Aus der Klinik für Anästhesiologie

Klinikum der Universität München

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Regulation der menschlichen Allostase durch sympathoadrenerge, glukokortikoide, purinerge und endocannabinoide Systeme unter Einfluss normo- und hypobarer Hypoxie und simulierter Schwerelosigkeit

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1 Hintergrund

Die Definition und Einteilung von Stress ist bis heute uneinheitlich und kann je nach Fokus unterschiedlich ausfallen. Zu Beginn der Stressforschung wurden damit vornehmlich Situationen beschrieben, die den bei Mensch und Tier von der Natur angelegten Überlebensmechanismus zur Kampf-oder-Flucht Reaktion *(fight-or-flight response)* auslösen (11). Die wichtigsten Wirkstoffe bilden hierbei zunächst vorrangig die stresspermissiven Hormone Adrenalin und Cortisol.
Eine erste umfassendere Definition und Konzeption des Begriffes wurde von Hans Selye in den 1930er Jahren eingeführt. Er erklärte **Stress** als eine Reaktion und Antwort des Körpers auf von außen auf ihn einwirkende Veränderungen (88). Dabei kam es im Verlauf zu einer Fehlprägung des Begriffes aufgrund sprachlicher Missverständnisse, da der Begriff „Stress" im physikalischen Sinne die Kraft beschreibt, die auf eine bestimmte Fläche eines Materials einwirkt. Im Sinne Selyes handelte es sich jedoch vielmehr um die proportional zur Masse einer einwirkenden Kraft (Stress) auftretende Deformation eines formbaren Gegenstandes, die im Englischen durch Hooke´s Law 1660 als *„Strain"* definiert wurde. Dieser Begriff hätte Selyes Verständnis seines Konzepts daher genauer wiedergegeben. Vor diesem Hintergrund formulierte Selye das allgemeine Anpassungssyndrom (86). Hierbei reagiert der Organismus auf länger einwirkende Stressoren zunächst mittels Alarmreaktion, die den Körper in gesteigerte Aktivität versetzt. Folgend etabliert sich die Widerstandsphase, um das Stressniveau des Organismus wieder zu reduzieren. Gelingt dies nicht oder hält der Stresszustand an, tritt das Erschöpfungsstadium ein, das dauerhafte Langzeitschäden des Organismus nach sich ziehen kann. Diesem Konzept liegt die Erkenntnis zugrunde, dass unterschiedliche Stressoren bei verschiedenen Individuen einheitliche Symptomkomplexe hervorrufen, die als unspezifische Antwort des Organismus auf Störungen des physiologischen Gleichgewichts, der **Homöostase**, zu werten sind. Ob und in welchem Ausmaß ein Organismus chronischen Stress toleriert, hängt von dessen Fähigkeit ab, sich an den Stressor anzupassen. Mangelnde Anpassungsfähigkeit führt zu einer Störung der Homöostase, die negative Auswirkungen auf alle Organsysteme hat und schließlich die Gesundheit des Organismus im Gesamten beeinträchtigen kann. Aufbauend auf diesem Konzept prägte Selye auch die Begriffe „**Eu- und Disstress**", die die Natur des Stressors im Sinne von „gutem" und „schlechtem" Stress definieren. Sie können jedoch unabhängig ihrer Definition im Langzeitverlauf Schäden am Organismus hinterlassen (87). In der weiteren Erforschung des Stresses verlagerte sich der Fokus dann zunächst vermehrt auf die Stressbewältigung *(Coping)* und seine Bewertung *(Appraisal)* und fand hauptsächlich hierin seine Definition (59).

<u>Vom Erhalt der Homöostase zur Allostase</u>

In den 1990ern stellte sich dann heraus, dass der Begriff der Homöostase die Komplexität des menschlichen Organismus und seiner Physiologie nicht umfassend genug abbildet, da er lediglich das Gleichgewicht durch die Aufrechterhaltung einiger weniger lebenswichtiger Parameter beschreibt, die für die Homöostase von Bedeutung sind (z.B. pH, Blutzucker, Körpertemperatur). Die an der Stressbewältigung beteiligten Systeme und Mechanismen (z.B. Hypothalamus-Hypophysen-Nebennierenrinden-Achse, Zytokine, Katecholamine) sorgen jedoch vielmehr für ein „Gleichgewicht durch Veränderung" (99) und stellen somit einen sich im Fluss befindenden Prozess dar, der sich den zu regulierenden Gegebenheiten anpasst und so letztendlich die Homöostase unterstützt. Diese Erkenntnis prägte den Begriff der **Allostase** (99). Eine Dauerbelastung des Organismus durch rezidivierende Aktivierung oder fehlende Adaptation an wiederkehrende Stressoren führt zu Dysregulation des empfindlichen Gleichgewichts und letztendlich zu Krankheit (*allostatic overload*) (65, 66) (Abbildung 1).

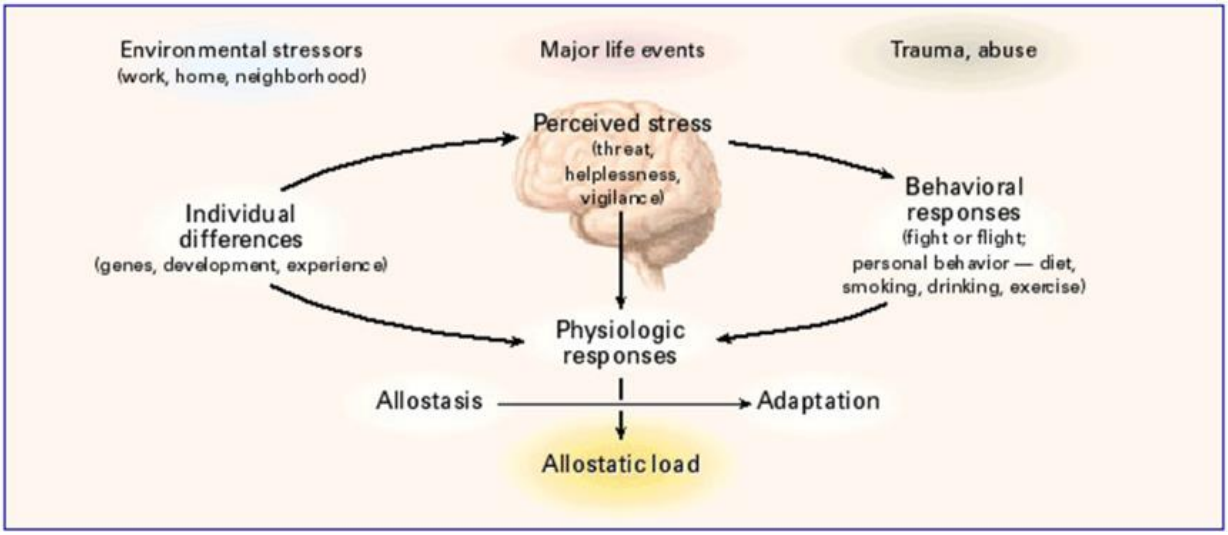

Abbildung 1: *Die Stressantwort und die Entstehung von allostatischer Last.* Die Wahrnehmung von Stress wird von individuellen Erfahrungen, Verhaltensmustern und genetischen Voraussetzungen beeinflusst. Wenn das Gehirn ein Ereignis als anstrengend und/oder aufreibend wahrnimmt, dann werden physiologische Reaktionen und Verhaltensmuster in Gang gesetzt, die zu Allostase und Adaptation führen. Über die Zeit akkumuliert die allostatische Last, so dass die dauerhafte Belastung mit Stressmediatoren negative Effekte auf die verschiedenen Organsysteme hat und konsekutiv krankmachend wirkt (modifiziert nach (65)).

Des Weiteren wandelte sich im Verlauf auch die Definition von „gutem“ und „schlechtem“ Stress aufgrund der Beobachtung, dass bei adäquater Adaptation Stress nicht zwangsläufig nur schädliche, sondern teils auch begünstigende Auswirkungen auf den Organismus haben kann (22, 23). Vor diesem Hintergrund gewannen die Begriffe „akuter“ versus „chronischer“ Stress mit ihrer zeitlichen Definition zunehmend an Bedeutung und stellen aktuell ein Hauptkriterium der Stresseinteilung dar (25). Zudem zeigte sich, dass individuell sehr große Unterschiede hinsichtlich Stresswahrnehmung, -bewertung, -verwertung und –bewältigung bestehen (41). Diese können ihre Ursache sowohl in unterschiedlichen genetischen Anlagen haben, als auch in verschiedenen Umwelteinflüssen, denen das Individuum ausgesetzt war (24).

In der Zusammenschau wird deutlich, wie umfassend die Thematik „Stress“ ist, und sie veranschaulicht und hebt auch deren allgemeine Bedeutung hervor.

Stress und seine Konsequenzen sind inzwischen ein wesentlicher Faktor in unserer Gesundheitsökonomie und sind damit von herausragendem Interesse in der Medizin und der öffentlichen Wahrnehmung. Zuletzt erläuterte ein Bericht in DER SPIEGEL, dass Stress die Zahl der Fehltage von Arbeitnehmern in Deutschland von 2012 bis 2016 auf das 1,5-fache pro Jahr verdoppelt hat (1, 44).

Dies unterstreicht die Notwendigkeit, die wissenschaftlichen Kenntnisse auf diesem Gebiet zu erweitern und die enge Verzahnung von Stressursache und –wirkung zu verstehen. Das Wissen kann dann genutzt werden, Präventions- und Therapiemaßnahmen zu etablieren und schädliche Auswirkungen von Stress auf den Menschen zu vermeiden oder zu lindern.

In der Medizin ist dieses Wissen in der täglichen Praxis aber v.a. auch in der Anästhesie und Intensivmedizin (Stressabschirmung) von Relevanz. Der Intensivpatient repräsentiert im Hinblick auf die physiologische Stressantwort das Maximalbeispiel allostatischer Last, da er sowohl externen (Lärm auf Intensivstation, Untersuchungen etc.) als auch internen (u.a. Infektion, Inflammation) Stressoren ausgesetzt ist. Für die Forschung auf diesem Gebiet bieten sich komplementäre Möglichkeiten. Sie kann einerseits im klinischen Rahmen am Patienten erfolgen, allerdings mit der

Einschränkung einer relativ hohen Variabilität der Bedingungen (v.a. der internen Stressoren). Andererseits können im Gegensatz hierzu standardisierte Bedingungen in einer experimentellen Umgebung genutzt werden, um per se gesunde Probanden in dieser Umwelt auf Stressreaktionen zu untersuchen. Die Standardisierung der Versuchsbedingungen trägt zu einer besseren Vergleichbarkeit bei und ermöglicht so auch Untersuchungen in geringerer Anzahl („n“). Auf diese Weise kann eine Beantwortung spezifischer Fragestellungen trotz individueller Variabilität eher gelingen. Die Forschung am Menschen im Weltraum bietet genau diese Rahmenbedingungen und kann dazu beitragen, herauszufinden, welche Mechanismen den menschlichen Organismus in extremer Umwelt unter Exposition spezifischer Stressoren vor Schäden und Krankheiten schützen. Astronauten erfahren bei ihrem Flug ins All den Einfluss zahlreicher Stressoren in maximaler Ausprägung, so dass Kenntnisse über die physiologischen Stressantworten essenziell und folglich auch entscheidend für den Erfolg oder Misserfolg einer Mission sind. Das Wissen um ihr physiologisches Stress-Antwortprofil kann prospektiv auch dazu beitragen, rechtzeitig Abwehr- und Bewältigungsmechanismen zu etablieren, um Beeinträchtigungen und Schäden vorzubeugen. Dies kann dann auch für die Medizin im klinischen Alltag von großem Nutzen sein.

Die im Einzelnen in dieser Umgebung wirkenden Stressoren sind vor allem die Langzeit-Isolation, veränderte Sauerstoff- und Druckverhältnisse (z.B. Hypoxie), eine veränderte zirkadiane Rhythmik, die Schwerelosigkeit sowie vermehrte Strahlen- und/oder Keimbelastung (Abbildung 2). Zur Erforschung ihrer Auswirkungen werden integrative Projekte auf der Internationalen Raumstation ISS sowie weitere Versuchs-anordnungen, die weltraum-ähnliche Bedingungen schaffen, genutzt. So z.B. Isolationsstudien wie das MARS500-Projekt, Bettruhe-Studien (*bed rest*), Feldversuche in der Antarktis oder in Unterwasser-Forschungsein-richtungen.

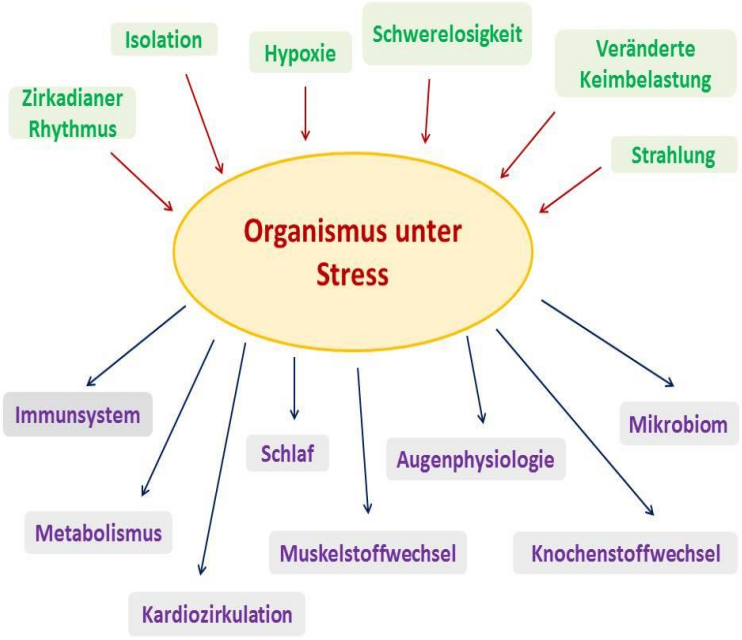

Abbildung 2: *Unterschiedliche weltraumassoziierte Stressoren und die betroffenen physiologischen Systeme des menschlichen Organismus*

In den letzten Jahren zeigten die Ergebnisse solcher systematischen Untersuchungen am Menschen, dass die Exposition gegenüber diesen Stressoren zu Veränderungen des Immunsystems (19, 31), der Kardiozirkulation (51, 85), des Knochen- und Muskelstoffwechsels (82, 117), des Metabolismus (107), der Augen- sowie der Schlafphysiologie (60, 76) und der mikrobiologischen Darmflora (13) führt (Abbildung 2). Und trotz der strengen Auswahlkriterien der Astronauten (z.B. guter Trainings-, gesunder Ernährungs- und Allgemeinzustand) existieren interindividuelle Unterschiede in der Anpassungsfähigkeit an das Leben im Weltraum und bei der Rückkehr auf die Erde (17). In diesem Kontext werden daher auch geschlechtsspezifische Anpassungsreaktionen genauer zu erforschen und einzuordnen sein.

Die Weltraumforschung bietet somit eine einzigartige Plattform, die physiologische Stressantwort zu evaluieren und zu quantifizieren, um dann die gewonnenen Erkenntnisse - mit entsprechender Vorsicht – auf den klinischen Alltag anzuwenden.

2 Fragestellung und Zielsetzung des Habilitationsprojektes

Die übergeordnete Zielsetzung dieser Arbeit bestand in der Evaluation und Quantifizierung der physiologischen Stressantwort hervorgerufen durch den Einfluss von Stressoren in extremen aber weitestgehend standardisierten Umwelten. Der Fokus lag auf den Auswirkungen von Hypoxie und simulierter Schwerelosigkeit (durch Bettruhe) und den dadurch hervorgerufenen Veränderungen spezifischer stressassoziierter Systeme (Abbildung 3).

Daraus ergaben sich folgende Fragestellungen:

1.) Verursachen Hypoxie und simulierte Schwerelosigkeit - einzeln oder in Kombination - eine Stressantwort im Sinne von Veränderungen des sympathoadrenergen, des glukokortikoiden oder purinergen sowie des endcannabinoiden Systems?

2.) Welches Ausmaß zeigt diese Stressantwort und wie verläuft ihre Dynamik und Kinetik auch hinsichtlich einer akuten oder chronischen Einwirkung dieser Stressoren?

3.) Ist die Stressantwort durch geschlechtsspezifische Unterschiede gekennzeichnet?

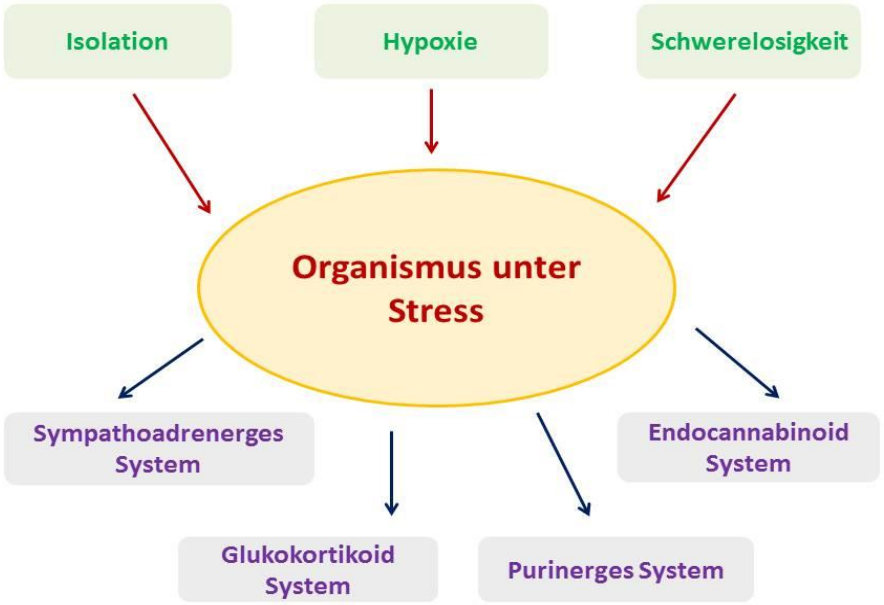

Abbildung 3: *Spezifische Stressoren in extremer Umwelt und Untersuchung spezieller stressassoziierter physiologischer Systeme*

3 Forschungsplattformen und Studienprotokolle

3.1 Olympia-Trainingszentrum Planica – Erforschung der Auswirkungen von Hypoxie und simulierter Schwerelosigkeit (Bettruhe)

Das Olympia-Trainingszentrum in Planica (Ratece, Slowenien) liegt nur 940 m über dem Meeresspiegel, aber durch Reduktion der inspiratorischen Sauerstofffraktion (FiO_2) können auf einem gesamten Stockwerk in allen Räumlichkeiten (Schlafzimmer, Bäder, Aufenthaltsraum etc.) hypoxische Luftverhältnisse erzeugt werden. Der atmosphärische Druck (P_{atm}) bleibt dabei konstant, so dass im Vergleich zu einem realen Höhenaufenthalt normobare statt hypobare Hypoxie entsteht. Auf diese Weise ist eine Simulation inspiratorischer Sauerstofffraktionen, die auf Höhen von bis zu 5400 m über dem Meeresspiegel (Höhe des Mount-Everest Basiscamps) herrschen, möglich. Das Zentrum wird daher zu Forschungszwecken, aber auch von Sportlern zum Training und zur Wettkampfvorbereitung genutzt.
In diesem Umfeld führten wir gemeinsam mit einem aus acht Partnern bestehenden Konsortium die EU- geförderte (7. Rahmenprogramm, FP07) Planetary Habitat Studie (***PlanHab***) zur Untersuchung der Auswirkungen von normobarer Hypoxie und simulierter Schwerelosigkeit auf die menschliche Physiologie durch. Schwerelosigkeit wurde durch Bettruhe simuliert, da Studien zeigten, dass es sich um ein adäquates Modell handelt, um ihre Auswirkungen auf die verschiedenen physiologischen Systeme nachzustellen (34, 71).

3.1.1 Studienprotokoll

Die Studie wurde von der Ethikkommission des Gesundheitsministeriums von Slowenien genehmigt, und das Studienprotokoll entsprach den Kriterien der Europäischen Raumfahrtorganisation (ESA) für Bettruhestudien (Standardisierung von Bettruhe-Bedingungen, Version 1.5, August 2009 mit internationaler Überarbeitung 2014 in den „Leitlinien zur Standardisierung von Bettruhe-Studien in Zusammenhang mit der Raumfahrt“ durch die Internationale Akademie der Astronautik (IAA)).
Die Studie unterlag einem prospektiven Cross-over Design, so dass die 14 gesunden, männlichen Probanden innerhalb des Studienjahres drei verschiedene Studienprotokolle durchliefen. Zwischen den Studienarmen lagen jeweils 4 Monate Pause, so dass von einer vollständigen Normalisierung der gemessenen Parameter ausgegangen werden kann. Die Zuteilung erfolgte randomisiert.
Der erste Studienarm bestand aus normoxischer Bettruhe (NBR), der zweite aus hypoxischer Bettruhe (HBR) und im dritten unterlagen die Probanden Hypoxie allerdings mit Bewegungsfreiheit in einem begrenzten Umfeld (HAMB – hypoxische Ambulation). Die Ausprägung der Hypoxie war einer Höhe von ~ 4000 m äquivalent und auch die weiteren Raum- bzw. Umweltbedingungen wurden standardisiert kontrolliert (Tabelle 1). Alle alltäglichen Abläufe und Tätigkeiten (Essen, Körperhygiene etc.) wurden in Bettruhe in horizontaler Position verrichtet. Außerdem erhielten die Probanden eine standardisierte Diät während der Interventionsphase.
Jeder Intervention ging eine 7-tägige Vorlaufphase zur Basisdatenerhebung voraus, daraufhin folgten 21 Tage unter Interventionsbedingungen und am Ende schloss sich eine 4-tägige Erholungsphase an.
Es wurden anonymisierte Blutproben zur Bestimmung von Endocannabinoiden und Purinen sowie Urin- und Speichelproben zur Analyse des sympathoadrenergen und glukokortikoiden Systems gesammelt.

	NBR	HBR	HAMB
FiO_2 [%]	0.209	0.141 ± 0.004	0.141 ± 0.004
PiO_2 [mmHg]	133.1 ± 0.3	90.0 ± 0.4	90.0 ± 0.4
		Alle Interventionen	
P_{atm} [mmHg]		684 ± 4	
RH [%]		53.5 ± 5.4	
Temperatur [°C]		24.4 ± 0.7	

Tabelle 1: *Sauerstoffverhältnisse der verschiedenen Interventionen;* FiO_2 = inspiratorische Sauerstofffraktion; PiO_2 = inspiratorischer Sauerstoffpartialdruck; P_{atm} = atmosphärischer Luftdruck; RH = relative humidity (relative Luftfeuchtigkeit); NBR = normoxische Bettruhe; HBR = hypoxische Bettruhe; HAMB = hypoxische Ambulation

3.2 Antarktis – Erforschung der Auswirkungen von Hypoxie und Isolation

Die Antarktis ist mit 14 000 000 km^2 der fünftgrößte Kontinent der Erde und ist zu 98% mit Eis bedeckt. Hier finden sich die im Durchschnitt kältesten Temperaturen, die größten Windstärken, und sie zählt zu den niederschlagärmsten, trockensten Regionen der Erde. Es existiert keine einheimische Bevölkerung. Die Jahreszeiten beschränken sich auf Sommer und Winter und sind den Jahreszeiten der Nordhalbkugel entgegengesetzt. Der antarktische Sommer dauert von Anfang November bis Anfang Februar und der antarktische Winter von Mai bis August. Der längste Tag (Mittsommer) liegt somit im Dezember und der kürzeste (Mittwinter) im Juni. Es kommt zum Verlust eines regelhaften Tag-Nacht-Rhythmus, da im antarktischen Sommer 24 Stunden Helligkeit und im antarktischen Winter 24 Stunden Dunkelheit herrschen. Diese extremen Wetterbedingungen machen eine An- oder Abreise zu oder von den antarktischen Forschungsstationen während des antarktischen Winters (Februar/März bis November) unmöglich. Außerdem ist die Telekommunikation (Internet, Telefon etc.) v.a. im Winter stark von den herrschenden Wetterbedingungen abhängig.

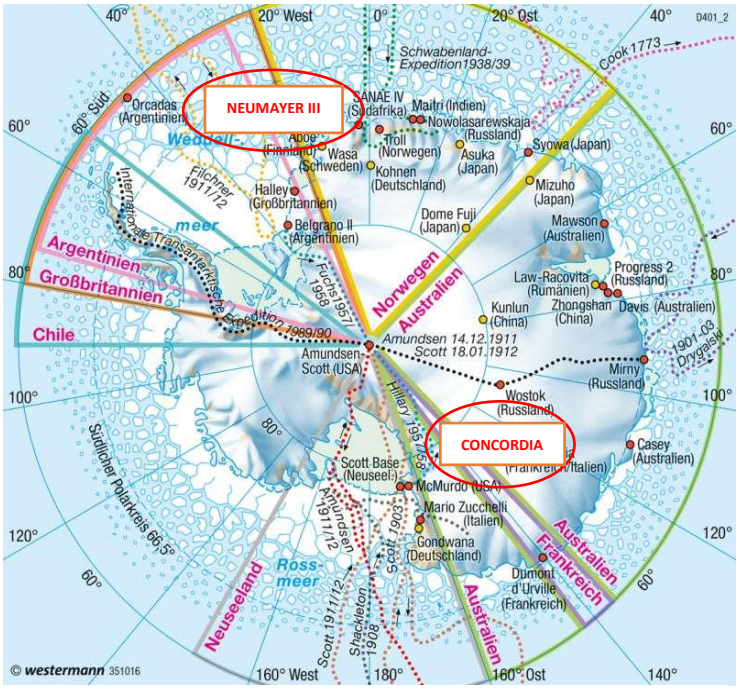

Abbildung 4: *Antarktis;* Lokalisation der verschiedenen internationalen Forschungsstationen (darunter Concordia und Neumayer III); Darstellung der sektorförmigen Hoheitsansprüche der verschiedenen Staaten (https://www.diercke.de/content/antarktis-hoheitsansprücheforschung-100849-169-6-1); modifiziert durch C. Strewe.

Die Antarktis ist mit ihren klimatischen Bedingungen eine menschenfeindliche Umwelt, die zahlreiche Stressoren vereint, die in der Stressbewältigung für den Menschen und seine physiologischen Adaptationsmechanismen große Herausforderungen darstellen.

3.2.1 Concordia Station

Die Forschungsstation befindet sich im Inneren der Ostantarktis auf einem Eis-Plateau (Dome C) 3233 m über dem Meeresspiegel (herrschender Luftdruck ~ 650 hPa) (Abbildung 4). Die geographischen Daten sind 75° 06' S Breite und 123° 21' E Länge. Die nächste Küstenstation ist ca. 1100 km entfernt.
Die Temperaturen schwanken im antarktischen Sommer zwischen -30 und -50 °C und im antarktischen Winter zwischen -60 und -80 °C.
Die Station wird seit 2005 ganzjährig in französisch-italienischer Kooperation betrieben. Im antarktischen Sommer bietet sie Platz für 50-70 Personen. In der antarktischen Wintersaison können 13-15 Personen auf der Station überwintern.

3.2.2 Neumayer III Station

Die küstennahe deutsche Forschungsstation liegt in der Atka-Bucht im nordöstlichen Weddellmeer auf dem Ekström Schelfeis und befindet sich auf Meereshöhe (Abbildung 4). Die geographischen Daten sind 70° 40' S Breite und 8° 16' W Länge. Die klimatischen Bedingungen und die Dauer der reinen Dunkel- oder Hellphasen im Winter und Sommer sind aufgrund der geographischen Lage weniger stark ausgeprägt als auf Concordia.
Die Durchschnittstemperatur im Sommer beträgt ~ -3 °C. Im Winter fallen die Temperaturen auf durchschnittlich -30 °C.

3.2.3 Studienprotokoll

Das Studienprotokoll wurde von der Ethikkommission der Universität München genehmigt [Protokolle #332-08, 524-15]. Alle Probanden gaben ihr schriftliches Einverständnis in Übereinstimmung mit der Erklärung von Helsinki.
Probanden der ersten Feldstudie waren die männlichen Teilnehmer von zwei Überwinterungskampagnen auf Concordia (2016/17; n = 15) und von drei Überwinterungskampagnen auf Neumayer III (2013-15; n = 16).
Die Probanden von Neumayer III galten als Kontrollgruppe für die Probanden von Concordia, da sie im Vergleich nicht hypobarer Hypoxie ausgesetzt waren.
Probanden der zweiten Feldstudie waren die männlichen und weiblichen Teilnehmer der drei Überwinterungskampagnen auf Neumayer III (Männer n = 16; Frauen n = 10).
Wir führten Blutentnahmen zur Bestimmung von Endocannabinoiden und Purinen durch und entnahmen Urin- und Speichelproben zur Analyse des sympathoadrenergen und glukokortikoiden Systems. Die Basisdatenerhebung der jeweiligen Expedition erfolgte in Europa 1-2 Monate vor Abreise. Während des einjährigen Antarktisaufenthaltes wurden dann monatlich (in der ersten Woche jedes Monats) und abschließend nochmals einmalig mehrere Monate nach Rückkehr aus der Antarktis nach Europa (April/Mai des Folgejahres) Daten akquiriert.

4 Einführung in die untersuchten physiologischen Systeme

4.1 Sympathoadrenerges & glukokortikoides System

Das sympathoadrenerge System (SAS) und die Hypothalamus-Hypophysen-Nebennierenrinden-Achse (HPA) sind vorrangig an der Regulation und Modulation von Stressantworten und der Aufrechterhaltung der Homöostase beteiligt (7, 47). Die Wirkung des SAS wird hauptsächlich durch die endogenen Katecholamine Adrenalin und Noradrenalin vermittelt, die an G-Protein-gekoppelte Adrenorezeptoren der Effektorzellen binden. Die beiden Hormone werden aus dem

Nebennierenmark und aus sympathischen Nerven freigesetzt und zum größten Teil nach Verstoffwechselung als Vanillinmandelsäure oder Metanephrine im Harn ausgeschieden.
Sie werden unter anderem mit arterieller Hypertension (69) und stressassoziierten kardialen Pathologien (z.B. Takotsubo-Kardiomyopathie) in Verbindung gebracht (18), regulieren im Sinne ihrer stresspermissiven Wirkung Stoffwechselprozesse (Lipolyse, Glykolyse etc.) (57), interagieren mit der Immunantwort und greifen modulierend und regulatorisch in inflammatorische Prozesse ein (10, 89). Ihre laborchemische Messung aus dem Urin erfolgt mittels Hochleistungsflüssigkeitschromatographie (HPLC). Wichtigster Botenstoff der HPA-Achse ist das Glukokortikoid Cortisol, das ebenfalls den katabolen Stoffwechsel fördert. Seine Wirkung am Glukokortikoidrezeptor vermittelt eine Regulation der Genexpression (111), so dass seine Wirkung langsamer aber länger andauernd zum Tragen kommt als die der Katecholamine. Auch Cortisol wirkt modulierend in zahlreichen physiologischen Prozessen, da Interaktionen zwischen glukokortikoider Antwort, Stress, Inflammation und Immunantwort sowie zirkadianer Rhythmik erwiesen sind (7, 46, 97, 110).
Die Bestimmung der Cortisolspiegel erfolgt mit einem Elektrochemilumineszenz-Immunoassay.

