Age-related loss of associations between acute exercise-induced IL-6 and oxidative stress

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Submitted 4 February 2005; accepted in final form 9 February 2006


STRENUOUS EXERCISE CAN PROVOKE skeletal muscle injury with a subsequent influx of leukocytes into the muscle tissue (34). Phagocytic cells remove tissue damaged by the initial injury but also produce reactive oxygen species that may, in some circumstances, cause secondary damage (44). These local reactions in muscle are often accompanied by a systemic acute-phase response, which includes increased hepatic production of C-reactive protein (CRP) and other acute-phase reactants, such as the metal chelators metallothionein, ferritin, and ceruloplasmin (28). The acute-phase response is initiated by several cytokines, including interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-6. IL-6 and IL-1β induce synthesis of CRP, a sensitive indicator of inflammation (6), whereas only IL-6 induces synthesis of certain acute-phase proteins with antioxidant properties such as ceruloplasmin (3, 43).

Monocytes, macrophages, and endothelial cells are important sources of IL-6 production (17), and there is evidence that skeletal muscle contributes to increased circulating concentrations of IL-6 with strenuous exercise (24). IL-6 binds to high affinity receptor complexes on target cells, consisting of one IL-6 receptor chain (IL-6R) and two signal-transducing glycoproteins (gp130) (46). Soluble forms of IL-6R (sIL-6R) retain affinity for IL-6 and enhance IL-6 activity. In contrast, soluble (s-)gp130 is devoid of signaling function and inhibits IL-6 activity.

Postexercise elevations in plasma IL-6 have been associated with exercised-induced circulating neutrophils (45). It has been suggested that IL-6 primes neutrophils for phagocytosis and oxidative burst activity (31). Other studies have reported age-related increases in mononuclear cell production and circulating concentrations of IL-6 in vivo (12, 39, 50), perhaps mediated in part by increased oxidative stress (23). Oxidative stress, as induced in vitro by xanthine and xanthine oxidase, promotes IL-6 production, and this IL-6 production is more pronounced in mononuclear cells isolated from old animals (27). Therefore, if the oxidative stress exceeds what is necessary and tolerable for normal cellular function, antioxidant interventions present a worthwhile area of investigation.

We (42) previously reported that an acute bout of downhill running increased circulating creatine kinase (CK, biomarker of muscle membrane permeability) and F2α-isoprostanes (bio- marker of lipid peroxidation and oxidative stress) and that vitamin E supplementation attenuated exercise-induced elevations in CK in the young and F2α-isoprostanes in the elderly. This strenuous exercise also increases muscle gene expression of certain cytokines, including IL-6, IL-1β, TNF-α, and TGF-β in the vastus lateralis muscle in these same healthy young and elderly men (21). As IL-6 promotes an antioxidant state through its induction of acute-phase proteins, it remains to be
determined whether IL-6 is associated with an antioxidant (or prooxidant) state in young or old men after eccentric exercise. Furthermore, it is uncertain how exercise and aging affects the circulating soluble receptors sIL-6R and s-gp130. Few studies have examined sIL-6R with respect to aging (2, 51), and no studies have examined the relationship among exercise, aging, and s-gp130.

In our earlier baseline studies we found, in young men only, strong relationships between exercise-induced oxidative stress and muscle CK release (42) and among changes in cytokine gene expression (21). To follow up these findings, we investigated here whether similar age-related associations exist among oxidative stress, IL-6, and the acute-phase response and longitudinally whether vitamin E supplementation affects these relationships. We hypothesized that, following an acute bout of downhill running, 1) both young and older men would experience elevations in circulating IL-6 and acute-phase reactants but that these responses would be exacerbated in older men; 2) the IL-6-mediated acute-phase response after exercise provides negative-feedback protection against exercise-induced oxidative stress and that exercise-induced responses would be associated with one another in the young men but not in the elderly; and 3) following vitamin E supplementation, older men would have an attenuated acute-phase response and regain associations between IL-6 and exercise-induced oxidative stress. The results of this study help elucidate whether IL-6 is associated with a pro- or antioxidant state within the context of intense exercise and aging.

