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Physical Training Modulates Proinflammatory Cytokines and the Soluble Fas/Soluble Fas Ligand System in Patients With Chronic Heart Failure

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OBJECTIVES We sought to investigate the effects of physical training on circulating proinflammatory cytokines and the soluble apoptosis mediators Fas (sFas) and Fas ligand (sFasL) in patients with chronic heart failure (CHF).

BACKGROUND Recent investigations have shown an overexpression of circulating proinflammatory cytokines and soluble apoptosis mediators in patients with CHF, which may be related to their exercise intolerance and clinical deterioration.

METHODS Plasma levels of tumor necrosis factor-alpha (TNF-alpha), soluble TNF receptors I and II (sTNF-RI and sTNF-RII, respectively), interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R), sFas and sFasL were measured in 24 patients with stable CHF (New York Heart Association functional class II/III; left ventricular ejection fraction 23.2 ± 1.3%) and in 20 normal control subjects before and after a 12-week program of physical training in a randomized, crossover design. Functional status of patients with CHF was evaluated by using a cardiorespiratory exercise test to measure peak oxygen consumption (Vo_2max).

RESULTS Physical training produced a significant reduction in plasma levels of TNF-alpha (7.5 ± 1.0 pg/ml vs. 4.6 ± 0.7 pg/ml, $p < 0.001$), sTNF-RI (3.3 ± 0.2 ng/ml vs. 2.7 ± 0.2 ng/ml, $p < 0.005$), sTNF-RII (2.6 ± 0.2 ng/ml vs. 2.3 ± 0.2 ng/ml, $p = 0.06$), IL-6 (8.3 ± 1.2 pg/ml vs. 5.9 ± 0.8 pg/ml, $p < 0.005$), sIL-6R (34.0 ± 3.0 ng/ml vs. 29.2 ± 3.0 ng/ml, $p < 0.01$), sFas (5.5 ± 0.7 ng/ml vs. 4.5 ± 0.8 ng/ml, $p = 0.05$) and sFasL (34.9 ± 5.0 pg/ml vs. 25.2 ± 4.0 pg/ml, $p < 0.05$), as well as a significant increase in Vo_2max (16.3 ± 0.7 ml/kg per min vs. 18.7 ± 0.8 ml/kg per min, $p < 0.001$). Good correlations were found between a training-induced increase in Vo_2max and a training-induced reduction in levels of the proinflammatory cytokine TNF-alpha ($r = -0.54$, $p < 0.01$) and the apoptosis inducer sFasL ($r = -0.57$, $p < 0.005$) in patients with CHF. In contrast, no significant difference in circulating cytokines and apoptotic markers was found with physical training in normal subjects.

CONCLUSIONS Physical training reduces plasma levels of proinflammatory cytokines and the sFas/sFasL system in patients with CHF. These immunomodulatory effects may be related to the training-induced improvement in functional status of patients with CHF. (J Am Coll Cardiol 2002;39:653–63) © 2002 by the American College of Cardiology

Recent investigations have shown an overexpression of proinflammatory cytokines and soluble apoptosis mediators in patients with chronic heart failure (CHF), which may be related to the cardiac and endothelial dysfunction characterizing this syndrome (1,2). Thus, an abnormal immunologic response appears to be an important factor in the development and progression of the syndrome of CHF (3). Proinflammatory cytokines, such as tumor necrosis factor (TNF)-alpha and interleukin-6 (IL-6), are capable of modulating cardiac and peripheral vascular functions by a variety of mechanisms, including abnormal regulation of nitric oxide synthase (NOS) expression, overproduction of oxygen free radicals and induction of cardiac myocyte and endothelial cell apoptosis (4,5). In addition, overexpression of the inducible form of NOS (iNOS) in the skeletal muscle of patients with CHF, possibly triggered by circulating proinflammatory cytokines, appears to be responsible for the attenuation of muscle contractile capacity and/or skeletal myocyte apoptosis, both associated with the severity of exercise tolerance limitation and the degree of CHF (6–8). It has also been reported that circulating apoptosis mediators, such as soluble Fas (sFas) and soluble Fas ligand (sFasL), are elevated in patients with CHF and correlated well with the severity of symptoms and prognosis of patients with heart failure (2,9,10).

To the best of our knowledge, no in vivo data exist on the effects of physical training on circulating proinflammatory cytokines and their soluble receptors, as well as the soluble apoptosis receptor sFas and the soluble apoptosis inducer

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Abbreviations and Acronyms

ANOVA	= analysis of variance
CHF	= chronic heart failure
ELISA	= enzyme-linked immunosorbent assay
IL-6	= interleukin-6
iNOS	= (inducible) nitric oxide synthase
NO	= nitric oxide
sFas	= soluble Fas
sFasL	= soluble Fas ligand
sIL-6R	= soluble intrerleukin-6 receptor
sTNF-RI	= soluble tumor necrosis factor receptor type I
sTNF-RII	= soluble tumor necrosis factor receptor type II
TNF	= tumor necrosis factor
V_{O_2}max	= peak oxygen consumption

sFasL, in patients with CHF. Therefore, we sought to investigate whether a physical training program affects the serum levels of the proinflammatory cytokines TNF-alpha and IL-6 and their soluble receptors, soluble tumor necrosis factor receptor type I (sTNF-RI), soluble tumor necrosis factor receptor type II (sTNF-RII) and soluble intrerleukin-6 receptor (sIL-6R), as well as the serum levels of apoptotic mediators expressed by the sFas/sFasL system, and whether these training-induced changes are associated with exercise tolerance, expressed by peak oxygen consumption (V_{O_2}max), in 24 patients with stable, moderate to severe CHF.

METHODS

Study group. Twenty-four patients with moderate to severe CHF (age 55 ± 2 years; New York Heart Association functional class II/III) gave informed, written consent and were studied in our Cardiology Department, in compliance with the approval of Ethics Committee. The mean left ventricular ejection fraction of patients with CHF, estimated by two-dimensional echocardiography, according to the modified Simpson's formula, was 23.2 ± 1.3%. Inclusion criteria were: stable CHF of at least three months' duration; ischemic etiology (n = 11, as evidenced by documented myocardial infarction and/or coronary arteriography and coronary artery bypass graft surgery) and idiopathic dilated cardiomyopathy (n = 13); a limitation of exercise by dyspnea or fatigue only and the ability to achieve a respiratory exchange ratio for at least unity; and the 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 anti-inflammatory or immunosuppressive agents for the last two weeks, were excluded from our study. The control subjects consisted of 20 age- and gender-matched volunteers without acute or chronic illness or any symptoms related to the cardiovascular system.

Patients and healthy control subjects underwent a 12-week, home-based, bicycle exercise training program or asked to avoid exercise (detraining) in a randomized, crossover design. The training program consisted of five days per week, 30 min per day; patients and normal control subjects were instructed to exercise at 50 rpm to keep their continuously monitored heart rate in the range of 60% to 80% of their previously determined maximal heart rate. Compliance with the program was assessed as the percentage of expected bicycle wheel revolutions achieved over the training period.

