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ORIGINAL ARTICLE

HIF1A P582S gene association with endurance training responses in young women

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Abstract Sequence variations in the gene encoding the hypoxia-inducible factor-1alpha, HIF1A, have been associated with physiologic function and could be associated with exercise responses. In the HIF1A P582S gene polymorphism (C1772T; rs 11549465 C/T), a single nucleotide transition from C → T alters the codon sequence from the usual amino acid; *proline* (C-allele), to *serine* (T-allele). This polymorphism was examined for association with endurance training responses in 58 untrained young women who completed a 6-week laboratory-based endurance training programme. Participant groups were defined as CC homozygotes versus carriers of a T-allele (CC vs. CT genotypes). Adaptations were examined at the systemic-level, by measuring $\dot{V}O_{2max}$, and the molecular-level by measuring enzymes determined from *vastus lateralis* ($n = 20$): 3-hydroacyl-CoA-dehydrogenase (HAD), which regulates mitochondrial fatty acid oxidation; cytochrome C oxidase (COX-1), a marker of mitochondrial density; and phosphofructokinase (PFK), a marker of glycolytic capacity. CT genotypes showed 45% higher training-induced gains in $\dot{V}O_{2max}$ compared with CC genotypes ($P < 0.05$). At the molecular level, CT increased the ratios PFK/HAD and PFK/COX-1 (47 and 3%, respectively), while in the CC genotypes these ratios were decreased (−26 and −54%, respectively). In conclusion, the T-allele of HIF1A P582S was associated with greater gains in $\dot{V}O_{2max}$ following endurance training in young women. In a sub-group we also provide preliminary evidence of differential muscle metabolic adaptations between genotypes.

Keywords Exercise · Hypoxia · Oxygen uptake · Polymorphism · Skeletal muscle

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Introduction

There is considerable inter-individual variability in adaptations to endurance training (Bouchard et al. 1999). In groups of individuals well matched for socioeconomic and environmental factors, the variability reflects differences in inherent '*trainability*', some of which has an underlying genetic basis (Bouchard and Rankinen 2001; Ahmetov et al. 2009). Several gene sequence variations have been associated with exercise training outcomes (e.g. see Bray et al. 2009). One candidate is a single nucleotide polymorphism (SNP) in the gene that transcribes the alpha subunit of the hypoxia inducible factor-1 (HIF-1α) transcription factor, termed HIF1A P582S (also known as C1772T; rs 11549465 C/T) (Prior et al. 2003). A nucleotide

transition from C → T alters the codon sequence from the usual amino acid *proline* (C-allele) to a *serine* (T-allele) in exon 12. In cell culture, the presence of serine instead of proline can disrupt HIF-1α degradation and thereby enhance HIF-1 transcriptional activity (Tanimoto et al. 2003; Fu et al. 2005; Yamada et al. 2005).

The maximal rate of oxygen uptake ($\dot{V}O_{2max}$) is an important indicator of cardiorespiratory fitness and endurance performance and is associated with mortality in humans (Blair et al. 1995; Erikssen 2001). It is widely used in laboratory-based training studies to quantify adaptation. Prior et al. (2003) reported that elderly male Caucasian carriers of a T-allele in HIF1A P582S showed attenuated training-induced gains in $\dot{V}O_{2max}$ compared with those homozygous for the C-allele (i.e., non-carriers of a T-allele).

In healthy young adults, the potential for aerobic metabolism of the quadriceps femoris muscle group exceeds the capacity to supply oxygen during whole-body exercise (Richardson et al. 1999) because $\dot{V}O_{2max}$ is primarily limited by the cardiovascular supply of oxygen to the working muscles (Saltin and Calbet 2006; Levine 2008). Training-induced gains in the cardiovascular system that promote oxygen supply to working muscles are strongly related to improvements in $\dot{V}O_{2max}$ (Schmidt and Prommer 2010). HIF-1 up-regulates the expression of genes that can lead to improvements in the supply of oxygen to the working muscles during exercise by promoting angiogenesis (Forsythe et al. 1996; Marti 2005) and erythropoiesis (Semenza and Wang 1992; Ameln et al. 2005). Therefore, if the T-allele confers greater HIF-1 transcriptional activity (Tanimoto et al. 2003; Fu et al. 2005; Yamada et al. 2005), it follows that greater training-induced gains in $\dot{V}O_{2max}$ could be expected in young adult carriers of a T-allele compared with CC homozygotes. Thus, the primary research hypothesis was that young women with at least one T-allele in HIF1A P582S would show greater training-induced gains in $\dot{V}O_{2max}$ following 6 weeks of supervised, laboratory-based endurance training.

Skeletal muscle adaptations to training do not necessarily correlate with changes in $\dot{V}O_{2max}$ (Vollaard et al. 2009; McPhee et al. 2010), so measuring only $\dot{V}O_{2max}$ does not give a full indication of the true extent of the muscle training adaptations. Therefore, a secondary aim of this study was to examine the association between the HIF1A P582S genotype and skeletal muscle adaptations. To address this, a single-leg cycling paradigm was used to measure the peak aerobic capacity of the leg muscles because in this mode of exercise the peak aerobic capacity is limited by the uptake and utilisation of oxygen by the working muscles (Davies and Sargeant 1974; Klausen et al. 1982; McPhee et al. 2009). In addition, muscle biopsies were obtained from a sub-group of subjects to measure the concentrations of enzymes involved in energy metabolism. Evidence from transgenic animals with tissue-specific HIF-1 deficiency and from cell culture show a HIF-1-related increase in glucose metabolism and associated enzymes, and in some instances this occurs concomitant with suppressed fatty acid oxidation and oxidative metabolism (Mason et al. 2004; Kim et al. 2006; Papandreou et al. 2006). Thus, it was hypothesised that carriers of a T-allele (possible greater HIF-1 transcriptional activity) would show smaller training-induced gains in the single-leg cycling peak aerobic capacity, but greater gains in glycolytic enzymes compared with non-carriers.

