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Rozprawa doktorska

Wpływ miesięcznej wyprawy wysokogórskiej na stan zdrowia, sposób żywienia, poziom wydolności fizycznej i strukturę mikrobiomu wspinaczy wysokogórskich

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Pragnę serdecznie podziękować Pani Promotor, prof. dr hab. Barbarze Frączek – za niezwykle cenne wskazówki, życzliwość i wsparcie na każdym etapie powstawania niniejszej pracy. Dziękuję za stworzenie atmosfery sprzyjającej samodzielności w pracy badawczej, za zaufanie, którym mnie Pani obdarzyła, oraz za możliwość rozwijania własnych pomysłów i inicjatyw naukowych. Ta swoboda, połączona z merytorycznym wsparciem, była dla mnie niezwykle cennym doświadczeniem, kształtującym mnie zarówno jako badacza, jak i człowieka.

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Wykaz skrótów i symboli

Skrót	Wyjaśnienie skrótu w języku angielskim	Wyjaśnienie skrótu w języku polskim
AAT	alpha-1-antitrypsin	alfa-1-antytrypsyna
ACSM	American College of Sports Medicine	Amerykańskie Kolegium Medycyny Sportowej
AI	adequate intake	wystarczające spożycie
ALAP	as low as possible	wartość tak niska, jak to możliwe
ALST	appendicular lean soft tissue	suma masy ciała szczupłego kończyn górnych i dolnych
ALT	alanine aminotransferase	aminotransferaza alaninowa
AMS	acute mountain sickness	choroba wysokościowa
AST	aspartate aminotransferase	aminotransferaza asparaginowa
ATP	adenosine triphosphate	adenozynotrifosforan
BASO	basophils	bazofile
BM	body mass	masa ciała
BMC	bone mineral content	zawartość składników mineralnych w kośćcu
BMD	bone mineral density	gęstość mineralna kości
BMI	body mass index	wskaźnik masy ciała
bw	body weight	kilogram masy ciała
CHO	carbohydrates	węglowodany
DHA	docosahexaenoic acid	kwas dokozaheksaenowy
DXA	dualenergy X-ray absorptiometry	metoda absorpcjometrii promieniowania X o dwóch energiach
ECG	electrocardiogram	elektrokardiogram
EFSA	European Food Safety Authority	Europejski Urząd ds. Bezpieczeństwa Żywności
EOS	eosinophils	eozynofile
EPA	alpha-linolenic acid	kwas alfa-linolenowy
EPO	erythropoietin	erytropoetyna
ESR	erythrocyte sedimentation rate	odczyn Biernackiego
FFM	fat free mass	beztłuszczowa masa ciała
FM	fat mass	masa tkanki tłuszczowej
GGTP	gamma-glutamyl transpeptidase	gamma-glutamylotranspeptydaza
GI	gastrointestinal	żołądkowo-jelitowy
HAPE	high altitude pulmonary edema	wysokogórski obrzęk płuc
HCT	hematocrit	hematokryt
HDL	high-density lipoprotein cholesterol	frakcja cholesterolu o wysokiej gęstości
HGB	hemoglobin	hemoglobina

HIF	hypoxia-inducible factor	czynnik indukowany niedotlenieniem
HPA	hypothalamic-pituitary-adrenal	podwzgórze-przysadka-nadnercza
HR	heart rate	częstość skurczów serca
HR_{max}	maximum heart rate	maksymalna wartość częstości skurczów serca
H_2S	hydrogen sulfide	siarkowodór
IECs	intestinal epithelial cells	komórki nabłonka jelitowego
I-FABP	intestinal fatty acid binding protein	białko wiążące kwasy tłuszczowe w jelicie
IL-6	interleukin 6	interleukina 6
IP	intestinal permeability	przepuszczalność jelitowa
IRCRA	The International Rock Climbing Research Association	Międzynarodowe Stowarzyszenie Badań nad Wspinaczką Górską
ISSN	The International Society for Sports Nutrition	Międzynarodowe Towarzystwo Żywienia Sportowego
LA	linoleic acid	kwas linolowy
LA (20')	lactate concentration at 20 minutes after aerobic capacity test	stężenie mleczanu 20 minut po teście wydolności tlenowej
LBM	lean body mass	masa ciała szczupłego
LDL	low-density lipoprotein cholesterol	frakcja cholesterolu o niskiej gęstości
LEA	low energy availability	niska dostępność energii
LEfSe	the Linear Discriminant Analysis Effect Size	wielkość efektu liniowej analizy dyskryminacyjnej
LPS	lipopolysaccharide	lipopolisacharyd
Lp299v	probiotic strain *Lactobacillus plantarum 299v*	szczep probiotyczny *Lactobacillus plantarum 299v*
LYMPH	lymphocytes	limfocyty
MAP	maximum anaerobic power	maksymalna moc anaerobowa
MCH	mean corpuscular hemoglobin	średnia masa hemoglobiny w czerwonej krwince
MCHC	mean corpuscular hemoglobin concentration	średnie stężenie hemoglobiny w erytrocytach
MCV	mean corpuscular volume	średnia objętość erytrocytów
Me	median	mediana
MM	muscle mass	masa tkanki mięśniowej
MON	monocytes	monocyty
MPV	mean platelet volume	średnia objętość płytek krwi
MRI	magnetic resonance imaging	rezonans magnetyczny
mTOR	mammalian target of rapamycin	ssaczy cel rapamycyny
MUFA	monounsaturated fatty acids	jednonienasycone kwasy tłuszczowe
NAC	N-acetylcysteine	N-acetylocysteina

NEU	neutrophils	neutrofile
NO	nitric oxide	tlenek azotu
p	statistical significance	istotność statystyczna
PAL	physical activity index	współczynnik aktywności fizycznej
P_{mean}	mean anaerobic power	średnia wielkość osiągniętej mocy
PUFA	polyunsaturated fatty acids	wielonienasycone kwasy tłuszczowe
Q1	lower quartile	dolny kwartyl
Q3	upper quartile	górny kwartyl
RDW	red blood cell distribution	wskaźnik zmienności objętości czerwonych krwinek
ROS	reactive oxygen species	reaktywne formy tlenu
RQ	respiratory quotient	współczynnik oddechowy
SCFA	short-chain fatty acids	krótkołańcuchowe kwasy tłuszczowe
SD	standard deviation	odchylenie standardowe
SFA	saturated fatty acids	nasycone kwasy tłuszczowe
T	Student's *t*-test for variables with parametric distribution	test t-Studenta dla zmiennych o rozkładzie parametrycznym
TC	total cholesterol	cholesterol całkowity
TG	triglycerides	triglicerydy
TJs	tight junctions	ścisłe połączenia
t_m	time of maintaining maximum power	czas utrzymania mocy maksymalnej
t_{max}	effort duration	czas trwania wysiłku
TNF-α	tumor necrosis factor	czynnik martwicy nowotworów
t_r	time of reaching maximum power	czas osiągnięcia mocy maksymalnej
U	urea	mocznik
UA	uric acid	kwas moczowy
$\dot{V}E$	pulmonary ventilation	wentylacja minutowa płuc
$\dot{V}E_{max}$	maximum minute pulmonary ventilation	maksymalna wartość wentylacji minutowej płuc
$\dot{V}O_{2max}$	maximum oxygen uptake	maksymalny minutowy pobór tlenu
VT2	second ventilatory threshold	II próg wentylacyjny
W	Wilcoxon rank test for variables with nonparametric distribution	test Wilcoxona dla zmiennych o rozkładzie nieparametrycznym
WSHL	rate of decrease from the maximum power of the lower limbs	wskaźnik spadku maksymalnej mocy kończyn dolnych
W_t	total work performed	globalna ilość wykonanej pracy
x	mean	średnia arytmetyczna
25(OH)D	25-hydroxyvitamin D	25-hydroksywitamina D

Wprowadzenie wraz z wykazem publikacji wchodzących w skład rozprawy

Alpinizm wysokogórski to dyscyplina łącząca wspinaczkę skałkową, lodową, skialpinizm oraz przemieszczanie się po lodowcach (Twight et al., 1999). Szczególną odmianą wspinaczki jest himalaizm, czyli wspinaczka uprawiana w najwyższych górach świata, których szczyty przekraczają 7000 m, tj. łańcuchy górskie Himalajów, Karakorum, Daxue Shan, Hindukusz, Kunlun, Pamir i Tienszan (Kotarba & Migoń, 2010). Zainteresowanie wspinaczką wysokogórską rośnie z każdym rokiem, także wśród alpinistów na poziomie amatorskim (Salisbury et al., 2021). Sport ten łączy wysiłek wytrzymałościowy z elementami siłowymi i obejmuje zarówno krótkotrwałe ćwiczenia o wysokiej intensywności, jak i dłuższe o intensywności submaksymalnej, co wymaga zaangażowania zarówno beztlenowych, jak i tlenowych ścieżek energetycznych (Bourrilhon et al., 2009). Podczas wspinaczki wzrasta zużycie tlenu i tętno, co sugeruje, że wymaga ona optymalnego poziomu wydolności tlenowej (Giles et al., 2006; Michael et al., 2019). Przygotowanie do wypraw wysokogórskich obejmuje zwykle cykl treningowy trwający od kilku miesięcy do roku, z regularnymi wyjściami w góry oraz treningami w przystosowanych do tego przestrzeniach sportowych (siłownie, ścianki wspinaczkowe, trenażery), a także w warunkach symulujących wysokość, które pozwalają na adaptację fizjologiczną i techniczną do działania w terenie wysokogórskim.

W środowisku wysokogórskim alpiniści muszą się mierzyć z intensywnym promieniowaniem słonecznym, obfitymi opadami deszczu lub śniegu, silnym wiatrem oraz spadkiem temperatury (średnio o 0,6° na każde 100 m wysokości) z dużymi wahaniami dobowymi, a także ryzykiem lawin oraz spadających kamieni (Apollo, 2014; Twight et al., 1999). Przebywanie w warunkach hipoksji hipobarycznej (>3000 m n.p.m.) wywołuje szereg szkodliwych skutków dla ludzkiego organizmu, takich jak pogorszenie zdolności wysiłkowych (Hoppeler et al., 1990), osłabienie regeneracji powysiłkowej (Milledge et al., 1977), zaburzenia snu (Lombardi et al., 2013), utratę masy ciała (Dünnwald et al., 2019), zaburzenia poznawcze (R. Su et al., 2024), rozwój procesów neurodegeneracyjnych, zmian patologicznych w strukturach mózgu, w tym obrzęku naczynioruchowego (Hackett & Roach, 2012), a także uszkodzenia bariery jelitowej (McKenna et al., 2022). Zaburzenia bariery jelitowej mogą prowadzić do translokacji bakterii i miejscowych lub ogólnoustrojowych reakcji zapalnych, co dodatkowo wpływa zarówno na ogólny stan zdrowia, jak i wydolność fizyczną (McKenna, et al., 2022). Uszkodzona bariera jelitowa może upośledzać wchłanianie składników odżywczych, co może przyczynić się do utraty masy ciała obserwowanej podczas długotrwałego przebywania na dużych wysokościach (Hamad & Travis, 2006; Karl et al., 2018). Spadek masy

ciała jest także spowodowany zmniejszonym spożyciem energii z powodu zaburzeń żołądkowo-jelitowych (Hamad & Travis, 2006), zwiększoną podstawową przemianą materii i wydatkiem energetycznym, zmienionymi stężeniami hormonów wpływających na sytość, w tym leptyny oraz utratą wody (Armellini et al., 1997; Pulfrey & Jones, 1996; Reynolds et al., 1999; Westerterp et al., 1992, 2000). Na dużych wysokościach zarówno apetyt, jak i zmysł smaku są upośledzone. Uczucie sytości występuje po mniejszych objętościowo posiłkach, dlatego spożywane są mniejsze ilości jedzenia. Zjawisko to, zwane „anoreksją górską", utrudnia alpinistom utrzymanie masy ciała już na wysokości 3600 m n.p.m., a dla większości osób powyżej 5000 m n.p.m. jest to niezwykle trudne (Westerterp, 2001). Zapotrzebowanie na płyny w warunkach wysokogórskich jest wyższe niż na poziomie morza ze względu na hiperwentylację, niską wilgotność, utratę potu (Butterfield, 1999; Gatterer et al., 2013) i zwiększoną diurezę w wyniku zmniejszenia ekspresji układu renina-angiotensyna-aldosteron, zwłaszcza w pierwszych dniach pobytu (Palubiski et al., 2020).

Odpowiednie przygotowanie do wyprawy, w szczególności zwrócenie uwagi na czynniki, na które wspinacze mają wpływ, np. odżywianie, nawodnienie oraz niezbędną suplementację, może przyczynić się do zapobiegania niedoborom żywieniowym i odwodnieniu, wpływającym na pogorszenie stanu zdrowia i wydolności organizmu. Niedostateczna podaż węglowodanów podczas aktywności wytrzymałościowych może skutkować hipoglikemią, obniżoną zawartością glikogenu i zmęczeniem mięśni (Nybo, 2003), a niewystarczająca podaż energii i białka, będąca przyczyną utraty beztłuszczowej masy ciała, negatywnie wpływa na wydolność tlenową, siłę mięśni i odporność (Hoppeler et al., 1990; Murdoch, 1995; Sergi et al., 2010). Niedotlenienie wysokościowe zwiększa utratę płynów i przyczynia się do odwodnienia, co zmniejsza wydolność tlenową w warunkach wysokościowych (Castellani et al., 2010). Optymalne nawodnienie może być ograniczone przez warunki środowiskowe, ale także przez czas i zasoby paliwa potrzebne do przygotowania wody pitnej, pozyskiwanej z lodowca (Ladd et al., 2016). Zmniejszone pragnienie na dużych wysokościach jest kolejnym czynnikiem przyczyniającym się do odwodnienia (Sharma et al., 2023). Przeprowadzono wprawdzie badania nad zmianami w składzie ciała wywołanymi pobytem na dużej wysokości (Dünnwald et al., 2019; Wing-Gaia, 2014), czy też podejściem do żywienia zespołów alpinistycznych w górach wysokich (Mariscal-Arcas et al. 2010; Kasprzak et al. 2015; Bondi et al. 2021) jednak nadal brakuje specyficznych i praktycznych wytycznych, dotyczących żywienia, nawodnienia oraz suplementacji w warunkach wysokogórskich, skupionych na rzeczywistych potrzebach i uwzględniających preferencje wspinaczy.

Na wysokości powyżej 5000 m n.p.m., aż 60%-70% utraty masy ciała stanowi beztłuszczowa masa ciała (Armellini et al., 1997; Reynolds et al., 1999). Redukcja beztłuszczowej masy ciała negatywnie wpływa na wydolność aerobową (Hoppeler et al., 1990), siłę mięśni (Sergi et al., 2010) i funkcje odpornościowe (Murdoch, 1995), co może zwiększać ryzyko chorób i urazów w tych ekstremalnych warunkach. Przebywanie na wysokości powyżej 3000 m n.p.m. może zmniejszać możliwość podejmowania intensywnych treningów i skutkować późniejszą utratą masy mięśniowej (Levine et al., 2008). Zmniejszenie masy mięśniowej, powierzchni przekroju poprzecznego włókien i gęstości mitochondriów we włóknach mięśniowych to główne przyczyny pogorszenia wydolności fizycznej po przewlekłej hipoksji (Hoppeler et al., 1990). Zdolność do wysiłku, w tym wydolność tlenowa są upośledzone w warunkach niedotlenienia, a maksymalne zużycie tlenu (VO_{2max}) spada o 6% na każde 1000 m przewyższenia (od 300 do 2800 m) (Wehrlin & Hallén, 2006). W niewielu badaniach poruszano zagadnienie zmian wydolności fizycznej przed i po pobycie na bardzo dużej wysokości (>3000 m n.p.m.) (Hoppeler et al., 1990; Szymczak et al., 2021). Nieprawidłowy stan zdrowia sportowca, m.in. niski poziom żelaza i ferrytyny czy wskaźników czerwonokrwinkowych wiąże się z obniżeniem wydolności fizycznej (Solberg & Reikvam, 2023), natomiast wzrost masy czerwonych krwinek poprawia wydolność tlenową (Mairbäurl, 2013). Człowiek wykazuje kompensacyjną odpowiedź fizjologiczną i biochemiczną na pobyt na dużych wysokościach, szczególnie we wczesnej fazie (B. Liu et al., 2023). Wzrost stężenia czerwonych krwinek we krwi jest ważną cechą aklimatyzacji do dużych wysokości (Richalet et al., 1994; Sawka et al., 1996). Niedotlenienie komórek nerek i wątroby powoduje uwolnienie hormonu erytropoetyny, która stymuluje szpik kostny do produkcji czerwonych krwinek (Sarkar et al., 2003). Długotrwała ekspozycja na duże wysokości (w zakresie od 2800 do 5800 m) zwiększa poziom hematokrytu, a wzrostowi temu towarzyszy wzrost poziomu hemoglobiny i lepkości krwi (Singh et al., 2003). Dotychczas przeprowadzono jednak niewiele badań (Kasprzak et al., 2015; Kurdziel et al., 2018), w których oceniano żywieniowe wskaźniki stanu zdrowia przed wyprawą oraz po wyprawie wysokogórskiej.

Mikrobiom jelitowy odgrywa kluczową rolę w utrzymaniu zdrowia człowieka oddziałując wieloaspektowo na procesy fizjologiczne organizmu. Ten złożony ekosystem drobnoustrojów uczestniczy, m.in. w metabolizmie składników odżywczych, modulacji układu odpornościowego i utrzymaniu integralności bariery jelitowej (Song et al., 2023; Takeuchi et al., 2023). W ostatnich latach mikrobiom jelitowy uznawany jest za istotny czynnik wpływający nie tylko na zdrowie, ale też wyniki sportowe. Zmiany w składzie drobnoustrojów jelitowych i zdolności metabolicznych zostały powiązane ze zmianami we wchłanianiu składników

odżywczych, metabolizmie energetycznym, stanach zapalnych i regeneracji, z których wszystkie mają kluczowe znaczenie dla optymalnej formy sportowej i dobrego samopoczucia (Clarke et al., 2014; Deehan & Walter, 2016; Hughes, 2020). Mikrobiom jelitowy może dynamicznie reagować na zmiany w diecie, a w czasie wypraw wysokogórskich często spożywana jest żywność przetworzona, liofilizowana lub wprowadzana jest suplementacja żywności ze względu na ograniczony dostęp do produktów spożywczych (Karpęcka-Gałka & Frączek, 2024). Żywność funkcjonalna stanowi istotny element diety sportowców z uwagi na jej wysoką wartość odżywczą, wygodę stosowania, łatwość transportu i przechowywania oraz atrakcyjne walory smakowe. Jednakże, spożywane w nadmiarze tego typu produktów może zaburzać równowagę mikroorganizmów jelitowych ze względu na przewlekłe działanie zawartych w nich dodatków do żywności (Álvarez-Herms et al., 2023; Ruiz-Ojeda et al., 2019; Smith et al., 2021). Wpływ warunków wysokogórskich na strukturę mikrobiomu alpinistów nie został dotąd wystarczająco zbadany (Guo et al., 2025; Quagliariello et al., 2019; Q. Su et al., 2024). Zrozumienie, w jaki sposób stresory żywieniowe i środowiskowe kształtują społeczności drobnoustrojów jelitowych, ma zasadnicze znaczenie dla opracowania świadomych strategii żywieniowych, które wspierają zdrowie, wydolność i optymalną aklimatyzację w trudnych warunkach.

Mając na uwadze nieustanny rozwój alpinizmu w Polsce i na świecie, istnieje wyraźna potrzeba pogłębienia wiedzy na temat adaptacji fizjologicznych i biochemicznych wspinaczy wysokogórskich oraz możliwości ich wykorzystania do zwiększenia wydolności sportowej, a także odpowiedniego programowania treningu. Identyfikacja specyficznych cech mikrobiomu i szlaków metabolicznych, które można modyfikować poprzez dietę i suplementację, może pomóc praktykom w opracowaniu dostosowanych interwencji żywieniowych i suplementacyjnych w celu poprawy zarówno zdrowia, jak i wyników sportowych wspinaczy działających w ekstremalnych warunkach środowiskowych.

Wykaz publikacji

Niniejszą rozprawę doktorską stanowi cykl publikacji pod wspólnym tytułem: „Wpływ miesięcznej wyprawy wysokogórskiej na stan zdrowia, sposób żywienia, poziom wydolności fizycznej i strukturę mikrobiomu wspinaczy wysokogórskich", na który składa się pięć prac opublikowanych w recenzowanych czasopismach naukowych o sumarycznej punktacji 14,9 Impact Factor i 390 pkt MNiSW.

I. **Ewa Karpęcka-Gałka**, Paulina Mazur-Kurach, Zbigniew Szyguła, Barbara Frączek. „Diet, Supplementation and Nutritional Habits of Climbers in High Mountain Conditions". *Nutrients* 2023; 15(19):4219. doi:10.3390/nu15194219

Impact Factor: 4,8; punktacja MNiSW: 140 pkt

II. **Ewa Karpęcka-Gałka** i Barbara Frączek. „Nutrition, hydration and supplementation considerations for mountaineers in high-altitude conditions: a narrative review". *Frontiers in Sports and Active Living* 2024; 6:1435494. doi: 10.3389/fspor.2024.1435494

Impact Factor: 2,6; punktacja MNiSW: 20 pkt

III. **Ewa Karpęcka-Gałka**, Marek Bawelski, Aleksandra Pięta, Paulina Mazur-Kurach, Paweł Pięta, Barbara Frączek. „The Impact of a High-Altitude Expedition on the Physical Performance and Nutritional Indices of Health Status of Alpinists". *Journal of Functional Morphology and Kinesiology* 2025; 10(2):143. doi:10.3390/jfmk10020143

Impact Factor: 2,5; punktacja MNiSW: 20 pkt

IV. **Ewa Karpęcka-Gałka,** Aleksandra Pięta, Katarzyna Świtała, Barbara Frączek. „Selected aspects of the habitual diet and levels of intestinal permeability markers of Polish alpinists". *Physical Activity Review* 2026; 14(1):1-13. doi:10.16926/par.2026.14.01

Impact Factor: 1,1; punktacja MNiSW: 70 pkt

V. **Ewa Karpęcka-Gałka**, Kinga Zielińska, Barbara Frączek, Paweł Łabaj, Tomasz Kościółek, Kinga Humińska-Lisowska. „High-altitude mountaineering induces adaptive gut microbiome shifts associated with dietary intake and performance markers". *Scientific Reports* 2025. doi: 10.1038/s41598-025-22848-9

Impact Factor: 3,9; punktacja MNiSW: 140 pkt

1. CEL PRACY, PYTANIA I HIPOTEZY BADAWCZE

Głównym celem badania była ocena wpływu wyprawy wysokogórskiej na sposób żywienia, stan zdrowia, wydolność fizyczną oraz strukturę mikrobiomu alpinistów.

Cele szczegółowe obejmowały:

1. przeglądową analizę literatury przedmiotu w zakresie zaleceń dotyczących żywienia, nawodnienia i suplementacji w warunkach wysokogórskich w celu sformułowania praktycznych rekomendacji żywieniowych dla alpinistów;
2. ocenę zwyczajowej diety alpinistów oraz nawyków żywieniowych, diety i suplementacji w czasie wyprawy wysokogórskiej w odniesieniu do zaleceń dedykowanych wspinaczom;
3. analizę wybranych wskaźników ogólnego stanu zdrowia (skład ciała, hematologia i biochemia krwi oraz badanie moczu, kału, przepuszczalności jelitowej) przed wyprawą wysokogórską oraz po jej ukończeniu;
4. ocenę zmian poziomu wydolności fizycznej (tlenowej i beztlenowej) wywołanych wyprawą wysokogórską;
5. ocenę wpływu wyprawy wysokogórskiej na taksonomię i funkcje mikrobiomu jelitowego alpinistów oraz zależności z dietą, wskaźnikami zdrowia i wydolności fizycznej;
6. opracowanie praktycznych rekomendacji dla wspinaczy uwzględniających zmianę diety podczas wyprawy oraz wpływ sposobu żywienia w warunkach wysokogórskich na mikrobiom jelitowy.

W związku z realizacją celów pracy sformułowano następujące pytania badawcze:

1. Jakie są aktualne rekomendacje dotyczące żywienia, nawodnienia i suplementacji w warunkach wysokogórskich?
2. Jaki jest bilans energetyczny diety alpinistów przed wyprawą i w czasie jej trwania?
3. W jaki sposób odżywiają się wspinacze podczas wyprawy?
4. Czy sposób żywienia sportowców uprawiających alpinizm w okresie poprzedzającym wyprawę wysokogórską i w czasie jej trwania, określany poprzez ilościową analizę wartości odżywczej i energetycznej diety jest zgodny z zaleceniami żywienia dla sportowców?
5. Czy suplementacja diety stosowana w czasie wyprawy, jeśli występuje, jest właściwa?
6. Czy wyprawa wysokogórska powoduje zmiany masy i składu ciała alpinistów, oceniane ilością tkanki mięśniowej, tłuszczowej, beztłuszczowej i gęstością kości?

7. Czy wyprawa wysokogórska powoduje zmiany wybranych wskaźników stanu zdrowia, tj.: morfologii krwi obwodowej, odczynu Biernackiego, metabolizmu węglowodanowego, lipidogramu, czynności nerek, enzymów wątrobowych, stężenia wybranych elektrolitów, żelaza, ferrytyny, witaminy D, witaminy B_{12}, fosfatazy kwaśnej, białka całkowitego, albumin i bilirubiny całkowitej oraz wskaźników ogólnej oceny moczu, kału i przepuszczalności jelitowej?
8. Jaki jest poziom wydolności fizycznej alpinistów i czy ekspozycja wysokogórska modyfikuje ich wydolność tlenową i beztlenową?
9. Jaki jest skład, obfitość gatunków oraz funkcjonalne właściwości mikrobiomu jelitowego alpinistów oraz jak zmieniają się one pod wpływem przebywania w warunkach wysokogórskich?
10. Czy cechy mikrobiomu (przed i po wyprawie) są modulowane zmianami w diecie wywołanymi ekspozycją na duże wysokości i korelują ze wskaźnikami stanu zdrowia i wydolności fizycznej?

Przyjęto następujące hipotezy badawcze:

1. Polscy alpiniści tylko w ograniczonym stopniu realizują zalecenia żywieniowe dla sportowców w swojej zwyczajowej diecie.
2. Dieta alpinistów wykazuje specyficzne dla dyscypliny nieprawidłowości związane z ekspozycją wysokogórską.
3. Wyprawa wysokogórska negatywnie wpływa na wskaźniki zdrowotne.
4. Przebywanie w warunkach wysokogórskich poprawia wydolność fizyczną wspinaczy poprzez korzystny wpływ na wskaźniki erytrocytarne.
5. Skład (taksony) i funkcje (alfa-/beta-różnorodności oraz obfitości wybranych szlaków) mikrobiomu jelitowego alpinistów zmieniają się w efekcie przebywania w warunkach wysokogórskich.

2. METODOLOGIA BADAŃ WŁASNYCH

2.1. Grupa badana i materiał badawczy

Badania zostały przeprowadzone w latach 2021-2024 zgodnie z zasadami Deklaracji Helsińskiej, a protokół badań został zatwierdzony przez Komisję Bioetyczną Okręgowej Izby Lekarskiej w Krakowie (67/KBL/OIL/2021 oraz 68/KBL/OIL/2022). Wszyscy uczestnicy zostali poinformowani o dobrowolnym przystąpieniu do procedury badawczej oraz wyrazili świadomą pisemną zgodę na uczestnictwo w projekcie.

Do udziału w projekcie zakwalifikowano 20 osób, jednak w trakcie jego trwania z różnych przyczyn 3 osoby zrezygnowały z dalszego udziału. Ostatecznie grupę badaną i opisaną w publikacjach 3, 4 i 5 stanowiło 17 polskich alpinistów płci męskiej w wieku od 23 do 40 lat (średnia wieku 30,29±5,8 lat) o średniej masie ciała 74,96±5,03 kg i wysokości ciała 180,47±8,36 cm. Na podstawie danych dotyczących wysokości ciała (cm) i masy ciała (kg) obliczono wskaźnik masy ciała (BMI), który wyniósł średnio 22,83±2,1 kg/m^2. Uczestnicy badania co najmniej raz w roku brali udział w wyprawach górskich, podczas których przebywali na wysokości ponad 3000 m n.m.p przez co najmniej trzy tygodnie. Osoby zakwalifikowane do badania były członkami klubów wysokogórskich zrzeszonych w Polskim Związku Alpinizmu.

Uczestniczący w badaniu alpiniści charakteryzowali się dużym doświadczeniem wspinaczkowym (10 ± 5 lat) we wspinaczce sportowej i górskiej. Średni poziom wspinaczkowy uczestników, mierzony skalą IRCRA (The International Rock Climbing Research Association), wynosił 20,69 ± 2,8, co zgodnie z zaproponowanymi kryteriami plasuje ich w kategorii zaawansowanych (poziom 3) (Draper et al., 2015). Wspinacze poświęcali na trening średnio 8 ± 3 godzin w tygodniu.

Alpiniści zakwalifikowani do badania przebywali w czasie odbytej wyprawy na wysokości 3000-8167 m n.p.m. Wyprawy wysokogórskie, zakwalifikowanych do badania alpinistów trwały średnio 34 ± 6 dni, z czego średnio 22 ± 4 dni wspinacze spędzili aktywnie wspinając się w górach, 11 ± 8 dni odpoczywając w obozach bazowych lub górskich miasteczkach. Ponieważ niektóre z tych miejsc znajdowały się powyżej 3000 m n.p.m., uczestnicy spędzili łącznie 26 ± 6 dni na wysokościach powyżej 3000 m n.p.m. Alpiniści zakwalifikowani do badania podróżowali w małych grupach lub indywidualnie, aby realizować swoje cele górskie w następujących masywach górskich: Kordyliera Biała (Peru), Karakorum (Pakistan), Himalaje Garhwalu (Indie), Himalaje (Nepal), Pamir-Ałaj (Kirgistan). Cele górskie alpinistów szczegółowo przedstawiono w publikacji 3 (Załącznik 1, Publikacja 3, Tabela 3).

Przed rozpoczęciem projektu, alpiniści odbyli konsultację z lekarzem medycyny sportowej, podczas której zakwalifikowano ich do udziału w projekcie na podstawie wyników wcześniej przeprowadzonych badań (EKG, badanie krwi i moczu). Obecność chorób przewlekłych oraz wiek powyżej 45 lat były czynnikami dyskwalifikującymi z udziału w projekcie.

Do badania ankietowego, które stanowiło część pierwszej publikacji z opisanego cyklu, zakwalifikowano 28 mężczyzn z Polski w wieku od 23 do 42 lat (średnia wieku 33,12±5,96 lat), o wysokości ciała 179,04±6.96 cm i masie ciała 71,48±5,43 kg oraz wskaźniku BMI wynoszącym 22,31±1,37 kg/m^2, biorących udział w wyprawach górskich co najmniej raz w roku, podczas których przebywali na wysokościach powyżej 3000 m n.p.m. przez co najmniej 3 tygodnie. Osoby zakwalifikowane do badania były członkami klubów wysokogórskich zrzeszonych w Polskim Związku Alpinizmu. Spośród badanych 42,9% zadeklarowało, że wspina się od ponad 10 lat, 10,7% - od 9 do 10 lat, 21,4% - od 7 do 8 lat, 17,9% - od 5 do 6 lat, a 7,1% - od 1 do 4 lat. Średni poziom wspinaczkowy uczestników, mierzony skalą IRCRA (The International Rock Climbing Research Association), wynosił 22,24 ± 2,66, co zgodnie z zaproponowanymi kryteriami plasuje ich w kategorii zaawansowanych (poziom 3) (Draper et al., 2015). Badani mieli duże doświadczenie wspinaczkowe, zarówno we wspinaczce skalnej, boulderingu, drytoolingu, jak i alpinizmie z własną asekuracją. Spośród respondentów 82,1% trenuje wspinaczkę sportową 2 razy w tygodniu lub więcej. Spośród 28 wspinaczy, 15 prowadziło dzienniki żywieniowe z trzech dni wyprawy podczas wspinaczek w Peru (Kordyliera Biała) i Pakistanie (Dolina Shimshal) w celu przeprowadzenia analizy sposobu żywienia.

2.2. Metody badań

2.2.1. Analiza przeglądowa literatury naukowej

Przegląd przeprowadzono z wykorzystaniem następujących baz danych: Pubmed, Scopus, ScienceDirect, dla badań opublikowanych w języku angielskim od stycznia 2000 r. do października 2023 r. W wyniku wstępnego wyszukiwania zidentyfikowało 2579 artykułów. Kryteria wykluczenia obejmowały badania z udziałem noworodków i niemowląt, osób lub zwierząt urodzonych na dużych wysokościach lub zwierząt, osób przebywających na stałe na dużych wysokościach i/lub osób cierpiących na niedotlenienie związane z chorobą. Przeszukano również Google Scholar w celu identyfikacji badań pominiętych w wymienionych bazach. Pobrano także listy literatury z artykułów i książek. Pozostałe artykuły przeszukano

ręcznie. Ostatecznie wybrano 207 artykułów. Pod uwagę wzięto badania randomizowane, z podwójną ślepą próbą i kontrolą placebo, artykuły przeglądowe, przeglądy systematyczne i metaanalizy.

2.2.2. Analiza sposobu żywienia, wartości energetycznej i odżywczej diety oraz nawyków żywieniowych alpinistów

Ocena ilościowa sposobu żywienia alpinistów obejmowała analizę wartości energetycznej i odżywczej diety, przeprowadzoną z wykorzystaniem 3-dniowego bieżącego notowania oraz metody wagowej. Alpiniści zostali poproszeni o odnotowanie wszystkich spożytych produktów, napojów i suplementów określając ich wielkość w miarach gramaturowych lub domowych (z zastosowaniem tabel miar domowych i gramatury produktów spożywczych i wybranych potraw) podczas 3-dniowego okresu w ciągu 2 tygodni poprzedzających wyprawę (2 dni aktywności sportowej, 1 dzień odpoczynku) (publikacja 4). Dni aktywności sportowej obejmowały wspinaczkę na ściance wspinaczkowej, wspinaczkę skałkową lub górską, bieganie i/lub trening siłowy. Wspinaczy poproszono o przeprowadzenie analogicznej procedury w czasie wyprawy - w ciągu 3 aktywnych dni (publikacja 1) oraz 2 dni aktywności górskiej oraz 1 dnia odpoczynku (publikacja 3). Aktywne dni opisywane były jako dni aklimatyzacji i wspinaczki wysokogórskiej w drodze na szczyt, a odnotowane w tym czasie aktywności obejmowały głównie wędrówki piesze z elementami wspinaczki. Szczegółową procedurę gromadzenia danych żywieniowych do analizy ilościowej sposobu żywienia przedstawiono w publikacji 1, 3 i 4.

Wypełnione dzienniki żywieniowe zostały przeanalizowane pod kątem dokładności i poprawności, a dane wprowadzono do programu „Aliant – Kalkulator dietetyczny" (Anmarsoft, Gdańsk, Polska; wersja: 85; baza danych: 6.2), opierającego się na „Tabelach składu i wartości odżywczej żywności" (Kunachowicz et al. 2017). Średnie spożycie makroskładników (białko, tłuszcze, węglowodany ogółem), błonnika pokarmowego, węglowodanów prostych, kwasów tłuszczowych nasyconych i sodu (publikacja 1) oraz dodatkowo, kwasów tłuszczowych (jedno- i wielonienasycone kwasy tłuszczowe) i cholesterolu oraz witamin (A, D, E i K, witamin z grupy B, kwasu foliowego oraz witaminy C) i składników mineralnych (wapnia, fosforu, żelaza, magnezu, cynku, miedzi, potasu, selenu i jodu) (publikacja 3 i 4) z 3 dni odniesiono do zaleceń dla sportowców (Benardot, 2012; EFSA (European Food Safety Authority), 2017; Kerksick et al., 2018; Lukaski, 2004; Thomas et al., 2016).

Analizę nawyków i postaw żywieniowych oparto na autorskiej ankiecie. Przeanalizowano odpowiedzi na 54 pytania (8 otwartych, 46 zamkniętych). Kwestionariusz

podzielono na 3 części uwzględniające: (1) dane antropometryczne oraz doświadczenie górskie i wspinaczkowe, a także stan zdrowia i pojawiające się dolegliwości w górach wysokich i po powrocie z gór; (2) przygotowanie do wyprawy związane z analizą stanu zdrowia; (3) zachowania żywieniowe w trakcie akcji górskiej, w tym: preferencje pokarmowe względem produktów spożywczych, suplementów, płynów; częstość spożycia grup produktów spożywczych w górach oraz odczuwanie apetytu. Kwestionariusz opracowano na podstawie literatury dotyczącej żywienia w warunkach wysokogórskich oraz kwestionariusza do badania poglądów i nawyków żywieniowych (Jeżewska-Zychowicz et al., 2014)

Pomiary wydatku energetycznego zostały wykonane przy użyciu monitora Polar M430 rejestrującego tętno (Polar-Electro, Kempele, Finlandia), które było rejestrowane 24/7 przez 3 dni (2 dni aktywności sportowej, 1 dzień odpoczynku) przed wyprawą i 3 dni podczas wyprawy (3 dni aktywności górskich – publikacja 1; 2 dni aktywności górskich i 1 dzień odpoczynku – publikacja 3). Wydatek energetyczny był rejestrowany w aplikacji Polar Flow. Szczegółową procedurę pomiaru wydatku energetycznego przedstawiono w publikacji 1, 3 i 4.

2.2.3. Analiza stanu zdrowia alpinistów

W celu oceny stanu zdrowia uczestników projektu przeprowadzono badanie krwi, moczu i kału oraz wykonano pomiary antropometryczne i analizę składu ciała.

Próbki krwi żylnej pobierano z żyły łokciowej w ramach analizy hematologicznej i biochemicznej, oznaczając: morfologię krwi obwodowej (hematokryt, stężenie hemoglobiny, objętość erytrocytów (MCV), zawartość hemoglobiny w erytrocytach (MCH), stężenie hemoglobiny w erytrocytach (MCHC), wskaźnik anizocytozy erytrocytów (RDW), liczbę erytrocytów, płytek krwi, objętość płytek krwi (MPV), liczbę leukocytów, różnicowanie leukocytów: limfocyty, monocyty, bazofile, eozynofile, neutrofile, odczyn Biernackiego (OB), glukozę, cholesterol całkowity, cholesterol lipoprotein o wysokiej gęstości (HDL), cholesterol lipoprotein o niskiej gęstości (LDL), trójglicerydy (TG), kreatyninę (Cr), mocznik (U), kwas moczowy (UA), białko całkowite, sód (Na), potas (K), chlorki, wapń (Ca), magnez (Mg), fosfor (P), żelazo (Fe), ferrytynę, witaminę D, witaminę B12, enzymy wątrobowe: aminotransferazę alaninową (ALT), aminotransferazę asparaginową (AST), fosfatazę kwaśną, gamma-glutamylotranspeptydazę (GGTP), albuminy i bilirubinę całkowitą. Oznaczenia przeprowadzono rano i na czczo, do dwóch tygodni przed wyprawą i w ciągu dwóch dni (1,41 ± 0,69 dnia) po zakończeniu wyprawy.

Analiza moczu obejmowała: badanie ogólne moczu (ciężar właściwy, pH, leukocyty, azotyny, białko, glukozę, ketony, urobilinogen, bilirubinę, krew (erytrocyty/hemoglobina),

barwę, przejrzystość), z kolei badanie kału zawierało: ogólne badanie kału, test na pasożyty, lamblie, Helicobacter pylori, poziomy zonuliny i antytrypsyny. Uczestnicy badania otrzymali jednolitą procedurę samodzielnego pobierania próbek moczu i kału. Po pobraniu próbki stolca wielkości śliwki, umieszczano ją w probówce i przechowywano w lodówce (temp. 2-4°C), a następnie dostarczano do laboratorium w ciągu 24 godzin od pobrania. Próbki moczu i kału zostały dostarczone do laboratorium w tym samym czasie, w którym pobrano krew. Analizy krwi i moczu wykonano metodą cytometrii przepływowej (morfologia krwi), kinetyczno-fotometryczną (OB.), potencjometrii pośredniej (Na, K, chlorki), spektrofotometrii (białko całkowite, Ca, P, Mg, cholesterol całkowity, HDL, triglicerydy, U, UA, Fe, AST, ALT, kreatynina, bilirubina całkowita, GGTP, glukoza), chemiluminescencji bezpośredniej (witamina B12, witamina D, ferrytyna), za pomocą testów paskowych (badanie ogólne moczu), metodą immunochromatograficzną z użyciem testu Giardia firmy Hydrex Diagnostics (Giardia lamblia w kale), metodą immunochromatograficzną z użyciem testu H. pylori Ag firmy nal von Minden (Helicobacter pylori w kale), techniką ELISA (zonulina i alfa-1-antytrypsyna w kale) i mikroskopowo (ogólne badanie kału i ocena resztek pokarmowych, badanie na obecność pasożytów, lamblii). Oznaczenia laboratoryjne wykonywano przy użyciu następujących urządzeń: Alinity HQ (Abbott, Singapore), Roller 20 (Alifax, Włochy), Alinity C (Abott, Singapur), Alinity I (Abbott, Singapur), Atellica 1500 (Siemens, Niemcy), oraz DYNEX DSX® (Dynex® Technologies, Chantilly, VA, USA) i zostały zlecone jako usługa zewnętrzna, wykonana przez Alab Laboratories (Warszawa, Polska).

Wysokość ciała badanych została zmierzona przed wyprawą za pomocą antropometru Seca 217 (Seca GmbH & Co. KG, Hamburg, Niemcy) z dokładnością do 1 mm. Masa ciała wspinaczy została określona za pomocą analizatora składu ciała InBody 120 (Inbody Bldg., Seul, Korea) rano po standaryzowanym posiłku między 7:45 a 8:30.

Analizę składu ciała wykonano z zastosowaniem metody absorpcjometrii promieniowania X o dwóch energiach (DXA), przy pomocy aparatu Lunar iDXA™ (GE HealthCare, Chicago, IL, USA). Każdorazowa analiza składu ciała dostarczyła informacji nt. masy ciała (BM) [kg] i wskaźnika wagowo- wzrostowego (BMI) oraz poszczególnych komponentów struktury ciała: zawartości tkanki tłuszczowej (FM) [% i kg], beztłuszczowej masy ciała (FFM) [% i kg], masy ciała szczupłego tj. masy ciała bez tkanki tłuszczowej oraz minerałów kostnych (LBM) [% i kg], składników mineralnych w kościach (BMC) [g] i gęstości mineralnej kości (BMD) [g/cm^2]. Oceniono także ilość LBM [kg] w kończynach górnych *(Arms*) i dolnych (*Legs*). Otrzymane wartości pozwoliły oszacować sumę masy ciała szczupłego (ALST, ang. *appendicular lean soft tissue*), a także obliczyć ilość masy mięśniowej

(MM) [% i kg]. Procedurę obliczenia masy mięśniowej opisano szczegółowo w publikacji 3. Analiza składu oraz pomiar masy ciała wykonywane były dwukrotnie. Pierwsze badanie wykonywano około dwóch tygodni przed wyprawą, drugie badanie wykonywano do czterech dni (3,88 ± 1,49 dni) od powrotu z wyprawy na wysokości ok. 383 m n.p.m.

2.2.4. Badanie wydolności aerobowej i anaerobowej alpinistów

Wydolność aerobową oceniano w trakcie klasycznego testu o stopniowo narastającej intensywności „do odmowy", natomiast wydolność anaerobową w trakcie testu Wingate dla kończyn dolnych i górnych. Testy wysiłkowe wykonywane były dwukrotnie. Pierwsze badanie wykonywano około dwóch tygodni przed wyprawą, drugie badanie wykonywano do czterech dni (3,88 ± 1,49 dni) od powrotu z wyprawy na wysokości ok. 383 m n.p.m.

Stopniowany test wysiłkowy „do odmowy" wykonano na bieżni mechanicznej h/p/Cosmos Saturn (h/p/Cosmos Sports & Medical GmbH, Nußdorf, Niemcy). Wskaźniki oddechowe monitorowano w sposób ciągły przy użyciu ergospirometru Cortex Metalyzer 3B (CORTEX Biophysik GmbH, Lipsk, Niemcy), a częstość skurczów serca rejestrowano przy pomocy kardiomonitora Polar S-410 (Polar-Electro, Helsinki, Finlandia). W trakcie testu rejestrowano szereg wskaźników krążeniowo oddechowych a w tym: częstość skurczów serca (HR), minutowy pobór tlenu (VO_2), minutowe wydalanie dwutlenku węgla (VCO_2), wentylację minutową płuc (VE), odsetek tlenu i dwutlenku węgla w powietrzu wydychanym (FEO_2 i $FECO_2$), iloraz oddechowy (RER). Na podstawie zmian wskaźników oddechowych w trakcie testu określono wielkość maksymalnego minutowego poboru tlenu (VO_2max) oraz poziom II progu wentylacyjnego. Jako kryterium wyznaczenia II progu wentylacyjnego przyjęto: maksymalną wartość odsetkową dwutlenku węgla w powietrzu wydychanym ($FECO_2$), minimalną wartość równoważnika oddechowego dla dwutlenku węgla (VE/VCO_2), gwałtowny wzrost wentylacji minutowej płuc (VE). Test Wingate wykonywano w pozycji siedzącej bez wstawania z siodełka na cykloergometrze Monark 894e dla kończyn dolnych (Monark Sports & Medical, Vansbro, Szwacja) i górnych Monark 834e (Monark Sports & Medical, Vansbro, Szwecja) w zmodyfikowanej 20 sekundowej wersji. W trakcie obu testów monitorowano w sposób ciągły przebieg zmian mocy, co pozwoliło na wyznaczenie wskaźników: maksymalnej mocy anaerobowej (MAP), pracy globalnej (W), mocy średniej z testu (Pśr), czasu uzyskania mocy maksymalnej ($t_{uz.}$) oraz czasu utrzymania mocy maksymalnej ($t_{utrz.}$). Metodykę przeprowadzenia obu testów szczegółowo opisano w publikacji 3.

2.2.5. Analiza mikrobiomu alpinistów

Stolec został pobrany i natychmiast umieszczony w pojemnikach do przechowywania kału, a następnie włożony do styropianowego pudełka z wkładem chłodzącym. Pojemniki przechowywano w lodówce (temp. 2-4°C), a w ciągu 24 godzin od pobrania przekazywano je do wykonawcy projektu (Akademia Kultury Fizycznej w Krakowie). Następnie próbki kału zostały umieszczone w zamrażarce (temp. -80°C) i przechowywane do czasu wykonania analiz. Próbki zebrano od uczestników zgodnie z opisem w sekcji dotyczącej analizy stanu zdrowia (przed i po wyprawie). Kolejne etapy analizy zostały przeprowadzone w Laboratorium Genetyki w Sporcie Akademii Wychowania Fizycznego i Sportu im. Jędrzeja Śniadeckiego w Gdańsku oraz w laboratoriach zewnętrznych współpracujących z AWFiS.

Około 200 mg każdej zamrożonej próbki kału wykorzystano do izolacji DNA przy użyciu zestawu QIAamp PowerFecal Pro DNA Kit (Qiagen, Niemcy), stosując zmodyfikowany protokół mający na celu zwiększenie efektywności lizy komórek mikroorganizmów, w tym bakterii Gram-dodatnich o grubszej ścianie komórkowej. Procedura obejmowała połączenie metod fizycznych (inkubacja w podwyższonej temperaturze), mechanicznych (rozdrobnienie i rozbijanie komórek przy użyciu homogenizacji z kulkami ceramicznymi w aparacie Bead Ruptor Elite; Omni International, USA) oraz chemicznych (zastosowanie buforów litycznych zestawu, w tym detergentów i proteinazy K, które wspomagały degradację błon komórkowych i białek). Homogenizacja obejmowała krótkie, szybkie cykle z przerwami na chłodzenie w celu ograniczenia przegrzewania i degradacji DNA. Szczegółowo procedurę homogenizacji opisano w opublikowanej publikacji nr 5. Wyizolowane DNA oceniono pod kątem stężenia i czystości za pomocą spektrofotometru NanoDrop (Thermo Scientific, USA), analizując stosunki absorbancji A260/A280 oraz A260/A230. Integralność DNA zweryfikowano poprzez elektroforezę na 1% żelu agarozowym. Do dalszych analiz zakwalifikowano wyłącznie próbki DNA o odpowiedniej jakości i wydajności. Stężenie DNA zostało następnie ponownie ocenione i znormalizowane przy użyciu fluorometru Qubit 3.0 wraz z zestawem Qubit DNA BR (Thermo Fisher Scientific, USA).

Biblioteki metagenomiczne przygotowano ze 100 ng wyjściowego DNA na próbkę, przy użyciu zestawu KAPA HyperPlus Library Preparation Kit (Roche, Bazylea, Szwajcaria), zgodnie z protokołem oraz opisem zawartym w publikacjach współautorów (Humińska-Lisowska et al., 2024). Etap fragmentacji został zoptymalizowany w celu uzyskania fragmentów o średniej wielkości 300-500 par zasad (bp – base pairs), co zapewniało równowagę pomiędzy głębokością sekwencjonowania a jakością odczytów. Następnie

przeprowadzono ligację z indeksowanymi adapterami przy użyciu zestawu KAPA Universal Dual-Indexed Adaprets (Roche, Szwajcaria). Adaptery zawierały unikalne sekwencje indeksowe (tzw. barcodes), które umożliwiały identyfikację poszczególnych próbek po ich połączeniu w jednej reakcji sekwencjonowania (multiplexing). Kontrolowane stężenie adapterów pozwoliło na utrzymanie wysokiej wydajności ligacji i ograniczenie powstawania dimerów adapterów.

