A common variation in the promoter region of interleukin-6 gene shows association with exercise performance

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Abstract

Skeletal muscle-derived interleukin-6 (IL-6) is a pleiotropic cytokine which regulates body metabolism during strenuous physical exercise. The effect of a potentially functional single nucleotide polymorphism (SNP) -174G/C of the IL6 gene (rs1800795) promoter was examined on maximal oxygen uptake (VO2max), body mass index (BMI) and plasma IL-6 levels in response to physical training. Fifty four male military conscripts were studied for 8 weeks during their basic training. At weeks 1, response to physical training. Fifty four male military conscripts were studied for 8 weeks during their basic training. At weeks 1, 5 and 8, VO2max and anthropometrics were measured, and individual responses to physical training (Macarthur and response to physical training. Fifty four male military conscripts were studied for 8 weeks during their basic training. At weeks 1, 5 and 8, VO2max and anthropometrics were measured, and anthropometrics were measured, and blood samples collected before and after acute aerobic exercise. Acute exercise increased plasma IL-6 in subjects with genotype CG. Moreover, during the 8-week training period, a tendency for increased plasma IL-6 was observed in subjects with this genotype. VO2max values increased in all genotype groups, but subjects with genotype CG made the greatest gains in VO2max. Training significantly decreased BMI only in subjects with genotype CG. Our findings suggest that the allele C may have an effect on plasma IL-6 response to acute exercise in healthy male subjects. Exercise training has a favourable effect on VO2max and BMI, with the most prominent effects in subjects with genotype CG. Thus we conclude that this SNP may account for individual response to exercise training.

Key words: Maximal oxygen uptake, IL-6, polymorphism, body mass index, training.

Introduction

Interleukin-6 (IL-6) has been considered as a marker of inflammation and an immunomodulatory cytokine, which is mainly produced by the immune cells (Febbraio and Pedersen, 2002). Recently, it has become evident that IL-6 plays a role in the regulation of metabolism during physical exercise (Febbraio and Pedersen, 2002). Efflux of IL-6 into blood increases up to 100-fold during strenuous exercise (Pedersen et al., 2003). IL-6 may improve skeletal muscle energy supply and assists in the maintenance of stable blood glucose levels by stimulating lipolysis in the adipose tissue and augmenting glycogenolysis in the liver (Pedersen and Febbraio, 2007; Pedersen et al., 2003).

Single nucleotide polymorphisms (SNPs) represent approximately 90% of the genetic differences between individuals (Brookes, 1999). In addition to physical training and nutrition, genetic factors have an effect on physical performance. SNPs located in the promoter regions may alter the function of genes and thereby explain individual responses to physical training (Macarthur and North, 2005). The potentially functional promoter SNP -174G/C (rs1800795) of the IL6 gene may influence its transcription (Brull et al., 2001; Fishman et al., 1998; Jones et al., 2001). Moreover, this SNP has also been associated with many health-related phenotypes (Halverstadt et al., 2005). To our knowledge, only one study has so far examined the effect of IL6 promoter polymorphism on physical working capacity in young male subjects (Ortlepp et al., 2003). In that study, the C allele was associated with elevated leukocyte, lymphocyte and monocyte counts, and a reduced physical fitness in smokers (Ortlepp et al., 2003). However, no follow-up was performed nor training responses were accounted.

In the present study, we hypothesized that the -174G/C SNP has an effect, not only on human exercise performance, but also on training responses. The information on individual genotype may have implications to design individual training plans for patients or athletes.

Methods

The subjects were 54 randomly chosen healthy voluntary male conscripts (age 19 ± 1 years, BMI 24.2 ± 2.3 kg·m⁻²) who were performing their normal military training. Throughout the 8-week study, the subjects were not allowed to use any nutritional supplements. The subjects performed physically demanding activities such as marching and combat training, and occasionally carried a full combat gear with 25 kg total weight including clothing. During the basic training season, the overall physical load of the subjects was according to the standard direction of the Finnish Army Defence Command. The research plan was approved by the University of Jyväskylä ethical committee. Informed consent was obtained from all subjects prior to inclusion in the study.