EXPERIMENTAL PROCEDURES

Human subjects. Healthy, physically active young (18–35 yr, n = 16) and elderly (65–80 yr, n = 16) men, with normal weight for their height, participated in the study, as presented in Table 1. Before admittance into the study, volunteers passed a physical examination and completed a maximal exercise test on a treadmill to ascertain maximal oxygen consumption (VO2 max). During this protocol, subjects were asked to run at a self-determined comfortable pace on a treadmill (High Speed 2–16 mph Treadmill; Warren E. Collins, Braintree, MA) with a 0% incline. The incline was increased by 3% every 2 min until two of the following criteria were achieved: 1) VO2 reached a plateau; 2) respiratory exchange rate was >1.1; 3) heart rate was within 5 beats/min of the age-predicted maximum (220 beats/min − age). VO2 and carbon dioxide expiration were assessed by indirect calorimetry (V6200 Autobox; SensorMedics, Yorba Linda, CA). Each subject was monitored for blood pressure and for heart function using an electrocardiogram. Potential volunteers were excluded from the study if they had coronary heart disease; congestive heart failure; diabetes; cancer other than melanoma skin cancer; thyroid problems; arthritis or other autoimmune conditions; severe anemia; liver or kidney disease; poorly accessible veins; or current use of addictive drugs, tobacco, or steroid hormone medications.

Study design. Subjects performed 45 min of downhill running to induce an acute exercise response on two occasions. The second bout was performed after 12 wk of administration of vitamin E, 1,000 IU/day, or vehicle placebo, in a double-blind, randomized, parallel design. Subjects refrained from taking vitamin C supplements for 1 mo and vitamin E supplements for 3 mo prior to the study and during the study period. Subjects also abstained from consuming caffeine and nonsteroidal anti-inflammatory drugs for 12 h before each blood draw. This study protocol was approved by the Tufts University-New England Medical Center Human Investigation Review Committee. The study was fully explained to each subject and written informed consent was obtained.

Acute exercise. As described previously (42), subjects ran downhill for three 15-min intervals at a speed equivalent to 75% of each subject’s predetermined VO2 max. Each 15-min interval was separated by a 5-min period of rest.

α-Tocopherol supplementation. The intervention was 1,000 IU/day of natural-source vitamin E (RRR-α-tocopherol) in soybean oil compared with an identical soybean oil placebo (Pharmavite, San Fernando, CA). Subjects were randomly assigned to receive one or the other compound in a randomized, double-blind fashion. Volunteers were asked to take one capsule daily with their largest meal for 12 wk.

Adherence to the supplementation regimen was confirmed by having subjects return to the study center for serum α-tocopherol checks and pill counts twice during the 12-wk supplementation period and for a third time during the final week of the study. Subjects continued to take their supplement during the final protocol evaluation.

Sample collection and analysis. As previously described (21), muscle biopsies were obtained 24 h before and 72 h after each exercise bout. Biopsies were taken from the superficial portion of the vastus lateralis of the nondominant leg (at the lower third portion of the thigh) using the percutaneous needle biopsy procedure described by Bergström (5). Special care was taken to extract the postexercise biopsy at least 10 mm from the preexercise biopsy site. Biopsies were snap-frozen in isopentane cooled to the temperature of liquid nitrogen and stored at −80°C until analysis.

Blood was drawn from an antecubital vein while the subject sat upright before the exercise test (baseline), immediately following exercise (0 h), and again at 6, 24, and 72 h after exercise. Subjects were asked to be in the fasting state for 12 h prior to each of the baseline and 24- and 72-h blood draws.

Clinical chemistries. As previously described (42), complete blood counts were measured using a Serono-Baker 9000 cell counter (Se-rono Laboratories, Norwell, MA). White blood cell differential counts were determined by microscopic examination of Wright-Giemsa-stained blood smears. As reported earlier (21), serum CRP was measured by latex particle-enhanced immunoturbidimetry using the high-sensitivity protocol of the kit CRP Ultra (Equal Diagnostics, Exton, PA). The lower detection limit of the CRP assay was 0.05 mg/l. Vitamin E (α-tocopherol) was measured as described previously (42). As serum vitamin E is influenced by lipid concentrations, vitamin E levels were expressed per 100 mg of serum total cholesterol plus triglycerides (Roche Diagnostic Systems, Nutley, NJ).

