Does the aging skeletal muscle maintain its endocrine function?

Running head: Muscle-derived interleukin-6 in elderly humans

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Abstract

Contracting skeletal muscles produce and release the cytokine interleukin (IL)-6 and this release is augmented by the presence of low muscle glycogen. Since muscle metabolism in elderly subjects relies on glycogen more than younger subjects, it is possible that aging is associated with an altered production of muscle-derived IL-6 during exercise. To test the relation between aging and muscle-derived IL-6, seven healthy elderly males, mean age 70 ± 1 (SEM) yr and six healthy young males, mean age 26 ± 2 (SEM) yr performed three hours of dynamic knee-extensor exercise at 50% of maximal work load (Wmax). IL-6 mRNA and glycogen in muscles were analysed and the IL-6 release were estimated before, during and after the exercise. Although the absolute work load in the elderly was less than half of that in the young, 41.1 ± 3.1 W and 92.5 ± 4.0 W, respectively, the muscle glycogen utilization after three hours of exercise did not differ between groups, 238.7 ± 52.4 and 245.2 ± 74.0 mmol/kg muscle in elderly and young, respectively. This could explain that the IL-6 release and the IL-6 mRNA amplification increased during exercise with no difference between groups, two-way ANOVA-P = 0.50 and 0.45, respectively.

In conclusion, elderly healthy people maintain the capacity to produce and release IL-6 in response to dynamic exercise, with no difference compared to young individuals furthermore, glycogen utilization expressed in changes of glycogen related to muscle mass was equal in elderly and young subject at 50% of Wmax.

Keywords: Interleukin-6, exercise and aging

Introduction

Plasma interleukin (IL)-6 levels increase up to 100 fold in response to exercise (42) (12;39-41;50;59). It has recently been shown that IL-6 is released from contracting skeletal muscles during dynamic knee extensor exercise and that this release may be responsible for the total increase in the systemic concentration (61). Studies in rats (26) (30) and humans (24;28;57;61) (10;15;45) demonstrate elevated levels of IL-6 mRNA in contracting skeletal muscle. Evidence exists that it is the muscle fibers per se, which are the source of muscle-derived IL-6 (14;16;44).

When muscle glycogen was depleted prior to exercise in one leg, this leg released IL-6 one hour prior the non-glycogen depleted leg (61). Furthermore, the transcription rate of the IL-6 gene in muscle nuclei (28), and in total muscle IL-6 mRNA increased when exercising with lower muscle glycogen compared with a trial where the same subjects exercised with normal muscle glycogen content (10;28;61), therefore low muscle glycogen has been suggested as a signal for IL-6 release from contracting muscles. This statement has been strengthened by a study showing an inverse correlation between high IL-6 release during exercise and muscle glycogen content at the end of exercise (21) and by the finding that training reduces the contraction-induced IL-6 mRNA expression in skeletal muscle (17).

Regular exercise offers protection against all cause mortality, primarily by protection against atherosclerosis and type 2-diabetes (3). In addition, physical training is effective in the treatment of patients with ischemic heart disease (25), hypertension (46) and hyperlipidemia (31).

Over the past decade, there has been much focus on the role of inflammation in the pathogenesis of atherosclerosis and its complications. Whereas most clinicians previously regarded atheroma as a bland lesion, the current notion that inflammation and immune response contribute to atherogenesis has created increased interest (32).

