Abstract  The molecular signalling pathways that regulate inflammation and the response to hypoxia share significant crosstalk and appear to play major roles in high-altitude acclimatization and adaptation. Several studies demonstrate increases in circulating candidate inflammatory markers during acute high-altitude exposure, but significant gaps remain in our understanding of how inflammation and immune function change at high altitude and whether these responses contribute to high-altitude pathologies, such as acute mountain sickness. To address this, we took an unbiased transcriptomic approach, including RNA sequencing and direct digital mRNA detection with NanoString, to identify changes in the inflammatory profile of peripheral blood throughout 3 days of high-altitude acclimatization in healthy sea-level residents (n = 15; five women). Several inflammation-related genes were upregulated on the first day of high-altitude exposure, including
a large increase in *HMGB1* (high mobility group box 1), a damage-associated molecular pattern (DAMP) molecule that amplifies immune responses during tissue injury. Differentially expressed genes on the first and third days of acclimatization were enriched for several inflammatory pathways, including nuclear factor-κB and Toll-like receptor (TLR) signalling. Indeed, both *TLR4* and *LY96*, which encodes the lipopolysaccharide binding protein (MD-2), were upregulated at high altitude. Finally, *FASLG* and *SMAD7* were associated with acute mountain sickness scores and peripheral oxygen saturation levels on the first day at high altitude, suggesting a potential role of immune regulation in response to high-altitude hypoxia. These results indicate that acute high-altitude exposure upregulates inflammatory signalling pathways and might sensitize the TLR4 signalling pathway to subsequent inflammatory stimuli.

(Received 4 February 2022; accepted after revision 22 June 2022; first published online 25 July 2022)

**Corresponding author** Erica C. Heinrich: 1126 Webber Hall, 900 University Avenue, Riverside, CA 92521, USA.
Email: erica.heinrich@medsch.ucr.edu

**Abstract figure legend** Acute high-altitude exposure (1–3 days) causes systemic hypoxic stress. In this study, we found that this is associated with upregulation of genes encoding damage-associated molecular pattern (DAMP) molecules, Toll-like receptors (TLRs) and chemotactic factors in peripheral blood. These changes are suspected to result in enhanced immune responses to subsequent inflammatory stimuli over this time frame.

**Key points**
- Inflammation plays a crucial role in the physiological response to hypoxia.
- High-altitude hypoxia exposure causes alterations in the inflammatory profile that might play an adaptive or maladaptive role in acclimatization.
- In this study, we characterized changes in the inflammatory profile following acute high-altitude exposure.
- We report upregulation of novel inflammation-related genes in the first 3 days of high-altitude exposure, which might play a role in immune system sensitization.
- These results provide insight into how hypoxia-induced inflammation might contribute to high-altitude pathologies and exacerbate inflammatory responses in critical illnesses associated with hypoxaemia.

**Introduction**

High altitude is a physiologically stressful environment owing to low oxygen availability, low temperatures and low humidity. Given that maintenance of oxygen homeostasis is essential for survival, rapid physiological adaptations occur upon high-altitude exposure to increase tissue oxygen delivery, including increased ventilation and red blood cell production (Beall, 2006; Moore, 2017; Scheinfeldt et al., 2012; Simonson, 2015). Although many of the mechanisms that modulate high-altitude acclimatization are well described, it remains unclear how acute high-altitude exposure influences inflammatory signalling and immune function.

In typical conditions in a healthy individual, cellular hypoxia is experienced primarily during infection or tissue injury. Local hypoxia triggers an inflammatory response that initiates tissue protection and repair mechanisms (Walmsley et al., 2014). Owing to this vital link between inflammation and hypoxia, the signalling pathways that control these responses have evolved to share significant crosstalk (Bandarra & Rocha, 2013; Corcoran & O’Neill, 2016; D’Ignazio et al., 2016; Görlach & Bonello, 2008; Pham et al., 2021).

The hypoxia-inducible factor (HIF) is a transcription factor that regulates gene expression in response to hypoxic stress (Semenza, 2009). Activity of HIF is regulated by oxygen-sensitive prolyl hydroxylase domain proteins (PHDs) and factor inhibiting HIF (FIH). As a result, in normal oxygen tensions, HIF-α is hydroxylated by PHD, leading to its degradation. However, decreased PHD activity in hypoxia allows HIF-α to accumulate, bind to HIF-β subunits, and translocate to the nucleus to initiate expression of hypoxia-response genes. One downstream target of HIF is nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), a master regulator of inflammation. Conversely, NF-kB expression also upregulates *HIFIA* mRNA expression (BelAiba et al., 2007; Van Uden et al., 2008). As a result, several studies have demonstrated that the HIF and NF-kB
Inflammatory gene expression during acute high-altitude exposure

paths share an interdependent relationship (BelAiba et al., 2007; Bonello et al., 2007; Frede et al., 2006; Taylor, 2008; Zhou et al., 2003). Furthermore, PHD is also involved in regulating NF-kB nuclear translocation through its action on I-kappaB kinase (IKK) activity, providing an additional direct mechanism by which hypoxia regulates inflammatory signalling (Cummins et al., 2006).

Owing to the demonstrated links between HIF and NF-kB signalling, it is reasonable to suspect that hypoxaemia induced by high-altitude exposure might result in a systemic inflammatory response. Indeed, hypoxaemia and systemic inflammation commonly occur simultaneously in critical illnesses, such as sepsis and acute respiratory distress syndrome (ARDS) and, although in vivo data are limited, preclinical data suggest a key role of oxygen status in modulating inflammatory and immunological outcomes in these cases (Kiers et al., 2016). Furthermore, acute increases in inflammatory cytokine expression might contribute to, or be a downstream consequence of, high-altitude illnesses, such as acute mountain sickness (AMS). Several previous studies have provided evidence that candidate inflammatory mediators are upregulated in peripheral blood during the first few days of acclimatization (Eltzschig & Carmeliet, 2011; Faquin et al., 1992; Hartmann et al., 2000; Heinrich et al., 2018; Lundeberg et al., 2018; Scholz & Taylor, 2013). However, whether these changes are associated with phenotypes including AMS severity remains inconclusive.

In the present study, we expand on this work with a broad, unbiased transcriptomic approach to improve our understanding of how inflammatory signalling is altered in peripheral blood mononuclear cells during acute exposure to high altitude. We use RNA sequencing (RNA-seq) and NanoString direct digital detection of mRNA to identify broad patterns in inflammatory gene expression. We hypothesized that pro-inflammatory gene expression would increase upon acute exposure to high-altitude hypoxia and that higher inflammatory cytokine expression levels would be associated with more severe hypoxaemia and AMS severity.

Methods

Ethical approval

This study was approved by the University of California, Riverside (UC Riverside) Clinical Institutional Review Board (HS19-076). All participants were informed of the purpose and risks of the study. Participants provided written informed consent in their native language (English). The work was conducted in accordance with the Declaration of Helsinki, except for registration in a database.

Participants

The study included 15 healthy participants (n = 5 women, 10 men) between 19 and 32 years of age. Participants were recruited by word of mouth and flyers on the UC Riverside campus. All participants reported no known history of cardiopulmonary disease or sleep disturbances, including obstructive sleep apnoea, and displayed no abnormal findings on ECG or pulmonary function testing. The mean age was 25 ± 4 years for men and 26 ± 5 years for women, and body mass index was 26.7 ± 5.4 kg/m² for men and 28.4 ± 6.9 kg/m² for women. Exclusion criteria included travel >2500 meters within 1 month of the first measurements, a previous history of high-altitude pulmonary or cerebral oedema, smoking and pregnancy.

Experimental design and physiological measures

In the 2 weeks before ascent to high altitude, participants completed initial screening for eligibility at UC Riverside, at ~400 m elevation (Riverside, CA, USA). Demographic information including age, height, weight and blood pressure were collected. Participants also answered questions about their ancestral background (to determine the presence of high-altitude ancestry) and medical history, including current medications. Participants then completed pulmonary function testing and ECG to verify the absence of lung or heart disease.