Peripheral blood mononuclear cell cytokine production. Peripheral blood mononuclear cells (PBMCs) were isolated from 20 ml of heparinized blood by centrifugation on a Ficoll-Hypaque gradient (40). Cells were consecutively washed three times in sterile, pyrogen-free saline and suspended at 5 × 10^6/ml in RPMI 1640 medium (Sigma Chemical, St. Louis, MO). Culture medium was supplemented with 100 µg/ml streptomycin (2% final concentration) and 100 U/ml penicillin (2% final concentration, Sigma) and contained 2% autologous heat-inactivated serum and 1% l-glutamine (GIBCO, Grand Island, NY). Cells were cultured in 96-well flat-bottom plates. The
final concentration of cells was $2.5 \times 10^6$ cells/ml in 250 μl of culture medium. Plates were incubated at 37°C in a 5% CO₂ atmosphere for 24 h and subsequently frozen at −80°C. Cells were lysed by three freeze/thaw cycles prior to analysis for total cytokine production.

**Cytokine assays.** Blood samples were collected in EDTA tubes and centrifuged at 1,000 g for 10 min at room temperature. Plasma was separated and frozen at −80°C for subsequent analysis. IL-6 and sIL-6Rs in mononuclear cell cultures and in plasma were measured using ELISA (Quantikine High Sensitivity Human IL-6, Human IL-6, and Human IL-6R ELISA; R&D Systems, Minneapolis, MN). In the case of s-gp130, separate antibodies and standards were purchased from R&D Systems. Detection limits were as follows: high-sensitivity IL-6 < 0.1 pg/ml, IL-6 < 0.7 pg/ml, sIL-6R < 7 pg/ml, and s-gp130 = 0.16 ng/ml.

Total RNA isolation from muscle biopsies and reverse transcription were performed as previously described by Hamada et al. (21). Real-time PCR primers and fluorochrome (FAM)-labeled probes for human IL-6 cDNA were predeveloped by and purchased from Applied Biosystems (Foster City, CA). Cytokine cDNA levels were quantified as reported elsewhere (21).

**Lactate, antioxidant, and oxidative stress assays.** Briefly, blood lactates were measured before and immediately after exercise for a biochemical measurement of exercise intensity. Assessment of lipid peroxidation was measured by plasma F₂-isoprostanes. Antioxidant status was assessed in serum by use of the oxygen radical absorbance capacity (ORAC) assay; the ORAC value (μM Trolox equivalent) represents the area under the quenching curve of β-carotene in the presence of serum antioxidants relative to that of the water-soluble vitamin E analog (10). Both the methods and the values for blood lactate, plasma F₂-isoprostanes, and serum ORAC were reported previously (42) and are used in this study to demonstrate the associations among the acute-phase response and oxidative stress.

**Data analysis.** Statistical analyses were performed using the SPSS statistical package (v. 12.0, SPSS, Chicago, IL), and reported values are means ± SE. To determine the effects of age, vitamin E supplementation, and exercise on measured parameters, a repeated-measures statistical package (v. 12.0; SPSS, Chicago, IL), and reported values were multiplied by the Bonferroni adjustment was used to control for the number of dependent variables. Resulting values were multiplied by the number of dependent outcome variables to make the appropriate correction. This yielded a $P$ value = 0.03, and the significant outcomes remained significant. $P$ values presented in RESULTS and in the figures are the unadjusted values. Pearson’s correlation coefficient was utilized to express bivariant relationships. The following skewed variables were log transformed prior to statistical analyses: serum CRP, plasma IL-6, plasma IL-6 bioavailability, and muscle IL-6 transcripts.

**RESULTS**

**Subject description.** As previously reported (42), both young and old subjects were able to complete the acute exercise and maintain the desired 75% of VO₂max exercise intensity. Heart rate, blood lactate, and serum CK levels were reported in detail previously (42). Both young and old men supplemented with vitamin E had higher serum α-tocopherol than the placebo groups at both compliance visits [mean serum α-tocopherol increased by 122 ± 5 and 141 ± 5% in the young and older men, respectively; both $P < 0.01$ (42)].

**Circulating neutrophils and serum CRP.** Prior to supplementation (visit 1), circulating neutrophils were unaffected by age, although there was a trend toward higher neutrophil counts in the elderly ($P = 0.055$; Fig. 1). As previously reported (21), serum CRP was 3.1-fold higher in the elderly at baseline and remained significantly higher than in young men after exercise ($P < 0.001$; Fig. 2). In young and old men, downhill running elevated circulating neutrophils immediately and 6 h after exercise ($P < 0.001$). Similarly, CRP increased approximately twofold 24 h after exercise ($P < 0.001$). For both neutrophil counts and serum CRP, there were no significant differences in the postexercise rise between age groups.

