RESEARCH ARTICLE

How to dismantle modern stressors: does a short trip to simulated Paleolithic conditions in the wild reduce cortisol levels? [version 1; peer review: 2 not approved]

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Abstract

Background: Chronic stress has become a central problem of our modern society. It appears plausible that our Paleolithic ancestors were more exposed to acutely life-threatening stress stimuli than chronically enduring psychosocial stressors. The aim of this study was to test whether it is possible to reduce physiological and psychological stress parameters by returning to simulated Paleolithic lifestyle conditions.

Methods: In total, 15 volunteers undertook a four-day hiking tour simulating hunter-gatherer lifestyle conditions in a National Park. Saliva samples were taken at 5, 6 and 7 pm prior to, during and after the intervention and evaluated for cortisol concentration. Perceived stress was measured by the Perceived Stress Questionnaire.

Results: The study was completed by 11 of the subjects. Mean body weight and fat mass decreased significantly during the intervention by 2.3 and 2.2 kg, respectively. There was a marked increase in cortisol levels on the first day of the intervention compared to baseline with a gradual return to baseline levels on the subsequent days. Individual cortisol responses were heterogeneous; in three subjects, cortisol concentrations generally decreased from 5 to 7 pm, while in four subjects the opposite trend was observed.

Conclusions: During this four-day outdoor intervention under simulated Paleolithic conditions, significant changes occurred almost exclusively at the first day of the study. The increase in cortisol levels

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

at this point supports the hypothesis that some individuals respond by an increased release of cortisol as a result of adaptation to new environmental conditions.

Keywords

Cortisol, Exercise, Paleolithic diet, Stress

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Introduction

Over the past 2.5 million years, humans, and the species preceding them, have continuously adapted to the corresponding environmental conditions¹. For the longest period of this development, humans subsisted through a hunter-gatherer lifestyle². However, environmental conditions changed ~12,000 years ago with the cultivation of plants and domestication of animals, and even more drastically ~150 years ago with the beginning of industrialization and 30 years ago with the advent of digitization^{3,4}.

Adaptation to stress signals is an integral part of human evolution⁵. The concept of the stress response is based on the idea that organisms activate protective mechanisms that allow them to survive unfamiliar and potentially dangerous situations⁶. Those who are resilient to internal and external danger signals increase their chance of survival, thereby also ensuring the survival of their genes⁷.

Chronic stress has become a central problem of our modern society, which is associated with numerous diseases, such as obesity, type 2 diabetes or depression⁸. It appears plausible that our Paleolithic ancestors were more exposed to acutely life-threatening stress stimuli than chronically enduring psychosocial stressors such as existential anxiety; indeed, many modern-day stressors including pressure to meet certain deadlines, social media stress etc. are a very recent phenomenon. Here, we report findings of a study that was designed to subject civilized humans to an imitation of the Paleolithic lifestyle for a short period of time. The research question was whether it is possible to reduce physiological and psychological stress parameters by returning to simulated Paleolithic conditions in a National Park over a four-day duration.

Methods

Design

The study was approved as a natural study by the ethics committee of the German Sports University Cologne (061-2018). All subjects signed a written informed consent concerning possible risks of participation before the start of the intervention. For a realistic simulation of Paleolithic conditions, the following basic requirements were defined:

1. During the intervention, subjects should remain exclusively in the wild.
2. In order to simulate hunting and gathering behavior, subjects should travel a distance of at least 20 km per day.
3. The first meal was not to be consumed before 12 am to simulate a period of hunting and gathering.
4. The diet should be based on the principles of a “Paleolithic diet” as defined by Cordain⁹, including lean meats, fish, fruits, vegetables, nuts, oils (coconut oil, olive oil) and sweet potatoes.
5. The sleep-wake cycle should be adapted to the natural circadian rhythm according to sunrise and sunset.

The study lasted a total of five days and four nights from 25th to 29th July 2018. However, the last day was for return only, so the intervention spanned a total of four full days. The tour was led by Sascha Krüger (SK), who himself was

a participant in our pilot studies published by Freese *et al.*^{3,4}, and offers these tours commercially under the name “BACK-2BASIC-Tour”. Our pilot studies took place in 2015 and 2016. The general study design was the same as in this follow-up study, but the research objective had focused on metabolic parameters.

