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Serum levels of cytokines and chemokines associated with cardiovascular disease in Brazilian patients treated with statins for dyslipidemia

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ABSTRACT

The anti-inflammatory effect of 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) has been investigated in dyslipidemic patients treated with these pharmacologic agents. The aim of this study was to investigate the serum levels of cytokines and chemokines that have been associated with atherosclerosis and cardiovascular disease in Brazilian patients treated for hypercholesterolemia with statin. The serum levels of the cytokines IL-1β, IL-6, IL-10, TNF-α and TGF-β, and the levels of the chemokines IL-8 (CXCL8) and MCP-1 were determined by enzyme-linked immunosorbent assay and tested for their association with cardiovascular disease. The suppression of circulating levels of TNF-α, MCP-1 and IL-8 and their enhancing effect on IL-10 and TGF-β production were more pronounced in male patients. Female patients treated with statins who had a previous myocardial infarction presented higher median levels of both TNF-α and IL-8 ($P < 0.05$) and a lower median level of IL-10 than female patients without MI ($P < 0.05$). Except in women with a previous myocardial infarction, the treatment of dyslipidemic Brazilian patients with statins down-modulates the production of atherogenic cytokines and chemokines and increases the circulating levels of anti-atherogenic cytokines.

1. Introduction

Atherosclerosis is the main cause of cardiovascular diseases (CVD) worldwide, including coronary arterial disease, myocardial infarction and stroke [1,2]. In Brazil, it is estimated that CVD mortality is approximately 52.7 cases per 100,000 inhabitants [3].

Cardiovascular disease has been associated with multiple risk factors including smoking, diabetes, obesity, hypertension, hypercholesterolemia, sedentary lifestyle and family history of CVD. However, the involvement of both inflammation and the adaptative immune response in the pathogenesis of atherosclerosis has been well documented. The role of monocytes and macrophages during the uptake of modified lipoproteins and the involvement of immune mediators such as cytokines and chemokines that are produced by macrophages, dendritic cells and T and B lymphocytes in atheroma development have demonstrated that atherosclerosis has strong characteristics of an immunological disease caused by dyslipidemia. Thus, it has been shown that tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), monocyte chemoattractant protein 1 (MCP-1), interleukin-6 and interleukin-8 (IL-8) exert pro-inflammatory activity on atherogenesis, whereas interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) down-modulate atheroma formation [2,4–6].

Some studies have demonstrated that statin treatment for dyslipidemia, in addition to decreasing hypercholesterolemia, can also down-modulate atherogenesis due to their effects on the production of some bioactive components involved in inflammation such as cytokines, chemokines and chemokine receptors, and C-reactive protein (CRP) [7–11]. However, the knowledge obtained from these studies still needs to be evaluated in different populations because genetic background and environmental factors can prevent its generalized application. This work investigated the serum levels of cytokines and chemokines associated with atherogenesis in patients who have been treated for dyslipidemia with statins in a Brazilian referral center for cardiovascular diseases in the city of Salvador (Bahia) using as reference the serum levels of these immune mediators in non-dyslipidemic healthy individuals.

2. Materials and methods

2.1. Subjects

A total of 162 patients, 80 of whom were women (age = 60 ± 10 years) and 82 of whom were men (age = 59 ± 8 years), from the Cardiology Service of the Hospital Ana Nery, located in Salvador (BA, Brazil) participated of this study after providing written informed consent. They had a clinical and laboratory diagnosis of dyslipidemia

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and were treated with statins for more than 12 months to control hypercholesterolemia (85% of patients were treated with simvastatin, and 15% of patients were treated with atorvastatin). One hundred of 162 (62.0%) patients had abnormal coronary angiography (CA). Thirty healthy volunteers (15 women and 15 men, mean age = 32.8 ± 8.4 years) were used as the control group. Pregnant women, patients with cancer or tuberculosis, those who were infected with HCV, HBV, HTLV-I or HTLV-II, and those suffering from Chagas' disease or autoimmune disease were excluded from the study. The study was approved by the Ethics Committee of the Climério de Oliveira Maternity (Hospital Complex Professor Edgard Santos, Federal University of Bahia).

