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Back to the Future. Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions – New Insights from The Eifel Study

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Back to the Future. Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions – New Insights from The Eifel Study

Abstract

Background: The observation that the emergence of common Western diseases takes place with much greater prevalence as societies migrate from natural-living cultures to modernized societies, has been well documented. For approximately 84,000 generations humans lived under hunter-gatherer conditions but recently endured dramatic change from our native lifestyle with the occurrence of the agricultural, industrial, and digital revolutions. The massive technological advancement that occurred within a relatively recent timeframe enabled humans to live in manner that is remarkably different than our pre-agricultural past. Consequently, the shift from a natural to a modern lifestyle likely promotes a gene-environment mismatch which causes metabolic dysregulation which causes disease.

Methods: Using a within-participant design, we examined whether, compared to baseline, changes in lifestyle towards a more Paleolithic-style pattern, for a four-day and four-night period related to changes in a variety of metabolic parameters. Two groups of 14 volunteers were isolated for a period of four days and four nights in the natural park Südeifel on the borders between Germany and Luxembourg. Participants lived outdoors without tents. The daily hiking performance was 16.4 km (≈ 24963 steps/day) and the daily activity time 5.49 h/day by a mean caloric intake of 1747 kcal/day.

Results: After four days of simulated Paleolithic conditions, body weight (-2.9%), body mass index (-2.7%), body fat (-10.4%), visceral fat (-13.6%) and waist-hip-ratio (-2.2%) significantly decreased, while muscle mass significantly increased (+2,3%). Additionally, fasting glucose (-6.5%), basal insulin (-44.4%), homeostasis model assessment-index (-49.3%) and fatty liver index (-41%) significantly dropped. In contrast, C-reactive protein, significantly increased (+67.1%).

Conclusion: Our study indicates that a short nature trip, where modern humans adjust their behavioral patterns to simulate a more Paleolithic-like condition, could serve as an effective strategy to help prevent or improve modern metabolic disease. Particularly, the major findings of an expeditious reduction of homeostasis model assessment-index and fatty liver index scores in only four days reveal the potential for meaningful benefits with such an intervention, even when compared to the effects of longer-term, single-intervention studies such as dietary or fitness programs on similar metabolic parameters.

Keywords

Western diseases, Metabolic syndrome, Type 2 diabetes, Insulin resistance, Fatty liver index, Physical activity, Paleo diet

Cover Page Footnote

The authors are indebted to Dr. Annette Quade, Head of the ambulatory health care center Dr. Quade & Kollegena in Cologne, who funded laboratory samples. The same holds for Hermann Widerhold, CEO of Weightcheckers GmbH, who provideds a Tanita Weight Management System (MC 780MA S) for the length of this study. They also thankSpecial thanks are given to all participants of this study for abandoning the comfort of their modern homes.

Background

The observation that the emergence of common Western diseases (WD) – from obesity to coronary heart disease to cancers [1-3] – takes place with much greater prevalence as societies migrate from natural-living cultures to those that increasingly assume the characteristics of wealthier, modernized societies, has been well documented [4-8]. This is highlighted clearly by, for example, the drastically increased prevalence of obesity and diabetes in recently urbanized vs rural-living indigenous peoples [9, 10]. For instance, in Nauru, since the 1920^{s}, royalties for the natural resource phosphate has allowed these people to become one of the world's richest per capita. This wealth, however, has also afforded a rapid change in lifestyle. In this population, the first case of type 2 diabetes (T2D) was noted only in 1925. Now, however, the Nauruans are the world's most obese people (92.8%), have the highest blood pressure in the Western Pacific region, and two-thirds of their population over age 55 suffer from T2D [11, 12].

For approximately 84,000 generations humans lived under hunter and gatherer conditions [13,14] but recently humans have endured dramatic change from their native lifestyle with the occurrence of the agricultural, industrial, and digital revolutions [15-18]. Despite the massive technological innovation that has taken place during these revolutions, they have all occurred within a relatively recent timeframe. These innovations have enabled humans to live in a manner that is discordant with expectancies of our genes, which were largely established during our pre-agricultural past.

The metabolic dysregulation that appears to accompany the rural-modern lifestyle transition is supported by an abundance of evidence indicting elements of the modern lifestyle as causative in WD. These include but are not limited to: overnutrition [19], low dietary fiber intake [20], sugar-rich diet [21], physical inactivity [22], vitamin D deficiency [23], psychosocial stress [24, 25], sleep deprivation and circadian rhythms disturbances [26, 27], and more. Therefore, the shift from a natural to a modern lifestyle likely promotes a gene-environment mismatch [28, 29] which causes metabolic dysregulation which causes disease.

In contrast to single-intervention studies, our study aimed to have participants emulate a modern-day, Paleolithic-like lifestyle pattern during a short nature trip – which included multiple alterations from the default lifestyle pattern of modern living – to assess signs of favorable metabolic changes. We hypothesize that adopting a more Paleolithic-like lifestyle pattern will yield favorable and observable effects on metabolism, even in the short term.

Methods

Design

Using a within-participant design, we examined whether, compared to baseline, changes in lifestyle towards a more natural-living pattern, "Paleolithic-style pattern", for a four-day and four-night period related to changes in a variety of metabolic parameters. Two groups of 14 volunteers were isolated for a period of four days and four nights in the natural park Südeifel on the borders between Germany and

Luxembourg. The protocol is in accordance with the declaration of Helsinki and was approved by the ethics committee of the German Sports University of Cologne.

Participants & Recruitment

Participants were recruited from advanced training courses of the German Trainer Academy in Cologne. Eligibility was determined by completion of a pre-admission questionnaire. The pool of participants comprised personal trainers and health professionals who were healthy and non-obese. People were excluded from the study if they were using any medication chronically, or had acute injuries or psychiatric disorders. All participants accepted Jens Freese and Sebastian Schwarz as the coordinators of this study and provided written informed consent prior to their participation.

