J Appl Physiol 102: 2033–2039, 2007.
First published 25 January 2007; doi:10.1152/japplphysiol.00871.2006.

Endurance training restores peritoneal macrophage function in post-MI congestive heart failure rats

M. L. Batista, Jr.,[1,4] R. V. T. Santos,[1,3] E. M. Oliveira,[2] M. C. L. Seelaender,[1] and L. F. B. P. Costa Rosa†

[1]*Molecular Biology of the Cell Group, Institute of Biomedical Sciences, and* [2]*Department of Biodynamic of the Human Body Movement, School of Sport and Physical Education, University of São Paulo;* [3]*Research Center of Psychobiology and Sports, Paulista School of Medicine, Federal University of São Paulo; and* [4]*School of Physical Education, University of Mogi das Cruzes, São Paulo, Brazil*

Submitted 7 August 2006; accepted in final form 18 January 2007

Batista ML Jr, Santos RV, Oliveira EM, Seelaender MC, Costa Rosa LF. Endurance training restores peritoneal macrophage function in post-MI congestive heart failure rats. *J Appl Physiol* 102: 2033–2039, 2007. First published 25 January 2007; doi:10.1152/japplphysiol.00871.2006.—Congestive heart failure (CHF) induces a state of immune activation, and peritoneal macrophages (Mϕs) may play an important role in the development and progression of one such condition. Moderate endurance training modulates peritoneal Mϕ function. We evaluated the effect of endurance training on different stages of the phagocytic process and in the production of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) after LPS stimulation. Either ligation of the left coronary artery or Sham operations were performed in adult Wistar rats. After 4 wk, control (Sham operated) and MI (ligation of the left coronary artery) animals were randomly assigned to either a sedentary (Sham-operated sedentary, $n = 7$ and MI sedentary, $n = 10$) or a trained group (Sham-operated trained, $n = 8$ and MI trained, $n = 8$). Trained rats ran on a treadmill (0% grade at 13–20 m/min) for 60 min/day, 5 days/wk, for 8–10 wk, whereas sedentary rats had only limited activity. Training increased maximal oxygen uptake normalized for body weight ($ml \cdot kg^{-1} \cdot min^{-1}$), as well as skeletal muscle citrate synthase maximal activity, when compared with sedentary groups. The resident and total cell number, the chemotaxis index, and the production of TNF-α stimulated by LPS were significantly higher in the MI sedentary group when compared with the Sham sedentary group. Moderate endurance training reversed these alterations promoted by post-MI. These results demonstrate that moderate intensity exercise training modulates peritoneal Mϕ function and induces beneficial metabolic effects in rats with post-MI CHF.

pro-inflammatory cytokines; migration; chemotaxis index; phagocytic capacity; exercise adaptation; myocardial infarction

MACROPHAGES (MΦS) are widely recognized as cells that play a central role in the regulation of immunity and inflammation, as well as in tissue remodeling. In response to endotoxin LPS, Mϕs secrete pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and interleukin-12 (IL-12) (1, 8, 9, 19). Chronic systemic inflammation may affect some of the other functions of Mϕs, namely adherence to tissue, chemotaxis, and digestion of foreign agents (40). The exact role of Mϕs in the progression of congestive heart failure (CHF) has been recently elucidated, showing that these cells may serve as an important source of cytokines and growth factors (2, 3, 15, 21). In addition to that, evidence has given force to the role of the innate immune system, mainly monocyte and Mϕ cells, in the pathogenesis of CHF (3, 37).

The beneficial effects of endurance exercise training on patients with CHF are well documented. These effects include increases in maximal exercise performance, maximal oxygen uptake ($\dot{V}O_{2\,max}$), maximal stroke volume, and maximal conductance to the working skeletal muscles (22, 25). Rats respond to endurance exercise training by increasing both $\dot{V}O_{2\,max}$ and skeletal muscle oxidative enzyme activities (22). In addition, the presently adopted rat model of coronary artery occlusion provided animals with histologically well-healed infarctions for determining the relation between infarct size and ventricular performance (21, 23). That procedure induces moderate to severe CHF within 21–42 days after myocardial infarction (MI) (6, 22). In the same way, moderate intensity exercise training enhances immune function, whereas intense exercise training depresses the immune system (27, 29). With regards to the influence of endurance training on the immune function, the results of animal experiments have shown an increase in phagocytic activity and in chemotaxis of peritoneal Mϕs, as well as of the proliferative response of T cells to mitogens (12, 14, 35, 36). On the other hand, few studies have evaluated the immunomodulatory effect of exercise training on peritoneal Mϕ function, since activated Mϕs are important sources of pro-inflammatory factors.

The aim of the present work was to study the effect of moderate endurance training on pro-inflammatory cytokine production and different stages of the phagocytic process of peritoneal Mϕs in rats with 14-wk post-MI CHF as induced by MI.

MATERIALS AND METHODS

Animals

A total of 33 male Wistar rats ranging in age from 6 to 8 wk (weighing ~250 g), obtained from the Animal Breeding Unit, Institute of Biomedical Sciences, University of São Paulo, were used. They were housed, five per cage, with food and water ad libitum, in an animal room under a 12-h light-dark cycle at a temperature of 22 ± 1°C and humidity of 60 ± 5%. The experiment was carried out after acclimation for a week. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Animal Use and Care Committee of

† Deceased May 19, 2005.

Address for reprint requests and other correspondence: Miguel Luis Batista Júnior, 1524 Ave. Lineu Prestes, CEP 05508-900, Butantã, São Paulo, SP, Brazil (e-mail: migueljr@usp.br).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

the Institute of Biomedical Sciences, University of São Paulo (Protocol No. 067/2002).

