Canadian Science Publishing | Applied Physiology, Nutrition, and Metal

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Journal:	*Applied Physiology, Nutrition, and Metabolism*
Manuscript ID	apnm-2023-0338.R4
Manuscript Type:	Article
Date Submitted by the Author:	05-Dec-2023
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Is the manuscript for consideration in a Special Issue or Collection?:	Not applicable (regular submission)
Keyword:	Hypoxia, altitude, bioimpedance, anthropometrics, ghrelin, leptin, sweat, wearable device

Nutritional physiology and body composition changes during a rapid ascent to high altitude

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Take-home message

Changes in satiety / appetite hormones occur during a 4-days hypoxic trek, not necessarily linked to body mass changes and dietary modifications

Highlights

- Short term exposure to hypobaric hypoxia has led to a weight loss non attributable to water losses;
- No changes in bioimpedance parameters were observed, but the trend in improvement of phase angle;
- Dietary intake in the target range and indication for water intake could minimize weight and water losses;
- Leptin levels increased, while those of ghrelin decreased, in response to a 4-day high-altitude trek;
- Interindividual variation in sweat dynamics were observed from two case studies

Abstract

Exposure to high altitude might cause the body to adapt with negative energy and fluid balance that compromise body composition and physical performance. In this field study involving 12 healthy adults, sex-balanced, aged 29±4 years with a body mass index 21.6±1.8 kg/m^2, we investigated the effects fo a 4-day trekking up to 4556 m a.s.l. on Monte Rosa (Alps, Italy). The food intake was recorded using food diaries and nutrients averages were calculated. The bio-impedance analysis was performed at low and high altitude, and a wearable biosensor (Swemax) was used to track hydro-saline losses in 2 participants. Daily total energy intake was 3348±386 kcal for males and 2804±415 kcal for females (13-14% protein, 35% fat, 44-46% carbohydrates). Although there was a significant body weight loss (65.0±9.3 vs 64.2±9.10 kg, p<0.001, d=1.398), no significant changes in body composition parameter were found but a trend in the increase of the bioelectrical phase angle in males (p=0.059, d=–0.991). Body water percentage significantly changed (p=0.026, η^2_p=0.440), but the absolute water did not, suggesting that the weight loss was not due to water loss. Salivary and urinary osmolality did not change. A reduction in sweat rate at higher altitudes was observed in both participants. Interestingly, salivary leptin increased (p=0.014, η^2_p=0.510), and salivary ghrelin decreased (p=0.036, η^2_p =0.403). Therefore, the 4-day trek under altitude hypoxia exposure induced changes in satiety and appetite hormones. High altitude expeditions require more specific nutritional guidance, and using multiplex analysis could help in monitoring fluid balance and body composition.

Keywords

Hypoxia; altitude; bioimpedance; anthropometrics; ghrelin; leptin; sweat; wearable device.

Introduction

The reduced partial pressure of oxygen causes the body to activate a set of compensatory processes, which collectively alter human physiology when exposed to altitude hypoxia. Among several adaptive pathways, there is evidence of severe effects on the endocrine systems, energy intake, resting metabolic rate and body mass (Stellingwerff et al., 2019). Weight loss - both fat and fat-free mass - and water loss are well-known impacts of high altitude (Hamad & Travis, 2006; Westerterp, 2001). Remarkable effects occur over ≈5,000 m of altitude (Westerterp, 2001), despite some effects, such as the increase in basal metabolic rate, manifest to a lesser extent at lower to moderate altitudes (Stellingwerff et al., 2019). Total daily energy expenditure has been recorded as quite large (e.g., about 5000 Kcal) in some studies below 4000 m of altitude, and expenditure reduces with rising altitude, according to economizing of oxygen consumption for metabolism (Viscor et al., 2023). The increased energy expenditure caused by the intensity and duration of physical effort contributes to the negative energy balance, concomitantly with the reduced appetite and lack of palatable food at altitude, which is influenced by the degree and duration of hypoxia exposure as well as individual adaptations (Dünnwald et al., 2019). Acute hypoxia has been shown to lower appetite via hormonal modulation; nevertheless, gaps in depicting the differential responses of various hormones in either acute or sustained exposure remain (Debevec, 2017).

Given the increased metabolic demand and the heterogeneity of physiological responses causde by a variety of factors, accurately deciding the dietary needs of expeditioners and athletes at altitude is challenging. Dietary needs in terms of solid foods and beverages, supplements and water, need to be deepened; within this context, dietary records, i.e. multiple-day food diaries supported by appropriate software and a related nutritional database (Turrini, D'Addezio, et al., 2022), represent an effective tool to quantify and detail dietary intakes, thus providing extensive nutritional data (Ortega, 2015). Monitoring water intake and fluid losses is fundamental for maintaining homeostasis, which represents a critical goal for preventing hydration status that can affect physical performance and endurance capacity (Castellani et al., 2010). Water requirements increase at high altitude due to increased water losses from increased ventilation and urinary excretion, which is due to the downregulation of the renin-angiotensin-aldosterone system at altitude and the dry air environment favoring perspiration (Mawson et al., 2000). Several studies have focused on body water balance and hypoxia since fluid loss was reported as an acute bodily measure preventing from acute mountain sickness (AMS) symptoms (Gatterer et al., 2013), although no associations of AMS with salt and water retention were found in humans passively exposed to hypoxia at 4,559 of altitude (Biollaz et al., 2021). Recent studies have highlighted that loss of bodily fluids occurs during prolonged altitude exposure, despite particular advice for limiting dehydration (Bondi et al., 2021).

Currently, Bioelectrical Impedance Analysis (BIA) represents an effective, rapid, and non-invasive tool to evaluate hydration status and body composition (Böhm & Heitmann, 2013). The estimated hydration parameters resulting from BIA measurements may occupy a valuable role in muscle performance assessment (Hetherington-Rauth et al., 2020). The method relies on body tissues' electrical properties consisting of resistances (R) and capacitive reactance (Xc) - respectively related to total body water (TBW), and membrane integrity and tissue interfaces - that determine the impedance (Z) of an electric current passing through the body (Kyle, 2004). Furthermore, the use of wearable sensors in the last years has allowed researchers to obtain relevant functionality on real-time monitoring in athletes even during high-altitude sports, thereby leading to better performance (Sbrollini et al., 2022). Among the physiological monitoring through wearable devices sweat and its chemical composition could be greatly useful in medicine and fitness, e.g. to keep athletes' dehydration under control (Angelucci et al., 2021). Current and emerging technologies to detect sweat rate and sweat-based biometrics and analytes, despite the advantages of easily accessible continuous monitoring, are challenged by several factors such as skin contamination and weather conditions (Seshadri et al., 2019).

Despite the existence of literature on high altitude, nutritional physiology, and body composition changes, there are still gaps in understanding of the effects during short-term hypoxic treks using an ecological approach, i.e., from a field study in which investigators do not modify contextual factors. Furthermore, the fieldwork of portable technologies and non-invasive approaches to assess bodily adaptations while taking into account valid protocols for monitoring dietary intakes in special environments merits implementations. Finally, sex differences in short-term nutritional physiology and body composition responses have not been elucidated. Within this background, the purpose of this study was to fill some of the current gaps in our comprehension of nutritional physiology and body composition changes during active exposure to altitude hypoxia by: 1) monitoring dietary intake during an altitude expedition up to highest European lodge, 2) verifying different adaptive physiological responses to a 4-days high altitude trekking in body composition and appetite/satiety signaling, 3) whether there were any sex differences in responses, and 3) providing perspectives on the use of novel wearable technologies to monitor salt and water balance.

Methods

Study design and participants

The project “Monte Rosa Exploration & Physiology” was carried out from August 29 to September 2, 2021, in Western Alps, Italy. A group of 15 healthy expeditioners (8 males and 7 females, age range: 24-to-63 years) performed a trek from Alagna Valsesia up to the highest European mountain lodge, i.e. Capanna Regina Margherita. Figure 1 shows the travel map. Participants were tested at low altitude (1,164 m, Alagna Valsesia) and at high altitude (4,556 m, Capanna Margherita). Climate conditions were evaluated and elsewhere reported, along with other results (Bondi et al., 2023). All participants signed an informed consent; the study was an ancillary project of wider studies approved by the local ethics committee (*Comitato Etico delle Province di Chieti e Pescara*, protocol n. 18, 29 July 2021).

AMS was verified with the Lake Louise AMS score, diagnosing AMS with ≥3 points in the total score and headache ≥1 point, at least 6 h after the ascent (Roach *et al.*, 2018). All participants took once daily half a pill of acetazolamide (125 mg) at 2,370 m and one pill (250 mg) at 3,647 and 4,556 m.

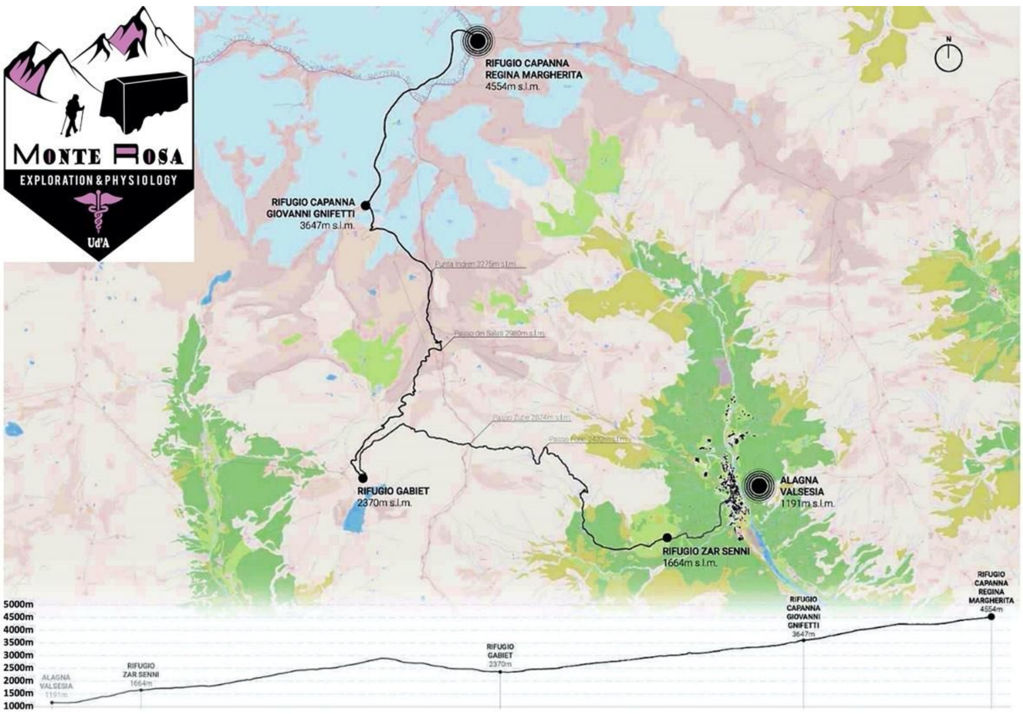

Figure 1. Travelling map of the “Monte Rosa Exploration & Physiology” project. The black line represents the effective trek. The plain data points represent the reached altitude at each day of walking and the name of the mountain lodge. The full biggest circular data points represent the start point (1191 m) at Alagna Valsesia (IT) and the highest altitude (4554 m) reached by participants at the end of the trek**.** Credits: Paolo Belnudo for the map and Antonello Vespoli for the logo (both agreed to publication). UTM coordinates of Capanna Regina Margherita: 5086575 412925 32T.