Baseline

All participants completed a 60-minute on-site introductory seminar about the main principles of our study design, and were then randomly divided into two cohorts. The intervention for both cohorts was identical. Each cohort had designated guides who had participated in the pilot study one year before, and were therefore experienced in the procedure, hiking tracks, and region.

Interventions

Diet

For the duration of the study, all participants followed a “Paleolithic” diet according to guidelines proposed by Cordain *et al.* [6, 17, 30-33] (Figure 1). The diet included lean meat, fish, eggs, vegetables, fruit, nuts and herbs. Foods were provided in limited quantity. For a complete list of food choices see Table 2. Seasonal, organic foods were bought locally in order to achieve optimal freshness. All processed, packaged foods, and all foods not in accordance with the Cordain guidelines for the Paleolithic diet, were excluded. Water was the only beverage allowed during the study period and was provided *ad libitum*. To quantify the total caloric intake and macronutrient ratios, we applied the USDA Nutrient Interactive Database [34].

Figure 1: A typical dinner of the intervention group according to the Paleo diet recommendations [6, 17, 31].

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Eating Schedule

In order to simulate what is estimated to be a more Paleolithic pattern of eating [6, 17, 31] food intake was only twice a day. The timing of the food availability was between noon and before sunset. Hence, each day the participants were provided no breakfast, snacks in the form of nuts and fruit after noon, and one main meal at dinner (Figure 2).

Groups hiked apart from each other, not knowing the direction, times of rest and times of food intake. Every participant carried a small day package of fruits and nuts with the instruction not to eat before noon. The intention of the present study was to guarantee an intermittent fasting period of at least 15 hours a day from last meal of the day to first meal of the next.

Recipes changed day by day. Before cooking, all foods were measured with a digital kitchen scale (Söhnle Food Control Easy, Leifheit AG, Nassau, Germany) by weight (grams). Each afternoon, coordinators prepared dinner in a nearby hotel and delivered the meals to the participants by car. Dinner plates and waste were collected after each meal by one of the study coordinators for disposal.

Figure 2: Foods distribution during the intervention.

Foods Distribution				
Before Noon →		Noon →		Evening
Spring Water		Fruits		Paleo Dinner
		Nuts		

Physical Activity and Sleep

To mimic foraging conditions, participants hiked for four hours each day starting after sunrise. Sleep period occurred during the natural day-night cycle, and morning awakening of participants happened naturally without the use of an alarm clock. Participants were also instructed not to expose themselves to artificial light.

For the quantification of the daily hiking distance, we used the portable navigation system Etrex Vista HCX (Garmin International, Olathe, USA). Comprised with a high-sensitive GPS receiver, the group's position was monitored in their respective environments. A built-in compass was used by the group leaders for navigation in the woods.

Measurement of the participants' daily energy expenditure and sleep behavior were collected with the SenseWear® armbands (BodyMedia Inc., Pittsburgh, USA). Due to the fact that the individuals within the two groups were together day and night throughout the intervention, we utilized armbands with only two participants to estimate physical activity and sleep for all participants. One male and one female subject wore an armband on the back of the upper left arm, facing upwards towards the shoulder with the sensors touching the skin. The SenseWear® system records physiological parameters and uses algorithms to report the average daily activity and

sleeping duration. Of the two samples, mean values were calculated and divided by the number of days in order to identify the approximate daily average for each measurement. Over the course of the intervention, participants lived outdoors without tents, but were provided tarps to protect participants from moisture at night.

Other Components of the Lifestyle Intervention

We also attempted to simulate non-food and activity-related Paleolithic conditions. As such, we implemented these conditions during the intervention:

- 24 hours in an open air, wooded environment
- Spending time with a tribe of 14 people
- Cut off from technology and modern-style work stress (e.g., notifications from mobile phones, email, time pressure, etc.)
- Exposure to natural 24-hour temperature variability (only modest amount of clothing was allowed; a sleeping bag was made available)

Blood Sample Collection and Anthropometric Data

Blood samples (lithium-heparin and potassium oxalate/sodium fluoride tubes) and EDTA-anticoagulated blood tests were drawn by venipuncture from fasted subjects the first morning and on day four immediately after the morning hike and during fasting conditions. Blood samples were drawn by a medical doctor and drained into three tubes: Sarstedt S-Monovette Serum-Gel (clinical chemistry), Sarstedt S-Monovette Kalium-EDTA (blood panel) and Sarstedt-S-Monovette Natrium-Fluorid (glucose). The tubes were stored in a cooling bag and immediately driven to the Dr. Quade & Kollegen laboratories in Cologne. Complete blood cell-count was analyzed with the XN 2000 Sysmex (Sysmex GmbH, Norderstedt, Germany). Quantitative and proportional analysis of the examined blood components were determined by electric impedance, laser light dispersion and dye binding. Gamma-glutamyltransferase, triglycerides, cholesterol, high density- and low-density lipoprotein were determined with the ADVIA 1800 Siemens (Siemens Healthcare GmbH, Erlangen, Germany) by the IFCC-method. Glucose was measured by the hexokinase reaction. Insulin was analyzed by chemical luminescence immunoassay reaction and high-sensitive C-reactive protein (CRP) by latex-enhanced-immunturbidimetric assay with the ADVIA 1800 Siemens. Body composition was measured by bioelectrical impedance analysis (BIA), using the Tanita Weight Management System MC 780MA S (Tanita Europe B.V, Amsterdam, The Netherlands) and carried out immediately after blood tests. BIA measurements were also drawn on the first and the last day of the intervention. Food was measured by the digital kitchen scale Söhnle Food Control Easy (Leifheit AG, Nassau, Germany). The homeostasis model assessment-index (HOMA) scores were calculated by the laboratory. To determine the fatty liver index (FLI), a diagnostic tool used to estimate the likelihood of fatty liver, we used the calculation method developed by Bedogni et al. [35].