At the beginning of the intervention, a 30-minute information session was held by the study director. Subjects were given extensive information about the exact procedure of the study as well as their tasks and were given the opportunity to receive answers to their open questions. During the entire intervention period, the study management team was available on site as scientific back-up. To analyze stress levels, saliva samples were taken before (t_0), during (t_{1-4}) and after (t_5 , t_7 , t_{11}) the intervention to determine cortisol levels.

Participants & recruitment

All participants in the commercially offered BACK-2BASIC tour were informed in advance about our study, and written informed consent was obtained. Exclusion criteria were any known diseases or the administration of medications that could affect cortisol levels. Participants completed a 30-minute on-site introductory seminar about the main principles and goals of the study.

Interventions

To simulate Paleolithic conditions, the following interventions were implemented during the study period:

- Provision of “Paleolithic foods”: At lunchtime participants received a food package consisting of two pieces of fruit and vegetables and 100 grams of nuts. Before sunset, we delivered a freshly prepared, Paleo-compliant meal to their place of residence.
- Spending 24 hours each day in an open arboreal environment
- Cut off from technology and modern-style work stress (e.g., notifications from mobile phones, email, time pressure, etc.)
- Exposure to the natural 24-hour temperature variability (only modest amount of clothing and sleeping bag was allowed)

Hiking for several hours each day starting after sunrise

The subjects were within the group at all times, supervised by the experienced tour guide SK. Simon Schnell (SS) was present each day at dinner to collect the saliva samples and hand out the new ones for the following day. Otherwise, there were no contacts during the interaction. Since subjects were constantly outside, all participants inevitably had to adhere to the study guidelines.

Measurements

Health Questionnaire and Anthropometric Data. Prior to the start of the intervention, each subject completed a health questionnaire that collected the following parameters: Personal,

anthropometric and physical activity data, dietary behavior, health complaints and diseases. In addition to the requested anthropometric data, body weight, fat-free mass, skeletal muscle mass, body fat mass, and body mass index (BMI) were determined using a bioimpedance scale (Tanita model DC 430 MA P). At the end of the intervention, measurements on the bioimpedance scale were repeated. A copy of the questionnaire is provided as extended data¹⁰.

Saliva samples. Three days before, during, and seven days after the 4-day intervention (i.e., from t_0 – t_{11}), three saliva samples were collected from each subject at 5, 6, and 7 pm, respectively. Saliva samples were collected in Salivettes Cortisol Code Blue (Sarstedt, Nümbrecht, Germany) and analyzed for cortisol concentrations in the laboratory of the Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, using a commercially available enzyme-linked immunosorbent assay (Cortisol ELISA, IBL International, Hamburg, Germany) according to the manufacturer's instructions as previously described^{11,12}. Intra- and inter-assay variance was 4.8% and 5.9% respectively, the detection limit was 0.005 µg/dL.

Psychological Stress Parameters. Perceived stress was measured every evening at the time of saliva sampling by the German version of the Perceived Stress Questionnaire¹³. The PSQ contains 20 items and comprises four subscales (worries, tension, joy and demands). The questionnaire's instruction asked the participants to rate how often an item applied to their life within the last four weeks or last four days, respectively. The rating scale ranges from 1 ("almost") to 4 ("usually"). An overall score of perceived stress can be built by a summation of the four subscales.

Mood was assessed with the help of the German version of the Multidimensional Mood Questionnaire¹⁴. The Multidimensional Mood Questionnaire contains 12 items and comprises three mood dimensions (pleasant–unpleasant, awake–sleepy, and calm–restless). The questionnaire's instruction asked participants to rate different adjectives regarding their current mood.

Physical measurements. Temperature was measured by the tour guide using a standard temperature meter. Daily hiking distance was measured by the smartphone app "Komoot – Cycling & Hiking Maps".

