OPINION ARTICLE

REVISED The inflammation paradox: Why are Tsimane protected against Western diseases while Westerners are not? [version 2; peer review: 2 approved, 1 approved with reservations]

Jens Freese[1], Rainer Johannes Klement[2], Helmut Lötzerich[1]

[1]Institute of Outdoor Sports and Environmental Science, German Sports University Cologne, Cologne, 50933, Germany
[2]Department of Radiotherapy and Radiation Oncology, Leopoldina Hospital Schweinfurt, Schweinfurt, 97422, Germany

v2 **First published:** 01 Mar 2018, **7**:252
https://doi.org/10.12688/f1000research.14052.1
Latest published: 06 Aug 2018, **7**:252
https://doi.org/10.12688/f1000research.14052.2

Abstract

We here describe two apparent paradoxes concerning high CRP levels and NCD risk. One has emerged from observational studies in the Amazon region showing that the indigenous Tsimane in Bolivia appear protected against non-communicable diseases (NCDs) such as obesity, type 2 diabetes, and cardiovascular diseases despite increased inflammatory markers. These findings stand in contrast to Western societies, where an increasing body of evidence demonstrates that low-grade-inflammation is the driver of NCDs. The second paradox has emerged from two field studies (Eifel studies) conducted in 2013 and 2014 with Westerners who returned to a simulated Palaeolithic lifestyle in a National park for 4 days. We had detected elevated inflammation markers, despite otherwise anti-inflammatory effects of these interventions as indicated by metabolic blood parameters. We here propose three hypotheses for this second inflammatory paradox.

Keywords

Tsimane, Low-Grade Inflammation, Eifel studies, Non-communicable diseases

Open Peer Review

Approval Status ✓ ? ✓

	1	2	3
version 2 (revision) 06 Aug 2018	✓		✓ view
version 1 01 Mar 2018	? view	? view	

1. **Michael D. Gurven**, University of California, Santa Barbara, Santa Barbara, USA
Caleb Finch, University of Southern California, Los Angeles, USA

2. **Colette Berbesque**, University of Roehampton, London, UK
Baptiste Sadoughi, University of Roehampton, London, UK

3. **J. Josh Snodgrass**, University of Oregon, Eugene, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Jens Freese (info@dr-freese.com)

Author roles: Freese J: Writing – Original Draft Preparation; **Klement RJ**: Writing – Review & Editing; **Lötzerich H**: Supervision

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Freese J, Klement RJ and Lötzerich H. **The inflammation paradox: Why are Tsimane protected against Western diseases while Westerners are not? [version 2; peer review: 2 approved, 1 approved with reservations]** F1000Research 2018, **7**:252 https://doi.org/10.12688/f1000research.14052.2

First published: 01 Mar 2018, **7**:252 https://doi.org/10.12688/f1000research.14052.1

REVISED **Amendments from Version 1**

In our revised manuscript, we strengthen the notion that Tsimane do not face the usual risk factors for NCDs that Westeners do. Moreover, we have been more careful with our formulations in the revised manuscript, refraining from making any causal claims.

We agree with the reviewers that our hypothesis concerning the forest bathing effect is currently only speculative until further measurements have been made. We have re-phrased the Outlook section accordingly. The same is true for our second hypothesis. Our future research is planned to more specifically address the forest bathing hypothesis.

In context of our third hypothesis, we have included the additional mechanism proposed by the reviewers in our discussion and re-wrote a large part of the third hypothesis. Our revision now also distinguishes more clearly between the chronic high CRP levels of the Tsimane and the acute elevations observed in the Eifel studies.

We agree that there are no data showing that chronic inflammation in Tsimans protects them against NCD (correlation is not causation). We have erased this claim. Rather, we agree that the best explantion is probably the absence of typical NCD risk factors. We also state in the revision that the data are not able to provide evidence for one of our hypotheses over any other, so that future studies should particularly put these hypotheses to test.

Finally, we agree that it is unclear if and when the elevated CRP levels found in the Eifel study participants would have decreased again. However, a similar study has found increased CRP levels after a 10-day trip through the wilderness. This is stated now in the last two sentences of the conclusions section.

See referee reports

Abbreviations

CRP, C-reactive protein; NCD, non-communicable diseases; NK cells, natural killer cells; LGI, low-grade inflammation; LPS, lipopolysaccharides; NFκB, nuclear-factor-kappa-B

Introduction

Recently, observational studies in the Amazon region showed that the indigenous Tsimane in Bolivia appear protected against non-communicable diseases (NCDs) such as obesity, type 2 diabetes, and cardiovascular diseases, despite increased inflammatory markers[1]. These findings stand in contrast to Western societies, where an increasing body of evidence demonstrates that low-grade-inflammation (LGI) is the driver of NCDs[2–4].

Report

Compared to US reference values, Tsimane exhibit markedly high levels of eosinophilic and neutrophilic granulocytes, B lymphocytes and natural killer cells. The leukocyte counts of Tsimans (8,600–12,000 cells/μL) are 1.5 times, and lymphocytes 1.2 to 1.6 times higher than in the US population (6,700–7,900 cells/μL)[5,6]. Eosinophilic granulocytes, primarily indicative of parasitic infections, are 7-fold elevated. Consequently, the immunoglobulin E values are also significantly higher (150–200-fold). Important biomarkers for inflammation, such as neutrophil granulocytes (1.2 to 1.6-fold), blood sedimentation (30 mm/h to 15–20 mm/h) and C-reactive protein (CRP) values (higher from infant to adolescence), are also upregulated[6]. The high CRP levels in Tsimane are in contrast to other indigenous people such as the Hadza hunter-gatherers in Tansania[7] or the Shuar hunter-horticulurists in Ecuador[8] who show no obvious signs of chronic low-grade inflammation. Thus, chronically high CRP levels in the Tsimane pose an apparent paradox, as high CRP levels due to LGI in Westernized societies correlate with NCD risk. The paradox could be resolved if one assumes that chronic systemic inflammation is a necessary, but not sufficient component in NCD development if certain other factors are simultaneously present. In particular, in the Tsimane the chronic state of infection is thought to stem from a high prevalence of intestinal worms which would i) decrease the absorption of macronutrient in the gut, ii) increase the amount of type 2 anti-inflammatory T helper cells and iii) raise basal metabolic rate, reducing the risk for obesity[9]. However, the major protective factor against NCD appears to be their lifestyle which prevents the development of typical NCD risk factors such as insulin resistance and adiposity.

