ORIGINAL RESEARCH

# Human physiological and metabolic responses to an attempted winter crossing of Antarctica: the effects of prolonged hypobaric hypoxia.

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## Abstract

An insufficient supply of oxygen to the tissues (hypoxia), as is experienced upon high-altitude exposure, elicits physiological acclimatization mechanisms alongside metabolic remodeling. Details of the integrative adaptive processes in response to chronic hypobaric hypoxic exposure remain to be sufficiently investigated. In this small applied field study, subjects ($n = 5$, male, age 28–54 years) undertook a 40 week Antarctica expedition in the winter months, which included 24 weeks residing above 2500 m. Measurements taken pre- and postexpedition revealed alterations to glucose and fatty acid resonances within the serum metabolic profile, a 7.8 ($\pm 3.6$)% increase in respiratory exchange ratio measured during incremental exercise (area under curve, $P > 0.01$, mean $\pm$ SD) and a 2.1($\pm 0.8$) % decrease in fat tissue ($P < 0.05$) postexpedition. This was accompanied by an 11.6 ($\pm 1.9$) % increase ($P > 0.001$) in $VO_2$ max corrected to % lean mass postexpedition. In addition, spine bone mineral density and lung function measures were identified as novel parameters of interest. This study provides, an in-depth characterization of the responses to chronic hypobaric hypoxic exposure in one of the most hostile environments on Earth.

## Introduction

At high altitudes, the inspired partial pressure of oxygen ($O_2$) is decreased as barometric pressure ($P_B$) falls, thus leading to hypobaric hypoxia. Survival in these conditions requires the combination of a robust physiological response alongside metabolic remodeling to ensure adenosine triphosphate (ATP) demand is met. Studies conducting in-depth investigation into physiological remodeling responses to chronic hypoxia are lacking. Indeed, the very definition of "chronic exposure" is unclear, with some defining it as ≥42 days (Horscroft and Murray 2014), some ≥3 months (Woolcott et al. 2015) and others as little as 2 weeks (Siebenmann et al. 2016).

Irrespective of the definition, evidence suggests metabolic responses to chronic hypoxia may differ from those apparent in acute exposure. For instance, an increase in blood lactate is observed upon acute hypoxic exposure both at rest (Siervo et al. 2014) and in response to submaximal exercise (Sutton et al. 1988), a response likely linked to hypoxic inducible factor-1α (HIF-1α)-dependent upregulation of glycolytic metabolism (Semenza et al. 1994; Behrooz and Ismail-Beigi 1999; Semenza 1999; López-Barneo et al. 2001). However, in altitude acclimatized individuals or high-land natives, decreasing lactate accumulation in response to exercise has been reported (Hochachka et al. 2002), suggesting metabolic shifts may occur with chronic compared to acute exposure (the lactate paradox). This is alongside alterations to the mitochondrial network, with exposure to ≥42 days being associated with decreasing mitochondrial density in skeletal muscle (Horscroft and Murray 2014). Further investigation into the effects of chronic hypobaric hypoxia is therefore warranted.

It is generally considered that the decreased $P_{I}O_2$ experienced at altitude is the main cause of the physiological acclimatization processes. However, high-altitude exposure usually coincides with cold ambient temperatures with the interaction between hypoxia and cold often being overlooked. This is especially pertinent when considering the metabolic effects of cold exposure.

Acclimatization to extreme cold undoubtedly relies heavily upon behavioral adaptations such as the wearing of warm clothing, but this can be accompanied by significant physiological changes that in turn influence substrate utilization, specifically increasing reliance upon carbohydrate and fat oxidation (Vallerand and Jacobs 1989).

The combination of cold exposure and hypoxia appears to modify responses to both conditions. While cold exposure is reported to increase the rate of $O_2$ consumption (Vallerand and Jacobs 1989), this is attenuated at altitude (3350 m and 4360 m), a response reported to be sustained over a 6 week period, only recovering upon descent (Blatteis and Lutherer 1976). Reliance upon on shivering thermogenesis for heat generation has also been reported to increase both in acute and chronic hypoxic exposure (Blatteis and Lutherer 1976; Robinson and Haymes 1990).

In this study, the focus is upon the effects of chronic hypobaric hypoxic exposure, with subjects being exposed to high altitudes (≤2500 m) for a 24 week period during a 33 week attempted winter crossing of Antarctica. Given that temperatures on the high plateau regularly fall below −60°C, exposure to extreme cold was an inevitable factor. However, as subjects had access to living cabins and extensive protective clothing for the duration of the expedition, cold exposure was intermittent and limited to peripheries. Assessment of anthropometric and functional physiological parameters were combined with serum metabolomics to provide a broad characterization of the responses to one of the world's most hostile environments.

## Methods

### Ethical approval

Prior to measurements being made, written informed consent was obtained from all subjects. All procedures were approved by the National Health Service Wandsworth Research Ethics Committee (reference number: 12/LO/0457) and conformed to the Declaration of Helsinki.

### Expedition overview and aims

This expedition was the first ever attempted winter crossing of Antarctica. The 5 male subjects (aged 28–54 years, BMI preexpedition 26.36 ± 3.87 kg/m$^2$) were assessed in the UK prior to and following their 40 week stay in Antarctica, with the attempted winter crossing lasting 33 weeks, including 24 weeks at or above 2500 m. Due to safety concerns, the crossing was halted on week 8, with subjects subsequently forced to set up winter camp, given difficulties in evacuation procedures in the winter months. Living cabins were taken along with the expedition on caterpillar trucks. Once winter camp was established, subjects spent the majority of their time residing within the cabins, only being exposed to the outside elements intermittently and whilst wearing insulating clothing.

Subjects recorded food diaries and body weight data throughout the expedition. An array of measurements were also made in the UK pre- and postexpedition including: assessment of metabolic profile, body composition, exercise, and lung function testing.

## Study design

A timeline of expedition and testing details are outlined in Figure 1A, with details on altitude exposure being outlined in Figure 1B. Pretesting was conducted at King's College London on 12th December 2012, after which subjects travelled to Antarctica arriving on January 22nd. The attempted winter crossing expedition began with departure from Crown Bay on 21st March and lasted 33 weeks. During this time, subjects spent weeks 5–29 above 2500 m and reached the highest altitude of 2824 m on week 8. The crossing was halted on the 9th May and winter camp established on 30th May (week 11) at 2752 m. Subjects remained here until week 28, after which they began descending. The final point near Belgian Princess Elizabeth Station (1367 m) was reached on week 32, being 1st November 2013. Subjects remained here until 23rd November, at which point they flew home. Posttesting was conducted at King's on 27th November, 2013.