Cardiopulmonary exercise testing. At each visit, after overnight fasting and before drug administration, the patients performed a cardiopulmonary exercise stress test to evaluate their exercise capacity by measuring V_{O_2}max (ml/kg per min). Exercise testing with respiratory gas exchange measurements was performed by using the Medgraphics CPX/MAX (Medical Graphics Corp., St. Paul, Minnesota) measuring system, while patients exercised on a treadmill according to the Dargie protocol (11). Blood pressure was measured with a mercury sphygmomanometer, and the electrocardiogram was continuously monitored with a computer-assisted system (Marquette Electronics Inc., Milwaukee, Wisconsin). All patients quit the test because of dyspnea or fatigue, and in all patients the gas exchange anaerobic threshold and a respiratory exchange ratio >1.0 were reached. Peak oxygen consumption during exercise was reported as the mean value during the last minute of exercise.

Laboratory measurements. Serum was obtained at baseline, after training and after detraining by centrifugation of vacutainer gel clotter tubes at 3,000 rpm for 10 min. All serum samples were stored at −70°C until the time of analysis, and samples were assayed in duplicate for TNF-alpha, IL-6, sTNF-RI, sTNF-RII, sIL-6R, sFas and sFasL concentrations, using commercially available enzyme-linked immunosorbent assay (ELISA) kits. For most of them (TNF-alpha, IL-6, sTNF-RI, sTNF-RII, sIL-6R and sFas), we used the R&D Systems kit (Minneapolis, Minnesota), whereas for sFasL, the Diaclone kit (Besancon, France) was used. All kits had the following sandwich ELISA format: the microtiter plates were already precoated with a murine monoclonal antibody against the human proinflammatory cytokine 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 for TNF-alpha, IL-6, sTNF-RI, sTNF-RII, sIL-6R and sFas measurements. Also, standards of the analyte and plasma samples-in-duplicate were added, along with another biotinylated antibody against another epitope of the analyte for sFasL measurement. The samples were incubated for 1.5 h for TNF-alpha, IL-6, sTNF-RI, sTNF-RII, sIL-6R and sFas and 2 h for sFasL at room temperature. In the case of sFasL determination, streptavidin-horse radish peroxidase was

Table 1. Demographic Data and Clinical Characteristics, as Well as Individual Values of the Various Proinflammatory Cytokines and Apoptotic Mediators at Baseline

Pt. No.	Age (yrs)	Drugs	EF (%)	Vo_2 max (ml/kg per min)	sFas (ng/ml)	sFasL (pg/ml)	TNF-alpha (pg/ml)	sTNF-RI (ng/ml)	sTNF-RII (ng/ml)	IL-6 (pg/ml)	sIL-6R (ng/ml)
1	65	D, ACE	27	13.8	3.57	33.4	6.39	5.2	2.185	3.95	26.2
2	61	D, ACE, Dig.	16	14.0	4.21	32.1	4.42	4.7	2.854	8.60	31.9
3	57	D, ACE, AA	30	15.8	6.88	42.9	4.11	5.3	2.236	4.90	47.6
4	50	D, ACE	35	15.4	3.98	67.8	0.55	5.4	3.354	9.90	37.0
5	68	D, ACE	21	12.4	16.24	56.0	7.67	4.9	4.823	13.60	31.0
6	59	D, ACE, Dig.	28	15.0	2.4	31.7	2.23	2.4	1.922	8.20	23.5
7	63	D, ACE	14	13.1	5.17	66.2	4.66	2.6	2.487	1.95	33.8
8	70	D, ACE	19	16.6	6.77	35.1	4.02	3.9	2.790	2.40	39.0
9	44	D, ACE	29	14.1	1.34	75.9	7.93	6.1	1.525	7.80	22.0
10	64	D, ACE, Dig.	20	12.2	2.08	9.5	11.64	4.23	3.174	4.50	34.3
11	60	D, ACE, AA	25	13.6	8.71	20.6	5.78	3.2	3.257	9.20	38.9
12	54	D, ACE	23	16.8	3.29	4.3	1.97	3.7	2.440	7.40	67.5
13	67	D, ACE, BB	18	11.2	4.90	3.6	10.03	2.78	2.322	8.40	26.2
14	47	D, ACE, Dig.	22	19.7	4.80	54.8	11.90	2.5	4.798	7.80	28.6
15	55	D, ACE	15	20.3	6.43	86.1	10.66	2.4	2.340	6.60	42.4
16	44	D, ACE, BB	17	18.3	7.96	79.8	9.42	2.3	4.469	10.4	49.2
17	62	D, ACE	28	11.7	2.87	11.2	3.61	2.9	1.981	3.1	21.7
18	65	D, ACE	25	20.4	7.51	44.8	12.36	2.65	2.033	16.4	37.2
19	57	D, ACE, Dig.	18	16.8	3.66	6.78	3.80	2.86	3.212	8.2	19.3
20	40	D, ACE, BB	23	23.7	4.25	4.78	6.34	1.92	1.790	8.6	ND
21	49	D, ACE	20	15.7	11.47	21.6	17.3	4.4	2.965	25.5	30.4
22	35	D, ACE, AA	17	13.2	5.45	13.7	10.48	3.35	2.686	3.3	51.2
23	30	D, ACE, BB	34	23.5	2.72	12.2	10.72	2.14	1.944	5.3	ND
24	59	D, ACE	32	22.6	10.98	25.31	10.22	2.22	2.538	7.7	41.1
Mean ± SE	55 ± 2		23.2 ± 1.3	16.2 ± 0.8	5.7 ± 0.7	35.0 ± 5.0	7.4 ± 0.8	3.5 ± 0.2	2.7 ± 0.2	8.1 ± 1.0	35.5 ± 3.0

AA = antiarrhythmics; ACE = angiotensin-converting enzyme inhibitors; BB = beta blockers; D = diuretics; Dig. = digoxin; EF = ejection fraction; IL-6 = interleukin-6; ND = nondetectable; sFas = soluble Fas; sFasL = soluble Fas ligand; sIL-6R = soluble interleukin-6 receptor; sTNR-RI and -RII = soluble TNF receptor types I and II; TNF-alpha = tumor necrosis factor-alpha; Vo_2max = peak oxygen consumption.

added for an extra 30 min, after the wells were washed. Finally, for all of them, the chromogen tetra-methyl benzidine was added and incubated for 30 min in the dark. After addition of 2N H_2SO_4, the optical densities at 450 nm (reference filter 620 nm) were read, and standard curves were plotted in an Organon Technika 530 (Turnhout, Belgium) microplate reader.

All tests were performed before daily medication had been taken and were conducted by a blinded observer.

Table 1 summarizes the demographic data and clinical characteristics, as well as the individual values of the various proinflammatory cytokines and apoptotic mediators, of patients with heart failure.