In 2013 and 2014, we carried out two field studies (Eifel studies) with Westerners who returned to a simulated Palaeolithic lifestyle in a National park for 4 days[10,11]. Contrary to our expectations, in both studies, CRP, the main liver-derived biomarker that displays nonspecific inflammation, had increased by 170% and 67%, respectively. The essential components of these interventions consisted of (i) the conversion to a paleo diet; (ii) the high range of locomotion (15 km/day in the Eifel study 2013, 16.4 km/day in the Eifel study 2014); (iii) a fasting period from 12 to 14 hours per day in conjunction with a low meal frequency resulting in undercaloric energy intake (1567 kcal in the Eifel study 2013, 1747 kcal in the Eifel study 2014). All mentioned factors have been shown to have anti-inflammatory effects[12–16].

Discussion

There are two interesting observations concerning the indigenous Tsimane in Bolivia and Westeners who returned to a paleolithic lifestyle in the Eifel studies: The former have *chronically* elevated CRP levels despite being practically free of NCDs, while the latter were found to have *acutely* elevated CRP levels despite other physiological changes that would usually be interpreted as protective against NCDs. We provide the following hypothetical explanations for the stimulation of the immune system in the Eifel studies, which are likely to influence one another:

1. Phyto-antibiotics (phytoncides), which plants release into the atmosphere to protect themselves against bacteria and insects, could have stimulated the innate immune system[17]. As studies from Japan and Korea have shown, so-called “forest bathing” (a multi-day hike through a forest) promotes the formation of high levels of natural killer cells (NK cells). This effect persists for up to 30 days after the intervention[18,19]. In addition, forest bathing also increases the activity of the cytolytic proteins perforin, granzyme A and granulysin in NK cells. Walks in the city, on the other hand, do not change the NK cell population or its activity[19]. These effects could have contributed to the increase in CRP levels in the

Eifel studies, as most of the time participants spent in a forest area.

2. The radical change from a near-sterile to a natural environment may have prompted the innate immune system to anticipate and prophylactically protect the organism against pathogens such as bacteria, parasites, fungi, and other microorganisms. Danger signals, called exogenous pathogen associated molecular patterns and endogenous danger associated molecular patterns, activate the innate immune system via Toll-like receptors, which can trigger a rapid antibacterial inflammatory response. This mechanism of action may have led to the development of an acute phase response. In contrast to LGI, substances such as lipoxins, resolvins and protectins are formed in acute inflammation in order to end the inflammatory process[20,21]. Since no follow-up measurements were made in the Eifel studies, this hypothesis is currently only speculative.

3. Despite the fact that the participants in the Eifel studies were in good mental and physical health, the level of physical stress due to the high workload combined with calorie restriction conditions could have induced a moderate acute phase response. Indeed, acute and transient elevations of CRP levels, inflammatory cytokines (in particular IL-6) and shifts in leucocyte patterns typically occur after strenuous exercise[22–24]. The latter include elevations in neutrophils and decreases in eopsinophils and lymphocytes, changes that have also been observed in our Eifel study participants[10,11]. Therefore, this hypothesis appears to be the best confirmed by the data. The acute increase in CRP levels could thereby be caused by several mechanisms including increased catecholamine and cortisol levels[23] or endotoxemia through bacterial lipopolysaccharides (LPS) caused by increased cell destruction and gut leakage[25]. LPS activate the innate immune system via Toll-like receptors and stimulate the activation of nuclear-factor-kappa-B (NFκB) intracellularly, leading to pro-inflammatory cytokine secretion[26]. A by-product of cell destruction is uric acid, which stimulates the release of CRP in the liver as part of the acute immune response[27,28]. In turn, CRP stimulates the production of antibodies from B lymphocytes to kill pathogens[29]. Due to the high range of locomotion in both Eifel studies, uric acid might have played a prominent role in stimulating the immune system. Since uric acid has not been measured, future studies should include this marker to provide a possible confirmation of this hypothesis.

Outlook

The fact that a chronic inflammatory situation in Tsimans persists despite practical non-existence of NCDs, while it is associated with NCDs in Westerners, appears paradoxical. A second apparent paradox concerns the acutely elevated CRP levels in Westeners returning to a Paleolithic lifestyle despite otherwise beneficial metabolic effects usually associated with a decrease in LGI. We have discussed three different hypotheses to solve this second paradox. While the "forest-bathing" and "danger anticipation" hypotheses are currently only speculative due to the lack of relevant measurements, a mild-to-moderate acute-phase response due to the strenuous physical activity is consistent with the available data. However, the data cannot clearly constitute evidence for one of these hypotheses against any other, in part because their likelihood under the "forest-bathing" and "danger anticipation" hypotheses remains to be determined. Finally it remains to be examined if, when and how the acute elevations in CRP levels would resolve if the participants would remain in Paleolithic living conditions. Interestingly, a study similar to the Eifel studies involving a 10-day trip through the Pyrenees also reported a significant mild increase in CRP levels after the trip, showing that the acute phase repsonse could be sustained at least up to 10 days[30,31].

