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Combined endurance/resistance training reduces plasma TNF-α receptor levels in patients with chronic heart failure and coronary artery disease

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Aims Physical reconditioning of patients with chronic heart failure (CHF) improves exercise capacity and restores endothelial function and skeletal muscle changes. The effects of 4 months combined endurance/resistance exercise training on cytokines and cytokine receptors in patients with CHF were studied. In addition, changes in sub-maximal and maximal exercise performance were addressed.

Methods and Results Twenty-three patients with stable CHF due to coronary artery disease (CAD, n=12) or idiopathic dilated cardiomyopathy (IDCM, n=11) were trained for 4 months. Blood sampling for measurement of plasma concentrations (ELISA) of interleukin (IL)-6, tumour necrosis factor (TNF)-*α*, soluble TNF receptor 1 (sTNFR1) and 2 (sTNFR2), as well as cardiopulmonary exercise testing were performed at baseline and after 4 months. Training induced a significant decrease in sTNFR1 ($P=0{\cdot}02$) for the total population, and in both sTNFR1 ($P=0{\cdot}01$) and sTNFR2 ($P=0{\cdot}02$) concentrations for the CAD group only. IL-6 and TNF-*α* levels were not altered. Cytokine concentrations remained unchanged in an untrained age- and sex-matched control group. NYHA functional class, submaximal and maximal workrate were significantly improved in both patient groups. Oxygen uptake at the anaerobic threshold ($P=0{\cdot}002$) and at peak exercise increased in the CAD patients only ($P=0{\cdot}008$).

Conclusion Besides an overall beneficial effect on exercise capacity, combined endurance/resistance exercise training has an anti-inflammatory effect in patients with CHD and CAD.

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Key Words: Chronic heart failure, inflammation, cytokines, exercise training.

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Introduction

Chronic heart failure (CHF) has been re-defined as a system disorder, comprising a central cardiac deficit and several peripheral manifestations, such as skeletal muscle changes[1,2] and endothelial dysfunction[3].

Both systemic and local inflammation have been suggested to play an important role in the pathogenesis and progression of the disease[4]. Moreover, circulating levels of cytokines and cytokine receptors have acquired prognostic significance[5–7]. Physical reconditioning has only recently been introduced as a valid treatment modality for patients with CHF. Endurance exercise training programmes improve exercise capacity, peripheral vascular reactivity and left ventricular systolic function[8–10]. The mechanisms by which cardiovascular function is improved through exercise training, however, have not been fully elucidated. Adamopoulos *et al.*[11,12] recently reported on the anti-inflammatory effect of a 3 month home-based bicycle training programme in patients with moderate to severe CHF. These authors demonstrated a significant decrease in peripheral markers reflecting monocyte-endothelial cell interaction[12] and in circulating cytokines and cytokine receptors[11]. In a similar approach, Larsen and colleagues were able to demonstrate that aerobic exercise training reduced increased TNF-*α* levels in patients with symptomatic CHF[13].

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Table 1 Baseline demographic and clinical characteristics of trained patients and untrained controls

	Trained HF group (n=23)	Untrained HF group (n=18)	*P*
Male/Female	16/7	12/6	ns
Age (years)	57 (27–78)	70 (28–80)	ns
CAD/IDCM	12/11	11/7	ns
NYHA (I–II/III–IV)	11/12	4/14	ns
LVEF (%)	27 (11–45)	28 (10–37)	ns

HF=heart failure, CAD=coronary artery disease, IDCM=idiopathic dilated cardiomyopathy, LVEF=left ventricular ejection fraction.

Physical rehabilitation for patients with CHF is almost exclusively based on aerobic exercises. Combined endurance/resistance training has been suggested, however, as a safe and effective alternative training mode. The latter mode offers the opportunity for exercising small muscle groups, without imposing the cardiovascular burden of whole body exercise. Maiorana and colleagues have shown that circuit weight training (cycle ergometry, treadmill walking and resistance weight training) induced a significant increase in peak oxygen consumption, exercise duration, muscular strength and improvement of peripheral vascular function, without any short-term adverse events[15].