Participants returned to UC Riverside in the early morning on the day of ascent. Baseline [sea-level (SL)] physiological measurements were collected at this time, including blood pressure, peripheral oxygen saturation (SPo2) by pulse oximetry, heart rate, end-tidal carbon monoxide (CO), and AMS scores via the 2018 Lake Louise scoring criteria, with an experimenter asking participants each question (Roach et al., 2018). Fasting blood samples were then collected via standard venipuncture procedures. Breakfast was provided to participants after blood sampling, before travel.

The group then travelled by car to Barcroft Station (elevation 3800 m) in the White Mountain Research Centre (Bishop, CA, USA) over a period of ~6.5 h. At the field station, fasting blood samples and morning measurements were collected each day within 1 h of waking and before 09.00 h to keep timing consistent with SL measurements. Physiological measurements and fasting blood samples were collected every morning for 3 days consecutively (HA1, HA2 and HA3), and end-tidal CO was measured at night. The end-tidal CO concentration was measured with a Micro+ Basic Smokerlyzer (CoVita, Santa Barbara, CA, USA). Participants held their breath for 15 s, then exhaled through the device to residual volume. Measurements were taken in triplicate and averaged. The analyser was calibrated with 50 ppm CO before the measurements each
Gene expression

RNA isolation. Peripheral blood was collected into PaxGene Blood RNA tubes (Qiagen, Germantown, MD, USA) using standard venipuncture procedures. Samples collected at SL were incubated at room temperature for 30 min, then frozen at −20°C and stored at −80°C until further processing. Samples collected at high altitude were stored at room temperature for 30 min, frozen at −20°C at the field station, then transported to UC Riverside in liquid nitrogen and frozen at −80°C until further processing.

Before RNA isolation, PaxGene Blood RNA tubes were allowed to thaw and incubate for 4 h at room temperature according to the manufacturer’s instructions. The RNA was isolated using a PaxGene Blood RNA Kit (Qiagen) following the manufacturer’s protocols. The quantity and quality of RNA were verified via a Nanodrop 2000 (ThermoFisher Scientific, Waltham, MA, USA) and Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA USA). RNA samples with RNA integrity number (RIN) values ≥8 were used for downstream sequencing.

NanoString. To obtain a more precise measure of inflammatory gene expression, we also measured mRNA levels of 250 key inflammation-related genes with the NanoString SPRINT Profiler (NanoString Technologies, Seattle, WA, USA). Given that we aimed to determine whether these expression levels were associated with phenotypes, this analysis was conducted on samples collected at SL and after one night at high altitude (HA1), because AMS scores were highest, $S_{P02}$ was lowest, and both were more variable across subjects on HA1. RNA profiling was conducted with 50 ng of total RNA, quantified using Nanodrop 2000. Samples were prepared for codelset hybridization with NanoString-prepared reporter and capture probes specific for the Inflammatory Panel CodeSet Human V2 following the manufacturer’s protocols. Paired participant samples were placed on the same cartridge to eliminate replicate bias.

Normalization and differential expression analysis were performed with the Advanced Analysis add-on to nSolver software (v.4.0). Counts were normalized to the following normalization probes: PGK1, CLTC, GADPH, GUSB, TUBB and HPRT1. These normalization probes are automatically selected using the geNorm algorithm, which selects probes that minimize the pairwise variation statistic during housekeeping gene selection. Genes within a sample with mRNA counts $\leq 2\times$ the maximum background (the average of the housekeeping gene count...
of negative controls) in >20% of all samples were excluded from analysis. Differential expression analysis included location (SL vs. HA1) as a predictor and subject as a confounder. The P-values were adjusted with the Benjamini–Yekutieli method. Adjusted P-values <0.05 were considered significant. Given that the NanoString gene expression panel included a preselected group of inflammation-related genes, we conducted enrichment analysis with GOrilla, which allowed a custom background gene set including only genes on the nCounter analysis with GOrilla, which allowed a custom background gene set including only genes on the nCounter Human Inflammation panel (Eden et al., 2009). The Probe Descriptive module of the nSolver Advanced Analysis software was used to examine gene-by-gene correlations, with location set as a covariate (SL or HA1) and interval identity, and participant as a series identity. Expression correlation for each pair of genes is expressed as the overall Pearson correlation coefficient and P-value.

### Statistical analysis

Statistical analyses were conducted in R (v.4.1.0; R Foundation for Statistical Computing). To identify changes in physiological variables at high altitude compared with baseline sea-level measures, we used repeated-measures ANOVA and post hoc Student’s paired t tests with Bonferroni corrections. To determine whether fold changes in gene expression were associated with physiological measures at high altitude (\(S_pO_2\) and AMS score), Pearson correlation coefficients and P-values were obtained with the \texttt{rcorr} function from the \texttt{Hmisc} package in R. Throughout the paper, data are presented as the mean ± SD.

### Results

#### Physiological measures

Table 1 provides an overview of physiological measures at sea level and over 3 days of acclimatization to high altitude. On the first morning at high altitude, eight of the 15 subjects indicated mild AMS (AMS score 3–5, with headache) and one participant indicated severe AMS (AMS score 6–9, with headache). By the third day, only one of the 15 subjects scored positive for AMS. The \(S_pO_2\) decreased by ~10 percentage points on the first day at high altitude and remained lower than SL values throughout day 3. This was coupled with an increase in heart rate at high altitude. There was no significant increase in diastolic or systolic blood pressure at high altitude. Exhaled end-tidal CO levels were elevated on average at high altitude, although this was not significant by repeated-measures ANOVA.

### Gene expression at high altitude

#### RNA sequencing

Three thousand nine hundred and fifty-eight genes were differentially expressed (adjusted \(P < 0.01\)) on HA1 (2177 upregulated and 1781 downregulated), and 4219 genes were differentially expressed on HA3 (2190 upregulated and 2029 downregulated) compared with SL. When applying a fold change threshold of one, 88 genes were upregulated and 75 downregulated on HA1, and 234 were upregulated and 51 downregulated on HA3. The top 20 differentially expressed genes on each day are provided in Table 2. Figure 1 demonstrates that the genes most differentially expressed on HA1 remained differentially expressed throughout HA3. However, the genes most differentially expressed on HA3 were slower responding and were typically not differentially expressed on HA1. Several genes associated with inflammation and the immune response were in the top 20 upregulated genes on HA1, including \(BCL2A1\), \(S100A8\), \(HMGB1\) and \(B2M\). Additional genes likely to be involved in the acclimatization process, including \(PDCD10\), which is involved in vascular development, were also in the top 20 differentially expressed genes. On HA3, several genes associated with acclimatization were upregulated, including \(BPGM\) (2,3-DPG), \(CA1\) (carbonic anhydrase 1), \(FECH\) (ferrochelatase), \(HEMGN\) (hemogen).
and PDCD10, in addition to several other genes associated with inflammation and immune function, including GYPA, IFIT1B and RIOK3.

A gene ontology analysis revealed that on HA1, differentially expressed genes were enriched for biological processes including regulation of autophagy, proteasome-mediated ubiquitin-dependent protein catabolic processes, endomembrane system organization and I-kappaB kinase/NF-kB signalling (Fig. 2A). There were also eight significantly enriched pathways, including apoptosis signalling, CCKR signalling, ubiquitin proteasome pathway, PDGF signalling, T-cell activation, Toll-like receptor (TLR) signalling, RAS and FAS signalling (Table 3). On HA3, the I-kappaB kinase/NF-kB signalling GO biological process remained enriched, in addition to other inflammation and immune function processes, including neutrophil activation involved in immune response (Fig. 2B). Nineteen pathways were significantly enriched on HA3, including several involved in inflammation and immune function and angiogenesis (VEGF signalling pathway) (Table 3).