Data availability

No data are associated with this article.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

1. Kaplan H, Thompson RC, Trumble BC, *et al.*: **Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study.** *Lancet.* 2017; **389**(10080): 1730–1739.
PubMed Abstract | Publisher Full Text | Free Full Text

2. Egger G: **Obesity, chronic disease, and economic growth: a case for "big picture" prevention.** *Adv Prev Med.* 2011; **2011**: 149158.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Ruiz-Núñez B, Pruimboom L, Dijck-Brouwer J, *et al.*: **Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context.** *J Nutr Biochem.* 2013; **24**(7): 1183–201.
PubMed Abstract | Publisher Full Text

4. Rodríguez-Hernández H, Simental-Mendía LE, Rodríguez-Ramírez G, *et al.*: **Obesity and inflammation: epidemiology, risk factors, and markers of inflammation.** *Int J Endocrinol.* 2013; **2013**: 678159.
PubMed Abstract | Publisher Full Text | Free Full Text

5. National Health and Nutrition Examination Survey (n.d.): **National health and nutrition examination survey**.
Reference Source

6. Blackwell AD, Trumble BC, Maldonado Suarez I, *et al.*: **Immune function in Amazonian horticulturalists.** *Ann Hum Biol.* 2016; **43**(4): 382–96.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Raichlen DA, Pontzer H, Harris JA, *et al.*: **Physical activity patterns and biomarkers of cardiovascular disease risk in hunter-gatherers.** *Am J Hum Biol.*

2017; **29**(2): e22919.
PubMed Abstract | Publisher Full Text

8. McDade TW, Tallman PS, Madimenos FC, *et al.*: **Analysis of variability of high sensitivity C-reactive protein in lowland Ecuador reveals no evidence of chronic low-grade inflammation.** *Am J Hum Biol.* 2012; **24**(5): 675–81.
PubMed Abstract | Publisher Full Text

9. Gurven MD, Trumble BC, Stieglitz J, *et al.*: **High resting metabolic rate among Amazonian forager-horticulturalists experiencing high pathogen burden.** *Am J Phys Anthropol.* 2016; **161**(13): 414–425.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Freese J, Ruiz-Núñez B, Heynck R, *et al.*: **To restore health, "do we have to go back to the future?" The impact of a 4-day paleolithic lifestyle change on human metabolism – a pilot study.** *J Evol Health.* 2016a; **1**(1): 12.
Publisher Full Text

11. Freese J, Pardi DJ, Ruiz-Nunez B, *et al.*: **Back to the future. Metabolic effects of a 4-day outdoor trip under simulated paleolithic conditions - new insights from the Eifel study.** *J Evol Health.* 2016b; **1**(1): 16.
Publisher Full Text

12. Inoue H, Sakai M, Kaida Y, *et al.*: **Blood lactoferrin release induced by running exercise in normal volunteers: antibacterial activity.** *Clin Chim Acta.* 2004; **341**(1–2): 165–72.
PubMed Abstract | Publisher Full Text

13. Konner M, Eaton SB: **Paleolithic nutrition: twenty-five years later.** *Nutr Clin Pract.* 2010; **25**(6): 594–602.
PubMed Abstract | Publisher Full Text

14. Lemke D, Klement RJ, Paul S, *et al.*: **The Paleolithic Diet and its Significance for the Prevention and Treatment of Chronic Diseases.** *Aktuel Ernahrungsmed.* 2016; **41**(06): 437–449.
Publisher Full Text

15. Longo VD, Mattson MP: **Fasting: molecular mechanisms and clinical applications.** *Cell Metab.* 2014; **19**(2): 181–92.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Weinhold M, Shimabukuro-Vornhagen A, Franke A, *et al.*: **Physical exercise modulates the homeostasis of human regulatory T cells.** *J Allergy Clin Immunol.* 2016; **137**(5): 1607–1610.e8.
PubMed Abstract | Publisher Full Text

17. Li Q, Nakadai A, Matsushima H, *et al.*: **Phytoncides (wood essential oils) induce human natural killer cell activity.** *Immunopharmacol Immunotoxicol.* 2006; **28**(2): 319–33.
PubMed Abstract | Publisher Full Text

18. Park BJ, Tsunetsugu Y, Kasetani T, *et al.*: **The physiological effects of *Shinrin-yoku* (taking in the forest atmosphere or forest bathing): evidence from field experiments in 24 forests across japan.** *Environ Health Prev Med.* 2010; **15**(1): 18–26.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Li Q: **Effect of forest bathing trips on human immune function.** *Environ Health Prev Med.* 2010; **15**(1): 9–17.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Serhan CN, Savill J: **Resolution of inflammation: the beginning programs the end.** *Nat Immunol.* 2005; **6**(12): 1191–7.
PubMed Abstract | Publisher Full Text

21. Serhan CN: **Novel lipid mediators and resolution mechanisms in acute inflammation: To resolve or not?** *Am J Pathol.* 2010; **177**(4): 1576–91.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Hoffman-Goetz L, Pedersen BK: **Exercise and the immune system: a model of the stress response?** *Immunol Today.* 1994; **15**(8): 382–7.
PubMed Abstract | Publisher Full Text

23. Gabriel H, Kindermann W: **The acute immune response to exercise: what does it mean?** *Int J Sports Med.* 1997; **18 Suppl 1**: S28–45.
PubMed Abstract | Publisher Full Text

24. Kasapis C, Thompson PD: **The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review.** *J Am Coll Cardiol.* 2005; **45**(10): 1563–9.
PubMed Abstract | Publisher Full Text

25. Bosenberg AT, Brock-Utne JG, Gaffin SL, *et al.*: **Strenuous exercise causes systemic endotoxemia.** *J Appl Physiol (1985).* 1988; **65**(1): 106–8.
PubMed Abstract | Publisher Full Text

26. Sun Y, Shang D: **Inhibitory Effects of Antimicrobial Peptides on Lipopolysaccharide-Induced Inflammation.** *Mediators Inflamm.* 2015; **2015**: 167572.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Kanellis J, Watanabe S, Li JH, *et al.*: **Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2.** *Hypertension.* 2003; **41**(6): 1287–93.
PubMed Abstract | Publisher Full Text

28. Chen CJ, Kono H, Golenbock D, *et al.*: **Identification of a key pathway required for the sterile inflammatory response triggered by dying cells.** *Nat Med.* 2007; **13**(7): 851–856.
PubMed Abstract | Publisher Full Text

29. Behrens MD, Wagner WM, Krco CJ, *et al.*: **The endogenous danger signal, crystalline uric acid, signals for enhanced antibody immunity.** *Blood.* 2008; **111**(3): 1472–1479.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Pruimboom L, Ruiz-Núñez B, Raison CL, *et al.*: **Influence of a 10-Day Mimic of Our Ancient Lifestyle on Anthropometrics and Parameters of Metabolism and Inflammation: The "Study of Origin".** *Biomed Res Int.* 2016; **2016**: 6935123.
PubMed Abstract | Publisher Full Text | Free Full Text