2.2. Lipid biomarkers

Serum levels of total cholesterol (TC) (reference value < 200 mg/dl), high-density lipoprotein (HDL, reference value < 40 mg/dl) and triglycerides (TG, reference value < 150 mg/dl) were determined using an automated biochemical analyzer (LABMAX 240, Labtest Diagnóstica SA, Brazil). Low-density lipoprotein (LDL, reference value < 130 mg/dl) levels were calculated by the Friedewald method when the triglyceride level was less than 400 mg/dl. Serum levels of apolipoprotein A (APOA, reference value 90–170 mg/dl) and apolipoprotein B (APOB, reference value 107–214 mg/dl) and ultrasensitive CRP (reference value < 3.0 mg/dl) were determined by nephelometry (IMMAGE™ System, Beckman-Coulter, USA).

2.3. Serum levels of cytokines

The serum levels of IL-1β, IL-6, IL-8, IL-10, MCP-1, TNF-α and TGF-β were determined by capture enzyme-linked immunosorbent assays (capture ELISA) using commercial immunological reagents (Ebioscience, USA).

2.4. Statistical analysis

The results are expressed as the median and interquartile range (IQR, Q1–Q3) in accordance with the non-Gaussian distribution of the data shown by the normality test of D'Agostino & Pearson. The difference between the medians of the two groups was compared with the Mann–Whitney test. Fisher's exact test was used to investigate the association between the two categorical groups, while their correlation was analyzed with Spearman correlation. The significance level was set at $P < 0.05$. GraphPad statistical software 5.0 was used for the statistical analysis.

3. Results

3.1. Cardiovascular risks

Arterial hypertension was the most prevalent risk factor for cardiovascular disease observed in the patients, which was followed by family history of CVD, sedentary lifestyle, diabetes, smoking and obesity. Although smoking was slightly more prevalent than obesity in men than in women, there was no significant difference in the prevalence of CVD risk factors between female and male patients (Table 1).

Table 1
Prevalence of cardiovascular risk factors in Brazilian patients treated for dyslipidemia with statins.

Risk factors	Female ($n = 80$)	Male ($n = 82$)	Total ($n = 162$)
Hypertension	71 (88.7%)	68 (82.9%)	139 (85.8%)
Familial CVD	52 (65.0%)	51 (62.2%)	103 (63.6%)
Sedentary lifestyle	40 (50.0%)	38 (46.3%)	78 (48.1%)
Diabetes	29 (36.2%)	23 (28.0%)	52 (32.1%)
Obesity	8 (10.0%)	5 (6.1%)	13 (8.0%)
Smoking	5 (6.2%)	13 (15.8%)	18 (11.1%)

3.2. Lipid profile

The serum levels of cholesterol, HDL, LDL, triglycerides, APOA and APOB were similar when male patients and their controls were compared ($P > 0.05$). However, female patients differed from female controls in their levels of cholesterol ($P < 0.01$), LDL ($P < 0.01$) and APOB ($P < 0.01$) (Table 2).

3.3. C-reactive protein

Patients and healthy controls showed similar levels of CRP (patient median = 2.7 mg/L, IQR 1.4 mg/L–5.8 mg/L; control median = 2.3 mg/L, IQR 1.6 mg/L–3.9 mg/L). Ninety of 162 (56.0%) patients had CRP levels lower than 3 mg/L, whereas 72 of 162 (44.0%) patients presented CRP levels above this reference value, which has been adopted for cardiac risk prediction in Brazil. In the control group, CRP levels above 3 mg/L were found in 11 of 30 (37%) individuals, whereas CRP levels <3 mg/L were observed in 19 of 30 (63.0%) subjects ($P > 0.05$). Female and male patients differed in their levels of CRP. Female patients had a median CRP of 3.5 mg/L (IQR = 1.8–6.4 mg/L), while male patients had a median of 2.4 mg/L (IQR = 1.2–5.0 mg/L; $P < 0.05$) (Fig. 1). There was no difference in the CRP levels when the patients were classified in accordance with their previous history of myocardial infarction (MI) or the presence of abnormal coronary angiography ($P > 0.05$).

3.4. Cytokine and chemokine levels

Levels of cytokines and chemokines above the lower limit of detection (LOD) of the immunoassays were observed with the following frequency: IL-1 (25/30, 83.3% controls and 49/162, 30.2% patients; $P < 0.0001$), IL-6 (15/30, 50% controls and 89/162, 54.9% patients; $P > 0.05$), IL-8 (25/30, 83.3% controls and 34/162, 21.0% patients, $P < 0.0001$), and TNF-α (29/30, 96.7% controls and 137/162, 84.7% patients). Levels of MCP-1, IL-10 and TGF-β above the LOD were observed in all controls and patients ($P > 0.05$). The serum levels of IL-1β, MCP-1, IL-6 and IL-10 in female patients were similar to those of their female controls. However, female patients had decreased serum levels of TNF-α and IL-8 ($P < 0.05$), but increased serum levels of TGF-β ($P < 0.05$). In male patients, the serum levels of IL-1β and IL-6 were similar to those of male controls, but they had decreased levels of MCP-1 ($P < 0.001$), IL-8 ($P < 0.0001$), TNF ($P < 0.01$) and IL-10 ($P < 0.01$). In contrast, male patients had the highest levels of TGF-β ($P < 0.0001$) (Table 3).

