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Decreased number of circulating endothelial progenitor cells in patients with Graves' hyperthyroidism

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ABSTRACT. ***Objective:*** **A relevant biological role of circulating endothelial progenitor cells (EPC) was recently demonstrated. EPC are generated in the bone marrow, and interact with damaged endothelium, restoring the integrity of the monolayer. Therefore, aim of the present study was to evaluate EPC in the blood of patients with untreated Graves' hyperthyroidism (GD), in whom an increased oxidative stress was observed.** ***Design and methods:*** **Twenty-three patients with untreated active GD and 18 matched normal controls (NC) were included in the study. Circulating EPC were isolated from peripheral blood. Mononuclear cells were cultured with endothelial basal medium supplemented with EGM SingleQuots, and were identified by positive double staining after 7 days in culture. Circulating levels of C reactive protein, total antioxidant power, interleukin (IL)-6, IL-18, monocyte chemoattractant protein-1, tumor necrosis facotr-α, soluble vascular cell adhesion molecule (VCAM) and intracellular adhesion molecule were evaluated by enzyme-linked immunosorbent assay kit. EPC number was also evaluated in a subgroup of GD patients after restoration of euthyroidism.** ***Results:*** **Systolic blood pressure resulted increased in GD patients compared with control subjects whereas diastolic blood pressure was not significantly different. Patients with GD showed an increase in circulating levels of IL-18 and VCAM-1 and a reduction of total antioxidant power ($p<0.05$) compared to NC. Moreover, a reduced number of EPC was observed in patients with GD compared to NC ($p<0.05$) which turned to NC values after restoring euthyroidism.** ***Conclusion:*** **Patients with GD showed a reduction in the physiological protective mechanisms against endothelial damage, probably induced by increased inflammation and oxidative stress.**

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INTRODUCTION

Graves' disease (GD) is a thyroid disorder caused by an antibody-mediated immune reaction against the receptor for TSH resulting in an abnormal chronic stimulation of the gland and consequent hyperthyroidism (1, 2). This, in turn, causes the clinical symptoms of hyperthyroidism. Indeed, thyroid hormones may exert multiple effects on the heart and vascular system, inducing an hyperkinetic status and the onset of isolated systolic hypertension (3, 4).

Activation of the sympathetic and of the renin-angiotensin-aldosterone (RAS) systems may elicit a major role in inducing cardiovascular changes in GD (5). Moreover, in hyperthyroidism, an unbalance between oxidant and antioxidant substances has been identified, thus leading to enhanced generation of reactive oxygen species (ROS), able to induce vascular dysfunction and damage (6). This may be the consequence of both an activation of RAS system and of a stimulation of energy metabolism induced by thyroid hormones (5). In addition, chronic inflammation has been shown in active GD, as well as in autoimmune thyroid disorders, and production of acute-phase proteins and proinflammatory cytokines [such as interleukin (IL)-12, IL-18, and tumor necrosis factor-α (TNF-α)] (7). Hemodynamic and metabolic changes may lead to vascular injury, as suggested by an increase of markers of endothelial damage observed in GD (8-10).

In the last years, endothelial progenitor cells (EPC) have been recognized to play important roles in post-natal neovascularization and vascular repair, and, therefore in maintaining endothelial integrity and function (11, 12). EPC are generated from bone marrow and represent precursors which preserve stem cell features, able therefore to proliferate, migrate, and differentiate in mature endothelial cells (11, 12). EPC number has been demonstrated to be correlated with the number of cardiovascular risk factors, with indices of endothelial dysfunction (13-15), and the incidence of cardiovascular events (16, 17). However, no data are presently available about the EPC number in hyperthyroidism caused by GD. Therefore the aim of the present study is to investigate the presence of oxidative stress and inflammation as well as the EPC number in patients with active GD compared with control subject with normal thyroid function (NC).

