RESEARCH ARTICLE

A pilot case study on the impact of a self-prescribed ketogenic diet on biochemical parameters and running performance in healthy and physically active individuals

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Abstract

Background: Ketogenic diets (KDs) have gained some popularity not only as effective weight-loss diets and treatment options for several diseases, but also among healthy and physically active individuals for various reasons. However, data on the effects of ketosis in the latter group of individuals are scarce. We therefore collected pilot data on the physiological response to a self-prescribed ketogenic diet lasting 5-7 weeks in a small cohort of healthy and physically active individuals.

Methods: Twelve subjects (7 males, 5 females, age 24-60 years) who followed moderate to intensive exercise routines underwent blood testing, bioelectrical impedance analysis (BIA) and spiroergometry during an incremental treadmill test. On the next day, they went on a self-prescribed KD for a median of 38 days (range 35-50 days), after which the same tests were performed again. Ketosis was self-monitored by urinary ketone strips. Subjective feeling during the diet was assessed by a questionnaire after the intervention. Due to the small and heterogenous sample, the results are interpreted in the context of the already existing literature.

Results: The KDs were tolerated well by the majority of individuals. Impaired recovery from exercise remained the most frequently reported side effect until the end of the study. Most blood parameters remained stable during the intervention. However, there were significant elevations of total and LDL cholesterol concentrations ($p<0.01$) and a trend towards increased HDL-cholesterol ($p=0.05$). The drastic reduction of carbohydrates had no statistically significant influence on running performance judged by the time to exhaustion, VO2max and respiratory compensation points. BIA measurements showed significant increases in phase angle ($p=0.01$) indicating improvements of body composition with an estimated decrease of 3.4 kg of fat mass ($p=0.002$) and gain of 1.3 kg of fat free mass. We discuss the validity of these estimates taking into account a possibly altered hydration status due to the KD.

Conclusions: Active healthy individuals will probably experience no major problems during a short term KD lasting several weeks. The drastically reduced carbohydrate content of the diet seems to be no limiting factor for running

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performance. In addition, improvements in body composition can be expected. While most biochemical parameters are not influenced by the diet, there seems to be an impact on the blood lipid profile that could be considered problematic with respect to cardiovascular disease risk. However, the predictive role of cholesterol levels alone in individuals undergoing regular physical activity remains to be elucidated.

Keywords:
High fat diet, exercise, bioimpedance analysis, blood lipids; fat loss; running performance

BACKGROUND

Ketogenic diets (KDs) are characterized by a very low carbohydrate (CHO, <30-50 g/day) and compensatory high fat content that lead to low insulin and subsequently increased ketone body levels in serum, also termed ketosis. Ketosis seems to have beneficial effects in certain pathological conditions, most notably epilepsy where it is the treatment of choice for patients with drug-resistant intractable seizures [1, 2], but also metabolic syndrome [3–5] , cancer [6] and possibly Alzheimer's and other neurodegenerative disorders [7]. In addition, KDs have gained much popularity as weight-loss diets, where they have been shown to be at least as effective or even superior to traditional low-fat diets in obese individuals [8–12] with the additional benefit of favourably influencing risk factors for cardiovascular disease.

But can a KD be recommended also for healthy individuals? This question is justified because we cannot a priori expect the same effects from a particular diet to happen in two groups of individuals that have different metabolism, lifestyle habits and health status. Surprisingly, the literature on the effects of KDs in healthy and physically active individuals is scarce, although in our experience such diets have some popularity among metabolically normal people and even athletes. First, formerly obese individuals who succeeded in losing weight on a KD might like to continue with this lifestyle change. Second, physically active people often aim at optimizing their lean- to fat-mass ratio or losing a few kilograms of weight in preparation for a competitive or social event. Third, many athletes are interested in improving their ability to mobilize their fat stores for fuel during exercise, especially in long endurance races. Indeed, studies suggest that KDs dramatically shift the substrate utilization from glucose to fat (and ketone bodies) at submaximal training intensities [13–18]. In their seminal study on trained cyclists, Phinney et al. further proved that at these intensities exercise performance can be conserved when allowing for a few weeks of adaption to a KD [15]. Similarly, Brinkworth et al. showed that an 8-week KD had no detrimental effects on exercise performance in a treadmill test to exhaustion in untrained obese subjects [18]. This contrasts to similar, but shorter-term studies that have reported reduced capacity for endurance exercise [19, 20]. Recently, Paoli et al. [21] demonstrated that a KD could be an option for athletes who want to lose fat mass without compromising muscle mass and strength. In this study, nine elite artistic gymnasts lost an average of 1.9 kg fat while maintaining their performance in several anaerobic strength tests over a period of 30 days. It should be noted, however, that these authors defined their diet as ketogenic despite a mean protein energy content of 40.7 % (i.e. 201 grams daily, surely above the protein needs of the athletes) and without providing information on ketone body measurements. High protein levels

diminish ketone production by favouring gluconeogenesis from abundant amino acids as already described by Hirschfeld in 1885 [22].

Only a few other studies have examined the metabolic and physiological responses to a KD lasting at least several weeks in healthy and physically active individuals. The first one was the study on the arctic explorer Vilhjalmur Stefansson and his comrade Karsten Andersen who consumed a diet of solely meat and fat for 12 months [23–25]. Besides an elevation in total cholesterol levels, no adverse effects on biochemical parameters, blood pressure or kidney function were noted and the two men "carried on their usual activities without any increase of fatigue while taking meat" [24]. In 1967 Krehl et al. [26] studied four normal-weight men who underwent supervised physical activity daily and ingested a KD of varying protein and fat content for a total of five months, whereby two of the men received an additional 50 g of CHO daily. The most significant changes were an increase in total cholesterol levels that was less pronounced in the men receiving the extra CHOs, and in blood urea nitrogen that increased in accordance with the protein content of the diet. 14 years later, Elliot et al. [27] confirmed these findings in a young female and male who ingested a KD for 24 days and showed that the increase in cholesterol levels was mainly due to an increase of LDL concentration. This led the authors to express deep concerns about the safety of a KD, because at that time LDL concentrations emerged as strong positive predictors of cardiovascular disease (CVD) risk. However, the results of an experiment published by Matthew Sharman, Jeff Volek and colleagues indicate that despite a rise in LDL concentrations, the overall cardio-vascular risk profile could change in a favourable way during a KD, e. g. by increased HDL and lowered triglyceride concentrations [28, 29]. This fits well with observations of an improvement of serum lipid patterns in obese adults after a one year KD despite increased LDL concentrations [12]. That LDL con-centrations levels stay elevated during long-term consumption of a KD is further suggested from a Polish cross-sectional study of 31 healthy individuals who ate according to the so-called "optimal diet" for at least one year [30]. Although not strictly ketogenic, the "optimal diet" consisted of about 75 % energy from fat. Most of the LDL and total cholesterol concentrations exceeded the reference ranges, yet the "optimal" dieters showed very low risk for CVD as judged by other predictive parameters.

The paucity of data regarding physiolo-gical changes that occur in healthy individuals on a KD implies a high uncertainty in predicting these changes; in addition, large individual differences seem to exist in the response to a KD. From our experience coun-selling cancer patients (UK) or athletes (JP), most individuals are highly interested in how the KD affects their ability to exercise. We therefore set out to collect new data on the effects of a KD in healthy and physically active individuals to add to and interpret in the context of the existing literature. We decided to study the effects of a KD as it would be adopted in a real-life situation by self-motivated, physically active and healthy individuals, focussing on changes of physical performance measures and biochemical parameters. In this paper we report on results from twelve subjects who were willing to adopt a KD for at least five weeks without financial compensation.

MATERIALS AND METHODS

Study design

This study was designed as an interventional case study without control group. The trial was conducted between 14 February 2012 and 10 August 2012. All testing procedures

were performed at PREDIA prevention and diagnostic center (PREDIA GmbH, Würzburg, Germany), which also provided the casualty insurance for our study subjects. All interventions were done in accordance with the International Conference on Harmonization guidelines and the Declaration of Helsinki and Good Clinical Practice guidelines [31].