The aim of the present study was to test the effect of a 4 month combined endurance/resistance training programme on circulating plasma levels of TNF-α, soluble TNF-receptors and IL-6 in patients with stable moderate to severe CHF due to ischaemic (CAD) and idiopathic cardiomyopathy (IDCM). In addition, the effect of this particular training modality on exercise capacity was studied.

Methods

Subjects

Twenty-three patients with CHF were enrolled into a non-randomized 4 months combined endurance/resistance exercise training programme. Patients were on standard medical treatment consisting of ACE-inhibitors (96%), diuretics (83%), spironolactone (70%), digoxin (39%), beta-blockers (61%), aspirin (30%) and amiodarone (17%), in varying dosages and combinations. All patients had been stable with regard to symptoms and medical therapy for at least 1 month. Medication remained unchanged throughout the study period. Eighteen age- and sex matched patients with comparable disease severity, attending the outpatient heart failure clinic, served as the untrained heart failure control group. Demographic and clinical characteristics are shown in Table 1.

Exclusion criteria were: (i) acute/chronic infections, allergies, rheumatoid disease, cancer, or treatment with anti-inflammatory drugs, (ii) severe exercise-induced myocardial ischaemia, exercise-induced malignant ventricular arrhythmia, recent myocardial revascularization (<6 weeks), acute myocarditis or pericarditis. The study was approved by the Local Ethical Committee. All patients had given written informed consent.

Training protocol

Patients exercised three times/week, for 1 h. The training session commenced and concluded with a 5 min warming-up or cooling-down and stretching period. Training involved a total of 30 min of resistive weight training and 20 min of cycling and/or jogging. Both training modalities were alternated. The resistance circuit consisted of nine predetermined resistance exercises, involving muscle groups of the lower and the upper limbs and torso. Resistance training intensity was set at 50% of pre-training 1-repetitive maximum (1-RM) tests. Per session, two sets, consisting of 10 repetitions each, were performed.

Endurance training intensity was aimed at a target heart rate, defined by cardiopulmonary exercise testing, as the heart rate reached at 90% of the ventilatory threshold. Patients were able to correct their training intensity according to individual pulse rate measurement (Polar® Heart Rate Monitor). Additionally, a central monitoring system permitted arrhythmia detection.

Laboratory measurements

Fasting blood samples were collected in patients with heart failure between 0800h and 0900h into ethylenediaminetetraacetic (EDTA) tubes (Vacutainer®, Becton and Dickinson, Meylan, France) at baseline and after 4 months. Plasma was separated by centrifugation and aliquots were stored at $-20\,^{\circ}C$. Ten age- and sex-matched subjects served as a healthy baseline control group (no chronic underlying disease, no cardiac history, no medication, no allergic condition). Concentrations of IL-6, TNF-α, sTNFR1 and sTNFR2 were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications (Quantikine, R&D Systems, sensitivity: 0·7 pg . ml^{-1} for IL-6, 1·5 pg . ml^{-1} for sTNFR1, 1 pg . ml^{-1} for sTNFR2). A high sensitivity kit (Quantikine HS, R&D Systems, sensitivity 0·18 pg . ml^{-1}) was used to measure TNF-α. All samples were run in duplicate.

Cardiopulmonary exercise testing

At baseline, monthly and at the end of the combined endurance/resistance training programme (4 months), treadmill exercise, using an incremental loading protocol, was performed up to exhaustion. Depending on age, NYHA class, left ventricular ejection fraction and prior exercise testing, two protocols were used, aiming at an exercise duration of 8 to 10 min. After 1 min of walking