PATIENTS AND METHODS

Forty-one subjects have been enrolled from the outpatient clinic of our Department and included in the study: 23 patients with newly diagnosed, active, untreated GD and 18 NC. GD patients had symptoms of hyperthyroidism (such as palpitation, insomnia, *etc.*) for at least 2 months before being evaluated in our Department. Diabetic patients, subjects with 2 or more cardiovas-

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cular risk factors or taking antihypertensive, lipid-lowering or antiplatelet drugs, as well as vitamins or diet integrators were excluded from the study. At entry, patients underwent a clinical evaluation; the presence of cardiovascular risk factors was recorded. A venous blood sample was obtained from each patient, for evaluation of EPC number, total antioxidant power (AOP) and circulating inflammatory markers, such as IL-6 and -18, soluble vascular cellular adhesion molecule-1 (sVCAM-1), intracellular adhesion molecule-1 (sICAM-1), and monocyte chemotactic protein-1 (MCP-1). Anti-TSH receptor antibody titer and thyroid ultrasound assessment were obtained in hyperthyroid patients to confirm diagnosis of GD. Clinic blood pressure was measured by standard mercury sphygmomanometer, according to the European Society of Hypertension-European Society of Cardiology Guidelines (18).

A subgroup of 7 patients with GD was then re-evaluated after at least 40 days of restoration of euthyroidism by anti-thyroid drug therapy (methimazole) and blood pressure and number of circulating EPC were measured.

The protocol of the study was approved by the Ethics Committee of our institution (Medical School, University of Brescia). The procedures followed were in accordance with institutional guidelines.

Isolation of EPC

The number of EPC was assessed using an *in vitro* assay using a previously described technique (19, 20). In brief, peripheral blood mononuclear cells were isolated from patients blood using density gradient centrifugation with Ficoll (Amersham) and seeded 10^7 cells on 6-well plates coated with human fibronectin (Sigma) in endothelial basal medium-2 (Clonetics) supplemented with growth factors [vascular endothelial growth factor (VEGF), fibroblast growth factor-2, epidermal growth factor, IGF-I), ascorbic acid, hydrocortisone, gentamicin and 10% fetal bovine serum. After 7 days in culture, we performed fluorescent chemical detection of the attached cells using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-labeled acetylated LDL (acLDL-DiI) (2.4 μg/ml; Molecular Probes) and fluorescein isothiocyanate-labeled Ulex europaeus agglutinin-1 (UEA-1) (10 μg/ml; Sigma) for cell staining. Samples were viewed with an inverted fluorescent microscope and counted (cell number/10^7 plated cells per 1.17 mm^2 of area). EPC were identified by positive double staining for both UEA-1 and acLDL-DiI. 4',6-diamidino-2-phenylindole (DAPI) were used to stain the nuclei.

Evaluation of circulating inflammatory markers and oxidative stress

Circulating levels of C reactive protein (CRP) (Bender MedSystem), total AOP (Oxford Biomedical Research), proinflammatory cytokines IL-6 and monocyte chemoattractant protein-1 (MCP-1), TNF-α, and IL-18, plasminogen activator inhibitor-1 (PAI-1), sVCAM, and sICAM (Bender MedSystem) have been measured in serum or plasma by enzyme-linked immunosorbent assay technique following the directions of the supplier company.

Statistical analysis

Results are expressed as mean±SEM. Comparison of continuous variables in the clinical study was performed by Student t-test and one-way analysis of variance. Relationships between different variables were assessed by calculating Pearson's correlation coefficient (r). The statistical significance was set at the conventional level of 5%.

RESULTS

Characteristics of patients are shown in Table 1. No differences in smoking habits, body mass index and history of dyslipidemia were observed between the groups. Patients with active GD presented, as expected, a marked increase in free T_3 (fT_3) and free T_4 (fT_4) values and subsequent suppression of TSH (fT_3: 17.7±2.0 pg/ml; fT_4: 31.4±2.9 pg/ml; TSH<0.01 mU/l) as well as positive anti-TSH receptor antibody titers (3.01±0.24 IU/l; normal values <1 IU/l). The thyroid ultrasound evaluation confirmed the diagnosis of GD showing thyroid hypoechogenicity and a widespread and marked increase in vascularization during color-doppler examination, considered a distinctive sign of the disease (21). Systolic blood pressure (SBP) values were significantly higher in patients with active GD, compared with control subjects, while a small, not statistically significant difference in diastolic blood pressure was observed between the 2 groups (Table 1). Patients with untreated GD showed a significantly higher heart rate compared with control subjects (Table 1). After restoration of euthyroidism, GD patients showed a reduction, in SBP, although the difference did not reach statistical significance, whereas heart rate was similar compared with controls (Table 1).