Procedures

Dietary assessment

The dietary plan was not established before the expedition because the study was conducted with an ecological field design in order to observe the natural relationship between the individual and the environment. Participants were instructed to record their intake of all foods, drinks and supplements in paper diaries structured into three main meals and four snacks during the 3 days of peak ascent. They recorded the time and place of consumption, exact description and quantity of each food and recipes, storage, and cooking methods. The quantities were specified referring to a dedicated picture atlas. In particular, the atlas included a picture series, whose participants pointed to the one that corresponded to the food portion actually consumed, for the determination of portion size (Turrini, Le Donne, et al., 2022). All food diaries were checked by the expert field worker who entered the data after the expedition into an ad hoc web-based software database, Food Consumption Database (FOODCONS). This software allows to code and process the data, transforming each recorded food and recipes into: total amount of food in grams, nutritional profile in terms of energy intake (EI), water, macronutrients (proteins, fat, saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), cholesterol, available and soluble carbohydrates (CHO)), fibres, alcohol, micronutrients (calcium (Ca), phosphorus (P), magnesium (Mn), potassium (K), iron (Fe), zinc (Zn) and vitamins such as vitamin C (vit C), thiamine, riboflavin, niacin, vitamin B6 (vit B6), vitamin B12 (vit B12), vitamin D (Vit D), vitamin E (Vit E), vitamin K (Vit K), retinol, vitamin A (expressed as retinol equivalents or REs)). The software allows clustering the intakes of 16 food categories and 90 food sub-categories.

The software FOODCONS and all related instruments (food composition database, the hard-copy food diaries, the picture atlas) were provided by the "Research Center for Food and Nutrition of Council for Agricultural Research and Economics" (CREA—Food and Nutrition) (https: //www.crea.gov.it/en/web/alimenti-e-nutrizione), developed for use in food consumption survey in Italy and adapted for this study. Energy, macronutrients and micronutrient intake were compared to the guidelines for specific physical activities of the "International Society of Sports Nutrition (ISSN)' (Kerksick et al., 2018) and of the 'Academy of Nutrition and Dietetics (Academy), Dietitians of Canada (DC), and the American College of Sports Medicine (ACSM)' (Thomas et al., 2016), and to the Recommended Daily Allowances (RDA) of nutrient intake for the general population (SINU, 2014).

Total Daily Energy Expenditure (TDEE) prediction

The Resting Energy Expenditure (REE) was calculated using the Harris-Benedict Equations (Harris & Benedict, 1918). The Total Daily Energy Expenditure (TDEE) was estimated considering the level of physical activity for pronounced muscle engagement (SINU, 2014), and was compared to the TDEE predicted using the Metabolic Equivalent for Task (MET), referred to hiking with a backpack (MET=7.8) as reported in the 2011 Compendium of Physical Activities (Ainsworth et al., 2011), for the duration of the activity in hours (minimum 2 hours, maximum 5 hours per day), and to the resting condition of MET=1.1 for the non-trek hours.

Body composition assessment - Anthropometry

Anthropometry was conducted by measuring the weight to the nearest 0.1 kg accuracy, using the Tanita BC-730 digital weighing scales (Tanita Corporation, Tokyo, Japan) properly calibrated, and the height was measured to the nearest 0.1 cm in an upright standing position using the Seca 206 mechanical measuring tape (Seca GmBH & Co Kg, Hamburg, Germany). The waist circumference was measured to the nearest 0.1 cm at the end of normal expiration using the Seca S 201 circumference measuring tape (Seca GmBH & Co Kg, Hamburg, Germany) following the standard procedures (WHO, 2008). Anthropometrics data were used to assess the Body Mass Index (BMI-Kg/h^2) and the Waist-to-Height Ratio (WtHR).

Body composition assessment - Bioelectrical Impedance Analysis (BIA)

Participants were tested for Bioelectrical Impedance Analysis (BIA) 2 times, before the expedition at low altitude (1164 m) and the day after reaching the summit (4556 m). BIA measures the resistance (Impedance – Z) of all tissues to a injected high-frequency (from 5 kHz to 250 kHz), low-amplitude alternating electric current (500 to 800 mA). It is based on electrical properties for which in biological system total body water, thus intra and extra-cellular electrolytic solutions, contributes to the resistance (R), and the integrity of the cell membranes acting as a capacitors contributes to the capacitive resistance (reactance - Xc) (Khalil et al., 2014). The storage of electrical energy on healthy cell membranes causes a delay in the current flow and the lag creates a phase difference between the current and voltage, which is expressed as the Phase Angle (PhA), specifically the arc- tangent value of the ratio of Xc to R, allowing this factor as an indicator of cellular health in terms of electrical integrity of vital cell membrane and cell function (Sardinha, 2018). PhA <5.95° and <5.02°, respectively for young males and females subjects, are useful cut-offs for muscle quality (Akamatsu et al., 2022), up to 9° in ideal physical conditions (Raschka et al., 2020). All these raw parameters, along with variables such as age, stature and weight, are included in the predictive equations provided by the BIA software that allows to estimate: Total Body Water (TBW), Intra and Extracellular Water

(ICW and ECW), Fat Mass (FM), Fat Free Mass (FFM), Fat Mass Index (FMI), Free Fat Mass Index (FFMI), Body Cellular Mass (BCM), Skeletal Muscle mass (SM), Skeletal Muscle mass Index (SMI). The predictive equation was chosen among those included in the software for each subject in each condition based on the bioelectrical values and on the software indication. These tasks were carried out by a specialist. In the current study, electrical parameters were used alternatively to derive raw variables to evaluate cell membrane function and water distribution; specifically, the ratio between Z at higher frequencies and Z at lower frequencies (extracellular water-ECW and intracellular water-ICW), contributing to the Impedance Ratio (IR) (Rinninella et al., 2018) was calculated. To evaluate muscle mass, the Levi's Muscle Index (LMI) (Levi Micheli et al., 2022) was used; the latter is an adjustment of the PhA for the subjects' height by dividing it by the R ((PhA*Height cm)/R 50kHz)), associating the phase variation with the variations of the muscle component, independently from the hydration variations. It has a high relationship with the BCM, and higher values correspond to greater muscle mass.

BIA measurements involve injecting current through electrodes-patch placed on the anterior surface of the skin; the two injector electrodes were located in the metacarpophalangeal and metatarsophalangeal joints; the sensing electrodes were placed at the wrist and ankle. All the measurements were carried out by a specialist using the HUMAN IM TOUCH multi-frequency analyzer (DS MEDICA, Milan, Italy). BIA measurement on the whole body was conducted in a supine position maintained for 5 minutes before the test, with legs and arms slightly abducted (respectively ~ 45° and 30°) to avoid contact between body segments. Any of the standard conditions for the measurements were met (empty bladder, cleaned skin, last beverages consumption and exercise at least 12 h before the measurement, fasting state > 3h (Walter-Kroker et al., 2011). Raw BIA parameters at 50 kHz were considered for the statistical analysis.

Wearable device

An innovative device called Swemax (Biometrica, Parma, Italy), was used to analyse the loss of liquids and salts during the ascent in two female participants. Swemax collects sweat drops during exercise. The technology is based on an electrical measurement, being the current proportional to the saline and liquid concentration. The patch, i.e. biosensor, represents the core of the technology as it collects liquids and salts in real time. The device must be kept in contact with the athlete's body: the bib and the bands have the function of pressing the patch on the skin without creating any disturbance. The device is implemented into a sleeveless garment or a thoracic band, and the biosensor remains in in the dorsal part. Through four pins the patch connects to the reference device, which transmits data into a customised smartphone app. The data are stored in a cloud and analysed through the reference algorithm. The device was specifically used to monitor participants MR2 (female, 24 years, 18.1 kg/m^2, who did not develop AMS at high altitude) and MR5 (female, 25 years, 20.2 kg/m^2, who developed AMS) in two consecutive days during the daily trek. Both participants wore the device before the start of the trekking, both on the third and fourth days of the expedition; the recording then began. The end of the recording was not figured out by the operator or the participants but by the device itself based on recording validity conditions. Valid time records were then analysed by the software associated.

Sample collection

Saliva and urine samples were collected in the early morning in a fasting state before (1164 m) and after reaching the summit (4556 m). Saliva sample was obtained using Salivette collection devices (Sarstedt Nümbrecht); samples were centrifuged for 2 minutes at 1,000×g and stored at –80°C for further analyses. Enzyme-linked immunosorbent assay (ELISA) technique was performed to evaluate salivary leptin (kit cat. No. EH0216; FineTest, Wuhan, China, ghrelin (kit cat. No. EH0355; FineTest, Wuhan, China), and Insulin-like Growth Factor-1 (IGF-1) levels (kit cat. No.EH0165; FineTest, Wuhan, China). The determinations were assessed in duplicate and the concentrations were measured spectrophotometrically at a wavelength of 450 nm by comparing the samples' optical density to standard curves; all the samples and standards were read by a microplate reader spectrophotometer (Infinite M200, Tecan Group Ltd., Männedorf, Switzerland); the inter-assay coefficient of variation was in the range indicated by the manufacturer.

Whole saliva was also collected by passive method, allowing the drooling of saliva into 15 ml conical centrifuge tubes. Urines from participants were collected in sterile containers on the first morning; at low altitude samples were stored in a freezer, at high altitude they were transported in boxes with ice packs down to low altitude; once arrived after some hours at low altitude, all the samples where transported in boxes with ice packs to Chieti for isothermal transport; before the night all the samples were stored at –80°C. The pH of the urine and saliva samples was analysed with XS 8 PRO pHmeter coupled with XS Sensor Micro electrode; the osmolality of each urine and saliva sample has been checked by using a Micro-Osmometer model 3300 (Advanced Instrument Inc., Norwood, MA, US).