Statistical analysis. The Shapiro-Wilk test was used to test for normally distributed anthropometric parameters and cortisol values. The pre-post intervention comparison was carried out by means of a dependent t-test for normally distributed variables, whereas differences between non-normally distributed pre-post parameters were checked for significance by means of the Wilcoxon rank sum test. Because of the lack of normal distribution, analysis of variance of the repeatedly measured cortisol data was performed using the Friedman test. In order to compare the individual intervention days, a post hoc test was used. Significance values were adjusted by Bonferroni correction. Parameters with p-value <0.005 were considered significant¹⁵, p <0.001 highly significant. We used Microsoft Excel 365 and IBM SPSS Statistics (version 25) for analysis.

A pre-post intervention comparison of the psychological stress parameters (perceived stress and mood) was conducted using dependent t-tests.

Results

Demographic profile

Out of 15 recruited volunteers, 11 completed the study (7 female and 4 male). Two were unable to attend for organizational reasons, while two others left during the intervention due to illness. The mean age of those who completed the study was 40.9 years (range 27–59 years). None of the participants was on medical treatment and all were considered mentally and physically healthy.

Paleolithic simulation conditions

Due to experiences of our studies from 2013–2014, we did not measure the average kcal intake per day and the macronutrient composition during the four nights and days in the wild. Average temperature was 33° Celsius at day one. On the other days, average temperatures ranged between 26–29° Celsius. The average daily hiking distance was 25.3 km.

Anthropometric and biochemical measurements

Four days of simulated Paleolithic conditions resulted in significant changes in subjects' body composition. Body weight dropped on average from 78.5 kg to 76.2 kg (- 2.9%, $p = 1.185 \times 10^{-13}$). Body fat mass was reduced from 20.8 kg to 18.6 kg (- 2.3 kg, $p = 0.0002$), which corresponds to a reduction of the body fat percentage from 26.3% to 24.2% (- 2.1%). All changes in body composition were highly significant (see Table 1¹⁰).

Cortisol measurements from t_0 – t_{11}

Friedman's multi-factorial analysis of variance confirmed that there were differences in cortisol levels between the individual measurement days ($p = 7.19 \times 10^{-16}$). As a result, post-hoc tests were performed taking the time of sampling into account. An initial analysis of average cortisol levels throughout the day (see Figure 1) revealed a marked increase in salivary cortisol at t_1 with a slight increase to t_4 . As it turned out, nearly all significant changes in cortisol levels were related to t_1 . Within 12 cases of significant changes, t_1 was involved ten times.

Figure 1 shows cortisol levels over the individual measurement days, according to the time of sampling. The curve at 5 pm shows at t_1 a lower increase in cortisol compared to 6 pm and 7 pm ($p=0.00146$ and $p=0.000706$, respectively). Nevertheless, cortisol level at 5 pm displays a significant difference between t_1 and t_7 and between t_4 and t_7 .

The mean cortisol level at t_1 increased almost threefold from 5 to 6 pm (from 10.82 to 29.5 ng/ml). The cortisol value at 6 pm on the first day (t_1) differed significantly from all other 6 pm cortisol values, except on t_4 . Consistent with the general trend, the 6 pm cortisol curve shows a significant increase from t_0 to t_1 , followed by a rapid drop at t_2 . On the other hand, the slight increase in salivary cortisol at t_4 does not differ significantly from the measurements on the other days at 6 pm.

Table 1. Changes in anthropometrical parameters from pre- to post-study.

	Pre	Post	Difference	p-value
Body weight [kg]	78.5±15.4	76.2±15.2	2.3	1.185×10 ⁻¹³
Body fat mass [kg]	20.8±6.5	18.6±6.1	2.2	0.000245
Body fat mass [%]	26.3±5.1	24.2±5.3	2.1	0.000466
BMI [kg/m ²]	25.4±3.4	24.7±3.4	0.8	1.575×10 ⁻¹¹

P-values have been Bonferroni-corrected.

Figure 1. Course of cortisol level (ng/ml) in relation to the measurements at 17:00, 18:00, 19:00 and the days of measurement.

At 7 pm, the highest cortisol levels of the study were measured at t_1 (mean 34.83 ng/ml, maximum 170 ng/ml). Most of the significant changes at 7 pm are related to t_1 . In addition, 7 pm cortisol levels differ significantly from t_4 to t_7 , as in the measurements at 5 pm. Regardless of sample-taking time, there were no significant differences in the pre-post comparison. Neither at t_5 , t_7 nor t_{11} significant changes from the initial value at t_0 could be found. The only significant changes from t_0 occurred at t_1 . Even during the intervention itself, there were hardly any significant changes in cortisol levels. Only between t_1 and t_3 (6 pm) and between t_1 and t_2 (7 pm) cortisol levels decreased significantly.

