



Applied nutritional investigation

## Assessment of micronutrients in a 12-wk ketogenic diet in obese adults

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### ABSTRACT

**Objective:** A 12-wk ketogenic diet was found to have many beneficial effects in healthy obese adults, but it is not clear if the supply of micronutrients is adequate.

**Methods:** In 35 adult individuals with body mass index >30, the intakes of minerals and their serum levels were analyzed at baseline and at weeks 4 and 12 of the ketogenic diet intervention. The intake of vitamins and serum antioxidative potential were also investigated.

**Results:** Throughout the diet the intakes of magnesium, calcium, iron, phosphorus, and potassium were less than recommended values, but serum levels always remained within the reference range. Nevertheless, the level of calcium decreased significantly (from  $2.52 \pm 0.10$  mmol/L at baseline to  $2.36 \pm 0.07$  mmol/L at week 12,  $P < 0.001$ ), which could be due to the omission of legumes and reduced dairy intake or because of the high fat intake alone. The levels of phosphate increased concomitantly. Calcium serum levels were negatively associated with  $\omega$ -6 but not with  $\omega$ -3 unsaturated fatty acid intake. The intakes of water-soluble vitamins were also too low. However, the antioxidative potential of serum did not change during intervention.

**Conclusions:** Careful choice of foods that will provide the necessary micronutrients is of utmost importance when consuming ketogenic diet. In the 12 wk study the decreased intakes were not reflected in serum values, but special attention to calcium should be advised if such diet is recommended for longer periods.

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### Introduction

Obesity is a large-scale global public health problem as it poses a major risk for many life-threatening non-communicable diseases, and despite the efforts of the medical community, its prevalence is reaching troubling numbers in different parts of the world [1,2]. In recent years a ketogenic diet (KD) was suggested as an efficient method of weight loss [3] and is thus gaining popularity. In healthy obese adults such diet was found to have no adverse effects on total cholesterol [4] or low-density lipoprotein levels [3] and was efficient in decreasing the levels of triacylglycerols [3,4]. Decreased emotional and external eating, decreased insulin levels, and improved physical performance also speak in its favor [5]. In patients with type 2 diabetes, the levels of fasting glucose were reduced more efficiently compared with low-calorie [6] or low glycemic index diet [7], whereas the changes in low-density

lipoprotein cholesterol were similar [6]. Favorable effects were reported also for specific groups of patients, such as those who had undergone adjustable gastric banding [8].

However, from the studies involving epileptic children, where KD has been used for decades and thus more information on long-term exposure is available, concerns related to the levels of micronutrients can be raised. There, reduced growth and progressive loss of bone mineral density were documented [9–11], and skeletal fractures were more common [12], all pointing to insufficient calcium levels. A decrease in mean plasma magnesium, selenium, and vitamin A levels was also found [13]. It is important to note that the KD used to treat epilepsy is even more severe in terms of limiting the carbohydrate consumption. In weight loss programs, diets with an intake of carbohydrates <26% and <10% of total energy intake (EI) are considered low carbohydrate and very low carbohydrate, respectively [14]. To ensure production of ketone bodies from fatty acids and prevent gluconeogenesis from amino acids, the intake of proteins should not exceed 20% of EI, and the intake of fat can therefore vary from 55% to 75% of EI. In the classic KD to treat epilepsy, the goal is to cover 90% of EI with fat, 6% to 8% with protein, and only the remaining 2% to 4% with carbohydrate [15,16].

SK, NM, and ZJP designed the study. SK, TPV, AP, NM, and ZJP conducted research. SK and ZJP analyzed the data. SK and ZJP wrote the manuscript. All authors read and approved the final manuscript. The authors declare no conflict of interest.