Patients with active GD had a significant reduction in the

Table 1 - *The table represents characteristics of control subjects and of patients with Graves' disease (GD).*

	Control subjects	Hyperthyroid GD patients	Euthyroid GD patients
No.	18	23	7
Gender (M/F)	7/11	5/18	2/5
Age (yr)	34.4±2.0	37.4±2.2	32.7±4.7
SBP (mmHg)	122.1±3	138.7±4[a]	122.0±7.0
DBP (mmHg)	76.9±2	82.4±2	79.0±3.8
HR (bpm)	70±1	88±2[c]	74±4[d]
fT_3 (pg/ml)	-	16.2±1.6[c]	-
fT_4 (pg/ml)	12.78±0.19	31.1±2.6[b]	8.4±1.05[d]
TSH (mU/l)	1.6±0.07	<0.01[c]	2.2±0.40[d]
TRAB (IU/l)	-	3.01±0.24	-

M: male; F: female; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; TRAB: anti-TSH receptor antibodies (normal values <1 IU/l). Data are presented as means±SEM. [a]p<0.01, [b]p<0.05, [c]p<0.001 compared to control subjects, [d]p<0.05 compared to euthyroid GD patients.

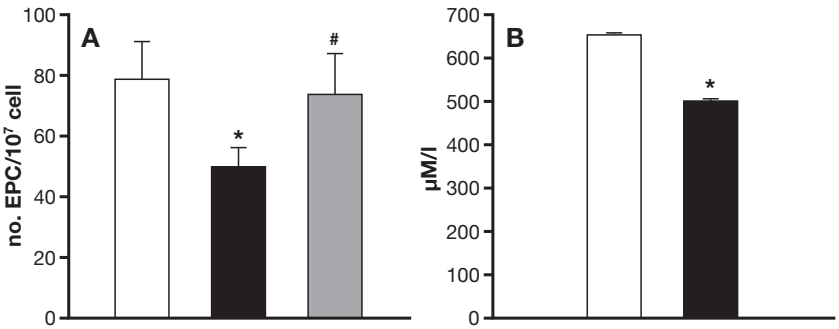

Fig. 1 - *Endothelial progenitor cells (EPC) number in Graves' disease (GD). A) Absolute number of EPC in patients with active GD (black bar) compared to controls (white bar), and to patients with GD after restoration of euthyroidism by methimazole (gray bar). EPC are expressed as absolute number of EPC/10^7 plated cells per 1.17 mm^2 of area. Right panel (B): plasma total antioxidant power in patients with active GD (black bars) compared to controls (white bar). Data are presented as means±SEM. *p<0.05 compared with controls, #p<0.05 compared with patients with active GD.*

number of EPC identified by immunofluorescence after 7 days of culture ($p<0.05$) (Fig. 1 and 2) which turned toward control values after restoration of euthyroidism by methimazole ($p<0.05$ *vs* GD, p=ns *vs* controls).
In addition, patients with untreated GD showed a significant increase in circulating levels of IL-18 and sVCAM-1 compared with controls, while no difference were observed in CRP, PAI-1, IL-6, and MCP-1 values (Table 2). The plasma total antioxidant power was significantly reduced in patients with active GD (Fig. 1, Table 2). No correlation was observed between the values of fT_3 and fT_4 and the EPC number in patients with GD.