Female patients with a documented history of myocardial infarction had higher serum levels of TNF-α and IL-8, but lower levels of serum IL-10 than female patients without previous MI ($P < 0.05$) (Fig. 1). No difference was observed in the serum levels of cytokines or chemokines when male patients were evaluated in accordance with their coronary angiography or previous documentation of MI ($P > 0.05$).

4. Discussion

In this work, we investigated serum levels of cytokines and chemokines that are associated with cardiovascular disease among Brazilian patients who were treated with statins for hypercholesterolemia. Most patients were individuals of African descent, who tend to consume food that is high in saturated and total fat. All patients included in this study had a previous documentation of hypercholesterolemia in their medical files, and most had abnormal coronary angiography. The cardiovascular risk factors in the patient group were the same as those

Table 2
Lipid profile of Brazilian patients treated for dyslipidemia with statins and of healthy controls.

	Female patients ($n = 80$)	Female controls ($n = 15$)	Male patients ($n = 82$)	Male controls ($n = 15$)
Cholesterol	193.0 (156.0–244.5)	156.0** (138.0–182.0)	168.5 (129.8–192.3)	169.0 (141.0–190.0)
LDL	122 (74.6–158.4)	82.00** (55.8–99.2)	87.4 (60.3–116.7)	93.4 (74.2–109.2)
HDL	43.50 (38.0–54.7)	50.0 (41.0–65.0)	37 (33.0–44.2)	40.0 (33.0–49.0)
Triglycerides	144.0 (110.5–208.3)	126.0 (79.0–164.0)	180.5 (133.0–237.8)	146.0 (109.0–196.0)
APOA	150.0 (131.3–165.0)	138.0 (126.0–170.0)	135.0 (119.8–154.3)	129.0 (112.0–145.0)
APOB	94.5 (69.6–119.5)	67.4** (61.2–80.8)	77.10 (61.2–97.1)	74.6 (65.7–89.7)

Lipid concentrations (mg/dl) are expressed as the median and interquartile range (IQR = Q1–Q3).
** $P < 0.01$.

reported in other population studies [12], and except for active smoking, they presented similar prevalence in both male and female patients.

Despite statin therapy, the lipid profile of female patients was abnormal in comparison to that of their controls, and it showed increased levels of total cholesterol, LDL and APOB. This finding may be justified by the rise in non-HDL-C and APOB levels with age in both men and women, but in this work only female patients differed from their controls. However, the serum levels of CRP were similar between healthy controls and male and female patients, and most patients had levels of this protein within the normal reference level due to the anti-inflammatory effects of statins, which decrease the level of CRP [11]. The observation of higher CRP levels in female patients than in male patients confirmed the report of a previous study showing the association of an African genetic background with high levels of this inflammatory biomarker in black women [13,14]. Nonetheless, the level of CRP in our study was not influenced by a previous history of MI or by the presence of abnormal CA in female patients.

We verified that statin treatment significantly decreased the serum levels of IL-1β and IL-8 in patients, and in an important proportion of patients, these levels were significantly below the lower detection limit of the immunoassays used to quantify these immune mediators. IL-1β is a cytokine that is involved in both inflammation and vascular injury in early atherosclerosis that stimulates the endothelial expression of cell adhesion molecules and promotes leukocyte recruitment and leukocyte transmigration. In addition, it also stimulates cell proliferation and differentiation, which causes vessel injury [5]. The circulating level of IL-1β and its expression in monocytes are decreased in patients with hypercholesterolemia, and additionally, there is suppression of its expression in peripheral blood mononuclear cells from patients with essential hypertension after simvastatin therapy, which justifies our results [15–17].

IL-8 or CXCL8 is a chemokine produced by nucleated cells, mainly macrophages, which is actively involved in inflammation by promoting the recruitment of monocytes and neutrophils. Its participation in atheroma formation has been well documented. Additionally, increased serum levels of this chemokine have been associated with cardiovascular events, which suggest that IL-8 is an important biomarker of atherosclerosis in patients with unstable coronary artery disease [18]. Our finding of low production of IL-8 in patients treated with statins is supported by previous observation showing the effect of this pharmacologic agent in down-regulating IL-8 production by endothelial cells and leukocytes [19].