Study participants

We searched for physically active adult study participants among private contacts and members of a local triathlon and weight lifting club. Exclusion criteria were defined as a previous diagnosis of the metabolic syndrome or usage of a KD during the six months prior to recruitment. After explaining the goals and course of the experiment as well as the possible side-effects associated with a KD and risks of the testing procedures, a total of ten individuals (six men and four women) volunteered to participate; in addition, two of the authors (RK and UK) took part in the study. All participants signed a written informed consent form prior to the first tests. Subjects had a high socioeconomic status and were physically active, non-smoking and – since we were not able to offer financial compensation – highly motivated. None of the subjects took any medication except for subject 11 who used a thyroxin substitute at 75µg/day. Subjects 1, 2, 4 and 11 had already tested ketogenic diets temporarily before, but in no case during the six months preceding the study initiation. The remaining eight persons had no experience with low CHO diets or ketogenic diets before starting the trial.

Details of our study participants concerning the type and duration of their training are provided in Table 1.

Testing procedure

On day zero (subsequently denoted PRE), participants reported to the lab after fasting for at least 6 hours. They were advised to restrain from strenuous exercise the day before as if preparing for a hard training session. Venous blood samples were collected into tubes containing either EDTA (blood count analysis) or sodium fluoride (glucose/lactate analysis) or no additive (serum generation for all other analyses). The serum tubes were stored at room temperature for 15 minutes to allow clotting of the blood samples and then centrifuged at 1500 g at 4 °C. The serum was then transferred to a new tube, and all tubes were sent immediately to the central laboratory of the University Hospital of Würzburg for routine laboratory analysis. Shortly, complete blood count without differential was performed on a XE 2100 automated hematology system (Sysmex Europe GmbH, Norderstedt, Germany). The chemistry/biochemistry panel was analyzed with the Cobas 8000 modular analyzer series (Roche Diagnostics; Mannheim, Germany).

Next, we performed bioelectrical impedance analysis (BIA) measurements following a standardized procedure according to the ESPEN guidelines described in Table 1 of Kyle et al. [32]. Electrodes were placed on hand and wrist and foot and ankle, and resistance R and reactance Xc to a 50 kHz current were measured by the BIA device (BIA 101; Akern Srl, Florence, Italy). The phase angle α, given as $\alpha = \arctan(Xc/R)$, was also recorded and uncertainties for α derived from the uncertainties of R and Xc given by the manufacturer as ±1 Ohm and ±2 Ohm, respectively. Each subject's height, weight and age were used together with R and Xc to estimate extracellular water (ECW), intracellular water (ICW), total body water (TBW = ECW+ICW), body cell mass (BCM), extracellular mass (ECM), fat free mass (FFM = BCM+ECM) and fat mass (FM = body weight-FFM) based on standard equations applicable to healthy adult individuals.

Tab. 1: Characteristics of our study subjects at PRE.

Subject	Gender	Age [years]	Weight [kg]	Height [cm]	BMI	Main sport	Training frequency [sessions week^{-1}]	Training time [hours week^{-1}]
1	male	32	74.9	182	22.6	Triathlon	10	11
2	male	29	78.5	183	23.4	Triathlon	6	6
3	male	29	77.5	180	23.9	Soccer	4	4
4	male	32	80.5	176	26.0	Tennis	2	3
5	male	24	87.4	186	25.3	Cycling	5	6
6	male	60	70.1	176	22.6	Running	2	2
7	male	36	93.5	172	31.6	Weight lifting	4	6
8	female	32	61	165	22.4	Triathlon	6	10
9	female	25	68.7	164	25.5	Triathlon	6	7
10	female	24	68.3	178	21.6	Running	2	1
11	female	46	72.4	171	24.8	Fitness training	3	4.5
12	female	35	47.5	158	19.0	Weight lifting	4	6

To take into account the intra-individual prediction errors in FFM given as 3.5-6 % by Kyle et al. [33], we generated for each subject 100000 Monte Carlo simulations of FFM and FM, whereby for calculation of the latter we neglected any uncertainties in weight measurements. Briefly, a new FFM measurement was simulated by drawing a random number with mean located at our measurement value and standard deviation equal to the assumed prediction error. Simulated p-values were obtained as the percentage of simulated datasets in which FM increased and FFM decreased, respectively.

Fifteen to thirty minutes after the BIA measurement, a graded running test to exhaustion was performed on a treadmill (Jaeger LE 580 C) to determine maximal oxygen consumption ($\dot{V}O_{2max}$) and substrate turnover at a given workload. During the test, air temperature was kept at 20-22 °C. Heart rate (HR) was monitored (Sport Tester; Polar Electro, Kempele, Finland) and oxygen consumption rate ($\dot{V}O_2$), carbon dioxide production rate ($\dot{V}CO_2$) and ventilation ($\dot{V}_E$) was recorded on a breath-by-breath basis (Jaeger Oxycon Pro, Carefusion Germany 234 GmbH, Höchberg, Germany). The gas analyser was calibrated with known gas mixtures (14.99 Vol% O_2 and 5.80 Vol% CO_2). Subjects started to run at 7.5 km/h. Every 3 min, velocity increased by 1.5 km/h until voluntary exhaustion was reached. $\dot{V}O_{2max}$ was determined at the point where (i) the oxygen consumption rate increased less than 1 ml kg^{-1} min^{-1} despite an increase in intensity, or (ii) the respiratory gas exchange quotient (RQ) reached 1.1. Alternatively, $\dot{V}O_{2peak}$ was estimated as the average oxygen consumption rate during the last 30 s if voluntary exhaustion was reached before conditions (i) and (ii). The respiratory compensation point (RCP) was determined from the minimum in the $\dot{V}_E/\dot{V}CO_2$ curve with

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relative heart rate as the reference [34]. After every three minutes, the treadmill slowed down for 30 s so that subjects could stand beside it for taking a 10 µl capillary sample from the ear lobe. In this way, samples were taken at rest, every three minutes during exercise, at time of exhaustion and three minutes after exercise and blood lactate and glucose concentrations were determined using an Super GL Ambulance Lactate Analyser (Dr. Müller Gerätebau GmbH, Freital, Germany) which according to the manufacturer has a 24 samples variation coefficient <1.5 % for glucose and <2.5 % for lactate.

The same testing routine was used for the final examination, which took place 35 to 50 days (median 38 days) after starting the KD (subsequently denoted as POST).

Study intervention

The duration of the study was initially chosen to last approximately from the beginning until the end of the paschal fasting period (~ 40 days), in part to enhance motivation for some individuals.

Subjects were provided with handouts similar to those used in the study of Cervenka et al. [35]. The handouts summarized the main aspects of a ketogenic diet and included a list of suitable foods with very low CHO content as well as ideas for suitable meals. Furthermore, subjects were provided with cooking recipes and an information brochure which had been created for a KD study with cancer patients [36]. We advised the participants to eat *ad libitum*, but limit their CHO intake to a maximum of 20 g/day with no more than 5-7 g digestible CHO per meal and to derive at least 75 % of energy from fat. Apart from that, subjects were free to design their KD according to their preferences without any further nutritional counselling. However, we offered to answer any questions that might arise in context with the KD via email, phone or personal contact. Recently, Cervenka et al. have shown that administering a KD over three months solely via email is feasible and effective in adults with relatively high socioeconomic status, which was also the case for all our participants [35].

Each subject also received 50 reagent strips for self-testing of urinary acetoacetate (Ketostix; Bayer, Basel, Switzerland). The handouts included a calendar to document daily measurements of urinary ketones and weight; the former ought to be taken preferably in the afternoon and not after exercise, the latter in the morning before breakfast. In addition, we obtained a random sample of three-day food diaries from five participants which were analysed using the software DGE-PC professional version 2.8.026.

Importantly, in order to not bias the results of the second treadmill test, we advised all participants to maintain their usual training frequency during the study intervention without increasing or decreasing the training load.

After the study, each subject received a short non-validated questionnaire designed by the authors via email addressing several aspects of their subjective feeling during the KD (see Additional file 1).

