Title

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Journal

Cytokine, 40(2)

ISSN

1043-4666

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Publication Date

2007-11-01

DOI

10.1016/j.cyto.2007.08.008

Published in final edited form as:
Cytokine. 2007 November ; 40(2): 75–81.

Serum from Exercising Humans Suppresses T-Cell Cytokine Production

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Abstract

Exercise affects t-cell cytokine production. Whether or not these effects are caused by circulating factors associated with physical activity (e.g., inflammatory mediators, acidosis) is unknown. To investigate this, we incubated sera (10%), obtained from 16 young-adults before (PRE) and after (END) 30-min of exercise, with commercially available Jurkat cells, a t-lymphocyte model, that, of course, had never been exposed to an exercise milieu. After 1-h and 6-h in culture, we measured in the supernatant four cytokines (each known to be altered by exercise and involved in disease pathophysiology): IL-2, TGF-β1, TNF-α, and IL-1ra. Cell proliferation was assessed with proliferating nuclear cell antigen (PNCA). Statistical analysis consisted of a linear mixed model for repeated measurement. There was no effect of exercise on t-cell production of either TGF-β1 or IL-1ra. In contrast, both IL-2 ($p=0.025$) and TNF-α ($p=0.031$) production was significantly suppressed in sera from the exercising participants. The suppression of these two cytokines occurred despite the fact that PNCA significantly increased ($p<0.0001$) in the END serum. In conclusion, exercise alters circulating factors that can, subsequently, influence t-cell cytokine production in vitro.

Keywords

Exercise; Inflammation; Jurkat; Cytokine; Cell culture

INTRODUCTION

Exercise can profoundly influence health [1], and there is mounting evidence that the mechanism of these beneficial effects is related, in part, to the impact of exercise on immune function [2]. Precisely how the various physiological perturbations that accompany physical activity (ranging from acidosis to heat to the secretion of potent endocrine hormones and neuroadrenergic mediators) actually alter immune function is still not well understood. One potential mechanism is that exercise changes the profile of mediators in the circulation which can, subsequently, influence cytokine secretion of circulating immune cells. In this study, we began to test this hypothesis by comparing the pattern of *in vitro* cytokine secretion from a commercially available immune cell-model (Jurkat) that had been co-cultured with sera obtained from young adults either before or immediately after a 30-min bout of heavy exercise. In this way, we hoped to determine whether or not exercise-associated immune mediators

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within the circulation could influence cytokine secretion in immune cells that had not been exposed to exercise *in vivo*.

T-lymphocytes (t-cells) can secrete a wide variety of cytokines and other factors that play a role in health and disease, particularly in diseases like asthma [3] and atherosclerosis [4] that are clearly tied to physical activity [5]. Circulating t-cell numbers and function are influenced by brief periods of exercise [6,7], and a number of mechanisms have been proposed to explain these effects. First, as noted, exercise alters circulating factors and mediators (e.g., lactate, IL-6, catecholamines, growth hormone) that can affect t-cells [8-10]. Secondly, lymphocytes could be influenced by more direct effects such as exercise associated neurostimulation of lymph nodes and other lymph tissues [11,12]. Finally, some of the immune effects of exercise are mechanical. For example, Hinton et al. [13] concluded that the most likely cause of their observed reduction in the mitogen response of cultured circulating leukocytes following exercise had been the mobilization into the circulation from marginal depots of large numbers of NK cells relative to t-cells.

Because of the profound changes in inflammatory and other mediators in the circulation that occur in response to even relatively brief exercise, we hypothesized that serum obtained from subjects who had just exercised would affect cytokine production by t-cells. We used the Jurkat cell line, an immortalized model of lymphoid cells, to isolate mediators in the circulating blood that could be altered by exercise and, subsequently, influence t-cell cytokine production [14]. The Jurkat cell line has been previously used to determine possible effects of serum factors on t-cell function in experiments in which Jurkat cells were incubated with serum derived from affected individuals (e.g., acute severe brain injury [15]).