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It is self-evident that there are differences in the metabolism and the nutritional requirements of children, lean or normal weight adults, and obese adults, and when evaluating KD, important differences were discovered. Opposite to the results in healthy obese adults [5] or patients with type 2 diabetes [4], de Lima et al. [17] reported negative changes in lipid profile, promoting atherogenic risk in epileptic children. In long-term studies these negative effects on cardiovascular health were found to be transient [18,19]. Even though some variables, such as growth rate, are no longer a concern in adulthood, the levels of micronutrients must nevertheless be maintained within reference values. Calcium and phosphate intake and their serum levels are particularly important for women approaching menopause, where inappropriate intake could pose an additional risk for osteoporosis. In the micronutrient analysis of hypothetical low-carbohydrate/high-fat (LCHF) meal plans, iron for women was less than reference values [20] and in the study evaluating such diet in epileptic adults, iron deficiency anemia was reported as one of the (rare) late-onset complications [21].

An insufficient intake of micronutrients may occur in all diets with low caloric intake [22] or when certain food groups are omitted [23]. Moreover, because of higher demands of obese adults, a diet following dietary reference intakes may not suffice to maintain constant serum micronutrient levels and fill tissue stocks [24]. To evaluate whether the KD is a suitable and safe dietary protocol in healthy obese adults with regard to micronutrient levels, we have investigated the intakes and serum levels of calcium, magnesium, phosphate, sodium, chloride, potassium, and iron during a 12-wk KD intervention. In addition, intakes of vitamins and the relation with serum antioxidative potential were evaluated.

## Materials and methods

### Study design and intervention

A thorough description of the study design and the dietary intervention was previously published [5]. Briefly, the study, which was conducted between March and June 2017, was a single-arm intervention study, where participants were assigned to a high-fat, low-carbohydrate KD for 12 wk. Medical ethical committee of the Republic of Slovenia approved the protocol (0120-100/2017); the study was also registered in [ClinicalTrials.gov](http://ClinicalTrials.gov) (NCT03338452). All procedures were conducted in accordance with the ethical guidelines of the Declaration of Helsinki. Inclusion criteria were absence of cardiovascular, inflammatory, or endocrine disease; body mass index > 30, stable weight in the last 3 mo, and willingness to consume the prescribed diet. Participants taking medications for lipid metabolism or psychiatric disorders were excluded. Thirty-eight out of 45 volunteers met the eligibility criteria and were enrolled in the study. Thirteen participants were men and 25 women, with an average age of  $37 \pm 7$  y and average body mass index of  $36.1 \pm 5.6$  kg/m<sup>2</sup> [5]. Participants were on a KD with the daily intake of carbohydrates between 5% to 10% of EI and at least 75% EI from fat. For the first 2 wk of intervention the EI was restricted to 1200 to 1500 kcal, after which *ad libitum* intake was allowed. Examples of the daily menu of a male and a female participant for week 1 (caloric restriction) and weeks 4 and 12 (*ad libitum*) are presented in Supplementary Table 1. Measurements reported here including blood withdrawal and analysis of dietary intake were performed at the beginning of the intervention and at weeks 1, 4, and 12. Three participants did not complete the study because of a clear drop in serum BHB, a sign of non-compliance, or not tolerating the prescribed diet. Results of the anthropometrical measurements, glucose and lipid profile, and hormonal status were published previously [5].

### Assessment of the intake of micronutrients

Participants recorded their food intake at baseline and after 4 and 12 wk of intervention for 3 consecutive days, 2 d during the week and 1 during the weekend. Food records were inspected and completed by the dietitian. Open Platform for Clinical Nutrition (OPEN, <http://opkp.si>) was used to analyze the nutrient composition; specifically intakes of calcium, phosphate, iron, sodium, potassium, chloride, magnesium, and vitamins were analyzed. Data from food records were automatically converted into EI, nutrients and food units. A food unit represents the net amount of food consumed without wastage, and each food unit from every selected food group contains similar amounts of carbohydrates, protein, total fat, and EI (Table 1).