During ageing circulating levels of a number of cytokines increase. Thus, increased plasma levels of TNFα (6;7;11;36), IL-6, IL-1ra (11) and sTNFR (5;6;8) have been demonstrated. In addition ageing is also associated with increased levels of acute phase proteins such as C reactive protein (CRP) and Serum Amyloid A (SSA)(2). Elevated levels of circulating IL-6 and TNFα have been associated with several disorders. Thus, increased levels of TNFα and IL-6 have been observed in obese individuals, in smokers and in non-insulin dependent diabetes mellitus (68) and levels of IL-6 have been shown to predict all-cause mortality as well as cardiovascular mortality (20;69). Furthermore, plasma concentrations of IL-6 and TNFα have been shown to predict the risk of myocardial infarction in several studies (52;53;64), and recently it was shown that the CRP level is a stronger predictor of cardiovascular events than the LDL cholesterol level and that CRP adds prognostic information to that conveyed by the Framingham risk score (54). It has been proposed that IL-6 is the mediator that links the acute phase response to visceral obesity, insulin resistance and atherosclerosis (72). High levels of IL-6 in patients with metabolic syndrome may be explained by the fact that IL-6 is produced in adipose tissue (18;35). Adipose tissue also produces and releases TNFα (65). However, in contrast to IL-6, the available data
suggests that TNFα plays a mechanistic role in insulin resistance. Thus, TNF-α
down-regulates GLUT-4 and inhibits insulin receptor activity (23). Since TNF-α
can trigger IL-6 release, one theory holds that adipose tissue derived TNF-α actually
is the „driver“ behind the metabolic syndrome and that TNF-α rather than IL-6
should be placed in the center as the cytokine that induces insulin resistance and
thereby initiates diabetes type 2 and atherosclerosis.

It is possible that exercise mediates its beneficial health effects by inducing
an anti-inflammatory environment. The cytokines, which are present in the circula-
tion following exercise is IL-6 and classical anti-inflammatory cytokines such as IL-10
and IL-1ra (39;43).

There has been much debate on how to classify the cytokine IL-6. Tilg et al
suggested that IL-6 should be classified as an anti-inflammatory cytokine (63).

The finding that rhIL-6 infusion enhances levels of IL-10 and IL-1ra (60)
and inhibits endotoxin-induced TNFα production lends support to the idea that
IL-6 has anti-inflammatory effects (56).

The fact that exercise alone inhibited endotoxin-induced TNFα increase in
the circulation (56) and that TNF-α overexpression returned to normal levels after
exercise in the TNF-α receptor knockout model (27) indicates that exercise mediates
anti-inflammation.

Given the biological profile of IL-6, it would be important to know whether
the aging skeletal muscle is able to produce IL-6. The purpose of the present
study, therefore, was to test whether elderly subjects maintain their capability of
producing and releasing IL-6, and whether this could be explained by an age
related change in muscle glycogen metabolism.

Matherials and Methods

Subjects: Seven healthy elderly males and six healthy young males (whose charac-
teristics are shown in table 1) participated in the study. All
subjects were recruited through earlier studies in our
department. Medical history, physical examination, clinical
chemistry profile, complete blood count, cell differential
count and CRP, and electrocardiogram demonstrated that
the subjects were completely healthy. The study was in accordance with the
Helsinki II Declaration and approved by the regional research ethical committee
(No. 01-216/00). All subjects gave their written consent before participation.

Studies of the effect of aging on metabolism or immune function during
exercise are complicated by large between-group differences in VO2max, which
raises the question of how to best standardize the exercise intensity. In the present
study, an exercise model was chosen which was not dependent upon the cardio-
respiratory capacity and therefore useful in studies including elderly untrained

subjects. Therefore, in this study we chose to investigate the two groups when
performing exercise at same relative percentage of maximal workload.

The subjects performed 3 h of dynamic two-legged knee extensor exercise
at 50 % of maximal workload (Wmax) on a modified Krogh cycle ergometer
as previously described (1). At least one week before the trial a two-legged knee
extensor exercise test was performed to determine Wmax. Resistance load was
increased every two minutes until a cadence of 60 extensions/min could no longer
be maintained. The highest workload that could be maintained for two minutes
was set as the maximum workload. After 1/4 hour rest the subjects performed 2 h
of two-legged knee extensor exercise at 50% of Wmax to familiarise themselves
with the apparatus.