Statistical analysis

Due to our heterogeneous sample population and small sample size, we use median, minimum and maximum values to characterize parameter distributions. The Wilcoxon signed rank sum test for paired samples is used to test the null hypotheses that no changes in a parameter of interest occurred from pre- to post-diet. Due to the explorative nature of this trial and our small sample size, we report two-sided p-values but refrain from using p-value corrections [37]. Thus, p-values <0.05 should be considered a trend and $p<0.01$ significant in the sense that further studies are needed to confirm our findings. As an alternative to post-hoc power

analysis, we provide 95 % confidence intervals (CIs) for all parameter changes. Descriptive statistics are used to summarize the results of our non-validated questionnaire. All statistical tests have been conducted using R version 2.15.2 (http://www.r-project.org).

RESULTS

All subjects completed the study with no major side effects. Median time on the diet was 38 days (range 35-50 days). Urinary ketone tests were recorded on 95 % of the days (92-100 %). Median time until the first appearance of acetoacetate in the urine was 4 days (1-5 days). Thereafter, a median of 95 % (59-100 %) of urinary ketone tests were positive, ranging between 0.5 and 16 mmol/l (median 1.5 mmol/l).

According to software-based analysis of three-day food records from five subjects the mean intake of CHOs was 16.5±1.1 g/day and therefore as low as prescribed (<20 g). Energy intake for the two males and three females was 3050±1360 kcal/day and 2030±330 kcal/day, respectively. The mean relative energy intake of CHOs, fats and protein was 3.0±0.4 %, 68±5 % and 29±5 %, respectively. Fat intake was composed of 69±33 g/day saturated, 93±55 g/day mono-unsaturated, 3.1±0.6 g/day omega 3 and 16.3±8 g/day omega 6 fatty acids as well as 1.2±0.6 g/day cholesterol, contributing 26±6 %, 34±7 %, 1.2±0.3 %, 6.1±1.1 % and 0.4±0.2 % to total energy intake, respectively.

Individual tolerance to a ketogenic diet

As already stated, study participants were highly motivated in the beginning. Some minor side effects like dizziness, headaches or general weakness disappeared within the initial 1-2 weeks after implementing the diet. However, subject 3 reported to be in a bad mood for most of the time and complained about limited food choices and bad practicability of the diet; nevertheless, he continued with the diet for a total of 37 days, of which he recorded elevated ketone bodies on 26 days.

Ten of our twelve subjects returned the questionnaires we sent out after the study (see Additional file 1). Practicability of the diet was judged as “very easy” or “easy” by four and five of the subjects, respectively, while subject 3 judged it as “difficult”. Effects on satiety were highly heterogeneous, with four subjects reporting no changes in hunger, four subjects reporting less hunger and two subjects more hunger than before. The most frequently missed food items were fruits (eight subjects) followed by unlimited amounts of vegetables (four subjects). Only two subjects reported a craving for other foods like baked goods. Most subjects were able to continue with their usual training, albeit with minor problems including increased perceived exertion and subjectively increased needed time of recovery. Three subjects had to decrease their training load due to impaired recovery and the inability to complete high intensity workouts. In particular, subject 1 lost a total of 12 training days due to an acute sinusitis starting on day 7 of the diet with subsequent relapse on day 29. For this reason, we decided to extend his diet period to 50 days. All participants consistently noted a subjective improvement in performance after 2-3 weeks and all but one reported feeling “very good” or “good” when not exercising. Positive reported effects that subjects related to the diet included less tiredness or improved power of concentration, respectively (four subjects) and feeling more balanced (three subjects). Impaired recovery after physical exercise at high intensities was reported to remain the strongest side effect until the end of the diet.

Anthropometric parameters

Most subjects experienced a decrease in body weight over the course of the study. The median self-monitored relative weight change

until day 35 for the whole group was -3.1 % (p=0.02). The difference between the first and last of the self-performed weight measurements was highly correlated to the difference between our laboratory measurements at PRE and POST (r=0.93, p<0.0001), proving the validity of the self-monitoring.

Our BIA measurements indicate that the KD induced pronounced changes in body composition (Table 2 and Figure 1). The phase angle α increased significantly from a median of 6.6° (4.8°-7.9°) to 6.9° (6.1°-8.6°) (p=0.01), leading to significant changes in other indirectly derived quantities: increases of intracellular water (ICW) from 24.8 l to 27.3 l (p=0.003) and body cell mass (BCM to which ICW contributes) from 32.3 kg to 35.8 kg (p = 0.003) as well as a decrease of fat mass (FM) from 16.4 kg to 13.0 kg (p = 0.002). Fat free mass (FFM) increased from 57.5 kg to 58.6 kg (p = 0.08) and extra-cellular water (ECW) decreased from 18.6 kg to 17.4 kg (p = 0.08) at POST. As an alternative representation, we have plotted these changes scaled to body height in Figure 1.

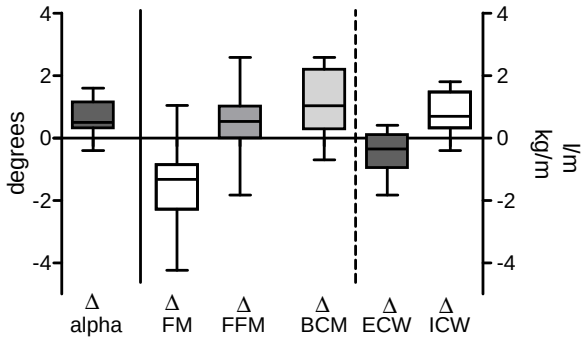

Figure 1: Box and whisker plot of changes in phase angle and body composition estimates from our bioimpedance measurements. The latter have been scaled to the individual's body height. Alpha: Phase angle; FM: Fat mass; FFM: Fat free mass; BCM: Body cell mass; ECW: Extracellular water; ICW: Intracellular water

Blood cell counts and serum minerals

No significant changes were observed in the count of blood platelets as well as leucocytes and their different subtypes (Table 3). There was a trend, however, for an increase in the percentage of eosinophil granulocytes. Hematocrit increased slightly, probably due to hemoconcentration because of water loss induced by the KD; the ratio between hematocrit and red blood cell count (MCV) remained fairly stable.

Despite not explicitly advising our subjects to supplement with extra minerals, the concentration of sodium, potassium and calcium remained stable and well within the reference ranges. Subjects 9, 10 and 11 initially had iron concentrations outside the reference range (31, 192 and 179 µg/dl, respectively) which normalized to 53, 67 and 120 µg/dl at POST. Ferritin concentrations for the whole group remained statistically unchanged, but in one of our female participants (subject 10) with a low value at PRE (26 ng/ml) ferritin had decreased further to 13 ng/ml at POST.

Liver and kidney parameters

The liver and kidney parameters are summarized in Table 4. We noted a trend towards an increase in the liver enzyme GPT. However, none of the subjects exceeded the reference range for this parameter. No changes between PRE and POST could be noted in concentrations of CRP, alkaline phosphatase and gamma-glutamyltransferase. Subject 9, however, initially showed an elevated CRP concentration of 3.46 mg/dl, possibly due to an acute infection. At POST this value had normalized to 0.23 mg/dl.

Renal function was assessed by measuring the concentrations of creatinine, uric acid and blood urea nitrogen (BUN). Increases occurred in all three parameters, the increase in creatinine levels showing a clear trend (p=0.016, 95 % CI for change from PRE to POST [0.03,0.16]).