## Measurements taken during the expedition

### Dietary intake and activity

Water (mL/day) and food intake were recorded during the expedition, the latter as total energy intake (kcal/day), broken down into contributions from: protein, fat and carbohydrate (g/day). These values were obtained from weighed food diaries in which the weight of all consumed food and water was input. Diaries were taken across a 7-day period at 17 points within the attempted crossing time (33 weeks). Body weights, following an overnight fast and voiding of urine, were also recorded at these time points using digital balance scales.

Outside activity data were obtained from diary records taken across a 7-day period at 8 time points within the attempted crossing time. A subjective scale was used to estimate duration of light and heavy work undertaken during these periods. Light work was roughly defined as walking or general camp maintenance and heavy work as, for instance, shoveling snow. Given the degree of subjectivity and lack of accuracy, this measure was merely used as a broad estimation and general overview of subject activity levels.

Both food and activity data are presented as an average over the 7-day periods to present intake or activity on a "typical" day.

### Peripheral capillary oxygen saturation (SpO$_2$)

SpO$_2$ was measured during a moderate exercise step test performed intermittently throughout the expedition. This 18 min test was performed using a 21.5 cm step with one step per beat of a metronome set to progressive beating rates, ending in 2 min at 160 beats per minute. SpO$_2$ and pulse were measured from the index finger at the end of this test.

## Measurements taken pre- and postexpedition

### Body composition

Subject height and mass were determined using a stadiometer and calibrated balance beam scales, respectively. Bone mineral density (BMD) of the whole body and lumbar spine was assessed by dual-energy X-ray absorptiometry (DXA) using a Hologic Discovery A scanner (Hologic, Bedford, MA). Fat mass and fat free mass were also determined from DXA scans.

### Resting cardiovascular function

Upon arrival at the laboratory resting cardiovascular function was assessed using a Finometer Pro (Finapres Medical Systems, Amsterdam, Netherlands) with a finger cuff attached to the middle finger of the right arm. Systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), and heart rate were recorded once every minute for 5 min at the end of a period of 15 min of quiet supine resting. Average values of each variable were calculated.

### Lung function

The greatest peak expiratory flow (PEF), forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV$_1$) and FEV$_1$/FVC% were recorded using an Oxycon Pro (CareFusion, Basingstoke, UK) calibrated prior to use. Subjects were instructed to inhale rapidly to total lung capacity and then without pausing exhale as forcefully as possible until no more air could be expired. Subjects were seated and required to maintain an upright posture during all maneuvers which were performed in accordance with the ATS/ERS Guidelines (Miller et al., 2005).

### Serum samples

Serum samples were taken from fasted subjects at rest (in the morning, prior to the exercise protocol) pre and postexpedition. They were obtained by venesection in serum separating tubes and immediately spun for 10 min at 520$g$ at 4$^{\circ}$C. The resulting supernatant was pipetted into 2 mL polypropylene cryotubes, and immediately frozen at $-80^{\circ}$C.
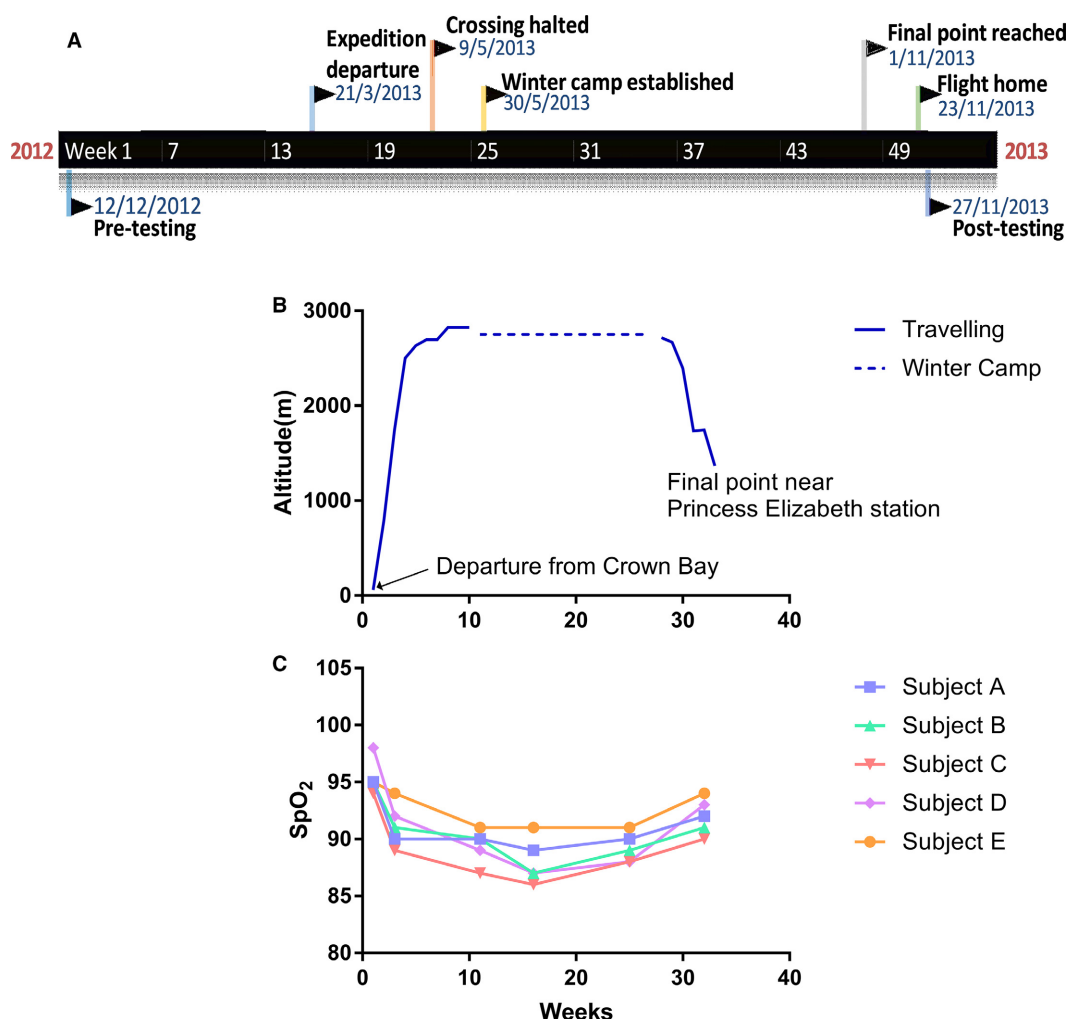
**Figure 1.** A timeline of testing and expedition details (A). A diagrammatic representation of altitude exposure during the attempted Antarctic winter crossing expedition (B) alongside corresponding SpO₂ recorded during a moderate intensity step test (C). The 33 week attempted crossing was measured from the point at which subjects departed from Crown Bay, Antarctica. SpO₂ was measured at intermittent points during the expedition following an 18 min moderate intensity exercise step test.