**Circulating IL-6, sIL-6R, and s-gp130.** At baseline, plasma IL-6 was higher in the old subjects than in the younger subjects (2.6-fold higher, $P < 0.001$; Fig. 3A). IL-6 concentrations increased at $6$ h postexercise to a similar extent in both groups of subjects ($P < 0.01$) and returned to baseline by $24$ h, with the IL-6 from the elderly men remaining higher at all times ($P < 0.01$). There were no effects of vitamin E on plasma IL-6 or on the magnitude of the exercise-induced increase in plasma IL-6 (data not shown).

sIL-6R did not differ between old and young subjects at visit 1 baseline (Fig. 3B). After exercise, sIL-6R became significantly higher in the older than in the younger subjects at both $6$ and $24$ h after exercise ($P < 0.05$). s-gp130, on the other hand, was $9\%$ lower at baseline in the older men (Fig. 3C, $P < 0.05$). Even though there was no significant effect of exercise on s-gp130, the difference between young and old groups was no longer significant by $24$ h after exercise. Baseline concentrations and exercise-induced changes in sIL-6R and s-gp130 during visit 2 were similar to those observed during visit 1 (data not shown).

The positive influence of sIL-6R and the negative influence of s-gp130 on IL-6 bioavailability are presented in Fig. 3D by plotting plasma IL-6 concentrations multiplied by the ratio of the receptor subunits (sIL-6R/s-gp130). As seen with plasma IL-6 alone, there was an increase in the calculated bioavail-

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**Fig. 1.** Young and old men experience similar neutrophil responses to acute exercise. Circulating blood neutrophil counts at baseline (BL), immediately postexercise (0hr), 6 h (6hr), and 24 h (24hr) postexercise for visit 1 (pre-supplementation) in young (○) and old (■) men. Vitamin E supplementation (1,000 IU/day for 12 wk) had no effect on circulating neutrophils; therefore, only visit 1 values are shown. Values are means ± SE. Exercise effect: increase over BL value for both Young and Old, *$P < 0.05$. AJP-Endocrinol Metab • VOL 291 • AUGUST 2006 • www.ajpendo.org
ability of IL-6 in the old compared with the young group at all
time points ($P < 0.01$).

**PBMC production of IL-6.** At visit 1 baseline, age had no
effect on mononuclear cell production of IL-6 (Fig. 4). Exer-
cise increased the production of IL-6 only during visit 1 in the
young group and during visit 2 for the old group ($P < 0.05$).
During visit 2, baseline values of PBMC IL-6 production were
elevated in the young placebo group compared with visit 1
values ($P < 0.05$), as was the exercise-induced production in
the older men supplemented with vitamin E ($P < 0.05$).

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**Fig. 2.** Vitamin E decreases exercise-induced C-reactive protein (CRP) in young men but not in old men. Serum CRP at BL and 24, and 72 h postexercise in young and old subjects before (visit 1/presupplementation, △) and after 12-wk supplementation with placebo or 1,000 IU/day vitamin E (visit 2/postsupple-
mentation, ■). Statistics were performed on log-transformed values of CRP. Values are means ± SE. Exercise effect: increase over BL value, *$P < 0.05$. Age
effect: values in the Old group are higher than in the Young group across time, $P < 0.001$. Vitamin E effect: decrease from presupplementation value, #$P <
0.05$.

**Fig. 3.** Old men have greater baseline and exercise-induced plasma IL-6 bioavailability. A: plasma IL-6; B: soluble IL-6 receptor (sIL-6R); C: soluble
glycoprotein 130 (s-gp130); D: IL-6 bioavailability [(IL-6 × sIL-6R)/gp130] at BL and 6 and 24 h postexercise for visit 1 (presupplementation) in young (△)
and old (■) subjects. Statistics were performed on log-transformed values of IL-6 and IL-6 bioavailability. Vitamin E supplementation (1,000 IU/day for 12 wk)
had no effect on these variables; therefore, only visit 1 values are shown. Values are means ± SE. Exercise effect: increase over BL value, *$P < 0.01$. Age effect:
values in the Old group are higher than in the Young group across time for both IL-6 and IL-6 bioavailability, $P < 0.01$. Old different from Young values, #$P <
0.05$. 

