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# Profile of inflammatory biomarkers in extreme conditions: Changes of serum amyloid A in a 7-week summer camp in Antarctica (*Short Communication*)

Michele Macedo Moraes[1,2], Leandro Borges[3], Thiago Teixeira Mendes[4], Elaine Hatanaka[3], Rosa Maria Esteves Arantes[1,2*]

[1] *Department of Pathology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[2] *Associate researcher of the Center for Newborn Screening and Genetics Diagnosis, Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil (NUPAD-FM/UFMG)*
[3] *Interdisciplinary Program in Health Sciences, Universidade Cruzeiro do Sul, São Paulo, SP, Brazil*
[4] *Department of Physical Education, Faculty of Education, Universidade Federal da Bahia, Salvador, BA, Brazil*

## Abstract
Isolated, confined, and extreme (ICE) conditions faced in Antarctica can influence immune system and inflammatory responses in humans. We evaluated the inflammatory biomarkers serum amyloid A (SAA), transforming growth factor-beta (TGF-β), and fatty acid-binding protein 2 (FABP2). Seven expeditioners took part in a 7-week Antarctic summer camp (Nelson Island) and were evaluated at Pre-Camp (i.e., at the beginning of the ship travel), Camp-Initial (*i.e*., 4$^{th}$ and 5$^{th}$ day in camp), Camp-Final (*i.e*., 45$^{th}$ – 46$^{th}$ day), and at the Post-Camp (on the ship on the return journey). Camping in Antarctica induced a bi-phasic change in SAA, with an increase found at Camp-Initial followed by a return to baseline levels. Such finding indicates a transient acute inflammation that does not imply a chronic inflammation condition in the long term.

***Key words:*** acute-phase protein, inflammation, SAA, stress-related responses, polar

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[*]Corresponding author: R. M. E. Arantes <rosa@icb.ufmg.br; rosa.esteves.arantes@gmail.com>
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## Introduction

Permanence in isolation, confinement, and extreme conditions (ICE) can result in psychophysiological and immunological changes (Palinkas et al. 2007, Moraes et al 2020, Moraes et al. 2023). In this context, clinical biomarkers can contribute to understanding the stress and systemic immune response in extreme conditions. We previously found a tendency to increase leptin and interleukin (IL)-8 in expeditioners during an Antarctic summer camp (Moraes et al. 2023). Herein in the follow-up study, we report complementary measures of the inflammatory biomarkers serum amyloid A(SAA), transforming growth factor-beta (TGF-β), and fatty acid-binding protein 2 (FABP2), due to their potential as indicators of pro-inflammatory condition, anti-inflammatory response, and installed tissue damage, respectively.

SAA is a nonspecific acute-phase inflammatory protein synthesized in the liver in response to the proinflammatory cascade triggered by infection, inflammation, injury, and stress; it is also implicated in several chronic inflammatory diseases (Hatanaka et al. 2007). Therefore, SAA assembles a fundamental contribution to understanding the temporal development of the inflammatory process (Hatanaka et al. 2007). TGF-β is a multifunctional pleiotropic cytokine that modulates cell differentiation, proliferation, apoptosis, and matrix production (Wahl et al. 1989). This anti-inflammatory cytokine protects the intestinal mucosal barrier and maintains a healthy microvasculature by regulating inflammation, clotting, and wound healing (Morikawa et al. 2016). FABP2 is a protein involved into fatty acids transport. Typically increase FABP2 levels in blood indicate intestinal mucosal damage. FABP2 is a biomarker for intestinal ischemia (Thuijls et al. 2011), located at the enterocyte and released as a response to increased intestinal permeability. FABP2 is also augmented in anxiety and depressive states (Stevens et al. 2018), and there is a relationship between FABP2 and insulin resistance (Weiss et al. 2002), which becomes attractive to investigate in an Antarctic camping context, especially considering the potential stressful ICE conditions and consumption of processed and ultra-processed foods (Moraes et al. 2023).

Therefore, we evaluated SAA, TGF-β, and FABP2 in a group of seven volunteers who remained camped on the Antarctica Peninsula (Nelson Island; S 53.178533°/ O 70.899750°) for seven weeks.

