Influence of Physical Activity on Serum IL-6 and IL-10 Levels in Healthy Older Men

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ABSTRACT

JANKORD, R., and B. JEMIOLO. Influence of Physical Activity on Serum IL-6 and IL-10 Levels in Healthy Older Men. *Med. Sci. Sports Exerc.*, Vol. 36, No. 6, pp. 960–964, 2004. **Introduction:** Chronic inflammation is thought to play a role in disease development and functional decline during aging. The purpose of this research was to examine the influence of regular physical activity, independent of disease and disability, on the levels of pro- and anti-inflammatory cytokines in older (65–74 yr) males. **Methods:** Subjects were carefully screened for participation in this study based upon the SENIEUR protocol. In addition, subjects were selected based upon their weekly volume of aerobic exercise. Twelve extremely healthy "SENIEUR" males (six very active, six less active) completed this study. Serum concentrations of MIP-1α, IL-1ra, IL-1β, IL-6, IL-10, and C-Reactive protein were measured by ELISA. **Results:** The very active group demonstrated significantly lower levels of IL-6 ($P = 0.016$) and significantly higher levels of IL-10 ($P = 0.016$) compared with the less active group. **Conclusions:** The higher volume of regular physical activity was associated with decreased IL-6 levels and increased IL-10 levels in very healthy older males. Thus, exercise may play a vital role in controlling inflammatory markers during the aging process. **Key Words:** CYTOKINES, AGING, SENIEUR PROTOCOL, CHAMPS, INFLAMMATORY MARKERS

The age-related decline in physical function has been associated with immunosenescence. These changes include a loss of phagocytic capacity, altered generation of radicals, reduced dendritic cell (DC) traffic, and increased pro-inflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-6 (IL-6) even in the absence of significant diseases (10,29). An elevation in pro-inflammatory cytokines also plays a central role in the hepatic production of C-reactive protein (CRP) and other acute-phase proteins involved in the inflammatory response (8).

Some diseases that are characterized by chronic inflammation and high levels of IL-6 cause a reduction in muscle strength and muscle mass that is often associated with physical disability in old and very old persons. Although this association has been shown we do not know if functional disability precedes or follows increases in IL-6 levels (6).

Conversely, it has been hypothesized that anti-inflammatory cytokines might be involved in successful aging and longevity. Recent studies suggest that IL-10, a key cytokine that can suppress cell mediated immunity and maturation of DC (12), is elevated in the very health elderly but declined in frail elderly along with DC antigen presenting function (27). IL-10 is deeply involved in the regulation of the inflammatory response and immune reaction. It has dominant suppressive effects on the production of pro-inflammatory cytokines from many cell types (12). IL-10 reduces serum levels of tumor necrosis factor-α (TNF-α), IL-6, IL-8, and IL-1β, as well as neutrophil accumulation, elastase production, and cortisone levels in human volunteers challenged with a low dose of endotoxin (15). Low levels of IL-6, IL-8, and IL-1β were also observed in rheumatoid arthritis patients undergoing IL-10 therapy (3).

The extent of interactions between exercise, aging and the immune system is not fully understood (28). The immune response to exercise is complex and dependent upon the frequency, intensity and volume of exercise. Active elderly males have demonstrated a significantly higher rate of interferon-γ (INF-γ) and anti-inflammatory interleukin-2 (IL-2) and interleukin-4 (IL-4) production (20). Also, moderate training can enhance the resting natural killer (NK) cell function of healthy elderly people, potentially increasing resistance to both viral infection and preventing malignant cell formations (14,28). Regardless of age, strenuous exercise can increase local and systemic cytokine (IL-1, IL-1ra, IL-6, IL-8, IL-10, INF, MIP-1) production (17), often exhibiting striking similarity to the cytokine response to trauma and infection. Moderate exercise results in minimal fluctuations of the various cytokine levels and regular physical activity can actually increase immunosurveillance and decrease risk of infection (13).