Statistical analysis. Statistical analysis was carried out according to the recommendations of Hills and Armitage(12) for crossover trials. Comparisons of values of the proinflammatory cytokines and apoptotic mediators, as well as Vo_2max at baseline, after training and after detraining, were done by using analysis of variance (ANOVA) for repeated measures, followed by Scheffé's procedure for post hoc comparisons of mean values. Simple regression analysis was performed to describe the relationship between the training-induced changes in circulating cytokines and apoptotic factors and Vo_2max. A p value <0.05 was accepted as statistically significant. Results are expressed as the mean value ± SE.

RESULTS

We demonstrated that physical training induced a significant reduction ($p < 0.005$ by ANOVA) in proinflammatory cytokines and apoptotic factors, as compared with the detraining period, in the group of patients with CHF. Thus, a significant decrease in serum concentrations of the circulating cytokine TNF-alpha was observed with training (7.5 ± 1.0 pg/ml vs. 4.6 ± 0.7 pg/ml, $p < 0.001$) (Fig. 1), as well as decreases in sTNF-RI (3.3 ± 0.2 ng/ml vs. 2.7 ± 0.2 ng/ml, $p < 0.005$) and sTNF-RII (2.6 ± 0.2 ng/ml vs. 2.3 ± 0.2 ng/ml, $p = 0.06$) (Fig. 2), the circulating cytokine IL-6 (8.3 ± 1.2 pg/ml vs. 5.9 ± 0.8 pg/ml, $p < 0.005$) and its soluble receptor sIL-6R (34.0 ± 3.0 ng/ml vs. 29.2 ± 3.0 ng/ml, $p < 0.01$) (Fig. 3) and the apoptotic mediators sFas (5.5 ± 0.7 ng/ml vs. 4.5 ± 0.8 ng/ml, $p = 0.05$) and sFasL (34.9 ± 5.0 pg/ml vs. 25.2 ± 4.0 pg/ml, $p < 0.05$) (Fig. 4). Exercise performance, expressed by Vo_2max, improved with the exercise training program (16.3 ± 0.7 ml/kg per min vs. 18.7 ± 0.8 ml/kg per min, $p < 0.001$). Significant correlations were found between the training-induced improvement in Vo_2max and the percent reduction in serum levels of the proinflammatory cytokine TNF-alpha ($r = -0.54$, $p < 0.01$) and the apoptosis mediator sFasL ($r = -0.57$, $p < 0.005$), indicating that the attenuation of proinflammatory cytokine activation and the downregula-

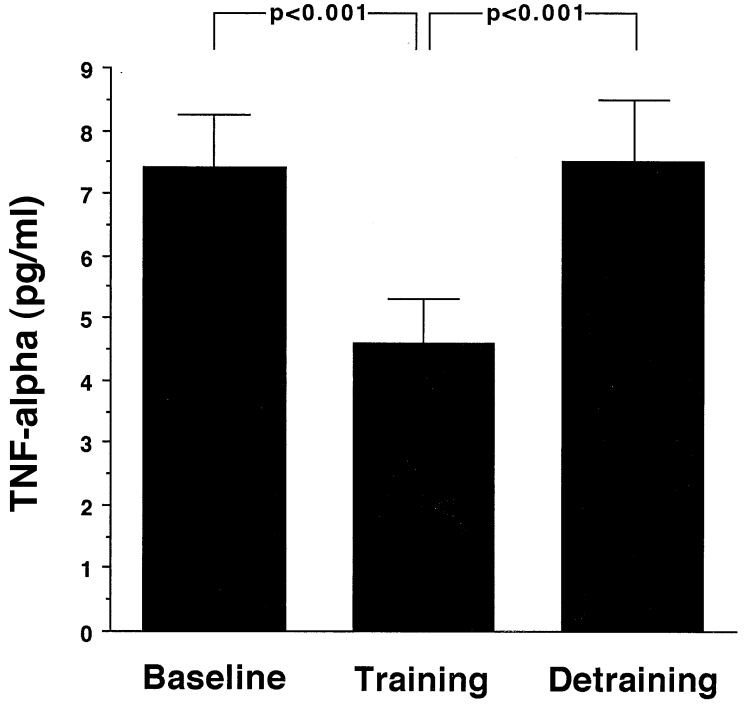

Figure 1. Effects of physical training on proinflammatory cytokine tumor necrosis factor (TNF)-alpha. Note the reduction in TNF-alpha levels with training in patients with chronic heart failure.

tion of the sFas/sFasL system may contribute to the improvement in exercise capacity achieved with physical training in patients with CHF (Fig. 5).

As with the detraining period, physical training produced a significant reduction ($p < 0.005$ by ANOVA) in proinflammatory cytokines and apoptotic factors, as compared with baseline, although no difference was detected between baseline and detraining values for exercise performance (16.2 ± 0.8 ml/kg per min vs. 16.3 ± 0.7 ml/kg per min for Vo_2max), the various proinflammatory cytokines (7.4 ± 0.8 pg/ml vs. 7.5 ± 1.0 pg/ml for TNF-alpha; 3.5 ± 0.2 ng/ml vs. 3.3 ± 0.2 ng/ml for sTNF-RI; 2.7 ± 0.2 ng/ml vs. 2.6 ± 0.2 ng/ml for sTNF-RII; 8.1 ± 1.0 pg/ml vs. 8.3 ± 1.2 pg/ml for IL-6; 35.5 ± 3.0 ng/ml vs. 34.0 ± and 3.0 ng/ml for sIL-6R) (Figs. 1 to 3) or the apoptotic factors (5.7 ± 0.7 ng/ml vs. 5.5 ± 0.7 ng/ml for sFas; 35.0 ± 5.0 pg/ml vs. 34.9 ± 5.0 pg/ml for sFasL) (Fig. 4).

No difference was detected in either baseline or training-induced changes in circulating proinflammatory cytokines, their soluble receptors and the sFas/sFasL system between patients with ischemic and dilated cardiomyopathy (data not shown).

Circulating proinflammatory cytokines and apoptotic mediators were significantly higher ($p < 0.01$) in patients with CHF, as compared with normal control subjects at baseline (7.4 ± 0.8 pg/ml vs. 3.6 ± 0.2 pg/ml for TNF-alpha; 3.5 ± 0.2 ng/ml vs. 1.2 ± 0.1 ng/ml for sTNF-RI; 2.7 ± 0.2 ng/ml vs. 1.6 ± 0.1 ng/ml for sTNF-RII; 8.1 ± 1.0 pg/ml vs. 3.0 ± 0.2 pg/ml for IL-6; 35.5 ± 3.0 ng/ml vs. 6.0 ± 0.3 ng/ml for sIL-6R; 5.7 ± 0.7 ng/ml vs. 2.5 ± 0.2 ng/ml for sFas; and 35.0 ± 5.0 pg/ml vs. 13.5 ± 1.0 pg/ml for sFasL). Despite the considerable improvement in all these variables with physical training in patients with CHF, their values still remain higher ($p < 0.01$) than the baseline values of the control subjects.

