Female reproductive, adrenal and metabolic changes during an Antarctic traverse

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Abstract

PURPOSE: To explore the effects of the first all-female transantarctic expedition on hormonal axes pertinent to reproductive and metabolic function.

METHODS: Six females (aged 28-36, BMI 24.2 ± 0.97 kgm^{-2}) hauled 80kg sledges 1700km in 61 days. Estimated average energy intake was

20.8 ± 0.103 MJ/day (4970 ± 25 kcal/day). Whole body and regional body composition was measured by DXA one and two months before, and

15 days after, the expedition. Body fat was also estimated by skinfold and bioimpedance analysis immediately before and after the expedition.

Blood tests comprised basal metabolic and endocrine markers followed by evening ingestion of 0.25mg dexamethasone and 1-hour, 10 µg Gonadorelin and 1.0 µg ACTH-(1-24) tests the following morning, 39-38 pre- and 4-5 and 15-16 days post-expedition. Cortisol was assessed before and after the expedition in hair (monthly average concentrations) and saliva (5-point day curves and two-point diurnal sampling).

RESULTS: Average body mass loss was 9.37 ± 2.31 kg ($p<0.0001$), comprising loss of fat mass only; total lean mass was maintained. Basal sex steroids, corticosteroids and metabolic markers were largely unaffected by the expedition except leptin, which decreased during the expedition and recovered after 11 days, the change being proportionately greater than change in body fat. LH reactivity was suppressed prior to and during the expedition, but recovered after 11 days, while FSH did not change during or after the expedition. Cortisol reactivity did not change during or after the expedition. Basal (suppressed) cortisol was 73.25 ± 45.23 mmol/L before, 61.66 ± 33.11 mmol/L 5 days post- and

54.43 ± 28.60 mmol/L 16 days post-expedition ($p=0.67$). Monthly average cortisol was elevated during the expedition.

CONCLUSION: The maintenance of reproductive function and the HPA axis in women following an extreme physical endeavor, and despite a modest energy deficiency, suggests the female biological capacity for extreme endurance exercise is greater than anticipated.

Keywords

Arduous exercise, pituitary function, adrenal function, energy deficit, female athlete triad

Introduction

Women undertake increasingly physically demanding sports and employment but sex-related biological consequences of arduous exercise are poorly understood. Over the past 20 years, emphasis on energy availability (EA, defined as energy intake minus exercise energy expenditure) has established low EA as a putative cause of the 'female athlete triad': hypothalamic pituitary gonad (HPG) axis suppression in athletes, leading to functional hypothalamic amenorrhoea (FHA) and/ or impaired bone health (1). The term 'female athlete triad' has been questioned, since these phenomena can also affect men (2), however women may have greater sensitivity to the effects of low EA than men, and there is a higher prevalence of disordered eating among women than men (3).

In the setting of military employment, it has been suggested that women may be at higher risk of psychological problems than men, such as post-traumatic stress disorder (1, 4). There appears to be evidence suggesting a greater incidence of primary infertility in military women than age-matched civilians (5). While these observations remain unexplored in terms of etiology, we recently proposed FHA in military women might contribute to menstrual dysfunction, hypothesizing this could be mediated by a complex alteration in hormonal milieu, including reduced EA (1). Aspects of military training and employment other than exercise and reduced EA may also be likely to contribute to HPG axis suppression, for example, sleep deprivation and psychological stress (1, 6, 7).

Field studies of military training generally measure the effects of multiple concurrent stressors, making it difficult to delineate the effects of individual components like low EA, sleep deprivation or psychological stress (6). One highly researched model of the endocrine effects of a multi-stressor environment is US Army Ranger Training. Predominantly undertaken by men, Ranger training involves 61 days of strenuous exercise, sleep deprivation, total energy expenditure of around 4000-5000kCal/ day,

routine energy deficit and widespread metabolic and hormonal deficiencies, e.g. elevated fasting cortisol, reduced total testosterone and IGF-1 (7, 8). Such changes have been demonstrated to be reversible upon re-feeding, cessation of stress and sleep derestriction (8). However, extremes of arduous exertion lasting this duration have not been widely researched in women.

We undertook an exploratory, observational study of the concurrent acute response and short-term recovery of female HPG and HPA axes (using basal and dynamic testing) in women undertaking an unprecedented, extremely arduous expedition to cross the Antarctic continental landmass, of similar duration to US Army Ranger training. The purpose of the crossing was to attempt to become the first all-female team to complete an unassisted Antarctic traverse using muscle power alone, and was not competitive, primarily research-focused or done to achieve a political or military training objective. The *a priori* hypothesis was that this expedition would induce an energy deficit, despite a comprehensive programme of physical and nutritional preparation, with concurrent disturbances in HPG and HPA axes.

Methods

Participants

Six women participating in an unassisted Antarctic ski traverse expedition were invited to participate in the study three months beforehand. This was the first all-female team to attempt an unassisted Antarctic traverse. Individuals planned to haul sledges weighing 80kg for 1700 km, expecting the crossing to take around 75 days. Selection and training for the expedition lasted 2 years, the final team being selected from a pool of 250 women. While none of the participants had been to Antarctica before, all had partaken in three preparatory expeditions in Norway, which aimed to simulate the crossing's intensity and

conditions (details can be found at http://exicemaiden.com/). Participation in the study was voluntary and independent of the expedition. All six women volunteered and provided written informed consent. Ethical approval was received form the Ministry of Defence Research Ethics Committee (827MoDREC/17). The study was conducted in accordance with the Declaration of Helsinki.

Experimental design

The study design consisted of two pre-expedition measurement sessions, 64 and 39 days prior to the expedition (visit pre-1 and pre-2, respectively) (figure 1A). Additional body composition measurements were undertaken separately from formal study visits, 16 days before and
5 days after the expedition. Follow-up visits were conducted 4 days after the expedition (immediately after arrival in Punta Arenas, Chile from Antarctica), and 15 days after the expedition, 36 hours after return to the UK (visits post-1 and post-2 respectively). As part of a broader preparation schedule, participants were advised to gain 0.5 kg of body mass per week between visit pre-1 and the expedition (64 days or 9 weeks; 4.5kg). The expedition altitude profile and distance are indicated in figure 1B. The maximum elevation above sea level (ASL) was 2950 m.

Dietary provision

Dietary provision for the expedition was estimated from changes in body mass during three training expeditions. During the expedition participants were provided with a complete diet providing average 20.9 ± 0.1 MJ per day (4970 ± 25 kcal per day, or 70.8 ± 0.35 kcal/kg/day), comprising ~45 % carbohydrate (7.7 ± 0.32 g/kg/day), ~45 % fat (3.6 ± 0.07 g/kg/day) and ~10 % protein (1.7 ± 0.35 g/kg/day). It is estimated (verbal communication)

that participants consumed median 85 % (range 70 % - 99 %) of the diet provided over the course of the expedition and did not share rations.

Procedures

The schedule of measurements is illustrated in Figure 1. At visit pre-1, information including ethnicity, education, smoking habits, alcohol consumption, and a comprehensive medical reproductive and medication history taken including use and type of, and indication for, hormonal contraceptives was recorded. Reproductive and medication history and use of contraceptive questions were repeated after the expedition (visit post-1).

Psychological assessment

Questionnaires comprising six validated self-rating items on a web-based application (SmartSurvey, Tewkesbury, UK) were completed at visits pre-2 and post-1 (figure 1). The psychosocial stress questionnaire was completed in an identical manner to Rosengren *et al*, assessing the sixmonth period prior to visit pre-2, and the four-month period prior to visit post-1 (9). Participants were asked to complete the Impact of Events Scale – Revised (IES-R) with reference to any major life event(s) identified (10). The Patient Health Questionnaire 9 (PHQ-9) (11) was chosen as a robust measure of depressive symptoms in military and civilian populations (12). We analyzed results on a continuous scale, to identify subtle differences in a low number of participants. The Beck Anxiety Inventory (BAI) and Connor Davidson Resilience Scale 10 (CDRISC 10) demonstrate similar consistency measuring anxiety and resilience, respectively, and were analyzed in the same manner (13, 14). The BEDA-Q assesses risk of disordered eating concisely and consistently,(15) and was scored according to the methods of Peric *et al.* (16). Total scores from each questionnaire were used for further analysis.

Weekly intra-expedition assessments

During the expedition, a weekly questionnaire was completed in the same manner as previous studies of female transantarctic expedition (figure 1) (17, 18). This documented average perceived exertion, psychological stress, restfulness of sleep and confidence the team would complete the expedition (all on a Likert type-scale ranging from 1 [not at all] to 10 [the most possible]), and the average number of hours slept per night.

Body composition

Stature was measured at visit pre-1 (SECA Stadiometer 213, Birmingham, UK) and body mass was measured at every study visit (SECA
Scales 874). Whole body and regional lean mass, fat mass and bone mineral content were measured using dual energy x-ray absorptiometry (DXA) was measured with participants wearing shorts and t-shirts at visits pre-1, pre-2 and post-2 (GE Lunar iDXA, GE Healthcare, Chalfont St Giles, UK) (figure 1).