Statistical analyses

Statistical comparisons were carried out with the R-based software Jamovi Version 2.3.21.0 (https://www.jamovi.org) and Prism Version 9 (GraphPad Software, San Diego, USA), the latter also used for creating the graphs. Accounting for the homogeneity of participants (i.e., maintaining a narrow range for age), the comparisons were carried out on a total of 12 (6 males and 6 females of age 29.25±4.16 years, within the range 24-to-36 years and similar age between groups, $p=0.558$) out of the whole sample of 15 participants. The assumptions were verified according to the Shapiro-Wilk test for the normality of the residuals, the evaluation of symmetry and kurtosis and the observation of the Q-Q graphs. To compare the whole sample in low vs high altitude, a paired sample t-test was carried out. To include sex as a dependent variable in some analyses, after verifying the assumption of homogeneity of the variances, comparisons with ANOVA for repeated measures were then applied, setting low vs high altitude as the within factor and sex as the between factor; alternatively, a linear mixed model analysis was carried out, setting altitude and sex as fixed factors and individuals as random factor. The level of significance was set for $p<0.05$ and the effect size (Cohen's d: d and partial eta squared: η^2_p) was calculated.

Results

As reported elsewhere (Bondi et al., 2023) AMS was diagnosed in 4 of 12 volunteers at high altitude. Among the 12 volunteers, one female reported abnormal value on bioimpedance analysis at high altitude and was removed post-hoc from the report of body composition analysis. That participant was one out of four who developed AMS.

Dietary profile

All participants completed a food diary and consumed their main meals (breakfast, lunch, and dinner) and snacks. The trained staff instructed all participants to pay attention to their water intake to avoid dehydration.

Table 1 reports the distribution as a percentage of EI, water and macronutrients intake according to the main meals and snacks. The highest energy intake was found at lunch and especially at dinner. Water was consumed especially at dinner and away from the main meals, during the trek (snacks). Protein was consumed similarly between lunch and dinner. Lipids were consumed mostly at dinner in the same percentage of carbohydrates. Carbohydrates were distributed similarly between the main meals, and were consumed in a small percentage during the snacks. Overall, the main meals were composed prevalently of carbohydrates and fats, inversely to the snacks. Alcohol beverages were drunk a little at dinner and even less during snack time as leisure time far from the trek.

Processing of food consumption revealed a mean daily energy intake of 3348 ± 386 kcal in males, and 2804 ± 415 kcal in females, in both cases in the range of the nutritional guidelines for moderate/high intensity training (Tr GL). Water intake was most pronounced in males (3286 ± 409 g/day), in both groups in line with general recommendation for the Italian population, but below the recommendation for training. Table 2 shows the aforementioned intake and the details in terms of grams of macro and micronutrients, in addition to the percentage contribution to the total energy intake. Protein intake represented for male and female respectively 13 and 14% of the total energy intake, specifically 1.5 ± 0.1 g/kg bw and 1.8 ± 0.4 g/kg bw, which matches the nutrient requirement for training. Fat contribution to the total EI was similar in both groups and adequate for the train recommendation (up to 50% of total EI); SFA exceeded a few of the suggested dietary target (SDT) for the general population in both groups (12%) and PUFA intake was far below. Comparing the Available carbohydrates intake with the training guidelines, the intake was below the recommendation in both sexes (respectively 5.4 ± 0.5 g/kg bw and 6.3 ± 1.3 g/kg bw), contributing to 44% and 46% of mean daily EI. Cholesterol intake was lower than the SDT. Fibre intake was greater in females (7.8 ± 1.2 g/1000 kcal/day) than males (6.8 ± 0.4 g/1000 kcal/day) participants but in both cases far from the Reference Intake. The mean alcohol % contribution to the EI was respectively for males and females 8 and 5%. Regarding micronutrients, the intake was compared to the recommended daily allowances for the Italian population (SINU, 2014). A higher intake than the recommendation stood out for potassium, phosphorus, magnesium, iron and zinc in both sexes. Only calcium intake was below the recommendation (<1000 mg/day). From this comparison also emerged that the intake of some vitamins was very lower than the recommended levels, such as vitamin D, in both sexes. The other vitamins intake provided by the FOODCONS computation were plenty above the recommendation (except for Vitamin A that was slightly below the Population Reference Intake). Details about the food categories consumed are reported in the supplementary materials. Energy intake by participants was adequate even if compared with the predictive equations for TDEE (Table 3), and considering the MET for trek. To support the TDEE predictions, we used the data registered from heart rate (HR) series during the trek in three female participants and reported elsewhere (Sbrollini et al., 2022) to obtain a prediction of EE from HR during submaximal exercise, as reported in (Keytel et al., 2005) for females:

$$EE\ (KJ/min) = -20.4022 + 0.4472 \times HR + 0.1263 \times weight + 0.074 \times age$$

We compared this prediction with the calculation based on the value of MET=7.8, thus obtaining very similar results: the percent differences of the energy expenditure during the trek in the three participants were 5.83%, 3.74%, and 0.78%.

Table 1. Percentage of energy, water, macronutrient and alcohol intake between main meals and snacks in the trekkers group, as obtained by food diaries.

	Breakfast	Lunch	Dinner	Snacks
Energy Intake %	18	27	41	14
Water %	15	18	36	31
Proteins %	2	5	6	1
Lipids %	5	9	15	6

Carbohydrates %	12	14	15	5
Alcohol%	0	0	5	1

Table 2. Data of the energy and nutrient intakes for male and female participants expressed as mean ± SD and percentage and as percentage contribution to the total energy intake, according to nutritional guidelines for moderate/high intensity training (Tr GL) and recommended daily intake for Italian population (It RDA).

	Male (n=6)		**Female (n=6)**		**Tr GL**[1,2,3]	**It RDA**[4]
	Mean	*SD**	*Mean*	*SD**		
Energy Intake_EI (MJ)	14	2	12	2		
Energy Intake_EI (kcal)	3348	386	2804	415	2000–7000	
Energy Intake (g/kg bw/day)	46	5	51	11	40-70[(1)]	
Water (g/day)	3286	409	2760	546	4000-5000[(2)]	2500[(AI)]
Protein (g/day)	110	9	99	18		63/54[(PRI)]
Protein (g/kg bw/day)	1.5	0.1	1.8	0.4	1.2 – 2.0[(1-2)]	0.9[(PRI)]
Fat (g/day)	130	19	108	18		
SFA (g/day)	43	7	39	9		
MUFA(g/day)	51	8	44	7		
PUFA (g/day)	23	6	16	2		
Cholesterol (mg/day)	237	49	200	40		<300[(SDT)]
Available carbohydrate (g/day)	390	37	343	47		
Available carbohydrate (g/kg bw/day)	5.4	0.5	6.3	1.3	8[(1)]–12[(2)]	
Simple carbohydrate (g)	116	32	107	35		
Dietary fibre (g/day)	23	3	22	3		>25[(SDT)]
Dietary fibre (g/1000 Kcal/day)	6.8	0.4	8.1	1.1		12.6-16.7[(RI)]
Alcohol (g/day)	39	13	20	13		
% Total energy from	**Male (n=6)**		**Female (n=6)**		**Tr GL**[1,2,3]	**It RDA**[4]
	Mean	*SD**	*Mean*	*SD**		
Protein (% EI)	13	0.5	14	0.7		
Fat (%EI)	35	2.2	35	2.3	up to 50[(1)]	≤30[(RI)]
SFA (%EI)	12	0.8	12	1.6		<10[(SDT)]
PUFA (%EI)	1	0	1	0	pRDA°	5-10[(RI)]
Available carbohydrate (%EI)	44	2.8	46	2.9		45-60[(RI)]
Simple carbohydrate (%EI)	13	2.8	14	3.0		<15[(SDT)]
Alcohol (%EI)	8	1.8	5	3.0		

Minerals	**Male (n=6)**		**Female (n=6)**		**Tr GL**[1,2,3]	**It RDA**[4]
	Mean	*SD**	*Mean*	*SD**		
Potassium (mg/day)	3920	573	4003	609	pRDA°	3900 (AI)
Phosphorus (mg/day)	1490	307	1573	270	pRDA°	700 (PRI)
Calcium (mg/day)	885	324	887	245	pRDA°	1000 (PRI)
Magnesium (mg/day)	390	38	417	67	pRDA°	240 (PRI)
Iron (mg/day)	14	3	16	3	Up to 70% of requirement	10 (PRI)
Zinc (mg/day)	13	2	14	2	pRDA°	12 (PRI)
Vitamins	**Male (n=6)**		**Female (n=6)**		**Tr GL**[1,2,3]	**It RDA**[4]
	Mean	*SD**	*Mean*	*SD**		
Thiamine (mg/day)	2	0	2	0	pRDA°	1.2 (PRI)
Riboflavin (mg/day)	2	1	2	1	pRDA°	1.6 (PRI)
Niacin (mg/day)	25	4	27	5	pRDA°	18 (PRI)
Vitamin C (mg/day)	127	35	147	45	pRDA°	105 (PRI)
Vitamin B6 (mg/day)	3	1	3	1	pRDA°	1.3 (PRI)
Vitamin A (REs µg/day)[§]	686	151	694	137	pRDA°	700 (PRI)
β-carotene (µg/day)	2528	459	2495	563		
Vitamin K (µg /day)	369	227	385	194	pRDA°	140 (AI)
Vitamin E (mg/day)	14	3	16	3	pRDA°	13 (AI)
Vitamin D (µg/day)	1	0	1	0	pRDA°	15 (AI)
Vitamin B12 (µg/day)	5	2	6	1	pRDA°	2.4 (PRI)

SD: standard deviation; bw: body weight
° Recommended daily allowances of nutrient intake for the general population (*SINU, 2014*)
§Vitamin A expressed as retinol equivalents (REs): 1 retinol equivalent (RE) = 1 µg retinol = 6 µg β-carotene
AI – Adequate Intake
AR- Average Requirement
PRI - Population Recommended Intake
RI- Reference intake range for nutrients
SDT- Suggested Dietary Targets
1 (Kerksick et al., 2018); 2 (Thomas et al., 2016); 3 (Carlsohn, 2016); 4 (SINU, 2014)

Table 3. Predicted energy expenditure and energy intake (EI) assessed by food diaries.