Otrzymane biblioteki oczyszczono za pomocą kulek magnetycznych KAPA Pure Beads (Roche, Szwajcaria), co pozwoliło usunąć fragmenty krótsze od zakładanych. Etap amplifikacji bibliotek ograniczono do sześciu cykli PCR, co minimalizowało ryzyko błędów amplifikacyjnych i artefaktów w procesie sekwencjonowania. Po amplifikacji przeprowadzono dodatkowy etap oczyszczania (również z wykorzystaniem kulek KAPA Pure Beads) w celu usunięcia pozostałości dimerów starterów i niespecyficznych produktów. Jakość bibliotek oraz rozkład wielkości fragmentów oceniono przy użyciu systemu 4150 TapeStation (Agilent Technologies, USA). Wszystkie biblioteki zostały następnie znormalizowane do 20 nM, połączone w równomolowe ilości i poddane sekwencjonowaniu w trybie pair-end (2x150 bp) na platformie Illumina NovaSeq 6000 (Illumina, CA, USA). Każda biblioteka wygenerowała średnio około 13 milionów odczytów sparowanych odczytów (pair-end), co zapewniło wysoką głębokość sekwencjonowania i umożliwiło kompleksową analizę społeczności drobnoustrojów i ich potencjału genomowego.

Uzyskane wyniki posłużyły do oceny zmian taksonomicznych (bogactwo gatunkowe, indeks Shannona, beta-różnorodność Braya-Curtisa, obfitości gatunków) oraz funkcjonalnych mikrobiomu alpinistów i ich modyfikacji pod wpływem przebywania w warunkach wysokogórskich.

2.2.6. Analiza statystyczna i bioinformatyczna

Do scharakteryzowania uzyskanych wyników badań wykorzystano podstawowe miary statystyczne w postaci średniej arytmetycznej ($\overline{x}$), odchylenia standardowego (SD), mediany (Me) oraz kwartyli (Q1, Q3). Uzyskane wyniki dotyczące zawartości makro- i mikroskładników w diecie, wartości wskaźników stanu zdrowia i określających poziom wydolności fizycznej testowano poprzez sprawdzenie normalności rozkładu zmiennych testem Shapiro-Wilka (publikacja 1, 3, 4). Dla rozkładów normalnych różnice w wartościach średnich obserwowanych wskaźników badano testem t-Studenta dla grup zależnych (publikacja 1, 3, 4), a dla pozostałych rozkładów (odbiegających od rozkładu normalnego) stosowano statystyki nieparametryczne, dla grup zależnych - test kolejności par Wilcoxona (publikacja 3).

Informacje nt. zastosowanego testu przedstawiono w tabelach 4-9 w publikacji nr 3 (Załącznik 1, Publikacja 3, Tabele 4-9). Korelacje pomiędzy między składnikami odżywczymi w codziennej diecie alpinistów a poziomem markerów przepuszczalności jelitowej analizowano za pomocą współczynnika korelacji Spearmana-Rho (publikacja 4). Za poziom istotności przyjęto wartość $p \leq 0{,}05$. Do analiz statystycznych wykorzystano pakiet Statistica 13.1 i 13.3 (StatSoft Inc., Tulsa, OK, USA) i pakiet JASP (University of Amsterdam, Amsterdam, Holandia; wersja: 0.19.3).

Do oceny realizacji ilościowych rekomendacji żywieniowych zastosowano klasyfikację binarną. Dla każdego składnika odżywczego przypisano wartość 1, jeśli spożycie danego składnika odżywczego przez uczestnika badania spełniało zalecaną normę i 0, jeśli nie. Następnie, w oparciu o te dane, obliczono odsetek alpinistów, którzy spełnili normy dla każdego składnika odżywczego. Analizę przeprowadzono przy użyciu arkusza kalkulacyjnego Excel (Microsoft Corporation, Washington, DC, USA). Medianę spożycia makroskładników odżywczych (białko, tłuszcz, węglowodany ogółem), błonnika pokarmowego, węglowodanów prostych, nasyconych, jednonienasyconych i wielonienasyconych kwasów tłuszczowych, cholesterolu oraz wybranych mikroskładników odżywczych w ciągu analizowanych dni zwyczajowego i wyprawowego żywienia odniesiono do zaleceń dla sportowców (Lukaski 2004; Benardot 2012; Thomas et al. 2016; EFSA (European Food Safety Authority) 2017; Kerksick et al. 2018). Wyniki przedstawiono w postaci procentowej, co pozwoliło ocenić stopień realizacji norm przez badaną grupę (publikacja 1 i 3).

Dane z analizy mikrobiomu poddano analizie bioinformatycznej, którą przeprowadzano we współpracy ze specjalistami z Małopolskiego Centrum Biotechnologii Uniwersytetu Jagiellońskiego w Krakowie oraz Sano Centre for Computational Medicine w Krakowie. Zestaw wskaźników uzyskanych w wyniku analizy mikrobiomu wykorzystano do porównań par przed wyprawą vs. po wyprawie (test Wilcoxona dla danych sparowanych) oraz do oceny różnic w strukturze społeczności na podstawie dystansów Braya-Curtisa. Korelacje cech mikrobiomu z metadanymi analizowano przy użyciu korelacji Spearmana z korektą Benjamini-Hochberga dla testów wielokrotnych, a różnicowe wzbogacenie taksonów oceniano z użyciem LEfSe. Cała analiza bioinformatyczna została przeprowadzona w środowisku Python 3.11.9 i wizualizowana przy użyciu pakietów matplotlib 3.7.2 i seaborn 0.12.2. Szczegółowy opis przeprowadzonej analizy bioinformatycznej przedstawiono w publikacji nr 5.

3. WYNIKI

3.1. Wyniki przeglądowej analizy literatury przedmiotu w zakresie zaleceń dotyczących żywienia, nawodnienia i suplementacji w warunkach wysokogórskich

W oparciu o przegląd literatury naukowej w publikacji nr 2 podsumowano aktualną wiedzę na temat zasad żywienia, nawodnienia i suplementacji w warunkach wysokogórskich oraz zaproponowano praktyczne zalecenia dla alpinistów.

Pomimo braku apetytu, należy zadbać o regularność spożywanych posiłków i przekąsek. Podaż węglowodanów w diecie alpinistów powinna być dostosowana do etapu wyprawy i intensywności wysiłku. Podczas pobytu w bazie, przy niskiej aktywności fizycznej, podaż ta powinna wynosić około 3-5 g węglowodanów na kg masy ciała/dzień, natomiast podczas trekkingu i/lub wspinaczki - minimum 6 g/kg masy ciała/dzień. Ze względu na wysiłek fizyczny o charakterze wytrzymałościowym, podaż białka w diecie powinna wynosić minimum 1,4 g/kg masy ciała/dzień, uwzględniając białko wysokiej jakości i rozdzielając je na główne posiłki i przekąski podczas wspinaczki i zdobywania szczytu. Alpiniści powinni opierać się na wartościowych źródłach tłuszczu, włączając do swojej diety produkty bogate w wielo- i jednonienasycone kwasy tłuszczowe, takie jak oleje roślinne (oliwa z oliwek, olej lniany), orzechy, nasiona. Zaleca się, aby podaż tłuszczu w diecie alpinistów stanowiła uzupełnienie energii po pokryciu zapotrzebowania na węglowodany oraz białko i powinna stanowić 20–35% całkowitej podaży energii. Alpiniści wybierając produkty zabierane w góry powinni kierować się przede wszystkim swoimi preferencjami i dostosować podaż każdego składnika odżywczego do indywidualnej tolerancji układu pokarmowego.

Ograniczony dostęp do świeżych owoców i warzyw oraz produktów bogatych w białko w warunkach wysokogórskich może prowadzić do niedoborów składników mineralnych i witamin. Przed wyprawą należy oznaczyć stężenie ferrytyny i witaminy D w surowicy krwi. W przypadku niedoboru mikroskładników odżywczych, takich jak żelazo i witamina D, należy wdrożyć suplementację w celu optymalizacji stanu zdrowia i kontynuować ją pod nadzorem lekarza. Jeszcze przed wyprawą warto włączyć do diety produkty bogate w żelazo i zapewnić odpowiednią biodostępność żelaza. Wskazane jest dostosowanie odpowiedniej dawki suplementacji witaminą D do aktualnego stężenia w surowicy i kontynuowanie suplementacji w trakcie wyprawy. Alpiniści powinni spożywać produkty bogate w przeciwutleniacze, w tym źródła witaminy C, beta-karotenu, witaminy E, selenu i cynku podczas pobytu na dużych wysokościach. Liofilizowane owoce, warzywa i ich sproszkowane formy mogą być cennym

źródłem witamin antyoksydacyjnych w warunkach wysokogórskich. Konieczne są dalsze badania w celu sformułowania zaleceń dotyczących podaży mikroelementów w warunkach wysokogórskich.

Ilość płynów na dużych wysokościach powinna być dostosowana indywidualnie ze względu na zróżnicowane tempo utraty płynów i elektrolitów i oparta na kontroli koloru moczu (odwodnienie powoduje ciemniejszy kolor moczu) oraz objawach AMS, która powoduje zatrzymanie wody w organizmie. Regularne uzupełnianie płynów jest niezbędne, aby zapobiec odwodnieniu.

Ze względu na ograniczony dostęp do produktów spożywczych na dużych i ekstremalnych wysokościach, suplementacja może być jedynym rozwiązaniem zapewniającym alpinistom odpowiednią podaż energii i niezbędnych składników odżywczych, a także poprawiającym funkcjonowanie organizmu w trudnych warunkach środowiskowych. Potencjalny ergogeniczny wpływ suplementowanych węglowodanów na wydolność wysiłkową w warunkach wysokogórskich może być modulowany przez aklimatyzację. Na podstawie przeglądu literatury przedmiotu uznano, że konieczne są dalsze badania w celu opracowania odpowiednich strategii podaży węglowodanów, uwzględniających wpływ niedotlenienia na wrażliwość na insulinę i utlenianie substratów, aby poprawić zdolności wysiłkowe na dużych wysokościach. Warto rozważyć suplementację białkiem w celu pokrycia dziennego zapotrzebowania na ten składnik ze względu na mniejszą dostępność produktów spożywczych będących źródłem białka w warunkach wysokogórskich, zwłaszcza powyżej bazy. Nadal brakuje badań oceniających wpływ suplementacji białkiem na uczucie sytości na dużych wysokościach. Równowaga prooksydacyjno-antyoksydacyjna ma kluczowe znaczenie dla aklimatyzacji wysokogórskiej, ale kwestia zaplanowania odpowiedniej strategii żywieniowej i suplementacyjnej w zakresie antyoksydantów pozostaje niejasna.

Spośród suplementów diety warto zwrócić uwagę na kofeinę, która podawana w małych dawkach (3 mg/kg mc.) może potencjalnie pozytywnie wpływać na funkcje poznawcze alpinistów. Wstępne dane sugerują, że kofeina może stanowić potencjalny środek terapeutyczny podczas nieprawidłowej aklimatyzacji do dużych wysokości prowadzącej do obrzęku płuc. Z kolei, azotany suplementowane w postaci soku z buraków mogą poprawiać funkcję śródbłonka na dużych wysokościach, ale nie są zalecane jako środek profilaktyczny lub ergogeniczny w przypadku występującej już choroby wysokościowej. W związku z dolegliwościami żołądkowo-jelitowymi zgłaszanymi podczas wspinaczki na dużych wysokościach, probiotyki i prebiotyki mogą potencjalnie chronić barierę jelitową alpinistów przed dysfunkcją wywołaną niedotlenieniem. W warunkach wysokogórskich, dostarczanie

odpowiedniej ilości kwasów tłuszczowych omega-3 może być niewystarczające, dlatego alpiniści powinni rozważyć suplementację szczególnie powyżej obozu bazowego, ze względu na ograniczony dostęp do żywności bogatej w ten składnik odżywczy. Glutamina może mieć pozytywny wpływ na funkcjonowanie enterocytów i komórek odpornościowych w jelitach. Ponadto może być substratem energetycznym dla mikrobiomu jelitowego, zmniejszając proces zapalny, któremu towarzyszy zwiększona przepuszczalność jelit, poprawiając w ten sposób funkcje poznawcze i nastrój. Brakuje dobrze zaprojektowanych badań badających związek między stosowaniem suplementów diety, takich jak: kofeina, azotany, probiotyki, kwasy tłuszczowe omega-3, glutamina a poprawą wyników sportowych i zdrowia na dużych i ekstremalnych wysokościach. Ponadto istnieje kilka suplementów, takich jak kurkumina, *Ginko biloba*, NAC i sok z cierpkiej wiśni, które wymagają dalszych badań w celu oceny ich skuteczności w warunkach wysokogórskich. Biorąc pod uwagę pozytywny wpływ soku z wiśni cierpkiej na jakość snu i wydolność fizyczną, stosowanie tego suplementu w warunkach wysokogórskich wydaje się interesującą strategią. Dlatego też istnieje potrzeba przeprowadzenia kontrolowanych, randomizowanych badań z udziałem alpinistów.

3.2. Wyniki analizy sposobu żywienia alpinistów

Oceniając wartość energetyczną i odżywczą zwyczajowej diety alpinistów przed wyprawą wysokogórską stwierdzono, iż mediana zapotrzebowania energetycznego wynosiła 2951 (2731; 3318) kcal, a podaż energii 3031,4 (2470,7; 3702,8) kcal. Suplementacja diety stanowiła 1,98% całkowitej podaży energii. Ponad połowa badanych wspinaczy (52,94%) utrzymywała ujemny bilans energetyczny. Mediana procentowego pokrycia zapotrzebowania energetycznego dietą u badanych wspinaczy wynosiła 92,64 (85,55; 113,62) %. Większość wspinaczy (70,59%) pokrywała zapotrzebowanie na węglowodany wraz z dietą, jednak podaż błonnika nie była zapewniona na odpowiednim poziomie u 23,53% osób (Załącznik 1, Publikacja 4, Tabela 1). Wszyscy wspinacze pokryli zapotrzebowanie na białka i tłuszcze wraz z dietą. Jednak 23,53% przekroczyło rekomendowane wartości spożycia tłuszczu, a 5,88% przekroczyło wartości spożycia białka. Mniej niż połowa ankietowanych wspinaczy realizowała zalecenia dotyczące spożycia nasyconych kwasów tłuszczowych, a nieco ponad jedna trzecia realizowała rekomendacje dotyczące spożycia cholesterolu wraz z dietą. Medianę wartości energetycznej i odżywczej diety uzyskanej z analizy dzienniczków żywieniowych przedstawiono w Tabeli 2 (Załącznik 1, Publikacja 4, Tabela 1). W odniesieniu do rekomendacji dla sportowców (Benardot 2012; Thomas et al. 2016; EFSA (European Food Safety Authority) 2017; Kerksick et al. 2018) alpiniści nie dostarczali odpowiednich

ilości selenu i jodu, a także witaminy D i K. Wszyscy wspinacze pokryli zapotrzebowanie na sód, fosfor, witaminy: A, B_1, B_2 i B_{12}. Na podstawie wyników uzyskanych z analizy autorskiego kwestionariusza do oceny nawyków żywieniowych wspinaczy i himalaistów w warunkach wysokogórskich stwierdzono, iż 85,7% alpinistów deklaruje, że ich dieta zwyczajowa znacząco różni się od typowej diety stosowanej podczas wypraw wysokogórskich, podczas gdy 14,3% nie zgłasza żadnych zmian w diecie w czasie pobytu w górach.

Oceniając wartość energetyczną i odżywczą 3 dni diety 15 alpinistów podczas górskich aktywności w czasie wyprawy wysokogórskiej stwierdzono, iż zapotrzebowanie energetyczne uczestników badania wynosiło 4559,5 ± 425 kcal, a podaż energetyczna 2776,8 ± 878 kcal. Wspinacze dostarczali wraz z suplementacją 588 kcal, co stanowiło 21,2% ich podaży energii. Jedynie 2 osoby, tj. 13,33% respondentów, pokryło zapotrzebowanie na energię zgodnie z rekomendacjami. Szczegółowe dane opisujące średnią wartość energetyczną (w odniesieniu do wydatku energetycznego) i odżywczą diety, uzyskaną na podstawie analizy dzienników żywieniowych, przedstawiono w Tabeli 2 i 3 (Załącznik 1, Publikacja 1, Tabela 2 i 3). Większość wspinaczy (93,3%) pokrywała zapotrzebowanie na węglowodany, a 46,7% na białko zgodnie z rekomendacjami dla sportowców. Wszyscy wspinacze pokryli zapotrzebowanie na tłuszcze, a 33,3% z nich przekraczało wartości rekomendowane.

Mediana wydatku energetycznego z 2 dni aktywności górskiej oraz 1 dnia odpoczynku wśród 17 uczestników badania podczas wyprawy wysokogórskiej wynosiła 3897,95 ± 521 kcal, a podaż energii 2715,71 ± 794 kcal. Wspinacze dostarczali średnio 325 ± 220 kcal wraz z suplementacją, co stanowiło 11,99% ich podaży energetycznej. Jedynie jedna osoba, tj. 5,88% respondentów, pokrywała swoje zapotrzebowanie na energię w stosunku do wydatku energetycznego. Większość wspinaczy (94,14%) pokrywała zapotrzebowanie na węglowodany, a 58,82% na białko. Wszyscy wspinacze pokryli zapotrzebowanie na tłuszcze, a 23,53% z nich przekraczało wartości rekomendowane. Medianę wartości energetycznej i odżywczej diety na podstawie analizy dzienników żywieniowych przedstawiono w Tabeli 10 (Załącznik 1, Publikacja 3, Tabela 10). W porównaniu do norm dla sportowców (Benardot 2012; Thomas et al. 2016; EFSA (EuSafety Authorityty Authority) 2017; Kerksick et al. 2018), alpiniści nie dostarczali odpowiedniej ilości potasu, wapnia, magnezu, żelaza, cynku, selenu i jodu, a także witamin A, D, C, kwasu foliowego i witaminy K. Większość wspinaczy pokrywała swoje zapotrzebowanie na makroskładniki odżywcze z dietą i suplementacją. Z kolei niewielki odsetek badanych (<50%) pokrywał zapotrzebowanie na żelazo, kwas foliowy, potas, wapń, witaminę D, witaminę C i witaminę A wraz z dietą i suplementacją. Żaden z badanych

wspinaczy nie dostarczał odpowiedniej ilości wapnia i witaminy K w odniesieniu do rekomendacji dla sportowców.

Na podstawie wyników uzyskanych z analizy autorskiego kwestionariusza do oceny nawyków żywieniowych wspinaczy i himalaistów w warunkach wysokogórskich 67,9% respondentów ocenia swój apetyt w górach powyżej 3000 m n.p.m. jako dobry, 14,3% jako średni, 14,3% jako bardzo dobry, a 3,6% osób ocenia swój apetyt jako słaby. Respondenci wybierali najczęściej batony czekoladowe, dania liofilizowane, czekoladę, żele energetyczne, żelki, słone przekąski, suszone mięso, musy owocowe, konserwy mięsne i rybne, kabanosy, kanapki, chałwę, suszone owoce, orzechy oraz żywność instant (galaretki, budynie, zupy) wśród produktów spożywanych w górach (Załącznik 1, Publikacja 1, Rysunek 1). Duży odsetek wspinaczy (75%) deklaruje zmęczenie słodkim smakiem, spowodowane dużą częstotliwością i ilością spożywanych przekąsek węglowodanowych, jednak mimo tego 78,6% respondentów preferuje słodkie przekąski w górach. Produkty słone lub kwaśne, takie jak mięso, ser, kiełbasy, kanapki i orzechy, preferuje 21,4% respondentów. W czasie pobytu w górach alpiniści najrzadziej spożywali warzywa, owoce, jaja, mleko i przetwory mleczne, masło i śmietanę, ryby i mięso i uważają te grupy produktów spożywczych za mało dostępne (Załącznik 1, Publikacja 1, Rysunek 2). Z kolei najczęściej spożywane są: cukier i słodycze (85,7%), orzechy i nasiona (67,9%), pełnoziarniste produkty zbożowe (64,3%) oraz rafinowane produkty zbożowe (57,1%). Spośród ankietowanych wspinaczy 7,1% realizowało prawidłowe zalecenia dietetyczne dotyczące częstotliwości spożycia olejów roślinnych (dzienne spożycie), 17,9% spożywało 1–2 porcje owoców dziennie, 17,9% spożywało produkty zbożowe codziennie, a 25% osób spożywało ryby 1–2 razy w tygodniu.

Najczęściej stosowanym rozwiązaniem żywieniowym po zakończeniu górskiej akcji z biwakiem (82,1%) są posiłki liofilizowane. Najczęściej spożywanymi przez wspinaczy podczas wypraw liofilizowanymi produktami są dania obiadowe, zupy, owsianki, desery i owoce. Żywność liofilizowaną chętnie spożywa 57,1% badanych. Aż 50% wspinaczy deklaruje występowanie dolegliwości żołądkowo-jelitowych (wzdęcia, bóle brzucha, biegunka) po spożyciu liofilizowanej żywności. Zdecydowana większość wspinaczy (92,9%) deklaruje, że ilość pożywienia wpływa na ich samopoczucie w górach, a 96,4% osób podkreśla, że jakość pożywienia ma również duże znaczenie. Mimo to, aż 57,1% wspinaczy nie zabiera ze sobą w góry odpowiedniej ilości jedzenia. Wynika to z błędów logistycznych lub próby zminimalizowania ciężaru plecaka. Według respondentów regeneracja po zakończeniu akcji górskiej trwa, w zależności od napotkanych trudności, od 1 do 7 dni. Po powrocie z wyprawy 73,3% respondentów zaobserwowało spadek masy ciała. Wyniki ankiety wskazują, że 85,7%

wspinaczy nie wykonuje badań krwi przed wyprawą, w przeciwieństwie do 14,3% ankietowanej grupy, która kontroluje swój stan zdrowia. Zdecydowana większość respondentów nie kontroluje również poziomu żelaza (92,9%) ani ferrytyny (96,5%). Jedynie 7,1% osób konsultuje się z dietetykiem przed wyjazdem na wyprawę.

Wspinacze najczęściej zabierają ze sobą w góry napoje izotoniczne (78,6%), herbatę (60,7%), wodę (53,6%), kawę (14,3%) lub soki (10,7%), a także piwo (3,6%), roztwór elektrolitów (3,6%), a także kisiel (3,6%). Ilość płynów spożywanych przez wspinaczy w górach przedstawiono na Rysunku 3 (Załącznik 1, Publikacja 1, Rysunek 3). Respondenci (57,1%) uważają, że ilość wypijanych w górach płynów jest niewystarczająca. W grupie respondentów 85,7% osób stosuje elektrolity jako dodatek do wody. Tylko 14,3% respondentów stosuje tabletki do uzdatniania wody podczas picia wody z lodowców. U 20% badanych wystąpiła ostra choroba wysokościowa. Aż 94,4% wspinaczy twierdzi, że jedną z przyczyn choroby wysokościowej może być niedostateczne nawodnienie organizmu.

Alpiniści w górach wysokich najczęściej suplementują dietę kofeiną (39,3%), suplementami białkowymi (28,6%), niektórymi witaminami i składnikami mineralnymi (multiwitaminami, witaminą B, witaminą D, witaminą C, witaminą K2, żelazem, magnezem – 28,6%), kwasami tłuszczowymi omega-3 (14,3%), suplementami białkowo-węglowodanowymi (7,1%), a także probiotykami (7,1%) (Załącznik 1, Publikacja 1, Rysunek 4). Z uwagi na ograniczony dostęp do żywności w górach wysokich, niską podaż energii i składników odżywczych wraz z dietą, stosowanie suplementów dostarczających białka, węglowodanów, składników mineralnych i witamin wydaje się być uzasadnione. Deklarowana przez respondentów suplementacja kofeiny i probiotyków podczas pobytu na dużych i ekstremalnych wysokościach jest zgodna z zaleceniami dla alpinistów, choć wciąż wymaga dalszych badań.

3.3. Wyniki analizy stanu zdrowia alpinistów

Długotrwały pobyt w warunkach wysokogórskich spowodował istotny statystycznie spadek masy ciała oraz masy mięśniowej. Nastąpił również istotny spadek masy ciała szczupłego w kończynach górnych oraz dolnych, a także wskaźnika ALST. Zgodnie z analizą wskaźnika BMI, masy tłuszczu, masy beztłuszczowej, masy mięśniowej [%], masy ciała szczupłego oraz składników mineralnych w kościach i gęstości mineralnej kości nie wykazano istotnych zmian po okresie przewlekłego niedotlenienia (Załącznik 1, Publikacja 3, Tabela 8).

Oceniając wartość wskaźników hematologicznych wykazano istotny wzrost liczby leukocytów, erytrocytów, płytek krwi oraz wzrost wartości hemoglobiny, hematokrytu, a także

neutrofili i monocytów po wyprawie wysokogórskiej (Załącznik 1, Publikacja 3, Tabela 9). Zaobserwowano istotny spadek poziomu cholesterolu całkowitego, HDL, bilirubiny całkowitej, albuminy oraz białka całkowitego. Wykazano istotny statystycznie wzrost poziomu fosfatazy kwaśnej całkowitej oraz fosforu nieorganicznego. Wszystkie wskaźniki moczu oraz wskaźniki kału (badanie ogólne kału, pasożyty, lamblie, Helicobacter pylori - dane nieopublikowane) i wskaźniki związane z mikrobiomem i przepuszczalnością jelit, oceniane na podstawie próbek kału, w większości pozostały w wartościach referencyjnych zarówno przed wyprawą jak i po wyprawie (Załącznik 1, Publikacja 5). W Tabeli 11 (Załącznik 1, Publikacja 3, Tabela 11) przedstawiono odsetek wspinaczy, których wyniki wskaźników stanu zdrowia po powrocie z wyprawy były zgodne z normami laboratoryjnymi i którzy pokrywali zapotrzebowanie na wybrane makro- i mikroskładniki odżywcze diety. Wyniki badań większości wspinaczy były zgodne z normami laboratoryjnymi. Wyjątkiem był poziom witaminy D u wspinaczy, który był zbyt niski u 52,94% przebadanych osób. Niewystarczająca podaż żelaza, kwasu foliowego i witaminy D wraz z dietą znajdowała odzwierciedlenie w wynikach badań krwi.

Na podstawie analizy wskaźników przepuszczalności jelitowej przed wyprawą wykazano, iż mediana wartości zonuliny w kale u wspinaczy wynosiła 28,40 (14; 193,7) ng/ml, podczas gdy wartość alfa-1-antytrypsyny w kale wynosiła 5,95 (4,10; 6,86) ng/ml. Wartości zonuliny w kale były podwyższone u 35,29% badanych wspinaczy, podczas gdy wartości alfa-1-antytrypsyny w kale były prawidłowe u wszystkich badanych. Analizowane korelacje między składnikami odżywczymi zwyczajowej diety alpinistów a poziomami markerów przepuszczalności jelitowej nie były istotne statystycznie (Załącznik 1, Publikacja 4, Tabela 2).

3.4. Wyniki analizy wydolności fizycznej alpinistów

Poziom wskaźników charakteryzujących wydolność tlenową organizmu alpinistów (maksymalny minutowy pobór tlenu w ujęciu globalnym i relatywnym, maksymalna wentylacja minutowa płuc, maksymalna częstość skurczów serca, czas testu stopniowanego) obniżył się po powrocie z wyprawy. Opisane zmiany były istotne statystycznie (Załącznik 1, Publikacja 3, Tabela 4). Wielkości wskaźników charakteryzujących poziom drugiego progu wentylacyjnego po wyprawie nie ulegały istotnym zmianom, co obrazuje Tabela 5 (Załącznik 1, Publikacja 3, Tabela 5). Jedyną istotną statystycznie zmianę odnotowano w poziomie częstości skurczów serca, który obniżył się podczas badań po powrocie z wypraw.

Poziom wskaźników charakteryzujących wydolność beztlenową organizmu badanych osób po powrocie z wyprawy generalnie obniżył się (Załącznik 1, Publikacja 3, Tabela 6). W teście dla kończyn dolnych maksymalna moc anaerobowa oraz moc średnia wyrażane w

wartościach globalnych i w ujęciu relatywnym obniżyły się. Również wartości pracy globalnej i czasu utrzymania MAP były niższe podczas badań prowadzonych po powrocie z wypraw. Czas osiągnięcia mocy maksymalnej obniżył się, jednak zmiana ta nie była istotna statystycznie w porównaniu do pozostałych wymienionych wskaźników. W teście dla kończyn górnych zmiany badanych wskaźników kształtowały się nieco odmiennie. Maksymalna moc anaerobowa zarówno w ujęciu globalnym jak i relatywnym uległa obniżeniu, ale obserwowane zmiany były nieistotne statystycznie. Podobnie zmiany czasu uzyskania i utrzymania mocy maksymalnej również nie były istotne statystyczne. W teście kończyn górnych w istotny statystycznie sposób zmniejszyły się wartości mocy średniej z testu i pracy globalnej (Załącznik 1, Publikacja 3, Tabela 7).

3.5. Wyniki analizy mikrobiomu alpinistów

Profil różnorodności taksonomicznej był wysoki zarówno przed, jak i po wyprawie (Załącznik 1, Publikacja 4, Ryc. 1a, Ryc. uzupełniająca 1). Podczas gdy względna obfitość dominujących gatunków nie zmieniła się znacznie przed i po wyprawie u niektórych osób, u innych odnotowano wyraźniejsze modyfikacje składu mikrobiomu. Zidentyfikowano średnio 200 ± 69 gatunków bakterii na uczestnika przed wyprawą i 179 ± 53 gatunków na uczestnika po wyprawie. Na poziomie funkcjonalnym zidentyfikowano średnio 76 ± 8 funkcji bakteryjnych na uczestnika przed wyprawą i 77 ± 8 funkcji po wyprawie. Różnice w liczbie gatunków i funkcji przed i po wyprawie nie były istotne statystycznie. Prewalencja (rozpowszechnienie) gatunków była zasadniczo stabilna po wyprawie (Załącznik 1, Publikacja 4, Ryc. 1a, po lewej). Dotyczyło to zwłaszcza taksonów o niskiej prewalencji (obecnych u <1/3 uczestników), gdzie odnotowane różnice wynikały z zmian u co najwyżej 1–2 osób. Wszelkie różnice można przypisać zmianom tylko u maksymalnie dwóch osób. W analizie gatunków najbardziej stabilne okazały się taksony o niskiej prewalencji, natomiast w analizie funkcji - te o wysokiej prewalencji w całej kohorcie. Jedynym wyjątkiem był szlak *GLUCOSE1PMETAB-PWY (degradacja glukozy i glukozo-1-fosforanu),* obecny u około połowy osób przed wyprawą, a wykrywany niemal u wszystkich po powrocie z wyprawy. Zaobserwowano statystycznie istotny wzrost obfitości tej funkcji w wyniku wyprawy (test Wilcoxona, $p < 0,05$). Analiza różnicowego wzbogacenia (LEfSe, tzn. porównanie względnej obfitości taksonów między próbami „przed" i „po") wykazała dwa gatunki o wyższej obfitości po ekspedycji: *Lactococcus lactis* oraz gatunek z rodzaju *Phocaeicola* (Załącznik 1, Publikacja 4, Ryc. 2). Zaobserwowano też trend spadkowy dla jednego gatunku z rodziny Clostridiaceae po ekspedycji. Nie wykryto statystycznie istotnych zmian na poziomie funkcji.

Korelacja cech mikrobiomu z danymi z analiz sposobu żywienia wykazała związek *szlaku PWY-6703: biosyntezy preQ0* (*wczesny etap biosyntezy 7-deazopuryn; preQ0 = 7-cyano-7-deazaguanina, prekursor modyfikacji nukleozydu queuozyny w bakteryjnym tRNA)* ze spożyciem witamin B_6 i C przed wyprawą (korelacje Spearmana odpowiednio 0,83 i 0,86, skorygowane wartości $p < 0,05$). Zaobserwowano spadek spożycia witamin po wyprawie, który był znaczący w przypadku witaminy C (witamina B_6: 6,1 mg do 2,6 mg, wartość *p* testu T > 0,05; witamina C: 325,7 mg do 149,2 mg, wartość *p* testu T < 0,01). Podobnie, średnia obfitość *szlaku biosyntezy PWY-6703: preQ0* wykazała znaczny, ale statystycznie nieistotny spadek (z 18% do 15%). Żadne inne powiązania funkcji lub gatunków ze składnikami odżywczymi diety przed lub po ekspedycji nie przeszły korekty na wielokrotne testowanie.

Ponieważ skala zmian mikrobiomu różniła się między uczestnikami, odległość Braya–Curtisa (miara różnic w składzie i obfitości) między próbkami przed i po ekspedycji była zróżnicowana (wyższe wartości oznaczają większą rozbieżność składu) (Załącznik 1, Publikacja 4, Ryc. 1a), przy czym próbki jednego z uczestników wykazywały największą rozbieżność.

Rozróżnienie osób na podstawie momentu osiągnięcia II progu wentylacyjnym (VT2) pozwoliło stwierdzić, że uczestnicy z poprawą wydolności charakteryzowali się większą modyfikacją mikrobiomu (Załącznik 1, Publikacja 4, Ryc. 3b). Ponadto u uczestników, u których odnotowano większe zmiany w mikrobiomie (odległość Braya-Curtisa ≥ 0,36, wybrana na podstawie mediany), stwierdzono istotnie większe bogactwo wyjściowe mikrobiomu w porównaniu z osobami o zmianach mniejszych (Załącznik 1, Publikacja 4, Ryc. 3c-e). Towarzyszyło temu nieistotne, ale wyraźnie wyższe spożycie manganu, wapnia, witaminy A, magnezu i fosforu (średnie spożycie manganu 9,2 vs. 3,9 mg, wapnia 1520,7 vs. 973,3 mg, witaminy A 2246,0 vs. 1291 µg, magnezu 687,7 vs. 397,6 mg, fosforu 2021,5 vs. 1545,7 mg u uczestników z istotnymi zmianami mikrobiomu i bez nich, $p < 0,05$, skorygowane wartości $p > 0,05$). Analiza różnicowego wzbogacenia (LEfSe) gatunków i funkcji na początku badania wykazała statystycznie istotne różnice między obiema grupami (Załącznik 1, Publikacja 4, Ryc. 3f). Próbki mikrobiomu od osób, u których stwierdzono zmiany, były wzbogacone w *Clostridium fessum, Streptococcus salivarius* i *szlak degradacji 5'-fosforanu inozyny*, a uboższe m.in. w taksony z rodziny Clostridiales oraz *szlaki metabolizmu folianów* i *biosyntezy aminoacylo-tRNA.*

W podgrupie z największymi zmianami mikrobiomu większa zmienność dotyczyła gatunków niż funkcji (Załącznik 1, Publikacja 4, Ryc. 4a–b). *Dorea sp. AF36_15AT* wzrosła z 57% do 100% próbek, a *GGB9758_SGB15368 (Firmicutes)* z 43% do 86%. Większość funkcji

utrzymała się przed i po wyprawie, choć dla kilku odnotowano spadek częstości: *tetrapyrrole biosynthesis I (from glutamate), UDP-N-acetyl-D-glucosamine biosynthesis I* oraz *O-antigen building blocks biosynthesis (E. coli)* (z 43% do 14% próbek).

Analiza różnicowego wzbogacenia (LEfSe) zidentyfikowała trzy gatunki o wyższej obfitości po wyprawie: *Blautia luti, Romboutsia timonensis* i *GGB4569_SGB6310* z rodziny Lactobacillaceae. Nie stwierdzono gatunków wzbogaconych przed wyprawą ani statystycznie istotnych zmian w liczebności funkcji.

Zidentyfikowano kilka korelacji cech mikrobiomu ze wskaźnikami krwi i wskaźnikami wydolności beztlenowej (Załącznik 1, Publikacja 4, Tabela 2). Przed wyprawą odnotowano dwa gatunki związane z potasem: *Prevotella copri clade A* (korelacja dodatnia) i *Bacteroides uniformis* (korelacja ujemna), a także 15 przypadków korelacji wskaźników z funkcjami drobnoustrojów. *Szlak biosyntezy dTDP-β-L-ramnozy* był jedyną funkcją związaną z dwoma różnymi wskaźnikami przed wyprawą: całkowitą wykonaną pracą kończyn dolnych (W_t) i średnią wielkością osiągniętej mocy kończyn dolnych (P_{mean}) (dodatnie w obu przypadkach). Po wyprawie odnotowano jedną korelację gatunku *Agathobaculum butyriciproducens* z wapniem (silna dodatnia) i osiem z funkcjami. *Biosynteza NAD de novo I (z asparaginianu)* była dodatnio skorelowana z bazofilami i wapniem a *ścieżka biosyntezy L-seryny i glicyny I* ujemnie z sodem i dodatnio z cholesterolem HDL). *Biosynteza rybonukleotydów guanozynowych de novo* była dodatnio skorelowana z sodem przed wyprawą i ujemnie ze średnią objętością płytek krwi (MPV) po wyprawie, a *degradacja mio-, chiro- i scyllo-inozytolu* była dodatnio skorelowana z sodem przed wyprawą i z bazofilami po wyprawie. Inne analizowane korelacje cech mikrobiomu ze wskaźnikami krwi, moczu, kału i wskaźnikami wydolności beztlenowej nie były istotne statystycznie.

Ponadto, znaleziono szereg korelacji funkcji drobnoustrojów z dietą po wyprawie (z których wszystkie przeszły korektę dla wielokrotnego testowania, Załącznik 1, Publikacja 4, Tabela uzupełniająca 1). Zidentyfikowano silne dodatnie korelacje (korelacja Spearmana > 0,90) *biosyntezy koenzymu A* z podażą energii i węglowodanów, jednonienasyconych kwasów tłuszczowych (MUFA), a także witamin C, B_{12}, B_1 i B_6. *Biosynteza L-argininy* była powiązana z ogólnym spożyciem węglowodanów i węglowodanami przyswajalnymi, podczas gdy *szlak fosforanu pentozy* z selenem, a białko roślinne z *biosyntezą sacharozy*. Stwierdzono ujemne korelacje między *adeniną* i *adenozyną* a sodem, a także między *biosyntezą L-ramnozy* a białkiem zwierzęcym.

4. MOCNE STRONY I OGRANICZENIA PROJEKTU

4.1. Mocne strony projektu i przyszłe kierunki

Badania przeprowadzone w ramach niniejszej rozprawy doktorskiej należą do nielicznych, które w sposób kompleksowy analizują wartość energetyczną i odżywczą diety, wydolność tlenową i beztlenową, masę ciała i skład ciała oraz wskaźniki hematologiczne i biochemiczne krwi, wskaźniki moczu, a także strukturę mikrobiomu jelitowego, zarówno przed, jak i po co najmniej 3-tygodniowym pobycie na wysokościach powyżej 3000 m n.p.m. Jednym z głównych atutów projektu jest przeprowadzenie badań w naturalnych warunkach wysokogórskich, co pozwala na uzyskanie wyników lepiej odzwierciedlających rzeczywiste reakcje organizmu niż w badaniach prowadzonych w sztucznych warunkach, np. z wykorzystaniem komory hipoksyjnej. Uzyskane wyniki stanowią podstawę do sformułowania wniosków dotyczących oddziaływania długotrwałej ekspozycji na niedotlenienie na sposób żywienia, wydolność fizyczną, stan zdrowia i mikrobiom na poziomie morza.

Z perspektywy praktycznego zastosowania uzyskane wyniki dostarczają cennych wskazówek dla alpinistów i turystów planujących wyprawy na wysokości powyżej 3000 m n.p.m., trenerów i dietetyków sportowych. Strategie przed wyprawą mogą obejmować optymalizację statusu mikroskładników odżywczych, w szczególności witamin i minerałów, które korelują z korzystnymi funkcjami drobnoustrojów i wskaźnikami wydolności. Podczas wypraw integracja pokarmów bogatych w składniki odżywcze, wspierających mikrobiom - nawet jeśli częściowo w postaci suplementów lub produktów liofilizowanych - może pomóc w utrzymaniu korzystnych profili mikrobiomu jelitowego i związanych z nimi funkcji metabolicznych. W szerszej perspektywie, poza kontekstem sportowym, przedstawione wyniki przyczyniają się do rozwijającej literatury dotyczącej znaczenia mikrobiomu jelitowego w adaptacji do ekstremalnych środowisk (Guo et al., 2025; Kleessen et al., 2005; Li et al., 2022; K. Liu et al., 2021; Ma et al., 2019; Q. Su et al., 2024; Zhao et al., 2023), podkreślając rolę interwencji żywieniowych jako potencjalnego narzędzia wspierania zarówno zdrowia, jak i wydolności.

Przyszłe badania powinny dążyć do potwierdzenia uzyskanych odkryć w większych kohortach oraz do oceny ukierunkowanych interwencji, takich jak prebiotyki, probiotyki, postbiotyki czy dostosowana suplementacja mikroskładnikami odżywczymi, mających na celu wspieranie korzystnej odpowiedzi mikrobiomu jelitowego w środowiskach wysokogórskich. Przyznać należy, że niektórzy uczestnicy spożywali suplementy sportowe i żywność funkcjonalną dla sportowców, które mogą wpływać na skład mikrobiomu jelitowego i

integralność bariery jelitowej. Chociaż w niniejszym projekcie odnotowano spożycie suplementów, nie analizowano szczegółowo potencjalnego wpływu dodatków powszechnie występujących w tych produktach (np. emulgatorów, sztucznych substancji słodzących, konserwantów) na mikrobiom jelita. Przyszłe badania powinny obejmować szczegółową ocenę składników suplementów, w tym dodatków nieodżywczych, aby lepiej zrozumieć ich wpływ w warunkach stresu wysokogórskiego. Integracja metagenomiki z transkryptomiką gospodarza, metabolomiką i profilowaniem immunologicznym, uzupełniona o kontrolowane badania żywieniowe, może dodatkowo wyjaśnić mechanizmy łączące mikrobiom jelitowy, dietę i adaptacje fizjologiczne w sportach ekstremalnych oraz poza nimi.

4.2. Ograniczenia projektu

Niniejszy projekt badawczy ma kilka potencjalnych ograniczeń. Jednym z nich jest niewielka liczebność grupy i brak grupy kontrolnej. W podejmowanych dotychczas badaniach w zakresie sposobu żywienia alpinistów w górach, a także medycyny i fizjologii górskiej liczebność badanych grup jest niska i opisuje najczęściej grupę alpinistów w ramach jednej wyprawy. Jednakże należy zauważyć, że znalezienie jednorodnej grupy uczestników stanowi spore wyzwanie, a doświadczeni alpiniści w Polsce stanowią stosunkowo niewielką populację, co dodatkowo ogranicza możliwości doboru próby. Alpiniści nie stanowili jednej grupy, której przyświecał jeden cel wspinaczkowy na terenie jednego kraju i pasma górskiego. Wspinacze, charakteryzujący się cechami opisanymi w badaniu, stanowią niewielką, ale elitarną grupę w Polsce, stąd do badania zakwalifikowano kilka odrębnych zespołów wyprawowych, wspinających się na podobnych wysokościach i z podobnymi celami sportowymi, ale w różnych częściach świata. Zebranie i zachęcenie uczestników było jednym z najtrudniejszych etapów projektu, dlatego ostatecznie grupę badaną stanowiło 17 osób (publikacja 3, 4 oraz 5) oraz 28 osób (publikacja 1).

W pierwszej publikacji z cyklu analizowano pod kątem wartości energetycznej i odżywczej diety jedynie dni dziennika żywieniowego, w czasie których podejmowano aktywność fizyczną w warunkach wysokogórskich. W kolejnym badaniu tego typu warto byłoby rozszerzyć analizę wartości energetycznej i odżywczej diety o dni w bazie (dni odpoczynku) i zwiększyć grupę badanych, co wykonano i opisano w publikacji 3. W przyszłości warto rozważyć uczestnictwo dietetyka na wyprawie, który miałby stały kontakt z kucharzami odpowiedzialnymi za przygotowywanie posiłków i kontrolował ilość spożywanego jedzenia, a także nadzorował prowadzone przez alpinistów dzienniczki żywieniowe. Ponadto, warto skupić się na jeszcze bardziej szczegółowej analizie suplementów stosowanych przez

wspinaczy w górach wysokich w kontekście ich wpływu na zdrowie, nastrój i wydolność fizyczną.

Wydolność fizyczna alpinistów była oceniana trzeciego lub czwartego dnia po powrocie z wyprawy, ponieważ musieli oni podróżować z innego kraju i odległych gór do odpowiedniego laboratorium w Polsce. Uniemożliwia to porównanie wyników z innymi badaniami, które różnią się liczbą dni między końcem ekspozycji na niedotlenienie a momentem wykonania pomiarów na poziomie morza. Poza niedotlenieniem, także inne czynniki, takie jak sposób żywienia i rodzaj aktywności fizycznej wykonywanej w górach, czy liczba dni aktywnych w porównaniu z liczbą dni odpoczynku, mogą znacząco przyczyniać się do obserwowanych zmian w składzie ciała. Grupy wspinaczkowe aktywne w Peru mogły wykorzystać przerwę między kilkoma dniami aktywności górskiej na zejście do miejscowości u podnóża gór, aby zrekompensować tam ujemny bilans energetyczny, podczas gdy grupy ekspedycyjne w Kirgistanie, Nepalu lub Pakistanie były uzależnione od pożywienia, które zabrały w góry lub które zaoferowała im agencja górska. W niniejszym badaniu nie mierzono dawki niedotlenienia, ale biorąc pod uwagę statystycznie istotną utratę masy mięśniowej u wspinaczy, można założyć, że dawka niedotlenienia była wystarczająca, aby wywołać opisaną zmianę. Warto byłoby zmierzyć ten wskaźnik w przyszłych badaniach, aby móc jednoznacznie ocenić wartość niedotlenienia, która koreluje ze spadkiem masy mięśniowej u osób badanych.

Ze względu na niewielką liczbę zaburzeń żołądkowo-jelitowych (n = 3) i AMS (n = 1), badanie nie miało wystarczającej mocy do wykrycia wiarygodnych korelatów mikrobiomu. Eksploracyjne skanowanie różnorodności i taksonomii nie ujawniło wartości odstających. Podobnie, eksploracyjne wskaźniki różnorodności alfa i beta oraz badanie LEfSe (gatunki i ścieżki MetaCyc, $p<0,05$) nie zidentyfikowały cech dyskryminujących. Potrzebne są większe kohorty, aby wyjaśnić wszelkie powiązania przyczynowe.

Ocena przepuszczalności jelitowej opisana w publikacji 4 była ograniczona do wybranych wskaźników kałowych, co może nie uchwycić pełnej złożoności dynamiki bariery jelitowej. Co ważne, nie uwzględniono innych uznanych markerów integralności jelitowej, takich jak lipopolisacharyd (LPS), białko wiążące kwasy tłuszczowe w jelitach (I-FABP) czy okludyna. Przyszłe badania powinny uwzględniać te ograniczenia, w tym inne determinanty mikrobiomu jelitowego, a także aspekty psychologiczne.

5. PODSUMOWANIE I WNIOSKI

Odpowiednie przygotowanie do wypraw wysokogórskich, obejmujące żywienie, nawodnienie i suplementację, może ograniczyć ryzyko niedoborów żywieniowych, pogorszenia stanu zdrowia i wydolności fizycznej. W związku z tym wprowadzeniem teoretycznym do cyklu publikacji naukowych był artykuł przeglądowy, w którym podsumowano aktualną wiedzę w tym zakresie oraz zaproponowano praktyczne zalecenia dla alpinistów. Alpiniści, przebywając na dużych wysokościach, są szczególnie narażeni na deficyt energii, a w konsekwencji utratę masy ciała i beztłuszczowej masy ciała, dlatego mimo braku apetytu kluczowe jest regularne spożywanie posiłków i przekąsek oraz dostosowanie podaży węglowodanów do etapu wyprawy. Zalecane jest spożycie minimum 1,4 g białka/kg masy ciała/dzień, z uwzględnieniem źródeł białka o wysokiej jakości, a także wartościowych źródeł tłuszczu włączając do swojej diety produkty bogate w wielo- i jednonienasycone kwasy tłuszczowe. Nawodnienie powinno być indywidualnie kontrolowane, m.in. poprzez obserwację koloru moczu i objawów AMS. Ograniczony dostęp do świeżej żywności w warunkach wysokogórskich może prowadzić do niedoborów składników mineralnych i witamin, dlatego jeszcze przed wyprawą warto oznaczyć stężenie ferrytyny i witaminy D w surowicy krwi oraz wdrożyć suplementację w przypadku niedoborów w celu optymalizacji stanu zdrowia i kontynuować ją pod nadzorem lekarza. W warunkach wysokogórskich szczególnie istotne są produkty bogate w antyoksydanty (witamina C, E, beta-karoten, selen, cynk), które mogą być dostarczane w formie liofilizatów (owoców, warzyw i ich sproszkowanych form).