Table 2 Cytokine and cytokine receptor levels at baseline

	Trained HF group (n=23)	*P*	Untrained HF group (n=18)	Trained CAD (n=12)	*P*	Trained IDCM (n=11)
IL-6	2·1 (0·0–16·7)	0·9	2·2 (0·0–23·6)	2·9 (0·5–7·4)	0·5	2·1 (0·0–16·7)
TNF-*α*	3·5 (1·33–7·2)	0·8	2·8 (1·5–7·1)	3·7 (1·8–7·2)	0·2	3·1 (1·3–5·5)
sTNFR1	1228 (461–2494)	0·2	1650 (475–2816)	1310 (461–2214)	0·2	1138 (633–2494)
sTNFR2	2239 (1067–4105)	0·6	2240 (362–4123)	2562 (1394–4105)	0·09	1559 (1067–3690)

HF=heart failure, CAD=coronary artery disease, IDCM=idiopathic dilated cardiomyopathy, IL-6=interleukin-6, TNF-*α*=tumor necrosis factor-*α*, sTNFR1=soluble TNF-*α* receptor 1, sTNFR2=soluble TNF-*α* receptor 2.

at 20 or 40 Watts, incremental load (1 min) was set at 10 or 20 Watts, respectively. Exhaled air was analysed to determine metabolic gas exchange with a respiratory mass spectrometer (Jaeger Medical, type EOS Sprint). Ventilation (VE), oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were determined on-line every 15 s. The anaerobic threshold was defined as the start of a systematic increase in the VE/VO_2 relation, without a concomitant increase in VE/VCO_2. The ventilatory threshold was defined as the compensatory increase to lactic acidosis in VE/VCO_2. Patients were continuously monitored for ECG, whereas automatic blood pressure measurement was performed every 2 min.

The monthly repeated cardiopulmonary exercise test was used to re-evaluate heart rate achieved at ventilatory threshold. Accordingly, aerobic exercise intensity was adjusted (see Training protocol).

Statistical analyses

Data are given as median and range. The Mann–Whitney U test was used for comparisons of numerical data between groups. Pairwise comparisons were carried out using the Wilcoxon matched pair signed rank test. Correlations were determined using Spearman's rank correlation test. *P*-value <0·05 was considered statistically significant.

Results

Cytokine levels in patients and controls

Plasma concentrations of cytokines and soluble TNF-*α* receptors were significantly elevated in patients versus healthy controls (IL-6: $P=0·005$; TNF-*α*: $P=0·0002$, sTNFR1: $P=0·0009$; sTNFR2: $P=0·01$). At baseline, there were no differences between the trained and the untrained heart failure group, nor between the trained CAD and the trained IDCM group (Table 2).

The effects of a 4 month combined endurance/resistance training programme on cytokine and cytokine receptors are shown in Table 3. TNF-*α*, sTNFR2 and IL-6 plasma concentrations were not significantly changed. Comparison of sTNFR1 levels before and after the training period, however, showed a significant decrease ($P=0·01$). When patients were divided according to the aetiology of heart failure, there was a significant decrease for both sTNFR1 ($P=0·01$) (Fig. 1a)) and sTNFR2 ($P=0·02$) (Fig. 1(b)) concentrations in the CAD group, but not in the IDCM patients. Cytokines and cytokine receptors remained unchanged throughout the study period in the untrained heart failure group and in both untrained subgroups (Table 4).

Exercise capacity and maximum oxygen uptake

At baseline, patients with IDCM had higher VO_2 at anaerobic threshold ($P=0·01$) and at peak exercise ($P=0·03$), and a higher maximal workrate ($P=0·03$) than patients with CAD. Improved New York Heart Association (NYHA) functional class was observed in both patient groups (Table 5). Submaximal exercise performance, as expressed by workrate at the anaerobic threshold, increased with the training programme for the total population and both subgroups. Maximum workrate and workrate/VO_2 were significantly enhanced in both patient populations. Peak VO_2 and VO_2 at the anaerobic threshold only improved in the CAD group (Table 5).