In this exploratory study, we focused on several key cytokines known to be produced by t-cells and that play a role in health conditions influenced by physical activity: interleukin-2 (IL-2) [16]; tumor-necrosis factor-α (TNF-α) [17]; transforming growth factor-β1 (TGF-β1) [18], and IL-1 receptor antagonist (IL-1ra) [19]. IL-2 is a proinflammatory cytokine and one of the most essential cytokines for regulating activation and proliferation of t-cells [20]. The TGF-β1 protein family is involved in muscle growth and atrophy [21] and has been implicated in both the inhibition and activation of t-cells [22,23]. TNF-α is a proinflammatory cytokine produced by t-cells typically following antigen stimulation and if chronically elevated can lead to a variety of diseases [24]. TNF-α, too, is involved in muscle atrophy [25]. IL-1ra is an anti-inflammatory cytokine that increases in the circulation with exercise. [26]. IL-1ra is produced by Jurkat cells [27].

METHODS

Subjects

Sixteen healthy, sedentary adult males (age range 20-30 y.o.) participated in this study (Table 1). Individuals participating in competitive sports and with a history of any chronic medical conditions or on any medications were excluded from participation. We excluded highly trained individuals because many physiological effects of exercise are influenced by training status. The Institutional Review Board at the University of California, Irvine approved the study. Written informed consent was obtained from all participants upon enrollment.

Anthropometric measurements

Standard, calibrated scales and stadiometers were used to determine height, body mass, and body mass index (BMI =wt/h^2).

Measurement of fitness

Each subject performed a ramp-type progressive cycle ergometer using the SensorMedics metabolic system (Ergoline 800S, Yorba Linda, CA). After resting on the cycle ergometer for 3 min and 1 min of unloaded pedaling, the work rate (WR) was incremented at a rate of 20-30 watts/min to the limit of the subject's tolerance. Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath-by-breath. Anaerobic (lactate) threshold and peak VO_2 was calculated using standard methods [28].

Exercise protocol

At least 48 hours, but not exceeding seven days following the completion of the ramp test, each subject performed 30-min of constant work rate cycle ergometry at a work rate equal to approximately 50% of the difference between the lactate threshold and the pre-determined peak VO_2. On average, this was approximately 80% of each individual peak work rate.

Blood sampling and analysis

30 minutes prior to the start of exercise, an indwelling catheter was inserted into the antecubital vein. Venous blood was drawn before exercise (PRE) and immediately following the exercise (END) and allowed to clot for 30 min on ice. PRE and END exercise serum was aliquoted and stored at -80°C until assayed.

Cell culture

Jurkat t-cells (ATCC, Manassas, VA) were grown at 37°C in a humidified atmosphere consisting of 5% CO_2/95% air in RPMI-1640 medium supplemented with 10%(v/v) heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1% (w/v) non-essential amino acids. Cells were kept in the exponential growth phase by passages at 2–3 day intervals. Prior to use, a washout period of FCS mediators was performed by growing cells in complete RPMI-1640 without FCS for 48 hours. Cell counts using trypan blue were performed in order to verify exponential growth. Jurkat cells were seeded in 12-well tissue culture plates at 5×10^5 cells/ml in RPMI-1640 supplemented with 10% heat-inactivated serum from PRE or END-exercise from each subject and grown at 5% CO_2 and 37°C. Supernatants were harvested at 1-h and 6-h and frozen at -80°C until use.

Effect of serum on cytokine production cell proliferation

Secreted cytokines levels at 1-h and 6-h after incubation with human serum were measured using commercially available ELISA kits manufactured by R&D Systems (Minneapolis, MN). Cytokines measured were: *Tumor Necrosis Factor Alpha; TNF-α*, with an intra-assay CV of 5.3 - 8.8%, inter-assay CV 10.8 - 16.7% and sensitivity ≤0.12 pg/ml; *Interleukin 1 receptor antagonist; IL-1ra*, with an intra-assay CV of 3.1–6.2%, inter-assay CV 4.4–6.7% and sensitivity 22 pg/ml; *Interleukin-2; IL-2*, with an intra-assay CV 3.2-4.3%, inter-assay CV 4.0 – 10.0% and sensitivity <7 pg/ml and *Transforming growth factor-β1; TGF-β1;* with an intra-assay CV of 2.4–3.4%, inter-assay CV 5.7–8.4% and sensitivity 4.6 pg/ml. The cytokine levels of the 10% serum solution were measure for each subject prior to incubation. Cell proliferation was determined by the Proliferating Nuclear Cell Antigen *(PNCA)* ELISA (CALBIOCHEM, USA). PNCA sensitivity is 0.2U/ml. In some cases, we did not have sufficient sample to perform all analysis, this is reflected in figures.