Materials and methods

Participants and study design

The study complied with the latest version of the Declaration of Helsinki and received approval from the ethics committee at Manchester Metropolitan University. This study was part of a larger study designed to investigate factors underlying the inter-individual variability in response to endurance training. Initially, 71 untrained but otherwise healthy young female university students gave written, informed consent to participate. Four of these did not provide a blood sample and nine withdrew from the training programme, so genotype and phenotype data were available from 58 participants (pre-training characteristics are presented in Table 1). All 58 participants completed measurements of two-leg cycling $\dot{V}O_{2max}$ before and after the training period. All participants were also asked to complete single-leg cycling tests and to provide a muscle biopsy before and after the training period, but these were described as "optional" tests, meaning that participants could decline participation in these tests yet still participate in the other aspects of the study. This approach permitted a more detailed examination of the skeletal muscle response to training in a sub-group of subjects. Fifty of the 58 participants volunteered to complete the single-leg cycling tests and 20 of the 58 participants gave a muscle biopsy before and after training. Details of the two-leg cycling, single-leg cycling, muscle biopsy procedures and training programme have been described in previous work (McPhee et al. 2009, 2010), but are described briefly below.

Cycle training

Subjects completed cycle training on mechanically or electrically braked cycle ergometers (Monark, Varberg, Sweden; Jaeger ergo-line 2000, Mindjhaart, Netherlands).

Table 1 Pre-training participant characteristics

	CC	CT
Age (years)	21 ± 1	21 ± 1
Height (m)	1.65 ± 0.01	1.67 ± 0.03
Body mass (kg)	66 ± 1.6	66 ± 3.1
BMI (kg/m^2)	24.3 ± 0.1	23.7 ± 0.3
Physical activity score[a]	7.7 ± 0.1	7.9 ± 0.3
$\dot{V}O_{2max}$ (L min^{-1})	2.43 ± 0.05	2.36 ± 0.12
$\dot{V}O_{2max}$ (mL kg min^{-1})	38.2 ± 1.2	37.3 ± 2.1
Single-leg $\dot{V}O_{2max}$ (L min^{-1})	1.88 ± 0.05	1.84 ± 0.11

CC group includes subjects homozygous for the C-allele (proline) at HIF1A P582S; CT group includes subjects with at least one T-allele (serine). All are $n = 45$ in CC group and 13 in CT group, except for single-leg $\dot{V}O_{2peak}$ where $n = 39$ in CC and 11 in CT. Data are mean ± SEM

[a] A physical activity score of ≤6 represents a sedentary lifestyle and ≥12 represents a high level of activity (Baecke et al. 1982)

Training sessions lasted 45 min and were scheduled three times per week for 6 weeks. A pedal frequency of around 85 rev min^{-1} was maintained at an intensity that elicited the required heart rate (HR), which ensured that the workload increased progressively throughout the training programme. The first week of training (sessions 1, 2 and 3) consisted of moderate intensity continuous exercise at 75% of maximal HR (HR_{max}). During the second week (training sessions 4, 5 and 6) subjects completed five repeats (without any rest between intervals) of 6-min moderate intensity exercise (75% of HR_{max}) followed immediately by 2-min higher intensity exercise (90% HR_{max}). The training intensity was again increased in the third week: subjects completed four repeats in succession (without any rest between intervals) of 6-min moderate intensity exercise (75–80% of HR_{max}) followed immediately by 3-min higher intensity exercise (90% of HR_{max}). Training sessions remained at this intensity through to the end of the 6-week programme (sessions 7–18, inclusive).

Cycling tests

Untrained people are usually unfamiliar with the type of incremental and high-intensity exercise necessary to attain $\dot{V}O_{2max}$, so these tests were performed twice before and twice after the training period. On all occasions the tests were carried out in an identical manner using an electronically braked cycle ergometer (Jaeger ergo-line 2000, Mindjhaart, Netherlands). In all tests, pulmonary gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) was measured using breath-by-breath analysis (Cosmed K4b^2, Rome, Italy), and HR was recorded telemetrically (Polar S810i, Kempele, Finland).

In the two-leg cycling tests, the workload was set at 30 W for 2 min and then increased by 20 W every minute until volitional exhaustion or until the required cadence of 80 rev min^{-1} could not be maintained despite strong verbal encouragement. The highest 30-s average oxygen uptake from either test was recorded and accepted as maximal ($\dot{V}O_{2max}$). In all participants, at least one of the standard criteria was achieved (respiratory exchange ratio >1.10, heart rate within 10 bpm of age-predicted maximum (220 − age in years) and/or a levelling off of $\dot{V}O_2$ despite an increase in workload).

Approximately 10 min after completing the second $\dot{V}O_{2max}$ test, subjects completed a familiarisation session to moderate-intensity single-leg cycling lasting for 6 min. On separate days, subjects also completed two further incremental exercise tests to the limit of tolerance for single-leg cycling $\dot{V}O_{2peak}$. There was at least 24 h rest between the single-leg cycling tests. The right foot was securely strapped to the right pedal, seat height optimised and the left foot rested on a stable platform. Following a 2-min warm-up at 20 W, the external work load was set at 40 W and then increased by 10 W every minute until volitional exhaustion or until the required cadence of 70 rev min^{-1} could no longer be maintained despite strong verbal encouragement. Single-leg $\dot{V}O_{2peak}$ was recorded as the highest 30-s average oxygen uptake during either of the two tests. The coefficient of variation between the first and second tests was 3% for two-leg and 6% for single-leg cycling.

