European Heart Journal (2001) **22,** 791–797
doi:10.1053/euhj.2000.2285, available online at http://www.idealibrary.com on IDEAL®

Physical training reduces peripheral markers of inflammation in patients with chronic heart failure

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Aims Previous studies have shown an abnormal expression of cellular adhesion molecules and cytokines in chronic heart failure, which may be related to endothelial dysfunction characterizing this syndrome. Our study investigates the effects of physical training on serum activity of some peripheral inflammatory markers associated with endothelial dysfunction, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage chemoattractant protein-1 (MCP-1), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) in patients with chronic heart failure.

Methods and Results Serum levels of GM-CSF, MCP-1, sICAM-1 and sVCAM-1 were determined in 12 patients with stable chronic heart failure (ischaemic heart failure: 6/12, dilated cardiomyopathy: 6/12, New York Heart Association: II-III, ejection fraction: $24 \pm 2\%$) before and after a 12-week programme of physical training in a randomized crossover design. In addition, the functional status of chronic heart failure patients was evaluated by using a cardiorespiratory exercise stress test to measure peak oxygen consumption. Physical training produced a significant reduction in serum GM-CSF (28 ± 2 vs 21 ± 2 pg . ml^{-1}, $P<0{\cdot}001$), MCP-1 (192 ± 5 vs 174 ± 6 pg . ml^{-1}, $P<0{\cdot}001$), sICAM-1 (367 ± 31 vs 314 ± 29 ng . ml^{-1}, $P<0{\cdot}01$) and sVCAM-1 (1247 ± 103 vs 1095 ± 100 ng . ml^{-1}, $P<0{\cdot}01$) as well as a significant increase in peak oxygen consumption ($14{\cdot}6 \pm 0{\cdot}5$ vs $16{\cdot}5 \pm 0{\cdot}5$ ml . kg^{-1} min^{-1}, $P<0{\cdot}005$). A significant correlation was found between the training-induced improvement in peak oxygen consumption and percentage reduction in soluble adhesion molecules sICAM-1 ($r = -0{\cdot}72$, $P<0{\cdot}01$) and sVCAM-1 ($r = -0{\cdot}67$, $P<0{\cdot}02$).

Conclusion Physical training affects beneficially peripheral inflammatory markers reflecting monocyte/macrophage-endothelial cell interaction. Training-induced improvement in exercise tolerance is correlated with the attenuation of the inflammatory process, indicating that inflammation may contribute significantly to the impaired exercise capacity seen in chronic heart failure.
(Eur Heart J 2001; 22: 791–797, doi:10.1053/euhj.2000.2285)

Key Words: Chronic heart failure, inflammation, endothelial dysfunction, adhesion molecules, physical training.

Introduction

Endothelial dysfunction is a key feature of chronic heart failure, contributing to enhanced peripheral vasoconstriction and impaired exercise capacity. Recent investigations have established the presence of endothelial dysfunction of peripheral resistance arteries and an impaired flow-dependent, endothelium-mediated dilation of conduit arteries in patients with chronic heart failure[1–3].

Immunological and inflammatory responses appear to play a pathogenic role in the development and progression of chronic heart failure[4]. Increased circulating levels of chemotactic cytokines (C-C chemokines), such as macrophage chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α), have been recently found in chronic heart failure. These chemokines are potent chemoattractants of monocytes and lymphocytes, with particularly high concentrations in those with the most severe heart failure[5]. Also,

Revision submitted 11 May 2000, and accepted 17 May 2000.

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0195-668X/01/090791+07 $35.00/0

human monocyte-endothelial cell interaction induces synthesis of granulocyte-macrophage colony-stimulating factor (GM-CSF), which plays an important role in the pathogenesis of atherosclerosis and inflammation by stimulating a range of functional activities of mature neutrophils, monocytes and eosinophils, including generation of free radicals and induction of cytokine production[6–8]. Cytokines and oxygen free radicals induce the expression of endothelial adhesion molecules such as intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1)[9]. These adhesion molecules are cleaved into the circulation by activated endothelial cells and can be measured as 'soluble adhesion molecules' (sICAM-1 and sVCAM-1)[10]. There is growing evidence that chronic heart failure is associated with elevated soluble adhesion molecules which may represent a marker of endothelial activation or damage[11].