It is worth mentioning that physical training produced no significant changes in the circulating proinflammatory cytokines (3.7 ± 0.3 pg/ml vs. 3.5 ± 0.3 pg/ml for TNF-alpha; and 2.9 ± 0.2 pg/ml vs. 2.8 ± 0.2 pg/ml for IL-6), their soluble receptors (1.17 ± 0.2 ng/ml vs. 1.14 ± 0.2 ng/ml for sTNF-RI; 1.5 ± 0.1 ng/ml vs. 1.4 ± 0.1 ng/ml for sTNF-RII; and 5.8 ± 0.2 ng/ml vs. 5.7 ± 0.3 ng/ml for sIL-6R) and the apoptosis mediators (2.4 ± 0.2 ng/ml vs. 2.3 ± 0.2 ng/ml for sFas; and 13.8 ± 2.0 pg/ml vs. 13.0 ± 2.0 pg/ml for sFasL) in the control group of normal subjects.

DISCUSSION

Proinflammatory cytokines (i.e., TNF-alpha, IL-1 and IL-6) cause myocardial and endothelial dysfunction in patients with CHF (13,14), either by increasing the production of oxygen free radicals, which, in turn, alter the production of nitric oxide (NO) in the cardiovascular system or by triggering apoptosis in myocardial and endothelial cells through oxidative stress (15–18). Interleukin-6 and related cytokines have also been implicated in the development of cardiac hypertrophy, through stimulation of their common receptor gp130 expressed in cardiac myocytes (19–21). Proinflammatory cytokine hyperactivation is also

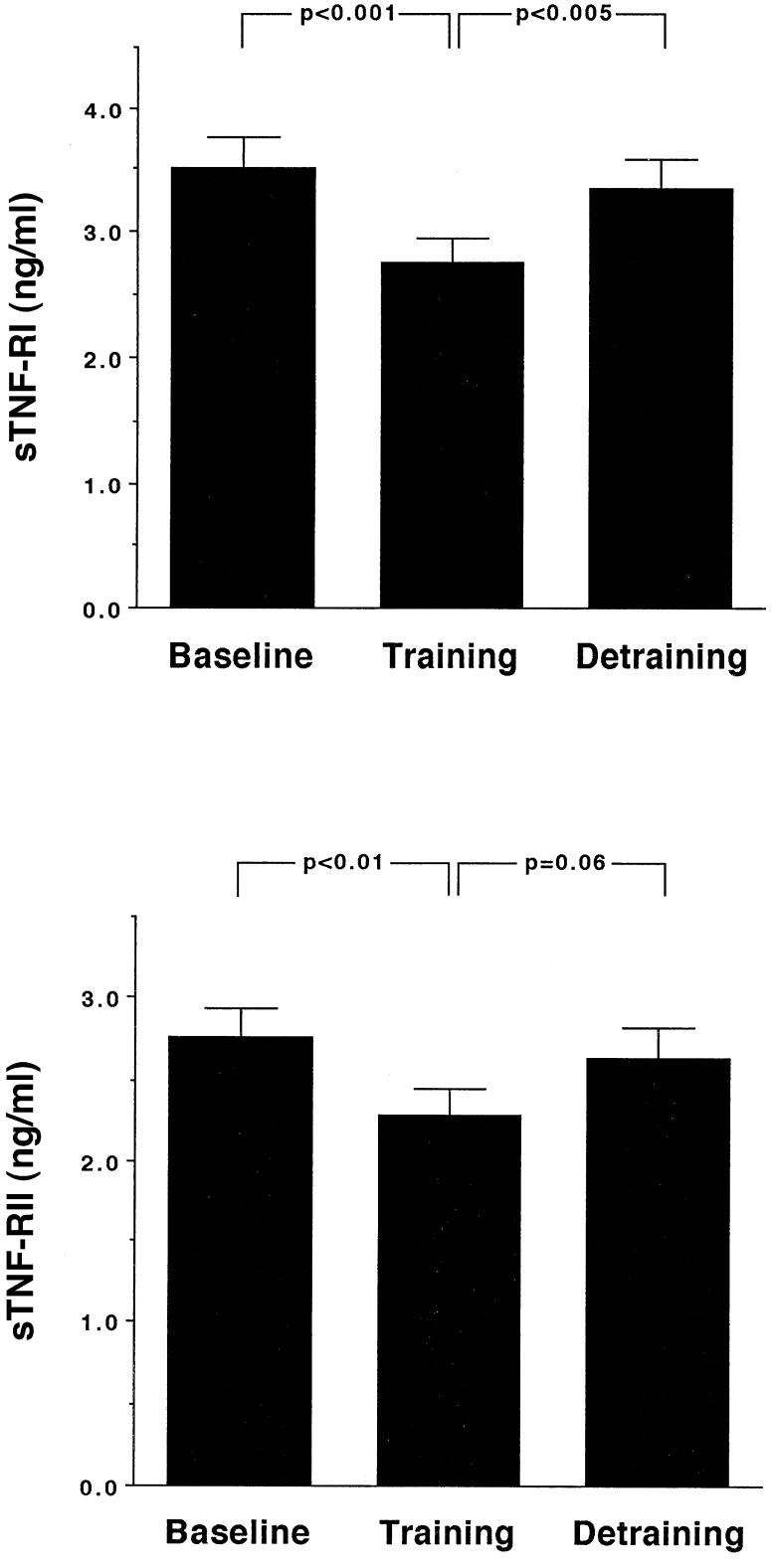

Figure 2. Effects of physical training on soluble tumor necrosis factor receptor type I (sTNF-RI) **(top)** and soluble tumor necrosis factor receptor type II (sTNF-RII) **(bottom)**. Note the reduction in sTNF-RI and sTNF-RII levels with training in patients with chronic heart failure.

associated with increased gene expression of iNOS in the skeletal muscle of patients with CHF, leading to attenuation of mitochondrial energy transfer (and, thus, attenuation of skeletal muscle contractile performance) and/or skeletal myocyte apoptosis (6,7). These deleterious, central and peripheral effects may be important pathophysiologic events

