ORIGINAL RESEARCH

The Lack of Associations Between Alleles at the Hypoxia-Inducible Factor 1A C1772T Loci and Responses to Acute Hypoxia

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Objective.—The aim of this study was to investigate the associations between alleles of the hypoxia-inducible factor 1A (*HIF1A*) C1772T polymorphism and several physiological responses to hypoxia, including the hypoxic ventilatory response (HVR), and serum erythropoietin (EPO), arterial oxygen saturation (Sao₂), and acute mountain sickness (AMS) responses during 8 hours of exposure to normobaric hypoxia.

Methods.—A total of 76 males participated in the study; 52 participants completed an 8-hour exposure to 12.7% oxygen, during which time Sao₂, EPO concentrations, and AMS scores were measured, while 62 individuals took part in an HVR trial (in total 38 individuals completed both protocols). DNA was obtained from leukocytes, and a 346-bp fragment of the *HIF1A* gene containing the C1772T polymorphism was amplified using polymerase chain reaction. Fragments were sequenced to reveal individual genotypes, and the associations between *HIF1A* genotype and EPO, Sao₂, AMS responses to hypoxia and HVR were examined.

Results.—The magnitude of the hypoxic responses was highly variable between individuals. The increase in participants' EPO responses ranged from 89% to 388% of baseline values following hypoxia, while Sao₂ values during the exposure ranged from 71% to 89%. The HVR ranged from -0.04 to $+2.18 \text{ L} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ among participants. No significant differences in EPO, Sao₂, AMS, or HVR results were observed between the *HIF1A* CC genotype and the combined CT/TT genotype group.

Conclusion.—In this study, the *HIF1A* C1772T polymorphism does not appear to influence EPO, Sao₂, or AMS responses during acute hypoxic exposure, or the magnitude of the HVR.

Key Words: arterial oxygen saturation, erythropoietin, genetics, hypoxia-inducible factor (HIF), hypoxic ventilatory response (HVR), polymorphism

Introduction

Exposure to hypoxic conditions, such as that found at high altitude, induces adaptations in various physiological systems that aid the maintenance of oxygen delivery to metabolically active tissue, and thereby mitigates the impact of the fall in atmospheric partial pressure of oxygen (Po₂). A critical response to acute hypoxia is the immediate increase in ventilation, which aids oxygen transport across the lung and helps maintain arterial

oxygen saturation (Sao₂). A large degree of variation is present between individuals' hypoxic ventilatory response (HVR)¹ and Sao₂ at altitude,^{2,3} and both contribute to the susceptibility to high altitude illness.² There is evidence that genetic factors strongly influence ventilatory responses to hypoxia,^{4,5} Sao₂ at high altitude in Tibetan natives,⁶ and susceptibility to altitude sickness.⁷ Another important response to hypoxia is the erythropoietin (EPO)-mediated expansion in erythrocyte mass, which helps to preserve oxygen delivery in the hypoxic environment. The rate of EPO production is also variable between individuals, and has been predicted to have a significant genetic component.⁸ Despite the evidence that

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genetic factors influence acclimation responses to hypoxia, very few studies have looked for associations between alleles of specific genetic polymorphisms and hypoxia acclimation phenotypes.

The transcription factor hypoxia-inducible factor-1 (HIF-1) is the key regulator of the cellular response to hypoxia.⁹ HIF-1 activates more than 100 genes, which encode proteins that facilitate cellular and systemic responses to hypoxia and promote the restoration of cellular oxygen homeostasis; these proteins are linked to the regulation of angiogenesis,¹⁰ erythropoiesis,¹¹ and energy metabolism.¹² HIF-1 is a heterodimer that is comprised of α and β subunits. Under hypoxic conditions, HIF-1 β protein levels remain unchanged, while HIF-1 α levels increase exponentially as oxygen tension decreases within the cell.¹³ HIF-1 DNA binding activity follows a similar trend to HIF-1 α protein levels, indicating that the HIF-1 transcriptional response is predominantly controlled by HIF-1 α protein concentration.¹³ In order for HIF-1 to activate transcription of a target gene, it must bind to a transcription regulating domain located within the promoter regions of target genes called the hypoxic response element (HRE).¹⁴