Statistical analysis

Since each subject provided both PRE- and END-exercise serum for Jurkat cell culture harvest, the cytokine levels obtained from serum of the same subject were considered to be correlated.

We applied a mixed linear model, which is designed for analyzing correlated repeated measurements, to assess the effect of exercise (PRE- to END-exercise) and harvest time (1-h vs. 6-h) using SAS 9.1 (Cary, NC) mixed procedure. Data were presented as mean ± standard error mean (SE) and test results were evaluated at 0.05 significance level.

RESULTS

Anthropometric and physiological characteristics

Anthropometric and physiological characteristics of the study participants are described in Table 1. The average BMI was within normal limits.

Serum lactate

The exercise bout caused a mean increase of 115±6.3 mg/dl in serum lactate levels [PRE (12.4 ±0.79 mg/dl) *vs.* END (127.5±6.5 mg/dl), p= p<0.0001].

Effects of serum factors on Jurkat t-cell viability and proliferation (Figure 1)

Cells placed in culture were 100% viable (using trypan blue exclusion dye). At both 1-h and 6-h, cells remained >99% viable. No ballooning of cell membranes, an early sign of apoptosis, was observed using light microscopy. END-exercise sera significantly increased proliferation (PNCA ELISA) of Jurkat t-cells as compare to PRE-exercise serum (p<0.0001). This stimulatory effect on proliferation was observed at both the 1-h and 6-h harvest.

Initial production of cytokines

As shown in Figure 2, there was very little or undetectable levels of the cytokines initially, but increased substantially by 60-min of incubation. (n.b., statistical analysis of this initial increase was not performed because levels of the cytokine in the initial aliquots were below the detection limits of the ELISA assays in many instances).

No effect of exercise serum on TGF-β1 and IL-1ra (Figure 3)

TGF-β1 secretion patterns in Jurkat t-cells were not influenced by PRE or END-exercise serum. TGF-β1 was significantly higher at 6-h of incubation, compared to the 1-h harvest (p<0.0001). Similarly, IL-1ra was significantly lower at 6-h of incubation (p<0.0001), compare to 1-h harvest.

Exercise serum suppressed TNF-α and IL-2 secretion

As shown in Figure 4, END-exercise serum significantly suppressed pro-inflammatory TNF-α (0.71±0.20 pg/ml at 1-h and 1.31±0.20 pg/ml at 6-h) secretion compared to PRE-exercise serum (1.31±0.31 pg/ml at 1-h and 1.9±0.31 pg/ml at 6-h, p=0.031). Exercise similarly suppressed IL-2 production (PRE, 5.93±0.72 pg/ml at 1-h and 4.84±0.44 pg/ml at 6-h compared with END, 4.77±0.73 pg/ml at 1-h and 3.71±0.47 pg/ml at 6-h, p=0.025).

