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Effects of long-term football training on the expression profile of genes involved in muscle oxidative metabolism

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ABSTRACT

We investigated whether long-term recreational football training affects the expression of health-related biochemical and molecular markers in healthy untrained subjects.

Five untrained healthy men trained for 1 h 2.4 times/week for 12 weeks and 1.3 times/week for another 52 weeks. Blood samples and a muscle biopsy from the *vastus lateralis* were collected at T0 (pre intervention) and at T1 (post intervention). Gene expression was measured by RTqPCR on RNA extracted from muscle biopsies. The expression levels of the genes principally involved in energy metabolism (PPARγ, adiponectin, AMPKα1/α2, TFAM, NAMPT, PGC1α and SIRT1) were measured at T0 and T1. Up-regulation of PPARγ ($p < 0.0005$), AMPKα1 ($p < 0.01$), AMPKα2 ($p < 0.0005$) and adiponectin was observed at T1 *vs* T0. Increases were also found in the expression of TFAM ($p < 0.001$), NAMPT ($p < 0.01$), PGC1α ($p < 0.01$) and SIRT1 ($p < 0.01$), which are directly or indirectly involved in the glucose and lipid oxidative metabolism. Multiple linear regression analysis revealed that fat percentage was independently associated with NAMPT, PPARγ and adiponectin expression. In conclusion, long-term recreational football training could be a useful tool to improve the expression of muscle molecular biomarkers that are correlated to oxidative metabolism in healthy males.

1. Introduction

Regular endurance exercise training can improve the physical capacity of inactive subjects and promote health. High-intensity interval training (HIT) significantly induces the expression of biomarkers related to performance and health in both healthy and unhealthy individuals and is therefore an effective alternative to traditional endurance training [1]. Less is known about the effects of low-volume HIT (LV-HIT), although evidence suggests that this type of training modifies health-related biomarker expression to an extent similar to that obtained with moderate-intensity continuous training. The latter finding is important from a public-health perspective given that "lack of time" and poor motivation remain the most common barriers to performing regular exercise [2].

In fact, LV-HIT significantly enhances maximal oxygen uptake in healthy and sedentary males [3,4]; it proved to be useful in improving body composition and muscle oxidative capacity in overweight women [5]. Lastly, it seems to be effective in reducing cardiovascular and dismetabolic risk factors [6,7].

Like HIT, football is an intermittent exercise that involves multiple intense actions with high aerobic loading interspersed with low-intensity recovery periods. Because football is one of most popular team sports in the world, it has been extensively studied as a health-promoting activity for healthy participants of all ages and for groups of patients [8,9].

Many recent evidences indicated that short-term recreational football training (12–24 weeks) exerts positive effects on different markers related to the health, i.e. improvement in glucose homeostasis, decrease in total fat mass and improvement in aerobic

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performance, in healthy and unhealthy individuals [10,11]. Several studies also indicate that football training is effective to reduce cardiovascular risk factors and improve bone mineral metabolism [12–14].

Furthermore, very recently, Randers et al. [15] demonstrated that 1 h of recreational football training 2.4 times/week for 12 weeks has significant cardiovascular and muscular-skeletal effects in untrained men and that these positive adaptations in cardiovascular fitness can be maintained over a 1-year period with reduced training frequency (1.3 times/week).

To the best of our knowledge, no studies have yet evaluated the effects of long-term (1-year) recreational football training on the expression level of messengers involved in oxidative metabolism. Therefore, the aims of the present study were:

a) to evaluate the mRNA expression levels of key biomarkers which are directly or indirectly involved in the energy balance and glucose and lipid oxidative metabolism in muscle; and b) to determine whether the expression profiles of the mRNA's examined were correlated with health-related clinical parameters.

2. Materials and methods

2.1. Subjects, protocol training and sample collections

The 5 male subjects analyzed in the present study belong to the Football Training Group (FG) enrolled at University of Copenhagen by Prof. Krustrup [15].

FG subjects carried out a training protocol as detailed in Ref. [15]. Briefly, subjects trained 2.4 times/week over the first 12 weeks, after which the training frequency was reduced to 1.3 times/week over the following 52 weeks. No differences in body mass, fat percentage and maximal oxygen uptake among the selected subjects were observed at the beginning of the training protocol. The subjects were non-smokers, did not take medication and had not been involved in any type of physical training for at least 2 years. A standardized 7-days recall questionnaire was administrated to all participants in order to evaluate diet habits. The football sessions lasted 1 h and consisted of ordinary four-a-side or five-a-side matches on a 25–40 m wide and 30–50 m long natural grass pitch. The total number of training sessions was 29 and 67 during the first 12 weeks and the following 52 weeks, respectively. In winter time, the training sessions were carried out on an indoor handball court with a wooden floor 20–40 m.