Suplementacja diety stanowi istotne uzupełnienie podaży energii i składników odżywczych, a także wspiera funkcjonowanie organizmu w trudnych warunkach środowiskowych. Potencjalnie korzystne działanie mogą wykazywać następujące suplementy: białko, węglowodany, kofeina, azotany, probiotyki, kwasy tłuszczowe omega-3, glutamina, kurkumina, Ginkgo biloba, NAC czy sok z wiśni cierpkiej, choć ich skuteczność w warunkach wysokogórskich wymaga dalszych badań. Ze względu na ograniczoną liczbę dobrze zaprojektowanych badań w tej dziedzinie, istnieje potrzeba przeprowadzenia kontrolowanych, randomizowanych badań z udziałem alpinistów, aby potwierdzić skuteczność stosowanych interwencji.

Ważnym zagadnieniem w cyklu publikacji była ocena wartości energetycznej i odżywczej diety alpinistów przed wyprawą wysokogórską. Stwierdzono, iż ponad połowa badanych wspinaczy utrzymywała ujemny bilans energetyczny. Wspinacze w większości dostarczali odpowiednie ilości makro- i mikroelementów wraz z dietą, z wyjątkiem selenu,

jodu, witaminy D i K. Na podstawie uzyskanych wyników wskazane jest prowadzenie edukacji żywieniowej alpinistów w celu zwiększenia ich świadomości dotyczącej zasad odżywiania, nawodnienia i suplementacji. Może to sprzyjać poprawie samopoczucia, utrzymaniu optymalnego stanu zdrowia i wydolności fizycznej, a tym samym zwiększać szanse na sukces podczas działań alpinistycznych oraz umożliwiać dostosowanie zaleceń żywieniowych do indywidualnych potrzeb już na etapie przygotowań do wyprawy.

Interesującym poznawczo zagadnieniem była analiza autorskiego kwestionariusza do oceny nawyków żywieniowych wspinaczy i himalaistów w warunkach wysokogórskich, a także analiza wartości energetycznej i odżywczej diety w czasie wyprawy wysokogórskiej. Wyniki wskazały na niską dostępność i rzadkie spożycie takich grup produktów jak: warzywa, owoce, nabiał, jaja, ryby i mięso, przy jednoczesnym częstym wyborze słodyczy, orzechów, nasion oraz produktów zbożowych. Najczęściej stosowana suplementacja obejmowała suplementy białkowe i białkowo-węglowodanowe, kofeinę, probiotyki, kwasy tłuszczowe omega-3 oraz witaminy i składniki mineralne. Analiza wartości energetycznej i odżywczej diety wspinaczy podczas wyprawy wysokogórskiej wykazała, iż była ona niskoenergetyczna w stosunku do ich wysokiego zapotrzebowania energetycznego, podaż białka była zbyt niska, a podaż tłuszczu zbyt wysoka. Wspinacze wysokogórscy powinni dążyć do zwiększenia podaży węglowodanów, a także mikroskładników odżywczych takich jak: potas, wapń, magnez, żelazo, cynk, selen i jod, a także witamin A, D, C, kwasu foliowego i witaminy K, a w sytuacji braku możliwości pokrycia zapotrzebowania wraz z dietą wskazane jest rozważenie suplementacji.

Kolejnym obszarem prezentowanych badań była analiza stanu zdrowia oraz wydolności fizycznej wspinaczy przed wyprawą wysokogórską oraz po jej ukończeniu. W wyniku przeprowadzonej analizy przepuszczalności jelitowej przed wyprawą, stwierdzono, iż u większości badanych wspinaczy była ona prawidłowa, chociaż u niektórych osób z prawidłowym stężeniem alfa-1-antytrypsyny zaobserwowano podwyższone wartości zonuliny. Długotrwały pobyt w warunkach wysokogórskich spowodował istotny statystycznie spadek masy ciała oraz masy mięśniowej. Nastąpił również istotny spadek masy ciała szczupłego w kończynach górnych oraz dolnych, a także wskaźnika ALST. Analiza wskaźników hematologicznych wykazała istotny wzrost liczby leukocytów, erytrocytów, płytek krwi oraz wzrost wartości hemoglobiny, hematokrytu, a także neutrofili i monocytów po wyprawie wysokogórskiej. Zaobserwowano istotny spadek poziomu cholesterolu całkowitego, HDL, bilirubiny całkowitej, albuminy oraz białka całkowitego. Wykazano istotny statystycznie wzrost poziomu fosfatazy kwaśnej całkowitej oraz fosforu nieorganicznego. Niewystarczająca

podaż żelaza, kwasu foliowego i witaminy D wraz z dietą alpinistów znajdowała odzwierciedlenie w wynikach badań krwi. Wszystkie wskaźniki moczu pozostały w wartościach referencyjnych zarówno przed wyprawą jak i po wyprawie. Na podstawie analizy wydolności fizycznej stwierdzono, iż ponad trzytygodniowa ekspozycja na niedotlenienie (>3000 m n.p.m.) miała niekorzystny wpływ na wydolność tlenową, wydolność beztlenową kończyn dolnych oraz niektóre wskaźniki wydolności beztlenowej kończyn górnych wspinaczy, pomimo zaobserwowanego efektu erytropoetycznego. Niekorzystne zmiany w składzie ciała wspinaczy mogły wpłynąć negatywnie na wydolność fizyczną.

Ostatnim obszarem badań była analiza struktury i cech funkcjonalnych mikrobiomu alpinistów przed wyprawą wysokogórską oraz po jej ukończeniu. Wykazano, iż mikrobiom jelitowy reaguje na ekspozycję wysokogórską. Przy stabilnej alfa-różnorodności i liczbie funkcji odnotowano wzrost obfitości szlaku *degradacji glukozy/glukozo-1-fosforanu GLUCOSE1PMETAB-PWY*, co sugeruje adaptację funkcjonalną do warunków wyprawowych. Skala zmian była silnie osobnicza, wskazując na zróżnicowane wzorce odpowiedzi. Relatywna stabilność funkcji przy zmianach taksonomicznych może wskazywać na funkcjonalną redundancję mikrobiomu jelitowego. Zaobserwowano korelacje między wybranymi cechami mikrobiomu a podażą makro- i mikroskładników diety (witamin B_6 i C przed wyprawą; energii, węglowodanów, MUFA, witamin C, B_{12}, B_1 i B_6 po wyprawie) oraz wskaźnikami stanu zdrowia (sodem, hemoglobiną, HDL przed wyprawą; wapniem, MPV, bazofilami, sodem, wapniem, magnezem, HDL, białkiem całkowitym po wyprawie) i wydolności fizycznej (całkowitą wykonaną pracą (W_t) kończyn dolnych i średnią wielkością osiągniętej mocy (P_{mean}) kończyn dolnych przed wyprawą). Korelacje te nie dowodzą związku przyczynowego, ale są zgodne z udziałem mikrobiomu w odpowiedzi adaptacyjnej na stres wysokogórski. Wyniki te wymagają potwierdzenia w większych kohortach oraz w badaniach interwencyjnych.

Analiza wyników uzyskanych w ramach przeprowadzonych badań pozwoliła na zweryfikowanie przyjętych hipotez badawczych.

Hipoteza pierwsza została częściowo potwierdzona w zakresie ograniczonej realizacji ilościowych zaleceń żywieniowych dla sportowców w zwyczajowej diecie alpinistów. Podaż energii nie była prawidłowa w stosunku do wydatku energetycznego, jednak wspinacze w większości dostarczali odpowiednie ilości makro- i mikroskładników pokarmowych wraz z dietą, z wyjątkiem selenu, jodu, witaminy D i K.

Hipoteza druga została potwierdzona w zakresie występowania nieprawidłowości dotyczących analizowanych aspektów sposobu żywienia alpinistów w czasie wyprawy wysokogórskiej oraz ograniczonej realizacji ilościowych zaleceń żywieniowych.

Hipoteza trzecia została w dużej części odrzucona. Przebywanie na dużych wysokościach nie miało negatywnego wpływu na zdrowie alpinistów, poza stwierdzonym obniżeniem poziomu cholesterolu HDL.

Hipoteza czwarta została częściowo potwierdzona. Wykazano, iż wyprawa wysokogórska miała niekorzystny wpływ na wydolność tlenową, wydolność beztlenową kończyn dolnych oraz niektóre wskaźniki wydolności beztlenowej kończyn górnych alpinistów, pomimo zaobserwowanego efektu erytropoetycznego.

Hipoteza piata została częściowo potwierdzona. Różnice w liczbie gatunków i funkcji mikrobiomu przed i po wyprawie nie były istotne statystycznie, a rozpowszechnienie gatunków wśród wszystkich uczestników badania pozostało niezmienne po wyprawie (stabilna prewalencja większości taksonów). Jednocześnie odnotowano adaptacyjne przesunięcie funkcjonalne (wzrost obfitości *szlaku GLUCOSE1PMETAB-PWY*) bez istotnego spadku alfa-różnorodności. Adaptacyjny („korzystny") charakter opisanej zmiany jest jednak nieweryfikowalny w opisanej grupie (mała liczebność, brak wskaźników przyczynowych) i wymaga potwierdzenia w większych kohortach.

Przeprowadzone w ramach niniejszej rozprawy doktorskiej badania pozwoliły na sformułowanie następujących wniosków:

1. Analiza przeglądowa literatury wykazała, że choć istnieje wiele prac naukowych dotyczących wpływu przebywania na wysokości na fizjologię człowieka, stosunkowo niewiele publikacji opisuje aspekty żywienia w warunkach wysokogórskich. Podkreśla to potrzebę pogłębienia świadomości sportowców w zakresie zasad odżywiania, nawodnienia i suplementacji diety, co może przyczynić się do poprawy samopoczucia, zapewnienia optymalnego stanu zdrowia, wydolności fizycznej oraz podniesienia szans na sukces podczas działań alpinistycznych. Istotne jest również zapewnienie odpowiedniej opieki dietetycznej, umożliwiającej edukację alpinistów i dostosowanie zaleceń żywieniowych do indywidualnych potrzeb już na etapie przygotowań do wyprawy.
2. Analiza zwyczajowej diety alpinistów wykazała, iż osoby uprawiające alpinizm są narażone na ryzyko wystąpienia ujemnego bilansu energetycznego i/lub niskiej dostępności energii.

3. Większość wspinaczy dostarczała w zwyczajowej diecie odpowiednie ilości makro- i mikroskładników, z wyjątkiem selenu, jodu, witamin D i K. Nieprawidłowości żywieniowe obserwowane podczas wyprawy wysokogórskiej dotyczyły przede wszystkim niewystarczającej ilości energii w stosunku do zwiększonego zapotrzebowania, zbyt niskiej podaży białka oraz nadmiernego udziału tłuszczów. Niedobory energetyczne powinny być uzupełniane poprzez większą podaż węglowodanów w diecie i/lub odpowiednią suplementację. Ze względu na stwierdzone niedobory wielu mikroskładników odżywczych zaleca się zwiększenie podaży potasu, wapnia, magnezu, żelaza, cynku, selenu i jodu, a także witamin A, D, C, kwasu foliowego i witaminy K, a w sytuacji braku możliwości pokrycia zapotrzebowania wraz z dietą wskazane jest rozważenie suplementacji.
4. Analiza częstości spożycia poszczególnych grup produktów w warunkach wysokogórskich wykazała, iż alpiniści podczas wyprawy najczęściej sięgali po cukier i słodycze, orzechy i nasiona, a także produkty zbożowe (pełnoziarniste i rafinowane). Z kolei, ze względu na ograniczoną dostępność, najrzadziej spożywali warzywa, owoce, jaja, mleko i przetwory mleczne, masło i śmietanę, ryby oraz mięso.
5. Suplementacja diety stosowana podczas wypraw wysokogórskich u większości uczestników wydaje się właściwa i zgodna z aktualnymi zaleceniami dla alpinistów, szczególnie w odniesieniu do węglowodanów, białka, witamin i składników mineralnych, kwasów tłuszczowych omega-3, kofeiny i probiotyków. Niemniej jednak, konieczne są dalsze badania mające na celu określenie optymalnych dawek, czasu stosowania suplementacji oraz jej długofalowego wpływu na proces adaptacji wysokogórskiej. Dobór właściwej suplementacji powinien być ustalany indywidualnie we współpracy ze specjalistami, tj. lekarzem i dietetykiem.
6. Wyprawy wysokogórskie spowodowały niekorzystne zmiany w składzie ciała, obejmujące spadek masy ciała, masy mięśniowej, masy ciała szczupłego w kończynach górnych oraz dolnych, a także wskaźnika ALST, które mogły negatywnie wpłynąć na wydolność fizyczną alpinistów. W związku z działaniem niekorzystnych czynników środowiskowych, charakterystycznych dla warunkach wysokogórskich oraz ze zwiększonym tempem metabolizmu, szczególny nacisk należy położyć na optymalizację podaży energii i indywidualnie dobraną suplementację, aby ograniczyć występowanie opisanych efektów.
7. Przebywanie na dużych wysokościach nie wpłynęło negatywnie na stan zdrowia alpinistów, za wyjątkiem stwierdzonego obniżenia poziomu cholesterolu HDL, jednak

niewystarczająca podaż żelaza, kwasu foliowego i witaminy D wraz z dietą znalazła odzwierciedlenie w wynikach badań krwi. U większości wspinaczy wskaźniki przepuszczalności jelitowej przed wyprawą mieściły się w zakresie wartości prawidłowych, chociaż u części osób, mimo prawidłowego stężenia alfa-1-antytrypsyny, zaobserwowano podwyższony poziom zonuliny.

8. Ponad trzytygodniowa ekspozycja na niedotlenienie (>3000 m n.p.m.) miała niekorzystny wpływ na wydolność tlenową, wydolność beztlenową kończyn dolnych oraz niektóre wskaźniki wydolności beztlenowej kończyn górnych wspinaczy, pomimo zaobserwowanego efektu erytropoetycznego.
9. Skład, obfitość gatunków i liczba funkcji mikrobiomu nie uległy istotnym zmianom pod wpływem przebywania w warunkach wysokogórskich, jednak odnotowano wzrost częstości i obfitości *szlaku degradacji glukozy/glukozo-1-fosforanu*, co wskazuje na ukierunkowaną adaptację funkcjonalną do zwiększonej dostępności węglowodanów i stresu środowiskowego.
10. Zaobserwowane korelacje między cechami mikrobiomu a wybranymi składnikami diety oraz wskaźnikami stanu zdrowia i wydolności fizycznej są zgodne z udziałem mikrobiomu w odpowiedzi adaptacyjnej na stres wysokogórski, jednak wymagają potwierdzenia w większych kohortach oraz w badaniach interwencyjnych.
11. Dalsze badania powinny obejmować długoterminową analizę zależności między sposobem żywienia a mikrobiomem jelitowym i wydolnością fizyczną w warunkach hipoksji wysokogórskiej, prowadzoną w większych kohortach oraz z wykorzystaniem kontrolowanych interwencji dietetycznych, takich jak zastosowanie pre-, pro-, synbiotyków, czy modulacja podaży węglowodanów i mikroskładników pokarmowych.

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Streszczenie

Alpinizm wysokogórski to dyscyplina łącząca wspinaczkę skałkową, wspinaczkę lodową, skialpinizm oraz przemieszczanie się po lodowcach (Twight et al., 1999), którą zainteresowanie rośnie z każdym rokiem, także wśród alpinistów na poziomie amatorskim (Salisbury et al., 2021). Przebywanie w warunkach hipoksji hipobarycznej (>3000 m n.p.m.) wywołuje szereg szkodliwych skutków dla ludzkiego organizmu, jednak wiedza na ich temat jest wciąż niepełna. Celem badania była ocena wpływu wyprawy wysokogórskiej na sposób żywienia, stan zdrowia, wydolność fizyczną oraz strukturę mikrobiomu alpinistów. Wprowadzeniem teoretycznym do cyklu publikacji naukowych był artykuł przeglądowy nt. żywienia, nawadniania i suplementacji diety alpinistów w warunkach wysokogórskich.

W badaniu wzięło udział 17 mężczyzn w wieku 30.29 ± 5.8 lat o stażu wspinaczkowym 10 ± 5 lat, trenujących średnio 8 ± 3 godzin w tygodniu i uczestniczących w wyprawach górskich na szczyty 5000-8000 m, trwających średnio 34 ± 6 dni (w tym 26 ± 6 dni powyżej 3000 m). W celu określenia wartości energetycznej i odżywczej zwyczajowej i wysokogórskiej diety alpinistów zastosowano metodę 3-dniowego bieżącego notowania i wagową. Analizę nawyków i postaw żywieniowych w czasie wyprawy wysokogórskiej oparto na autorskiej ankiecie. Przeprowadzono analizę składu ciała z zastosowaniem metody absorpcjometrii promieniowania X o dwóch energiach oraz ocenę wybranych wskaźników ogólnego stanu zdrowia (hematologia i biochemia krwi oraz badanie moczu, badanie kału, przepuszczalność jelitowa). Wydolność aerobową oceniano w trakcie klasycznego testu o stopniowo narastającej intensywności „do odmowy", natomiast wydolność anaerobową w trakcie testu Wingate dla kończyn dolnych i górnych. W celu przeprowadzenia analizy mikrobiomu wykonano sekwencjonowanie metagenomiczne. Analizy bioinformatyczne i statystyczne pozwoliły ocenić zmiany w składzie i funkcjach mikrobiomu oraz ich korelacje z parametrami fizjologicznymi i dietetycznymi. Analiza stanu zdrowia, składu ciała, wydolności fizycznej oraz mikrobiomu jelitowego alpinistów przeprowadzona została dwukrotnie – przed wyprawą oraz po jej ukończeniu.

Analiza przeglądowa literatury wykazała, że choć istnieje wiele prac naukowych dotyczących wpływu przebywania na wysokości na fizjologię człowieka, stosunkowo niewiele publikacji opisuje aspekty żywienia w warunkach wysokogórskich. Podkreśla to potrzebę pogłębienia świadomości sportowców w zakresie zasad odżywiania, nawodnienia i suplementacji diety, co może przyczynić się do poprawy samopoczucia, zapewnienia optymalnego stanu zdrowia, wydolności fizycznej oraz podniesienia szans na sukces podczas

działań alpinistycznych. Istotne jest również zapewnienie odpowiedniej opieki dietetycznej, umożliwiającej edukację alpinistów i dostosowanie zaleceń żywieniowych do indywidualnych potrzeb już na etapie przygotowań do wyprawy.

Analiza zwyczajowej diety alpinistów wykazała, iż osoby uprawiające alpinizm są narażone na ryzyko wystąpienia ujemnego bilansu energetycznego i/lub niskiej dostępności energii. Większość wspinaczy dostarczała w zwyczajowej diecie odpowiednie ilości makro- i mikroskładników, z wyjątkiem selenu, jodu, witamin D i K. Nieprawidłowości żywieniowe obserwowane podczas wyprawy wysokogórskiej dotyczyły przede wszystkim niewystarczającej ilości energii w stosunku do zwiększonego zapotrzebowania, zbyt niskiej podaży białka oraz nadmiernego udziału tłuszczów. Niedobory energetyczne powinny być uzupełniane poprzez większą podaż węglowodanów w diecie i/lub odpowiednią suplementację. Ze względu na stwierdzone niedobory wielu mikroskładników odżywczych zaleca się zwiększenie podaży potasu, wapnia, magnezu, żelaza, cynku, selenu i jodu, a także witamin A, D, C, kwasu foliowego i witaminy K, a w sytuacji braku możliwości pokrycia zapotrzebowania wraz z dietą wskazane jest rozważenie suplementacji. Analiza częstości spożycia poszczególnych grup produktów w warunkach wysokogórskich wykazała, iż alpiniści podczas wyprawy najczęściej sięgali po cukier i słodycze, orzechy i nasiona, a także produkty zbożowe (pełnoziarniste i rafinowane). Z kolei, ze względu na ograniczoną dostępność, najrzadziej spożywali warzywa, owoce, jaja, mleko i przetwory mleczne, masło i śmietanę, ryby oraz mięso. Suplementacja diety stosowana podczas wypraw wysokogórskich u większości uczestników wydaje się właściwa i zgodna z aktualnymi zaleceniami dla alpinistów, szczególnie w odniesieniu do węglowodanów, białka, witamin i składników mineralnych, kwasów tłuszczowych omega-3, kofeiny i probiotyków. Niemniej jednak, konieczne są dalsze badania mające na celu określenie optymalnych dawek, czasu stosowania suplementacji oraz jej długofalowego wpływu na proces adaptacji wysokogórskiej. Dobór właściwej suplementacji powinien być ustalany indywidualnie we współpracy ze specjalistami, tj. lekarzem i dietetykiem.

Wyprawy wysokogórskie spowodowały niekorzystne zmiany w składzie ciała, obejmujące spadek masy ciała, masy mięśniowej, masy ciała szczupłego w kończynach górnych oraz dolnych, a także wskaźnika ALST, które mogły negatywnie wpłynąć na wydolność fizyczną alpinistów. W związku z działaniem niekorzystnych czynników środowiskowych, charakterystycznych dla warunkach wysokogórskich oraz ze zwiększonym tempem metabolizmu, szczególny nacisk należy położyć na optymalizację podaży energii i indywidualnie dobraną suplementację, aby ograniczyć występowanie opisanych efektów.

Przebywanie na dużych wysokościach nie wpłynęło negatywnie na stan zdrowia alpinistów, za wyjątkiem stwierdzonego obniżenia poziomu cholesterolu HDL, jednak niewystarczająca podaż żelaza, kwasu foliowego i witaminy D wraz z dietą znalazła odzwierciedlenie w wynikach badań krwi. U większości wspinaczy wskaźniki przepuszczalności jelitowej przed wyprawą mieściły się w zakresie wartości prawidłowych, chociaż u części osób, mimo prawidłowego stężenia alfa-1-antytrypsyny, zaobserwowano podwyższony poziom zonuliny. Ponad trzytygodniowa ekspozycja na niedotlenienie (>3000 m n.p.m.) miała niekorzystny wpływ na wydolność tlenową, wydolność beztlenową kończyn dolnych oraz niektóre wskaźniki wydolności beztlenowej kończyn górnych wspinaczy, pomimo zaobserwowanego efektu erytropoetycznego.

Skład, obfitość gatunków i liczba funkcji mikrobiomu nie uległy istotnym zmianom pod wpływem przebywania w warunkach wysokogórskich, jednak odnotowano wzrost częstości i obfitości *szlaku degradacji glukozy/glukozo-1-fosforanu*, co wskazuje na ukierunkowaną adaptację funkcjonalną do zwiększonej dostępności węglowodanów i stresu środowiskowego. Zaobserwowane korelacje między cechami mikrobiomu a wybranymi składnikami diety oraz wskaźnikami stanu zdrowia i wydolności fizycznej są zgodne z udziałem mikrobiomu w odpowiedzi adaptacyjnej na stres wysokogórski, jednak wymagają potwierdzenia w większych kohortach oraz w badaniach interwencyjnych. Dalsze badania powinny obejmować długoterminową analizę zależności między sposobem żywienia a mikrobiomem jelitowym i wydolnością fizyczną w warunkach hipoksji wysokogórskiej, prowadzoną w większych kohortach oraz z wykorzystaniem kontrolowanych interwencji dietetycznych, takich jak zastosowanie pre-, pro-, synbiotyków, czy modulacja podaży węglowodanów i mikroskładników pokarmowych.

Abstract

High-altitude mountaineering is a discipline that combines rock climbing, ice climbing, ski mountaineering, and glacier travel (Twight et al., 1999). Interest in high-mountain climbing is growing every year, including among mountaineers at the amateur level (Salisbury et al., 2021). Exposure to hypobaric hypoxia conditions (>3,000 MASL) induces a range of adverse effects on the human body; however, knowledge of these mechanisms remains incomplete. The aim of the study was to assess the impact of a high-altitude expedition on dietary habits, health status, physical performance, and gut microbiome composition in alpinists. The theoretical introduction to this series of scientific publications was a review article on nutrition, hydration, and dietary supplementation of mountaineers in high-altitude environments.

The study included 17 male participants aged 30.29 ± 5.8 years, with a climbing experience of 10 ± 5 years, training on average 8 ± 3 hours per week, and participating in expeditions to peaks ranging from 5,000 to 8,000 MASL, lasting on average 34 ± 6 days (including 26 ± 6 days above 3,000 MASL). To determine the energy and nutrient value of both habitual and high-altitude diets, a 3-day weighed food record method was used. Dietary habits and attitudes during the expedition were assessed using a custom-designed questionnaire. Body composition was analyzed using dual-energy X-ray absorptiometry (DEXA), and selected indicators of general health status were assessed (hematology and blood biochemistry, urinalysis, stool testing, and intestinal permeability). Aerobic performance was evaluated using a graded exercise test to exhaustion, while anaerobic performance was assessed using the Wingate test for upper and lower limbs. Metagenomic sequencing was used for microbiome analysis. Bioinformatic and statistical analyses enabled the evaluation of changes in microbiome composition and function, as well as their correlations with physiological and dietary parameters. Assessments of health status, body composition, physical performance, and gut microbiome were performed twice - before and after the expedition.

The literature review indicated that, although numerous studies have examined the effects of high-altitude exposure on human physiology, relatively few publications address nutritional aspects under high-altitude conditions. This highlights the need to raise athletes' awareness of proper nutrition, hydration, and supplementation strategies, which may contribute to improved well-being, optimal health, enhanced physical performance, and greater chances of success during alpine expeditions. It is also important to ensure appropriate dietary support, including nutrition education and the individualization of dietary recommendations, even during the preparation stage.

The analysis of habitual diets showed that alpinists are at risk of negative energy balance and/or low energy availability. Most climbers provided adequate amounts of macro- and micronutrients in their habitual diet, except for selenium, iodine, and vitamins D and K. Nutritional issues observed during high-altitude expeditions primarily involved insufficient energy intake relative to increased demands, inadequate protein intake, and excessive fat consumption. Energy deficits should be compensated by increasing carbohydrate intake and/or appropriate supplementation. Due to observed deficiencies in many micronutrients, increased intake of potassium, calcium, magnesium, iron, zinc, selenium, iodine, as well as vitamins A, D, C, folate, and K is recommended. If dietary intake cannot meet requirements, supplementation should be considered. The analysis of food group consumption at high altitude showed that climbers most frequently consumed sugar and sweets, nuts and seeds, and cereal products (both whole-grain and refined). Conversely, due to limited availability, they least often consumed vegetables, fruits, eggs, milk and dairy products, butter and cream, fish, and meat. Supplement use during the expeditions appeared appropriate and consistent with current recommendations for climbers, particularly regarding carbohydrates, protein, vitamins and minerals, omega-3 fatty acids, caffeine, and probiotics. Nonetheless, further research is needed to determine optimal dosages, timing, and long-term effects of supplementation on high-altitude adaptation. Supplementation should always be tailored individually in consultation with healthcare professionals such as physicians and dietitians.

High-altitude expeditions resulted in unfavorable changes in body composition, including reductions in body weight, muscle mass, lean mass in both upper and lower limbs, and appendicular lean soft tissue index (ALST), which could negatively affect physical performance. Given the adverse environmental factors characteristic of high-altitude conditions and the elevated metabolic rate, special attention should be paid to optimizing energy intake and individualized supplementation to mitigate these effects. Staying at high altitude did not negatively affect climbers' health status, except for a decrease in HDL cholesterol levels. However, insufficient dietary intake of iron, folate, and vitamin D was reflected in their blood test results. Most climbers presented normal intestinal permeability markers before the expedition, although some showed elevated zonulin levels despite normal alpha-1-antitrypsin concentrations. More than three weeks of hypoxia exposure (>3000 MASL) negatively affected aerobic capacity, anaerobic performance of the lower limbs, and selected indices of anaerobic performance of the upper limbs, despite a noticeable erythropoietic effect.

The composition, species abundance, and number of microbiome functions did not change significantly under high-altitude conditions; however, an increase in the frequency and

abundance of the *glucose and glucose-1-phosphate degradation pathway* was observed, indicating a functional adaptation to increased carbohydrate availability and environmental stress. Correlations observed between microbiome characteristics and selected dietary components, as well as health and performance indicators, support the involvement of the gut microbiome in the adaptive response to high-altitude stress. Nevertheless, these findings require confirmation in larger cohorts and interventional studies. Future research should include long-term analyses of the relationships between diet, gut microbiome, and physical performance under high-altitude hypoxia, conducted in larger populations and through controlled dietary interventions such as the use of pre-, pro-, and synbiotics, or modulation of carbohydrate and micronutrient intake.

Aneks

Załącznik 1. Kopie publikacji wchodzących w skład rozprawy doktorskiej

Publikacja 1 „Diet, Supplementation and Nutritional Habits of Climbers in High Mountain Conditions”

nutrients

MDPI

Article

Diet, Supplementation and Nutritional Habits of Climbers in High Mountain Conditions

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Abstract: Appropriate nutritional preparation for a high-mountain expedition can contribute to the prevention of nutritional deficiencies affecting the deterioration of health and performance. The aim of the study was to analyze the dietary habits, supplementation and nutritional value of diets of high mountain climbers. The study group consisted of 28 men (average age 33.12 ± 5.96 years), taking part in summer mountaineering expeditions at an altitude above 3000 m above sea level, lasting at least 3 weeks. Food groups consumed with low frequency during the expedition include vegetables, fruits, eggs, milk and milk products, butter and cream, fish and meat. The energy demand of the study participants was 4559.5 ± 425 kcal, and the energy supply was 2776.8 ± 878 kcal. The participants provided 79.6 ± 18.5 g of protein (1.1 ± 0.3 g protein/kg bw), 374.0 ± 164.5 g of carbohydrates (5.3 ± 2.5 g/kg bw) and 110.7 ± 31.7 g of fat (1.6 ± 0.5 g/kg bw) in the diet. The climbers' diet was low in calories, the protein supply was too low, and the fat supply was too high. There is a need to develop nutritional and supplementation recommendations that would serve as guidelines for climbers, improving their well-being and exercise capacity in severe high-mountain conditions, which would take their individual taste preferences into account.

Keywords: mountaineering; nutrition; diet; mountains; himalaism

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1. Introduction

There is a dynamic development and interest in mountain sports, including mountaineering and Himalayan mountaineering [1]. Due to the negative effects of weather and environmental conditions, mountain climbers are exposed to the risk of health deterioration and even loss of life [2]. Both short- and long-term exposure to hypoxia in high mountains causes extensive physiological changes [3]. Due to hypoxia, the partial pressure of oxygen in the blood and tissues decreases. The human body, in response to unfavorable conditions in terms of oxygen availability, responds with hyperventilation and activation of the sympathetic nervous system and causes an increase in cardiac output, which is associated with an increase in metabolic demand. Due to the conditions prevailing in the high mountain environment—i.e., reduced atmospheric pressure, leading to the development of hypobaric hypoxia, increased UV radiation, lack of access to fresh food, and thus low supply of antioxidant ingredients with food, heavy physical effort and mental stress—an increased production of reactive oxygen species (ROS) is observed, which is referred to as oxidative stress [4]. The effect of ROS in climbers returning from high-altitude expeditions is the development of many diseases, including neurodegenerative processes [5] and damage to the intestinal barrier, leading to consequences such as bacterial translocation and local/systemic inflammatory reactions [6].

At high altitude, both appetite and sense of taste are impaired. The feeling of satiety occurs after smaller meals in terms of volume, therefore smaller amounts of food are eaten. This phenomenon, called "mountain anorexia", makes it difficult for some people to maintain body weight at an altitude of 3600 m and extremely difficult for the majority of people over 5000 m above sea level [7]. In addition, weight loss is caused by reduced energy intake due to gastrointestinal disorders [8], increased basal metabolism, altered concentrations of hormones affecting satiety, including leptin, and water loss [9–15]. Fluid requirements in high altitude conditions are higher than at sea level due to hyperventilation, low humidity, sweat losses [16,17] and increased diuresis as a result of downregulation of the renin-angiotensin-aldosterone system, especially during the first days of stay [18].

Appropriate preparation for the expedition, in particular attention to the factors that climbers have an influence on, e.g., nutrition and hydration, can contribute to the prevention of nutritional deficiencies and dehydration, affecting the deterioration of health and fitness of the body. Inadequate supply of carbohydrates during endurance activities may result in hypoglycemia, lower glycogen content and muscle fatigue [19]. On the other hand, inadequate supply of energy and protein, which is the cause of the loss of lean body mass, has a negative effect on aerobic capacity, muscle strength and immunity [20–22]. High-altitude hypoxia increases fluid loss and contributes to dehydration, which reduces aerobic capacity in high-altitude conditions [23]. Optimal hydration can be limited by environmental conditions, but also by the time and fuel resources needed to prepare drinking water, sourced from the glacier [24]. Reduced thirst at high altitudes is another factor contributing to dehydration [25].

Many studies have been conducted on changes in body composition caused by staying at high altitude [26,27], or approaches to feeding teams of mountaineers in the high mountains [28–30]. There is still a lack of precise guidelines on nutrition in high-mountain conditions, focused on the real needs and referring to the preferences of climbers. The following research hypothesis was put forward: climbers' diets are characterized by discipline-specific inaccuracies resulting from being in high-altitude conditions. If the hypothesis of a deficient diet in high altitude conditions among Polish climbers is confirmed, this study will provide a starting point for creating dietary recommendations. This article aims to indicate what, from the point of view of Polish climbers, is the most difficult to achieve in high mountains in terms of nutrition and to provide data from the analysis of food diaries kept during the expedition.

2. Materials and Methods

2.1. Study Participants

The study group consisted of 28 men from Poland aged 23 to 42 years, taking part in mountain expeditions at least once a year during the summer period, during which they stayed at altitudes above 3000 m above sea level for at least 3 weeks. The persons qualified for the survey and supplementing the surveys were members of alpine clubs associated with the Polish Mountaineering Association. The anthropometric data and BMI of the group are presented in Table 1.

Table 1. Anthropometric data and BMI of climbers participating in the survey (n = 28) and supplementing food diaries (n = 15).

	Men (n = 28)		**Men (n = 15)**	
	$\overline{x}$	**SD**	$\overline{x}$	**SD**
Age [years]	33.12	5.96	29.6	5.25
Body height [cm]	179.04	6.96	179.87	7.37
Body weight [kg]	71.48	5.43	71.39	6.80
BMI [kg/m^2]	22.31	1.37	22.06	1.61

Abbreviations: $\overline{x}$ —mean; SD—standard deviation; BMI—body mass index.

Among the subjects, 42.9% of them said they had been mountain climbing for more than 10 years, 10.7% of the subjects 9–10 years, 21.4% of the subjects 7–8 years, 17.9% 5–6 years, and 7.1% 1–4 years. The subjects had extensive climbing experience, both in rock climbing (sport climbing levels between 7b and 8c), bouldering, drytooling, and mountaineering on their own belay. Among the respondents, 82.1% train sport climbing 2 or more times a week. In addition to climbing, those surveyed (92.86%) introduce complementary training: running, biking, swimming, weightlifting, skydiving or skiing, among others.

Within the group of 28 climbers, 15 kept food diaries from three days of expedition while climbing in Peru (Cordillera Blanca) and Pakistan (Shimshal Valley). Subjects who participated in mountain expeditions, where the time spent at an altitude above 3000 m above sea level was not less than 3 weeks, were enrolled in the study. The macronutrient content of the diet was assessed in mountaineers belonging to the Polish Mountaineering Association between the ages of 22 and 40. The anthropometric data and BMI of the group are presented in Table 1. At the time when the food diaries were kept, the climbers were at an altitude of 4000–6000 m. The goal of the climbing group in Peru was to ascend, among others, the 800 m Cruz del Sur route on the rock monolith La Esfinge (5325 m). A new route was established on Ocschapalca (5888 m) and Nevado Churup (5495 m), and ascents were made on Artesonraju (6025 m) and Alpamayo (5947 m). Climbers targeting peaks in the Shuijerab Mountain Group in Pakistan carved out a route on the west face of the pristine Trident Peak (6150 m).

Before participating in the project, the climbers had a consultation with a sports medicine doctor. On the basis of previously performed examinations (ECG, blood and urine tests), the ability to practice mountaineering, as well as to participate in the project, was assessed. The presence of chronic diseases and age over 45 were disqualifying factors from participating in the project.

The Bioethics Committee of the Regional Medical Chamber of Krakow granted approval for the research project (68/KBL/OIL/2022), which was carried out in accordance with the Declaration of Helsinki. After understanding the risks and benefits of participating in the study, all climbers gave written informed consent to participate in this study.

2.2. Study Design

2.2.1. Nutrition Survey

The analysis of dietary habits and attitudes was based on the author's survey. Responses to 54 questions (8 open, 46 closed) were analyzed. The questionnaire was divided into 3 parts concerning the following: anthropometric data and mountain experience and climbing experience, as well as health status and emerging ailments in the high mountains and after returning from the mountains; preparation for the expedition related to health analysis; eating behavior during the mountain action (including preferred foods, supplements, liquids, frequency of consumption of food groups in the mountains, appetite). The questionnaire was prepared on the basis of literature related to nutrition in high mountain conditions and a questionnaire to study dietary views and habits [31]. Responses to the questions on the sheet, which were analyzed, are included in the Supplementary Materials.

2.2.2. Nutritional Analysis of the Diet

The supply of selected macronutrients during the mountaineering expedition was determined by analyzing the whole-day rations obtained using the 3-day food diary method. The expedition participants were asked to note down all the foods, meals and supplements eaten, and fluids drank during the 3 active days of the expedition. These were days of acclimatization and alpine climbing on the way to the summit, and the activities noted during this time were mainly hiking with climbing elements. Climbers were given detailed instructions on how to keep food diaries before leaving for the expedition in order to minimize recording errors. The diaries contained commercially available products, so the necessary data for analysis was read from labels and nutritional tables. In the case of

food products such as bars, energy gels and freeze-dried products, climbers were asked to provide the product name, manufacturer and product weight.

The data from the diaries were meticulously entered into the program Aliant Dietetic Calculator (Anmarsoft, Gdańsk, Poland; version: 85; database: 6.2) for quantitative analysis of the climbers' diets during the active days of the expedition. The program used the "Tables of food composition and nutritional value" [32] as a database, taking into account food recipes and losses due to product processing. The average intake of macronutrients (protein, fat, total carbohydrates), dietary fiber, simple carbohydrates, saturated fatty acids, and sodium from 3 days was referred to recommendations for athletes [33–37].

2.2.3. Anthropometric Measurements

The subjects' body heights were measured before the expedition using a Seca 217 anthropometer (Seca GmbH & Co. KG, Hamburg, Germany) with an accuracy of 1 mm. The athletes' body weight was determined using InBody 120 body composition analyzer (Inbody Bldg., Seoul, Republic of Korea) in the morning after a standardized meal between 7:45 and 8:30 a.m.

2.2.4. Measurement of Energy Expenditure

Measurements of energy expenditure during the expedition were made by the researchers using a monitor recording heart rate Polar M430 (Polar-Electro, Kempele, Finland), which was recorded 24/7 for 3 active days. Pulsometers consisted of watches (receiver) and straps with transmitters Polar H10 sensor (Polar-Electro, Kempele, Finland), worn on the chest at the level of the gladius process in the anterior midline. The watches were placed on the hand so that they were adjacent to the body just below the wrist bone. Before the measurement began, the required data were entered into the device for each athlete: gender, age, body weight and height. Before the start of physical activity, the subjects were instructed to put on the transmitter strap, start the physical activity recording option on the device (climbing outdoors), and when the physical activity was over, to turn off the recording by pressing the corresponding button on the watch. In the case of recording activity during acclimatization or when reaching the climbing start point, the training option was not enabled. All climbers were advised not to remove the devices from their wrists for the duration of the energy expenditure recording being carried out (sleep, daytime activity, physical activity). Energy expenditure was recorded in the Polar Flow app. For those whose measurements were not taken using heart rate monitoring, an averaged physical activity index (PAL) value was calculated based on the measurements of the other climbers, which was 2.6, and then the total metabolism value was calculated by multiplying the basal metabolism value, calculated using the Harris Benedict formula, by the PAL value.

2.2.5. Statistical Analysis

Analyses were carried out using Statistica 13.3 statistical package (StatSoft Inc., Tulsa, OK, USA) by determining the parameters of descriptive statistics, i.e., mean value of macronutrient intake, standard deviation (SD) and significance level (p). Differences between energy supply and demand were analyzed using Student's t-test for related variables. A test probability of $p < 0.05$ was considered significant and the statistical significance of $p < 0.01$ was considered highly significant.

3. Results

3.1. Survey Analysis

The results of the survey indicate that 85.7% of climbers do not perform blood tests before the expedition, in contrast to 14.3% of the surveyed group who control their health. The vast majority of respondents also do not control the level of iron (92.9%) or ferritin (96.5%). Only 7.1% of people consult a dietician before departure. Before leaving for an expedition, 75% of the respondents follow a normal diet, while 25% report using a diet other than the usual one, e.g., gluten-free, semi-vegetarian, vegan and vegetarian.

Of the climbers, 85.7% declare that their diet significantly differs from the usual diet in the mountains, while 14.3% of climbers do not report any changes in their diet in the mountains. Of the respondents, 67.9% assess their appetite in the mountains 3000 m above sea level as good, 14.3% as average, 14.3% as very good, while 3.6% of people assess their appetite as low.

The most common choices in terms of products consumed in the mountains among the respondents include chocolate bars, freeze-dried dishes, chocolate, energy gels, gummies, salty snacks, dried meat, fruit mousses, canned meat and fish, kabanos sausages, sandwiches, halva, dried fruit, nuts and instant food (jellies, puddings, soups) (Figure 1).

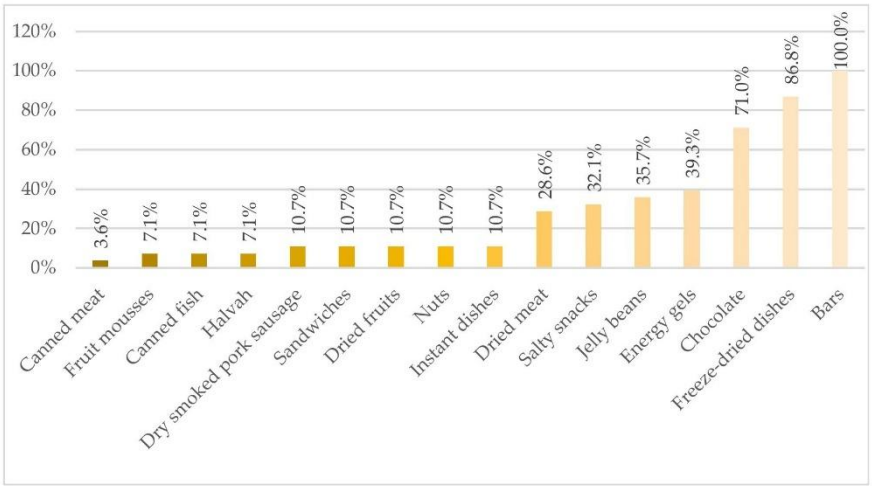


Figure 1. Food products most often taken on a mountain action with a bivouac above the base camp by climbers (percentage of climbers).

Cereal bars, date bars, high-protein bars and chocolate coated bars are the most popular options among climbers. Freeze-dried meals are the most frequently used nutritional solution after the end of a mountain action with bivouac (82.1%). A large percentage of climbers (75%) declare an overabundance of sweet taste, caused by the high frequency and quantity of carbohydrate snacks consumed, yet 78.6% of respondents prefer sweet snacks in the mountains as their first choice. Salty or acidic products, including meat, cheese, sausages, sandwiches and nuts are preferred by 21.4% of respondents.

Food groups consumed during the expedition with low frequency include vegetables, fruit, eggs, milk and milk products, butter and cream, fish and meat (Figure 2).

In turn, the following products are most often consumed by climbers: sugar and sweets (85.7%), nuts and seeds (67.9%), wholegrain cereal products (64.3%) and refined cereal products (57.1%). Among the climbers surveyed, 7.1% of them realized the correct dietary recommendations regarding the frequency of consumption of vegetable oils (daily intake), 17.9% took 1–2 servings of fruit daily, 17.9% took cereal products daily, and 25% of people took fish 1–2× a week.

The freeze-dried products most often consumed by climbers during the expedition include lunch dishes, soups, porridges, oatmeal, desserts and fruit. Freeze-dried food is willingly eaten by 57.1% of the respondents, and as many as 50% of climbers declare the occurrence of digestive discomfort (flatulence, abdominal pain, diarrhea) after eating freeze-dried food. The vast majority of climbers (92.9%) declare that the amount of food affects their well-being in the mountains, and 96.4% of people emphasize that the quality of food is also very important. Despite this, as many as 57.1% of climbers do not take the right

amount of food with them to the mountains. This is due to logistical errors or an attempt to minimize the weight of the backpack. Regeneration after the end of the mountain action lasts, depending on the difficulties the climbers encountered, from 1 to 7 days.

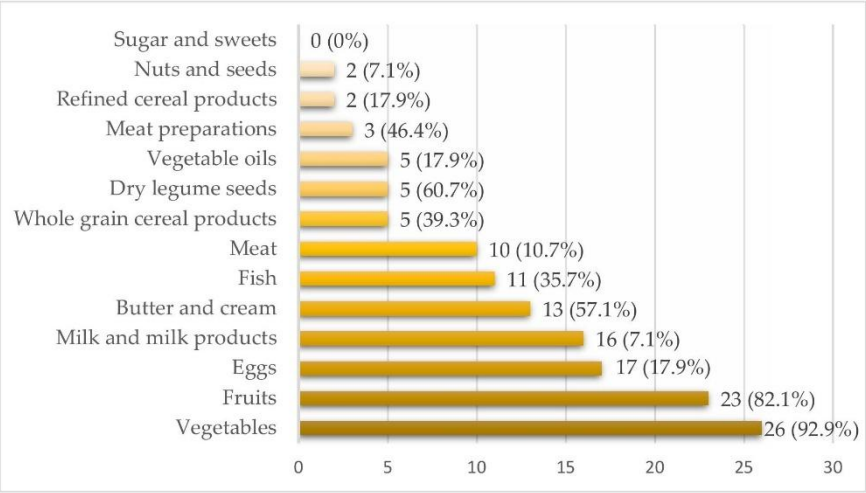


Figure 2. Declarations (percentage of climbers) concerning food groups consumed in high mountain conditions with low frequency.

Climbers most often take isotonic drinks (78.6%), tea (60.7%), water (53.6%), coffee (14.3%) or juices (10.7%) with them in the mountains, as well as beer (3.6%), electrolyte solution (3.6%) and also kissel (3.6%). The amount of fluids consumed by climbers in the mountains is shown in Figure 3.

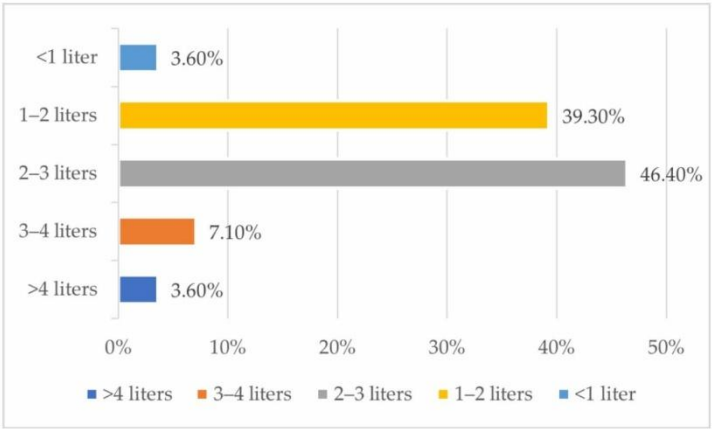


Figure 3. The amount of fluids consumed by climbers in the mountains (per day).

The respondents (57.1%) believe that the amount of fluids they drink in the mountains is not sufficient. Among the group of respondents, 85.7% of people use electrolytes as an addition to water. Only 14.3% of respondents use water purification tablets when drinking

water from glaciers. 20% of the respondents experienced acute mountain sickness. As many as 94.4% of climbers claim that one of the causes of their high-altitude sickness could be inadequate hydration of the body.

Dietary supplements used by climbers include protein and protein-carbohydrate supplements, caffeine, probiotics, omega-3 fatty acids, as well as some vitamins (multivitamins, vitamin B, vitamin D, vitamin C, vitamin K2) and minerals (iron, magnesium) (Figure 4).

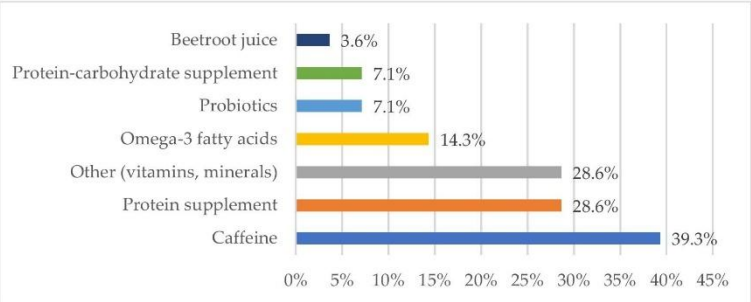


Figure 4. Percentage of climbers using dietary supplements during the expedition.

After returning from the expedition, 73.3% of the respondents observed a decrease in body weight.

3.2. *Analysis of Nutritional Value of Diet*

The energy demand of the study participants was 4559.5 ± 425 kcal, and the energy supply was 2776.8 ± 878 kcal. Climbers were supplied with supplementation an average of 588 kcal, which accounted for 21.2% of their energy supply. Generally, only 2 people, i.e., 13.33% of the respondents, covered their needs in accordance with the standard (Table 2).

Table 2. Differences between energy supply and demand analyzed with the Student's *t*-test for related variables.