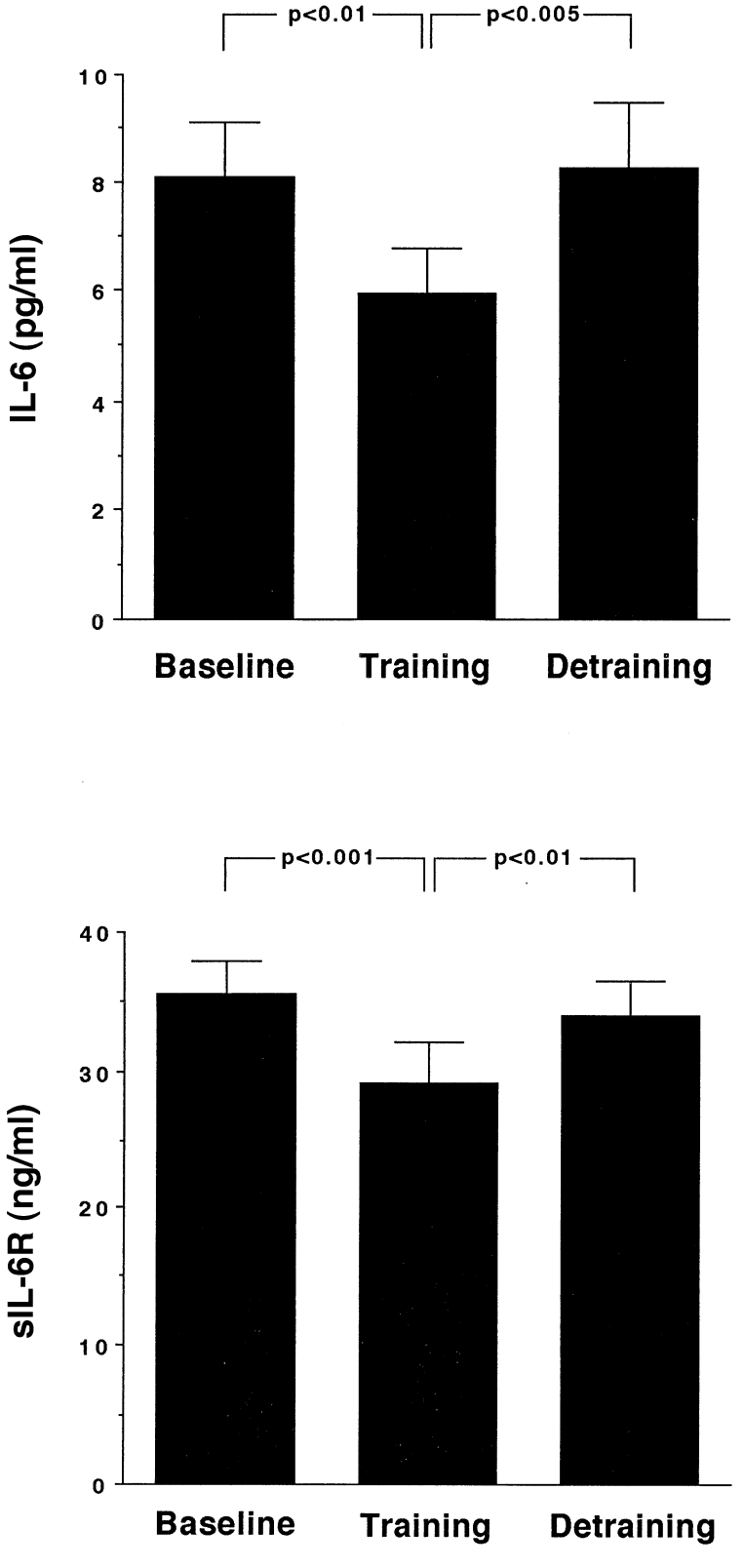

Figure 3. Effects of physical training on interleukin-6 (IL-6) **(top)** and soluble interleukin-6 receptor (sIL-6R) **(bottom)**. Training produced a significant reduction in IL-6 and sIL-6R levels in patients with chronic heart failure.

associated with the impaired exercise capacity of patients with heart failure (8,22,23).

It is known that programs of physical training, by causing sustained, pulsatile increases in peripheral blood flow, affect the release of prostaglandins in the skeletal muscle microvasculature (24), induce the expression of NOS and cyto-

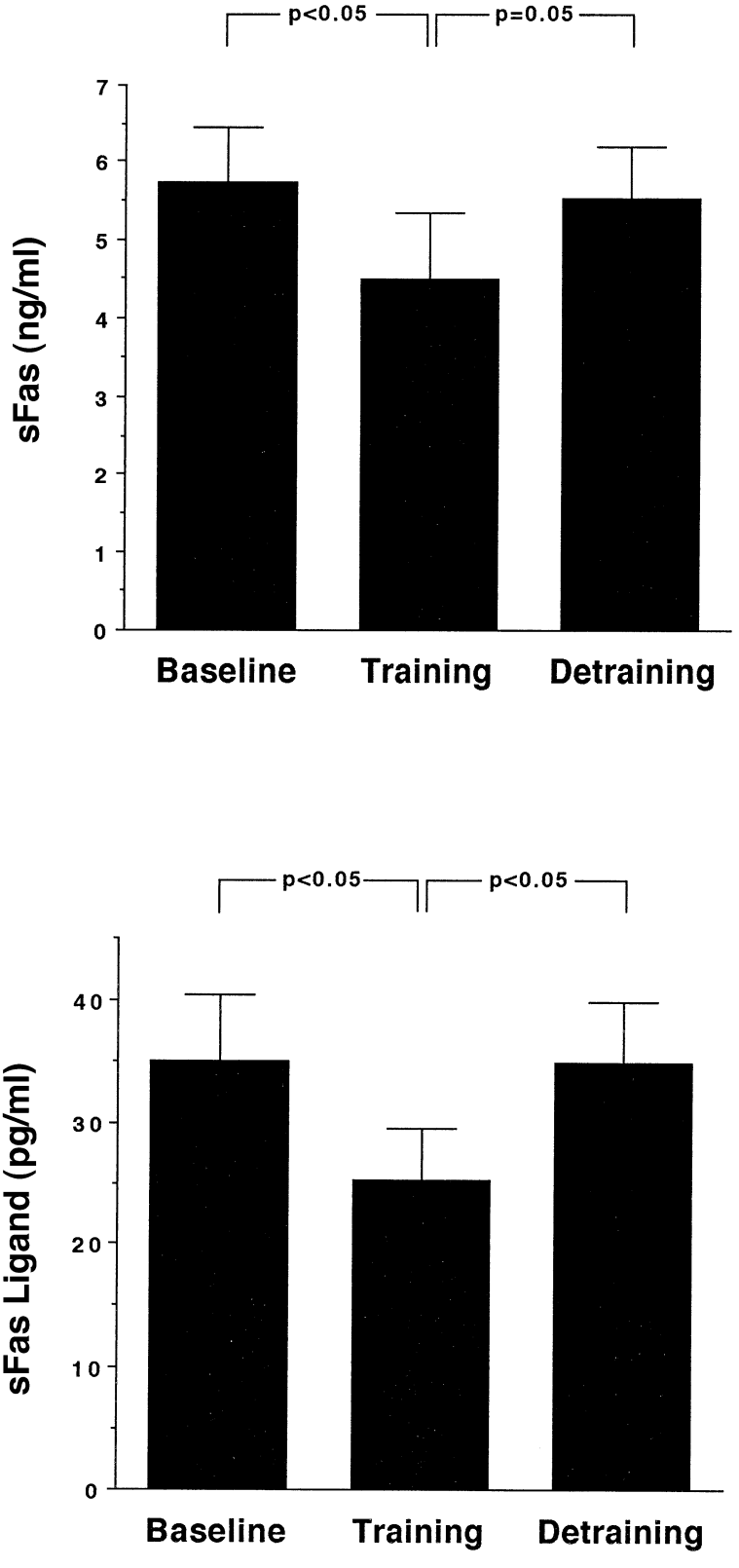

Figure 4. Effects of physical training on the soluble apoptosis mediators soluble Fas (sFas) **(top)** and sFas ligand **(bottom)**. Note the decrease in both apoptotic variables with training in patients with chronic heart failure.

solic superoxide dismutase (25), a free radical scavenger, and enhance Ca^{++} influx in endothelial cells, which is necessary for both NO and prostaglandin synthesis (26).