## Proton nuclear magnetic resonance spectroscopy (¹H-NMR) for serum metabolomics

For analysis, serum samples were defrosted at room temperature and centrifuged at 16,000$g$ for 10 min. A 300 $\mu$L aliquot of the resulting supernatant was then mixed with 300 $\mu$L of NMR buffer (250 $\mu$L DSS plus phosphate buffered saline (PBS) at a concentration of 5 mmol/L added to 50 $\mu$L 99.9% $D_2O$). The final solution contained 8 % $D_2O$ for the magnetic lock. The resulting mixture was transferred to 5 mm NMR tubes within a 96-tube rack ready for spectral acquisition, which was conducted using a Bruker Avance III 700 MHz spectrometer (Bruker Biospin, Karlsruhe, Germany) as described previously (Curtis et al. 2015).

## ¹H-NMR data processing and analysis

Data were processed using serial processing in Topspin (Bruker Topspin Software), applying an exponential window function with a line broadening of 0.3 Hz in the frequency domain prior to Fourier transformation. Each spectrum was subsequently phase corrected and aligned, with chemical shifts being manually referenced to DSS (at $\delta$ = 0 ppm), in ACD labs (ACD Labs Software.Ink), before being imported into Matlab (Mathworks, Natick, MA) at full resolution. Following this, spectra were normalized using probabilistic-quotient normalization (Dieterle et al. 2006) and binned using adaptive intelligent binning (De Meyer et al. 2008), both in Matlab. Principal component analysis (PCA) was

conducted and identification of the metabolites associated with the peaks undergoing significant changes was undertaken using Chenomx software (Chenomx NMR Suite 7.1).

## Cardio pulmonary exercise testing

Maximal oxygen uptake ($\dot{V}O_2$max) was determined using a continuous exercise test on a cycle ergometer (Lode Coriva, Lode, Groningen, Netherlands). Breath-by-breath measurements of $O_2$ and $CO_2$ concentrations as well as volume of expired air were recorded continually throughout the test (Oxycon Pro; CareFusion). Subjects wore a face mask to which the volume and gas sensors were attached. The $O_2$ and $CO_2$ analyzers were calibrated on each testing day with known gases in accordance with the manufacturers' guidelines. A 3 L syringe was used to calibrate the volume sensor before testing commenced.

Subjects initially cycled at a work rate of 50 W for 3 mins after which the power output continually increased until the subject could no longer continue despite strong verbal encouragement. The rate of increase in power output (1–2 W every 3–5 sec) was estimated for each subject such that maximal effort would be reached within 10–12 min. The subjects cycled at constant self-selected rate typically between 75 and 80 rpm. Heart rate was continually monitored throughout the test using a 12 lead ECG. $VO_2$max was determined as the greatest $O_2$ uptake recorded over a 20 sec period at the end of the test. To ensure a valid $\dot{V}O_2$max was attained subjects had to meet at least two of the following criteria: (1) achievement of maximum heart rate greater than age predicted maximum (220–Age), (2) a respiratory exchange ratio of >1.15 and (3) a plateau in $VO_2$ indicated by an increase in $VO_2$ of no more than 100 mL/min in the final two 20 sec periods of the test. Ventilatory threshold (VT) was determined using a combination of the v-slope method (the point where a clear steeper increase in $\dot{V}CO_2$ compared to $\dot{V}O_2$ occurs) and the ventilatory equivalent method (the point where $\dot{V}E/\dot{V}O_2$ rises without a concomitant rise in $\dot{V}E/\dot{V}CO_2$). VT was assessed by 2 independent investigators with any differences between the two resolved by a third investigator when necessary (Gaskill et al. 2001).

## Respiratory exchange ratio

Calculated from breath by breath measurements of $CO_2$ and $O_2$ concentrations taken during the $VO_2$max test. To capture changes in RER across each percentile of the $VO_2$max test, the area under the curve for was assessed for each subject for the full exercise duration.

## Maximal voluntary muscle strength

Maximal voluntary strength (MVC) was assessed in the knee extensors of the dominant limb. Subjects were seated upright with their arms folded in a custom-built dynamometer with their knee in 90° of flexion. Their lower leg (~3 cm proximal to the ankle) was strapped in a padded steel brace attached by a rigid bar to a strain gauge. Signals from the strain gauge were recorded on Spike 2 software via an analogue-to-digital converter (Cambridge Electronic Design (CED) 1401, UK) at a sampling rate of 2 kHz. Waist and shoulder straps were used to minimize any hip or upper body movement. The distance from the center of rotation of the knee joint to the steel brace was measured to allow torque to be calculated.

Subjects performed 3 MVCs during which maximal force as quickly as possible and hold the contraction for 3–4 sec. Strong verbal encouragement and visual feedback from a monitor placed in front of the subject were given. At least 1 min of rest was given between each contraction. The greatest MVC was used for analysis.

## Statistical analysis

The low subject numbers in this study mean that the emphasis of data analysis and interpretation is in the observed trends. However, statistical tests were still performed in order to gauge the degree of change from pre- to postexpedition. As this data set contains insufficient numbers for normality testing, a Gaussian distribution was assumed and differences in physiological variables pre- to postexpedition were identified using a paired Student T test. Statistical analysis and generation of graphs was conducted in Graphpad Prism.