After the GO analysis, we examined key genes of interest that play significant roles in the top enriched GO pathways. In particular, we were interested in genes involved in immune pathway activation, such as TLR4, HMGB1, IL8 and interleukin-8 (IL8) (Fig. 3). We found that there was a significant upregulation of TLR4 and HMGB1 mRNA counts across all participants. However, IL8 was not found to be significantly differentially expressed and had low counts. Additionally, we were interested in IL8 gene expression because neutrophil activation was highly enriched. We found there was significant IL8 upregulation upon high-altitude exposure in all but one participant.

**NanoString analysis of inflammatory pathway genes.**
To examine specific changes in inflammation-related gene expression further, we conducted a NanoString analysis on samples collected on HA1. Of the 250 genes on the human inflammation panel, 18 genes showed significant upregulation (Figs 4 and 5). Of the 18 genes identified as differentially expressed by NanoString, 13 were also detected with RNA sequencing. However, five differentially expressed genes were identified by NanoString alone, including LY96. This might be because the NanoString nCounter targets all isoforms of each gene and does not need to convert RNA to complementary DNA for amplification. Seven of the 18 significantly differentially expressed genes are involved in the significantly enriched GO pathway 'Regulation of binding (GO: 00 51098)' (adjusted P < 0.0001; TGFBR1, IFIT2, IFIT1, MAPK8, DDIT3, HMGB2 and HMGB1).

Other processes in which significantly upregulated genes are involved include positive regulation of apoptotic processes (MAPK8, DDIT3, HMGB1, TGFBR1 and IFIT2), regulation of DNA binding (MAPK8, HMGB2 and HMGB1), DNA conformational change (HMGB2 and HMGB1) and positive regulation of endothelial cell proliferation (HMGB2, HMGB1 and TGFBR1).

Given that our inflammation gene panel included the key hypoxia-response gene, HIF1A, we looked for significant correlations between HIF1A expression and expression of our top 18 differentially expressed

| Table 2. Top 20 differentially expressed genes on days 1 and 3 at high altitude |
|------------------------|------------------------|
| Gene                  | FC         | Adjusted P-value |
| BCL2A1                | 1.73      | 1.46 × 10^{-24 } |
| EVI2A                 | 1.52      | 7.87 × 10^{-20 } |
| ERGIC2                | 0.98      | 5.60 × 10^{-18 } |
| PPIG                  | 0.66      | 3.25 × 10^{-15 } |
| PDCD10                | 1.22      | 7.17 × 10^{-15 } |
| TXNDC9                | 1.20      | 1.28 × 10^{-14 } |
| RGS18                 | 1.04      | 3.09 × 10^{-14 } |
| SUB1                  | 1.09      | 3.09 × 10^{-14 } |
| TAF7                  | 0.66      | 9.58 × 10^{-14 } |
| S100A8                | 1.58      | 1.88 × 10^{-13 } |
| NFIX1                 | 0.97      | 2.53 × 10^{-13 } |
| CCDC82                | 1.02      | 2.79 × 10^{-13 } |
| RSL24D1               | 1.63      | 4.17 × 10^{-13 } |
| HMGB1                 | 0.80      | 4.71 × 10^{-13 } |
| MAN1A1                | 0.89      | 7.26 × 10^{-13 } |
| NDUFA5                | 1.35      | 7.26 × 10^{-13 } |
| ANKRD12               | 0.76      | 1.20 × 10^{-12 } |
| B2M                   | 0.94      | 1.59 × 10^{-12 } |
| NORAD                 | 0.55      | 1.59 × 10^{-12 } |
| BLOC1S2               | 0.88      | 1.70 × 10^{-12 } |
| Sl vs. HA day 3       |           |                   |
| BPGM                  | 2.86      | 3.65 × 10^{-28 } |
| HEMGN                 | 2.18      | 3.33 × 10^{-24 } |
| GYPA                  | 4.64      | 9.63 × 10^{-24 } |
| IFIT1B                | 2.91      | 1.35 × 10^{-22 } |
| CA1                   | 3.09      | 1.35 × 10^{-22 } |
| XK                    | 2.45      | 1.65 × 10^{-22 } |
| SAC5                  | 1.51      | 2.85 × 10^{-21 } |
| TENT5C                | 1.69      | 1.61 × 10^{-19 } |
| FECH                  | 1.95      | 1.61 × 10^{-19 } |
| EVI2A                 | 1.44      | 5.45 × 10^{-18 } |
| MBNL3                 | 1.47      | 8.16 × 10^{-18 } |
| PDCD10                | 1.30      | 2.45 × 10^{-17 } |
| ZNF292                | 1.04      | 4.31 × 10^{-17 } |
| RIOK3                 | 1.34      | 4.92 × 10^{-17 } |
| CAPZB                 | −0.56     | 9.42 × 10^{-17 } |
| YOD1                  | 1.34      | 1.97 × 10^{-16 } |
| PI3                   | −1.61     | 6.91 × 10^{-16 } |
| CREG1                 | 0.99      | 8.01 × 10^{-16 } |
| NORAD                 | 0.62      | 1.02 × 10^{-15 } |
| LOC644285             | −1.35     | 1.12 × 10^{-15 } |
genes to identify possible relationships between HIF signalling and inflammatory gene expression in vivo. Expression of HIF1A was significantly associated with expression of PTK2, MAPK1, HMGB1, TLR8 and NFE2L2 (Fig. 6).

**Phenotype associations**

Given that the highest AMS scores and lowest $S_{\text{po2}}$ levels were observed on the first day at high altitude, we looked for significant correlations between phenotypes on the first day at high altitude and inflammatory genes.
Table 3. Significantly enriched pathways on days 1 and 3 at high altitude

| Term                                                                 | Adjusted P-value | Overlap |
|----------------------------------------------------------------------|------------------|---------|
| **High altitude day 1 vs. sea level**                               |                  |         |
| Apoptosis signalling pathway Homo sapiens P00006                     | 0.0003           | 40/102  |
| CCKR signalling map ST Homo sapiens P06959                          | 0.0003           | 57/165  |
| Ubiquitin proteasome pathway Homo sapiens P00060                    | 0.0024           | 20/43   |
| PDGF signalling pathway Homo sapiens P00047                         | 0.0033           | 39/112  |
| T cell activation Homo sapiens P00053                               | 0.0033           | 28/73   |
| Toll receptor signalling pathway Homo sapiens P00054                 | 0.0033           | 21/49   |
| Ras Pathway Homo sapiens P04393                                     | 0.0064           | 26/69   |
| FAS signalling pathway Homo sapiens P00020                          | 0.0156           | 14/31   |
| **High altitude day 3 vs. sea level**                               |                  |         |
| Apoptosis signalling pathway Homo sapiens P00006                     | 0.0000           | 49/102  |
| CCKR signalling map ST Homo sapiens P06959                          | 0.0000           | 62/165  |
| T cell activation Homo sapiens P00053                               | 0.0011           | 31/73   |
| Toll receptor signalling pathway Homo sapiens P00054                 | 0.0011           | 23/49   |
| PDGF signalling pathway Homo sapiens P00047                         | 0.0011           | 42/112  |
| VEGF signalling pathway Homo sapiens P00056                         | 0.0053           | 23/54   |
| Interleukin signalling pathway Homo sapiens P00036                   | 0.0068           | 32/86   |
| Glycolysis Homo sapiens P00024                                      | 0.0103           | 10/17   |
| Ras Pathway Homo sapiens P04393                                     | 0.0141           | 26/69   |
| Integrin signalling pathway Homo sapiens P00034                      | 0.0162           | 49/156  |
| Ubiquitin proteasome pathway Homo sapiens P00060                    | 0.0162           | 18/43   |
| B cell activation Homo sapiens P00010                               | 0.0171           | 22/57   |
| AngiotsinII-stimulated signalling through G proteins and beta-arrestin Homo sapiens P05911 | 0.0176 | 15/34 |
| Inflammation mediated by chemokine and cytokine signalling pathway Homo sapiens P00031 | 0.0232 | 56/188 |
| p53 pathway Homo sapiens P00059                                     | 0.0302           | 25/71   |
| Parkinson disease Homo sapiens P00049                               | 0.0464           | 27/81   |
| General transcription regulation Homo sapiens P00023                 | 0.0464           | 12/18   |
| mRNA splicing Homo sapiens P00058                                   | 0.0464           | 4/5     |
| Alzheimer disease-amyloid secretase pathway Homo sapiens P00003      | 0.0464           | 20/56   |
| EGF receptor signalling pathway Homo sapiens P00018                  | 0.0464           | 34/109  |