Muscle biopsy sampling and analysis

Subjects were instructed to refrain from strenuous exercise and alcohol for at least 24 h, and from caffeinated drinks and food consumption for at least 4 h before giving a biopsy. All samples were obtained in the early evening between 18:00 and 20:00 hours. Samples were obtained under local anaesthesia (2% Lignocaine) from the mid-section of the *vastus lateralis* muscle of the right leg using the conchotome technique (Dietrichson et al. 1980). The sample was immediately flash-frozen in liquid nitrogen and stored at −80°C until analysis.

Western blot analysis was used to determine the protein concentrations of cytochrome c oxidase subunit 1 (Invitrogen, California, USA), which was used as a marker of mitochondrial density; 3-hydroxyacyl CoA dehydrogenase (HAD; Abcam, Cambridge, UK), which was used as a marker of fatty acid β-oxidative capacity; and phosphofructokinase (PFK; Santa Cruz Biotechnology, Inc., California, USA), which was used as a marker of glycolytic capacity. Muscle samples were powdered on dry ice, and 150 μL of ice-cold sucrose lysis buffer was added (50 mM Tris pH 7.5, 250 mM Sucrose, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 50 mM NaF, 1 mM

$NaVO_4$ 5 mM $Na_4P_2O_7$ and 0.1% DTT). The samples were shaken at 4°C for 30 min at 1,200 rpm. The lysed samples were cleared by centrifugation and protein concentrations were determined using a DC protein assay (Bio-Rad, Hercules, CA). Equal aliquots of protein were boiled for 5 min in Laemmli sample buffer and separated on a 10% gel by SDS-polyacrylamide gel electrophoresis. Following electrophoresis, proteins were transferred to a Protran nitrocellulose membrane (Whatman, Dassel, Germany) at 100 V for 1 h. The membrane was blocked for 1 h in 3% milk in Tris-buffered saline + 0.1% Tween (TBST), rinsed and then incubated overnight at 4°C with the appropriate primary antibody in TBST at 1:1,000. The following day, membranes were washed in TBST and incubated with the appropriate secondary antibody (in 3% milk) for 1 h. Imaging and band quantification were carried out using a Chemi Genius Bioimaging Gel Doc System (Syngene, Cambridge, UK). Samples were run for each protein so stripping of membranes was not necessary. Protein values were internally standardised against the protein content of the house keeping proteins, eukaryotic translation elongation factor 2 (eEF2; Cell Signalling Technology, Danvers, MA, USA) and annexin 2 (annexin2; Santa Cruz Biotechnology, Inc., California, USA), proteins that remain stable regardless of interventions (Huang et al. 2006). Data are presented as arbitrary units.

HIF1A P582S genotyping

Whole blood was collected from a superficial forearm vein into EDTA collection tubes and then transferred into eppendorf tubes and stored at −80°C until analysis. For genotyping analysis, DNA was isolated from 200 µL of whole blood using the Qiagen QIAcube spin column technique following the manufacturer's instructions (Qiagen, West Sussex, UK). Briefly, whole blood was lysed leaving the genomic DNA free to bind with a silica-gel membrane, the samples were then centrifuged, impurities washed away and the remaining DNA was eluted into 200 µL low-salt buffer.

To determine the HIF1A P582S genotype, a 346-bp fragment of the *HIF1A* gene, containing the P582S polymorphism, was amplified using the polymerase chain reaction (PCR) technique. 50 µL reactions contained 50 ng genomic DNA; 200 ng of each primer: 5′-AAGGTGTGGC CATTGTAAAAACTC-3′ (forward) and 5′-GCACTAGT AGTTTCTTTATGTATG-3′ (reverse) (Ollerenshaw et al. 2004); 0.2 mM of each deoxynucleotide triphosphate (Sigma-Aldrich, Gillingham, England); 2 mM $MgCl_2$ (Promega, Madison, USA); 10 µL GoTaq® Flexi Buffer; and 2.5 units GoTaq DNA polymerase (Promega, Madison, USA). After an initial denaturation at 94°C for 6 min, 35 cycles of 94°C for 60 s, 60°C for 90 s and 72°C for 120 s were performed. A final extension at 72°C for 5 min was then carried out.

The PCR products were run in 1.5% agarose gels which were stained with ethidium bromide. The DNA was extracted from the gels using a commercial purification kit (QIAquick®, Qiagen, Crawley, England) and sent to a separate lab for sequencing analysis (MWG Biotech, London, England). Once sequencing had been performed, the genotypes could be discerned by visual analysis of the chromatogram: the T-allele showed as a red peak and the C-allele showed as a blue peak at position 582. Genotyping analysis was performed retrospectively, after the training programme had been completed.

Statistical analyses

A repeated measures ANOVA was used to assess the training effect and the genotype-training interaction. Analyses were performed both with and without the inclusion of the pre-training height, body mass and the pre-training value for the variable of interest as covariates (e.g. pre-training $\dot{V}O_{2max}$, height and body mass were used when testing the genotype-training interaction for $\dot{V}O_{2max}$). Data were analysed using SPSS (version 16.0) (SPSS Inc., Chicago, IL, USA) and statistical significance was accepted at $P \leq 0.05$. Mean ± standard error of the mean (SEM) are presented.

Results

Participants generally found the training programme challenging, but all were able to complete the required training intensities. Only one participant was homozygous for the T-allele so statistical analyses were conducted on T-allele carriers (CT, $n = 13$) versus non-carriers (CC, $n = 45$). Genotype data were in Hardy–Weinberg equilibrium. Genotype groups did not differ significantly for age, height, body mass, BMI or habitual physical activity levels (Table 1).