Surgical Preparation

Rats were initially anesthetized with 3% halothane, intubated via tracheotomy, placed under a rodent respirator apparatus (Harvard model 680), and maintained on a 2% halothane-oxygen mixture. The heart was exposed through left thoracotomy between the fifth and sixth ribs (1.5 cm in diameter), and the pericardium was opened. In animals in which a MI was produced, a 9-0 Ethilon suture was placed under the left main coronary artery at a point 1–2 mm distal to the edge of the left atrium, and the artery was ligated (30). Sham-operated animals underwent the same procedure, except that the suture under the coronary artery was left untied. Muscle and skin incisions were closed with separate purse-string silk sutures (size 0), and the lungs were fully expanded. The heart was then returned to its normal position and the thorax immediately closed. Each animal was allowed a minimum of 4 wk of recovery.

Experimental design. After MI preparation, animals were subjected to surgery. After 4 wk (this being the necessary time to achieve the development to chronic heart failure state) (31), they were randomly assigned to either a sedentary or training group. Some of them were then kept in sedentary control groups (Sham-operated sedentary, $n = 7$ and MI sedentary, $n = 10$) and some underwent an endurance training protocol (Sham-operated trained, $n = 8$ and MI trained, $n = 8$).

Determination of Endurance Exercise Capacity

As running on a treadmill requires adaptation, all rats were exercised on the treadmill (Columbus Instruments, Columbus, OH) before the experiment at a speed of 20 m/min and 5° elevation for 5 min/day, 3 days/wk, for 2 wk. Such workload induces no training adaptations (18) but familiarizes the rats with treadmill running. Six weeks after MI or Sham operation, all rats performed a treadmill exercise test until fatigue, which was initiated between 9:00 and 10:00 AM. The exercise protocol consisted of a graded running test in which each rat ran initially at 0% grade, and at a speed of 15 m/min, for 10 min. Thereafter, the treadmill speed was increased 5 m/min every 10 min until each animal reached the point of fatigue. The criterion for fatigue was the rat's inability to keep pace with the treadmill, thus being unable to avoid the electric stimulus (volts) for a period longer than 15 s. The same investigator was responsible for the exercising of the rats 12–14 wk after infarction and did not know whether the animal being tested was a post-MI rat or a Sham-operated rat.

Treadmill Testing ($\dot{V}O_{2\,max}$)

$\dot{V}O_{2\,max}$ was determined by having each rat perform a maximal exercise test adapted from Musch et al. (22). The parameters were measured using the Oxymax gas analyzing system for small animals (Columbus Instruments). The test was always carried out after a 1-day recovery period. The volume of the supplied air was 4.5 l/min. The gas analyzer was calibrated with a reference gas mixture before each test. The $\dot{V}O_{2\,max}$ test protocol involved stepwise increasing of the treadmill speed as follows: after a 15-min period of acclimation, the treadmill was then started at 10 m/min, and the speed was incrementally increased 5 m/min every 3 min until the rat reached exhaustion. Exhaustion was defined as spending time on the shocker plate without attempting to reengage the treadmill within 15 s. The highest $\dot{V}O_{2\,max}$ measured at each workload was taken as a measure of each rat's running economy ($\dot{V}O_{2submax}$) for that workload, and at the last step, as $\dot{V}O_{2\,max}$.

Aerobic Training Program

Rats in the training groups ran for 5 days/wk for 8 wk at a work rate that ranged between 55 and 65% $\dot{V}O_{2\,max}$. $\dot{V}O_{2\,max}$ was determined for each rat at the end of 2 wk of training, and work loads were adjusted upward according to the increase found in $\dot{V}O_{2\,max}$. On the first day of training, all the rats ran for 30 min. On the subsequent days of training the running time was extended 10 min each day until all the rats were running 60 min/day. Familiarization with the treadmill was maintained also in the sedentary groups by having each rat run on the treadmill (0% grade) for 10 min/day, 2 days/wk at a speed of 15 m/min. After a rest period of 48 h after the last workout session, the animals were killed by decapitation without anesthesia.

Tissue Weight Determination

To obtain the wet/dry weight ratio of the lungs and liver, these organs were removed and freed from adhering tissues. In each case, the sample tissue was weighed, dissected into smaller pieces, and placed in the oven at 65° until a constant weight was obtained, which was usually after ~24 h.

Determination of Left Ventricular Infarct Size

After fixation in formalin for a minimum of 24 h, the left ventricle was separated into four transverse sections from base to apex in parallel with the atrioventricular groove. The four sections of the left ventricle were then dehydrated in alcohol, cleaned in xylene, and embedded in paraffin. Transverse sections (10 μm thick) were obtained, mounted, and stained with Masson's trichrome stain from which hematoxylin was omitted to provide maximum discrimination between fibrous area of infarct and muscle. These sections were then measured with a planimeter Digital Image Analyzer (Carl Zeiss), according to the technique described by Pfeffer et al (30).

Collection of Peritoneal Suspensions

The abdomen was cleansed with 70% ethanol, the abdominal skin was carefully dissected without opening the peritoneum, and 6 ml of PBS adjusted to pH 7.4 were injected intraperitoneally. The abdomen was massaged, and 90–95% of the injected volume was recovered. The peritoneal resident Mϕs, identified by morphology and nonspecific esterase staining, were counted and then adjusted in the same medium to 5×10^6 Mϕs per ml. Cell viability was confirmed by the Trypan-blue exclusion test (>95%). At least 92% of the peritoneal exudate cells were Mϕs.

Preparation of Zymosan (Saccharomyces cerevisiae)

Thirty-five milligrams of zymosan in 100 ml PBS (Sigma, St Louis, MO, U.S.A.) were boiled for 30 min and washed twice with PBS prior to use. Subsequently, Zymosan particles were resuspended in PBS containing Ca^{2+} and Mg^{2+} to provide a concentration of 14 mg/ml, and the solution was stored at 4°C. Normal serum used for opsonization was stored at −20°C. For opsonization, 0.5 ml of zymosan particles (14 mg/ml PBS) were mixed in 0.5 ml rat serum and incubated for 30 min at 37°C. The opsonized zymosan particles were then washed, resuspended in PBS at a concentration of 1 mg/ml, and stored for up to 7 days at 4°C.