The evening before the experiment, all subjects were provided with a fixed
diet to ensure that there was no difference in diet between the two groups. The
subjects reported to the laboratory the following day after an overnight fast.
Catheters were placed in the femoral vein and artery of one leg under local anesthe-
thesia (lidocaine 20 mg/ml). Blood samples were drawn into pre-cooled tubes
containing EDTA at 0, 30, 60, 90, 120 and 180 min of exercise and at 60 and 120
min post-exercise. Blood samples were immediately spun at 4°C and plasma was
isolated and stored at -80°C until analyses were performed. Blood flow in the femoral
artery at each time point was measured with Doppler ultrasound tech-
nique as described in details previously (51). In addition, muscle biopsies were
obtained from the vastus lateralis at 0, 30, 90 and 180 min of exercise and at 120
min post-exercise using the percutaneous needle biopsy technique with suction.
Biopsy samples were obtained from the left leg (0 and 90 min during exercise
and 120 min post-exercise) and from the right leg (30 and 180 min). Muscles were
cleaned of connective tissue and blood, and quickly frozen in liquid nitrogen for
later analysis.

IL-6 measurements: Commercially available enzyme-linked immunosor-
bent assay (ELISA) kits were used (Quantikine HS, R&D systems, Minneapolis,
MN, USA) to measure plasma IL-6. According to R&D systems the detection
limit is less than 0.094 pg/ml. All samples were run in duplicates and mean values
were used. Net IL-6 release was calculated according to Fick’s principle (70) by
multiplying the arterial-femoral venous (a-fv) difference by blood flow.

Measurements of catecholamines: High Performance Liquid radioim-
munoassay Chromatography described in detail elsewhere (4), was used to deter-
mine systemic concentrations of adrenaline and noradrenaline.

Measurements of lactate: Plasma lactate were measured in arterial and
venous blood using an automatic analyser (Cobas Fara, Roche, France) and
release was calculated according to Fick’s principle.

IL-6 mRNA analyses: Total RNA was isolated from 9-86 mg of muscle tis-
sue by a modified guanidinium thiocyanate phenol-chloroform extraction method
adapted from Chomczynski and Sacchi (9) as previously described (48). Final
pellets were dissolved in 0.1 mM EDTA (2 µl/mg wet weight). Reverse transcription
reactions were carried out on 11 µl sample using the Superscript II Rnase H-
system (Gibco-BRL) in a reaction volume of 20 µl. All samples were diluted to a
final volume of 150-200 µl with nuclease free water.

β-actin mRNA and IL-6 mRNA levels were determined by real-time PCR,
which determines the amount of cDNA amplification after each PCR cycle. With
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Results:

The area of QF in each slide by the distance between the slides and summed for all images and the muscle mass was calculated assuming a muscle density of 1.04 kg/l (22).

Statistical methods: SYSTAT version 8.0 (SPSS Inc., Chicago, USA) was used as software. Plasma IL-6 and IL-6 mRNA values were not normally distributed and were therefore square root transformed. Absolute changes in response to exercise were evaluated by analysis of variance (ANOVA) for repeated measurements (model parameter = time + age + time x age). If a significant (time x age) was found, a two-sample t test (Bonferroni-adjusted) for independent groups was used to detect age-related differences in absolute changes from baseline levels. If only time turned out to be significant, age group was pooled and a paired t-test (Bonferroni-adjusted) was performed to detect changes from baseline levels. A P-value < 0.05 was considered as significant.

Figure 1: Blood flow in femoral artery in 7 elderly subjects (♀) and 6 young controls (●) measured before, during and after (+60 and +120) 3 h of dynamic knee-extensor exercise. Data are presented as median and S.E.M.

* P< 0.05 vs. pre-ex
† P< 0.05 vs. elderly subjects

Blood flow: The blood flow increased in both groups, being more pronounced in the young subjects (Two-way ANOVA for repeated measurements, P<0.001) (Fig. 1).

Plasma IL-6: The a-v difference for IL-6 increased in the end of exercise in both groups (P < 0.05) (Fig. 2A). When the net IL-6 release was calculated there was a gradual increase during exercise (P<0.05). However, over time there was no difference between the groups (Fig. 2B). Furthermore, when the net IL-6 release was related to the QF muscle mass (MRI) there was no differ.