DISCUSSION

These data support the hypothesis that one mechanism through which exercise can alter immune function is by changing circulating mediators that are, in turn, capable of influencing cytokine secretion of t-cells. The targeted cytokines in the supernatant increased dramatically in the first hour of the cell culture conditions when the Jurkat cells were exposed to either PRE or END serum (Figure 1). This suggests that the increased cytokine levels in the supernatant had most likely resulted from secretory activity of the Jurkat cells as they were exposed to the growth promoting conditions of cell culture. Of the 4 cytokines we chose to study, there was no effect of the co-cultured human sera on TGF-β1 or IL-1ra secretion (Figure 3). In contrast,

we found a significant suppressing effect of the sera obtained immediately following exercise (END) on the supernatant concentrations of IL-2 and TNF-α (Figure 4). The data show that a relatively brief bout of heavy exercise alters the circulating milieu in healthy subjects sufficiently that Jurkat cell production of key cytokines was different even when the cells were exposed to media that contained only a 10% solution of the sera obtained from the resting and exercising research participants. Finally, these effects were not the result of reduced Jurkat cell proliferation as PNCA was increased when cells were incubated with the serum obtained from participants who had just exercised (Figure 1).

There are several circulating factors and physiological conditions that both change rapidly and robustly with exercise and are also known to influence t-cells. These “candidate” factors include, as noted, catecholamines, growth hormone, and the cytokine IL-6, and the parallel dramatic changes in local muscle and body temperature [29,30], oxygenation and acid-base status [31]. Glucocorticoids also increase with exercise, but usually after much more intense periods of exercise than used in this study [32].

In 2001, Starkie and coworkers [33] examined the effect of adrenergic blockade on the lymphocyte response to exercise in humans. Using flow cytometry and intracellular staining, these workers noted that although exercise increased the number of circulating t-lymphocytes staining positive for IL-2 (following stimulation), the relative cellular concentration of IL-2 decreased with exercise. Moreover, although adrenergic blockade significantly reduced the magnitude of the increase of IL-2 positive t-cells in the circulation, blockade had no effect on the apparent suppression of intracellular IL-2 caused by exercise. In humans, the suppressive effect of exercise on the IL-2 response to mitogens in lymphocytes has been additionally corroborated by Gleeson and Bishop [34].

Recently, Kohut and coworkers [35] using a mouse model also showed that exercise supressed t-cell production of IL-2. Intriguingly, while their data demonstrated that adrenergic blockade did not influence exercise suppression of t-cell production of IL-2, they did find that glucocorticoids (produced with heavy, prolonged exercise) might actually *attenuate* the exercise suppression of IL-2. The data presented here corroborate the observations in humans made by Starkie and coworkers and Gleeson and Bishop and the mouse-model results of Kohut and coworkers. There appear to be as yet unidentified circulating factors associated with exercise that specifically reduce t-cell production of the cytokine IL-2.

There is a remarkable paucity of data on the effects of exercise on t-cell production of TNF-α, the other inflammatory cytokine whose production in Jurkat cells was suppressed by exercise serum in our study (Figure 4). Most investigations have focused on macrophage or monocyte cell lines and demonstrated that catecholamines like epinephrine inhibit cellular production of TNF-α (e.g., [36]). Using intracellular fluorescent staining and flow cytometry, we found that the numbers of t-cells positive for TNF-α increased following exercise, but not the mean fluorescent intensity [6]. Goebel and coworkers [37] using stimulated whole blood obtained from human participants after exercise and other psychological stressors showed that TNF-α production increased only with exercise but was blunted by isoproterenol infusion. Thus, the effect of exercise on t-cell TNF-α results from the interplay of known and as yet undiscovered circulating factors.

Although sparse, current data on the interaction between the other major exercise mediators that we noted (IL-6 and growth hormone), also suggest the existence of as yet unidentified circulating factors that influence t-cell production of IL-2 and TNF-α. For example, circulating IL-6 is increased with exercise (likely due to production by working muscle cells [38]), but, in tissue culture, IL-6 enhances, rather than suppresses, t-cell production of IL-2 [39]. The role of IL-6 with regards to TNF-α remains controversial, with some investigators promoting the

idea that IL-6 inhibits TNF-α production [40]. There is also evidence that growth hormone suppresses t-cell production of IL-2 [41] while having the opposite effect on peripheral blood mononuclear cell production of TNF-α [42].