Figure 2. Enriched gene ontology terms with highly variable genes

Clustering of significantly enriched gene ontology (GO) terms into representative subsets using semantic similarities. Significantly enriched GO terms are provided for genes differentially expressed on day 1 (HA1; A) and day 3 (HA3; B) at high altitude compared with a sea-level (SL) baseline. Bubble colour indicates the log\(^{10}\) P-value for each term, and bubble size indicates the frequency of the GO term in the underlying GOA database (more general terms are larger). [Colour figure can be viewed at wileyonlinelibrary.com]
measured by NanoString. Resting oxygen saturation (SpO2, as a percentage) was significantly associated with FASLG, SMAD7, PTGER4 and TRAF2, with a trend towards a correlation with IL8 (P = 0.05; Table 4). The AMS score was significantly associated with TNFSF14, FASLG, IL18, CD40LG, PTGER4, MAPKAPK2, HLADRB1, SMAD7, AGER, MAFK and IRF5 (Table 4).

Discussion
This study examined how high-altitude acclimatization influences inflammatory signalling by use of RNA sequencing and NanoString transcriptome analyses in whole blood of healthy participants. Our results demonstrate that upon acute high-altitude exposure, many inflammation-related genes are significantly upregulated. These upregulated genes are enriched in pathways involved in stress responses and regulation of inflammatory signalling (Fig. 2; Table 3), and the expressions of several inflammation-related genes, including FASLG and SMAD7, were associated with AMS scores and SpO2 levels (Table 4). The release of cellular stress markers during tissue hypoxia is known to trigger downstream mechanisms that promote host defence, such as the TLR signalling pathway (Schaefer, 2014). Therefore, it is not surprising that we found TLR signalling pathways to be impacted by acute high-altitude exposure. Many of the differentially expressed genes we identified also included damage-associated molecular patterns (DAMPs; HMGB1, HMGB2 and S100A8), interferon-stimulated genes (IFIT1, IFIT1B, IFIT2 and IFI44) and markers of DNA damage (DDIT3; Table 2; Fig. 5; Diamond, 2014; Schaefer, 2014; Yang et al., 2017). Here, we will discuss the impact of DAMPs, particularly HMGB1 and its synergistic role with LY96 in TLR4 activation.

Upregulation of the TLR4 pathway at high altitude
LY96 is a potential indicator of immune system priming (Kim et al., 2010; Park & Lee, 2013). LY96 encodes for MD-2, a coreceptor with TLR4, a key player in innate immunity defense. LY96 plays an essential role in the TLR4-mediated inflammatory response to lipopolysaccharide (LPS; da Silva Correia et al., 2001; Park et al., 2009). Activation of TLR4 by LPS, a pathogen-associated molecular pattern (PAMP), triggers the expression of inflammatory cytokines and chemokines (Park & Lee, 2013). Given that hypoxia has been shown to increase TLR4 expression, this might lead to exacerbated inflammation in response to subsequent inflammatory stimuli (Kim et al., 2010). In the present in vivo study, both TLR4 and LY96 gene expression were significantly upregulated after 1 day of high-altitude exposure (Fig. 5). Although TLR4 upregulation was found to be significant only in the RNA-seq data, TLR4 expression approached significance in the NanoString data (adjusted P = 0.057).
These data suggest that over 1–3 days of exposure to hypoxia, hypoxic stress primes the innate immune response and might increase the inflammatory response to infection. This is supported with in vitro work by Kim et al. (2010), in which hypoxia exacerbated the TLR4-mediated inflammation in response to LPS in murine RAW 264.7 macrophages. However, the duration of exposure to hypoxia, in addition to the model used, have varying effects on TLR4 expression. With short-term exposure (2–4 h) in macrophages, TLR4 was noted to have increased expression (Kim et al., 2010). With longer hypoxic exposure (>24 h) in murine dendritic cells, there was no significant change in TLR4, but there was a significant increase in TLR2 and TLR6 expression (Kuhlicke et al., 2007). Therefore, although it is clear that hypoxic stress is likely to impact TLR4 signalling in peripheral blood cells, the details and time domains of this effect require further study.

To complement LY96 and TLR4 upregulation, HMGB1 also showed one of the strongest signals of increased expression at high altitude (Table 2). HMGB1 encodes the high mobility group Box 1 protein, which interacts directly with TLR4 and functions as a DAMP mediator of inflammation. Activity of HMGB1 is dependent on its location and cell type. Intracellular cytosolic HMGB1 has been shown to inhibit apoptosis and to activate the autophagic response, particularly in response to oxidative stress (Tang et al., 2011; Zhu et al., 2015). Upon release...

### Table 4. Relationships between phenotypes and log2 fold changes in gene expression

| Gene   | \(R\)  | \(P\)-value |
|--------|--------|-------------|
| \(S_{\text{PO}_2}\) | | |
| FASLG  | 0.68   | 0.005       |
| SMAD7  | 0.63   | 0.011       |
| PTGER4 | 0.56   | 0.028       |
| TRAF2  | 0.55   | 0.035       |
| IL8    | −0.51  | 0.051       |
| AMS score | | |
| TNFSE14 | −0.74  | 0.002       |
| FASLG  | −0.71  | 0.003       |
| IL18   | −0.62  | 0.013       |
| CD40LG | 0.61   | 0.015       |
| PTGER4 | −0.60  | 0.019       |
| MAPKAP2| −0.58  | 0.024       |
| HLA-DRB1| 0.57   | 0.027       |
| SMAD7  | −0.57  | 0.027       |
| AGER   | −0.55  | 0.035       |
| MAFK   | −0.54  | 0.037       |
| IRF5   | −0.54  | 0.037       |

The \(P\)-values for Pearson correlations are provided. Negative values of \(R\) indicate that increased expression levels at high altitude were associated with lower acute mountain sickness (AMS) scores or peripheral oxygen saturation (\(S_{\text{PO}_2}\)) levels.
after cell death or active secretion, extracellular HMGB1 can act as a pro-inflammatory mediator by binding to other pro-inflammatory molecules, such as LPS or interleukin-1β, to activate TLR4 receptors and initiate downstream inflammatory signalling (Yang et al., 2020). This mode of TLR4 activation is particularly important in immune cell types, such as monocytes, macrophages and neutrophils. In monocytes and macrophages, extracellular HMGB1 can bind to MD-2, which forces two TLR4 chains to form a complex and initiate downstream signalling and induces production of cytokines and chemokines, such as tumour necrosis factor (TNF; Yang et al., 2015, 2020). In neutrophils, HMGB1–TLR4 signalling increases production of reactive oxygen species through activated neutrophil NADPH oxidase activity (Billiar et al., 2021; Fan et al., 2010; Yang et al., 2020).