The present study was undertaken to determine whether regular weekly participation in aerobic activity of 65- to 75-yr-old men in perfect health can play a role in regulation of pro- and anti-inflammatory cytokines. In particular, we were interested in determination of IL-6 (pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) levels in serum of well-functioning, very healthy men whose partic-

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ipation in aerobic activities differed in intensity and frequency. Could self imposed regular weekly aerobic activity be beneficial to an otherwise "healthy" immune system of older men?

MATERIALS AND METHODS

Subjects. A total of 188 men, ages 65–75 yr, were selected out of an existing pool of volunteers from the Ball State University Adult Physical Fitness Program database. An additional 40 subjects were recruited from the Muncie, IN, area by use of newspaper advertisements. All potential subjects were initially screened over the phone. The completion of a health history questionnaire and the CHAMPS physical activity questionnaire for older adults (24) resulted in 16 very healthy male subjects being chosen to participate in this study. Acceptable volunteers met the clinical and pharmacological requirements of the SENIEUR protocol and were 1) without acute or chronic diseases: 2) nonsmokers (at least for last 2 yr): and 3) without infection, inflammation, or malignancy (11). Subjects were given both oral and written information about study conditions before they gave their written informed consent. The study was approved by the Institutional Review Board for Human Subjects Research at Ball State University, Muncie, Indiana.

The CHAMPS physical activity questionnaire was used to place the subjects into one of two groups based upon their weekly volume of aerobic activity. Aerobic activities included 1) jogging or running, 2) incline waking or hiking, 3) fast walking, 4) riding bicycle or stationary cycle, 5) use of aerobic machines (rowing, step, elliptical, etc.), 6) swimming at a moderate or fast pace, and 7) aerobics or aerobic dancing. The CHAMPS questionnaire estimates weekly participation (over last 4 wk) and weekly energy expenditure in physical activities that could lead to health benefits. Scoring of the CHAMPS survey gave us two measurements: estimated kilocalories per week and frequency per week spent in low- or greater-intensity (intensity of 3 METs or greater) aerobic activities. This questionnaire has been shown to be a valid tool for assessing physical activity in older adults (9). To qualify for the very active (VA) group our study subjects had to be exercising most days of the week ($4+$ d·wk^{-1}) and expending at least 1000 kcal·wk^{-1} in moderate- to high-intensity activity. Subjects who were not exercising most days of the week qualified for the less active (LA) group.

The SENIEUR selection scheme aims to select only those individuals in perfect health in order to distinguish any alternations of immune system function caused by aging *per se*, not by disease (16). Using the SENIEUR protocol to select subjects to the present study resulted in the exclusion of the majority of individuals from initial group of 228 men, age 65–75 yr. The use of the CHAMPS physical activity questionnaire to select very active and less active individuals also contributed to lowering the number of potential subjects. However, as the result of this rigorous screening, we had selected two groups that were homogenous in respect to subject health status and heterogeneous in respect to weekly physical activity and energy expenditure status.

Clinical values. Whole blood samples were collected from subjects after 12-h fast and 5 min of rest in seated position between 7 and 8 a.m. at the Human Performance Laboratory, Ball State University. Blood samples from each subject were collected from the antecubital vein using one 5-mL Vacutainer tube with EDTA and one 10-mL serum separator tube (SST). In addition, approximately 20 mL of urine was collected into a plastic collection container. All blood and urine samples were delivered to Pathologists Associated laboratory, Muncie, IN, to be analyzed for the biological markers such as total cholesterol, glucose, liver enzymes: aspartate and alanine aminotransferase (AST and ALT), albumin, white blood cells, and platelets.