Sixteen days prior to the expedition (separately from main study visits), and at visit post-1, skinfolds were measured at four sites (bicep, triceps, sub-scapular, supraspinatus) to the nearest mm by the same examiner using Harpenden calipers (BodyCare, UK) according to the method of International Society for the Advancement of Kinanthropometry (19). The average of three measurements taken from each site was used to calculate percentage body fat (19).

Body fat was measured by four-point bioimpedance (Omron BF511, Milton Keynes, UK) upon waking in the morning, 1, 5, 10, 15 and 18-24 days after the expedition.

Basal blood samples

After an overnight fast, a venous blood sample was collected at visits pre-2, post-1 and post-2 for measurements of metabolic, nutritional, reproductive and adrenal function.

Dynamic reproductive and adrenal cortex function

Dynamic reproductive and adrenal cortex function was measured at visits pre-2, post-1 and post-2. Participants first ingested 0.25 mg dexamethasone at 2200h before a second overnight fast. This dose has been used to assess the sensitivity of the HPA axis to a nearphysiological level of central negative feedback and to attempt to reduce the baseline variation in morning fasting cortisol prior to the prestimulation test cortisol.(20, 21) At 0800 the following morning, a 21-gauge cannula was inserted into an antecubital or dorsal hand vein and a baseline blood sample was obtained before 10 µg Gonadorelin hydrochloride (Intrapharm, Maidenhead UK), followed by 1.0 µg ACTH-(1-24) (tetracosactrin acetate as Synacthen®, Mallinckrodt, Dublin, Ireland), were injected followed by a 10mL saline flush. ACTH-(1-24) was freshly diluted using 249ml 0.9% NaCl (Baxter, UK), to which Synacthen ® 250 µg in 1mL had been added, shaken thoroughly and 1 ml of this mixture was injected using a 5 ml syringe to minimise contact with plastic. Venous blood was sampled through the cannula in EDTA-containing tubes 20, 30, 40 and 60 min after drug administration. The doses of Gonadorelin, dexamethasone and ACTH-(1-24) were selected to mimic physiological levels of stimulation, as opposed to stimulation tests used clinically (and recommended in various clinical practice guidelines) which are intended to induce maximal axis stimulation and exclude endocrine insufficiency (e.g. 100 µg, 1 mg and 250 µg, respectively) (20).

Hair and saliva cortisol

A 0.5cm diameter hair sample was taken close to the scalp for measurement of cortisol at visit pre-2 (6 x 1cm segments) and visit post-1 (4 x

1cm segments). Hair grows at 1cm per month, thus 1cm represents 1 month of cortisol exposure (22).

Saliva was sampled by chewing on a synthetic swab for 1 minute, which was placed in a plastic collection tube (Salivette®; Sarstedt, Nümbrecht, Germany). A detailed saliva day curve was measured at visits pre-2 and post-2 as follows: participants were woken at 07:00 and saliva sampled at 07:10, 08:20, 09:00, 09:30, 12:15, 13:30, 17:20 and 21:50. Evening and morning saliva sampling (last thing at night before going to sleep and immediately after waking the following morning) were also measured 1, 5 and 10 days after the expedition.

Laboratory methods

Blood was collected in EDTA, serum-separating gel and fluoride oxalate tubes (Monovette®, Sarstedt, Nümbrecht, Germany) and centrifuged at 5,000 rpm for 5 minutes. Plasma and serum were stored at −80°C (after dry ice shipment to the UK of samples taken in Chile) until measurement.

Metabolic and nutritional markers

Thyroid stimulating hormone (TSH), unbound thyroxine (fT4) and total T3 (tT3) were measured from gel-separated serum using Abbott ®

Architect analyzer (Abbott, Maidenhead, UK) according to manufacturer's instructions. Insulin-like growth factor 1 (IGF-1), ferritin, insulin and Cpeptide were determined from gel-separated serum using Roche ® Cobas e411 analyzer (Roche Diagnostics, Welwyn

Garden City, UK) according to manufacturer's instructions. Creatinine, albumin, transferrin, calcium, zinc, iron and magnesium were determined from gelseparated serum and glucose and lactate from plasma containing fluoride oxalate using commercial kits (Alpha Laboratories, Eastleigh, UK) adapted for use on a Cobas Fara centrifugal analyzer (Roche, UK). Leptin was measured by ELISA (Quantikine, USA). Urea was determined from gel-separated serum using a commercial kit (Randox laboratories, UK) adapted for use on a Cobas Fara centrifugal analyzer. Plasma pH was detected in using a blood gas analyzer (Siemens RapidLab 348EX, Camberley, UK). Homeostatic modelling assessment (HOMA) for beta cell function (HOMA-B) insulin sensitivity (HOMA-S) and insulin resistance (HOMA-IR) were calculated according to the methods of Levy *et al*.
(23).

Additional data including resting energy expenditure and substrate utilization from direct calorimetry pre- and post-expedition are being published elsewhere.

Reproductive markers

Luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone and estradiol were determined from plasma containing EDTA using

Abbot Architect ® analyzer according to the manufacturer's instructions. Inhibin B was measured by ELISA (Beckman Coulter, High Wycombe,

UK). Sex hormone binding globulin (SHBG) and anti-müllerian hormone (AMH) were determined from gel-separated serum using Roche ®

Cobas e411 analyzer according to manufacturer's instructions. The rationale for these methods are summarized in supplementary box 1.

Adrenal markers

Cortisol, 17OH progesterone, testosterone, dihydroepiandrostenedione (DHEA) and androstenedione were measured using liquid chromatography mass spectrometry (LC/ MS), by modifying internal standards from a protocol described previously (24). Hair was divided into 1cm segments and powdered prior to cortisol extraction in each segment, representing 1 month averages, for a total of 10 months. Extraction and analysis by LC/ MS was completed as described by Kirschbaum *et al*. (25). Saliva was stored at -80 C within 7 days of collection and was extracted and analyzed by LC/ MS as described by Miller *et al. (26).*

Inter-assay %CV was <4% for Architect ®, e411, Fara assays and blood gas analyzer, and intra-assay %CV <10% for all ELISAs.

Statistical Analysis

Data are presented as individual data, or mean ± SD or median (IQR) for group comparison. Normality was assessed using Shapiro-Wilk test and non-normally distributed data were log transformed prior to statistical analysis. Due to the small sample size, variables are presented as mean (95% confidence interval [CI]). Repeated measures ANOVA was used to compare change in variables over time and pairwise comparisons were used where appropriate for statistically significant results. Paired t tests were used to compare the two pre-expedition DXA scans, and single post-expedition variables with baseline. Pre- and post-expedition dichotomous questionnaire data were compared using Chi squared test. One individual was excluded from analyses of basal reproductive hormones as she had commenced a combined contraceptive pill immediately prior to the expedition. Serum LH and FSH concentrations following injection of GnRH and ACTH were described as absolute values, and as percentage change, by dividing concentrations

after injection by the baseline concentration. This was done to allow comparison of within-subject change, since hormone-containing contraceptive use influenced baseline values. Area under the curve (AUC) was calculated using the trapezoidal rule. Within-subject changes in peak and AUC of cortisol and fold-rise in LH and FSH from baseline were compared from before to after the expedition.

Statistical analysis was performed using SPSS version 23.0 for Mac (IBM, USA). Significance was set at $p<0.05$. For multiple variables assessed in the same domain, Bonferroni adjustment was made as follows: body composition, $p<0.01$; basal reproductive markers, $p<0.005$, adrenal markers $p<0.05$, metabolic markers, $p<0.002$.

Results

Description of participants

Baseline characteristics of the cohort are shown in table 1. The median (range) age was 32.8 (28.6 to 36.1) years. Baseline questionnaires demonstrated high resilience, low depression and anxiety scores and normal patterns of eating behaviour. Fasting TSH, free T4, total T3, prolactin, LH:FSH ratio, androstenedione, total testosterone, DHEA, 17-OH progesterone, urea, sodium, potassium, chloride and creatinine were within normal limits prior to the expedition (table 2).

All participants used hormonal contraceptives during the expedition, intending to induce amenorrhoea. One individual commenced levonogestrel 150 mcg/ ethinylestradiol 30 mcg immediately prior to the expedition. One individual used Nexplanon ® contraceptive implant while all others used a Mirena ® intrauterine device. Five participants were amenorrhoeic during the expedition and one menstruated twice, stating this was less frequent than normal, within 4-10 days of due date.

Intra-expedition rating scales

Average scores for physical exertion scale were 5.5 ± 2.3 /10 and stress level 3.7 ± 1.94 /10, and level of confidence the team would complete the expedition 6.73 ± 1.81)/10; (6.35 ± 1.93 in weeks 1-3 and 7.11 ± 1.32 in weeks 5-8, p=0.09). Average duration of sleep was 6.73 ± 1.75 hours and rating of restfulness of sleep was 5.53 ± 2.05 /10. Questionnaires following the expedition suggested moderately lower levels of psychosocial stress and financial stress, and fewer significant adverse events than prior to the expedition (p=0.079, supplementary table 2).