4.2 Endocannabinoid System (ECS)

Das Endocannabinoid System ist ein evolutionär alter Teil des Nervensystems, dessen Name auf die Entdeckung der Wirkstoffe der Cannabispflanze, die Cannabinoide, zurückgeht. Seine endogenen Mediatoren, die Endocannabinoide 2-Arachidonoylglycerol (2-AG) und Anandamid (AEA) (77), wirken über die zugehörigen zentralen und peripheren G-Protein-gekoppelten Cannabinoidrezeptoren CB_1 und CB_2 (78) und hemmen konsekutiv die intrazelluläre Adenylylcyclase. Die Endocannabinoide sind Derivate der Arachidonsäure, einer vierfach ungesättigten Fettsäure, und werden durch Spaltung mit Hilfe der Fettsäureamid-Hydrolase (FAAH) und des Enzyms Monoacylglycerolipase (MAGL) abgebaut.
Es existieren noch weitere biologisch aktive Moleküle, die mit dem Endocannabinoid System interagieren. Sie gehören zur selben chemischen Gruppe der N-Acylethanolamide (NAEs). Ihre wichtigsten Vertreter sind Palmitoylethanolamid (PEA), Oleoylethanolamid (OEA) und Stearoylethanolamid (SEA).
Die Endocannabinoide als auch die NAEs haben Einfluss auf zahlreiche physiologische Funktionen und spielen eine wichtige Rolle in der Vermittlung, Regulation und Modulation zentraler und peripherer Stressantworten (37). Sie sind unter anderem an neuroendokrinen und neurochemischen Prozessen, die das Verhalten, die Stimmung als auch Gedächtnisfunktionen steuern, beteiligt (50, 93). Des Weiteren greifen sie modulierend in das Immunsystem ein (5, 67), vermitteln Schmerzantworten (112) und regulieren vegetative Funktionen wie den Appetit, die gastrointestinale Motilität oder die myokardiale Kontraktilität (4, 79).
Diese Steuerungsmechanismen können durch äußere Einflüsse wie z.B. Hypoxie oder Temperaturveränderungen beeinflusst werden (72, 105). Das Endocannabinoid System steht zur Stressregulation außerdem in Verbindung mit der Hypothalamus-Hypophysen-Nebennierenrinden-Achse. Ihre Interaktionen sind allerdings noch nicht vollständig verstanden, denn die gegenseitige Einflussnahme zeigt sowohl negative als auch positive Korrelationen (49, 68, 83).
Die Bestimmung der Konzentration der Endocannabinoide wird mit Hilfe der hochauflösenden Flüssigchromatographie in Verbindung mit der Massenspektrometrie (LC-MS/MS) durchgeführt.

4.3 Purinerges System

Das endogene Nukleosid Adenosin findet sich ubiquitär im intra- und extrazellulären Raum im Gewebe der verschiedenen Organsysteme. Extrazelluläres Adenosin entsteht hauptsächlich durch Phosphohydrolyse von 5´-Adenosintri-/monophosphat (ATP/AMP). Es bindet an vier G-Protein-gekoppelte Adenosinrezeptoren (A_1, A_{2A}, A_{2B}, A_3), die die Adenylylcyclase entweder stimulieren (A_{2A}, A_{2B}) oder inhibieren (A_1, A_3) und so den sekundären Botenstoff zyklisches Adenosinmonophosphat (cAMP) entweder vermehren oder reduzieren (74, 95). Das extrazelluläre Adenosin wird nach Wirkung schnell in die Zellen aufgenommen und entweder vom Enzym Adenosindeaminase (ADA) zu Inosin abgebaut oder von der Adenosinkinase in Adenosinmonophosphat umgewandelt.
Es wurde nachgewiesen, dass Adenosin an zahlreichen verschiedenen physiologischen Prozessen beteiligt ist und vor allem in inflammatorische und immunologische Abläufe regulierend eingreift (16, 108). Die vorherrschenden Sauerstoffverhältnisse spielen hierbei eine wichtige Rolle (53, 70), so dass die purinerge Signalkaskade in der Tumorbiologie von besonderer Bedeutung ist (58, 94).
Die Plasmakonzentration von Adenosin wird mit der Hochleistungsflüssigkeitschromatographie (HPLC) bestimmt.

5 PlanHab - Hypoxie und simulierte Schwerelosigkeit

5.1 Sympathoadrenerges & glukokortikoides System

Unter simulierter Schwerelosigkeit durch Bettruhe zeigten sich Veränderungen der neuroendokrinen Stressantwort vor allem durch Veränderungen der Noradrenalinmenge. Während der Intervention fiel die Noradrenalinmenge im 12 Stunden Tages-Sammelurin in beiden Bettruhe-Gruppen signifikant an Tag 14 im Vergleich zur Basisdatenerhebung (*baseline data collection*, BDC) ab. Außerdem zeigten sich signifikante Unterschiede zur ambulatorischen Gruppe HAMB an den Tagen 14 und 21. Auffallend war ein starker Anstieg der Noradrenalinmenge in den Bettruhe-Gruppen nach Ende der Intervention in der Erholungsphase (Abbildung 5A). Die Ergebnisse im 12 Stunden Nacht-Sammelurin waren ähnlich, allerdings leicht abgeschwächt (Abbildung 5B) (103).
Die Bettruhe ist hier maßgeblich und unabhängig von der Hypoxie für diese Veränderungen verantwortlich und bewirkt die gezeigte Abschwächung der sympathoadrenergen Aktivität. Ähnliche Effekte wurden zuvor von Goldstein et al. (36) und Sigaudo et al. (91) nachgewiesen. Vor diesem Hintergrund repräsentiert der Anstieg der Noradrenalinmenge in der Erholungsphase sehr wahrscheinlich die orthostatische Regulation nach Positionswechsel zur Kompensation der kardiozirkulatorischen Einschränkungen (sog. *deconditioning*) unter Bettruhe. Das Ausmaß der Gegenregulation hängt nach Eckberg und Fritsch (28) von der Barorezeptorsensitivität ab, die nach Bettruhe im Vergleich zum ambulanten Probanden reduziert zu sein scheint und daher exzessiver ausfällt.
Die Konzentration von Cortisol im Speichel überstieg weder morgens noch abends die normalen Referenzwerte, die zirkadiane Rhythmik blieb erhalten, und es wurden keine signifikanten Unterschiede der Cortisolkonzentrationen an den Interventionszeitpunkten zu BDC oder zwischen den drei Interventionsgruppen gemessen. Es war keine gesteigerte Aktivität des glukokortikoiden Systems im Speichel nachweisbar (Daten nicht gezeigt).

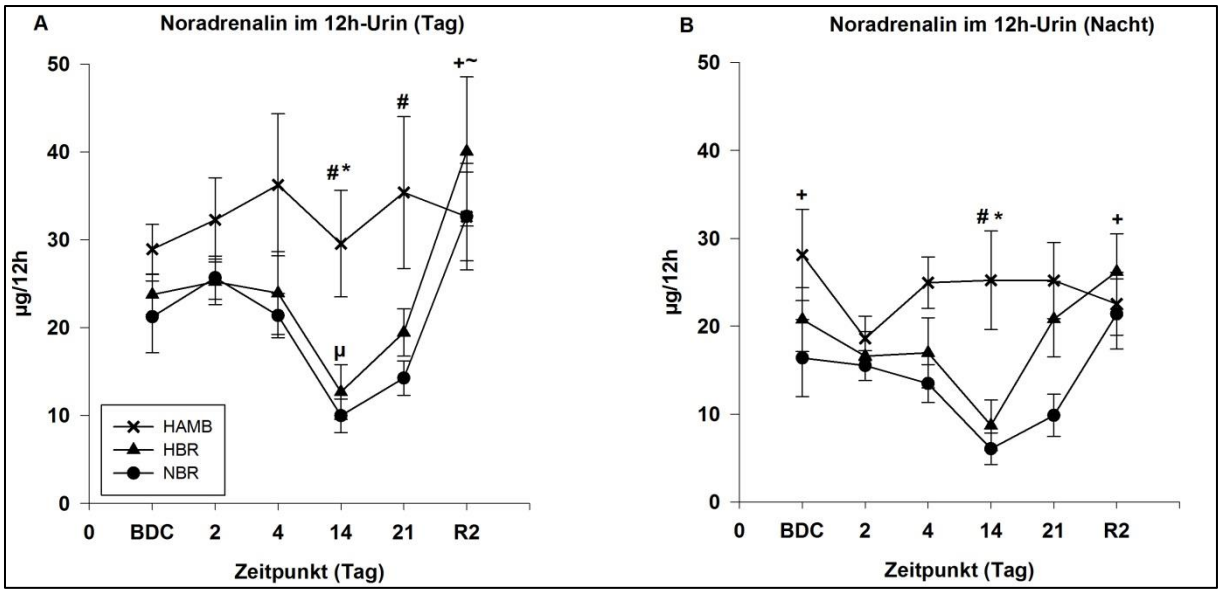

Abbildung 5: *Noradrenalin im 12h-Urin Tag (**A**) oder Nacht (**B**);* Daten sind dargestellt als Mittelwerte ± SEM; Einheit ist µg/12h; HBR: hypoxische Bettruhe (n = 10-14); NBR: normoxische Bettruhe (n = 8-13); HAMB: hypoxische Ambulation (n = 12); BDC: Baseline Data Collection; R2: 2 d nach Ende der Intervention; Noradrenalin Tag: # signifikanter Unterschied zwischen HAMB und NBR; * signifikanter Unterschied zwischen HAMB und HBR; µ signifikanter Unterschied zwischen BDC und Tag 14 in HBR/NBR; + signifikanter Unterschied zwischen R2 und Tag 14 in HBR/NBR; ~ signifikanter Unterschied zwischen R2 und Tag 21 in NBR; Noradrenalin Nacht: # signifikanter Unterschied zwischen HAMB und NBR; * signifikanter Unterschied zwischen HAMB und HBR; + signifikanter Unterschied zwischen R2/BDC und Tag 14 in HBR/NBR ($p < 0.05$) (103).

5.2 Endocannabinoid System (ECS)

Das Endocannabinoid System zeigte keine Veränderungen während den verschiedenen Interventionen.

In früheren Studien wurde nachgewiesen, dass Hypoxie einen dysregulierenden Einfluss auf neuroendokrine Funktionen hat (14, 73) und dass die Modulation dieser Mechanismen auch andere physiologische Funktionen beeinflusst (54, 113). Eine frühere Studie unserer Forschungsgruppe (33) wies erhöhte EC-Konzentrationen unter kurzzeitiger physischer Anstrengung nach, die durch Hypoxie noch gesteigert wurden, wohingegen allerdings der alleinige Einfluss von Hypoxie ebenfalls keine Auswirkungen auf die EC-Konzentrationen hatte.

5.3 Purinerges System

Adenosin stieg in allen drei Interventionsgruppen mit jeweils signifikanten Unterschieden zu BDC (HBR Tag 21; HAMB Tag 5 und NBR Tag 14, 21 und R2) stark an (Abbildung 6 A/B) (104). Überraschenderweise war der Anstieg in der HBR Gruppe am größten, aber er wurde auch in der reinen Bettruhe Gruppe NBR nachgewiesen. Bettruhe verstärkte nicht nur die Effekte der Hypoxie auf die Adenosinkonzentration, sondern war per se verantwortlich für ihre Steigerung.

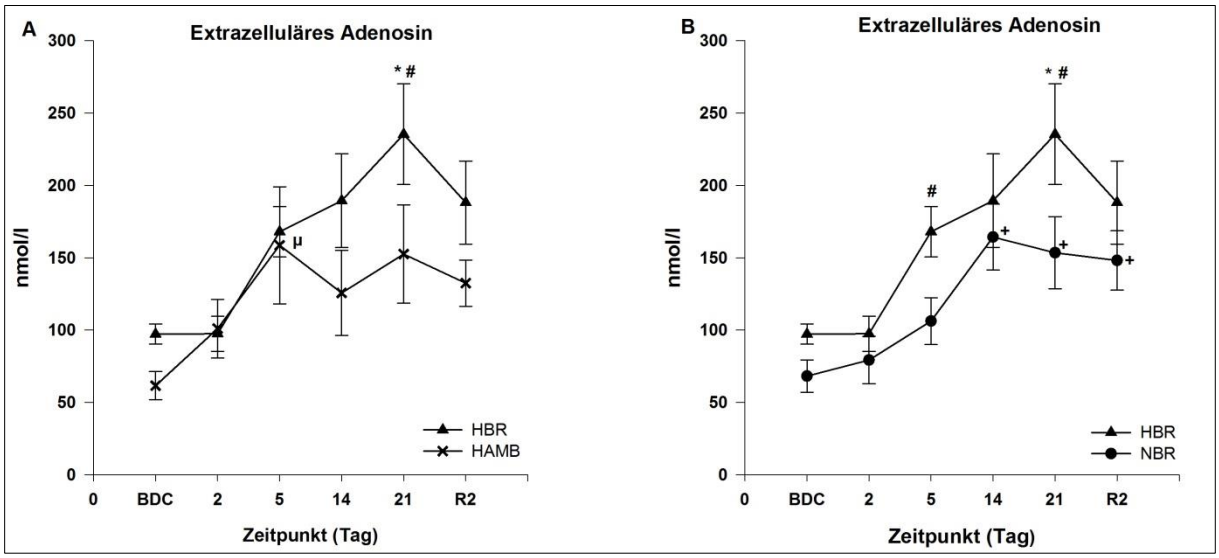

Abbildung 6: *Extrazelluläre Adenosinkonzentrationen im Plasma (**A**,**B**);* Daten sind dargestellt als Mittelwerte ± SEM; Einheiten sind nmol/l; HBR: hypoxische Bettruhe (n = 12–14); NBR: normoxische Bettruhe (n = 11–13); HAMB: hypoxische Ambulation (n = 12); BDC: Baseline Data Collection; R2: 2 Tage nach Ende der Intervention; # signifikanter Unterschied zwischen HBR und HAMB oder NBR; * signifikanter Unterschied zu BDC in HBR; μ signifikanter Unterschied zu BDC in HAMB; + signifikanter Unterschied zu BDC in NBR ($p < 0.05$) (104).

Erythrozyten speichern ATP zur Aufrechterhaltung der Energieversorgung, und das purinerge System spielt für ihren Metabolismus und ihre Reaktionsfähigkeit bei Hypoxie eine wichtige Rolle (20, 62, 96). Eine erhöhte ATP-Freisetzung aus Erythrozyten unter Hypoxie mit konsekutiver Steigerung der extrazellulären Adenosinkonzentration wird vor allem durch Hämolyse verursacht (63, 92). Obwohl wir erhöhte Erythropoetinspiegel mit konsekutiver Retikulozytose in beiden Hypoxie-Gruppen (HAMB, HBR) nachwiesen, fand sich nur in der HBR Gruppe eine höhere Hämolyserate, so dass Hypoxie allein kein ausreichender Trigger für eine gesteigerte Hämolyse zu sein scheint (104).
Unsere Forschungsgruppe hatte in einer früheren Bettruhe-Studie (32) beschrieben, dass Bettruhe zu einer veränderten Flüssigkeitsverteilung mit konsekutiver Zunahme von Scherstress der Zellen im Gefäßbett führt, der seinerseits ein vermehrtes Abscheren von Adhäsionsmolekülen (*shedding*) verursacht. Da Hypoxie die Verformbarkeit von Erythrozyten beeinträchtigt und sie anfälliger für Zellschäden macht (64, 90), könnte erhöhter Scherstress durch Flüssigkeitsverschiebungen den Ausschlag für das Einsetzen einer Hämolyse geben. Solche Flüssigkeitsverschiebungen wurden in der PlanHab-Studie tatsächlich am stärksten für die HBR Gruppe beschrieben (52), die zugleich auch den höchsten Adenosinanstieg zeigte. Allerdings kam es auch in den beiden anderen Gruppen HAMB und NBR zu einem Adenosinanstieg, dessen Ursache unabhängig von einer Zerstörung von Erythrozyten war, denn in beiden Gruppen war nur eine geringe Hämolyse und Flüssigkeitsumverteilung nachweisbar (52, 104). Der fehlende Nachweis einer erhöhten Hämolyse könnte i) einer reduzierten Sensitivität der Detektionsmethodik geschuldet sein, da wir auch in HAMB und NBR eine Retikulozytose fanden, die zumindest für NBR eine positive Korrelation zur Hämolyserate zeigte, oder

ii) metabolisch verursacht worden sein. Die Aussagen zur oxidativen Kapazität und des Metabolismus des Muskels unter körperlicher Anstrengung sind vielfältig (35, 45) und Untersuchungen hierzu unter körperlicher Inaktivität bisher selten (39). Gram et al. (39) legen jedoch nahe, dass purinabhängige Metabolismen bei körperlicher Inaktivität wahrscheinlich reduziert werden, allerdings schließen sie konkordante purinerge Veränderungen wie nach körperlicher Anstrengung nicht aus. Unsere Kollegen Debevec et al. (21) wiesen in der PlanHab Studie nach, dass die Gesamtkörper- sowie die reine Muskelmasse nach allen drei Interventionen signifikant im Vergleich zu BDC verringert war, so dass auch hier ein veränderter purinerger Metabolismus für den allgemeinen Adenosinanstieg verantwortlich sein kann. Die Hypoxie zeigte entgegen der Vermutung keine additive Wirkung. Dass diese Veränderungen auch in der ambulatorischen Gruppe vorlagen erklärten Debevec et al. mit einem möglichen allgemein verminderten Appetit mit konsekutiv niedriger Energiezufuhr bzw. einem allgemein niedrigen Aktivitätslevel und Isolationseffekten.
Andere (ggf. additive) Ursachen wie beispielsweise eine Malperfusion des abhängigen Gewebes durch die anhaltende Bettruhe mit konsekutiver Hypoxie und Purinfreisetzung wurden nicht bestätigt, da wir mit den angewandten Methoden keine steigenden Konzentrationen von Endothelfunktionsmarkern (Zonulin, sICAM-1) messen konnten (104). Die Hypothese beruhte dabei auf einer früheren Studie unserer Forschungsgruppe (34), die eine Schrankenstörung des Endothels mit konsekutiver Permeabilitätssteigerung und Erhöhung von Zonulin nachwies und diese in Verbindung zu mit Bettruhe assoziierten Kopfschmerzen gebracht hatte.

6 Antarktis - Hypoxie und Isolation

6.1 Sympathoadrenerges & glukokortikoides System

In der Concordia Gruppe stieg unter hypobarer Hypoxie die nächtliche Noradrenalimenge im Urin am Anfang des einjährigen Aufenthaltes signifikant im Vergleich zu BDC an und fiel im Verlauf bis zum Ende der Expedition auf das Ausgangsniveau ab (Abbildung 7A) (102). Die Cortisolkonzentration im Speichel stieg ebenso an, aber zeigte keine signifikanten Veränderungen zu BDC. Allerdings wurden fast über den gesamten Jahresverlauf in der Concordia Gruppe höhere Cortisolkonzentrationen als in der Neumayer III Gruppe gemessen (Daten nicht gezeigt).
Die Werte des nächtlichen Adrenalins verhielten sich ähnlich, zeigten jedoch keinen signifikanten Anstieg zu BDC, und fluktuierten im Jahresverlauf stärker als bei Noradrenalin (Abbildung 7B).
Im Vergleich hierzu blieb die exkretorische Noradrenalinmenge in der Neumayer III Gruppe (Meereshöhe) zunächst niedrig und stieg erst am Ende der Expedition leicht an (Abbildung 7A) (102).
Einen ähnlichen Verlauf wie in der Concordia Gruppe hatten wir in einer früheren Antarktisstudie (ebenfalls auf Concordia) gezeigt, bei der die erhöhten Katecholaminwerte mit veränderten Immunfunktionen einhergingen (30). Und auch Studien anderer Kollegen in simulierter Höhe, wiesen einen steigernden Effekt von hypobarer Hypoxie auf die sympathoadrenerge und glukokortikoide Antwort nach (3, 26, 114). Der Einfluss ist allerdings variabel und hängt von zusätzlich einwirkenden Faktoren ab, denn die Kombination von Hypoxie und Bettruhe in unserer PlanHab-Studie modulierte diese Wirkung und rief eine gegensätzliche Antwort mit reduzierter sympathoadrenerger Reaktion hervor (103). Und obwohl Woods et al. (114) nachwiesen, dass die Auswirkungen von simulierter normo- und hypobarer Hypoxie auf sympathoadrenerge und glukokortikoide Antworten nach physischer Anstrengung denen von realer hypobarer Hypoxie ähneln, stellten wir in der PlanHab-Studie keine signifikanten sympathoadrenergen oder glukokortikoiden Veränderungen unter reiner normobarer Hypoxie fest (103). Aufgrund der unterschiedlichen Dauer der Hypoxieexposition von

akut (wenige Stunden – bei Woods et al.) über subakut (21 Tage - hypoxische Bettruhe in der PlanHab-Studie) bis hin zu chronisch (1 Jahr – Antarktis), könnten Adaptationsprozesse für diese unterschiedlichen Ergebnisse verantwortlich sein.

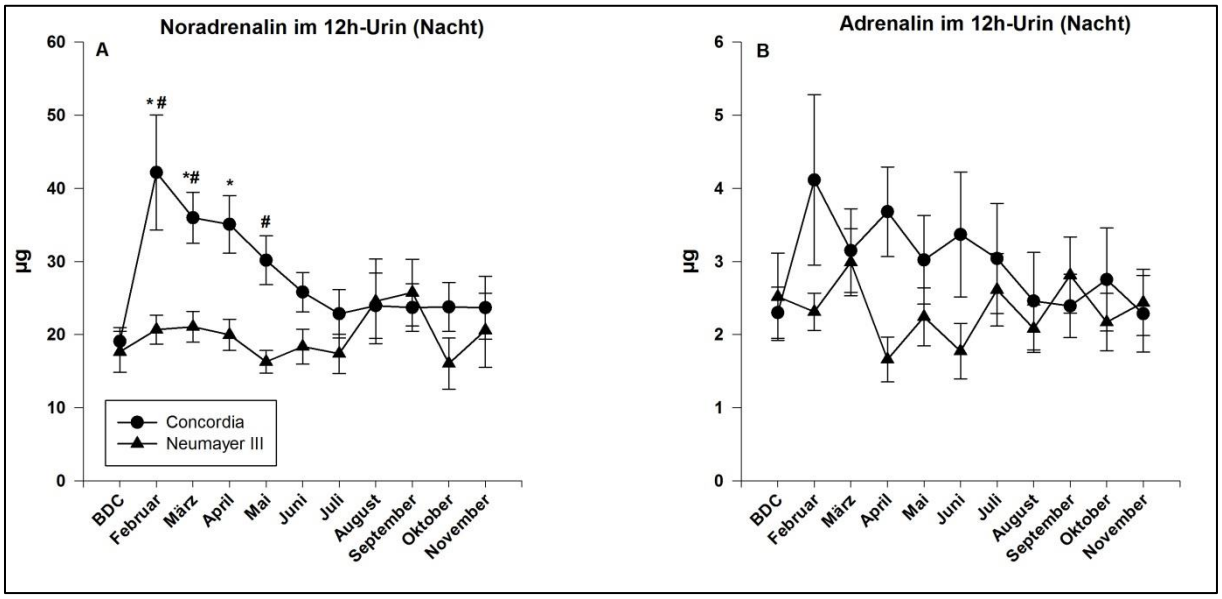

Abbildung 7: *Noradrenalin (**A**) und Adrenalin (**B**) im 12h-Urin (Nacht) auf Neumayer III (n =11-16) und auf Concordia (n =11-15);* Daten sind dargestellt als Mittelwerte ± SEM; Einheiten sind µg; BDC: Baseline Data Collection; * signifikanter Unterschied zu BDC; # signifikanter Unterschied zwischen den Gruppen auf Neumayer III und Concordia (102).

6.2 Endocannabinoid System (ECS)

Auf Meereshöhe (Neumayer III) fand sich gleich zu Beginn der Expedition eine signifikante Erhöhung der Endocannabinoide und der NAEs. Die Konzentrationen blieben über den Jahresverlauf deutlich erhöht und fielen erst gegen Ende des Jahres ab. Im Gegensatz hierzu wurden unter hypobarer Hypoxie (Concordia) stets niedrige ECs und NAEs gemessen, die erst am Ende des Jahres signifikant anstiegen (Abbildung 8 A-E) (102).

Scheinbar führt die extreme Umwelt der Antarktis per se zu einer gesteigerten Aktivität des ECS und die additiv wirkende hypobare Hypoxie auf Concordia moduliert diese Antwort im Sinne einer Herunterregulierung.

Ein möglicher erklärender Mechanismus dieser Herunterregulierung bzw. des mangelnden Anstiegs der EC-Konzentrationen auf Concordia ist die hypoxische Signalvermittlung und der Metabolismus der ECs. Es ist bekannt, dass Hypoxie die Expression von HIF-1α und darüber die Expression des Enzyms Cyclooxygenase-2 (COX-2) steigert (40). COX-2 ist unter anderem am Metabolismus der ECs beteiligt (105), und AEA und 2-AG sind ihre Hauptsubstrate (15). Eine Erklärung wäre eine hypoxie-vermittelte Steigerung von COX-2, die zu einem gesteigerten Metabolismus von ECs mit vermehrter Bildung von Molekülen wie Prostaglandin-Ethanolamiden und Hydroxy-Anandamiden führte (15).

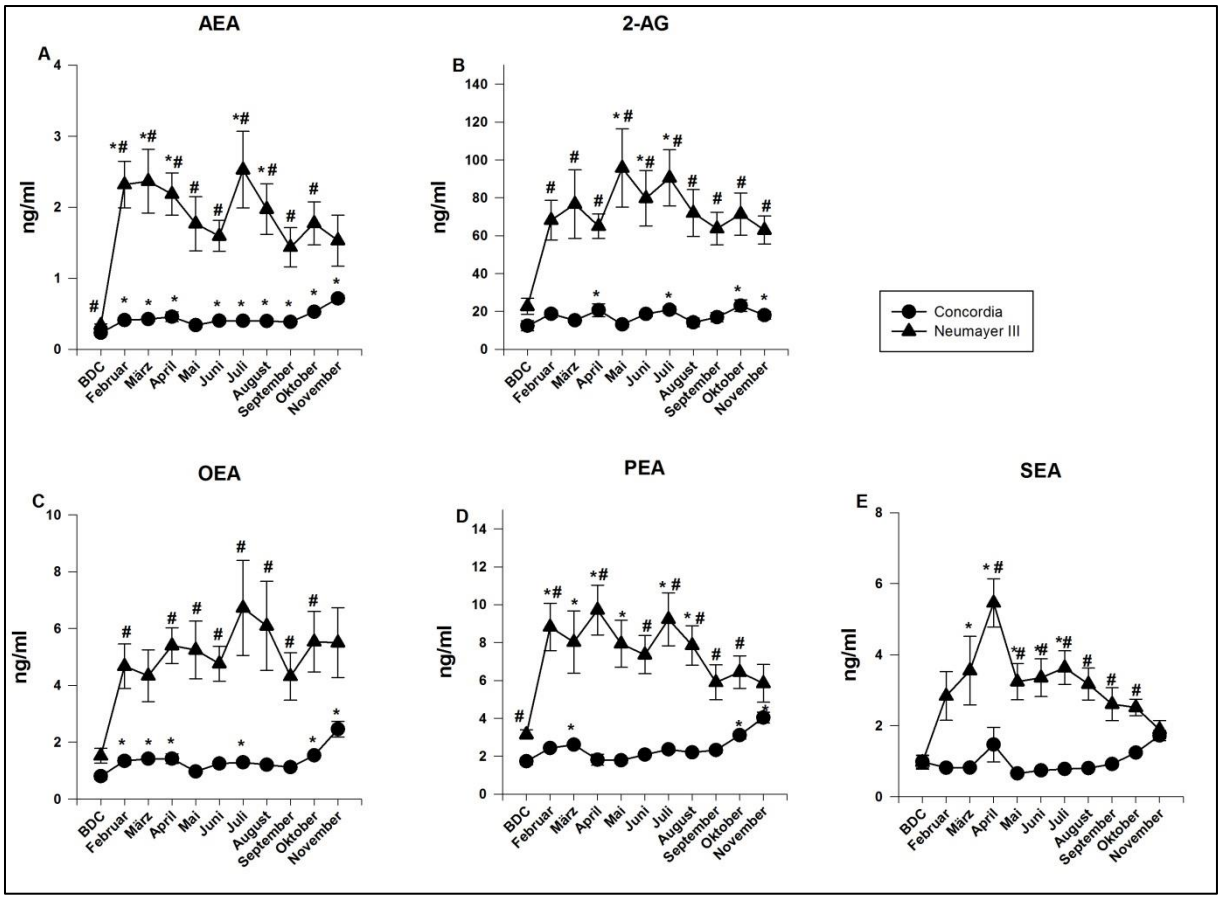

Abbildung 8: *Endocannabinoid (EC) und N-Acylethanolamid Konzentrationen auf Neumayer III (n = 15-16) und Concordia (n = 7-15);* Daten sind dargestellt als Mittelwerte ± SEM; Einheiten sind ng/ml; BDC: Baseline Data Collection; AEA: Anandamid (**A**); 2-AG: 2-Arachidonoylglycerol (**B**); OEA: Oleoylethanolamid (**C**); PEA: Palmitoylethanolamid (**D**); SEA: Stearoylethanolamid (**E**); * signifikanter Unterschied zu BDC; # signifikanter Unterschied zwischen den Gruppen auf Neumayer III und Concordia (102).

Besonders erwähnenswert ist, dass die Expression von HIF-1α unter chronischer Hypoxie eine Zeitabhängigkeit zeigt. Sie steigt nach Exposition von hypobarer Hypoxie zunächst an (8, 9) und nimmt im zeitlichen Verlauf wieder ab (80). Mit zunehmender Akklimatisierung an die chronische hypobare Hypoxie verliert dieser Weg der Signalvermittlung an Wichtigkeit (6). In Übereinstimmung wiesen Feuerecker et al. in einer früheren Studie (31) auf Concordia eine Reduktion der Expression von HIF-1α unter chronischer hypobarer Hypoxie nach. Goyal et al. (38) fanden ähnliche Ergebnisse im Tiermodell. Vor dem Hintergrund, dass COX-2 den EC-Metabolismus beeinflusst, lässt sich auch vermuten, dass dieser Mechanismus zum Anstieg der EC-Konzentrationen auf Concordia am Ende der Expeditionen beitrug.