Assessment of physical performance

International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) was applied to determine subjects’ physical activity prior to entering military training. Additional questionnaire were applied for determination of participant’s physical strenuousness of work, subjective health and smoking habits. Body mass was recorder by a precision scale (Sartorius F 150S-D2, Goettingen, Germany). Percent body fat (F%) was estimated from the thickness of four skinfolds (triceps, biceps, subcapular and supraspinale) using skinfold calipers (Model 98.610, Holtain Ltd., Dyfed, Wales, UK). Fat free mass was cal-
calculated using the following formula: body mass × (100-
F%)/100. Body height was measured to the nearest 0.5 cm
using a wall-mounted stadiometer. Body mass index
(BMI, kg·m⁻²) was calculated as body mass divided by the
body height squared.

To determine VO₂max, the subjects performed a
maximal treadmill test using the following criteria: a
plateau in VO₂max despite an increase in grade and/or
speed, a respiratory exchange ratio (RER) >1.1, and blood
lactate levels higher than 8 mM one min after completing
the test (American College of Sports Medicine, 2001).
Every test was preceded by a brief (3 min) warm-up at 4.6
km/h (1% slope). Thereafter exercise intensity was
increased every 3 min to induce an increase of 6 mL
O₂/kg/min in VO₂ demand during running (American
College of Sports Medicine, 2001) until exhaustion. Pul-
monary ventilation and respiratory gas exchange were
measured by breath-by-breath method (Jaeger Oxygen
Pro, VIASYS Healthcare GmbH, Hoechberg, Germany).
Blood lactate levels were determined 1 min after comple-
tion of the exercise from fingertip blood samples (Lac-
tatePro®, Arkray, Japan). Follow-up tests were done at the
same time of day and with same food ingested at
weeks 5 and 8.

**Blood samples and genotyping**

Blood samples were collected before and immediately
after a 45 min sub-maximal march test which was per-
formed on an outdoor track at 70% level of participant’s
maximal workload in the beginning (baseline), and at
weeks 4 and 7 of the training period. Plasma and whole
blood samples were stored at -80°C until analysis. Plasma
IL-6, TNF-α and IL-1β were measured by immunoassay
(ELISA) according to manufacturer’s protocol (Sanquin
Reagents, Amsterdam, The Netherlands). Assay specifica-
tions for IL-6, TNF-α, IL-1β were as follows: sensitivity
limits; 0.4 pg/ml, 1.4 pg/ml and 1.5 pg/ml, respectively,
 intra-assay CV% were 3.6%, 7.2% and 5.3%, respec-
tively, and that of inter-assay CV% were 8.4%, 7.2% and
9.7%, respectively. Creatine kinase (CK) was measured
using Vitros CK DT Slides and a Vitros DT60 Analyzer
(Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA).
At high and low concentrations the intra-assay CV% were
3.1% and 1.7%, respectively, and inter-assay CV% were
6.1% and 3.0%, respectively. Percentage change in
plasma volume (%ΔPV) was calculated from changes in
haemoglobin and hematocrit according to the method of
Dill and Costill (1974). To adjust for hemoconcentration,
IL-6, TNF-α, IL-1β and CK post exercise values were
normalised as follows: Post-EXad = Post-EX + (Post-EX
× %ΔPV /100).