## Results

### SpO$_2$, food intake, activity, and body weight data recorded during the expedition

SpO$_2$ measures reported in response to a moderate intensity step test revealed a progressive decrease with increasing altitude, from 95.4 ± 1.5% (±SD) at 58 m to 89.4 ± 1.5% at 2752 m (Fig. 1C). This decrease was sustained, remaining at 89.2 ± 1.6 following 14 weeks at 2752 m and increasing to 92 ± 1.6% upon descent to 1700 m.

The nutritional intake of the subjects, obtained from analysis of food diaries is detailed in Figure 2A. Total protein intake was 108.67 ± 18.17 g/day, carbohydrate intake 350 ± 101 g/day and fat intake 122 ± 25 g/day. The mean total energy intake was 2871.8 ± 543.1 kcal/
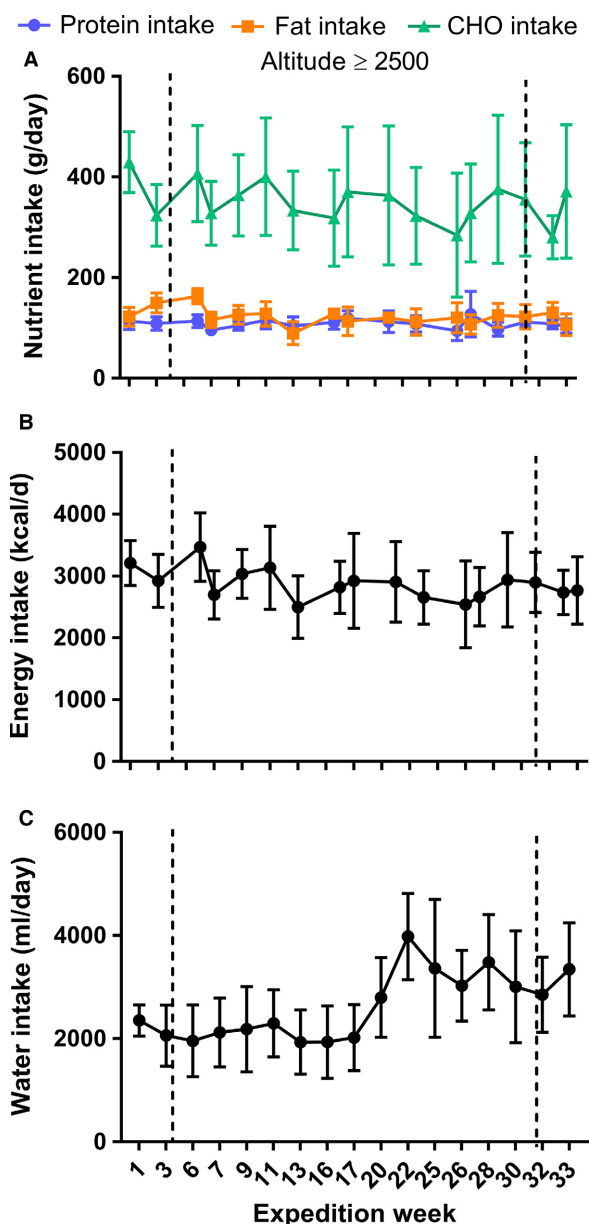
**Figure 2.** Nutrient (A), energy (B) and water (C) intake expressed as daily values throughout the winter expedition duration. Nutrient intake is broken down into protein, fat and carbohydrate (CHO) intake. Data were collected each day over the 7-day period on the expedition week specified and is expressed as average + SD, *n* = 5.

day (Fig. 2B). Water intake was relatively constant (2167.0 ± 649.6 mL/day) between weeks 1 and 20 (Fig. 2C), after which it increased as did the inter-individual differences, the latter reflected by the increasing SD (3294.2 ± 934.0 mL/day).

Outdoor exposure varied throughout the expedition and between subjects. Total time spent outside averaged

153 ± 94 mins/day (Fig. 3A) with light and heavy work (Fig. 3B) accounting for 128 ± 80 and 44 ± 48 min/day, respectively. A drop in average activity levels can be observed following week 11, coinciding with the point at which winter camp was established, before increasing once more upon descent from winter camp (week 28). Light work was performed indoors and remained constant at 60 min/day for each subject throughout the duration of the expedition.

Body weight data obtained during the expedition revealed an average decrease in 2.56 ± 2.15 kg from week 1 to week 32 (Fig. 3C). Statistically, this difference was close to significance (*P* = 0.063).

## Pre: postexpedition measurements

### Serum metabolic profile changes

Multivariate statistics revealed a reduction in principal component 2 (PC2) postexpedition, which captured ~30% of variance in the metabolic profiling data (Fig. S1A). The separation of variables by PC2 is demonstrated in (Fig. S1B). Within PC2, peaks corresponding to glucose and a fatty acid $CH_2$ resonance underwent the most prominent alterations, decreasing by 35.9 (±22.9)% and 39.6 (±20.6)% respectively (*P* < 0.05) (Fig. 4). Unfortunately, it was not possible to identify the subtype of fatty acid from which this resonance was derived.

### Anthropometric and functional physiological measurements

The anthropometric and physiological measurements taken pre- and postexpedition and are summarized in Table 1. No significant changes in body weight (Fig. 5A) or BMI (pre = 26.4 ± 3.9 vs. post = 26.0 ± 3.5 kg/m$^2$) were observed postexpedition. However, an increase in % lean and a decrease in % fat tissue, by 2.1 (±0.8) % (*P* < 0.05) were observed postexpedition (Fig. 5B and C). This change was also reflected through a 2.0 (±1.1 kg) decrease in body fat (*P* < 0.05). In addition, a 1.8 (±0.9) % decrease (*P* < 0.05) was observed in spine BMD (Fig. 5D).

Lung function measurements revealed no significant change in either $FEV_1$ (L) or FVC (L). However, FVC numerically increased in all subjects postexpedition, a change that was close to significant (*P* = 0.06) and that is reflected in the decrease in the $FEV_1$/FVC% by 5.8 (±3.3) % postexpedition (*P* < 0.05) (Fig 5E).