DISCUSSION

Our study demonstrated, for the first time, that patients with untreated GD, compared with controls, show a reduced number of EPC isolated from venous peripheral blood after 7 days of culture. The reduction in EPC is associated with an increase of SBP and of circulating IL-18 and sVCAM-1 levels and with a significant reduction of plasma total antioxidant power. After restoration of euthyroidism by anti-thyroid drug therapy (methimazole) circulating EPC number in GD patients was similar to controls as well as heart rate and SBP, albeit SBP was not significantly different from untreated GD.
Hyperthyroidism is commonly associated with isolated systolic hypertension and is characterized by an hyperkinetic status. It has been suggested that vascular endothelium is a specific target of thyroid hormones; in hyperthyroidism, increase of endothelial hypersensitivity to vasodilator stimuli contributes to reduced peripheral vascular resistance and the maintenance of a hyperkinetic state (22) with consequent increase in shear stress (23). Shear stress induces vasodilatation through an increased release of nitric oxide production; however, when chronically or excessively stimulated, it may also promote endothelial activation and damage (24). Accordingly, increased plasma concentrations of von Willebrand Factor (vWF), which is an indicator of endothelial damage and dysfunction (25, 26), were reported in patients with hyperthyroidism, and were demonstrated to decrease below normal limits in patients who became euthyroid after anti-thyroid treatment (8). Increased circulating levels of vWF may be observed already in patients with subclinical hyperthyroidism (10).
Recently, it has been suggested that the number of EPC may represent an indicator of the presence of vascular injury and also an expression of reparative mechanisms and vascular protection, which are of primary importance for the integrity of the endothelium. EPC have been identified as $CD34^+$ mononuclear cells taken from peripheral blood that have the ability *in vitro* to differentiate into cells with endothelial phenotype and to participate to neovascularization in ischemic tissues (11, 12). EPC number can be reduced in the presence of cardiovascular risk factors such as smoke, hypercholesterolemia, and high blood pressure (14). In patients with stable coronary artery disease, essential hypertension is associated with an impaired ability of *in vitro* EPC migration (13). However, at present, evidences about alterations in EPC number or functional activity in essential hypertension are still controversial (27, 28). In addition, EPC number has been previously demonstrated to be related to other indica-

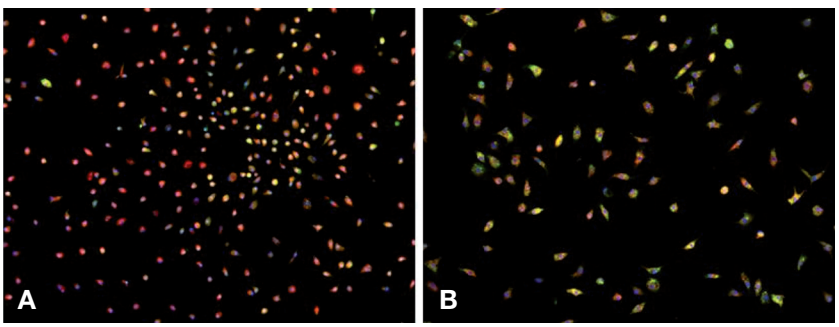

Fig. 2 - *Representative image of endothelial progenitor cells (EPC). Representative image of EPC in normothyroid control subjects (panel A) and patients with active Graves' disease (panel B). EPC were identified by positive double staining for both fluorescein isothiocyanate-labeled Ulex europaeus agglutinin I (green fluorescence) and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-labeled acethylated LDL (red fluorescence). 4',6-diamidino-2-phenylindole (DAPI) were used to stain the nuclei (blue fluorescence). p<0.05 between groups. See Method section for more details.*

Table 2 - *The table shows circulating levels of total antioxidant power (AOP), C reactive protein (CRP), pro-inflammatory cytokines [monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor α (TNF-α), interleukin (IL)-6 and IL-18], plasminogen activator inhibitor-1 (PAI-1) and adhesion molecules [soluble vascular cellular adhesion molecule-1 (sVCAM-1), intracellular adhesion molecule-1 (sICAM-1)] in control subjects and in patients with Graves' disease.*