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Statistical Analysis

Statistical analysis of all measurements were made using exploratory student t-tests for dependent samples using Microsoft Excel 2013 and SPSS (version 16.0). P-values shown are uncorrected. For all cases, $p < 0.05$ was considered statistically significant.

Results

Demographic Profile

A total of 28 participants were enrolled in the study. Three participants dropped out after one night due to the perceived stress load concerning the study's program design. In total, 25 participants (12 females; 13 males) completed the protocol. Subjects were classified as exercise-trained if they regularly performed three hours per week of anaerobic and aerobic exercise with moderate to high intensity. Collectively, subjects were relatively healthy, and did not currently take any prescription drugs. For additional participant demographics, please see Table 1.

Table 1: Demographic and anthropometric features of all participants.

Items	Participants
Race	Caucasian
N	25
Female	12 (48%)
Male	13 (52%)
Age (years)	40 (+/- 13)
Normal weight BMI < 25 (kg m^2)	20 (80%)
Overweight BMI > 25 (kg m^2)	5 (20%)
Exercised trained (> 3 h/week)	12 (48%)
Not exercised trained (< 3 h/week)	13 (52%)
Smoker	0

Food and Calorie Profile

Daily energy intake for participants averaged 1747 kcal/day. The ratio of macronutrient intake during the intervention were calculated to be 26% carbohydrates, 49% fat and 25% protein. The available foods, and the total amount eaten from all participants across the duration of the intervention is shown in Table 2.

Table 2. Offered and consumed foods over 4 days.

Foods	Weight (kg)	Kcal	Protein (g)	Fat (g)	Carbo-hydrate (g)
Almond	1.5	8685	317.25	748.95	323.25
Apple	19.9	1040	5.2	3.4	276.2
Apple Cider Vinegar	0.3	63	0	0	2.79
Apricot	4.9	2352	68.6	19.11	544.88
Eggplant	3.45	1225	29.05	8.05	305.55
Avocado	0.4	640	8	58.64	34.12
Banana	16.13	14356	175.82	53.23	3684.09
Broccoli	1.4	476	2.57	5.18	92.96
Canola Oil	0.65	5693	0	644	0
Carrot	13.5	5535	125.5	32.4	1293.3
Celery	0.6	96	4.14	1.02	17.82
Cashew	0.4	2212	72.88	175.4	120.76
Chicken thigh	10.12	23664	2372.52	1500.42	0
Cabbage	5	1250	64	5	290
Dark chocolate	0.8	4632	48.96	306.48	419.36
Eggs	100	7150	625.5	473.5	35.5
Ginger	0.1	80	1.82	0.75	17.77
Ground Beef	6	13800	1707	722.4	0
Honey	0.22	1216	1.2	0	329.6
Leek	2	620	16.2	4	152.4
Beef, lean, cooked	6.28	13608	1924.02	658.98	0
Mushrooms	3.6	792	111.24	12.24	117.36
Nut mix	4.87	24255	1568	1862	539
Olive Oil	0.95	7638	0	864	0
Onion	3.9	1560	42.9	3.9	364.26
Brazil nut	0.8	5248	114.56	531.44	98.16
Peach	3	1170	27.3	7.5	286.2
Pepper sweet	2.3	713	22.77	6.9	138.69
Radishes	3.2	512	21.76	3.2	108.8
Salmon, wild, cooked	6	11040	1641.6	450	0
Spinach	1	230	28.6	3.9	36.3
Strawberry	1.9	608	12.73	5.7	145.92
Swiss Chard	0.35	70	6.58	0.28	14.46
Turnip	4.7	1316	42.3	4.7	302.21
Walnut	1.27	8034	312.78	767	128.83
Watermelon	24	7200	146.4	36	1812
Zucchini, squash	4	760	40.4	10.8	155.2
kcal/total (4 days)		**179,539**			
kcal/person/day		**1,747**			
Macronutrient ratios			**Protein: 25%**	**Fat: 49%**	**Carbohydrate 26%**

All foods were measured by weight (grams). To estimate total caloric intake and macronutrient ratios, we used the USDA Nutrient Interactive Database [34].

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Physical Activity and Sleep

Measurements revealed that the participants averaged 5.49 h of physical activity (non-sedentary time), 24,962 steps, and 16.21 km of hiking per day. At night, it is estimated that participants averaged 7.15 h time in bed for sleep.

Anthropometric and Biochemical Measurements

After 4 days of simulated Paleolithic conditions we found decreased body weight (-2.9 kg), BMI (-2.7%), body fat (-10.4%), visceral fat (-13.6%) and waist/hip-ratio (-2.2%) (see Table 3). Muscle mass increased (+2.3%), although participants performed mainly low impact movement and their daily caloric intake (1747 kcal/day) was above the basal metabolic rate. We also observed decreases in the following metabolic parameters: total cholesterol (-6.1%), LDL/HDL-Quotient (-16.1), fasting glucose (-6.5%), basal insulin (-44.4%), HOMA (-49.3%), FLI (-41%) (Table 4). In contrast to all measured metabolic parameters, CRP, which represents the first immunological response to inflammatory conditions, increased (+67.1%) significantly (see Figures 3-7).

Table 3. Changes in anthropometric data over the course of the intervention.