Regarding cardiovascular measures, no significant changes were observed in resting blood pressure (sBP, dBP or MAP) or heart rate.
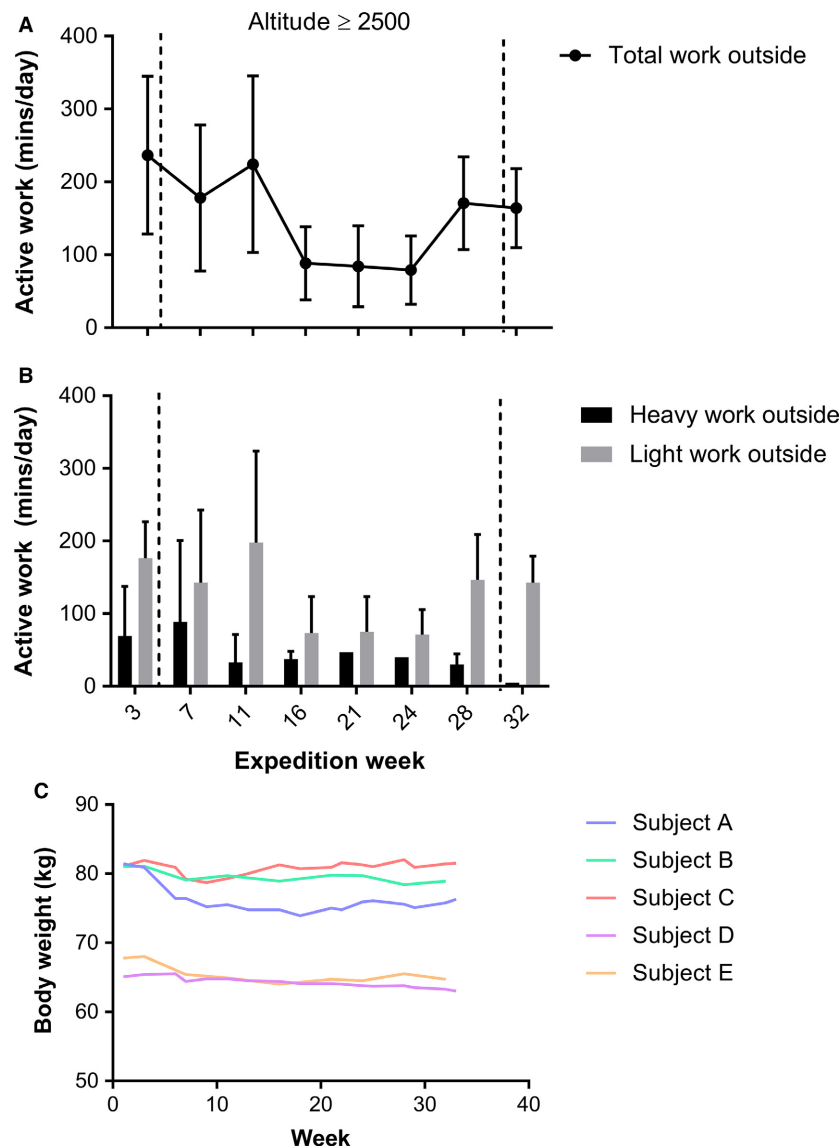
**Figure 3.** Work performed outside and body weight recorded during the 33 week attempted Antarctica winter crossing. Depicted as total work performed (A) and as an estimate of the division between heavy and light work (B) and body weight (C). Work data were collected each day over the 7-day period on the expedition week specified and is expressed as average of this +SD, *n* = 5.

### Exercise data

Physiological measures related to exercise are displayed in Table 2. An 11.6 ($\pm$1.9) % increase ($P > 0.001$) was observed in $VO_2$max corrected to lean mass postexpedition (Fig 6A). A 7.8 ($\pm$3.6) % increase ($P > 0.01$) in AUC for RER was observed postexpedition compared to pre ($P < 0.01$). The average (mean) RER of the group through each percentile of the $VO_2$max test is depicted in Fig 6C, with individual differences displayed in Fig 6D.

Examination of maximal muscle strength in the dominant leg revealed an increase in all subjects bar one postexpedition (Fig 6B). The trend towards an increase was reflected in the *P* value that was close to significance ($P = 0.06$).

## Discussion

The Antarctic winter is amongst the most extreme environments on earth and so presents significant physiological challenges. Up on the Antarctic plateau, high altitudes (and so hypobaric hypoxia) are combined with monthly mean temperatures falling below −60°C, strong magnetic fields, high UV radiation and wind velocities as well as
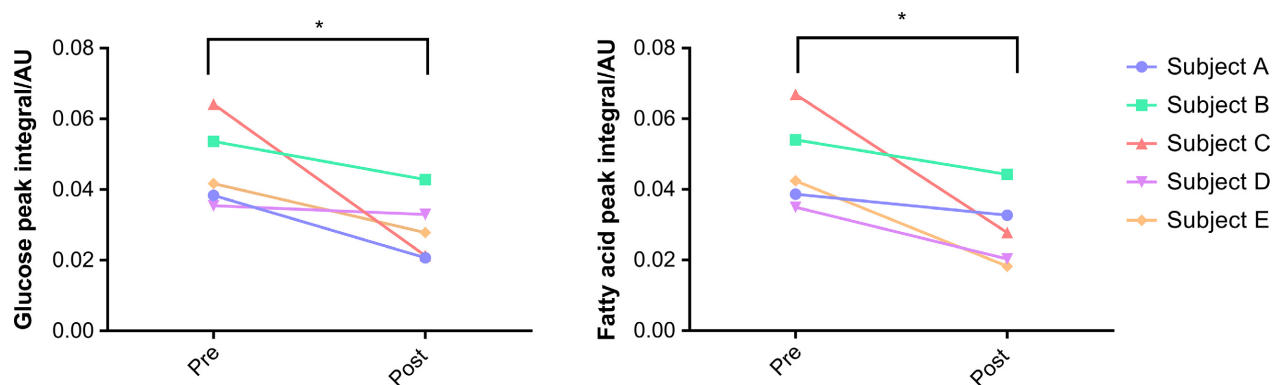
**Figure 4.** Serum metabolic profile analysis. Peak integrals undergoing significant decreases within principal component 2. This includes the peaks of glucose and the shoulder of the fatty acid CH2 resonance, displayed as arbitrary units (AU), * $P \leq 0.05$.