Physical training programmes improve both basal endothelial nitric oxide formation and agonist-mediated endothelium-dependent vasodilation of the skeletal muscle vasculature in patients with chronic heart failure, probably because of endothelial relaxing factors released in response to cell membrane shear stress induced by pulsatile blood flow[12,13].

To our knowledge, no in vivo data exist regarding the effects of physical training on peripheral inflammatory markers associated with endothelial dysfunction in patients with chronic heart failure. We therefore sought to investigate the effects of a physical training programme on some indices representative of inflammation (with particular emphasis on GM-CSF described for the first time in chronic heart failure) and possibly involved in monocyte–endothelial cell interaction, as well as to examine their role in impaired exercise tolerance characterizing chronic heart failure. These peripheral markers may represent an alternative non-invasive approach to describe endothelial cell activity in conditions of impaired endothelial function.

Methods

Study population

All subjects gave informed consent for this trial, which was approved by the Local Research Ethics Committee. Twelve patients with moderate to severe chronic heart failure (age: 59·6 ± 2 years, New York Heart Association: class II-III; ejection fraction: 24 ± 2%) were included in the study and compared with ten age–sex matched healthy control subjects who undertook regular physical activity. Inclusion criteria were stable chronic heart failure of at least 3 months' duration; ischaemic aetiology (six patients, as evidenced by documented myocardial infarction and/or coronary arteriography and coronary artery bypass surgery) and idiopathic dilated cardiomyopathy (six patients); limitation of exercise by dyspnoea or fatigue only and ability to achieve a respiratory exchange ratio of at least unity; absence of Holter monitoring evidence of ventricular tachycardias or other serious arrhythmias. Patients with infections, malignancies, collagen or other inflammatory diseases as well as patients taking antiinflammatory or immunosuppressive agents for the last 2 weeks were excluded from the study. After two initial visits to the hospital to acquire baseline measurements and to be familiarized with the laboratory environment, patients underwent a 12-week home-based bicycle exercise training programme or were asked to avoid exercise (detraining) in a randomized crossover design. The training programme consisted of 30 min exercise 5 days per week and patients were instructed to exercise at 50 rpm in order to keep their continuously monitored heart rate in the range of 70%–80% of their previously determined maximal heart rate. All patients were on angiotensin-converting enzyme inhibitors, whereas no patient was on angiotensin II receptor type I blockers. Baseline medication remained unchanged throughout the entire study. Detailed medication, demographic and clinical characteristics as well as individual values of the various inflammatory parameters of the patients are shown in Table 1.

Laboratory measurements

Serum was obtained before and after training by centrifugation of vacutainer gel clotter tubes at 3000 rpm for 10 min. All serum samples were stored at − 70 °C until analysis, and samples were assayed in duplicate for sICAM-1, sVCAM-1, GM-CSF and MCP-1 concentrations using commercially available enzyme-linked immunosorbent assay kits. For all samples we used the R&D Systems kits that have the following sandwich ELISA format: microtitre plates already pre-coated with a murine monoclonal antibody against the human adhesion molecule being measured. Standards of the analyte and plasma samples-in-duplicate were added, along with another antibody against another epitope of the analyte conjugated to horse radish peroxidase. To test the validity of the assays, we also used a control serum that contains known concentrations of all four molecules. The samples were incubated for 1·5 h at room temperature. The wells were then washed and the chromogen tetramethyl benzidine was added and incubated for 30 min in the dark. After addition of 2 N H_2SO4, the optical densities at 450 nm (reference filter 620 nm) were read and standard curves were plotted in an Organon Technika 530 microplate reader.

A cardiopulmonary exercise stress test was performed to evaluate exercise capacity by measuring peak oxygen consumption (VO_2max). A 2-D echocardiogram was used to estimate ejection fraction according to the modified Simpson's formula. All tests were performed before daily medication had been taken and were conducted by a 'blinded' observer.