Table 2: BIA measurements at PRE and POST

Subject	Weight [kg]		Phase angle (α) [°]		Extracellular water [l]		Intracellular water [l]		Fat free mass [kg]		Fat mass [kg]		Body cell mass [kg]	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
1	75.0	72.0	7.9	8.6	17.9	18.2	28.9	32.0	64.0	68.7	11.0	3.3	39.8	44.2
2	78.5	78.0	7.1	7.6	19.5	19.4	27.9	29.7	64.7	67.1	13.8	10.9	38.1	40.7
3	77.5	73.7	6.8	8.4	18.9	16.5	25.8	28.4	61.0	61.4	16.5	12.3	35.1	39.2
4	80.5	75.0	6.4	7.6	21.5	18.3	27.4	28.3	66.8	63.6	13.7	11.4	37.2	38.8
5	87.4	86.8	6.8	7.2	21.0	20.5	28.6	30.0	67.8	69.0	19.6	17.8	38.9	41.0
6	70.2	67.8	6.1	7.1	19.4	18.3	23.7	26.3	53.9	55.7	16.2	12.1	29.4	32.8
7	93.5	92.1	7.0	6.6	21.1	21.8	29.7	29.0	69.5	69.5	24.0	22.6	40.6	39.4
8	61.0	59.0	5.9	6.3	15.3	14.7	17.9	18.5	45.3	45.3	15.7	13.7	24.1	25.0
9	67.7	70.1	6.1	6.4	16.8	16.2	20.3	20.8	50.7	50.4	18.0	19.7	27.5	27.8
10	68.4	66.3	4.8	6.1	18.3	16.4	16.8	20.0	48.0	49.8	20.3	16.5	22.5	27.1
11	72.4	70.8	5.8	6.3	16.9	16.3	19.3	20.4	49.5	50.2	22.9	20.6	26.1	27.7
12	48.5	48.8	6.7	6.5	12.2	12.8	16.4	16.7	39.1	40.3	9.4	8.5	22.3	22.6
Median	73.7	71.4	6.6	6.9	18.6	17.4	24.8	27.3	57.5	58.6	16.4	13.0	32.3	35.8
95% CI	[-3.0,-0.5]		[0.2,1.0]		[-1.5,0.05]		[0.6,2.3]		[-0.7,2.5]		[-4.1,-1.4]		[0.9,3.3]	
p-value	0.02		0.01		0.08		0.003		0.08		0.002		0.003	

Table 3: Blood parameters measured at PRE and POST

Parameter	PRE [median (range)]	POST [median (range)]	Reference range	p-value	95 % CI
Leucocytes (10^3/mm^3)	5.6 (4.3 – 7.5)	5.1 (4.7 – 6.4)	4.0-10.0	0.61	[-0.8,0.4]
Erythrocytes (10^6/µl)	4.7 (4.4 - 5.2)	4.8 (4.4 – 5.4)	4.4-5.9	0.19	[-0.1,0.3]
Lymphocytes (%)	28 (17 - 48)	31 (20 - 45)	20-40	0.78	[-5,8]
Monocytes (%)	6 (4 - 9)	6 (4 - 10)	0-10	0.52	[-1.5,1]
Thrombocytes (10^3/µl)	230 (138 - 468)	250 (174 - 404)	150-400	0.88	[-31,28]
Granulocytes					
Basophils (%)	1 (0 - 1)	1 (0 - 1)	0-1	n.a.	
Eosinophils (%)	2 (1 - 5)	3 (1 - 7)	0-4	0.02	[1,3]
Neutrophils (%)	59 (43 - 74)	59 (43 - 64)	40-70	0.48	[-11,6]
Hemoglobin content (g/dl)	14.1 (12.9 – 16.0)	14.3 (12.5 – 15.8)	13.3-17.7	0.29	[-0.3,0.6]
Hematocrit (%)	42.8 (38.6 – 46.8)	43.3 (39.0 – 47.7)	40.0-52.0	0.06	[-0.2,2.6]
MCV (fl)	89 (86 - 95)	90 (87 - 94)	81-100	0.33	[-1,2]
MCH (pg)	30.0 (28.3 – 31.0)	29.8 (28.0 – 30.3)	27.0-34.0	0.27	[-0.85,0.55]
MCHC (g/dl)	33.6 (32.4 – 34.7)	33.2 (31.9 – 34.1)	31.5-36.3	0.08	[-1.1,0.15]
HbA1c (%)	5.6 (5.3 – 5.7)	5.4 (5.0 – 5.8)	<6.1	0.11	[-0.35,0.05]

Parameter	PRE [median (range)]	POST [median (range)]	Reference range	p-value	95 % CI
Sodium (mmol/l)	140 (137 - 142)	140 (137 - 144)	135-148	0.76	[-3,2]
Potassium (mmol/l)	3.97 (3.73 – 4.46)	4.07 (3.71 – 4.39)	3.70-5.00	0.73	[-0.13,0.15]
Calcium (mval/l)	4.73 (4.36 – 5.14)	4.70 (4.45 – 5.09)	4.20-5.20	0.52	[-0.18,0.15]
Iron (µg/dl)	90 (31 - 192)	72 (42 - 120)	59-158	0.10	[-60,7]
Ferritin (ng/ml)	54 (25 - 411)	88 (14 - 381)	30-350	0.57	[-9,41]

Table 4: Liver and kidney parameters

Parameter	PRE [median (range)]	POST [median (range)]	Reference range	p-value	95 % CI
CRP (mg/dl)	0.07 (0.04 – 3.46)	0.10 (0.04 – 0.82)	<0.50	0.92	[-1.6,0.3]
GOT (U/l)	25 (14 - 40)	27 (15 - 53)	<51	0.31	[-2,7]
GPT (U/l)	22 (10 - 115)	29 (11 - 39)	<51	0.05	[0,10]
Gamma GT (U/l)	13 (8 - 33)	13 (8 - 20)	<66	0.23	[-10,1]
Alkaline Phosphatase (U/l)	56 (39 - 70)	54 (37 - 67)	40-142	0.43	[-7,4]
Total protein (g/dl)	7.14 (6.86 – 7.44)	7.08 (6.59 – 7.34)	6.50-8.00	0.18	[-0.38,0.17]
Creatinine (mg/dl)	0.92 (0.69 – 1.23)	1.02 (0.74 – 1.36)	<1.20	0.02	[0.03,0.16]
Blood Urea Nitrogen (mg/dl)	33 (22 - 47)	39 (19 - 48)	<50	0.16	[-2,11]
Uric acid (mg/dl)	4.5 (2.8 – 6.3)	4.7 (3.1 – 6.9)	<7.0	0.15	[-0.3,1.2]

Table 5: Blood glucose, lipids and TSH

Parameter	PRE [median (range)]	POST [median (range)]	Reference range	p-value	95 % CI
Glucose (mg/dl)	94 (86 - 110)	88 (74 - 108)	60-110	0.21	[-14,3]
Triacylglycerides (mg/dl)	64 (26 - 91)	76 (35 - 110)	<170	0.11	[-2.5,28.0]
TC (mg/dl)	204 (137 - 518)	277 (199 - 479)	<200	0.007	[21,90]
HDL-C (mg/dl)	92 (46 - 141)	104 (52 - 164)	>40	0.05	[0,23]
LDL-C (mg/dl)	116 (64 - 369)	157 (76 - 327)	<160	0.01	[11,78]
TC/HDL-C	2.6 (1.7 – 4.6)	3.1 (1.6 – 6.4)	<4.0	0.11	[-0.1,0.7]
LDL-C/HDL-C	1.5 (0.6-3.3)	2.0 (0.6-4.7)	<4.5	0.11	[0.0,0.7]
TSH (UIE/ml)	1.56 (0.21 - 5.12)	2.33 (0.19 - 4.61)	0.35-4.50	0.15	[-0.20,1.02]

However, in contrast to the observations made by Phinney et al. in 9 healthy men after a 4-week KD [14], none of our subjects exceeded the reference ranges for BUN and uric acid. The same was true for creatinine except for subject 1 whose creatinine concentration increased slightly above the reference range from 1.23 mg/dl at PRE to 1.36 mg/dl at POST.

Energetic substrates and cholesterol levels

Changes in the levels of blood glucose, triacylglycerides (TAG), lipoproteins and the thyroid-stimulating hormone (TSH) are summarized in Table 5. Blood glucose concentrations remained within the reference range with a non-significant decrease during the study intervention. TAG concentrations increased non-significantly and stayed within the lower reference range. There was a significant increase in LDL-cholesterol (LDL-C) and total cholesterol (TC) concentrations, accompanied by a trend for an increase in HDL-C (Figure 2). We found that TC levels were already elevated above the current reference range (considered to be at 200 mg/dl) at PRE in 7 of our 12 subjects. After the study, however, TC levels exceeded the reference range in all but one of the subjects (subject 11 had a POST measurement of 199 mg/dl). In particular, subject 2 initially showed very high levels of total and LDL -C and was immediately informed about his blood analysis. He subsequently reduced his self-reported intake of eggs and meat and increased the amount of plant fats.