Auch Veränderungen der Abbau- und Metabolisierungssysteme der ECs (z.B. Fettsäure-Amid-Hydrolase (FAAH), intrazelluläre Transportsysteme) kommen ursächlich für den mangelnden EC Anstieg auf Concordia in Frage, waren aber nicht Bestandteil der Studienuntersuchungen.

Im Gegenzug waren als Ursachen für eine Aktivitätssteigerung des ECS bereits in früheren Studien physische Herausforderungen (z.B. physische Anstrengung, Schlafentzug) identifiziert worden (12, 33, 43), die unsere Hypothese, dass die extreme Umwelt der Antarktis Ursache des EC-Anstiegs auf Neumayer III ist, unterstützen. Auch bei den Astronauten der Internationalen Raumstation ISS, also ebenso unter chronischen Bedingungen aber in einem anderen extremen Habitat, maßen wir erhöhte EC-Werte (100).

Im Zusammenhang mit der gesteigerten ECS-Aktivität würde man komplementär eine gesteigerte sympathoadrenerge Antwort erwarten, die wir jedoch auf Meereshöhe auf Neumayer III nicht detektierten. Stattdessen fand sich eine niedrige exkretorische Katecholaminmenge (Abbildung 7A/B). Dieser inverse Zusammenhang der beiden Systeme wurde auch schon mehrfach in Studien anderer Kollegen belegt (81, 106). Unter künstlichen Isolationsbedingungen auf Meereshöhe (116) existiert andererseits aber auch der Nachweis reduzierter EC-Werte assoziiert mit einer erhöhten sympathoadrenergen Antwort und Cortisolkonzentration. Dies ist trotz inverser Kinetik ein konträrer Befund zu den Ergebnissen auf Neumayer III.
Des Weiteren gibt es allerdings auch Studien mit einer positiven Korrelation der beiden Systeme (75, 84), so dass die Variabilität der Antworten groß und wahrscheinlich den unterschiedlichen Studienbedingungen geschuldet ist.
Unter Einfluss von hypobarer Hypoxie (Concordia) waren die niedrigen EC-Werte assoziiert mit signifikant erhöhten Noradrenalinmengen, die allerdings gegen Ende der Expedition abfielen (Abbildung 7A). Zu diesem Zeitpunkt wurde wiederum ein Anstieg der EC-Konzentrationen gemessen. Dieser inverse Verlauf stützt die Hypothese, dass die sympathoadrenerge Antwort unter hypobarer Hypoxie heraufreguliert und dadurch die EC-Antwort herunterreguliert wurde.
Im Einklang mit dem Anstieg der Konzentration der ECs unter hypobarer Hypoxie auf Concordia am Ende der Expedition steht der Nachweis von Alarcon-Yaquetto et al. (2), dass Bewohner großer Höhen mit erhöhtem Hämoglobinspiegel und erniedrigten Sättigungswerten ebenso erhöhte NAE-Konzentrationen zeigten.

6.3 Purinerges System

Die Analyse früherer Daten bei einer kleineren Studiengruppe (Probanden der Concordia-Antarktiskampagne 2009) zeigten moderate Änderungen der Adenosinplasmakonzentrationen nach einer Woche unter Einfluss von hypobarer Hypoxie (30) (Abbildung 9). Viel deutlicher und statistisch signifikant erhöht waren die Adenosinkonzentrationen hingegen in der PlanHab-Studie in der ambulatorischen Gruppe (HAMB) am Tag 5 unter Interventionsbedingungen im Vergleich zu BDC (Abbildung 6A). Danach sanken die Adenosinkonzentrationen im Verlauf der 21-tägigen Intervention bis zur Messung in der Erholungsphase wieder ab. Die Hypoxie scheint einen akuten aber temporären Effekt auf die Adenosinfreisetzung auszuüben, da er im Zeitverlauf rückläufig und somit möglicherweise ein Hinweis für eine Akklimatisierung ist. Das Verhalten der Adenosinkonzentration unter chronischer Exposition (1 Jahr Aufenthalt auf Concordia) muss mit zukünftigen Messungen und bei höherer n-Zahl festgestellt werden.
Genetische Analysen zusammen mit der quantitativen Polymerase-Kettenreaktion der Proben der Concordia-Crews der Jahre 2009 und 2010 wiesen im Langzeitverlauf der Expeditionen nach, dass im Transkriptom die Adenosindeaminase (ADA) sowie der Adenosinrezeptor A_{2B} im Vergleich zu BDC signifikant reduziert und die Rezeptoren A_1 und A_3 unbeeinträchtigt waren. Nach vier Monaten Aufenthalt unter Hypoxie zeigte sich eine signifikante Herunterregulation von HIF-1α im Vergleich zu BDC (31). Ähnliche Ergebnisse fanden sich im Tiermodell (6, 38). Petousi et al. (80) berichteten zudem von einer verzögerten physiologischen Antwort auf Hypoxie im adaptierten Menschen.
Weitere Langzeitbeobachtungen sind notwendig, um diese Veränderungen genauer einordnen zu können.

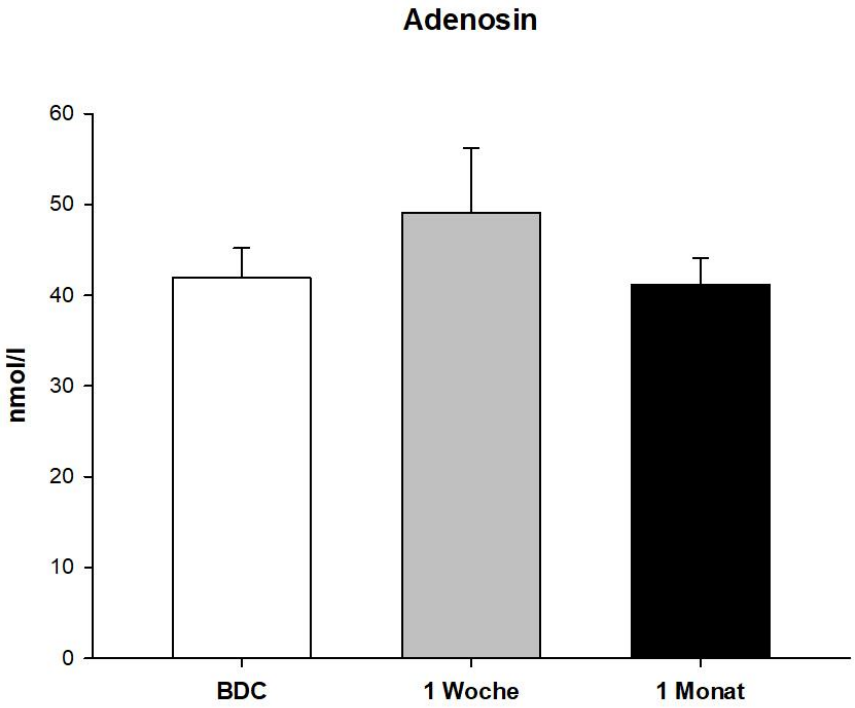

Abbildung 9: *Adenosinplasmakonzentrationen;* vorher (BDC in Europa), 1 Woche und 1 Monat nach Exposition unter hypobarer Hypoxie in der Antarktis. Daten sind als Mittelwerte ± SEM dargestellt (n=9, gepaarter t-Test, einseitig, 1 Woche vs. BDC, p = 0.12) (30) (Mary Ann Liebert, Inc., New Rochelle, NY).

7 Geschlechtsspezifische Unterschiede in der Stressantwort

Die Untersuchung der geschlechtsspezifischen Unterschiede in der Stressantwort erfolgte ausschließlich an den Probanden der drei Kampagnen 2013 - 2015 auf Neumayer III (10 Frauen, 16 Männer), da die Anzahl weiblicher Probanden auf Concordia pro Überwinterung bisher nur sehr gering ist (max. 2). Daher ist eine Beurteilung der Auswirkungen der hypobaren Hypoxie auf die Stressantwort in weiblichen Probanden zurzeit nicht möglich. Des Weiteren erfolgte die Beurteilung des purinergen Systems bisher nur auf Concordia zur Identifizierung des Einflusses der hypobaren Hypoxie, so dass die Identifizierung möglicher geschlechtsspezifischer Unterschiede noch aussteht.

7.1 Sympathoadrenerges & glukokortikoides System

In beiden Geschlechtern war die Noradrenalinausscheidung tagsüber im Urin in den ersten Monaten nach Ankunft auf Neumayer III höher als bei BDC. Ein Abfall der Noradrenalinausscheidung fand sich sowohl bei Männern als auch bei Frauen zu Beginn des antarktischen Winters im April/Mai als auch erneut zu Beginn des Sommers im Oktober/November. Während der Nacht fluktuierte die Noradrenalin- als auch die Adrenalinausscheidung bei beiden Geschlechtern. Insgesamt war die Katecholaminausscheidung über den gesamten Expeditionszeitraum bei den Männern meist höher als bei den Frauen (Abbildung 10 A-D) (101). Eine Erklärung hierfür liegt wohl in der Tatsache, dass physisch stark beanspruchende Arbeit auf antarktischen Forschungsstationen meist von Männern (z.B. Techniker) verrichtet wird. Der saisonale Abfall der Katecholaminausscheidung in beiden Geschlechtern spiegelt wahrscheinlich zu Beginn des Winters die allgemeine Reduktion physischer Arbeit, wenn die Crew aufgrund der Wetterbedingungen in der Station bleiben muss. Zu Beginn des Sommers ist er wahrscheinlich mit der Ankunft des Sommerteams und damit mit einer Aufteilung der Arbeitslast zu erklären.

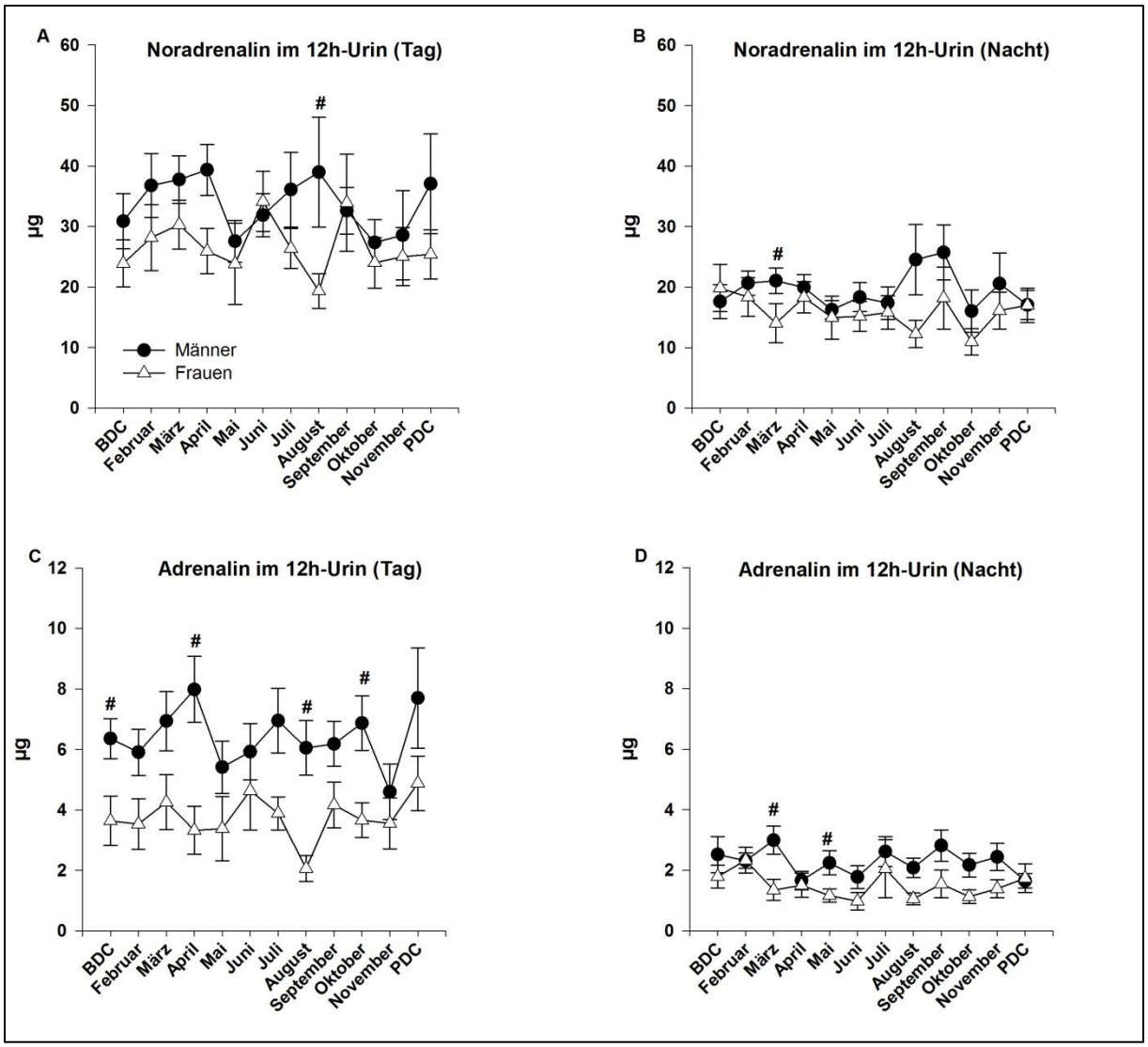

Abbildung 10: *Noradrenalin (**A**-**B**) und Adrenalin (**C**-**D**) im Tag- und Nachturin (Sammelzeit jeweils 12 Stunden);* Daten sind als Mittelwerte ± SEM dargestellt; Einheiten sind µg; Frauen n = 6-10; Männer n = 11-16; BDC: Baseline Data Collection; PDC: Post Data Collection; # signifikanter Unterschied zwischen Männern und Frauen (101).

Die Cortisolkonzentrationen am Morgen zeigten signifikante Unterschiede zwischen den Geschlechtern in den Wintermonaten April ($p = 0.009$), Mai ($p = 0.02$) und Juli ($p = 0.036$) mit höheren Konzentrationen bei den Frauen (Abbildung 11A). Die Cortisolkonzentrationen am Abend fluktuierten in beiden Geschlechtern während des Beobachtungszeitraums, allerdings ohne signifikante Unterschiede zwischen den Geschlechtern aufzuweisen (Abbildung 11B). Das Verhältnis zwischen Cortisolkonzentrationen am Morgen und Abend zeigte einen ähnlichen Verlauf, wobei die Werte während der Expeditionen im Vergleich zu BDC bei beiden Geschlechtern konstant höher waren (Abbildung 11C).

Frühere Studien haben gezeigt, dass die Cortisolfreisetzung durch verschiedene sowohl psychische, soziale als auch bewegungsassoziierte Stressfaktoren beeinflusst und reguliert wird (29, 48). Zudem scheint die Cortisolantwort auf spezifische z.B. psychische Stressfaktoren geschlechterabhängig (109) als auch individuell einem sog. Chronotypus (Morgen- oder Abendtypus) zuzuordnen zu sein (27, 56), Dabei ist die Antwort der HPA Achse auf spezifische Stressfaktoren morgens stärker ausgeprägt als abends und korreliert so mit der zirkadianen Rhythmik der Cortisolsynthese (115). Des Weiteren wurden höhere Cortisolkonzentrationen im Zusammenhang mit teilweisem oder totalem Schlafentzug, einer kürzeren Schlafzeit bzw. einer schlechteren Schlafeffizienz beobachtet (61). Steinach et al. (98) berichteten von einer verminderten Schlafqualität (Zunahme der Zeit, die im Bett verbracht wird, Abnahme der Schlafeffizienz, Steigerung der Anzahl der Wachphasen) bei weiblichen Probanden während insgesamt sieben Überwinterungskampagnen auf Neumayer III im Zeitraum von 2008 bis 2014. Vor dem Hintergrund des nachgewiesenen Zusammenhangs von Schlaf und Aktivität der HPA Achse, stützt diese Studie daher unsere Ergebnisse höherer Cortisolkonzentrationen am Morgen bei Frauen im Vergleich zu Männern während des Winters bei jedoch erhaltener zirkadianer Rhythmik. Steinach et al. (98) regten an, dass Umweltfaktoren wie die Isolation, die extreme Kälte vor Ort und die monotone Umwelt einen stärkeren Einfluss auf Frauen als auf Männer haben könnten und diskutierten, dass Frauen möglicherweise sensibler auf psychosozialen Stress reagieren, was folglich für die abnehmende Schlafqualität ursächlich sein könnte (ihre allgemeine physische Aktivität blieb unverändert). Dem widersprachen allerdings unsere Ergebnisse, da wir weder bei Männern noch Frauen eine durch Fragebögen verifizierbare erhöhte psychische Belastung feststellten. Zudem kehrten die Cortisolkonzentrationen von Frauen am Ende des antarktischen Winters wieder auf die Höhe der Ausgangskonzentrationen bei BDC zurück. Dies unterstützt, ebenso wie die Ergebnisse der Untersuchung der EC und NAE Daten auf den beiden Antarktisstationen sowie nachfolgend zwischen den beiden Geschlechtern auf Neumayer III, unsere Hypothese, dass der beobachtete Cortisolanstieg bei Frauen durch die Umwelt vermittelt wird.

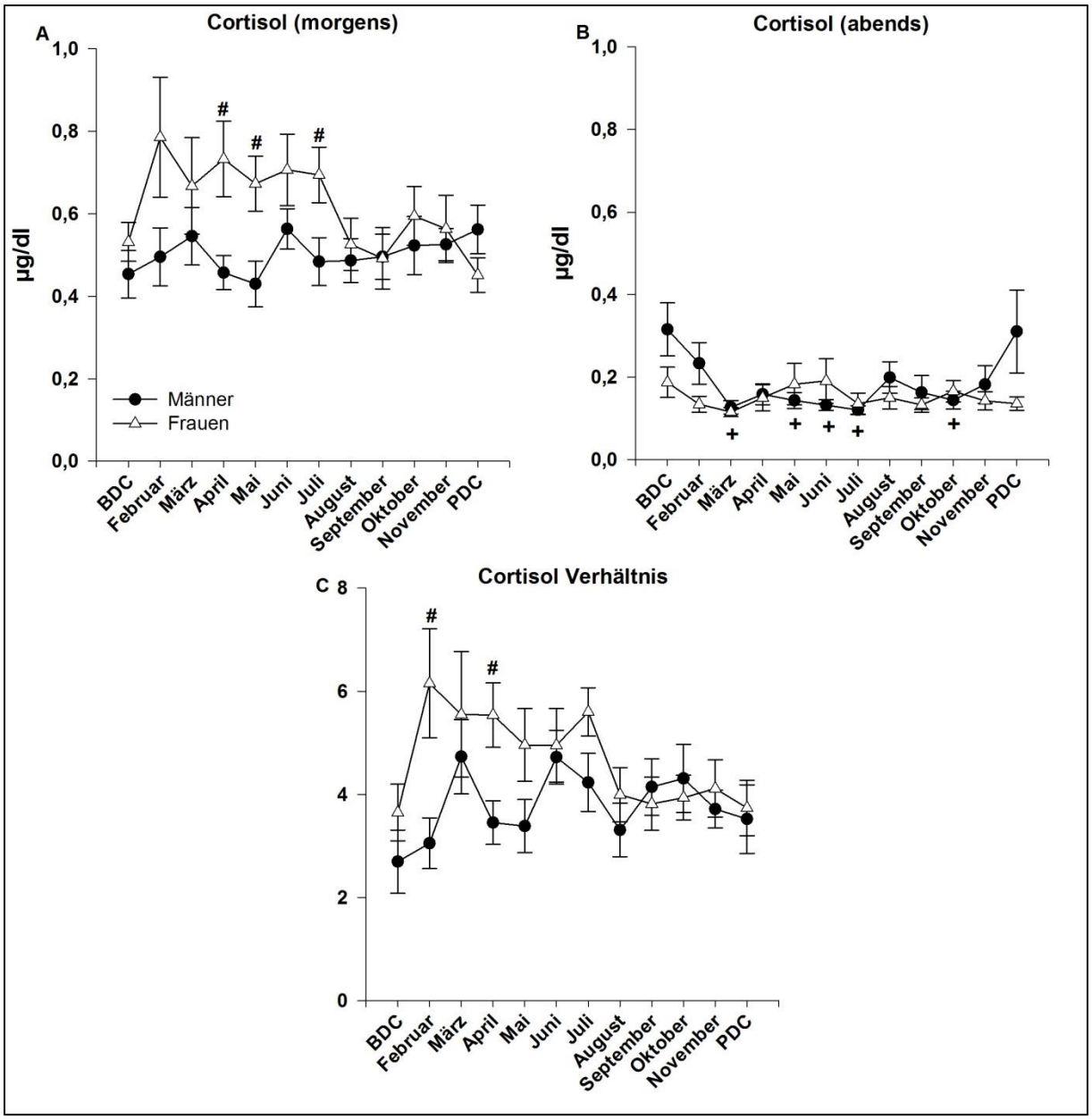

Abbildung 11: *Cortisol im Speichel morgens (**A**) und abends (**B**) und das Verhältnis der Cortisolwerte von morgens zu abends (**C**);* Daten sind als Mittelwerte ± SEM dargestellt; Einheiten sind µg/dl; Frauen n = 8-10; Männer n = 13-16; BDC: Baseline Data Collection; PDC: Post Data Collection; # signifikanter Unterschied zwischen Männer und Frauen; + signifikanter Unterschied zu BDC bei Männern (101).

7.2 Endocannabinoid System (ECS)

Die Analyse der weiblichen EC und NAE Daten in den drei Kampagnen auf Neumayer III zeigten signifikant erhöhte Konzentrationen während der Expedition und damit dasselbe Reaktionsmuster, das wir bei ihren männlichen Kollegen festgestellt hatten (Abbildung 12 A-E). Diese Aktivierung des ECS konnte in den mit identischen Verfahren gemessenen Proben männlicher Probanden auf Concordia unter hypobarer Hypoxie nicht quantifiziert werden (Abbildung 8), so dass wir postulierten, dass die extreme Umwelt der Antarktis für diese Aktivierung verantwortlich ist und die auf Concordia herrschende hypobare Hypoxie die ECS Aktivierung über die auf S. 16 und 17 beschriebenen hypoxie-sensitiven Signalwege verminderte und hemmte. Die Ähnlichkeit der

Ergebnisse der weiblichen EC und NAE Daten stützt diese Hypothese, allerdings fehlt der Nachweis des Verlaufs weiblicher EC und NAE Konzentrationen unter hypobarer Hypoxie aufgrund der wie oben beschriebenen geringen Anzahl von Frauen während Überwinterungskampagnen auf Concordia.

Des Weiteren scheint der negative Kontrollmechanismus zwischen der HPA Achse und dem EC System bei Frauen vor dem Hintergrund der gemessenen erhöhten weiblichen Cortisolkonzentrationen unter diesen Experimentbedingungen nur eingeschränkt wirksam zu sein. Obwohl zumeist in Tiermodellen erforscht, könnten die geschlechtlichen Unterschiede in der Stressantwort durch Modulation der HPA Achse bei Frauen mit einer stärkeren Aktivierung und einer ausgeprägteren Hormonantwort erklärt werden, die durch gonadale Hormone wie Estradiol und Testosteron reguliert wird (42). Bei Menschen sind die Forschungsergebnisse weniger konsistent und widersprüchlicher, aber der weibliche Menstruationszyklus scheint eine wichtige Rolle zu spielen (55).

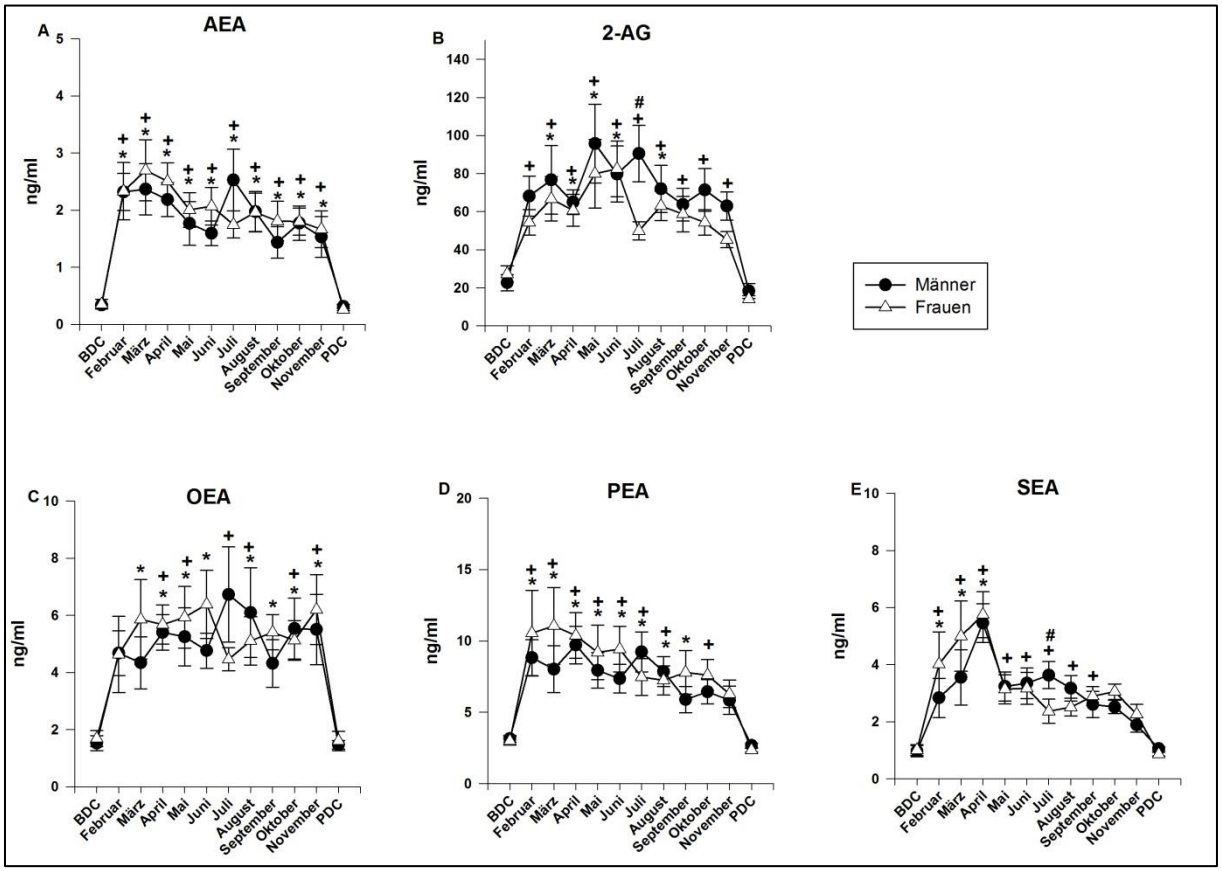

Abbildung 12: *Endocannabinoid und N-Acylethanolamid Konzentrationen;* **A/B** = ECs; **C-E** = NAEs; Daten sind dargestellt als Mittelwerte ± SEM; Einheiten sind ng/ml; Frauen n = 9-10; Männer n = 15-16; BDC: Baseline Data Collection; PDC: Post Data Collection; AEA: Anandamid (**A**); 2-AG: 2-Arachidonoylglycerol (**B**); OEA: Oleoylethanolamid (**C**); PEA: Palmitoylethanolamid (**D**); SEA: Stearoylethanolamid (**E**); # signifikanter Unterschied zwischen Männern und Frauen; + signifikanter Unterschied zu BDC bei Männern; * signifikanter Unterschied zu BDC bei Frauen (101).

8 Zusammenfassung

Die menschliche Allostase dient - als Funktion der Art und Intensität verschiedenster Stressoren - der Erhaltung des physiologischen Gleichgewichts des Menschen durch Veränderung und Anpassung unterschiedlicher physiologischer Funktionen und Reaktionen. Diese variieren je nach individueller Entwicklung, Erfahrung, genetischer Grundlage und Verhaltensweisen.
Ziel des Habilitationsprojektes war die Untersuchung der Regulation der menschlichen Allostase durch verschiedene an der Stressantwort beteiligte physiologische Systeme und deren Evaluation und Quantifizierung unter dem Einfluss spezifischer Stressoren in extremer Umwelt.
Dies erfolgte zum einen in einer Studie (**PlanHab**), die unter hoch standardisierten kontrollierten Bedingungen die Stressoren extremer Umwelt simulierte (Hypoxie und Bettruhe) und zum anderen in zwei Feldstudien direkt in extremer Umwelt in der **Antarktis** auf zwei verschiedenen Forschungsstationen.

Die **PlanHab**-Studie umfasste drei unterschiedliche Studienbedingungen (NBR, normobare normoxische Bettruhe; HBR, normobare hypoxische Bettruhe; HAMB, normobare hypoxische Ambulation), die alle im Sinne eines cross-over Designs von 14 männlichen Probanden in drei aufeinanderfolgenden Kampagnen mit jeweils viermonatigem Abstand durchlaufen wurden.
Die Quantifizierung der Freisetzung stresspermissiver Substanzen (Katecholamine, Cortisol, Endocannabinoide, Purine) zeigte keinen Einfluss der normobaren Hypoxie auf die neuroendokrine Stressachse, aber eine signifikante Reduktion der sympathoadrenergen Antwort durch Bettruhe (NBR; Noradrenalin Tag 21.23 ± 14.16 auf 9.98 ± 6.88 µg), die durch Hypoxie aber nicht additiv beeinflusst wurde (HBR; Noradrenalin Tag 23.77 ± 8.29 auf 12.68 ± 11.63 µg). Die normobare Hypoxie (HAMB) führte allerdings zu einer signifikanten Steigerung der Adenosinkonzentration (61.52 ± 33.81 auf 158.61 ± 140 nmol/l), die durch simultane Bettruhe (HBR) noch erhöht wurde (97.15 ± 25.92 auf 235.37 ± 125.61 nmol/l), jedoch auch unter normoxischen Bedingungen (NBR) nachweisbar war (68.14 ± 37.0 auf 164.29 ± 81.92 nmol/l).