In addition to HMGB1, S100A8 was also significantly upregulated after 1 day of acute high-altitude exposure (Fig. 1). Like HMGB1, S100A8 is an endogenous DAMP that is actively secreted from phagocytes in response to stress (Ehrchen et al., 2009). S100A8, along with S100A9, are highly expressed in neutrophils and in phagocytes in inflammatory conditions, such as inflammatory bowel and lung diseases (Rugtveit et al., 1994; Zavadlo et al., 1988). The S100A8/S100A9 heterodimer has previously been found to be an endogenous activator of TLR4 (Ehrchen et al., 2009; Foell et al., 2007; Ma et al., 2017; Vogl et al., 2007). Furthermore, S100A8 has been found to interact directly with the TLR4–MD-2 complex to initiate downstream signalling (Vogl et al., 2007). Previous research has also found that S100A8/S100A9 significantly increases secretion of pro-inflammatory cytokines, such as TNF-α and interleukin-6, in cultured BV-2 microglial cells. When TLR4 was inhibited, the pro-inflammatory cytokine secretion was blunted after S100A8/S100A9 stimulation.
Acute mountain sickness commonly manifests in sojourners to high altitude (>2500 m; Gallagher & Hackett, 2004; Hackett, 2000; Julian et al., 2011; Luks et al., 2017). Most individuals affected by AMS develop headaches, nausea, insomnia and shortness of breath that resolve on their own within a few days. Current research has noted that AMS develops as a result of a complex network of physiological responses to hypoxia (i.e. inflammation, hypoxaemia, vasogenic oedema and acidosis) in addition to anatomical factors (i.e. insufficient cerebrospinal fluid production, varied cerebral venous blood flow; Hackett, 2000; Luks et al., 2017). Several studies support the role of inflammation in development of AMS. For example, multiple groups report that in individuals acutely exposed to high altitude select pro-inflammatory cytokine and inflammatory marker (most notably C-reactive protein, interleukin-1β and interleukin-6) concentrations are increased compared with their sea-level concentrations (Hartmann et al., 2000; Song et al., 2016). Some groups have also found associations between the incidence of AMS and expression of these candidate inflammatory markers (Boos et al., 2016; Kammerer et al., 2020; Klausen et al., 1997; Liu et al., 2017; Malacrida et al., 2019; Song et al., 2016). Accordingly, Dumont et al. (2000) showed a reduction in AMS incidence and severity with anti-inflammatory drug treatment. Both steroids and non-steroidal anti-inflammatory drugs reduced the incidence of AMS, despite their different mechanisms of action (Gertsch et al., 2010, 2012; Kanaan et al., 2017; Lipman et al., 2012; Nepal et al., 2020; Rock et al., 1989; Tang et al., 2014; Zheng et al., 2014). However, other studies demonstrated no significant association between pro-inflammatory cytokine concentration and AMS incidence (Lundeberg et al., 2018; Swenson et al., 1997). As a result, questions remain about how high-altitude-induced inflammation might contribute to AMS.
In our study, many typical inflammatory markers were not included in the top differentially expressed genes via RNA-seq or NanoString after 1 or 3 days at high altitude and were not associated with AMS severity. This is not unexpected, because many studies have shown that inflammatory markers, such as interleukin-6 and TNF-α, resolve rapidly after expression. Previous studies have also reported that although the protein expressions of interleukin-6 and TNF-α in plasma increased significantly after acute vigorous exercise, there was no change in mRNA expression in peripheral blood mononuclear cells (Bernecker et al., 2013; Ostrowski et al., 1998, 1999). Thus, although protein levels can remain elevated after 24 h of exposure, mRNA expression levels might have resolved by this time. Nonetheless, we identified other significant components of inflammatory signalling and immune system regulation that were activated at high altitude and significantly associated with AMS severity at these time points.

Of the genes most significantly associated with AMS scores, we found that FASLG is not only negatively associated with AMS severity ($R = -0.071; P < 0.01$), but also positively associated with $S_{pO_2}$ ($R = 0.68; P < 0.01; \text{Table 4}$). Although most of the participants in our study had mild AMS symptoms, this might indicate that FASLG and the FAS/FASLG pathway might play a role in modulating the physiological response to acute high-altitude hypoxia exposure. FASLG encodes the Fas ligand, and the FAS/FASLG pathway plays a crucial role in protection against autoimmunity. In addition to tightly regulating immune system activation by activation-induced cell death (Brunner et al., 2003; Griffith et al., 1995; Nagata & Golstein, 1995; Strasser et al., 2009), this process is crucial to dampen the immune response. Our findings show that participants with lower AMS scores had increased FASLG mRNA expression compared with their baseline SL expression. Furthermore, we also found that the FAS signalling pathway was enriched with significantly differentially expressed genes after the first day of high-altitude exposure (Table 3). This might have multiple implications. First, evidence suggests that endothelial cells produce soluble Fas ligand in hypoxia, which protects them from activation-induced cell death (Mogi et al., 2001). Second, pro-inflammatory cytokines (such as TNF-α) can induce expression of Fas ligand on tissue cells and, in turn, trigger apoptosis in activated T cells by binding to the FAS receptor on the T-cell surface (Brunner et al., 2003). Indeed, protein levels of pro-inflammatory cytokines have been found to be elevated in plasma upon hypoxic exposure (Boos et al., 2016; Julian et al., 2011; Song et al., 2016). Thus, this initial elevation in pro-inflammatory cytokine concentration upon high altitude exposure might induce Fas ligand expression on tissue cells and cause apoptosis in activated T cells to dampen the immune response. However, given that the majority of our RNA sample should be derived from peripheral blood mononuclear cells, this might not explain the increased FASLG expression we observed. A final interpretation is that elevated FASLG expression might be reflective of T cells inducing mutual activation-induced cell death amongst the T-cell population (Brunner et al., 2003).

Our data also show that FASLG mRNA expression is also positively correlated with $S_{pO_2}$. Mogi et al. (2001) showed that hypoxia stimulates the release of the soluble Fas ligand. In contrast to the membrane-bound Fas ligand, soluble Fas ligand would inhibit the apoptotic signal in FAS+ cells. Upon high-altitude exposure, Higher $S_{pO_2}$ and elevated FASLG expression could indicate that their physiological response to hypoxia quickly adapts and appropriately blunts the immune response. This would also protect tissue cells from apoptosis. However, there is conflicting research regarding the role of Fas ligand in hypoxia. Kosanovic et al. (2019) showed that there was a significant reduction of circulating Fas ligand in sojourners to high altitude and in native highlanders. It is important to note that circulating plasma protein can come from multiple sources, such as endothelial cells, and we were measuring gene expression in immune cells. Additionally, it was shown that highlanders with pulmonary hypertension had a significantly lower circulating levels of Fas ligand in plasma compared with lowlander control subjects (Kosanovic et al., 2019; Sydykov et al., 2021). Overall, these results indicate a potential role of FAS signalling in high-altitude acclimatization and adaptation.

The Journal of Physiology

Concurrent anti-inflammatory profile

Our data also demonstrate a concurrent upregulation of anti-inflammatory elements. One such element is NFE2L2, which encodes for nuclear factor erythroid 2-related factor 2 (NRF2), an important transcription factor involved in regulating and attenuating oxidative damage and toxic insults by regulating the expression of cytoprotective genes (Kobayashi et al., 2004; Fig. 5). Previous studies clearly illustrate effects of hypobaric hypoxia exposure on production of reactive oxygen species and biomarkers of oxidative damage (Chandel et al., 1998; Malacrida et al., 2019; Waypa & Schumacker, 2002). One downstream gene target of the NRF2 pathway is haem oxygenase-1 (HO-1), a cytoprotective rate-limiting enzyme that is crucial for degradation of haem into equimolar amounts of
Fe\(^{2+}\), biliverdin and CO, all of which play roles as antioxidants and regulators of inflammation, apoptosis and angiogenesis (Tift et al., 2020). Our study shows a non-significant trend for increased end-tidal CO after 1 day at high altitude (Table 1). Given that the only known source of endogenous CO is the haem oxygenase pathway, increased CO upon acute high-altitude exposure might indicate increased haem degradation. Furthermore, elevated CO levels might provide tissue-protective effects, such as those described in models of acute inflammation in ischaemia–reperfusion injury, vascular injury or disease, and sepsis (Knauert et al., 2013; Minamino et al., 2001; Ryter & Choi, 2016; Tift et al., 2020).