31. Pruimboom L, Ruiz-Núñez B, Raison CL, *et al.*: **Corrigendum to "Influence of a 10-Day Mimic of Our Ancient Lifestyle on Anthropometrics and Parameters of Metabolism and Inflammation: The "study of Origin"".** *Biomed Res Int.* 2017; **2017**: 1641589.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 2

Reviewer Report 06 August 2019

https://doi.org/10.5256/f1000research.17155.r50908

✔ **J. Josh Snodgrass**
Department of Anthropology, University of Oregon, Eugene, OR, USA

I have the luxury of reading this article after revisions based on two thorough reviews, both of which raise important points and make excellent suggestions for improvement. The new version of the article has been substantively revised in response to these reviews. The present version of the article, while highly speculative, does raise some interesting and important issues and starts a conversation that will generate additional testing and data collection.

Several points are worth discussing in more detail. I appreciate that the authors are much more clear in the revised article that chronic inflammation in the Tsimane is not protective against NCDs and instead that it is a result of the absence of certain NCD risk factors that are common in other populations. My research group has published Shuar CRP data cited in the article as well as comparative immune studies of Tsimane and Shuar. A key point that's mentioned in the present article yet not elaborated on here or really elsewhere is the divergent CRP values in Shuar and Tsimane despite both being subsistence-based populations in the Amazon. It seems likely that differences in disease ecology between the two groups, especially related to helminths, influences inflammatory profile and other immune differences. Although baseline CRP is low among Shuar, they do experience acute CRP elevations in response to various challenges. It would be useful to look at helminth species (extent of helminth coinfection) and intensity in each of the populations coupled with immune work to see the extent to which chronically elevated inflammation is reflective of repeated or constant helminth infections. Clearly more work needs to be done to identify underlying contributors to inflammation in these types of populations, and their contribution to disease risk. I appreciate that this article drives that conversation forward.

The Eifel Studies, while interesting and potentially useful for unraveling contributions to inflammation, are very brief in duration and with very small sample sizes. The studies need to be of longer duration to fully understand the time-course of the various biomarkers (it's impossible to interpret changes in a small sample in only 4 days), as well as need to include measurement of more variables of interest. I think I would feel more comfortable if the authors made it more clear that these studies are very preliminary. And of course then make plans for additional research!
As for the proposed explanations, while highly speculative, they do advance the conversation and

potentially lead to testable hypotheses that can be examined using new data collected in studies like those of Eifel as well as in populations like the Tsimane and Shuar.

Is the topic of the opinion article discussed accurately in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

Are arguments sufficiently supported by evidence from the published literature?
Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: human biology; global health; biomarkers; immunology; cardiovascular disease

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 23 October 2018

https://doi.org/10.5256/f1000research.17155.r36833

Michael D. Gurven
Department of Anthropology, University of California, Santa Barbara, Santa Barbara, CA, USA

Is the topic of the opinion article discussed accurately in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

Are arguments sufficiently supported by evidence from the published literature?
Yes

Are the conclusions drawn balanced and justified on the basis of the presented arguments?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 06 April 2018

https://doi.org/10.5256/f1000research.15283.r31345

?

Colette Berbesque
Centre for Research in Evolutionary, Social and Inter-Disciplinary Anthropology, University of Roehampton, London, UK
Baptiste Sadoughi
Centre for Research in Evolutionary, Social and Inter-Disciplinary Anthropology, University of Roehampton, London, UK

The authors statement is organised around two discoveries: the highly inflammatory immune profile in Tsimane associated with low prevalence of NCDs, and the increased inflammation in westerners whom participated in a 'Paleo-trek'[1]. From these observations, FKL reach the conclusion that chronic inflammation in the Tsimane protects against NCDs and propose explanations for why this might be the case. We will discuss this assumption in the following sections.

First, we disagree with the assumption that Tsimane people stand as an adequate model for the study of the relationship between Low-grade inflammation (LGI) and non-communicable diseases (NCDs). LGI, associated with coronary diseases and other NCDs in westerners, is usually not triggered by specific stimuli such as infection or injury[2], but strongly associated with other risk factors such as stressful situations[3] and excess of adipose tissue[4]. Tismanes are less mobile than most hunter-gatherers, such as the Hadza - who demonstrate low CPR[5]. This increased sedentisation may be part of the reason they face a heavy parasitic load and bacterial infections[6] likely responsible for their hightened inflammatory response[7], along with low levels of cholesterol, LDL and HDL. It is also noteworthy that the major comparative study on Tsimane immune function failed to show that after childhood there was any significant increase in CRP, a major low-grade inflammatory marker[7]. The hypothesis that the parasitic burden triggering Th2 response may enhance anti-inflammatory cytokine levels and thus protect against NCDs is interesting. However, the absence of major NCDs risk factors, such as chronic stress, insulin-

resistance and elevated fatty acid levels, brings into question the underlying assumption that the Tsimane may face common NCDs.

Finally, we strongly reject the authors' conclusion that inflammation may protect against NCDs in Tsimane. Indeed, CRP levels were found to be a positive predictor of non-null coronary artery calcium scores[8], suggesting CRP might actually be predictive of atherosclerosis, a major NCD, in Tsimane as well.

According to the authors, the inflammatory state of the Tsimane could be partly explained by environmental factors also responsible for the pro-inflammatory profile observed in the participants of the Eifel experiment. We will review and discuss two of the hypotheses proposed by FKL to explain this result.

First, a 'forest bathing' effect would have enhanced innate immune response and therefore result in an increase in CRP levels. Although we do not wish to undermine the potential role of the 'forest bathing' effect, to the best of our knowledge no evidence so far suggests that it could have an impact on CRP levels. Most studies focus on NK cells, cortisol levels and bio-markers of endothelial function. As CRP elevation in the Eifel participants failed to reach significance and NK cells were not measured, the 'forest bathing' effect remains speculative.