An equally complex issue focuses on how physiological perturbations associated with exercise (heat, hypoxia, and acidosis) could alter serum factors with the potential to regulate t-cell cytokine production. Soluble heat shock proteins, namely, Hsp 70 increase with exercise [43] as do a number of genes in the Hsp family in PBMCs [44], and the heat shock proteins are considered by some investigators as endogenous, almost surrogate antigens capable of activating immune cells [45]. There is little currently known about whether or not Hsp alters t-cell cytokine production; Breloer and coworkers [46] showed that in certain t-cells, Hsp stimulated IFN-γ production, but had no influence on IL-2. Zanin-Zhorov and coworkers [18] showed that Hsp led to reduced TNF-α secretion in t-cells through toll-like receptor-2. Chronic hypoxia can reduce t-cell production of cytokines like IL-2 [47], but the influence of relatively brief periods of intramuscular hypoxia such as occurs with exercise [31] on t-cell function has not been fully studied. Chronic lactic acidosis can also reduce t-cell production of IL-2 [8], but, again, whether the brief lactic acidosis that accompanies exercise similarly attenuates t-cell cytokine production is not known. In the current study, no correlations were observed between end-exercise lactic acid levels and measured cytokines.

In summary, this exploratory study showed that there are factors in the circulating blood following 30 min of exercise than suppress t-cell production of IL-2 and TNF-α. The suppression of the production of these particular pro-inflammatory cytokines is consistent with previous observations by other investigators using different methods to assess the immediate immune consequences of physical activity. Moreover, the data are consistent with the notion that any pro-inflammatory stimulation by exercise is balanced in healthy people by activation of mediators and factors that ultimately attenuate the inflammatory cascade [48]. We were able to identify these responses using only a 10% concentration of human serum and by incubating cells that were “naïve” to the exercise. Thus, the data strongly support the idea that exercise alters circulating factors that can, subsequently, influence immune cells. The effect is not global, as not all the cytokines we studied were influenced (namely, IL-1ra and TGF-β1 were produced by the Jurkat cells co-cultured with human serum, but not affected by exercise). The approach described here may prove useful in uncovering some of the mechanisms through which exercise influences the function of circulating immune cells.

Acknowledgements

This work was supported in part by National Institutes of Health grants RO1-HL080947, P01HD-048721 and the UCI Satellite GCRC:MO1 RR00827.

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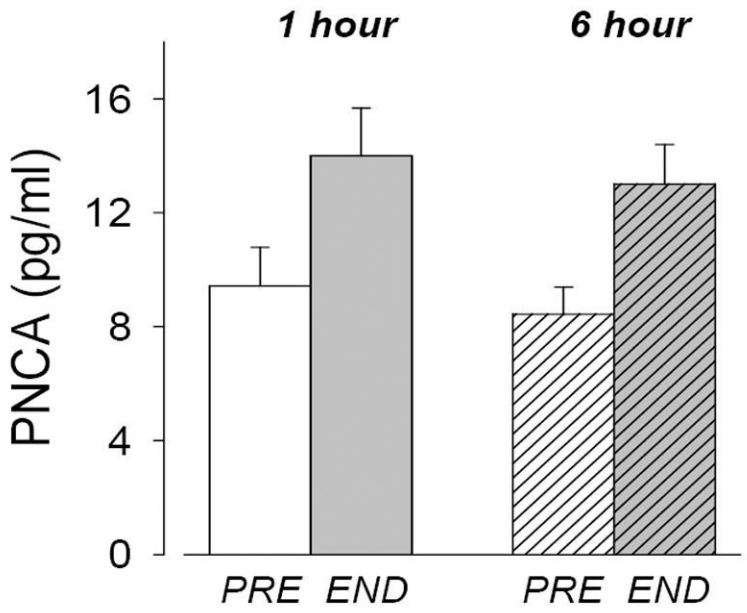

Figure 1.
Effects of serum obtained from resting subjects (PRE--white bars) and immediately after 30-min of exercise (END--grey bars) harvested at 1-h (no hatch mark) and 6-h (hatch mark) on t-cell proliferation. T-cells incubated with exercise serum significantly increased cell proliferation ($p=0.0004$, $n=9$).