Interestingly, there was a significant gender difference in the LDL-C concentration at POST and the TC/HDL-C ratio at PRE and POST (U-test, all p=0.005) with women having lower values than men (Figure 3).

There are studies suggesting a connection between serum lipoprotein concentrations and TSH even across the euthyroid range [38, 39].

We observed a non-significant increase of TSH which is displayed graphically in Figure 3. However, subject 11 is not representative for the effects of the KD on TSH as she took a thyroxin substitute. Omitting her from the analysis, the change in TSH remained non-significant (p = 0.15).

Performance tests

The results of our performance tests at PRE and POST are shown in Figure 4 with dashed and solid lines, respectively, for each individual. Black and red lines indicate the rise of heart rate and lactate concentrations, respectively, over time and with increasing running speeds. The vertical lines denote the time of exhaustion.

A comparison of test results between PRE and POST is given in Table 6.

It revealed no significant differences in either time to exhaustion, maximum heart rate, absolute and relative peak power output, absolute and relative $\dot{V}O_{2max}$ or heart rate at RCP. While maximum lactate concentrations showed no significant differences, RQ values at exhaustion tended to be lower at POST compared to PRE (1.06 (1.0-1.07) vs. 0.98 (0.92-1.02), p=0.03).

DISCUSSION

By studying a series of twelve cases we investigated the effects of a self-administered KD over at least 5 weeks in healthy and physically active adults. This reflects the conditions probably encountered in real life when an individual decides to implement a KD for a restricted period of time. We would expect such an individual to be highly motivated, physically active and well informed about which foods to eat and which to avoid.

The excellent compliance of our subjects is proven by the fact that they quickly reached ketosis after 1-5 days and remained therein

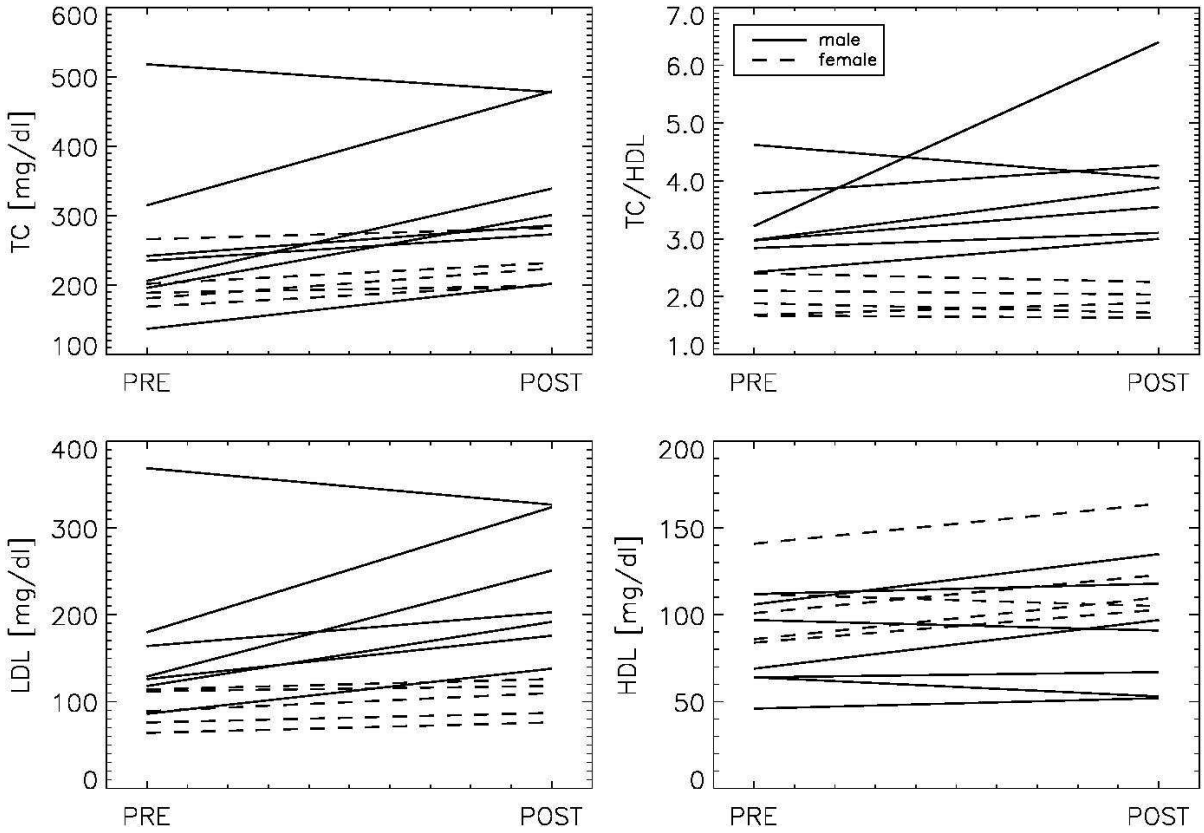

Figure 2: Changes in total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol as well as the TC/LDL ratio. Note the difference between male (solid lines) and female (dashed lines) subjects.

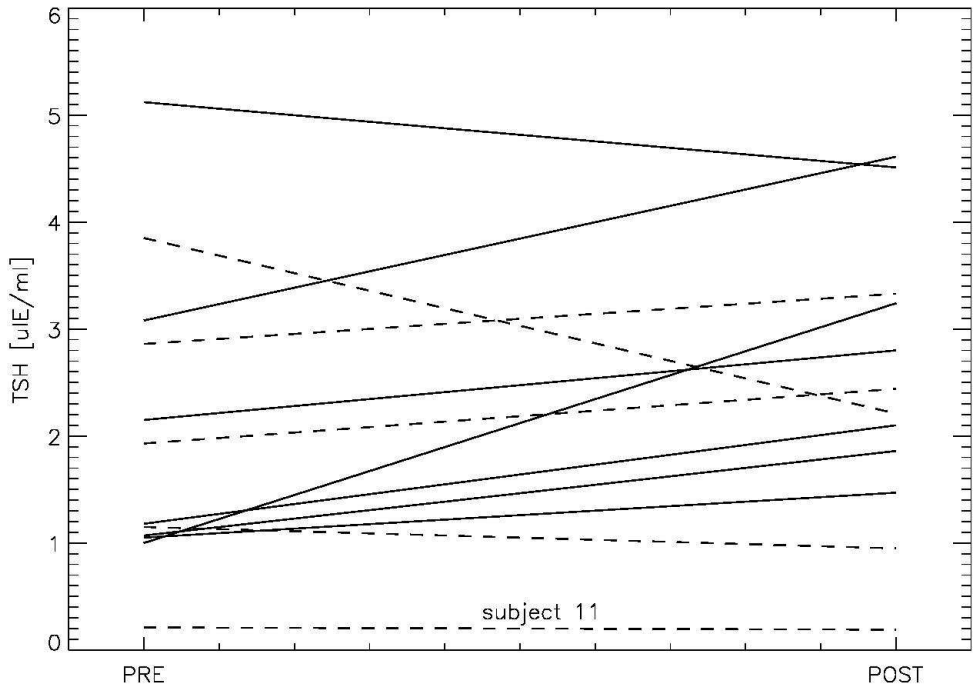

Figure 3: Changes in TSH levels. Gender is coded according to Figure 2. Note that subject 11 controlled her TSH level by taking a thyroxin substitute.