Body composition, metabolic and nutritional changes

Physical changes during the study are presented in figure 2 and supplementary tables 1 and 2. All participants gained body mass during the two months prior to the expedition, (average increase 2.56 ± 0.79 kg, or 3.69 ± 1.12 % of body weight, p=0.006), consisting of body fat

(average increase 4.05 ± 0.96 %, p<0.0001), and lost body mass during the expedition (average loss 9.37 ± 2.31 kg, or 12.9 ± 3.17 % of body weight, p<0.0001). Body composition measured by DXA demonstrated a significant increase in total fat mass before (13.2 ± 2.11 vs 17.5 ± 2.52 kg, p<0.001) and loss during the expedition (fat mass at visit post-2 was 12.1 ± 1.37 kg, p<0.001), with these changes reflected in most regions (supplementary table 2). However, there was no difference in total lean mass or bone mineral content between visit pre-2 and visit post-2 (52.3 ± 2.10 vs 51.5 ± 3.04, p=0.27), despite a 6.10% loss in lean mass from the legs. In the 15 days between the expedition and the follow-up DXA scan, fat mass estimated by bioimpedance tended to increase (supplementary table 1). Regional DXA analysis showed statistically significant but modest decreases in android (area between the ribs and pelvis), gynoid (pelvis and upper thighs) and leg lean mass between visits pre-1

and pre-2, and loss of leg lean mass during the expedition (average 6.05 ± 1.11 % decrease), but these did not impact the change in total lean mass (supplementary table 2). There was a small but statistically significant increase in total bone mineral content prior to the expedition (2.75 ± 0.13 kg vs 2.80 ± 0.13) kg, p=0.005, but no change between visits pre- 2 and post- 2 (2.77 ± 0.12, p=0.19 (supplementary table 2).

Leptin decreased significantly following the expedition, thereafter increasing two-fold from visits post-1 to post-2 (table 2). Post-hoc tests showed the change between visit pre-2 and post-1 was significant (p=0.005), while there was no difference between pre-2 and post-2 (p=0.39). Thyroid stimulating hormone, free T4 and total T3 were normal pre-expedition and remained unchanged after the expedition (table 2). Fasted glucose, HOMA-B, HOMA-S and HOMA-IR, adjusted calcium, magnesium and phosphate did not change during or after the expedition (table
2).

Questionnaire data demonstrated a marginal increase in BEDA-Q scores after the expedition, consistent with higher markers of disordered eating risk (supplementary table 3). Markers of nutritional status (albumin, magnesium, phosphate, iron, zinc), urea (Ln transformed) and electrolytes did not change during or after the expedition (table 2).

Reproductive function

Basal markers of reproductive function are displayed in table 2. Estradiol tended to be lower at visit post-1, with a recovery noted by visit post2. No differences between other sex steroids, LH or FSH were shown. Inhibin B and AMH did not differ between baseline and immediately after the expedition (p=0.71 and p=0.15, respectively, table 2).

Dynamic LH and FSH responses before and after the expedition are shown in figure 3. Fold rise in FSH and FSH AUC were log transformed prior to statistical analysis. LH and FSH fold rise and AUC during the test did not differ between visit pre-2 and visit post-1. At visit post-2, FSH had not changed from visit pre-1 (figure 3C, supplementary table 4), while there was a marked upward trend in LH, measured by AUC fold rise and peak fold rise ($p=0.055$ and $p=0.071$, respectively; figure 3D, supplementary table 4).

Adrenal cortex function

Basal plasma cortisol did not change significantly during or after the expedition (table 2).

Average hair cortisol before and during the expedition is shown in figure 4b. Mean values are shown in supplementary table 4. Most participants demonstrated a significant increase in average cortisol levels during the expedition.

Individuals' dynamic plasma cortisol responses before and after the expedition are shown in figures 4A. Both AUC and peak cortisol did not change between the three time points ($p=0.12$ and $p=0.45$, respectively, figure 4B). Subjects demonstrated marked suppression of early morning cortisol following low-dose dexamethasone administration

One participant demonstrated a more suppressed baseline in plasma cortisol than others (filled square symbol, figure 4). This individual also demonstrated markedly higher hair cortisol concentration through the expedition and two months beforehand.

Salivary cortisol in the days immediately following the exercise was blunted but by day 10 had recovered (figure 4C), reflected in a normal day curve which was unchanged from baseline (figure 4D).

Discussion

With on-going debate as to whether women can endure extreme physical activity without detrimental effects on hormonal axes, given the finding of HPA and HPG axis suppression in extremely arduous exercise in men (e.g. in US Army ranger training (7, 8)), we exploited the opportunity to examine the HPA and HPG axes among six women who completed a 1700km ski expedition hauling 80kg sledges up to 2950m elevation. In doing so, the team broke several records including being the first all-female team to cross the Antarctic unsupported. Our data demonstrate HPG and HPA axis resilience during extreme exertion despite significant fat loss. HPA axis basal function, sensitivity to central suppression and adrenal reactivity to ACTH did not change during or after the expedition, but demonstrated greater sensitivity to suppression from dexamethasone than anticipated from other studies using a similar protocol in older participants (20, 21). Hair cortisol rose during the expedition as would be expected with sustained arduous exercise (27).

Coincidentally, the expedition duration (61 days) was identical to US Army Ranger training. Trainee Rangers are expected to cover around 322 km, carrying 30-41 kg. While the expedition comprised a different form of exercise (skiing rather than walking or running), it was arguably noninferior in terms of effort or endeavor. One crucial difference is the 0-5 hours of sleep per day expected during Ranger training,(7) and deliberate psychological stress (28). which contrasts with the average 6.73 ± 1.75 hours of sleep per night, albeit with poor perception of restfulness (in 24-hour daylight), and modest weekly and whole-expedition stress ratings.

The primary drivers of adverse endocrine and metabolic changes in Ranger training appear to be nutritional deprivation (with loss of lean mass), psychological stress, sleep deprivation and exercise intensity. Nindl *et al.* showed a 12.6% loss of body mass, 6% lean mass and 50% fat mass (7, 28). The endocrine effects of negative energy balance are

well-documented adaptations for survival and include suppression of the HPG axis and hypercortisolemia (1). In their meta-regression of field studies of arduous training, Murphy *et al.* showed that the combination of training duration and low EA were inversely associated with physical performance (29), although it is difficult to delineate EA as a cause from the other factors described here.

A carefully calculated provision of approximately 21 MJ/ day (5000 kcal/ day; ~45% carbohydrate, ~45% fat and ~10% protein), with significant fat gain prior to the expedition, plus a relatively low altitude and preservation of sleep, meant participants lost only fat mass, not lean mass. Sustained, submaximal exertion appears to have had the effect of preserving total lean mass, although leg lean mass reduced by 6.10%. This may relate to muscle fiber pennation rather than reduced mass *per se;* we were unable to confirm this by biopsy. Thus, weight loss was healthy, reinforcing the importance of appropriate nutrition preventing loss of lean mass and/ or hormonal disturbances, as has been shown in overtraining syndrome.(30) As insufficient nutrition has been shown to cause multiple endocrine deficiencies in sports and exercise,(2) we hypothesize that sufficient and appropriate nutrition had an important role in preventing changes to the HPA and HPG axes.

Calbet *et al.* demonstrated that exercise maintains lean mass, during a 4-day extreme energy deficit in overweight men (31). Protein supplementation alone (1.5g/kg body mass/day) did not preserve lean mass, compared with carbohydrate. However, as demonstrated by Smith *et al*. in obese, sedentary women, a protein intake of 1.2 g/kg/day mitigated loss of lean mass, compared with low protein intake (0.8 g protein/kg/day) during 10% weight loss over 27 weeks (32). In men undertaking arduous military training, a mixed dietary supplement
(5.1MJ/day (1220 kcal/day); ~45% carbohydrate, ~40% fat, ~15% protein) prevented 2 kg loss in lean mass, over 8 weeks, compared with nonsupplemented controls (33). Despite a

caloric deficit (indicated by weight loss), our participants maintained total lean mass, with an average protein intake of around 1.6 g/kg/day.

Low ambient temperatures induce brown adipose tissue (BAT) thermogenesis, mediated by catecholamine upregulation, acting as a sink for glucose and fatty acid uptake.(34) Adaptive thermogenesis is upregulated by ß-3 adrenergic receptors, which are expressed in fat but not in muscle.(35) Thus, the cold Antarctic environment could partially explain the high selectivity of substrate.

In mixed sex Norwegian Ranger training involving seven-day food and sleep deprivation, women demonstrated greater fat utilization and glycogen preservation than men, implying greater capacity for endurance exercise.(36) Estrogens appear to be responsible for this substrate dimorphism,(37) while women subjectively claim better patrolling performance than men perhaps because of this metabolic advantage.

In addition to exercise and nutrition, the modest altitude of the expedition environment could have mitigated the loss in lean mass, compared with arduous expeditions at extreme altitudes, where hypobaric hypoxia contributes to loss of lean mass (38). Likewise, insufficient sleep, whether at altitude or as a programmed part of arduous training, could impede absorption of macronutrients and reduces gut readiness for daytime absorption (39), and it could be postulated that preservation of sleep contributed to the maintained total lean mass we observed.