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Lactate release: There was a small but significant increase in lactate release over time (P = 0.002) with no difference between groups. After 30 min of exercise the release peaked in the elderly group to 1.06 ± 0.21 mmol/min and in the young group after 120 min of exercise to 0.35 ± 0.27, (Fig. 5).

Muscle Glycogen Content: The muscle glycogen content decreased during exercise with no difference between groups, either before or during the exercise (Fig. 4A). After 3 h of exercise the glycogen utilization was 238.7 ± 52.4 and 245.2 ± 74.0 mmol/kg muscle in elderly and young, respectively. When energy from glycogen utilization was calculated, there was no difference between groups (Fig. 4B).

Effect of mechanical work: When the total energy from muscle glycogen breakdown was related to the total mechanical energy performed, it was demonstrated that the elderly had a higher (P <0.05) glycogen breakdown, indicating that the elderly individuals may rely more on glycogen breakdown than the young subjects (Fig. 4C).

Catecholamines: Adrenaline and noradrenaline increased (P<0.01) as expected during exercise, with no difference between the two age groups, although there was a tendency to higher levels in the young subjects after three hours of exercise (Table 2).
which it declined, makes this assumption valid since it indicates that lactate production was negligible and unlikely to contribute to anaerobic glycogenolysis and/or affect IL-6 production.

In this study, the groups differed with regard to both maximal workload performance as well as muscle mass. However, the difference between groups with regard to maximal workload performance was more pronounced than the difference in muscle mass. This may be explained by an overestimation of muscle mass in the elderly group, as a result of a higher water and fat content in aging muscles (19;49;66).

It has been debated whether catecholamines stimulate IL-6 production during exercise (62).

However, the exercise-induced increase in plasma IL-6 could not be mimicked by epinephrine infusion. Although epinephrine induced a small increase in IL-6 and may, therefore, partly influence the plasma levels of IL-6 during exercise, it could not account for the massive increase in IL-6 during exercise (58).

As skeletal muscle is the major source of IL-6 during exercise (61) it is not likely that adrenaline is a major stimulator of muscle-IL-6, although it stimulates adipose tissue IL-6 production (29).

In the present study there was no significant difference between the two age groups with regard to the neither the catecholamine responses nor the IL-6 release. Therefore, the present study does not really shed much light on the role of epinephrine in the regulation of muscle-IL-6.

IL-6 has been placed in the center of modern internal medicine as the link between inflammation, obesity, stress and coronary heart disease (72). Given the finding that during exercise skeletal muscles produce and release large amounts of IL-6 into the systemic circulation and given the many beneficial effects of physical exercise on health, it is possible that during moderate regular exercise, IL-6 may mediate some of these effects. Recently, it was demonstrated that IL-6 knockout mice develop late onset obesity and impaired glucose tolerance (71). IL-6 may mediate some of these effects. Recently, it was demonstrated that IL-6 knockout mice develop late onset obesity and impaired glucose tolerance (71). Moreover, mice bearing IL-6-producing tumours lose weight (34) and rhIL-6 knockout mice develop late onset obesity and impaired glucose tolerance (71). IL-6 may mediate some of these effects. Recently, it was demonstrated that IL-6 knockout mice develop late onset obesity and impaired glucose tolerance (71). However, the exercise-induced increase in plasma IL-6 could not be mimicked by epinephrine infusion. Although epinephrine induced a small increase in IL-6 and may, therefore, partly influence the plasma levels of IL-6 during exercise, it could not account for the massive increase in IL-6 during exercise (58).

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We therefore suggest that muscle-derived IL-6 may work in a hormone like fashion mediating exercise-induced lipolysis and anti-inflammation (13;33;43). These findings further classify skeletal muscle as an endocrine organ.

In conclusion, the present study demonstrates that aging skeletal muscle maintains its endocrine functions and specifically demonstrates that healthy elderly subjects maintain a normal capability of producing and releasing IL-6 from contracting muscle compared to young subjects.

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