Code	**Sex**	**REE**	**TDEE Larn***	**TDEE MET**		**EI**		**Mean EI/kg body weight**
				Min	*Max*	*Min*	*Max*	
MR1	M	1719	3455	2850	4290	2530	4015	43
MR2	F	1342	2322	2226	3350	2692	3546	60
MR3	M	1661	3338	2753	4145	3009	3890	48
MR4	M	1788	3593	2964	4461	2963	4386	46

MR5	F	1424	2862	2217	3337	2611	4240	60
MR8	F	1421	2458	2356	3546	1932	2565	35
MR9	M	1740	3498	2886	4344	2926	4565	53
MR10	F	1308	2263	2169	3265	2427	3795	61
MR11	M	1867	3752	3096	4659	2897	3921	47
MR12	F	1324	2291	2196	3305	2501	3055	51
MR13	F	1392	2408	2308	3474	2052	2767	41
MR15	M	1766	3550	2929	4409	1814	4030	39

Data are expressed as Kcal. REE: Resting Energy Expenditure calculated using the Harris-Benedict equation (Harris & Benedict, 1918); TDEE: Total Daily Energy Expenditure; MET: Metabolic Equivalent of Task for trek hiking backpack; EI: Energy Intake. In the columns of TDEE MET, Min refers to 2 h/day of trek, and Max to 5h/day of trek, computing MET=1.1 for non-trek hours and MET=7.8 for hiking (Ainsworth et al. 2011).

**Larn: Livelli di Assunzione Raccomandata dei nutrienti per la popolazione italiana (translation: Recommended nutrient intake levels for the Italian population; SINU, 2014)*

Body composition

Table 4 shows the mean of anthropometrics data and indices in both sexes. Anthropometric assessment revealed statistically significant changes of body weight before and after the trekking (p=0.005, d=1.009). As shown in Figure 2, at high altitude BMI slightly decreased, on average from 21.65±1.82 to 21.42±1.63 (p=0.005, η^2_p=0.557), similarly in both females and males (p=0.312, η^2_p=0.102). Parallelly, waist-to-height ratio decreased at high altitude, despite over the threshold for statistical significance, on average from 0.45±0.02 to 0.44±0.03 (p=0.059, η^2_p=0.313), similarly in both females and males (p=0.573, η^2_p=0.033).

Table 4. Comparison of mean values of the anthropometric measure and indices. The p-values in the last column refer to the comparison between low and high altitude through a series of paired-samples t-tests

	LA (1164 m)		**HA (4556 m)**		**LA vs HA**
	M (n=6)	**F (n=6)**	**M (n=6)**	**F (n=6)**	
Weight (kg)	72.5 ± 2.7	55.4 ± 4.5	71.5 ± 2.4	54.9 ± 4.2	p=0.005
Height (cm)	179 ± 4	164 ± 3			
BMI (kg/m^2)	22.6 ± 0.9	20.7 ± 2.1	22.3 ± 0.8	20.6 ± 2.0	p=0.005
Waist girth (cm)	84 ± 1	70 ± 2	83 ± 2	69 ± 4	p=0.029
WtHR	0.47 ± 0.01	0.43 ± 0.01	0.46 ± 0.02	0.42 ± 0.02	p=0.050

Data are expressed as mean ± standard deviation. LA: low altitude; HA: high altitude; M: male; F: female; BMI: body mass index; WtHR: waist-to-height ratio.

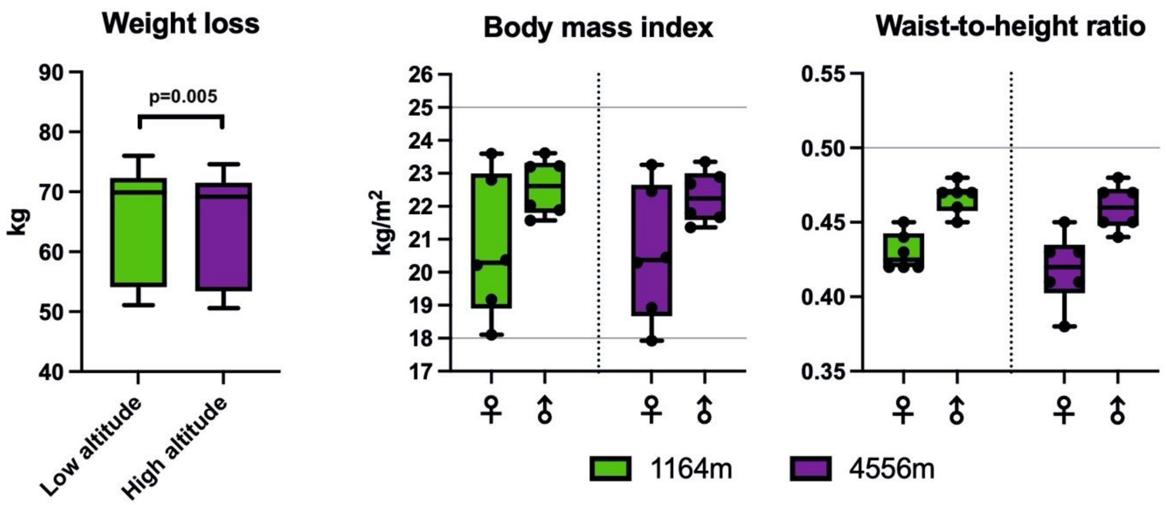

Figure 2. Box (quartiles) and whiskers (min to max) plots of weight loss, body mass index (BMI) and Waist-to-height ratio (WtHR) differences at low and high altitude. ***p<0.001

PhA value highlighted healthy muscle quality in both sexes, and a trend on increase in PhA (p=0.093, d=0.560), confirmed for male participants (p=0.059, d=0.991), was found. No differences of reactance and resistance normalized by height and an increase of LMI (p=0.032, d=0.753) were observed, but not confirmed after grouping by sex. Table 5 shows the mean value of raw BIA parameters (R, Xc, PhA) and derived variables (IR and LMI).

Total body water did not change at high altitude, but the TBW% did (p=0.019, d=0.839). The absolute values of fat mass were slightly lower at high altitude (p=0.071, d=0.610), and a similar trend was found to a lower extent for fat free mass (p=0.093, d=0.559). Table 6 shows the mean value of body composition predicted by BIA equations. The two-way analysis revealed significant differences between low and high altitude were found for TBW/weight% (p=0.026, η^2_p=0.440), not confirmed looking at liters of TBW (p=0.403, η^2_p=0.079) and in terms of ECW% and ICW% on TBW (respectively p=0.170, η^2_p=0.198 and p=0.296, η^2_p=0.120), suggesting the dehydration process did not occur during acute exposure to high altitude (see also Figure 3). No statistically significant changes were found for FM/weight%, FFM (kg), FMI, FFMI, BCM/FFM%, SM/FFM%, and SMI. Figure 4 shows the comparison of FFM and FM/Weight% before and after the expedition.

Table 5. Raw Bioimpedance parameters and derived raw variables before and after the expedition in both group of participants. The p-values in the last column refer to the comparison between low and high altitude through a series of paired-samples t-tests

	LA (1164 m)		HA (4556 m)		LA vs HA
	M (n=6)	F (n=5)	M (n=6)	F (n=5)	

Xc/h (Ω)	36 ± 2	38 ± 2	38 ± 3	38± 2	p=0.308
R/h (Ω)	308 ± 15	386 ± 45	309 ± 11	379 ± 41	p=0.586
PhA (degree)	6.7 ± 0.4	5.7 ± 0.5	6.9 ± 0.5	5.8± 0.5	p=0.093
IR (kHz)	0.8 ± 0.02	0.8± 0.02	0.7 ± 0.04	0.8 ± 0.01	p=0.102
LMI (◦·cm·Ω^{-1})	2.2 ± 0.2	1.5 ± 0.3	2.2 ± 0.2	1.5 ± 0.3	p=0.032

Data are expressed as mean ± standard deviation. LA: low altitude; HA: high altitude; M: male; F: female; Xc/h: capacitive reactance/height; R/H: resistance/height; PhA: phase angle; IR: impedance ratio; LMI: Levi's muscle index (Levi Micheli et al., 2022).

Table 6. Results of body composition by predictive BIA equations. The p-values in the last column refer to the comparison between low and high altitude through a series of paired-samples t-tests

	LA (1164 m)		**HA (4556 m)**		**LA vs HA**
	M (n=6)	**F (n=5)**	**M (n=6)**	**F (n=5)**	
Weight	72.5 ± 2.7	56.0 ± 4.8	71.5 ± 2.4	55.3 ± 4.5	p=0.001
TBW (L)	41.3 ± 1.9	30.5 ± 2.1	41.4 ± 1.2	30.8 ± 2.1	p=0.390
TBW/weight (%)	54.1 ± 1.5	52.3± 1.8	55.1 ± 2.0	53.6 ± 1.4	p=0.019
ECW/TBW (%)	37.8± 0.8	38.8 ± 1.0	36.9 ± 1.3	39.0 ± 1.4	p=0.172
ICW/TBW (%)	62.3 ± 0.8	61.3 ± 1.1	63.1 ± 1.3	61.1 ± 1.4	p=0.280
ECW/ICW (%)	60.6± 2.1	63.5 ± 1	58.5 ± 3.1	63.9 ± 3.7	p=0.576
FM/weight (%)	19.2± 1.1	23.7± 3.4	18.8± 1.6	23.4± 3.1	p=0.288
FM (kg)	13.9 ± 1.0	13.4 ± 3.1	13.4 ± 1.3	13.0 ± 2.7	p=0.071
FFM (kg)	58.6± 2.2	42.6± 1.8	58.1± 2.1	42.3± 2.2	p=0.093
FMI	4.4± 0.4	5.0± 1.3	4.2± 0.5	4.8± 1.1	p=0.047
FFMI	18.2± 0.5	15.8± 1.1	18.1± 0.3	15.7± 1.2	p=0.221
BCM/FFM (%)	55.5± 1.8	51.1± 2.5	56.7± 2	51.4± 2.4	p=0.106
SM/FFM (%)	51.6± 0.9	47.5± 2.6	51.7± 0.7	48.5± 1.9	p=0.120
SMI	9.4± 0.4	7.5± 0.9	9.4± 0.3	7.6± 0.8	p=0.818

LA: low altitude; HA: high altitude; M: male; F: female; TBW: total body water); ECW: extracellular water; ICW (intracellular water); FM: fat mass; FFM: fat free mass; FMI: fat mass index, FFMI: free fat mass index, BCM: body cellular mass; skeletal muscle mass (SM), skeletal muscle mass index (SMI).