**Table 1.** Summary of changes in anthropometric variables, resting lung function and cardiovascular parameters pre- and postexpedition.

| Variable | Mean pre (±SD) | Mean post (±SD) | Difference | *P* value |
|---|---|---|---|---|
| % Lean tissue | 79.3 (3.7) | 81.4 (3.0) | 2.1 (0.8) | 0.015[1] |
| % Fat tissue | 20.7 (3.7) | 18.6 (3.0) | −2.1 (0.8) | 0.015[1] |
| Body fat (kg) | 16.0 (4.7) | 14.0 (3.7) | −2.0 (1.1) | 0.034[1] |
| Body weight (kg) | 77.9 (9.58) | 76.9 (9.3) | −1.02 (2.5) | 0.41 |
| Spine BMD g/cm$^2$ | 1.2 (0.05) | 1.13 (0.04) | −0.02 (0.01) | 0.035[1] |
| FVC (L) | 6.38 (1.29) | 7.11 (1.88) | 0.72 (0.62) | 0.060 |
| FEV1 | 4.29 (0.65) | 4.34 (0.71) | 0.05 (0.22) | 0.661 |
| FEV$_1$/ FVC% | 68.2 (9.7) | 62.4 (8.4) | −5.8 (3.3) | 0.016[1] |
| Heart rate (bpm) | 60 (6) | 63 (7) | 3 (7) | 0.39 |
| sBP | 123 (6) | 121 (5) | −2 (8) | 0.61 |
| dBP | 72 (8) | 75 (9) | 2 (6) | 0.37 |
| MAP | 93 (9) | 93 (7) | −0.04 (5.7) | 0.99 |

BMD, bone mineral density; FVC, forced vital capacity; FEV$_1$,forced expiratory volume in 1 sec, bpm, beats per minute, sBP , systolic blood pressure, dBP, diastolic blood pressure, MAP , mean arterial blood pressure.

*n* = 4–5, values presented as mean ± SD.

[1]Denotes significance, $P < 0.05$.

altered dark/light cycles and psychological stressors. In this small applied field study, the use of protective clothing and cabin shelter meant exposure to the majority of these environmental extremes beyond peripheries was kept to a minimum. In addition, nutritional intake remained within recommended daily levels (Trumbo et al. 2002) and a moderate level of physical activity was maintained. Changes observed in the array of physiological parameters and metabolic profile measures obtained pre- and postexpedition are therefore most likely a result of acclimatization to chronic hypobaric hypoxia as opposed to other perturbations. The stress of this moderate hypoxic exposure is apparent in the progressive decrease in SpO$_2$ reported with increasing altitude, an effect sustained throughout the expedition.

Amongst these physiological changes were a number of measures that were suggestive of metabolic remodeling.

This includes anthropometric measures revealing a loss of body fat and assessment of resting serum metabolic profiles, which highlighted changes in glucose and fatty acid resonances. In relation to physical exertion, these changes were accompanied by an increase in RER, thus indicating an increased reliance upon carbohydrate utilization during incremental exercise.

The exact causes or consequences of these shifts are unclear. Alterations in carbohydrate and fat metabolism have both been identified as having key roles in the response to short term hypobaric hypoxia in skeletal muscle (Horscroft and Murray 2014). It is therefore possible that changes in substrate utilization are occurring in response to chronic hypoxia at a whole-body level and that this is reflected both in anthropometric measures and substrate utilization during exercise. Indeed, the increase in RER is in line with previous reports of humans undergoing
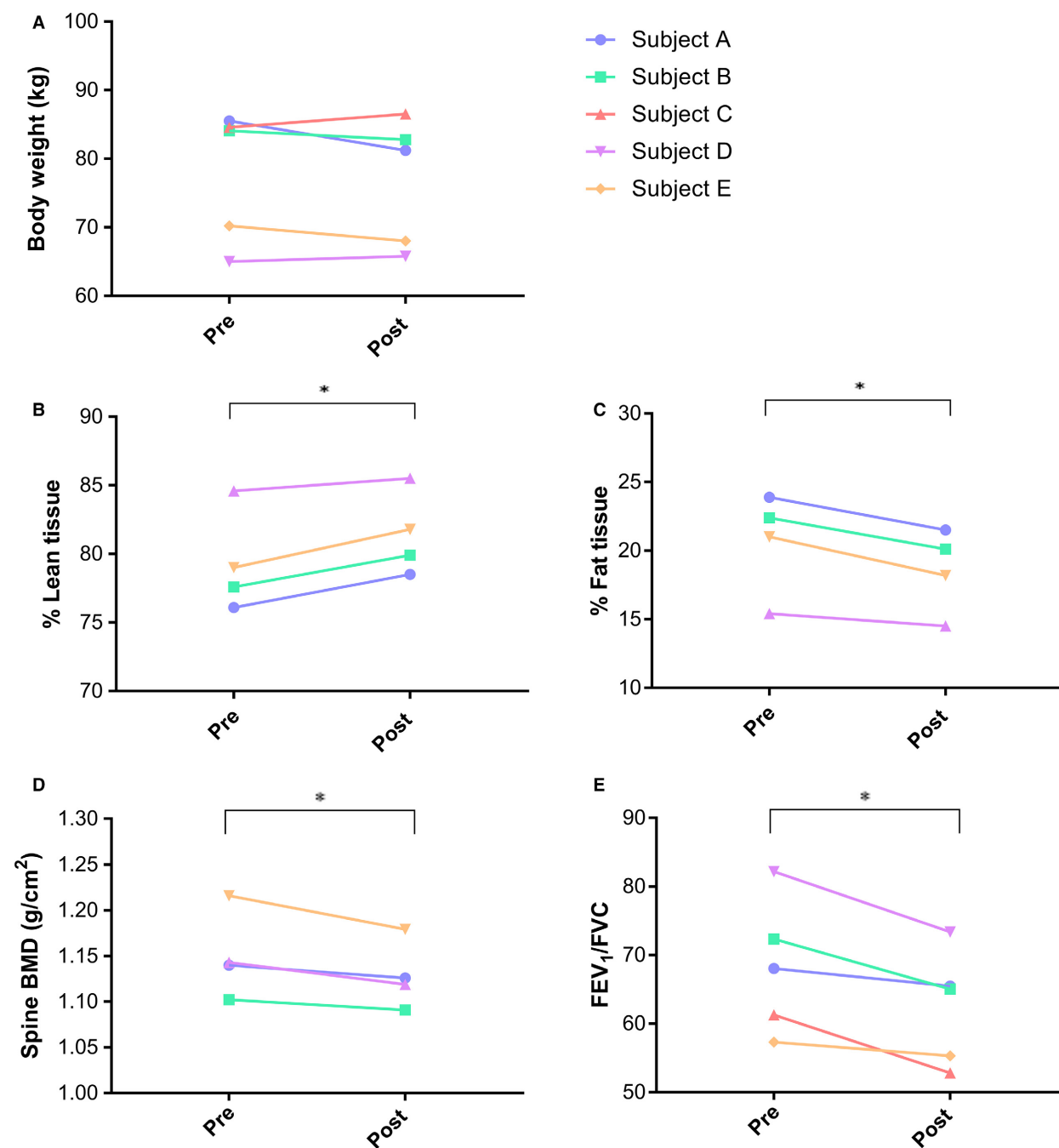
**Figure 5.** Anthropometric and physiological measurements taken pre- and postexpedition. Body weight (kg) (A), % lean tissue (B), % fat tissue (C), spine BMD (bone mineral density) (D), the ratio of forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) (E). Data presented as individual subject values, * $P \leq 0.05$, $n = 4$–5.