No suppression of metabolic parameters such as thyroid hormones or elevated cortisol were seen during or after the expedition. Lean mass exerts a greater effect on resting metabolic rate and appetite than fat mass (40), and demonstrates a greater bidirectional relationship with androgens, and to a lesser extent estrogens, than fat mass (41). Thus, preservation of total lean mass might mitigate against some of the endocrine sequelae of

negative energy balance. The decrease in leptin, followed by recovery post-expedition, was more pronounced than the changes we observed in body fat. Cold exposure itself may reduce leptin in women,(42) but this appears to become effective only when cold exposure is sustained.(43) The change in HPG axis function we observed did not correlate with leptin, as has been reported previously.(44)

Dynamic attenuations in LH and sex steroids following an energy deficit may confer immediate survival benefits but may be associated with maladaptive suppression of hormonal axes and reproductive, bone or psychological sequelae if sustained.(2) Luteinizing hormone was relatively suppressed prior to and during the expedition (reflecting hormonal contraception usage), but recovered by post-exercise visit pre-2. There was no change in FSH before, during or after the expedition; this is consistent with studies of overtraining syndrome which generally demonstrate relatively normal FSH levels when LH is suppressed (reviewed in Cadegiani *et al.* (45)), and laboratory studies of reduced EA, which show normal levels relative to suppression of LH in response (46).

Cortisol reactivity and diurnal salivary cortisol were blunted relative to other studies, and may be an appropriate response to a high intensity of training (20, 21). Alternatively, similar responses have been noted in dynamic testing of athletes during dysfunctional overtraining, also associated with elevated basal cortisol (reviewed in Cadegiani *et al.) (45)*. Elevated hair cortisol concentrations are associated with exercise *per se;* whether the marked elevation during the expedition may represent an overtraining syndrome would be a pertinent question for future studies (27). The response of the HPA axis to central negative feedback is greater than has been described elsewhere (4, 20). Yehuda *et al.* reviewed the use of low-dose dexamethasone suppression in post-traumatic stress disorder (PTSD), showing PTSD was associated with increased central axis sensitivity (4). No suggestion of PTSD was noted from the psychological stress or IES-R assessments

before or after the expedition, thus this may relate simply to age, fitness and lower volume of distribution of these participants compared with previous studies.

Similar exercise-associated patterns in the HPA and HPG axis were seen following restricted carbohydrate intake with aerobic and resistance activity (average 46 ± 9.1 MET and 4.7 ± 0.7 sessions per week, respectively), in normal BMI women over 20 weeks (47). This regimen achieved a 11.9% weight loss with unchanged lean mass, and was associated with increased menstrual dysfunction, reduced testosterone, estradiol, free T3 and TSH and unchanged cortisol compared with weight-stable, exercising controls. While the degree of weight loss was similar to the present study, this intervention was achieved primarily through dietary restriction, since the exercise was less intense. The investigators also assessed recovery, demonstrating partial normalization of sex and thyroid hormones and leptin after 18 weeks. As in the current study, mood profile was unaffected by the intervention, which might possibly account for the apparently stable cortisol responsiveness we observed.

Other correlates of overtraining syndrome include sleep deprivation and psychological stress.(48) Psychological stress is a prominent feature of extreme physical endeavor. Therefore, while both stress and reduced EA may be shown to cause reproductive endocrine dysfunction independently, their impact in this context may be synergistic and it may be impossible to draw a distinction between them (1). The expedition required both significant mental and physical exertion, although perceived stress levels were modest through the expedition and anxiety, depression and psychosocial risk factor assessments did not change after the expedition.

It has been suggested the psychological stress of Ranger training results from nutrient and sleep deprivation, which serve to increase the arduousness of many military training formats (6). Sleep deprivation in isolation is associated with elevated evening cortisol,

flattened cortisol day curve, reduced androgen secretion and higher sympathetic nervous system activity (39). Female sex hormones appear to be protective of the effect of sleep deprivation on cortisol blunting after psychosocial stress (49). The sustained moderate to high exercise intensity needed for a polar traverse represents a different form of exertion compared to US Ranger training, including its sustained, repetitive nature, austere environment, safety concerns and isolation. The stress and physical exertion scores reported during the expedition were consistent with previous arduous expeditions (17, 18), while sleep diaries showed significantly longer sleep duration than would be expected in Ranger training (7, 28), albeit of low perceived restfulness. Both increased sleep and the sustained, submaximal intensity of exercise could also account for the biological resilience we observed. Together with the nutritional strategy taken, and relatively reduced energy expenditure in women compared with men, these factors might have contributed to mitigating some of the negative psychological effects.

The major strength of our study is the unique nature of the expedition; this likely represents the first opportunity to study a cohort of female participants complete an endeavor of such a prolonged, arduous nature. Mitigating against low EA in women is important, since women appear to be at greater risk of low EA and its consequences than men (1, 2). Previous studies of prolonged, arduous training have focused on male cohorts and recovery rates in women have not been studied. Furthermore, the effects of exercise or low EA on the dynamic of the HPA and HPG axes have not previously been studied in either sex.

Limitations to our study include the small number of participants. This is unavoidable on such extreme expeditions; we have attempted to mitigate this by a comprehensive characterization of the participants. The team is larger than any previous female-only transantarctic attempts, increasing the number of women who have skied across the continent from four to 10 (17, 18). Other limitations include the natural limitations of a field

study, such as four day delay in testing after the expedition. Every effort was made to overcome these using study visits shortly after the expedition arrived in Chile with imaging undertaken as soon as reasonably possible following the participants return to the UK. It was not logistically possible to repeat imaging immediately before and after the expedition, or use the same examiner to perform skinfolds in the UK and Chile, so we used the best feasible measures of body composition. The use of hormonal contraceptives, while representative of real-world hormonal milieu, do limit the interpretation of LH responses. For logistical and ethical reasons, dynamic tests of the HPA axis at a higher level (e.g. insulin tolerance test, corticotrophin releasing hormone test, desmopressin test) were not possible, however in future studies a maximal or two-bout exercise test could be considered. Calculation of cortisol awakening response would add merit to our study, but was not possible since participants were woken 10 minutes before the first saliva sample taken in the pre- and post-expedition day curves.

In conclusion, no short term adverse effects were demonstrated from an unprecedented, successful transantarctic expedition in women. Cortisol reactivity and pituitary gonadotrophin reactivity were not impaired. We hypothesize these findings related to and pre- and intraexpedition nutrition, sleep provision, on the background of desirable selection characteristics, so that participants did not rate the expedition as subjectively stressful and lean mass was maintained.

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Conflicts of interest

None of the authors has conflicts of interest to declare, including professional relationships with companies or manufacturers who will benefit from the results of the present study. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Tables, figures and Appendices.

Appendices

Table 1. Characteristics of participants at visit pre-1

Age, years; median (range)	32.7 (28.6 to 36.1)
Reproductive characteristics	
Age at menarche, years; median (range)	13 (11-16)
Medical suppression of menstruation Levonorgestel 20mcg per 24h intrauterine device (Mirena ®) only – 4 (67%) Mirena ® plus ethinylestradiol 30 mcg/ levonorgestrel 50 mcg – 1* (17%) 68 mg subcutaneous implant (Nexplanon ®) – 1 (17%)	
Body composition	
Body mass, kg Mean (SD)	72.8 (4.00)
BMI, kgm^{-2} Mean (SD)	24.2 (0.97)
% fat by DXA, kg	20.92 (2.12)

mean (SD)	
Lean mass by DXA, kg Mean (SD)	53.5 (3.06)
Psychological assessments	
Several periods of psychological stress Mean (SD)	5 (83)
Permanent, psychosocial stress N (%)	0
Some periods of psychological stress N (%)	1 (17)
Never experienced psychological stress N (%)	0
One or more adverse events N (%)	4 (67)
High or severe financial stress N (%)	0

IES-R	36 (9 – 52)**

Median (range)	
PHQ-9 Median (range)	3 (0 – 11)
BAI Median (range)	11 (2 – 15)
CDRISC 10 Median (range)	34 (31 – 36)
BEDA-Q	
Score Median (range)	4 (0-6)
BEDA-Q part B	
“Are you trying to lose weight now?”	
Yes N (%)	0
“Have you ever tried to lose weight?”	

Yes N (%)	3 (50)

“If so, how many times?”	
Number of times (n [%])	3-5 (2 [33]) >5 (1 [17])

Caption. Data are mean (SD) unless otherwise stated.

BEDA-Q brief eating disorders in athletes questionnaire. IES-R Impact of events scale (revised), PHQ-9 adjusted patient health questionnaire 9, BAI Beck Anxiety Inventory, CDRISC10 Connor Davidson Resilience Scale 10, N/A not applicable

* One participant using Mirena ® also commenced ethinylestradiol 30 mcg/ levonorgestrel 50 mcg once daily immediately prior to expedition until after testing was completed.

** Applies to four subjects who experienced a significant event

Table 2. Biochemical and hormonal parameters at baseline, 4 and 14 days after the expedition.