Table 1 Demographic and clinical characteristics as well as individual values of the various inflammatory parameters of the patients at baseline

Patients	Drugs	Age (years)	Aetiology	EF (%)	VO_2max (ml . kg^{-1} min^{-1})	GM-CSF (pg . ml^{-1})	MCP-1 (pg . ml^{-1})	sICAM-1 (ng . ml^{-1})	sVCAM-1 (ng . ml^{-1})
1	D; I	65	ICM	27	13·8	29·3	150	462	1598
2	D; I; Dig	61	ICM	16	14·0	20·9	186	367	1279
3	D; I; A	57	DCM	30	17·1	34·0	219	276	1132
4	D; I	50	DCM	35	15·4	31·4	178	480	1626
5	D; I	68	ICM	21	12·4	31·5	189	567	1947
6	D; I; Dig	59	DCM	28	15·0	26·6	202	237	963
7	D; I	63	ICM	14	13·1	22·4	203	408	1444
8	D; I	70	DCM	19	16·6	39·7	209	180	764
9	D; I	44	DCM	29	14·1	26·1	200	362	941
10	D; I; Dig	64	DCM	20	12·2	20·4	211	339	1290
11	D; I; A	60	ICM	25	13·6	36·0	218	372	1133
12	D; I	54	ICM	23	16·8	28·0	228	248	1054

EF=ejection fraction; VO_2max=peak oxygen uptake; GM-CSF=granulocyte-macrophage colony-stimulating factor; MCP-1=macrophage chemoattractant protein-1; sICAM-1=soluble intercellular adhesion molecule-1; sVCAM-1=soluble vascular cell adhesion molecule-1; D=diuretics; I= angiotensin converting enzyme inhibitors; A=antiarrhythmics; ICM=ischaemic cardiomyopathy; DCM= dilated cardiomyopathy.

Statistical analysis

Statistical analysis was carried out according to the recommendations of Hills and Armitage for crossover trials[14]. Comparison of values of the inflammatory markers and peak oxygen consumption at baseline, after training and after detraining was conducted by using analysis of variance (ANOVA) for repeated measures followed by Scheffe's procedure for post hoc comparisons of means. Simple regression analysis was performed to describe the relationship between the various peripheral inflammatory markers. Simple regression analysis was also used to examine the relationship between the training-induced changes in inflammatory factors and peak oxygen consumption. A value of $P<0·05$ was accepted as statistically significant. Results are expressed as mean ± SE.

Results

We demonstrated that physical training induced a significant reduction (ANOVA, $P<0·001$) in peripheral inflammatory markers compared with the detraining period: a significant decrease was, thus, observed with physical training in inflammatory factors GM-CSF (28 ± 2 vs 21 ± 2 pg . ml^{-1}, $P<0·001$) and MCP-1 (192 ± 5 vs 174 ± 6 pg . ml^{-1}, $P<0·001$), both involved in monocyte/macrophage endothelial cell interaction and possibly contributing to endothelial dysfunction in chronic heart failure (Fig. 1(a) and (b) respectively), and in soluble adhesion molecules sICAM-1 (367 ± 31 vs 314 ± 29 ng . ml^{-1}, $P<0·01$) and sVCAM-1 (1247 ± 103 vs 1095 ± 100 ng . ml^{-1}, $P<0·01$) both reflecting inflammation-induced activation of endothelial cells (Fig. 2(a) and (b), respectively). Exercise performance, expressed by VO_2max, improved with the exercise training programme ($14·6 \pm 0·5$ vs $16·5 \pm 0·5$ ml . kg^{-1} min^{-1}, $P<0·005$). A significant correlation was found between the training induced improvement in VO_2max and the percentage reduction in soluble adhesion molecules sICAM-1 ($r=-0·72$, $P<0·01$) and sVCAM-1 ($r=-0·67$, $P<0·02$), indicating that the attenuation of inflammatory activation may contribute to the improvement in exercise capacity achieved with physical training in patients with chronic heart failure (Fig. 3(a) and (b), respectively).

There was also a significant correlation between chemokine MCP-1 and adhesion molecules sICAM-1 ($r=0·6$, $P<0·05$) and sVCAM-1 ($r=0·7$, $P<0·02$) at baseline, suggesting an important role of chemokines in inducing adhesion molecules on the endothelial surface in patients with chronic heart failure.