Venous blood samples were collected from each participant after an overnight fast to obtain serum used for determining blood biochemical parameters. A muscle biopsy from the *vastus lateralis* muscle was also obtained from the m. *vastus lateralis* under local anesthesia using the Bergstrom technique as described in Ref. [15].

Biochemical and clinical investigations and muscle biopsies were performed as described in Randers et al. [15].

2.2. RNA extraction and RTqPCR

Total RNA was extracted from the muscle biopsies of the subjects enrolled in the study using an RNAspin mini kit (GE Healthcare, Little Chalfont, UK) according to the manufacturer's protocol. Real-time quantitative PCR (RTqPCR) was performed using the iQ5-iCycler Optical System (Bio-Rad, Hercules, USA). IQ SYBR (100 mM KCl, 40 mM Tris HCl pH 8.4, 0.4 mM dNTPs, iTaq DNA polymerase 50 U/ml, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein, stabilizer) (Bio-Rad) was used according to the manufacturer's instructions. Reaction mixtures were incubated at 95 °C for 30 s, followed by 2 cycles at 95 °C for 30 s and 95 °C for 3 min and by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Finally, 80 cycles were run starting at 55 °C and increasing the temperature by 5 °C every 10 s up to 95 °C. Fluorescence signals were measured during the elongation step. All reactions were performed in triplicate. The target mRNA levels were examined and normalized to the levels of the peptidylprolyl isomerase A cyclophilin A (PPIA) gene using the $2^{-\Delta\Delta CT}$ method [16].

The oligonucleotide primer sequences used were:

silent mating type information regulation 2 homolog 1 (SIRT1)
SIRT1 Fw TAATTCAGTGTCATGGTTCC
SIRT1 Rev ACTTCATCTTTGTCATACTTC
Adiponectin Fw GAAAGGAGATCCAGGTCTTATTG
Adiponectin Rev TCAGCAAAACCACTATGATGG
Peroxisome proliferator-activated receptor gamma (PPARγ)
PPARγ Fw GGTTGACACAGAGATGCCAT
PPARγ Rev CTCCATAGTGAAATCCAGAAG
Transcription Factor A, Mitochondrial (TFAM)
TFAM Fw CGCTCCCCCTTCAGTTTTGT
TFAM Rev CACTCCGCCCTATAAGCATC
nicotinamide phosphoribosyl transferase (NAMPT)
NAMPT Fw CAGCAGAACACAGTACCATA
NAMPT Rev CTCTAAGATAAGGTGGCAGC
Peroxisome proliferator-activated receptor-coactivator-1α (PGC-1α)
PGC1α Fw ACAACACTTACAAGCCAAACC
PGC1α Rev CCCTTTCTTGGTGGAGTTAT
5′-AMP-activated kinase α1 and α2 (AMPKα1 and AMPKα2)
AMPKα1 Fw GATAGCTGATTTTGGTCTTTC
AMPKα1 Rev GTTTCAAAAGGCTAATCACAG
AMPKα2 Fw CATGGACGGGTTGAAGAGAT
AMPKα2 Rev CATACAAGATAACACCACAGC
PPIAFw CTGAGCACTGGAGAGAAAGG
PPIARev AGGAATGATCTGGTGGTTAAG

2.3. Statistical analysis

The data relative to RTqPCR are shown as mean ± SD and were analyzed with Student's *t*-test. Pearson's correlation was used to determine the correlation coefficient between molecular biomarkers and clinical variables. Multiple linear regression analysis was performed to determine the association between variables. Differences were considered statistically significant at a *p*-value of $p < 0.05$.

3. Results

We first evaluated the mRNA expression levels of some biomarkers directly or indirectly involved in glucose and lipid metabolism in muscle biopsies, namely, PPARγ and adiponectin. As shown in Fig. 1A, PPARγ mRNA was significantly higher ($p < 0.0005$) at T1 *vs* T0.

Also the expression of adiponectin mRNA was increased, albeit not statistically significant ($p = 0.07$), at T1 *vs* T0.

These results suggest that long-term recreational football training leads to an increase in the expression level of messengers involved in oxidative metabolism.

Our results are in line with the improvement of some clinical-biochemical and fitness parameters i.e.: significant increase in maximal oxygen uptake by 2.0 mL/kg/min at T1 *vs* T0; improvement of body composition: a significant reduction in fat percentage by 3.3% from 23.8 to 20.5%, together with a positive trend in the lipid profile – reduction in triglycerides and LDL-cholesterol. We also observed a significant improvement of resting heart rate and MAP (mean arterial pressure) as reported in Table 1.

Furthermore, as shown in Fig. 1B, the mRNA expression levels of the two catalytic isoforms of AMPK (AMPKα1 and AMPKα2) were