Studies conducted on HIF-1 α “knock-out” mice have illustrated the influence of HIF-1 α on acclimation responses to hypoxia. Homozygous knock-out (*Hif1a*^{-/-}) mice have an embryonic lethal phenotype due to malformations of the heart and vasculature; however, heterozygous knock-out (*Hif1a*^{+/-}) mice, which are partially deficient in HIF-1 α , are indistinguishable from wild-type mice in normoxic conditions.¹⁵ Compared to wild-type mice, the *Hif1a*^{+/-} mice have impaired acclimation responses to hypoxia; these include impaired carotid body function and ventilatory responses,¹⁶ reduced right ventricular hypertrophy,¹⁵ an absent EPO response,¹¹ and delayed erythropoiesis.¹⁵

The human gene that encodes HIF-1 α , *HIF1A*, contains a cytosine (C) to thymine (T) polymorphism at position 1772 (C1772T), which causes a proline to serine amino acid change at residue 582 of the HIF-1 α protein.¹⁷ In the COS 7 cell line, the serine variant has more than 2-fold greater HRE binding activity than the proline variant.¹⁷ In addition, the level of HIF-1 α binding to the HRE during exposure to severe hypoxic conditions (1% oxygen) was approximately 20-fold higher than baseline values in the serine variant compared to the approximately 7-fold increase observed for the proline variant.¹⁷ This polymorphism has also been shown to influence several human phenotypes, demonstrating the functional effect of the polymorphism in vivo. These phenotypes include, but are not limited to, greater susceptibility to various forms of cancer^{18–20} and reduced coronary collateral formation in patients with ischemic heart dis-

ease.²¹ All of the phenotypes previously studied in relation to the *HIF1A* C1772T polymorphism take a long time to progress and the polymorphism interacts with the environmental conditions associated with the phenotype (eg, hypoxia) over a long period. At this time, it is not known whether the *HIF1A* polymorphism influences acute responses to hypoxia. However, in mice, HIF-1 α deficiency has been shown to alter responses to hypoxia after only 1 hour.¹¹ Furthermore, the C1772T polymorphism influences HIF-1 α activity in normoxic conditions,¹⁷ which may subsequently alter physiological responses to acute hypoxia. Therefore, it is plausible that the *HIF1A* C1772T polymorphism will have an impact on acute physiological responses to hypoxia in humans.

The influence of the *HIF1A* C1772T polymorphism on human responses to atmospheric hypoxia has only been studied twice previously. Droma et al²² observed no difference in the frequency of the *HIF1A* genotypes between Sherpas who were and were not susceptible to acute mountain sickness (AMS). A further study looked to evaluate the influence of the *HIF1A* C1772T polymorphism on acclimation responses to intermittent hypoxia; however, only the CC genotype was identified in the small study population (n = 26), which limited analysis.²³ At this time no study has been conducted to investigate whether this polymorphism accounts for some of the variability in ventilatory and hematological responses to atmospheric hypoxia. As such, here we have studied whether the *HIF1A* T allele, which is associated with higher HIF-1 α activity and increased transcription of target genes,^{17,24} is linked to a higher HVR and a greater EPO response, a higher Sao₂, and a decreased occurrence of AMS symptoms during acute exposure to 12.7% oxygen.

Methods

PARTICIPANTS

Following local human ethical committee approval and written informed consent, 76 healthy Caucasian males were recruited for the study. The study was separated into 2 trials. In the first trial, 52 participants (age 24 ± 6 years, height 1.77 ± 0.07 m, body mass 76.1 ± 9.6 kg) completed an 8-hour exposure to 12.7% oxygen. In the second trial, 62 individuals (age 24 ± 5 years, height 1.80 ± 0.07 m, body mass 78.8 ± 8.4 kg) completed an HVR trial. Of the study population, 38 participants (age 23 ± 4 years, height 1.79 ± 0.06 m, body mass 77.6 ± 8.7 kg) completed both protocols. The 38 participants first completed the 8-hour exposure trial then, after a “wash-out” period of 1 month, took part in the HVR trial. All individuals were native low-landers who had not

been exposed to hypoxic conditions in the 3 months prior to participation. Participants completed a health screen questionnaire prior to the first trial, and individuals were excluded from the study if they reported current or previous cardiovascular, respiratory, or renal problems.