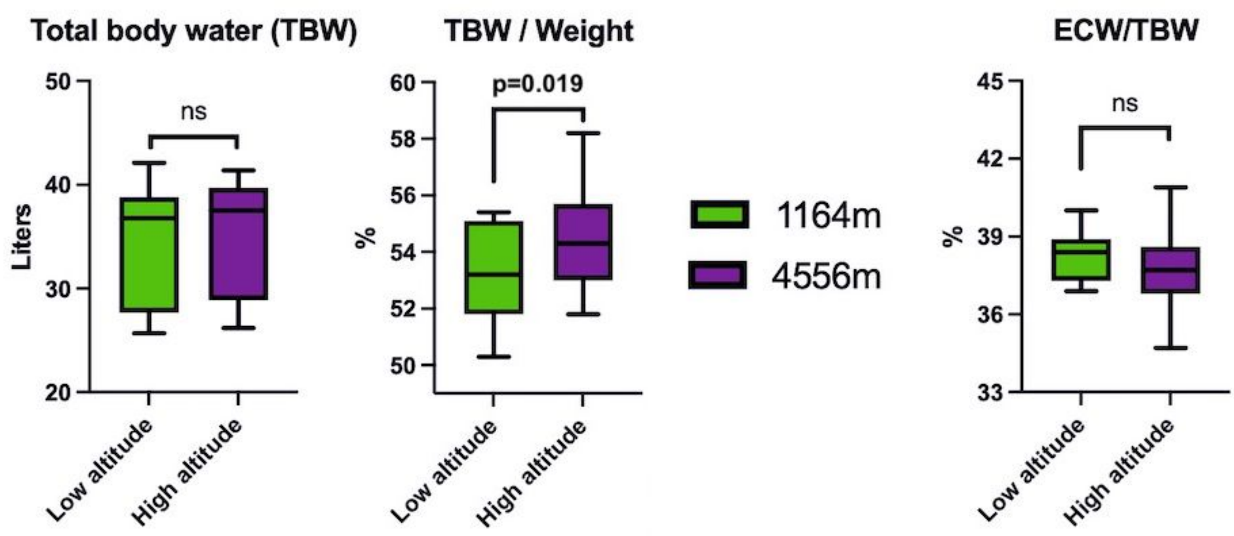

Figure 3. Box (quartiles) and whiskers (min to max) plots of absolute liters of total body water (TBW), TBW/weight % and extracellular water (ECW)/TBW at low and high altitude, derived by Bioelectrical Impedance Analysis regression equations. Urinary and salivary osmolality are shown. ns: non-significant

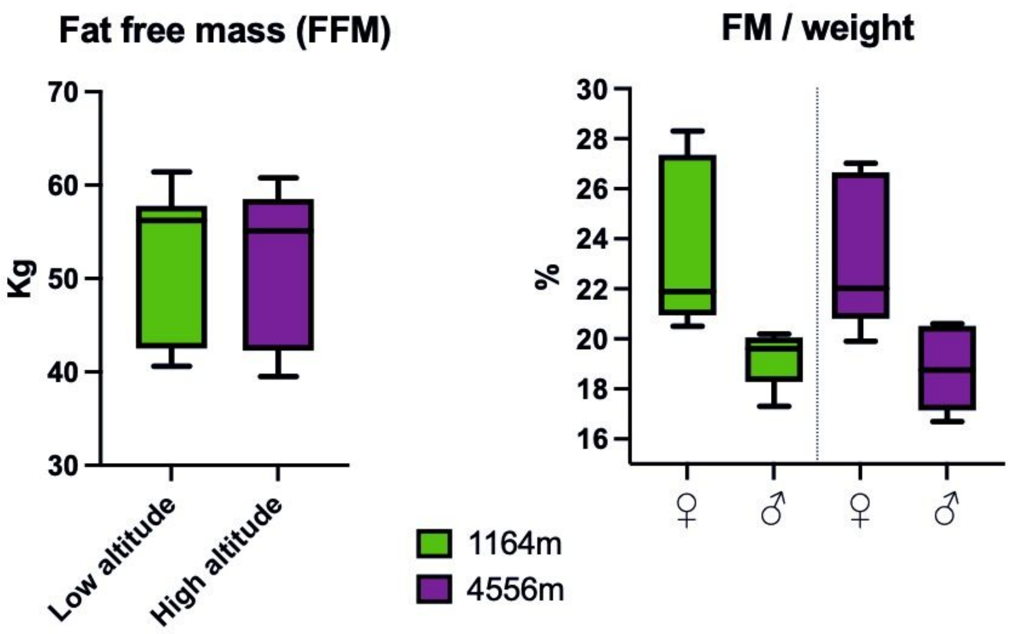

Figure 4. Box (quartiles) and whiskers (min to max) plots of absolute Fat free mass (FFM) and % of Fat mass (FM)/weight data at low and high altitude, derived by Bioelectrical Impedance Analysis regression equations.

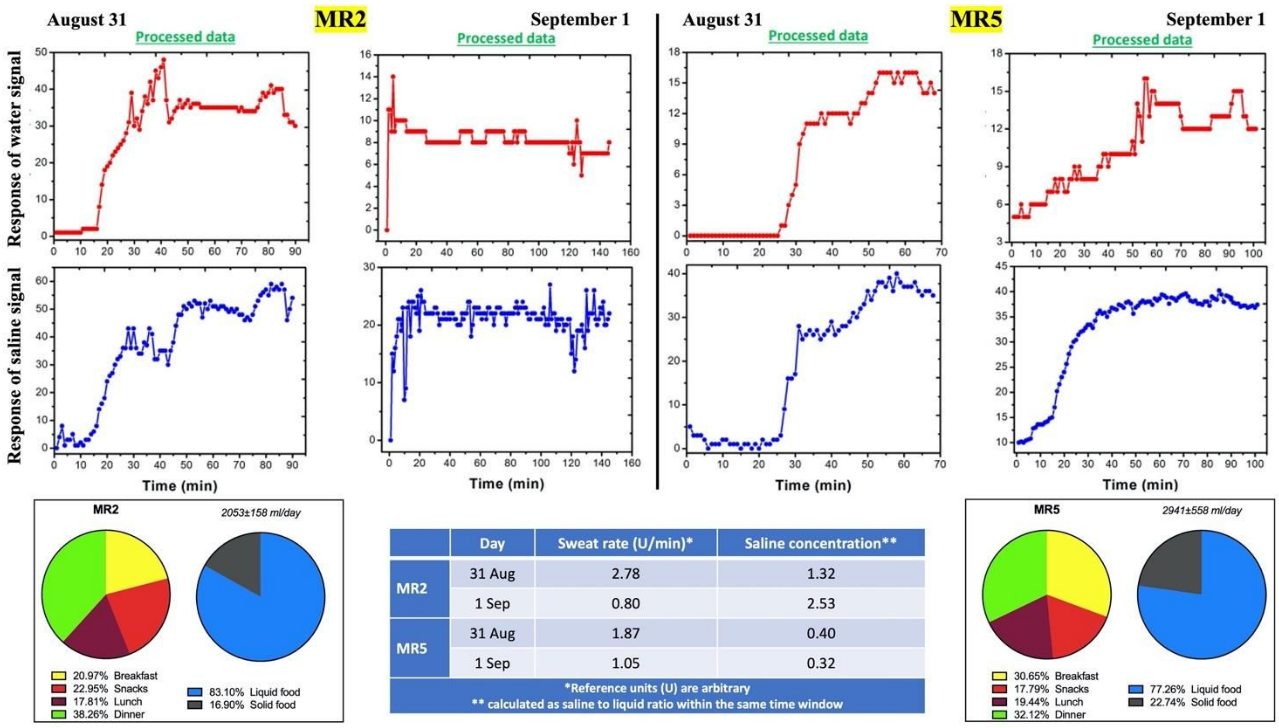

Figure 5. Sweat dynamics assessment of 2 female participants by wearable sensors in terms of response of water and saline signals, and sweat rate (U/min) and saline concentration. Mean water intake and % of meals and food water distribution. Credits: "Biometrica" company for the original graphs of processed data (authors: MB, PLR, DS, RM, TB).

Fluid monitoring on two female participants revealed an increased sweat production followed by regular fluid and salts excretion, and a reduction in sweat rate was observed in both participants at higher altitude (Figure 5). The variability of saline concentration trend after ascent was noted. The fluid intake from liquid and solid sources and the distribution of water in meals obtained from the analysis of food diaries was also shown. The differences in the duration of time records were due to the challenging environmental conditions.

Hormonal salivary analysis - Leptin, ghrelin, Insulin-like Growth Factor-1

At high altitude salivary leptin increased (p=0.014, η^2_p=0.510), with a greater, although non statistically significant, increase in males (time × sex, p=0.125, η^2_p=0.241). Ghrelin decreased at high altitude (p=0.036, η^2_p=0.403), similarly in both females and males were observed. No robust changes in IGF-1 were observed (p=0.198, η^2_p=0.250) (Figure 6).

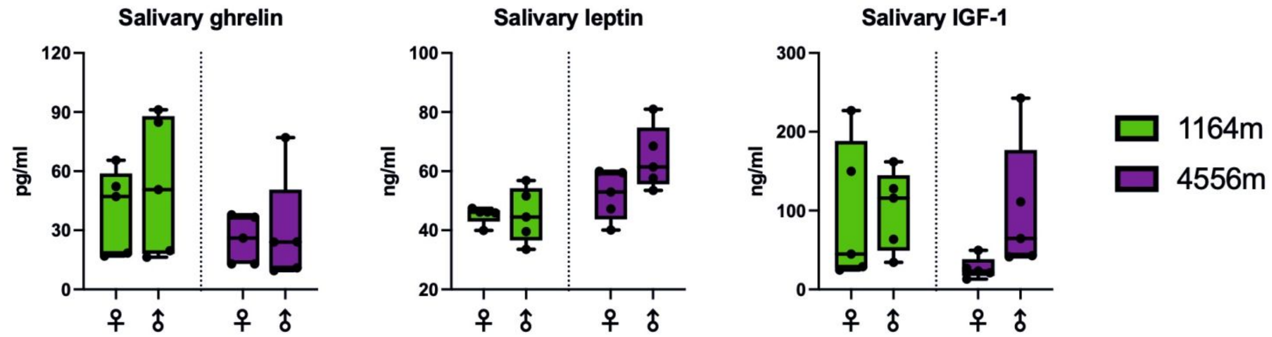

Figure 6. Box (quartiles) and whiskers (min to max) plots of salivary hormones' data at low and high altitude in male and female participants.

Salivary pH remained similar when comparing low vs high altitude (p=0.709, η^2_p=0.021). Salivary osmolality also did not change at high altitude (p=0.581, η^2_p=0.046), although a slight tendency for a differential response in males could be observed (time × sex, p=0.171, η^2_p=0.250). Data on urine samples confirmed no difference in biological fluid osmolality at high altitude (p=0.499, η^2_p=0.052). Salivary and urinary osmolality results confirmed dehydration did not occur. Figure 7 shows the data distribution in male and female participants.