Second, as suggested by the authors, exposure to exogenous pathogen associated-molecules could have caused a shift towards Th1 response in Eifel participants. However, there is no evidence to support the assumption that this shift was 'prophylactic' as participants may have well indeed face acute infection from small wounds or respiratory pathogens in a forest environment. In order to strengthen their theoretical argument, the authors could provide references regarding the resolution of chronic inflammation following an episode of acute inflammation.

Finally, we would like to suggest another potential origin for the pro-inflammatory immune profile in the Eifel participants. It seems participants exhibit the typical leukocyte count induced by stress and exercise[9]: neutrophilia, lymphopenia and eosinopenia. Such leukocyte pattern is supposedly the result of cortisol-induced cell-migration into peripheral tissue[10] and does not fit with Tsimane leukocyte pattern[7]. Furthermore, exercise-induced response has been shown to gradually decrease in trained athletes. Long-term training is eventually followed by the release of anti-inflammatory cytokines[11,12]. Such difference between initial-shock immune response to intense exercise, as undertaken by untrained Eifel participants, and long-term immune adaptation to physical activity brings into question the reliability of the leukogram patterns measured on the fourth day of trek in the Eifel study. A shift towards a more anti-inflammatory pattern could therefore be expected in the Eifel participants after acclimatisation to this new lifestyle, regardless of environmental factors. It is therefore highly speculative that the Eifel participants' immune profile would be a good model for the immune pattern of a Paleo-population.

References

1. Freese J, Pardi D, Ruiz-Núñez B, Schwarz S, et al.: Back to the Future. Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions – New Insights from The Eifel Study. *Journal of Evolution and Health*. 2016; **1** (1). Publisher Full Text
2. Rohleder N: Stimulation of systemic low-grade inflammation by psychosocial stress.*Psychosom Med*. 2014; **76** (3): 181-9 PubMed Abstract | Publisher Full Text
3. Hänsel A, Hong S, Cámara RJ, von Känel R: Inflammation as a psychophysiological biomarker in

chronic psychosocial stress.*Neurosci Biobehav Rev*. 2010; **35** (1): 115-21 PubMed Abstract | Publisher Full Text
4. Festa A, D'Agostino R, Williams K, Karter AJ, et al.: The relation of body fat mass and distribution to markers of chronic inflammation.*Int J Obes Relat Metab Disord*. 2001; **25** (10): 1407-15 PubMed Abstract | Publisher Full Text
5. Raichlen DA, Pontzer H, Harris JA, Mabulla AZ, et al.: Physical activity patterns and biomarkers of cardiovascular disease risk in hunter-gatherers.*Am J Hum Biol*. 2017; **29** (2). PubMed Abstract | Publisher Full Text
6. Page AE, Viguier S, Dyble M, Smith D, et al.: Reproductive trade-offs in extant hunter-gatherers suggest adaptive mechanism for the Neolithic expansion.*Proc Natl Acad Sci U S A*. 2016; **113** (17): 4694-9 PubMed Abstract | Publisher Full Text
7. Blackwell AD, Trumble BC, Maldonado Suarez I, Stieglitz J, et al.: Immune function in Amazonian horticulturalists.*Ann Hum Biol*. 2016; **43** (4): 382-96 PubMed Abstract | Publisher Full Text
8. Kaplan H, Thompson R, Trumble B, Wann L, et al.: Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study. *The Lancet*. 2017; **389** (10080): 1730-1739 Publisher Full Text
9. Suzuki K, Yamada M, Kurakake S, Okamura N, et al.: Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. *Eur J Appl Physiol*. 2000; **81** (4): 281-7 PubMed Abstract | Publisher Full Text
10. Davis A, Maney D, Maerz J: The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*. 2008; **22** (5): 760-772 Publisher Full Text
11. Suzuki K, Totsuka M, Nakaji S, Yamada M, et al.: Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage.*J Appl Physiol (1985)*. 1999; **87** (4): 1360-7 PubMed Abstract | Publisher Full Text
12. Weinhold M, Shimabukuro-Vornhagen A, Franke A, Theurich S, et al.: Physical exercise modulates the homeostasis of human regulatory T cells.*J Allergy Clin Immunol*. 2016; **137** (5): 1607-1610.e8 PubMed Abstract | Publisher Full Text

Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Yes

Are arguments sufficiently supported by evidence from the published literature?

Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

No

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 26 Jul 2018

Jens Freese, Deutsche Sporthochschule Köln, Germany

The authors statement is organised around two discoveries: the highly inflammatory immune profile in Tsimane associated with low prevalence of NCDs, and the increased inflammation in westerners whom participated in a 'Paleo-trek'[1]. From these observations, FKL reach the conclusion that chronic inflammation in the Tsimane protects against NCDs and propose explanations for why this might be the case. We will discuss this assumption in the following sections.

First, we disagree with the assumption that Tsimane people stand as an adequate model for the study of the relationship between Low-grade inflammation (LGI) and non-communicable diseases (NCDs). LGI, associated with coronary diseases and other NCDs in westerners, is usually not triggered by specific stimuli such as infection or injury[2], but strongly associated with other risk factors such as stressful situations[3] and excess of adipose tissue[4]. Tismanes are less mobile than most hunter-gatherers, such as the Hadza - who demonstrate low CPR[5]. This increased sedentisation may be part of the reason they face a heavy parasitic load and bacterial infections[6] likely responsible for their hightened inflammatory response[7], along with low levels of cholesterol, LDL and HDL. It is also noteworthy that the major comparative study on Tsimane immune function failed to show that after childhood there was any significant increase in CRP, a major low-grade inflammatory marker[7]. The hypothesis that the parasitic burden triggering Th2 response may enhance anti-inflammatory cytokine levels and thus protect against NCDs is interesting. However, the absence of major NCDs risk factors, such as chronic stress, insulin-resistance and elevated fatty acid levels, brings into question the underlying assumption that the Tsimane may face common NCDs.