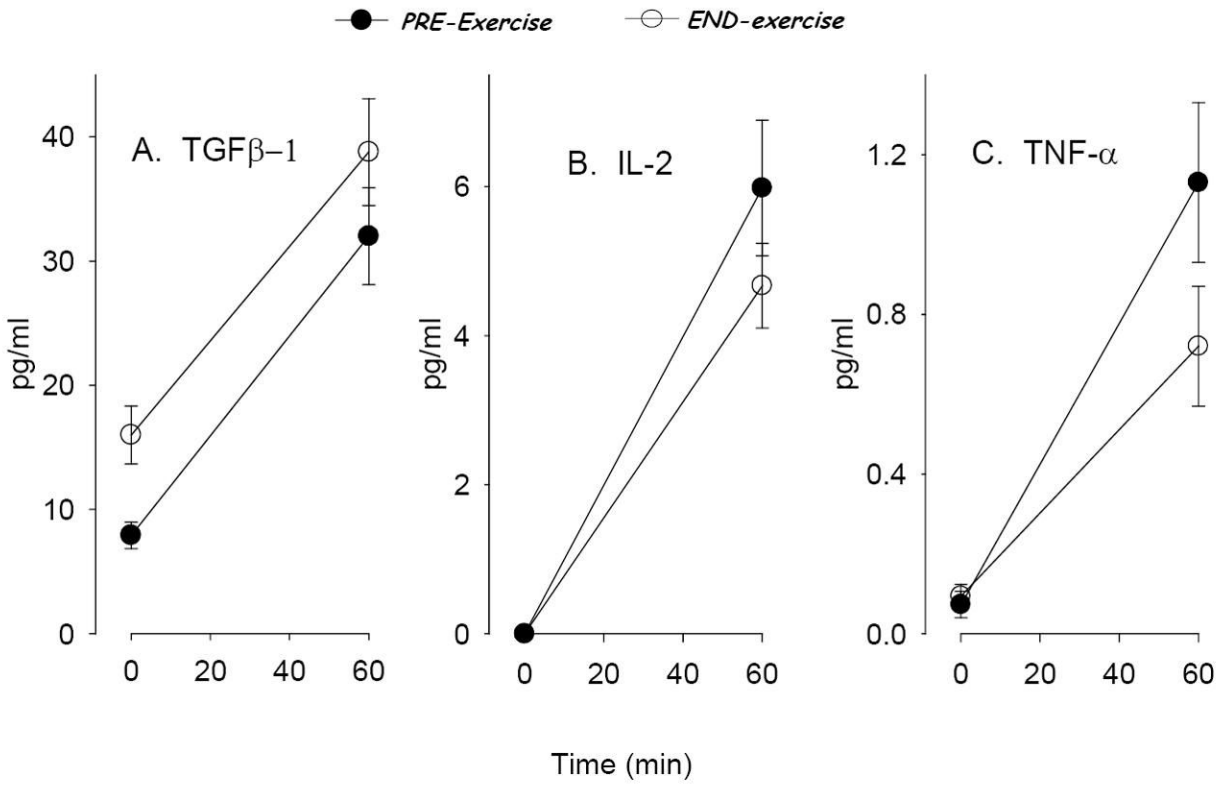

Figure 2.

Effects of exercise serum on cytokine secretion patterns in Jurkat t-cells incubated with PRE (●) and END-exercise (○) serum at time zero and 1-h after incubation (n=13). As noted in the results, in some cases, the time zero values were below the detection limits of the assay. TGF-β1, IL-2 and TNF-α were higher at 1-h after incubation compared to time zero. IL-1ra not assayed due to sample limitation.