Cytokine measurements. Blood samples for cytokine measurements were collected from subjects that passed the initial clinical screening (as described above). After a 12-h fast, 24-h refraining from moderate to high level physical activity, and 5-min rest in the seated position, blood was collected from the antecubital vein in two 10-mL SST tubes, between 7 and 8 a.m. Blood samples were centrifuged at 3200 rpm for 10 min at 4°C and aliquoted into Eppendorf tubes. Samples were kept frozen at −80°C until analysis (within 2 months). Each sample was analyzed two times, each time in duplicate using Quantikine HS colorimetric sandwich ELISA kits (R&D Systems, Minneapolis, MN). The minimum detection dose of the measured immune markers, accordingly to the manufacturer's information, were typically less then the given values: IL-10, 0.5 pg·mL^{-1}; IL-6, 0.7 pg·mL^{-1}; IL-1ra, 22 pg·mL^{-1}; IL-1β, 1.0 pg·mL^{-1}; MIP-1α, 49.9 pg·mL^{-1}. Optical density (OD) was measured by Wallac 1420 Victor2 multilabel counter/plate reader (EG&G Wallac, Finland) set to 450 nm with wavelength correction set to 540 nm. Standard curve was created by reducing the data using computer software (WorkOUT for Victor2, Wallac, Finland) capable of generating a four parameter logistic (4-PL) curve-fit. Average OD for each sample, control, and standard was also corrected by subtracting the average zero standards OD. The level of CRP was determined by ACTIVE CRP ELISA (DSL, Webster, TX); an enzymatically amplified "two-step" sandwich-type immunoassay. OD was measured by Wallac 1420 Victor2 set to 450 nm. Standard curve employed log values of OD and CRP concentration for each standard in linear curve-fit (WorkOUT for Vicror2 software). According to information provided by R&D System, the ELISA kits used in the study determine the relative mass value of natural human IL-1β, IL-1ra, IL-6, IL-10, and MIP-1α. The intra-assay coefficient of variance (%CV) for all ELISA kits was between 0.1 and 9.8.

Statistical analysis. Because of the small subject number and their unknown population characteristics (1), two-tailed Wilcoxon Mann-Whitney U tests were used to determine differences in MIP-1α, IL-1β, IL-1ra, IL-6, and IL-10 concentrations between the very active and less active groups. All other comparisons of subject data between the two groups involved two-tailed independent *t*-tests (small sample size but the population characteristic was relatively symmetric). Statistics were run using Statistical Packages

TABLE 1. Characteristics of subject groups.

Parameter	Very Active (VA)	Less Active (LA)
N	6	6
Age (yr)	68.0 ± 1.5	70.3 ± 1.7
BMI ($kg \cdot m^{-2}$)	25.3 ± 1.4	28.1 ± 1.5
Aerobic exercise frequency ($d \cdot wk^{-1}$)	5.0 ± 0.5	1.3 ± 0.6**
Aerobic energy expenditure ($kcal \cdot wk^{-1}$)	2671.0 ± 574	541.0 ± 268*

Date represent mean ± SE; * $P < 0.01$, ** $P < 0.001$.

for the Social Sciences, version 8.0 (SPSS Inc., 1997). Power analysis was performed using StatPower program. The statistical power was greater than 0.80 at $P < 0.05$ with our sample size of 6.

RESULTS

On the basis of data received from analysis of whole blood (disease markers) and serum (cytokine levels) samples, we had to exclude four subjects from the study. Thus, each subject included in the data analysis was carefully selected and met all the requirements of the SENIEUR protocol. One subject exhibited very high levels of IL-6, another had elevated levels of IL-1ra, and two subjects had abnormal concentrations of liver enzymes. According to R&D ELISA information, a high level of high-affinity autoantibodies to IL-6 in the serum of normal blood donors have the potential to interfere with the measurement of IL-6 by this ELISA immunoassay. Also, recombinant human IL-1 sR1 can sometimes cross-react in the ELISA assays and interfere in quantification of IL-1ra, particularly when its concentration is > 1000 $pg \cdot mL^{-1}$.

Table 1 shows the general features of the less active (LA) and very active (VA) groups. The two groups differed significantly in weekly participation in exercise ($P < 0.001$) and in weekly energy expenditure ($P < 0.01$). Table 2 shows no significant differences between the VA and LA groups in mean serum concentrations of inflammatory markers (white cells, MIP-1α, and CRP), liver enzymes (AST and ALT), platelets, total cholesterol, and glucose. However, a significantly lower level ($P < 0.05$) of albumin was found in the serum of LA men when compared with the VA subjects.