	Dietary Energy Supply [kcal]	Energy Requirements [kcal]
Arithmetic mean	2776.84	4559.50
Standard error	234.67	113.60
Standard deviation	908.87	439.96
−95% CI (confidence interval)	2273.53	4315.86
+95% CI	3280.15	4803.14
Mean of differences	−1782.66	
Standard error	262.4959	
Standard deviation	1016.6423	
−95% CI	−2345.6578	
+95% CI	−1219.6623	
T statistic	−6.7912	
Degrees of freedom	14	
Two-sided *p*-value	<0.0001	

A highly significant ($p < 0.01$) difference was found between energy supply and demand. Consumption is much lower than the theoretical requirement.

The average energy and nutritional value of the diet obtained from the analysis of the food diaries is shown in Table 3. Most climbers (93.3%) met the carbohydrate requirement, and 46.7% met the protein requirement. All climbers met the requirements for fats, and 33.3% of them exceeded the normative values.

Table 3. Energy and nutritional value of the daily food ration of mountain climbers in relation to the recommendations for athletes [34–37].

	Nutritional Recommendations for Athletes	Diet of Climbers during the Expedition	
		$\bar{x}$	SD
Energy [kcal]	Daily energy expenditure	2776.8	878
Protein [%]	15–20 [1]	12.1	2.8
Protein [g]	-	79.6	18.5
Protein [g/kg bw]	1.2–2.2 [1]	1.1	0.3
Carbohydrates [%]	45–65 [2]	52.7	8.1
Carbohydrates [g]	-	374.0	164.5
Carbohydrates [g/kg bw]	6–12 [3]	5.3	2.5
Simple carbohydrates [g]	-	147.3	67.1
Fiber [g]	38 [2]	24.8	8.2
Fats [%]	20–35 [2,3,4]	36.8	7.2
Fats [g]	-	110.7	31.7
Fats [g/kg bw]	0.5–1.5 [1]	1.6	0.5
Saturated fatty acids [%]	ALAP [4]	13.8	5.4
Saturated fatty acids [g]	ALAP [4]	40.5	16.5
Sodium [mg] (AI)	1500–>10,000 [2]	3177.0	855.7

[1]—[37]; [2]—[34]; [3]—[36]; [4]—[35]. Abbreviations: $\bar{x}$—mean; SD—standard deviation; bw—body weight; AI—Adequate Intake; ALAP—as low as possible.

4. Discussion

Covering energy requirements while climbing high mountains is very challenging for participants in high altitude expeditions. Our study revealed that most climbers did not meet their energy needs with diet (Table 2). This result is consistent with other studies. Kasprzak et al. [30] analyzed the diet of nine men participating in an expedition to the Alps and staying at an altitude of 3200 to 3616 m above sea level. Climbers were supplied with a diet of 1919.3 ± 771.88 kcal/day, which significantly differed from dietary recommendations for this group of physically active persons. In turn, Mariscal-Arcas et al. [29] conducted a quantitative analysis of the diet of seven climbers in an expedition base camp at an altitude of 4500 m above sea level. The average energy supply with the diet was 2833 kcal/day, which provided an insufficient supply of energy relative to demand. The results of another study showed that a group of Italians and Nepalese during an expedition to the Himalayas supplied, respectively, 2793 kcal/day and 2775 kcal/day, which was not enough in relation to the demand [28]. The dietary energy supply in our study was similar to the results presented above (Table 2). According to the recommendations and the level of physical activity, the energy demand of athletes may range from 40 to 70 kcal/kg bw/day (2000–7000 kcal/day for a 50–100 kg athlete) [37]. It has also been shown that estimated energy expenditure increases with increasing climb angle by 1.5–2.0 additional kcal/min for climbs of 80–90° and 5 additional kcal/min for climbs of 102° [38]. Mountain experience can affect energy expenditure. Energy expenditure of experienced climbers during mountain climbing was found to be lower [39].

Increased energy expenditure during long high-altitude hikes may be responsible for a negative energy balance leading to changes in body composition, namely loss of fat mass and lean body mass [8,11,40]. Analysis of energy expenditure conducted using the double labeled water method with Mount Everest climbers indicate that energy expenditure during physical activity is 1.85–3.0 times higher than resting energy expenditure at sea level [11]. Negative energy balance is the main cause of muscle protein catabolism. After depletion of glycogen and fat stores, protein stores are catabolized to cover energy expenditure [41]. Climbers lose at least 3% of their body weight after eight days at 4300 m and 15% after three months at 5300–8000 m [42]. The results of a survey we conducted also showed weight loss in 73.3% of climbers returning from an expedition. Loss of lean body mass negatively

affects aerobic capacity [20], muscle strength [21], immune functions of the body [22] at high altitudes, which may increase the risk of disease and injury in these extreme conditions. However, muscle breakdown can be beneficial as an adaptation in high altitude conditions because it increases the density of capillaries relative to the muscle cell [43]. The density of capillaries within a given area of muscle tissue will greatly contribute to matching the delivery of oxygen and nutrients with the myofibers' metabolic needs, particularly during contractile activity [44].

Mountaineers need to provide carbohydrate sources with their diet during high-altitude physical activity to maintain body weight, recover adequately and replenish glycogen stores [45,46]. At sea level, carbohydrate intake before and during prolonged moderate-to-high-intensity physical activity conserves endogenous carbohydrate stores and delays the onset of fatigue [47,48]. Nutritional recommendations for people staying at high altitudes say that carbohydrate intake should account for at least 60% (6–8 g/kg bw/day) of total energy intake [49,50]. These guidelines are based on research showing that total carbohydrate oxidation during high-altitude exercise is greater than exercise of the same intensity at sea level [51], which leads to a faster use of carbohydrate stores in the body (i.e., muscle and liver glycogen and blood glucose). In our study most climbers met the carbohydrate requirement (Table 3). The carbohydrate supply was similar to the results reported by Bondi et al. [28], describing the nutrition of Italian trekkers during an expedition to the Himalayas. The Nepalese porters consumed larger amounts of carbohydrates (7 ± 2 g carbohydrates/kg bw/day) [28]. In a study by Mariscal-Arcas et al. [29] the level of carbohydrates in the diet of climbers was too low (39.5%). The climbers in our study provided more simple sugars with their diet (Table 3) than the group of Italians and Nepalese in the study by Bondi et al. [28] (45 and 90 g/day, respectively). While total daily carbohydrate requirements may be elevated when staying at high altitudes, it is unclear whether additional carbohydrate intake provides an ergogenic performance benefit. At high altitude, the potential ergogenic effect of carbohydrate supplementation on exercise performance may be partially modulated by the acclimatization state [52–54]. Bradbury et al. [55] showed that carbohydrate supplementation during steady-state exercise does not improve exercise performance in lowland residents acutely exposed to hypoxia or residing at high altitude (4300 m) for 22 days.

An adequate supply of fiber with the diet is a major challenge for participants in high altitude expeditions due to limited access outside base camp. The results of the Bondi et al. [28] study showed that the Nepalese porter group provided 23 g/day of fiber, while the Italian trekker group provided 43 g/day. The results of our study indicate similar fiber supply values to the Nepalese group (Table 3). The fiber supply provided with the diet of the mountaineers in the Kasprzak et al. [30] study was even lower (12.2 g/day). Low intake of dietary fiber and resistant starch may lead to decreased bowel movements resulting in decreased bowel function, and also decreased the diversity of gut microbiota [56].

A high-carbohydrate, low-fat diet at altitude increases the respiratory quotient (RQ). If only fats are used for energy production, this coefficient is 0.7, while using carbohydrates (or proteins) increases to a value close to 1. The effect of this change in RQ is to increase the partial pressure of oxygen, resulting in an increase in arterial blood oxygen saturation [43,57]. Carbohydrate utilization is also a more efficient process in terms of energy yield per unit of O_2 consumed [58]. In the high mountains, a high-fat diet can lead to chronic muscle fatigue due to insufficient amounts of readily available carbohydrates. Diets rich in fatty acids require more oxygen during metabolism, slowing down acclimatization [59]. In our study all climbers met the requirements for fats and part of them exceeded the normative values (Table 3). The dietary fat supply of the climbers from our study is similar to the dietary fat supply (38%) of the Nepalese porters studied by Bondi et al. [28]. The percentage of fats in the diet during the expedition was lower among the Italians trekkers (24%) [28]. In a study by Mariscal-Arcas et al. [29] the level of fat in the diet of climbers was too high (45.5%). The diet prepared by the Sherpas at the base during the expedition was characterized by a high content of saturated fatty acids (derived mainly from meat, butter and cheese) [29]. The

supply of saturated fatty acids provided with the diet of the mountaineers in our research was also high (Table 3). Specialists from the Academy of Nutrition and Dietetics, Dietitians of Canada, American College of Sports Medicine (ACSM) and the International Society of Sports Nutrition (ISSN) recommend that fat intake by athletes should be in line with public health guidelines [36,37]. According to the recommendations of the European Food Safety Authority (EFSA), saturated fatty acids intake should be as low as is possible within the context of a nutritionally adequate diet [35].

An adequate supply of protein should be provided with the diet during physical activity at high altitudes to prevent weight loss and to ensure an adequate amount of this nutrient for tissue construction and repair [45]. The dietary protein intake necessary to support metabolic adaptation, repair, remodeling and protein turnover typically ranges from ISSN 1.2 to 2.0 g/kg bw/day to prevent loss of lean mass (FFM) [36], which is confirmed by the guidelines, which give a range of 1.7–2.2 g/kg bw/day [37]. Protein intake, especially branched-chain amino acids, is crucial for regulating muscle protein synthesis [60]. In the study by Bondi et al. [28], a group of Italians and Nepalese during an expedition to the Himalayas provided 1.2 ± 0.4 g of protein/kg of body weight, respectively, and 1.3 ± 0.3 g protein/kg bw/day. These results are similar to those we presented (Table 3). In contrast in a study by Mariscal-Arcas et al. [29] climbers were taking in 1.5–2.5 g protein/kg body weight, which is in line with the recommendations for athletes.

Increasing the protein content in the diet during caloric restriction to 1.6 g/kg bw/day improves nitrogen balance and allows for maintaining lean body mass [61]. These assumptions are particularly important for athletes who often need to prevent the loss of lean body mass. This approach was tested during a stay at high altitude [62]. However, protein increases the feeling of satiety [63], which is not a beneficial effect at high altitude, where climbers face high-altitude anorexia [64]. A diet richer in protein may also not align with the dietary preferences of high-altitude climbers. There are no studies that would evaluate the effect of protein supplementation on the feeling of satiety at high altitude. The most practical strategy to protect the body from lowering lean body mass at altitude seems to be to stimulate MPS and/or reduce proteolysis with a low-volume protein supplement rich in branched-chain amino acids that promote MPS, especially leucine [27]. A high-protein diet (2 g protein/kg bw/day) did not spare lean body mass in 4300-metre-altitude military personnel because doubling protein intake (compared to the standard diet group) resulted in a parallel increase in whole-body protein oxidation, suggesting that the additional protein consumed in the high-protein group was mainly used as an energy source during the 21 days of energy deficit at high altitude [65]. Proper nutrition can offset the loss of muscle mass, but it is not possible to completely prevent muscle atrophy [27].

The increased loss of breathing water and diuresis often seen in the early response to altitude exposure can result in a significant increase in water requirements, while reduced thirst and changes in fluid availability in a new environment can alter habitual drinking practices [45,66,67]. According to ACSM experts, the daily requirement for fluids is 4 to 5 L during altitude training and competition [36], while the ISSN encourages individual monitoring of hydration status to determine athletes' fluid needs [37]. At moderate altitudes up to 4000 m above sea level, water loss from the respiratory tract can be increased to 1900 mL/day in men [45] and in addition, urinary excretion can be increased by 500 mL/day [59]. Our results show that nearly half of the respondents drink 2–3 L of fluids per day in the mountains, while the rest of the respondents take in even smaller amounts of fluids per day (Figure 3), which can lead to dehydration and have a negative impact on acclimatization. A study by Ladd et al. [24] based on two methods of measuring hydration status (urine specific gravity and ultrasound measurements of the inferior vena cava size and collapsibility index) found that about half of the surveyed climbers who had climbed Denali in Alaska were dehydrated. Water supply was monitored in a group of Italians and Nepalese during an expedition to the Himalayas, reaching 3099 ± 462 g/day and 3240 ± 310 g/day, respectively [28]. A common custom of the group of Italians and the group of Nepalese porters participating in the Himalayan expedition was to consume

tea frequently throughout the trek, more than once a day [28]. The study conducted by Scott et al. [68] shows that even when drunk at high altitude where fluid balance is stressed, there is no evidence that tea acts as a diuretic when consumed through natural routes of ingestion by regular tea drinkers, but that it does have a positive effect on mood. Only 14.3% of respondents in our study use water purification tablets when drinking water from glaciers. Available ice and snow may be contaminated and unfit for consumption. Giardia lamblia, an intestinal parasite that causes diarrhea, is found in high-altitude regions [69]. In the absence of water treatment tablets, there is a high risk of parasitic infection.

The use of supplementation by climbers in high mountains can improve exercise capacity and recovery, concentration, provide deficient dietary components and also have a positive effect on immunity. The most commonly used supplement by climbers in our survey is caffeine (Figure 1). The ergogenic effect of caffeine in sports has been demonstrated in many scientific studies. Caffeine influences cognitive and physical functions by blocking adenosine A1 and A2a receptors in the central nervous system and peripheral tissues. Doses of 1–4 mg/kg body weight improve alertness, concentration, and reaction time [70], while doses of 3–6 mg/kg bw caffeine can enhance cognitive performance, motor skills, and physical performance in many types of sports [71,72]. These features are especially important in technically demanding places when climbing and when descending mountains or rappelling. Another common dietary supplement chosen by climbers in our survey is protein supplement (Figure 1). The use of a protein supplement rich in leucine seems to be a reasonable solution to protect the body against the reduction of lean body mass at high altitude [27]. Other dietary supplements used by climbers in our study include protein-carbohydrate supplements, probiotics, omega-3 fatty acids, as well as some vitamins (multivitamins, vitamin B, vitamin D, vitamin C, vitamin K2) and minerals (iron, magnesium). Overall, a positive association between omega-3 fatty acids supplementation and reaction time, skeletal muscle recovery, inflammatory markers and cardiovascular dynamics was reported [73]. Supplementation has also been shown to influence muscle protein synthesis, especially in conditions such as immobilization and energy restriction, or when consumed with other nutrients [74,75]. Substances with antioxidant activity, e.g., vitamin C, α-tocopherol and α-lipoic acid have a modulating effect on oxidative stress and AMS symptoms at high altitudes [76]. In order to reduce the frequency of upper respiratory tract infections, improve intestinal barrier function and reduce inflammation, probiotic supplementation is recommended [37]. Current evidence points to a potential improvement in endothelial function at high altitude after NO_3 supplementation [77]. Beetroot juice (and more precisely the nitrates it contains) is not a good prevention of AMS. Nitrates are not recommended as a prophylactic or ergogenic agent for already existing AMS [78].

A decrease in appetite is most often observed in the first few days after arriving at altitude. Staying at an altitude below 5000 m above sea level after a few days of acclimatization, the appetite increases again, while at extreme altitudes the phenomenon of high-altitude anorexia is particularly acute [43,79]. Our results show that the majority of respondents assess their appetite in the mountains 3000 m above sea level as good. It is worthwhile for climbers to also manage their appetite and select snacks in the mountains according to their own preferences and gastrointestinal tolerance. Karl et al. [80] in their study proved that exposure to high altitude was closely related to the preference for sweet foods and the preference for low-protein foods over higher-protein foods. These results suggest that sweet foods low in protein may be particularly appealing during the initial period at high altitude. After weight loss and acclimatization, study participants preferred high-fat foods [80]. In our research, a large percentage of climbers report an excess of sweet taste due to the high frequency and quantity of carbohydrate snacks consumed. However, there is still a majority of respondents (78.6%) that mostly prefer sweet snacks in the mountains. The Aeberli et al. [81] study examined changes in appetite and food preference after a rapid ascent to 4559 m with a starting altitude of 446 m. Liking of all food items except for sweet food was reduced after a meal at baseline and at high altitude. There was a significant increase in palatability of spicy foods at high altitude

and an interaction between food preference and susceptibility to acute mountain sickness (AMS). Subjects that develop AMS seem to prefer sweet foods over savory foods [81]. In our study, salty or acidic products, including meat, cheese, sausages, sandwiches, and nuts are preferred by 21.4% of respondents. Reducing the available amount of sodium in humans leads to an increase in salt palatability [82], and high-altitude climbing has been shown to lead to sodium diuresis associated with suppression of the renin-angiotensin-aldosterone system [6]. Our results show that climbers are most likely to consume both sweet and salty snacks and dried or freeze-dried products in the mountains (Figure 1). The freeze-dried products most often consumed by climbers during the expedition include lunch dishes, soups, porridges, oatmeal, desserts and fruit. As many as half of climbers report experiencing digestive discomfort (bloating, abdominal pain, diarrhea) after eating freeze-dried food. Gastrointestinal complaints are frequently reported during high altitude climbing (> 2500 m above sea level), although their etiology is unknown. Initial data also suggest that prolonged hypoxic exposures can compromise the intestinal barrier through alterations in immunological function, microbiota, or mucosal layers [83].

Among the climbers surveyed in our study, 7.14% followed the correct dietary recommendations regarding the frequency of consumption of vegetable oils (daily intake), 17.85% took in 1–2 servings of fruit per day, 17.85% took in cereal products several times a day, and 25% took in fish 1–2× per week with reference to the Swiss food pyramid for athletes [84]. None of the climbers followed the recommendations for adequate frequency of consumption of vegetables and dairy products. Vegetables and fruits are the main and very important sources of vitamins and minerals in a diet of athletes. However, consuming these products outside of base camp is difficult to accomplish. Taken by mountaineers on the summit attack or used during climbing, it should be as light as possible and easy to prepare, and additionally it should not freeze. It is worth considering the use of vegetables and fruits in the form of lyophilisates. Especially in the base camp, where the availability of products may be greater, it is worth taking care of the supply of these products.

Our research shows that the vast majority of climbers do not perform blood tests before an expedition, and do not monitor their iron or ferritin levels. At high altitude, the process of erythropoiesis is intensified, which increases the need for iron [85]. Therefore, even before the expedition, it is worth taking blood tests and consulting the result with a sports medicine doctor who would help choose the right dose of the supplement [86]. Current recommendations suggest assessing the iron level 8–10 weeks before the start of activity in high mountains [87] and starting appropriate oral supplementation 2–3 weeks before exposure to altitude and continuing this supplementation [88–90]. Of the climbers surveyed, only 7.1% of them work with a nutritionist before an expedition. Evidence-based nutritional strategies and a nutrition plan provided by a dietitian can lead to improved performance and recovery [91].

The results of the present study may have significance in the cooperation of sports nutritionists and trainers with climbers, allowing them to prepare more effectively for high altitude expeditions. The results obtained may also serve as practical guidance for high altitude climbers themselves.

Limitations and Directions for Future Research

We acknowledge that this study has several potential limitations. In the next such study, it would be worthwhile to expand the analysis of the energy value of the diet to include days at base camp (rest days) and to increase the group of subjects. However, it should be noted that the study group was homogeneous in terms of mountain goals and level of climbing, so finding more subjects is a challenge. It would be worthwhile for a nutritionist to also participate in the expedition, so that he or she is in constant contact with the cooks responsible for preparing the meals and has control over the amount of food consumed. We also recommend that future studies analyze the micronutrient content of foods used by climbers so that the analysis is more accurate. In addition, it is worth

focusing on analyzing the supplements used by climbers in the high mountains and their effects on health, mood and physical performance.

5. Conclusions

1. The climbers' diet was low in calories in relation to the high energy requirements, the protein supply was too low, and the fat supply was too high. Mountain climbers should aim to increase the supply of carbohydrates.
2. During climbing, climbers usually take snacks (bars, energy gels, gummies), which are a great supplement to carbohydrates. Providing adequate fiber outside base camp during a climb can be a problem, so especially at base camp, where food availability is greater, it is worthwhile to ensure a supply of foods abundant in this dietary component.
3. The use of a protein supplement at high altitudes seems to be a practical strategy that can reduce the loss of lean body mass. There are many supplements with well-documented effects in sports (e.g., beetroot juice, caffeine, probiotics, omega-3 fatty acids, essential minerals and vitamins) that are worth considering as a supplement to the diet.
4. There is a need to develop nutritional and supplementation recommendations that would serve as guidelines for climbers, improving their well-being and exercise capacity in severe high-mountain conditions, which would take into account their individual taste preferences.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/nu15194219/s1. Questionnaire for assessing the dietary habits of climbers and Himalayan climbers in high altitude conditions.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Nutrition, hydration and supplementation considerations for mountaineers in high-altitude conditions: a narrative review

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Staying and climbing in high mountains (>2,500 m) involves changes in diet due to poor access to fresh food, lack of appetite, food poisoning, environmental conditions and physiological changes. The purpose of this review is to summarize the current knowledge on the principles of nutrition, hydration and supplementation in high-altitude conditions and to propose practical recommendations/solutions based on scientific literature data. Databases such as Pubmed, Scopus, ScienceDirect and Google Scholar were searched to find studies published from 2000 to 2023 considering articles that were randomized, double-blind, placebo-controlled trials, narrative review articles, systematic reviews and meta-analyses. The manuscript provides recommendations for energy supply, dietary macronutrients and micronutrients, hydration, as well as supplementation recommendations and practical tips for mountaineers. In view of the difficulties of being in high mountains and practicing alpine climbing, as described in the review, it is important to increase athletes' awareness of nutrition and supplementation in order to improve well-being, physical performance and increase the chance of achieving a mountain goal, and to provide the appropriate dietary care necessary to educate mountaineers and personalize recommendations to the needs of the individual.

KEYWORDS
recommendations, mountaineering, nutrition, diet, mountains, himalaism, hydration, supplementation

1 Introduction

High altitude climbing is alpinism defined as ascending difficult mountain terrain using appropriate technique and equipment. It is a sport combining rock climbing, ice climbing, ski mountaineering and glacier travel (1). A special variety of climbing is Himalayanism i.e., climbing practiced in the highest mountains in the world, whose peaks exceed 7,000 m the (Himalayan mountain ranges, Karakorum, Daxue Shan, Hindu Kush, Kunlun, Pamir and Tienshan) (2). Interest in high-mountain climbing is growing every year, including among mountaineers at the amateur level. The Himalayan Database describes all known attempts to climb Nepalese and border peaks between 1905 and 2019, where 10,363 expeditions were recorded, involving 60,162 climbers and 28,587 high-altitude porters (3, 4). Table 1 shows the characteristics of altitude ranges referred to in the article (5, 6).

Staying and climbing in high mountains involves a change in diet due to poor access to fresh food, lack of appetite, food poisoning, harsh environmental conditions and

TABLE 1 Definitions of altitude and associated physiological changes (5, 6).

Altitude	Definition	Physiological changes
1,500–2,500 m	Intermediate altitude	Physiological changes detectable. Arterial oxygen saturation >90%. Altitude illness rare but possible with rapid ascent, exercise, and susceptible individual.
2,500–3,500 m	High altitude	Altitude illness common when individuals ascend rapidly.
3,500–5,800 m	Very high altitude	Altitude illness common. Arterial oxygen saturation <90%. Marked hypoxemia during exercise. 5,800 m is altitude of the highest permanent habitation.
>5,800 m	Extreme altitude	Marked hypoxaemia at rest. Progressive deterioration despite maximal acclimatisation. Permanent survival is not thought to be possible.
>8,000 m	"Death zone"	Prolonged acclimatisation (>6 weeks) is essential. Most mountaineers require supplementary oxygen to climb safely. Arterial oxygen saturations about 55%. Rapid deterioration is inevitable and time spent above this altitude is strictly limited.

physiological changes (7, 8). According to the definition of altitude above 2,500 m altitude illness common when individuals ascend rapidly (Table 1) (5, 6). Mountaineers have to deal with reduced atmospheric pressure, leading to the development of hypobaric hypoxia, intense solar radiation, heavy rain or snow, strong winds and low temperatures with large diurnal fluctuations, as well as the risk of avalanches and falling rocks (1, 9). Physical performance and aerobic capacity are impaired under hypoxia, and maximal oxygen consumption (VO_2max) decreases by 6% for every 1,000 m of elevation gain (10). Acclimatization to prolonged hypoxia is regulated at the molecular level by hypoxia-inducible factor (HIF). HIF-induced genetic reprogramming includes increased erythropoiesis, angiogenesis, increased glycolytic enzyme activity and inhibition of oxidative phosphorylation (11, 12). Physical or mental stressors activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of glucocorticoids that induce the activity of cellular reduction and oxidation systems (13).

Due to the conditions that prevail in the high mountain environment, low supply of antioxidant components with food, heavy physical exertion and mental stress there is an increased production of reactive oxygen species (ROS), which is referred to as oxidative stress (14–19). The effects of ROS include the development of neurodegenerative processes, which are recognized in mountaineers returning from high-altitude expeditions, as well as cognitive impairment and the appearance of pathological changes in brain structures, indicating the development of vasogenic edema (20) and damage to the intestinal barrier, leading to consequences such as bacterial translocation and local/systemic inflammatory reactions (21). Visitors to high mountains often experience gastrointestinal distress and loss of appetite, which can result from hypoxia itself, but can be exacerbated by infection. Acute short-term exposure to altitude appears to increase leptin levels through hypoxia-inducible factor HIF 1α and contributes to decreased appetite (22, 23). In addition, several other hormonal markers may contribute to this effect (24). This appears to be largely dependent on the duration of exposure to hypoxia, with the greatest weight loss, due primarily to loss of free fat mass, observed during the first weeks at altitude (8, 25–27), so monitoring energy intake to limit weight loss should be a priority in mountaineers.

Staying in high-altitude conditions poses a risk to mountaineers' health and life, and requires them to have adequate knowledge, preparation and experience to prevent the negative effects of climbing at high altitude, as well as the ability to make quick decisions and manage the risks of staying in exposed terrain. One mistake can cost the loss of life, hence full focus on the tasks performed during climbing under conditions of increasing fatigue is essential. Adequate nutrition, hydration and appropriately selected supplementation (of micronutrients that are deficient in high-mountain conditions, antioxidants and ingredients that improve exercise performance) help optimize mountain activities and can help prevent nutritional deficiencies that negatively affect health, cognition and physical performance. Developing nutritional and supplementation recommendations requires an understanding of the physiological adaptations to altitude that occur in the human body, as well as the consequences of staying in an unfavorable high-altitude environment, which are weight loss and decreased appetite. The purpose of this review is to summarize current knowledge on nutrition, hydration and supplementation in high-mountain (>2,500 m) conditions. Previously published studies have described recommended solutions for mountaineers in the context of nutrition and hydration. Our review completes this information with data on supplementation in high mountains in order to create a compendium of practical knowledge necessary to develop an optimal nutritional and supplementation strategy to help mountaineers.

2 Materials and methods

The review was conducted using the following databases: Pubmed, Scopus, ScienceDirect for studies published in English, from January 2000 to October 2023. Search terms included the following keywords: "mountains", "mountaineers", "himalayans", "hypoxia", "high altitude", "nutrition", "hydration", "diet", "energy expenditure", "macronutrients", "micronutrients", "minerals", "vitamins", "supplements", "nitrates", "beetroot juice", "N-acetylcysteine", "Ginko biloba", "tart cherry", "glutamine", "curcumin", "omega-3 fatty acids", "carbohydrates", "proteins", "fats", "probiotics", "prebiotics", "short-chain fatty acids", "caffeine" which were combined with Boolean operators ("AND" and "OR"). A preliminary search identified 2,579 articles. Exclusion criteria included studies involving neonates, newborns, high altitude natives or animals, permanent sojourns at altitude and/or disease related hypoxia. An additional Google Scholar search was

conducted to identify other additional studies not identified in the above databases. Literature lists of articles and books were also downloaded. The remaining articles were searched manually. Reviewed articles related to the search terms were evaluated based on the titles, abstracts and content of the articles, and in the end, 207 articles were selected. Articles considered were randomized, double-blind, placebo-controlled trials, narrative review articles, systematic reviews and meta-analyses.

3 Discussion

3.1 Energy supply and energy expenditure in the context of weight loss of mountaineers during high altitude expeditions

Prolonged exposure to high altitudes is associated with an imbalance between energy expenditure and energy intake, resulting in a decrease in body fat stores, and this relationship increases as altitude increases (28). At altitudes above 5,000 meters above sea level, negative energy balance may result from reduced energy intake due to reduced appetite (29) or partly due to intestinal disorders (28, 30, 31). To offset heat loss and maintain proper body temperature, there may be an additional increase in energy expenditure due to involuntary shivering, activated to increase heat production and use fat stores (32). Changes in body composition may not only be based on an adaptive response to hypoxia, but may also depend on genetic factors (33, 34). Reduction of brown adipose tissue during high-altitude sojourns does not appear to be desirable due to its thermoregulatory functions (maintenance of body temperature) (35, 36) and resulting impairment of physical performance (37). Natural stressors, such as cold, physical exercise, leptin expression and interleukin-6 expression, initiate adipocyte browning towards beige fat, triggering a higher thermogenesis (38). Especially in long-term altitude sojourns, this might be a factor which strongly increases energy expenditure. Both increased leptin expression and increased energy expenditure decrease with the degree of altitude acclimatization and presumably subside after complete acclimatization. This initial period during high-altitude sojourns is characterized by greater consumption of fatty acids recruited from the body's reserves stored in adipocytes (37).

Increased ventilation, due to being in a cold and dry high altitude environment, can also contribute to increased water loss (i.e., non-perceptible water loss), also leading to weight loss (27). Mountaineers lose at least 3% of their body weight after eight days at 4,300 m above sea level and 15% after three months at 5,300–8,000 m above sea level (29). In women, altitude-induced weight changes are smaller compared to men. In a study involving 12 women who climbed to an altitude of 5,050 m above sea level and stayed there for 21 days, no changes were observed in body weight and lean mass compared to baseline values (39).

Negative energy balance leading to depletion of glycogen and fat stores is the main cause of muscle protein catabolism (40). Above 5,000 m above sea level, 60%–70% of weight loss is lean body mass (41, 42). Its loss negatively affects aerobic capacity (43), muscle strength (44), and immune function (45), which can increase the risk of disease and injury in these extreme conditions. At altitudes above 8,000 m, the total number of muscle fibers decreases by about 17% (46, 47). The mechanisms of muscle atrophy are largely unknown, but can be attributed in part to malnutrition and reduced exercise intensity, as well as loss of appetite and impaired digestion or absorption (48, 49). On the other hand, the breakdown of muscle tissue may be beneficial as an adaptation under high-altitude conditions because it increases the density of capillaries in relation to the muscle cell (50). Being in hypobaric hypoxia decreases leucine turnover and uptake from muscle cells (51), which results in the arrest of protein synthesis and is associated with a decrease in mammalian target of rapamycin (mTOR) and is not dependent on food intake (52). This suggests that proper nutrition can offset the loss of muscle mass, but it is not possible to completely prevent muscle atrophy when climbing at high altitudes over time (22).

Analyses using the double-labeled water method in mountaineers ascending Mount Everest indicate that energy expenditure is 1.85–3.0 times that at sea level (53). The energy requirements of mountaineers while climbing in the Himalayas were 4,634 ± 287 kcal/day (5,900–8,046 m) (54), 5,394 ± 1,565 kcal/day (5,300–8,848 m) (42) and 3,248 ± 407 kcal/day (5,300–8,872 m) (53), while the energy supply was, respectively, 3,296 ± 478 kcal/day (54), 2,928 ± 968 kcal/day (42) and 1,791 ± 358 kcal/day (53). In the analyzed studies conducted in the Alps (2,400–3,800 m), the energy requirements of professional climbers from the French Military Group during an ultraendurance alpine climbing race were 10,413 ± 287 kcal/day, and energy supply 3,530 ± 165 kcal/day (55), while in the Cascade Mountains (2,500–3,100 m) involving soldiers during military field training, 4,037 ± 387 kcal/day and 2,357 ± 860 kcal/day, respectively (56). In our own research among Polish mountaineers during an expedition to Pakistan in the Shuijerab Mountain Group and Peru in the Cordillera Blanca (4,000–6,000 m), energy requirements were 4,560 ± 425 kcal/day and energy supply was 2,777 ± 878 kcal/day (57). In a study by Miller et al. the energy requirement of mountaineers summiting a Pangaea Peak in Karakoram Mountains (2,230-6,170 m) was 4,173 ± 848 kcal/day, but energy supply was not determined (58). The majority of studies used the gold standard technique of energy expenditure measurement, the doubly labeled water method (42, 53, 54, 56). An alternative method used by many authors to estimate total daily energy expenditure during mountaineering activities is heart rate recording (55, 57, 59).

A study by Pulfrey and Jones found that mountain climbers staying at extreme altitudes (5,900–8,046 m) covered 70% of their daily energy requirements with diet (54). The results of Watts et al. study conducted in the Cascade Mountains (1,636–3,266 m) showed that technical ice climbing was characterized by generating the highest energy expenditure of all mountaineering activities studied (59). A trail hike with a load averaging 45% of body weight led to an energy expenditure of 7.5 ± 1.9 kcal/min in

2.2 h, with an average peak of 13.4 ± 3.0 kcal/min. On the other hand, technical ice climbing consisting of moving up and down 12 meters in an average time of 14.3 ± 2.6 min required an average energy expenditure of 9.5 ± 3.4 kcal/min, and the peak average value was 14.6 ± 3.5 kcal/min (59). Energy expenditure of experienced climbers during mountaineering was lower than that of inexperienced climbers (59). It was also shown that the estimated energy expenditure increases with increasing slope angle by additional 1.5–2.0 kcal/min for overhangs at 80°–90° and additional 5 kcal/min for overhangs at 102° (60). According to the recommendations and the level of physical activity, the energy requirements of athletes can range from 40 to 70 kcal/kg of body weight/day (kg of b.w.) (2,000–7,000 kcal/day for a 50–100 kg athlete) (61).

Many of the factors influencing weight loss during a high-altitude expedition are beyond the mountaineers' control (loss of appetite, frequent gastrointestinal distress, increased energy expenditure due to increased thermogenesis and physiological processes determined by being in hypoxia, water loss due to constricted ventilation, loss of lean body mass). The only factor that mountaineers have a real influence on is energy supply. It is therefore worth aiming to increase energy supply to cover energy requirements. This is an important element of an expedition to plan, especially during the acclimatisation phase and in difficult technical terrain (ice climbing), when energy expenditure is higher.

3.2 Recommendations for macronutrient dietary supply while at high altitude

Staying in high-altitude conditions alters substrate oxidation for a given level of exercise intensity (62, 63). Modification in energy substrate utilization at high altitude toward carbohydrates (CHO) (64) influences increased dietary carbohydrate requirements for muscle glycogen storage and replenishment, maintenance of body mass, avoidance of hypoglycemia, adequate recovery and optimal glycogen resynthesis (65, 66). A higher intake of CHO is important at every stage of trekking and climbing in high mountains and has a beneficial effect on performance (67, 68). Consuming enough carbohydrates is especially important when there is cold stress and chills (69). Heat can be produced by shivering, which is an unconscious mechanism activated by the central nervous system, triggered by a decrease in body temperature (70). Increased muscle contractions during shivering result in a 2.5-fold increase in energy expenditure, most of which is due to increased carbohydrate oxidation. Cold exposure also results in increased muscle glycogen utilization, resulting from increased plasma catecholamine concentrations (71).

According to dietary recommendations (72), carbohydrate supply during low-intensity and technical exercise, should be 3-5 g/kg of b.w./day. Athletes doing moderate amounts of high-intensity training (about 1 h/day) should provide 5–7 g carbohydrates/kg of b.w./day, while endurance trainers doing moderate to high-intensity training (about 1–3 h/day) should take in 6–10 g carbohydrates/kg of b.w./day. Extreme endurance training (about 4–5 h/day) of moderate to high intensity requires 8–12 g of carbohydrates/kg of b.w./day. In order to improve glycogen resynthesis, it is recommended to consume 1–1.2 g of carbohydrates/kg of b.w. in the first 4–6 h after the end of activity.

Carbohydrate supply should be adjusted not only to the intensity of the exercise, but also to the stage of the expedition. During base camp at low physical activity, this supply should be about 3–5 g CHO/kg of b.w./day, while during trekking and/or climbing a minimum of 6 g/kg of b.w./day. According to the study by Friedlander et al. (73), carbohydrate intake in people at high altitude should be at least 60% (6–8 g/kg b.w./day) of total energy intake. These guidelines are based on studies showing that total carbohydrate oxidation during high-altitude exercise is greater than during exercise of the same intensity at sea level (74) leading to faster utilization of the body's carbohydrate stores (i.e., muscle and liver glycogen and blood glucose). A high-carbohydrate, low-fat diet at altitude increases the respiratory quotient (RQ). If only fats are used for energy production, this coefficient is 0.7, while using carbohydrates (or proteins) increases to a value close to 1. The effect of this change in RQ is to increase the partial pressure of oxygen, resulting in an increase in arterial blood oxygen saturation (75–77). In addition, at high altitudes, carbohydrate oxidation was considered the preferred metabolic pathway for aerobic exercise because it provides the highest adenosine triphosphate (ATP) yield per mole of oxygen (78). The study by McClelland et al. shows that carbohydrate utilization does not change after altitude acclimatization and that metabolic fuel utilization is mainly influenced by relative exercise intensity, as at sea level (79). In women, unlike men, carbohydrate utilization decreased while at 4,300 m above sea level (80). McKay et al. (81) showed in a study that short-term CHO restriction increased concentrations of the iron-regulating hormone, hepcidin, thereby potentially reducing post-exercise iron absorption. These studies were not conducted in mountainous terrain, so it would be appropriate to conduct an analogous study among mountaineers to assess the impact of a low-carbohydrate diet on the athletes' health. Analysis of the carbohydrate supply in the diet of mountaineers during an expedition in the Himalayas indicates either too low [39.5% of the energy supplied (82)] or the right amount [52.7% (57), 52% (83), 51.8% and 67.7% (84)].

From a practical point of view, environmental conditions in high mountains should be taken into account, especially low temperatures, which may prevent the consumption of carbohydrate snacks such as bars due to their freezing. Based on our experience, we recommend that snacks taken to the mountains are first tested at low temperatures. While food preparation is not a problem in base camp conditions (it is possible to take more time to cook and consume the meal unhurriedly, relying on valuable sources of complex carbohydrates), during a summit attack, digestive comfort and ease of meal consumption is a primary requirement. Snacks must also be convenient to eat with warm gloves.

Mountaineers practicing high altitude climbing should provide adequate amounts of protein to prevent weight loss and ensure adequate amounts of this nutrient to build and repair tissues

(85). An adequate supply of protein, especially branched-chain amino acids, is key to regulating muscle protein synthesis (86). International sports organizations recommend that the protein intake of physically active individuals should be between 1.2 and 2.0 g/kg of b.w./day, as recommended by the Academy of Nutrition and Dietetics, Dietitians of Canada and the American College of Sports Medicine (ACSM) (72), which is confirmed by the guidelines of The International Society for Sports Nutrition (ISSN), which give a range of 1.4–2.2 g/kg of b.w./day (61). The protein supply of mountaineers' diets should be adapted to the stage of the expedition and the intensity of the effort. During an energy deficit, it is recommended to increase the protein content of the diet up to 1.6 g/kg of b.w./day, which improves nitrogen balance and maintains lean body mass (87). The protective effect of higher protein supply on muscle and whole-body protein homeostasis is impaired when the energy deficit is more than 40% of daily energy requirements. It was found that a high-protein diet (2 g protein/kg of b.w./day) did not spare lean body mass in military personnel at an altitude of 4,300 m during 21 days of energy deficit. The doubling of protein intake (compared to the standard diet group) resulted in a parallel increase in protein oxidation, suggesting that a greater proportion of dietary amino acids were then oxidized for energy production. Consequently, the availability of amino acids to support protein balance was limited (88). In studies analyzing the nutrient content of mountaineers' diets in high-altitude conditions, the protein supply was most often too low in relation to recommendations for athletes i.e., 1.1 g/kg of b.w./day (57), 1.2 and 1.3 g/kg of b.w./day (84) or an appropriate 1.5–2.5 g/kg of b.w./day (82). In view of the endurance exercise undertaken by mountaineers, the dietary protein supply should be a minimum of 1.4 g/kg of b.w./day, taking into account high-quality protein and distributing it to the main meals and snacks during the climb or approach.

Foods rich in fat are high in calories, which in the mountains can help prevent weight loss. According to recommendations, athletes should consume a moderate amount of fats from 20% to 35% of their daily caloric needs, while during regular high-volume training they can safely take in up to 50% (61, 72, 89). A high-fat diet appears to be beneficial during high-altitude sojourns (37), due to increased leptin expression, resulting in decreased appetite and increased β-oxidation of fatty acids. It is worthwhile for mountaineers to also be guided by their appetite and select snacks in the mountains according to their own preferences and tolerance of their digestive system. In the Karl et al. study, mountain climbers preferred high-fat foods after weight loss and acclimatization (90). Prolonged exposure to altitude reduces the consumption of free fatty acids during physical activity (62). In the studies conducted so far that analyzed the nutrient content of mountaineers' diets, the fat content was too high in relation to the recommendations for athletes i.e., 45.5% (82), 38% (84), 36.8% (57), 36.1% (83), and only the Italian group described in the study by Bondi et al. (84) provided relatively little fat (24%). From our perspective mountaineers staying in high-mountain conditions should rely on valuable sources of fat, including in their diet products rich in poly- and monounsaturated fatty acids, such as vegetable oils (olive oil, flaxseed oil), nuts, seeds. Mountaineers choosing products in the mountain diet should be guided primarily by appetite and adjust the supply of this component to the individual tolerance of the digestive system.

3.3 Prevention of micronutrient deficiencies in mountaineers' diets

Due to limited access to fresh vegetables, fruits and many protein-containing foods, the supply of minerals and vitamins in mountaineers' diets may be too low. To our knowledge, there is a lack of research indicating what the micronutrient requirements of the diet are in high-altitude conditions, and studies conducted so far emphasize the role of iron, B vitamins, antioxidant vitamins and minerals, and vitamin D. Further research is needed to formulate recommendations for the supply of micronutrients in high altitude conditions.

Given the importance of haemoglobin levels for aerobic power, optimal iron content is particularly important for endurance athletes. Furthermore, they are at risk of increased iron loss from sweat, urine, through the gastrointestinal tract and due to haemolysis and exercise-related blood loss, through injury and menstruation (91). At high altitudes, iron requirements are increased due to erythropoiesis (92). This process may be impaired in athletes with low iron levels (93). Adequate iron stores are required to sustain the hypoxia-induced increase in heme synthesis and iron-dependent enzyme production during prolonged exposure at altitude (94). Iron intake was insufficient during the stay in the Alps (3,200–3,616 m) and was 10.4 ± 1.73 mg/day (83) and 14 ± 4 mg/day during an expedition in the Himalayas (1,070–5,143 m) in the Nepalese group and 24 ± 3 mg/day in the Italian group (84). According to the recommendations of the International Olympic Committee, 8–10 weeks before the start of training activities above 2000m, ferritin levels should be assessed (95). It is suggested to increase iron intake or oral supplementation when serum ferritin levels are less than 30 nmol/L in women and 40 nmol/L in men (95), however, these pre-altitude ferritin cut-offs, in combination with iron supplementation, have not been scientifically validated. According to Stellingwerff et al. (67) it is recommended to perform pre-altitude blood tests about 4–6 weeks prior to allow for a more precise assessment of ferritin levels, so that appropriate oral supplementation can be started about 2 weeks before and during altitude exposure, if necessary. Recommendations for iron supplementation before mountain activity were established on ferritin cut-offs of <100, ~100–130 and >130 ng/ml and are based on interpolation and/or extrapolation of existing data (94, 96–98). Current findings suggest that most athletes in hypoxic environments increase hemoglobin mass by taking ~100–200 mg of elemental iron per day orally, with most of the evidence related to iron salts. We recommend involving a sports medicine physician in the process of assessing the need for iron supplementation prior to high-altitude exposure and determining its dose in the event of iron deficiency or low ferritin concentrations. Excessive iron

supplementation and clinically elevated endogenous iron stores can have negative health consequences (67, 99). Ferritin limits need further scientific validation, as there is currently a lack of studies evaluating the body's response to iron intake in mountaineers staying above 3,000 m above sea level. It is important for mountaineers to include rich sources of iron (meat, fish, pulses, whole grains, dairy products) in their diet before and during the expedition. When consuming iron-containing products, it is also important to consider its bioavailability. Heme iron (from meat) has a higher absorption capacity (~5%–35%) than non-heme sources (~2%–20%) (100). The presence of vitamin C and meat, poultry and fish can increase the absorption of nonheme iron, while substances such as polyphenols, phytates or calcium that are part of tea, coffee, whole grains, legumes and dairy products can decrease the amount of nonheme iron absorbed from a given meal (101). In addition, it is worth considering a probiotic (102, 103) and lactoferrin (104–106) to improve iron bioavailability. Lactoferrin increases intestinal iron absorption by binding to iron and improves hemoglobin and serum iron levels, thus maintaining iron homeostasis in the body and cells (106). Optimal levels of vitamin D (30–50 ng/ml) and B_{12} (400–700 pg/ml) can probably help improve iron status and thus avoid anemia (107–109).

Hypobaric hypoxia leads to reduced oxygen delivery to the brain, and can subsequently contribute to cognitive impairment and increased risk of dementia, including Alzheimer's disease (110). High altitude cerebral edema and hypoxia cause cognitive impairment and dementia associated with white matter pathology (111). Whether exposure to hypoxia at high altitudes causes irreversible brain damage is debatable. In an magnetic resonance imaging (MRI) study of 35 mountaineers, the authors concluded that there is sufficient evidence of brain damage after high altitude climbing, and that amateur climbers are at higher risk of brain damage than professional climbers (112). In the study, Garrido et al. showed that Sherpas highlanders have better brain protection when exposed to extreme altitude compared to a group of elite lowland mountaineers who had climbed more than 8,000 m, and a control group (113). A study by Tunali et al. describes the case of a 57-year-old man who developed symptoms of acute mountain sickness after climbing 5,416 m in Nepal. Several months after descending the mountain, he developed symptoms such as loss of empathy, impaired speech, problems with perceiving and expressing emotions, and an increased interest in sugary foods. The patient's MRI and PET/CT results showed frontotemporal neurodegeneration (111). Metabolic functions of B vitamins and their role in neurochemical synthesis can be seen as having a particular impact on brain function (114). Folic acid, vitamin B_{12} and iron play a key role in erythropoiesis (115). Krzywanski et al. recommended that vitamin B12 serum concentrations in athletes training at sea level should be maintained between 400 and 700 pg/ml to support haemoglobin synthesis and improve red blood cell markers, with regular monitoring to consider supplementation when vitamin B12 levels are <400 pg/ml (108). However, there is a lack of studies assessing the optimum serum concentration of B vitamins and the requirement for these vitamins in high-altitude conditions. Currently, there is insufficient evidence to recommend supplementation of B vitamins in high-altitude conditions, given their role in erythropoiesis and to improve cognitive functions.

Vitamin D plays an important role in immune, muscle and cardiovascular function, inflammatory response, protein synthesis, cell growth and regulation of the musculoskeletal system (116–118). Many athletes have insufficient levels of vitamin D, and most often serum levels are below 20 ng/ml, especially during the winter months (119). According to Zhang et al. (120), to achieve optimal anti-inflammatory effects of vitamin D, it is important to maintain serum vitamin D levels >30 ng/ml. According to current recommendations, in normal-weight adults, cholecalciferol or calcifediol supplementation should be implemented and continued under supervision of a medical doctor until an optimal serum concentration of >30–50 ng/ml of 25-hydroxyvitamin D (25(OH)D) is achieved and maintained (116). Kasprowicz et al. (109) have shown that high doses of vitamin D (10,000 IU/day) can prevent the decline in serum iron levels after exercise. Kasprzak et al. (83) have observed a decrease in 25(OH)D serum concentrations among mountaineers during a 14-day stay at 3,200-3,616 m above sea level, associated with modulation of immune processes. This is consistent with a study conducted at an altitude of 5,400-6,700 m, for about 4 months, involving 221 male volunteers serving in the Indian Army (121), in whom a decrease in serum 25(OH)D concentrations was associated with skeletal deterioration at extreme altitudes (121). Mountaineers staying in high-altitude conditions with low temperatures and UV radiation, need to protect their skin from burns, and additionally use down suits, both of which prevent skin synthesis of vitamin D. Given how important the role of vitamin D is in the context of the immune system and the fact that its deficiency is associated with a higher risk of anemia (107), it is important to monitor the concentration of this vitamin before the expedition, adjust the appropriate dose to the current serum concentration, and continue supplementation during the expedition.

Staying at high altitudes, associated with reduced oxygen pressure, can result in increased production of reactive oxygen and nitrogen species and the development of oxidative stress (15). Excessive production of RONS, which exceeds endogenous antioxidant defenses, can lead to damage to lipids, proteins and DNA, thereby impairing cellular and immune function, causing delayed post-workout recovery (122). The supply of antioxidant-rich dietary components can be a helpful intervention in the fight against altitude-induced oxidative stress. It seems most prudent to include a large amount of antioxidant-rich foods, including vitamin C, beta-carotene, vitamin A, vitamin E, selenium and zinc in the daily diet of mountaineers. Freeze-dried fruits, vegetables and their powdered forms can be valuable sources of antioxidant vitamins in high-altitude conditions. An intervention at 2,320 meters above sea level to increase the intake of antioxidant-rich foods, served as snacks between meals, not only increased the intake of antioxidants, but also improved the overall amount of macro- and micronutrients in the athletes'

diets (123). It would be advisable to test such a strategy in the higher parts of the mountains as well.