Determination of Citrate Synthase Activity

Citrate synthase (CS) activity, an index of oxidative capacity, was determined for the soleus muscle of each rat. Tissue samples were homogenized at 0°C in a volume of 100 mM KPO_4 buffer so that a 1:20 (wt/vol) homogenate was obtained. CS activity was measured according to the spectrophotometric method described by Srere (34). All assays were linear with respect to time and dilution, and each sample was analyzed in duplicate.

Assays and Tests of Macrophage Function

Adherence index. For the quantification of adherence capacity, aliquots of 100 μl of peritoneal suspension (5×10^5 Mϕs/ml of RPMI

1640 medium) were enriched with glutamine (2 mM) and 10% homologous serum. After 1 h of incubation at 37°C and 5% CO_2, 10 μl of each sample were removed after gently shaking to resuspend sediment cells and the number of non-adhered Mϕs was determined. The adherence index was calculated according to the equation proposed by De la Fuente et al (11).

Chemotaxis index. Chemotaxis was evaluated by a modification (11) of the original technique described by Boyden (5), which consists basically of the use of chambers with two compartments separated by a Millipore filter with a pore diameter of 3 μm. Aliquots of 100 μl of the peritoneal suspension (5×10^5 Mϕs/ml of RPMI 1640 medium) were deposited in the upper compartment of a Boyden chamber. Aliquots of 300 μl of N-Formyl-Met-Leu-Phe (FMLP; Sigma, 10^{-8} M) were placed in the lower compartment to induce chemotaxis. After 3 h of incubation at 37°C and 5% CO_2, the filter was fixed and stained. The chemotaxis index, representing the total number of Mϕs counted at random in 16 fields of the lower face of the filters, was calculated.

Phagocytosis. Resident Mϕs were incubated with 10 ml PBS containing opsonized zymosan and in the presence of glucose (5 mM) and glutamine (2 mM) for 30 min at 37°C, so that phagocytosis could be quantified by counting (in a counting chamber) the percentage of cells that had phagocytosed more than four particles of zymosan (10).

Production of IL-6, IL-1β, and TNF-α. To determine the cytokine production, peritoneal Mϕs (100 μl) at a concentration of 5×10^5 Mϕs/ml of RPMI 1640 medium were resuspended in RPMI 1640 medium enriched with glutamine (2 mM) and 10% homologous serum and incubated at 37°C with 5% CO_2 in humidified air for 48 h with 50 μl of LPS (10 μg/ml). The samples were frozen and stored at −80°C. TNF-α, IL-1β, and IL-6 activity in culture supernatants was measured by ELISA using a commercial rat kit for TNF-α, IL-1β, and IL-6 detection (Quantikine Elisa Kits, R&D System, Abingdon, UK).

Statistical Analysis

The statistical analysis was performed by using a commercially available statistical package from SigmaStat (version 3.1, SigmaStat, SYSTAT, Point Richmond, CA). Data were expressed as means ± SD. A primary observation indicated the results of experiments to be distributed normally. Posttraining measurements were analyzed by 2-way ANOVA of 2 × 2 design (sedentary/trained vs. sham-operated/MI). With this type of analysis, the data were partitioned into main effects (sedentary vs. trained group effects, A; and MI vs. Sham group effects, B). The interaction effects consisted of A × B.

When a significant *F* value was found by 2-way ANOVA, a Holm-Sidak post hoc test was performed to demonstrate all pairwise multiple comparisons between the means. The 0.05 probability level was considered to indicate statistical significance.

RESULTS

All the animals were weighed at the end of experiment: Sham-operated (sedentary: 319 ± 36 g, $n = 7$ and trained: 332 ± 35 g, $n = 8$) and MI (sedentary: 377 ± 65 g, $n = 10$ and trained: 321 ± 26 g, $n = 8$). Among the four groups, no differences could be detected regarding the mean weight at each measurement time (Table 1).

Left ventricle (LV) infarct size, as an indicator of a structural index of LV dysfunction and post-MI, was assessed for all rats in the different groups. For MI sedentary and MI trained groups, it was 38.7 ± 5.3% and 37.2 ± 4.8%, respectively (Table 1). No detectable infarcts were found in the sedentary and trained sham-operated rats. Heart weight showed significant MI vs. Sham-operated group effects with no difference in sedentary vs. trained group effects. These results indicate a greater heart weight in the MI sedentary group when compared with the Sham-operated sedentary group (1.12 ± 0.17 and 0.90 ± 0.04 mg, respectively; $P < 0.05$). Although there was a strong trend for greater heart/body wt ratios in the MI sedentary group when compared with the Sham-operated sedentary group, this difference was not significant ($P = 0.07$). Moreover, these increases in heart weight coincided with increases in lung and liver wet/dry weight ratios (7% and 4%, respectively; $P < 0.05$), demonstrating that these animals had significant pulmonary and liver congestion.

Decrements in exercise performance (endurance exercise capacity) were found in the MI sedentary (25%, $P < 0.05$) rats compared with their Sham-operated counterparts after the training protocol period (14 wk post-MI). These decrements were not reflected in $\dot{V}O_{2\,max}$ (no difference in MI vs. Sham-operated), although a strong trend toward ($P = 0.076$) reduction in the MI sedentary group was found.

$\dot{V}O_{2\,max}$ for the different groups showed significant sedentary vs. trained group effects (Fig. 1*A*). $\dot{V}O_{2\,max}$ was 13% and 15% higher in Sham-operated trained and MI trained rat groups, respectively, than in their sedentary counterparts after moderate endurance training ($P < 0.05$).

The decrements in exercise performance that were found in the MI sedentary group were restored in the MI trained group, demonstrating a significant sedentary vs. trained group effect (81%, $P < 0.001$) after this period of training. An effect of moderate endurance training was found in the Sham-operated trained group, whose performance increased by 66% ($P < 0.01$) compared with their sedentary counterpart ($P < 0.01$; Fig. 1*B*).