In der ersten Feldstudie in der **Antarktis** verglichen wir die Stressantwort von männlichen Teilnehmern von zwei bzw. drei Überwinterungsexpeditionen (Dauer je 11 Monate) auf zwei verschiedenen Antarktisstationen (Neumayer III (Meereshöhe), n = 16; Concordia (3233 m), n = 15) zur Differenzierung des Einflusses von hypobarer Hypoxie.
Dabei zeigte sich auf Neumayer III direkt nach Beginn der Expeditionen ein signifikanter Anstieg der EC und NAE Konzentrationen (z.B. AEA 0.34 ± 0.07 auf 2.53 ± 2.09 ng/ml; PEA 3.15 ± 0.98 auf 9.72 ± 5.25 ng/ml), wohingegen die Katecholaminwerte niedrig blieben. Im Gegensatz hierzu kam es auf Concordia unter hypobarer Hypoxie zum selben Zeitpunkt zu einem signifikanten Anstieg der nächtlichen Noradrenalinmenge (19.07 ± 7.04 auf 42.18 ± 30.47 µg) bei gleichzeitig niedrigen EC und NAE Konzentrationen.
In der zweiten Feldstudie untersuchten wir geschlechtsspezifische Differenzen der Stressantwort in den drei Expeditionsteams auf Neumayer III (weibliche Probanden n = 10; männliche Probanden n = 16).
Wir wiesen nach, dass die EC und NAE Konzentrationen der Frauen auf Neumayer III einen ähnlichen Verlauf mit signifikantem Anstieg wie bei ihren männlichen Kollegen zeigen.
Des Weiteren waren die morgendlichen Cortisolkonzentrationen der Frauen im antarktischen Winter signifikant höher als die der Männer (z.B. April: Frauen 0.73 ± 0.29; Männer 0.46 ± 0.16), ohne dass eine erhöhte psychische Belastung nachweisbar war.

Zusammenfassend lässt sich aus den Untersuchungen und hier dargestellten Ergebnissen schließen, dass verschiedene Stressoren definierte und differenzierte Veränderungen in der Stressantwort

einzelner physiologischer Systeme hervorrufen, die mess- und quantifizierbar und in ihrem Verlauf variabel sind.
Unsere Ergebnisse präsentieren Veränderung und Anpassung der verschiedenen physiologischen Systeme ganz im Sinne des Konzepts der menschlichen Allostase dahingehend, dass die verursachten Veränderungen der jeweiligen Umwelt entsprechend spezifisch ausfallen, aber zugleich eine hohe individuelle Variabilität aufweisen. Aufgabe zukünftiger Forschungsbemühungen muss es daher sein, die individuellen Faktoren, die hierfür ursächlich sind, zu identifizieren und zu differenzieren.
Neben der spezifischen Umwelt kommt dem Einflussfaktor „Zeit“ eine entscheidende Rolle zu, denn die akute bzw. subakute Exposition während der PlanHab-Studie rief ein anderes physiologisches Reaktionsmuster hervor als die chronische Langzeitexposition in der Antarktis. Die Ergebnisse der systematischen Untersuchungen am Menschen bringen zum Ausdruck, dass die physiologischen Veränderungen unter chronischer Exposition wahrscheinlich eher einer Akklimatisierung an die Verhältnisse entsprechen als Folge pathologischer Veränderungen sind, da sie im zeitlichen Verlauf allmählich rückläufig sind und sich wieder der „Normal“- Zustand etabliert. Die Exploration und Klassifikation der Zeitspannen „akut“, „subakut“ und „chronisch“ wird hier zukünftig neue Erkenntnisse bringen.
Vor dem Hintergrund einer zunehmend individualisierten Medizin, identifizierten wir nur geringe Differenzen bei geschlechtsspezifischer Betrachtung der Stressantwort. Diese scheinen allerdings ebenso durch die spezifische Umwelt bedingt, so dass weitere Forschung und Aufklärung auch hinsichtlich möglicher Konsequenzen notwendig sind.
Langfristig kann die Erforschung der Regulation der menschlichen Allostase in extremer Umwelt helfen, potenziell schädliche Auswirkungen von Veränderungen durch adäquate Gegenmaßnahmen im besten Fall zu verhindern oder zumindest zu kontrollieren. Diese Erkenntnisse können dann auch im klinischen Umfeld umgesetzt werden und am Patienten Anwendung finden.

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10.2 Fallbericht

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10.3 Übersichtsartikel

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10.4 Buchbeitrag

Aus Choukèr, A. Stress Challenges and Immunity in Space: From Mechanisms to Monitoring and Preventive Strategies (Second Edition, in press):

Stress, Hypoxia, and Immune Responses
Claudia Strewe, Manfred Thiel, Michail Sitkovsky, Alexander Choukèr and Matthias Feuerecker

10.5 Sonstige Veröffentlichungen

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10.6 Kongressmitteilungen/Vorträge

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C. Strewe, M. Feuerecker, J. Pagel, I. Mekjavic, M. Hoerl, S. Matzel, K. Biere and A. Choukèr
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Life in Space for Life on Earth Symposium, Aberdeen, Schottland, 2012

Consequences of long-term Confinement and Hypobaric Hypoxia on Immunity in the Antarctic Concordia Environment (CHOICE). A Hypoxia controlled field Study to prepare for manned exploration class Mission
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12 Anhang

Open Access

- **Strewe C, Moser D,** Buchheim J-I, Gunga H-C, Stahn A, Crucian B E, Fiedel B, Bauer H, Gössmann-Lang P, Thieme D, Kohlberg E, Choukèr A, Feuerecker M
  „Sex differences in stress and immune responses during confinement in Antarctica“
  Biol Sex Differ. 2019 Apr 16;10(1):20. doi: 10.1186/s13293-019-0231-0.

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Biology of Sex Differences

RESEARCH Open Access

Sex differences in stress and immune responses during confinement in Antarctica

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Abstract

Background: Antarctica challenges human explorers by its extreme environment. The effects of these unique conditions on the human physiology need to be understood to best mitigate health problems in Antarctic expedition crews. Moreover, Antarctica is an adequate Earth-bound analogue for long-term space missions. To date, its effects on human physiology have been studied mainly in male cohorts though more female expeditioners and applicants in astronaut training programs are selected. Therefore, the identification of sex differences in stress and immune reactions are becoming an even more essential aim to provide a more individualized risk management.

Methods: Ten female and 16 male subjects participated in three 1-year expeditions to the German Antarctic Research Station *Neumayer III*. Blood, saliva, and urine samples were taken 1–2 months prior to departure, subsequently every month during their expedition, and 3–4 months after return from Antarctica. Analyses included cortisol, catecholamine and endocannabinoid measurements; psychological evaluation; differential blood count; and recall antigen- and mitogen-stimulated cytokine profiles.

Results: Cortisol showed significantly higher concentrations in females than males during winter whereas no enhanced psychological stress was detected in both sexes. Catecholamine excretion was higher in males than females but never showed significant increases compared to baseline. Endocannabinoids and *N*-acylethanolamides increased significantly in both sexes and stayed consistently elevated during the confinement. Cytokine profiles after in vitro stimulation revealed no sex differences but resulted in significant time-dependent changes. Hemoglobin and hematocrit were significantly higher in males than females, and hemoglobin increased significantly in both sexes compared to baseline. Platelet counts were significantly higher in females than males. Leukocytes and granulocyte concentrations increased during confinement with a dip for both sexes in winter whereas lymphocytes were significantly elevated in both sexes during the confinement.

Conclusions: The extreme environment of Antarctica seems to trigger some distinct stress and immune responses but—with the exception of cortisol and blood cell counts—without any major relevant sex-specific differences. Stated sex differences were shown to be independent of enhanced psychological stress and seem to be related to the environmental conditions. However, sources and consequences of these sex differences have to be further elucidated.

Keywords: Sex differences, Neuroendocrine response, Immunity, Extreme environment, Antarctica, Confinement

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Introduction

Antarctica is one of the most remote regions on Earth with a generally misanthropic environment. Expeditioners are exposed to a very cold and harsh climate with a mainly monotonic landscape of snow and ice. This leads, especially during winter, to a complete isolation of sojourners on Antarctic research stations as visiting the sites or an evacuation here are almost impossible. Additionally, the natural day-night rhythm is abrogated as during the summer months the sun never sets which leads to 24 h of daylight and during winter it never rises causing 24 h of almost complete darkness.

It has been demonstrated that this inhospitable environment represents also a suitable and standardized Earth-bound analogue to mimic some factors like monotonous surroundings, repetitive daily routine and confinement, and its possible resulting psycho-social stress that humans are subjected to during long-term spaceflights [1–3]. General findings in respective studies describe hormonal changes due to an altered biological rhythm [4] which directly affects sleep pattern and quality [5–7] as well as psycho-social behavior [8]. Furthermore, endocrine and metabolic dysfunctions and negative mood patterns have been related to these adverse conditions [9, 10], and also altered functions of the innate and adaptive immunity were identified [11–15].

Until recently, most efforts in the field of Antarctic and space research concentrated principally on identifying the impact of such adverse, extreme environment on different aspects of human physiology but did not necessarily focus on identifying potential sex-specific differences or reaction patterns. This was historically due to the male dominance in Antarctic missions. Also in human spaceflight, male dominance has persisted, but in Antarctica, more and more female applicants and crews are recorded. Hence, despite the fact that the quantity of human studies with focus on sex still remains little, research revealed sex-based specificities in different domains of interest in regard to both Antarctic and space exploration missions.

Against the background of stress-related reactivation of latent viruses [16] and an assumed general increased risk for adverse health effects during spaceflight, the sex differences in immune answers already identified in "normal" life might be important to assure crew health. Herein, women present higher $CD4^+$ T cell counts, a higher CD4/CD8 ratio, a higher number of B cells, greater antibody responses, and higher immunoglobulin levels [17, 18]. Therefore, women seem to be provided with a greater resistance to and in case of an infection with a more solid and potent immune response but on the other hand more susceptible to autoimmune diseases [19–21]. These findings together with the increasing number of women in Antarctica and in space emphasize the necessity to multiply studies that focus on sex-specific reactions, mechanisms, and outcome and subsequently to install distinctive countermeasures.

Therefore, our study focused on identifying potential sex differences in psychological, neuroendocrine, and immune reactions to the exposition of women and men to the extreme environment of Antarctica as ground-based space analogue at the German *Neumayer III* station during three overwintering campaigns.

On the basis of previous research, we hypothesized that (i) women show a higher susceptibility to psycho-social stress in isolated extreme environments that subsequently triggers an enhanced neuroendocrine response and (ii) thus results in a decreased immune answer in women compared to their male companions.

Material and methods

Study environment

The coastal Antarctic research station *Neumayer III* is located in the Atka Bay in the northeast Weddel sea on the Ekström shelf ice at sea level (70° 40′ S/8° 16′ W) and is operated by the German Polar Institute "Alfred Wegener Institute for Polar and Marine Research", Bremerhaven, Germany. Expeditioners see no sunlight for about 60 days around midwinter (21 June, complete darkness from mid-May to mid-July). Outside temperatures during midwinter phase drop to ~ − 40 °C, while summertime brings temperatures of ~ − 3 °C. These harsh and extreme conditions restrict access to and exit from the station during winter and lead to a complete isolation period of almost 9 months from mid-February till mid-October as also communication via phone or Internet is dependent on current weather conditions. Therefore, all supply goods for the over-wintering period must be provided during summer and stored adequately.

Study participants

During the expeditions in 2013, 2014, and 2015, in total, 10 women and 16 men took part in the study. Their demographic data are given in Tables 1 and 2. These expeditioners were primarily employed as scientists,

Table 1 Demographic data of the participants of the campaigns 2013, 2014, 2015

	Female	Male
n	10	16
Age [years]	31.8 ± 6.1 (24 - 44)	37.7 ± 9.1 (26 - 55)
Height [m]	1.66 ± 0.06 (1.59 – 1.74)	1.80 ± 0.05 (1.72 – 1.88)#
Weight [kg]	67.8 ± 10.0 (59.1 - 88)	86.8 ± 10.7 (71 - 107.7)#
BMI [kg/m^2]	24.5 ± 3.5 (20.5 - 32.3)	26.9 ± 3.4 (22.5 - 33.6)

n = number of participants; BMI = body mass index; data are mean ± SD (range); # significant difference between male and female; $p \leq 0.001$ respectively.

Table 2 Demographic data of participants separated by sex and winter-over season (WO)

	WO 2013		WO 2014		WO 2015	
Sex	female	male	female	male	female	male
n	4	5	2	6	4	5
Age [years]	30.0 ± 3.7	33.8 ± 2.05	26.5 ± 3.54	36.2 ± 8.95	36.3 ± 6.55	43.4 ± 11.85
Height [m]	1.64 ± 0.07	1.80 ± 0.04	1.69 ± 0.07	1.82 ± 0.05	1.67 ± 0.05	1.76 ± 0.04
Weight [kg]	60.9 ± 1.44	88.6 ± 15.17	64 ± 0	87.2 ± 7.68	76.5 ± 11.24	84.5 ± 10.54
BMI [kg/m^2]	22.6 ± 1.68	27.4 ± 4.82	22.5 ± 1.88	26.3 ± 2.50	27.4 ± 3.63	27.2 ± 3.22

n = number of participants; BMI = body mass index; data are mean ± SD

cooks, engineers (including IT), electricians, and medical doctors. On-site data acquisition and sample processing were performed by the respective crew surgeon (BF, HB, PG-L). As the medical position in the crew did not necessarily require a scientific background, they all received training in carrying out research protocols and in monitoring the study participants during the experimental period before their deployment.

Sample schedule and collection

Baseline data collection (BDC) was conducted in October of the previous year (e.g., October 2012 for the over-wintering campaign 2013) at the Center for Space Medicine and Extreme Environments at the Charité, Berlin, Germany. Generally, all crew members arrived at *Neumayer III* in December of the respective season. First data and sample collection was always performed in February after an "acclimatization" phase and then on a monthly basis till November. Sampling took place in the first week of each month, in the morning around 7:00 am after an overnight fasting period. Physical exercise was not allowed for 24 h prior to sample collection. Psychological data was acquired by paper questionnaires. Saliva samples for cortisol measurements were taken in the morning (7 am) fasted after awakening and in the evening (7 pm) before dinner. Urine samples were collected for 12 h during the day (7 am to 7 pm) and for 12 h during the night (7 pm to 7 am) for catecholamine determination. Blood draw included the sampling of blood into EDTA and lithium-heparinized tubes. After each expedition, all study samples were shipped back to Munich, Germany, at a temperature of at least − 25 °C. Three to 4 months after return from Antarctica, a post data collection (PDC) was conducted at the Center for Space Medicine and Extreme Environments, Charité, Berlin, Germany, or at the Alfred-Wegener-Institute, Bremerhaven, Germany.

Study outcome parameters and data

The primary outcome parameter to state a potentially enhanced neuroendocrine response was cortisol concentration. Endocannabinoids, catecholamines, the blood cell count, and the scores assessed by psychological tests were defined as secondary outcome measures. Additionally, the cytokine release after various stimulations was defined as secondary outcome parameter to evaluate the immune response.

Parts of the data have been used for a comparison with data from another Antarctic research station in a recent publication [22].

Study parameters

Psychological stress response

Three different paper questionnaires were performed to evaluate and quantify the participants' emotional stress level and the intensity of perceived anxiety.

Current Stress Test (CST) The test is conceived to assess a person's acute emotional stress level [23] and mirrors sensitively acute situational changes in subjective stress. It consists of six questions with paired positive and negative adjectives monitoring the perception of current stress (e.g., "tense–calm"). The subjects give their ratings on a 6-point Likert scale. The range for total item means is 1–6, with higher values indicating an increased stress experience. The design of the test reduces the possibility of carry-over effects upon frequent application. The test was applied at BDC, every month during the confinement and at PDC in the morning and evening, respectively.

Spielberger State Trait Anxiety Inventory (STAI) The STAI [24] distinguishes two kinds of anxiety: state anxiety (perceived in a specific situation) and trait anxiety (referring to one's character). It consists of two parts with each 20 questions rating the answers on a 4-point scale. Global test score ranges between 40 and 160 points. The STAI was evaluated at BDC, every month during the confinement and at PDC.

Post-Traumatic Stress Scale-10 (PTSS-10) The test detects feelings associated with anxiety and depression (e.g., nightmares, sleep disorders, pain). It consists of two parts with part A including yes/no answers to mirror their existence in the last month and part B grading 10 negative feelings in the past few days on a 7-point scale with a total score between 10 and 70 points. The PTSS-10 was applied at three time points (BDC, July, and PDC).

Neuroendocrine stress response

Cortisol in saliva Saliva was collected using a Salivette® (Sarstedt, Nümbrecht, Germany). Participants chewed on the cotton swab for 30–45 s which was subsequently frozen and stored at a temperature of at least − 25 °C at *Neumayer III*. Cortisol concentrations were then quantified by an electrochemiluminescence immunoassay (Elecsys 2010, Roche, Mannheim, Germany) at the Institute of Clinical Chemistry, Hospital of the University of Munich, Germany.

Urine catecholamines Norepinephrine and epinephrine were measured from the pooled daytime and nighttime urine by taking a 10-ml sample respectively. The first voided urine volume was discarded before starting the collection. The samples were immediately frozen, stored, and subsequently transported at a minimum temperature of at least − 25 °C. Quantification of catecholamine concentrations was executed at the Institute of Clinical Chemistry, University of Munich, Munich, Germany, using HPLC (Chromsystems, Martinsried, Germany). The absolute mass of excreted catecholamines was determined by multiplication of urine catecholamine concentrations with the respective urine volume.

Endocannabinoid (EC) and *N*-acylethanolamide (NAE) measurements from lithium-heparinized blood EC concentrations were measured from lithium-heparinized whole blood. Samples were drawn from the fastened subject, immediately placed on ice water to prevent temperature effects [25] and after transfer into Eppendorf tubes frozen at a temperature of at least − 25 °C without any delay. Such sample treatment ensures EC stability for at least 6 months [26]. Quantification of the EC concentrations of anandamide (AEA), 2-arachidonoylglycerol (2-AG), and the NAEs oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and stearoylethanolamide (SEA) was executed at the Institute of Doping Analysis und Sports Biochemistry, Kreischa, Germany. The exact method has been described previously [22].

Blood analyses and immune cell functions

Complete blood cell count from EDTA-anti-coagulated blood EDTA-anti-coagulated blood samples were used to determine the complete blood cell count using the on-site QBC Autoread plus automated analyzing system (QBC Diagnostics, Port Matilda, PA, USA). Hematocrit, hemoglobin concentration, platelet, and differential white blood cell counts, as well as the percentages of granulocytes and lymphocytes, were quantified.

Recall antigen- and mitogen-stimulated cytokine profiles from lithium-heparinized blood For analysis of secreted T helper type 1/2 (Th1/Th2) cytokine profiles, lithium-heparinized blood was incubated in assay tubes with the same volume of RPMI-1640 (Sigma-Aldrich) and a fungal antigen mixture containing Candida lysate (10 mg/mL; Allergopharma, Reinbeck, Germany) and trichophyton lysate (10 mg/ml; Allergopharma, Reinbeck, Germany) or RPMI-1640 and pokeweed mitogen (PWM) (5 mg/mL; Sigma-Aldrich). PWM acts as a strong "polyclonal" activator, inducing mitosis in lymphocytes in a non-receptor-specific manner. The assay tubes were incubated for 48 h at 37 °C. After incubation, 200 μl of the supernatant were transferred into Eppendorf tubes and frozen immediately at a temperature of at least − 25 °C for future cytokine analyses.

Assessment of cytokine production from lithium-heparinized blood After thawing of the supernatants, concentrations of the cytokines IFN-γ, IL-10, IL-2, and TNF were analyzed by LuminexxMAP® technology (Bioplex®) with commercially available reagents from BioRad Laboratories Inc. (Hercules, CA, USA), according to the manufacturer's guidelines. Data were analyzed using Bioplex software; the sensitivity threshold was at 2 pg/ml.

Statistical analysis

The data was tested for normal distribution using the Shapiro-Wilk test. To realize within-group comparisons (e.g. changes in hematocrit over time in one sex), one-way repeated measure analysis of variances (one--way RM-ANOVA) was applied followed by the post hoc Dunnett or Holm-Sidak test to correct for multiple comparisons. Significant differences were determined by comparing baseline time point (BDC) to the deployed and recovery values. Between-group comparisons (between the two sexes) were executed using a t test for normally distributed data and the Mann-Whitney U test for non-parametric data. The mean differences between time points were considered significantly different if $p < 0.05$ and are indicated as such on each data table and figure. Statistical inferences regarding the interaction of sex and time were based on mixed linear models with a random intercept for each subject, the fixed effects sex, time-point (entered as a categorical variable), interaction of time and sex, age, and BMI and a first-order autoregressive covariance structure. Correlations between parameters were quantified using Spearman's rank correlation coefficient. Statistical calculations were performed using SigmaPlot® 12.5 (Systat Software, Chicago, IL, USA), IBM SPSS Statistics V.25 (Armonk, NY, USA), and the Statistical Analysis System release 9.4 for Windows (SAS Institute, Cary, NC, USA).

Results

Psychological stress response

Current Stress Test (CST)

The CST mirrored a low stress level with constant score values below 3 in both sexes in the morning and evening respectively, with no significant differences between the sexes or in the course of the observation period to BDC (Table 3).

Spielberger State Trait Anxiety Inventory (STAI)

Scores for both sexes were low throughout the entire observation period and showed no significant differences between the two sexes or within each group. Threshold values indicating anxiety in a specific situation (36.83 ± SD 9.82 [27]) were moderately exceeded by men at the end of the deployment and back in Europe and by females at the onset of the Antarctic winter and in November. Mean total scores displaying anxiety as a character trait (34.45 ± SD 8.83 [27]) were almost never exceeded (Table 3).

Table 3 Current Stress Test (CST) and Spielberger State Trait Anxiety Inventory (STAI)

	CST morning		CST evening		STAI state		STAI trait	
	Female	Male	Female	Male	Female	Male	Female	Male
BDC	2.2 ± 0.55	2.4 ± 0.73	2.2 ± 0.79	2.1 ± 0.90	30.0 ± 4.1	32.4 ± 9.9	35.3 ± 6.3	33.8 ± 7.9
February	2.5 ± 1.11	2.7 ± 0.96	2.3 ± 0.99	2.1 ± 0.90	36.4 ± 9.5	36.4 ± 9.6	34.7 ± 7.7	32.6 ± 9.2
March	2.3 ± 0.73	2.3 ± 0.59	2.2 ± 0.94	2.3 ± 0.86	31.2 ± 6.8	32.7 ± 6.4	31.1 ± 6.8	30.9 ± 8.1
April	2.1 ± 0.54	2.7 ± 1.13	2.6 ± 0.76	2.6 ± 1.17	31.7 ± 6.0	36.6 ± 10.8	29.9 ± 4.7	32.3 ± 9.5
May	2.5 ± 1.12	2.2 ± 1.08	2.5 ± 1.28	2.4 ± 1.02	37.6 ± 12.1	33.6 ± 9.7	32.0 ± 7.4	30.9 ± 10.0
June	2.3 ± 0.79	2.4 ± 1.18	2.1 ± 0.71	2.4 ± 1.19	34.4 ± 7.9	33.3 ± 10.6	30.8 ± 7.3	31.7 ± 11.6
July	2.5 ± 1.1	2.4 ± 0.78	2.4 ± 1.0	2.3 ± 0.96	36.3 ± 12.1	34.9 ± 9.3	31.7 ± 6.7	32.3 ± 10.0
August	2.5 ± 1.17	2.3 ± 1.14	2.5 ± 1.23	2.1 ± 0.95	33.4 ± 10.0	34.2 ± 12.0	32.1 ± 8.4	31.6 ± 10.0
September	2.1 ± 0.82	2.3 ± 1.0	2.1 ± 0.9	2.2 ± 1.07	33.4 ± 6.6	34.5 ± 11.8	30.9 ± 5.0	31.8 ± 9.4
October	2.5 ± 1.38	2.4 ± 1.19	2.4 ± 1.02	2.6 ± 1.29	35.5 ± 13.6	35.7 ± 11.7	29.2 ± 5.8	31.8 ± 11.5
November	2.7 ± 1.36	2.7 ± 1.39	2.7 ± 1.55	2.6 ± 1.28	41.0 ± 16.2	38.5 ± 12.2	32.1 ± 10.1	35.5 ± 12.4
PDC	2.3 ± 1.24	2.4 ± 1.06	2.2 ± 1.2	2.4 ± 1.35	35.6 ± 14.0	36.9 ± 12.6	29.4 ± 8.5	32.6 ± 11.6

Data are mean ± SD; units are scores (CST) or points (STAI); female n = 8-10; male n = 13-16; BDC = Baseline Data Collection, PDC = Post Data Collection; no significant changes between females and males or within each group to BDC

Post-Traumatic Stress Scale-10 (PTSS-10)

Part A indicated no increased feelings of anxiety in either sex at all time points (data not shown). Part B demonstrated in both sexes a small but not significant increase in negative feelings at each time point (*females* $n = 10$; BDC 20.9 ± 5.09; July 23.8 ± 10.14; PDC 23.3 ± 12.70; *males* $n = 16$; BDC 18.88 ± 9.20; July 19.5 ± 7.56; PDC 23.13 ± 10.09).

Neuroendocrine stress response

Cortisol in saliva

Cortisol concentrations in the morning were higher in both sexes at the beginning of the expedition than at BDC even though these changes missed statistical significance. Values in females were consistently higher in April ($p = 0.009$), May ($p = 0.02$) and July ($p = 0.036$) compared to their male colleagues. In the evening, cortisol concentrations dropped in both sexes until March, then fluctuated with no significant differences between males and females. Values in males differed significantly to BDC in March, May to July and October ($F(11,160) = 2.531$, $p = 0.006$) (Fig. 1a, b).

Cortisol ratio between morning and evening values fluctuated but showed constantly higher values throughout the expedition compared to BDC for both sexes. Values in females peaked in February and July and decreased afterwards to baseline values. Values in males peaked in March and June and fluctuated in between. Within-group comparisons showed no statistical significance in males or females, respectively. Between groups comparisons showed a higher cortisol ratio in females than males in February ($p = 0.02$) and April ($p = 0.017$) (Fig. 1c).

Urine catecholamines

Norepinephrine In both sexes, norepinephrine excretion during the day showed higher values in the first months of deployment compared to BDC. A drop was found in April/May for both sexes at the onset of the Antarctic winter. In the following months, norepinephrine excretion constantly rose in males to peak again in August whereas female values peaked already in June to subsequently drop to their lowest values in August with a significant difference to their male colleagues' values (August: *females* 19.35 ± 8.63 μg; *males* 39 ± 36.35 μg; p = 0.01). During the night, norepinephrine excretion was

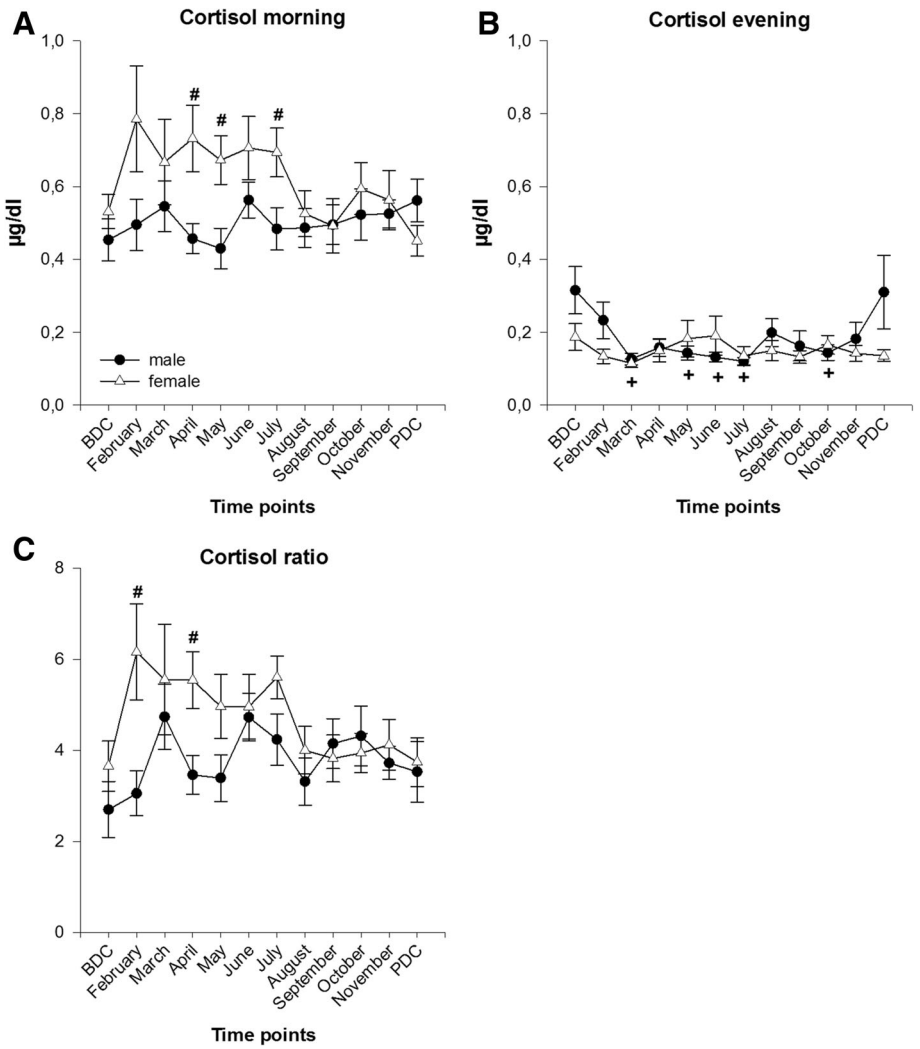

Fig. 1 Cortisol in saliva in the morning (**a**) and evening (**b**) and its ratio between morning and evening samples (**c**); data are means ± SEM; units are µg/dl; female $n = 8–10$; male $n = 13–16$. BDC, baseline data collection; PDC, post data collection; #, significant difference between male and female; +, significant difference to BDC in males

significantly lower in females than males in March (*females* 14.05 ± 10.22 µg; *males* 21.07 ± 8.47 µg; $p = 0.023$). A rise of norepinephrine excretion albeit without any statistical significance was found in both sexes in September (*females* 18.20 ± 16.17 µg; *males* 25.75 ± 18.27 µg) at the end of the Antarctic winter season. In general, the mass of norepinephrine excretion in female expeditioners during the day and the night remained below the values of their male colleagues (Fig. 2a, b).

Epinephrine Total mass epinephrine excretion during the day and the night fluctuated during the deployment period in both sexes. Significant differences between males and females were found at four time points during the day and at two during the night (*epinephrine day*: BDC $p = 0.02$; April $p = 0.003$; August $p = 0.002$; October $p = 0.014$; *epinephrine night:* March $p = 0.014$ and May $p = 0.047$). In general, the mass of epinephrine excretion in female expeditioners during the day and the night remained below the values of their male colleagues (Fig. 2c, d).