Correlation between cytokines and exercise capacity

For the trained population, TNF-*α* and sTNFR2 were significantly correlated with peak VO_2 ($r=-0·58$, $P=0·007$; $r=-0·67$, $P=0·002$), VO_2 at anaerobic threshold ($r=-0·53$, $P=0·01$; $r=-0·77$, $P=0·0003$), maximal workrate ($r=-0·51$, $P=0·02$; $r=-0·69$, $P=0·001$) and workrate at anaerobic threshold ($r=-0·50$, $P=0·02$; $r=-0·81$, $P=0·0002$). sTNFR2 was correlated to workrate/VO_2 ($r=-0,51$, $P=0·02$).

In the trained CAD group, sTNFR1 changes significantly correlated with changes in work efficiency (watt/VO_2) ($r=-0·78$, $P=0·009$, Fig. 2). TNF-*α* and sTNFR2 changes correlated with changes in maximal workrate ($r=-0·64$, $P=0·03$; $r=-0·65$, $P=0·03$). TNF-*α* changes also correlated with changes in submaximal workrate ($r=-0·64$, $P=0·04$) and work efficiency (watt/VO_2) ($r=-0·71$, $P=0·02$).

Table 3 Cytokine and cytokine receptor levels at baseline and after 4 months training

	Total population (n=23)			CAD (n=12)			IDCM (n=11)		
	Basal	*P*	4 months	Basal	*P*	4 months	Basal	*P*	4 months
IL-6	2·1 (0·0–16·7)	0·7	1·3 (0·1–12·5)	2·9 (0·5–7·4)	0·8	2·1 (0·1–12·4)	2·1 (0·0–16·7)	0·4	1·2 (0·1–12·5)
TNF-*a*	3·5 (1·3–7·2)	0·5	3·3 (1·4–8·3)	3·7 (1·8–7·2)	0·8	3·8 (1·9–8·3)	3·1 (1·3–5·5)	0·3	2·2 (1·4–5·6)
sTNFR1	1228 (461–2494)	0·02	1039 (418–1860)	1310 (461–2214)	0·01	990 (418–1860)	1138 (633–2494)	0·6	1043 (523–1657)
sTNFR2	2239 (1067–4105)	0·2	1961 (982–4287)	2562 (1394–4105)	0·02	2025 (982–2658)	1559 (1067–3690)	0·5	1753 (1173–4287)

CAD=coronary artery disease, IDCM=idiopathic dilated cardiomyopathy, IL-6=interleukin-6, TNF-*a*=tumor necrosis factor-*a*, sTNFR1=soluble TNF-*a* receptor 1, sTNFR2=soluble TNF-*a* receptor 2.