HIF1A P582S association with maximal oxygen uptake

Pre-training $\dot{V}O_{2max}$ did not differ significantly between genotype groups (Table 1). CC and CT groups both showed significant improvements in $\dot{V}O_{2max}$ following training. Figure 1 shows individual data points for the training-induced change in $\dot{V}O_{2max}$ for CC and CT groups. The increase in CT was significantly greater than the increase in CC genotypes, both when expressed as the absolute (mL min^{-1}) and percentage changes from the original units of measurement ($P = 0.050$; Fig. 1) and

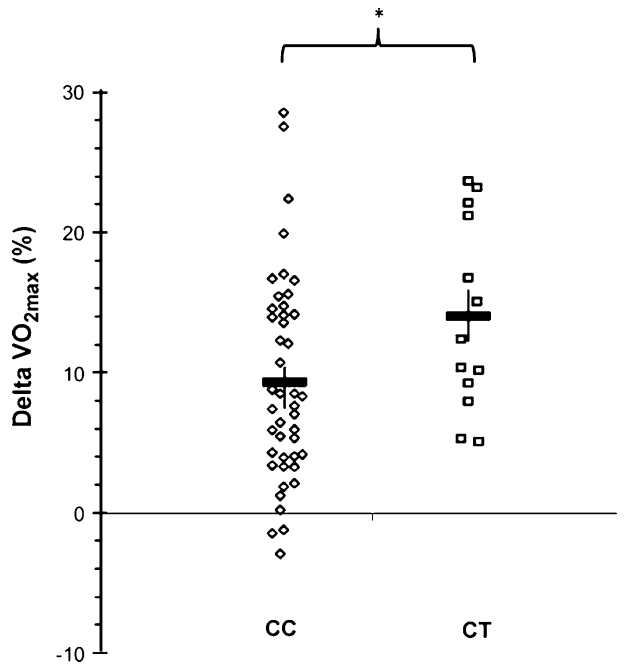

Fig. 1 Training-induced change in two-leg cycling $\dot{V}O_{2max}$. Individual data points are presented within CC (*open diamonds*, $n = 45$) and CT (grey squares, $n = 13$). *Black horizontal rectangles* indicate mean ± SEM within groups. *Indicates significant difference between genotype groups ($P = 0.05$)

when expressed relative to body mass: in mL kg min^{-1} ($P = 0.030$). When considering the pre-training values for $\dot{V}O_{2max}$, height and body mass as covariates using ANCOVA analysis, the statistical significance of the genotype-training interaction was reduced slightly when data were expressed as L min^{-1} ($P = 0.057$), but remained significant when data were expressed as mL kg min^{-1} ($P = 0.040$).

HIF1A P582S association with single-leg cycling

Genotype groups did not differ significantly in their pre-training single-leg $\dot{V}O_{2peak}$ ($P = 0.968$; Table 1). Both groups showed significant improvements in single-leg $\dot{V}O_{2peak}$, with no difference between groups for the training-induced improvements (12.5 ± 2.2% in CC vs. 10.0 ± 3.7% in CT; $P = 0.575$).

HIF1A P582S association with muscle enzymes

CC ($n = 14$) and CT ($n = 6$) genotype groups had similar pre-training values for COX-1 (0.54 and 0.44 in CC and CT, respectively; $P = 0.464$), a marker of mitochondrial density; HAD (0.84 and 0.66 in CC and CT, respectively; $P = 0.497$), a marker of fatty acid oxidation; and PFK (1.01 and 0.84 in CC and CT groups, respectively; $P = 0.454$), a marker of glycolytic capacity.

The CC group showed significant 60 ± 20% increase in COX-1 ($P = 0.006$) and 84 ± 25% increase in HAD ($P = 0.031$), and non-significant 18 ± 24% increase in PFK ($P = 0.551$). The CT group showed non-significant 31 ± 19% increase in COX-1 ($P = 0.122$), 25 ± 32% increase in HAD ($P = 0.291$) and 65 ± 46% increase in PFK ($P = 0.255$). These responses were not significantly different between genotypes.

Discussion

We examined the HIF1A P582S gene polymorphism for genotype-training interactions following a 6-week laboratory-based, supervised endurance training programme in young women. The main finding was that CT genotypes showed 45% higher training-induced gains in $\dot{V}O_{2max}$ compared with CC genotypes.

HIF1A P582S and $\dot{V}O_{2max}$ response to training

In a previous investigation of the HIF1A P582S genotype and endurance training interaction, Prior et al. (2003) reported greater gains in $\dot{V}O_{2max}$ in a sub-group of the "oldest" male Caucasian subjects homozygous for the C-allele compared with carriers of a T-allele. This is contrary to our findings in young women. The disagreement might be due to differences in training programme; the subjects in the study of Prior et al. (2003) worked at lower training intensity and trained for 24 weeks. It is also possible that the different subject populations (young women vs elderly men) adapt differently to training (McGuire et al. 2001; Murias et al. 2010), because elderly people have reduced skeletal muscle mass, muscle function and $\dot{V}O_{2max}$ compared with young adults (Ogawa et al. 1992; Booth et al. 1994; Stratton et al. 1994).

$\dot{V}O_{2max}$ is dependent both on cardiovascular supply of oxygen to the working muscles and uptake and utilisation of that oxygen by the working muscles. The balance of evidence suggests that in young adults $\dot{V}O_{2max}$ is limited by the supply of oxygen (Saltin and Calbet 2006; Levine 2008), meaning that exercise to establish $\dot{V}O_{2max}$ is terminated before the full aerobic capacity of the individual muscle groups has been reached (McPhee et al. 2009). In the present study, we included a test to estimate the local muscle aerobic capacity by utilising a single-leg cycling paradigm. Single-leg cycling recruits a relatively smaller muscle mass compared with whole-body exercise and is limited by the uptake and utilisation of oxygen by the working muscles (Davies and Sargeant 1974). It has been shown previously that improvements in $\dot{V}O_{2max}$ are often not correlated with those of the local musculature (Vollaard