CS maximal activity measured in the soleus muscle showed a significant sedentary vs. trained group effect (Fig. 2). CS activity in the Sham-operated trained and MI trained was 16.7% and 29.4% greater than in their sedentary counterparts, respectively ($P < 0.05$).

Total cell and resident Mϕ counts were evaluated from peritoneal suspensions. Total cell count in the 14th week of the experiment showed significant MI vs. Sham-operated group

Table 1. *Left ventricular infarct sizes, body weights, heart weights, heart weight-to-body weight, lung wet-to-dry weight, and liver wet-to-dry weight ratios in MI and Sham-operated sedentary and trained rats*

	Infarct Size, %	Body Wt, g	Heart Wt, g	Heart Wt/Body Wt, mg/g	Lung Wet/Dry Wt	Liver Wet/Dry Wt
Sham-operated rats						
Sedentary ($n = 7$)		319.3±35.9	0.90±0.04	2.99±0.31	4.12±0.17	3.04±0.07
Trained ($n = 8$)		332.3±35.2	0.98±0.05	2.98±0.51	4.18±0.18	3.00±0.08
MI rats						
Sedentary ($n = 10$)	38.7±5.3	377.2±65.3	1.12±0.15†	3.31±0.45	4.41±0.15†	3.24±0.07†
Trained ($n = 8$)	37.2±4.8	321.9±26.9	1.18±0.18	3.68±0.78	4.37±0.20	3.12±0.08

Values are means ± SD; n = number of rats. MI, myocardial infarction. †$P < 0.05$: MI effect, MI vs. Sham-operated (two-way ANOVA).

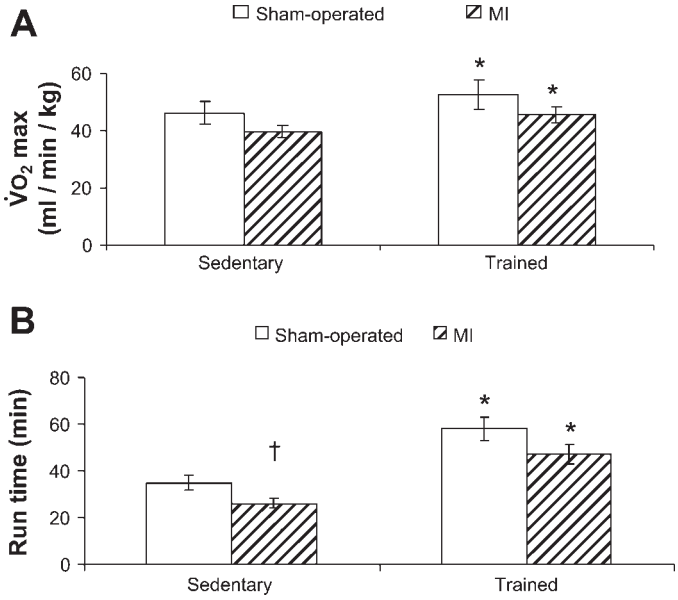

Fig. 1. Maximal oxygen uptake ($\dot{V}O_{2\,max}$) measures of Sham-operated (sedentary, $n = 7$ and trained, $n = 8$) and myocardial infarction (MI) (sedentary, $n = 10$ and trained, $n = 8$) rats were performed (*A*). Similarly, run time to fatigue was determined for the same animals (*B*). Values are mean ± SD. *$P <$ 0.05: training effect, sedentary vs. trained (two-way ANOVA). †$P < 0.05$: MI effect, MI vs. Sham-operated (two-way ANOVA).

effects. The total cell count was 69.2% higher in MI sedentary rats in relation to the Sham-operated counterparts ($P < 0.05$); however, no significant sedentary vs. trained group effects were found. Post-MI also increased the resident Mϕ count in the peritoneal cavity, the MI sedentary group having shown an increase in peritoneal Mϕ count of approximately 2.1-fold compared with the Sham-operated sedentary rats ($P < 0.05$; Table 2). The Resident Mϕ number was reduced by 33.5% in the MI trained group compared with the sedentary group ($P <$ 0.01). There was, however, no difference in the Sham-operated trained animals when compared with their sedentary counterparts.

Adherence and phagocytosis were determined in peritoneal Mϕs (Table 2). No differences in adherence were found regarding training or group effects.

The chemotaxis index obtained in peritoneal Mϕs incubated with FMLP was 1.3-fold greater in the MI sedentary group than in the Sham-operated sedentary group ($P < 0.001$). Significant sedentary vs. trained group effects and MI vs. Sham-operated group effects were found. Moderate endurance training reduced the chemotaxis index by 54.8% ($P < 0.001$) in MI trained animals, compared with MI sedentary animals, with no difference in the Sham-operated groups (sedentary vs. trained) (Fig. 3).

Regarding peritoneal Mϕ phagocytosis capacity (Table 2), we report significant sedentary vs. trained group effects and MI vs. Sham-operated group effects. The MI group showed a higher percentage of phagocytosis index when compared with the Sham-operated groups, and the trained groups also showed a higher percentage when compared with their sedentary counterparts.

Figure 4 shows TNF-α (*A*), IL-1β (*B*) and IL-6 (*C*) production by peritoneal Mϕs cultured for 48 h and stimulated with LPS. TNF-α production by peritoneal Mϕs yielded MI vs. Sham-operated group differences of statistical significance, as well as sedentary vs. trained effects. TNF-α production of the MI sedentary group cells was significantly higher (2.6-fold) than that of the Sham-operated sedentary group ($P < 0.001$). After a multiple comparison procedure to isolate which groups differed from the others (Holm-Sidak method), moderate endurance training was found to be significantly effective in reducing (80.1%) TNF-α production in the MI trained animals, compared with their sedentary counterparts ($P < 0.05$). IL-6 production of peritoneal Mϕs stimulated by LPS also showed MI vs. Sham-operated group effects. However, there was no effect of training. IL-6 production by cells of the MI sedentary group was significantly higher (1.5-fold) than that of the Sham-operated sedentary group ($P < 0.001$). There was no difference in IL-1β production in any of the conditions evaluated.