3.4 Importance of hydration and electrolyte supply during high altitude sojourn

Hypoxia occurring in high-mountain conditions increases fluid loss through hyperventilation and contributes to dehydration, which reduces aerobic capacity (124). Dehydration causes changes in electrolyte concentrations in the body, resulting in stress on the cardiovascular system as plasma volume decreases. Impaired cardiovascular function reduces cutaneous blood flow and the body's ability to dissipate heat to the environment (125). In addition to these physiological effects, dehydration can impair cognitive function and concentration (126).

Dehydration has not been shown to increase the risk of acute mountain sickness (AMS) (124), although control of fluid output is thought to be important in preventing the onset of AMS, as clinically, measures to prevent excessive fluid retention are likely to reduce the symptoms of AMS (127). Fluid requirements in high-altitude conditions are higher than at sea level also due to hyperventilation, low humidity, sweat losses (127, 128) and increased diuresis as a result of down-regulation of the renin-angiotensin-aldosterone system, especially during the first days of the stay (129). Optimal hydration may also be limited by the time and fuel resources needed to prepare potable water, sourced from the glacier (130). Another factor that contributes to dehydration is reduced thirst at high altitudes (131).

Urine colour analysis is a simple and convenient method of determining the whole-body hydration status (132, 133). Numerous studies using the subjective eight-point colour scale have shown that an increase in dehydration of the subject results in a darker urine colour (134). Fluid supply should be responsive to thirst and sufficient to prevent dehydration and avoid infrequent dark-coloured urination. Attempting to take in large amounts of fluids in a short period of time should be avoided (75). At 4,300 m water loss from the respiratory tract can be increased to 1,900 ml/day in men (85) and 850 ml/day in women (135), and urinary excretion by 500 ml/day (136). Water loss in mountaineers of Mount Everest (5,000–8,872 m) was 3.3 ± 0.6 L/day (53), while in people with low physical activity, engaged in cooking, melting snow, repairing tents, stationed at 6,542 m for 21 days, 3.0 ± 0.5 L/day (137). Another study analyzed water balance at sea level and at an altitude of 4,350 meters. Water loss decreased from ~4.5 to 3.5 L/day, mainly as a result of a decrease in ambient temperature of ~10°C (138). There was also a study among mountaineers who climbed Denali in Alaska. Based on two methods of measuring hydration status (urine specific gravity and ultrasound measurements of inferior vena cava size and IVC-CI collapse index), it was found that about half of the mountaineers in the study were dehydrated (130). In contrast, in a survey conducted by Karpęcka-Gałka et al. (57), almost half of mountain climbers drank 2-3 L of fluids per day in the mountains, while the rest of the respondents took even smaller amounts of fluids per day, which can lead to dehydration. Average water supply (per person per day) was monitored in a study conducted by Bondi et al. (84) in a group of Italians and Nepalese during an expedition in the Himalayas (1,070–5,143 m), reaching 3,099 ± 462 g/day and 3,240 ± 310 g/day, respectively.

Mountaineers in high-mountain conditions use glacier water as a source of hydration and for cooking, characterized by low mineral content, so it is worth considering electrolyte supplementation. An additional complication that arises in high mountains is the reduced boiling point, as a result of which prepared meals must take longer to cook (139). In the absence of water treatment tablets, there is a high risk of parasite infection. *Giardia lamblia*, an intestinal parasite that causes diarrhea, is found in high-altitude regions (140). It should be emphasized that recommendations for fluid replenishment at high altitude should be individualized due to different rates of fluid and electrolyte loss, urine color control and symptoms of AMS, which causes water retention in the body. According to ACSM expert recommendations, the daily fluid requirement is 4–5 L during altitude training and competition (72), while ISSN encourages individual monitoring of hydration status to determine athletes' fluid requirements (61). General recommendations during mountain activities include intake of 400–800 ml/h fluids with 0.5–1 g Na/L water (139).

3.5 Recommendations on the use of dietary supplements during high altitude expeditions

Due to poor access to food products at high and extreme altitudes, supplementation may be the only solution to provide adequate energy and essential nutrients, as well as to improve the functioning of the body in harsh environmental conditions. To date, there have been many studies confirming the effects of ergogenic agents during physical activity (141), however, there are a limited number of reports on supplementation in hypoxic or high-altitude conditions. Supplements with potential beneficial effects on exercise capacity, sleep quality, the immune system, the intestinal barrier, the gut microbiome and more in high-altitude conditions are described below. However, most of them require further research.

3.5.1 Carbohydrate supplements

Carbohydrate supplementation during exercise by non-acclimatized men has improved performance in a test conducted after 3 days of exposure at an altitude of 4,300 m, with a simultaneous 30% deficit in delivered energy applied (68). In a study involving men acclimated to altitude and in a state of energy balance, there was no benefit of carbohydrate supplementation on time-trial performance during the first and third days of altitude exposure (142). From the above studies, despite many differences in methodology, it can be concluded that the potential ergogenic effect of supplemented carbohydrates on exercise capacity may be modulated by acclimatization. In a

recent study evaluating the effect of carbohydrate supplementation on aerobic exercise performance in non-acclimated men after 5 h of exposure to an altitude of 4,300 m and after 22 days of acclimatization with an accompanying 40% energy deficit, there was no improvement in aerobic capacity (143). Based on the results of the study by Bradbury et al. (143), it is recommended that people planning to stay or engage in physical activity (e.g., alpine climbing) in high mountains prioritize carbohydrate intake while still at sea level in order to optimize glycogen stores before going into the mountains.

Although carbohydrate requirements to meet energy expenditure at high altitude increase, there is a paradoxical impairment in the ability to utilize exogenous carbohydrates for, at least, the first day after ascent. The inability to oxidize and functionally benefit from exogenous carbohydrate intake during high-altitude activity coincides with hyperinsulinemia, accelerated glycogenolysis and reduced peripheral glucose uptake. These responses are consistent with hypoxia-mediated deregulation of metabolism, reflecting insulin resistance. The findings also suggest a role for the gut microbiome in host metabolism, bioenergetics and physiological responses at high altitude, suggesting that the gut microbiome is a potential mediator of hypoxia-mediated metabolic deregulation (144). Pasiakos et al. (144) have indicated a lack of ergogenic effects of carbohydrate supplementation at altitude, which questions the effectiveness of recommending carbohydrate supplementation to maintain physical performance after ascending to high altitude. Thus, more research is needed to develop appropriate carbohydrate supply strategies that take into account the effects of hypoxia on insulin sensitivity and substrate oxidation to optimize the activities performed in high-altitude conditions. From our perspective, given the challenging conditions during high-mountain climbing, it is worthwhile for mountaineers to consider carbohydrate-supplying supplements (energy gels, gummies, carbohydrate jellies, carbohydrate bars, isotonic drinks) that are low in weight and easy to open at low temperatures in order to increase dietary carbohydrate supply, as well as to increase energy intake.

3.5.2 Protein supplements

If the protein supply does not cover the body's need for this nutrient, supplementation with a protein can be considered. Stimulation of muscle protein synthesis and/or reduction of proteolysis with a low-volume protein supplement rich in branched-chain amino acids, especially leucine, seems to be the best strategy to protect the body of a high altitude climber from an undesirable decrease in lean body mass (22). However, the results of studies conducted so far have not shown a benefit in this aspect when mountaineers supplemented their diet with a preparation of branched-chain amino acids (145, 146). Protein increases feelings of satiety (147), which is not a beneficial effect at high altitude, where mountaineers struggle with altitude anorexia (148). There is still a lack of studies evaluating the effect of protein supplementation on feelings of satiety at high altitude. Based on our experience, a diet rich in protein may not meet the expectations and dietary preferences of high altitude climbers, so when staying in high mountains above base camp, it is worth considering supplementation with a protein supplement to cover the daily requirement of this component, which also gains justification given the lower availability of food products that are sources of protein.

3.5.3 Antioxidants

Exogenous antioxidants neutralize free radicals, so it would seem logical that supplementation with these compounds could be a helpful intervention against altitude-induced antioxidant stress. While some studies have shown that antioxidant supplements have a modulating effect on oxidative stress and AMS symptoms at high altitude (149), more recent studies indicate that there is no such effect (150). After all, supplementation with antioxidants, especially in the early phase of the altitude exposure, can be counterproductive and potentially weaken or delay acclimatization to altitude (151). On the other hand, the results of the study of Koivisto et al. indicate that increasing the intake of antioxidant-rich foods during altitude exposure caused the expected increase in antioxidant capacity and attenuated some altitude-induced systemic inflammatory biomarkers in athletes (152). Undeniably, pro-oxidant-antioxidant balance is paramount for altitude acclimatization, but the question of planning an appropriate antioxidant-related nutritional and supplementation strategy remains unclear.

3.5.4 Caffeine

Caffeine is a supplement with a very well-documented effect, used to improve exercise capacity, neuromuscular function, alertness and concentration, as well as reduce perceived fatigue during exercise (153). The benefits of its supplementation are observed in sports of an endurance nature, requiring dexterity and precision, as well as short-duration, supramaximal or repeated high-intensity efforts (141). Mountaineering is an endurance sport with elements of intensive effort of a strength nature (55). Thus, it is worth considering caffeine supplementation among mountaineers, given the nature of the sport and the extreme fatigue and impaired concentration resulting from the harsh high-altitude conditions. Caffeine supplementation can also have a positive effect on cognitive function, and according to a study by Zhang et al. (154), low doses of caffeine (3 mg/kg of b.w.) have better effects in terms of improving cognitive function and brain activation. In a study conducted by Tian et al. (155), caffeine alleviated high altitude pulmonary edema (HAPE) and reduced oxidative stress and stabilized mitochondrial morphology in alveolar type 1 cells under hypoxic conditions. The results of the study presented here suggest that caffeine may be a potential treatment for abnormal acclimatization resulting in HAPE (155). Due to the need for mountaineers to remain focused despite extreme exhaustion throughout the expedition, caffeine could be used in high-altitude conditions. It is worth considering caffeine supplementation about 60 min before situations requiring sharpened attention (blood caffeine concentrations peak at this time) (156) such as technically

difficult sections of the route, before rappelling after the climb or on the way back from the summit. However, further research is needed to confirm this hypothesis.

3.5.5 Beetroot juice

Beetroot juice (the nitrates it contains) is a supplement that has attracted a lot of interest in recent years for its effects in improving the ability to perform long-term moderate-intensity exercise and repeated high-intensity short-term exercise. The mechanism of action of nitric oxide (NO) is based on improved blood flow through blood vessels, lower ATP consumption during force generation by muscles, reduced aerobic cost of effort, and increased biogenesis and mitochondrial performance (141, 157–159), which can potentially improve athletic performance. It is recommended to consume a dose of 6–8 mmol (~350–500 mg) of nitrates 2–3 h before exercise. Another possible strategy is to consume 6–8 mmol of nitrates per day for several days prior to competition (160).

There are studies confirming increased exercise economy by about 5%–10% in hypoxic situations and/or better performance in recreational athletes after nitrate supplementation (161–163). In high-performance endurance athletes in hypoxic conditions, beetroot juice supplementation was not beneficial (164–167), but there is also a study supporting its effectiveness (168). Current evidence suggests potential improvement in endothelial function at high altitude after nitrate supplementation (169). In another study carried out in hypoxia assessed that the nitrates in beet juice do not prevent altitude sickness and should not be recommended as a preventive or ergogenic measure for already existing altitude sickness (170). Hannis et al. (171) in a study conducted with young men while trekking to Everest Base Camp showed that oral nitrate supplementation using beetroot juice was safe and feasible at altitude, and that dietary nitrate did not change mountain sickness symptoms or alter physiology. These results are consistent with a study performed by Cumpstey et al. (172) in the Alps (4,559 m), in which dietary nitrate supplementation was shown to be well tolerated at high altitude and to significantly increase lung NO availability and NO metabolite concentrations in saliva and exhaled breath condensate, and was not associated with changes in haemodynamics, oxygen saturation or the development of AMS. Similarly, a study conducted by Marshall et al. (173) with the British Military during a trek in the Himalayas (1,400–5,755 m) confirmed that dietary nitrate supplementation may ameliorate the decline in physical performance associated with staying at high altitude and has no effect on the incidence of high altitude illness. In the study by Bakker et al. (174), acute dietary nitrate ingestion offset the decline in endothelial function in healthy young participants during an expedition in Nepal (28 days >2,500 m, including a peak of 3,700 m).

Most of the published studies have examined the effects of nitrate supplementation at simulated altitude (161–168), which is often considered an incomplete substitute for "real" altitude. There is ongoing debate about potential differences in the physiological response to normobaric hypoxia and terrestrial altitude (175), including potential disparities in NO metabolism (176). The research conducted so far at terrestrial altitude is promising (171–174). Further studies are needed to evaluate the effects of nitrate–at terrestrial altitude and/or contrasting the effects of nitrate–supplementation between terrestrial and simulated altitude.

3.5.6 Prebiotics and probiotics

Gastrointestinal complaints are often reported during climbing at high altitudes (>2,500 m), although their etiology is unknown. Hypoxia and oxidative stress can damage the intestinal barrier, consequently leading to bacterial translocation and local/systemic inflammatory reactions. Preliminary data suggest that prolonged exposure to hypoxia can damage the intestinal barrier through changes in immune function, microbiota or mucosal layers. Exercise may worsen the intestinal damage associated with high altitude through additional reductions in visceral circulation and greater hypoxemia (21). Kleessen et al. analyzed fecal samples and serum from 7 mountaineers participating in a 47-day expedition to the Himalayas. The results of the study indicate a change in the composition of the gut microbiota after exposure to very high altitudes (>5,000 m). The number of bifidobacteria and species belonging to the *Atopobium*, *Coriobacterium* and *Eggerthella lenta* groups decreased, while the number of potential pathogenic bacteria from the gamma Proteobacteria subgroup and specific *Enterobacteriaceae*, such as *Escherichia coli*, increased. Changes in the composition of the microbiota may be associated with changes in indicators of the immune system and result in the deterioration of mountaineers' health (177). Karl et al. (178) have observed increased intestinal permeability in a group of healthy, physically active but non-acclimated men after a rapid (transported by airplane and car), 22-day exposure to an altitude of 4,300 m. The findings are consistent with Dinmore et al. (179); however, they should be interpreted with caution due to the limitations of the ongoing projects.

Acetate, propionate and butyrate, short-chain fatty acids (SCFAs) that are produced by some gut microbes mainly during the fermentation of dietary fiber, are key mediators of intestinal permeability and may protect the intestinal barrier from hypoxia-induced dysfunction (180). Adequate dietary fiber supply is a major challenge for participants in high altitude expeditions due to limited access to fresh food outside base camp. In the studies conducted so far, mountaineers most often did not cover the dietary requirements for this component for adults i.e., the values did not exceed 25 g/day (57, 83, 84). Recommended dietary fiber supply in athletes is 38 g (71). Insufficient intake of dietary fiber can result in a deterioration of intestinal function, as well as a reduction in the diversity of the microbiota (181). Prebiotics (galacto-oligosaccharides, fructo-oligosaccharides, human milk oligosaccharides, xylo-oligosaccharides, mannano-oligosaccharides, inulin), which are one type of fiber, are substrates selectively utilized by host microorganisms (182, 183). Prebiotic supplementation in high-altitude conditions is worth considering in view of the low dietary fiber supply, and the well-documented effect of prebiotics in promoting SCFA production.

Similarly, probiotics, or live microorganisms, when consumed in adequate amounts, can provide health benefits (184). Long-term

intake of probiotics *Bulgaricus 2038* and *Streptococcus thermophilus 1131* can increase the abundance of *Bacteroides* closely related to butyrate and propionate production (185). Increasing iron absorption may be a valuable strategy for improving iron status while at high altitudes and before going on an expedition. The probiotic strain *Lactobacillus plantarum 299v* (Lp299v, LP299V®) has been shown to improve iron absorption in meal studies (186, 187). The results of the study indicate that ingestion of Lp299v with 20 mg of iron may result in a more significant and faster improvement in iron levels compared to iron alone (102). The study was conducted in female athletes with low iron stores at sea level, so the effectiveness of the strategy would also need to be tested in high-altitude conditions. More studies using prebiotics and probiotics in high-altitude conditions are needed to assess their effectiveness in protecting the intestinal barrier against hypoxia-induced stress.

3.5.7 Omega-3 fatty acids

Long-chain omega-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA), show anti-inflammatory effects (188). According to the recommendations of the European Food Safety Agency (189), their intake by adults should be 250 mg for EPA and DHA and 0.5% of total energy intake for ALA. EPA/DHA are considered safe in doses of up to 5 g/day (190). The results of some studies suggest that EPA/DHA may improve endurance, delay the onset of muscle soreness and promote recovery in athletes (191, 192). It has also been shown that supplementation affects muscle protein synthesis, especially under conditions of immobilization and energy restriction or when taken with other nutrients (193, 194). A study using cell cultures assessed that DHA attenuates the increase in total ROS production and lipid peroxidation in mature adipocytes. The importance of such changes observed in response to DHA may reduce the deleterious effects of excessive ROS production caused by severe hypoxia and prevent lipid peroxidation and damage (195). In high-altitude conditions, the provision of adequate omega-3 fatty acids may be inadequate especially above the base camp due to limited access to foods abundant in omega-3 fatty acids. However, further human studies conducted under hypoxic conditions and at terrestrial altitude are needed to unequivocally recommend omega-3 fatty acid supplementation in high-altitude conditions.

3.5.8 Curcumin

Polyphenol curcumin derived from the *Curcuma longa* plant provides protection of the intestinal barrier by reducing oxidative stress and apoptosis (196). In the situation of HAPE, curcumin supplementation increases the integrity of the alveolar epithelial barrier (197). Curcumin supplementation may lead to lower levels of pro-inflammatory cytokines or indirect markers of muscle damage under normoxia (198–200). However, more studies conducted under hypoxic conditions and at terrestrial altitude are needed to demonstrate the efficacy of curcumin supplementation to justify its use in mountaineers.

3.5.9 Glutamine

Glutamine is an amino acid with potent anti-inflammatory effects, a key energy fuel for rapidly growing cells (such as immune cells and enterocytes), a precursor to glucose, proteins and nucleic acids (201). It has a variety of functions in the body, including being a key component for the synthesis of cytokines, hormones, maintenance of acid-base balance, ammonia transport and cell proliferation (202). Under hypoxic conditions, glutamine synthesis in the body may be insufficient to meet the increased demand for this amino acid. Findings indicate that glutamine supplementation reduces the risk of upper respiratory tract infections after prolonged low-altitude (1,640 m) exercise, thereby improving immunocompetence in hypoxia (203). We evaluated the effects of glutamine supplementation on the regulation of lymphocyte-mediated immune responses and inflammation in humans exposed to 6 h of simulated hypoxia at 4,500 m in a normobaric chamber, with and without exercise (204). Glutamine (at a dose of 20 g) was administered to study participants for 6 days before exposure to altitude. Glutamine supplementation stimulated both T helper 1 lymphocyte responses, inhibiting T helper 2 lymphocyte responses, and attenuated the release of IL-6 and TNF-α (204). Glutamine may have a positive effect on the function of enterocytes and immune cells in the gut and improve defense mechanisms. Moreover, it can be an energy substrate for the intestinal microbiota, reducing the inflammatory process accompanied by increased intestinal permeability, thereby improving cognitive function and mood (205, 206). Glutamine administered to rats at a dose of 5 g/kg of b.w. 3 days before and 5 days during a simulated 7,000 m altitude had the effect of reducing hypoxia-induced damage to the intestinal structure and regulating the intestinal microbiota (207). Glutamine can directly modify neurotransmitter activity in the brain, affecting mood and cognitive function (208). McMorris et al. showed in a meta-analysis that hypoxia harms cognitive processes (209), and this condition can probably be alleviated by glutamine supplementation (210), but there is a lack of human studies using glutamine supplementation in high-altitude conditions to confirm this hypothesis. Studies in hypoxia and at terrestrial altitude, with and without glutamine supplementation, should be performed to experimentally correlate the relationship between glutamine and cognitive function, mood, immune response in hypoxia.

3.5.10 N-acetylcysteine (NAC)

N-acetylcysteine (NAC) is used for its antioxidant and anti-inflammatory effects. NAC supplementation appears to be safe and may regulate glutathione homeostasis, have antioxidant effects and improve physical performance (211). In studies conducted to date, NAC has increased resistance to fatigue (212), improved immune function (213), as well as hemodynamics and muscle blood flow (214) and modulated EPO production (215). The dosage of NAC in the conducted studies ranged from 600 to 1,200 mg per day, and supplementation was applied for 5–9 days (214, 215). There is a lack of studies confirming the validity of NAC supplementation in hypoxic conditions. Moreover, there

are also studies available that do not confirm a positive effect of NAC on EPO production (216) and even indicate a pro-oxidant effect when taken in high doses before a prolonged period of time (i.e., 1,200 mg/day for 4 weeks, then 2,400 mg/day for another 2 weeks) (217). Thus, further research is needed to evaluate NAC as a useful supplement for use before or during altitude exposure.

3.5.11 Tart cherry juice

Supplementation with tart cherry taken in many forms can improve muscle recovery from exercise-induced damage (218–220), physical performance (221, 222) and sleep quality (223–225.) It is recommended to use tart cherry juice at a dose of about 250–350 ml (30 ml for concentrate), twice a day, for 4–5 days before sports competition or for 2–3 days afterwards to support recovery (141). The results of a study conducted by Horiuchi et al. (226) indicate that a 5-day supplementation with tart cherry (a capsule containing 100 mg of anthocyanins) improves exercise tolerance in hypoxia. However, more studies are needed to unequivocally recommend supplementation with this preparation in hypoxia.

New arrivals to altitude commonly experience poor-quality sleep. At high altitudes, reduced oxygen content in the blood can cause sleep disturbances with frequent awakenings and feelings of shortness of breath (227). De Aquino Lemos et al. (228) have found that hypoxia reduces total sleep time, sleep efficiency and causes rapid eye movements. Changes in sleep patterns can affect mood and cognitive function after 24 h (228). In mountaineers rapidly climbing to high altitudes, sleep quality is initially impaired, but improves with acclimatization due to improved oxygen saturation (229). Given the positive effects of tart cherry juice on sleep duration and efficiency, the use of this supplement in high-altitude conditions seems an interesting strategy. Further studies need to be conducted in hypoxia and at terrestrial altitude, with and without tart cherry juice supplementation, to experimentally correlate the relationship between tart cherry juice and physical performance and sleep quality in hypoxia.

3.5.12 Ginko biloba

Ginko biloba herb extract has been found to reduce tissue hypoxia, induce vasodilation, and reduce free radical generation and lung leakage, which in turn may prevent AMS (230). A meta-analysis conducted by Tsai et al. yielded inconclusive results regarding the effect of *Ginko biloba* on AMS prevention (230). In the study, Moraga et al. showed that 24 h pretreatment with *Ginko biloba* and subsequent maintenance during high-altitude exposure was sufficient to reduce the incidence of AMS in participants with no prior high-altitude experience (231).

3.6 Practical tips for nutrition at different stages of a high altitude expedition

Staying at high altitudes in the mountains requires advance planning of nutrition, hydration and supplementation. Depending on the direction of activities, expeditions vary in the location of the base camp and its supplies, so it is necessary to have knowledge of the expedition's logistics. Mountaineers can procure food on their own or use the help of an expedition agency, which organizes kitchen facilities at base camp and provides food, and is responsible for obtaining drinking water (139). Much of the food that will be consumed during the expedition is obtained in the country where the mountain activities are undertaken. Cultural differences, religious aspects and the availability of products in a country should be taken into account. In Buddhist countries, such as Nepal, it can be difficult to purchase meat products.

Depending on the stage of the expedition, mountaineers face some difficulties regarding nutrition and hydration. The first is trekking and acclimatization. This stage usually ends with an approach to base camp at an altitude of about 3,500–4,500 meters above sea level, depending on the mountain range. Mountaineers during this time usually enjoy overnight accommodations in high mountain villages. Eating in local restaurants, tasting street food and drinking water can cause food poisoning and contagious diarrhea. Therefore, extreme caution should be applied and care should be taken to ensure the hygiene of the meals consumed.

At base camp (for peaks >5,000 m), mountaineers are usually located at an altitude between 4,500 and 5,000 meters above sea level. This is the place where mountain climbers can enjoy a well-balanced diet, laden with fresh foods for the last time before setting off on a summit attack. Expeditions without the help of mountain travel agencies and hired porters are forced to properly plan the distribution of transported food. Food supplies should secure the mountaineers' stay at altitude for several weeks, which requires an appropriate weight and volume strategy, such as low-weight high-energy foods and freeze-dried products, to transport the maximum amount of high-quality and tasty food. Trying to avoid diet monotony is hard work, but it helps motivate mountaineers to eat. Storage of fruits and vegetables is a problem due to low temperatures. Animal products, such as eggs and meat (poultry and yak meat), are good sources of protein during the expedition, but their availability is limited. Another source of protein is fish, usually in canned form. In Buddhist countries, base camps usually use vegetable protein from pulses such as lentils and chickpeas. To maintain nutritional hygiene, latrines should be placed away from kitchens and water sources.

Above the base camp, mountaineers use glacial water for hydration and for cooking. At this stage, mountain climbers move to the next point, the intermediate base camp, gaining more and more altitude on their way to the summit. Mountaineers also descend to these points after gaining altitude to acclimatize above base camp for better recovery and sleep. It is essential to properly plan and pack freeze-dried and dehydrated foods, carbohydrate snacks and supplements to ensure an adequate supply of energy on the way to the summit. Reduced appetite, low temperatures and the problem of cooking meals and water at high altitude in an unfavorable environment pose major challenges for mountaineers, which increase with the altitude gained. Regardless of appetite, it is necessary to eat regular meals and high-energy snacks throughout the day.

During a summit attack there is an accumulation of unfavorable factors. Fatigue and weakness increase, aerobic capacity decreases due to staying at very high or extreme altitudes (often in the death zone i.e., >8,000 m), and in addition, mountaineers are exposed to very low temperatures, the consequence of which is sometimes frostbite (232). Throughout the year, the typical chill equivalent temperature near the summit of Everest is always <−30°C, and the typical facial frostbite time is less than 20 min (232). Bad weather is estimated to contribute to 25% of deaths in the mountains (233). The summit attack, including the return to intermediate camp, may take 12–24 h (139), and this time depends on the mountain range and the logistics of this stage. The food taken by mountaineers must be at this stage as light as possible, high-energy, and also easily digestible in order not to burden the weakened digestive system. Drinks and food taken should be properly protected from freezing, preferably by keeping them close to the body in the inner pockets of the suit. It is worth considering energy gels, gummies, carbohydrate jellies and water with added carbohydrates i.e., high-energy, high-carbohydrate, low-weight products and supplements. Due to the described difficulties of staying in high mountains and climbing, it is important to increase athletes' awareness of nutrition, hydration and supplementation in order to improve body function, general wellbeing, exercise performance and increase their chances of success.

4 Summary and further directions

Due to the negative effects of weather and environmental conditions in high mountains, mountaineers are at risk of compromised health and even loss of life. Adequate preparation for an expedition, especially attention to factors that mountaineers have control over, including nutrition, hydration and supplementation, can help prevent the development of nutritional deficiencies that affect the deterioration of health and performance. Energy requirements during climbing in high-altitude conditions can vary significantly depending on the load in the form of equipment transported in the backpack, the experience of the climber, as well as the difficulties encountered during the climb. Mountaineers, while at high-extreme altitudes, are at risk of energy deficiency, and consequently loss of body weight and lean body mass. Despite the lack of appetite, care should be taken to ensure regularity in the meals eaten and snacks taken.

The supply of carbohydrates in the diet of mountaineers should be adapted to the stage of the expedition and the intensity of the effort. Staying at base camp, with low physical activity, this supply should be about 3-5 g CHO per kg of b.w./day, while during trekking and/or climbing—a minimum of 6 g/kg of b.w./day. In view of the endurance physical effort mountaineers undertake, dietary protein supply should be a minimum of 1.4 g/kg of b.w./day, taking into account high-quality protein and distributing it to main meals and snacks during climbing and ascent to the summit. Mountaineers should rely on valuable sources of fat, including in their diet products rich in poly- and monounsaturated fatty acids such as vegetable oils (olive oil, flaxseed oil), nuts, seeds. It is recommended that the supply of fat in the diet of mountaineers should be in addition to energy after the carbohydrate and protein requirements are met, and should be 20%–35% of the total energy supply. Mountaineers choosing products in the mountain diet should be guided primarily by appetite and adjust the supply of this component to the individual tolerance of the digestive system.

Limited access to fresh fruits and vegetables and protein-containing foods in high-mountain conditions may be associated with an inadequate supply of minerals and vitamins. Ferritin and vitamin D serum concentration should be assessed before the expedition. If there is a deficiency of micronutrients, such as iron and vitamin D, supplementation should be implemented to optimize health and continued under supervision of a medical doctor. It is worth including foods rich in iron in the diet and ensuring adequate iron bioavailability. It is advisable to adjust the appropriate dose of vitamin D supplementation to the current serum concentration and continue supplementation during the expedition. Mountaineers should consume products rich in antioxidants, including sources of vitamin C, beta-carotene, vitamin E, selenium and zinc while at high altitude. Freeze-dried fruits, vegetables and their powdered forms can be valuable sources of antioxidant vitamins in high-altitude conditions. Further research is needed to formulate recommendations for the supply of micronutrients in high altitude conditions.

Fluid supply at high altitude should be individualized due to different rates of fluid and electrolyte loss, urine color control (dehydration results in a darker urine colour) and symptoms of AMS, which causes water retention in the body. A regular supply of fluids is necessary to prevent dehydration.

Due to poor access to food products at high and extreme altitudes, supplementation may be the only solution to provide adequate energy and essential nutrients, as well as to improve the functioning of the body in harsh environmental conditions. The potential ergogenic effect of supplemented carbohydrates on exercise capacity in high-mountain conditions may be modulated by acclimatization. More research is needed to develop appropriate carbohydrate supply strategies that take into account the effects of hypoxia on insulin sensitivity and substrate oxidation to optimize the activities performed in high-altitude conditions. It is worth considering supplementation with a protein supplement to cover the daily requirement of this ingredient due to the lower availability of food products that are sources of protein in high-altitude conditions, especially above base camp. There is still a lack of studies evaluating the effect of protein supplementation on feelings of satiety at high altitude. Pro-oxidant-antioxidant balance is paramount for altitude acclimatization, but the question of planning an appropriate antioxidant-related nutritional and supplementation strategy remains unclear.

Caffeine administered in low doses (3 mg/kg of b.w.) has the potential to positively affect mountaineers' cognitive function. Preliminary data suggest that caffeine may be a potential treatment for high altitude pulmonary edema. However, further studies are needed to assess the efficacy of caffeine

supplementation in hypoxic conditions. Nitrates supplemented in the form of beet juice may improve endothelial function at high altitude, but nitrates are not recommended as a prophylactic or ergogenic agent for already existing altitude disease. Further studies are needed to evaluate the validity of beet juice supplementation in high-altitude conditions. In view of the gastrointestinal complaints reported during high altitude climbing, probiotics and prebiotics have the potential to protect mountaineers' intestinal barrier from hypoxia-induced dysfunction. However, further research focusing on the use of specific probiotic strains and their effects on the state of the gut microbiota in high-mountain conditions is needed. In high-altitude conditions, the provision of adequate omega-3 fatty acids may be inadequate especially above base camp due to limited access to foods abundant in omega-3 fatty acids. Further research is needed to assess the validity of omega-3 supplementation in hypoxic conditions. Glutamine may have a positive effect on the function of enterocytes and immune cells in the gut. Moreover, it may be an energy substrate for the intestinal microbiota, reducing the inflammatory process accompanied by increased intestinal permeability, thereby improving cognitive function and mood. Further research is needed with humans using glutamine supplementation in high-altitude conditions. There are several supplements, such as curcumin, *Ginko biloba*, NAC and tart cherry juice, that need further research to evaluate their effectiveness in high-altitude conditions. Given the positive effect of tart cherry juice on sleep quality and physical performance, the use of this supplement in high-altitude conditions seems an interesting strategy. There is a lack of well-designed studies examining the relationship between the use of dietary supplements and improved athletic performance and health at high-extreme altitudes. Therefore, there is a need for controlled, randomized studies involving mountaineers.

5 Conclusions

In view of the difficulties of being in high mountains and practicing alpine climbing, as described in the review, it is important to increase athletes' awareness of nutrition, hydration and supplementation in order to improve well-being, physical performance and increase the chance of success in high altitude action, and to provide the appropriate dietary care necessary to educate mountaineers and personalize recommendations to the needs of the individual.

Author contributions

EK-G: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. BF: Funding acquisition, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

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

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Publikacja 3 „The Impact of a High-Altitude Expedition on the Physical Performance and Nutritional Indices of Health Status of Alpinists”

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Article

The Impact of a High-Altitude Expedition on the Physical Performance and Nutritional Indices of Health Status of Alpinists

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Abstract: Objective: The aim of the study was to determine the effect a mountain expedition (>3000 m) would have on the physical performance and nutritional indices of alpinists' health status. **Methods:** The study included 17 men aged 30.29 ± 5.8 years participating in mountain expeditions to peaks of 5000–8000 m, lasting an average of 34 ± 6 days. The following were assessed: aerobic and anaerobic capacity, body composition and the values of selected biochemical and hematological indices of blood and urine before and after returning from the expeditions and a quantitative analysis of the alpinists' diet. **Results:** There was a statistically significant decrease ($p \leq 0.05$) in aerobic capacity, anaerobic capacity, subjects' body mass, muscle mass and the lean body mass of the upper and lower extremities. There was a significant increase ($p \leq 0.05$) in erythrocytes, hemoglobin, hematocrit, leukocytes, platelets, neutrophils, monocytes and a significant decrease ($p \leq 0.05$) in total and high-density lipoprotein cholesterol, total bilirubin, albumin and total protein. A small percentage of the subjects met the requirements for iron (29.41%), folate (35.29%) and vitamin D (17.65%) supply with diet, as reflected in the blood test results. **Conclusions:** Despite the observed positive effect of three-week hypoxic exposure on the climbers' health, the deterioration of aerobic and anaerobic capacity was shown, which, in addition to environmental conditions and systemic inflammation, may have been influenced by adverse changes in body composition. To improve the nutritional status of the body during the expedition and upon return, alpinists should consider including the necessary supplementation of deficient components.

Keywords: alpinism; body composition; climbing; health status; mountaineering; physical performance

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1. Introduction

Alpinism is a discipline combining rock climbing, ice climbing, ski mountaineering and moving on glaciers [1]. A special variety of climbing is Himalayanism—that is, climbing practiced in the highest mountains in the world whose highest peaks exceed 7000 m—these are the mountain ranges of the Himalayas, Karakorum, Daxue Shan, Hindu Kush, Kunlun, Pamir and Tianshan [2]. Interest in mountaineering is growing every year, also among amateur climbers [3]. It is an endurance sport with elements of high-intensity

strength training. Exercise typically involves short periods of high intensity and long periods of submaximal intensity that require anaerobic and aerobic energy pathways [4]. During climbing, oxygen consumption and heart rate increase, suggesting that it requires an optimal level of aerobic capacity [5,6]. In the high-altitude environment, mountaineers must contend with intense sunlight, heavy rain or snow and strong winds and temperature drops (an average of 0.6° for every 100 m of altitude) with large fluctuations throughout the day, as well as the risk of avalanches and falling rocks [1,7]. Staying in hypobaric hypoxia (>3000 m) causes a number of deleterious effects on alpine climbers, such as a decreased exercise capacity [8], impaired post-exercise recovery [9], sleep deprivation [10], cognitive impairment [11] and body mass loss [12]. Acclimatization to long-term hypoxia is regulated at the molecular level by hypoxia-inducible factor-1 (HIF-1), which is responsible for mobilizing hundreds of genes in response to oxygen limitation [13]. Table 1 shows the characteristics of the altitude ranges referred to in the article [14,15].

Table 1. Definitions of altitude and associated physiological changes [14,15].

Altitude	Definition	Physiological Changes
1500–2500 m	Intermediate altitude	Physiological changes detectable. Arterial oxygen saturation > 90%. Altitude illness rare but possible with rapid ascent, exercise and a susceptible individual.
2500–3500 m	High altitude	Altitude illness common during rapid ascents.
3500–5800 m	Very high altitude	Altitude illness common. Arterial oxygen saturation < 90%. Marked hypoxemia during exercise. A total of 5800 m is the highest altitude of permanent human settlements.
>5800 m	Extreme altitude	Marked hypoxemia at rest. Progressive deterioration despite maximal acclimatization. Permanent survival is considered impossible.
>8000 m	"Death zone"	Prolonged acclimatization (>6 weeks) is essential. Most mountaineers require supplementary oxygen to climb safely. Arterial oxygen saturations about 55%. Rapid deterioration is inevitable and time spent above this altitude is strictly limited.

A number of studies have evaluated the aerobic and anaerobic capacity of Polish professional athletes [16–20], as well as the effect of training in hypoxia on aerobic and anaerobic capacity [21,22]. On the other hand, few studies have addressed the issue of changes in physical performance before and after a stay at high altitude (>3000 m) [8,23]. Poor athlete health, including low levels of iron and ferritin or red blood cell indices, is associated with decreased physical performance [24], while an increase in red blood cell mass improves aerobic capacity [25,26]. Altitude training, due to the intake of less oxygen during respiration, can be conducted to improve cardiorespiratory indices. The optimal altitude for this type of training is unknown; however, most scientific studies have been performed at intermediate and high altitudes (2000–3000 m) [27], where increases in erythropoietin levels have been observed with minimal side effects [28,29]. Altitudes above 3000 m can result in the loss of training intensity and subsequent muscle atrophy [30]. The main reasons for hypoxia-induced reduction in body and muscle mass are low energy intake, increased energy expenditure, unfavorable climatic conditions and the type of muscle fiber structure [31–33]. At altitudes above 5000 m above sea level, 60–70% of weight loss concerns lean body mass [34,35]. A reduction in lean body mass negatively affects aerobic capacity [36], muscle strength [37] and immune function [38], which can increase the risk of disease and injury under these extreme conditions. A decrease in muscle mass, the reduction of the fiber cross-sectional area and mitochondrial density in muscle fibers are the main causes of deterioration in physical performance after chronic hypoxia [8].

HIF-1, in response to hypoxia and increased free radical levels, initiates mechanisms to reduce free radicals in mitochondria [39]. Mechanisms induced by HIF-1 reduce oxidative stress, along with the apoptosis of muscle fibers [39]. However, compensation for the effects of hypoxia by HIF-1 is limited by the dose of hypoxia. Exercise capacity, including aerobic capacity, is impaired under hypoxic conditions, and maximal oxygen consumption ($\dot{V}O_{2max}$) decreases by 6% for every 1000 m of elevation (from 300 to 2800 m) [40]. Humans demonstrate a compensatory physiological and biochemical response to staying at high altitudes, especially in its early stages [41]. An increase in the concentration of red blood cells in the blood is an important feature of acclimatization to high altitudes [42,43]. Hypoxic kidney and liver cells release the hormone erythropoietin, which, in turn, stimulates the bone marrow to produce red blood cells [44]. Prolonged exposure to high and very high altitudes (in the range of 2800 to 5800 m) increases hematocrit levels, and this increase is accompanied by an increase in hemoglobin levels and blood viscosity [45]. Proper nutrition, hydration and appropriately selected supplementation (of micronutrients that are deficient in high-mountain conditions, antioxidants and ingredients that improve exercise performance) all help optimize mountain activities and can help prevent nutritional deficiencies that negatively affect health, cognition and physical performance [46–48].

The purpose of the study was to determine the effect of a 3-week stay at an altitude above 3000 m on the physical performance and nutritional indices of the health status of mountaineers participating in high-altitude expeditions. The following research hypotheses were put forward: (1) staying in high altitude conditions improved the performance of climbers due to improved red blood cell indices and (2) the high-altitude expedition negatively affected health indices. The results of the study will serve to deepen the knowledge of physiological and biochemical adaptations of high-altitude climbers and the possibility of using these adaptations to increase athletic performance, as well as appropriate training programming.

2. Materials and Methods

2.1. Study Participants

The study group consisted of 17 Polish male mountaineers between the ages of 23 and 40, who participated in mountain expeditions at least once a year, during which they stayed at altitudes above 3000 m for at least 3 weeks. Individuals who qualified for the study were members of high-mountain clubs affiliated with the Polish Mountaineering Association. The group's anthropometric and BMI data are shown in Table 2.

Table 2. Anthropometric data and BMI of climbers participating in the research (n = 17).

	Men (n = 17)	
	$\bar{x}$	**SD**
Age [years]	30.29	5.8
Body height [cm]	180.47	8.36
Body weight [kg]	74.96	5.03
BMI [kg/m^2]	22.83	2.1

Abbreviations: $\bar{x}$—mean; SD—standard deviation; BMI—body mass index.

The climbers participating in the study were characterized by extensive climbing experience (10 ± 5 years) in sport climbing and mountaineering. The average climbing level of participants, as measured by the IRCRA (The International Rock Climbing Research Association) scale, was 20.69 ± 2.8, which, according to the proposed criteria, places them in the advanced (level 3) category [49]. The climbers who qualified for the study spent an average of 8 ± 3 h per week training. The climbers stayed at an altitude of 3000–8167 m

above sea level during their expedition. In total, the high-altitude expeditions lasted an average of 34 ± 6 days, of which participants spent an average of 22 ± 4 days actively climbing in the mountains and 11 ± 8 days resting in base camps or mountain towns. Because some of these sites were located above 3000 m, the participants spent a total of 26 ± 6 days at altitudes above 3000 m.

The mountaineers recruited for the study traveled in small groups or individually to pursue their mountain aims, which are shown in Table 3.

Table 3. The mountain goals of mountaineers.

Number of Climbers Participating in the Expedition	Mountain Massif	Country	Mountain Goal
6	Cordillera Blanca	Peru	the 800-m Cruz del Sur route on the La Esfinge rock monolith (5325 m); new route on Ocschapalca (5888 m); Nevado Churup (5495 m); ascents on Artesonraju (6025 m) and Alpamayo (5947 m)
3	Shuijerab, North Karakorum	Pakistan	virgin peaks: Trident Peak (6150 m) and Sakwa Sar (6050 m)
3	Gangotri Valley, Garhwal Himalaya	India	attempted virgin peaks, but failed due to weather conditions (the highest point of the expeditions—5000 m)
2	Himalaya	Western and Northern Nepal	unsuccessful attempt to climb a virgin peak (due to weather); the highest point—5000 m
1	Himalaya	Nepal	Annapurna (8091 m) and Dhaulagiri (8167 m)
1	Himalaya	Nepal	Ama Dablam (6812 m)
1	Lailak Valley, Pamir-Alai	Kyrgyzstan	ascent via Troschenko route on the north face of Ak-su (5217 m)

Prior to the start of the project, the mountaineers had a consultation with a sports medicine doctor, during which they were qualified to participate in the project based on the results of previous tests (ECG, blood and urine tests). The presence of chronic diseases and age over 45 were disqualifying factors for participation in the project. The Bioethics Committee of the Cracow Regional Medical Chamber approved the research project (67/KBL/OIL/2021), which was conducted in accordance with the Declaration of Helsinki. After learning about the risks and benefits of taking part in the study, all climbers gave written informed consent to participate.

2.2. Study Design

2.2.1. Analysis of Aerobic and Anaerobic Capacity

Aerobic capacity was assessed during a test with gradually increasing load, performed until failure due to extreme fatigue, while anaerobic capacity was assessed during the Wingate test for the lower and upper extremities. Exercise tests were performed twice. The first test was performed about two weeks before the expedition, and the second up to four days after returning from the expedition at an altitude of about 383 m above sea level. The study was conducted as soon as possible after return to catch hypoxia-induced changes in health, body composition and physical performance.

1. Aerobic performance

The test was performed on an h/p/Cosmos Saturn mechanical treadmill (h/p/Cosmos Sports & Medical GmbH, Germany) according to the following scheme: a 2 min recording of baseline cardiorespiratory indices in a standing position, a 4 min run at a constant speed of 8 km/h and a treadmill incline angle of 1°, followed by a 1.1 km/h increase in running speed every 2 min until a running speed of 16.8 km/h was reached. In the remaining part of the test, the running speed did not change, and an increase in exercise intensity was obtained by raising the angle of the treadmill by 1° every 2 min. The test was continued until exhaustion, subjective to the test participant. Respiratory indices were monitored continuously using a Cortex Metalyzer 3B ergospirometer (CORTEX Biophysik GmbH, Leipzig, Germany), and heart rate was recorded using a Polar S-410 cardiac monitor (Polar-Electro, Helsinki, Finland). A number of cardiorespiratory indices were recorded during the test, including heart rate (HR), minute oxygen uptake ($\dot{V}O_2$), minute carbon dioxide excretion ($\dot{V}CO_2$), minute pulmonary ventilation ($\dot{V}E$), percentage of oxygen and carbon dioxide in exhaled air (FEO_2 and $FECO_2$) and respiratory quotient (RER). Based on the changes in respiratory indices during the test, the magnitude of maximum minute oxygen uptake ($\dot{V}O_{2max}$) and the level of ventilation threshold II were determined. The maximum percentage of carbon dioxide in exhaled air ($FECO_2$), the minimum value of the respiratory equivalent for carbon dioxide ($\dot{V}E/\dot{V}CO_2$) and a sharp increase in minute pulmonary ventilation ($\dot{V}E$) were used as criteria for determining the second ventilation threshold.

2. Anaerobic performance

This test was performed in a sitting position without getting up from the saddle on a Monark 894e cycloergometer for the lower limbs (Monark Sports & Medical, Vansbro, Sweden) and Monark 834e for the upper limbs (Monark Sports & Medical, Vansbro, Sweden) in a modified 20-s version. The test participants' task was to perform crank turns at maximum rhythm throughout the test. Both tests were preceded by warm-ups, with the lower limb test lasting 5 min with a 100-watt load, and the upper limb test lasting 4 min with a 60-watt load. The pedaling rhythm was 60 rpm. During the warm-up for the lower limb test, brief 5 s accelerations of the pedaling rhythm to about 80% of subjective maximum capacity occurred in the 2nd, 4th and 5th min, and in the warm-up for the upper limb test in the 2nd and 4th min, respectively. After the warm-up, the participants performed stretching exercises for 2–3 min, after which the test proper was started. The test load was set at 7.5% and 4.5% of body weight for the lower and upper limb tests, respectively. During both tests, the course of power changes was monitored continuously, which allowed the determination of the following indices: maximum anaerobic power (MAP), total work (W_t), mean anaerobic power (P_{mean}), time to reach maximum power (t_r) and time to maintain maximum power (t_m).

2.2.2. Analysis of Health Status

1. Anthropometric measurements and body composition analysis

The body height of the participants was measured before the expedition using a Seca 217 anthropometer (Seca GmbH & Co., KG, Hamburg, Germany) with an accuracy of 1 mm. The climbers' body weight was determined using an InBody 120 body composition analyzer (Inbody Bldg., Seoul, Republic of Korea) in the morning after a standardized meal between 7:45 and 8:30 a.m.

Body composition analysis was performed using dual-energy X-ray absorptiometry (DXA), with the Lunar iDXA™ instrument (GE HealthCare, Chicago, IL, USA). The DXA method used the phenomenon of attenuation occurring when a beam of ionizing radiation passes through various tissues of the body. The radiation dose was very low, so the test

was safe. The Lunar iDXA used fan beam technology with a 64-channel solid-state detector. Each body composition analysis provided information on body mass (BM) [kg] and body mass index (BMI), as well as individual components of body structure: body fat (FM) [% and kg], fat free mass (FFM) [% and kg], lean body mass, i.e., body mass without body fat and bone mineral (LBM) [% and kg], bone mineral content (BMC) [g] and bone mineral density (BMD) [g/cm^2]. The amount of LBM [kg] in both the upper limbs (arms) and lower limbs (legs) was also assessed. The values obtained additionally allowed for the estimation of the sum of appendicular lean soft tissue (ALST) weight. The ALST index was the sum of the lean body mass of the upper and lower limbs (ALST = LBM arms + LBM legs). With this, muscle mass (MM) [kg and %] was calculated. Kim's formula was used to calculate muscle mass in kilograms, with a value of 1 for the male gender: MM = (1.13 × ALST) − (0.02 × age) + (0.61 × gender) + 0.97 [50]. Composition analysis and weight measurement were performed twice. The first analysis was performed about two weeks before the expedition, and the second analysis was performed up to four days after returning from the expedition at an altitude of about 383 m above sea level.

2. Blood and urine tests

For health status analysis, blood samples were taken from veins in the elbow pit to determine hematological and biochemical blood indices, i.e., peripheral blood count (hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution (RDW), erythrocyte count, platelets count, mean platelet volume (MPV), white blood cell count—leukocytes, leukocyte differentiation: lymphocytes (LYMPH), monocytes (MON), basophils (BASO), eosinophils (EOS), neutrophils (NEU), erythrocyte sedimentation rate (ESR), glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), creatinine, urea (U), uric acid (UA), total protein, sodium, potassium, chloride, calcium, magnesium, phosphorus, iron, ferritin, vitamin D, vitamin B12, liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase, gamma-glutamyl transpeptidase (GGTP), albumin and total bilirubin. The determinations were carried out in the morning and on an empty stomach, about two weeks before the expedition and within two days after the expedition.