et al. 2009; McPhee et al. 2010), so adaptations of different body systems should not be assumed to occur equally within subjects following training. In line with this we found no significant difference between genotypes in single-leg $\dot{V}O_{2peak}$ training responses. The finding of similar gains in local $\dot{V}O_{2peak}$ suggests that greater gains in $\dot{V}O_{2max}$ in the CT group following training could have been due to differences in training-induced and HIF-1-related expression levels of genes involved in oxygen supply. One candidate is HIF-1 activation of VEGF (Forsythe et al. 1996; Marti, 2005), leading to improved muscle capillarisation. However, this would also improve the potential for oxygen uptake and thus increase the single-leg $\dot{V}O_{2peak}$, so capillarisation probably was not the main factor influencing the genotype difference in $\dot{V}O_{2max}$ training response. Other HIF-1 activated candidate genes include those involved in vasodilation such as eNOS (Kimura et al. 2000; Coulet et al. 2003), and erythropoiesis, such as erythropoietin which leads to improved oxygen carrying capacity of the blood (Semenza and Wang 1992; Ameln et al. 2005). Up-regulation of these genes would improve the supply of oxygen to the working muscles during whole-body exercise and should be investigated in future studies.

HIF1A P582S and skeletal muscle metabolic response to training

At the skeletal muscle molecular level, CC genotypes in the present study showed improved potential for fatty acid oxidation through increased protein concentrations of mitochondrial enzymes COX-1 and HAD, but in the CT group these adaptations were attenuated. Further investigation revealed significant genotype differences in the training-induced change in the ratio PFK:COX-1 ($P = 0.005$) and PFK:HAD ($P = 0.004$): the CC group decreased both ratios, while CT increased both ratios (Fig. 2). Ratios such as these have been used previously to indicate changes in the glycolytic capacity relative to the mitochondrial density and between glycolytic and fatty acid enzymes, respectively (Linossier et al. 1997; Melby et al. 2000; Ukkola et al. 2003; Swallow et al. 2007; Goldsmith et al. 2009). Although these data were collected from a relatively small subgroup, they are in line with findings from animal studies and cell cultures showing that HIF-1 can differentially affect glycolytic and mitochondrial pathways (Mason et al. 2004; Pisani and Dechesne 2005; Kim et al. 2006; Papandreou et al. 2006).

A small, but significant tendency for greater frequency of the HIF1A P582S C-allele has been found in endurance athletes compared with non-athlete controls (Doering et al. 2010). This might at first seem contrary to our results of greater gains in $\dot{V}O_{2max}$ in CT genotypes, since endurance athletes are characterised by a high $\dot{V}O_{2max}$. But, of equal importance is the ability to work at a high fraction of $\dot{V}O_{2max}$ without incurring lactate accumulation and fatigue, and this is achieved through high mitochondrial content and fatty acid oxidation (Holloszy 1967). Therefore, the possibility of greater mitochondrial adaptations in CC genotypes compared with carriers of a T-allele might help to explain the higher frequency of C-alleles in an endurance athlete population. In relation to high-level sprint and power athletes, a higher frequency of the T-allele has been found compared with non-athletic controls (Ahmetov et al. 2008). Sprint and power performance are dependent on rapid supply of ATP, which can be fuelled by phosphocreatine and glycolytic pathways. We report here that carriers of a T-allele tended to show greater adaptation of the glycolytic pathway (Fig. 2). More work is needed to

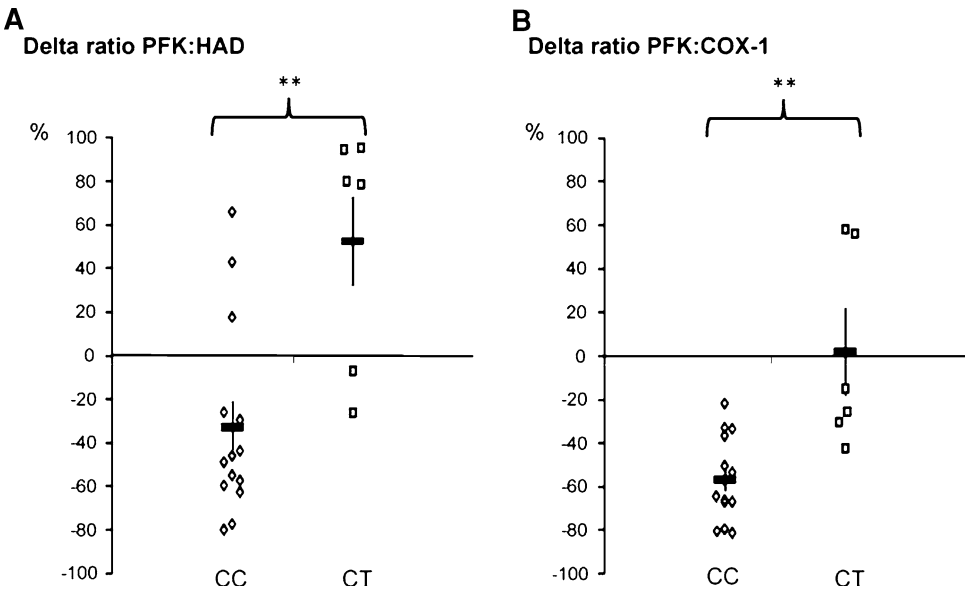

Fig. 2 Training induced change in muscle enzymes. **a** Ratio between PFK:HAD and, **b** PFK:COX-1. Individual data points are presented within CC (*open diamonds*, $n = 14$) and CT (*grey squares*, $n = 6$). *Black horizontal rectangles* indicate mean ± SEM within groups. **Indicates significant difference between genotype groups ($P < 0.01$)

confirm the apparent differences between genotype groups in the muscle molecular training adaptations and to further characterise the physiological relevance of HIF1A P582S gene polymorphism.