As with the detraining period, physical training produced a significant reduction (ANOVA, $P<0·001$) in peripheral markers of inflammation compared with baseline, whereas no difference was found between baseline and detraining values of exercise performance ($14·5 \pm 0·5$ vs $14·6 \pm 0·5$ ml . kg^{-1} min^{-1} for VO_2max) or the various inflammatory factors (29 ± 2 vs 28 ± 2 pg . ml^{-1} for GM-CSF, 199 ± 6 vs 192 ± 5 pg . ml^{-1} for MCP-1, 358 ± 32 vs 367 ± 31 ng . ml^{-1} for sICAM-1, 1264 ± 98 vs 1247 ± 103 ng . ml^{-1} for sVCAM-1, baseline and detraining respectively, Figs 1 and 2).

No difference was detected in either baseline or training-induced changes in peripheral inflammatory markers between patients with ischaemic and dilated cardiomyopathy (data not shown).

Peripheral markers were significantly higher ($P<0·001$) in patients with chronic heart failure at baseline than in normal control subjects who undertook regular physical activity (29 ± 2 vs $3·1 \pm 0·2$ pg . ml^{-1} for GM-CSF, 199 ± 6 vs 118 ± 5 pg . ml^{-1} for MCP-1, 358 ± 32 vs 177 ± 7 ng . ml^{-1} for sICAM-1, 1264 ± 98 vs 583 ± 10 ng . ml^{-1} for sVCAM-1). Despite the

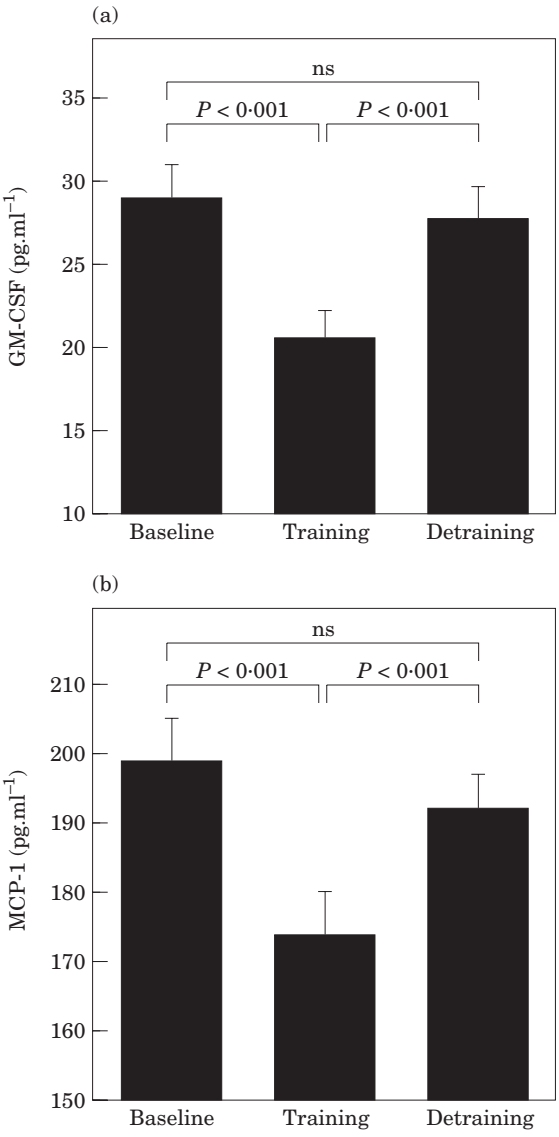

Figure 1 **Effects of physical training on inflammatory markers granulocyte–macrophage colony-stimulating factor (GM-CSF) and macrophage chemoattractant protein-1 (MCP-1). Note the significant reduction of GM-CSF (a) and MCP-1 (b) with exercise training.**