In this report, we have shown that an exercise training program intervenes in the various stages of inflammatory and apoptotic processes in patients with CHF, by reducing

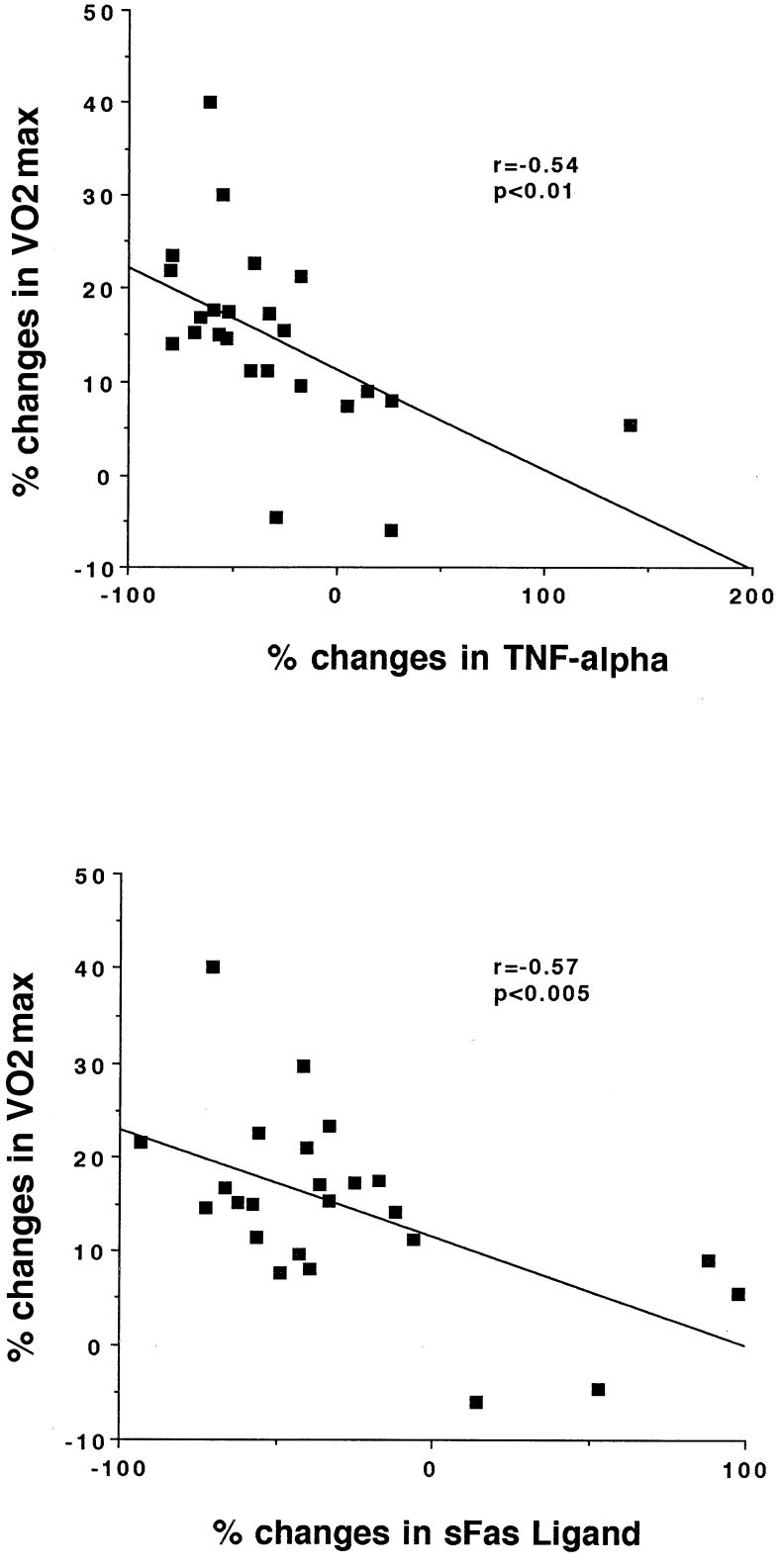

Figure 5. Correlations between training-induced changes in peak oxygen consumption (VO_2max) and reductions in tumor necrosis factor (TNF)-alpha **(top)** and soluble Fas (sFas) ligand **(bottom)** levels in patients with chronic heart failure.

not only the major proinflammatory cytokines TNF-alpha and IL-6, which enhance the cytokine cascade and are potent inducers of apoptosis (27), but also the soluble receptors of TNF-alpha and IL-6, which are products of a monocyte-myocyte/endothelial cell interaction and biologic modulators of circulating cytokine actions (28); sFasL, a

newly discovered potent cytokine that is homologous to TNF-alpha and induces apoptosis by binding to its membrane receptor Fas through the activation of caspases (29); and the soluble apoptotic receptor sFas, which represents an important signal for the initiation of the apoptotic process in the cardiovascular system and may have prognostic importance in the syndrome of CHF (2,8).

Proinflammatory cytokines and physical training. Enhanced immune activation, reflected in increased circulating levels of various proinflammatory cytokines (i.e., TNF-alpha, IL-1 and IL-6), has been recently recognized to play a significant role in the pathophysiology of CHF (1,14). Although the mechanism for increased elaboration of IL-6 is not known, TNF-alpha is sufficient to induce IL-6 gene (by releasing the nuclear binding protein nuclear factor-κB) and protein expression in a variety of cell types, suggesting that there may be a "cytokine cascade" in the setting of CHF (30). Despite the growing interest in the contribution of cytokine activation to the central (myocardial contractility and remodeling) and peripheral (skeletal muscular and endothelial function) pathophysiology of CHF, few reports exist regarding the influence of traditional pharmacologic and nonpharmacologic medications on cytokine levels in human CHF. For example, in patients with severe CHF, high-dose angiotensin-converting enzyme therapy is associated with a significant decrease in IL-6 activity (31), and in patients with dilated cardiomyopathy, a reduction in levels of IL-6 has been proposed to be important for the beneficial effect of amlodipine on mortality in the Prospective Randomized AmlodIpine Survival Evaluation heart failure study (32). In addition, an important immunoregulatory role of beta-blockers in modifying the dysregulated cytokine network has been recently reported in patients with dilated cardiomyopathy (33). For the first time, to the best of our knowledge, our study reports on the immunomodulatory role of physical training in patients with CHF, as demonstrated with significant reductions in circulating levels of major proinflammatory cytokines (TNF-alpha and IL-6), all inducers of myocyte (both cardiac and skeletal) and endothelial cell apoptosis, all actively involved in cardiovascular maladaptive remodeling (34), all associated with progression of the syndrome and all powerful, independent predictors of new heart failure episodes, death or the need for heart transplantation (28,35–37).