exercise in hypoxia following a prolonged (40 day) exposure (Sutton et al. 1988). However, comparison to the present study is limited given the differences in duration and severity of exposure and the fact that the measures of this study were obtained at sea level. The in-depth investigation into metabolic function that included use of metabolomics did not, therefore, highlight novel alterations. Instead, the changes identified do suggest that the effects of chronic hypobaric hypoxic exposure are in line with those reported in previous shorter term investigations.

**Table 2.** Summary of changes in exercise parameters pre- and postexpedition.

| Variable | Mean pre (±SD) | Mean post (±SD) | Difference (±SD) | *P* value |
|---|---|---|---|---|
| $VO_2$ max (L/min) | 3.2 (0.6) | 3.5 (0.4) | 0.2 (0.3) | 0.163 |
| $VO_2$ max (mL/kg/min) | 42.1 (4.9) | 45.5 (3.8) | 3.38 (5.3) | 0.187 |
| $VO_2$ max (mL/kg lean tissue) | 50.4 (5.1) | 56.1 (5.0) | 5.8 (0.6) | 0.0003[2] |
| Max heart rate (bpm) | 191.6 (5.6) | 189.6 (5.1) | −2.0 (6.8) | 0.546 |
| AUC RER | 8.85 (0.30) | 9.54 (0.26) | 0.68 (0.31) | 0.007[1] |
| MVC (Nm) | 204.5 (16.7) | 226.5 (33.9) | 22.0 (27.7) | 0.062 |

$VO_2$, pulmonary oxygen uptake; bpm-beats per minute; bpm-beats per minute RER, respiratory exchange ratio; AUC, area under the curve, MVC, maximal voluntary contraction; Nm, Newton meters. $n = 4$–5, values presented as mean ± SD.
[1]Denotes significance, $P < 0.01$,
[2]Denotes significance $P < 0.001$.
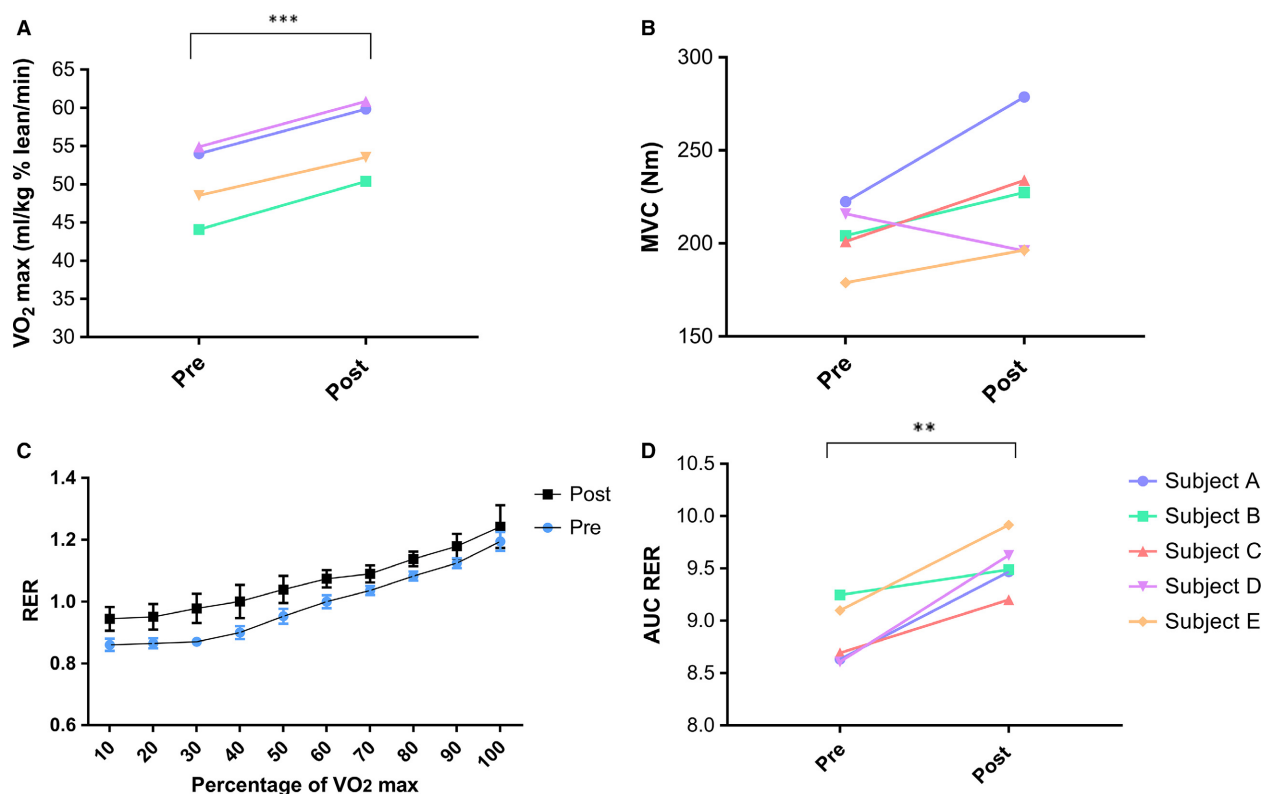


**Figure 6.** Exercise parameters measured pre- and postexpedition. $VO_2$ max corrected to % lean mass (A), Maximal voluntary contraction (MVC) (B), average RER (+SD) presented at each 10th percentile of a $VO_2$ max test (C), area under the curve (AUC) for RER (D). Data presented as individual subject values, **$P < 0.01$, ***$P < 0.001$, $n = 4$–5.