**Limitations**

One limitation of our study is our moderate sample size (n = 15), although the paired design allowed us to identify changes in numerous inflammatory pathway markers. Also, although our study group included both men and women, we did not examine sex-specific changes in inflammatory gene expression patterns owing to the low number of women in our sample (n = 5 women). Future work exploring potential differences in the impact of high altitude on immune function in women and men will be essential. Furthermore, only one participant developed severe AMS after acute high-altitude exposure. Therefore, although we were still able to identify significant associations between expression of select inflammation-related genes and AMS scores, a larger sample size with a wider range of AMS severity will provide stronger power to identify and validate these potential associations. Finally, the RNA samples used in our study were collected from peripheral whole blood; therefore, our data are representative only of changes occurring in peripheral blood cells. However, these findings are significant because they shed light on the impact of high altitude on immune cell function and inflammatory status.

**Conclusion**

In conclusion, we demonstrate that acute exposure to high-altitude hypoxia triggers significant changes in inflammation-related gene expression. Specifically, our analysis has led to the identification of several inflammatory-related genes that might be involved in immune system sensitization, such as components of the TLR4 signalling pathway. Clearly, hypoxaemia and high-altitude exposure have significant impacts on inflammatory signalling, but further studies are essential to elucidate the mechanism behind hypoxia-induced inflammation in vivo and how high-altitude exposure impacts immune cell function. Future research studies should investigate how concurrent hypoxic and inflammatory stimuli might exacerbate pro-inflammatory cytokine production in peripheral blood mononuclear cells in vitro. Additionally, investigating the potential role of LY96 and HMGB1 in immune system sensitization might expand our understanding of how hypoxia and inflammatory response pathways lead to an exacerbated response to subsequent inflammatory stimuli. This work will provide valuable insights into how hypoxaemia modulates inflammatory responses in critical and chronic illnesses, such as ARDS and coronavirus disease 2019.

**References**

Bandarra, D., & Rocha, S. (2013). A tale of two transcription factors: NF-κB and HIF crosstalk. OA Molecular and Cell Biology, 1(1), 6.

Basaran, K. E., Villongco, M., Ho, B., Ellis, E., Zarndt, R., Antonova, J., Hopkins, S. R., & Powell, F. L. (2016). Ibuprofen blunts ventilatory acclimatization to sustained hypoxia in humans. *Plos One*, 11(1), e0146087. https://doi.org/10.1371/journal.pone.0146087.

Beall, C. M. (2006). Andean, Tibetan, and Ethiopian patterns of adaptation to high-altitude hypoxia. *Integrative and Comparative Biology*, 46(1), 18–24.

BelAiba, R. S., Bonello, S., Zähringer, C., Schmidt, S., Hess, J., Kietzmann, T., & Görlach, A. (2007). Hypoxia up-regulates hypoxia-inducible factor-1α transcription by involving phosphatidylinositol 3-kinase and nuclear factor κB in pulmonary artery smooth muscle cells. *Molecular Biology of the Cell*, 18(12), 4691–4697.

Bernecker, C., Scherr, J., Schinner, S., Braun, S., Scherbaum, W. A., & Halle, M. (2013). Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scandinavian Journal of Medicine & Science in Sports*, 23(2), 207–214.

Billiar, T. R., Wilson Hackam, M. A., Vodovotz, Y., Yang, H., Tracey, K. J., Fan, J., Li, Y., Levy, R. M., & Fan, J. J. (2021). HMGB1-TLR4 signaling oxidase activation in neutrophils: Role of hemorrhagic shock induces NAD(P)H. *J Immunol References*, 178, 6573–6580.

Bone, R. C. (1992). Toward an epidemiology and natural history of SIRS (Systemic Inflammatory Response Syndrome). *Jama*, 268(24), 3452–3455.

Bonello, S., Zähringer, C., BelAiba, R. S., Djordjevic, T., Hess, J., Michiels, C., Kietzmann, T., & Görlach, A. (2007). Reactive oxygen species activate the HIF-1α promoter via a functional NFκB site. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(4), 755–761.

Boos, C. J., Woods, D. R., Várias, A., Biscocho, S., Heseltine, P., & Mellor, A. J. (2016). High altitude and acute mountain sickness and changes in circulating Endothelin-1, Interleukin-6, and Interleukin-17a. *High Altitude Medicine and Biology*, 17(1), 25–31.

Brunner, T., Wasem, C., Torgler, R., Cima, I., Jakob, S., & Corazza, N. (2003). Fas (CD95/Apo-1) ligand regulation in T cell homeostasis, cell-mediated cytotoxicity and immune pathology. *Seminars in Immunology*, 15(3), 167–176.
Chandel, N. S., Maltepe, E., Goldwasser, E., Mathieu, C. E., Simon, M. C., & Schumacker, P. T. (1998). Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proceedings of the National Academy of Sciences of the United States of America, 95*(20), 11715–11720.

Chakraborty, R. K., & Burns, B. (2022). 2021 Jul 28. Systemic inflammatory response syndrome. In: *StatPearls [Internet]. StatPearls Publishing; PMID: 31613449.*

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., Clark, N. R., & Ma’ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *Bmc Bioinformatics [Electronic Resource], 14*(S18), 1–14.

Corcoran, S. E., & O’Neill, L. A. J. (2016). HIF1α and metabolic reprogramming in inflammation. *Journal of Clinical Investigation, 126*(10), 3699–3707.

Cummins, E. P., Berra, E., Comerford, K. M., Ginouves, A., Fitzgerald, K. T., Seeballuck, F., Godson, C., Nielsen, J. E., Moynagh, P., Pouysségur, J., & Taylor, C. T. (2006). Prolyl hydroxylase-1 negatively regulates IκB kinase-β, giving insight into hypoxia-induced NFκB activity. *Proceedings of the National Academy of Sciences of the United States of America, 103*(48), 18154–18159.

Diamond, M. S. (2014). IFIT1: A dual sensor and effector molecule that detects non-2′-O methylated viral RNA and inhibits its translation. *Cytokine & Growth Factor Reviews, 25*(5), 543.

D’Ignazio, L., Bandarra, D., & Rocha, S. (2016). NF-κB and HIV crosstalk in immune responses. *FEBS Journal, 283*(3), 413–424.

Dinarello, C. A., Gelfand, J. A., & Wolff, S. M. (1993). Anti-cytokine strategies in the treatment of the systemic inflammatory response syndrome. *Jama, 269*(14), 1829–1835.

Dubois, C., Marcé, D., Faivre, V., Lukašzewicz, A. C., Junot, C., Fenaille, F., Simon, S., Becher, F., Morel, N., & Payen, D. (2019). High plasma level of S100A8/S100A9 and S100A12 at admission indicates a higher risk of death in septic shock patients. *Science Reports, 9*(1), 15660.

Dumont, L., Mardirosoff, C., & Tramèr, M. R. (2000). Efficacy and harm of pharmacological prevention of acute mountain sickness: Quantitative systematic review. *British Medical Journal, 321*(7256), 267–272.

Eden, E., Navon, R., Steinfeld, I., Lipson, D., & Yakhini, Z. (2009). GOrilla: A tool for discovery and visualization of enriched GO terms in ranked gene lists. *Bmc Bioinformatics [Electronic Resource], 10*, 1–7.

Ehren, J. M., Sundekötter, C., Foell, D., Vogl, T., & Roth, J. (2009). The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. *Journal of Leukocyte Biology, 86*(3), 557–566.