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Muscle IL-6 mRNA. There was no age-related difference in baseline IL-6 transcript levels. However, age reduced the accumulation of IL-6 transcripts after exercise by the elderly, as exercise failed to increase IL-6 mRNA levels in old subjects (1.3-fold, \( P = 0.44 \)) but tended to increase IL-6 mRNA levels in young subjects (3.6-fold increase, \( P = 0.057 \)) (21). We found no effect of vitamin E supplementation on muscle expression of IL-6 in either group at baseline or after exercise (Young-visit 1: baseline 6.0 ± 1.4, 72 h 34.3 ± 15.6; Young-visit 2 after vitamin E supplementation: baseline 4.0 ± 0.9, 72 h 32.9 ± 11.7; Old-visit 1: baseline 8.5 ± 3.1, 72 h 13.5 ± 4.8; Old-visit 2 after vitamin E supplementation: baseline 6.1 ± 1.0, 72 h 15.5 ± 4.4).

Membrane permeability, IL-6, and oxidative stress. As previously reported (42), downhill running resulted in elevations in serum CK, biomarkers of oxidative stress (plasma F2-isoprostanes and malondialdehyde), and decreases in antioxidant status (as measured by ORAC), and these changes were all highly correlated with one another in young men but not in the elderly. We now report strong associations in the young men between exercise-induced peak levels of plasma IL-6, serum CK, serum CRP, circulating neutrophils (Table 2), and blood lactate (\( P < 0.001; R^2 = 0.74 \)). Exercise-induced changes in circulating neutrophils, CRP, and CK were also all highly correlated in the young (\( R^2 = 0.47–0.67, P < 0.01 \)), but only CK and neutrophils were associated in the old men (\( R^2 = 0.32, P < 0.05 \)). The only other identified relationship in the older men was between the exercise-induced rise in plasma IL-6 and the rise in circulating neutrophils (\( R^2 = 0.29, P < 0.05 \); Table 2). No marker of IL-6 status (plasma, PBMC production, or muscle mRNA) correlated with another at baseline or following exercise. The associations seen among plasma IL-6 and biomarkers of oxidative stress were also present if plasma IL-6 was replaced by the IL-6 bioavailability value.

Not only was there a strong relationship among circulating CK, CRP, and IL-6 in the young but also between biomarkers of oxidative stress and IL-6 (Table 2). Peak exercise-induced changes in IL-6 at 6 h correlated directly with peak elevations in F2-isoprostanes at 72 h, whereas PBMC production of IL-6 increased at 24 h, and muscle IL-6 mRNA at 72 h was associated with lower with F2-isoprostanes at this time. IL-6 is associated with antioxidant status as well. The greater the rise in postexercise plasma IL-6, the greater the fall in serum ORAC at 72 h, whereas elevations in IL-6 mRNA at 72 h were associated with increases in serum ORAC (Table 2). Exercise-induced plasma IL-6 appears to be associated with a prooxidant state, whereas muscle IL-6 transcripts are linked with an antioxidant state. Furthermore, a high baseline antioxidant status correlated with greater postexercise circulating IL-6 in both young and old men (Fig. 5A), lower PBMC IL-6 production at 24 h in the young and an increased production in the old (Fig. 5B), and decreased muscle IL-6 mRNA content at 72 h in the young men only (Fig. 5C).

Table 2. Exercise-induced increases in IL-6 correlate with other markers of the acute-phase response and oxidative stress after downhill running in young men

<table>
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<tr>
<th>Exercise-Induced Increases in IL-6</th>
<th>Exercise-Induced Increases in IL-6</th>
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<tr>
<td><strong>Acute-Phase Response and Oxidative Stress</strong></td>
<td><strong>Plasma IL-6</strong></td>
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<td>Neutrophils (+) ( R^2 = 0.41 )</td>
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<td>CK (+) ( R^2 = 0.42 )</td>
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<td>CRP (+) ( R^2 = 0.31 )</td>
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<tr>
<td>F2-IsoP (+) ( R^2 = 0.64 )</td>
<td>(−) ( R^2 = 0.35^* )</td>
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<td>ORAC (−) ( R^2 = 0.47 )</td>
<td>(−) ( R^2 = 0.44 )</td>
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Correlations between exercise-induced changes in IL-6 (in plasma at 6 h, peripheral blood mononuclear cell production (PBMC) at 24 h, or muscle mRNA expression at 72 h) and exercise-induced changes in circulating neutrophil counts (at 6 h), serum creatine kinase (CK, at 24 h), serum C-reactive protein (CRP at 24 h), plasma F2-isoprostanes (F2-IsoP at 72 h), or decrease in antioxidant status [oxygen radical absorbance capacity (ORAC) at 72 h]. Associations were for visit 1 (unsupplemented) only. Correlations were run on log-transformed values for plasma and muscle IL-6 and serum CRP. Associations are positive (+) or negative (−) as indicated and are significant at \( P < 0.01 \) unless noted. *\( P < 0.05 \). Circulating neutrophils also correlated with plasma IL-6 in the older men (\( P < 0.05, R^2 = 0.29 \)).
DISCUSSION