In this work, the levels of IL-6 in patients were similar to those presented by the controls. IL-6 regulates different aspects of the immune response, including B lymphocyte differentiation and the production of acute phase proteins such as CRP, and participates in atheroma formation by increasing the expression of adhesion molecules on endothelial and smooth muscle cells. Additionally, IL-6 level has been demonstrated to be a predictor of future cardiovascular events [5,20–23]. Although a lowering effect of statin on the production of IL-6 has been well documented by measuring the serum levels of IL-6 in hypercholesterolemic patients before and after treatment [19,24], our finding of normal IL-6 levels in dyslipidemic patients treated with statin was similar to that observed in a large cross-sectional study conducted in Lausanne (Switzerland). Thus, such discrepancy suggests that difference in therapeutic dose of this medication could influence the results of these studies on its anti-inflammatory effects in dyslipidemic patients [25].

Interestingly, circulating MCP-1 levels were inhibited by statin only in male patients. MCP-1 is a chemokine that promotes monocyte migration into the arterial wall to form foam cells. Also, high levels of this chemokine have been observed in patients with obesity and have been

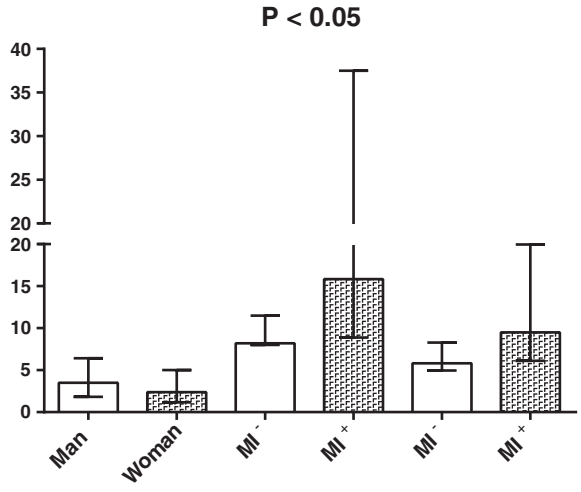

Fig. 1. Levels of C-reactive protein (men and women), and IL-8 and TNF-α in female patients treated for dyslipidemia with statins without a previous myocardial infarction or having MI documentation. Serum levels are shown as the median and interquartile range (IQR = Q1–Q3).

Table 3
Serum levels of cytokines and chemokines in dyslipidemic women and men treated with statins and their controls.

	Controls	Patients	*P* value
MCP-1	143.4 (119.0–166.9)	98.4 (54.4–155.8)	>0.05
	242.3 (169.0–296.1)	125.3 (74.5–183.2)	<0.001
IL-1β	5.1 (3.9–5.9)	4.7 (4.0–5.4)	>0.05
	7.9 (5.5–11.5)	6.1 (4.7–7.9)	>0.05
TNF-α	9.5 (7.5–12.7)	6.4 (4.8–10.2)	<0.05
	11.4 (8.4–17.8)	6.9 (4.9–10.6)	<0.01
IL-8	14.7 (13.1–12.0)	9.2 (8.1–13.6)	<0.05
	27.3 (17.2–73.4	9.4 (8.3–12.0)	<0.0001
IL-6	2.5 (2.1–3.1)	2.2 (1.7–2.9)	>0.05
	2.2 (1.8–2.7)	1.9 (2.2–30.7)	>0.05
IL-10	7.3 (5.8–8.6)	6.1 (5.1–7.4)	>0.05
	8.4 (7.1–10.2)	6.3 (5.1–7.9)	<0.01
TGF-β	127.4 (76.9–188.6)	213.7 (146.4–295.9)	<0.05
	131.0 (78.6–182.9)	196.3 (144.8–288.7)	<0.001

Serum levels are shown as the median and IQR.

associated with a worse prognosis in diseases caused by atherosclerosis such as myocardial infarction and stroke, as well as CVD mortality [26]. It has been demonstrated that MCP-1 levels are normally higher in men than in women, mainly in patients in their fifth decade, which is in agreement with our results [27]. In addition, an increased serum level of MCP-1 has been demonstrated to be associated with traditional cardiovascular risk factors and subclinical atherosclerosis [28]. However, our findings regarding MCP-1 in male patients are supported by a previous report of significant reduction of the serum levels of these immune mediators in hypercholesterolemic individuals after simvastatin treatment [19,29]. On the other hand, the absence of low levels of this chemokine in female patients treated with statin could be justified by their menopausal or postmenopausal status (mean age = 60 ± 10 years) and their higher level of CRP, which is a bioactive protein that promotes MCP-1 production [30,31].