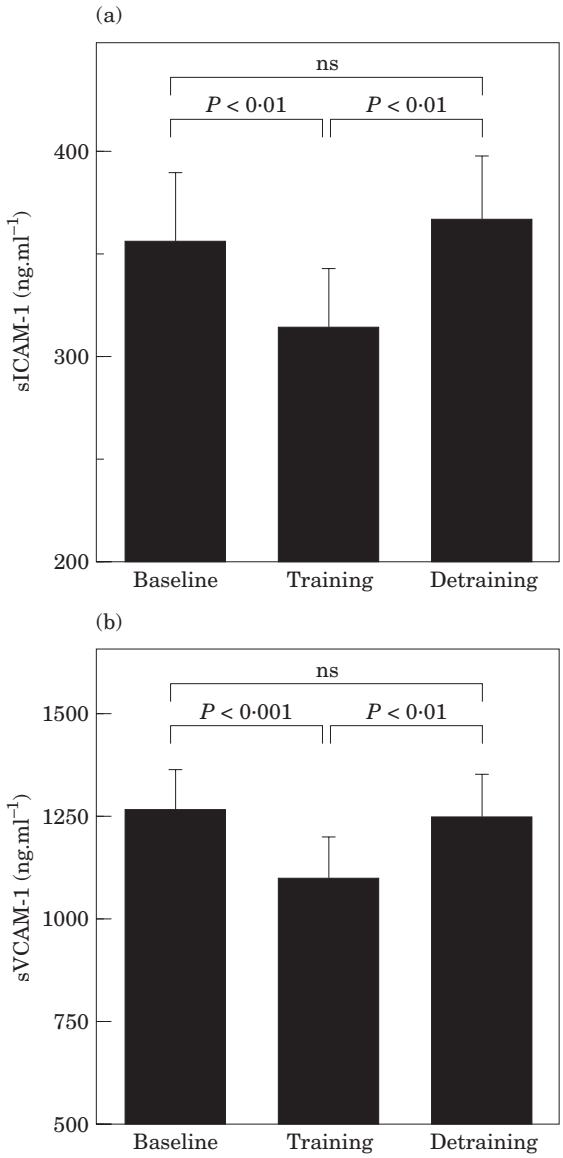

Figure 2 **Effects of physical training on soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1). Note the significant reduction of sICAM-1 (a) and sVCAM-1 (b) with exercise training.**

significant reduction in inflammatory indices with training, values of these parameters were still higher ($P<0{\cdot}001$) when compared with the control subjects.

Discussion

Accumulating data suggest that immunological and inflammatory responses appear to be important factors in the development and progression of the syndrome of chronic heart failure. Recent investigations suggest that proinflammatory cytokines (tumour necrosis factor alpha and interleukin-1) are capable of modulating cardiac and peripheral vascular functions, by a variety of mechanisms including production of oxygen free radicals and apoptosis[15,16]. Activation of leukocytes and migration of these cells from the circulation to areas of inflammation may play a significant role in immunological responses in chronic heart failure[17].

It is becoming apparent that the vascular endothelium has a pivotal role in orchestrating the behaviour of the peripheral circulation and, therefore, in determining tissue perfusion. Under basal conditions the endothelium continuously releases endothelium-derived relaxing factors, thus providing a constant force to counteract vasoconstrictor substances including noradrenaline, angiotensin II and endothelins[18]. Endothelium-derived relaxing factors, the biological