## Material and Methods

The study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of the Universidade Federal de Minas Gerais (protocol code 19092819.8.0000.5149/3.744.162); for studies involving humans. The volunteers were informed about the research objectives and all the experimental procedures before giving their written informed consent for participation in this study.

The data were collected on a ship before the beginning of the camp period (*i.e.*, "Pre-Camp"), at the beginning (*i.e.*, "Camp-Initial"; the 4$^{th}$ day of camp) and at the end of this period (*i.e.*, "Camp-Final"; the 45$^{th}$ day in camp), and on a ship after the Post-Camp period. Unstimulated whole saliva samples (1.5 mL) were collected in the morning, with the volunteers fasting, stored in liquid nitrogen during the camp period, and, after the days in the camp, transferred to the −80°C freezer inside the ship. The samples were kept frozen (−80°C) until the analysis. Before salivary analysis, the mucins and precipitants were removed by centrifugation (14.000g

for 20 min. at 4°C). Enzyme-linked immunosorbent assay (ELISA) was used according to the respective manufacturer's protocols to measure SAA, FABP2, and TGF-β (DuoSet Kit; Quantikine, R&D Systems, Minneapolis, MN, USA) and cortisol (Salimetrics, State College, PA, USA, dilution 1:5). The optical density (OD) of each sample contained in the well was immediately assessed with a microplate reader set to 450 and 540 nm (wavelength correction) (FLUOstar Omega, BMG LabTech). The data were linearized by plotting the log of the levels *vs*. the log of the OD, and the fitting line was defined by regression analysis. The detailed methods were previously presented (Moraes et al. 2023).

*Statistical Analysis*

For statistical analysis, the Shapiro-Wilk test revealed normal distribution, except TGF-β, submitted to log-transform. One-way repeated-measures analysis of variance (ANOVA) was applied (SigmaPlot; Systat Software, Inc.). Outlier test: ROUT (Q = 1%). Pearson's correlation was used to evaluate the association between two variables by determining the r coefficient. The α level was set at 0.05. Considering the limited number of subjects joining the expedition, we also calculated Cohen's d effect size (*ES*) for ANOVAs as a supplementary analysis using the following equation: $\eta 2$ = Effect SQ/Total SQ; SQ = sum of squares. The $\eta 2$ values were converted into *d* values (Fritz et al. 2012). The ES values were classified as trivial ($ES < 0.20$), small (*ES* 0.20–0.59), medium (*ES* 0.60–1.20), or large ($ES \geq 1.20$) (Hopkins et al. 2009).

## Results

Camping in Antarctica changed SAA with a large effect size (ES) ($F = 3.566$; $p = 0.042$, ES = 1.6), resulting in higher values at Camp-Initial (Fig. 1A). Curiously, at the end of the camp, SAA returned to baseline levels and was not different from Pre-Camp values. Additionally, SAA correlated with cortisol (Fig. 2). There were no differences found for TGF-β ($F = 0.408$, $p = 0.750$; ES = 0.4), and FABP2 ($F = 0.878$; $p = 0.472$; ES = 0.7) (Figs. 1B and 1C).

## Discussion

The observed acute increase in SAA after four field days in ICE, followed by the reduction to baseline levels, indicates a transient acute inflammation that does not imply a chronic inflammation condition in the long term. This increase in SAA concentration may be associated with worsening sleep patterns in the field, as showed by a reduction in sleep efficiency in the first days of camp (Moraes et al. 2023), since sleep deprivation triggers SAA production in humans and mice (de Oliveira et al. 2017). Physical activities in the field may have contributed to the observed SAA response. It is worth noting that previous studies reported a reduction in SAA with physical exercise in different populations (Ogawa et al. 2020, Kolahdouzi et al. 2019); however, some studies did not find changes in SAA with physical exercise (Campbell et al 2009).