Body Composition	Pre	Post	Change	p
Body Fat (%)	20.17 (± 7.38)	18.07 (± 7.99)	-2.10 (- 10.4 %)	< 0.001*
Visceral Fat (cm²)	4.58 (± 2.9)	3.96 (± 2.77)	-0.63 (- 13.6 %)	< 0.001*
Weight (kg)	74.55 (± 13.5)	72.42 (± 13.01)	-2.13 (- 2.9 %)	< 0.001*
BMI (kg/m²)	23.68 (± 2.82)	23.03 (± 2.72)	-0.65 (- 2.7 %)	< 0.001*
Fat Free Mass (kg)	59.43 (± 11.77)	59.35 (± 12.43)	-0.08 (- 0.1 %)	0.83
Muscle Mass (%)	75.64 (± 6.78)	77.83 (± 7.63)	2.18 (+2.3 %)	< 0.001*
Total Body Water (%)	56.73 (± 5.29)	58.54 (± 6.06)	1.81 (+ 3.2 %)	< 0.001*
Waist (cm)	83.67 (± 9.32)	81.79 (± 9.22)	-1.88 (- 2.2 %)	< 0.001*
Waist/Hip-Ratio	0.83 (± 0.08)	0.814 (± 0.08)	-0.02 (- 2.2 %)	< 0.001*

Values represent the mean ± SD. P-values shown are uncorrected. *Significant difference between the values before and after the intervention.

Abbreviations: BMI (body mass index), ICW (intracellular water), ECW (extracellular water)

Table 4. Changes in biochemical data over the course of the intervention.

Biochemical Data	Pre	Post	Change	p
Leukocytes (nl)	6.11 (± 1.54)	5.91 (± 1.49)	-0.20 (- 3.2 %)	0.558
Erythrocytes (PL)	4.85 (± 0.36)	4.67 (± 0.41)	-0.18 (- 3.7 %)	< 0.001*
Hemoglobin (g/dl)	14.82 (± 0.93)	14.20 (± 1.02)	-0.62 (- 4.2%)	< 0.001*
Hematocrit (%)	42.63 (± 2.47)	41.00 (± 2.98)	-1.63 (- 3.8 %)	< 0.001*
MCV (fl)	88.10 (± 3.5)	88.00 (± 3.59)	-0.09 (- 0.1 %)	0.729
MCH (pg)	30.61 (± 1.42)	30.50 (± 1.45)	-0.12 (- 0.4 %)	0.328
MCHC (g/dl)	34.76 (± 0.66)	34.65 (± 0.72)	-0.10 (- 0.3 %)	0.289
RDW (%)	12.78 (± 0.68)	12.53 (± 0.7)	-0.25 (- 2 %)	< 0.001*
Thrombocytes (nl)	240.13 (± 47.34)	242 (± 48.39)	1.88 (+ 0.8 %)	0.672
Neutrophils (%)	54.83 (± 8.81)	58.58 (± 6.69)	3.74 (+ 6.8 %)	0.031*
Lymphocytes (%)	33.27 (± 8.14)	29.69 (± 6.46)	-3.58 (- 10.8 %)	0.022*
Monocytes (%)	8.54 (± 1.94)	9.11 (± 1.79)	0.58 (+ 6.8 %)	0.088
Eosinophils (%)	2.67 (± 1.93)	1.93 (± 1.18)	-0.73 (- 27.5 %)	0.014*
Basophils (%)	0.70 (± 0.24)	0.69 (± 0.29)	-0.01 (- 1.2 %)	0.865
Fasting glucose (mg/dl)	80.34 (± 8.84)	75.10 (± 7.87)	-5.25 (- 6.5 %)	0.017*
Gamma GT (U/L)	20.71 (± 15.22)	20.13 (± 12.64)	-0.58 (- 2.8 %)	0.365*
Total cholesterol (mg/dl)	200.50 (± 32.93)	188.25 (± 33.21)	-12.25 (- 6.1 %)	< 0.001*
HDL (mg/dl)	75.57 (± 21.4)	78.30 (± 19.83)	2.73 (+ 3.6 %)	0.056*
Triglycerides (mg/dl)	95.63 (± 78.71)	49.04 (± 18.7)	-46.58 (- 48.7 %)	0.004*
LDL (mg/dl)	113.11 (± 28.28)	101.53 (± 23.43)	-11.58 (- 10.2 %)	< 0.001*
LDL/HDL-Quotient	1.61 (± 0.58)	1.36 (± 0.4)	-0.24 (- 15.1 %)	0.002*
CRP high sensitive (mg/l)	1.334 (± 2.62)	2.23 (± 2.96)	0.895 (+ 67.1%)	0.121
Insulin (uU/ml)	8.943 (± 7.52)	4.97 (± 2.11)	-3.97 (- 44.4 %)	0.016*
HOMA-Index	1.847 (± 1.83)	0.94 (± 0.46)	-0.91 (- 49.3 %)	0.022*
Fatty Liver Index	20.51 (± 23.9)	12.10 (± 15.3)	-8.41 (- 41 %)	< 0.001*

Values represent the mean ± SD. P-values shown are uncorrected. *Significant difference between the values before and after the intervention.

Abbreviations: MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution), Gamma GT (gamma-glutamyl transpeptidase)

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Figure 3: Boxplots showing fasting glucose at baseline (Pre) and post intervention (Post).