DISCUSSION

The purpose of the present study was to evaluate whether or not a program of treadmill exercise training would produce immunomodulatory effects on peritoneal Mϕ function in an experimental model of post-MI. The results provide evidence that Mϕ function was effected by the protocol, which induced an overproduction in pro-inflammatory cytokines stimulated by LPS, as well as a decrease in the chemotaxis index and in the Mϕ count in the peritoneal cavity and pro-inflammatory cytokine production, all parameters which were increased by MI and attenuated by exercise training.

Quantification of Post-MI CHF

Musch et al. (22) showed that the rat may compensate much of the tissue loss caused by a large MI through cellular hypertrophy of the remaining tissue, but when the size of the MI reaches 31 to 45% of the myocardium, the amount of hypertrophy that can occur may not always be enough to return the index of systolic functions back to normal, inducing elevated left ventricular end-diastolic pressures at rest and during exercise, lower mean arterial pressure at rest, lower maximal heart rate, and lower $\dot{V}O_{2\,max}$ values. Previous study (3) demonstrated that a MI size higher than 35% is positively correlated with accentuated left ventricular dysfunction, as noted by progressive left ventricular dilatation accompanied by a marked decrease in fractional ejection and shortening.

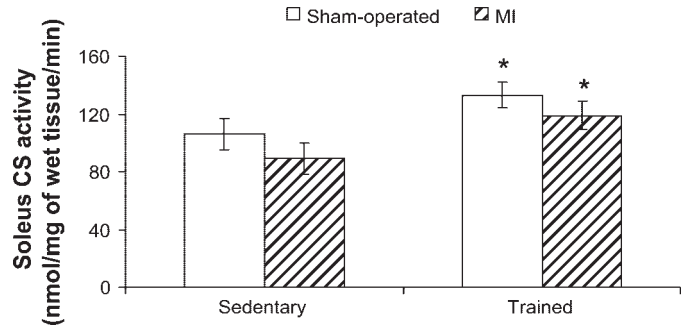

Fig. 2. Citrate synthase (CS) activity measures in the soleus muscle of Sham-operated (sedentary, $n = 7$ and trained, $n = 8$) and MI (sedentary, $n =$ 10 and trained, $n = 8$) rats were performed. Values are mean ± SD. *$P < 0.05$: training effect, sedentary vs. trained (two-way ANOVA).

Table 2. *Total cell number, number of macrophages, and adherence and phagocytosis indexes in MI and Sham-operated sedentary and trained rats*

	n	Total Cell Count, × 10^4	Mϕ Count, × 10^4	Adherence, %	Phagocytosis, %
Sham-operated rats					
Sedentary	7	1.274±0.304	0.817±0.225	61±7	41±4
Trained	8	1.240±0.431	1.020±0.321	69±7	43±5
MI rats					
Sedentary	10	2.155±0.431	1.682±0.340†	57±6	52±2†
Trained	8	1.867±0.325	1.118±0.220*	68±7	67±2†

Values are means ± SD; *n* = number of rats. Mϕ, macrophage. *$P < 0.05$, training effect, sedentary vs. trained (two-way ANOVA); †$P < 0.05$, MI effect, MI vs. Sham-operated (two-way ANOVA).

Aspects of our data suggest that MI rats have moderate CHF. To support this conclusion we point to several facts. First, the LV infarct size in the MI group was 39% and 37% (sedentary and trained rats) of the total LV, respectively (positive correlation with left ventricular dysfunction). MI rats showed higher heart weight, suggesting progressive LV dilatation. Second, the lung and liver wet/dry weight ratio was elevated, indicating lung and liver congestion (failure stage). Finally, the maximal exercise capacity was lowered, as indicated by the early onset of fatigue compared with sham-operated control animals. At this point, it's interesting to note that, even with no changes in $\dot{V}O_{2\,max}$ induced by MI, there was a strong trend toward reduction of this parameter in MI sedentary rats ($P = 0.076$), as shown also by other studies (22, 23). This may be due to different protocols, since we have not used grade increment to increase the intensity in the maximal exercise test. In this way, we may assume that the post-MI rats used in the present study may be classified as having moderate CHF.

Effects of Endurance Exercise Training

In the animal model of CHF induced by MI, the rats respond to moderate endurance training by increasing both $\dot{V}O_{2\,max}$ and skeletal muscle oxidative enzyme activities (metabolic changes) (22). However, these effects of training have been proposed to be dependent on training variables (intensity, volume). Furthermore, higher intensity, up to 70% $\dot{V}O_{2\,max}$ (moderate to high), has been shown to cause, besides metabolic adaptations, central cardiac adaptation such as decrement of maximal heart rate and downregulation of cardiac myosin isozyme V_1 isoform towards the slower ATPase (V_2 and V_3) isoforms (23, 24).

The current training protocol should be classified as one of light to moderate intensity (50–60% $\dot{V}O_{2\,max}$) and duration (60 min/day, 5 days/wk) if compared with those used in previous protocols (22, 24); however, it was indeed intense enough to induce some training effects in trained rats, both in those with infarction and in those that underwent sham operations, when compared with their sedentary counterparts. Such effects included increases in relative $\dot{V}O_{2\,max}$ values, along with in-

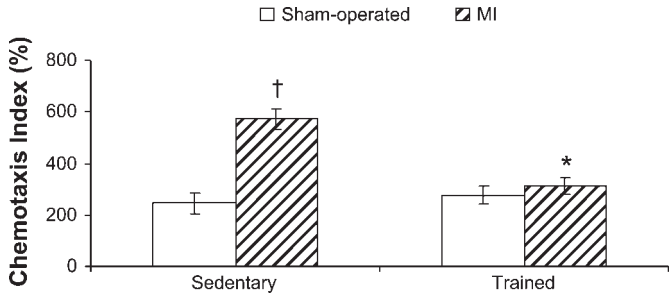

Fig. 3. Chemotaxis capacity of Sham-operated (sedentary, $n = 7$ and trained, $n = 8$) and MI (sedentary, $n = 10$ and trained, $n = 8$) rats from peritoneal macrophages (Mφs) incubated with N-Formyl-Met-Leu-Phe (FMLP; 10^{-8} M) for 3 h. Values are means ± SD. *$P < 0.05$: training effect, sedentary vs. trained (two-way ANOVA). †$P < 0.05$: MI effect, MI vs. Sham-operated (two-way ANOVA).