All of the elderly males from both groups showed no pathological levels of either IL-1β or IL-1ra in the serum.

TABLE 2. Concentrations of various blood parameters in the very active and less active groups.

Parameter	Very Active (VA)	Less Active (LA)
Total cholesterol ($mg \cdot dL^{-1}$)	212.0 ± 10.9	186.0 ± 9.2
Glucose ($mg \cdot dl^{-1}$)	94.2 ± 3.4	94.5 ± 3.4
AST ($U \cdot L^{-1}$)	21.2 ± 1.7	18.8 ± 1.2
ALT ($U \cdot L^{-1}$)	16.8 ± 1.6	17.8 ± 2.1
Albumin ($g \cdot dL^{-1}$)	4.5 ± 0.1	4.2 ± 0.1*
WBC ($k \cdot \mu L^{-1}$)	4.7 ± 0.2	5.7 ± 0.7
Platelets ($k \cdot \mu L^{-1}$)	180.8 ± 12.6	220.3 ± 30.5
MIP-1α	36.1 ± 0.8[a]	35.5 ± 0.6[a]
C-Reactive protein ($mg \cdot L^{-1}$)	2.0 ± 0.3	2.2 ± 0.5

Data represent mean ± SE.
* Significant difference at $P < 0.05$.
[a] Mean concentration values are on the edge of ELISA sensitivity.

The serum level of IL-1β in all elderly subjects was below the ELISA kit sensitivity level (<1.0 $pg \cdot mL^{-1}$). This agrees with previous studies that have also shown remarkably low levels of IL-1β in circulation of healthy humans. The concentration of IL-1ra was not significantly ($P > 0.05$) different between the LA group (433 ± 59 $pg \cdot mL^{-1}$) and the VA group (338 ± 28 $pg \cdot mL^{-1}$). Low serum levels of IL-1ra (<800 $pg \cdot mL^{-1}$) have been detected in healthy humans; however, this cytokine is drastically increased in pathological conditions such as rheumatoid arthritis and osteoarthritis (7).

We found significant differences between VA and LA groups with mean serum levels of IL-6 and IL-10. The LA group of healthy older men demonstrated a mean IL-6 level more than three times greater than the VA group (Fig. 1) and an IL-10 level approximately half that of the VA group (Fig. 2.).

DISCUSSION

The present study demonstrates that higher levels of regular physical activity are associated with decreased levels of IL-6 and increased levels of IL-10 in the blood of healthy older males. The decreased IL-6 concentration in the VA group agrees with previous studies that have shown decreased levels of this cytokine in elderly individuals participating in a higher volume of moderate and strenuous physical activity (5,26). In addition, functional ability (2) and the ability to walk at a faster speed (26) have also been associated with decreased levels of IL-6 in older adults. The increased concentration of IL-10 in the very active and healthy older subjects has not been shown in previous studies. However, an increase of IL-10 in the healthy elderly in comparison with frail elderly has recently been shown (27). Even though all subjects had normal healthy albumin levels, this level was significantly lower ($P < 0.05$) in the LA group when compared with the VA group. A high serum level of albumin has been shown to have a protective effect in healthy older persons who do not have evidence of cytokine-mediated inflammation (18).

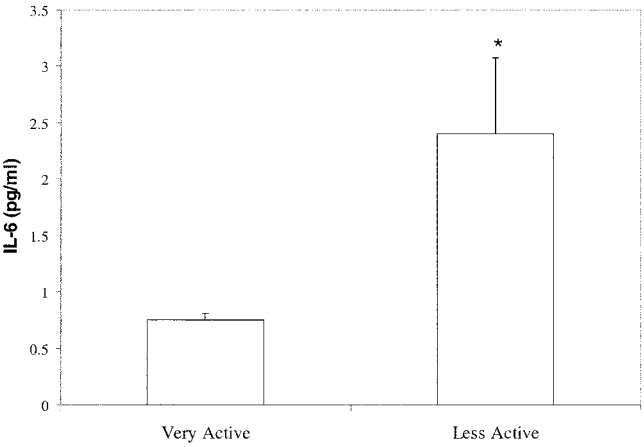

FIGURE 1—Influence of increased levels of aerobic exercise on serum IL-6 concentration in healthy older males. Values represent mean ± SE; *Wilcoxon Mann-Whitney U test; $P < 0.02$.