For SNP analysis, a molecular beacons assay was
employed (Marras et al., 1999). Briefly, genomic DNA
was first isolated from blood mononuclear cells using
QIAamp DNA Blood kit (Qiagen, Hilden, Germany). Next,
15 nanograms of the DNA was amplified using
Brilliant QPCR Master Mix (Stratagene, La Jolla, CA)
with gene-specific primers and each molecular beacon on
a Mx3000P Real-time PCR System (Stratagene) The
oligonucleotides were as follows (shown in 5’- 3’ orienta-
tion):

forward primer AAGAGTGGTTCTGTGTTTACAG,
reverse primer GTGAGGGTGGGCAGAGAG
Allele C Beacon FAM-CCGGATCAGTTGTGTTTCG
CATCGTAAAGGACGATCAGG-BHQ1 and
Allele G Beacon HEX-CCGGATCAGTTGTGTTTCG
GGATCGTAAAGGACGATCAGG-BHQ1.

**Energy intake**

To estimate possible variations in energy intake during the
study, the subjects kept pre-filled dietary records for
3-4 days in four phases. Altogether, habitual food intake
was obtained for 15 days. The pre-filled dietary records
provided detailed information regarding the food in-
gested. Any questions, ambiguities or omissions were
individually resolved and controlled via personal inter-
views, where under-eating or mis-recordings were ques-
tioned. Nutrient use was calculated using Nutrica® soft-
ware (version 3.11, The Social Insurance Institution of
Finland, Helsinki, Finland).

**Statistical analysis**

Calculations were performed using SPSS software (SPSS
Inc, Chicago, IL, USA). Genotype frequencies were ana-
lyzed by chi-square with Yates’ correction between the
different groups. Analysis of variance (ANOVA) was
used to compare the effect of SNP on continuous vari-
ables. Interaction between exercise training and genotype
was examined using repeated measures ANOVA. Corre-
lations were analysed with Pearson’s product moment
method. Statistical significance was set at P<0.05. Data
are presented as mean values with 25-75 percentile range
shown.

**Results**

**Genotype frequency**

The allele distribution was in Hardy-Weinberg’s equilib-
rium in study population (p = 0.197). Allele frequencies
were 0.78 for allele C and 0.76 for allele G, and the geno-
type distributions were as follows: GG: 22% (n=12), CG:
54% (n = 29), and CC 24% (n = 13).

**Plasma levels of IL-6**

At baseline, there were no statistically significant differ-
ences in resting plasma levels of IL-6 protein between
genotypes (Figure 1). However, acute exercise increased
plasma IL-6 in subjects with genotype CG at weeks 1 and
7 (p = 0.037 and p = 0.042, respectively) (Figure 1). No
interaction between training and acute exercise was ob-
woven within genotype groups.

**Maximal oxygen uptake**

VO₂max values were calculated as ml O₂·kg⁻¹·min⁻¹, and
also as mlO₂/fat free mass/min. Because these alternative
calculations gave comparable outcome, we used the
common expression of VO₂max, the ml O₂·kg⁻¹·min⁻¹, to
present final results. At baseline, the VO₂max values were
different between genotype groups with highest values in
subjects with genotype CC and lowest with genotype CG
(Figure 2).
VO2max values increased in all genotype groups during the study period, and the major improvements were observed during the first 5 weeks (Figure 2). After 8 weeks of training, subjects with genotype CG made greatest gains in their VO2max (10.8% increase; from 42 to 47 ml·kg⁻¹·min⁻¹; p < 0.001), followed by subjects with genotype GG (6.7% increase; from 45 to 48 ml/kg/min; p = 0.02), and the smallest improvements were observed in subjects with genotype CC (5.1% increase; from 49 to 52 ml·kg⁻¹·min⁻¹; p = 0.17). When VO2max was defined in absolute values (litres of O₂/min), the rank-order for gains was as follows: genotype CG (8.5% increase; from 3.18 to 3.45 L·min⁻¹; p < 0.001), genotype GG (6.2% increase; from 3.41 to 3.62 L·min⁻¹; p = 0.015), and genotype CC (4.6% increase; from 3.70 to 3.87 L·min⁻¹; p = 0.19).
IL-6 gene variation and exercise performance

Body composition and energy intake

There was a non-significant increase in BMI with respect to number of C alleles (Figure 3). Interestingly, subjects with genotype CG significantly reduced their BMI during the training period (p = 0.02 from week 1 to week 5, p < 0.001 from week 1 to week 8 and p < 0.001 from week 5 to week 8), whereas in other genotypes, the changes were non-significant (Figure 3). In addition, fat-free mass decreased significantly only in subjects with genotype CG (-1.2%; p = 0.036) (data not shown).