Female patients with a previous history of MI had higher IL-8 and TNF-α levels but lower IL-10 levels in comparison with female patients without MI history. This finding may suggest that female patients with MI history have a diminished capacity to down-modulate atherogenesis or are more resistant to the anti-inflammatory effects of statins. Nonetheless, the association of high levels of both IL-8 and TNF-α and low IL-10 serum levels may be an evidence of atheroma instability in these patients and may also indicate a bad prognosis as previously suggested, as male and female patients did not differ in CVD risk prevalence [18,32,33].

An important increase in the circulating levels of TGF-β was observed in both male and female patients, which was probably caused by statin medication, as previously documented [34]. Serum concentration of TGF-β is decreased in advanced atherosclerosis [35]. TGF-β down-regulates atherogenesis by inhibiting the migration and proliferation of endothelial cells and smooth muscle cells, is involved in down-modulation of lymphocyte activation and acts as a stabilizing factor for human atherosclerotic plaques. Furthermore, an increasing effect of statin on TGF-β production has been experimentally demonstrated in lung cancer cells treated with this medication and simvastatin promotes the *FoxP3* expression in regulatory T cells and their proliferation [36–41].

In conclusion, important effects on the production of cytokines and chemokines involved in atherogenesis can be observed in Brazilian patients treated for dyslipidemia with statins. Despite the consistent documentation showing the efficacy of statins in the primary and secondary prevention of cardiovascular events in both men and women [42], the effects of this medication on atherogenic and anti-atherogenic immune mediators seem to be lower in female patients with a previous history of myocardial infarction, and this finding requires future investigation.