Elzschig, H. K., & Carmeliet, P. (2011). Hypoxia and inflammation holger. *New England Journal of Medicine, 364*(7), 656–665.

Fan, J., Xiang, M., & Fan, J. (2010). Association of toll-like receptor signaling and reactive oxygen species: A potential therapeutic target for posttrauma acute lung injury. *Mediators of Inflammation, 2010*, 1–8. https://doi.org/10.1155/2010/916425.

Faquin, W. C., Schneider, T. J., & Goldberg, M. A. (1992). Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. *Blood, 79*(8), 1987–1994.

Foell, D., Wittkowski, H., Vogl, T., & Roth, J. (2007). S100 proteins expressed in phagocytes: A novel group of damage-associated molecular pattern molecules. *Journal of Leukocyte Biology, 81*(1), 28–37.

Fredes, S., Stockmann, C., Freitag, P., & Fandrey, J. (2006). Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-κB. *Biochemical Journal, 396*(3), 517–527.

Gallaher, S. A., & Hackett, P. H. (2004). High-altitude illness. *Emergency Medicine Clinics of North America, 22*(2), 329–355.

Gallucci, S., & Matzinger, P. (2001). Danger signals: SOS to the immune system. *Current Opinion in Immunology, 13*(1), 114–119.

Gertsch, J. H., Corbett, B., Holck, P. S., Mulcahy, A., Watts, M., Stillwagon, N. T., Casto, A. M., Abramson, C. H., Vaughan, C. P. A., Macguire, C., Farzan, N. N., Vo, B. N., Norvelle, R. J., May, K., Holly, J. E., Irons, H., Stutz, A. M., Chapagain, P., Yadav, S., … Basnayat, B. (2012). Altitude sickness in climbers and efficacy of NSAIDs trial (ASCENT): Randomized, controlled trial of ibuprofen versus placebo for prevention of altitude illness. *Wilderness and Environmental Medicine, 23*(4), 307–315.

Gertsch, J. H., Lipman, G. S., Holck, P. S., Merritt, A., Mulcahy, A., Fisher, R. S., Basnayat, B., Allison, E., Hanzelka, K., Hazan, A., Meyers, Z., Odegaard, J., Pook, B., Thompson, M., Slomovic, B., Wahlberg, H., Wilshaw, V., Weiss, E. A., & Zafren, K. (2010). Prospective, double-blind, randomized, placebo-controlled comparison of acetazolamide versus ibuprofen for prophylaxis against high altitude headache: The headache evaluation at altitude trial (HEAT). *Wilderness and Environmental Medicine, 21*(3), 236–243.

Görlach, A., & Bonello, S. (2008). The cross-talk between NF-kappaB and HIF-1: Further evidence for a significant liaison. *The Biochemical journal, 412*(3), e17-e19.

Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R., & Ferguson, T. A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science, 270*(5239), 1189–1192.

Hackett, P. H. (2000). High altitude cerebral edema and acute mountain sickness: A pathophysiology update. *Advances in Experimental Medicine and Biology, 474*, 23–45.

Hartmann, G., Tschöp, M., Fischer, R., Bidlingmaier, C., Riepl, R., Tschöp, K., Hautmann, H., Endres, S., & Toepfer, M. (2000). High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine, 12*(3), 246–252.

Heinrich, E. C., Anza-Ramirez, C., Macarlpuno, J.-L., Corante, N., Vizzardo-Galindo, G., Villafuerte, F. C., Powell, F. L., & Simonson, T. S. (2018). Increased levels of interleukin-6 (IL-6) in andean males with chronic mountain sickness and sea-level participants after one day at high altitude may reflect differences in IL-6 regulation. *The FASEB Journal, 32*(S1), lb479–lb479.
Mogi, M., Fukuo, K., Yang, J., Suhara, T., & Ogihara, T. (2001). Hypoxia stimulates release of the soluble form of fas ligand that inhibits endothelial cell apoptosis. *Laboratory Investigation, 81*(2), 177–184.

Moore, L. G. (2017). Human genetic adaptation to high altitudes: Current status and future prospects. *Quaternary International, 461*, 4–13.

Nagata, S., & Golstein, P. (1995). The fas death factor. *Science, 267*(5203), 1449–1456.

Nepal, G., Yadav, J. K., Rehrig, J. H., Bhandari, N., Baniya, S., Ghimire, R., & Mahotra, N. (2020). Efficacy and safety of inhaled budesonide on prevention of acute mountain sickness during emergent ascent: A meta-analysis of randomized controlled trials. *BMC Emergency Medicine, 20*(1), 38.

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., & Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of Physiology, 515*(1), 287–291.

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., & Pedersen, B. K. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *The Journal of Physiology, 508*(3), 949–953.

Park, B. S., & Lee, J.-O. (2013). Recognition of lipopolysaccharide pattern by TLR4 complexes. *Experimental & Molecular Medicine, 466*, 45.

Park, B. S., Song, D. H., Kim, H. M., Choi, B. S., Lee, H., & Lee, J. O. (2009). The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature, 458*(7242), 1191–1195.

Pham, K., Parikh, K., & Heinrich, E. C. (2021). Hypoxia and inflammation: Insights from high-altitude physiology. *Frontiers in Physiology, 12*, 712.

Pugin, J. (2007). Dear SIRS, the concept of “alarmins” makes a lot of sense! *Intensive Care Medicine, 342*(34), 218–221.

Pugin, J. (2012). How tissue injury alarms the immune system and causes a systemic inflammatory response syndrome. *Ann Intensive Care, 2*(1), 1–6.

Roach, R. C., Hackett, P. H., Oelz, O., Bärtsch, P., Luks, A. M., MacInnis, M. J., Baillie, J. K., Achatz, E., Albert, E., Andrews, J. S., Anholm, J. D., Ashraf, M. Z., Auerbach, P., Basnyat, B., Beidleman, B. A., Berendes, R. R., Berger, M. M., Bloch, K. E., … Brugger, H. (2018). The 2018 lake louise acute mountain sickness score. *High Altitude Medicine and Biology, 19*(1), 4–6.

Rock, P. B., Johnson, T. S., Larsen, R. F., Fulco, C. S., Trad, L. A., & Cymerman, A. (1989). Dexamethasone as prophylaxis for acute mountain sickness: Effect of dose level. *Chest, 95*(3), 568–573.

Rugtveit, J., Brandtzæg, P., Halstensen, T. S., Fausa, O., & Scott, H. (1994). Increased macrophage subset in inflammatory bowel disease: Apparent recruitment from peripheral blood monocytes. *Gut, 33*(5), 669.

Ryter, S. W., & Choi, A. M. K. (2016). Targeting heme oxygenase-1 and carbon monoxide for therapeutic modulation of inflammation. *Translational Research, 167*(1), 7–34.

Schaefer, L. (2014). Complexity of danger: The diverse nature of damage-associated molecular patterns. *The Journal of Biological Chemistry, 289*(51), 35237.

Scheinfeldt, L. B., Soi, S., Thompson, S., Ranciaro, A., Wolfemeskel, D., Beggs, W., Lambert, C., Jarvis, J. P., Abate, D., Belay, G., & Tishkoff, S. A. (2012). Genetic adaptation to high altitude in the Ethiopian highlands. *Genome Biology, 13*(1), R1.

Scholz, C. C., & Taylor, C. T. (2013). Targeting the HIF pathway in inflammation and immunity. *Current Opinion in Pharmacology, 13*(4), 646–653.

Semenza, G. L. (2009). Regulation of oxygen homeostasis by hypoxia-Inducible factor 1. *Physiology, 24*(2), 97–106.

da Silva Correia, J., Soldau, K., Christen, U., Tobias, P. S., & Ulevitch, R. J. (2001). Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex: TRANSFER FROM CD14 TO TLR4 AND MD-2. *Journal of Biological Chemistry, 276*(24), 21129–21135.