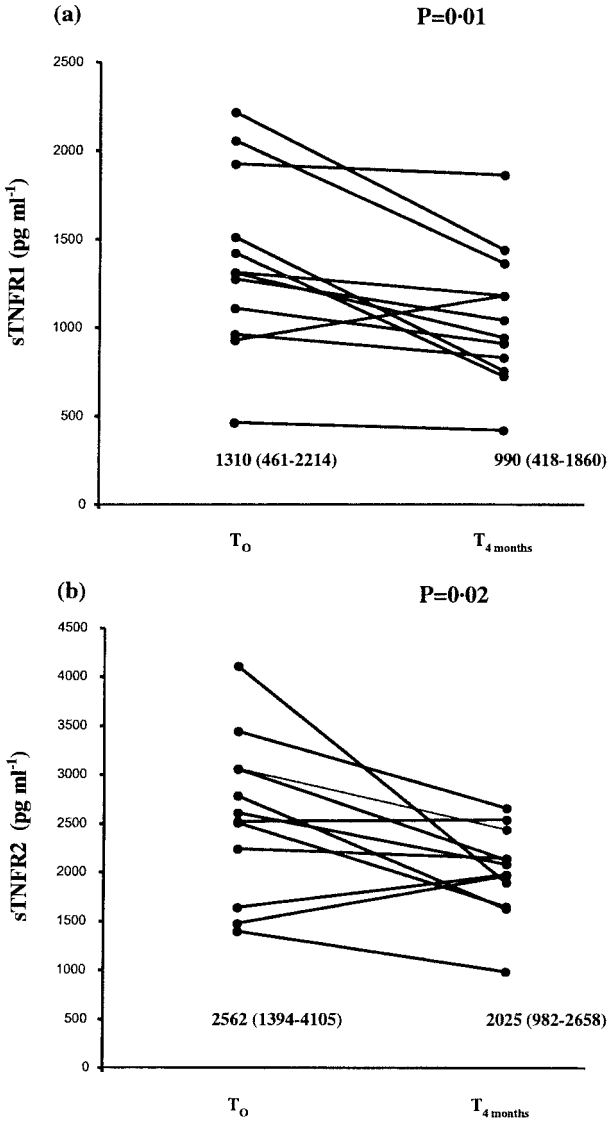

Figure 1 **(a) sTNFR1 levels before and after 4 months training in the CAD group. (b) sTNFR2 levels before and after 4 months training in the CAD group. CAD= coronary artery disease, sTNFR1: soluble tumor necrosis factor-*α* receptor 1. sTNFR2: soluble tumor necrosis factor-*α* receptor 2. T_0=baseline, T_{4mths}=after 4 months training.**

Conversely, in the trained IDCM group, changes in cytokines and cytokine receptors were not related to changes in exercise capacity.

Discussion

The present findings indicate that a 4-month combined endurance/resistance training programme has a favourable effect on circulating soluble TNF-receptors in patients with CHF due to CAD, but no effect in the case of IDCM. An improvement in submaximal and peak VO_2 was seen in the CAD group only. However, this particular training modality induced a highly significant

Table 4 Cytokine and cytokine receptor levels at baseline and after 4 months in the control group

	Untrained controls (n=18)			Controls with CAD (n=11)			Controls with IDCM (n=7)		
	Basal	*P*	4 months	Basal	*P*	4 months	Basal	*P*	4 months
IL-6	2·2 (0·0–23·6	0·6	1·5 (0·1–10·1)	3·7 (0·1–14·6)	0·6	3·5 (0·1–10·1)	1 (0·0–23·6)	1	0·4 (0·1–2·1)
TNF-*α*	2·8 (1·5–7·1)	0·5	3·1 (1·6–10·2)	2·3 (1·5–7·1)	0·6	2·3 (1·6–10·2)	4·5 (2·8–6·6)	0·7	4·4 (1·7–7·3)
sTNFR1	1650 (475–2816)	0·5	1319 (455–5732)	2028 (475–2816)	0·6	1490 (1167–5732)	956 (828–1729)	0·9	1161 (455–1492)
sTNFR1	2240 (362–4123)	0·1	1923 (564–4543)	2439 (1176–4123)	0·2	2159 (1038–4543)	1296 (362–2359)	0·3	1201 (564–1387)

CAD=coronary artery disease, IDCM=idiopathic dilated cardiomyopathy, IL-6=interleukin-6, TNF-*α*=tumor necrosis factor-*α*, sTNFR1=soluble TNF-*α* receptor 1, sTNFR2=soluble TNF*α* receptor 2.

improvement in NYHA functional class, submaximal and maximal workrate, and work efficiency in both subgroups.

Both systemic and local inflammation are pertinent players in the pathogenesis of CHF[5–7,16–18]. Strong prognostic power has been attributed to cytokines and more so, to their soluble receptors[5–7].

