM-1) expression by human vascular endothelial cells and increases soluble ICAM-1 release in vivo. Circulation 1999;100:1646–1652.
7 Wagner OF, Jilma B: Putative role of adhesion molecules in metabolic disorders. Horm Metab Res 1997;29:627–630.
8 Blann AD, Lip GY: Cell adhesion molecules in cardiovascular disease and its risk factors – what can soluble levels tell us? J Clin Endocrinol Metab 2000;85:1745–1747.
9 Oishi Y, Wakatsuki T, Nishikado A, Oki T, Ito S: Circulating adhesion molecules and severity of coronary atherosclerosis. Coron Artery Dis 2000;11:77–81.
10 Hwang SJ, Ballantyne CM, Sharett AR: Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases. Circulation 1997;96:4219–4225.
11 Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, Meyer J: Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation 2001;104: 1336–1342.
12 Blüher M, Unger R, Rassoul F, Richter V, Paschke R: Relation between glycaemic control, hyperinsulinaemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or type II diabetes. Diabetologica 2002;45:210–216.
13 Mazzone A, Cusa C, Mazzucchelli J, Vezzoli M, Ottini E, Ghio S, Tossini G, Pacifici R, Zuccaro P: Cigarette smoking and hypertension influence nitric oxide release and plasma levels of adhesion molecules. Clin Chem Lab Med 2001;39:822–826.
14 Morisaki N, Saito J, Tamura K, Tashiro J, Masuda M, Kanzaki T, Watanabe S, Masuda Y, Saito Y: New indices of ischemic heart disease and aging: Studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease. Atherosclerosis 1997;131:43–48.
15 Miles EA, Thies F, Wallece FA, Powell JR, Hurst TL, Newsholme EA, Calder PC: Influence of age and dietary fish oil on plasma soluble adhesion molecule concentrations. Clin Sci (Colch) 2001;100:91–100.
16 Richter V, Rassoul F, Purschwitz K, Wittek M, Vierig G, Kuntze T, Hentschel B, Reuter W: Circulating cell adhesion molecules in dependence on aging. Gerontology 2001;47(suppl 1): 393–394.
17 Richter V, Purschwitz K, Bohusch A, Seim H, Weisbrich C, Reuter W, Sorger D, Rassoul F: Lipoproteins and other clinical-chemistry parameters under the conditions of lacto-ovo-vegetarian nutrition. Nutr Res 1999;19:545–554.
18 Richter V, Rassoul F, Reuter W, Purcz T, Julius U, Gläser V, Hentschel B, Wagner O: Effect of extracorporeal low-density lipoprotein elimination on circulating cell adhesion molecules in patients with hypercholesterolemia. Am J Cardiol 2001;87:1111–1113.
19 Purschwitz K, Rassoul F, Reuter W, Purschwitz R, Jahn N, Kellert D, Richter V: Soluble leukocyte adhesion molecules in vegetarians of various ages. Z Gerontol Geriatr 2001;34:476–479.
20 Rink L, Cakman J, Kirchner H: Altered cytokine production in the elderly. Mech Ageing Dev 1998;102:199–209.
21 Mascaruccil P, Taub D, Saccani S, Paloma MA, Dawson H, Roth GS, Ingram DK, Lane MA: Age-related changes in cytokine production by leukocytes in rhesus monkeys. Aging (Milano) 2001;13:85–94.
22 Sadeghi HM, Schnelle JF, Thoma JK, Nishanian P, Fahey JL: Phenotypic and functional characteristics of circulating monocytes of elderly persons. Exp Gerontol 1999;34:959–970.
23 Bruunsgaard H, Skinhoj P, Pedersen AN, Schroll M, Pedersen BK: Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. Clin Exp Immunol 2000;121:255–260.
24 Zohlnhöfer D, Brand K, Schipek K, Pogatsa-Murray G, Schömig A, Neumann FJ: Adhesion of monocyte very late antigen-4 to endothelial vascular cell adhesion molecule-1 induces interleukin-1-β-dependent expression of interleukin-6 in endothelial cells. Arterioscler Thromb Vasc Biol 2000;20:353–359.
25 Schulze-Osthoff K, Bauer MK, Vogt M, Wesselborg S: Oxidative stress and signal transduction. Int J Vitam Nutr Res 1997;67:336–342.
26 Weber C, Erl W, Pietsch A, Ströbel M, Ziegler-Heitbrock HW, Weber PC: Antioxidants inhibit monocyte adhesion by suppressing nuclear factor-kappa B mobilization and induction of vascular cell adhesion molecule-1 in endothelial cells stimulated to generate radicals. Arterioscler Thromb 1994;14:1665–1673.
27 Meydani M: Dietary antioxidants modulation of aging and immune-endothelial cell interaction. Mech Ageing Dev 1999;111:123–132.
28 Dansky HM, Barlow CB, Lominska C, Sikes JL, Kao C, Weinsaft J, Cybulsky MJ, Smith JD: Adhesion of monocytes to arterial endothelium and initiation of atherosclerosis are critically dependent on vascular cell adhesion molecule-1 gene dosage. Arterioscler Thromb Vasc Biol 2001;21:1662–1667.
29 Cybulsky MJ, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS: A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 2001;107:1255–1262.