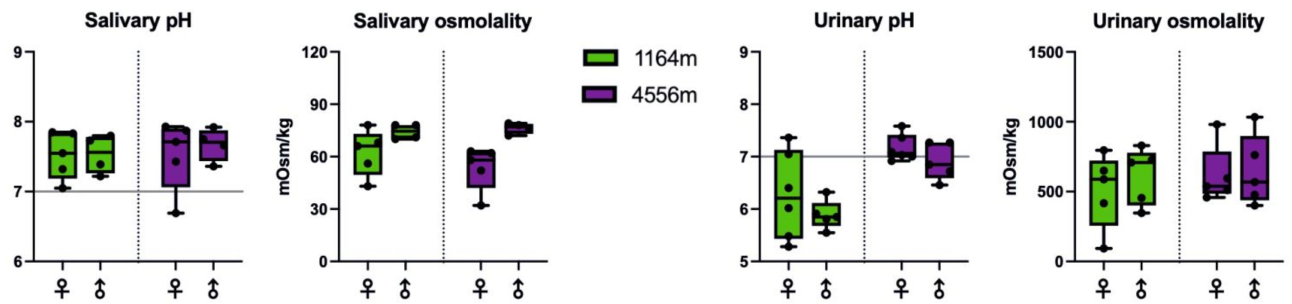

Figure 7. Salivary and urinary pH and osmolality before and after the trekking assessed in male and female participants.

Discussion

Mountaineering has become a popular exploration and sporting activity, with an increasing number of amateur mountaineers attempting ascents of the highest peaks. The degree and duration of altitude exposure have a strong influence on expeditions; thus, acclimatization is a current physiological challenge. Signals stimulating ventilation and sympathetic activation, as well as impairment of fuel substrate utilization occur during exposure to hypobaric hypoxia (Kara et al., 2003). A metabolic shift seems to promote glycolysis and gluconeogenesis as an adaptive response to low oxygen availability, leading to increased lactate uptake and ATP wasting after glucose conversion, thus resulting in inefficient energy production after prolonged exposure (Palmer & Clegg, 2014). The main driver of these adaptations at the molecular level is the Hypoxia-Inducible Factor (HIF) which transactivates genes thus triggering the adaptive response to limited oxygen availability. Specifically, the HIF acts at several levels, by 1) regulating key metabolic enzymes (pyruvate dehydrogenase kinase 1 (PDK1), lactate dehydrogenase A (LDHA), and glycogen phosphorylase L (PYGL)), and 2) playing a pivotal role in the hypothalamic nutrient and hormonal sensor function, regulating leptin gene expression and appetite (Gaspar & Velloso, 2018). The hypothalamic regulation of energy expenditure is a complex integrated process and the effect of hypoxia on leptin secretion is highly controversial (Sierra-Johnson et al., 2008). Leptin is an adipocyte secretory product, directly proportional to the amount of body fat (Perakakis et al., 2021), and possibly involved in triggering reduced appetite and food intake occurring at high altitudes. Previous studies showed leptin increase under acute exposure to hypoxia (24 h) independently of nutritional intake and induced higher leptin secretion after acclimatization to altitude independently from the body weight changes (Snyder et al., 2008). Other studies reported decreased leptin levels regardless of weight changes (Lippl et al., 2010).

In this study, analysis on salivary samples, a reliable alternative non-invasive tool for measuring blood biomarkers (Abdalla & Choo, 2018), confirmed a rise in leptin under acute high altitude hypoxia exposure. This increase was not due to a weight gain, as our participants lost significant amounts of weight. It was also unlikely to be due to physical exercise, as decreased or unchanged leptin concentrations have been reported in literature as affected by physical exercise (Azizi, 2012; Bouassida et al., 2006; Murawska-Ciałowicz et al., 2022). Indeed, the leptin gene contains HIF response elements providing a molecular mechanism for increased leptin gene expression under conditions of hypoxia (Rausch et al., 2018). Furthermore, anorectic effects and increasing thermogenesis and energy expenditure are provided by the HIF response elements located at POMC (proopiomelanocortin) gene (Gaspar & Velloso, 2018). In addition to leptin, the hypoxia-induced anorexia is possibly mediated by ghrelin, which has been observed to be reduced after 7 days of exposure at 4,300 m and 5,200 m (Benso et al., 2007; Shukla et al., 2005). Our results agree with this finding, confirming a reduction in salivary ghrelin after 4 days of high-altitude exposure. Based on other evidence reported in the literature, it is unlikely that physical activity during hypoxic exposure had an additive influence on hormonal markers of appetite (Debevec, 2017). Appetite dysregulation disrupts energy balance and may be a driver of body weight loss and related body composition changes observed during mountain travel. Our insights add to the body of knowledge about hormonal appetite regulation via ghrelin and leptin following altitude hypoxia exposure (Debevec, 2017).

A variety of studies have reported high-altitude-induced weight loss as a result of 1) negative energy balance (reduced energy intake and increased energy expenditure), 2) inadequate water intake with exacerbated water losses (via expiration and skin perspiration), and 3) gastrointestinal distress and malabsorption (Dünnwald et al., 2019). Furthermore, due to expedition-related logistical challenges such as carried load and lack of palatable food, energy intake can be as low as 50–70% of the daily requirements; such a reduction does not correspond to either the increase in basal metabolic rate (10–28% at 4000–6000 m) - secondary to the adaptation of breathing and thermoregulation - or the increase in energy expenditure (Viscor et al., 2023). High altitude sports require to produce effort in harsh environments and can be related to as high-volume training, whose intensity depends on the daily trek and the altitude (2–3/3–6 h per day performed 5–6 times per week). The caloric needs may range between 40–70 kcal/kg bw/day (2000–7000 kcal/day for a 50–100 kg athlete) (Kerksick et al., 2018); carbohydrates requirement for athletes involved in high-volume training may approach 8–10 g/kg bw/day (Kerksick et al., 2018), until 12 g/kg bw/day (Thomas et al., 2016). Optimal protein intake necessary to maintain muscle mass, supporting metabolic adaptation to training, repair and remodelling processes, and for protein turnover generally ranges from 1.2 to 2.0 g/kg/day up to 2 g/kg bw/day (Thomas et al., 2016), to prevent loss of fat-free mass (FFM), also included in the ISSN guidelines which report a range of 1.7–2.2 g/kg bw/day (Kerksick et al., 2018). Regarding fat intake, it should be in line with public health guidelines (Kerksick et al., 2018; Thomas et al., 2016), up to 50% of daily calories safely ingested by athletes during regular high-volume training (Kerksick et al., 2018). Our results showed that the energy intake was adequate to training nutritional guidelines in both male and female participants (respectively, 3348 ± 386 kcal and 2804 ± 415 kcal), supporting the predicted TDEE. Furthermore, matched protein intake to the dietary guidelines was found in both sexes (respectively, 1.5 ± 0.1 g/bw/day and 1.8 ± 0.4 g/bw/day). Dietary macronutrient content is of particular importance for the maintenance of optimal body mass and composition. According to previous studies, the weight and body composition changes observed during expeditions are a mixture of water, fat and free fat mass losses (Wing-Gaia, 2014). In this context, the most affected is the FFM that represents even more than 60% of the weight lost, compromising the muscle performance and the adaptations to extreme environments (Wandrag et al., 2017). The weight loss observed in this study could not be attributed to a specific body compartment. In fact, differences in absolute body water were negligible and only minor changes in estimated mass were observed using BIA-specific equations. In fact, although a statistical trend in net fat mass and net fat-free mass loss was observed, the weight loss cannot be completely attributed to one compartment because: compartment-specific loss was not homogeneous among participants, and fat mass and lean mass obtained from BIA are based on estimates. However, the average estimated losses of fat-free mass (0.41 kg) and fat mass (0.46 kg) are not trivial for a 4-day expedition and deserves further study. Overall, the results suggest that participants may experience a reduction in fat mass and, to a lesser percent extent, lean mass following a 4-day high-altitude trek. Previous research has found that muscle mass loss is related to low initial body fat percentages (Ocobock, 2017). In this study, both male and female participants had an initial body fat percentage in the good ranges (19.2± 1.1 and 23.7± 3.4), which may have a protective effect on lean body mass loss. Indeed, the minimum healthy required amount of fat is around 4–8% in males and 8–19% in females to meet physiological processes (Ocobock, 2017). It should be considered that fat and fat-free mass as estimated by BIA may result in changes when some assumptions are violated (hydration status, voided bladder, food intake, exercise). Although very small (Randhawa et al., 2021), these changes may have determined in our study, together with the inter-individual differences in the bodily response, the absence of notable changes in the different body compartments. Interestingly, looking at the PhA, which is independent of estimations unlike other BIA parameters, a value of 6.7 ± 0.4 degree in males and 5.7 ± 0.5 degree in females demonstrated a healthy quality of muscle mass; usually, PhA decreases when muscle atrophy occurs; PhA did not decrease at high altitude, thus suggesting the FFM loss did not occur in our study. Moreover, the PhA can be interpreted as an indicator of membrane integrity and intra- and extracellular water distribution (Marini et al., 2020). Further investigations on raw BIA parameters as indicators of mass changes at altitude should be considered. Carefully looking at estimated body water, differences were found in terms of % of TBW/Weight after the expedition, not confirmed in terms of absolute liters of water, and no significant differences in the water cellular distributions were observed. In our study, participants were informed about the risk of dehydration and consumed an amount of water adequate to the recommendation for a healthy population (SINU, 2014); however, water intake was below the specific recommendation for endurance training (Thomas et al., 2016). We hypothesize that the dehydration process was not pronounced as a result of short-term exposure, and unchanged salivary and urinary osmolality as hydration biomarkers supported these results.

Hydrosaline dynamics were also observed through the wearable device SWEMAX. In the first place, the feasibility was successful in terms of comfort, anatomical location, and adhesion. The two case reports indicated a great and partially unexpected inter-individual difference, both in the absolute sweat rate and saline concentration, and in the longitudinal changes across two days of trekking. This opens new intriguing perspectives for monitoring expeditioners and athletes by means of wearable devices and detects early predictors, if any, of further health threats. Recommendations for sweat monitoring while doing physical activity and sports include controlling of background contamination, patch saturation and skin contamination (Seshadri et al., 2019). Longitudinal monitoring of participants' sweating should be combined, whenever possible, with nutritional data, micturition analysis, and meteorological data, thus providing a comprehensive picture of integrated physiological systems and external factors. Sweat sensing prototypes are currently undergoing a transition to smartphone-based data storage and real-time analysis, multiplexed biophysical and biochemical sensor arrays, engineered estimation techniques, and trace biomarker sensors (Saha et al., 2022). Furthermore, researchers and practitioners will benefit from developing algorithms to accurately estimate water and saline loss from raw electrical data collected during field studies, as well as validating sweat-borne biomarker levels and biophysical parameters with physiological state transition.