Basal (fasting) variable	**Visit pre 2** *(39 days preexpedition) mean (SD)*	**Visit post 1** *(Expedition + 4 days mean (SD)*	**Visit post 2** *(Expedition + 15 days mean (SD)*	**Mean (95% CI) difference visit pre 2 versus visit post 1**	**Mean (95% CI) difference visit pre 2 versus visit post 2**	**P value**
Reproductive and HPA axis markers						
Estradiol mmol/L	227 (176)	163 (144)	394 (183)	64.3 (-156, 284)	-168 (-427.4, 91.7)	0.043
LH IU/L	5.36 (2.03)	5.13 (3.70)	3.42 (1.43)	0.23 (-4.15, 4.61)	1.94 (-1.02, 4.91)	0.332
FSH IU/L	5.83 (1.09)	5.30 (1.36)	3.50 (0.48)	0.53 (-1.47, 2.53)	2.33 (-0.82, 5.48)	0.161
Androstenedione mmol/L	9.56 (2.98)	7.33 (2.61)	8.91 (2.78)	2.23 (0.45, 4.01)	0.65 (-2.92, 4.22)	0.148
Total testosterone mmol/L	1.46 (1.56)	0.59 (0.81)	0.54 (0.4)	0.87 (-0.8, 2.55)	0.92 (-0.39, 2.22)	0.178
Dihydrotestosterone mmol/L	2.93 (2.12)	2.18 (1.24)	1.33 (0.78)	0.75 (-0.5, 1.99)	1.6 (-1.09, 4.28)	0.182

DHEA	360.24 (85.34)	370.37 (46.32)	412 (42.3)	-10.1 (-81.9, 61.6)	-52.4 (-114, 9.62)	0.127

mmol/L						
17-OH Progesterone mmol/L	3.81 (5.04)	1.54 (1.51)	7.97 (4.53)	2.27 (-2.18, 6.72)	-4.15 (-13.1, 4.74)	0.071
SHBG nmol/L	59.5 (25.4)	100 (53.0)	69.0 (25.0)	-40.7 (24.6, -104)	13.8 (-44.9, 25.9)	0.132
Prolactin mU/L (60 to 500)	338 (45)					N/A
LH:FSH ratio	1.05 (0.22)	0.93 (0.32)	1.32 (0.57)	0.12 (-1.10, 1.34)	-0.26 (-2.12, 1.60)	0.791
AMH pmol/L	12.2 (3.85)	9.44 (2.61)		2.79 (-1.38, 6.97)		0.147
Cortisol (unsuppressed) mmol/L	552 (67.3)	434 (74.2)	519 (19.4)	117 (-123, 358)	32.2 (-122, 186)	0.279
Cortisol (suppressed by 0.25 mg dexamethasone 10 hours before) mmol/L	73.3 (45.2)	61.7 (33.1)	54.4 (11.7)	11.6 (-28.1, 51.3)	18.8 (-5.21, 42.9)	0.302
Basal metabolic and nutritional markers						

Albumin g/L	35.4 (2.06)	33.1 (0.84)	34.9 (1.58)	0.94 (-0.14, 4.71)	0.92 (-1.88, 2.84)	0.075
Glucose mmol/L	4.93 (0.62)	4.57 (0.25)	4.42 (0.75)	0.37 (-0.2, 0.97)	0.52 (-0.2, 1.19)	0.132

HOMA						
%B	96.4 (27.8)	114 (39.6)	118 (63.0)	15.8 (-59.1, 22.5)	21.6 (-77.1, 34.0)	0.634
%S	113 (31.3)	130 (82.2)	140 (39.5)	42.8 (-127, 92.8)	18.5 (-75.3. 19.8)	0.712
IR	0.94 (0.24)	1.00 (0.46)	0.76 (0.22)	0.26 (-0.74, 0.62)	0.10 (-0.08, 0.43)	0.537
Leptin ng/mL	10.8 (4.84)	2.71 (1.57)	4.93 (3.58)	8.09 (3.64, 12.5)	5.87 (1.04, 10.7)	**0.002***
IGF-1 ng/mL	46.0 (18.8)	29.1 (11.9)	46.0 (18.8)	33.1 (-44.4, 110)	-19.5 (-79.3, 40.3)	0.116
Iron µmol/L	23.3 (6.05)	28.3 (8.87)	19.0 (4.29)	2.50 (-11.4, 1.43)	1.76 (-0.20, 8.87)	0.091
Ferritin ng/mL	59.7 (22.8)	55.3 (35.3)	55.2 (26.7)	4.33 (-11.1, 19.8)	4.50 (-14.6, 23.6)	0.801
TSH mU/L	2.51 (0.57)	3.53 (1.84)		-1.02 (-2.69, 0.66		0.180

Total T3 nmol/L	1.43 (0.08)	1.42 (0.09)	1.38 (0.07)	-0.17 (-0.40, 0.37)	0.05 (-0.20, 0.30)	0.924
Free T4 pmol/L	12.5 (0.34)	11.8 (0.54)		0.67 (-0.42, 1.75)		0.181
Zinc µg/dL	134 (6.15)	122 (12.3)	137 (15.7)	5.60 (-1.91, 26.9)	4.31 (-13.7, 8.41)	0.041
Urea	4.53 (0.65)	5.37 (1.07)	4.63 (0.9)	-0.83 (-1.7, 0.05)	-0.1 (-1, 0.81)	0.243

mmol/L						
Sodium mmol/L	141 (10.2)	143 (8.04)	139 (5.09)	-2.67 (-15.1, 9.73)	1.17 (-9.5, 11.84)	0.822
Potassium mmol/L	4.27 (0.34)	4.45 (0.24)	4.08 (0.33)	-0.18 (-0.4, 0.05)	0.18 (-0.4, 0.74)	0.144
Magnesium mmol/L	0.79 (0.06)	0.81 (0.04)	0.80 (0.73)	0.02 (-0.06, 0.17)	0.01 (-0.04, 0.25)	0.360
Chloride mmol/L	105.17 (6.97)	107.5 (5.01)	103.83 (3.19)	-2.33 (-10.3, 5.65)	1.33 (-5.3, 7.93)	0.381
Creatinine µmol/L	63.7 (5.89)	63.3 (3.98)	69.0 (5.66)	0.33 (-4.8, 5.42)	-5.33 (-9.9, -0.8)	0.023

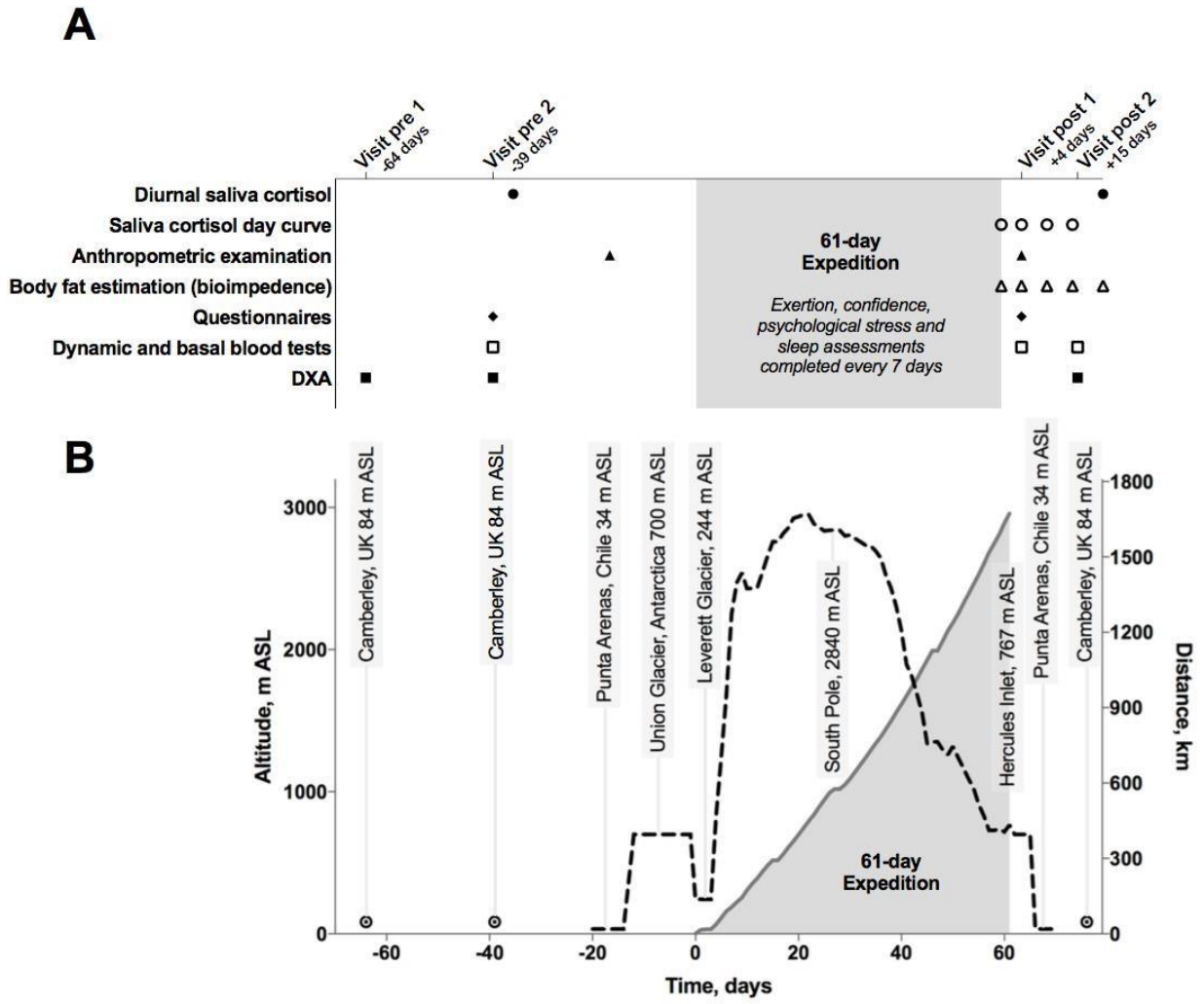
Creatine kinase U/L	130 (16.94)	137 (33.2)	153 (71.8)	77.3 (-145.6, 300)	-118 (-371, 134)	0.357
Lactate mmol/L	0.75 (0.17)	0.85 (0.25)	0.57 (0.12)	-0.1 (-0.4, 0.17)	0.04 (-0.4, 0.44)	0.491
pH	7.43 (0.05)	7.35 (0.01)	7.40 (0.01)	-0.02 (-0.3, 0.24)	-0.08 (-0.4, 0.19)	0.642
Calcium (adjusted) mmol/L	2.56 (0.08)	2.55 (0.06)	2.56 (0.04)	0.01 (-0.06, 0.09)	-0.01 (-0.11, -0.01)	0.813