In addition to the blood test, a general urine test was performed, i.e., specific gravity, pH, leukocytes, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, blood (erythrocytes/hemoglobin), color and transparency. The urine for testing was delivered to the laboratory at the same time as the blood draws were performed. Measurements were made by flow cytometry (blood count), kinetic-photometric (ESR), indirect potentiometry (Na, K, chloride), spectrophotometry (total protein, calcium, phosphorus, magnesium, TC, HDL, TG, U, UA, iron, AST, ALT, creatinine, total bilirubin, GGTP, glucose), direct chemiluminescence (vitamin B12, vitamin D, ferritin) and by strip testing (urinalysis). Laboratory determinations were performed using the following devices: Alinity HQ (Abbott, Singapore, Singapore), Roller 20 (Alifax, Polverara, Italy), Alinity C (Abott, Singapore, Singapore), Alinity I (Abbott, Singapore, Singapore) and Atellica 1500 (Siemens, Munich, Germany), and were commissioned as an external service, performed by Alab Laboratories (Warsaw, Poland).

2.2.3. Nutritional Analysis of Diet

The supply of selected macronutrients during the mountaineering expedition was determined by analyzing the whole-day rations obtained using the 3-day food diary method. The expedition participants were asked to note down all the foods, meals and supplements eaten and fluids drunk during 3 days during the expedition (2 days of sports activity, 1 day

of rest). These were days of acclimatization and alpine climbing on the way to the summit, and the activities noted during this time were mainly hiking with climbing elements. Climbers were given detailed instructions on how to keep food diaries before leaving for the expedition in order to minimize recording errors. The diaries contained commercially available products, so the necessary data for analysis were read from labels and nutritional tables. In the case of food products such as bars, energy gels and freeze-dried products, climbers were asked to provide the product name, manufacturer and product weight.

The data from the diaries were meticulously entered into the Aliant Dietetic Calculator program (Anmarsoft Marcin Olech, Gdańsk, Poland; version: 85; database: 6.2) for quantitative analysis of the climbers' diets during the expedition. The program used the "Tables of food composition and nutritional value" [51] as a database, taking into account food recipes and losses due to product processing.

2.2.4. Measurement of Energy Expenditure

Measurements of energy expenditure during the expedition were made by the researchers using a Polar M430 monitor recording heart rate (Polar-Electro, Kempele, Finland), which was recorded 24/7 for 3 days (2 days of sports activity, 1 day of rest). The pulsometers consisted of watches (receiver) and transmitter straps with the Polar H10 sensor (Polar-Electro, Kempele, Finland), worn on the chest at the level of the metasternum in the anterior midline. The watches were placed on the wrist, so that they were adjacent to the body just below the wrist bone. Before the measurement began, the required data were entered into the device for each athlete: gender, age, body weight and height. Before the start of physical activity, the subjects were instructed to put on the transmitter strap, start the physical activity recording option on the device (climbing outdoors), and when the physical activity was over, to turn off the recording by pressing the corresponding button on the watch. In case of recording activity during acclimatization or when reaching the climbing starting point, the training option was not enabled. All climbers were advised not to remove the devices from their wrists for the entire duration of the energy expenditure recording (sleep, daytime activity, physical activity). Energy expenditure was recorded in the Polar Flow app.

2.2.5. Statistical Analysis

Basic statistical measures in the form of arithmetic mean ($\bar{x}$), standard deviation (SD), median (Me) and quartiles (Q1, Q3) were used to characterize the received results. The obtained research results were tested by checking for normal distribution of variables with the W Shapiro–Wilk test. For normal distributions, differences in the mean values of the observed indicators were tested using the Student's t-test for dependent groups, and for other distributions (deviating from the normal distribution), non-parametric statistics were used, with the Wilcoxon paired rank-order test for dependent groups. The significance level was taken as $p \leq 0.05$. The statistical analysis package Statistica 13.1 (StatSoft, Tulsa, OK, USA) was used for statistical analyses.

A binary classification approach was used to assess the implementation of nutritional standards. For each nutrient, a value of 1 was assigned if the subject's intake of the nutrient met the recommended standard, and 0 if it did not. Then, based on these data, the percentage of subjects who met the standards for each nutrient was calculated. The analysis was carried out using an Excel spreadsheet (Microsoft Corporation, Washington, DC, USA). The median intake of macronutrients (protein, fat, total carbohydrates), dietary fiber, simple carbohydrates, saturated fatty acids, and micronutrients over 3 days was referred to recommendations for athletes [52–56]. The results were presented in the form of percent-

ages, which made it possible to assess the degree to which the study group implemented the standards.

3. Results

The level of indices characterizing the aerobic capacity of climbers (maximum minute oxygen uptake globally and relatively, maximum minute pulmonary ventilation, maximum heart rate, effort duration) decreased after returning from the expedition. The described changes were statistically significant [Table 4].

Table 4. Aerobic performance indices measured before and after a stay at high altitude > 3000 m.

Aerobic Performance Indices	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$	SD	$\bar{x}$	SD		
$\dot{V}O_{2max}$ [L/min]	4.07	0.37	3.85	0.39	T	0.0006
$\dot{V}O_{2max}$ [mL/kg/min]	54.53	5.96	52.38	5.49	T	0.0232
$\dot{V}E_{max}$ [L/min]	149.34	15.88	138.05	14.43	T	0.0003
HR_{max} [beats/min]	190	10	187	11	T	0.0397
t_{max} [min]	20.38	1.95	18.82	2.16	T	0.0001

Abbreviations: $\bar{x}$—mean; SD—standard deviation; T—Student's *t*-test for variables with parametric distribution; *p*—statistical significance; $\dot{V}O_{2max}$—maximum oxygen uptake; $\dot{V}E_{max}$—maximum minute pulmonary ventilation; HR_{max}—maximum heart rate; t_{max}—effort duration.

The indices characterizing the level of the second ventilatory threshold after the expedition did not change significantly, as shown in Table 5. The only statistically significant change was observed in the level of heart rate, which decreased during the study, as measured after the return from the expeditions.

Table 5. Aerobic performance indices associated with the reaching of second ventilatory threshold measured before and after a stay at high altitude > 3000 m.

Aerobic Performance Indices at VT2	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$	SD	$\bar{x}$	SD		
$\dot{V}O_2$ at VT2 [L/min]	2.96	0.24	2.9	0.34	T	0.2545
$\dot{V}O_2$ at VT2 [mL/kg/min]	39.65	3.50	39.45	4.62	T	0.7722
$\%\dot{V}O_{2max}$ at VT2	73.06	5.65	75.37	4.87	T	0.0642
$\dot{V}E$ at VT2 [L/min]	78.35	8.81	80.71	8.46	T	0.2824
HR at VT2 [beats/min]	159	10	155	10	T	0.0358
$\%HR_{max}$ at VT2	73.06	5.65	75.36	4.87	T	0.6418
t at VT2 [min]	11.11	2.15	11.08	2.33	T	0.9330

Abbreviations: $\bar{x}$—mean; SD—standard deviation; T—Student's *t*-test for variables with parametric distribution; *p*—statistical significance; VT2—second ventilatory threshold; $\dot{V}O_2$ at VT2—maximum oxygen uptake at VT2; $\%\dot{V}O_{2max}$ at VT2—percentage of maximum oxygen uptake at VT2; $\dot{V}E$ at VT2—pulmonary ventilation at VT2; HR at VT2—heart rate at VT2; $\%HR_{max}$ at VT2—percentage of maximum heart rate at VT2; t at VT2—effort duration to reach VT2.

The level of indices characterizing the participants' anaerobic capacity generally decreased after returning from the expedition [Table 6]. In the lower limb test, maximum anaerobic power and mean anaerobic power expressed in global and relative values decreased. The values of total work and time of maintaining maximum power were also lower during the tests conducted after returning from the expeditions. The time of reaching maximum power decreased, but this change was not statistically significant compared to the other indicators mentioned

Table 6. Anaerobic performance indices of the lower limbs measured before and after a stay at high altitude > 3000 m.

Anaerobic Performance Indices (Legs)	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$/Me	SD/(Q1; Q3)	$\bar{x}$/Me	SD/(Q1; Q3)		
MAP [W]	807.46	73.66	767.83	78.52	T	0.0015
MAP [W/kg]	10.73	0.77	10.4	0.71	T	0.0130
P_{mean} [W]	668.24	49.57	629.66	45.85	T	0.0004
P_{mean} [W/kg]	8.89	0.64	8.54	0.49	T	0.0022
W_t [kJ]	13.36	0.99	12.59	0.91	T	0.0004
t_r [s]	5.67	0.63	5.46	1.06	T	0.3053
t_m [s]	3.69	(2.90; 4.42)	3.44	(2.51; 3.62)	W	0.0024

Abbreviations: $\bar{x}$—mean; SD—standard deviation; Me—median; Q1—first quartile; Q3—third quartile; T—Student's *t*-test for variables with parametric distribution; W—Wilcoxon rank test for variables with nonparametric distribution; *p*—statistical significance; MAP—maximum anaerobic power; P_{mean}—mean anaerobic power; W_t—total work performed; t_r—time of reaching maximum power; t_m—time of maintaining maximum power.

In the upper limb test, the changes in the analyzed indices were slightly different. Maximal anaerobic power, both globally and relatively, decreased but the observed changes were not statistically significant. Similarly, changes in the time to reach and maintain maximal power were also not statistically significant. In the upper limb test, the values of mean anaerobic power from the test and total work decreased in a statistically significant way [Table 7].

Table 7. Anaerobic performance indices of the upper limbs measured before and after a stay at high altitude > 3000 m.

Anaerobic Performance Indices (Arms)	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$/Me	SD/(Q1; Q3)	$\bar{x}$/Me	SD/(Q1; Q3)		
MAP [W]	556.64	79.45	536.67	90.53	T	0.1346
MAP [W/kg]	7.38	0.8	7.25	0.86	T	0.4472
P_{mean} [W]	439.51	44.65	414.32	47.45	T	0.0015
P_{mean} [W/kg]	6.02	(5.55; 6.15)	5.64	(5.41; 5.81)	W	0.0245
W_t [kJ]	8.79	0.89	8.29	0.95	T	0.0015
t_r [s]	5.56	(3.99; 8.46)	4.07	(3.46; 7.20)	W	0.0683
t_m [s]	3.95	(2.23; 5.02)	2.53	(2.16; 4.55)	W	0.1239

Abbreviations: $\bar{x}$—mean; SD—standard deviation; Me—median; Q1—first quartile; Q3—third quartile; T—Student's *t*-test for variables with parametric distribution; W—Wilcoxon rank test for variables with nonparametric distribution; *p*—statistical significance; MAP—maximum anaerobic power; P_{mean}—mean anaerobic power; W_t—total work performed; t_r—time of reaching maximum power; t_m—time of maintaining maximum power.

A prolonged stay in high-altitude conditions resulted in a statistically significant decrease in body weight and muscle mass. There was also a significant decrease in lean body mass in the upper and lower limbs, as well as the ALST index. Analysis of BMI, fat mass, lean mass, muscle mass [%], lean body mass and bone mineral content and bone mineral density showed no significant changes after chronic hypoxia [Table 8].

Table 8. Anthropometric indices measured before and after a stay at high altitude > 3000 m.

Anthropometric Indices	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$/Me	SD/(Q1; Q3)	$\bar{x}$/Me	SD/(Q1; Q3)		
BM [kg]	74.96	5.03	73.79	5.53	T	0.0149
BMI [kg/m^2]	22.83	2.10	22.43	1.43	T	0.1606
FM [kg]	9.64	(8.42; 12.62)	9.30	(7.99; 10.47)	W	0.1024

Table 8. *Cont.*

Anthropometric Indices	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$/Me	SD/(Q1; Q3)	$\bar{x}$/Me	SD/(Q1; Q3)		
FM [%]	12.80	(11.50; 17.60)	12.90	(11.60; 16.20)	W	0.1626
FFM [kg]	64.51	5.09	63.99	4.90	T	0.1330
FFM [%]	87.78	(83.05; 88.89)	87.66	(84.46; 88.99)	W	0.1488
MM [kg]	33.72	2.91	33.10	2.73	T	0.0071
MM [%]	44.97	2.05	44.88	2.15	T	0.7851
LBM [kg]	61.35	4.83	61.01	4.74	T	0.4437
LBM [%]	83.39	(79.00; 84.64)	83.43	(80.73; 84.69)	W	0.1626
LBM Arms [kg]	8.25	0.76	8.08	0.73	T	0.0224
LBM Legs [kg]	20.72	2.00	20.35	1.80	T	0.0391
ALST [kg]	28.98	2.58	28.43	2.43	T	0.0071
BMC [g]	3.16	0.33	3.16	0.33	T	0.7562
BMD [g/cm^2]	1.30	0.07	1.29	0.05	T	0.1054

Abbreviations: $\bar{x}$—mean; SD—standard deviation; Me—median; Q1—first quartile; Q3—third quartile; T—Student's *t*-test for variables with parametric distribution; W—Wilcoxon rank test for variables with nonparametric distribution; *p*—statistical significance; BM—body mass; BMI—body mass index; FM—fat mass; FFM—fat-free mass; MM—muscle mass; LBM—lean body mass; ALST—appendicular lean soft tissue; BMC—bone mineral content; BMD—bone mineral density.

The analysis of the hematological indices showed a significant increase in the number of leukocytes, erythrocytes and platelets and an increase in the values of hemoglobin and hematocrit, as well as neutrophils and monocytes Table 9. A significant decrease in total cholesterol, HDL cholesterol, total bilirubin, albumin and total protein was observed. There was a statistically significant increase in the levels of total acid phosphatase and inorganic phosphorus. All of the urine indicators remained within the reference values both before and after the expedition.

Table 9. Health status indices measured before and after a stay at high altitude > 3000 m.

Health Status Indices	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$/Me	SD/(Q1; Q3)	$\bar{x}$/Me	SD/(Q1; Q3)		
Blood counts						
Leukocytes [10^9/L]	5.65	1.05	6.68	2	T	0.0066
Erythrocytes [10^{12}/L]	5.18	0.31	5.43	0.36	T	0.0007
HGB [g/dL]	15.39	0.86	16.16	0.88	T	0.0004
HCT [%]	47.26	2.72	49.61	3.41	T	0.0025
MCV [fL]	91.34	2.84	91.53	2.8	T	0.7862
MCH [pg]	29.79	1.43	29.87	1.41	T	0.7134
MCHC [g/dL]	32.61	1.08	32.64	1.28	T	0.9402
RDW [%]	13.25	1.00	13.25	0.90	T	0.9986
Platelets [10^9/L]	203.8	(193.2; 256.8)	240	(222; 260)	W	0.0075
MPV [fL]	8.6	1.56	8.51	1.37	T	0.8161
NEU [%]	55.10	(46.10; 57.27)	57.16	(49.58; 61.15)	W	0.7583
NEU [10^9/L]	3	(2.75; 3.19)	3.62	(2.5; 5.5)	W	0.0442
LYMPH [%]	34.45	6.51	29.55	10.06	T	0.1687
LYMPH [10^9/L]	1.81	(1.61; 2.00)	2	(1.83; 2.15)	W	0.0929
MON [%]	8.18	1.79	8.13	2.92	T	0.9267
MON [10^9/L]	0.45	(0.40; 0.51)	0.54	(0.49; 0.71)	W	0.0064
EOS [%]	3.11	2.12	2.31	1.44	T	0.1393

Table 9. *Cont.*

Health Status Indices	Before Chronic Hypoxia		After Chronic Hypoxia		Statistical Test	*p*
	$\bar{x}$/Me	SD/(Q1; Q3)	$\bar{x}$/Me	SD/(Q1; Q3)		
EOS [10^9/L]	0.11	(0.08; 0.27)	0.15	(0.10; 0.24)	W	0.9382
BASO [%]	1.11	0.34	0.84	0.58	T	0.1018
BASO [10^9/L]	0.06	(0.05; 0.07)	0.06	(0.04; 0.08)	W	0.6592
ESR [mm/h]	2	(2; 2)	2	(2; 5)	W	0.0759
Electrolytes						
Sodium [mmol/L]	140.41	1.09	140.71	2.02	T	0.6071
Potassium [mmol/L]	4.33	0.16	4.42	0.24	T	0.1139
Chlorides [mmol/L]	102	(100; 104)	104	(102; 104)	W	0.2115
Calcium [mmol/L]	2.36	0.12	2.36	0.13	T	0.9379
Magnesium [mmol/L]	0.85	0.04	0.85	0.08	T	0.8599
Lipid profile						
TC [mmol/L]	4.49	0.79	4.13	0.77	T	0.0197
LDL [mmol/L]	2.59	0.66	2.37	0.6	T	0.0942
HDL [mmol/L]	1.56	0.25	1.38	0.3	T	0.0162
TG [mmol/L]	0.70	(0.57; 0.81)	0.62	(0.55; 0.99)	W	0.2659
Kidney parameters						
Creatinine [µmol/L]	78.83	9.8	75.20	10.48	T	0.1855
Urea [mmol/L]	5.23	0.7	4.85	1.2	T	0.3155
Uric acid [µmol/L]	308.17	57.15	327.12	62.90	T	0.1317
Urine pH	5.88	0.47	6.26	0.79	T	0.072
Urine specific gravity	1.02	(1.02; 1.03)	1.02	(1.01; 1.02)	W	0.125
Liver parameters						
AST [U/L]	23	4.42	20.06	4.49	T	0.0710
ALT [U/L]	21	(16; 23)	21	(16; 26)	W	0.3636
Total bilirubin [µmol/L]	19.81	7.98	11.66	5.41	T	0.0001
GGTP [U/L]	17	(16; 19)	19	(17; 20)	W	0.0501
Albumin [g/L]	47	(45;4; 49.3)	45.9	(43.5; 46.6)	W	0.0171
Total acid phosphatase [U/L]	3.6	(3; 4)	4.3	(4.0; 4.4)	W	0.0151
Another indices						
Total protein [g/L]	72.36	3.52	68.62	5.74	T	0.0029
Glucose [mmol/L]	5.01	(4.68; 5.24)	4.88	(4.72; 5.04)	W	0.2659
Inorganic phosphorus [mmol/L]	1.09	0.14	1.26	0.12	T	0.0003
Iron [µmol/L]	26.67	9.01	21.02	7.85	T	0.0567
Ferritin [ng/mL]	70.82	32.19	66.82	36.29	T	0.4492
Vitamin D (25(OH)D) total [ng/mL]	31.76	7.93	31.92	7.31	T	0.8941
Vitamin B12 [pg/mL]	402	(329; 449)	409	(337; 433)	W	0.4074

Abbreviations: $\bar{x}$—mean; SD—standard deviation; Me—median; Q1—first quartile; Q3—third quartile; T—Student's *t*-test for variables with parametric distribution; W—Wilcoxon rank test for variables with non-parametric distribution; *p*—statistical significance; HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RDW—red blood cell distribution; MPV—mean platelet volume; NEU—neutrophils; LYMPH—lymphocytes; MON—monocytes; EOS—eosinophils; BASO—basophils; ESR—erythrocyte sedimentation rate; TC—total cholesterol; LDL—low-density lipoprotein cholesterol; HDL—high-density lipoprotein cholesterol; TG—triglycerides; AST—aspartate aminotransferase; ALT—alanine aminotransferase; GGTP—gamma-glutamyl transpeptidase.

The median energy demand of the study participants was 3897.95 ± 521 kcal, and the energy supply was 2715.71 ± 794 kcal. The climbers were equipped with supplementation of 325 ± 220 kcal on average, which accounted for 11.99% of their energy supply. Generally,

only one person, i.e., 5.88% of the respondents, covered their needs in accordance with the standard. Most climbers (94.14%) met the carbohydrate requirement, and 58.82% met the protein requirement. All climbers met the requirements for fats, and 23.53% of them exceeded the normative values. The median energy and nutritional value of the diet based on analysis of the food diaries is shown in Table 10. Compared to the standards for athletes [53–56], mountaineers did not provide adequate amounts of potassium, calcium, magnesium, iron, zinc, selenium and iodine, as well as vitamins A, D, C, folate and vitamin K.

Table 10. Energy and nutritional value of the daily food ration of mountain climbers in relation to the recommendations for athletes [53–56].

	Diet of Climbers During the Expedition	Nutritional Recommendations for Athletes
	Me (Q1; Q3)	RDA/AI
	Energy and macronutrients intake	
Energy [kcal]	2700.8 (2023.6; 3036.9)	Daily energy expenditure
Protein [%]	13.8 (11.7; 15.5)	15–20 [1]
Protein [g]	90.2 (72.4; 105.4)	-
Plant protein [g]	48.90 (36.9; 51.6)	-
Animal protein [g]	33.3 (32.3; 54.4)	-
Protein [g/kg bw]	1.16 (1.01; 1.34)	1.2–2.2 [1]
Carbohydrates [%]	52.2 (49.8; 54.4)	45–65 [2]
Carbohydrates [g]	332.2 (268.2; 443.4)	-
Carbohydrates [g/kg bw]	4.37 (3.81; 5.91)	6–12 [3]
Simple carbohydrates [g]	102 (84.8; 136.3)	-
Simple carbohydrates [%]	16.77 (13.26; 23.33)	<20 [4]
Fiber [g]	26.7 (21.8; 33.2)	>25 [4]
Fats [%]	32.8 (27.9; 36)	20–35 [2,3,4]
Fats [g]	96.6 (78.6; 114.8)	-
Fats [g/kg bw]	1.23 (1.04; 1.56)	0.5–1.5 [1]
SFA [%]	11.56 (8.42; 14.4)	<10 [3]; ALAP [4]
SFA [g]	34.7 (28.3; 38.9)	ALAP [4]
MUFA [g]	23.2 (10.1; 32.6)	-
PUFA [g]	12.4 (10; 12.8)	ALA 0.5%, LA 4%
Cholesterol [mg]	225.2 (144; 271.5)	<300 [5]
	Micronutrients intake	
Sodium [mg] (AI)	2832 (2070; 2897.3)	1500–>10,000 [2]
Potassium [mg] (AI)	2039.7 (1358.1; 2715.4)	3500 [4]–4700 [2]
Calcium [mg]	445.3 (349.4; 611)	1000 [1]–2000 [3]
Phosphorus [mg]	866.2 (587.2; 901.9)	700 [1]–1500 [2]
Magnesium [mg]	278.8 (201.5; 398.7)	420 [1]
Iron [mg]	7.8 (4.8; 10.4)	11 [4]
Zinc [mg]	5.4 (4.8; 7.1)	11–15 [2]
Copper [mg]	1 (0.7; 1.7)	0.9 [2]–1.6 [4]
Selenium [µg]	5.1 (1.6; 12.2)	50–55 [2]
Iodine [µg]	22.8 (13.8; 39.1)	120–150 [2]
Vit. A [µg]	729.2 (473.1; 1570.8)	900 [1]
Vit. D [µg]	2.6 (1.2; 4.8)	15 [2,4]
Vit. E [mg]	14.2 (9.9; 17.8)	15 [1,2]
Vit. C [mg]	90.5 (74.5; 164.5)	110 [4]–200 [2]

Table 10. *Cont.*

	Diet of Climbers During the Expedition	Nutritional Recommendations for Athletes
Vit. B_1 [mg]	1.4 (1.2; 1.7)	1.2 [1]
Vit. B_2 [mg]	1.4 (0.8; 2.2)	1.3 [1,4]
Vit. B_3 [mg]	13.7 (5.1; 20.1)	14–20 [2]
Vit. B_6 [mg]	1.8 (1.2; 2.3)	1.5–2 [2]
Vit. B9 [µg]	325.4 (203.9; 477.1)	400 [1,2]
Vit. B_{12} [µg]	2.8 (1.6; 4.1)	2.4 [1]–4 [4]
Vit. K [µg]	4.2 (2.2; 14.4)	120 [1]–700 [2]

Abbreviations: [1]—[56]; [2]—[53]; [3]—[55]; [4]—[54]; [5]—[57]; Me—median; Q1—first quartile; Q3—third quartile; RDA—recommended dietary allowance; AI—adequate intake; ALA—α-linolenic acid; ALAP—as low as possible; bw—body weight; LA—linoleic acid; MUFA—monounsaturated fatty acid; PUFA—polyunsaturated fatty acid; SFA—saturated fatty acids.

Table 11 shows the percentages of climbers whose results of nutritional indices of health status after chronic hypoxia were in accordance with laboratory standards and which were realizing the requirements for selected macro- and micronutrient components of the diet. Most climbers had test results in line with the laboratory standard. The exception is the climbers' vitamin D levels, which were too low in 52.94% of those tested. Most climbers covered their macronutrient requirements with diet and supplementation. A small percentage of the subjects (<50%) covered the needs for iron, folate, potassium, calcium, vitamin D, vitamin C and vitamin A. If there was an inadequate supply of iron, folate and vitamin D, this was reflected in the blood test results. None of the climbers surveyed provided adequate calcium and vitamin K relative to standards.

Table 11. Percentage of climbers whose selected test results of nutritional indices of health status after chronic hypoxia were in accordance with laboratory standards and percentage of climbers realizing the requirements for selected macro- and micronutrient components of the diet.

Nutritional Indices of Health Status After Chronic Hypoxia	Percentage of Climbers Whose Test Results of Health Indicators Were Consistent with Laboratory Standards [%]	Macro- and Micronutrients of the Diet	Percentage of Climbers Realizing the Requirement for a Given Dietary Nutrient [%]
Erythrocytes [10^{12}/L]	100		
HGB [g/dL]	100		
HCT [%]	76.47		
MCV [fL]	94.12	Iron [mg]	29.41
MCH [pg]	94.12	Vitamin B9 [µg]	35.29
MCHC [g/dL]	70.59	Vitamin B12 [µg]	58.82
Iron [µmol/L]	94.12		
Ferritin [ng/mL]	76.47		
Vitamin B12 [pg/mL]	94.12		
Sodium [mmol/L]	100	Sodium [mg]	100
Potassium [mmol/L]	100	Potassium [mg]	23.53
Calcium [mmol/L]	82.35	Calcium [mg]	0
Magnesium [mmol/L]	100	Magnesium [mg]	11.76
Vitamin D (25(OH)D) total [ng/mL]	52.94	Vitamin D [µg]	17.65

Table 11. *Cont.*

Nutritional Indices of Health Status After Chronic Hypoxia	Percentage of Climbers Whose Test Results of Health Indicators Were Consistent with Laboratory Standards [%]	Macro- and Micronutrients of the Diet	Percentage of Climbers Realizing the Requirement for a Given Dietary Nutrient [%]
TC [mmol/L]	82.35	Cholesterol [mg]	82.35
LDL [mmol/L]	82.35	Fibre [g]	58.82
HDL [mmol/L]	88.24	Simple carbohydrates [%]	70.59
TG [mmol/L]	88.24	Vitamin C [mg]	35.29
Glucose [mmol/L]	100	Vitamin E [mg]	52.94
		Vitamin A [µg]	41.18

Abbreviations: HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; TC—total cholesterol; LDL—low-density lipoprotein cholesterol; HDL—high-density lipoprotein cholesterol; TG—triglycerides.

4. Discussion

4.1. Main Findings

In our study, a decrease in aerobic capacity in climbers was observed, expressed as a statistically significant decrease in maximal oxygen uptake ($\dot{V}O_{2max}$), maximum minute pulmonary ventilation ($\dot{V}E_{max}$), a decrease in the duration of the performance test (t_{max}) and a decrease in the value of the maximal heart rate (HR_{max}). Similar results were observed by Hoppeler et al. [8], describing a 5% decrease in aerobic capacity expressed as $\dot{V}O_{2max}$ in subjects after a minimum of 6 weeks of exposure above 5200 m. A study by Szymczak et al. [23] showed no significant changes in aerobic capacity parameters in alpinists after 4 weeks of being in hypoxia at an average altitude of 4900 m. In our study, there were no significant changes in the indices characterizing the level of second ventilatory threshold. Changes in oxygen uptake at the threshold, minute pulmonary ventilation and time of threshold onset were small and not statistically significant. Different results were obtained in their study by Szymczak et al. [23], who reported a significant decrease in anaerobic threshold and heart rate levels after a month of staying at an average altitude of 4900 m; similarly, in our study, a significant decrease in the value of heart rate at the level of the second ventilation threshold was observed. An interesting observation was made in a study by other authors [40], showing that exercise capacity and aerobic capacity are impaired under conditions of simulated hypoxia, with $\dot{V}O_{2max}$ decreasing linearly by 6% for every 1000 m of elevation (from 300 to 2800 m). In our study, $\dot{V}O_{2max}$ levels were determined before and after the expeditions, and a 3.95% decrease was noted.

The level of indices characterizing the anaerobic capacity of the subjects generally decreased after returning from the expedition. Such changes are confirmed by studies of other authors [23], who indicate a decrease in total anaerobic work and maximum anaerobic power after 1 month at altitudes above 3500 m (average 4900 m) [23]. Doria et al. [58] observed a decrease in the mechanical and metabolic indices of the Wingate test after a 43-day expedition to the Himalayas (23 days above 5000 m). Grassi and co-workers observed that, after 10 days of acclimatization to 5050 m, muscle power output and blood lactate accumulation were unaffected by chronic hypoxia during short supra-maximal exercise [59,60]. Other studies have shown decreases in lower limb peak power at altitude and after return from high altitude when compared to before departure [61,62]. In our study, there was a statistically significant decrease in MAP (4.91%), average power (5.77%) and total work (5.77%) performed in the test. The change in the time to reach MAP was not statistically significant, while the time to maintain maximum power significantly decreased (6.98%). In the upper limb power tests, the only significant changes were noted in the values of mean anaerobic power and total work, which decreased by 5.73% and 5.74%, respectively.

Kayser et al. [63] measured the upper and lower limb maximum alactic anaerobic power of healthy Caucasians both at sea level and during a one month stay at 5050 m. Anaerobic alactic performance was unaltered by a sojourn at very high altitude in this study [63].

In the study, the participants were exposed to adverse environmental conditions while staying at high altitudes (>3000 m) for relatively long periods (>3 weeks). The average climbing speed corresponded to an exercise intensity of 50% $\dot{V}O_{2max}$ [64]. This type of effort and the type of mountain activities performed may explain the reduction in muscle strength during the Wingate test [65]. The muscle fibers of the vastus lateralis likely changed from fast-twitch (type 2) to slow-twitch (type 1) fibers [65]. In general, type 1 fibers have lower mechanical power than type 2 fibers, which is the reason behind the reduced ability of leg muscles to perform extremely powerful movements that require mainly anaerobic sources [66]. A reduction in the number of fast-twitch fibers rebuilt at altitude may result in a decrease in anaerobic capacity at sea level, which we observed in climbers returning from high-altitude expeditions. The lower strength of contraction after staying at high altitude would explain the reduced maximal anaerobic power and total anaerobic work observed in the Wingate test in our study. Additionally, the decrease in muscle mass observed in our study may have led to weaker muscles and reduced muscle function [67] and power [61].

In our own study, there was a statistically significant decrease in body mass (1.56%) and muscle mass (1.84%) in a group of alpinists staying a minimum of 3 weeks at altitudes above 3000 m. These results are consistent with a study by Hoppeler et al. [8], in which the researchers noted a 5% decrease in size of body mass in climbers after a minimum 6-week stay at altitudes > 5200 m. A significant reduction in muscle fiber size (−20%) and loss of muscle oxidative capacity (−25%) were observed. Oxygen supply to muscle mitochondria was found to improve after exposure to high altitude. Body mass loss has been considered to be due to both the loss of muscle mass and loss of total body fat [8]. Hypoxia occurring at high altitudes (>5500 m) leads to a loss of lean body mass of up to 15% [68], with consequent muscle weakness and reduced muscle function [67] and power [61]. Also, Bondi et al. [69] showed a reduction in muscle mass in participants trekking in the Himalayas at an altitude of 1070–5143 m over 19 days. A study by Szymczak et al. [23] showed no significant changes in lean body mass in alpinists after 4 weeks of being in hypoxia at an average altitude of 4900 m. The dose of hypoxia experienced by the mountaineers was likely insufficient to cause significant deterioration of their muscle structure, which, in turn, may explain the slight changes in their aerobic indices [23]. In our study, there was a significant decrease in muscle mass (MM), which may have affected the magnitude of maximal minute oxygen uptake. Other data from the literature also showed no significant loss of muscle mass [70,71]. Differences in hypoxic doses may explain this discrepancy. It has been suggested that a minimum hypoxic exposure of 5000 km hours (km·h) [72] is required for the development of hypoxia-induced muscle atrophy [70]. Given the statistically significant loss of muscle mass in climbers, it can be supposed that the hypoxic dose was sufficient to induce the described change. This appears to be largely dependent on the duration of exposure to hypoxia, with the greatest body mass loss observed during the first weeks at altitude [73,74]. The factors responsible for changes in body composition mainly include increased basal metabolism and negative energy balance (i.e., a mismatch between energy intake and energy expenditure, often increased due to intensive hiking or alpine climbing) [75,76]. In addition to increased basal metabolism, at least at altitudes above 5000 m, a negative energy balance may result from decreased energy intake due to reduced appetite [77] or, in part, from impaired gut function [33]. Meta-analysis of data on body composition and body mass changes at different altitudes revealed that diet (21%) and duration of altitude exposure (20%) determined most of the heterogeneity in weight changes between studies [12]. The studies that revealed the largest cumulative average weight reductions at high altitude

ranged from 22 to 42 days [12]. In our study, as many as 94.12% of the subjects did not meet the energy supply requirement with diet and supplementation.

Our study showed a statistically non-significant decrease in fat mass (from 14.51% to 13.82%) in climbers after a 3-week stay at altitudes above 3000 m. These data are consistent with the results of Kasprzak et al. [78], who conducted a study with men participating in an expedition in the Alps at an altitude of 3200–3616 m for 16 days. The observed decrease in body fat in the study above (from 16.54% to 16.28%) was not statistically significant. Similar results have also been described in other studies [79,80]. On the other hand, the data obtained in our own study are inconsistent with the results of Szymczak et al. [23], who showed a significant reduction in fat mass from 14% of body weight before the expeditions to 11.5% after 1 month at altitudes above 3500 m (average 4900 m). Other studies have also shown a decrease in body fat after a sojourn at high altitudes [12,34,81,82]. According to the results of a meta-analysis of data on body composition and body mass changes at different altitudes, it is diet and type of exposure (active/passive) that explain 25% and 30% of the heterogeneity in fat mass changes between studies, respectively [12]. The statistically insignificant decrease in body fat observed in our study may have been due to the negative caloric balance observed in climbers.

The analysis of bone mineral content and bone mineral density showed no significant changes after a period of chronic hypoxia in our study. The analysis was based on dual energy X-ray absorptiometry (DXA) measurements. Other studies to assess bone density using the method above were conducted using laboratory animals (mice and rats) [83,84]. Further studies involving humans would need to be performed to compare the results. A study involving mountaineers showed that exposure to a very high altitude (>3700 m) reduced bone mineral density and that the deleterious effects of hypobaric hypoxia on the skeleton were not completely eliminated after 12 months [85].

HIF-induced genetic reprogramming includes, among other things, increased erythropoiesis [86]. In our study, we reported a significant increase in erythrocyte count (4.78%) and an increase in hemoglobin (5%) and hematocrit values (4.98%) in climbers staying above 3000 m for a minimum of 3 weeks. The results obtained are consistent with the study of Reynafarje et al. [87], whose results show a 10% increase in red blood cells after 4 weeks at 4500 m. Similarly, Ferretti et al. [88] noted that staying at altitudes above 5200 m for a minimum of 6 weeks resulted in a significant increase in hemoglobin of 14.1% and hematocrit of 12.2%. Also, the results of Kurdziel et al. show a significant increase in red blood cell, hematocrit and hemoglobin levels in climbers after staying at altitudes above 4900 m for 36 to 44 days [89]. The results of a meta-analysis by Ramussen et al. indicate that, at altitudes above 4000 m, exposure time must exceed 2 weeks to produce a significant effect, and the magnitude of the erythropoietic response depends on the initial volume of red blood cells [90]. In addition, hemoglobin mass returns to baseline values at sea level within 2–3 weeks after descending to sea level [91].

Iron requirements are increased at high altitudes due to enhanced erythropoiesis [92]. Adequate iron stores are necessary to sustain the hypoxia-induced increase in heme synthesis and iron-dependent enzyme production during prolonged exposure at altitude [93]. Our study showed a statistically insignificant decrease in iron and ferritin levels. Kasprzak et al. [78] noted a significant decrease in iron levels in climbers staying at altitudes of 3200–3616 m for 2 weeks. In turn, Kurdziel et al. [89] showed a significant increase in iron and ferritin levels after staying above 4900 m for 36 to 44 days. The researchers also observed significantly reduced hemoglobin content in red blood cells, indicating functional iron deficiency among climbers participating in expeditions to K2 and Broad Peak. This change may be a result of prolonged exposure to a hypoxic environment [89]. The results of our own study indicate a non-significant increase in the hemoglobin content of red

blood cells, and these data are inconsistent with the results of the above studies. This may be due to the shorter duration of the expedition and the lower altitudes at which the mountaineers stayed, compared to the results of the Kurdziel et al. study [89]. The iron supply with diet and supplementation in 29.41% of the climbers surveyed in our study was not in accordance with recommendations. The blood iron concentration in 94.12% of climbers after the expedition was normal, while ferritin levels were not adequate in 23.53% of subjects. Due to an insufficient supply of iron and folic acid and a low percentage of people covering their vitamin B12 requirements (58.82%) with diet and supplementation, there is a risk of iron deficiency during or after returning from a high-mountain expedition. Iron deficiency is associated with reduced physical performance in athletes [94], so it is important to monitor iron and ferritin levels after returning from expeditions.

Monocytes, neutrophils and tissue macrophages are responsible for the phagocytosis processes of pathogens [95]. In our study, we observed a statistically significant increase in monocyte (20%) and neutrophil levels (20.67%) after high-altitude expeditions. Similar results were obtained in a study by Pham et al. [96], who showed that the total frequency of monocytes as a percentage of the total number of white blood cells was significantly elevated on the first day of acute exposure at very high altitude (after a night of arrival at 3800 m), compared to values at sea level. However, these studies differed significantly in the time of exposure to altitude. Long-term hypoxia led to adaptive changes in the immune system, including increased numbers of monocytes and neutrophils, which can be interpreted as a response to altitude stress and hypoxia. A recent study showed that exposure to high altitudes with pre-acclimation induces the expression of genes involved in neutrophil activation. This finding indicates that neutrophil activation contributes to an important biological function that promotes human acclimatization at high altitudes [97].

A statistically significant increase in platelet count (17.76%) was observed in our study. These results are consistent with those obtained by Rocke et al. in a study involving 63 healthy lowland volunteers acclimatizing to very high altitude (5200 m) [98] and with those of Hudson et al. who showed a significant and sustained increase in platelet count in 26 healthy individuals 48 h and one week after ascending to very high altitude (3600 m) [99]. Possible explanations for the increase in platelet count that potentially need to be considered include increased physical exertion while at altitude, medication or a change in diet, all of which can interfere with platelet production and function [100]. In contrast, other researchers have observed a decrease in platelet counts after exposure to altitudes of 3200 m and 3771 m [101]. The results of a data meta-analysis by Wang et al. [102] showed that chronic high-altitude hypoxia ($\geq$1 month) results in a significant decrease in platelet count, while acute high-altitude exposure has no significant effect on platelets.

In our study, there was a significant decrease in the serum albumin concentration (0.88%) in climbers after returning from expeditions. These results are consistent with those of Kurdziel et al. [89], who studied climbers participating in expeditions to K2 and Broad Peak. The Smolichev study also confirms the above results [103]. In this study, the relative and absolute content of serum albumin decreased immediately after the ascent and remained low for a month after the descent from 4200 m to 850 m [103]. The body responds to each extreme and abnormal stimulus with a decrease in serum albumin and an increase in serum globulin. The transition from normal atmospheric pressure to conditions of reduced atmospheric pressure and back again is considered a stress, which causes an internal functional reorganization of the entire physiological system of the organism [103]. Apparently, albumin synthesis is impaired under these conditions. Inflammatory conditions, particularly high concentrations of the cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF-alpha), reduce serum albumin levels [104]. The results of a study conducted by Hartmann et al. [100] indicate that circulating IL-6, IL-1ra and CRP

are up-regulated in response to hypobaric hypoxic conditions at high altitude, and moderate increases in these inflammatory markers may reflect significant local inflammation. Our own study showed a statistically significant reduction in total blood protein concentration (5.17%) after 26 ± 6 days above 3000 m, so these results are inconsistent with those of the study described above. The results of the study [103] indicate that, during the first month of the mountaineers' stay in the Pamir, the protein concentration increased significantly. After four months at an altitude of 4200 m, the concentration of protein in the blood decreased slightly, but remained above the initial level. In the first month after the descent, serum protein concentrations remained high [103]. The levels of acid phosphatase and organic phosphorus, determined in our study, increased significantly, while bilirubin levels decreased (41.15%) after 26 ± 6 days above 3000 m. However, all of the aforementioned indicators remained within reference values both before and after the expedition. The results of the study by Liu et al. [41], on the other hand, indicate an increase in bilirubin levels in subjects subjected to acute exposure from an altitude of 200 m to an altitude of 3648 m (36 h of train travel).

Our study showed a statistically significant reduction in total cholesterol (8.04%) and HDL cholesterol (11.49%), as well as a decrease in LDL cholesterol, but the change was not substantial. Verratti et al. [105] in their study involving 12 climbers who spent 6 days at 900–5895 m observed similar changes, namely, a significant decrease in total cholesterol and LDL levels, while HDL changes were not considerable. Ferezou et al. [106] studied the effects of a high-altitude sojourn on plasma lipids in subjects who spent 3 weeks at base camp below Annapurna IV (4800 m) after 12 days of hiking. The researchers observed a marked and sustained reduction in plasma cholesterol and phospholipids. The low plasma cholesterol levels at high altitude were mainly due to a reduction in low-density lipoprotein (C-LDL) cholesterol [106]. The effects described above likely depend on both altitude hypoxia and physical activity as consequences of hypoxia- and exercise-related adaptations [107]. In our study, the levels of total cholesterol, HDL, LDL fractions and triglycerides in most of the subjects were in accordance with the laboratory standard. This is confirmed by the supply of cholesterol levels with the diet, which was adequate in 82.35% of the subjects. The lipid profile may also have been influenced by the supply of fiber, as well as SFAs, PUFAs and MUFAs. The median fiber supply in the self-reported study was 26.7 g, which is in line with the standards for this nutrient, while the requirement for this nutrient was met by only 58.82% of the subjects. Dietary fiber reduces the absorption of gastric acid molecules, and this is the main mechanism of fiber's blood cholesterol-lowering effect [108]. The median supply of saturated fatty acids was 11.56%. Current recommendations from The European Food Safety Authority suggest that the supply of SFAs should be as low as possible [54]. In contrast, the position statement of the Academy of Nutrition and Dietetics, Dietitians of Canada and the American College of Sports Medicine [55] suggests limiting SFA supply to <10% of energy requirements. However, the supply of monounsaturated and polyunsaturated fatty acids in the mountaineers' diets slightly exceeded the supply of saturated fatty acids, which may have affected lipid indices. When replacing 1% of energy from SFAs with PUFAs, MUFAs, complex carbohydrates and/or plant protein, a 4% to 8% reduction in cardiovascular disease (CVD) risk has been reported [109]. The most important thing for reducing LDL cholesterol in the blood is not to reduce dietary cholesterol dietary cholesterol, but to replace SFAs (animal fats) with vegetable fats. Kim et al. [110], in their study, estimated that the risk of death from cancer increased by 4% for every 5% increase in energy from SFAs. Therefore, in the diet of healthy adults, the intake of saturated fatty acids should be limited in cancer prevention [111]. The median supply of simple carbohydrates with the climbers' diet and supplementation was 16.77% of the diet's energy value, and in

70.59% of them, the supply was within the norm. All of the subjects had normal fasting glucose results after returning from the expedition.

Our study showed a statistically significant increase in leukocyte levels (18.14%) and a slight increase in vitamin D levels, but these changes were not statistically significant. These data are inconsistent with the results of Kasprzak et al. [78] who observed a significant decrease in 25(OH)D concentrations and a statistically insignificant decrease in leukocyte levels in men after they returned from an expedition to the Alps, during which they stayed at altitudes of 3200–3616 m. In our study, an adequate blood concentration of vitamin D was observed in a small percentage of climbers (52.94%). The supply of this vitamin with the diet was too low (median intake: 2.6 µg), and the percentage of subjects covering the requirement according to standards was only 17.65%. Given how important a role vitamin D plays in the context of the immune system and the fact that its deficiency is associated with a higher risk of anemia [24,112,113], it is important to monitor the concentration of this vitamin before the expedition, adjust the appropriate dose to the current serum concentration and continue supplementation during the expedition [46].

Compared to standards for athletes [50–53], mountaineers did not provide adequate amounts of potassium, calcium, magnesium, iron, zinc, selenium and iodine, as well as vitamin A, D, C, folate and vitamin K. Moreover, a small percentage of the subjects (<50%) met their needs for iron, folic acid, potassium, calcium, vitamin D, vitamin C and vitamin A, and none of the participating climbers provided adequate amounts of calcium and vitamin K relative to standards. While staying in high-mountain conditions, alpinists should consider including the necessary supplementation of deficient components, especially iron, calcium, potassium, magnesium, zinc, selenium, iodine, B vitamins, as well as vitamin D, C, A, E and K. Appropriate supplementation should be determined individually with specialists—a physician and a nutritionist.

4.2. Limitations and Strengths

Our study is one of the few to analyze aerobic and anaerobic capacity, body weight and composition and the hematological and biochemical parameters of climbers' blood and urine before and after a minimum 3-week stay at altitudes above 3000 m. Our results show how prolonged exposure to hypoxia affects physical performance at sea level. These data can be used by many climbers and alpine tourists planning their expeditions at altitudes above 3000 m.

Limitations of the study include the fact that the climbers' fitness was tested on average on the third or fourth day after their return from the expedition, as they had to travel from another country and distant mountains to the corresponding laboratory in Poland. This makes it impossible to compare the results with other studies, which differ in the number of days between the end of exposure to hypoxia and when the sea level measurements were taken. The climbers did not constitute a single group, led by a single climbing goal in the territory of a single country and mountain range. Climbers, characterized by the characteristics described in the study, constitute a small but elite group in Poland, hence the several distinct expedition groups, climbing at similar altitudes and with similar sporting goals, but in different parts of the world that qualified for the study. Collecting and encouraging participants was one of the most challenging stages of the ongoing project.

In addition to the level of hypoxia, other factors, such as nutrition and the type of physical activity performed in the mountains, as well as the number of active days versus the number of rest days, may contribute significantly to the observed changes in body composition. Climbing groups active in Peru could have used the downtime between several days of mountain activity to descend to a town at the foot of the mountains to compensate for the negative energy balance there, while expedition groups in Kyrgyzstan,

Nepal or Pakistan were dependent on the food they took into the mountains or that the expedition agency offered them. The dose of hypoxia was not measured in our study, but, given the statistically significant loss of muscle mass in climbers, it can be assumed that the hypoxic dose was sufficient to induce the described change. It would be worthwhile to measure this indicator in future studies to be able to clearly assess the value of hypoxia that correlates with the decrease in muscle mass in test subjects.

In the future, it would also be advantageous to improve the conduct of dietary analysis. It would also be worthwhile for a nutritionist to participate in the expedition, so that he or she is in constant contact with the cooks responsible for preparing the meals and has control over the amount of food consumed.

5. Conclusions

More than three weeks of hypoxic exposure (>3000 m) had adverse effects on aerobic capacity, lower limb anaerobic capacity and some indices of upper limb anaerobic capacity of the climbers, despite the observed erythropoietic effect. Being in high altitude conditions did not adversely affect the alpinists' health other than by lowering HDL cholesterol levels. Adverse changes in the body composition of climbers may have worsened physical performance, so in view of the negative environmental factors observed in high altitude conditions and increased metabolism, emphasis should be placed on optimizing energy supply and tailored supplementation to reduce the described effects. To improve the nutritional status of the body both during the expedition and upon return, alpinists should consider necessary supplementation of deficient components. Appropriate supplementation should be determined individually with specialists—a physician and a nutritionist.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Bioethics Committee of the Cracow Regional Medical Chamber (protocol code 67/KBL/OIL/2021 and date of approval: 19 March 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ALST	appendicular lean soft tissue
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BASO	basophils
BM	body mass

BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
bw	body weight
EOS	eosinophils
ESR	erythrocyte sedimentation rate
FFM	fat free mass
FM	fat mass
GGTP	gamma-glutamyl transpeptidase
HCT	hematocrit
HDL	high-density lipoprotein cholesterol
HGB	hemoglobin
HR	heart rate
HR_{max}	maximum heart rate
LBM	lean body mass
LDL	low-density lipoprotein cholesterol
LYMPH	lymphocytes
MAP	maximum anaerobic power
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MM	muscle mass
MON	monocytes
MPV	mean platelet volume
NEU	neutrophils
p	statistical significance
P_{mean}	mean anaerobic power
RDW	red blood cell distribution
SD	standard deviation
T	Student's *t*-test for variables with parametric distribution
TC	total cholesterol
t_m	time of maintaining maximum power
t_{max}	effort duration
tr	time of reaching maximum power
$\dot{V}E$	pulmonary ventilation
$\dot{V}E_{max}$	maximum minute pulmonary ventilation
$\dot{V}O_{2max}$	maximum oxygen uptake
VT2	second ventilatory threshold
W	Wilcoxon rank test for variables with nonparametric distribution
W_t	total work performed
$\bar{x}$	mean

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Publikacja 4 „Selected aspects of the habitual diet and levels of intestinal permeability markers of Polish alpinists”

Original Article

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Selected aspects of the habitual diet and levels of intestinal permeability markers of Polish alpinists

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Authors' Contribution: A – Study Design, B – Data Collection, C – Statistical Analysis, D – Manuscript Preparation, E – Funds Collection

Abstract: The purpose of this study is to investigate selected aspects of habitual diet and levels of intestinal permeability markers in a group of Polish mountaineers. The study included 17 men aged 30.29 ± 5.8 years characterized by long climbing experience (10 ± 5 years) in sport climbing and mountaineering. The study included a quantitative analysis of the alpinists' diet and the determination of zonulin and alpha-1-antitrypsin levels in fecal samples using the ELISA technique. Less than half of the group covered their energy needs in accordance with the recommendations. Most climbers (70.59%) met the carbohydrate requirement, however, the fiber supply was not provided at an adequate level in 23.53% of individuals. All climbers met the requirements for protein and fats. More than one-fifth (23.53%) of them exceeded the normative values for fats, while 5.88% for protein. Mountaineers did not provide adequate amounts of selenium and iodine, as well as vitamins D and K. Fecal zonulin values were elevated in 35.29% of the climbers tested, while fecal alpha-1-antitrypsin values were normal in all subjects. Individuals engaged in climbing and alpine activities are at risk of energy restriction and/or low energy availability. The climbers mostly provided adequate amounts of macro- and micronutrients with their diets. In most of the climbers studied, intestinal permeability was adequate, although elevated zonulin values were observed in some individuals with normal AAT concentrations. These preliminary findings indicate the need for nutritional education and further studies in larger cohorts.