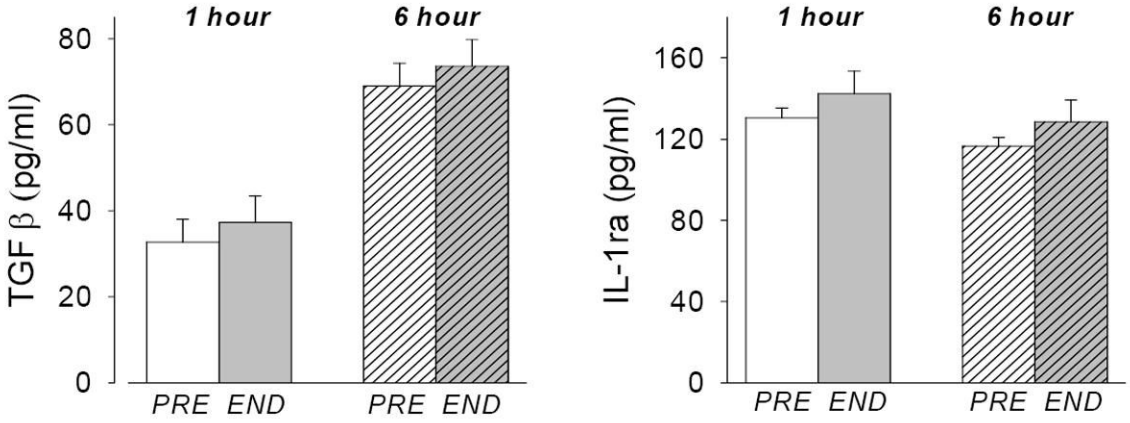

Figure 3.

Effects of serum obtained from resting subjects (PRE--white bars) and immediately after 30-min of exercise (END--grey bars) harvested at 1-h (no hatch mark) and 6-h (hatch mark) on cytokine secretion patterns. TGF-β1 (n=16) and IL-1ra (n=9) secretion patterns in Jurkat t-cells were not affected by exercise but were significantly affected by duration of incubation ($p<0.0001$).

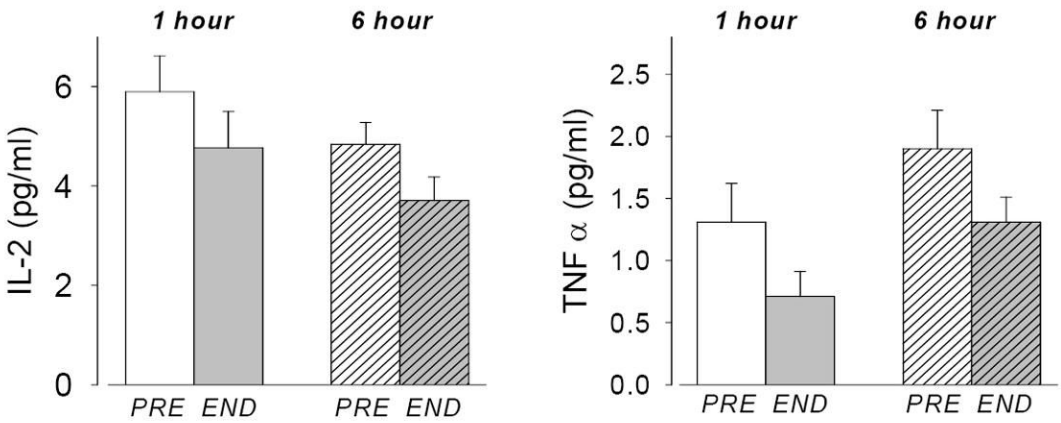

Figure 4.

Effects of serum obtained from resting subjects (PRE--white bars) and immediately after 30-min of exercise (END--grey bars) harvested at 1-h (no hatch mark) and 6-h (hatch mark) on cytokine secretion patterns (n=16). IL-2 secretion was significantly decreased by exercise ($p=0.025$). TNF-α secretion was affected both by exercise ($p=0.031$) and duration of incubation ($p=0.042$).

Table 1

Anthropometric and physiological characteristics of the 16 subjects

Age (years)	24.6 ± 0.7
Height (cm)	176.3 ± 1.8
Body Mass (kg)	74.2 ± 2.3
BMI (kg/m2)	23.9 ± 0.8
Peak VO2 (ml/kg/min)	38.7 ± 1.5
% peak work rate during the constant work rate protocol	78.7 ± 3.4

Values are means ± SE; BMI, body mass index; Peak VO_2, peak oxygen uptake