Limitations

We cannot rule out the possibility that the diuresis caused by acetazolamide has biased the fluid balance results. Therefore, future studies evaluating body composition changes during high-altitude treks for may include a micturition diary to control for this variable. Considering the fact that we analysed data from 11 participants, of which only 3 who developed AMS at high altitude, we were unable to make robust statistical comparisons to evaluate the association of body water changes and AMS (Biollaz et al., 2021; Gatterer et al., 2013). Because the SWEMAX sensor has yet to be validated in comparison to a gold standard for sweating analysis during field studies, only speculations and perspectives could emerge from that study branch. Another limitation was the lack of a control group that performed the same physical activity and had the same food intake at low altitude, though such a design could produce different results depending on whether the external or internal load was mirrored. With the data from this study, it was not possible to precisely establish whether and to what extent food intake was reduced during the trek because of hormonal changes. Additionally, since a similar evaluation of participants' dietary habits during their regular life prior to the expedition was not conducted, it was not possible to determine any significant variations compared to their usual diet.

Conclusions

This is one of the few studies that investigated nutrition and body composition during a few days of trekking in high-altitude hypoxia. Our findings show that a 4-days trek in hypobaric hypoxia resulted in significant weight loss in both female and male participants that was not attributable to TBW losses; no changes in bioimpedance parameters were observed, but the trend in improvement of PhA can be interpreted as a surrogate for body composition changes at altitude. Despite increased leptin levels and decreased ghrelin levels, dietary intake was found to be within the target range for healthy people and sports activity; although speculatively, these hormones likely changed due to hypoxia. Furthermore, the water intake recommendation may have limited weight loss and dehydration, which are common at altitude. We demonstrated that changes in satiety / appetite hormones can occur at during a short-term (4 days) altitude expeditions; further studies may define whether these changes occur prior and independently to body mass changes.

Feeding at high altitudes by following a pre-planned dietary plan based on recommendation for various sports or other physical activities may encourage body mass preservation, counteracting the possible detrimental effects of hypoxia. Finally, the two participants involved in the sweat study showed inter-individual differences in their sweating response. New analytical and instrumental tools that must be validated in field studies for continuous and thorough sweat assessments under physiologically relevant conditions could yield valuable insights into the physiological reactions of humans to both acute and long-term exposure to harsh environments.

Competing interest statement

Matteo Beccatelli, Pierluigi Lodi Rizzini, Davide Seletti, Rocco Mecca, and Tommaso Beccatelli were employee or collaborators of the company Biometrica S.r.l., Parma, Italy (which provided the device SWEMAX and the technical assistance) while engaged in the research project. The other authors declare there are no competing interests.

Author Contributions

Conceptualization, C.S., D.B.; methodology, C.S., D.B., V.V., S.M-S., S.P., S.M., A.F., R.P., C.L.D., M.B., P.L.R., D.S.; formal analysis, C.S., D.B., S.M-S., S.P., S.M., R.P., C.L.D., M.B., P.L.R., D.S., R.M., T.B.; investigation, C.S., D.B., F.C., S.B., P.P.; resources, S.M-S., A.F., R.P., C.L.D., M.B., P.L.R., D.S., R.M., T.B. V.V.; writing—original draft preparation, C.S., D.B.; writing—review and editing, V.V., S.M-S., F.C., S.B., P.P., T.P., S.P., S.M., A.F., R.P., C.L.D., L.L., M.B., P.L.R., D.S., R.M., T.B.; visualization, C.S., D.B., M.B., P.L.R., D.S., R.M., T.B.; supervision, V.V. and T.P..; project administration, D.B. and V.V.; funding acquisition, L.L. and V.V. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

Authors thank Biometrica S.r.l. (Parma, Italy) for the technical support and constructive discussion of the results obtained. Map and logo design was the work of Antonello Vespoli and Paolo Belnudo.

Funding

This work was supported by University "G. d'Annunzio" of Chieti-Pescara, Italy. In particular, the School of Medicine and Health Sciences supported F.C. and S.B. The funders had no role in the design of the study, data collection, analyses, or interpretation of the data, writing of the manuscript, or in the decision to publish the results.

Data availability statement

The data that support the findings of this study are available from the corresponding author C.S. upon reasonable request.

References

Abdalla, M. M. I., & Choo, S. S. (2018). Salivary Leptin Level in Young Adult Males and its Association with Anthropometric Measurements, Fat Distribution and Muscle Mass. *European Endocrinology*, *14*(2), 94. https://doi.org/10.17925/EE.2018.14.2.94

Ainsworth, B. E., Haskell, W. L., Herrmann, S. D., Meckes, N., Bassett, D. R., Tudor-Locke, C., Greer, J. L., Vezina, J., Whitt-Glover, M. C., & Leon, A. S. (2011). 2011 Compendium of Physical Activities: A Second Update of Codes and MET Values. *Medicine & Science in Sports & Exercise*, *43*(8), 1575–1581. https://doi.org/10.1249/MSS.0b013e31821ece12

Akamatsu, Y., Kusakabe, T., Arai, H., Yamamoto, Y., Nakao, K., Ikeue, K., Ishihara, Y., Tagami, T., Yasoda, A., Ishii, K., & Satoh-Asahara, N. (2022). Phase angle from bioelectrical impedance analysis is a useful indicator of muscle quality. *Journal of Cachexia, Sarcopenia and Muscle*, *13*(1), 180–189. https://doi.org/10.1002/jcsm.12860

Angelucci, A., Cavicchioli, M., Cintorrino, I. A., Lauricella, G., Rossi, C., Strati, S., & Aliverti, A. (2021). Smart Textiles and Sensorized Garments for Physiological Monitoring: A Review of Available Solutions and Techniques. *Sensors (Basel, Switzerland)*, *21*(3), 814. https://doi.org/10.3390/s21030814

Azizi, M. (2012). Serum leptin and ghrelin changes-induced aerobic training in healthy young females. *International Journal of Collaborative Research on Internal Medicine and Public Health*, *4*.

Benso, A., Broglio, F., Aimaretti, G., Lucatello, B., Lanfranco, F., Ghigo, E., & Grottoli, S. (2007). Endocrine and metabolic responses to extreme altitude and physical exercise in climbers. *European Journal of Endocrinology*, *157*(6), 733–740. https://doi.org/10.1530/EJE-07-0355

Biollaz, J., Buclin, T., Hildebrandt, W., Décosterd, L. A., Nussberger, J., Swenson, E. R., & Bärtsch, P. (2021). No renal dysfunction or salt and water retention in acute mountain sickness at 4,559 m among young resting males after passive ascent. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *130*(1), 226–236. https://doi.org/10.1152/japplphysiol.00382.2020

Böhm, A., & Heitmann, B. L. (2013). The use of bioelectrical impedance analysis for body composition in epidemiological studies. *European Journal of Clinical Nutrition*, *67*(S1), S79–S85. https://doi.org/10.1038/ejcn.2012.168

Bondi, D., Aloisi, A. M., Pietrangelo, T., Piccinelli, R., Le Donne, C., Jandova, T., Pieretti, S., Taraborrelli, M., Santangelo, C., Lattanzi, B., & Verratti, V. (2021). Feeding Your Himalayan Expedition: Nutritional Signatures and Body Composition Adaptations of Trekkers and Porters. *Nutrients*, *13*(2), 460. https://doi.org/10.3390/nu13020460

Bondi, D., Lobefalo, L., Ciampini, F., Rapagnani, A., Santangelo, C., Pignatelli, P., Bonan, S., & Verratti, V. (2023). Clinical features and health-threatening conditions of the trek to Capanna Margherita. *The Journal of Sports Medicine and Physical Fitness*, *63*(8). https://doi.org/10.23736/S0022-4707.23.14859-6

Bouassida, A., Zalleg, D., Bouassida, S., Zaouali, M., Feki, Y., Zbidi, A., & Tabka, Z. (2006). Leptin, its Implication in Physical Exercise and Training: A Short Review. *Journal of Sports Science & Medicine*, *5*(2), 172–181.

Carlsohn, A. (2016). Aktuelle Ernährungsempfehlungen für Ausdauersportler. *Deutsche Zeitschrift Für Sportmedizin*, *2016*(01), 7–12. https://doi.org/10.5960/dzsm.2015.193

Castellani, J. W., Muza, S. R., Cheuvront, S. N., Sils, I. V., Fulco, C. S., Kenefick, R. W., Beidleman, B. A., & Sawka, M. N. (2010). Effect of hypohydration and altitude exposure on aerobic exercise performance and acute mountain sickness. *Journal of Applied Physiology*, *109*(6), 1792–1800. https://doi.org/10.1152/japplphysiol.00517.2010

Debevec, T. (2017). Hypoxia-Related Hormonal Appetite Modulation in Humans during Rest and Exercise: Mini Review. *Frontiers in Physiology*, *8*, 366. https://doi.org/10.3389/fphys.2017.00366

Dünnwald, T., Gatterer, H., Faulhaber, M., Arvandi, M., & Schobersberger, W. (2019). Body Composition and Body Weight Changes at Different Altitude Levels: A Systematic Review and Meta-Analysis. *Frontiers in Physiology*, *10*, 430. https://doi.org/10.3389/fphys.2019.00430

Gaspar, J. M., & Velloso, L. A. (2018). Hypoxia Inducible Factor as a Central Regulator of Metabolism – Implications for the Development of Obesity. *Frontiers in Neuroscience*, *12*, 813. https://doi.org/10.3389/fnins.2018.00813

Gatterer, H., Wille, M., Faulhaber, M., Lukaski, H., Melmer, A., Ebenbichler, C., & Burtscher, M. (2013). Association between Body Water Status and Acute Mountain Sickness. *PLoS ONE*, *8*(8), e73185. https://doi.org/10.1371/journal.pone.0073185

Hamad, N., & Travis, S. P. L. (2006). Weight loss at high altitude: Pathophysiology and practical implications. *European Journal of Gastroenterology & Hepatology*, *18*(1), 5–10. https://doi.org/10.1097/00042737-200601000-00002

Harris, J. A., & Benedict, F. G. (1918). A Biometric Study of Human Basal Metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, *4*(12), 370–373.