Changes in cortisol Measurements between 5 pm, 6 pm and 7 pm

To investigate differences in cortisol values between 5–6 pm, 6–7 pm and 5–7 pm, a variance analysis was performed. The outcome was positive ($p=7.19 \times 10^{-16}$). Post-hoc tests

showed significant differences in cortisol levels over several days, depending on the time of the day, but not on all days. At t_0 , t_1 , t_2 , t_3 and t_{11} , cortisol changed significantly in the course of time. In contrast, there were no significant differences in cortisol measured at the different times of the day at t_4 , t_5 and t_7 .

In two out of eight days, there was a significant change in cortisol levels between 5 and 6 pm (at t_1 and t_3). Between 6 pm and 7 pm cortisol levels did not vary significantly. Most of the changes were detected between 5 and 7 pm (at t_0 , t_1 , t_2 , t_3 and t_{11}), especially during the intervention period lasting from t_1 – t_4 .

As Figure 1 shows, cortisol levels decreased from 5 to 6 pm and from 6 to 7 pm on six out of eight days, except for t_1 and t_4 . Nevertheless, as shown in Figure 2, mean salivary cortisol levels showed a continuous increase from 5–6 pm (4.32 ng/ml to 5.9 ng/ml) and 6–7 pm (5.9 ng/ml to 6.33 ng/ml).

Figure 2. Course of the cortisol level (ng/ml) according to time and day of measurement.

Cortisol measurements of individual subjects

Cortisol levels were also examined individually in all subjects. For this purpose, subjects were divided into “pro”-responders and non-responders. The former were comprised of subjects whose cortisol levels generally decreased from 5 to 7 pm (on all days), whereas subjects whose cortisol levels generally increased from 5 to 7 pm were rated as non-responders. In total, three subjects were classified as pro-responders, four subjects as non-responders. Noteworthy, cortisol levels of pro-responders increased significantly at the beginning of the study (see Figure 3). At baseline (t_0), the average concentration of salivary cortisol was 8.27 ng/ml at 5 pm, 5.25 ng/ml at 6 pm and 4.01 ng/ml at 7 pm. Non-responders had already low cortisol levels at the beginning of the study (see Figure 4).

In a pre-post comparison, pro-responders achieved a significant reduction in cortisol levels. From t_0 to t_{11} , average cortisol decreased from 8.27 to 2.18 ng/ml at 5 pm, from 5.25 to 1.37 ng/ml at 6 pm, and from 4.01 to 1.95 ng/ml at 7 pm. In contrast, cortisol levels of non-responders increased between t_0 and t_{11} , albeit to a lesser extent than pro-responders decreased. Four subjects could not be classified as pro- or non-responders. These neither had a consistent increase nor a reduction of cortisol levels in the pre-post comparison.

Psychological Stress Parameters

Analyses showed a trend for a change regarding the overall stress score ($t(9) = 2.45$, $p = 0.04$) and the perceived worries ($t(9) = 2.51$, $p = 0.03$) and demands ($t(9) = 3.03$, $p = 0.01$) from pre to post intervention.

No significant changes concerning participants' mood were found from pre to post intervention (pleasant–unpleasant:

$t(9) = 0.20$, $p = 0.85$, awake–sleepy: $t(9) = 0.63$, $p = 0.55$, and calm–restless: $t(9) = -1.05$, $p = 0.32$).

Discussion

The aim of our study was to test how living in the wild for a short period of time affects markers of stress, which is an important research question given that nowadays many people spend significant amounts of time in highly artificial environments.