	AOP (μMol/l)	MCP-1 (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)	IL-18 (pg/ml)	sICAM-1 (ng/ml)	sVCAM-1 (ng/ml)	PAI-1 (ng/ml)	CRP (μg/ml)
Control subjects	654.89±3.55	446.32±4.62	0.19±0.29	3.15±0.49	891.21±7.18	224.10±2.60	617.93±6.48	114.27±1.92	0.538±0.420
Graves' disease	500.79±3.30[a]	407.62±3.53	0.48±0.33	3.44±0.42	2353.58±10.99[a]	254.40±2.73	1105.83±6.28[a]	117.02±1.67	0.480±0.229

[a]$p<0.05$ *vs* controls.

tors of endothelial damage, such as vWF circulating levels (29), flow-mediated dilatation of the brachial artery (15) as well as to adverse clinical events (16, 17), suggesting that endothelial injury in the absence of sufficient repair by circulating EPC promotes the progression of vascular disease.

In our study, the number of EPC was reduced in patients with active GD compared with control subjects and it turned to control values after restoration of euthyroidism. Therefore, it cannot be ruled out that the reduction of EPC number observed in patients with untreated GD may be influenced by the presence of higher blood pressure values in these patients. However, the reduction in the number of EPC in patients with active GD might also depend on other factors and recognize several mechanisms. Besides thyroid hormones' direct effects on the heart and vascular system, it has been reported that RAS acts as mediator in some of the cardiovascular effects of thyroid hormones. Indeed, a tissue-specific modulation of ACE was identified in experimental hyperthyroidism (with the demonstration of increased serum, pulmonary, and renal ACE levels, and reduced levels in the heart) (30). Thyroid hormones activate some components of cardiac RAS inducing hypertrophy and fibrosis which may be attenuated or prevented by RAS inhibition (31). In addition, in hyperthyroidism, a resetting of the pressure-natriuresis relationship is present, in relation to activation of the RAS, which, in addition to its pressure effects (3, 5), may lead to development and progression of vascular damage.

The effects of RAS are partly mediated by its pleiotropic activities, including cytokines release and increased expression of pro-inflammatory transcription factors such as nuclear factor-κB which, in turn, regulate the expression of adhesion molecules such as VCAM-1 and ICAM-1 (32). In particular, it has been previously shown that angiotensin II increases the proliferation of EPC-mediated VEGF, but also accelerates EPC senescence through an increase in oxidative stress (33), thus leading to impairment of endothelial repair mechanisms which may indeed be restored by angiotensin II receptor antagonists, such as irbesartan and olmesartan (17). In addition, an activation of the sympathetic nervous system by thyroid hormones has also been reported (5). In this regard, in our study we observed a significant increase in heart rate in patients with active GD compared to control subjects which turned to control values after restoration of euthyroidism.

Indeed sensitivity to catecholamines is enhanced in hyperthyroidism (22). Most importantly, in hyperthyroid - ism, an increased oxidative stress has been demonstrated (6) which, together with the activation of inflammatory processes, is one of the main pathophysiological mechanisms involved in the clinical manifestations of GD. Stimulation of energy metabolism by thyroid hormones implies an enhanced generation of ROS in target tissues, which determines a greater consumption of cellular antioxidants and inactivation of enzymatic mechanisms providing antioxidant protection, thus inducing oxidative stress (6). In fact, in patients with hyperthyroidism and with untreated GD, a significant increase in circulating lipid peroxidation activity indices has been described (34), which are suppressed by propylthiouracil treatment (35). In addition, T_3 administration induces an increase in ROS generation by mononuclear cells (36). This pro-oxidant condition has been shown both in experimental animals and in man, and it has been associated with cellular dysfunctions in several target tissues (5). An increase in oxidative stress has been observed also in hypothyroidism, even if subclinical, due to both an impaired antioxidant status and the altered lipid metabolism (37).

Furthermore, GD is an inflammatory condition with the production of acute-phase proteins and several proinflammatory cytokines, including IL-12, IL-18, and TNF-α (7). High levels of adhesion molecules such as sICAM-1 have been observed in sera from GD patients (16, 38). In this regard, we observed a significant increase in the levels of oxidative stress (as expressed by the reduction of plasma total antioxidant power) as well as an increased in circulating proinflammatory markers IL-18 and sVCAM-1 in patients with active GD compared with control subjects, whereas no significant differences were observed in sICAM-1 and IL-6 levels between the 2 groups of patients. In accordance with another study, serum CRP levels were not found to be significantly affected by the degree of thyroid dysfunction (39).