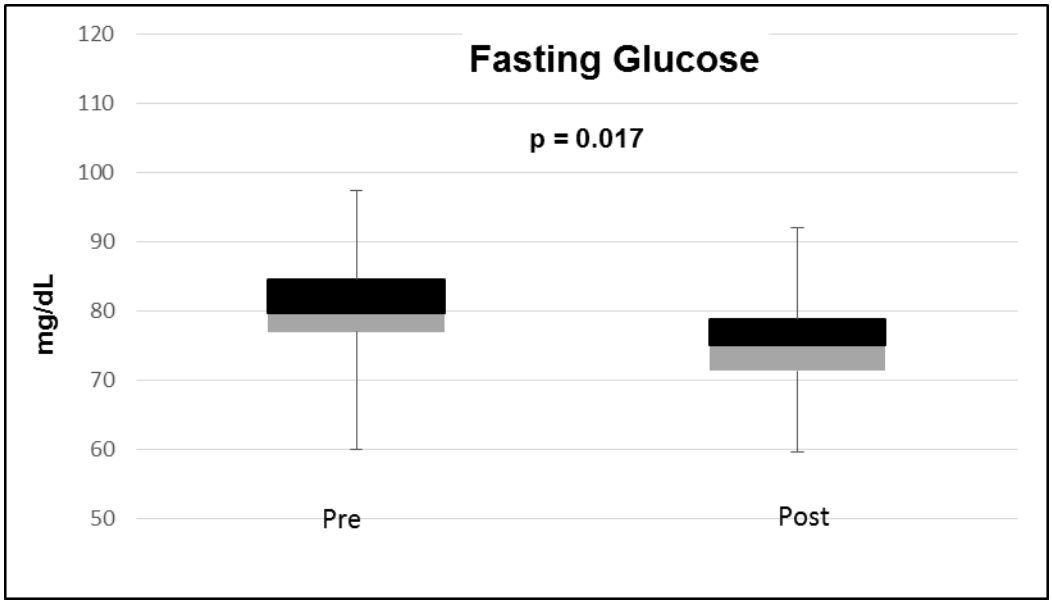

Figure 4: Boxplots showing insulin at baseline (Pre) and post intervention (Post).

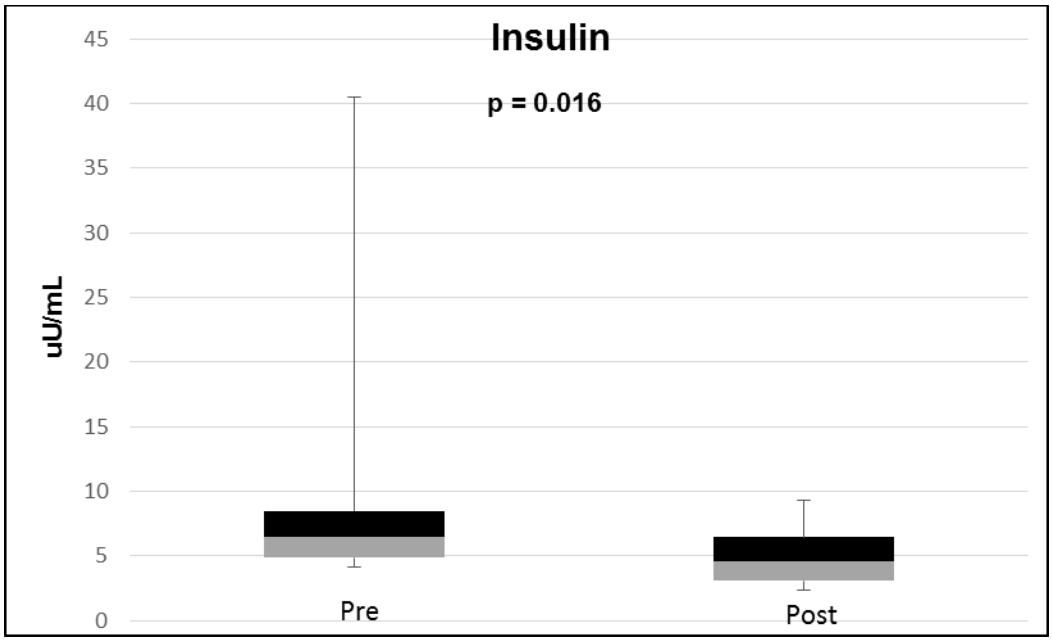

Figure 5: Boxplots showing HOMA-Index at baseline (Pre) and post intervention (Post).

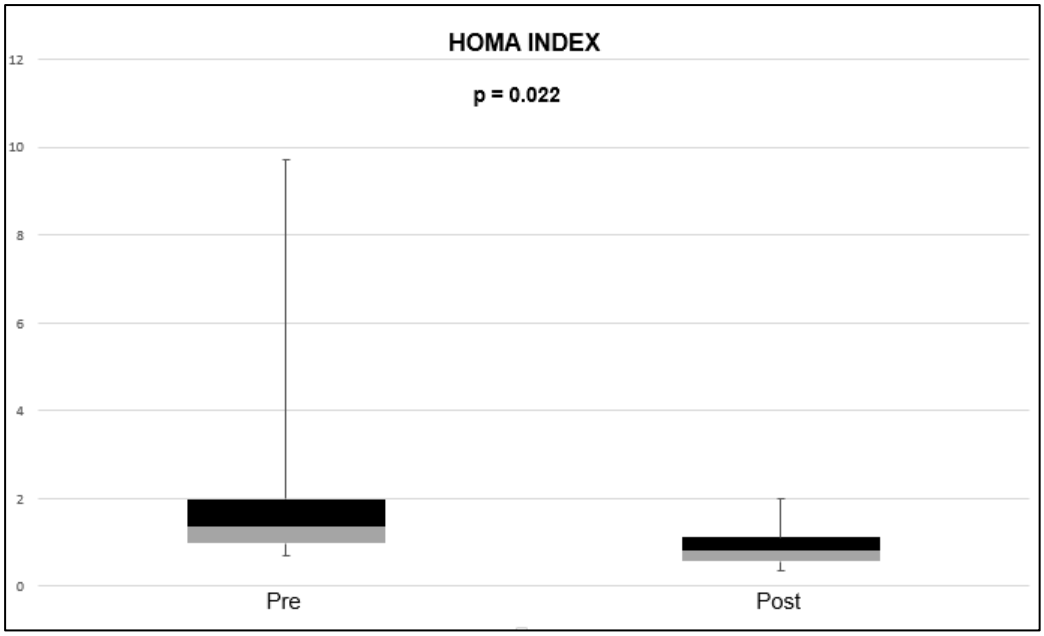

Figure 6: Boxplots showing Fatty Liver Index at baseline (Pre) and post intervention (Post).