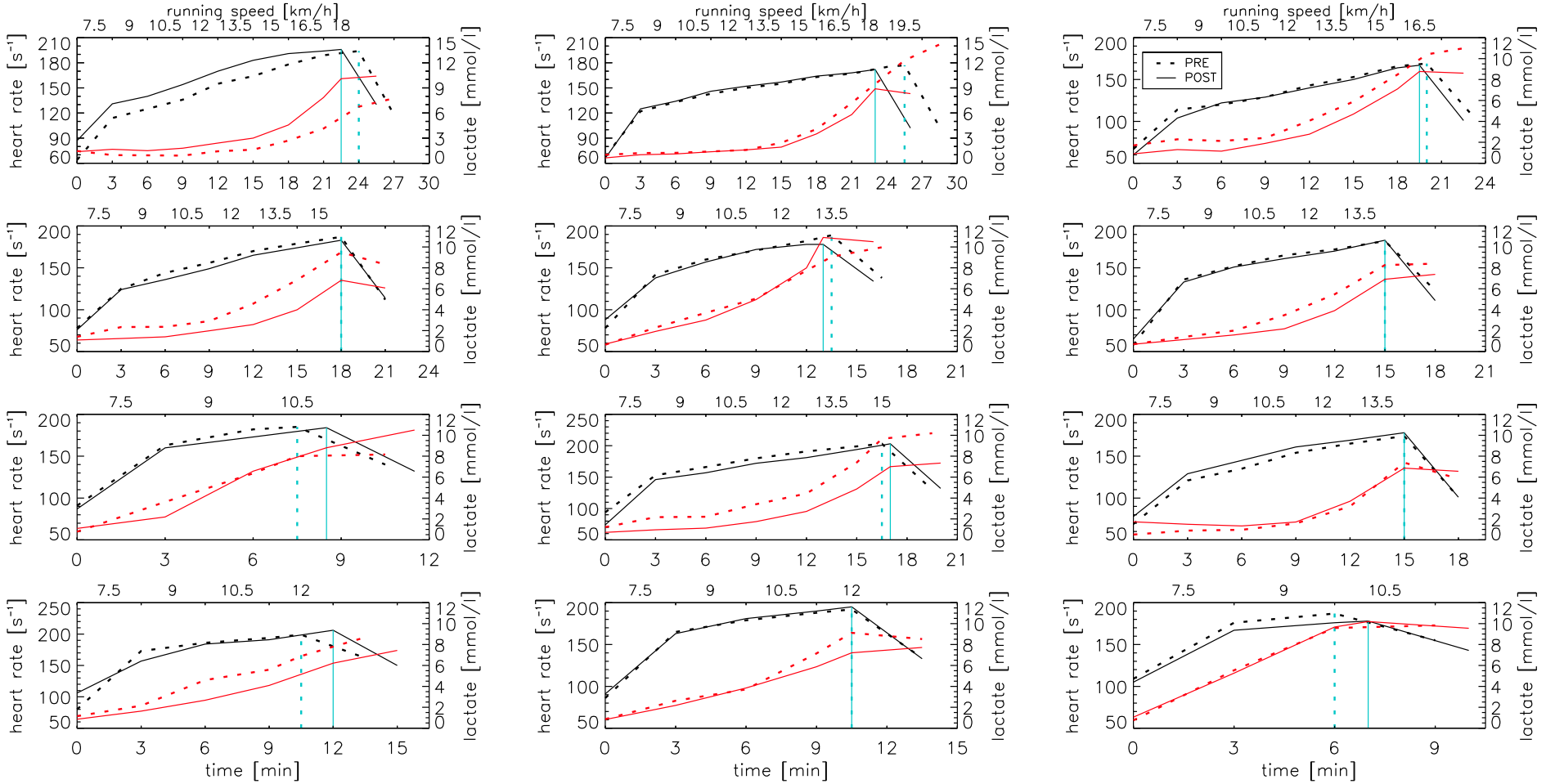

Figure 4: Lactate (red) and heart rate (red) curves at PRE (dashed lines) and POST (solid lines) for all subjects in ascending order from top left to bottom right. The vertical lines denote the time at exhaustion.

Table 6: Treadmill test data

Subject	Time to Exh. [s]		HR_{max} [s^{-1}]		Peak Power [Watt]		Peak Power rel [Watt kg^{-1}]		VO_{2max} [ml min^{-1}]		VO_{2max} rel [ml min^{-1} kg^{-1}]		HR at RCP [s^{-1}]		HR at RCP/HR_{max} [%]		RQ_{max}	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
1	1440	1350	198	195	272	261	3.6	3.6	4643	4132	61.9	57.4	170	172	85.9	88.2	1.06	0.91
2	1530	1380	174	173	285	284	3.6	3.6	5164	5154	65.8	66.1	160	158	92.0	91.3	1.00	0.91
3	1200	1170	167	165	259	245	3.3	3.3	4159	3866	53.7	52.5	150	138	89.8	83.6	1.08	0.92
4	1080	1080	187	182	246	227	3.1	3.0	4267	4196	53.0	55.9	165	168	88.2	92.3	1.13	0.97
5	810	780	189	187	238	240	2.7	2.8	4583	4704	52.4	54.2	171	165	90.5	88.2	0.91	1.02
6	900	900	181	179	190	183	2.7	2.7	3629	3264	51.7	48.1	154	148	85.1	82.7	1.00	0.99
7	450	510	183	183	203	199	2.2	2.2	3608	3351	38.6	36.4	160	158	87.4	86.3	1.07	1.03
8	990	1020	203	201	182	176	3.0	3.0	3436	3420	56.3	58.0	180	181	88.7	90.0	1.06	1.05
9	900	900	174	177	186	189	2.7	2.7	3205	3260	47.3	46.5	158	163	90.8	92.1	1.06	1.02
10	630	720	195	206	164	159	2.4	2.4	2618	2859	38.3	43.1	175	180	89.7	87.4	1.04	0.99
11	630	630	195	193	173	170	2.4	2.4	3375	3201	46.6	45.2	170	175	87.2	90.7	0.91	0.93
12	360	420	184	175	81	98	1.7	2.0	1640	1737	33.8	35.6	171	162	92.9	92.6	1.07	0.97
Median	900	900	186	183	197	194	2.7	2.8	3619	3386	52.1	50.3	168	164	89.2	89.1	1.06	0.98
95% CI	[-60,90]		[-4,3]		[-10,1]		[-0.1,0.3]		[-261,55]		[-1.8,1.8]		[-5,3]		[-2.3,1.7]		[-0.1,-0.01]	
p-value	0.95		0.21		0.08		0.79		0.20		0.97		0.50		0.91		0.03	

for most of the time as judged by the 59-100 % of positive urinary tests.

They also reported that these measurements had been taken temporally offset from any exercise, so that we can exclude ketone production being due solely to exercise. We point out, however, that urinary ketone measurements neither reflect actual endogenous ketone production nor our subject's adherence to the target CHO restriction as ingested protein above ones needs can attenuate ketone production through an increased gluconeogenesis and can increase ketone excretion by elevation of insulin levels. Analysis of available food diaries indicates that adherence to our prescribed CHO intake was excellent (<20 g/day), but protein intake might indeed have been high (29±5 % of energy intake). We hypothesize that this may reflect more protein-rich food choices of physically active individuals on self-prescribed KDs.

The KD was well tolerated by the majority of our subjects as judged by personal contact, email exchanges and the questionnaires they returned after the study. Apart from exercise, most subjects were in a good mood and even reported improvements of several subjective feeling like less tiredness and improved ability for mental concentration. Although all subjects noted a subjective improvement in their exercise capacity after 2-3 weeks impaired recovery thereafter, and increased perceived exertion during more intensive workouts remained the strongest side effects until the end of the study. This can be seen as problematic for individuals undergoing high-intensity exercise in the context of competitive training as in the case of subjects 1, 2, 7 and 12. These side-effects could reflect shortcomings in the self-prescribed diet plans to meet the required micronutrient intakes [40, 41] or to compensate for urinary mineral losses [42] or transient energy deficits due to protein-induced satiety [43] (one third of our subjects reported less hunger during the intervention). Most importantly, however, we think that the KDs resulted in a chronic depletion of muscular glycogen stores [13] that could have caused symptoms of exercise-related fatigue and delayed recovery [44–46]. Low glycogen availability during exercise exacerbates the release of the myokine interleukin-6 that either directly or indirectly through interaction with immune cells may act on the central nervous system to induce fatigue [47]. Although ample protein ingestion after exercise could be sufficient to maximally stimulate muscle protein synthesis [48, 49], additional CHO intake seems necessary to maximize the speed of recovery and attenuate exercise-induced immune suppression [44], at least in subjects not fully adapted to ketosis.