It is also possible that, in our study, the presence of autoimmune disease (hallmark of GD) could have a crucial role in the increased oxidative stress and inflammation. Presumably, oxidative stress and inflammation could directly influence the mobilization or half-life of EPC. In particular, it has been suggested that cytokines, growth factors, and other proinflammatory elements, which are involved in the vascular repair process originating from the marrow, may lead to progressive amplification of inflammatory reactions and of vascular damage in presence of incompetent blood marrow (40). Therefore, it is possible that the coexistence of several factors, such as high blood pressure and thyroid dysfunction, resulting in the amplification of the mechanisms involved in vascular damage, may play a role in the observed decrease in EPC number.

In conclusion, patients with untreated GD show a reduc-

tion in the number of EPC, thus meaning the reduction of a protective physiological mechanisms against endothelial damage, probably caused by an increase of inflammation and oxidative stress. Indeed, the regression toward normal thyroid function is associated to a normalization of the number of EPC. Further studies are needed in order to establish whether these alterations may be present also in other hyperthyroid conditions.

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Disclosure statement

The Authors have nothing to disclose.

REFERENCES

1. Weetman AP. Graves' disease. N Engl J Med 2000, 343: 1236-48.
2. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endocr Rev 1998, 19: 673-716.
3. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. N Engl J Med 2001, 344: 501-9.
4. Biondi B, Palmieri EA, Lombardi G, Fazio S. Effects of thyroid hormone on cardiac function: the relative importance of heart rate, loading conditions, and myocardial contractility in the regulation of cardiac performance in human hyperthyroidism. J Clin Endocrinol Metab 2002, 87: 968-74.
5. Vargas F, Moreno JM, Rodríguez-Gómez I, et al. Vascular and renal function in experimental thyroid disorders. Eur J Endocrinol 2006, 154: 197-212.
6. Venditti P, Di Meo S. Thyroid hormone-induced oxidative stress. Cell Mol Life Sci 2006, 63: 414-34.
7. Miyauchi S, Matsuura B, Onji M. Increased levels of serum interleukin-18 in Graves' disease. Thyroid 2000, 10: 815-9.
8. Rogers JS 2nd, Shane SR, Jencks FS. Factor VIII activity and thyroid function. Ann Intern Med 1982, 97: 713-6.
9. Baumgartner-Parzer SM, Wagner L, Reining G, et al. Increase by tri-iodothyronine of endothelin-1, fibronectin and von Willebrand factor in cultured endothelial cells. J Endocrinol 1997, 154: 231-9.
10. Coban E, Aydemir M, Yazicioglu G, Ozdogan M. Endothelial dysfunction in subjects with subclinical hyperthyroidism. J Endocrinol Invest 2006, 29: 197-200.
11. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997, 275: 964-7.
12. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999, 85: 221-8.
13. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001, 89: E1-7.
14. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003, 348: 593-600.
15. Quyyumi AA. Circulating endothelial progenitor cells as novel biological determinants of vascular function and risk. Can J Cardiol 2004, 20 (Suppl B): 44B-8B.
16. Schmidt-Lucke C, Rossig L, Fichtlscherer S, et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. Circulation 2005, 111: 2981-7.
17. Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005, 353: 999-1007.
18. European Society of Hypertension-European Society of Cardiology Guidelines Committee. European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. J Hypertens 2003, 21: 1011-53.
19. Dimmeler S, Aicher A, Vasa M, et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J Clin Invest 2001, 108: 391-7.