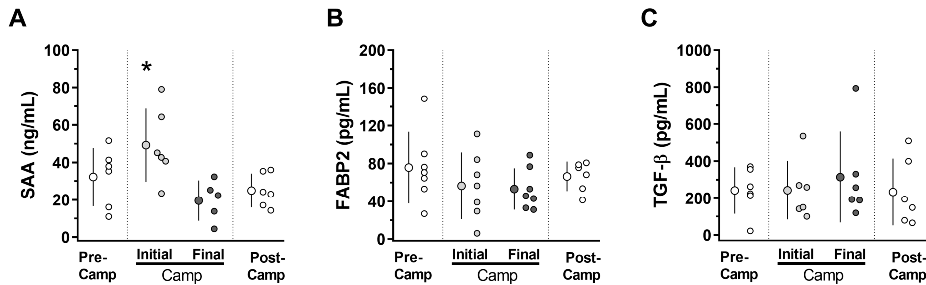


**Fig. 1.** Salivary cytokine and signal proteins at the Pre-Camp (*i.e.*, 2nd and 3rd days on the ship, before camp), at Camp [Initial (*i.e.*, 4th day in camp), and Final (*i.e.*, 45th day in camp) moments at camp] at the Post-Camp (*i.e.*, 4th day on the ship, after camp) during an Antarctic expedition. **A**) Serum amyloid A protein (SAA). **B**) Fatty acid-binding protein 2 (FABP2). **C**) Transforming growth factor-beta (TGF-β). For TGF-β and SAA, n=6, except SAA in the Camp-Final moments, n=5, due to the limited sample volume for one volunteer. For FABP2, n=7, except in the Post-Camp, n=6, due to outlier data. The data are expressed as mean ± standard deviation (SD). Each point represents an individual individual datum. *Difference $p < 0.05$ revealed by the ANOVA for SAA, being different from Camp-Final ($p$=0.047) and Post-Camp ($p$=0.048) and tending to difference from Pre-Camp ($p$=0.085), as revealed by the post-hoc of Student-Newman-Keuls.

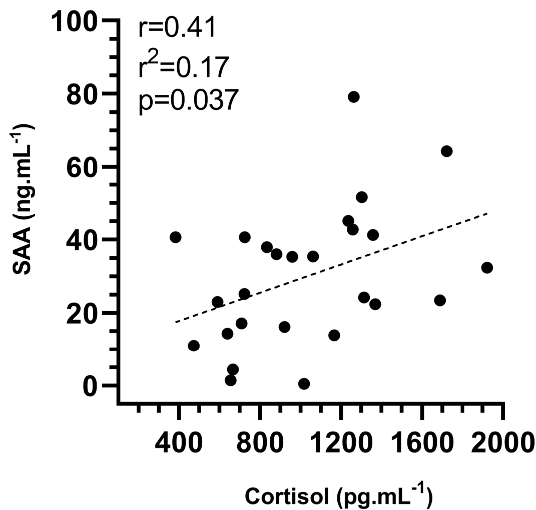


**Fig. 2.** Correlation between serum amyloid A protein (SAA) and cortisol. Each point represents an individual.

The present results expand the understanding of the inflammatory responses during the stay in the Antarctic ICE, showing the transience of the increase of SAA, which indicates a positive sign in the face of possible clinical consequences (Kisilevsky and Tam 2002). Considering the complex interrelationships between proinflammatory responses and the interaction between physiological stress and environmental factors, we suggest that more studies be carried out in camps with dif-

ferent groups to find the most robust biomarkers with a greater propensity for indications of inflammatory changes in ICE. In the face of the scarcity of data in extreme environments, we suggest adding basal levels of inflammatory biomarkers a few months before moving to and after the return from Antarctica in future evaluations. Additionally, investigating these biomarkers during long periods of stay in polar environments (such as 'overwinter,' *i.e.*, staying in research stations over a year) can contribute to understanding possible health issues and events presented during the stay in different ICE in Antarctica and beyond.

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**Data sharing**

The datasets generated during and/or analysed during the current study are available from the authors upon reasonable request.

**Geolocation information**

Nelson Island, South Shetland Islands (S 53.178533°/O 70.899750°).