TRIAL 1: 8-HOUR EXPOSURE TO 12.7% OXYGEN

Participants were instructed to abstain from caffeine and exercise in the 12 hours leading up to the trial and during the trial. On arrival at the laboratory, located at approximately 50 m altitude, participants assumed the supine position and maintained this for 10 minutes before baseline measurements were taken. Baseline SaO_2 was measured continuously for 5 minutes, after which a 15-mL blood sample was taken from a vein located at the antecubital fossa using standard venepuncture techniques. At 0900 hours, participants entered a nitrogen dilution chamber (Design Environmental, Gwent, Wales) with atmospheric oxygen maintained at 12.7%. This produced a PO_2 of 97 mm Hg, which is equivalent to 4000 m altitude. Participants remained in the chamber for 8 hours, during which they adopted the supine position from the 45th to the 60th minute of each hour. During the last 5 minutes, SaO_2 was measured continuously using a finger pulse oximeter (model 8600, Nonin, Plymouth, MN) connected to a personal computer, and the mean value for each time point was calculated. Every 2 hours participants reported AMS symptoms from sections of the Lake Louise questionnaire,²⁵ and at 4-hour intervals venous blood samples were taken. The severity of AMS symptoms was calculated by totaling the scores for 4 questions from the self-report section of the Lake Louise worksheet. Question 5 of the Lake Louise AMS questionnaire asks whether individuals had suffered from sleeping difficulties over the previous night. This question was not used as the hypoxic exposure only began at 0900 hours and, therefore, it was not relevant. This modified version of the questionnaire has been validated against other AMS questionnaires and against the clinical assessment section of the Lake Louise form.²⁶ During the trial, participants ate and drank ad libitum.

Hematological analysis was conducted on 50 of the 52 participants. From a 15-mL blood sample, 10 mL was aliquoted into a tube that promotes coagulation (Sarstedt, Numbrecht, Germany). After a 20- to 30-minute incubation at room temperature, the coagulated blood was centrifuged to obtain serum, which was stored frozen (-80°C) until used for analysis. From the remaining whole blood, hematocrit (Hct) values were determined in triplicate using microcentrifuge techniques (Hawksley,

Sussex, England). The cyanmethemoglobin method of analysis was used to determine hemoglobin (Hb) concentration. Hct and Hb levels were subsequently used to estimate plasma volume changes using equations originally proposed by Dill and Costill.²⁷ Serum EPO concentration was determined using an EPO enzyme-linked immunosorbent assay (ELISA) (Biomerica, Hannover, Germany) and the values obtained were corrected for changes in plasma volume. The ELISA procedure was performed in duplicate according to the manufacturer's instructions. The intra-assay and interassay coefficients of variation for this method were 5.4% and 6.5%, respectively.

TRIAL 2: HYPOXIC VENTILATORY RESPONSE

On arrival at the laboratory, pulmonary function [forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and FEV_1/FVC (%)] was measured and assessed using a portable spirometer (Micro Plus, Micro Medical, Kent, England) in accordance with guidelines published by the British Association of Sport and Exercise Sciences.²⁸ An exclusion criterion was put in place in which participants would not be allowed to take part in this aspect of the study if either their FEV_1 or FEV_1/FVC value was below 70% of the predicted value. However, no participants were excluded from the trial according to these criteria.

During the isocapnic HVR trial, respiratory variables were measured breath by breath (ZAN 600USB, Nspire Health, Oberthulba, Germany) and values were displayed continuously on a personal computer allowing constant monitoring of ventilatory parameters, which included the partial pressure of end tidal carbon dioxide (P_{ETCO_2}). Participants wore a facemask (model 7940, Hans Rudolph, Kansas City, KS) connected to a pneumotachograph, previously calibrated using a 3-L syringe. Before each trial, gas analyzers were calibrated using certified gases of known concentration (BOC Gases, Guildford, UK). A 2-way non-rebreathing valve (model 2730, Hans Rudolph) was located distal to the pneumotachograph and the inspiratory port was connected, via a 1.5-m length of corrugated tubing, to a 200-L Douglas bag (Harvard Apparatus Ltd, Edenbridge, England) containing hypoxic air ($9.1\% \pm 0.8\% O_2$, balanced with nitrogen). A single-channel tubing valve (model 50-1021, Harvard Apparatus Ltd) was located between the corrugated tubing and the Douglas bag, which allowed the inspire to be quickly changed from ambient air to hypoxic air. The inspired oxygen fraction was continuously measured 10 cm upstream of the 2-way non-rebreathing valve by a paramagnetic transducer (Servomex 1400, Crowborough, England), which produces an analogue signal relative to the measured fraction of inspired oxygen (F_{IO_2}). The analogue output was changed to a digital signal via an

analogue to digital converter (LabPro, Vernier, Sarasota, FL), and the $F_{I}O_2$ data were viewed and stored on a personal computer. Inspired gas fractions were altered manually through the addition of oxygen and carbon dioxide into the corrugated tubing 1 m upstream of the 2-way non-rebreathing valve. The addition of oxygen and carbon dioxide to the inspired air allowed hyperoxic and isocapnic conditions, respectively, to be produced.