Daily hiking tours averaging 25.3 km per day exposed subjects to physical stress. Research has shown that medium to high intensity exercise (60–80% VO_2max) leads to an increased cortisol output, whereas moderate exercise (40% VO_2max) reduces cortisol¹⁶. Walking is generally classified as a low-intensity exercise. However, due to the long hiking distances and the uncommon high ambient temperatures, individual activity levels may have been strenuous for most of the subjects. Brenner *et al.*¹⁷ revealed a cumulative effect of moderate- to high-intensity exercise and heat on cortisol output. In addition, they pointed out that repetitive physical stress at high temperature conditions causes elevated stress reactions, which last for longer periods after cessation of exercise. This outcome may explain the sharp and progressive increase of cortisol levels at t_1 .

Due to the high ambient temperature and the above-average intensity for most subjects on the first day of the intervention, it should be considered that the large increase in cortisol levels at t_1 may be due to synergistic effects of heat and exercise. In three of 11 subjects, cortisol levels at t_1 showed no changes at all. Obviously, these subjects may be accustomed to prolonged stress in the heat. Health questionnaires confirmed this assumption: two of these subjects regularly participated

Figure 3. Course of cortisol and cortisol levels in pre-post comparison (t_0 versus t_{11}) in pro-responders.

in similar events, especially the tour guide who completed this intervention for the third time with other groups that year. One subject regularly performed endurance training. According to Martikainen *et al.*¹⁸ endurance training results in a reduced activity of the hypothalamus-pituitary-adrenal (HPA) axis with correspondingly lower cortisol release. For all other subjects, who were not accustomed to the extensive physical workload in combination with heat and intermittent fasting conditions, the cumulative lifestyle changes would likely have been a strong stressor.

Conclusions

During this four-day outdoor intervention under simulated Paleolithic conditions, significant changes occurred almost exclusively at the first day of the study (t_1). The increase

in cortisol levels at this point supports the hypothesis that non-responders respond by an increased release of cortisol as a result of adaptation to new environmental conditions. Although we could not find an average pre-post change in this small sample size, the individual analysis shows encouraging results. The distinction between pro- and contra-responders displays that subjects with high psychosocial stress could benefit from such an intervention. Future studies should recruit a larger number of subjects with more relevant inclusion criteria, such as subjects, who are permanently exposed to high levels of psychosocial stress.

As a limitation of this study, all analyses should be considered with caution, due to the small sample size and heterogeneity of the study participants.

Figure 4. Course of cortisol and cortisol levels in pre-post comparison (t_0 versus t_{11}) in non-responders.

Data availability

Underlying data

Zenodo: How to dismantle modern stressors: does a short trip to simulated Paleolithic conditions in the wild reduce cortisol levels? (Study data). <http://doi.org/10.5281/zenodo.4549516>¹⁰

This project contains the following underlying data:

- Distance.png
- Confirmation Ethics Application Eifel Study 2018.pdf
- Cortisol Evaluation 26.7. with Time Comparison per Day.spv
- Output Body Data.spv
- Evaluation Questionnaires Eifel Study.sav
- Data Sheet Body Data.sav

Extended data

Zenodo: How to dismantle modern stressors: does a short trip to simulated Paleolithic conditions in the wild reduce cortisol levels? (Study data). <http://doi.org/10.5281/zenodo.4549516>¹⁰

This project contains the following underlying data:

- Health Questionnaire & Informed Consent.pdf

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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How to dismantle modern stressors: does a short trip to simulated Paleolithic conditions in the wild reduce cortisol levels? [version 1; peer review: 1 not approved]

The article investigates the effect of 4 days of hiking approximately 25 km/ day in a forest setting. Paleo style snacks and a paleo meal are provided. Salivary cortisol is obtained at 5, 6, and 7 pm and analyzed. Cortisol is elevated most significantly at 5 pm on the first day but is also elevated at 6 and 7. Over time the cortisol elevations are lessened. Psychological stress parameters lessened over time, no significant change in mood over time was observed.

Introduction

- What were your aims and hypotheses?
- What were the eligibility inclusion/ exclusion criteria?
- Recruitment strategy?

Methods

- Item #3 first meal not be consumed before 12 am is probably a typographical error.
- What foods were provided in the paleo compliant meal?
- Were people allowed to eat additional foods ad libitum?
- Where was the residence?
- The participants usual residence or with the group in tents, shelters, cabins, hotels, or their homes?
- Were these foods a change from the usual dietary intake of participants from your health

questionnaire? Please describe as a table.