Endocannabinoids and NAEs The ECs AEA and 2-AG and all NAEs increased significantly already in the first months of the Antarctic stay with mean values reaching up to sixfold their basic values (*AEA females* $F(11,97) = 6.144$, $p < 0.001$; *AEA males* $F(11,164) = 5.78$, $p < 0.001$; *2-AG females* $F(11,97) = 5.224$, $p < 0.001$; *2-AG males* $F(11,163) = 4.08$, $p < 0.001$; *OEA females* $F(11,97) = 3.392$, $p < 0.001$; *OEA males* $F(11,163) = 2.980$, $p < 0.001$; *PEA*

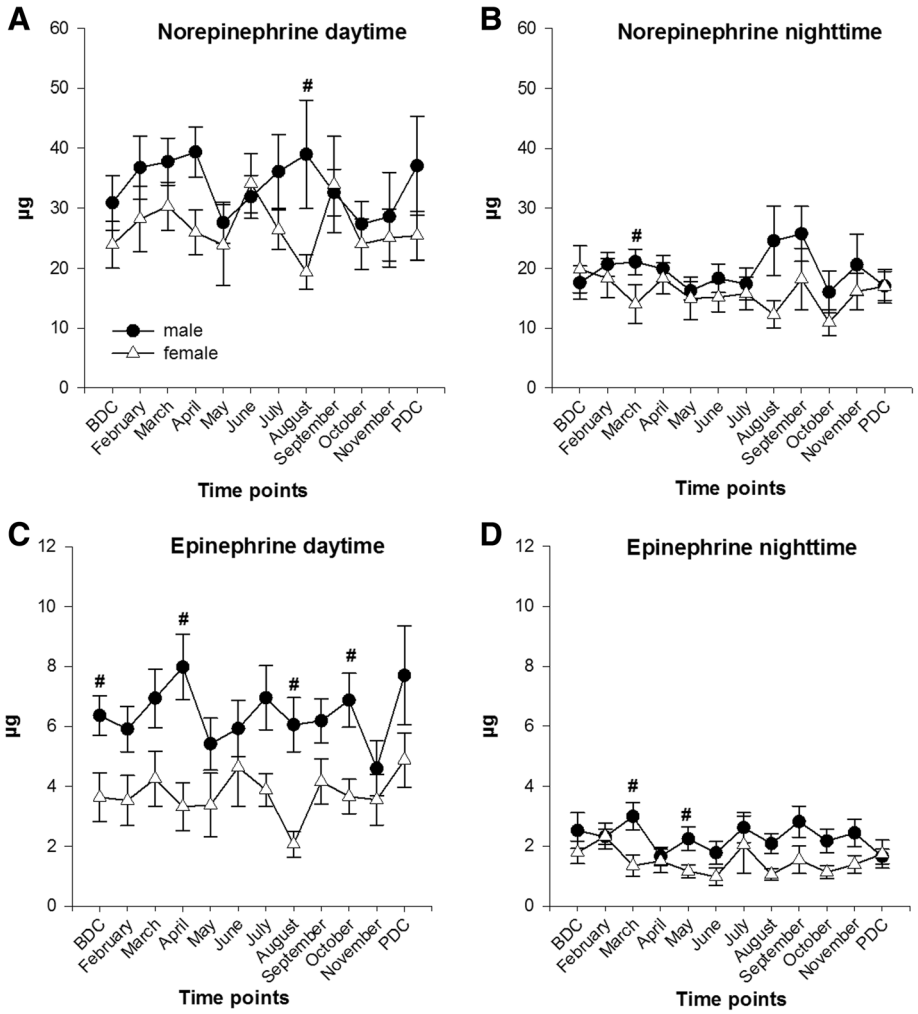

Fig. 2 Norepinephrine (**a**, **b**) and epinephrine (**c**, **d**) in urine during the day and night (collection time 12 h); data are means ± SEM; units are µg; female $n = 6–10$; male $n = 11–16$. BDC, baseline data collection; PDC, post data collection; #, significant difference between males and females

females $F(11,97) = 4.776$, $p < 0.001$; *PEA males* $F(11,162) = 5.751$, $p < 0.001$; *SEA females* $F(11,97) = 6.361$; $p < 0.001$; *SEA males* $F(11,163) = 7.068$; $p < 0.001$). This increase was consistent in both sexes. Throughout the observation period, EC and NAE concentrations fluctuated in males and females but always on a highly elevated level and returned to BDC values only when back in Europe. Significant differences between the two sexes were stated for 2-AG and SEA in July (2-AG $p = 0.016$; SEA $p = 0.048$) (Fig. 3a–e).

Blood analyses and immune cell functions

Complete blood cell count (Fig. 4a–f)

Hemoglobin Hemoglobin increased significantly in both sexes during the whole stay in Antarctica and returned to BDC values back in Europe (*females* BDC 13.6 ± 0.6; peak in March 15 ± 1.08 g/dl; $F(11,93) = 3.008$, $p = 0.002$; *males* BDC 15.4 ± 0.73 g/dl; peak in September 16.6 ± 1.43 g/dl; $F(11,157) = 7.654$, $p < 0.001$). Significant differences between males and females were found at all time points with female values being always lower than male values ($p \leq 0.001$ to 0.005).

Hematocrit Hematocrit showed a similar course to hemoglobin regarding sex differences (significantly different at all time points; $p \leq 0.001$ to 0.002), but significant changes to BDC were found in females in the months March, July, September, and October ($F(11,93) = 3.988$, $p < 0.001$).

Thrombocytes Thrombocytes increased higher in females with significant differences to males from April to November ($p = 0.004$ to 0.032). Significant differences to BDC were

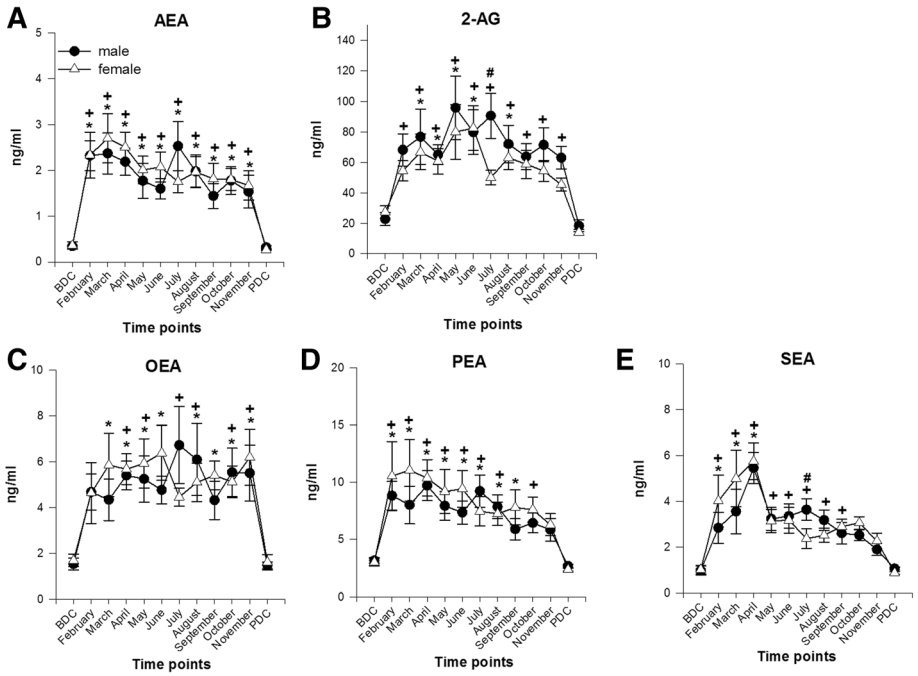

Fig. 3 Endocannabinoid and *N*-acylethanolamide concentrations in lithium-heparinized blood. **a**, **b** ECs. **c**–**e** NAEs. Data are means ± SEM; units are ng/ml; female $n = 9$–10; male $n = 15$–16. BDC, baseline data collection; PDC, post data collection; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; SEA, stearoylethanolamide; #, significant difference between male and female; +, significant difference to BDC in males; *, significant difference to BDC in females

found in females in June and July when their thrombocyte count peaked ($F(11,93) = 2.961$, $p = 0.002$) and for males in March and July ($F(11,157) = 2.549$, $p = 0.005$).

Leukocytes Leukocytes showed a similar course in males and females. They increased in February then fluctuated on an elevated level with male values being significantly different to BDC from February to May and from July to October ($F(11,158) = 5.862$, $p < 0.001$) and returned to baseline values at PDC but stayed elevated in females.

Percentage of granulocytes and lymphocytes The percentage of granulocytes and lymphocytes rose in both sexes but showed almost no statistical in-between differences. In general, percentage of granulocytes was higher in females whereas percentage of lymphocytes was higher in males. Significant differences to BDC for granulocytes were found in males throughout the whole deployment ($F(11,158) = 8.455$, $p < 0.001$) and for lymphocytes from April to November ($F(11,158) = 6.115$, $p < 0.001$) and for females from June to November (except October) ($F(11,93) = 2.238$, $p = 0.018$).

Recall antigen- and mitogen-stimulated cytokine profiles

Basal cytokine release Basal release of the cytokines INF-γ, IL-10, IL-2, and TNF showed a significant difference to BDC only for IL-2 in female participants in April ($F(6,53) = 2.287$, $p = 0.049$). Female values were consistently lower than male values (Table 4).

Cytokine profile after stimulation with fungal antigens (Fig. 5a–d)

INF-γ The concentration of IFN-γ rose continuously in both sexes and peaked in June (males) or August (females) but showed significant changes to BDC only for males (June and August). Afterwards, IFN-γ concentrations declined gradually in both sexes to reach baseline levels at PDC.

IL-10 At BDC, a significant sex difference ($p = 0.027$) was stated that could not be detected in the further course of the observation period. Both sexes showed significant increases in IL-10 concentrations at PBC (females $F(6,29) = 4.888$; $p = 0.001$).

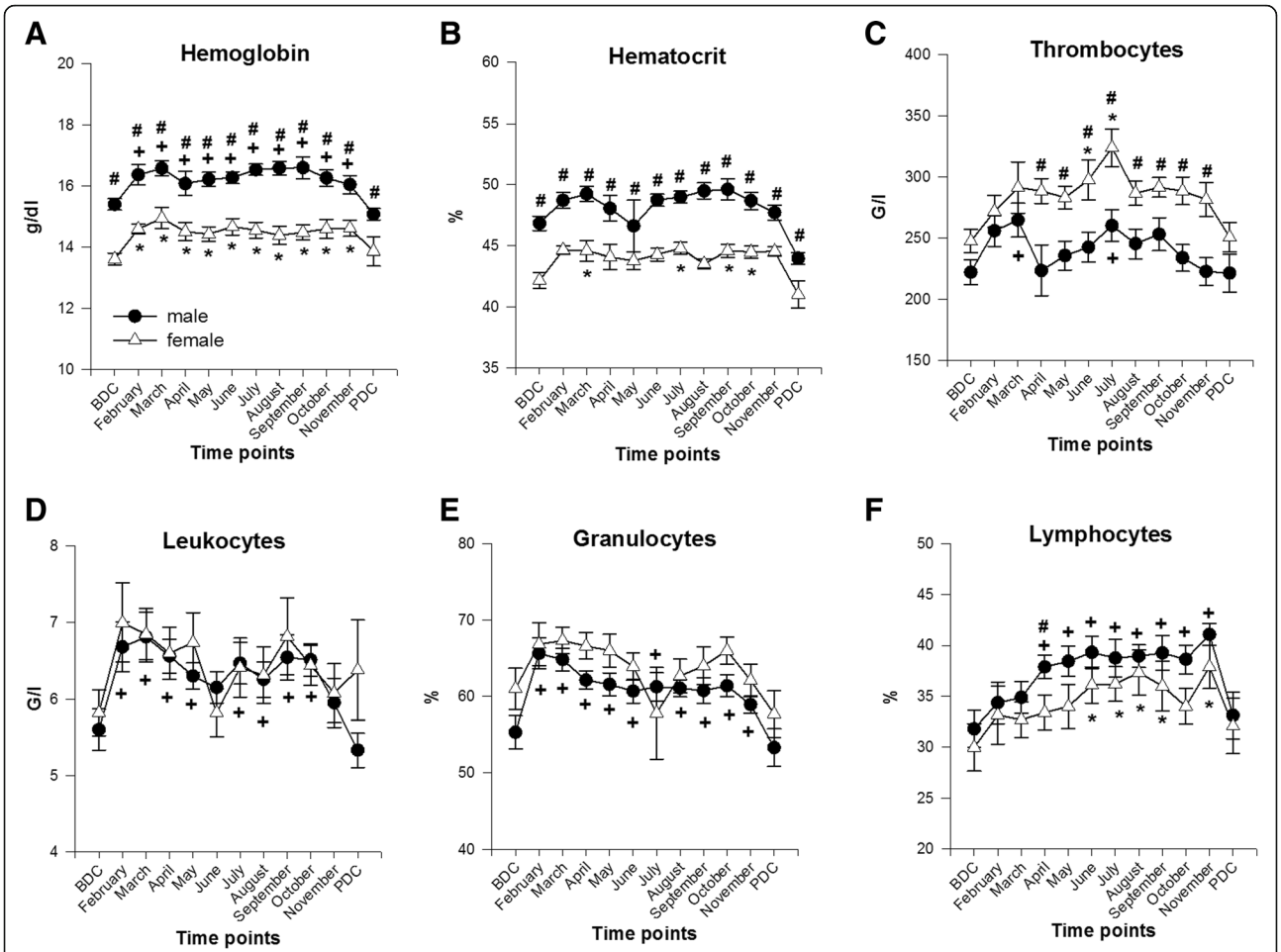

Fig. 4 Blood cell count from EDTA anti-coagulated blood. **a** Hemoglobin [units are g/dl]. **b** Hematocrit [units are %]. **c** Thrombocytes [units are G/l]. **d** Leukocytes [units are G/l]. **e** Percentage of granulocytes [units are %]. **f** Percentage of lymphocytes [units are %]; data are means ± SEM; female $n = 6–10$; male $n = 11–16$. BDC, baseline data collection; PDC, post data collection; #, significant difference between male and female; +, significant difference to BDC in males; *, significant difference to BDC in females

IL-2 After stimulation with fungal antigens, the concentration dropped in both sexes in February before it rose to maximum levels in June and declined afterwards with minimum levels at PDC (significant decline to BDC in males). In both sexes, IL-2 levels—although peaking—did only moderately exceed BDC values and never reached statistical significance.

TNF In comparison to BDC, TNF concentration declined during deployment with no difference between sexes. At PDC, TNF levels rose again, however, without reaching BDC values.

Cytokine profile after stimulation with the mitogen PWM (Fig. 6a–d)

INF-γ IFN-γ concentrations peaked in April (females) and June (males), but significant differences were only observed in females (April and June, $F(6,52) = 5.218$, $p < 0.001$) compared to BDC.

IL-10 Concentrations increased in females till February and in males till April but only in females the increase was statistically significant ($F(6,29) = 4.115$, $p = 0.004$). Subsequently, concentrations declined continuously with the lowest levels at PDC. No sex differences were observed.

IL-2 After an initial drop in February in males, IL-2 concentrations showed continuously higher values with a peak in April (females) and in June (males) compared to BDC, then fluctuated in both sexes and decreased till PDC. No significances were stated.

TNF Till February (females) and April (males), TNF concentrations decreased to subsequently peak in June

Table 4 Basal cytokine release for male and female participants

	IFN-γ [pg/ml]		IL-10 [pg/ml]		IL-2 [pg/ml]		TNF [pg/ml]	
	female	male	female	male	female	male	female	male
BDC	0.13 ± 0.10	4.41 ± 10.50	1.14 ± 1.57	7.71 ± 13.63	0.25 ± 0.50	0.15 ± 0.19	0.42 ± 0.48	1.07 ± 1.43
February	0.66 ± 1.78	6.09 ± 17.16	0.88 ± 1.46	11.31 ± 22.80	0.48 ± 1.09	2.87 ± 5.79	0.48 ± 1.23	2.29 ± 6.52
April	0.21 ± 0.35	6.40 ± 15.56	1.97 ± 0.91	#7.89 ± 9.24	*2.76 ± 4.21	3.01 ± 7.33	1.36 ± 1.36	1.84 ± 3.78
June	3.54 ± 7.09	9.80 ± 32.27	6.34 ± 9.57	15.73 ± 26.45	1.21 ± 2.16	2.46 ± 6.37	1.87 ± 3.32	13.20 ± 35.62
August	3.65 ± 7.19	7.54 ± 22.55	2.45 ± 1.34	16.81 ± 28.51	0.76 ± 0.99	2.60 ± 5.65	1.25 ± 1.0	3.73 ± 10.78
October	5.15 ± 8.20	5.68 ± 13.51	2.56 ± 1.55	10.91 ± 18.62	0.98 ± 1.90	3.07 ± 6.28	1.08 ± 0.88	2.30 ± 5.56
PDC	0.10 ± 0	12.73 ± 49.98	2.77 ± 1.34	18.94 ± 34.38	0.33 ± 0.72	1.76 ± 5.19	1.24 ± 0.91	8.80 ± 30.89

Data are mean ± SD; units are pg/ml; IL-2: female n = 9-10; male n = 15-16; IL-10: female n = 5-6; male n = 10-11 ;TNF: female n = 9-10; male n = 15-16; IFN-γ: female n = 9-10; male n = 14-16; BDC = Baseline Data Collection; PDC = Post Data Collection; IL = interleukin; TNF = tumor necrosis factor; IFN-γ = interferon γ; # significant difference between male and female (p = 0.037); * significant difference to BDC in females (p = 0.017)

and then decline below BDC levels till the end of mission albeit without statistical significance.

General effects of sex, time, and the interaction of sex and time

Factor sex

A significant influence of sex was stated for epinephrine (daytime: $p = 0.0032$; nighttime: $p = 0.008$), hemoglobin ($p < 0.0001$), hematocrit ($p < 0.0001$), the percentage of granulo- and lymphocytes ($p = 0.046$; $p = 0.044$), and thrombocytes ($p = 0.0001$).

Factor time

All parameters were significantly influenced by the time (p ranged between < 0.0001 and 0.04) except epinephrine during the day, cortisol in the morning, IFN-γ after PWM stimulation, the CST (morning and evening), and the STAI (trait).

Interaction of sex and time

A significant influence of the interaction of sex and time was found for cortisol in the morning ($p = 0.012$) and for IL-10 after fungal stimulation ($p = 0.001$).

Correlations between psychological evaluation, neurohormones, and cytokines

Correlations between CST and morning cortisol

A positive statistical correlation was found in males ($R = 0.298$; $p < 0.001$) but not in females.

Correlations between CST and endocannabinoids

A positive statistical correlation with different endocannabinoids was detected in males (AEA: $R = 0.183$; $p = 0.022$; OEA: $R = 0.178$; $p = 0.027$) and females (OEA: $R = 0.204$; $p = 0.047$; PEA: $R = 0.216$; $p = 0.035$).

Correlations between CST and cytokines

A positive statistical correlation with the cytokine IL-10 was found in both sexes (males: IL-10 fungal stimulation $R = 0.486$; $p < 0.001$; females: $R = 0.604$; p = 0.001; males: IL-10 PWM stimulation $R = 0.36$; $p = 0.007$; females: $R = 0.465$; $p = 0.013$).

Correlations between morning cortisol and cytokines

A positive statistical correlation with the cytokine IL-10 was detected in males (IL-10 after fungal stimulation $R = 0.427$; $p = 0.001$; after PWM stimulation $R = 0.428$; $p = 0.001$).

Discussion

The present study focused on the investigation of sex-specific differences in psycho-neuroendocrine and immune responses of humans who were subjected to a 1-year isolation period in the harsh and inhospitable environment of Antarctica at the German Antarctic Research Station *Neumayer III*. Against our hypotheses, sex differences in general were only little. Interestingly, cortisol concentrations in the morning were higher in women than men throughout the winter period however with maintenance of the diurnal rhythm. Opposite to our hypothesis, these elevated cortisol levels in females were not correlated to increased psychological stress and did not specifically result in decreased immune answers in women as based on the assays that could be performed on-site. The few stated positive statistical correlations between psychological evaluation, neurohormones, and inflammatory markers might potentially display their general positive interaction but must be evaluated and interpreted with caution due to the small sample size.

Differential neurohumoral response pattern and psychological evaluation in males and females

In previous studies, it has been demonstrated that cortisol excretion is influenced by different regulating triggers such as psychological, social, or exercise stress [28–30] and that the cortisol response to specific

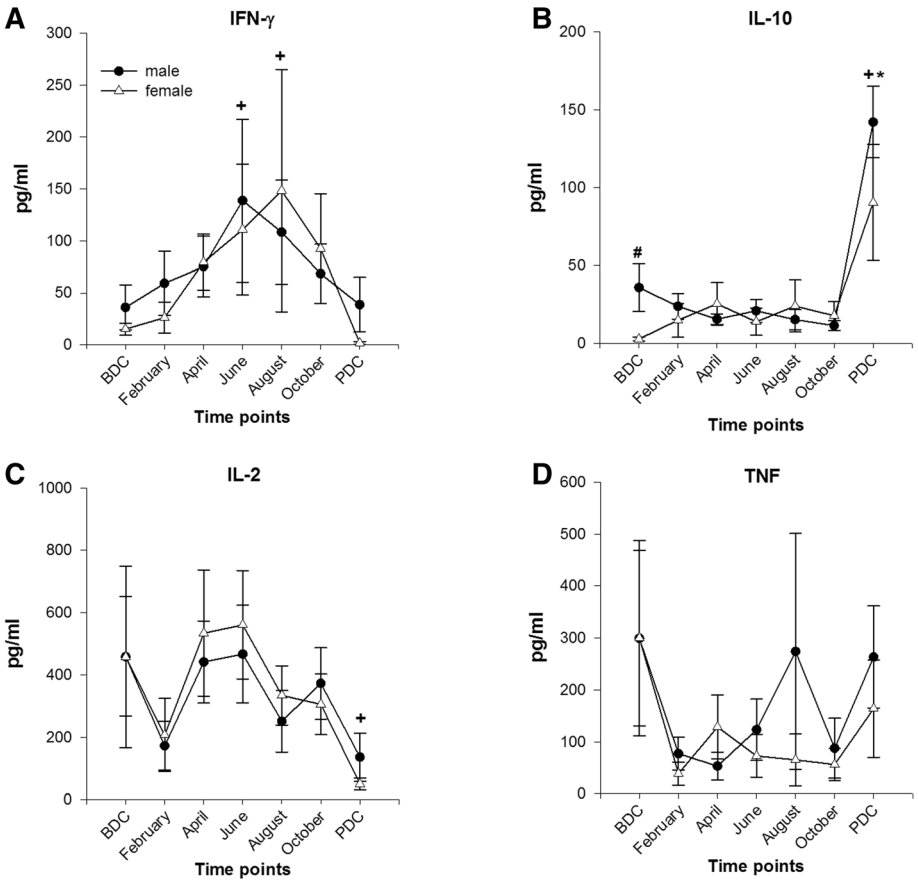

Fig. 5 Cytokine release after stimulation with fungal antigens from lithium-heparinized blood. **a** Interferon γ (IFN-γ); female $n = 9–10$; male $n = 16$. **b** Interleukin 10 (IL-10); female $n = 5–6$; male $n = 11$. **c** Interleukin 2 (IL-2); female $n = 9–10$; male $n = 16$. **d** TNF (tumor necrosis factor); female $n = 9–10$; male $n = 16$; data are means ± SEM; units are pg/ml. BDC baseline data collection; PDC, post data collection; #, significant difference between male and female; +, significant difference to BDC in males; *, significant difference to BDC in females

triggers (e.g., psychological stress) seems to be sex-specific [31]. Furthermore, diurnal cortisol profile, cortisol awaking response and total cortisol excretion over the day are associated with each individual's chronotype classifying, one as rather a morning or evening type [32–34]. Additionally, recent studies observed that the hypothalamic-pituitary-adrenal (HPA) response to acute psychological and to high-intensity exercise stress is more pronounced in the morning than in the evening, correlating with the circadian rhythm of cortisol synthesis [35, 36]. This finding is in line with our observations of a maintained circadian cortisol rhythm despite higher morning concentrations in women.

Besides psychological and exercise stress, partial or total sleep deprivation, a shorter sleep duration, and also a lower sleep efficiency were observed to be responsible for higher cortisol concentrations [37–39]. Wright et al. [40] assumed the cortisol increase due to sleep deprivation to be a consequence of the absent sleep-induced decrease in cortisol. Moreover, a negative feedback mechanism seems to be serviced by sleep fragmentation inducing increased cortisol levels which, in turn, leads to an increased activation of the HPA axis promoting further sleep fragmentation [41, 42].

Recently, Steinach et al. [43] notably observed a declined sleep quality (increase of time in bed, decline in sleep efficiency, increase in number of arousals) in females during a total of seven overwintering campaigns at the German Antarctic *Station Neumayer II/III* from 2008 to 2014. Against the background of the demonstrated interaction of sleep and HPA activity, these findings support our results of higher cortisol concentrations in females during three overwintering campaigns at *Neumayer III*. Steinach et al. [43] suggested that environmental factors such as isolation, extreme cold, and absence of environmental stimuli might have a higher impact on females than males and discussed a higher susceptibility of women to psycho-social stress to be responsible for the reduction in sleep quality since their physical activity remained unchanged.

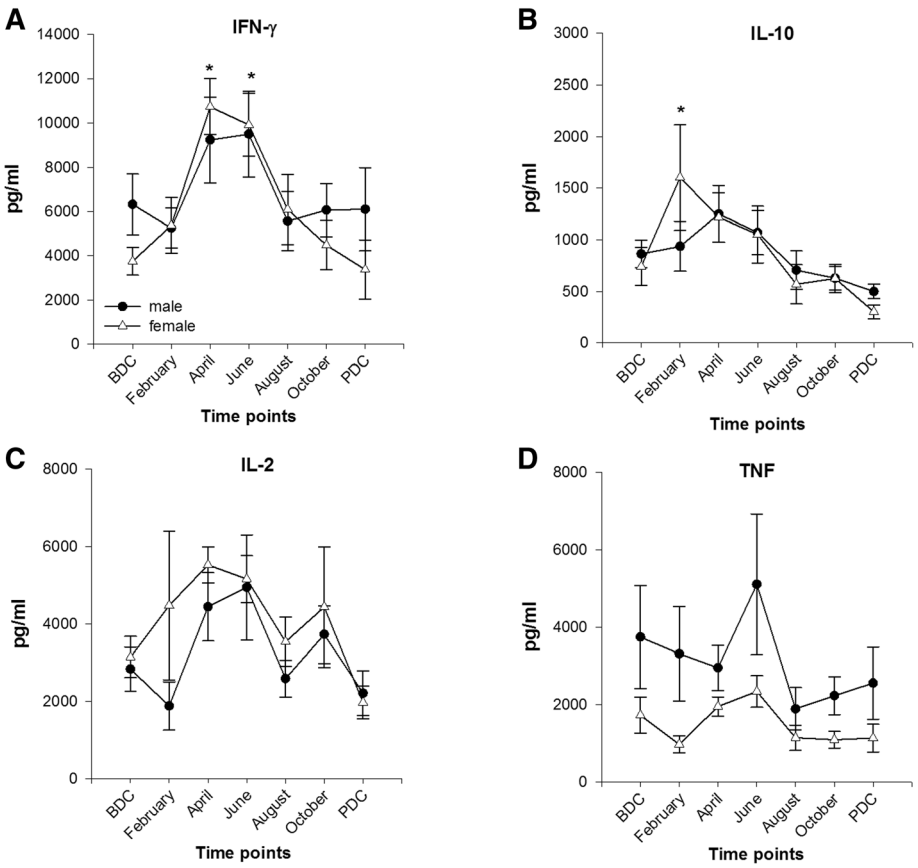

Fig. 6 Cytokine release after stimulation with pokeweed mitogen (PWM) from lithium-heparinized blood. **a** Interferon γ (IFN-γ); female $n = 9–10$; male $n = 16$. **b** Interleukin 10 (IL-10); female $n = 5–6$; male $n = 11$. **c** Interleukin 2 (IL-2); female $n = 9–10$; male $n = 16$. **d** TNF (tumor necrosis factor); female $n = 9–10$; male $n = 16$; data are means ± SEM; units are pg/ml. BDC, baseline data collection; PDC post data collection; *, significant difference to BDC in females

However, the psychometric data in our study did not point out any psychological strain of the participants independent of their sex. Furthermore, the hypothesis of an environment triggered cortisol increase and simultaneous decrease in sleep quality is corroborated (i) by the fact of a return of cortisol concentrations in females to baseline levels at the end of the Antarctic winter and (ii) by the results of the examination of the EC system, another stress-related system. It represents a neurobiological mechanism that acts as a regulator in stressful situations and conditions. Activated by physical or emotional stress, it supports the organism in its adaptation to new environmental and physiological challenges [44–46]. In a recently published study of our group [22], we examined the course of EC and NAE concentrations in exclusively male participants of overwintering campaigns at either *Neumayer III* at sea level or *Concordia Station* at high altitude of 3200 m to discern the effects of hypobaric hypoxia. As we detected significantly elevated EC and NAE concentrations during the confinement in the participants at *Neumayer III* but not at *Concordia*, we assumed an activation of the EC system by exposure to the physically challenging environment of Antarctica that is altered and diminished by hypobaric hypoxia at *Concordia.* The actual analysis of the female EC and NAE data at *Neumayer III* revealed the same reaction pattern as stated in their male colleagues which strengthens the assertion of a potential environmental influence. However, in regard to the simultaneously high cortisol concentrations in females, the negative feedback control between the HPA axis and the EC system [47, 48] seems to be blunted in women in this experimental set-up. Sex differences in the HPA axis response to stress have been identified on each level of the regulatory pathway although mostly in animal models [49, 50]. It has been demonstrated that females show a greater HPA axis activation with a greater hormonal response to stress [51, 52], potentially due to a regulatory effect of the gonadal hormones estradiol and testosterone [53, 54]. They have been evidenced to exert both long-term

differentiation effects during key developmental periods and also immediate modulatory effects [55, 56]. In humans, research results are more inconsistent and contradicting [57, 58] but the female menstrual cycle seems to play an important role and also the type of stressor or age might have an effect [55, 59, 60].

Additionally, physiological parameters such as the peripheral capillary oxygen saturation (SpO_2) also seem to succumb these sex-specific influences as Ricart et al. [61] measured at sea level slightly but significantly higher SpO_2 values in women than in men. Levental et al. [62] confirmed these findings and additionally found no such sex differences in newborns. Thus, the authors assumed that age-related hormonal differences are involved and attributed a possible indirect effect of sex on SpO_2 to the influence of reduced dead space due to women's smaller airways. Overall, sex differences seem to appear when specific coexisting influences operate together. In our study, the Antarctic environment apparently creates conditions in which HPA axis activity in women is stimulated.