There were no significant differences in physical activity by IPAQ questionnaire or physical strenuousness of work, subjective health status, and smoking habits between genotype groups before the military training (data not shown). In addition, no major differences were found in dietary habits of the subjects (data not shown).

Muscle damage and inflammation

Muscle damage was evaluated using plasma CK, whereas TNF-α and IL-1β served as markers of inflammation. Throughout the study, no statistically significant differences were observed in the levels of CK, TNF-α and IL-1β between the genotypes (Table 1).

Nevertheless, mean CK values tended to decrease in all genotype groups towards the end of training period (Table). In subjects with genotype CC, plasma IL-6 and TNF-α did not correlate with each other. IL-6, however, showed a strong positive correlation with IL-1β at baseline (r = 0.919, p < 0.001) and after acute exercise (r = 0.951, p < 0.001), as well as after 7 weeks of training, before (r = 0.909, p < 0.001) and after acute exercise (r = 0.939, p < 0.001). In subjects with genotype CG, IL-6 showed a strong positive correlation with TNF-α at baseline (r = 0.794, p < 0.001) and after acute exercise (r = 0.801, p < 0.001), but not after 7 weeks of training. Plasma IL-6 and IL-1β correlated at baseline (r = 0.466, p = 0.022) and after acute exercise (r = 0.497, p = 0.014), but not after 7 weeks of training. In subjects with genotype GG, TNF-α or IL-1β did not correlate with IL-6 at baseline or after 7 weeks of training.

Discussion

The main finding of the present study was that the IL6 promoter SNP -174G/C showed association with VO₂max and BMI responses to physical training. This

Table 1. Markers of muscle damage and inflammation before and after acute exercise during the study period. Values are shown as means (± SD).

<table>
<thead>
<tr>
<th>Genotype CC</th>
<th>Genotype CG</th>
<th>Genotype GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Week 7</td>
<td>Week 1</td>
</tr>
<tr>
<td>CK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ex</td>
<td>Post-ex</td>
<td>Pre-ex</td>
</tr>
<tr>
<td>(U/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>536***</td>
<td>441</td>
</tr>
<tr>
<td>(228)</td>
<td>(255)</td>
<td>(260)</td>
</tr>
<tr>
<td>(pg/ml)</td>
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</tr>
<tr>
<td>600</td>
<td>600</td>
<td>603</td>
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<td>640</td>
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<td>617</td>
</tr>
<tr>
<td>598</td>
<td>655</td>
<td>655</td>
</tr>
<tr>
<td>IL-1β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ex</td>
<td>Post-ex</td>
<td>Pre-ex</td>
</tr>
<tr>
<td>(pg/ml)</td>
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<tr>
<td>58</td>
<td>57</td>
<td>59</td>
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<tr>
<td>(61)</td>
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<td>(54)</td>
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</table>

Repeated measures ANOVA: * p < 0.05; **p < 0.01; ***p < 0.001 between pre- and post-exercise values. † p < 0.05; †† p < 0.01; ††† p < 0.001 between pre-exercise values at week 1 and week 7. No significant differences were observed between the genotypes at any data points.
polymorphism also showed association with plasma IL-6 response to acute exercise in subjects with genotype CG. Although circulating IL-6 may not precisely reflect the expression pattern and biological significance at the tissue level, our finding that allele C was associated with elevated IL-6 plasma levels only in response to acute stressor is supported by earlier studies (Brull et al., 2001; Jones et al., 2001). On the other hand, elevated levels of plasma IL-6 have been found in type 2 diabetic patients with allele G (Vozarova et al., 2003), while decreased levels in patients with juvenile chronic arthritis patients with allele C (Fishman et al., 1998), yet some studies have suggested no association (Nauck et al., 2002). In the study by Obergach et al. (2008), the subjects carrying allele C, significantly reduced their IL-6 levels in serum after long-term exercise training. Furthermore it was concluded that genetic variations are important determinants for individual response to anti-inflammatory effects of exercise training. Evidently, the net effect of this SNP on circulating IL-6 is remains unclear and may be attributable to the characteristics of the population investigated.