Keywords: intestinal permeability, zonulin, alpha-1-antitrypsin, alpinists, diet, climbing

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INTRODUCTION

Diet is a key component of lifestyle influencing both gut microbiota composition and intestinal permeability. Dietary factors and exposure to psychological stress are among the causes of intestinal disorders [1]. Dietary patterns rich in fiber, polyphenols, and omega-3 fatty acids tend to support barrier integrity by fostering beneficial microbial populations and anti-inflammatory pathways. In contrast, diets high in saturated fats, refined sugars, and food additives have been associated with increased gut permeability, microbial imbalance, and endotoxemia [2]. The habitual diet, therefore, plays a central role in shaping both the microbial environment and the function of the intestinal barrier.

The gut microbiota exerts a critical influence on the preservation of human health through its multifaceted interactions with host physiological processes. This complex microbial ecosystem contributes to essential functions such as nutrient metabolism, modulation of the immune system, and maintenance of intestinal barrier integrity [3]. Alterations in the composition and metabolic activity of the gut microbiota have been associated with changes in nutrient absorption, energy metabolism, inflammatory responses, and recovery - all of which are fundamental to achieving optimal athletic performance and overall well-being [4].

The intestinal epithelium acts as a selective barrier, permitting nutrient absorption while preventing penetration of pathogens and harmful components of the external environment [5]. Disturbances in microbial diversity and function, collectively referred to as dysbiosis, have been implicated in a wide range of health disorders, including gastrointestinal diseases, metabolic syndrome, and systemic inflammation [6]. One of the critical consequences of dysbiosis is an increase in intestinal permeability (IP), increasing the risk of leaky gut syndrome, stimulates the immune system, increasing the risk of developing inflammation and allergic and autoimmune diseases [5]. Intestinal permeability is assessed using zonulin and alpha-1-antitrypsin (AAT), among other tests. Zonulin is a physiological modulator of tight intercellular junctions in the gut, reversibly regulating intestinal barrier permeability [7]. AAT is a protease inhibitor that would be increased during the disruption of the mucosal barrier and increased permeability [8].

In athletes, particularly those exposed to extreme environmental or physiological stress, such as endurance athletes or alpinists, gastrointestinal function is of particular interest. Intense physical activity, heat stress, hypoxia, and energy deficits have all been linked to impaired gut barrier function and elevated intestinal permeability markers [9, 10]. Increased gut permeability in athletes has been associated not only with gastrointestinal symptoms but also with systemic inflammation and impaired recovery, potentially affecting training adaptations and performance outcomes [11]. Despite growing recognition of these associations, data on intestinal permeability markers and their correlation with nutrient intake from the habitual diet among Polish alpinists remain limited.

The purpose of this study is to investigate selected aspects of the habitual diet and the levels of intestinal permeability markers - zonulin and alpha-1-antitrypsin-in a group of Polish mountaineers. The following research hypotheses were put forward: (1) Polish alpinists are at risk of low energy availability, even though their macronutrient intake is within range and (2) intestinal permeability is increased in alpinists. Nutritional knowledge empowers athletes to meet their energy and nutrients requirements [12]. Understanding the interaction between diet and intestinal barrier integrity in this unique population of athletes may offer new insights into optimizing dietary habits and overall health to enhance performance and well-being at high altitudes.

MATERIAL AND METHODS

Study Participants

The study group consisted of 17 male mountaineers from Poland between the ages of 23 and 40 (mean 30.29±5.8 years) with a body weight of 74.96±5.03 kg and a height of 180.47±8.36 cm. A body mass index (BMI) was calculated from the height (cm) and weight (kg) data and was 22.83±2.1 kg/m^2. The study participants took part in mountain expeditions at least once a year, during which they stayed at an altitude of over 3,000 meters for at least three weeks. Individuals qualified for the study were members of high-mountain clubs affiliated with the Polish Mountaineering Association.

The alpinists participating in the study were characterized by long climbing experience (10 ± 5 years) in sport climbing and mountaineering. The average climbing level of participants, as measured by the IRCRA (The International Rock Climbing Research Association) scale, was 20.69 ± 2.8, which according to the proposed criteria, places them in the advanced (level 3) category [13]. The climbers spent an average of 8 ± 3 hours per week training.

Before the start of the study, all participants underwent a consultation with a sports medicine physician and were deemed eligible to participate based on the results of the previous standard medical tests, including ECG, and blood and urine analyses. The presence of chronic diseases and age over 45 were disqualifying factors for participation in the project. The Bioethics Committee of the Cracow Regional Medical Chamber approved the research project (67/KBL/OIL/2021), which was conducted in accordance with the Declaration of Helsinki. After learning about the risks and benefits of participating in the study, all climbers gave written informed consent to participate in this study.

Study Design

Nutritional Analysis of Diet

The supply of selected macronutrients was determined by analyzing whole-day rations obtained using the 3-day food diary method. Mountaineers were asked to note all food, meals and supplements consumed and fluids drunk during a standard 3-day period in the 2 weeks prior to the expedition (2 days of sports activity, 1 day of rest). Sports activity days included climbing in a climbing wall, rock or mountain climbing, running and weight training. Climbers were given detailed instructions on how to keep food diaries before leaving for the expedition in order to minimize recording errors. The diaries also included market foods, so the data needed for analysis was read from labels and nutritional tables. For food products such as bars, energy gels and isotonic drinks, among others, climbers were asked to provide the product name, manufacturer and weight of the product.

Data from the diaries were meticulously entered into the Aliant Diet Calculator program (Anmarsoft Marcin Olech, Gdańsk, Poland; version: 85; database: 6.2) to perform a quantitative analysis of the climbers' habitual diet. The program used the "Tables of food composition and nutritional value" [14] as a database, taking into account food recipes and losses due to product processing.

Measurements of energy expenditure were made by the researchers using a monitor recording heart rate Polar M430 (Polar-Electro, Kempele, Finland), which was recorded 24/7 for 3 days (2 days of sports activity, 1 day of rest). Pulsometers consisted of watches (receiver) and straps with transmitters Polar H10 sensor (Polar-Electro, Kempele, Finland), worn on the chest at the level of the gladius process in the anterior midline. The watches were placed on the hand so that they were adjacent to the body just below the wrist bone. Before the measurement began, the required data were entered into

the device for each athlete: gender, age, body weight and height. Before the start of physical activity, the subjects were instructed to put on the transmitter strap, start the physical activity recording option on the device (climbing indoors, outdoors, running oraz gym), and when the physical activity was over, to turn off the recording by pressing the corresponding button on the watch. All climbers were advised not to remove the devices from their wrists for the duration of the energy expenditure recording being carried out (sleep, daytime activity, physical activity). Energy expenditure was recorded in the Polar Flow app.

Body height of participants was measured using a Seca 217 anthropometer (Seca GmbH & Co., KG, Hamburg, Germany) with an accuracy of 1 mm. The climbers' body weight was determined using InBody 120 body composition analyzer (Inbody Bldg., Seoul, Republic of Korea) in the morning after a standardized meal between 7:45 and 8:30 am.

Intestinal permeability analysis

In order to determine the levels of intestinal permeability markers (alpha-1-antitrypsin and zonulin), a stool sample was collected from the athletes. The athletes were informed about the correct collection of material (faeces) from 3 different locations to standardise the samples. The material was placed in sterile containers and stored in a cool place until delivery to the ALAB laboratory. The correct reference range for the intestinal permeability markers analysed is, for alpha-1-antitrypsin below 27.5 ng/ml and for zonulin below 60 ng/ml. The tests were performed using the ELISA technique and commissioned as an external service by ALAB Laboratories (Warsaw, Poland).

Statistical analysis

The Shapiro-Wilk test was performed indicating non-normality of the distribution. Basic statistical measures in the form median (Me) and quartiles (Q1, Q3) were used to characterize the received results. Correlations between quantitative variables were analyzed using the Spearman-rho correlation coefficient. The significance level was taken as $p \leq 0.05$. The statistical analysis package JASP (University of Amsterdam, Amsterdam, The Netherlands; version: 0.19.3) was used for statistical analyses.

A binary classification approach was used to assess the implementation of nutritional standards. For each nutrient, a value of 1 was assigned if the subject's intake of the nutrient met the recommended standard, and 0 if it did not. Then, based on these data, the percentage of subjects who met the standards for each nutrient was calculated. The analysis was carried out using an Excel spreadsheet (Microsoft Corporation, Washington, DC, USA). The median intake of macronutrients (protein, fat, total carbohydrates), dietary fiber, simple carbohydrates, saturated fatty acids, and micronutrients from 3 days was referred to recommendations for athletes [15–20]. The results were presented in the form of percentages, which made it possible to assess the degree to which the study group implemented the standards.

RESULTS

The median energy demand of the study participants was 2951 (2731; 3318) kcal, and the energy supply was 3031.4 (2470.7; 3702.8) kcal [Table 1]. Dietary supplementation accounted for 1.98% of the total energy supply. More than half of the climbers surveyed (52.94%), maintained a negative energy balance [Figure 1]. The median percentage coverage of energy requirements with diet in the surveyed climbers was 92.64 (85.55; 113.62) %. Most climbers (70.59%) met the carbohydrate requirement; however, the fiber supply was not provided at an adequate level in 23.53% of individuals [Figure 1]. All climbers met the recommended intake levels for protein and fats [Figure 1]. However, 23.53% exceeded the recommended values for fat intake, and 5.88% exceeded those for protein. Less than half of the climbers surveyed met the recommendations for saturated fatty acid intake, and slightly more than a third met the recommendations for dietary

cholesterol intake [Figure 1]. The median energy and nutritional value of the diet obtained from the analysis of the food diaries is shown in Table 1. Compared to standards for athletes [16–20], mountaineers did not provide adequate amounts of selenium and iodine, as well as vitamins D and K. All climbers met the requirements for sodium, phosphorus, vitamins: A, B_1, B_2 and B_{12} [Figure 2].

Table 1. Energy and nutritional value of the daily food ration of mountain climbers in relation to the recommendations for athletes [16–20]

Nutrients		Diet of alpinists	Nutritional recommendations for athletes
		Me (Q1; Q3)	RDA/AI
Energy and macronutrients, fiber, fatty acids and cholesterol intake	Energy [kcal]	3031.4 (2470.7; 3702.8)	EnExp*
	Protein [%]	15.5 (14.1; 18.4)	15–20 [1]
	Protein [g]	111.8 (104.5; 147.9)	-
	Protein [g/kg bw]	1.53 (1.33; 1.91)	1.2–2.2 [1]
	Plant protein [g]	47.90 (39.5; 56.2)	-
	Animal protein [g]	68.4 (49.8; 81.0)	-
	Carbohydrates [%]	47.6 (43.7; 53.2)	45–65 [2]
	Carbohydrates [g]	406.6 (292.6; 446.8)	-
	Carbohydrates [g/kg bw]	5.39 (3.72; 5.67)	6–12 [3]
	Simple carbohydrates [g]	98.8 (70.1; 126.2)	-
	Simple carbohydrates [%]	13.34 (10.14; 14.86)	<20 [4]
	Fiber [g]	36.3 (24.2; 48)	>25 [4]
	Fats [%]	33.5 (29.6; 36.5)	20–35 [2,3,4]
	Fats [g]	105.3 (87.1; 131.4)	-
	Fats [g/kg bw]	1.44 (1.17; 1.52)	0.5–1.5 [1]
	SFA [%]	11.32 (9.7; 12.57)	<10 [3]; ALAP [4]
	SFA [g]	36.6 (34.3; 40.4)	ALAP [4]
	MUFA [g]	32.1 (25.2; 37.9)	-
	PUFA [g]	14.5 (10.4; 19)	ALA 0,5%, LA 4%
	Cholesterol [mg]	403.6 (321.9; 611.6)	<300 [5]
Micronutrients intake	Sodium [mg] (AI)	2990.3 (2464.3; 3344.4)	1500 – >10,000 [2]
	Potassium [mg] (AI)	4488.4 (2882.6; 4780.7)	3500 [4] – 4700 [2]
	Calcium [mg]	1160.6 (912; 1493.9)	1000 [1] – 2000 [3]
	Phosphorus [mg]	1693 (1600.6; 2023)	700 [1] - 1500 [2]
	Magnesium [mg]	504.7 (432.2; 612.8)	420 [1]
	Iron [mg]	17 (14; 20)	11 [4]
	Zinc [mg]	13.5 (11.6; 16.1)	11 - 15 [2]
	Copper [mg]	2.0 (1.5; 2.5)	0.9 [2] -1.6 [4]
	Selenium [µg]	13.2 (7.7; 22.7)	50 - 55 [2]
	Iodine [µg]	67.2 (45.9; 76.6)	120 - 150 [2]
	Vit. A [µg]	1323.2 (1072.7; 2140.5)	900 [1]
	Vit. D [µg]	3.8 (2.7; 7)	15 [2,4]
	Vit. E [mg]	16.8 (13.7; 26.5)	15 [1,2]
	Vit. C [mg]	247 (179.9; 327.3)	110 [4] – 200 [2]
	Vit. B_1 [mg]	1.8 (1.5; 2.1)	1.2 [1]
	Vit. B_2 [mg]	2.5 (2.3; 2.9)	1.3 [1,4]
	Vit. B_3 [mg]	22.8 (16.6; 29.7)	14-20 [2]
	Vit. B_6 [mg]	3 (1.6; 3.9)	1.5 - 2 [2]
	Vit. B_9 [µg]	494.8 (375.1; 751.4)	400 [1,2]
	Vit. B_{12} [µg]	5.3 (3.2; 7.5)	2.4 [1] - 4 [4]
	Vit. K [µg]	27.8 (11.4; 65.7)	120 [1] – 700 [2]

Abbreviations: *- energy expenditure; 1—[18]; 2—[19]; 3—[17]; 4—[16]; 5—[20]; ME – median; Q1 – first quartile; Q3 – third quartile; RDA - recommended dietary allowance; AI—adequate intake; ALA - α-linolenic acid; ALAP—as low as possible; bw—body weight; LA - linoleic acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; SFA - saturated fatty acids

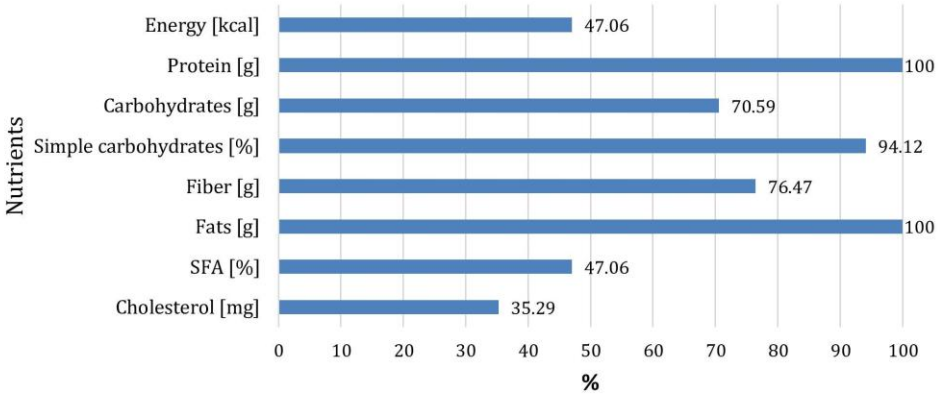


Figure 1. Percentage of alpinists realizing the requirement for energy, macronutrients, fiber, saturated fatty acids (SFA) and cholesterol [%].

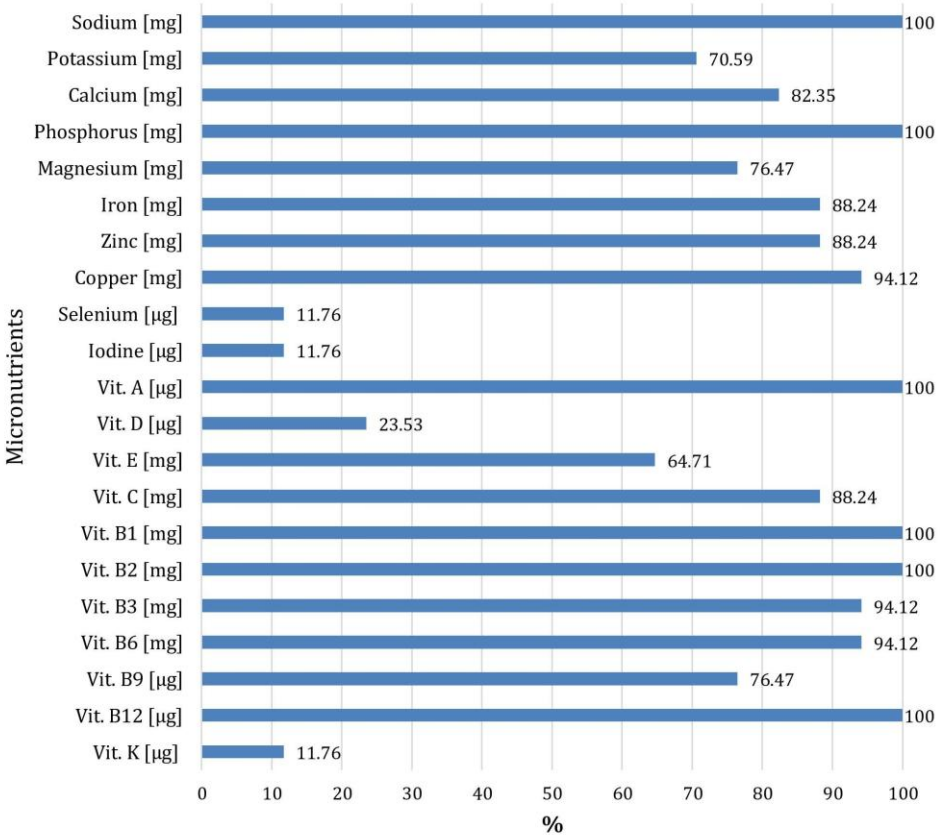


Figure 2. Percentage of alpinists realizing the requirement for specific micronutrients [%].

The median value of fecal zonulin in climbers was 28.40 (14; 193.7) ng/ml, while fecal alpha-1-antitrypsin was 5.95 (4.10; 6.86) ng/ml. Fecal zonulin values were elevated in 35.29% of the climbers tested, while fecal alpha-1-antitrypsin values were normal in all subjects. All correlations between nutrients of the habitual diet of alpinists and levels of intestinal permeability markers among alpinists were not statistically significant [Table 2].

Table 2. Correlations between nutrients of the habitual diet of alpinists and levels of intestinal permeability markers among alpinists

Nutrients	Intestinal permeability markers			
	Zonulin		Alpha-1-antitrypsin	
	S-rho	*p*	S-rho	*p*
Energy [kcal]	-0.036	0.920	0.371	0.291
Protein [%]	-0.268	0.454	-0.237	0.510
Protein [g]	-0.600	0.073	0.067	0.865
Plant protein [g]	0.139	0.707	0.079	0.838
Animal protein [g]	-0.152	0.682	-0.127	0.733
Protein [g/kg bw]	-0.539	0.113	0.261	0.470
Carbohydrates [%]	-0.188	0.608	0.212	0.560
Carbohydrates [g]	-0.006	1.000	0.321	0.368
Carbohydrates [g/kg bw]	-0.079	0.838	0.380	0.279
Simple carbohydrates [g]	0.210	0.560	0.140	0.707
Fiber [g]	0.236	0.514	0.224	0.537
Fats [%]	0.333	0.349	-0.236	0.514
Fats [g]	0.152	0.682	-0.055	0.892
Fats [g/kg bw]	0.200	0.584	0.000	1.000
SFA [g]	0.224	0.537	0.079	0.838
MUFA [g]	0.200	0.584	-0.127	0.733
PUFA [g]	0.219	0.544	-0.164	0.657
Cholesterol [mg]	-0.236	0.514	0.030	0.946
Sodium [mg] (AI)	-0.115	0.759	0.067	0.865
Potassium [mg] (AI)	0.042	0.919	0.079	0.838
Calcium [mg]	-0.018	0.973	0.309	0.387
Phosphorus [mg]	-0.042	0.919	-0. 176	0.632
Magnesium [mg]	0.055	0.892	-0.127	0.733
Iron [mg]	0.055	0.892	-0.103	0.785
Zinc [mg]	0.127	0.733	-0.248	0.492
Copper [mg]	0.139	0.707	-0.067	0.865
Selenium [µg]	-0.321	0.368	-0.370	0.296
Iodine [µg]	0.176	0.632	-0. 576	0.088
Vit. A [µg]	-0.261	0.470	0.176	0.632
Vit. D [µg]	-0.370	0.296	-0.018	0.973
Vit. E [mg]	0.030	0.946	-0.115	0.759
Vit. C [mg]	-0.152	0.682	-0.067	0.865
Vit. B_1 [mg]	-0.213	0.554	-0.116	0.750
Vit. B_2 [mg]	-0.486	0.154	-0.012	0.973
Vit. B_3 [mg]	-0.455	0.191	-0.012	0.973
Vit. B_6 [mg]	-0.055	0.881	-0.286	0.424
Vit. B_9 [µg]	-0.079	0.838	-0.115	0.759
Vit. B_{12} [µg]	-0.333	0.349	-0.224	0.537
Vit. K [µg]	0.273	0.448	-0.503	0.143

Abbreviations: bw—body weight; MUFA - monounsaturated fatty acid; *p* - statistical significance; PUFA - polyunsaturated fatty acid; S-rho - Spearman's correlation coefficient; SFA - saturated fatty acids

DISCUSSION

Main Findings

The present study evaluated the habitual dietary intake and selected markers of intestinal permeability in a group of Polish alpinists. More than half of the alpinists surveyed maintained a negative energy balance, indicating a notable discrepancy between energy demand and intake, and suggesting a risk of chronic low energy availability (LEA). Low energy intake can result in loss of muscle mass and bone density, as well as an increased risk of fatigue, injury, and illness [21]. The failure to meet energy requirements may exacerbate gut barrier dysfunction [22]. Inadequate energy supply relative to demand can potentially have a negative impact on recovery, immune function and overall performance during training, as well as during planned high-altitude activities [23, 24]. The results of another study involving sport climbers also indicate an inadequate dietary energy intake. Overall, 77.5% of the climbers studied did not meet the predicted energy requirements necessary to maintain a "moderate" level of physical activity [25]. Individuals engaged in climbing and alpine activities are at risk of energy restriction and/or low energy availability, evidenced by suboptimal energy intakes, and a lack of adjustment of energy intake in relation to exercise volume.

Carbohydrate (CHO) intake was adequate in the majority of study participants (70.59%), aligning with the recommendations for athletes engaged in endurance sports [17]. However, dietary fiber intake was insufficient in almost a quarter of respondents. Dietary fiber can significantly influence the composition, diversity, and richness of the microbiome, offering a wide array of substrates for fermentation processes conducted by particular microbial species that possess the requisite enzymatic capabilities to break down these intricate carbohydrates [26]. Low intake of dietary fiber and resistant starch may lead to decreased bowel movements resulting in decreased bowel function, and also decreased the diversity of gut microbiota [27]. Given the role of dietary fiber in supporting gut microbiota diversity and intestinal barrier function, this may have implications for gut health, especially in the context of exposure to hypoxic conditions and physical stress. A proper intestinal barrier is crucial for mountaineers, as hypobaric hypoxia can increase permeability and lead to gastrointestinal distress [28]. Maintaining gut health before expeditions helps prevent symptoms like nausea and diarrhea, ensuring better performance and overall well-being at high altitudes [29].

All mountaineers met the requirements for protein and fats with their diets, with 23.53% of them exceeding the normative values for fats and 5.88% for protein. These findings hold physiological relevance when considering the impact of macronutrient intake on intestinal permeability - a key factor in maintaining gut barrier integrity under environmental and physical stressors. Excessive supply of protein with the diet, though observed in a minority, is less directly implicated in intestinal permeability but may modulate gut microbial activity in ways that affect mucosal health. High protein intake increases the delivery of undigested proteins to the colon, where they undergo microbial fermentation. This process produces potentially harmful metabolites such as ammonia, hydrogen sulfide (H_2S), and phenolic compounds, which have cytotoxic and pro-inflammatory properties. These metabolites can impair epithelial cell respiration, damage DNA, and compromise tight junction integrity, contributing to gut barrier dysfunction [30, 31]. Currently, an increasing body of evidence suggests that the ingestion of excessive dietary lipids can differentially augment intestinal permeability. For instance, dietary lipids influence the expression and localization of tight junctions (TJs), promote a transition towards barrier-compromising hydrophobic bile acids, and additionally provoke oxidative stress and apoptosis in intestinal epithelial cells (IECs) [32]. Another study shows that 6-day ketogenic low-CHO, high-fat dietary intervention combined with a high-fat preexercise meal resulted in greater exercise-associated perturbations to markers of intestinal integrity than a high CHO diet and even low energy availability [33].

The median intake of saturated fatty acids (SFA) with the mountaineers' diet was 11.32%, exceeding the established recommendations. Current guidelines from the European Food Safety Authority advise that SFA intake should be as low as possible [16]. In contrast, the position statement of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine [17] recommends limiting SFA intake to less than 10% of total energy intake. However, the correlation between SFA intake and fecal zonulin and alpha-1-antitrypsin levels was not statistically significant. The intake of monounsaturated and polyunsaturated fatty acids in the mountaineers' diets slightly exceeded that of saturated fatty acids. Available scientific evidence indicates that a diet with high nutrient density - rich in polyphenols, omega-3 fatty acids, dietary fiber, and probiotics - has a beneficial effect on the composition and function of the intestinal microbiota. In contrast, a diet rich in highly processed foods, saturated fatty acids, and simple sugars is associated with adverse changes in the gut microbiota [34].

Micronutrient analysis revealed dietary inadequacies in selenium, iodine, and vitamins D and K - nutrients essential for immune function, antioxidant defense, and bone health. Emerging evidence suggests that deficiencies in these micronutrients may contribute to increased intestinal permeability, often referred to as "leaky gut", which can lead to systemic inflammation and impaired immune defense - factors particularly detrimental during high-altitude exposure. Vitamin D, beyond its well-established role in calcium homeostasis and immune regulation, has been shown to modulate the expression of tight junction proteins (e.g., occludin and claudins) in intestinal epithelial cells [35]. Its deficiency has been associated with compromised mucosal barrier function and increased translocation of endotoxins into the bloodstream, which may exacerbate systemic inflammation [35]. Given the important role of vitamin D in immune function and its association with an increased risk of anemia when deficient [36], it is essential to monitor serum vitamin D levels before an expedition and adjust supplementation accordingly. Selenium, a crucial cofactor for antioxidant enzymes such as glutathione peroxidase, also plays a protective role in gut health by counteracting oxidative stress-induced epithelial damage [37]. Inadequate selenium intake may impair redox balance and increased epithelial permeability, thereby contributing to greater susceptibility to infections and reduced tolerance to hypoxic stress [38]. Similarly, iodine is a critical micronutrient for thyroid hormone synthesis, which affects metabolic rate and energy balance, but also modulates mucosal immunity and epithelial turnover. Subclinical hypothyroidism, often observed with low iodine intake, can impair gastrointestinal motility and barrier maintenance [39]. Vitamin K has also been implicated in the regulation of inflammation and mucosal immunity. By downregulating expression of cytokines such as TNF-α and IL-6, vitamin K may help mitigate inflammation-induced disruption of tight junction proteins (e.g., occludin, claudin-1, zonula occludens 1), which are critical to the structural integrity of the gut barier [40]. The observed deficiencies in the habitual diets of the studied alpinists raise concerns about suboptimal gut resilience and systemic immune competence prior to and during expeditions.

Referring to the levels of the analysed intestinal permeability markers, elevated levels of zonulin (28.40 (14; 193.7) ng/ml) were found in the studied group of alpinists, with concentrations exceeding the reference values in approximately 35.29%. The results may indicate a disturbed intestinal microbiota and a potential state of dysbiosis, associated with increased permeability of the intestinal barrier. Similar trends of increased intestinal permeability and elevated levels of relevant markers in athletes have also been found in other studies. Increased intestinal permeability and elevated levels of zonulin have been observed in Polish athletes [41], and increased levels of circulating zonulin (tight junction modulator), occludin (tight junction protein) and LPS translocation have been confirmed in elite Italian soccer players [42]. In a study involving Finnish runners, a significant increase in intestinal permeability (as determined by zonulin and LPS levels) was observed after 90 minutes of intense exercise [43]. Elevated levels of zonulin were also observed in Polish triathletes after completing a race [44]. Changes in

markers of muscle cell damage correlated strongly with changes in zonulin concentration at different stages of the study [44]. The elevated zonulin levels observed in some alpinists may suggest disturbances in the gut microbiota and increased intestinal permeability; however, our analyses did not show significant correlations with nutrient intake. This interpretation should be treated with caution and requires confirmation in studies with larger groups and the use of additional permeability markers.

Another less common marker of intestinal permeability is fecal alpha-1-antitrypsin, the levels of which were normal in the group of alpinists studied. AAT is a protease inhibitor that would be increased during the disruption of the mucosal barrier and increased permeability [8]. It was shown that AAT reduces the production of pro-inflammatory cytokines, inhibits apoptosis and inflammatory reactions [45]. Several studies reported that AAT activity is increased post-exercise [46–48]. Another study reported post-exercise increase of both IL-6 concentration and AAT activity, which may confirm that the release of pro-inflammatory cytokines stimulates the release of acute-phase proteins [49]. There is a lack of studies in athletes in which intestinal permeability was determined using alpha-1-antitrypsin. The anti-inflammatory and immunomodulatory properties of AAT can be used therapeutically to treat pulmonary, infectious diseases and autoimmune diseases [50]. In the studied group, alpha-1-antitrypsin levels remained within the normal range. Possible explanations include insufficient intensity of training stimuli to induce an increase in this marker, or substantial inter-individual variability that may have masked subtle changes. Further studies with larger samples are needed, including additional markers of inflammation and intestinal barrier integrity.

Limitations

The limitations of the study include the limited sample size and lack of a control group and limited statistical power. Moreover, the assessment of intestinal permeability was limited to selected fecal biomarkers, which may not capture the full complexity of gut barrier dynamics. Importantly, other established markers of intestinal integrity, such as lipopolysaccharide (LPS), intestinal fatty acid binding protein (I-FABP), or occludin, were not included.

Another limitation is the lack of detailed validation data regarding the 3-day food diaries, which may be subject to potential reporting errors. Furthermore, possible confounding variables, such as vitamin D supplementation or previous gastrointestinal complaints, were not considered. Future studies should address these limitations, including a more comprehensive set of intestinal permeability markers, validated dietary assessment methods, and the evaluation of additional determinants of gut microbiota as well as psychological aspects.

CONCLUSION

1. Individuals engaged in climbing and alpine activities are at risk of energy restriction and/or low energy availability. The climbers mostly provided adequate amounts of macro- and micronutrients with their diets in exception to selenium, iodine, vitamin D and K.
2. In most of the climbers studied, intestinal permeability was predominantly within the normal range. In some individuals, elevated zonulin values were observed, while AAT concentrations remained within the normal range. Due to the small sample size and the lack of significant correlations with diet, the results should be considered preliminary and require further verification in studies with larger groups.
3. There is a need for nutritional and supplementation education by qualified sports nutritionists to increase awareness among this group of athletes about energy balance and sports nutrition in the preparatory stage before trips to the high mountains.
4. Future research should explore the longitudinal relationship between diet, gut health, and performance at altitude in larger cohorts.

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Publikacja 5 „High-altitude mountaineering induces adaptive gut microbiome shifts associated with dietary intake and performance markers”

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High-altitude mountaineering induces adaptive gut microbiome shifts associated with dietary intake and performance markers

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This study examined how high-altitude exposure and expedition-specific dietary changes influence gut microbiome composition, functional pathways, and their relationships with performance and health markers in alpinists. Seventeen male mountaineers (age 30.29 ± 5.8 years) participating in multi-week expeditions (> 3,000 MASL) were assessed before and after their climbs. Assessments included dietary intake analysis, blood and urine biomarkers, aerobic and anaerobic performance tests, and metagenomic sequencing of the gut microbiome. Bioinformatic and statistical analyses evaluated changes in microbiome composition and function and their correlations with physiological and dietary parameters. High-altitude exposure was associated with significant shifts in gut microbial composition and functional capacity. While the total number of bacterial species and functions remained stable, the glucose degradation pathway increased post-expedition. Participants with greater microbiome shifts showed improved performance and had richer baseline microbiomes. Pre-expedition, certain microbial functions were associated with vitamin B_6 and C intake, while post-expedition correlations involved specific macronutrients and micronutrients. Additionally, some microbiome changes correlated with blood markers, indicating links to nutrient metabolism and electrolyte balance. The gut microbiome of alpinists adapts to extreme environmental stress and dietary changes, influencing metabolic, immune, and performance-related processes. Optimizing dietary strategies to support a beneficial microbiome profile may enhance resilience and performance in challenging high-altitude environments.

Keywords Gut microbiome, High-altitude mountaineering, Dietary intake, Performance markers, Metagenomics, Physiological adaptation

During climbing at high altitudes (> 2,500 MASL), alpinists must deal with reduced atmospheric pressure, leading to the development of hypobaric hypoxia, intense solar radiation, variable weather conditions, strong winds and low temperatures with large fluctuations throughout the day, as well as the risk of avalanches and falling rocks[1,2]. During high-altitude expeditions, dietary changes occur due to poor access to fresh food, lack of appetite, food poisoning, harsh environmental conditions, and physiological changes[3,4]. Reactive oxygen species (ROS) production is accelerated with mountainous elevation, which may play a role in developing serious health crises. Exposure to increasing altitude leads to a reduction in ambient O_2 availability in cells, contributing to hypoxic oxidative stress and disturbed redox homeostasis, potentially impairing exercise performance and recovery[5,6]. As a result of increased ROS production, alpinists may experience cognitive decline, development of neurodegenerative processes, pathological changes in brain structures, including vasogenic edema[7], as well as damage to the intestinal barrier. This intestinal barrier disruption may lead to bacterial translocation and local or systemic inflammatory reactions, further impacting both general health and physical performance[8]. A damaged intestinal barrier may impair absorption of nutrients, which could explain some of the weight loss observed during prolonged exposures to high altitude[9,10].

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In addition to these physiological stresses, blood parameters and body composition can be altered by high-altitude conditions and changes in dietary intake. This may affect red blood cell counts, iron status, and markers of metabolic and immune function[11–15]. Similarly, adjustments in macronutrient and micronutrient intake before and during the expedition can affect an athlete's energy availability, leading to shifts in body mass and composition[16], and potentially affecting maximum oxygen uptake (VO_{2max}), anaerobic power, and other key performance indicators. Such high-altitude exposures thus serve as a natural experimental setting to assess how nutrition, physiology, and microbial ecology interact to shape athletic performance, health status, and adaptation to extreme environments.

In recent years, the gut microbiome has emerged as a critical factor influencing human health, immune function, and athletic performance. Alterations in gut microbial composition and metabolic capacity have been linked to changes in nutrient absorption, energy metabolism, inflammation, and recovery, all of which are central to optimal athletic performance and well-being[17–19]. In addition, the gut microbiota can respond dynamically to dietary changes, and high-altitude expeditions often require consumption of processed, freeze-dried, or supplemental foods due to limited access to fresh produce[20]. The use of functional foods has important advantages for athletes, such as the fact that they can be easily ingested, transported and stored, and they also taste good. However, when consumed in excess, these products may disrupt gut microbiota balance due to the chronic effects of their food additives[21–24]. Understanding how these dietary and environmental stresses shape gut microbial communities is essential for informed nutritional strategies that support health, performance, and safe acclimatization to challenging conditions.

The present study aimed to analyze the structure and functional characteristics of the microbiome of climbers before and after a high-mountain expedition. By integrating detailed dietary records, blood and urine analyses, and measures of aerobic and anaerobic exercise capacity, we sought to determine how high-altitude exposure and associated dietary changes affect the gut microbiome and, in turn, how these microbial shifts correlate with health markers and performance outcomes. Such insights could provide evidence-based guidance for optimizing dietary composition, supplementation, and overall expedition planning. Ultimately, identifying specific microbial signatures and metabolic pathways that are modifiable through diet and supplementation may help practitioners develop tailored nutritional interventions to improve both health and athletic performance of climbers operating under extreme environmental conditions.

Materials and methods

Study participants

The study group consisted of 17 male mountaineers from Poland between the ages of 23 and 40, participating in mountain expeditions at least once a year, during which they stayed at altitudes above 3,000 m for at least 3 weeks. Individuals qualified for the study were members of high-mountain clubs affiliated with the Polish Mountaineering Association. The group's anthropometric and BMI (body mass index) data are shown in Table 1.

The climbers participating in the study were characterized by extensive climbing experience (10 ± 5 years) in both sport climbing and mountaineering. The average climbing level of participants, as measured by the IRCRA scale, was 20.69 ± 2.8, which according to the proposed criteria, places them in the advanced (level 3) category[25]. The climbers spent an average of 8 ± 3 h per week training. Climbers qualified for the study stayed at an altitude of 3,000–8,167 m above sea level at the time of their expedition. In total, the high-altitude expeditions lasted an average of 34 ± 6 days, of which climbers spent an average of 22 ± 4 days actively climbing in the mountains and 11 ± 8 days resting in base camps or mountain towns. Because some of these sites were located above 3,000 m, participants spent a total of 26 ± 6 days at altitudes above 3,000 m.

The mountaineers recruited for the study traveled in small groups or individually to pursue their mountain goals. Specifically, the objective of the group of climbers ($n = 6$) exploring the White Cordillera massif in Peru was to climb the 800-meter Cruz del Sur route on the La Esfinge rock monolith (5,325 m). A new route was also established on Ocschapalca (5,888 m) and Nevado Churup (5,495 m), and ascents were made on Artesonraju (6,025 m) and Alpamayo (5,947 m). Climbers ($n = 3$) targeting peaks from the Shuijerab mountain group in North Karakorum, Pakistan, made a route on the west face of the virgin peak of Trident Peak (6,150 m), and climbed the virgin peak of Sakwa Sar (6,050 m). Climbers ($n = 3$) exploring the Gangotria Valley in India's Garhwal Himalaya and mountaineers ($n = 2$) climbing in the Himalayas in Western Nepal and Northern Nepal attempted virgin peaks, but failed due to weather conditions (the highest point of the expeditions – 5,000 m). There were also two independently active climbers in the Himalayas - one of them made ascents of Annapurna (8,091 m) and Dhaulagiri (8,167 m) in Central Nepal, while the other climbed Ama Dablam (6,812 m). In the western Pamir-Alay in Kyrgyzstan's Lailak Valley, an ascent was made by the Troschenko route on the north face of Ak-su (5,217 m).

	Men ($n = 17$)	
	value	SD
Age [years]	30.29	5.8
Body height [cm]	180.47	8.36
Body weight [kg]	74.96	5.03
BMI [kg/m^2]	22.83	2.1

Table 1. Anthropometric data and BMI of climbers participating in the research ($n = 17$). Abbreviations: BMI - body mass index.

Before taking part in the project, the climbers had a consultation with a sports medicine doctor. The climbers' fitness for mountaineering and participation in the project was assessed based on previous examinations (ECG, blood and urine tests). The presence of chronic diseases and age over 45 were disqualifying factors for participation in the study. The Bioethics Committee of the Cracow Regional Medical Chamber approved the research project (68/KBL/OIL/2022; date: 11/04/2022), which was conducted in accordance with the Declaration of Helsinki. After learning about the risks and benefits of taking part in the project, all climbers gave written informed consent to participate in this study.

Study design

Nutritional analysis of diet

The supply of selected macronutrients before and during the mountaineering expedition was determined by analyzing the whole-day rations obtained using the 3-day food diary method. The expedition participants were asked to note down all the foods, meals and supplements eaten and fluids drunk during the 3 days before the expedition (2 days of sports activity, 1 day of rest) and 3 days during the expedition (2 days of sports activity, 1 day of rest). In the mountain conditions, these were days of acclimatization and ascending towards the summit, and the activities noted during this time included mainly hiking with elements of climbing. Climbers were given detailed instructions on how to keep food diaries before leaving for the expedition in order to minimize recording errors. The instructions included written guidelines (the table of household measures of popular foods with equivalent weights to make it easier to determine product measures and an example of a completed diary) along with a briefing session explaining how to record all foods, beverages, and supplements consumed. Participants were asked to specify product names, manufacturers, quantities (in grams, milliliters, or standard household measures), preparation methods (if applicable), and the time of consumption. Special attention was given to recording commercially packaged products such as energy gels, bars, and freeze-dried meals, for which climbers were instructed to note the exact product name, brand, and weight to allow analysis based on the product's label data and nutritional table.

The data from the diaries were meticulously entered into the Aliant Dietetic Calculator program (Anmarsoft, Gdańsk, Poland; version: 85; database: 6.2) for quantitative analysis of the climbers' diets before and during the expedition. The program used the "Tables of food composition and nutritional value"[26] as a database, taking into account food recipes and losses due to product processing.

Anthropometric measurements

Body height of participants was measured before the expedition using a Seca 217 anthropometer (Seca GmbH & Co. KG, Hamburg, Germany) with an accuracy of 1 mm. The climbers' body weight was determined using InBody 120 body composition analyzer (Inbody Bldg., Seoul, Korea) in the morning after a standardized meal between 7:45 and 8:30 am.

Health status analysis

For health status analysis, blood samples were taken from veins in the elbow pit to determine hematological and biochemical blood indices, i.e. peripheral blood count (hematocrit, hemoglobin concentration, erythrocyte volume (MCV), erythrocyte hemoglobin content (MCH), erythrocyte hemoglobin concentration (MCHC), erythrocyte anisocytosis index (RDW), erythrocyte count, platelets, platelet volume (MPV), white blood cell count - leukocytes, leukocyte differentiation: lymphocytes, monocytes, basophils, eosinophils, neutrophils), erythrocyte sedimentation rate (ESR), glucose, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), creatinine (Cr), urea (U), uric acid (UA), total protein, sodium, potassium, chloride, calcium, magnesium, phosphorus, iron, ferritin, vitamin D, vitamin B_{12}, liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase, gamma-glutamyltranspeptydase (GGTP), albumin and total bilirubin. The determinations were carried out in the morning and on an empty stomach, up to two weeks before the expedition and within two days (1.41 ± 0.69 days) after the expedition.

In addition to the blood determinations, a general urinalysis (specific gravity, pH, leukocytes, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, blood (erythrocytes/hemoglobin), color, clarity) was performed, as well as a fecal examination (general fecal examination, test for parasites, lamblia, *Helicobacter pylori*, levels of zonulin and antitrypsin). Study participants were given a uniform procedure for collecting urine and stool samples on their own. After a plum-sized stool sample was gathered, it was placed in a tube and stored in a refrigerator (temp. 2–4° C) and then delivered to the laboratory within 24 h after the collection. The samples of urine and feces were submitted for testing to the laboratory at the same time as blood was collected.

Measurements were made using flow cytometry (blood count), kinetic-photometric method (ESR), indirect potentiometry (sodium, potassium, chloride), spectrophotometry (total protein, calcium, phosphorus, magnesium, total cholesterol, HDL, triglycerides, U, UA, iron, AST, ALT, creatinine, total bilirubin, GGTP, glucose), direct chemiluminescence (vitamin B_{12}, vitamin D, ferritin), strip tests (general urinalysis), immunochromatographic method with Giardia test from Hydrex Diagnostics (*Giardia lamblia* in feces), and by H. pylori Ag by nal von minden (*Helicobacter pylori* in feces) and microscopically (general fecal examination and evaluation of food debris, testing for parasites, lamblia). All laboratory analyses described here were performed using the following equipment: Alinity HQ (Abbott, Singapore), Roller 20 (Alifax, Italy), Alinity C (Abott, Singapore), Alinity I (Abott, Singapore) and Atellica 1500 (Siemens, Germany) and were commissioned as an external service, performed by Alab Laboratories (Warsaw, Poland).

Clinical event monitoring

Throughout each expedition, the climbers prospectively logged gastrointestinal (GI) complaints and symptoms suggestive of acute mountain sickness (AMS) in individual food diaries. Two participants reported transient diarrhea that was treated with a short course of oral nifuroxazide, and one additional episode occurred after returning to low altitude. One climber experienced AMS during the ascent. After an immediate descent to base camp, he received a single intramuscular dose of dexamethasone. No other antibiotics, proton pump inhibitors, or nonsteroidal anti-inflammatory drugs were taken during the study period.

Aerobic and anaerobic performance testing

Aerobic capacity was assessed during a test with gradually increasing load performed until exhaustion, while anaerobic capacity was assessed during the Wingate test for the lower and upper limbs. Exercise tests were performed twice. The first test was performed about two weeks before the expedition, and the second test was performed up to four days (3.88 ± 1.49 days) after returning from the expedition at an altitude of about 383 m above sea level.

Graded treadmill test

The test was performed on a mechanical treadmill h/p/Cosmos Saturn (h/p/Cosmos Sports & Medical GmbH, Germany) according to the following scheme: 2-minute recording of baseline cardiorespiratory indices in a standing position, a 4-minute run at a constant speed of 8 km/h with a treadmill angle of 1°, followed by a 1.1 km/h increase in running speed every 2 min until a running speed of 16.8 km/h was reached. In the remaining part of the test, the running speed did not change and an increase in exercise intensity was obtained by raising the angle of the treadmill by 1° every 2 min. The test continued until exhaustion, subjective to the test participant. Respiratory indices were monitored continuously using a Cortex Metalyzer 3B ergospirometer (CORTEX Biophysik GmbH, Germany), heart rate recorded using a Polar S-410 cardiac monitor (Polar-Electro, Finland).

Wingate anaerobic test

This test was performed in a sitting position without getting up from the saddle on a Monark 894e cycloergometer for the lower limbs (Monark Sports & Medical, Sweden) and Monark 834e for the upper limbs (Monark Sports & Medical, Sweden) in a modified 20-second version. The participants' task was to perform crank turns at a maximally high rhythm throughout the test. Both tests were preceded by warm-ups, with the lower limb test lasting 5 min with a load of 100 watts and the upper limb test lasting 4 min with a load of 60 watts. The pedaling rhythm was 60 rpm. During the warm-up for the lower limb test, brief 5–second accelerations of the pedaling rhythm to about 80% of subjective maximum capacity occurred in 2nd, 4th and 5th minute and in the warm-up for the upper limb test in 2nd and 4th minute, respectively. After the warm-up, the participants performed stretching exercises for 2 to 3 min, after which the test proper was started. The test load was set at 7.5% and 4.5% of body weight in the lower and upper limb tests, respectively.

Assessment of lactate concentration

Before the start (5 min before exercise) and at the end of all tests (3 and 20 min after exercise), blood was drawn from the fingertip capillaries into tubes containing a glycolysis inhibitor to determine plasma lactate concentration. Lactate concentration was determined using LC2389 reagent (Randox Laboratories Ltd., UK) by an enzymatic colorimetric method, which allows the determination of biochemical markers by using a specific enzymatic reaction that yields a colored product. The concentration of lactate was determined by the color change of the solution, assessed by reading the absorbance at 550 nm using a Thermo Scientific Nicolet Evolution 201 PC Control dual-beam UV/VIS spectrophotometer (Thermo Fisher Scientific, USA). The absorbance results were recorded electronically, and were then used to calculate the concentration of lactate in each sample.

Microbiome analysis

Sample collection

Stool was collected and immediately placed in stool storage containers, and these were placed in a Styrofoam box with a cooling insert. The box with the stool sample was kept in the refrigerator (temp. 2–4° C) and then transferred within 24 h of collection to the project contractor. The stool was then placed in the freezer (temp. −80° C). The fecal samples were collected from participants in the same rounds as described in the section describing the health analysis (before and after the expedition).