The isocapnic HVR protocol was based on the steady-state methods proposed by Powell,²⁹ which was designed to bring continuity in HVR methodology between laboratories. As such, this HVR protocol was chosen in the current study so that results would be comparable with other studies in the area. This steady-state method includes a period of hyperoxia before the hypoxic exposure. The use of a hyperoxic period prior to HVR measurement is implemented as it has been reported that at sea level and high altitude an increase in $F_{I}O_2$ can decrease ventilatory drive from oxygen-sensitive chemoreceptors and cause a reduction in ventilation.²⁹ Thus a hyperoxic period was used in the current study to reduce the potential for intra-individual variation in baseline chemoreceptor drive from influencing the interpretation of the HVR data.

Individuals were asked to abstain from caffeine, alcohol, and exercise for 12 hours prior to the trial, and to fast for 2 hours before the trial, which was performed in a darkened room between 0830 and 1100 hours. The trial was conducted after 5 minutes of rest and with the participants in a semi-recumbent, 30° head-up position. Participants kept their eyes closed and listened to quiet music (the same for each individual) for the duration of the trial. Participants were fitted with a facemask that was attached to the HVR apparatus. SaO_2 was measured continuously throughout the trial using a pulse oximeter (Model 8600, Nonin). The isocapnic HVR test was conducted over a 30-minute period. For the first 10 minutes, participants breathed ambient air (partial pressure of inspired oxygen $[P_{I}O_2] = 159$ mm Hg) to become accustomed to the apparatus. $P_{I}O_2$ was then increased to 200 mm Hg and maintained at this level for 15 minutes. After 5 minutes of hyperoxia ($P_{I}O_2 = 200$ mm Hg), $P_{I}CO_2$ was adjusted to increase $P_{ET}CO_2$ 1 to 2 mm Hg above the level attained during the first 5 minutes of hyperoxia (as assessed from the displayed $P_{ET}CO_2$ value); this value was then held constant for the remainder of the trial. After 25 minutes, the inspire was changed from hyperoxic air to a hypoxic gas reservoir containing $9.1\% \pm 0.7\%$ O_2 , which reduced SaO_2 to $78.1\% \pm 4.6\%$. The hypoxic gas was breathed for the rest of the test although, due to a high level of ventilation achieved by 2 participants, the hypoxic intervention was aborted in these cases after 4 minutes as the volume of the hypoxic gas was low.

Ventilatory parameters, $F_{I}O_2$ and SaO_2 were calculated to give 5-second averages. The mean of the data collected over the last 2 minutes of hyperoxia was taken as the resting value, while the mean value calculated from the final 30 seconds of the trial represented the hypoxic response. HVR was determined by dividing Δ minute ventilation ($\dot{V}_E$) by ΔSaO_2 . The same protocol was followed to determine the breathing frequency (f_R) and tidal volume (V_T) responses to hypoxia, termed in this study the hypoxic breathing frequency response ($Hf_{R,R}$) and the hypoxic tidal volume response ($HV_{T,R}$), respectively. The interday coefficient of variation for this method was 29%, which is comparable with other isocapnic HVR methods.¹ In addition, the reliability of the method described here was high (intraclass correlation coefficient [ICC] = 0.921).

HIF1A GENOTYPE IDENTIFICATION

Genomic DNA was generally extracted from 300 μ L of whole blood using a DNA purification kit (Promega, Madison, WI). However, a mouthwash protocol (Oragene DNA kit, DNA Genotek, Ottawa, Canada) was implemented for 7 participants to obtain buccal cells, from which DNA was subsequently isolated in accordance with manufacturers' guidelines.