In relation to body composition changes, the proportionate increase in % lean mass and decrease in fat mass may be indicative of alterations to lipid storage capacity as hypoxic exposure has been associated with fat store mobilization (Young et al. 1989; Wood et al. 2011; Suzuki et al. 2014). This concept would align well with the shifts away from fat towards carbohydrate utilization, as indicated by the RER results of this study.

Previous high altitude studies reveal mixed results regarding body composition changes, with reports of an initial loss of fat mass followed by lean and vice versa (Boyer and Blume 1984; Guilland and Klepping 1985; Rose et al. 1988). Interestingly, these prior studies tend to observe that changes in body composition are accompanied by a loss of body weight, the degree of which increases with increasing altitude (Boyer and Blume 1984;

Guilland and Klepping 1985; Rose et al. 1988). For instance, Caucasian subjects exposed to 5400 m demonstrated a 1.5–1.9 kg loss in body weight, which increased to 3.4–4 kg at 7100 m (Boyer and Blume 1984; Guilland and Klepping 1985). However, weight loss at altitude is not reported across all studies, with subjects being exposed to 3,454 m for 28 days reporting no change in either body weight or fat mass (Jacobs et al. 2012). This suggests that maintaining body weight becomes an issue at more extreme altitudes (above ~3500 m) and may result from the commonly reported loss of appetite (Vats et al. 2004). The lack of body weight change in subjects of the present study, both during the expedition and measured pre and post, may therefore be due to the altitude exposure being at the lower end of the spectrum (~2500 m). The consistent nutrient intake suggests loss of appetite was not experienced. The lack of body weight change may also be reflective of the prolonged nature of the study, with body weight stabilizing over the duration of the expedition as subjects acclimatized.

At a functional level, the apparent shifts in metabolic signature and body composition were accompanied by significant improvements in $VO_2$max corrected to % lean tissue. Although not significant, trends were also observed for increasing MVC and FVC in most subjects postexpedition, the latter contributing to the significant increase in $FEV_1$/FVC. Together, these changes could be a reflection of a training effect. However, this is unlikely given that the majority of heavy physical exertion was undertaken during the first 11 weeks, after which levels declined. It is also not possible to draw such conclusions given that there is an absence of baseline activity levels nor accurate measures of exercise severity. In particular, the improvements in $VO_2$max could reflect potential benefits of hypoxic acclimatization upon $O_2$ delivery, for instance resulting from increasing erythropoiesis and angiogenesis (Shweiki et al. 1992; Rodriguez et al. 1999).

Given previous reports of both cold and high altitude exposure having detrimental effects upon lung function (Welsh et al. 1993; Cotes et al. 2006; Ziaee et al. 2008), it may perhaps appear surprising that FVC tended towards an increase. However, the translation of this prior work to this study is limited given differences in the degree and severity of altitude exposure and the fact that spirometry measures of this study were conducted at sea level, meaning altitude induced obstruction is not relevant.

A novel measure amongst high altitude studies is the decrease in spine BMD. Whilst slight, the decline reported here is in line with that associated with increasing fracture risk (Nguyen et al. 1993). Whilst the precise etiology of this is not clear, it may be related to the effect of hypoxia upon the regulation of bone density (Arnett et al. 2003;

Utting et al. 2006) or HIF-1α regulation on osteoclasts (Knowles 2015). Other factors known to impact BMD include decreases in activity levels (Mazzeo et al. 1998; Vuori 2001), although the maintenance of activity levels throughout and reported improvement in functional measures suggest this is unlikely to be the case. It could also relate to the altered day-night cycle experienced during the Antarctic winter in turn impacting altering vitamin D intake. Sunlight exposure is required to satisfy vitamin D requirements (Holick 2004), therefore, the prolonged lack of sunlight exposure resulted in subjects having insufficient vitamin D levels. Similarly dietary changes in $Ca^{2+}$ (Kelly et al. 1990), vitamin C (Aghajanian et al. 2015) and D (Bischoff-Ferrari et al. 2004) can also influence BMD. However, as details of $Ca^{2+}$, vitamin C and D intake were not recorded, it is not possible to draw any meaningful conclusion.

## Study limitations

This study presents a unique and novel exploration of the effects of an extreme environment upon human physiology. However, as the main aim of the expedition was to cross Antarctica as opposed to conducting a robust scientific experiment, there are a number of limitations in the study design and subsequent data interpretation.

Firstly, the small subject numbers severely limits statistical power and there was no control group. Secondly, for logistical reasons, the majority of physiological measures were taken 6 weeks prior to and 4 days postexpedition. This is particularly limiting for those measures prone to rapid fluxes, including metabolic profile and to a lesser extent body composition and lung function. This is less of a concern for measures of exercise performance given that previous studies have demonstrated maintenance of muscle energetics in response to altitude exposure 1 week postexposure (Edwards et al. 2010).

## Conclusion

We have presented the first in-depth examination of human physiology and metabolism in response to prolonged exposure to high altitude (~2500 m) in the Antarctic winter. It has highlighted key areas of interest for future investigations, particularly in the context of chronic hypoxic exposure and prolonged expeditions of a similar nature. This includes a change in metabolic signature, involving alterations to both glucose and fatty acid homeostasis with a shift towards increased reliance on carbohydrate metabolism during exercise and a reduction in body fat. This was accompanied by an improvement in $VO_2$max. In addition, spine BMD and lung function measures were identified as novel parameters of interest.

## Acknowledgments

## Conflict of Interests

None declared.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Principal component analysis of serum metabolic profile. Scree plot (A) demonstrating the variance explained by each principal component. Scores plot of principal components 1 and 2 where green corresponds to pre expedition and red post (B).