Simonson, T. S. (2015). Altitude adaptation: A glimpse through various lenses. *High Altitude Medicine & Biology, 16*, 125–137.

Song, T. T., Bi, Y. H., Gao, Y. Q., Huang, R., Hao, K., Xu, G., Tang, J. W., Ma, Z. Q., Kong, F. P., Coote, J. H., Chen, X. Q., & Du, J. Z. (2016). Systemic pro-inflammatory response facilitates the development of cerebral edema during short hypoxia. *Journal of Neuroinflammation, 13*(1), 63.

Strasser, A., Jost, P. J., & Nagata, S. (2009). The many roles of FAS receptor signaling in the immune system. *Immunity, 30*(2), 180.

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *Plos One, 6*(7), e21800.

Swenson, E. R., MacDonald, A., Vatheuer, M., Maks, C., Treadwell, A., Allen, R., & Schoene, R. B. (1997). Acute mountain sickness is not altered by a high carbohydrate diet nor associated with elevated circulating cytokines. *Aviation, Space, and Environmental Medicine, 68*, 499–503.

Sydykov, A., Mamazhakovypov, A., Maripov, A., Kosanovic, D., Weissmann, N., Ghofrani, H. A., Sarybaev, A. S., & Schermuly, R. T. (2021). Pulmonary hypertension in acute and chronic high altitude maladaptation disorders. *International Journal of Environmental Research and Public Health, 18*(4), 1–33.

Tang, D., Kang, R., Livesey, K. M., Zeh, H. J., & Lotze, M. T. (2011). High mobility group box 1 (HMGB1) activates an autophagic response to oxidative stress. *Antioxidants and Redox Signaling, 15*(8), 2185–2195.

Tang, E., Chen, Y., & Luo, Y. (2014). Dexamethasone for the prevention of acute mountain sickness: Systematic review and meta-analysis. *International Journal of Cardiology, 173*(2), 133–138.

Taylor, C. T. (2008). Interdependent roles for hypoxia inducible factor and nuclear factor-κB in hypoxic inflammation. *Journal of Physiology, 586*(17), 4055–4059.

Tift, M. S., Alves de Souza, R. W., Weber, J., Heinrich, E. C., Vilhuergue, F. C., Mallotra, A., Otterbein, L. E., & Simonson, T. S. (2020). Adaptive potential of the heme oxygenase/carbon monoxide pathway during hypoxia. *Frontiers in Physiology, 11*, 886.

Van Uden, P., Kenneth, N. S., & Rocha, S. (2008). Regulation of hypoxia-inducible factor-1α by NF-κB. *Biochemical Journal, 412*(3), 477–484.
Valenty, L. M., Longo, C. M., Horzempa, C., Ambesi, A., & Mckeown-Longo, P. J. (2017). TLR4 ligands selectively synergize to induce expression of IL-8. *Advances in Wound Care, 6*(10), 309.

Vogl, T., Tenbrock, K., Ludwig, S., Leukert, N., Ehrhardt, C., Van Zoenen, M. A. D., Nacken, W., Foell, D., Van Der Poll, T., Sorg, C., & Roth, J. (2007). Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nature Medicine, 13*(9), 1042–1049.

Walmansley, S., Harris, A., Thompson, A. A. R. & , & MoiraKB W. (2014). HIF-mediated innate immune responses: Cell signaling and therapeutic implications. *Hypoxia, 2*, 47–58.

Waypa, G. B., & Schumacker, P. T. (2002). O2 sensing in hypoxic pulmonary vasoconstriction: The mitochondrial door re-opens. *Respiratory Physiology and Neurobiology, 132*(1), 81–91.

Xie, Z., Bailey, A., Kuleshov, M. V., Clarke, D. J. B., Evangelista, J. E., Jenkins, S. L., Lachmann, A., Wojciechowicz, M. L., Kropiwnicki, E., Jagodnik, K. M., Jeon, M., & Ma‘ayan, A. (2021). Gene set knowledge discovery with enrichr. *Current Protocols, 1*(3), e90.

Yang, H., Wang, H., Ju, Z., Ragab, A. A., Lundbäck, P., Long, W., Valdes-Ferrer, S. I., He, M., Pribis, J. P., Li, J., Lu, B., Gero, D., Szabo, C., Antoine, D. J., Harris, H. E., Golenbock, D. T., Meng, J., Roth, J., Chavan, S. S., … Al-Abed, Y. (2015). MD-2 is required for disulfide HMGB1-dependent TLR4 signaling. *Journal of Experimental Medicine, 212*(1), 5–14.

Yang, H., Wang, H., & Andersson, U. (2020). Targeting inflammation driven by HMGB1. *Frontiers in Immunology, 11*, 484.

Yang, Y., Liu, L., Naik, I., Braunstein, Z., Zhong, J., & Ren, B. (2017). Transcription factor C/EBP homologous protein in health and diseases. *Frontiers in Immunology, 8*, 1612.

Zheng, C. R., Chen, G. Z., Yu, J., Qin, J., Song, P., Bian, S. Z., da Xu, B., Tang, X. G., Huang, Y. T., Liang, X., Yang, J., & Huang, L. (2014). Inhaled budesonide and oral dexamethasone prevent acute mountain sickness. *American Journal of Medicine, 127*(10), 1001–1009.e2.

Zhou, J., Schmid, T., & Brüne, B. (2003). Tumor necrosis factor-α causes accumulation of a ubiquitinated form of hypoxia inducible factor-1α through a nuclear factor-κB-dependent pathway. *Molecular Biology of the Cell, 14*(6), 2216–2225.

Zhu, H., Guo, Y., Yang, J., Yexie, Z., Bai, Y., & Cao, T. (2020). Association of high altitude polycythemia with an increased risk of systemic inflammatory response syndrome in acute pancreatitis. *Japanese J Gastroenterol Hepatol, 3*, 1–6.

Zhu, X., Messer, J. S., Wang, Y., Lin, F., Cham, C. M., Chang, J., Billiar, T. R., Lotze, M. T., Boone, D. L., & Chang, E. B. (2015). Cytosolic HMGB1 controls the cellular autophagy/apoptosis checkpoint during inflammation. *Journal of Clinical Investigation, 125*(3), 1009–1110.

Zwadlo, G., Brüggen, J., Gerhards, G., Schlegel, R., & Sorg, C. (1988). Two calcium-binding proteins associated with specific stages of myeloid cell differentiation are expressed by subsets of macrophages in inflammatory tissues. *Clinical and Experimental Immunology, 72*, 510–515.

### Additional information

#### Data availability statement

The data supporting these findings is available in the Dryad data repository at: [https://datadryad.org/stash/dataset/doi:10.6086/D1XM45](https://datadryad.org/stash/dataset/doi:10.6086/D1XM45).

#### Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

#### Author contributions

K. Pham and E.C.H. conceived and designed the research. K. Pham, S.F., N.P., B.O. and E.C.H. assisted in sample collection. K. Pham, K. Parikh and E.C.H. analysed data, interpreted the results of experiments and prepared figures. K. Pham drafted the manuscript. K. Pham, K. Parikh and E.C.H. edited and revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

#### Funding

This work was supported by the White Mountain Research Center Mini-Grant 2020 and the Mildred E. Mathias Graduate Student Research Grant 2019 from the University of California Natural Reserve System (Barcroft Station), and by the University of California Riverside School of Medicine. E.C.H. is supported by a UCR Regents Faculty Fellowship. This publication includes data generated at the UC San Diego IGM Genomics Center using an Illumina NovaSeq 6000 that was purchased with funding from a National Institutes of Health SIG grant (#S10 OD026929).

#### Acknowledgements

We thank the University of California, Natural Reserve System, the UCLA White Mountain Research Center, the Barcroft Station staff and the participants for their support.

#### Keywords

acute mountain sickness, high altitude, hypoxia, inflammation

#### Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

- Statistical Summary Document
- Peer Review History