We hypothesized that after an intense bout of muscle-damaging exercise older men would experience exacerbated acute-phase and oxidative stress responses and lack IL-6 negative-feedback protection against exercise-induced oxidative stress and that these differential responses could be attenuated by supplementation with the lipid-soluble antioxidant vitamin E. Our data do not support these hypotheses. The healthy older men examined in the present study experienced similar exercise-induced oxidative stress responses to both young and old men.

Fig. 5. Both young and old men demonstrate associations between BL antioxidant status and peak levels of exercise-induced IL-6. A: peak plasma IL-6 (6 h postexercise) was associated with BL antioxidant status (oxygen radical absorbance capacity (ORAC)) in both young and old men (P < 0.05). B: total PBMC of IL-6 24 h after exercise was negatively associated with BL ORAC in Young (P < 0.05) and positively associated with BL ORAC in Old (P < 0.05). C: muscle IL-6 mRNA 72 h after exercise was negatively associated with BL antioxidant status in Young (P < 0.01) but not in Old (P = 0.12). cDNA levels were quantified by real-time PCR and normalized to 18S cDNA levels. All values are visit 1 (prior to supplementation).
changes to the young men in biomarkers of muscle membrane permeability and oxidative stress (42) as well as with acute-phase markers and plasma and PBMC production of IL-6. However, both before and after exercise, the older men had elevated serum CRP and plasma IL-6 along with greater bioavailability of IL-6 (as measured by the IL-6 ratio). Furthermore, only in the young men did biomarkers of muscle membrane permeability (serum CK), acute-phase response (serum CRP), and oxidative stress (plasma F2\textsubscript{\alpha}-isoprostanes) correlate with one another. This observation suggests a certain degree of dysregulation in the cascade from “damage” to “repair” following intense exercise in the elderly. Furthermore, these age-related differences were not ameliorated by 12 wk of vitamin E supplementation.

The acute-phase response was similar between the two groups of men, although plasma IL-6 and serum CRP were higher in the older men both before and for the 72 h after exercise. Even though there was a comparable exercise-induced increase in neutrophils, there was a trend toward the elderly having higher circulating neutrophils over time, which is in contrast to earlier studies that had shown a blunted neutrophil response in the elderly (7, 9, 47). These studies also found blunted CK responses in older individuals. In the present study, however, our young and elderly men experienced similar postexercise changes in CK (42), neutrophil, and CRP responses, and we have demonstrated that postexercise CRP and neutrophils are both associated with exercise-induced serum CK. We believe that these findings may be due to the higher relative fitness of our older subjects compared with those studied previously; even though both young and old men exercised at similar relative intensities (~75% V\textsubscript{O2 max}), our older subjects may have worked at a higher absolute intensity [based on higher postexercise blood lactate values in these older men (42)], which would decrease the difference between the young and old groups in the actual amount of work performed during the exercise bout.

Vitamin E also decreased the exercise-induced rise in CRP in the young men. Serum CRP has been shown to be associated with oxidative stress as a result of disease (14, 22), and likewise we found in this study that exercise-induced F2\textsubscript{\alpha}-isoprostanes are also associated with postexercise elevations in CRP ($R^2 = 0.25, P < 0.01$). Because of this association with oxidative stress, one would expect vitamin E to decrease serum CRP, as has been shown by others in individuals with diabetes and in healthy subjects (14); or it may be that vitamin E impacts serum CRP via a non-oxidant-mediated mechanism (53). We had hypothesized that old men, who theoretically are exposed to greater amounts of oxidative stress and inflammation, would be more responsive to this antioxidant intervention. This was not the case. It is plausible that older men are simply unable to respond to supplementation due to J) age-associated alterations in one or more signaling events downstream of vitamin E or 2) age-related factors independent of oxidative stress, such as increased levels of glycosylated proteins (49) or decreased levels of dehydroepiandrosterone resulting from an aging endocrine system (13).