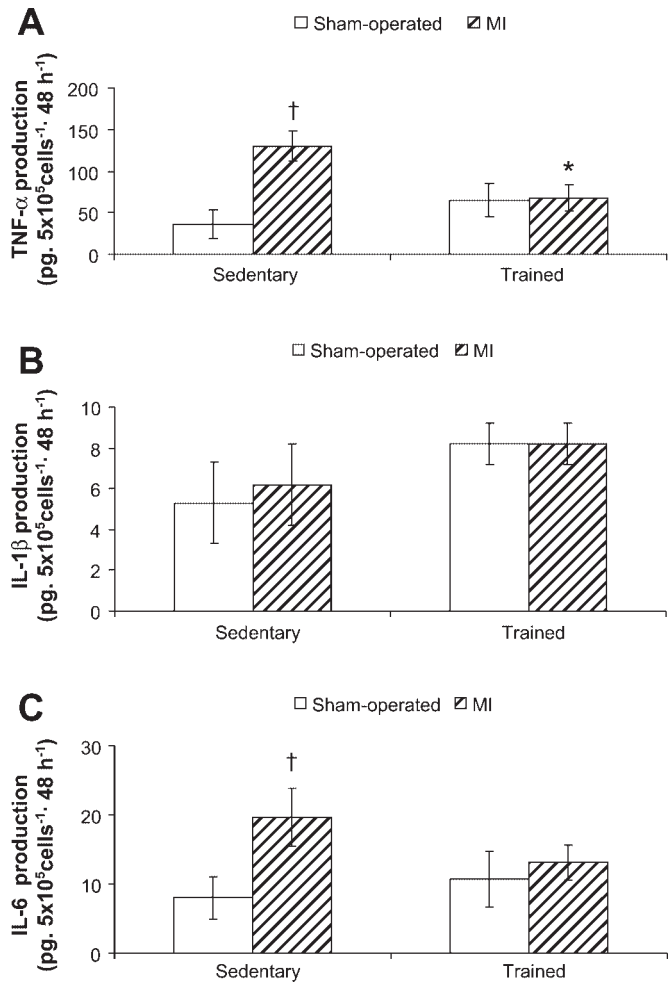

Fig. 4. Cytokines production of TNF-α (*A*), interleukin-1β (IL-1β) (*B*), and interleukin-6 (IL-6) (*C*) of Sham-operated (sedentary, $n = 5$ and trained, $n = 6$) and MI (sedentary, $n = 7$ and trained, $n = 6$) rats from peritoneal Mϕs incubated with LPS (10 μg/ml) for 48 h. *$P < 0.05$: training effect, sedentary vs. trained (two-way ANOVA). †$P < 0.05$: MI effect, MI vs. Sham-operated (two-way ANOVA). Values are means ± SD.

creases in CS enzyme activitiy in the soleus muscles. Besides the cardiovascular benefits, regular exercise training seems able to induce immunomodulation and enhanced immune competence (13, 16, 25, 27) in some pathological conditions, notably in the presence of an abnormal inflammatory response and immune activation (3, 16, 20, 25).

Effects on Macrophage Function

In the experiments with this animal model of post-MI CHF we found increased total cell count and migration. Neibauer et al., (26) suggests that the bacterial LPS (endotoxin) contributes to immune activation in CHF. Acute venous congestion could lead to altered gut permeability for bacteria, endotoxin, or both, and consequent translocation into circulation. Additionally, other studies with a smaller number of patients found a trend towards a cellular (monocyte) hypersensitivity to LPS in patients with stable CHF (14, 38). In addition to that, increases in peritoneal Mϕ count and in the total number of cells in the post-MI CHF animal model suggest an important role of these cells in the presence of a possible chronic inflammatory condition, as well as the effectiveness of endurance training in restoring peritoneal Mϕ count, yielding values close to those presented by Sham-operated sedentary rats. These facts suggest a specific training effect in the MI group that was not present in the Sham-operated group. However, whether this is possibly correlated with LPS concentration in the blood or peritoneal cavity is a matter requiring further investigation.

Reports on peritoneal Mϕ function following exercise training are rather controversial. The response depends on the variables of physical exercise such as intensity, volume and frequency, along with the exercise type. Several studies, however, have shown that regular moderate exercise stimulates many aspects of the immune response (27, 29, 33, 36).

Chemotaxis refers to the directed movement of cells along a concentration gradient of a "chemotactic factor." Chemotactic factors, or chemottractants, initiate mononuclear phagocyte migration after binding to specific receptors on the cell surface, and they are vital for host defense. Increased expression of surface receptors is known to be one of the earliest events in Mϕ activation, preceding other functional changes such as superoxide anion production or degranulation (7, 39). We have previously reported changes in Mϕ chemotactic activity, having shown significant increases in chemotactic activity, together with higher hydrogen peroxide production and phagocitosis index in CHF rats (3). The altered chemotaxis could be a facilitating factor for the development of bacterial (endotoxin) or fungal infections and life-threatening host defense problems (39).

The chemotatic capacity (in vitro) of peritoneal Mϕs was higher in MI rats than in the Sham-operated animals, corroborating with the data showing increase of total Mϕ count in peritoneal cavity, altogether suggesting presence of chronic inflammatory state in the post-MI CHF rats. In the present study, enhancement of chemotaxis capacity by peritoneal Mϕs in response to the sensing of FMLP was reversed by moderate endurance training, reestablishing the values to levels near those found in the Sham-operated sedentary animals. Some studies (14, 35, 36) have shown an increase in the chemotaxis index induced by exercise training stress; however, it appears that the modulatory effect of exercise on the Mϕs is dependent on the parameter being measured, the timing, the intensity and duration of the effort, and the concentration and type of chemokines being utilized (40).