Hetherington-Rauth, M., Baptista, F., & Sardinha, L. B. (2020). BIA-assessed cellular hydration and muscle performance in youth, adults, and older adults. *Clinical Nutrition (Edinburgh, Scotland)*, *39*(8), 2624–2630. https://doi.org/10.1016/j.clnu.2019.11.040

Kara, T., Narkiewicz, K., & Somers, V. K. (2003). Chemoreflexes - physiology and clinical implications: **Chemoreflexes in health and disease**. *Acta Physiologica Scandinavica*, *177*(3), 377–384. https://doi.org/10.1046/j.1365-201X.2003.01083.x

Kerksick, C. M., Wilborn, C. D., Roberts, M. D., Smith-Ryan, A., Kleiner, S. M., Jäger, R., Collins, R., Cooke, M., Davis, J. N., Galvan, E., Greenwood, M., Lowery, L. M., Wildman, R., Antonio, J., & Kreider, R. B. (2018). ISSN exercise & sports nutrition review update: Research & recommendations. *Journal of the International Society of Sports Nutrition*, *15*(1), 38. https://doi.org/10.1186/s12970-018-0242-y

Keytel, L. R., Goedecke, J. H., Noakes, T. D., Hiiloskorpi, H., Laukkanen, R., van der Merwe, L., & Lambert, E. V. (2005). Prediction of energy expenditure from heart rate monitoring during submaximal exercise. *Journal of Sports Sciences*, *23*(3), 289–297. https://doi.org/10.1080/02640410470001730089

Khalil, S. F., Mohktar, M. S., & Ibrahim, F. (2014). The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. *Sensors (Basel, Switzerland)*, *14*(6), 10895–10928. https://doi.org/10.3390/s140610895

Kyle, U. (2004). Bioelectrical impedance analysis?part I: review of principles and methods. *Clinical Nutrition*, *23*(5), 1226–1243. https://doi.org/10.1016/j.clnu.2004.06.004

Levi Micheli, M., Cannataro, R., Gulisano, M., & Mascherini, G. (2022). Proposal of a New Parameter for Evaluating Muscle Mass in Footballers through Bioimpedance Analysis. *Biology*, *11*(8), 1182. https://doi.org/10.3390/biology11081182

Lippl, F. J., Neubauer, S., Schipfer, S., Lichter, N., Tufman, A., Otto, B., & Fischer, R. (2010). Hypobaric hypoxia causes body weight reduction in obese subjects. *Obesity (Silver Spring, Md.)*, *18*(4), 675–681. https://doi.org/10.1038/oby.2009.509

Marini, E., Matias, C. N., Toselli, S., Sardinha, L. B., & Silva, A. M. (2020). Phase angle and bioelectrical impedance vector analysis in the evaluation of body composition in athletes. *Clinical Nutrition*, *39*(2), 447–454. https://doi.org/10.1016/j.clnu.2019.02.016

Mawson, J. T., Braun, B., Rock, P. B., Moore, L. G., Mazzeo, R., & Butterfield, G. E. (2000). Women at altitude: Energy requirement at 4,300 m. *Journal of Applied Physiology*, *88*(1), 272–281. https://doi.org/10.1152/jappl.2000.88.1.272

Murawska-Ciałowicz, E., Kaczmarek, A., Kałwa, M., & Oniszczuk, A. (2022). Influence of Training and Single Exercise on Leptin Level and Metabolism in Obese Overweight and Normal-Weight Women of Different Age. *International Journal of Environmental Research and Public Health*, *19*(19), 12168. https://doi.org/10.3390/ijerph191912168

Ocobock, C. J. (2017). Body fat attenuates muscle mass catabolism among physically active humans in temperate and cold high altitude environments. *American Journal of Human Biology: The Official Journal of the Human Biology Council*, *29*(5). https://doi.org/10.1002/ajhb.23013

Ortega, R. M. (2015). Métodos de evaluación de la ingesta actual: Registro o diario dietético. *NUTRICION HOSPITALARIA*, *3*, 38–45. https://doi.org/10.3305/nh.2015.31.sup3.8749

Palmer, B. F., & Clegg, D. J. (2014). Ascent to altitude as a weight loss method: The good and bad of hypoxia inducible factor activation: Ascent to Altitude as a Weight Loss Method. *Obesity*, *22*(2), 311–317. https://doi.org/10.1002/oby.20499

Perakakis, N., Farr, O. M., & Mantzoros, C. S. (2021). Leptin in Leanness and Obesity. *Journal of the American College of Cardiology*, *77*(6), 745–760. https://doi.org/10.1016/j.jacc.2020.11.069

Randhawa, A. K., Jamnik, V., Fung, M. D. T., Fogel, A. S., & Kuk, J. L. (2021). No differences in the body fat after violating core bioelectrical impedance measurement assumptions. *BMC Public Health*, *21*(1), 495. https://doi.org/10.1186/s12889-021-10552-y

Raschka, C., Koch, H. J., & Käsebieter, J. (2020). Change of hydration parameters in moderate altitude: Measurements in healthy volunteers by means of bioelectric impedance analysis. *Papers on Anthropology*, *29*(1), Article 1. https://doi.org/10.12697/poa.2020.29.1.03

Rausch, L. K., Hofer, M., Pramsohler, S., Kaser, S., Ebenbichler, C., Haacke, S., Gatterer, H., & Netzer, N. C. (2018). Adiponectin, Leptin and Visfatin in Hypoxia and its Effect for Weight Loss in Obesity. *Frontiers in Endocrinology*, *9*, 615. https://doi.org/10.3389/fendo.2018.00615

Rinninella, E., Cintoni, M., Addolorato, G., Triarico, S., Ruggiero, A., Perna, A., Silvestri, G., Gasbarrini, A., & Mele, M. (2018). Phase angle and impedance ratio: Two specular ways to analyze body composition. *Annals of Clinical Nutrition*, *1*. https://doi.org/10.33582/2638-1370/1003

Saha, T., Del Caño, R., la De Paz, E., Sandhu, S. S., & Wang, J. (2022). Access and Management of Sweat for Non-Invasive Biomarker Monitoring: A Comprehensive Review. *Small (Weinheim an Der Bergstrasse, Germany)*, e2206064. https://doi.org/10.1002/smll.202206064

Sardinha, L. B. (2018). Physiology of exercise and phase angle: Another look at BIA. *European Journal of Clinical Nutrition*, *72*(9), 1323–1327. https://doi.org/10.1038/s41430-018-0215-x

Sbrollini, "Agnese, Bondi, D., Romagnoli, S., Morettini, M., Marcantoni, I., Pietrangelo, T., Verratti, V., & Burattini", L. (2022, December 31). *Segmented-Beat Modulation Method-Based Procedure for Extraction of Electrocardiogram-Derived Respiration from Data Acquired by Wearable Sensors During High-Altitude Activity*. 2022 Computing in Cardiology Conference. https://doi.org/10.22489/CinC.2022.096

Seshadri, D. R., Li, R. T., Voos, J. E., Rowbottom, J. R., Alfes, C. M., Zorman, C. A., & Drummond, C. K. (2019). Wearable sensors for monitoring the internal and external workload of the athlete. *NPJ Digital Medicine*, *2*, 71. https://doi.org/10.1038/s41746-019-0149-2

Shukla, V., Singh, S. N., Vats, P., Singh, V. K., Singh, S. B., & Banerjee, P. K. (2005). Ghrelin and leptin levels of sojourners and acclimatized lowlanders at high altitude. *Nutritional Neuroscience*, *8*(3), 161–165. https://doi.org/10.1080/10284150500132823

Sierra-Johnson, J., Romero-Corral, A., Somers, V. K., & Johnson, B. D. (2008). Effect of altitude on leptin levels, does it go up or down? *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *105*(5), 1684–1685. https://doi.org/10.1152/japplphysiol.01284.2007

SINU. (2014). *SINU, The Italian Society of Human Nutrition (SINU) Tables.* https://sinu.it/tabelle-larn-2014

Snyder, E. M., Carr, R. D., Deacon, C. F., & Johnson, B. D. (2008). Overnight hypoxic exposure and glucagon-like peptide-1 and leptin levels in humans. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *33*(5), 929–935. https://doi.org/10.1139/H08-079

Stellingwerff, T., Peeling, P., Garvican-Lewis, L. A., Hall, R., Koivisto, A. E., Heikura, I. A., & Burke, L. M. (2019). Nutrition and Altitude: Strategies to Enhance Adaptation, Improve Performance and Maintain Health: A Narrative Review. *Sports Medicine (Auckland, N.Z.)*, *49*(Suppl 2), 169–184. https://doi.org/10.1007/s40279-019-01159-w

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. *Journal of the Academy of Nutrition and Dietetics*, *116*(3), 501–528. https://doi.org/10.1016/j.jand.2015.12.006

Turrini, A., D'Addezio, L., Ferrari, M., Le Donne, C., Mistura, L., Piccinelli, R., & Sette, S. (2022). Editorial: Emerging topics in dietary assessment – Edition II. *Frontiers in Nutrition*, *9*, 984915. https://doi.org/10.3389/fnut.2022.984915

Turrini, A., Le Donne, C., Piccinelli, R., D'Addezio, L., Mistura, L., Sette, S., Martone, D., Comendador Azcarraga, F. J., Ferrari, M., & Catasta, G. (2022). Italian national dietary survey on adult population from 10 up to 74 years old – IV SCAI ADULT. *EFSA Supporting Publications*, *19*(9), 7559E. https://doi.org/10.2903/sp.efsa.2022.EN-7559

Viscor, G., Corominas, J., & Carceller, A. (2023). Nutrition and Hydration for High-Altitude Alpinism: A Narrative Review. *International Journal of Environmental Research and Public Health*, *20*(4), 3186. https://doi.org/10.3390/ijerph20043186

Walter-Kroker, A., Kroker, A., Mattiucci-Guehlke, M., & Glaab, T. (2011). A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease. *Nutrition Journal*, *10*, 35. https://doi.org/10.1186/1475-2891-10-35

Wandrag, L., Siervo, M., Riley, H. L., Khosravi, M., Fernandez, B. O., Leckstrom, C. A., Martin, D. S., Mitchell, K., Levett, D. Z. H., Montgomery, H. E., Mythen, M. G., Stroud, M. A., Grocott, M. P. W., Feelisch, M., & Caudwell Xtreme Everest Research Group. (2017). Does hypoxia play a role in the development of sarcopenia in humans? Mechanistic insights from the Caudwell Xtreme Everest Expedition. *Redox Biology*, *13*, 60–68. https://doi.org/10.1016/j.redox.2017.05.004

Westerterp, K. R. (2001). Energy and water balance at high altitude. *News in Physiological Sciences: An International Journal of Physiology Produced Jointly by the International Union of Physiological Sciences and the American Physiological Society*, *16*, 134–137. https://doi.org/10.1152/physiologyonline.2001.16.3.134

WHO. (2008). *WHO STEPwise approach to surveillance (STEPS): Guide to physical measurements.* http://www.who.int/chp/steps/Part3_Section3.pdf

Wing-Gaia, S. L. (2014). Nutritional Strategies for the Preservation of Fat Free Mass at High Altitude. *Nutrients*, *6*(2), 665–681. https://doi.org/10.3390/nu6020665