HIF1A P582S and training interactions

The interaction between HIF1A P582S and exercise appears to be important. Differences were not found between genotype groups in characteristics of untrained people either in the present study or in other similar studies (Prior et al. 2003; Ahmetov et al. 2008; Doering et al. 2010), and a study of Chuvash Polycythemia, a genotype-dependent disorder resulting in considerably greater HIF-1 stability, found differences in HIF-1 activated mRNA transcripts in untrained patients compared with controls, but no differences in activity levels of muscle enzymes that are regulated by HIF-1 (Formenti et al. 2010).

Muscle PO_2 is relatively high in rested muscle (approx. 25 Torr: Richardson et al. 2001), so it is assumed that in both HIF1A P582S genotypes the HIF-1α would be quickly degraded (Ameln et al. 2005) due to its half-life of <1 min in the presence of oxygen (Yu et al. 1998). During bouts of muscular activity (i.e. endurance exercise) the muscle PO_2 can be reduced to as low as 5 Torr (Richardson et al. 2001), which is far below the half-maximal PO_2 necessary for HIF-1α stabilisation (around 10–15 Torr: Jiang et al. 1996). Thus, training would result in repetitive activation of HIF-1 with repeated sessions in untrained subjects (Ameln et al. 2005) and could result in differences between genotypes in accumulation of the proteins coded by genes under the control of HIF-1.

Evidence from cell-culture models suggests the presence of serine (T-allele) instead of proline (C-allele) in HIF1A P582S slows HIF-1α degradation and thereby enhances the HIF-1 transcriptional activity (Tanimoto et al. 2003; Fu et al. 2005; Yamada et al. 2005), and this provides a possible mechanistic explanation for the reported genotype associations. The present study was not designed to investigate these mechanisms. A complicating factor is that the muscle specimens were obtained at rest (and prior exercise was avoided) where muscle PO_2 would be high. Future studies could directly measure HIF-1 activity during or immediately after exercise or, indirectly, investigate the possibility that carriers of a T-allele might show greater overall expression of HIF-1 activated genes after exercise.

Multiple genes contribute to training-induced gains in $\dot{V}O_{2max}$

It is evident that there still exists considerable interindividual variability in $\dot{V}O_{2max}$ training responses, even after stratifying groups by HIF1A genotype (Fig. 1). This is probably indicative of the complex and polygenic nature of the training response. A gene polymorphism that has received a lot of attention in relation to endurance phenotypes in recent years is the ACE I/D polymorphism, but there was no association between ACE I/D and $\dot{V}O_{2max}$ in our participants (results not shown) which is in line with several other studies (Bouchard et al. 2000; Rankinen et al. 2000; Sonna et al. 2001; Woods et al. 2002; Timmons et al. 2005; Day et al. 2007; Timmons et al. 2010). There is the possibility that the most "(dis)advantageous" genotypes might aggregate to confer the extreme *high* or *low* responses (Williams and Folland 2008; Bouchard et al. 2010), but our study population was not sufficiently powered for this type of investigation.

Human training adaptation is likely to be highly polygenic (Williams and Folland 2008). A recent genome-wide association study identified 21 SNPs that collectively accounted for 49% of the age, gender and baseline-adjusted variance in $\dot{V}O_{2max}$ training response in Caucasian participants in the HERITAGE Family Study, although only four of these individually accounted for ≥4% of the variability (Bouchard et al. 2010). From our data it was calculated that HIF1A P582S accounted for 5.6% of the variability in $\dot{V}O_{2max}$ training response. Multiple regression analysis, as used on the data from the genome-wide association study (Bouchard et al. 2010), can produce lower values for the predictive potential of SNPs compared with the individual-gene approach used in the present study. For example, the ACSL1 (rs6552828) gene accounted for 3.5% in the multiple regression model and 7% in the individual gene model (Bouchard et al. 2010). Thus, it is likely that larger replication studies will produce a more conservative estimate of the predictive value of HIF1A P582S for $\dot{V}O_{2max}$ training response.

HIF1A P582S was not identified as significantly associated with $\dot{V}O_{2max}$ training response in the genomic scan that was carried out on the data of the HERITAGE Family Study (Bouchard et al. 2010). This could be due to the longer duration (20 weeks) and lower training intensity used in the HERITAGE study compared with ours. It is known that the exercise-induced reduction in PO_2 (and hence, HIF-1α stability) is intensity dependent (Richardson et al. 2001) and long-term endurance training can lead to down-regulation of HIF-1α expression (Ameln et al. 2005). Different studies often identify different gene sets (cf. Bouchard et al. 2000, 2010; Timmons et al. 2010), and reasons for the discrepancies could include: false positive results; differences in training duration and intensity; participant characteristics and the use of gene transcripts (Timmons et al. 2010) or genomic scans (Bouchard et al. 2010) to identify the gene sets.

In conclusion, the T-allele of HIF1A P582S was associated with greater gains in $\dot{V}O_{2max}$ following 6 weeks of endurance training in previously untrained young women. In a sub-group we also provide preliminary evidence of differential muscle metabolic adaptations between genotypes.

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Conflict of interest There are no conflicts of interest to declare.

References

Ahmetov II, Hakimullina AM, Lyubaeva EV, Vinogradova OL, Rogozkin VA (2008) Effect of HIF1A gene polymorphism on human muscle performance. Bull Exp Biol Med 146:351–353

Ahmetov II, Williams AG, Popov DV, Lyubaeva EV, Hakimullina AM, Fedotovskaya ON, Mozhayskaya IA, Vinogradova OL, Astratenkova IV, Montgomery HE, Rogozkin VA (2009) The combined impact of metabolic gene polymorphisms on elite endurance athlete status and related phenotypes. Hum Genet 126(6):751–761

Ameln H, Gustafsson T, Sundberg CJ, Okamoto K, Jansson E, Poellinger L, Makino Y (2005) Physiological activation of hypoxia inducible factor-1 in human skeletal muscle. FASEB J 19:1009–1011

Baecke JA, Burema J, Frijters JE (1982) A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 36:936–942