A 346-bp fragment of the *HIF1A* gene containing the C1772T polymorphism was amplified using the polymerase chain reaction (PCR) technique. The 50- μ L reactions contained 50 ng genomic DNA, 200 ng of each primer, 5'-AAGGTGTGGCCATTGTAAAACTC-3' (forward) and 5'-GCACTAGTAGTTTCTTTATGTATG-3' (reverse) previously used by Ollerenshaw et al,³⁰ 0.2 mmol/L of each deoxynucleotide triphosphate (Sigma-Aldrich, Gillingham, England), 2 mmol/L $MgCl_2$ (Promega), 10 μ L GoTaq Flexi Buffer (Promega), and 2.5 units GoTaq DNA polymerase (Promega). After an initial denaturation at 94°C for 6 minutes, 35 cycles of 94°C for 60 seconds (denaturation), 60°C for 90 seconds (annealing), and 72°C for 120 seconds (extension) were performed. This was followed by a final extension at 72°C for 5 minutes. PCR products were analyzed in 1.5% (wt/vol) agarose gels, which were stained with ethidium bromide. Each 346-bp DNA fragment was then extracted using a commercial purification kit (QIAquick, Qiagen, Crawley, England) and sequenced (MWG Biotech, London, England) using the forward primer.

STATISTICAL ANALYSIS

SPSS 15.0 (SPSS Inc, Chicago, IL) was used for statistical analysis and the level of significance was set at $P < .05$. Data are presented as the mean $\pm$ 1 SD. Chi-

Table 1. *HIF1A* C1772T genotype frequencies for the different study populations

	<i>HIF1A</i> C1772T genotype		
	CC	CT	TT
Whole population (n = 76)	81% (n = 62)	16% (n = 12)	3% (n = 2)
8-hour hypoxia (n = 52)	79% (n = 41)	17% (n = 9)	4% (n = 2)
HVR (n = 62)	79% (n = 49)	18% (n = 11)	3% (n = 2)

HIF1A, hypoxia-inducible factor 1A gene; HVR, hypoxic ventilatory response.

square analysis was performed to test whether the distribution of the *HIF1A* C1772T alleles was in Hardy-Weinberg equilibrium. Because the *HIF1A* TT genotype was only identified in 2 individuals, data for the CT and TT genotypes were combined to form a single group, and statistical analysis was made between the CC and CT/TT groups. This practice is in accord with previous studies^{21,31} in which the results for the *HIF1A* CC genotype were compared with those for the combined CT/TT genotype group. In trial 1, independent-samples *t* tests were used to test for differences between the *HIF1A* genotypes in respect to participants' physical characteristics, and environmental conditions. Sao_2 , EPO, and AMS responses over time for the *HIF1A* genotypes were analyzed using 2-way repeated-measures factorial analysis of variance (ANOVA) (group – time). When a significant main effect (time) was evident, differences between time-points were identified using dependent-samples *t* tests, with the *P* value corrected for multiple comparisons using the Bonferroni calculation. In addition, analysis of covariance (ANCOVA) was used to assess whether differences in EPO responses after 8 hours of hypoxia were present between the *HIF1A* C1772T genotypes after removing the variance caused by differences in mean Sao_2 levels by assigning it as a cofactor. In trial 2, differences in ventilatory parameters between *HIF1A* genotypes were assessed using 2-way repeated-measures factorial ANOVA (group – time). When a significant main effect for time was obtained, multiple dependent *t* tests were used to establish the position of the difference, with the *P* value adjusted to acknowledge multiple measures via the Bonferroni calculation. Physical characteristics of the participants, ventilatory parameters (HVR, $\text{Hf}_{\text{R}}\text{R}$, $\text{HV}_{\text{T}}\text{R}$) and spirometry data (FVC, $\text{FEV}_{1\text{s}}$, $\text{FEV}_{1\text{s}}/\text{FVC}$) for the *HIF1A* genotypes were compared using independent *t* tests. Correlation analyses were performed using the Pearson product moment coefficient.

Results

The distribution of the *HIF1A* C1772T genotypes in the population that completed the 8-hour exposure to 12.7% oxygen, the HVR population, and the whole study population, are shown in Table 1. In each study population, the frequencies of the *HIF1A* C1772T genotypes were in Hardy-Weinberg equilibrium. Due to the small number of participants identified with the TT genotype (n = 2), the CT and TT genotypes were combined and analyzed together.

TRIAL 1: 8-HOUR EXPOSURE TO 12.7% OXYGEN

Table 2 shows the age, height, and body mass of the individuals who participated in the 8-hour exposure, according to *HIF1A* genotype, all of which were not significantly different between genotypes. Atmospheric $\text{F}_{\text{I}}\text{O}_2$, $\text{F}_{\text{I}}\text{CO}_2$, temperature, and humidity were also not different between the genotype groups.