The extracellular domains of the TNF-alpha receptors (soluble proteins TNF-RI and TNF-RII) provide more complete information on TNF-alpha activation in patients with CHF (28). Analysis of our data confirmed a parallel reduction of cytokines and all of their soluble receptors with training, except for sTNF-RII, which showed a nonsignificant trend ($p = 0.06$) toward reduced levels in trained patients. This partially discordant training effect on TNF-alpha and sTNF-RII might provide stronger evidence for a less pro-apoptotic serum for endothelial cells of the vasculature and for myocytes of the striated muscles. We believe, however, that reduced sTNF receptor levels after physical training reflect deactivation of the whole TNF system, possibly indicating that trained patients with CHF no longer "require" protection and, therefore, recruitment of TNF-alpha receptors to counterbalance the TNF-induced detrimental effects. Moreover, there is growing evidence underlining the prognostic significance of the enhanced levels of the sTNF receptors (especially sTNF-RII) in the syndrome of CHF (28,37). Previous reports have shown that the increase in circulating TNF-alpha in patients with CHF was associated with a substantial decrease in myocardial TNF-alpha receptors and an increase in sTNF-alpha receptors (28,38). Physical training, therefore, by virtue of its anti-inflammatory and anti-apoptotic effects, may attenuate myocyte and endothelial cell TNF-alpha expression. Thus, we hypothesize that tissue TNF-alpha receptors may be upregulated with training into the cardiovascular system, offering an additional explanation of the training-induced reduction in sTNF-alpha receptor levels.

The extracellular domain of the human IL-6 receptor can be detected as the soluble form (sIL-6R) into the circulation of patients with CHF. Although the clinical significance of elevated sIL-6R in CHF remains uncertain, it has been postulated that, similar to the TNF-alpha system, overactivation mechanisms may be responsible for sIL-6R release into the circulation of patients with CHF (39). A training-induced decrease in sIL-6R levels, associated with a parallel reduction in IL-6 levels, reflects a deactivation of the IL-6 system, another multifunctional cytokine that mediates both the immune and inflammatory responses.

The Fas/FasL system and physical training. To the best of our knowledge, we are the first to demonstrate that exercise training induces a significant reduction in sFas and sFasL, two major soluble signaling molecules implicated in the pathophysiology of the apoptotic process in the cardiovascular system (2,9).

Circulating sFas levels are increased in patients with dilated cardiomyopathy in proportion to the severity of heart failure and may provide prognostic information independent of left ventricular geometry (40). Serum concentrations of sFas are also elevated in patients with myocarditis and correlated with the soluble interleukin-2 receptor, a marker of T-cell activation (41).

The soluble form of the apoptosis inducer FasL (converted from membrane-bound FasL to a soluble form by a metalloproteinase [42]) is increased in patients with advanced CHF and may not be derived only from circulating T lymphocytes and natural killer cells; endothelial cells, as well as the myocardium, should be considered as important contributors of sFasL in human CHF (29,43).

The Fas/FasL system, therefore, may play a significant role in the regulation of central and peripheral immune responses in CHF. Physical training downregulates the Fas/FasL system and resets homeostasis at a lower level. The detrimental overfunction of the system, expressed mainly with the FasL-induced tissue (endothelial cells and myocytes) apoptosis, therefore, is attenuated.

A protective role of the soluble form of the Fas molecule from FasL-mediated apoptosis has been recently described; therefore, one could argue that physical training facilitates apoptosis by causing a reduction in the sFas concentration. We have demonstrated, however, that training also causes a significant reduction in a broad spectrum of immunologic variables characterized as major inducers of apoptosis (e.g., TNF-alpha, IL-6, sFasL), re-establishing the balance of the Fas/FasL system. Therefore, the training-induced decrease in sFas levels simply reflects re-establishment of a more optimal balance of the Fas/FasL system, as well as reversal of the ongoing "immune activation" associated with CHF.

Immunologic responses and exercise tolerance. The good correlation between training-induced changes in exercise capacity and the proinflammatory cytokine TNF-alpha and the apoptotic inducer sFasL indicates that exercise training may exert its beneficial effects, at least partially, by suppressing the proinflammatory cytokine activation characterizing the progress and clinical deterioration of CHF.

We have recently shown that exercise training programs reduce inflammatory markers (e.g., granuloctye macrophage colony-stimulating factor, monocyte chemoattractant protein-1, soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1) indicative of a macrophage-endothelial cell adhesive interaction, and that inflammatory process may underlie exercise intolerance associated with CHF (44). Our study extends this initial observation, not only by interpreting a possible epiphenomenon, but also by shedding light on the fundamental mechanisms implicated in the pathogenesis of exercise intolerance, a cardinal characteristic of the syndrome. Physical training, therefore, by virtue of its anti-inflammatory and anti-apoptotic effects, seems to beneficially regulate peripheral immune responses, resulting in improvement in exercise capacity. Proinflammatory cytokines and apoptotic inducers have been implicated in the pathogenesis, not only of myocardial dysfunction and cardiomyocyte death, but also of skeletal myopathy (through their skeletal myocyte apoptotic properties) (7) and endothelial dysfunction (through endothelial activation or damage) (45).

Clinical implications. These observations support the notion that immunologic and inflammatory responses are important pathophysiologic features in CHF, and physical training may improve exercise performance by modifying the inflammatory status and the subsequent apoptotic process of patients with this syndrome, as well as by possibly reversing inflammation-induced deleterious effects on the cardiovascular system. Thus, our study provides new insight into the pathophysiologic mechanisms underlying the beneficial effects of physical training on symptoms and exercise tolerance in the complex syndrome of CHF.

It is, therefore, tempting to hypothesize that modulation of immunologic variables is emerging as a major therapeutic goal in the treatment of patients with CHF, and that physical training may represent an important immunomodulatory option that may possibly intervene in the progression of the disease.

Conclusions. We found that physical training causes a significant decrease in the circulating proinflammatory cytokines TNF-alpha and IL-6 and their soluble receptors (TNF-RI, TNF-RII and IL-6R), as well as in the soluble apoptosis inducer FasL and the soluble apoptosis receptor Fas, and that these beneficial effects may be related to the training-induced improvement in functional status of patients with CHF, suggesting that persistent immune activation appears to be involved in the impaired exercise capacity characterizing this syndrome.