To gain insight into the relative bioavailability of IL-6, we have expanded our findings to include measurements of the circulating soluble receptors sIL-6R and s-gp130 and calculated the ratio (IL-6 × sIL-6R)/s-gp130 as an index of IL-6 bioavailability (19). Plasma sIL-6R was similar between the young and elderly at rest, whereas s-gp130 was lower in the elderly. Together, this may indicate greater basal IL-6 bioavailability in the elderly. In contrast to these results, one study found an increase sIL-6R and s-gp130 with age up until the seventh decade in women (20). These differences may be due to the good health of our subjects and/or potential sex differences in circulating levels of these receptors. Furthermore, after exercise in the old men, the levels of sIL-6R became higher than those observed in the young men and were coupled with lower concentrations of s-gp130. This resulted in greater exercise-induced bioavailability of IL-6 in the old men.

Both PBMC production and muscle mRNA expression of IL-6 also increased after exercise; yet neither of these measurements of IL-6 was associated with changes in muscle CK release or acute-phase biomarkers. Only exercise-induced plasma IL-6 was associated with changes in CK, neutrophils, and CRP. Furthermore, associations between blood lactate and CK with plasma IL-6 were observed only in young men. In addition to our findings, others have associated plasma IL-6 with exercise intensity and muscle membrane permeability (35, 36), and one study also found that exercise-induced plasma IL-6 correlated with CK only in young men (47). Thus plasma IL-6 may serve as a marker for exercise intensity and muscle damage in the young, but loses this association with age. We did not measure muscle IL-6 mRNA production until 72 h after the eccentric exercise. However, others have found earlier postexercise elevations in muscle IL-6 mRNA (36), which may partially explain the lack of association between IL-6 mRNA and CK in our study. The current results indicate, at least in young men, that circulating IL-6 (which peaks at 6 h) is more indicative of the changes in muscle membrane permeability and acute-phase response occurring at 24 h than cellular production of IL-6 at 24 h or muscle expression of this cytokine at 72 h (timeline of peak responses depicted in Fig. 6). This compartmentalization of cytokine responses is similar to our previous observations in patients with HIV infection (1) and rheumatoid arthritis (38).

We had also previously reported that elderly men do not exhibit the associations among serum CK, oxidative stress, and antioxidant status that are present in the younger men (42). Here, we extend these findings by showing that, additionally, only in younger men do changes in IL-6 and increased oxidative stress after exercise correlate with one another. For example, elevations in lipid peroxidation (F2\textsubscript{\alpha}-isoprostanes) and decreases in antioxidant status (ORAC) are tightly associated with changes in IL-6. Others have shown correlations between urinary F2\textsubscript{\alpha}-isoprostanes and serum IL-6 with certain disease states (11, 33), although the nature of this association remains unclear. Oxidative stress increases IL-6 (27), but F2\textsubscript{\alpha}-isoprostanes, which are a biomarker for lipid peroxidation, do not increase IL-6 production in vitro (52). To the best of our knowledge, there are no studies demonstrating that IL-6 increases lipid peroxidation. It is possible that IL-6 stimulates F2\textsubscript{\alpha}-isoprostane synthesis through a prostaglandin (PG)-mediated mechanism. For example, even though IL-6 does not increase mononuclear cell production of PGE\textsubscript{2} (15), it increases cerebrospinal PGE\textsubscript{2} levels (15). Our results suggest that in the young men there is a strong interplay between circulating biomarkers of muscle membrane permeability, inflammation, antioxidant status, and oxidative stress with the IL-6 response (Table 2 and Fig. 6).
We have demonstrated a strong correlation between IL-6 and oxidative stress after exercise in young men; yet there are limited studies examining the effect of vitamin E on exercise-induced IL-6 production (32, 37), and these studies have shown no effect of vitamin E on circulating IL-6. In the present study, even with a sufficient duration of supplementation to reach high steady-state levels of vitamin E in plasma and most likely within the muscle (30), vitamin E did not affect circulating IL-6, sIL-6R, s-gp130, or muscle IL-6 gene expression. On the other hand, supplementation with 1,000 IU/day of vitamin E increased IL-6 production by mononuclear cells from the older men after exercise but had no effect on baseline production, which was similar between the young and older men. The similarities in baseline PBMC IL-6 production among different age groups have been supported by others (4), whereas the effects of vitamin E supplementation on enhanced cytokine production in the healthy elderly have also been demonstrated in earlier research (8, 25). These results suggest that PBMC IL-6 production may be inhibited by reactive oxygen species following exercise in old age. This is consistent with the hypothesis that oxidative stress increases with aging and that the effects of this stress can be partially ameliorated by vitamin E supplementation, at least in the circulating cellular compartment.