Influence on body composition

Consistent with previous studies, there was a mild weight loss of 1.7 kg. A remarkable finding was the increase of the phase angle α in ten of our twelve subjects, which was due to a decrease of resistance R (reflecting fluid losses) and increase of reactance Xc (reflecting higher capacitive resistance indicative of a better integrity of cell walls). The median increase of 0.3 ° was larger than the individual measurement uncertainties of 0.1 °. α has also been interpreted as a "muscle index" [50], and larger values are related to better outcomes in many clinical situations, e.g. in cancer and malnourished patients [50–53]. Single-frequency BIA at 50 kHz seems to primarily measure extracellular water (ECW) [54], while changes in intracellular water (ICW), which contributes to body cell mass, cannot be accurately measured [33]. Body composition estimates rely on a stable relationship between ECW, ICW and fat free mass (FFM) that could have been altered during the KD. In addition, we have to take into account intra-individual prediction errors that Kyle et al. summarized as 3-8 % for total body water and 3.5-6 % for FFM

[33]. Our Monte Carlo simulations showed that, assuming a prediction error of 3.5 %, in only 2.2 % of all simulated datasets FM increased (i.e. its median change was greater than 0) while in 12 % FFM decreased. The simulated p-values for changes in FM remained less than 0.05 for assumed FFM prediction errors up to 4 %.

While this confirms the non-significant gains in FFM, the observed loss of FM seems fairly robust even taking into account typical measurement uncertainties. This is further supported by the indication of ECW losses at POST through the slightly higher haematocrit values. Because FM is estimated from the difference between total body weight and FFM, a rapid loss of water (which contributes to FFM) would even result in an over-estimation of FM at POST. However, similar to fasting, this effect would be most severe during the first week of the diet, while after at least five weeks we could expect a more stable situation [55]. In fact, none of our BIA measurements indicated an abnormal hydration status at either PRE or POST according to the RXc graph of Piccoli et al. [56] (not shown here). This graphical interpretation has the advantage of assessing an individual's nutritional health status based on the directly measured impedance vector without relying on predictive equations or models. It confirms that the KD favourably affected body composition as indicated by higher phase angles at POST, even if the exact amounts of FFM gained and FM lost are somewhat uncertain due to the limitations of BIA. We point out that these findings have important implications for the nutrition of critically ill patients, who often suffer from muscle wasting and whose survival time strongly and positively correlates with phase angle [51–53].

We can rule out strength training as the general cause of the observed increase in α, because only subjects 1, 2, 7, 11 and 12 conducted strength training during the study period and α actually decreased slightly in subjects 7 and 12 (the two weight lifters). An alternative explanation could be - at least in part - the muscle-sparing effect of ketosis that is essential for surviving longer periods of starvation [57]. Rat studies provided evidence that physiological levels of ketone bodies inhibit oxidation of the branched chain amino acids in muscle [58] and decrease the release of the gluconeogenic amino acid alanine [59]. Interestingly, the cyclists studied by Phinney et al. had significant elevations of fasting plasma leucine, isoleucine and valine levels that were most pronounced during the first week of their KD [14]. Furthermore, it has been shown that the hyperaminoacidemia resulting from ample protein ingestion after exercise is sufficient to maximally stimulate muscle protein synthesis and maximally impair protein breakdown by increasing insulin to sufficient levels of ≥30 mU/l without the need for additional CHO [48, 49].

Lipid profile and cardiovascular disease risk

Consistent with the findings of Phinney et al. (14), most blood parameters in our subjects remained fairly stable and within the reference ranges, although large individual differences existed (Table 2). An exception was the significant elevation in total cholesterol (TC) and LDL-C concentrations in all of our subjects (Fig.2). This is consistent with the significant elevations of TC and LDL-C levels reported by previous studies of normolipidemic and normal-weight healthy [23, 26–28] as well as epileptic [35] adults after a few weeks to a few months on a KD. In obese adults on a one-year KD, a significant increase of LDL-C was observed as well [12]. Studies in athletes reported either increased [14, 60] or unchanged [17, 61–63] TC and LDL-C concentrations after high-fat, partly ketogenic diets of various duration. Apparently, elevated LDL-C levels are a consequence of increased saturated fat intake [64, 65] and

can be measured as early as after three days [66], but we refrained from correlating saturated fat intake with individual LDL changes because of the small number of available food diaries. Our study subjects at POST compared well to the "optimal" dieters from Poland who showed median LDL-C and TC concentrations of 177.1 and 268.7 mg/dl, respectively, after at least one year on this very low CHO diet [30]. Hypercholesterolemia was also observed in Vilhjalmur Stefansson and Karsten Andersen during their one year exclusive meat diet, but TC levels returned to normal after discontinuation of the diet [23]. Notably, the maximum TC level of Andersen had been measured as 800 mg/l. Thus it could be questioned whether KDs increase cardiovascular disease (CVD) risk.

Elevated LDL-C is seen as a risk factor for atherosclerosis and CVD, for which reason fat-enriched diets are regarded as critical. There is evidence, however, that CHO-restricted and fat-enriched diets do not necessarily raise the number of total LDL particles, but rather induce a change in the size of these particles [28, 66–68] so that the number of small dense LDL particles decreases while that of large LDL particles increases. It has been argued that large LDL particles have a lower atherogenic potential as they are less able to migrate from the lumen into the subendothelial space of blood vessels and seem to be less susceptible to oxidation [69]. Although a larger number of small and intermediate LDL particles has often been correlated with an increased risk for CVD in univariate analyses, the predictive role of LDL particle size is questioned through multivariate analyses that established plasma triacylglycerides (TAG) and the TC/HDL-C ratio as more reliable independent predictors of CVD (see [70] and references therein).

Our measurements indicate a blood lipid profile (elevated TC, HDL-C and LDL-C, unchanged low TAG) that would be consistent with the mechanisms mentioned above. Large LDL-particles carry more total cholesterol while the TAG content seems to decrease or only change slightly. This gives rise to increased LDL-C with stable or decreased TAG as shown by Guay et al. in normo-lipidemic men [66]. Because we have no assessment of lipoprotein subclasses in our subjects, we can only speculate about an atherogenic potential of the KD based on the risk estimates that adopt CRP, TC, LDL-C and HDL-C [62]. In 11 and 9 of our 12 subjects, respectively, the LDL-C/HDL-C and TC/HDL-C ratios remained within the reference range (Table 2), whereby subject 2 already had a TC/HDL-C ratio of 4.6 at PRE and reached 4.1 at POST. Combined with the low TAG and CRP levels, this indicates that the KD does not necessarily increase the risk for CVD.

It is interesting to note that TAG levels did not decrease, but slightly increased in our participants. This is unexpected in light of previous studies usually showing a reduction in TAG when CHOs are restricted [12]. However, this effect is also most prominent in insulin-resistant individuals with elevated TAG levels. Our healthy subjects had already low TAG values initially and as athletes are expected to be insulin sensitive. This could explain the lack of reduction of TAG levels.

In subjects 1 and particularly 2, very high TC and LDL-C concentrations were measured PRE and POST. In these cases, a genetic predisposition must be considered. At least for subject 2 we cannot rule out a familial hypercholesterolemia since a maternal dys-lipidemia was known.

Another mechanism for the rise in cholesterol levels in our subjects could be related, at least in part, to a potential drop in the circulating thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), as indicated by the rise in TSH levels. T_3 (and to a lesser extent T_4) stimulates the expression of the LDL receptor on hepatic and peripheral cells through binding to its nuclear receptor and subsequent activation of thyroid response

elements on the promoter region of the LDL receptor gene [71–73]. Less T_3 would therefore result in less LDL receptors and decreased clearance of LDL particles from the circulation. Both in vitro [74] and in vivo [14, 75–79] studies have indeed shown that severe CHO restriction reduces T_3 levels within several days similar to fasting by impairing the conversion of T_4 to T_3. Although in these experiments, which lasted a maximum of two weeks, TSH was either not measured, remained stable or decreased, our observations indicate that in the longer term TSH levels might increase in response to lower circulating thyroid hormones. Interestingly, subject 11 who took a thyroxin substitute experienced the least dramatic rise in LDL levels. Furthermore, positive associations between TSH and LDL as well as total cholesterol levels have been found in cross-sectional studies in euthyroid healthy subjects, and the strength of these associations seems to depend on an individual's insulin sensitivity [38, 39]. We therefore hypothesize that the KD has diminished the production of T_3 from T_4, thereby reducing the number of LDL receptors and thus reducing LDL particle clearance which might be further impaired due to the missing stimulating effect of insulin on LDL uptake into cells [71].