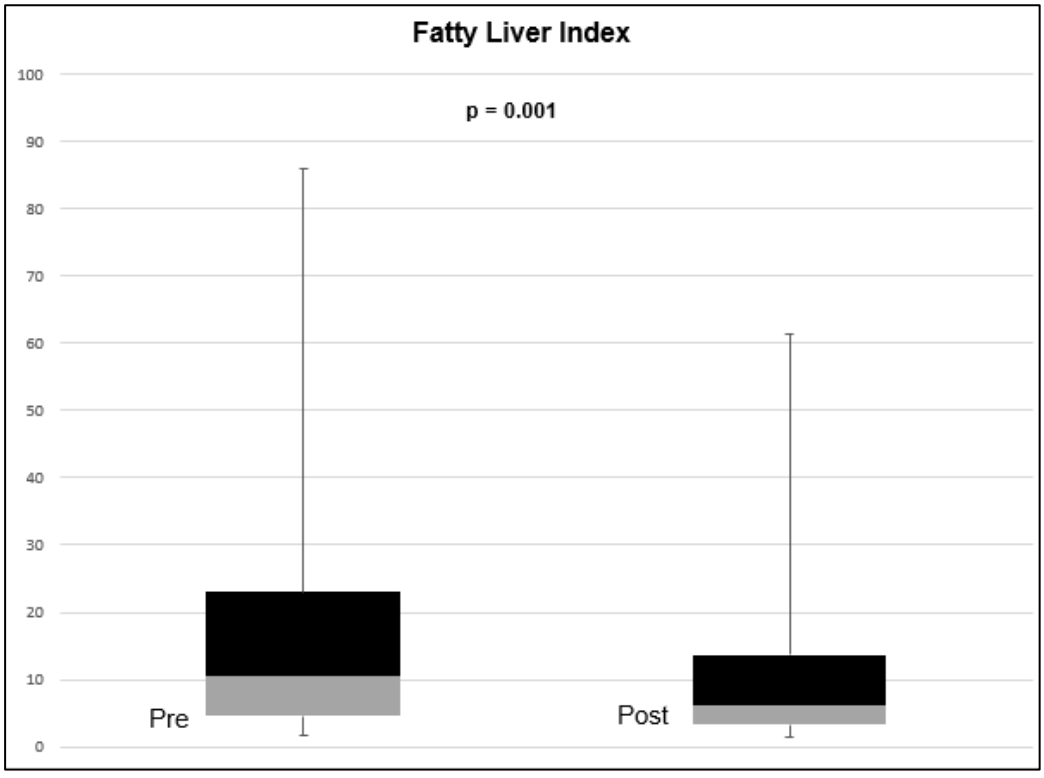

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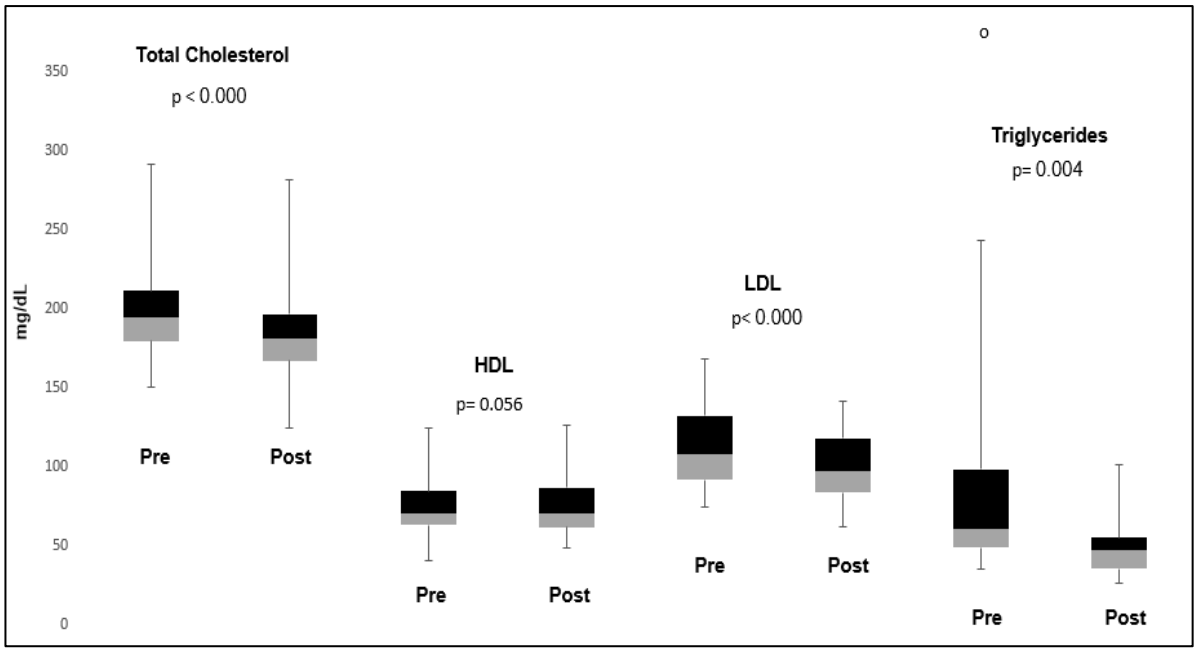
Figure 7: Boxplots showing total cholesterol, HDL, LDL and triglycerides at baseline (Pre) and post intervention (Post).