Answer: We apologize if we seemed to have suggested the Tsimane as an adequate model for studying the connection between LGI and NCD. This was not our aim. Rather, our aim was to point out the Tsimane as an interesting case of indigenous people who are apparently free from the NCD that plague the Western societies despite having chronically elevated CRP levels. This should make clear that they are not an adequate model, but rather an exceptional case worth further studying. In our revised manuscript, we strengthen the notion that Tsimane do not face the usual risk factors for NCDs that Westeners do, agreeing with your argument above. This is probably the best explanantion for their low incidence of NCDs.

Finally, we strongly reject the authors' conclusion that inflammation may protect against NCDs in Tsimane. Indeed, CRP levels were found to be a positive predictor of non-null coronary artery calcium scores[8], suggesting CRP might actually be predictive of atherosclerosis, a major NCD, in Tsimane as well.

Answer: We are sorry for making causal claims where the data do not clearly support any. We have been more careful with our formulations in the revised manuscript, refraining from making any causal claims.

According to the authors, the inflammatory state of the Tsimane could be partly explained

by environmental factors also responsible for the pro-inflammatory profile observed in the participants of the Eifel experiment. We will review and discuss two of the hypotheses proposed by FKL to explain this result.

First, a 'forest bathing' effect would have enhanced innate immune response and therefore result in an increase in CRP levels. Although we do not wish to undermine the potential role of the 'forest bathing' effect, to the best of our knowledge no evidence so far suggests that it could have an impact on CRP levels. Most studies focus on NK cells, cortisol levels and bio-markers of endothelial function. As CRP elevation in the Eifel participants failed to reach significance and NK cells were not measured, the 'forest bathing' effect remains speculative.

Answer: We agree that this hypothesis is currently only speculative until further measurements have been made which are able to distinguish it from other hypotheses such as the acute phase response due to strenuous physical activity. We have re-phrased the Outlook section accordingly. The same is true for our second hypothesis. Nevertheless, we consider them possible explanations that should be considered in data interpretation. Our future research is planned to more specifically address the forest bathing hypothesis.

Second, as suggested by the authors, exposure to exogenous pathogen associated-molecules could have caused a shift towards Th1 response in Eifel participants. However, there is no evidence to support the assumption that this shift was 'prophylactic' as participants may have well indeed face acute infection from small wounds or respiratory pathogens in a forest environment. In order to strengthen their theoretical argument, the authors could provide references regarding the resolution of chronic inflammation following an episode of acute inflammation.

Answer: We had already noted that this hypothesis is currently only speculative. However, since we are mainly interested in explaining the acute rise of CRP levels, we have deleted any hypothesizing that this acute phase response could have led to the resolution of chronic LGI. An example for the resolution of chronic inflammation after acute stimulation of the immune system (although not neccessarily translatable to all NCDs) is the spontaneous remission of cancer after an acute episode of fever that is now more or less officially acknowledged (e.g. Jessy T (2011): Immunity over inability: The spontaneous regression of cancer. J Nat Sci Biol Med; 2(1): 43–49). Other examples from medical practice include treatment of chronic tendonitis through re-injury or using low-dose ionizing radiation to treat chronic inflammatory conditions.

Finally, we would like to suggest another potential origin for the pro-inflammatory immune profile in the Eifel participants. It seems participants exhibit the typical leukocyte count induced by stress and exercise[9]: neutrophilia, lymphopenia and eosinopenia. Such leukocyte pattern is supposedly the result of cortisol-induced cell-migration into peripheral tissue[10] and does not fit with Tsimane leukocyte pattern[7]. Furthermore, exercise-induced response has been shown to gradually decrease in trained athletes. Long-term training is eventually followed by the release of anti-inflammatory cytokines[11,12]. Such difference between initial-shock immune response to intense exercise, as undertaken by untrained Eifel participants, and long-term immune adaptation to physical activity brings into question

the reliability of the leukogram patterns measured on the fourth day of trek in the Eifel study. A shift towards a more anti-inflammatory pattern could therefore be expected in the Eifel participants after acclimatisation to this new lifestyle, regardless of environmental factors. It is therefore highly speculative that the Eifel participants' immune profile would be a good model for the immune pattern of a Paleo-population.

Answer: Thank you for these thoughts which go along the lines of our third hypothesis, namely that the sudden physical activity increase would be responsible for the acute CRP increase. We have included this additional mechanism in our discussion and re-wrote a large part of the third hypothesis. We think that the revision now also distinguishes more clearly between the chronic high CRP levels of the Tsimane and the acute elevations observed in the Eifel studies. In no way did we intent to propose that the immune profile of the latter is a good model for the former.

Competing Interests: No competing interests were disclosed.

Reviewer Report 12 March 2018

https://doi.org/10.5256/f1000research.15283.r31343

?

Michael D. Gurven
Department of Anthropology, University of California, Santa Barbara, Santa Barbara, CA, USA
Caleb Finch
Leonard Davis School of Gerontology & Dornsife College, University of Southern California, Los Angeles, CA, USA

We appreciate that Freese and colleagues [FKL] attempt to understand what they refer to as the "inflammation paradox", i.e. that people may experience high chronic levels of circulating inflammation (as measured by biomarkers such as high sensitivity C-reactive protein (CRP)), yet not succumb to the diseases such as heart disease and diabetes usually attributed to such high exposure in urban settings. We first documented this pattern among Tsimane Amerindians in Bolivia in 2009 when we found no cases of peripheral arterial disease among older adults despite high average levels of CRP, and no relationship between CRP and the continuous ankle-brachial index used to assess peripheral arterial disease risk[1]. More recently, we reported minimal coronary artery calcification that progressed 25 years more slowly than in the US,, and again, without any relationship with CRP[2].

FKL assert that the typically high inflammation the Tsimane experience may be protective against

non-communicable diseases (NCDs), and that their healthy hearts may not be due simply to their having protective diets, high moderate-to-vigorous activity levels and other lifestyle factors. We interpret their position that high inflammation is protective based on their discussion of field Eifel studies which showed CRP elevations after an attempt to mimic a more traditional "Paleo" lifestyle on a 4 day forest trek with low-carb diet, caloric restriction and semi-fasting. They propose three reasons for why CRP might be elevated under these (and presumably Tsimane conditions), but yet have no relationship with NCDs and their inflammation-related risk factors like smoking and obesity: 1) forest bathing may induce innate immune activity from plants releasing phyto-antibiotics into the atmosphere; 2) trekking in the "natural environment" (but not the city) may induce anticipatory immune defenses; and 3) caloric restriction combined with vigorous activity may have led to "cell depletion" and increased innate immune activity, including CRP, via uric acid byproducts.