DNA isolation, quantitation and quantification

Approximately 200 mg of each frozen stool sample was processed for DNA isolation using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Germany) following a modified protocol designed to enhance microbial cell disruption. This procedure combined physical, mechanical (bead-beating with a Bead Ruptor Elite; Omni International, USA), and chemical lysis steps. Homogenization involved short, high-speed cycles with cooling intervals. Key modifications included the following steps: after adding Solution CD1, the sample was incubated at 55 °C for 10 min with 500 rpm shaking and vortexing every 2 min. Further, bead beating was performed for 15 s at 6 m/s, repeated 4 times with a 2-minute break after each cycle, followed by a short spin. Next, 40 µl of proteinase K was added, and the mixture was incubated at 25 °C for 10 min. A final, 5th round of bead beating was performed for 15 s at 6 m/s. The extracted DNA was assessed for concentration and purity using a NanoDrop spectrophotometer (Thermo Scientific, USA), and the integrity of the DNA was verified on 1% agarose gels. Only the DNA samples with sufficient quality and yield were selected for further processing. The

DNA concentration was then re-evaluated and standardized using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA) and the Qubit DNA BR assay, ensuring accurate normalization prior to library preparation.

Library preparation and sequencing

Metagenomic libraries were prepared from 100 ng of input DNA per sample, using the KAPA HyperPlus Library Preparation Kit (Roche, Basel, Switzerland) as described in our previous papers[27,28]. The fragmentation step was optimized to produce fragments with an average size of 300–500 bp. Indexed adapter ligation was performed using KAPA Universal dual-indexed adapters at a controlled concentration to maintain efficient ligation and minimize adapter dimer formation. Libraries were then purified with KAPA Pure Beads (Roche, Switzerland), and library amplification was limited to six PCR cycles to reduce amplification bias. After PCR, a final clean-up step was performed to remove any residual primer dimers and non-target fragments. The resulting libraries were assessed for quality and fragment size distribution using a 4150 TapeStation system (Agilent Technologies, USA).

All libraries were then normalized to 20 nM, pooled in equimolar amounts, and subjected to 150 bp paired-end sequencing on an Illumina NovaSeq platform (Illumina, CA, USA). Each library generated approximately 13 million paired-end reads. This deep sequencing strategy provided a comprehensive snapshot of the microbial communities and their genomic characteristics.

Statistics and bioinformatics analysis

The raw fastq sequences were subjected to quality control with TrimGalore version 0.6.10[29]. Three samples were rejected from further analysis due to poor reading quality. To remove human contaminants, any reads mapping to the human reference genome build 38 with bowtie2 version 2.2.3[30] were removed. Taxonomic and functional profiles were calculated via MetaPhlAn 4.0 and HumanN 3.7[31]. Differential enrichment analysis was performed using the Linear Discriminant Analysis Effect Size analysis (LEfSe)[32]. The LEfSe analysis was implemented in Python, leveraging functions from the scipy.stats module (version 1.11.1) for non-parametric statistical tests, specifically kruskal for identifying features with significant differential abundance. Subsequently, sklearn. discriminant_analysis.LinearDiscriminantAnalysis (Scikit-learn version 1.3.0) was utilized to calculate the Linear Discriminant Analysis (LDA) score, which estimates the effect size of each differentially abundant feature. Alpha diversity (Shannon, Simpson, species richness) was calculated using the scipy module (version 1.11.1). Beta diversities (Bray-Curtis) were calculated using the QIIME 2[33] amplicon-2024.2 version diversity packages and the PERMANOVA test was used to identify statistical significance. Finally, to investigate relationships between microbiome features and metadata variables, Spearman's rank correlation coefficients were calculated. This analysis was performed using the spearmanr function from the scipy.stats module (version 1.11.1). To control for the false discovery rate arising from multiple comparisons, the resulting p-values were adjusted using the Benjamini-Hochberg (BH) correction. This multiple testing correction was applied via the multipletests function from the statsmodels.stats.multitest submodule, part of the statsmodels package (version 0.14.1)[34]. The entire analysis was conducted within a Python 3.11.9 environment and visualized using the matplotlib 3.7.2 and seaborn 0.12.2 packages.

Results

Cohort overview

In this study, we investigated 17 experienced Polish male mountaineers (23–40 years old) who undertook high-altitude expeditions lasting more than three weeks at altitudes above 3,000 MASL. Before and after their expeditions, we collected comprehensive data including dietary intake, blood and urine parameters, aerobic and anaerobic performance measures, and detailed metagenomic analyses of their gut microbiomes. By integrating these multidimensional datasets, our goal was to characterize how exposure to extreme mountain environments and dietary changes influence gut microbial community structure, functional pathways, and potential health and performance outcomes.

Clinical events during the expeditions

Two participants experienced self-limited diarrhea that resolved within 48 h after taking nifuroxazide orally. One climber reported diarrhea upon returning to a lower altitude. One participant developed acute mountain sickness, which was managed by descending to base camp and administering a single intramuscular dose of dexamethasone. No additional gastrointestinal complaints were recorded.

Microbiome changes after the mountaineering expedition

All participants exhibited generally diverse microbiomes both before and after the expedition (Fig. 1a, Supplementary Fig. 1). While the relative abundance of the most plentiful species did not change much in some individuals (A3), others experienced greater shifts in the microbiome (A10). There were on average 200 ± 69 bacterial species identified per participant before and 179 ± 53 species per participant after the expedition. In contrast, we found 76 ± 8 bacterial functions per participant before and 77 ± 8 functions after the expedition. The differences in the numbers of species and functions before and after the expedition were not significant.

Across all participants, the prevalence of species remained stable after the travel (Fig. 1b, left). This was particularly true for species occurring with low prevalence (present in less than a third of individuals), and any differences could be attributed to changes in only up to two individuals. We observed a slightly different trend in the comparison of functions - this time, the most stable functions were highly prevalent. There was one outlier, however; it was the *GLUCOSE1PMETAB-PWY: glucose and glucose-1-phosphate degradation* pathway. This function, present in a half of individuals before the expedition, was found in almost everyone upon their return.

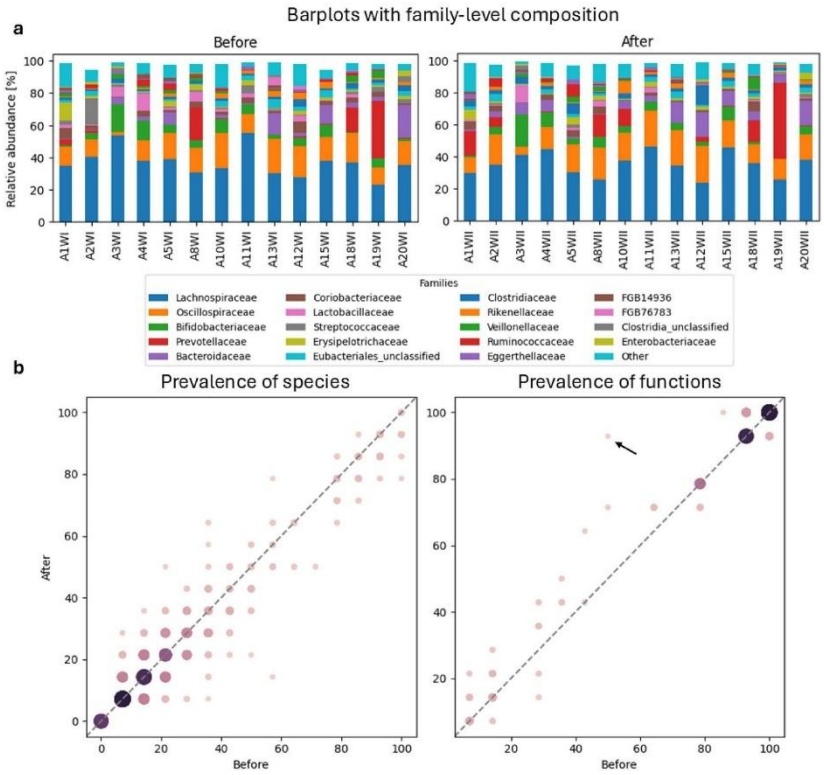


Fig. 1. Comparison of gut microbiome compositions before and after the expedition. (**a**) Barplots with family-level composition of each sample before and after the expedition. (**b**) Prevalence of species (left) and functions (right) before versus after the expedition. The *GLUCOSE1PMETAB-PWY*: *glucose and glucose-1-phophate degradation pathway*, which substantially changed in prevalence as a result of the travel, is marked with a black arrow.

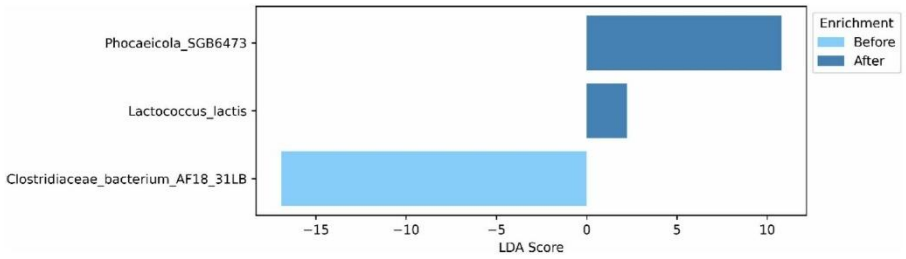


Fig. 2. Species enriched in the participants before and after the expedition.

We observed a statistically significant increase in the abundance of this function as a result of the expedition (Wilcoxon signed-rank test, $p < 0.05$).

A differential enrichment analysis performed using LEfSe revealed two species enriched after the expedition and one before (Fig. 2). The two species which increased in abundance after the expedition were *Lactococcus*

lactis and a species belonging to the *Phocaeicola* genus. On the other hand, there was a depletion trend of the *Clostridiaceae* bacterium. We found no statistically significant alterations in microbial functions.

Correlating microbiome features with dietary information revealed an association of a *PWY-6703: preQ0 biosynthesis pathway* with vitamin B_6 and C intake before the expedition (Spearman correlations of 0.83 and 0.86, respectively, adjusted p-values < 0.05). We observed a decrease in the intake of the vitamins after the expedition, which was significant for vitamin C (vitamin B_6: 6.1 mg to 2.6 mg, T-test p-value > 0.05; vitamin C: 325.7 mg to 149.2 mg, T-test p–value < 0.01). Similarly, the average abundance of the *PWY-6703: preQ0 biosynthesis pathway* showed a notable, but statistically insignificant decrease (from 18% to 15%). No other associations of functions or species with dietary parameters before or after the expedition passed multiple testing corrections.

Varying degrees of Microbiome alterations among participants

Following our observation that the microbiomes were altered in individuals to different degrees (Fig. 1a), we calculated Bray-Curtis distances between matched samples from the same individual, collected before and after the intervention (Fig. 3a, Supplementary Fig. 2). We observed a range of distances between the samples, with samples from the individual A12 being visibly more differentiated than the rest.

Separating the individuals based on their second ventilatory threshold (VT2) pace change after the expedition revealed that individuals who experienced an improvement in fitness experienced a notably yet not significantly greater microbiome alteration (Fig. 3b). In addition, participants whose microbiomes changed more (Bray-Curtis distance ≥ 0.36, chosen based on the median) had significantly richer microbiomes before the intervention in comparison to those who experienced subtle shifts (Fig. 3c-e). This was accompanied by insignificant but notably higher intakes of manganese, calcium, vitamin A, magnesium and phosphorus (mean manganese intake 9.2 and 3.9 mg, calcium 1520.7 and 973.3 mg, vitamin A 2246.0 and 1291 µg, magnesium 687.7 and 397.6 mg, phosphorus 2021.5 and 1545.7 mg in participants with and without substantial gut alterations, $p < 0.05$, adjusted p-values > 0.05).

A LEfSe differential enrichment analysis of species and functions at baseline revealed statistically significant differences between the two groups (Fig. 3f). The microbiomes of individuals who experienced alterations were

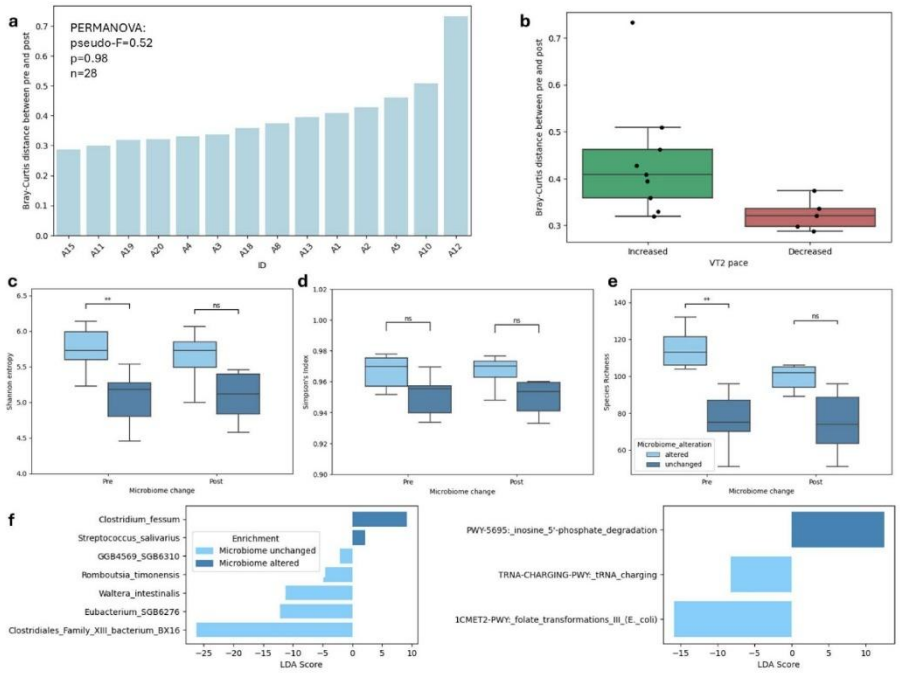


Fig. 3. A comparison of participants with greater and smaller gut microbiome alterations after the high-altitude travel. (**a**) Bray-Curtis distance between matched samples (before and after the expedition) from the same participant. (**b**) Individuals who observed an improvement in the VT2 pace after the expedition experienced greater microbiome alterations. (**c**),(**d**),(**e**) The microbiome of individuals who experienced greater microbiome alterations was significantly richer before the expedition. (**f**) LEfSe differential enrichment analysis comparing species and functions in individuals with and without large microbiome alterations at baseline.

enriched in *Clositridium fessum*, *Streptococcus salivarius* and the *inosine 5'-phosphate degradation pathway*. On the other hand, they appeared to have lower abundances of, among others, a bacterium from the Clostridiales family as well as the *folate transformations* and *tRNA charging pathways*.

Microbiome correlations with blood markers, anaerobic capacity physiological markers and diet in participants exhibiting the strongest Microbiome shifts

Investigating the subset of individuals with large microbiome alterations revealed shifts in prevalence of species were more varied than that of functions (Fig. 4a and b). The taxa with the greatest alterations included *Dorea sp AF36_15AT*, present in 57% of individuals before and in 100% after the expedition, as well as *GGB9758_SGB15368* from the Firmicutes phylum, found in 43% before and 86% of individuals after. While the prevalence of functions remained relatively stable, with most functions being present both before and after the expedition, a few functions experienced a substantial prevalence drop. They were *tetrapyrrole biosynthesis I (from glutamate)*, *UDP-N-acetyl-D-glucosamine biosynthesis I* and *O-antigen building blocks biosynthesis (E.coli)*, all present in 43% before and 14% of individuals after the expedition.

A differential enrichment analysis with LEfSe identified 3 species, namely *Blautia luti*, *Romboutsia timonensis* and *GGB4569_SGB6310* from the Lactobacillaceae family, enriched in abundance after the expedition. We found no species enriched before, as well as no statistically significant alterations of abundance in functions.

We identified several microbiome feature correlations with blood markers and anaerobic indices (Table 2). Before the expedition, there were two species associated with potassium, specifically *Prevotella copri clade A* (positive correlation) and *Bacteroides uniformis* (negative correlation), as well as 15 cases of marker associations with microbial functions. *dTDP-β-L-rhamnose biosynthesis pathway* was the only function associated with two different markers before the expedition, specifically total work performed of lower limbs (W_l) and mean anaerobic power of lower limbs (P_{mean}) (positively in both cases). After the investigation, we found one association with species *Agathobaculum butyriciproducens* with calcium (strong positive correlation) and 8 with functions. This time, two functions were associated with at least two markers (*NAD de novo biosynthesis I (from aspartate)* positively with basophils and calcium; and *superpathway of L-serine and glycine biosynthesis I* negatively with sodium and positively with cholesterol HDL). Finally, two functions had different associations both before and after the expedition. *Guanosine ribonucleotides de novo biosynthesis* was positively correlated with sodium before and negatively with mean platelet volume (MPV) after, while *myo-*, *chiro-* and *scyllo-inositol degradation* was positively associated with sodium before and with basophils after. Other analyzed correlations of microbiome features with blood, urine, fecal markers and anaerobic indices were not statistically significant.

Finally, we found a number of microbial function associations with diet after the expedition (all of which passed the correction for multiple testing, Supplementary Table 1). We identified strong positive correlations (Spearman correlation > 0.90) of *coenzyme A biosynthesis* with overall calorie and carbohydrate intake, monounsaturated fatty acid (MUFA) as well as vitamins C, B_{12}, B_1 and B_6. *L-arginine biosynthesis* was associated with overall carbohydrate intake and digestible carbohydrates, while the *penthose phosphate pathway* with selenium and plant protein with *sucrose biosynthesis*. Negative correlations were found between *adenine and adenosine salvage* and sodium, as well as between *L-rhamnose biosynthesis* and animal protein.

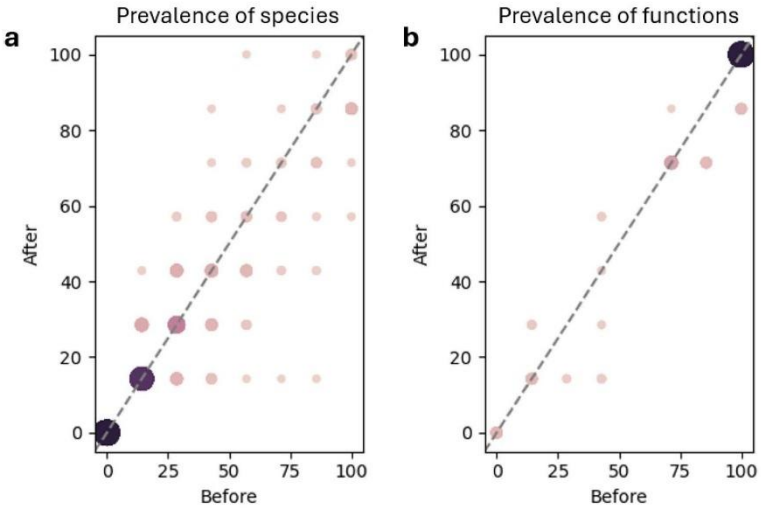


Fig. 4. Prevalence comparison of species (**a**) and functions (**b**) in individuals with large microbiome alterations as a result of the expedition.

Timepoint	Parameter	Microbiome feature	Spearman correlation
Before	Potassium [mmol/l]	*Prevotella copri clade A*	0.98
		Bacteroides uniformis	−0.97
	LA (20') [mmol/l]	*GGB9642 SGB15119*	−0.99
	HGB [g/dl]	*RIBOSYN2-PWY: flavin biosynthesis I (bacteria and plants)*	0.96
	Sodium [mmol/l]	*ANAGLYCOLYSIS-PWY: _glycolysis_III_(from_glucose)*	0.94
		GLUTORN-PWY: L-ornithine biosynthesis I	0.94
		PWY-1042: glycolysis IV	0.94
		PWY-2942: L-lysine biosynthesis III	0.94
		PWY-5097: L-lysine biosynthesis VI	0.94
		PWY-7221: guanosine ribonucleotides de novo biosynthesis	0.94
		PWY-7237: myo-, chiro- and scyllo-inositol degradation	0.94
	HDL [mmol/l]	*ARGSYN-PWY: L-arginine biosynthesis I (via L-ornithine)*	−0.96
		ARGSYNBSUB-PWY: L-arginine biosynthesis II (acetyl cycle)	−0.96
	W_t [J · kg^{-1}]	*DTDPRHAMSYN-PWY: dTDP-β-L-rhamnose biosynthesis*	0.96
	P_{mean} [W · kg^{-1}]	*DTDPRHAMSYN-PWY: dTDP-β-L-rhamnose biosynthesis*	0.96
	t_m [s]	*PWY0-1296: purine ribonucleosides degradation*	0.96
	WSHL [W/kg/s]	*ILEUSYN-PWY: L-isoleucine biosynthesis I (from threonine)*	0.96
After	Calcium [mmol/l]	*Agathobaculum butyriciproducens*	−0.99
	MPV [fL]	*PWY-7221: guanosine ribonucleotides de novo biosynthesis*	−0.96
	Basophils [10^9/L]	*PWY-7237: myo-, chiro- and scyllo-inositol degradation*	0.96
		PYRIDNUCSYN-PWY: NAD de novo biosynthesis I (from aspartate)	0.96
	Sodium [mmol/l]	*SER-GLYSYN-PWY: superpathway of L-serine and glycine biosynthesis I*	−0.96
	Calcium [mmol/l]	*PYRIDNUCSYN-PWY: NAD de novo biosynthesis I (from aspartate)*	0.99
	Magnesium [mmol/l]	*PWY-6151: S-adenosyl-L-methionine salvage I*	−0.98
	HDL [mmol/l]	*SER-GLYSYN-PWY: superpathway of L-serine and glycine biosynthesis I*	0.96
	Protein total [g/l]	*HISTSYN-PWY: L-histidine biosynthesis*	0.96

Table 2. Statistically significant correlations (adjusted p-values < 0.05) of blood markers and anaerobic indices with Microbiome features in individuals with Microbiome alterations, before and after the expedition ($n = 7$). Abbreviations: HDL – high-density lipoprotein cholesterol; HGB – haemoglobin; LA (20') – lactate concentration at intake at 20 min after aerobic capacity test; MPV - mean platelet volume; P_{mean} – mean anaerobic power (lower limbs); t_m – time of maintaining maximum power (lower limbs); WSHL - rate of decrease from the maximum power of the lower limbs; W_t – total work performed (lower limbs).

Discussion

Microbiome and nutritional adaptations to High-Altitude stress

The observed changes in gut microbiome compositions before and after the high-altitude mountaineering expedition indicate adaptive shifts in the gut microbiome and host physiology in response to extreme environmental and physiological stressors. Our findings demonstrate that exposure to low atmospheric pressure, hypoxic conditions, and challenging dietary changes can reshape the gut microbiome, potentially affecting host metabolism, immune function, and overall performance. Similar to previous studies showing that altitude-induced hypoxia alters energy metabolism and physiological adaptations in athletes[35–37], we identified functional shifts in the gut microbiome, such as increased prevalence of the *glucose and glucose-1-phosphate degradation pathway*, which was present in half of individuals before the expedition and in almost all participants upon their return. In addition, the abundance of this pathway increased significantly. The presence of this pathway likely reflects not only the elevated intake of simple carbohydrates during the expedition - through consumption of energy gels, sweetened bars, isotonic drinks, and freeze-dried meals[38,39] - but also an adaptive microbial response to environmental and physiological stressors, including hypoxia and increased energy demand. While the specific *glucose and glucose-1-phosphate degradation pathway* has not been directly reported in earlier hypoxia studies, a number of investigations in altitude-adapted populations suggest broader functional shifts in microbial carbohydrate metabolism under environmental stress[40–42]. The observed increase in the prevalence of the *glucose and glucose-1-phosphate degradation pathway* may indicate both an increase in substrate availability and a microbiome strategy to maximize ATP yields in the presence of restricted oxygen.

From a nutritional perspective, our results highlight the importance of vitamin intake, particularly vitamins C and B_6, in modulating microbial metabolic pathways such as the *preQ0 biosynthesis pathway*. The observed association between the *preQ0 biosynthesis pathway* and dietary intake of vitamins B_6 and C prior to the high-altitude mountaineering expedition highlights the intricate interplay between nutrition, microbiome dynamics, and metabolic adaptation. Vitamin B_6, known for its role as a cofactor in amino acid metabolism[43], is essential for the synthesis of nucleotides and neurotransmitters. This vitamin may enhance the efficiency of metabolic processes involved in preQ0 production, as the pathway is likely reliant on amino acid precursors. Additionally,

vitamin C serves as a potent antioxidant, crucial for mitigating oxidative stress that can be exacerbated in high-altitude environments[44–46]. Its antioxidant properties may also support beneficial gut microbial populations capable of metabolizing this vitamin and subsequently influencing pathways like the *preQ0 biosynthesis pathway*. The dietary intake of these vitamins may modulate gut microbiome composition, ensuring the availability of substrates necessary for microbial metabolism, which in turn supports the *preQ0 biosynthesis pathway*. This association underscores the significance of adequate vitamin intake in preparing the body for the metabolic demands of high-altitude exposure, where increased energy expenditure and altered oxygen availability necessitate robust cellular adaptations[47].

An analysis of Bray-Curtis distances between baseline and post-expedition samples revealed varying degrees of microbiome alterations among the participants. Individuals with greater microbiome shifts responded to the high-altitude conditions better through improvements in fitness markers such as a higher pace at the VT2. This observation suggests that greater microbiome plasticity might be associated with improved metabolic or cardiorespiratory adaptation under environmental stress, such as hypobaric hypoxia[19,48]. Interestingly, participants with larger microbiome shifts also had significantly richer and more diverse baseline microbiomes. Greater microbial diversity has been previously linked with enhanced resilience and adaptive capacity of the gut ecosystem, potentially allowing for more dynamic functional reorganization in response to stressors like high altitude, changes in diet, or physical exertion[49–51]. This was accompanied by insignificant but notably higher intakes of macronutrients, such as manganese, calcium, vitamin A, magnesium and phosphorus before the expedition, which have been implicated in supporting microbial diversity, intestinal barrier integrity, and immune function[52–56]. Finally, their microbiomes were enriched in *Clostridium fessum*, *Streptococcus salivarius* and the *inosine 5'-phosphate degradation pathway*. *S. salivarius* has been identified as a commensal species with immunomodulatory properties and the ability to produce bacteriocins and anti-inflammatory compounds[57,58], while *Clostridium fessum* may contribute to short-chain fatty acid production, although its role remains less well understood. SCFAs (e.g., acetate, propionate, butyrate) are key microbial metabolites derived from fermentation of dietary fibers and play important roles in host energy metabolism, immune regulation, and gut barrier integrity[59]. The presence of the *inosine 5'-phosphate degradation pathway* may reflect increased nucleotide turnover and energy metabolism potential, which has been observed in microbiomes adapting to metabolic stress[60]. These preliminary findings suggest that baseline microbiome composition and its capacity to dynamically respond to environmental stressors may influence the physiological outcomes of high-altitude exposure. Future studies with larger cohorts are needed to explore these associations further and confirm causality. A similar performance-oriented plasticity is emphasized in our recent narrative review of athletic gut microbiomes[61], which shows that elite performers consistently harbour SCFA- and bile-acid-producing consortia that safeguard energy supply, immunity and barrier function during heat, hypoxia and prolonged exertion - paralleling the altitude-driven shifts observed here.

Importantly, several microbial features that differed within our cohort showed significant correlations with physiological read-outs, suggesting that the compositional shifts we observed may have functional - rather than purely cosmetic - consequences. Before the expedition, several microbiome-host links converged on the same theme: a *Prevotella*-rich, rhamnose-active community was associated with superior lower-limb power and tighter electrolyte control. Specifically, the *dTDP-β-L-rhamnose-biosynthesis pathway* showed a strong positive correlation with both total work and mean anaerobic power of the lower limbs ($\rho = 0.96$ for each; Table 2), while serum potassium related positively to *Prevotella copri* ($\rho = 0.98$) and negatively to *Bacteroides uniformis* ($\rho = -0.97$). Taken together, these baseline patterns indicate that athletes entering the expedition with a *Prevotella*-rich enterotype may enjoy more efficient mineral absorption and rapid carbohydrate utilisation[19] - an interpretation consistent with endurance cohorts in which *Prevotella* enrichment tracks high-carbohydrate, fibre-dense diets and long weekly training volumes[62]. In our climbers, the same triad - *P. copri*, elevated potassium, and the rhamnose nucleotide-sugar route - suggests a microbiome "fitness reserve" that supports energy flux and electrolyte stability when oxygen becomes limited at altitude.

Consistent with the idea that pre-existing gut configurations modulate later physiological responses, our recent case-control study in collegiate men performing maximal Wingate and Bruce tests revealed a different, but conceptually similar, pattern: baseline enrichment of *Clostridium phoceensis* and *Catenibacterium* spp. predicted blunted IL-1α and TIMP-1 responses to the aerobic (Bruce) protocol, whereas anaerobic testing elicited distinct microbiome–cytokine couplings driven by ethanolamine- and pyrimidine-related pathways[28]. After the climb, a distinct post-expedition signature emerged. Enrichment of the *NAD^+de-novo-biosynthesis I pathway* correlated positively with circulating calcium ($\rho = 0.99$) and with basophil counts ($\rho = 0.96$), whereas the *super-pathway of L-serine/glycine biosynthesis* related inversely to sodium ($\rho = -0.96$) but positively to HDL cholesterol ($\rho = 0.96$). In parallel, the *myo-,chiro- and scyllo-inositol degradation pathway* also tracked with basophils ($\rho = 0.96$), and *guanosine-ribonucleotide biosynthesis* showed an inverse relationship with mean platelet volume ($\rho = -0.96$). Post-expedition functional read-outs therefore realigned earlier associations: at baseline both *guanosine-ribonucleotide biosynthesis* and the *myo-/chiro-/scyllo-inositol degradation pathway* tracked positively with serum sodium, whereas after altitude exposure the same routes coupled to MPV and basophils, respectively. This directional reversal underscores a flexible microbiome contribution to nutrient flux, electrolyte balance and immune signalling under chronic hypoxia. Taken together, these links connect post-expedition shifts in nucleotide, redox-cofactor and amino-acid metabolism to haematological (basophils, MPV), electrolyte (sodium, calcium) and lipid (HDL) read-outs, supporting a role for the gut microbiome in coordinating metabolic and immune adaptation under prolonged hypoxia and high energy demand[63]. Importantly, intestinal-permeability markers (zonulin and α_1-antitrypsin) remained unchanged, consistent with recent reports that well-acclimatized alpinists can maintain epithelial integrity under hypobaric stress[8].

A differential enrichment analysis with LEfSe in our study identified 3 species, namely *Blautia luti*, *Romboutsia timonensis* and *GGB4569_SGB6310*, enriched in abundance after the expedition. Similarly, Su et al. showed

a relative and absolute increase in *Blautia A* abundance in men who were exposed to high altitudes (> 3,600 MASL) for more than 2 months[41]. *Blautia A* species were shown to be involved in a module of "cobalamin biosynthesis" and butyric acid production that are both beneficial to microbial ecosystems and intestinal epithelial cells[41,64]. An in vivo animal experiment supported a crucial role of *Blautia A* species in facilitating host fitness to hypoxia environments, likely via anti-inflammation and intestinal barrier protection to maintain intestinal health, thereby suggesting a high translational potential of *Blautia A* species as a candidate probiotic agent for the prevention or treatment of hypoxia-associated maladaptation or disorders. Taken together, these observations of increased abundance of *Blautia A* imply this genus is a beneficial high-altitude bacterial group that may play an important role in promoting acclimatization and adaptation to high-altitude conditions[41].

We observed significant microbial functional associations with dietary intake after the expedition. Notably, strong positive correlations with *coenzyme A biosynthesis* and various dietary components suggest that these nutrients may enhance microbial metabolic pathways essential for energy production and lipid metabolism. Similarly, positive associations of *L-arginine biosynthesis* with overall carbohydrate intake and the assimilation of carbohydrates indicate that dietary carbohydrates may support microbial synthesis of this amino acid, which is crucial for various physiological functions[18,19,63]. Furthermore, associations of the *pentose phosphate pathway* with selenium and plant protein with sucrose biosynthesis highlight the potential role of specific micronutrients and macronutrients in modulating microbial metabolic pathways. On the other hand, negative associations of *adenine and adenosine salvage* pathways with sodium intake, as well as between *L-rhamnose biosynthesis* and animal protein consumption could suggest competitive inhibition or substrate limitation in these metabolic processes. Overall, the results emphasize complex interactions between dietary components and microbial metabolic activities in the gut. However, the sample size for this analysis was limited (n = 7); thus, future validation of these findings in a larger cohort is necessary.

Practical Implications, Limitations, and future perspectives

From an applied perspective, these findings provide valuable guidance for mountaineers, coaches, and sports nutritionists. Pre-expedition strategies might include optimizing micronutrient status, particularly for vitamins and minerals shown here to correlate with beneficial microbial functions and performance markers. During expeditions, integrating nutrient-dense, microbiome-supportive foods – even if partially in the form of supplements or freeze-dried products – may help maintain beneficial gut microbiota profiles and associated metabolic functions. Beyond the athletic context, our findings contribute to a growing body of literature on the role of gut microbiome in adaptation to extreme environments[40,41,65–69], highlighting opportunities to leverage nutritional interventions to support both health and performance.

Climbers should avoid taking non-prescribed antibiotics when possible, as agents such as nifuroxazide can temporarily reduce gut microbial diversity. Gradual acclimatization, along with a diet rich in fiber, polyphenols, and adequate carbohydrates, may further support gut barrier integrity and mitigate AMS-related inflammation[61].

Future research should aim to confirm these findings in larger cohorts and explore targeted interventions-such as prebiotics, probiotics, postbiotics, or tailored micronutrient supplementation to promote a beneficial gut microbiome response in high-altitude environments. Integrating metagenomics with host transcriptomics, metabolomics, and immune profiling, as well as conducting controlled feeding studies, could further elucidate the mechanisms linking the gut microbiome, diet, and physiological adaptation in extreme sports and beyond.

Our study has some limitations. The climbers did not constitute a single group, led by a single climbing goal in the territory of a single country and mountain range. Climbers, characterized by the characteristics described in the study, constitute a small but elite group in Poland, hence several distinct expedition groups, climbing at similar altitudes and with similar sporting goals, but in different parts of the world, qualified for the study. Gathering and encouraging participants was one of the most challenging stages of the project. While all expeditions involved high-altitude exposure above 3000 MASL, other factors such as temperature, humidity, and radiation levels were not controlled for and may have contributed to interindividual differences in microbiome responses. Future studies conducted in more standardized settings are warranted to disentangle the specific environmental drivers of microbiome changes at altitude.

Due to the small number of GI disturbance (n=3) and AMS (n=1) events, the study lacked the power to detect reliable microbiome correlates. Exploratory diversity and taxonomic scans did not reveal outliers (see Supplementary Fig. 3). Likewise, exploratory alpha- and beta-diversity metrics and LEfSe screening (species and MetaCyc pathways, $p<0.05$) failed to identify discriminant features. Larger cohorts are needed to clarify any causal links.

In addition to the level of hypoxia, other factors such as nutrition and the type of physical activity performed in the mountains, as well as the number of active days versus the number of rest days, may contribute significantly to the observed changes in microbiome. Climbing groups active in Peru could have used the downtime between several days of mountain activity to descend to a town at the foot of the mountains to compensate for the negative energy balance there, while expedition groups in Kyrgyzstan, Nepal or Pakistan were dependent on the food they took into the mountains or that the expedition agency offered them.

We acknowledge that some participants consumed sports supplements and functional foods for athletes, which - according to Álvarez-Herms et al.[23] - may influence gut microbiota composition and gut barrier integrity in athletes. Although supplement intake was recorded in our study, we did not specifically analyze the potential impact of additives commonly found in these products (e.g., emulsifiers, artificial sweeteners, preservatives) on the gut microbiota. Furthermore, we did not observe a clear association between general supplement use and microbiome shifts or performance outcomes. Nevertheless, considering the small sample size, individual variability, and heterogeneous supplement composition, we cannot fully exclude a modulatory effect. Future studies should incorporate detailed assessments of supplement ingredients, including non-nutritive additives, to better understand their influence under high-altitude stress conditions.

Conclusions

In conclusion, our study demonstrates that the gut microbiome is responsive and potentially malleable to the dietary and environmental stresses encountered during high-altitude mountaineering. The observed changes in microbial composition and function, their associations with micronutrient intake, and their potential links to improved athletic performance underscore the integral role of the microbiome as an adaptive partner in extreme human endeavors[61].

Data availability

The raw fastq sequences have been deposited to the European Nucleotide Archive (ENA) under the accession PRJEB84244.

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Author contributions

EKG, BF and KHL conceptualized the study; EKG, KZ and KHL conducted the data collection and processing; EKG, KHL and KZ produced metadata; KZ, PPŁ and TK performed the statistics and bioinformatics analysis; EKG and BF obtained funding; EKG and BF provided of study materials; EKG and KHL developed methodology; KZ visualized the data; EKG, BF and KHL managed the project; BF and KHL supervised the project; EKG, KZ and KHL drafted the manuscript. All authors authors edited the manuscript and contributed to the final version of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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Declarations

Competing interests
The authors declare no competing interests.

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Załącznik 2. Oświadczenia współautorów publikacji

Oświadczenie współautorów publikacji

Karpęcka-Gałka, E.; Mazur-Kurach, P.; Szyguła, Z.; Frączek, B. Diet, Supplementation and Nutritional Habits of Climbers in High Mountain Conditions. *Nutrients*. **2023**;15(19):4219. https://doi:10.3390/nu15194219

Niniejszym oświadczamy, że indywidualny wkład w powstanie ww. publikacji jest następujący:

Autor	Wkład [%]	Udział Autorów określony w publikacji czasopisma (Author Contributions)*	Podpis
Ewa Karpęcka-Gałka	65	K, M, Fa, B, T, Wr, Wo, Zp, F	Ewa Karpęcka-Gałka
Paulina Mazur-Kurach	15	Fa, Wo, S	Paulina Mazur-Kurach
Zbigniew Szyguła	5	S	Zbigniew Szyguła
Barbara Frączek	15	M, Wo, S, F	Barbara Frączek

***K** – koncepcja projektu (conceptualization), **B** – przeprowadzenie badań (investigation), **M** - opracowanie metodologii badań (methodology), **Fa** – analiza formalna (formal analysis), **Z** – zasoby (resources), dostarczanie materiałów badawczych (provision of study materials), **T** – tworzenie metadanych i zachowywanie danych badawczych (data curation), **Wr** – przygotowanie wstępnej wersji artykułu (writing – orginal draft preparation), **Wo** – współuczestniczenie w przygotowaniu manuskryptu na etapie recenzji/korekty (writing – review and editing), **S** – superwizja (supervision), **Zp** – zarządzanie projektem (project administration), **F** – pozyskiwanie finansowania (funding acquisition).

Ewa Karpęcka-Gałka
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Podpis Doktorantki

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Oświadczenie współautorów publikacji

Karpęcka-Gałka, E.; Frączek B. Nutrition, hydration and supplementation considerations for mountaineers in high-altitude conditions: a narrative review. *Front. Sports Act. Living.* **2024**; 6:1435494. https://doi:10.3389/fspor.2024.1435494

Niniejszym oświadczamy, że indywidualny wkład w powstanie ww. publikacji jest następujący:

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Ewa Karpęcka-Gałka
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Podpis Doktorantki

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Podpis Promotora

Oświadczenie współautorów publikacji

Karpęcka-Gałka, E.; Bawelski, M.; Pięta, A.; Mazur-Kurach, P.; Pięta, P.; Frączek, B. The Impact of a High-Altitude Expedition on the Physical Performance and Nutritional Indices of Health Status of Alpinists. *J. Funct. Morphol. Kinesiol.* **2025**, *10*, 143. https://doi.org/10.3390/jfmk10020143

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Autor	Wkład [%]	Udział Autorów określony w publikacji czasopisma (Author Contributions)*	Podpis
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Aleksandra Pięta	10	Fa, B	
Paulina Mazur-Kurach	10	Fa,	
Paweł Pięta	5	B	
Barbara Frączek	5	K, M, Wo, S, F	

***K** – koncepcja projektu (conceptualization), **B** – przeprowadzenie badań (investigation), **M** - opracowanie metodologii badań (methodology), **Fa** – analiza formalna (formal analysis), **Z** – zasoby (resources), dostarczanie materiałów badawczych (provision of study materials), **T** – tworzenie metadanych i zachowywanie danych badawczych (data curation), **Wr** – przygotowanie wstępnej wersji artykułu (writing – orginal draft preparation), **Wo** – współuczestniczenie w przygotowaniu manuskryptu na etapie recenzji/korekty (writing – review and editing), **S** – superwizja (supervision), **Zp** – zarządzanie projektem (project administration), **F** – pozyskiwanie finansowania (funding acquisition).

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Oświadczenie współautorów publikacji

Karpęcka-Gałka, E.; Pięta, A.; Świtała, K.; Frączek, B. Selected aspects of the habitual diet and levels of intestinal permeability markers of Polish alpinists. *Physical Activity Review*. **2026**; 14(1):1-13. doi:10.16926/par.2026.14.01

Niniejszym oświadczamy, że indywidualny wkład w powstanie ww. publikacji jest następujący:

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Katarzyna Świtała	15	D	Katarzyna Świtała
Barbara Frączek	5	A, E	Barbara Frączek

*__A__ – przygotowanie projektu badania (study design), __B__ – gromadzenie danych (data collection), __C__ – analiza statystyczna (statistical analysis), __D__ – przygotowanie manuskryptu (manuscript preparation), __E__ – gromadzenie funduszy (funds collection)

Ewa Karpęcka-Gałka
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Podpis Promotora

Oświadczenie współautorów publikacji

Karpęcka-Gałka, E.; Zielińska, K.; Frączek, B.; Łabaj, P.P.; Kościółek, T.;, Humińska-Lisowska, K. High-altitude mountaineering induces adaptive gut microbiome shifts associated with dietary intake and performance markers. *Scientific Reports* **2025**. doi: 10.1038/s41598-025-22848-9

Niniejszym oświadczamy, że indywidualny wkład w powstanie ww. publikacji jest następujący:

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Barbara Frączek	5	K, Z, S, Wo, Zp, F	
Paweł Łabaj	5	Fa, Wo	
Tomasz Kościółek	5	Fa, Wo	
Kinga Humińska-Lisowska	20	K, B, M, T, Wr, Wo, S, Zp	Humińska-Lisowska

***K** – koncepcja projektu (conceptualization), **B** – przeprowadzenie badań (investigation), **M** - opracowanie metodologii badań (methodology), **Fa** – analiza formalna (formal analysis), **W** – wizualizacja graficzna (visualization), **Z** – zasoby (resources), dostarczanie materiałów badawczych (provision of study materials), **T** – tworzenie metadanych i zachowywanie danych badawczych (data curation), **Wr** – przygotowanie wstępnej wersji artykułu (writing – orginal draft preparation), **Wo** – współuczestniczenie w przygotowaniu manuskryptu na etapie recenzji/korekty (writing – review and editing), **S** – superwizja (supervision), **Zp** – zarządzanie projektem (project administration), **F** – pozyskiwanie finansowania (funding acquisition).

Ewa Karpęcka-Gałka
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Podpis Promotora

Przebieg pracy naukowo-zawodowej – informacje dodatkowe

Publikacje naukowe (spoza cyklu)

1. Szot M, Karpęcka-Gałka E, Dróżdż R, Frączek B. Can Nutrients and Dietary Supplements Potentially Improve Cognitive Performance Also in Esports?. *Healthcare (Basel)*. 2022;10(2):186. Published 2022 Jan 18. doi:10.3390/healthcare10020186
2. Balwierz R, Jasiński K, Osowski M, Grela, K, Karpęcka-Gałka E, Kusakiewicz-Dawid A, Skotnicka-Graca U. Rola diety i metod leczenia żywieniowego w ograniczeniu dolegliwości zespołu jelita drażliwego. *Farmacja Polska*. 2021;77. doi:10.32383/farmpol/138856
3. Frączek B, Gacek M, Karpęcka E. Alcoholic beverage preferences and consumption among Polish professional athletes. Human Movement. 2020;21(2):71-77. doi:10.5114/hm.2020.89917.
4. Frączek B, Gacek M, Pięta A, Tyrała F, Mazur-Kurach P, Karpęcka E. Dietary mistakes of Polish athletes in relation to the frequency of consuming foods recommended in the Swiss food pyramid for active people. *Rocz Panstw Zakl Hig*. 2020;71(1):97-104. doi:10.32394/rpzh.2020.0104
5. Karpęcka E, Fraczek B. Macronutrients and water – do they matter in the context of cognitive performance in athletes? Balt J Health Phys Act. 2020;12(3):114-135. doi: 10.29359/BJHPA.12.3.11

Staże naukowe

Staż naukowy krajowy pod opieką dr Kingi Humińskiej-Lisowskiej na Akademii Wychowania Fizycznego i Sportu im. Jędrzeja Śniadeckiego w Gdańsku (Wydział Kultury Fizycznej, Katedra Zdrowia i Nauk Przyrodniczych, Zakład Biologii Molekularnej) w terminie 25.07.-28.07.2023. Głównym jego celem było podniesienia kwalifikacji naukowych i praktycznych w tematyce analiz mikrobiomu (izolowanie DNA).

Udział w projektach naukowych

W charakterze kierownika

Projekt w ramach Konkursu Młodych Naukowców AWF Kraków pt.: „Wpływ miesięcznej wyprawy wysokogórskiej na stan zdrowia, sposób żywienia i poziom wydolności fizycznej himalaistów” (nr: 147/MN/INB/2021).

W charakterze wykonawcy

1. Projekt w ramach grantu Regionalna Inicjatywa Doskonałości AWF Kraków pt.: „Wybrane psychologiczne i genetyczne uwarunkowania preferencji pokarmowych w grupie osób rekreacyjnie uprawiających sport” (nr: 30/RID/2025).
2. Projekt w ramach grantu Regionalna Inicjatywa Doskonałości AWF Kraków pt.: „Analiza sposobu żywienia, podaży probiotycznych źródeł pokarmowych oraz suplementacji probiotycznej jako czynników modulujących przepuszczalność jelitową w grupie studentów aktywnych fizycznie” (nr: 31/RID/2025).

3. Projekt w ramach grantu Regionalna Inicjatywa Doskonałości AWF Kraków pt: „Wpływ miesięcznej wyprawy wysokogórskiej na strukturę mikrobiomu wspinaczy" (nr: 39/PB/RID/2022).
4. Projekt w ramach badań statutowych AWF Kraków pt.: „Ocena poziomu wydolności fizycznej, kosztów energetycznych treningów oraz sposobu żywienia grupy polskich wspinaczy wyczynowych" (nr: 238/BS/INB/2020).

Aktywne uczestnictwo w konferencjach naukowych

1. Karpęcka-Gałka E, Frączek A, Pięta A. *Antioxidant Capacity and Oxidative Stress Response to a Paleolithic Diet in Professional Athletes*, konferencja „Free radicals in biology, medicine, sport and nutrition", Gdańsk, 27–28.06.2025 r. (sesja posterowa).
2. Tyrała F, Pięta A, Szot M, Karpęcka-Gałka E, Mazur-Kurach P, Frączek B. *Wartość odżywcza niskoenergetycznych diet roślinnych w kontekście wpływu żywienia na zdrowie.* XXX Ogólnopolskie Sympozjum Bromatologiczne oraz III Ogólnopolska Konferencja Naukowa „Żywność i Żywienie w pigułce". Gdańsk, 16-18. 09.2024 r. (sesja posterowa).
3. Karpęcka-Gałka E, Frączek B. *Nutritional habits of climbers in high mountain conditions.* 4th World Scientific Congress "Quality of Life in Interdisciplinary Approach", Kochcice, 25-27.10.2023 r. (sesja posterowa).
4. Karpęcka-Gałka E. *Dieta wspinaczy w czasie wyprawy wysokogórskiej.* Międzynarodowa Konferencja Naukowa Dni Medycyny Społecznej i Zdrowia Publicznego Polska 2022. Kraków, 15.09.2022 r. (wystąpienie ustne).
5. Karpęcka-Gałka E, Frączek B. *Eating habits and food attitudes of climbers in high mountain conditions*. 27th Annual Congress of the European College od Sport Science. Sewilla, 30.08.– 2.09.2022 r. (e-poster).
6. Karpęcka-Gałka E. *Podaż wybranych makroskładników w diecie wspinaczy podczas wyprawy wysokogórskiej.* II Kongres Młodej Nauki. Gdańsk, 7-10.07.2022 r. (sesja posterowa).
7. Karpęcka E, Tyrała F, Pięta A, Frączek B. *Assessment of antioxidant micronutrient intake in the group of Polish professional athletes.* 25th ECSS Anniversary Congress. Online, 28-30.10.2020 r. (e-poster).

Praca dydaktyczna

Prowadzenie zajęć dydaktycznych w Zakładzie Medycyny Sportowej i Żywienia Człowieka Akademii Kultury Fizycznej w Krakowie od października 2024 r.

Dodatkowo działalność środowiskowa

Działalność popularyzatorska

Wybrane prelekcje, prezentacje, warsztaty:

1. Webinar dla firmy Norsan, „Żywienie i suplementacja w warunkach wysokogórskich oraz zastosowanie omega-3", 11.09.2025 r.
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Działalność organizacyjna

Współorganizacja „Maratonu Wykładów - Dietetycy dla Ukrainy”, działanie charytatywne, cykl wykładów prowadzonych przed dietetyków, którego celem było zebranie środków na pomoc dla walczących o swój kraj Ukrainek i Ukraińców. „Żywienie w warunkach wysokogórskich”, 8-22.03.2022.

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