25 OH D	112 (25.3)	75.8 (21.3)		35.8 (14.3, 57.4)		**0.008***
Phosphate mmol/L	1.19 (0.05)	1.08 (0.04)	1.34 (0.09)	0.07 (-0.06, 0.29	0.01 (-0.35, 0.025)	0.017

Caption. AMH, antimüllerian hormone; DHEA, Dehydroepiandrosterone; βCTX, β-carboxyl-terminal cross-linked telopeptide of type I collagen; P1NP Amino-terminal propeptide of type 1 procollagen; FSH, follicle stimulating hormone; HPA, hypothalamic-pituitary-adrenal; HOMA, homeostatic modelling assessment; IGF-1 insulin-like growth factor 1; %S insulin sensitivity; %B –beta cell function; -IR- insulin resistance; LH, Luteinizing hormone; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; 25 OH D 25 hydroxyl vitamin D. P value for repeated measures ANOVA. * denotes statistical significance after Bonferroni adjustment: $p<0.005$ for reproductive markers, $p<0.05$ for cortisol, $p<0.002$ for metabolic and nutritional markers.

Figure captions.

Figure 1. Overview of experimental design and expedition

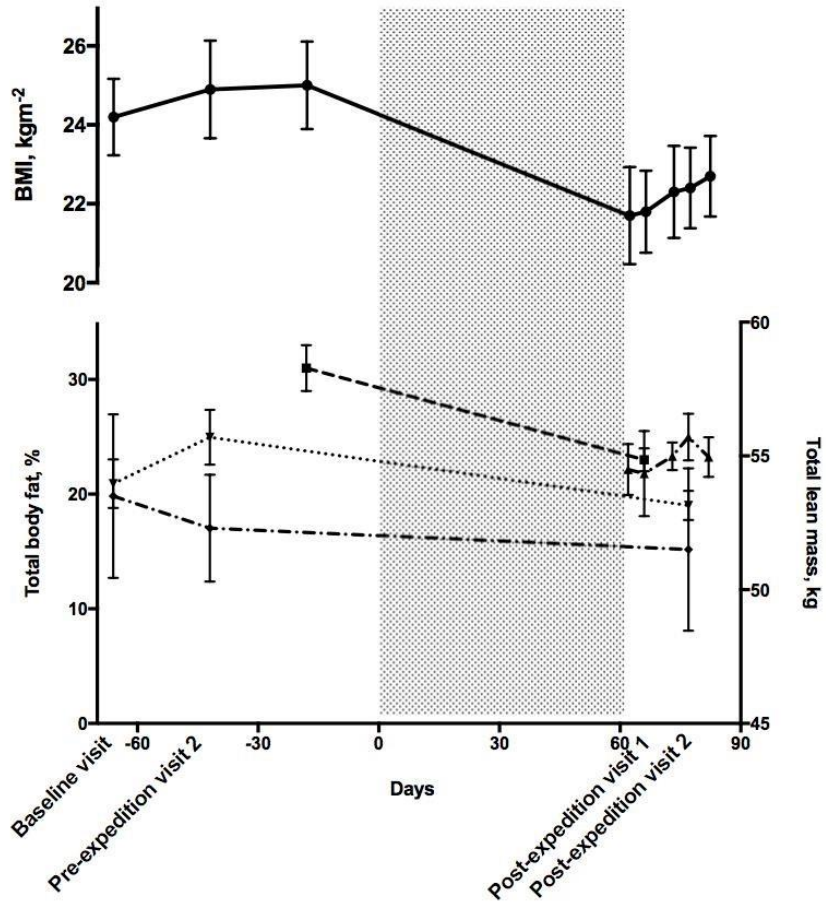


A. Timeline summary of major investigations. Saliva cortisol: 5-point day curve was measured 40-34 days pre- and 18-24 days post-expedition (filled circle); morning and evening sampling undertaken 1, 5 and 10 days post-expedition (unfilled circle).

Anthropometric examination: weight and skinfolds were undertaken 16 days pre- and 5 days post-expedition (filled triangle). Body fat was estimated by bioimpedance 1,5,10 and 20 days post-expedition (unfilled triangle). Questionnaires were undertaken 39 days pre- and 5 days post-expedition (diamond). Dynamic and basal blood tests: fasted blood sampling and dexamethasone-suppressed combined GnRH and ACTH-(1-24) test, 39 days pre, and 4-5 and 15-16 days post-expedition (unfilled square). Body composition measured by DXA scan 64 and

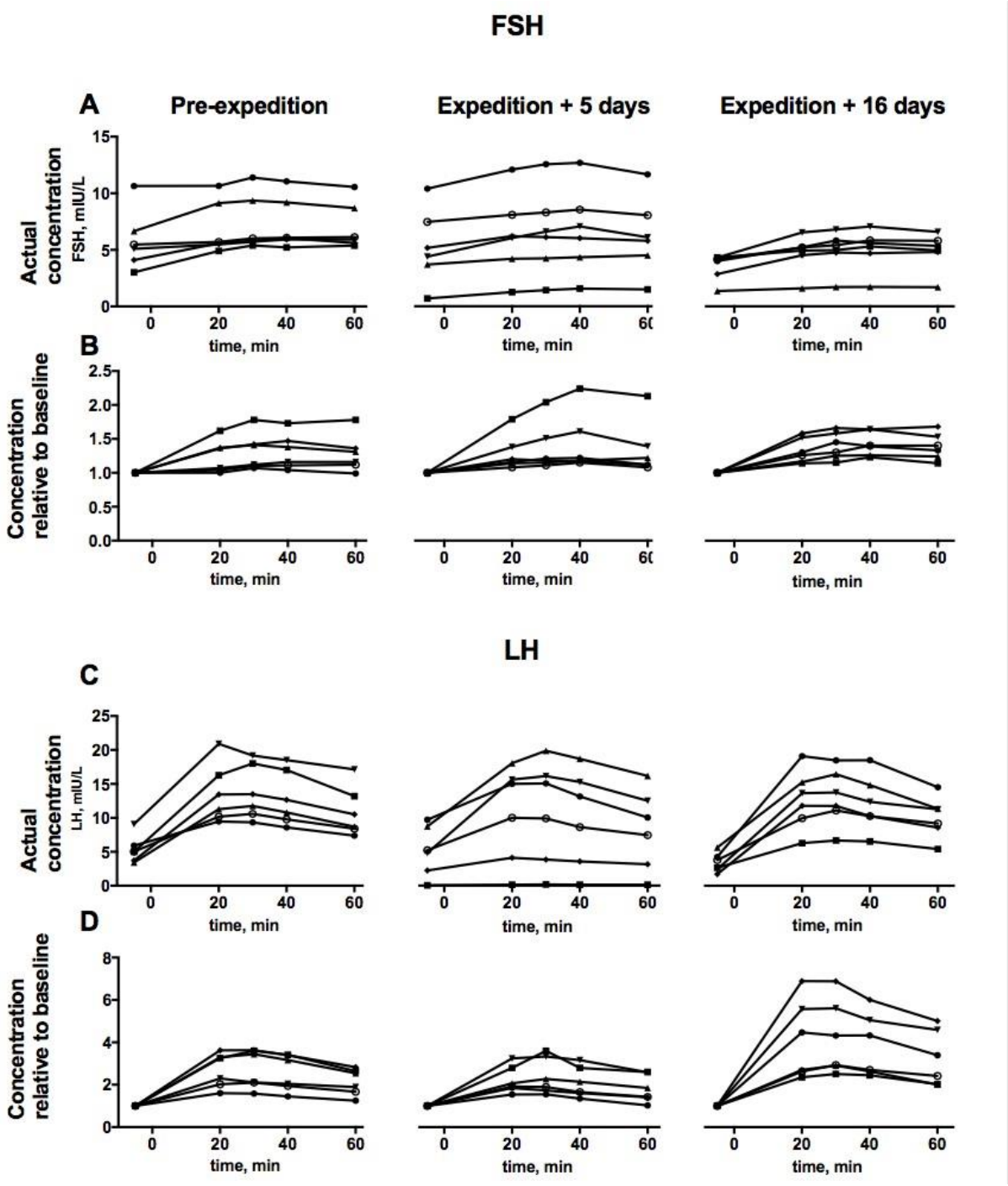
39 days pre and 15 days post-expedition (filled square). *B*: Altitude profile of study. Dashed line: altitude. Solid line: elapsed ski distance. Target icons indicate altitude of study visits in Camberley, UK. GnRH, gonadotrophin releasing hormone; ACTH adrenocorticotrophic hormone, DXA dual-energy x-ray absorptiometry, ASL above sea level

Figure 2. Anthropometric changes during the expedition.