We found no evidence for increased skeletal muscle damage between the genotype groups which could explain the observed differences in IL-6 response to acute exercise. Moreover, in subjects with at least one C allele, plasma IL-6 levels correlated with the inflammatory cytokines TNF-α or IL-1β, although this association was shown to be highly variable. Therefore, despite the metabolic factors that may induce IL-6 secretion in response to physical exercise (Pedersen et al., 2001), we can not completely rule out the possibility that the increase in plasma IL-6 levels is associated with inflammation in subjects with allele C.

Our results are partially supported by previous findings in healthy male smokers, where allele C was associated with reduced physical performance, although no such association was observed in the corresponding non-smoking group (Ortliapp et al., 2003). Furthermore, other studies have not found association between physical performance and the present SNP (Halverstadt et al., 2005; McKenzie et al., 2004). However, in these studies the populations were not homogenous, the genotype frequencies were not in Hardy-Weinberg equilibrium, and the subjects were seemingly older than in our study. Moreover, no comparable follow-up studies have previously been performed. We observed that subjects with genotype CC had the highest baseline VO2max, although no significant associations between a single allele and physical performance were found. These results should, however, be interpreted with caution, because the number of subjects in our study was not large enough to achieve adequate statistical power. Furthermore, voluntary physical activity before the military training could not explain the observed high VO2max values in the genotype CC group. On the other hand, the self-reported physical activity values prior the study period were comparable in all genotype groups. Moreover, during eight weeks of training, no major differences were found in the dietary habits. Therefore, the different VO2max values at baseline cannot be explained through preceding physical activity or nutritional factors. Cigarette smoking and general perceived health were also evaluated, but no factor that could explain the difference in baseline VO2max measurement, was found.

With respect to training response, subjects with genotype CG made the greatest gains in VO2max, and this improvement can be explained to a great extent by the fact that their baseline values were the lowest. Indeed, the baseline VO2max strongly determines the magnitude of an individual response to physical training (McArdle et al., 2006). On the other hand, subjects with genotype GG also improved their VO2max during the 8-week training, suggesting a possible association of allele G with exercise training. Whether the present IL6 promoter SNP is an independent determinant of basal physical/aerobic performance should be elucidated further in a larger cohort.

We also found a trend of higher BMI values with respect to the number of C alleles at baseline. Consistent with these findings, it has been previously suggested that the C allele is a risk factor for obesity and weight gain (Klipstein-Grobusch et al., 2006). Furthermore, in the present study, the BMI values significantly decreased in response to training only in subjects with genotype CG. It is thus plausible to speculate whether these subjects had a more negative energy balance and thus greater BMI reduction than those with other genotypes.

**Conclusion**

Altogether our results demonstrate that the IL6 promoter SNP -174G/C may have an effect on plasma IL-6 levels in response to acute exercise and shows association with aerobic physical performance (VO2max). Training improved VO2max and decreased BMI in all study subjects, but this effect seems to be more pronounced in the individuals with genotype CG.

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**References**


**Key points**

- Allele C of the IL6 promoter SNP -174G/C may have an effect on plasma IL-6 response to acute exercise.
- All subjects responded to physical exercise, but the improvement in VO2max and decrease in BMI after training are more pronounced in the individuals with genotype CG, hence the IL6 promoter SNP -174G/C may have an influence on training responses.
- The small number of subjects investigated in the present study warrants further research to confirm these findings in large cohorts.

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