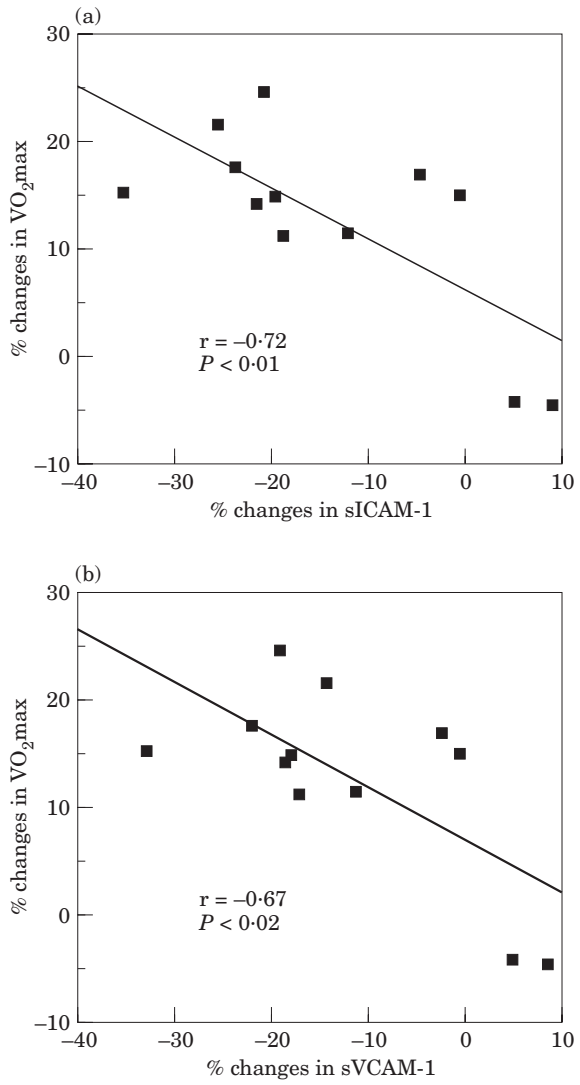

Figure 3 **Correlation between training-induced improvement in exercise tolerance, assessed by peak oxygen consumption (VO_2max), and percentage changes in soluble forms of intercellular adhesion molecule-1 (sICAM-1, (a)) and vascular cell adhesion molecule-1 (sVACM-1 (b)).**

activity of which is provided by nitric oxide, can also be released in response to either endocrine mediators, such as acetylcholine and bradykinin or as a result of physical stimulation, such as changes in blood flow velocity and endothelial shear stress, reflecting flow-dependent, endothelium-mediated vasodilation[19]. Experimental and clinical data have also demonstrated decreased vasodilation in response to acetylcholine in epicardial coronary arteries in idiopathic dilated and ischaemic cardiomyopathy[20,21]. The blunted endothelium-mediated vasodilation, related to the severity of chronic heart failure and independent of the underlying aetiology, could be attributed to impaired intracellular availability of the precursor of nitric oxide L-arginine, decreased expression of nitric oxide synthase, impaired receptor-mediated release of nitric oxide in response to pharmacological or mechanical stimuli, or increased degradation of nitric oxide as a result of increased endothelial and/or vascular smooth muscle production of oxygen free radicals[22].

Programmes of physical training, by causing chronic, pulsatile increases in peripheral blood flow, affect the release of prostaglandins in skeletal muscle microvasculature[23], induce the expression of nitric oxide synthase and cytosolic superoxide dismutase, a free radical scavenger[24], and enhance Ca^{2+} influx in endothelial cells, which is necessary for both nitric oxide and prostaglandin synthesis[25].

Our study provides insight into the pathophysiological mechanisms underlying the beneficial effects of physical training on exercise performance. We are the first, to our knowledge, to demonstrate that an exercise training programme intervenes with the various stages of inflammatory processes in patients with chronic heart failure. This is done by reducing the growth factor GM-CSF, which differentiates and proliferates myeloid progenitor cells, the chemoattractant cytokine MCP-1, which represents an important signal for the accumulation of mononuclear leukocytes in the endothelial cells and the adhesion molecules sICAM-1 and sVCAM-1, which are expressed on activated endothelial cells as a result of the monocyte–endothelial cell adhesive interaction. In particular, training produced a significant reduction in GM-CSF, a glycoprotein that, in addition to its growth promoting effects on myelopoietic progenitor cells, stimulates a constellation of peripheral activities, such as regulation of leukocyte adhesion, generation of superoxide anions and induction of cytokine production[6,7]. GM-CSF is also produced locally by monocyte–endothelial cell interaction, further contributing to the recruitment of monocytes/macrophages and their proliferation in activated endothelial cells[8]. It is therefore likely that GM-CSF, which we show for the first time to be elevated in patients with chronic heart failure, may contribute to the pathophysiological events involved in inflammation and associated with endothelial dysfunction, an important characteristic of this syndrome. Proinflammatory cytokines (e.g. macrophage-derived tumour necrosis factor alpha) may cause endothelial dysfunction in chronic heart failure, either by increasing the production of oxygen free radicals that, in turn, decrease nitric oxide synthase mRNA half-life and/or destroy the nitric oxide produced by the endothelium, or by triggering apoptosis in endothelial cells through oxidative stress[26]. GM-CSF, by virtue of its characteristic of modulating monocyte/macrophage function in vivo and subsequently of generating free radicals and enhancing cytokine production, may represent the common denominator of the pathophysiological sequelae leading to reduced nitric oxide synthase expression and increased nitric oxide synthase deactivation in patients with chronic heart failure. Training-induced improvement in endothelial function has recently been attributed to improvement in endothelial nitric oxide formation, both basal and agonist-mediated[13]. By therefore

attenuating the effects of GM-CSF with a physical training programme we may interfere beneficially with the inflammatory process affecting endothelial function, and contribute to the improvement in functional work capacity of patients with chronic heart failure.