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References

[1] Hansson GK, Robertson AKL, Söderberg-Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol Mech Dis 2006;1:297–329.
[2] Kuiper J, van Puijvelde GH, van Wanrooij EJ, et al. Immunomodulation of the inflammatory response in atherosclerosis. Curr Opin Lipidol 2007;18:521–6.
[3] Ministério da Saúde/SVS. Sistema de Informações de Mortalidade (SIM) e IBGE. Brasil: Indicadores e Dados Básicos; 2011.
[4] Girn HR, Orsi NM, Homer-Vanniasinkam S. An overview of cytokine interactions in atherosclerosis and implications for peripheral arterial disease. Vasc Med 2007;12:299–309.
[5] Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. Annu Rev Immunol 2009;27:97–165.
[6] Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Cytokine network and T cell immunity in atherosclerosis. Semin Immunopathol 2009;31:23–33.
[7] Romano M, Diomede L, Sironi M, et al. Inhibition of monocyte chemotactic protein-1 synthesis by statins. Lab Invest 2000;80:1095–100.
[8] Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;22:1194–9.
[9] Waehre T, Damås JK, Gullestad L, et al. Hydroxymethylglutaryl coenzyme a reductase inhibitors down-regulate chemokines and chemokine receptors in patients with coronary artery disease. J Am Coll Cardiol 2003;41:1460–7.
[10] Waehre T, Yndestad A, Smith C. Increased expression of interleukin-1 in coronary artery disease with downregulatory effects of HMG-CoA reductase inhibitors. Circulation 2004;109:1966–72.
[11] Ridker PM, Cannon CP, Morrow D, Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) Investigators, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
[12] Khot UN, Khot MB, Bajzer CT, et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 2003;290:898–904.
[13] Kelley-Hedgepeth A, Lloyd-Jones DM, Colvin A, et al. Ethnic differences in C-reactive protein concentrations. Clin Chem 2008;54:1027–37.
[14] Shah T, Newcombe P, Smeeth L, et al. Ancestry as a determinant of mean population C-reactive protein values: implications for cardiovascular risk prediction. Circ Cardiovasc Genet 2010;3:436–44.
[15] Ferroni P, Martini F, Cardarello CM, Gazzaniga PP, Davi G, Basili S. Enhanced interleukin-1beta in hypercholesterolemia: effects of simvastatin and low-dose aspirin. Circulation 2003;108:1673–5.
[16] Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. J Am Coll Cardiol 2000;36:427–31.
[17] Zhao S, Li Q, Liu L, Xu Z, Xiao J. Simvastatin reduces interleukin-1beta secretion by peripheral blood mononuclear cells in patients with essential hypertension. Clin Chim Acta 2004;344:195–200.
[18] Apostolakis S, Vogiatzi K, Amanatidou V, Spandidos DA. Interleukin 8 and cardiovascular disease. Cardiovasc Res 2009;84:353–60.
[19] Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;22:1194–9.
[20] Kanda T, Takahashi T. Interleukin-6 and cardiovascular diseases. Jpn Heart J 2004;45:183–93.
[21] Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. Thromb Haemost 2009;102:215–22.
[22] Danesh J, Kaptoge S, Mann AG, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med 2008;5(4):e78. http://dx.doi.org/10.1371/journal.pmed.0050078.
[23] Nishida H, Horio T, Suzuki Y, et al. Interleukin-6 as an independent predictor of future cardiovascular events in high-risk Japanese patients: comparison with C-reactive protein. Cytokine 2011;53:342–6.
[24] Musial J, Undas A, Gajewski P, Jankowski M, Sydor W, Szczeklik A. Anti-inflammatory effects of simvastatin in subjects with hypercholesterolemia. Int J Cardiol 2001;77:247–53.
[25] Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: a population-based Colaus study. Atherosclerosis 2011;219:253–8.
[26] Panee J. Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. Cytokine 2012;60:1–12.
[27] Inadera H, Egashira K, Takemoto M, Ouchi Y, Matsushima K. Increase in circulating levels of monocyte chemoattractant protein-1 with aging. J Interferon Cytokine Res 1999;19:1179–82.
[28] Deo R, Khera A, McGuire DK, et al. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. J Am Coll Cardiol 2004;44:1812–8.
[29] Rallidis LS, Hamodraka ES, Fountoulaki K, Moustogiannis G, Zolindaki MG, Kremastinos DT. Simvastatin exerts its anti-inflammatory effect in hypercholesterolaemic patients by decreasing the serum levels of monocyte chemoattractant protein-1. Int J Cardiol 2008;124:271–2.
[30] Park KS, Ahn KJ, Kim BJ, et al. Circulating concentrations of monocyte chemoattractant protein-1 are associated with menopause status in Korean women. Clin Chim Acta 2009;403:92–6.
[31] Pasceri V, Cheng JS, Willerson JT, Yeh ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 2001;103:2531–4.
[32] Welsh P, Woodward M, Rumley A, Lowe G. Associations of circulating TNF-α and IL-18 with myocardial infarction and cardiovascular risk markers: the Glasgow Myocardial Infarction Study. Cytokine 2009;47:143–7.
[33] Heeschen C, Dimmeler S, Hamm CW, et al. Serum level of the anti-inflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 2003;107:2109–14.
[34] Porreca E, Di Febbo C, Baccante G, Di Nisio M, Cuccurullo F. Increased transforming growth factor-beta(1) circulating levels and production in human monocytes after 3-hydroxy-3-methyl-glutaryl-coenzyme a reductase inhibition with pravastatin. J Am Coll Cardiol 2002;39:1752–7.
[35] Grainger DJ, Kemp PR, Metcalfe JC. The serum concentration of active transforming growth factor-beta is severely depressed in advanced atherosclerosis. Nat Med 1995;1:74–9.
[36] Mallat Z, Tedgui A. The role of transforming growth factor beta in atherosclerosis: novel insights and future perspectives. Curr Opin Lipidol 2002;13:523–9.

[37] Robertson AK, Rudling M, Zhou X, Gorelik L, Flavell RA, Hansson GK. Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. J Clin Invest 2003;112:1342–50.
[38] Grainger DJ. TGF-beta and atherosclerosis in man. Cardiovasc Res 2007;74:213–22.
[39] Lee KJ, Moon JY, Choi HK. Immune regulatory effects of simvastatin on regulatory T cell-mediated tumour immune tolerance. Clin Exp Immunol 2010;161:298–305.
[40] Kim YC, Kim KK, Shevach EM. Simvastatin induces Foxp3 + T regulatory cells by modulation of transforming growth factor-beta signal transduction. Immunology 2010;130:484–93.
[41] Meng X, Zhang K, Li J, et al. Statins induce the accumulation of regulatory T cells in atherosclerotic plaque. Mol Med 2012;18:598–605.
[42] Kostis WJ, Cheng JQ, Dobrzynski JM, Cabrera J, Kostis JB. Meta-analysis of statin effects in women versus men. J Am Coll Cardiol 2012;59:572–82.