Aerobic exercise, as an adjunct to the pharmacological therapy of patients with CHF, enhances exercise capacity through improved peripheral vascular function and resolution of skeletal muscle abnormalities. Interestingly, Adamopoulos *et al.*[11] observed a reduction of granulocyte-macrophage-colony stimulating factor (GM-CSF), MCP-1, soluble intercellular adhesion molecule (sICAM)-1 and soluble vascular cell adhesion molecule (sVCAM)-1, after a 3 month home-based bicycle training programme in patients with moderate to severe CHF. These results were called upon by the investigators, to explain the beneficial effects on vascular reactivity and exercise performance[19]. The decrease in circulating levels of TNF-*α*, sTNFR1, sTNFR2, IL-6, soluble IL-6 receptor, soluble Fas and soluble Fas ligand, observed in another study[11], in which a similar training protocol was applied, could be secondary to this inhibition of monocyte-endothelial cell interaction. Recently, Larsen and colleagues demonstrated a significant reduction in elevated TNF-*α* levels in patients with symptomatic CHF, after a period of 3 months endurance training[13]. Taken together, these observations suggest that, besides shear stress-induced upregulation of endothelial nitric oxide (NO) synthase (eNOS)[20,21], the attenuation of inflammatory mediators through exercise training could be an important player in the reversal of peripheral vascular endothelial dysfunction. TNF-*α* induces the formation of oxygen free radicals, which rapidly scavenge and destroy NO, produced at the endothelial level. Anker *et al.*[22] have shown a relation between TNF-*α* concentration and impaired peak leg blood flow in patients with CHF. Moreover, serum of heart failure patients downregulates eNOS and increases endothelial apoptosis[23]. These effects are at least partly mediated by TNF-*α*

It has been shown that circuit weight training (cycle ergometry, treadmill walking and resistance weight training) induces significant improvements in exercise capacity, muscle strength[14], and peripheral vascular function[15]. In contrast to aerobic training, resistance training focuses on daily encountered activities, by means of improving submaximal endurance and enhancement of muscle mass and strength. A tailored resistance programme offers the opportunity to selectively train small muscle groups, thereby allowing relatively high loading, without imposing the stress of whole body exercise to the cardiovascular system.

We decided, therefore, to assess the influence of a combined endurance/resistance training programme on proinflammatory cytokines and cytokine receptors. For the total population, there were strong correlations between TNF-*α* and sTNFR2 on the one hand and submaximal and maximal exercise levels on the other hand. Concentrations of sTNFR1 and sTNFR2 showed a significant decrease after 4 months of training in the CAD group, whereas no changes were measured in the IDCM patients. Although at the present time hypothetical, one could presume that an intervention, aimed at increasing shear stress, could result in more profound inflammatory changes in patients, whose atherosclerotic peripheral conduit vessels are heavily infiltrated with inflammatory cells. Moreover, an increase in peak VO_2, a parameter depending on restoration of vascular reactivity[24], was only observed in the CAD group. The fact that exercise capacity at baseline was higher in the IDCM patients, possibly indicating that this was a subgroup with less severe disease, could also have influenced the observed less pronounced training-induced changes in this group.

Another interesting observation is the fact that changes in TNF-*α* and soluble TNF-receptors were correlated with workrate and work efficiency, whereas there was no relationship with changes in oxygen uptake. Since the former parameters depend on muscle mass and strength, it is tempting to presume that the anti-inflammatory effect of exercise training could reverse the detrimental consequences of heart failure-induced muscular adaptations. Indeed, Adams and colleagues[25,26] have shown a relation between the local induction of iNOS and IL-1*β* and the occurrence of apoptosis in skeletal muscle biopsies of patients with CHF. In addition, the same authors found that higher TNF-*α* levels correlated with a reduced cross-sectional area of the musculus quadriceps and with earlier muscle fatigue.

Why the beneficial effect of the implemented training programme in the present study is not reflected in lower

Table 5 Exercise capacity and maximum oxygen uptake at baseline and after 4 months training