Moreover, concordant to Steinach et al. [43], physical activity in females stayed balanced during the study period as displayed on the hormonal level by continuously low excreted masses of norepinephrine and epinephrine and therefore has to be excluded as potential explanation for the high cortisol concentrations. The nearly constantly higher masses of both catecholamines in males compared to females, particularly in the daytime urine collection, may be explained by the fact that the physically most strenuous work on-site Antarctic research stations is predominantly done by men (e.g., technicians). A decrease in catecholamine excretion can be stated for both sexes at the onset of the Antarctic winter in April/May and again at the beginning of summer in October/November. This seasonal drop may be explained by a lapse of physical work at the beginning of winter when the crew is more and more restricted to the inside of the station due to weather conditions and at the beginning of summer by the arrival of the summer team that entails a parting of the workload to a larger group of people.

Sex-related HPA changes and immune effects

To verify our second hypothesis that higher HPA axis activity in women results in a decreased immune response, we analyzed the basal and stimulated cytokine profile after in vitro stimulation with fungal recall antigen and the T and B cell-specific mitogen PWM. A former study of our group [63] demonstrated that this test reflects well the suppressive effects of glucocorticoids on immune responses upon stimulation. However, after analysis, we had to reject our hypothesis as the cytokine profiles between men and women did not vary but rather showed similar courses during confinement. A potential explanation might be that cortisol concentrations were indeed higher after arrival in Antarctica in females compared to BDC but nevertheless never exceeded normal ranges (reference values according to the manufacturer: morning < 0.87 μg/dl; evening < 0.35 μg/dl).

In the stimulation assay, basal values of all measured cytokines were close to detection limit and demonstrated no systemic inflammation for either sex (stated statistical significances are probably due to single identified outliers). Furthermore, no episodes of clinical infection or acute illness were stated or reported during each of the three campaigns. Remarkably, stimulation with fungal antigens and PWM resulted in a significant increase of the pro-inflammatory cytokine IFN-γ in both sexes during the Antarctic winter and a subsequent decrease and return to baseline values till PDC. Simultaneously, the anti-inflammatory cytokine IL-10 showed no peculiar reaction pattern upon stimulation during the confinement but fungal recall antigen exposition resulted in a significant increase in both sexes after the return to Europe at PDC. A former study presented similar results with elevated plasma IFN-γ and suppressed IL-10 concentrations however without a specific time point after return to "normal life" conditions [64]. Furthermore, lately, we [12] published data showing increases in IFN-γ, IL-2, and TNF concentrations after the same recall antigen stimulation assay with peak concentrations after 4 to 7 months of confinement in expeditioners at the Antarctic Research Station *Concordia*. In difference to the present study, cytokine release remained elevated during the whole expedition and did not decrease after winter which might be explained by the immune modulating effect of hypobaric hypoxia that reigns at *Concordia Station* due to its high altitude location.

Therefore, in addition to the EC measurements, also our observations concerning the reactivity of the immune system corroborate the assumption that the Antarctic environment and in particular the winter period seem to affect the human immune system. However, in contrast to the HPA axis reaction, sex differences do not seem to play a significant role here. During winter when expeditioners are confined to the station and immunological challenge and sensitization is low, the immune system seems to stand at attention which might lead to its boost reactivity after re-exposure to a "normal" microbial load.

Restrictively, it has to be stated that the immune answers were not totally consistent to that effect that other pro-inflammatory cytokines such as IL-2 and TNF showed less distinct profiles than IFN-γ. TNF showed a reduction upon fungal stimulation during confinement. However, due to the high inter-individual variability especially at BDC, the data has to be evaluated with caution. One might only speculate that monocytes and macrophages—the main TNF-producing cell lines [65,

66]—are restricted in their functioning after fungal stimulation in this setting. This could also explain a less effective answer after stimulation with the T and B cell-specific mitogen PWM. Additionally, blood cell counts showed an increase in leukocytes and granulocytes in both sexes (statistically significant only for males) with an m-shaped curve during the expedition with a dip during midwinter. Simultaneously, lymphocytes increased significantly in both sexes over time. Similar results were presented by Yi et al. [67], and they speculated that in the absence of immune challenges during isolation, the immune system might release a disproportionately high number of lymphocytes in the peripheral system to prepare for a potential infection. Moreover, different stressors (e.g., low temperature, isolation) seem to activate the physiological stress response to a different extent [68]. Although an animal model, Bowers et al. [68] stated stressor-specific alterations in corticosterone and immune responses with low temperature leading to a significant increase of corticosterone and trafficking of lymphocytes and monocytes but without an enhanced delayed-type hypersensitivity (DTH) response. Additionally, recent human studies [69, 70] demonstrated that sex-specific cold responses induce different neuroendocrine, immune, and memory responses in men and women. However, the effect of these alterations (immune suppression vs. activation) is not distinct and varies depending on the respective study [71–73].

In summary, our observations give further hints to support the assumption of a generally alerted immune system with a shift towards a lymphocyte-mediated answer even though the absolute count of the different cell lines remained in normal ranges.

Sex differences in blood cell count

Expected sex differences were found for hemoglobin and hematocrit with higher values in males than females as of common knowledge [74, 75]. However, a definite plausible explanation for the significant increase in hemoglobin in both sexes during the isolation period remains missing. Low air pressure and subsequently reduced oxygen partial pressure can be excluded as potential reason, as *Neumayer III* is situated at sea level with an air pressure of ~ 980 mbar. One potential cause could be fluid shifts as hematocrit also increases albeit not constantly significant which might indicate a relative volume loss.

Interestingly, platelet count in women increased significantly in winter compared to BDC and values were significantly higher than in males nearly throughout the whole observation period. Former studies already demonstrated higher platelet counts in women [76, 77] but showed discordant results with higher or lower platelet reactivity in women dependent on the existent general disease [76–78]. Furthermore, there seems to be a relation between endogenous corticoid concentrations and the risk of venous thromboembolism [79, 80]. Here, one might speculate that thrombocyte count plays perhaps a role. Against this background, the sex differences in platelet count might be associated with the higher cortisol concentrations in females but might also be due to a sex-specific reactive sensitivity under exposure to the environmental challenges in our study set-up.

For verification and more substantiated statements, further and more detailed and precise examination of the different cell lines and immune components with focus on sex differences are necessary. The same accounts for the identification of their triggers.

Due to blood volume restrictions and overall difficult research conditions in this field study, a closer look in cell differentiation and more specific immune cell functional states was unfortunately not possible.

Conclusion

In summary, we conclude that Antarctica with its extreme environment as an Earth-bound analogue for long-term space exploration class missions seems to trigger some distinct physiological answers but without eliciting major relevant sex-specific differences in these answers. Detected sex differences were related to a higher HPA axis activity and a higher platelet count in females than males, even though their consequences remain unknown and need to be identified in future studies. Moreover, we had to reject our hypotheses of higher cortisol concentrations in females (i) being caused by higher psychological stress and (ii) resulting in a decreased immune answer (as detectable with the performed tests) as women showed the same psychological resilience and immune responses as males. Instead, the examination of other stress-related systems such as the EC system provided further evidence to support the explanation of the environment being the trigger for our sex-specific and general observations.

On the long run, more detailed and precise sex- and trigger-oriented studies will support a better individual risk assessment and the development of appropriate countermeasures for both male and female Antarctic expeditioners and space travelers.

Limitations

Sample collection, processing, and functional tests were performed by the expedition's medical doctors who were no specialists in laboratory research and had to perform under extreme experimental conditions. A more cell-differentiated immune analysis and, further, more detailed functional tests with regard to potential sex differences were not possible due to tight blood volume

and financial restrictions that entailed the absence of a flow cytometer on-site. Due to overall limited cases, a one-to-one correlation was also not possible.

To validate the assumed environmental effects on human physiological answers and to verify the stated sex differences a control group exposed to normal life conditions or even a cross-over study design would have been necessary and ideal. However, such study designs are barely feasible in extreme environment field studies with regard to particularly logistics, costs, participants, and others. Additionally, it must be stated that the participants of such long-term, difficult, and also dangerous expeditions pass through a rigorous selection process and experience a specific training before departure which aims to prepare them for potential conflict situations not only physically but also psychologically. Therefore, the study group misses to be an exact representative of the general population which might have an influence on measured outcome parameters. Furthermore, a more specific and defined study set-up concerning sex differences would improve the outcome of isolating and distinguishing them from general alterations.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
MF and AC designed the work. AS, BF, HB, PG-L, and AC collected the data. Data analysis and interpretation as well as drafting the article and critical revision for important intellectual content were performed by all authors (CS, DM, J-IB, H-CG, AS, BF, HB, PG-L, DT, BEC, EK, AC, MF). All authors gave final approval of the version to be submitted.

Ethics approval and consent to participate
The participation in the study was voluntary and every subject gave its written informed consent which was approved by the Ethical Board of the University of Munich, Germany (Reference Nr.: 332-08, 524-15). During the whole season, they retained the right to end their participation without any explanation. Applied procedures and techniques were performed in accordance to the Declaration of Helsinki and met the criteria of standard laboratory guidelines and regulations.

Consent for publication
Not applicable.

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The authors declare that they have no competing interests.

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Modulations of Neuroendocrine Stress Responses During Confinement in Antarctica and the Role of Hypobaric Hypoxia

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The Antarctic continent is an environment of extreme conditions. Only few research stations exist that are occupied throughout the year. The German station Neumayer III and the French-Italian Concordia station are such research platforms and human outposts. The seasonal shifts of complete daylight (summer) to complete darkness (winter) as well as massive changes in outside temperatures (down to −80°C at Concordia) during winter result in complete confinement of the crews from the outside world. In addition, the crew at Concordia is subjected to hypobaric hypoxia of ∼650 hPa as the station is situated at high altitude (3,233 m). We studied three expedition crews at Neumayer III (sea level) (n = 16) and two at Concordia (high altitude) (n = 15) to determine the effects of hypobaric hypoxia on hormonal/metabolic stress parameters [endocannabinoids (ECs), catecholamines, and glucocorticoids] and evaluated the psychological stress over a period of 11 months including winter confinement. In the *Neumayer III* (sea level) crew, EC and *n*-acylethanolamide (NAE) concentrations increased significantly already at the beginning of the deployment ($p < 0.001$) whereas catecholamines and cortisol remained unaffected. Over the year, ECs and NAEs stayed elevated and fluctuated before slowly decreasing till the end of the deployment. The classical stress hormones showed small increases in the last third of deployment. By contrast, at *Concordia* (high altitude), norepinephrine concentrations increased significantly at the beginning ($p < 0.001$) which was paralleled by low EC levels. Prior to the second half of deployment, norepinephrine declined constantly to end on a low plateau level, whereas then the EC concentrations increased significantly in this second period during the overwintering ($p < 0.001$). Psychometric data showed no significant changes in the crews at either station. These findings demonstrate that exposition of healthy humans to the physically challenging extreme environment of Antarctica

(i) has a distinct modulating effect on stress responses. Additionally, (ii) acute high altitude/hypobaric hypoxia at the beginning seem to trigger catecholamine release that downregulates the EC response. These results (iii) are not associated with psychological stress.

Keywords: endocannabinoids, catecholamines, glucocorticoids, hypobaric hypoxia, high altitude, Antarctica

INTRODUCTION

Humans have an amazing ability to survive in extreme environments and to acclimatize to varying environmental conditions ranging from humid tropical forests to polar deserts (Burtscher et al., 2018; Ilardo and Nielsen, 2018). Stress hormones and other stress activated systems play an important role as mediators of acclimatization responses to changes in the environment (Dhabhar, 2018). To measure these hormones and evaluate the function of the corresponding and interacting physiological systems, helps to better understand such acclimatization processes and thus offers to explore options to prevent and counteract possible negative responses with detrimental effects on human physiological integrity.

It was demonstrated that catecholamine and cortisol responses to physical exercise differ under conditions of normoxia or hypoxia with higher concentrations under hypoxia (Mazzeo et al., 1994; Woods et al., 2017). However, the question whether normobaric and hypobaric hypoxia elicit the same reactions remains contradictory (Girard et al., 2012; Millet et al., 2012; Mounier and Brugniaux, 2012). Furthermore, to date, most studies investigated the consequences of acute or intermittent hypoxia (Xie et al., 2001; Calbet, 2003; Lusina et al., 2006; Sander, 2016) but data and knowledge about the effect of prolonged and chronic exposure to hypoxic conditions are rather scarce (Dhar et al., 2014).

Moreover, the exposure often compasses not only one environmental stressor (e.g., hypoxia) but demands the acclimatization to several interacting and combined stressors (e.g., hypobaric hypoxia and cold) (Burtscher et al., 2018). In this context, the acclimatization to one stressor was found to be able to modify and influence the response to the other, which is called cross-adaptation (Chauhan et al., 2015). It was evidenced for cold/heat and hypoxia (Launay et al., 2006; Lunt et al., 2010; Keramidas et al., 2015; Gibson et al., 2017) which modified their interaction but broad data in this field of research is still missing due to difficulties of the measure so that a general conclusion cannot be drawn to date. The same accounts for the acclimatization response of each individual that seems to be affected by distinct differences (Bartone et al., 2018).

Besides the sympathoadrenal and glucocorticoid system, the endocannabinoid system (ECS) plays an important role in coping with such stress reactions. Its lipid mediators the endocannabinoids (ECs) and chemically related *N*-acylethanolamides (NAEs) are very related with acclimatization processes at several physiological lines (e.g., psychological, metabolic, peripheral, and central nervous system) in response to environmental factors such as hypobaric hypoxia and temperature to reach physiological homeostasis (Campolongo et al., 2009, 2013; Richard et al., 2009; Chouker et al., 2010; Dlugos et al., 2012; Feuerecker et al., 2012; Hauer et al., 2013, 2014; Morena et al., 2014; Neumeister et al., 2015; Hanlon et al., 2016).

We investigated a cohort of healthy male individuals over 12 months including a 9-month overwintering period at two Antarctic research stations: Neumayer III near the Antarctic coast in the Queen Maud land and Concordia in Inner East Antarctica.

In general, the Antarctic environment is characterized by extreme shifts in daylight from 24 h light during the Antarctic summer to complete 24 h darkness during the winter period as well as massive changes in outside temperatures at onset of the Antarctic winter requiring complete confinement of the crew to the protective research stations. Thus, expeditioners suffer from sensory deprivation by a monotone environment with lack of stimuli, restricted food variation, and a distinct social narrowing with high potential for conflicts (Das et al., 2018). Furthermore, a disruptive circadian rhythm may influence the hormonal stress response (Chen et al., 2016; Vitale et al., 2018).

In detail, living conditions at Concordia are more severe and thus ensue more rigorous changes than at Neumayer III due to its inland location. Additionally, in contrast to Neumayer III which is situated at sea level, Concordia is situated at 3,233 m above sea level inducing an environment of hypobaric hypoxia to acclimatize to.

We hypothesized that (i) the extreme environmental conditions would lead to an increase of stress-related hormones and that (ii) this increase would be aggravated and sustained in the crew of Concordia Station as of their exposition to long-term, chronic hypobaric hypoxia.

The aim of our study was to investigate and precise the modulations of the stress-related ECS in humans exposed to harsh and extreme environmental living conditions as represented in Antarctica. The main focus hereby was set on the changes induced by chronic hypobaric hypoxia.

To confirm our hypothesis, we therefore determined hormonal/metabolic stress parameters (ECs, catecholamines, and glucocorticoids) and evaluated the psychological stress level in three expedition crews at Neumayer III (sea level) and in two at Concordia (high altitude), respectively.

MATERIALS AND METHODS

Group of Study

In this prospective field study, in total, 15 healthy male participants were included for the *Concordia* (high altitude) crew investigation (seven individuals during the expedition period

2016 and eight volunteers in 2017) and 16 healthy male crew members for the *Neumayer III* (sea level) investigation (five individuals in 2013, six in 2014, and five in 2015). The crews at both stations changed in every expedition period so that every participant was included only once in the study. Demographic data of the participants are given in **Table 1**.

Concordia and Neumayer III Research Station

This study took place at two different Antarctic research stations: (i) the French-Italian inner-continental station *Concordia*, situated at 3,233 m above sea-level (high altitude; pressure level ∼640 to 650 hPa). It is located at a latitude/longitude of 75° 06′ S/123° 21′ E on an inland high ice plateau area called Dome C. The closest coastal region is approx. 1,100 km away (ii) the German coastal Antarctic station *Neumayer III* which is situated in the Atka Bay in the northeast Weddell sea on the Ekström shelf ice at sea level with the coordinates 70° 40′ S/8° 16′ W. Seasons in Antarctica are opposite to the northern hemisphere with Antarctic summer lasting from beginning of November to beginning of February and winter from May to August. The longest day (mid-summer) is in December and the shortest day in June (mid-winter). During the Antarctic summer the lack of a light/dark cycle results in 24 h of constant sunlight. During this period, average outside temperatures are around −50°C (Concordia, high altitude) and −3°C (Neumayer III, sea level). By contrast, during the winter season no sunlight is present and outside temperatures range around −60°C and can drop to −80°C at Concordia (high altitude) and −30°C at Neumayer III (sea level). In addition to the extreme outside temperatures, humidity is very low especially in inner Antarctica (Concordia, high altitude) leading to a very dry environment. Here, precipitation is very little throughout the year.

In order to maintain a regular circadian rhythm in this environment, a normal day–night cycle is tried to be respected by keeping regular hours for common meals, working duties, and evening activities.

In summary, these extreme conditions lead to a complete isolation (no access/exit possible) from the outer world during almost 9 months (mid-February to mid-October). Telecommunication with the outside world from the stations is possible *via* phone which can depend on current weather conditions. The same applies for internet access that is possible but not always reliable. All supply goods for the over-wintering period are stored in different areas inside the two stations, including different fridges and freezers (+4 to −25°C).

Study Protocol

Data from two expedition campaigns at Concordia (high altitude) (2016 and 2017) and from three expedition campaigns at Neumayer III (sea level) (2013–2015) were analyzed. Data collection and blood sampling for stress hormone analyses in the study groups were performed on a monthly basis during the first week of the month and in the morning around 7:00–8:00 am starting in January/February after arrival of the crew members and continued through the whole year until October/November when the station was prepared for the next seasonal change of crew. One of the authors (CS and MF) was present at Concordia (high altitude) during December/January (summer season) of each campaign when the station was accessible by aircraft to help with the setup of the study protocol and for training of the crew to maximize study compliance. To establish procedural processing at Neumayer III (sea level) external assistance from the study team (AC) was present during the first Antarctic summer season. The training of the crew surgeons took place 1–3 months before deployment. During the actual overwintering period, data collection, blood sampling, and processing of the samples was performed by one of the crew surgeons (FvdB, CD, BF, HB, and PG-L) who have received several weeks of training in carrying out research protocols and in the long-term assessment and monitoring of the crew members during the winter period.

In addition to data collection and blood sampling in Antarctica, a baseline data collection (BDC) including blood sampling was performed in Europe. BDC took place approximately 2–4 months prior to departure to Antarctica and was performed close to sea level in Bremerhaven, Cologne, or Berlin, Germany.

This study was carried out in accordance with the recommendations of the local Ethics Committee of the University of Munich with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the local Ethics Committee of the University of Munich [Protocols# 332-08, 524-15]. All samples were collected after an 8 h fasting period in the morning around 7:00–8:00 am. Physical exercise was not allowed for 24 h prior to sample collection.

Biochemical Measurements

Biochemical measurements were performed from blood samples taken in EDTA tubes for blood cell count, in lithium-heparinized tubes (S-Monovette®, Sarstedt, Nümbrecht, Germany) for EC and NAEs measurements, from saliva (Salivette®, Sarstedt,

TABLE 1 | Demographic data of participants of the respective campaign and Antarctic station at BDC.

	Crew 2016 Concordia (high altitude)	Crew 2017 Concordia (high altitude)	Total Concordia (high altitude)	Crew 2013 Neumayer III (sea level)	Crew 2014 Neumayer III (sea level)	Crew 2015 Neumayer III (sea level)	Total Neumayer III (sea level)
Number (*n*)	7	8	15	5	6	5	16
Age (years)	43.4 ± 4.6	35.3 ± 3.2	39.1 ± 2.8	33.8 ± 0.9	36.2 ± 3.7	43.4 ± 5.3	37.7 ± 2.3
Body mass index (kg/m^2)	24.7 ± 0.6	24.8 ± 1.3	24.7 ± 0.8	27.37 ± 2.2	26.3 ± 1.0	27.1 ± 1.4	26.9 ± 0.8

Data are mean ± SEM.

Nümbrecht, Germany) for the determination of free cortisol and from 12 h pooled urine [from 19:00 to 7:00 (nighttime)] for catecholamine analyses.

All samples were taken in the early morning and after an overnight fasting period. Blood samples for blood cell count were stored at ambient temperature until further processing the same day. Blood samples drawn for ECs were immediately placed on ice water to prevent temperature effects (Vogeser et al., 2006). Blood, urine, and saliva samples were frozen immediately after processing and stored throughout the expedition year at a minimum temperature of at least −25°C. The return of the frozen samples from Concordia (high altitude) and Neumayer III (sea level) back to Germany was effected by ship and plane with a strict temperature monitoring assuring a temperature of at least −25°C or colder. Samples were stored at −60°C (freezer) till further processing and analyses after arrival at Munich.

Blood Cell Count

EDTA-anti-coagulated blood samples were used to determine the blood cell count using the on-site QBC Autoread plus automated analyzing system (QBC Diagnostics, Port Matilda, PA, United States). Hemoglobin concentration and hematocrit were quantified.

Endocannabinoid and NAEs Measurements

After sampling, blood samples were immediately centrifuged, the plasma transferred into Eppendorf tubes and frozen at −60°C without any delay (Hauer et al., 2013). This procedure allows the storage of EC samples for at least 6 months (Di Marzo et al., 2009).

Plasma concentrations of the ECs anandamide (AEA), 2-arachidonoylglycerol (2-AG) as well as the *N*-acyl-ethanolamides (NAEs) palmitoylethanolamide (PEA), oleoylethanolamide (OEA), and stearoylethanolamide (SEA) were determined using a LC-High Resolution LC-MS/MS instrument combination (Agilent 1290/Sciex Triple TOF 6600) in positive high-resolution MRM (multiple reaction monitoring, MS resolution ∼16,000) mode. Chromatographic separation was achieved on a RP-C18 column (Zorbax C8, 2.1 mm × 50 mm, 3.5 μm; Agilent). Both components of the binary gradient contained a mixture of 2 mmol aqueous ammonium acetate and ACN at a mixing ratio of 95+5 (A) and 5+98 (B). Both buffers were moderately acidified with 0.1% acetic acid. The separation gradient started at an amount of 10% B which was linearly increased to 100% B at 10 min and was finally kept constant for another 2 min at a flow rate of 250 μl/min.

Urine Catecholamines

The catecholamines norepinephrine and epinephrine were determined from pooled nighttime urine samples reflecting the required conditions of physical inactivity. Nighttime samples were used to obtain baseline secretion of catecholamines in contrast to daytime urine samples which reflect physical challenges and activity throughout the awake phase.

Samples were stored at −60°C till final processing in Europe. After defrosting, quantification of catecholamine concentrations was performed at the Institute of Clinical Chemistry, University of Munich, Germany, using HPLC (Chromsystems, Martinsried, Germany). In order to determine the absolute mass of excreted catecholamines, urine catecholamine concentrations were multiplied by respective urine volume.

Saliva Cortisol

Until final measurement, samples were stored frozen at −60°C. Cortisol concentrations were quantified by an electrochemiluminescence immunoassay (Elecsys 2010, Roche, Mannheim, Germany) at the Institute of Clinical Chemistry, University of Munich, Germany.

Psychological Evaluation and Measurements

Two different paper questionnaires were performed to quantify and analyze each participant's emotional stress level:

CST (Short Questionnaire on Current Stress)

The test is validated to reflect the level of acute emotional stress (Current Stress Test, CST) (Müller and Basler, 1993). It consists of six questions and is highly sensitive to acute situational changes in subjective stress experience. Six items of paired positive and negative adjectives referring to perceptions of current stress and strain or relaxation (e.g., "tense–calm," "uneasy–relaxed") must be rated on a six-point Likert scale. The range for total item means is 1–6, with higher values indicating an increased stress experience. The composition of the questionnaire entails that the subject usually does not remember the previous ratings, thus preventing carry-over effects.

Spielberger State Trait Anxiety Inventory (STAI)

It consists of two parts (each 20 questions) rating the answers on a 4-point scale. The score for the global test may range between 40 and 160 points. It evaluates state anxiety (in a specific situation) or trait anxiety (as part of a person's character) (Spielberger et al., 1970).

Statistical Analyses

Normal distribution of sample data was tested using the Shapiro–Wilk test. Within group comparisons (e.g., changes in EC levels over time) were performed by one way repeated measures analysis of variances (one-way RM-ANOVA) followed by a *post hoc* Holm–Sidak test to determine which measurements were significantly different and to correct for multiple comparisons. Between-group comparisons were performed using a *t*-test for normally distributed data and the Mann–Whitney *U* test for non-parametric data followed by a Bonferroni correction for multiple comparisons, respectively. For all testing, a p-value <0.05 was regarded as statistically significant. Data are displayed as mean ± SEM. Statistical calculations were performed using SigmaPlot® (Systat, Software, Chicago, IL, United States) and IBM SPSS Statistics v24, United States.

RESULTS

Biochemical Measurements

Blood Cell Count (Table 2)

Neumayer III (sea level)

Hemoglobin concentrations were moderately elevated during the isolation period in February, March, and June to October when compared to BDC (15.4 ± 0.18 g/dl, $p < 0.05$) but still fluctuated in the normal range. The hematocrit always stayed on a normal level.

Concordia (high altitude)

Hemoglobin concentrations and hematocrit were significantly elevated in every month throughout the deployment compared to BDC with highest values at the end of the stay (Hb BDC 14.4 ± 0.35 g/dl to November 18.5 ± 0.28 g/dl; $p < 0.001$ versus Hct BDC 0.42 ± 0.01 to November 0.54 ± 0.01; $p < 0.001$).

Neumayer (sea level) versus Concordia (high altitude)

Hemoglobin concentrations were significantly higher in the Concordia (high altitude) crew from February to June, in August, October, and November ($p < 0.05$). Hematocrit values were significantly elevated in the crew at Concordia (high altitude) compared to the crew at Neumayer III (sea level) in May and at the end of the stay in October and November ($p < 0.001$).

Endocannabinoid (EC) and NAEs Plasma Concentrations (Figures 1A–E)

At BDC in Europe before deployment of the crews to Antarctica, EC and NAEs values were within the normal range in both crews.

Neumayer III (sea level)

The NAEs and ECs (AEA and 2-AG) increased their concentrations significantly up to 10-fold from BDC already at the beginning of the deployment, fluctuated throughout the year but always on a highly elevated level compared to BDC, and finally decreased slowly till the end of the deployment but not returning to BDC values [*AEA* increased from BDC 0.34 ± 0.02 to 2.53 ± 0.54 ng/ml; $p < 0.001$ (July). *2-AG* from BDC 22.7 ± 4.23 to 95.74 ± 20.71 ng/ml; $p < 0.001$ (May). *PEA* from BDC 3.15 ± 0.25 to 9.72 ± 1.31 ng/ml; $p < 0.001$ (April). *OEA* from BDC 1.52 ± 0.26 to 6.73 ± 1.67 ng/ml, no significance (July). *SEA* from BDC 0.97 ± 0.2 to 5.46 ± 0.68 ng/ml; $p < 0.001$ (April)].

Concordia (high altitude)

The NAEs and ECs (AEA and 2-AG) fluctuated on a constantly low level with intermittent small significant increases. Significant increases were found at the end of the deployment [*AEA* increased from BDC 0.23 ± 0.01 to 0.72 ± 0.04 ng/ml; $p < 0.001$ (November). *2-AG* from BDC 12.43 ± 2.65 to 23.04 ± 3.09 ng/ml; $p < 0.001$ (October). *PEA* from BDC 1.73 ± 0.18 to 4.03 ± 0.29 ng/ml; $p < 0.001$ (November). *OEA* from BDC 0.8 ± 0.12 to 2.46 ± 0.28 ng/ml; $p < 0.001$ (November). *SEA* from BDC 0.97 ± 0.17 to 1.72 ± 0.14 ng/ml; no significance (November)].

Neumayer III (sea level) versus Concordia (high altitude)

The differences in NAEs and EC (AEA and 2-AG) values between the crews at Neumayer III (sea level) and Concordia (high altitude) were highly significant for all neurotransmitters and nearly throughout the whole isolation period and duration of stay in Antarctica (*AEA* June $p < 0.05$, for all other months except the end of the stay $p < 0.001$. *2-AG* for all months $p < 0.001$ except BDC. *PEA* October $p < 0.05$, for all other months except March, May, and the end of the stay $p < 0.001$. *OEA* May and June $p < 0.05$, for all other months except BDC, March, and November $p < 0.001$. *SEA* June $p < 0.05$, for all other months except BDC, February, March, and November $p < 0.001$). At the end of the stay, EC and NAEs scores of the crews at both stations converged since their concentrations at Neumayer III (sea level) decreased but increased at Concordia (high altitude).

TABLE 2 | Hemoglobin concentrations (g/dl) and hematocrit (%).