The present study is, to our knowledge, the first to show the capacity of exercise to revert post-MI effects toward baseline values for chemotaxis index in peritoneal Mϕs in post-MI CHF rats. We also show that training may exert beneficial effects in the exercise performance of MI rats by modifying the inflammatory status of rats with this syndrome, possibly preventing the recruitment, migration, and activation of Mϕs in areas of inflammation in the peritoneum.

The phagocytic capacity was significantly different between the MI and the Sham-operated groups, which indicates that this function was enhanced by post-MI CHF. Peritoneal Mϕs (resident) show low functional capacity, whereas inflammatory Mϕs are upregulated and sensitized (primed) for further activation (8, 9). Acquisition of full function usually requires a complex set of signals dependent on the nature of the triggering stimulus (i.e., bacterial products and cytokines) (4, 40). Therefore, under the presence of a chronic stimulatory condition (i.e., post-MI CHF) and/or chronic stress (i.e., exercise training), peritoneal Mϕs would be more susceptible to changes, and when subsequently stimulated, they would improve their phagocytic capacity and produce increased amounts of inflammatory mediators.

Pro-inflammatory cytokines such as IL-6 and tumor TNF-α were recently identified as contributors to the syndrome of CHF (35, 37), to the underlying myopathic processes (cardiac and skeletal muscle) of adverse LV remodeling, and to progressive ventricular dysfunction (28), as well as to alterations in the skeletal muscle (atrophy; increased fiber type switching: type I to type IIb) (20).

Our findings show that post-MI CHF rats showed enhanced IL-6 and TNF-α production by peritoneal Mϕs stimulated by LPS, indicating that an overexpression of those cytokines may be due to change in tissue Mϕ sensitivity to LPS. Additionally, LPS hypersensitivity of monocytes/macrophages has been reported in patients with stable CHF, resulting in increased TNF-α production (17).

Interestingly, post-MI CHF animal model studies provide evidence for an overexpression of pro-inflammatory cytokines in tissues as cardiac and skeletal muscles (28, 32). Serum levels of pro-inflammatory cytokines tend toward an increase in the concentration of TNF-α without significant difference in the levels of IL-1β and IL-6, most probably the result of a state of compensated heart failure in the animal experimental model (32). High local expression of pro-inflammatory cytokines, even with no alteration of normal serum levels, is consistent with the notion that local inflammation may induce hypersensitivity of peritoneal Mϕs, followed by systemic cytokine spillover (20, 32). Consequently, our study showed that, beyond tissue pro-inflammatory cytokine overexpression, peritoneal Mϕs seems to be an important site of cytokines production in post-MI CHF rats.

Additionally, our study reports the immunomodulatory role of moderate endurance training in post-MI CHF rats, as demonstrated by the significant reduction in TNF-α production by peritoneal Mϕs under LPS stimulation. Thus, we hypothesize that peritoneal Mϕ sensitivity to LPS may be reduced with training, offering an additional explanation of training-induced reduction of TNFα production.

Taken together, our results show that under the present stimulatory condition (i.e., post-MI CHF rats) there is a modification of hypersensitivity to LPS of peritoneal Mϕs such that, when they are subsequently stimulated, they produce increased amounts of inflammatory mediators. Moderate endurance training was efficient, at least partially, in reducing this chronic stimulatory condition, as evaluated by the assessment of peritoneal Mϕs function.

In addition to these effects, our protocol also demonstrated restorative (or anti-inflammatory) effects that trend toward the normalization of Mϕ function (i.e., Sham-operated sedentary values), notably of pro-inflammatory cytokine production (TNF-α) under LPS stimulation, chemotaxis index, and Mϕ count in the peritoneal cavity. The current study has proven moderate endurance training is adequate to reestablish innate immune-cell function after the setting of post-MI CHF.

ACKNOWLEDGMENTS

The authors thank the Laboratory of Molecular and Cellular Physiology of Exercise, EEFE-USP, and Katt Mattos and Marcele Coelho for technical assistance.

GRANTS

This work was supported by FAPESP Grant 01/11448-5.