Impact on kidney function

Previous studies have also shown that KDs lasting for at least a few weeks can result in significant elevations of uric acid [14, 26], blood urea nitrogen (BUN) [26, 28] and creatinine [14]. Increases in uric acid have been attributed to the effect of ketone bodies competing with tubular uric acid transport in the kidneys [80, 81]. This would result in a retention of uric acid when ketone bodies are excreted, leading to a positive association between serum uric acid levels and urinary ketone concentrations as already observed by Krehl et al. [26]. Elevations of BUN have been interpreted as a physiological consequence of increased protein intake [26, 28]. It should further be noted that especially during the initial phase of ketosis, protein breakdown and amino acid catabolism are increased to enable ATP and glucose production which also leads to increases in BUN. Finally, elevated ketone bodies could give rise to higher serum creatinine concentrations by interfering with the Jaffé reaction [82]. Consistent with these mechanisms, we observed elevations of uric acid, BUN and creatinine in our subjects, the latter with a clear trend ($p=0.016$). Estimated glomerular filtration rate according to the MDRD equation [83] revealed a decrease from a median of 91.8 ml/min/1.73 m^2 to 82.9 ml/min/1.73 m^2 ($p=0.021$). However, given that elevated creatinine concentrations could simply reflect relative muscle mass and considering the uncertainties in that equation for healthy individuals, a negative impact of the KDs on kidney function seems unlikely but cannot be ruled out with certainty. Unfortunately, from our data, we are neither able to track the exact changes in kidney blood parameters over time nor to relate them to precise measurements of macronutrient intake.

Influence on running performance

According to our test results, several weeks of a KD did not impair running performance in our study cohort, although great inter-individual differences existed. In particular, there were no significant differences in $\dot{V}O_{2max}$ as well as the HR at RCP. The RCP can be interpreted as a threshold towards the high intensity zone. As anaerobic energy production begins to increase disproportionate to intensity, H^+-ions start to accumulate in the extra-cellular space (metabolic acidosis), leading to a respiratory compensation through increased ventilation. This point is therefore defined as the respiratory compensation point. In our study design, the RCP might be a better indicator of

running intensity than the anaerobic threshold usually derived from the lactate-curve, because it is known that lactate kinetics are influenced by the amount of stored glycogen while the minimum in the $\dot{V}_E/\dot{V}CO_2$ curve is not (Fig.7 in Hughes et al. [84]).

In this regard, we do not judge lactate- or RQ-kinetics as relevant performance indicators, because they are influenced by diet composition. It is interesting to note, however, that similarly high lactate concentrations were reached at POST compared to PRE despite the ketogenic nature of the diets. This indicates that glycogen stores were not fully depleted during the exercise protocol. Nevertheless, maximum RQ values tended to be lower at POST compared to PRE reflecting a shift in fuel utilization towards fat oxidation in line with the findings of previous studies on obese [13, 18] or lean [15] subjects after at least three weeks on a KD. In these studies, no detrimental effects of the KD on $\dot{V}O_{2max}$ values have been found, contrary to shorter-term studies allowing a maximum of two weeks for adaption. Phinney [42] summarized these findings to conclude that three main factors may explain the conflicting results: extra sodium and potassium intake (that was not controlled for in this study); ample protein ingestion (that probably was given in our subjects based on the available food diaries); and most importantly sufficient time for metabolic adaption to chronically low CHO intake, probably two weeks and more. This coincides with the subjective feeling of improved exercise performance and recovery after 2-3 weeks reported by our subjects, and would explain the restoration of exercise capacity at POST.

Relative $\dot{V}O_{2max}$ values are frequently used in the literature to compare exercise capacity after an intervention [18, 19]. However, these values should be interpreted with caution since our subjects were lighter at POST than at PRE. The same caveat applies to the relative peak power values. Nevertheless, changes in the absolute measures of these variables did not reach statistical significance.

In conclusion, our treadmill test results imply that drastically reducing the amount of CHOs in the diet was no limiting factor for running performance in our 12 subjects. Intensity was maintained with increased fat oxidation rates. In principle, spiroergometric parameters can be used for interpreting ($\dot{V}O_{2max}$) as well as controlling (RCP) the training load.

Limitations of the study

Our study has several limitations. The most obvious is the heterogeneity of our sample with respect to age, gender, main sport as well as amount and intensity of training. However, we see this more as an advantage to the statistical modelling since our goal was to infer general changes that would occur in healthy individuals independent from the factors listed above. Nevertheless, our conclusions should be interpreted as hypotheses that warrant further testing with larger sample sizes. As a small interventional case study, there was no control group against which we could have compared any changes.

Another point for critique could be the lack of control regarding energy intake as well as quality and quantity of macro-nutrients. For example, fat quality has a large impact on cholesterol levels and we are not able to disentangle these effects from others like CHO restriction or energy intake. However, our goal was to study the effects of a KD as it would be practiced in a free-living population, where inter-individual variations in diet compositions naturally occur; again, this can be seen as an advantage for identifying common effects that are due to some general mechanisms of a KD. In this context, the somewhat high protein consumption derived from analyzing a sample of three-day food diaries from five

subjects might simply reflect the real life situation. It did not, however, diminish nutritional ketosis as judged by the daily urinary ketone measurements.

Regarding the performance tests, several confounding factors exist. First, women were not in the same menstrual cycle phase for all measurements. Second, only a few subjects monitored their training, and we cannot rule out the possibility that the others increased their training volume and/or intensity during the intervention, although subjects affirmed this not to be the case. Third, diet composition on the days before the two tests was not standardized. Also, we cannot infer how the second test would have been if subjects would have been allowed to eat a high-CHO diet on the day before. Fourth, the lack of monitoring training opens the possibility that changes in running performance simply reflect the influence of altered physical training. Nevertheless, our results indicate that for most subjects, exercise capacity was not compromised by the very low CHO content of their diet, given several weeks of adaptation.

Finally, we did not measure hormonal changes except TSH that might underlie some of the observed effects of the KD.

CONCLUSIONS

Several weeks of a self-prescribed KD did not cause major health problems, and even improved some subjective parameters like mood state, in twelve active individuals from a variety of sports, although subjective recovery from exercise was impaired.

The drastically reduced carbohydrate content of the diet seems to be no limiting factor for running performance. In addition, there was an improvement in body composition indicated through significantly higher phase angles, and loss of FM. While most biochemical parameters were not influenced by the diet, there was an impact on the blood lipid profile that can be considered problematic with respect to cardiovascular disease risk. As we discussed, however, the predictive role of cholesterol levels alone in healthy, physically active individuals remains to be elucidated.

ADDITIONAL FILES

Additional file 1 is an English version of the questionnaire sent to our study subjects after the intervention period. Where there was a choice from a list of possible answers, each "x" indicates that one subject chose the corresponding answer. We have also included individual remarks in the file with the corresponding subject number given in front of each remark.

LIST OF ABBREVIATIONS

BCM = Body cell mass; BUN = Blood urea nitrogen; BW = Body weight; CI = Confidence interval; CHO = Carbohydrate; ECM = Extracellular mass; ECW = Extracellular water; FFM = Fat free mass; FM = Fat mass; HDL-C = HDL cholesterol; ICW = Intracellular water; KD = Ketogenic diet; LDL-C = LDL cholesterol; RCP = Respiratory compensation point; TAG = Triacylglyceride; TBW = Total body water; TC = Total cholesterol

COMPETING INTERESTS

None of the authors has any competing or financial interest to declare.

AUTHORS CONTRIBUTIONS

RK and UK participated in the study design, assistance of study subjects, data analysis and drafting of the manuscript. TF conducted the treadmill tests and provided all testing equipment. JP, TA and SF helped with the analysis and interpretation of the data and drafting of the manuscript.

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