Data are mean ± SD. Shaded rectangle: Duration of expedition. Circle with solid line: BMI. Square with dashed: total body fat (%) by skinfold. Triangle pointing upwards with dash-dotdot line: total body fat (%) by bio-electrical impedance. Triangle pointing downwards with dotted line: Total body fat (%) by dual energy x-ray absorptiometry. Diamond with dash-dot line: total lean mass (kg) by dual energy x-ray absorptiometry. BMI, body mass index

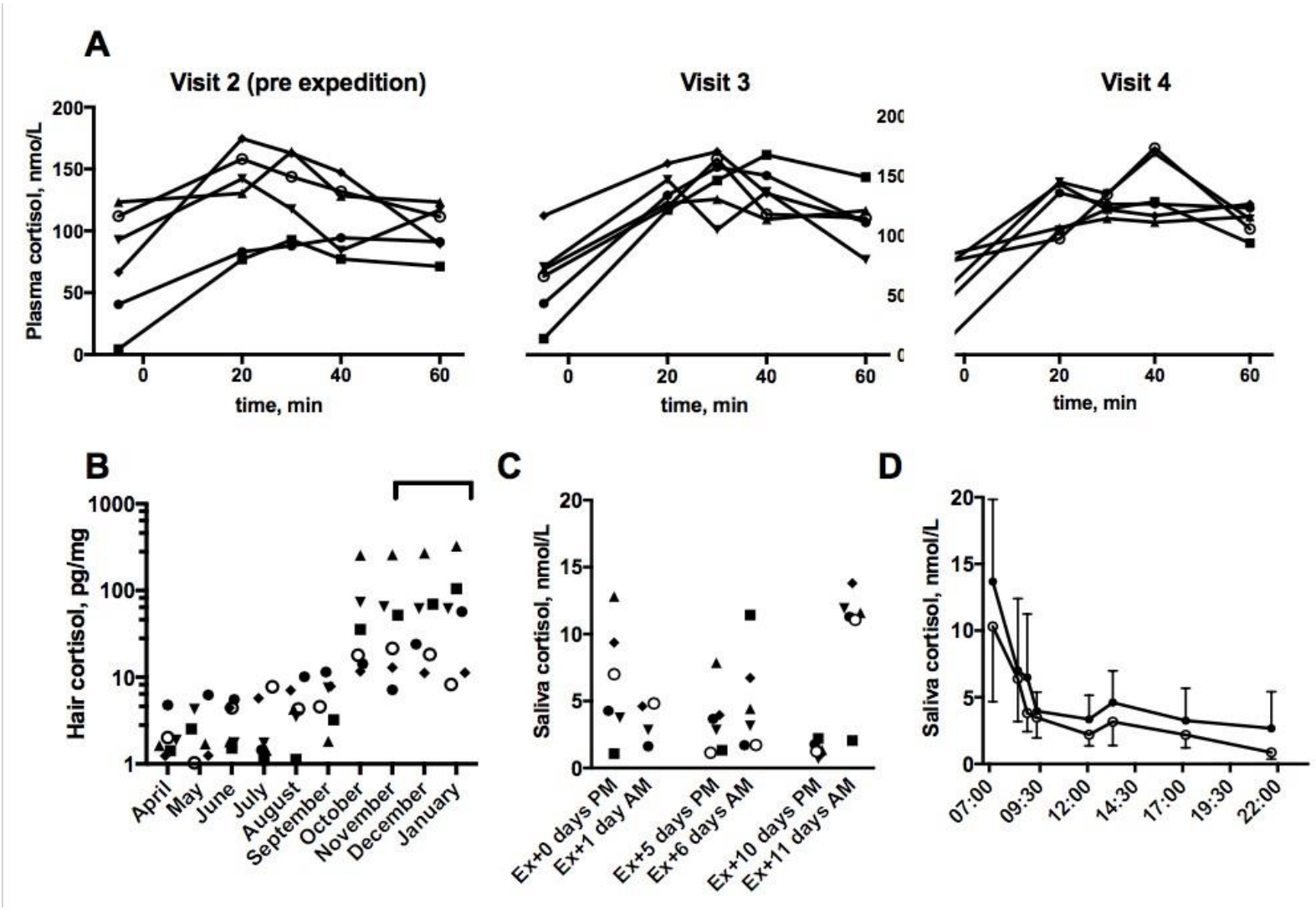
Figure 3. Dynamic gonadotrophin function before and after the expedition.



Individuals represented by symbols. Actual concentrations (top row) and fold difference from baseline concentrations (middle row) after 10μg GnRH administration before, 5 and 16 days after the expedition for FSH (A) and LH (B). The bottom row shows change in AUC and peak concentrations following 10μg GnRH administration at the same 3 time points for FSH (C) and LH

(D). FSH AUC fold rise and peak fold rise did not change across visits (p=0.71 and p=0.55, respectively). There was an upward trend in LH AUC fold rise and peak fold rise (p=0.055 and p=0.071, respectively). FSH, follicle stimulating hormone; LH, luteinizing hormone; GnRH, gonadotrophin releasing hormone; AUC, area under the curve. One individual (filled square) commenced levonogestrel 150 mcg/ ethinylestradiol 30 mcg immediately prior to the expedition. One individual (unfilled circle) used Nexplanon ® contraceptive implant while all others used a Mirena ® intrauterine device

Figure 4. Dynamic, monthly average hair and diurnal saliva cortisol concentrations



A: adrenal response to (1-24) adrenocorticotrophin, 10 hours after central suppression with 0.25mg dexamethasone, before, and 5 days and 16 days after the expedition. Top row: cortisol concentrations. Bottom row: fold difference in cortisol from baseline. Area under the curve and peak cortisol did not change between the three time points ($p=0.12$ and $p=0.45$, respectively, figure 4B). B: average monthly cortisol from 1cm hair segments prior to and during the expedition (expedition represented by bracket). C: change in AUC and peak concentrations during the dynamic test before, and 5 and 16 days after the expedition. D: Saliva cortisol 36-40 days pre-expedition and 18-24 days post expedition (left panel) and diurnal cortisol 1,4 and 10 days post-expedition (right panel).

Individuals represented by symbols. Time: after ACTH-(1-24) administered. ACTH, adrenocortiocotrophin; F, cortisol. ** $p<0.001$

Supplementary table 1. Anthropometric changes during the expedition.

	Pre-expedition			**Post-expedition**				
	Visit pre-1 *(-64 days)*	**Visit pre-2** *(-39 days)*	**- 16 days**	**+1 day**	**Visit post-1** *(+5 days)*	**+ 10 days**	**Visit post-2** *(+15 days)*	**+ 18 to 24 days**
BMI, kgm^{-2} Mean (SD) [range]	24.2 (0.97) [22.8 - 25.45]	24.9 (1.24) [23.11 - 26.72]	25.0 (1.11) [23.48 - 26.73]	21.7 (1.23) [19.68 - 22.91]	22.3 (1.03) [20.59 - 23.27]	22.3 (1.17) [20.37 - 23.53]	22.4 (1.02) [20.56 - 23.42]	22.7 (1.02) [20.81 - 23.72]
Body mass, kg Mean (SD) [range]	70.47 (4.51) [62.7 - 74.2]	72.6 (1.75) [65.85 - 76.83]	72.8 (3.99) [65.8 - 77.2]	63.2 (4.74) [55.5 - 68.4]	64.9 (4.20) [58.0 - 69.2]	65.0 (4.03) [58.4 - 68.9]	65.3 (4.21) [58.3 - 69.1]	66.2 (4.11) [59.5 - 69.7]
% fat, DXA mean (SD)	20.92 (2.12) [19.2 to 25.4]	24.97 (2.39) [23.3 to 30.3]					19.02 (1.28) [17.2 to 20.4]	

% fat, skinfold mean (SD)			31.0 (2.0) [0.28 - 0.33]		23.0 (1.0) [0.21 - 0.24]			
%fat, BIA mean (SD)				22.15 (2.22) [19.4 - 26.0]	21.8 (3.71) [18.1 - 28.8]	23.3 (1.20) [22.5 - 25.7]	25.0 (2.04)	23.2 (1.74) [22.2 - 28.1]
Total body mass, DXA, kg Mean (SD) [range]	70.6 (4.5) [62.68 to 74.26]	72.6 (3.9) [65.85 to 76.83]					66.4 (4.2) [59.67 to 70.76]	
Total lean mass, DXA, kg Mean (SD) [range]	53.5 (3.06) [48.81 to 57.27]	52.3 (2.00) [48.85 to 54.72]					51.5 (3.04) [47.25 to 54.24]	
Total bone mineral content, DXA, kg Mean (SD) [range]	2.75 (0.13) [2.62 to 2.97]	2.80 (0.13) [2.67 to 3.02]					2.77 (0.12) [2.64 to 2.97]	

BMI, body mass index. DXA, dual x-ray absorptiometry; BIA, bio-electrical impedance.