First, we disagree with their conclusion that a "chronic inflammatory situation in Tsimans [sic] protects against NCD". This has never been shown. Rather, we have just shown that Tsimane do not appear to suffer from NCDs like heart disease and diabetes, despite their elevated systemic inflammation. Elsewhere, we have proposed that high inflammation may not lead to increased heart disease risk under certain conditions that may be particular for traditional subsistence populations like the Tsimane[3,4]. High systemic inflammation might not lead to greater NCD risk when combined with: 1) low LDL, a physically demanding lifestyle and minimal obesity; 2) chronic helminthic infections that: a) modulate immune function toward more anti-inflammatory Th2 activity that helps prevents systemic inflammation from damaging arteries, b) increase basal metabolic rate and lower obesity risk; c) reduce blood lipids such as LDL cholesterol and triglycerides, and blood glucose. Also notable is their limited exposure to tobacco smoke.

Second, while the Eifel study results suggest that adopting a more "Paleo"-like lifestyle can improve cardiometabolic biomarkers, we disagree that they show that higher inflammation (or innate immune activity in general) typifies hunter-gatherers or other subsistence populations. While CRP increased by 67% after the four day forest trek of healthy adults, this increase was not statistically significant (see Table 4). Thus, given the small sample size (n=25), these speculations have limited basis and are premature. Moreover, we see other issues with generalizing from the Eifel study. Half of the study participants did not engage in regular physical activity (52% <3 hrs/week). Heavy physical activity could lead to acute increases in inflammation, whereas long-term effects of regular exercise are usually associated with reduced chronic low grade inflammation. It is also possible that during the experiment, some participants incurred minor injuries or acute infections, both of which could result in higher CRP. Psychosocial stress, as might occur under the Eifel study conditions of food restriction for example, can also increase inflammation, especially IL-6, IL-1B and CRP[5].

While Tsimane show evidence of chronic, elevated CRP across much of the life course[6,7], it is yet to be determined how representative this pattern is among small-scale subsistence populations. Shuar forager-horticulturalists of Ecuador show elevated CRP only when infected, but otherwise have low baseline CRP levels[8]. A small sample of Hadza hunter-gatherers also showed reasonably low CRP levels (74% of 23 adults had CRP < 3 mg/L)[9].

Anticipatory stimulation of the innate immune system in the forest is an interesting idea; even visual exposure to photographs with disease cues (e.g. sneezing) can increase IL-6 by 24% compared to controls shown neutral stimuli[10]. However, while short-term changes in other

biomarkers like blood lipids and blood glucose may be generalizable from the Eifel study to a long-term study, it is unclear whether any acute innate immune activity observed over a 4 day period would sustain in a longer-term study after habituation. As FKL argue, there are many other reasons CRP can be elevated that have little to do with inflammation, but might still be related to tissue stress or injury[11].

References

1. Gurven M, Kaplan H, Winking J, Eid Rodriguez D, et al.: Inflammation and infection do not promote arterial aging and cardiovascular disease risk factors among lean horticulturalists.*PLoS One*. 2009; **4** (8): e6590 PubMed Abstract | Publisher Full Text
2. Kaplan H, Thompson R, Trumble B, Wann L, et al.: Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study. *The Lancet*. 2017; **389** (10080): 1730-1739 Publisher Full Text
3. Gurven MD, Trumble BC, Stieglitz J, Blackwell AD, et al.: Cardiovascular disease and type 2 diabetes in evolutionary perspective: a critical role for helminths?. *Evol Med Public Health*. 2016. PubMed Abstract | Publisher Full Text
4. Vasunilashorn S, Crimmins EM, Kim JK, Winking J, et al.: Blood lipids, infection, and inflammatory markers in the Tsimane of Bolivia.*Am J Hum Biol*. **22** (6): 731-40 PubMed Abstract | Publisher Full Text
5. Steptoe A, Hamer M, Chida Y: The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis.*Brain Behav Immun*. 2007; **21** (7): 901-12 PubMed Abstract | Publisher Full Text
6. Gurven M, Kaplan H, Winking J, Finch C, et al.: Aging and inflammation in two epidemiological worlds.*J Gerontol A Biol Sci Med Sci*. 2008; **63** (2): 196-9 PubMed Abstract
7. Blackwell AD, Trumble BC, Maldonado Suarez I, Stieglitz J, et al.: Immune function in Amazonian horticulturalists.*Ann Hum Biol*. 2016; **43** (4): 382-96 PubMed Abstract | Publisher Full Text
8. McDade TW, Tallman PS, Madimenos FC, Liebert MA, et al.: Analysis of variability of high sensitivity C-reactive protein in lowland Ecuador reveals no evidence of chronic low-grade inflammation.*Am J Hum Biol*. **24** (5): 675-81 PubMed Abstract | Publisher Full Text
9. Raichlen DA, Pontzer H, Harris JA, Mabulla AZ, et al.: Physical activity patterns and biomarkers of cardiovascular disease risk in hunter-gatherers.*Am J Hum Biol*. 2017; **29** (2). PubMed Abstract | Publisher Full Text
10. Schaller M, Miller GE, Gervais WM, Yager S, et al.: Mere visual perception of other people's disease symptoms facilitates a more aggressive immune response.*Psychol Sci*. 2010; **21** (5): 649-52 PubMed Abstract | Publisher Full Text
11. Kushner I, Rzewnicki D, Samols D: What does minor elevation of C-reactive protein signify?. *Am J Med*. 2006; **119** (2): 166.e17-28 PubMed Abstract | Publisher Full Text

Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Yes

Are arguments sufficiently supported by evidence from the published literature?

Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?
No

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 26 Jul 2018

Jens Freese, Deutsche Sporthochschule Köln, Germany

We appreciate that Freese and colleagues [FKL] attempt to understand what they refer to as the "inflammation paradox", i.e. that people may experience high chronic levels of circulating inflammation (as measured by biomarkers such as high sensitivity C-reactive protein (CRP)), yet not succumb to the diseases such as heart disease and diabetes usually attributed to such high exposure in urban settings. We first documented this pattern among Tsimane Amerindians in Bolivia in 2009 when we found no cases of peripheral arterial disease among older adults despite high average levels of CRP, and no relationship between CRP and the continuous ankle-brachial index used to assess peripheral arterial disease risk[1]. More recently, we reported minimal coronary artery calcification that progressed 25 years more slowly than in the US,, and again, without any relationship with CRP[2].

FKL assert that the typically high inflammation the Tsimane experience may be protective against non-communicable diseases (NCDs), and that their healthy hearts may not be due simply to their having protective diets, high moderate-to-vigorous activity levels and other lifestyle factors. We interpret their position that high inflammation is protective based on their discussion of field Eifel studies which showed CRP elevations after an attempt to mimic a more traditional "Paleo" lifestyle on a 4 day forest trek with low-carb diet, caloric restriction and semi-fasting. They propose three reasons for why CRP might be elevated under these (and presumably Tsimane conditions), but yet have no relationship with NCDs and their inflammation-related risk factors like smoking and obesity: 1) forest bathing may induce innate immune activity from plants releasing phyto-antibiotics into the atmosphere; 2) trekking in the "natural environment" (but not the city) may induce anticipatory immune defenses; and 3) caloric restriction combined with vigorous activity may have led to "cell depletion" and increased innate immune activity, including CRP, via uric acid byproducts.

First, we disagree with their conclusion that a "chronic inflammatory situation in Tsimans [sic] protects against NCD". This has never been shown. Rather, we have just shown that Tsimane do not appear to suffer from NCDs like heart disease and diabetes, despite their elevated systemic inflammation. Elsewhere, we have proposed that high inflammation may not lead to increased heart disease risk under certain conditions that may be particular for traditional subsistence populations like the Tsimane[3,4]. High systemic inflammation might not lead to greater NCD risk when combined with: 1) low LDL, a physically demanding lifestyle and minimal obesity; 2) chronic helminthic infections that: a) modulate immune

function toward more anti-inflammatory Th2 activity that helps prevents systemic inflammation from damaging arteries, b) increase basal metabolic rate and lower obesity risk; c) reduce blood lipids such as LDL cholesterol and triglycerides, and blood glucose. Also notable is their limited exposure to tobacco smoke.

Answer: We agree that there are no data showing that chronic inflammation in Tsimans protects them against NCD (correlation is not causation). We have erased this claim. Rather, we agree that the best explantion is probably the absence of typical NCD risk factors. Therefore, the Tsimane are noteworthy for being an example that chronic high inflammation is not sufficient for increasing NCD risk, given that other conditions are satisfied as you mentioned. This is now emphasized in the revision.

Second, while the Eifel study results suggest that adopting a more "Paleo"-like lifestyle can improve cardiometabolic biomarkers, we disagree that they show that higher inflammation (or innate immune activity in general) typifies hunter-gatherers or other subsistence populations. While CRP increased by 67% after the four day forest trek of healthy adults, this increase was not statistically significant (see Table 4). Thus, given the small sample size (n=25), these speculations have limited basis and are premature. Moreover, we see other issues with generalizing from the Eifel study. Half of the study participants did not engage in regular physical activity (52% <3 hrs/week). Heavy physical activity could lead to acute increases in inflammation, whereas long-term effects of regular exercise are usually associated with reduced chronic low grade inflammation. It is also possible that during the experiment, some participants incurred minor injuries or acute infections, both of which could result in higher CRP. Psychosocial stress, as might occur under the Eifel study conditions of food restriction for example, can also increase inflammation, especially IL-6, IL-1B and CRP[5].

Answer: **You are correct that high-grade inflammation is not a general hallmark of hunter-gatherer populations, and we apologize if we seem to have made such a statement. Our goal was simply to propose several possible hypotheses fort he observed acute elevations in CRP in the Eifel studies, partly in preparation for a new study that is going to start this summer. We also state in the revision that the data are not able to provide evidence for one of our hypotheses over any other, so that future studies should particularly put these hypotheses to test.**

While Tsimane show evidence of chronic, elevated CRP across much of the life course[6,7], it is yet to be determined how representative this pattern is among small-scale subsistence populations. Shuar forager-horticulturalists of Ecuador show elevated CRP only when infected, but otherwise have low baseline CRP levels[8]. A small sample of Hadza hunter-gatherers also showed reasonably low CRP levels (74% of 23 adults had CRP < 3 mg/L)[9].

Answer: Thank you for pointing this out. We added the references to these studies that indicate that the Tsimane CRP levels are apparently higher than that of other indegenous people.

Anticipatory stimulation of the innate immune system in the forest is an interesting idea; even visual exposure to photographs with disease cues (e.g. sneezing) can increase IL-6 by

24% compared to controls shown neutral stimuli[10]. However, while short-term changes in other biomarkers like blood lipids and blood glucose may be generalizable from the Eifel study to a long-term study, it is unclear whether any acute innate immune activity observed over a 4 day period would sustain in a longer-term study after habituation. As FKL argue, there are many other reasons CRP can be elevated that have little to do with inflammation, but might still be related to tissue stress or injury[11].

Answer: We agree that it is unclear if and when the elevated CRP levels found in the Eifel study participants would have decreased again. However, a similar study has found increased CRP levels after a 10-day trip through the wilderness. This is stated now in the last two sentences of the conclusions section: "In particular it remains to be determined, if, when and how the acute elevations in CRP level resolve if the participants would remain in Paleolithic living conditions. Interestingly, a study similar to the Eifel studies involving a10-day trip through the Pyrenees also reported a significant mild increase in CRP levels after the trip, showing that the acute phase repsonse could be sustained at least up to 10 days (29-30)."

Competing Interests: No competing interests were disclosed.