Even though vitamin E does not appear to impact muscle IL-6 expression directly, a greater baseline antioxidant capacity in the young men was associated with a lower level of postexercise IL-6 mRNA (Fig. 5C). These results suggest that circulating antioxidant status (representative of mainly water-soluble antioxidants and independent of vitamin E supplementation; see Ref 42), may reduce exercise-induced changes in IL-6 gene expression in muscle. It could be that the roles of antioxidants differ within the compartments considered, i.e., the systemic compartment vs. muscle. The absence of this association in older men may also be due to the lack of IL-6 transcript accumulation at old age and could result in greater inflammation via a negative-feedback loop, as suggested by transgenic models of IL-6 knockout mice (16). On the other hand, chronic high circulating IL-6 in older men [as found in this study and by others (47)] may reflect an age-related effort to defend against an underlying inflammatory state. Antioxidants may work to attenuate underlying inflammation via an oxidative stress-mediated mechanism and, in turn, this chronic elevation in circulating IL-6. In this model, both circulating IL-6 and muscle mRNA would increase following intense exercise in elderly men to counter inflammation. Then, due to the reestablishment of a negative-feedback loop, basal levels of circulating IL-6 would return to those levels observed in the young.

In conclusion, an acute bout of eccentric exercise elicits similar changes in the acute-phase response and oxidative stress in healthy young and elderly men. However, our results illustrate that the progression of the adaptive acute-phase cascade and its relationship to oxidative stress in response to eccentric exercise is dysregulated in healthy older men, whereas there are multiple relationships among these parameters in healthy young men (Fig. 6). In the young, changes in circulating IL-6 are closely associated with a prooxidant state, whereas changes in muscle IL-6 mRNA correlate with an antioxidant state. These results therefore highlight the compartmental nature of cytokine kinetics and the dangers of overgeneralizing about cytokine status from a single source. Further-

Fig. 6. Time line of the exercise-induced relationships among IL-6, inflammation, and oxidative stress that exist in young men only. Positive (A) and negative associations (B) between IL-6, muscle membrane permeability [serum creatine kinase (CK)], acute-phase response [serum CRP and circulating neutrophils], antioxidant status (ORAC), and biomarkers of oxidative stress [F_2-isoprostanes (F_2IsoP)] in young men after 45 min of downhill running compiled from results presented in Table 2 and Fig. 5. Arrows represent positive associations (solid lines, $P < 0.05$) in A and negative associations (dashed lines, $P < 0.05$) in B.
more, supplementation with vitamin E for 3 mo did not reestablish any of the apparent dissociations in the elderly. A larger subject pool in the protocol might have added greater statistical power to these observations; nonetheless, these findings provide useful information for those interested in further examining hypotheses about the interrelationships between aging, exercise, and cytokines. Future studies should incorporate a full assessment of pro- and anti-inflammatory cytokines in the circulation, cells, and tissues to further elucidate age-related dysregulation of tissue injury and inflammatory responses and whether antioxidants may modulate these changes.

ACKNOWLEDGMENTS

We appreciate the contributions made to this study by the staff of the Metabolic Research Unit and Nutrition Evaluation Laboratory from the National Institute of Aging at Tufts University. We are grateful for the technical support of Leslie Abad and Russell Parker throughout the study and the statistical contributions made by Dr. Gerald Dallas.

GRANTS

This study was supported by U.S. Department of Agriculture Cooperative Agreement 58-1950-9-001 (R. Roubenoff), Otsuka Pharmaceuticals (R. Roubenoff), American College of Sports Medicine (J. M. Sacheck), and LifeFitness (J. M. Sacheck).

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