Results

- Reasons for dropouts?
- Please provide demographic information from your health questionnaire.
- Please provide the perceived stress, multidimensional mood questionnaire score data as a table, mean SD pre and post, change value and P value in the text or in a table.
- Did the cortisol response differ according to the prior exercise level or dietary pattern? Define how you determined pro responder and non-responder. Identify this as a post hoc analysis.

I am willing to review after revisions.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: paid to speak BioCeuticals, Genova Diagnostics, Metagenics.

Reviewer Expertise: Dietary intervention studies of the modified paleolithic diet in the setting of multiple sclerosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 20 April 2021

<https://doi.org/10.5256/f1000research.53879.r82127>

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In this paper, Freese and colleagues describe weight loss and afternoon salivary cortisol levels before, during and after a 4-day hiking tour. This 4-day activity was supposed to simulate Paleolithic conditions with a walking distance of at least 20 km per day, cut off from technology, exposure to the natural 24-hour temperature variability and a Paleolithic diet with the first meal consumed at 12 a.m. at the earliest. This 4-day intervention caused weight loss and a marked increase in salivary cortisol at the first day of intervention. Salivary cortisol returned to baseline values at day 2, 3 and 4 of the hiking tour. This is a well-conducted small study with interesting results on day 1 of the intervention. However, the statistical presentation of the results (see 4 and 5 below) needs to be improved until the paper can be recommended to a wider audience.

1. Could the authors describe the rational why they decided to measure cortisol at 5, 6 and 7 p.m.? In my opinion, these are quite unusual time points. It would therefore be important to know the reason for this choice of time points.
2. In this paper, higher salivary cortisol levels at day 1 of the hiking tour compared to before the tour but also compared to day 2 of the hiking tour are found. This finding is discussed very well in the paper. However, also cortisol levels at 6 p.m. and 7 p.m. at day 1 are significantly higher than the cortisol levels at 5 p.m. at the same day. I suggest that the authors discuss possible explanations for this finding. To find an explanation, it would be important to know what study participants did during 5 p.m. and 7 p.m. at day 1.
3. The authors describe in the methods that they used the Wilcoxon rank sum test to compare the non-normally distributed pre- and post-test variables. However, the Wilcoxon rank sum test is usually used to compare two independent groups which is not the case in this study. I would suggest using the Wilcoxon signed rank test instead.
4. The file with the original data is published together with the paper. Because of the very low p-values in table 1, I calculated all statistical test for table 1 and I have to state that all p-values in table 1 are wrong. Maybe the authors applied the Bonferroni correction in the wrong direction? An easy way to apply the Bonferroni correction: You decide for which number of analyses you want to correct for; some authors choose to correct for the number of analyses in one table, other authors correct for the number of analyses in the whole paper. In this paper, table 1 has four analyses, so the Bonferroni corrected significance value is $p = 0.05/4 = 0.0125$. Comparing weight before and after the intervention with an independent t-test results in $p = 4.26 \times 10^{-7}$. So, weight loss is significant in this study because this p-value is below the significance value of $p = 0.0125$.
5. I would suggest adding a measurement of variability to all figures, e.g. standard deviation, standard error of the mean or 95% confidence intervals.
6. Whenever exact numbers are depicted in the text of the results section or the abstract, a

measurement of variability, e.g. standard deviation, should be added. However, if the exact numbers are described in the table, you do not need to repeat those numbers in the text of the results section.

7. It should be added to the figure legend of table 1 that 'mean $\pm$ standard deviation' is depicted in this table. In table 1, the standard deviation should be added for the difference.
8. Because of the significant weight loss in this study, it would be valuable to report calorie intake.
9. Several participants left the intervention early. Are there weight and cortisol measurements available for those participants? Did those participants differ from the other participants that completed the intervention?
10. It is highly debated if salivary cortisol is a good biomarker for stress; for chronic psychological stress it may not be a biomarker at all. The authors should add a couple of sentences about the usefulness of their biomarker to the discussion section. An example of a paper that discusses this: Hellhammer DH *et al*: Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* (2009) 34, 163-171¹.

References

1. Hellhammer DH, Wüst S, Kudielka BM: Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*. 2009; **34** (2): 163-171 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: clinical trials, statistics, nutrition, diabetes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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