Chemotactic cytokines may represent not only a 'new' parameter of enhanced immune activation in chronic heart failure but may also reflect an important pathogenic mechanism in the development of this syndrome. Thus, increased MCP-1 secretion from endothelial and smooth muscle cells may lead to infiltration of monocytes into the arterial wall and generation of reactive oxygen species in monocytes, which may be involved in the pathogenesis of atherosclerosis[27] and increased apoptosis of cardiomyocytes and endothelial cells observed in patients with chronic heart failure[28–30]. A good correlation between MCP-1 and the adhesion molecules sICAM-1 and sVCAM-1 confirms the role of chemokines in attracting macrophages in inflammatory areas and subsequently activating endothelial cells, on the surface of which adhesion molecules are expressed. Training, by reducing MCP-1, inactivates, at least partially, an important chemoattractant signal which seems to play a role in the recruitment and activation of monocytes/macrophages and migration to areas of inflammation in the arterial wall of patients with chronic heart failure. This finding further supports the notion that immunological and inflammatory responses are important pathophysiological features in chronic heart failure and that training may exert beneficial effects on exercise performance by modifying the inflammatory status of patients with this syndrome and possibly reversing inflammation-induced endothelial dysfunction. Creatine levels and clearance remained within normal limits throughout the study and, therefore (a) the increased levels of GM-CSF and MCP-1 at baseline are not due to renal dysfunction and (b) the training-induced beneficial changes in these factors cannot be attributed to alterations in renal function.

Finally, training significantly reduced soluble adhesion molecules ICAM-1 and VCAM-1, which may be characterized as the 'end products' of the interaction between activated monocytes/macrophages and endothelial cells. Thus, training reverses, in part, a broad spectrum of serum monocyte–endothelial cell adhesive interaction markers. In parallel, in the present study changes in exercise tolerance were significantly correlated with changes in soluble adhesion molecules ICAM-1 and VCAM-1, indicating that peripheral inflammation may underlie the impaired exercise capacity in patients with chronic heart failure and that training-induced improvement in exercise tolerance may be attributed to the attenuation of the peripheral inflammatory process, possibly via reversing the deleterious effects of endothelial dysfunction. It should, however, be emphasized that the correlation between changes in peak oxygen consumption and plasma concentration of adhesion molecules does not establish a cause–effect relationship. Other mechanisms unrelated to endothelial dysfunction and blood flow, such as neurohormonal derangements and intrinsic skeletal muscle metabolic abnormalities[31], have been proposed to explain exercise intolerance, seen in chronic heart failure, and to mediate the beneficial effects of exercise training.

Plasma levels of adhesion molecule sVCAM-1 were significantly decreased after long-term ACE inhibition, which was associated with a beneficial long-term effect on endothelium-dependent muscarinic receptor mediated vasodilation[11]. Plasma levels of adhesion molecule sICAM-1 and endothelin-1 increased with the severity of chronic heart failure and may, therefore, provide additional prognostic information[32]. Measurements of adhesion molecules (inflammatory indices expressed on activated endothelial cells) may offer an easy, non-invasive way to assess the clinical severity of the syndrome and to evaluate the severity of endothelial dysfunction and its modification with various pharmacological and non-pharmacological interventions in patients with chronic heart failure.

Clinical implications

This study throws more light onto the mechanisms underlying peripheral abnormalities of patients with chronic heart failure, suggesting that inflammatory activation may contribute to impaired exercise capacity characterizing this syndrome, possibly by causing or enhancing endothelial dysfunction. In our study, only indirect measurements concerning inflammation were made and our data can neither prove a direct action of the inflammatory parameters on the endothelial function nor can they exclude the possibility that other up- or downstream immune molecules are responsible for the observed effects.

Furthermore, the good correlation between training-induced changes in exercise capacity and soluble adhesion molecules indicate that exercise training may exert its beneficial effects, at least in part, by modifying the inflammatory status associated with heart failure. In addition, these peripheral inflammatory markers, reflecting monocyte–endothelial cell adhesive interaction, may provide a simple way for longitudinal description of the degree of endothelial dysfunction in chronic heart failure.

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