REFERENCES

1. **Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJ.** Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. *Am J Cardiol* 79: 1426–1430, 1997.
2. **Arai AE, Greenberg BH.** Medical management of congestive heart failure. *West J Med* 153: 406–414, 1990.
3. **Batista ML Jr, Santos RV, Cunha LM, Mattos K, Oliveira EM, Seelaender MC, Costa Rosa LF.** Changes in the pro-inflammatory cytokine production and peritoneal macrophage function in rats with chronic heart failure. *Cytokine* 34: 284–290, 2006.
4. **Bergman M, Salman H, Bessler H, Omanski M, Punsky I, Djaldetti M.** Interaction between phagocytosis and IL-1beta production by rat peritoneal macrophages. *Biomed Pharmacother* 56: 159–162, 2002.
5. **Boyden S.** The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *J Exp Med* 115: 453–466, 1962.
6. **Chimenti S, Carlo E, Masson S, Bai A, Latini R.** Myocardial infarction: animal models. *Methods Mol Med* 98: 217–226, 2004.
7. **Coleman DL, Liu J, Bartiss AH.** Recombinant granulocyte-macrophage colony-stimulating factor increases adenylate cyclase activity in murine peritoneal macrophages. *J Immunol* 143: 4134–4140, 1989.
8. **Costa Rosa LF, Cury Y, Curi R.** Hormonal control of macrophage function and glutamine metabolism. *Biochem Cell Biol* 69: 309–312, 1991.
9. **Costa Rosa LF, Safi DA, Cury Y, Curi R.** Effect of epinephrine on glucose metabolism and hydrogen peroxide content in incubated rat macrophages. *Biochem Pharmacol* 44: 2235–2241, 1992.
10. **Costa Rosa LF, Safi DA, Cury Y, Curi R.** The effect of insulin on macrophage metabolism and function. *Cell Biochem Funct* 14: 33–42, 1996.
11. **De la Fuente M, Martin MI, Ortega E.** Changes in the phagocytic function of peritoneal macrophages from old mice after strenuous physical exercise. *Comp Immunol Microbiol Infect Dis* 13: 189–198, 1990.
12. **Fehr HG, Lotzerich H, Michna H.** The influence of physical exercise on peritoneal macrophage functions: histochemical and phagocytic studies. *Int J Sports Med* 9: 77–81, 1988.
13. **Fehrenbach E, Schneider ME.** Trauma-induced systemic inflammatory response versus exercise-induced immunomodulatory effects. *Sports Med* 36: 373–384, 2006.
14. **Ferrandez MD, De la Fuente M.** Effects of age, sex and physical exercise on the phagocytic process of murine peritoneal macrophages. *Acta Physiol Scand* 166: 47–53, 1999.
15. **Frangogiannis NG, Smith CW, Entman ML.** The inflamatory response in myocardial infarction. *Cardiovasc Res* 53: 31–47, 2002.
16. **Gielen S, Adams V, Mobius-Winkler S, Linke A, Erbs S, Yu J, Kempf W, Schubert A, Schuler G, Hambrecht R.** Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. *J Am Coll Cardiol* 42: 861–868, 2003.
17. **Kruger S, Kunz D, Graf J, Stickel T, Merx MW, Hanrath P, Janssens U.** Endotoxin sensitivity and immune competence in chronic heart failure. *Clin Chim Acta* 343: 135–139, 2004.
18. **Lambert MI, Noakes TD.** Dissociation of changes in VO2 max, muscle QO2, and performance with training in rats. *J Appl Physiol* 66: 1620–1625, 1989.
19. **Laskin DL, Pendino KJ.** Macrophages and inflammatory mediators in tissue injury. *Annu Rev Pharmacol Toxicol* 35: 655–677, 1995.
20. **Mann DL, Reid MB.** Exercise training and skeletal muscle inflammation in chronic heart failure: feeling better about fatigue. *J Am Coll Cardiol* 42: 869–872, 2003.
21. **Mann DL.** Tumor necrosis factor-induced signal transduction and left ventricular remodeling. *J Card Fail* 8: S379–S386, 2002.
22. **Musch TI, Moore RL, Leathers DJ, Bruno A, Zelis R.** Endurance training in rats with chronic heart failure induced by myocardial infarction. *Circulation* 74: 431–441, 1986.
23. **Musch TI, Moore RL, Smaldone PG, Riedy M, Zelis R.** Cardiac adaptations to endurance training in rats with a chronic myocardial infarction. *J Appl Physiol* 66: 712–719, 1989.
24. **Musch TI, Nguyen CT, Pham HV, Moore RL.** Training effects on the regional blood flow response to exercise in myocardial infarcted rats. *Am J Physiol Heart Circ Physiol* 262: H1846–H1852, 1992.
25. **Niebauer J, Clark AL, Webb-Peploe KM, Coats AJ.** Exercise training in chronic heart failure: effects on pro-inflammatory markers. *Eur J Heart Fail* 7: 189–193, 2005.
26. **Niebauer J, Volk HD, Kemp M, Dominguez M, Schumann RR, Rauchhaus M, Poole-Wilson PA, Coats AJ, Anker SD.** Endotoxin and immune activation in chronic heart failure: a prospective cohort study. *Lancet* 353: 1838–1842, 1999.
27. **Nieman DC.** Exercise immunology: practical applications. *Int J Sports Med* 18: S91–S100, 1997.
28. **Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S.** Cytokine gene expression after myocardial infarction in rat hearts: possible implication in left ventricular remodeling. *Circulation* 98: 149–156, 1998.
29. **Pedersen BK, Hoffman-Goetz L.** Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 80: 1055–1081, 2000.
30. **Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E.** Progressive ventricular remodeling in rat with myocardial infarction. *Am J Physiol Heart Circ Physiol* 260: H1406–H1414, 1991.
31. **Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E.** Myocardial infarct size and ventricular function in rats. *Circ Res* 44: 503–512, 1979.
32. **Schulze PC, Gielen S, Adams V, Linke A, Mobius-Winkler S, Erbs S, Kratzsch J, Hambrecht R, Schuler G.** Muscular levels of pro-inflammatory cytokines correlate with a reduced expression of insulinlike growth factor-I in chronic heart failure. *Basic Res Cardiol* 98: 267–274, 2003.
33. **Shephard RJ, Verde TJ, Thomas SG, Shek P.** Physical activity and the immune system. *Can J Sport Sci* 16: 169–185, 1991.
34. **Srere PA.** Citrate synthase. *Methods Enzymol* 13: 3–5, 1969.
35. **Sugiura H, Nishida H, Inaba R, Mirbod SM, Iwata H.** Immunomodulation by 8-week voluntary exercise in mice. *Acta Physiol Scand* 168: 413–420, 2000.
36. **Sugiura H, Nishida H, Sugiura H, Mirbod SM.** Immunomodulatory action of chronic exercise on macrophage and lymphocyte cytokine production in mice. *Acta Physiol Scand* 174: 247–256, 2002.
37. **Torre-Amione G.** Immune activation in chronic heart failure. *Am J Cardiol* 95: 3–8, 2005.
38. **Vonhof S, Brost B, Stille-Siegener M, Grumbach IM, Kreuzer H, Figulla HR.** Monocyte activation in congestive heart failure due to coronary artery disease and idiopathic dilated cardiomyopathy. *Int J Cardiol* 63: 237–244, 1998.
39. **Wolach B, Gavrieli R, Ben-Dror SG, Zigel L, Eliakim A, Falk B.** Transient decrease of neutrophil chemotaxis following aerobic exercise. *Med Sci Sports Exer* 37: 949–954, 2005.
40. **Woods JA, Davis JM, Smith JA, Nieman DC.** Exercise and cellular innate immune function. *Med Sci Sports Exerc* 31: 57–66, 1999.