	Total population (n=23)			CAD (n=12)			IDCM (n=11)		
	Basal	*P*	4 months	Basal	*P*	4 months	Basal	*P*	4 months
NYHA I–II/III–IV	11/12	0·0003	19·4	3/9	0·02	8/4	8/3	0·005	11·0
Peak VO_2	18·7 (9·7–28·7)	0·008	20·1 (13·3–28·2)	16·4 (9·7–25·0)	0·008	19·2 (13·3–28·2)	21·5 (15·3–28·7)	0·5	22·1 (15·1–26·6)
Max Watt	100 (60–200)	0·0001	160 (90–240)	80 (60–140)	0·002	140 (90–180)	100 (80–200)	0·02	160 (90–240)
Watt/VO_2	5·2 (4·1–8·5)	0·0005	6·6 (5·1–9·3)	5·1 (4·1–6·4)	0·008	6·8 (5·1–8·8)	5·2 (4·3–8·4)	0·03	6·5 (5·5–9·3)
VO_2 at AT	13·3 (9·0–22·1)	0·07	15·2 (9·8–19·8)	12·0 (9·0–16·4)	0·002	15·4 (9·8–19·0)	14·5 (10·4–22·1)	0·4	14·1 (10·1–19·8)
Watt at AT	60 (30–120)	0·0002	100 (40–160)	55 (30–80)	0·003	80 (50–100)	80 (40–120)	0·03	100 (40–160)

CAD=coronary artery disease, IDCM=idiopathic dilated cardiomyopathy, VO_2=oxygen uptake (ml . kg^{-1} min^{-1}), AT=anaerobic threshold.

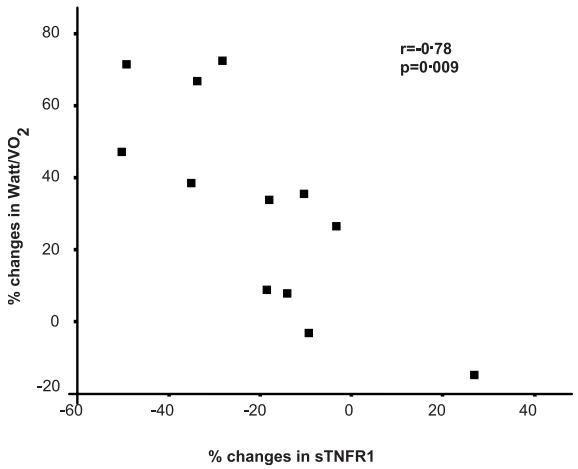

Figure 2 **Relation between changes in sTNFR1 concentration and changes in work efficiency in the CAD group. sTNFR1=soluble TNF-α receptor 1, CAD=coronary artery disease.**

concentrations of TNF-α and IL-6 immunoactivity warrants further discussion. First, one should recognize that commercially available TNF-α enzyme-linked immunoassays are variably influenced by the presence of soluble TNF-α receptors[27,28]. The ELISA system we used (Quantikine, R&D System) measures the immunoactivity of free trimeric TNF-α and trimeric TNF-α bound to soluble receptors. It follows, that from our measurements, we cannot exclude lowering of circulating free bioactive trimeric TNF-α. Second, the investigation of the TNF-α and IL-6 system in plasma is hazardous. As was shown by Dibbs *et al.*[29], natural variability in serum concentrations of TNF-α and IL-6 in patients with CHF is considerable. It follows that greater sample sizes are required to reliably evaluate the influence of an intervention on TNF-α and IL-6 concentrations than for both soluble TNF-receptors.

Since TNF-receptors are shed upon binding with their ligand TNF-α, it is hypothesized that these circulating TNF-receptors thus reflect tissue exposure to TNF-α. In addition, it has been suggested that circulating TNF-receptors could serve as a temporary buffer for circulating TNF-α, but in the long term could act as a TNF-reservoir, providing a slowly released continuous source of biologically active cytokine.

Conclusion

In the present study, we documented the anti-inflammatory effect of a 4 month combined endurance/resistance training programme in patients with moderate to severe heart failure due to CAD. Although no effect was demonstrated on concentrations of cytokines and TNF-receptors in patients with IDCM, further investigation of larger patient groups with more advanced chronic heart failure is warranted to clarify this issue. The observed highly significant increase of submaximal

and maximal exercise parameters and the improvement in NYHA functional class should provide a stimulus for the implementation of this particular training modality in CHF patients.

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