Blair SN, Kohl HW III, Barlow CE, Paffenbarger RS Jr, Gibbons LW, Macera CA (1995) Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. JAMA 273:1093–1098

Booth FW, Weeden SH, Tseng BS (1994) Effect of aging on human skeletal muscle and motor function. Med Sci Sports Exerc 26:556–560

Bouchard C, Rankinen T (2001) Individual differences in response to regular physical activity. Med Sci Sports Exerc 33:S446–S451 (discussion S452–443)

Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, Perusse L, Leon AS, Rao DC (1999) Familial aggregation of *V*O(2max) response to exercise training: results from the HERITAGE Family Study. J Appl Physiol 87:1003–1008

Bouchard C, Rankinen T, Chagnon YC, Rice T, Perusse L, Gagnon J, Borecki I, An P, Leon AS, Skinner JS, Wilmore JH, Province M, Rao DC (2000) Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE Family Study. J Appl Physiol 88:551–559

Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, Rao DC, Rankinen T (2010) Genomic predictors of maximal oxygen uptake response to standardized exercise training programs. J Appl Physiol. doi:10.1152/japplphysiol.00973.2010

Bray MS, Hagberg JM, Perusse L, Rankinen T, Roth SM, Wolfarth B, Bouchard C (2009) The human gene map for performance and health-related fitness phenotypes: the 2006–2007 update. Med Sci Sports Exerc 41:35–73

Coulet F, Nadaud S, Agrapart M, Soubrier F (2003) Identification of hypoxia-response element in the human endothelial nitric-oxide synthase gene promoter. J Biol Chem 278:46230–46240

Davies CT, Sargeant AJ (1974) Physiological responses to one- and two-leg exercise breathing air and 45 percent oxygen. J Appl Physiol 36:142–148

Day SH, Gohlke P, Dhamrait SS, Williams AG (2007) No correlation between circulating ACE activity and VO_{2max} or mechanical efficiency in women. Eur J Appl Physiol 99:11–18

Dietrichson P, Mellgren SI, Skre H (1980) Muscle biopsy with the percutaneous conchotome technique. J Oslo City Hosp 30:73–79

Doering F, Onur S, Fischer A, Boulay MR, Perusse L, Rankinen T, Rauramaa R, Wolfarth B, Bouchard C (2010) A common haplotype and the Pro582Ser polymorphism of the hypoxia-inducible factor-1alpha (HIF1A) gene in elite endurance athletes. J Appl Physiol. doi:10.1152/japplphysiol.01165.2009

Erikssen G (2001) Physical fitness and changes in mortality: the survival of the fittest. Sports Med 31:571–576

Formenti F, Constantin-Teodosiu D, Emmanuel Y, Cheeseman J, Dorrington KL, Edwards LM, Humphreys SM, Lappin TR, McMullin MF, McNamara CJ, Mills W, Murphy JA, O'Connor DF, Percy MJ, Ratcliffe PJ, Smith TG, Treacy M, Frayn KN, Greenhaff PL, Karpe F, Clarke K, Robbins PA (2010) Regulation of human metabolism by hypoxia-inducible factor. Proc Natl Acad Sci USA 107:12722–12727

Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL (1996) Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 16:4604–4613

Fu XS, Choi E, Bubley GJ, Balk SP (2005) Identification of hypoxia-inducible factor-1alpha (HIF-1alpha) polymorphism as a mutation in prostate cancer that prevents normoxia-induced degradation. Prostate 63:215–221

Goldsmith R, Joanisse DR, Gallagher D, Pavlovich K, Shamoon E, Leibel RL, Rosenbaum M (2009) Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. Am J Physiol Regul Integr Comp Physiol 298:R79–R88

Holloszy JO (1967) Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242:2278–2282

Huang YC, Dennis RG, Baar K (2006) Cultured slow vs. fast skeletal muscle cells differ in physiology and responsiveness to stimulation. Am J Physiol Cell Physiol 291:C11–C17

Jiang BH, Semenza GL, Bauer C, Marti HH (1996) Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. Am J Physiol 271:C1172–C1180

Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177–185

Kimura H, Weisz A, Kurashima Y, Hashimoto K, Ogura T, D'Acquisto F, Addeo R, Makuuchi M, Esumi H (2000) Hypoxia response element of the human vascular endothelial growth factor gene mediates transcriptional regulation by nitric oxide: control of hypoxia-inducible factor-1 activity by nitric oxide. Blood 95:189–197

Klausen K, Secher NH, Clausen JP, Hartling O, Trap-Jensen J (1982) Central and regional circulatory adaptations to one-leg training. J Appl Physiol 52:976–983

Levine BD (2008) What do we know, and what do we still need to know? J Physiol 586:25–34

Linossier MT, Dormois D, Perier C, Frey J, Geyssant A, Denis C (1997) Enzyme adaptations of human skeletal muscle during bicycle short-sprint training and detraining. Acta Physiol Scand 161:439–445

Marti HH (2005) Angiogenesis—a self-adapting principle in hypoxia. EXS 94:163–180

Mason SD, Howlett RA, Kim MJ, Olfert IM, Hogan MC, McNulty W, Hickey RP, Wagner PD, Kahn CR, Giordano FJ, Johnson RS (2004) Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. PLoS Biol 2:e288

McGuire DK, Levine BD, Williamson JW, Snell PG, Blomqvist CG, Saltin B, Mitchell JH (2001) A 30-year follow-up of the Dallas Bedrest and Training Study: II. Effect of age on cardiovascular adaptation to exercise training. Circulation 104:1358–1366

McPhee JS, Williams AG, Stewart C, Baar K, Perez Schindler J, Aldred S, Maffulli N, Sargeant AJ, Jones DA (2009) The training stimulus experienced by the leg muscles during cycling in humans. Exp Physiol 94:684–694

McPhee JS, Williams AG, Degens H, Jones DA (2010) Inter-individual variability in adaptation of the leg muscles following a standardised endurance training programme in young women. Eur J Appl Physiol 109:1111–1118