Serum EPO was $49\% \pm 43\%$ higher than sea level values after 4 hours of exposure to 12.7% oxygen ($P < .001$), and continued to increase and reached values $125\% \pm 66\%$ greater than baseline after 8 hours ($P < .001$). The magnitude of the EPO increase was highly variable between individuals; values ranged from 89% to 388% of baseline values after 8 hours. The magnitude of this increase correlated with the individuals' mean Sao_2 during the exposure ($r = -0.474$, $P < .001$). The coefficient of determination ($R^2 = 0.22$) indicates that the mean Sao_2 during the exposure accounts for approximately 22% of the interindividual variation in EPO response after 8 hours. The *HIF1A* CT/TT genotype group was not associated with a greater EPO response during hypoxia when analyzed in either absolute values or as a percentage of baseline ($P = .170$ and

Table 2. Participants' characteristics and environmental conditions during the 8-hour hypoxic exposure according to *HIF1A* genotype

	<i>HIF1A</i> C1772T genotype		
	CC	CT/TT	<i>P</i> value
Age (yr)	23 $\pm$ 7	24 $\pm$ 5	.849
Height (m)	1.77 $\pm$ 0.07	1.77 $\pm$ 0.06	.972
Mass (kg)	76.3 $\pm$ 10.4	75.4 $\pm$ 5.9	.902
$\text{F}_{\text{I}}\text{O}_2$ (%)	12.7 $\pm$ 0.2	12.7 $\pm$ 0.1	.582
$\text{F}_{\text{I}}\text{CO}_2$ (%)	0.12 $\pm$ 0.10	0.09 $\pm$ 0.04	.100
Temperature ($^{\circ}\text{C}$)	25.1 $\pm$ 0.2	25.0 $\pm$ 0.0	.360
Humidity (%)	40.1 $\pm$ 0.2	40.0 $\pm$ 0.2	.796

Data are mean $\pm$ 1 SD; *P* values are from independent-samples *t* tests. *HIF1A*, hypoxia-inducible factor 1A gene; $\text{F}_{\text{I}}\text{O}_2$, fraction of inspired oxygen; $\text{F}_{\text{I}}\text{CO}_2$, fraction of inspired carbon dioxide.

Figure 1. Erythropoietin (EPO) profile according to *HIF1A* genotype during the 8-hour exposure to 12.7% oxygen. EPO values are represented as a percentage of baseline values; CC (□), CT/TT (◆). Values are the mean $\pm$ 1 SD. ^aWhole population significantly different from baseline ($P < .001$). ^bWhole population significantly different from the 4-hour time point. ($P < .001$).

$P = .485$, respectively; Figure 1). In addition, when the variation in EPO levels due to differences in SaO_2 were accounted for using ANCOVA no differences in EPO responses were present between the *HIF1A* genotypes ($P = .612$). Plasma volume did not alter significantly from sea level values during the 8-hour hypoxic exposure ($P = .110$).

Figure 2. Arterial oxygen saturation (SaO_2) profile according to *HIF1A* genotype during the 8-hour exposure to 12.7% oxygen; CC (□), CT/TT (◆). Values are the mean $\pm$ 1 SD. ^aWhole population significantly different from baseline at this time point ($P < .001$). ^b SaO_2 at this time point higher than at the 1-hour time point ($P = .031$).

Figure 3. Acute mountain sickness (AMS) scores during an 8-hour exposure to 12.7% oxygen according to *HIF1A* genotype. Values are the mean $\pm$ 1 SD. ^{aa}Whole population significantly different from baseline at this time point ($P < .05$). ^aWhole population significantly different from baseline at this time point ($P < .001$).

During the exposure, SaO_2 decreased significantly from baseline levels of $98.0\% \pm 1.0\%$ to mean exposure levels of $81.3\% \pm 3.4\%$ ($P < .001$). SaO_2 was lower than pre-exposure values from the first hour of exposure until the last; however, SaO_2 increased significantly during the exposure from $80.6\% \pm 4.0\%$ after 1 hour to $82.7\% \pm 4.3\%$ after 8 hours ($P = .031$). The magnitude of the decrease in SaO_2 was highly variable between individuals; the mean SaO_2 ranged from 71% to 89%. The SaO_2 profile of both *HIF1A* genotype groups was similar to that of the whole population, a steep decrease from pre-exposure values, followed by a gradual increase during the exposure with saturation remaining below baseline values (Figure 2). The magnitude of the decrease in SaO_2 was not significantly different between participants with either the CC or the CT/TT genotype ($P = .843$).