	Neumayer III (sea level)	Concordia (high altitude)	Neumayer III (sea level)	Concordia (high altitude)
	Hb (g/dl)	Hb (g/dl)	Hct (%)	Hct (%)
BDC	15.4 ± 0.18	14.4 ± 0.35	47 ± 0.6	42 ± 1$^{\#}$
February	16.4 ± 0.33*	17.9 ± 0.28*,$^{\#}$	49 ± 0.6	52 ± 1.2*
March	16.6 ± 0.24*	18.0 ± 0.24*,$^{\#}$	49 ± 0.6	51 ± 2.1*
April	16.1 ± 0.39	17.9 ± 0.20*,$^{\#}$	48 ± 1.1	53 ± 2*
May	16.2 ± 0.23	17.8 ± 0.20*,$^{\#}$	49 ± 0.6	53 ± 0.6*,$^{\#}$
June	16.3 ± 0.19*	17.5 ± 0.17*,$^{\#}$	49 ± 0.5	51 ± 0.8*
July	16.5 ± 0.18*	17.7 ± 0.33*	49 ± 0.5	53 ± 1.1*
August	16.6 ± 0.23*	18.0 ± 0.27*,$^{\#}$	50 ± 0.7	53 ± 0.8*
September	16.6 ± 0.36*	17.7 ± 0.22*	50 ± 0.8	52 ± 0.9*
October	16.3 ± 0.26*	18.4 ± 0.19*,$^{\#}$	49 ± 0.7	53 ± 0.6*,$^{\#}$
November	16.1 ± 0.30	18.5 ± 0.28*,$^{\#}$	48 ± 0.6	54 ± 1*,$^{\#}$

*Data are mean ± SEM. Hb, hemoglobin; Hct, hematocrit; BDC, baseline data collection. *Significant difference to BDC. $^{\#}$Significant difference between Neumayer III (sea level) and Concordia (high altitude).*

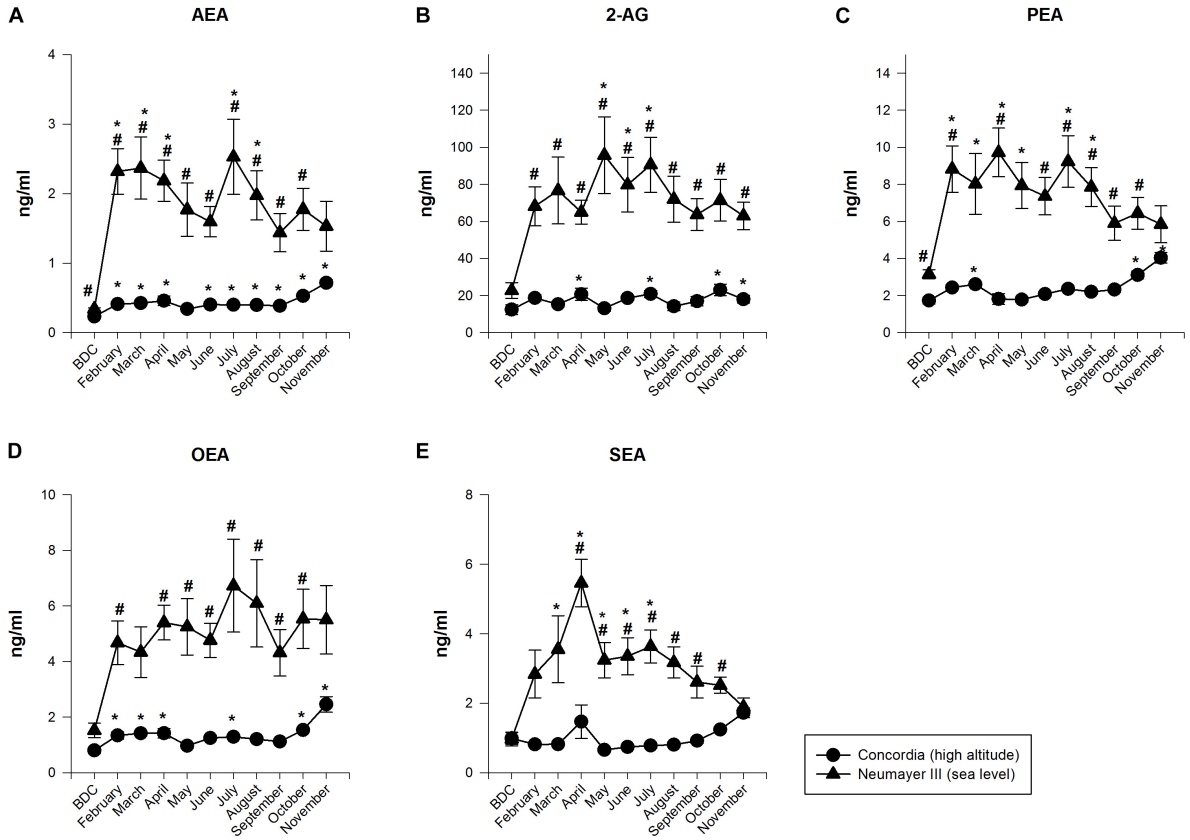

FIGURE 1 | Endocannabinoid (EC) and *N*-acylethanolamide plasma concentrations at Neumayer III (sea level) (n = 7–15) and Concordia (high altitude) (n = 15–16); data are means ± SEM; units are ng/ml; BDC, baseline data collection; AEA, anandamide **(A)**; 2-AG, 2-arachidonoylglycerol **(B)**; PEA, palmitoylethanolamide **(C)**; OEA, oleoylethanolamide **(D)**; SEA, stearoylethanolamide **(E)**; *significant difference to BDC; #significant difference between Neumayer III (sea level) and Concordia (high altitude) crews.

Urinary Catecholamine Excretion

Norepinephrine (Figure 2A)

Neumayer III (sea level)

Norepinephrine levels during the night stayed on a consistent level throughout the confinement and showed no significant changes. Only toward the end of the months of August and September, the levels raised albeit not significantly.

Concordia (high altitude)

Nighttime norepinephrine amount increased significantly from BDC to February (BDC 19.07 ± 1.88 μg to February 42.18 ± 7.87 μg; $p < 0.001$) and stayed on a significantly elevated high level until April. Peak values were already reached in February before levels decreased slowly and constantly till July. In July, norepinephrine mass plateaued until the end of the stay at levels similar to BDC.

Neumayer III (sea level) versus Concordia (high altitude)

A significant difference between the nighttime norepinephrine masses in the crews of the two stations was found in February, March, and May ($p \leq 0.001$ to $p < 0.05$). The mass of excreted norepinephrine was constantly higher in the Concordia (high altitude) crew except for the months August and September.

Calculated SEMs were higher in the Concordia (high altitude) than in the Neumayer III (sea level) crew and exceeded at the beginning of the expedition when significant differences between both stations were stated.

Epinephrine (Figure 2B)

Neumayer III (sea level)

In the Neumayer III (sea level) crew, epinephrine levels during the night varied inconsistently during the stay and ended at almost the same level as at BDC.

Concordia (high altitude)

Epinephrine excretion increased in February (from BDC 2.30 ± 0.35 to 4.11 ± 1.17 μg), although without statistical significance and decreased slowly over the year with little fluctuations to finally reach BDC levels at the end of the year.

Neumayer III (sea level) versus Concordia (high altitude)

Epinephrine masses started in both crews at the same level. During the year, levels were always higher at Concordia (high altitude) except at the end in the month September but showed no significant differences between the two crews.

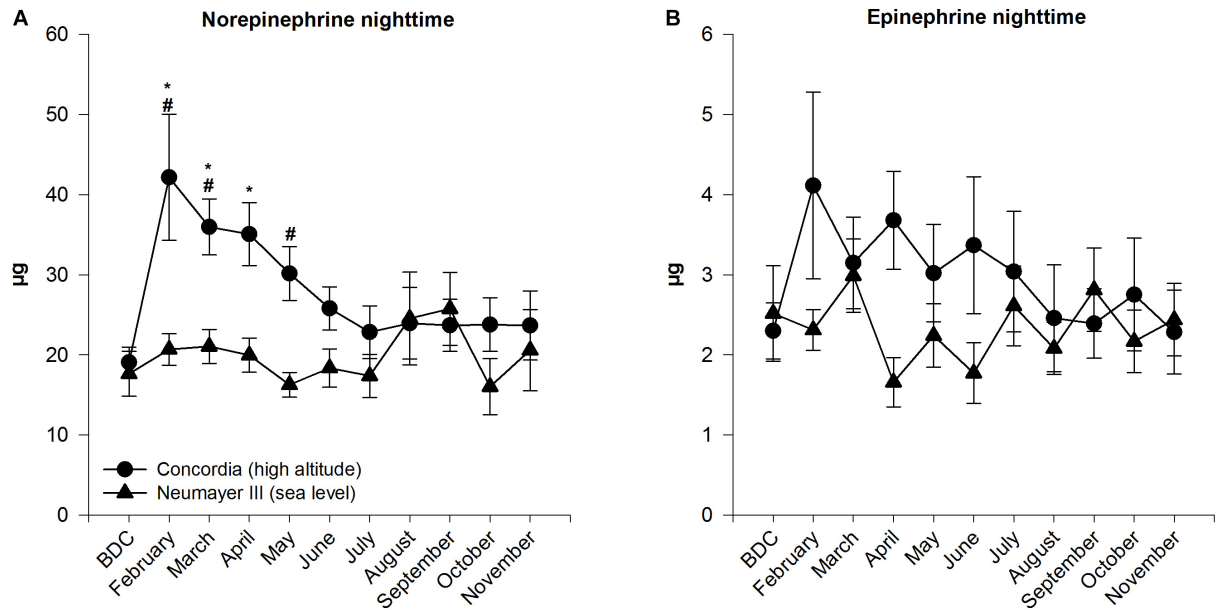

FIGURE 2 | Norepinephrine **(A)** and epinephrine **(B)** in 12-h nighttime urine at Neumayer III (sea level) (n = 11–16) and Concordia (high altitude) (n = 11–15); data are means ± SEM; units are µg (collection time 12 h); BDC, baseline data collection; *significant difference to BDC; #significant difference between Neumayer III (sea level) and Concordia (high altitude) crews.

Cortisol in Saliva

Morning Cortisol (Figure 3)

Neumayer III (sea level)

Morning cortisol concentrations increased slightly in the first 3 months but without statistical significance and then fluctuated around the baseline level.

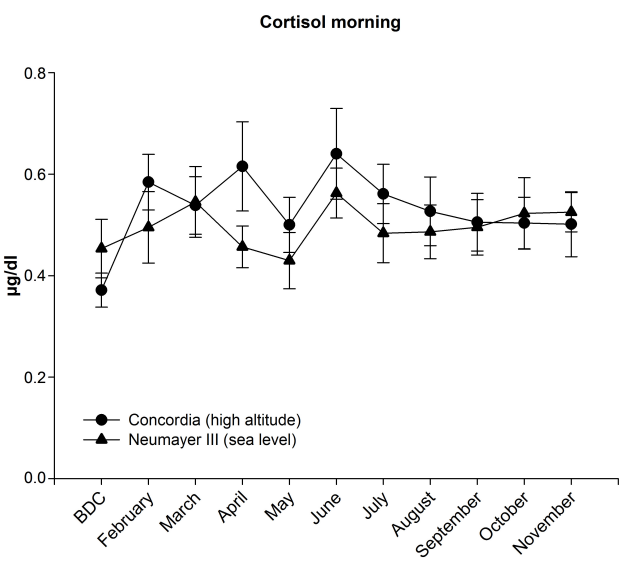

FIGURE 3 | Cortisol in saliva in the morning at Neumayer III (sea level) (n = 13–16) and Concordia (high altitude) (n = 12–15); data are means ± SEM; units are µg/dl; BDC, baseline data collection.

Concordia (high altitude)

Morning cortisol concentrations increased until April albeit not significantly then dropped in May before reaching the maximum level in June (from BDC 0.37 ± 0.03 to 0.64 ± 0.09 µg/dl). Afterward, cortisol declined constantly but stayed at a level higher than at BDC.

Neumayer III (sea level) versus Concordia (high altitude)

During the deployment, cortisol concentrations were nearly constantly higher in the Concordia (high altitude) crew but without any significant differences between the two crews.

Evening Cortisol (Data Not Shown)

Evening cortisol concentrations were constantly lower than in the morning and did not exceed normal values at either station (Neumayer III (sea level) between 0.12 and 0.22 µg/dl; Concordia (high altitude) between 0.13 and 0.17 µg/dl). At Concordia (high altitude), values ranged minimally higher than at Neumayer III (sea level) during the deployment except at the end of the stay (August-November). The circadian rhythm was maintained.

Psychological Evaluation and Measurements

CST (Short Questionnaire on Current Stress) (Table 3)

Neumayer III (sea level)

The acute stress test (CST) revealed no significant changes throughout the deployment period. Mean CST values in the morning ranged between 2.2 ± 0.3 and 2.7 ± 0.4 points. Evening results displayed the same pattern (data not shown).

TABLE 3 | Psychometric data of CST.

	CST m Neumayer III (sea level)	CST m Concordia (high altitude)
BDC	2.4 ± 0.2	1.9 ± 0.2
February	2.7 ± 0.2	2.0 ± 0.1
March	2.3 ± 0.1	1.9 ± 0.1
April	2.7 ± 0.3	2.0 ± 0.2
May	2.2 ± 0.3	1.9 ± 0.1
June	2.4 ± 0.3	2.0 ± 0.2
July	2.4 ± 0.2	1.7 ± 0.1
August	2.3 ± 0.3	1.9 ± 0.2
September	2.3 ± 0.2	1.9 ± 0.1
October	2.4 ± 0.3	1.9 ± 0.1
November	2.7 ± 0.4	1.8 ± 0.2

Data are mean ± SEM. CST, Current Stress Test; BDC, baseline data collection.

Concordia (high altitude)

No significant changes throughout the deployment period were detectable, with mean CST values ranging in the morning between 1.7 ± 0.1 and 2.0 ± 0.1 points. The same results were measured in the evening (data not shown).

Neumayer III (sea level) versus Concordia (high altitude)

CST scores were consistently higher at Neumayer III (sea level) than at Concordia (high altitude) independent of the time point and the time during the day but without any significant differences between the two crews (evening data not shown).

Spielberger State Trait Anxiety Inventor (STAI) (Table 4)

Neumayer III (sea level)

Mean state evaluation scores ranged from 32.4 ± 2.5 to 38.5 ± 3.4 points whereas the trait assessment showed mean values between 32.3 ± 2.5 and 35.5 ± 3.4 points. No significant changes were stated.

Concordia (high altitude)

State mean scores at Concordia (high altitude) ranged from 27.3 ± 1.3 to 30.4 ± 2.8 points; trait means ranged between 28.4 ± 1.0 and 29.9 ± 1.5 points. These findings were not statistically significant.

Neumayer III (sea level) versus Concordia (high altitude)

Between the two crews, the anxiety level (state and trait) was consistently higher at Neumayer III (sea level) but without any significant differences.

DISCUSSION

The present study investigated humans during long-term acclimatization to the highly aversive environment of two Antarctic research stations. Main focus of the research was to investigate the effects of hypobaric hypoxia on stress-related metabolites. Interestingly and contrary to expectations, a massive increase of these metabolites was observed under normoxic conditions (Neumayer III, sea level) while concentrations under hypobaric hypoxia (Concordia, high altitude) stayed low during the isolation period but enhanced significantly at the end. Conversely, catecholamines showed an increase at the beginning of exposure to high altitude being prolonged for norepinephrine whereas cortisol showed no changes.

It seems that the exposure of humans from a physiologically familiar to an unknown and physically very challenging environment triggers this enhanced EC response and that hypobaric hypoxia modulates this response in terms of a downregulation. Downregulated expression or lack of increased expression of ECs in the Concordia (high altitude) crews may originate in hypoxia signaling and EC metabolism. In the tumor microenvironment, hypoxia is known to upregulate the expression of cyclooxygenase-2 (COX-2) *via* hypoxia-inducible factor-1 (HIF-1) signaling (Greenhough et al., 2009). COX-2 is one of the enzymes responsible for metabolism of ECs (Sugimoto et al., 2017) with the main substrates being AEA and 2-AG (Chiurchiu et al., 2015). Therefore, missing upregulation of ECs at Concordia (high altitude) might be due to hypoxia-induced upregulation of COX-2 (and possibly other enzymes) which in turn oxidizes ECs into compounds (e.g., prostaglandin-ethanolamides, hydroxy-AEAs) (Chiurchiu et al., 2015) that were beyond the scope of our investigations. Furthermore, other dysregulations of degrading and metabolizing systems of the ECs [e.g., fatty acid amide hydrolase (FAAH), intracellular transporters] that were not focus of our study might also have contributed to the stated downregulation of the ECS.

However, HIF-1α expression under long-term hypobaric hypoxia seems to be time-dependent. A short-lived transient activation of HIF-1α-dependent pathways was observed at the beginning of exposition to high altitude (Bigham and Lee, 2014; Bigham, 2016) but seems to elapse gradually over time (Petousi et al., 2014). Thus, this regulation pathway seems to be less significant in acclimatization processes upon long-term exposition to hypobaric hypoxia (Baze et al., 2010). This is in good accordance with findings of Feuerecker et al. (2018) who observed in a former study at Concordia a downregulation of HIF-1α expression under chronic hypobaric hypoxia. Goyal and Longo (2014) observed similar results albeit in an animal model. Against the background of the assumed COX-2 influence on EC metabolism, one might speculate that this mechanism might have also contributed to the rising EC levels at Concordia (high altitude) at the end.

The hypothesis that an unknown and physically challenging environment triggers enhanced EC responses is in good accordance with recent studies which demonstrated that the consequences of strenuous physical conditions (e.g., sleep restrictions, physical exercise) are able to induce a heightened EC response (Cedernaes et al., 2016; Hanlon et al., 2016).

In the light of such stressful conditions, we also expected enhanced catecholamines and cortisol levels due to an elevated sympathetic tone but surprisingly the stress hormone answers stayed normal in the Neumayer III (sea level) expeditioners. One possible explanation for these unexpected observations derives from the interaction between the EC and the sympathetic nervous system and the glucocorticoids. During an acute stress

TABLE 4 | Psychometric data of STAI.

	STAI state Neumayer III (sea level)	STAI state Concordia (high altitude)	STAI trait Neumayer III (sea level)	STAI trait Concordia (high altitude)
BDC	32.4 ± 2.5	28.8 ± 2.0	33.8 ± 2.0	28.4 ± 1.0
February	36.4 ± 2.4	28.6 ± 1.4	32.6 ± 2.3	29.9 ± 1.5
July	34.9 ± 2.3	27.3 ± 1.3	32.3 ± 2.5	29.4 ± 2.0
November	38.5 ± 3.4	30.4 ± 2.8	35.5 ± 3.4	29.6 ± 1.3

Data are mean ± SEM. STAI, Spielberger State Trait Anxiety Inventory; BDC, baseline data collection.

model realized by parabolic flights, subjects who experienced no motion sickness showed similar results to ours presenting high EC concentrations and simultaneously low cortisol levels. By contrast, in subjects with motion sickness, the hormones' balance was inversed (Chouker et al., 2010). Increasing EC levels were also described in a chronic stress model when examining astronauts on the International Space Station (Strewe et al., 2012).

On the other hand, Yi et al. (2016) reported during a 520 days lasting confinement in the course of a simulated mission to Mars a reduction in circulating 2-AG but a significant increase in urinary norepinephrine and saliva cortisol. The chronic stressor of confinement resulted here in an interaction of these systems that is in contrast to our findings albeit it also displays its apparent inverse character. Moreover, the hypothesis of an inverse interaction is also supported by Ishac et al. (1996) who stated an inhibitory effect of presynaptic cannabinoid CB_1 receptors on noradrenaline release in peripheral sympathetic nerves and Surkin et al. (2018) who evidenced that pharmacological augmentation of EC signaling reduces the neuroendocrine response to stress. Niederhoffer and others also found this inhibitory effect of ECs on noradrenaline to be the cause of cardiovascular depression (Wagner et al., 1998; Niederhoffer et al., 2003; Pfitzer et al., 2005). However, opposite reactions were also described dependent on the acting EC (Kurihara et al., 2001). Furthermore, the way of interaction seems to vary dependent on the physiological system that is examined. Indeed, Simkins et al. (2016) described a positive correlation when they detected a reduced noradrenergic signaling in the spleen capsule in the absence of cannabinoid receptors. Additionally, cannabinoid receptor agonists were shown to induce peripheral antinociception by activating the noradrenergic system (Romero et al., 2013) and they increased norepinephrine efflux in the frontal cortex in an animal model (Page et al., 2008).

By contrast, when analyzing the data of the Concordia (high altitude) crew, we stated that low EC levels were associated with significantly increased norepinephrine concentrations and increased cortisol levels albeit not significant. A former study of our research group in the same Antarctic environment already evidenced similar results with elevated catecholamine levels and assumed their association with stated immune alterations (Feuerecker et al., 2014). Furthermore, several other studies in (simulated) high altitude investigated well the impact of hypobaric hypoxia on sympathoadrenal and adrenocortical stress responses and found a positive correlation (Calbet, 2003; Simeoni et al., 2011; Dhar et al., 2014; Aliyev et al., 2017; Woods et al., 2017). Woods et al. (2017) showed that simulated altitude under normobaric or hypobaric hypoxia (equivalent to 3,375 m) appears to induce similar effects in humans than genuine high altitude. Nevertheless, these effects of hypoxia are also variable when combined with other co-(stress) factors such as, e.g., immobilization in bed rest. Thus, we demonstrated in a former study exposing subjects to 21 days of normobaric hypoxia (simulated altitude ∼ 4,000 m; 14% O_2) and/or bed rest a reduced sympathoadrenal answer after exposition to this combination of stressors. Normobaric hypoxia alone did not induce any significant changes in the catecholamine or cortisol answer (Strewe et al., 2017). However, the results of this study suggest a different mechanism of regulation due to the hypobaric hypoxic environment at Concordia. This mechanism seems to be based on an inverse interaction of the sympathoadrenal with the EC response. Hypobaric hypoxia would therefore either downregulate the EC response or upregulate the sympathoadrenal answer with subsequently raised norepinephrine levels which, in turn, would downregulate and suppress the other system, respectively. The opposite action could be stated in the Neumayer III (sea level) crew.

The hypothesis of an upregulated sympathoadrenal answer is supported by the findings while the mission approaches its end. In the course of time, the sympathoadrenal response declined gradually and stagnated on a low level at the end. Here, interestingly, the EC response started to increase and concentrations of, e.g., AEA and 2-AG were significantly higher than at BDC.

The increase of ECs and NAEs at Concordia (high altitude) at the end with the adjustment of the physiological answers is in line with the hypothesis of a recent study reported by Alarcon-Yaquetto et al. (2017) who demonstrated significant higher NAE concentrations in natives of high altitude (3,830 m) with higher hemoglobin concentration and lower pulse oxygen saturation. Apparently, the time course and the long-term impact of the physical conditions seem to play an important role in the development of the physiological answer. In addition, Feuerecker et al. (2012) evidenced no changes in the human ECS under short-term hypobaric hypoxia (altitude of 3,196 m) but only in its combination with physical effort. The opposite effect of an enhanced EC response was probably due to the study design that displayed an acute stress model. Furthermore, Strewe et al. (2017) found no changes in the ECS when exposing humans for 21 days to normobaric hypoxia (14% O_2 and a simulated altitude of ∼4000 m) either in combination with bed rest or in an ambulatory setting. Here, the different results may be explained by the different physical stressors and the shorter time frame.

Furthermore, differences in reaction and regulation patterns of the ECS may also vary dependent of the status and type of cell that is examined (tumor versus healthy cells as mentioned above).

Additionally, in the Concordia (high altitude) crew, great SEMs for norepinephrine (especially in the first months of exposition to hypobaric hypoxia), epinephrine, and cortisol displayed a great inter-individual variability in the concentrations measured that was not found in the Neumayer III (sea level) crew. This might express the individual responses of acclimatization to high altitude in the subjects (Siques et al., 2009; Hermand et al., 2015; Richalet and Lhuissier, 2015).

In summary, our findings at both Antarctic stations evidence that human exposure to a physically challenging and physiologically new and unknown environment with extreme conditions generates a neuroendocrine reaction pattern of interacting physiological systems. Herein, high altitude exposure evokes a significant increase of norepinephrine at the beginning in contrast to other neuroendocrine parameters. Furthermore, there seems to be a great variability in the inter-individual acclimatization responses to high altitude.

As seen by the results in contrast to the baseline data, the reaction pattern seems to display and express a long-term acclimatization process of the human body and physiology to these conditions rather than a pathological process as its intensity and efficiency diminishes over time to finally return to a nearly "normal" status.

Furthermore, our results attribute and relate these reactions to the physical challenge in this environment and do not support an explanation based on increased psychological stress. As all inquired stress questionnaires on both stations negated its presence. However, as a limiting factor, it must be considered that all participants in such expeditions are highly motivated and well prepared which might be a reason for the absence of a distinct psychological effect. Furthermore, such psychological evaluation tests depend on the motivation and honesty (manifested self-reflection) of the subject. If one wants to mask his real feelings and psychological state of mind, any emotional impairment might just be negated. Moreover, a constant higher score level in these tests in the Neumayer III (sea level) crew, albeit in the normal range, might also just display national differences in carrying out the tests or depend on the way how the tests were explained to the subjects in the first place.

Limitations

The Neumayer III crew served as control group at sea level to identify the delta effects of hypobaric hypoxia compared to the crew at Concordia. This is not an ideal control group as (i) groups differ in their demographics (BMI) and (ii) not only hypoxia varies but also all other environmental conditions are alleviated at the Antarctic coast (e.g., temperatures, wild life, etc.) compared to Concordia located in inner Antarctica. A better control would have been either a cross-over study design where the same subjects would have been exposed to both, low and high altitude with the same light exposure time, same food, seasonal variations, and duration of expedition or a longitudinal study design with the same subjects. However, even in these two design models, either the same study year (cross-over design) or the different environmental conditions (low versus high altitude) would not be applied to the respective investigated subjects. Moreover, both designs seem however not feasible in this environment from many perspectives (participants, logistics, costs, and others).

Additionally, daily physical activity and challenge of the participants as well as their physical conditions and fitness might interfere with the measures taken so that in an ideal setting their monitoring would be warranted. Moreover, an investigation of each individuals' peculiar physiological response could be of interest and importance to define personal differences and potentially identify corresponding groups with similar reaction patterns.

Furthermore, even though Antarctic climate only changes slowly due to global climate change and environmental conditions at both stations stay relatively stable from 1 year to the other, natural undulations in temperature, humidity, day–night cycle, etc. exist, and thus, a possible influence on the measures made cannot fully be excluded.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

MF, AC, and GS designed the work. CS, MF, AC, CD, FvdB, BF, HB, and PG-L collected the data. All authors (CS, DT, CD, BF, FvdB, HB, AS, PG-L, PC, DM, RQ, MM, SB, EK, GS, AC, and MF) performed data analysis and interpretation as well as drafting the article and critical revision for important intellectual content. All authors gave final approval of the version to be submitted and any revised version.

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SCIENTIFIC REPORTS

OPEN

PlanHab Study: Consequences of combined normobaric hypoxia and bed rest on adenosine kinetics

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Adenosine plays a role in the energy supply of cells and provokes differential, hormone-like functions in circulating cells and various tissues. Its release is importantly regulated by oxygen tension. This renders adenosine and its kinetics interesting to investigate in humans subjected to low oxygen conditions. Especially for space exploration scenarios, hypoxic conditions – together with reduced gravity - represent two foreseen living conditions when planning manned long-duration space missions or planetary habitats. The PlanHab study investigated microgravity through inactivity in bed rest and normobaric hypoxia to examine their independent or combined effect on adenosine and its kinetics. Healthy male subjects (n = 14) completed three 21-day interventions: *h*ypoxic *b*ed *r*est (HBR); *h*ypoxic *amb*ulatory confinement (HAMB); *n*ormoxic *b*ed *r*est (NBR). The interventions were separated by 4 months. Our hypothesis of a hypoxia-triggered increase in adenosine was confirmed in HAMB but unexpectedly also in NBR. However, the highest adenosine levels were noted following HBR. Furthermore, the percentage of hemolysis was elevated in HBR whereas endothelial integrity markers stayed low in all three interventions. In summary, these data suggest that neocytolysis accounts for these effects while we could reduce evidence for microcirculatory changes.

The endogenous nucleosid adenosine exists ubiquitously in the intra- and extracellular space of the different organ tissues. Extracellular adenosine is generated predominantly through phosphohydrolysis of 5′-adenosine tri-/monophosphate (ATP/AMP).

Over the last years it has been demonstrated that adenosine influences and modulates multiple different physiological processes[1,2] and that hypoxia plays an important regulatory role[3–6]. Adenosine acts through four G protein-coupled adenosine receptors that either stimulate (A_{2A}, A_{2B}) or inhibit (A_1, A_3) adenylate cyclase and thus enhance or decrease the second messenger cyclic AMP (cAMP). Finally, extracellular adenosine is rapidly taken up into the cells and metabolized either into inosine by adenosine deaminase or into AMP by adenosine kinase. As hypoxia does not allow sufficient and oxygen dependent re-phosphorylation of ADP and AMP, adenosine concentrations could increase as a function of hypoxia. Environmental hypoxia is not only a natural condition in higher altitudes or in aviation, but is also strongly considered as an atmospheric condition for future manned missions to outer space. Therefore, research on the effects of hypoxic environmental and living conditions in addition to reigning microgravity is of emerging interest for planning possible planetary habitats on the moon or Mars, or for long-duration space exploration class missions. In light of these scientific questions, the Planetary Habitat Simulation Study (PlanHab) was performed. We investigated the changes in adenosine release and its

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kinetics under simulated space conditions by subjecting 14 male subjects to 21 days of hypoxia and/or horizontal bed rest to mimic microgravity effects. We specifically investigated the course of the adenosine release, its concentrations and that of its metabolite inosine and were further interested, if and to what degree the adaptation to these standardized living conditions are linked to adenosine profiles and different physiologic states. We hypothesized that adenosine concentrations would be enhanced during the early stages of both hypoxic interventions. Our unexpected additional finding of hypoxia-independent adenosine increase has initiated further analysis to i) identify a potential role of hemolysis as a source of adenosine in our experimental interventions, ii) to evaluate the interaction of adenosine and endothelial barrier function, and iii) to investigate the impact of physical activity on adenosine concentrations.

Material and Methods

The Planetary Habitat Simulation Study (PlanHab) was an EU-funded research program (Call FP7-SPACE-2011-1; project number 284438) ministered by several research groups investigating physiological and psychological effects of life in simulated planetary habitat conditions. The study protocol has been registered at ClinicalTrials.gov with the identifier NCT02637921 on December 1st, 2015. It was approved by the Committee for Medical Ethics at the Ministry for Health of the Republic of Slovenia. The Declaration of Helsinki was respected and bed rest protocols met the criteria of the European Space Agency (Standardization of bed rest study conditions, Version1.5, August 2009; revised on an international basis in 2014 in the 'Guidelines for Standardization of Bed Rest Studies in the Spaceflight Context' by the International Academy of Astronautics (IAA) (www.nasa.gov/sites/default/files/atoms/files/bed_rest_studies_complete.pdf)). All methods used were performed in accordance to standard laboratory guidelines and regulations.

Study design and participants. The PlanHab Study took place at the Olympic Sport Centre Planica (Ratece, Slovenia) which is situated at an altitude of 940 m above sea level. In total, 65 healthy males were screened and 14 were included in the trial. Individuals with a recent exposition (<2 months) to altitudes above 2000 m and individuals normally residing at altitudes higher than 500 m were excluded. All participants gave written informed consent. 11 participants finished all three campaigns; 3 dropped out before the last campaign due to personal reasons. The participants' demographical data were as follows: age 26.4 ± 5.2 years; body mass 75.9 ± 10.6 kg; stature 1.8 ± 0.05 m; BMI 23.5 ± 2.8 kg/m^2 (mean ± SD).

The participants were exposed to three different protocols: normobaric normoxic horizontal bed rest (NBR: $FiO_2 = 0.209\%$; $PiO_2 = 133.1 \pm 0.3$ mmHg), normobaric hypoxic horizontal bed rest (HBR: $FiO_2 = 0.141 \pm 0.004\%$; $PiO_2 = 90.0 \pm 0.4$ mmHg; equivalent to ~4000 m) and normobaric hypoxic ambulatory confinement (HAMB: $FiO_2 = 0.141 \pm 0.004\%$; $PiO_2 = 90.0 \pm 0.4$ mmHg). In three campaigns executed between October 2012 and October 2013 every participant consecutively passed each protocol (cross-over design). The campaigns were separated by an approximate 4-month wash-out period and each campaign lasted 32 days. In the first 7 days after the participants' arrival the baseline data was collected (BDC). During the next 21 days the participants were confined to the respective condition (HAMB, HBR or NBR) followed by a 4-day recovery period to obtain post-confinement measurements (R). Each campaign was entered sequentially in a fixed order by two participants per day.