Melby CL, Ho RC, Jeckel K, Beal L, Goran M, Donahoo WT (2000) Comparison of risk factors for obesity in young, nonobese African-American and Caucasian women. Int J Obes Relat Metab Disord 24:1514–1522

Murias JM, Kowalchuk JM, Paterson DH (2010) Mechanisms for increases in VO_{2max} with endurance training in older and young women. Med Sci Sports Exerc. doi:10.1249/MSS.0b013e3181dd0bba

Ogawa T, Spina RJ, Martin WH III, Kohrt WM, Schechtman KB, Holloszy JO, Ehsani AA (1992) Effects of aging, sex, and physical training on cardiovascular responses to exercise. Circulation 86:494–503

Ollerenshaw M, Page T, Hammonds J, Demaine A (2004) Polymorphisms in the hypoxia inducible factor-1alpha gene (HIF1A) are associated with the renal cell carcinoma phenotype. Cancer Genet Cytogenet 153:122–126

Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3:187–197

Pisani DF, Dechesne CA (2005) Skeletal muscle HIF-1alpha expression is dependent on muscle fiber type. J Gen Physiol 126:173–178

Prior SJ, Hagberg JM, Phares DA, Brown MD, Fairfull L, Ferrell RE, Roth SM (2003) Sequence variation in hypoxia-inducible factor 1alpha (HIF1A): association with maximal oxygen consumption. Physiol Genomics 15:20–26

Rankinen T, Perusse L, Gagnon J, Chagnon YC, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C (2000) Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study. J Appl Physiol 88:1029–1035

Richardson RS, Grassi B, Gavin TP, Haseler LJ, Tagore K, Roca J, Wagner PD (1999) Evidence of O_2 supply-dependent VO_{2max} in the exercise-trained human quadriceps. J Appl Physiol 86:1048–1053

Richardson RS, Newcomer SC, Noyszewski EA (2001) Skeletal muscle intracellular PO(2) assessed by myoglobin desaturation: response to graded exercise. J Appl Physiol 91:2679–2685

Saltin B, Calbet JA (2006) Point: in health and in a normoxic environment, VO_{2max} is limited primarily by cardiac output and locomotor muscle blood flow. J Appl Physiol 100:744–745

Schmidt W, Prommer N (2010) Impact of alterations in total hemoglobin mass on VO_{2max}. Exerc Sport Sci Rev 3:68–75. doi:10.1097/JES.0b013e3181d4957a

Semenza GL, Wang GL (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12:5447–5454

Sonna LA, Sharp MA, Knapik JJ, Cullivan M, Angel KC, Patton JF, Lilly CM (2001) Angiotensin-converting enzyme genotype and physical performance during US Army basic training. J Appl Physiol 91:1355–1363

Stratton JR, Levy WC, Cerqueira MD, Schwartz RS, Abrass IB (1994) Cardiovascular responses to exercise. Effects of aging and exercise training in healthy men. Circulation 89:1648–1655

Swallow EB, Gosker HR, Ward KA, Moore AJ, Dayer MJ, Hopkinson NS, Schols AM, Moxham J, Polkey MI (2007) A novel technique for nonvolitional assessment of quadriceps muscle endurance in humans. J Appl Physiol 103:739–746

Tanimoto K, Yoshiga K, Eguchi H, Kaneyasu M, Ukon K, Kumazaki T, Oue N, Yasui W, Imai K, Nakachi K, Poellinger L, Nishiyama M (2003) Hypoxia-inducible factor-1alpha polymorphisms associated with enhanced transactivation capacity, implying clinical significance. Carcinogenesis 24:1779–1783

Timmons JA, Jansson E, Fischer H, Gustafsson T, Greenhaff PL, Ridden J, Rachman J, Sundberg CJ (2005) Modulation of extracellular matrix genes reflects the magnitude of physiological adaptation to aerobic exercise training in humans. BMC Biol 3:19

Timmons JA, Knudsen S, Rankinen T, Koch LG, Sarzynski M, Jensen T, Keller P, Scheele C, Vollaard NB, Nielsen S, Akerstrom T, MacDougald OA, Jansson E, Greenhaff PL, Tarnopolsky MA, van Loon LJ, Pedersen BK, Sundberg CJ, Wahlestedt C, Britton SL, Bouchard C (2010) Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J Appl Physiol 108:1487–1496

Ukkola O, Joanisse DR, Tremblay A, Bouchard C (2003) Na+-K+-ATPase alpha 2-gene and skeletal muscle characteristics in response to long-term overfeeding. J Appl Physiol 94:1870–1874

Vollaard NB, Constantin-Teodosiu D, Fredriksson K, Rooyackers OE, Jansson E, Greenhaff PL, Timmons JA, Sundberg CJ (2009) Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance. J Appl Physiol 106:1479

Williams AG, Folland JP (2008) Similarity of polygenic profiles limits the potential for elite human physical performance. J Physiol 586:113–121

Woods DR, World M, Rayson MP, Williams AG, Jubb M, Jamshidi Y, Hayward M, Mary DA, Humphries SE, Montgomery HE (2002) Endurance enhancement related to the human angiotensin I-converting enzyme I–D polymorphism is not due to differences in the cardiorespiratory response to training. Eur J Appl Physiol 86:240–244

Yamada N, Horikawa Y, Oda N, Iizuka K, Shihara N, Kishi S, Takeda J (2005) Genetic variation in the hypoxia-inducible factor-1alpha gene is associated with type 2 diabetes in Japanese. J Clin Endocrinol Metab 90:5841–5847

Yu AY, Frid MG, Shimoda LA, Wiener CM, Stenmark K, Semenza GL (1998) Temporal, spatial, and oxygen-regulated expression of hypoxia-inducible factor-1 in the lung. Am J Physiol 275:L818–L826