20. Bahlmann FH, de Groot K, Mueller O, Hertel B, Haller H, Fliser D. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonist. Hypertension 2005, 45: 526-9.
21. Cappelli C, Pirola I, De Martino E, et al. The role of imaging in Graves' disease: a cost-effectiveness analysis. Eur J Radiol 2008, 65: 99-103.
22. Napoli R, Biondi B, Guardasole V, et al. Impact of hyperthyroidism and its correction on vascular reactivity in humans. Circulation 2001, 104: 3076-80.
23. Davies PF. Flow-mediated endothelial mechanotransduction. Physiol Rev 1995, 75: 519-60.
24. Cunninghan K, Gotlieb A. The role of shear stress in the pathogenesis of atherosclerosis. Lab Invest 2005, 85: 9-23.
25. Jensen T, Bjerre-Knudsen J, Feldt-Rasmussen B, Deckert T. Features of endothelial dysfunction in early diabetic nephropathy. Lancet 1989, 1: 461-3.
26. Kario K, Matsuo T, Kobayashi H, Matsuo M, Sakata T, Miyata T. Activation of tissue factor-induced coagulation and endothelial cell dysfunction in non-insulin-dependent diabetic patients with microalbuminuria. Arterioscler Thromb Vasc Biol 1995, 15: 1114-20.
27. Delva P, Degan M, Vallerio P, et al. Endothelial progenitor cells in patients with essential hypertension. J Hypertens 2007, 25: 127-32.
28. Pirro M, Schillaci G, Menecali C, et al. Reduced number of circulating endothelial progenitors and HOXA9 expression in CD34 cells of hypertensive patients. J Hypertens 2007, 25: 2093-9.
29. Junhui Z, Xingxiang W, Guosheng F, Yunpeng S, Furong Z, Junzhu C. Reduced number and activity of circulating endothelial progenitor cells in patients with idiopathic pulmonary arterial hypertension. Respir Med 2008, 102: 1073-9.
30. Carneiro-Ramos MS, Silva VB, Santos RA, Barreto-Chaves ML. Tissue-specific modulation of angiotensin-converting enzyme (ACE) in hyperthyroidism. Peptides 2006, 27: 2942-9.
31. Barreto-Chaves ML, Carrillo-Sepúlveda MA, Carneiro-Ramos MS, Gomes DA, Diniz GP. The crosstalk between thyroid hormones and the Renin-Angiotensin System. Vascul Pharmacol 2010, 52: 166-70.
32. Touyz RM, Schiffrin EL. Signal transduction mechanism mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev 2000, 52: 639-72.
33. Imanishi T, Hano T, Nishio I. Angiotensin II accelerates endothelial progenitor cells senescence through induction of oxidative stress. J Hypertens 2005, 23: 97-104.
34. Komosinska-Vassev K, Olczyk K, Kucharz EJ, Marcisz C, Szczotka KW, Kotulska A. Free radical activity and antioxidant defense mechanisms in patients with hyperthyroidism due to Graves' disease during therapy. Clin Chim Acta 2000, 300: 107-17.
35. Videla LA, Sir T, Wolff C. Increased lipid peroxidation in hyperthyroid patients: suppression by propylthiouracil treatment. Free Radic Res Commun 1988, 5: 1-10.
36. Magsino CH Jr, Hamouda W, Ghanim H, Browne R, Aljada A, Dandona P. Effect of triiodothyronine on reactive oxygen species generation by leukocytes, indices of oxidative damage, and antioxidant reserve. Metabolism 2000, 49: 799-803.
37. Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. Clin Endocrinol (Oxf) 2009, 70: 469-74.
38. De Bellis A, Bizzarro A, Gattoni A, et al. Behaviour of soluble intercellular adhesion molecule-1 and endothelial-leukocyte adhesion molecule-1 concentrations in patients with Graves' disease with or without ophthalmopathy and in patients with toxic adenoma. J Clin Endocrinol Metab 1995, 80: 2118-21.
39. Lee WY, Suh JY, Rhee EJ, Park JS, Sung KC, Kim SW. Plasma CRP, apolipoprotein A-1, apolipoprotein B and Lpa levels according to thyroid function status. Arch Med Res 2004, 35: 540-5.
40. Dong C, Crawford LE, Goldschmidt-Clermont PJ. Endothelial progenitor obsolescence and atherosclerotic inflammation. J Am Coll Cardiol 2005, 45: 1458-60.