Furthermore, the participants were subjected to a standardized diet that was strictly applied throughout each campaign.

Bed rest and maintenance of hypoxic conditions. During bed rest conditions (HBR and NBR) the participants were strictly restricted to a horizontal position avoiding any strenuous physical activity. These restrictions implicated all daily routines (e.g. showering/toilet) though a change of position from lateral to supine or prone was allowed as well as passive stretching by a physiotherapist.

In contrast, when being assigned to the hypoxic ambulatory condition (HAMB), subjects were encouraged to maintain a level of physical exercise comparable to their usual daily routine by moving around in the restricted area (110 m^2) and performing exercise sessions (e.g. cycling).

The circadian rhythm of all subjects was regulated by a standardized wake/sleep cycle (wake-up 7:00 am; lights off 11:00 pm). In the facility, environmental conditions were controlled and maintained at the same level through all campaigns (ambient temperature: 24.4 ± 0.7 °C; relative humidity: 53.5 ± 5.4%; ambient pressure: 684 ± 4 mmHg).

Hypoxic conditions were maintained with an O_2 dilution system and have been reported in detail previously[7]. The maintenance of a sufficient O_2 level during hypoxic conditions was surveyed continuously with portable O_2 sensors (RAE PGM-1100, California, USA) with an alarm triggered at a pre-set O_2 level of 13.5%.

Blood sampling, processing and analysis. Blood collection took place 2 days before the beginning of the intervention period (baseline data collection, BDC), at days 2, 5, 14 and 21 during the confinement and 2 days after the end of confinement (R2). The blood was drawn from an antecubital vein from the fasted subjects in a horizontal, supine position at 7:30 in the morning.

For the assessment of hemolysis, the quantitative measurement of sICAM-1 and Zonulin, EDTA-anticoagulated blood was centrifuged at 3500 RPM for 5 minutes, the supernatants transferred into Eppendorf tubes and directly frozen at −80 °C. For the analysis, plasma samples were thawed in ice water and subsequently processed.

Purine analysis. A 5 ml syringe prefilled with 2 ml of an ice-cooled stop solution (composed of 6.3 mg EHNA, 7.446 g Na_2EDTA, 7.608 g EGTA, 6.482 g D,L-α-glycerophosphate, 100 mg dipyridamole and NAOH to titrate a ph = 6 in 1 l of NaCl 0.9%) to prevent any supplementary formation or degradation of adenosine was used to draw venous blood samples. After transfer into a gel-vacutainer and centrifugation at 4000 RPM for 5 minutes, the samples were frozen in an upright position at −80 °C. Plasma concentrations of the purine nucleoside adenosine and

inosine were analyzed by dual-column switching high-affinity performance/reversed-phase high performance liquid chromatography (HPLC, Chromosystem, Martinsried, Germany) as described previously[8,9].

Assessment of hemolysis. Before assessing hemolysis in the subjects' samples standard curves were established. Here, 2 ml of whole blood were centrifuged at 4000 RPM for 5 minutes, the supernatant discarded and the pellet relocated with 1 ml Aqua dest. and incubated for 1 hour at room temperature to induce hemolysis. After further centrifugation at 4000 RPM for 10 minutes, the supernatant was kept as 100% lysate and the pellet was discarded. A dilution series was prepared by serial dilution of the 100% lysate with Hanks buffered salt solution (HBSS) to acquire 2.5, 1.0, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125 and 0.00390625% hemolysed samples. After centrifugation at 4000 RPM for 10 minutes the supernatants were measured with the Thermo Scientific™ Nano Drop 2000/2000c Spectrophotometer (Erlangen, Germany) at 414 nm to establish a standard curve.

Subsequently, the plasma samples from EDTA-anticoagulated blood were thawed, mixed and 1 µl of the sample measured at 414 nm (Thermo Scientific™ Nano Drop 2000/2000c Spectrophotometer, Erlangen, Germany). Its percentage of hemolysis was then detected via the established standard curve.

Erythropoietin analysis. Lithium-heparinized blood (2 ml) was transferred into a gel-vacutainer, centrifuged and frozen at −80 °C. Erythropoietin concentrations were determined by sandwich enzyme-linked immunoassay as described previously by Keramidas *et al.*[10].

Cell blood count. Hemoglobin, hematocrit, mean corpuscular volume (MCV), erythrocytes, reticulocytes and thrombocytes were analyzed with an automated laser-based hematology analyzer (Advia 120; Siemens, Munich, Germany) within 8 h after blood sampling.

Body mass and composition. Body mass and composition were assessed daily in the supine position using a calibrated, custom-made gurney incorporating load cells (Sigma 6 C, Libela ELSI, Celje, Slovenia) and a fan-beam dual energy X-ray absorptiometer (DXA; Discovery W-QDR series, Hologic, Bedford USA) as described previously[7].

Soluble intercellular adhesion molecule-1 (sICAM-1). Quantitative measurement of sICAM-1 was carried out by using the commercially available abcam® ICAM1 Human ELISA Kit (abcam®, Cambridge, MA, USA) according to the manufacturer's guidelines. Plasma samples and a 1:10 dilution was used for the measurement.

Zonulin. Quantitative measurement of Zonulin was carried out by using the commercially available IDK® Zonulin ELISA Kit (Immundiagnostik AG, Bensheim, Germany).

Data availability. The datasets generated and/or analysed during the current study are available from the corresponding author upon request.

Statistics. Data were analyzed and plotted with SPSS 23.0 (IBM, Armonk, NY) and Sigma Plot 12.5 (Systat Software Inc., San Jose, CA) as reported previously[11]. Outcome variables and residuals were tested for deviations from the normal distribution using Kolmogorov–Smirnov tests followed by a Box–Cox Transformation where residuals were not normally distributed. Statistical inferences regarding the effects of different conditions and time points during the same campaign were imputed through mixed linear models (LME). We included fix effects for campaign, sequence of campaigns, potential carryover from the previous campaign, condition, time within the respective campaign, and the interaction of condition and time. Random effects were included for subject and carry-over using a covariance matrix with variance components structure. A p value of <0.05 was regarded as statistically significant.

Results

Purine concentrations and kinetics. *Adenosine.* The concentration of extracellular adenosine was significantly augmented during the intervention period in all three conditions, with a first peak on day 5, and a maximum value occurring mostly between days 14 and 21. The highest adenosine concentrations of over 200 nmol/l were observed in HBR with a high statistical significance to BDC at day 21 ($p < 0.05$ (in the comparison between HBR vs. HAMB) and $p < 0.01$ (in the comparison between HBR vs. NBR)). After the end of the interventions adenosine concentrations remained elevated in all three groups with a statistical significance at R2 in NBR compared to BDC ($p < 0.05$). A difference between the condition HBR and NBR was found at day 5 and 21 and between HBR and HAMB at day 21 ($p < 0.05$, respectively) (Fig. 1A,B).

Inosine. Inosine concentrations were significantly augmented during the intervention period in all three conditions, with a peak occurring at day 21. The highest inosine concentrations of ~300 nmol/l were attained in HBR at day 21. A significant difference was observed between HBR and NBR at day 21 ($p < 0.05$). During the recovery period, the inosine concentrations declined in all three interventions, but were sustained at a level which was significantly different compared to BDC at R2 (each intervention respectively $p < 0.01$) (Fig. 1C,D).

Assessment of hemolysis. The percentage of hemolysis was significantly different between HBR and HAMB at day 5 ($p = 0.041$), and HBR and NBR at day 5, 14 and 21 ($p = 0.044$; $p = 0.018$; $p = 0.031$). In each intervention group no significant differences were detected versus BDC. In HAMB and NBR the percentage of hemolysis sustained on a constantly low level (Fig. 2).

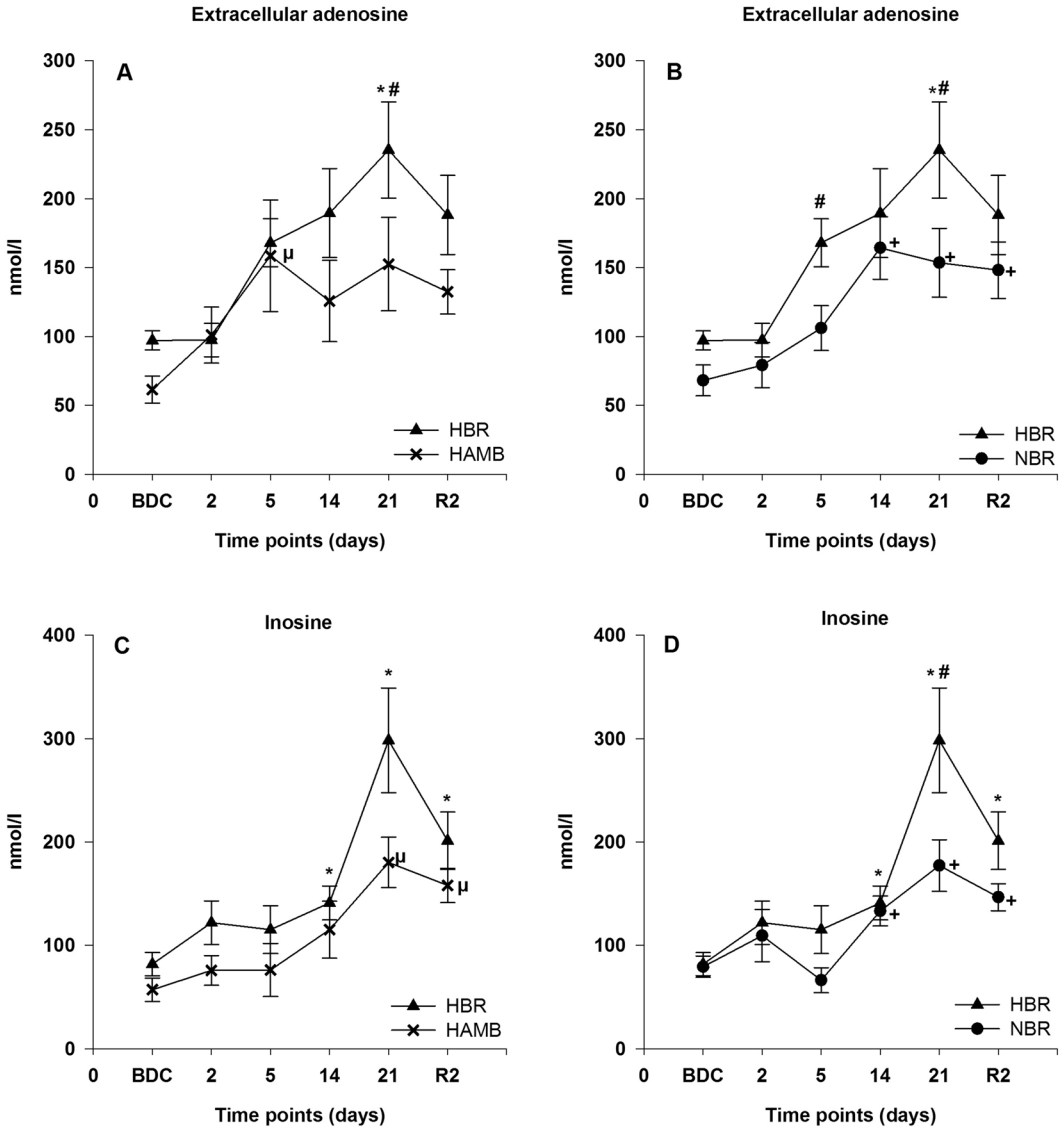

Figure 1. Extracellular adenosine (**A**,**B**) and inosine (**C**,**D**) concentrations in plasma; data are means ± SEM; units are nmol/l; HBR = hypoxic bed rest (n = 12–14); NBR = normoxic bed rest (n = 11–13); HAMB = hypoxic ambulation (n = 12); BDC = Baseline Data Collection; R2 = 2 days after the end of condition; #Significant difference between HBR and HAMB or NBR; *Significant difference to BDC in HBR; μSignificant difference to BDC in HAMB; +Significant difference to BDC in NBR ($p < 0.05$).

Serum erythropoietin. Highest erythropoietin concentrations were attained in the acute phase of exposition to HBR or HAMB at day 2, respectively, with a rapid decline to baseline or even lower values at the end of the intervention period and R2. A statistical significant difference to BDC was detected in HAMB at day 2 and 5 and in HBR at day 2 ($p < 0.01$ respectively). A significant difference between HAMB and HBR was detected at day 2 and 5 ($p = 0.018$ respectively) during the intervention period (Fig. 3).

Cell blood count. During the intervention periods all three conditions exhibited a significant hemoconcentration with elevated hematocrits. Hemoglobin was also augmented significantly during all conditions, whereas the mean corpuscular volume stayed nearly constant. A significant thrombocytosis was detected during HBR and HAMB (Table 1).

Reticulocytes increased significantly during HBR and HAMB with their maximum peak at day 5 (Fig. 4).

Body mass and composition. Body mass and whole body fat free mass were significantly reduced in the 4-day recovery phase after each intervention compared to BDC. Data has been published previously[7].

Soluble intercellular adhesion molecule-1 (sICAM-1). No significant changes between the three interventions or between the intervention period and BDC in each intervention were detected (Table 2).

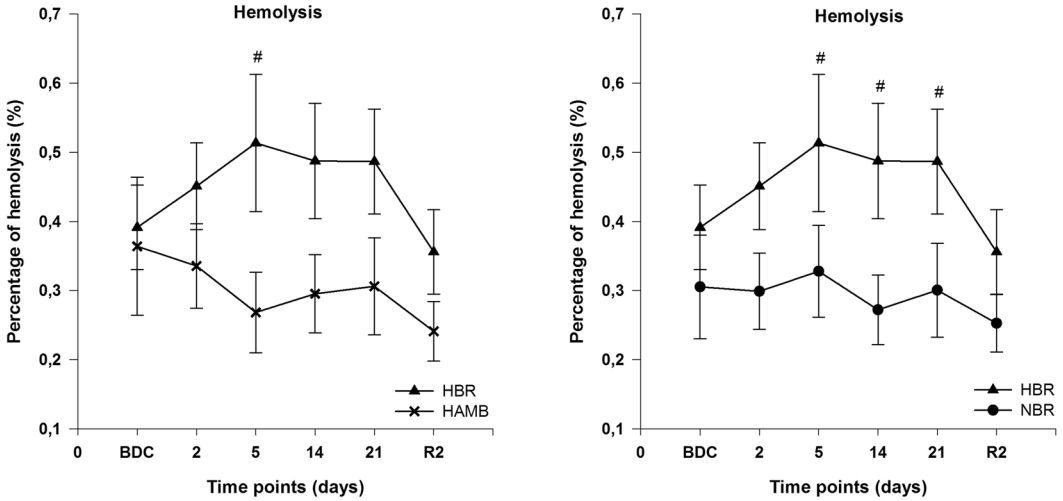

Figure 2. Assessment of hemolysis; data are means ± SEM; units are percent (%); HBR = hypoxic bed rest (n = 12–14); NBR = normoxic bed rest (n = 11–13); HAMB = hypoxic ambulation (n = 12); BDC = Baseline Data Collection; R2 = 2 days after the end of condition; #Significant difference between HBR and HAMB or NBR ($p < 0.05$).

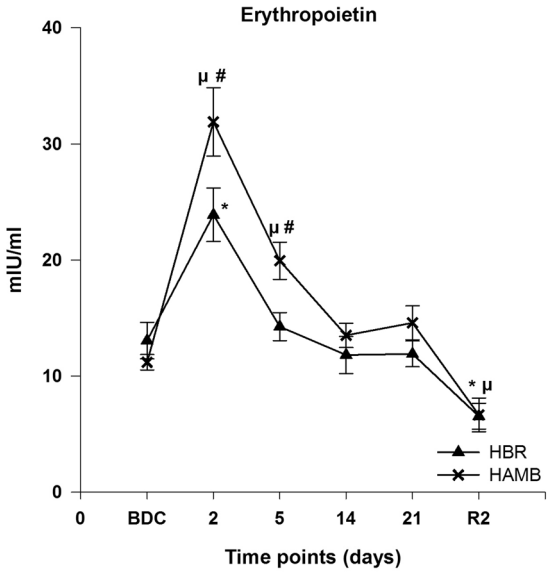

Figure 3. Absolute values of erythropoietin concentrations in serum; data are means ± SEM; units are mIU/ml; HBR = hypoxic bed rest (n = 11); HAMB = hypoxic ambulation (n = 11); BDC = Baseline Data Collection; R2 = 2 days after the end of condition; #Significant difference between HBR and HAMB; *Significant difference to BDC in HBR; μSignificant difference to BDC in HAMB ($p < 0.05$).

Zonulin. No significant changes between the three interventions or between the intervention period and BDC in each intervention were detected (Table 2).

Discussion

The PlanHab study was designed to fulfill the highest possible level of standardization to examine in humans the adaptation of organ systems to defined environmental factors anticipated for human exploration missions. Two main environmental factors were implemented in PlanHab: hypoxia to verify effects of the anticipated atmospheric conditions, and bed rest as model to mimic reduced gravitational forces. Therefore, PlanHab and its high fidelity conditions offered, for the first time, the possibility to investigate the independent or combined effects of these extreme conditions on the kinetics of the purine adenosine and its metabolite inosine, both recognized as important markers of cell stress and metabolism especially as a function of hypoxia[3,6,12]. Hence, we hypothesized a hypoxia-triggered early-stage increment in their kinetics with increased adenosine and inosine concentrations.

This hypothesis was confirmed during PlanHab as purine concentrations increased in both hypoxic groups during the intervention period, but interestingly two unexpected findings were noted: i) bed rest seemed to boost

	Time points	HBR	NBR	HAMB
Hemoglobin (g/dl)	BDC	14.63 ± 0.75	14.60 ± 0.91	14.67 ± 0.56
	2	$^{\mu}$15.84 ± 0.95	$^{\mu}$15.83 ± 1.03	$^{\mu}$15.65 ± 0.59
	5	$^{\mu}$16.54 ± 1.06	$^{\mu}$15.80 ± 1.03	$^{\mu}$15.69 ± 0.83
	14$^{\#,+}$	$^{\mu}$17.50 ± 1.03	$^{\mu}$16.05 ± 1.13	$^{\mu}$16.12 ± 0.57
	21$^{\#}$	$^{\mu}$17.09 ± 1.13	$^{\mu}$15.72 ± 1.26	$^{\mu}$16.42 ± 0.83
	R2	15.33 ± 1.15	15.37 ± 2.04	15.05 ± 0.92
Hematocrit (%)	BDC	43.93 ± 2.12	44.15 ± 2.62	43.86 ± 2.21
	2	$^{\mu}$47.31 ± 2.55	$^{\mu}$47.92 ± 2.95	$^{\mu}$47.10 ± 1.72
	5	$^{\mu}$49.63 ± 2.97	$^{\mu}$47.49 ± 2.71	$^{\mu}$47.18 ± 3.09
	14$^{\#,+}$	$^{\mu}$51.11 ± 2.84	$^{\mu}$47.41 ± 3.21	$^{\mu}$48.09 ± 2.40
	21$^{\#}$	$^{\mu}$50.58 ± 3.31	46.42 ± 3.38	$^{\mu}$48.90 ± 2.96
	R2	44.91 ± 3.71	45.47 ± 6.31	44.98 ± 3.18
MCV (fl)	BDC	84.64 ± 4.66	85.99 ± 4.68	87.89 ± 3.48
	2	84.44 ± 5.10	85.99 ± 4.52	87.45 ± 3.50
	5	84.06 ± 4.62	85.39 ± 4.55	87.80 ± 3.36
	14^{+}	$^{\mu}$82.36 ± 5.11	$^{\mu}$84.62 ± 3.84	87.31 ± 4.38
	21	83.51 ± 5.34	$^{\mu}$84.13 ± 3.81	87.40 ± 4.86
	R2	83.63 ± 4.97	$^{\mu}$84.11 ± 4.20	88.17 ± 4.65
Thrombocytes (G/l)	BDC	206.14 ± 76.65	197.92 ± 45.50	201.00 ± 42.36
	2	233.86 ± 68.12	204.77 ± 49.37	229.83 ± 49.13
	5	$^{\mu}$245.21 ± 51.08	215.08 ± 64.21	215.08 ± 56.22
	14	$^{\mu}$260.29 ± 66.18	221.08 ± 75.07	$^{\mu}$238.17 ± 40.30
	21	$^{\mu}$237.36 ± 82.19	200.00 ± 69.38	212.92 ± 46.74
	R2	210.14 ± 80.03	171.39 ± 73.27	196.58 ± 36.86

Table 1. Cellular blood parameters; data are means ± SD; MCV = mean corpuscular volume; HBR = hypoxic bed rest (n = 14); NBR = normoxic bed rest (n = 13); HAMB = hypoxic ambulation (n = 12); BDC = Baseline Data Collection; R2 = 2 days after the end of condition; $^{\#}$Significant difference between HBR and NBR; $^{+}$Significant difference between HAMB and HBR; *Significant difference between HAMB and NBR at the respective time points; $^{\mu}$Significant difference vs. BDC in the respective group ($p < 0.05$).

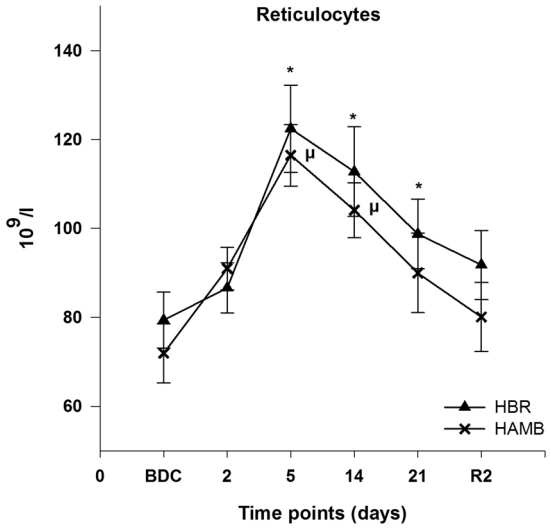

Figure 4. Reticulocyte count; data are means ± SEM; units are 10^9/l; HBR = hypoxic bed rest (n = 11); HAMB = hypoxic ambulation (n = 11); BDC = Baseline Data Collection; R2 = 2 days after the end of condition; *Significant difference to BDC in HBR; $^{\mu}$Significant difference to BDC in HAMB ($p < 0.05$).

adenosine release in combination with hypoxia as highest purine levels were attained during HBR, and ii) bed rest *per se* does seem to induce – although to a much lower degree - adenosine release since its concentrations were also augmented under normoxic conditions (NBR). To investigate and explain the hypoxic-enhanced release of adenosine, we further examined potential physiological mechanisms that may have contributed to these

	Time points	HBR	NBR	HAMB
sICAM-1 (ng/ml)	BDC	100.75 ± 45.12	71.75 ± 10.12	85.57 ± 45.82
	2	105.65 ± 46.57	77.62 ± 6.20	86.05 ± 30.26
	14	101.28 ± 47.40	67.06 ± 15.13	84.27 ± 45.93
	21	114.62 ± 49.99	71.25 ± 13.59	74.56 ± 9.91
Zonulin (ng/ml)	BDC	10.99 ± 1.88	13.01 ± 8.56	13.39 ± 4.91
	2	14.55 ± 6.02	12.93 ± 2.95	20.01 ± 8.35
	14	10.91 ± 2.32	11.69 ± 2.77	10.50 ± 2.65
	21	10.85 ± 3.45	11.94 ± 4.51	14.86 ± 3.10

Table 2. sICAM-1 and zonulin; data are means ± SD; units are ng/ml; HBR = hypoxic bed rest (n = 6); NBR = normoxic bed rest (n = 6); HAMB = hypoxic ambulation (n = 6); BDC = Baseline Data Collection.

adenosine changes: we verified its potential source of release from red blood cells, from mal-perfused tissues, or as function of physical activity or nutritional changes.

Red blood cells are well known to store adenosin-5-triphosphate (ATP) to maintain energy supply and it was demonstrated that adenosine signaling plays an essential role in their metabolism as well as in their capacity to react to hypoxia[13–15]. ATP release from erythrocytes occurs in response to hypoxia and hypercapnia as well as from deformation[16,17]. Beside specific release mechanisms, hemolysis was found to be at its origin dependent on the oxygenation status of the cells[18]. Sikora *et al.*[19] even stated that hemolysis seems a primary ATP-release mechanism in human erythrocytes as they observed that ATP release, independent of the stimulus, was exclusively triggered by hemolysis. That is why we quantified hemolysis during PlanHab and looked for its relation to other well-known hypoxia-triggered pathways as the erythropoietin- neo-erythrocytosis- axis. The glycoprotein hormone erythropoietin (EPO) represents an essential factor for the viability and proliferation of erythrocytic progenitors. Its release is known to be triggered by hypoxia via hypoxia-inducible transcription factors and induces a reticulocytosis. Significantly elevated EPO concentrations with a subsequent reticulocytosis were also observed in both hypoxic groups during PlanHab. However, this reticulocytosis resulted in a quantifiable hemolysis only during HBR and not HAMB. This was confirmed by a significant ($p < 0.01$) positive correlation for hemolysis and reticulocytes in the HBR group (data not shown). Apparently, only the combination of hypoxia with bed rest seems to be a sufficient trigger for a measurable hemolysis in this set-up and not hypoxia alone. To explain this phenomenon, we searched for mechanisms peculiar to bed rest. Feuerecker *et al.*[20] suggested that bed rest modifies blood flow via fluid redistribution and thus causes shear forces that induce shedding of adhesion molecules. When these shear forces now encounter instead erythrocytes that were already primed by hypoxia in the sense that their deformability was decreased and their susceptibility to cell damage increased[21,22], subsequent hemolysis potentially accounts for the extracellular adenosine increase. In this context, fluid redistribution under bed rest conditions might be one possible explanation for the combined effect of bed rest and hypoxia concerning hemolysis. Our findings during PlanHab support this hypothesis as fluid redistribution was most prominent in the HBR intervention[10]. Nevertheless, the question why the purine concentration was enhanced in all three interventions remains unresolved. During NBR and HAMB fluid redistribution and hemolysis were low, excluding these mechanisms as sole explanation. Against the background of the *in vitro* study results of Sikora *et al.*[19], focusing on hemolysis as explanation, enhanced hemolysis should have been detected in each intervention to explain the increased adenosine concentrations. Thus, perhaps the degree of hemolysis could not linearly be quantified with the sensitivity of the analysis method of hemolysis, which is supported by the fact that a reticulocytosis was present also during HAMB and NBR (data not shown), but no subsequent enhanced hemolysis could be detected in these two interventions. However, a positive correlation for hemolysis and reticulocytes was observed in NBR ($p < 0.01$; data not shown) supporting this hypothesis. Additionally, further yet unidentified influences might be responsible for the stated adenosine release. Evidence exists that adenosine promotes the permeability of the blood-brain-barrier[23,24] and that sustained adenosine exposure causes lung endothelial barrier dysfunction[25]. We hypothesized that a general mal-perfusion and subsequent hypoxic state in certain tissues is generated as a consequence of the constant ground reaction force-induced compression of dependent tissues during bed rest. Thus, an adenosine release is induced that impacts on the endothelial barrier function increasing its permeability, which is finally reflected in an increase of endothelial functional markers. Therefore, we quantified i) zonulin, a known physiological modulator of intercellular tight junctions that plays an important role in regulating intestinal permeability and is linked to the development of chronic inflammatory diseases[26–28], and also ii) intercellular adhesion molecule-1 and its soluble form (sICAM-1), another functional endothelial marker present on endothelial cells that facilitates leukocyte adhesion and migration[29] and is raised in inflammatory states[30,31]. Both markers have been implicated in a variety of pathological states (headache, typ-II-diabetes)[32,33]. However, despite the stated fluid redistribution that also hints to possible endothelial barrier dysfunction, we could not detect any increase in those markers which led to the rejection of this hypothesis to explain the observed adenosine release.

We further examined the effects of inactivity realized through bed rest on body composition and muscle mass. A battery of existent literature deals with the effect of physical training on muscle oxidative capacity, but conflicting results are demonstrated with either enhanced or reduced release of purines to plasma[34,35]. However, studies investigating the inverse state, physical inactivity, especially in humans are rather scarce as already stated by Gram *et al.*[36]. They seem to suggest the inactivity-induced down-regulation of the multiple purine-dependent pathways but query at the same time that these adaptions may not necessarily be opposite to that of physical training. Against this background, the analysis of our study colleagues Debevec *et al.*[7] on the body composition

of the PlanHab subjects provides evidence that muscle catabolism in this setting might be one possible explanation for the significant increase of purine concentrations. Body mass and whole body fat free mass were significantly reduced from BDC to recovery. Against what was hypothesized, hypoxia did not aggravate these findings. Interestingly, these results were found in all three interventions and not only in the bed rest groups. Debevec *et al.* suggested that this might be underlined appetite reduction and subsequent insufficient energy intake, low activity levels and/or effects of confinement *per se*.

In summary, our results confirm our initial hypothesis and clearly demonstrate that environmental hypoxia provokes a significant adenosine increase. Our analysis further suggests that this increase might be associated to neocytolysis. Unexpectedly, increased adenosine concentrations under normoxic conditions indicated that hypoxia-associated changes cannot be the sole explanation for our findings. However, other adenosine-related changes such as increased endothelial permeability could not be detected with the data given. In conclusion, our results can only partly be explained through hypoxic effects alone and other explanations remain to be provided. In this context, it has to be stressed that the one common factor of all three intervention groups in this study was the strictly controlled and standardized diet applied through all three campaigns. This might be one influential factor for the observations made.

Limitations. The unexpected findings during the PlanHab study prompted us to verify different pathways to search for explanations. Unfortunately, due to restrictions in the volume and frequency of blood sampling, we were limited in the number of analyses we could conduct. A supplemental normobaric normoxic ambulatory confinement (NAMB) intervention would have been relevant in this cross-over designed study, but could not be realized due to financial limitations. Furthermore, the impact of nutrition could have been investigated by introducing a NAMB intervention with and without an applied diet.

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Author Contributions

C.S. and A.C. wrote the main manuscript, C.S., A.C., R.Z., M.H., I.K. and S.M. realized sample collection on-site and were mainly responsible for sample processing. Specifically, M.T. and M.H. established the methodology for plasma purine nucleosides measurements. ACr and B.J. helped to realize and interpret statistics. T.D., I.M. and O.E. were involved in planning the study, its on-site realization and are authors of publications of results of the same study that are also part of our present study. C.S., A.C., M.F., M.T. and G.S. were mainly responsible for the interpretation of the results. All authors reviewed the manuscript.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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