The symptoms of AMS increased progressively during the 8-hour exposure to 12.7% oxygen, and were significantly greater than resting levels from 2 hours onwards (2 hours, $P = .014$; 4 hours, $P = .002$; 6 hours, $P = .001$; 8 hours, $P < .001$). The AMS score for the CC genotype group was consistently higher than the CT/TT genotype group during hypoxia (Figure 3), although no significant association between alleles at the *HIF1A* C1772T loci and AMS symptoms was observed ($P = .137$).

TRIAL 2: HYPOXIC VENTILATORY RESPONSE

The physical characteristics of the 62 participants who took part in the HVR trial, sorted by *HIF1A* genotype, are shown

Table 3. Physical characteristics and spirometry data of the HVR participants according to *HIF1A* genotype

	HIF1A C1772T genotype		P value
	CC	CT/TT	
Age (yr)	24 ± 5	23 ± 5	.538
Height (m)	1.80 ± 0.06	1.79 ± 0.07	.118
Mass (kg)	79.5 ± 9.3	76.1 ± 5.9	.677
FVC (L)	5.4 ± 0.7	5.4 ± 0.4	.811
FEV ₁ (L)	4.5 ± 0.6	4.5 ± 0.4	.877
FEV ₁ /FVC (%)	83.4 ± 5.3	84.5 ± 7.1	.615

Data are mean ± 1 SD; P values are from independent-samples *t* tests. *HIF1A*, hypoxia-inducible factor 1A gene; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second.

in Table 3. During the hyperoxic phase of the isocapnic HVR trial Sao_2 was $98.9\% \pm 0.6\%$ and it decreased to $78.1\% \pm 4.6\%$ during 5 minutes of hypoxia ($P < .001$). $\dot{V}_E$ increased significantly from hyperoxic values of $9.9 \pm 1.7 \text{ L} \cdot \text{min}^{-1}$ to $23.2 \pm 7.2 \text{ L} \cdot \text{min}^{-1}$ during hypoxia ($P < .001$), due primarily to the significant rise in V_T from resting values of $0.84 \pm 0.22 \text{ L} \cdot \text{Sao}_2\%^{-1}$ to $1.67 \pm 0.48 \text{ L} \cdot \text{Sao}_2\%^{-1}$ ($P < .001$). Although, the significant increase in f_R from $12.9 \pm 3.4 \text{ breaths} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ to $14.7 \pm 4.4 \text{ breaths} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ ($P < .001$) also contributed to the elevation in $\dot{V}_E$. P_{ETCO_2} was $38.2 \pm 2.8 \text{ mm Hg}$ prior

to the hypoxic phase of the trial and it decreased to $37.7 \pm 2.8 \text{ mm Hg}$ by the end of the intervention ($P = .001$). However, P_{ETCO_2} was maintained within 2 mm Hg of pre-hypoxic levels during each trial. The mean isocapnic HVR was $0.69 \pm 0.41 \text{ L} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$. Figure 4 shows an example of $\dot{V}_E$, Sao_2 , and F_{IO_2} responses to a 30-minute isocapnic HVR test.

HVR was not influenced by *HIF1A* C1772T genotype ($P = .565$). Values were $0.70 \pm 0.44 \text{ L} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ and $0.63 \pm 0.30 \text{ L} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ for the CC and CT/TT genotype groups, respectively. Similarly, $\text{Hf}_{\text{R}}R$ was independent of genotype ($0.096 \pm 0.125 \text{ breaths} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ and $0.101 \pm 0.195 \text{ breaths} \cdot \text{min}^{-1} \cdot \text{Sao}_2\%^{-1}$ for CC and CT/TT, respectively [$P = 0.096$]), as was $\text{HV}_{\text{T}}R$ ($0.048 \pm 0.044 \text{ L} \cdot \text{Sao}_2\%^{-1}$ and $0.044 \pm 0.027 \text{ L} \cdot \text{Sao}_2\%^{-1}$ for CC and CT/TT, respectively [$P = 0.743$]). Spirometry data according to genotype are presented in Table 3; no significant differences were found between the CC and CT/TT genotypes for FVC, FEV₁, or FEV₁/FVC.

Discussion

The main findings of this study were that the *HIF1A* C1772T polymorphism does not influence isocapnic HVR or acute EPO and Sao_2 responses to hypoxia.