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REFERENCES

1. Mann DL, Young JB. Basic mechanisms in congestive heart failure: recognizing the role of proinflammatory cytokines. Chest 1994;105:897–904.
2. Nishigaki K, Minatogouchi S, Seishima M, et al. Plasma Fas ligand, an inducer of apoptosis, and plasma soluble Fas, an inhibitor of apoptosis, in patients with chronic congestive heart failure. J Am Coll Cardiol 1997;29:1214–20.
3. Devaux B, Scholz D, Hirche A, Klovekorn WP, Schaper J. Upregulation of cell adhesion molecules and the presence of low grade inflammation in human chronic heart failure. Eur Heart J 1997;18:470–9.
4. Meldrum DR. Tumor necrosis factor in the heart. Am J Physiol 1998;274:R577–95.
5. Ferrari R, Angoletti L, Comini L, et al. Oxidative stress during myocardial ischemia and heart failure. Eur Heart J 1998;19 Suppl B:B2–11.
6. Hambrecht R, Adams V, Gielen S, et al. Exercise intolerance in patients with chronic heart failure and increased expression of inducible nitric oxide synthase in the skeletal muscle. J Am Coll Cardiol 1999;33:174–9.
7. Vescovo G, Zennaro R, Sandri M, et al. Apoptosis of skeletal muscle myofibers and interstitial cells in experimental heart failure. J Mol Cell Cardiol 1998;30:2449–59.
8. Vescovo G, Volterrani M, Zennaro R, et al. Apoptosis in the skeletal muscle of patients with heart failure: investigation of clinical and biochemical changes. Heart 2000;84:431–7.
9. Parissis J, Venetsanou K, Ziras N, et al. Clinical correlates and prognostic value of apoptosis mediator soluble forms in patients with congestive heart failure (abstr). J Am Coll Cardiol 1998;32 Suppl A:329A.
10. Okuyama M, Yamaguchi S, Nozaki N, Yamaoka M, Shirakabe M, Tomoike H. Serum levels of soluble form of Fas molecule in patients with congestive heart failure. Am J Cardiol 1997;79:1698–701.
11. Riley M, Northidge DB, Henderson E, et al. The use of an exponential protocol for bicycle and treadmill exercise testing in patients with chronic cardiac failure. Eur Heart J 1992;13:1363–7.
12. Hills M, Armitage P. The two-period cross-over clinical trial. Br J Clin Pharmacol 1979;8:7–20.
13. Katz SD, Rao R, Berman JW, et al. Pathophysiologic correlates of increased serum tumor necrosis factor in patients with congestive heart failure: relation to nitric oxide-dependent vasodilation in the forearm circulation. Circulation 1994;90:12–6.
14. Ferrari R. Tumor necrosis factor in CHF: a double-faced cytokine. Cardiovasc Res 1998;37:554–9.
15. Belch JJF, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. Br Heart J 1991;64:245–8.
16. Sasayma S, Matsumori A, Kihara Y. New insights into the pathophysiological role for cytokines in heart failure. Cardiovasc Res 1999;42:557–64.

17. Vanderheyden M, Kersschof E, Paulus WJ. Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm: serial assessment in patients with congestive heart failure. Eur Heart J 1998;19:747–52.
18. Keith M, Geranmayegan A, Sole MJ, et al. Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998;31: 1352–6.
19. Yoshida K, Taga T, Saito M, et al. Targeted disruption of gp130, a common signal transducer for the interleukin-6 family of cytokines, leads to myocardial and hematological disorders. Proc Natl Acad Sci U S A 1996;93:407–11.
20. MacGowan GA, Mann DL, Kormos RL, et al. Circulating interleukin-6 in severe heart failure. Am J Cardiol 1997;79:1128–31.
21. Hunter J, Chien KR. Signaling pathways for cardiac hypertrophy and failure. N Engl J Med 1999;341:1276–83.
22. Katz SD. The role of endothelium-derived vasoactive substances in the pathophysiology of exercise intolerance in patients with congestive heart failure. Prog Cardiovasc Dis 1995;38:23–50.
23. Hambrecht R, Fiehn E, Weigl C, et al. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation 1998;98:2709–15.
24. Koller A, Huyang A, Sun D, Kaley G. Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles: role of endothelial nitric oxide and prostaglandins. Circ Res 1995;76:544–50.
25. Inoue N, Ramascamy S, Fukai T, Nereri RM, Harrison DG. Shear stress modulates expression of Cu/Zn superoxide dismutase in human aortic endothelial cells. Circ Res 1996;79:32–7.
26. Falcone JC, Kuo L, Meininger GA. Endothelial cell calcium increases during flow-induced dilatation in isolated arterioles. Am J Physiol 1993;264:H653–9.
27. Mann DL. Mechanisms and models in heart failure: a combinatorial approach. Circulation 1999;100:999–1008.
28. Ferrari R, Bachetti T, Confortini R, et al. Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. Circulation 1995;92:1479–86.
29. Yamaguchi S, Yamaoka M, Okuyama M, et al. Elevated circulating levels and cardiac secretion of soluble Fas ligand in patients with congestive heart failure. Am J Cardiol 1999;83:1500–3.
30. Paulus WJ. How are cytokines activated in heart failure? Eur J Heart Fail 1999;1:337–52.
31. Gullestad L, Aukrust P, Ueland T, et al. Effect of high- versus low-dose angiotensin converting enzyme inhibition on cytokine levels in chronic heart failure. J Am Coll Cardiol 1999;34:2061–7.
32. Mohler ER, Sorensen LC, Ghali JK, et al. Role of cytokines in the mechanism of action of amlodipine: the PRAISE heart failure trial. J Am Coll Cardiol 1997;30:35–41.
33. Ohtsuka T, Hamada M, Hiasa G, et al. Effect of beta-blockers on circulating levels of inflammatory and anti-inflammatory cytokines in patients with dilated cardiomyopathy. J Am Coll Cardiol 2001;37: 412–7.
34. Bozkurt B, Kribbs SB, Clubb FJ, et al. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation 1998;97: 1382–91.
35. Orus J, Roig E, Perez-Villa F, et al. Prognostic value of serum cytokines in patients with congestive heart failure. J Heart Lung Transplant 2000;19:419–25.
36. Tsumamoto T, Hisanaga T, Wada A, et al. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and high plasma of interleukin-6 is an important predictor in patients with congestive heart failure. J Am Coll Cardiol 1998;31: 391–8.
37. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the VESnarinone Trial (VEST). Circulation 2001;103:2055–9.
38. Torre-Amione G, Kapadia S, Lee J, et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation 1996;93:704–11.
39. Testa M, Yeh M, Lee P, et al. Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension. J Am Coll Cardiol 1996;28:964–71.
40. Kawakami H, Shigematsu Y, Ohtsuka T, et al. Increased circulating soluble form of Fas in patients with dilated cardiomyopathy. Jpn Circ J 1998;62:873–6.
41. Toyozaki T, Hiroe M, Saito T, et al. Levels of soluble Fas in patients with myocarditis, heart failure of unknown origin, and in healthy volunteers. Am J Cardiol 1998;81:798–800.
42. Kayagaki N, Kawasaki A, Ebata T, et al. Metalloproteinase-mediated release of human Fas ligand. J Exp Med 1995;182:1777–83.
43. Sata M, Walsh K. TNF-alpha regulation of Fas ligand expression on the vascular endothelium modulates leukocyte extravasation. Nat Med 1998;4:415–20.
44. Adamopoulos S, Parissis J, Kroupis C, et al. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J 2001;22:791–7.
45. Ferrari R, Bachetti T, Agnoletti L, Comini L, Curello S. Endothelial function and dysfunction in heart failure. Eur Heart J 1998;19 Suppl G:G41–7.