Discussion

The aim of our study was to investigate if a 4-day period simulating a modern day version of Paleolithic conditions has the potential to demonstrate signs of favorable metabolic and inflammatory effects on already healthy and fit adults. In 2012, the first author of this paper followed a hunter and gatherer-type lifestyle over 10 days in the Spanish Pyrenees. Afterwards, our study group initiated the current Eifel study starting with a small pilot group in 2013 [36], and followed by this larger 25-person cohort in this follow-up study presented here. For this study, we estimated that the 10-day intervention performed by Freese in 2012 would be too demanding for sedentary modern people not acclimated to living in this manner. Additionally, considering that the Asian forest bath studies [37-40] impressively impacted several health markers after short hiking trips in woodlands compared to walks in a city, we estimated that favorable effects on metabolic and inflammation parameters would be observable after a shorter intervention period.

Although humans' metabolic system depends on a sufficient replenishment of macro- and micronutrients, it is flexible to produce sufficient energy under multiple environmental circumstances. The range of challenges to produce sufficient energy include everything from seasonal abundance and deprivation of food(s), to even sustaining of energy over long journeys without food to the rapid production of energy to escape predators [41, 42]. Hence, over millennia, humans evolved to develop

metabolic flexibility to survive the variety of situations likely faced over the span of time required to successfully reproduce.

The organ demanding the most protection from metabolism is the human brain [43, 44]. Neurons, however, do not store energy substrates but do show an extraordinary flexibility in producing energy from a variety of substrates including glucose, their primary fuel source, ketone bodies, and lactate. This flexibility guarantees adequate energy provision in the face of fluctuating environment and physiological circumstances. Thus, the human brain does not depend on continuous fueling with a single source of fuel.

Today, overnutrition is a major issue affecting health [19, 45]. Due to the learned ability of humans to create foods that leverage the preferences of our central nervous system, we are not only constantly surrounded by food in our modern environment, but we are also surrounded by foods that encourage us to eat in the absence of hunger [46]. Therefore, the obesogenic environment of modern humans has led to, among other things, an internal condition with impaired capability to allocate energy from alternative fuel sources other than glucose, especially for the needs of neurons. This is due to the fact that these alternative energy systems are rarely, if ever, required to be the dominant source of energy provisions [47, 48]. In affluent societies, food is not only consumed in order to maintain energy balance but also for hedonic attributes independent of energy status, and this might be one of the reasons for calorie excess [49, 50]. This situation is then compounded by prevalent physical inactivity [22, 51] and sleep disturbance [52], all combining to lead to severe metabolic disturbances such as metabolic syndrome and T2D mellitus over time [21, 53].

Calories

It has been hypothesized that human body composition and selected metabolic parameters are able to return to a more ancestral-like state, when diet and lifestyle resemble hunter-gatherer conditions. More than 30 years ago, O´Dea *et al.* showed that returning diabetic, urbanized Aboriginal Australians [41] to their natural habitat for 7-weeks of a hunter-gatherer lifestyle could normalize glucose metabolism and reverse insulin resistance. The favorable health outcomes of that study, however, were likely impacted by the substantial negative energy balance observed in participants, making it hard to determine if it was the lifestyle, the caloric restriction, or both that were the cause of the metabolic improvements.

In our study, participants were asked to imitate life as a hunter-gatherer. With this goal in mind, our protocol implemented a daily shortened eating window (i.e., intermittent fasting) allowing only two meals per day. All provided foods complied with the tenets of the Paleo diet offered by Cordain and colleagues [6, 17, 31]. Physical activity, most of which was conducted under fasting conditions, also aimed to emulate the amount, intensity, and modality of hunter-gatherers [14, 54]. Lastly, the environment with which the study was conducted was also considered natural. The participants were kept in the wild, observing plants and animals, and exposed to the natural vicissitudes of environmental light and temperature. Modern day technologies that minimize the fluctuations in these natural signals – such as sunglasses during

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the day, artificial light at night, a range of thermo-neutralizing clothing – were also excluded or minimized (Table 6).

Table 6: Simulated Paleolithic conditions over the course of the 4-day intervention.

Conditions	Activities
Physical	Hiking
	Swimming in a river
	Climbing
	Collecting and lifting wood
External	Reduced calories
	Less frequent food intake
	Intense sunlight
	Exposure to bugs
	High temperatures during the day
	Cool temperatures morning and nights
Physiological	Thirst
	Hunger
	Sweating and freezing
	Aching muscles
Other	Building a fire
	Group socializing
	Searching for suitable night camp
	Orientating in the forest
	Looking for wild foods
	Watching wildlife
	Sunbathing

In our study, the average daily food allotment per person per day was 1,747 kcal. Because the participants in our study were given a definitive amount of food, and because of the high degree of physical activity per day, we do not know how much food these people would have eaten if food was provided ad libitum. Thus, like the O'Dea study mentioned above, our study suffered from the same limitation of not knowing whether our findings were a result of negative calorie balance or other aspects of the lifestyle intervention we implemented. Additionally, it is likely that our daily calorie total was artificially high. As mentioned, 28 participants were originally enrolled but only 25 participants completed the protocol. Daily calorie intake, however, was estimated based on the total amount of food provided to participants over the four days – without the benefit of any food not consumed being factored into the calculation – divided by the number of participants who completed the study. The three participants who dropped out did consume one daily fruit pack and one dinner before dropping out. Those calories have been factored into our equation for average daily energy intake per participant.