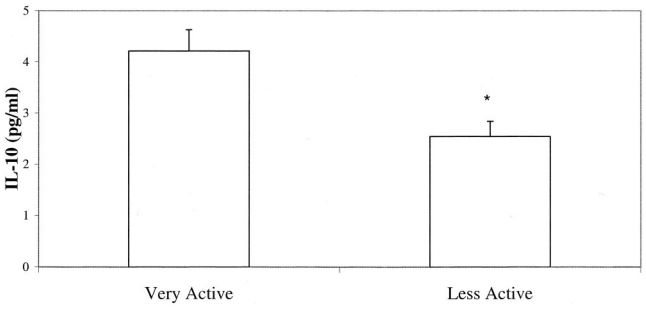

FIGURE 2—Influence of increased levels of aerobic exercise on serum IL-10 concentration in healthy older males. Values represent mean ± SE; *Wilcoxon Mann-Whitney U test; $P < 0.02$.

Lowering levels of IL-6 in the elderly is very important because an age-related increase in IL-6 has been associated with the development of disability (6), disease (4), and mortality (10). Ferrucci and colleagues (6) have proposed two possible explanations for the pathophysiological role of IL-6 in functional disability: direct causal role of cytokines in sarcopenia or the role of inflammation in the development of medical conditions leading to disability. However, Ferrucci and colleagues (6) pointed out that even though the association between IL-6 and disability has been shown, no information is available about whether high levels of IL-6 precede or follow the development of disability.

Roubenoff and Hughes (19) noted that physical activity is probably the most important environmental stimulus for maintaining muscle mass and function. Our results support the idea that regular exercise may also indirectly affect the process of sarcopenia through influencing circulating levels of IL-6.

In addition to differences in serum IL-6, we found a significantly higher level of IL-10 in the very active group compared with the less active group. This finding is important because IL-10 has enormous clinical implications for the cardiovascular (23), immune and neuroendocrine systems (21). Interleukin-10 is the most potent anti-inflammatory cytokine and has been shown to promote the survival of neurons (25).

In the cardiovascular system, IL-10 plays a protective role against atherosclerosis (22). Exercise training has been shown to increase mononuclear cell production of IL-10 and other atheroprotective cytokines (IL-4, TGF-β1) while decreasing production of atherogenic cytokines (IL-1α, TNF-α, IFN-γ) (23). Through increasing or maintaining "healthy" IL-10 levels, it appears that physical activity may help to provide a protective environment for tissue during the aging process.

Our study is the first, to our knowledge, to report an influence of physical activity on basal levels of IL-10. This study also provides additional evidence supporting previous studies that have shown a role of physical activity in decreasing IL-6 levels in healthy older men. Because physical activity plays an important role in maintaining health and preventing disease, which will affect cytokine levels, subjects must be carefully selected if one wants to look directly at the influence of physical activity on cytokine levels. Through applying the SENIEUR protocol this study was able to obtain a very homogenous subject pool that varied only in weekly physical activity. This very selective, extremely healthy subject pool allowed us to show a more direct influence of physical activity on IL-6 and IL-10 levels because subjects were eliminated for the presence of any disease, disability or abnormal clinical value. Our results strongly indicate a beneficial effect of physical activity on cytokine levels in older males and provide the basis for further studies of the physiological mechanisms that mediate this adaptation. In addition to providing an anabolic stimulus, regular physical activity in older men may also indirectly attenuate the effects of aging by playing an important role in maintaining healthy levels of pro- and anti-inflammatory cytokines.

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