The decrease in Sao_2 and the elevation in serum EPO concentration in response to atmospheric hypoxia are

Figure 4. Example of a 30-minute isocapnic hypoxic ventilatory response (HVR) test showing minute ventilation; $\dot{V}_E$, arterial oxygen saturation; Sao_2 , and fraction of inspired oxygen; F_{IO_2} .

well documented,^{32,33} and in the current study these findings were replicated during exposure to an $F_{I}O_2$ of 12.7%. Here, SaO_2 decreased from 98% in normoxia to 81.3% during the hypoxic bout and EPO concentration increased to 125% after 8 hours of exposure. In addition, AMS results from the current study are also in agreement with the literature³⁴; symptoms of AMS were present early into the exposure and increased progressively during the 8-hour duration.

The C1772T variation in exon 12 of the *HIF1A* gene causes a proline to serine amino acid change at residue 582 of the HIF-1 α protein. The serine variant has been shown in vitro to be more stable than the proline variant and the former is associated with greater transactivational activity during normoxic and hypoxic conditions.¹⁷ The *HIF1A* C1772T polymorphism has also been shown to influence a number of disease phenotypes that relate to hypoxia,^{18–20} demonstrating a functional effect of the polymorphism in vivo. As HIF-1 α has been shown to have an important role in ventilatory, cardiovascular, and hematological acclimation responses to atmospheric hypoxia,^{15,16} we hypothesized that the higher HIF-1 α activity associated with the *HIF1A* T-allele would be associated with greater acclimation to hypoxia, which would include an increased EPO response, a higher level of SaO_2 , a decreased occurrence of AMS, and a greater HVR. However, in the current study the *HIF1A* C1772T polymorphism did not influence the magnitude of participants' HVR, or their EPO, SaO_2 , or AMS responses during the 8-hour exposure to 12.7% oxygen.

Because this is the first study to investigate the associations between the alleles at the *HIF1A* C1772T loci and the HVR, EPO, and SaO_2 response during hypoxic exposure, it is not known if the lack of associations is because of the study conditions employed here or if the polymorphism has no influence on these particular phenotypes. An aspect of the current study that is in stark contrast to those that have identified a significant association between the alleles of the *HIF1A* C1772T polymorphism and a specific phenotype is the time-frame. The *HIF1A* polymorphism has been associated with various disease phenotypes, including type 2 diabetes,³⁵ ischemic heart disease,²¹ and the occurrence and severity of various forms of cancer.^{18–20} Each of these diseases is a chronic condition that takes many years to become established; therefore, the polymorphism is influencing these phenotypes over a prolonged period of time. Here the influence of the polymorphism was evaluated over 8 hours of hypoxic exposure, which may have been an insufficient period of time to influence the various phenotypes studied. However, there is evidence that supports the notion that the *HIF1A* polymorphism could influence acute responses to hypoxia. Firstly, in het-

erozygous HIF-1 α knock-out mice, renal EPO mRNA levels were significantly lower than wild-type mice after only 1 hour of intermittent hypoxic exposure.¹¹ This result shows that *HIF1A* deficiency alters acute responses to hypoxia and justifies the time frame used in the 8-hour hypoxic exposure trial of the current study. Second, the *HIF1A* C1772T polymorphism alters HIF-1 α activity in normoxic, as well as in hypoxic conditions,¹⁷ and thus the influence of altered HIF-1 α activity during normoxia could influence very acute responses to hypoxia, such as the HVR. Despite this evidence, future studies in this area should investigate associations between the alleles of the *HIF1A* C1772T polymorphism and chronic acclimation responses to atmospheric hypoxia, such as erythropoiesis and the progressive increase in HVR over weeks of hypoxic exposure. Increasing the duration of hypoxic exposure would make the time-frame more similar to those studies that found the *HIF1A* polymorphism to influence a particular phenotype^{18,35} and would also more closely mimic the exposure profiles of individuals traveling to high altitude, increasing the external validity of the study.

A limitation commonly found in human genetic studies is having low power to detect differences between genotypes due to limited participant numbers, and the current study is not an exception. In the present study, the lack of associations between the alleles of the *HIF1A* C1772T polymorphism and the various responses to hypoxia may in part be due to the moderate number of participants studied, despite participant numbers being comparable with other published studies that have investigated the influence of other genetic polymorphisms on these phenotypes.^{3,36} Future studies could overcome this limitation by genotyping a large number of individuals before measuring a particular phenotype and preselecting individuals to take part in the physiological aspect of the study based on their *HIF1A* genotype. This would ensure a larger number of TT individuals would be included in the study and would increase the power to detect differences between the genotypes.

In conclusion, we observed no association between the alleles of the *HIF1A* C1772T polymorphism and either HVR or acute acclimation responses to an 8-hour normobaric hypoxic exposure (12.7% oxygen).

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