Body Measurements

The average change in body fat was -10.4% and the average change in visceral fat was -13.6%. While the changes in these measurements are impressive, it is critical to note that our methods of measuring both parameters, bioelectrical impedance, is subject to large fluctuations depending upon hydration status and is generally not considered accurate when compared to gold standard measurement methods. It is also likely that our subjects were less hydrated after the four-day intervention which would explain some or all change in both parameters.

We also observed a 48.7% reduction in triglycerides and a 41% reduction in the FLI. Given that 10-35% of Western people suffer from a non-alcoholic fatty liver disease [55], the remarkable reduction of the triglyceride and FLI in only four days intrigues us that brief outdoor trips based on humans' primal behavior patterns should be investigated further to help the spreading metabolic epidemic.

Insulin

Previous research by Lindeberg observed a lower mean insulin (-50%) concentration in primal living Kitava islanders aged between 50-74 years when compared to a sample of age-matched Swedish people characterized by a typical Western lifestyle pattern [15]. In line with those findings, our data show a 44.4% decrease in mean fasting insulin from baseline to the end of the intervention (from 8.94 ± 7.52 uU/ml to 4.97 ± 2.11 uU/ml; p=0.016), as well as a 6.5% decrease in mean fasting glucose. Together, this lead to a remarkable reduction of HOMA (-49.3%; from 1.85 ± 1.83 to 0.94 ± 0.46; p=0.022). Also notable is the reduced variance in the post-intervention values, compared to baseline, for both fasting insulin and HOMA scores. This reduction in variance indicates that most of the people in the study clustered around the lower, presumably healthier values for both measures in response to the intervention. Similarly, a study by Frassetto et al. [55] in people following a Paleolithic diet for three days also showed markedly reduced mean values and reduced variance in response to the intervention for both fasting insulin and HOMA. In light of a worldwide expanding T2D epidemic, it is striking that such a short intervention presented here, even with healthy subjects, has yielded comparable figures to the findings by Lindeberg in the natural habitat and Frassetto in a clinical setting [15, 55].

Inflammation

Intriguingly, the acute phase protein CRP increased by 67.1%. This protein is produced by the liver in response to elevated concentrations of interleukin-6, which is distributed by macrophages and adipocytes in order to orchestrate pathogen-induced inflammation, among other functions. There are several plausible explanations for this large increase in this inflammatory signal.

The radical change from a more sterile modern environment into a wild habitat – replete with bacteria, parasites, fungi, and phytoncides (wood essential oils) - could have stimulated a response of the innate immune system [36, 57, 58], possibly explaining the large increase in CRP observed in our study. Recently, a study by Gurven et al. [59] of the Tsimane, a Bolivian tribe in the Amazon, showed that these forager-horticulturalists have a level of white blood cells that is ten times the level of

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the US population. This is unsurprising given the fact that approximately 70% of the Tsimane people are infected by parasitic helminthes. We did not test our participants for parasitic infections but these infections are rare in our study population. Future studies should evaluate whether people who are exposed to nature on a regular basis show lower inflammatory reactions during a nature trip similar to what was used in our study. Indeed, evidence suggests that those who take regular walks in forested areas show reduced inflammatory cytokines [39].

Despite the fact that our participants were healthy and fit, it is possible that the amount and type of physical work - mostly under fasting conditions - could have stimulated a significant stress response and cell damage. Animals in starvation or under severe stress load show elevated uric acid levels [60] - a by-product of cell destruction - which can promote the release of CRP as a part of an acute immune response [61-63], which further stimulates the production of antibodies from B-lymphocytes to fight against pathogens [64]. This stress marker has also been shown to increase after long-distance runs [65, 66]. While our study did not measure uric acid levels, future studies should monitor this marker directly and do a correlation analysis with it and CRP.

Conclusion

At this stage, our case and pilot studies indicate that there could be a positive health effect during a short-term implementation of a Paleo-like nature trip. We believe our findings justify more advanced investigations into this method to influence health. Future studies should aim to use better controls to investigate several things. First, what aspects of this lifestyle, if any, are most impactful in promoting health? Second, are the effects of a short-term Paleo-like nature trip durable? Third, are there meaningful side effects to such an intervention, and what populations might benefit most? Fourth, what is the ideal time and number of days needed to maximize the benefit of this style of health intervention?

Different components of a lifestyle pattern – food, physical activity, sleep, etc. – all independently influence the internal metabolic milieu. Assessing attribution of potential effects from a multifactorial health program, given the complexities of eliminating confounds, does generate important questions as to what factor, factors or combination of factors is having an influence. We must acknowledge, however, that potential synergies exist in improving several health influences simultaneously, even when it’s hard to determine why. Additionally, focusing on the net impact of a dynamic lifestyle pattern on health and disease - even if it’s implementation is only short term, like with our protocol – still has great merit.

This study indicates that a short trip, where modern humans adjust their behavioral patterns to simulate a more Paleolithic-like condition, could serve as an effective strategy to help prevent the dangers of metabolic diseases. Particularly, the major findings of an expeditious reduction of HOMA and FLI in only four days may reveal the potential of exceptional benefits even when compared to long-term, single interventions such as dietary or fitness programs.

Abbreviations
BIA: bioelectrical impedance analysis; CRP: High-sensitive C-reactive protein; FLI: Fatty liver index; HOMA: Homeostasis model assessment-index; T2D: Type 2 diabetes; WD: Western diseases.

Conflicts of interest
The authors declare that they have no conflicts of interest.

Authors´ contributions
JF conceived and designed the experiment. JF, SS performed the experiment. JF, RH, BR analyzed the data and performed statistical analysis. JF and DP wrote the manuscript. All authors read and approved the final manuscript.

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