Supplementary table 2.

Regional lean, fat and bone mass changes during the expedition

	Visit pre-1	**Visit pre-2**	**Visit post-1**
Lean mass (kg)			
Arms	5.44 (0.57)	5.15 (0.45)*	4.99 (0.36)
Legs	18.79 (1.05)	18.08 (0.98)*	16.98 (1.22)*
Trunk	26.15 (1.96)	26.06 (1.5)	26.52 (1.84)
Android	3.52 (0.24)	8.33 (0.61)*	53.57 (3.06)
Gynoid	3.73 (0.25)	8.4 (0.59)	52.34 (1.99)
Total	3.67 (0.31)	8.22 (0.54)	51.55 (3.04)
Fat mass (kg)			
Arms	1.44 (0.12)	1.94 (0.16)*	1.49 (0.19)*
Legs	6.4 (1.13)	7.26 (1.47)*	5.05 (0.79)*
Trunk	5.59 (1.09)	7.47 (1.16)*	4.79 (0.66)*
Android	0.61 (0.17)	0.9 (0.15)*	0.49 (0.08)*
Gynoid	2.92 (0.52)	3.71 (0.59)*	2.49 (0.43)*
Total	14.23 (2.11)	17.5 (2.52)*	12.13 (1.37)*
Total (%)	1.44 (0.12)	1.94 (0.16)*	1.49 (0.19)*
Bone mineral content (kg)			
Arms	0.34 (0.02)	0.35 (0.03)	0.35 (0.03)
Legs	1.05 (0.06)	1.05 (0.06)	1.05 (0.05)
Trunk	0.83 (0.07)	0.86 (0.07)*	0.83 (0.07)

Android	0.05 (0.00)	0.06 (0.01)*	0.05 (0.01)
Gynoid	0.30 (0.02)	0.30 (0.02)*	0.30 (0.02)
Total	2.75 (0.13)	2.80 (0.13)*	2.77 (0.12)

Android: the area between the ribs and pelvis, gynoid: pelvis and upper thighs.

* $p<0.05$ vs previous visit (paired t test)

Supplementary table 3 Comparison of Pre and Post- Expedition psychological testing

	Score Pre	Score Post ('during expedition')	*p*
Several periods of psychological stress	5	2	0.079
Permanent psychosocial stress	0	0	1.0
Some periods of psychological stress	1	3	0.2
Never experienced psychological stress	0	1	1
One or more adverse events	4	1	0.079
High or severe financial stress	0	0	1.0
IES-R Median (range)	36 (9 – 52)*	41**	N/A
PHQ-9 Median (range)	3 (0 – 11)	4 (1 – 6)	1.0
BAI Median (range)	11 (2 – 15)	6 (2 – 11)	0.386
CDRISC 10 Median (range)	34 (31 – 36)	31 (29 – 35)	0.076
BEDA-Q			

Score Median (range)	4 (0-6)	7 (2-8)	0.009

BEDA-Q part B			
“Are you trying to lose weight now?”			
Yes n (%)	0 (0.0)	1 (16.7)	1.00
“Have you ever tried to lose weight?”			
Yes n (%)	3 (50)	4 (66.7)	0.558
“If so, how many times?”			
	3-5 (2) >5 (1)	>1 (1) 3-5 (2) >5 (1)	

BEDA-Q: brief eating disorders in athletes questionnaire, IES-R: Impact of events scale (revised), PHQ-9 adjusted patient health questionnaire 9, BAI Beck Anxiety Inventory, CDRISC10 Connor Davidson Resilience Scale 10, N/A not applicable

* Applies to four subjects who experienced a significant event

**Applies to one subject who experienced a significant event

Supplementary table 4. Mean values for dynamic endocrine function tests

FSH concentration (figure 3A)					
	Baseline	20 min	30 min	40 min	60 min
Pre-Ex	5.83 (2.66)	6.91 (2.38)	7.28 (2.49)	7.25 (2.33)	7.05 (2.1)
Ex + 5 days	5.3 (3.33)	6.31 (3.65)	6.55 (3.77)	6.71 (3.79)	6.27 (3.42)
Ex + 16 days	3.5 (1.18)	4.67 (1.65)	4.91 (1.73)	5.03 (1.8)	4.86 (1.68)
FSH concentration relative to baseline (figure 3B)					
Pre-Ex		1.24 (0.25)	1.32 (0.28)	1.31 (0.26)	1.29 (0.28)
Ex + 5 days		1.29 (0.26)	1.37 (0.36)	1.43 (0.44)	1.34 (0.4)
Ex + 16 days		1.33 (0.18)	1.40 (0.20)	1.43 (0.18)	1.39 (0.19)
LH concentration (figure 3C)					
Pre-Ex	5.36 (2.04)	13.59 (4.35)	13.73 (4.03)	12.90 (4.03)	10.89 (3.68)
Ex + 5 days	5.13 (3.70)	10.49 (7.10)	10.84 (7.63)	9.92 (7.12)	8.25 (5.95)
Ex + 5 days	3.42 (1.43)	12.67 (4.42)	13.03 (4.18)	12.12 (4.15)	10.04 (3.08)
LH concentration relative to baseline (figure 3D)					
Pre-Ex		2.68 (0.82)	2.74 (0.91)	2.57 (0.86)	2.14 (0.63)
Ex + 5 days		2.24 (0.64)	2.40 (0.87)	2.12 (0.72)	1.82 (0.65)
Ex + 5 days		4.10 (1.86)	4.19 (1.75)	3.86 (1.49)	3.24 (1.31)
Cortisol concentration (figure 4A)					
Pre-Ex	73.25 (45.23)	127.54 (39.77)	128.14 (33.82)	110.53 (28.95)	100.60 (19.9)
Ex + 5 days	61.66 (33.11)	130.53 (14.62)	140.09 (23.50)	131.77 (19.47)	110.16 (21.39)
Ex + 5 days	54.43 (28.60)	122.21 (21.50)	125.79 (8.02)	137.61 (26.91)	113.19 (12.03)

Hair cortisol by month (figure 4B)										
Month*	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
	8.36 (2.89)	11.40 (1.78)	10.83 (3.43)	11.96 (4.89)	11.58 (4.68)	9.50 (4.94)	35.22 (29.31)	38.86 (36.2)	41.48 (48.56)	54.79 (75.41)

Diurnal Hair cortisol post-Ex (figure 4C)					
Ex+1 day PM	Ex+2 days AM	Ex+4 days PM	Ex+5 days AM	Ex+10 days PM	Ex+11 days AM
6.38 (4.24)	6.89 (7.74)	3.47 (2.45)	4.86 (3.73)	1.37 (0.55)	10.29 (4.15)

Hair cortisol day curve (figure 4D)								
Time	07:10	08:30	09:00	09:30	12:15	13:30	17:20	21:50
Pre-Ex	13.68 (6.16)	7.03 (5.37)	6.48 (4.76)	3.97 (1.43)	3.36 (1.79)	4.6 (2.39)	3.26 (2.42)	2.68 (2.75)
Post-Ex	10.31 (5.63)	6.39 (3.2)	3.82 (1.39)	3.47 (1.52)	2.2 (0.85)	3.17 (1.79)	2.18 (0.97)	0.86 (0.50)

Baseline: immediately prior to dynamic function test, LH: luteinizing hormone, FSH: follicle stimulating hormone, Ex: expedition

Supplementary box 1.

Hormonal markers tested
17 OH Progesterone is an important steroid precursor hormone and is elevated in common forms of congenital adrenal hyperplasia (CAH). It is commonly checked to exclude CAH. **Androstenedione** is a weak adrenal androgen and precursor of testosterone and estradiol. It is also produced in the ovaries under influence of gonadotrophins and higher levels may predict recovery from FHA. {falsetti 2002} **Anti-müllerian hormone** is a biomarker of ovarian reserve. It peaks during puberty, then correlates inversely with age from around age 25 years.{Lie Fong 2012} **Cortisol** is a glucocorticoid produced by the hypothalamic-pituitary-adrenal axis. It has important roles in mobilizing energy stores and may be released in response to external stimuli, such as physical or psychosocial threats or challenges.

Estradiol is the major feminizing sex hormone, responsible for the development of secondary sexual characteristics. It is produced from estrone or testosterone, predominantly but not exclusively in the ovaries.

Inhibin B is produced in the ovaries in response to FSH and is reflects early-follicular phase follicle activity.{McNeilly 2012}

LH, FSH are secreted in a pulsatile manner by the anterior pituitary in response to GnRH, and serve to control gonad function. The **LH:FSH ratio** is elevated in conditions with elevated androgen levels, such as polcystic ovarian syndrome.

Prolactin is secreted by the anterior pituitary and is included as part of a complete anterior pituitary function test.

Sex hormone binding globulin is produced by the liver and binds androgens and estrogens, limiting the amount of biologically available hormone. It is produced in response to estrogens while its production is reduced by androgens and IGF-1.

Testosterone is the main androgen, produced in in men and to a lesser extent women. It is activated to **dihydrotestosterone**, which has higher androgenic effect, by 5□reductase