**Table 1**  
Nutritional composition and energy value of one food unit from each group

Food group	Carbohydrates (g)	Proteins (g)	Fats (g)	Energy (kJ)	Energy (kcal)
Milk and milk products	10	7	3	400	95
Vegetables	5	2	0	118	28
Fruit	15	0	0	250	60
Starchy food	15	2	0	300	70
Legumes	15	5	0	370	83
Meat and replacements	0	7	7	390	93
Fat and fatty food	0	0	5	200	48
Sugars	10	0	0	170	40

### Biochemical measurements

At the beginning of the intervention and at weeks 4 and 12, venous blood was withdrawn into vacuum tubes (Becton Dickinson, Rutherford, NJ, USA) between 07.00 and 10.00 h in a fasting state. Serum samples were prepared immediately, and aliquots were stored at  $-80^{\circ}\text{C}$  until further analysis could be performed. Serum levels of calcium, magnesium, iron, sodium, potassium, chloride, and phosphate were measured on a Cobas c111 analyzer (Roche, Basel, Switzerland) using corresponding Cobas reagents or electrodes.

### Total antioxidative capacity

Total antioxidative capacity of serum was measured on a Photochem instrument (Jena Analytik, Jena, Germany), following the manufacturers' instructions for the hydrophilic substances. Serum aliquots were centrifuged and diluted 1:10 in the supplied buffer solution. Results are expressed as nanomole equivalents of ascorbic acid per microliter of serum.

### Data Analysis

For the statistical analysis, IBM SPSS Version 23 (IBM Corp., Armonk, NY, USA) was used. Normal distribution of the variables was tested with Kolmogorov-Smirnov test. Means  $\pm$  SD are given for all the parameters. A repeated measure analysis of variance was used to assess differences between serum parameters and dietary intakes (between baseline, week 4, and week 12). In addition, a paired *t* test was used to compare the intakes of micronutrients between two time points (between baseline and week 4; between baseline and week 12, and between week 4 and week 12). Values of  $P < 0.05$  were considered statistically significant. Relative differences in serum concentrations and dietary intakes were calculated for the Pearson and Spearman association analysis. Pearson's *r* before and after the adjustment for the relative difference in EI is shown in Table 5.

## Results

### Daily food units

To ensure the synthesis of ketone bodies, the omission of certain food groups containing a high proportion of carbohydrates is necessary. In our intervention the participants consumed no starchy food and legumes and almost no fruit at week 4 (Table 2), strictly following the instructions of the dietitian. At week 12 the intake of these foods somewhat increased ( $0.02 \pm 0.07$  units for legumes,  $0.17 \pm 0.55$  units for starchy food). The intake of vegetables, fat and fatty foods, and meat and its replacements increased at week 4 but returned to baseline at week 12 when the participants spontaneously decreased the daily EI further from the initially prescribed restriction. The intake of milk and milk products was decreased at week 4 and even further at week 12 (baseline:  $2.54 \pm 1.43$  units, week 4:  $1.57 \pm 1.01$  units,  $P = 0.024$ ; week 12:  $0.93 \pm 1.01$  units,  $P < 0.001$ ). Eggs, olive oil, butter, avocado, nuts, green vegetables, and chicken were the most commonly selected foods during the intervention (Supplementary Table 1). There were no major differences in selected foods among men and women.

**Table 2**  
Mean (SD) number of daily food units of intake evaluated from FR

Food groups*	At baseline	At week 4	At week 12
Milk and milk products	2.54 ± 1.43	1.57 ± 1.01 <sup>†</sup>	0.93 ± 1.01 <sup>†‡§</sup>
Vegetable	1.90 ± 1.45	2.43 ± 1.14	1.69 ± 1.37
Fruit	0.64 ± 0.79	0.02 ± 0.01 <sup>†</sup>	0.05 ± 0.10 <sup>†</sup>
Starchy food	6.51 ± 4.14	0.00 ± 0.00 <sup>†</sup>	0.17 ± 0.55 <sup>†</sup>
Legumes	0.22 ± 0.50	0.00 ± 0.00 <sup>†</sup>	0.02 ± 0.07 <sup>†</sup>
Meat and replacements	6.39 ± 4.11	8.47 ± 2.60	5.91 ± 1.76 <sup>‡</sup>
Fats and fatty food	15.1 ± 7.6	23.6 ± 5.4 <sup>†</sup>	15.5 ± 6.4
Sugars	2.53 ± 2.71	0.00 ± 0.00 <sup>†</sup>	0.04 ± 0.01 <sup>†</sup>

KD, ketogenic diet; FR, 3-d weighed food record.

Means ± SD are given, and repeated measures analysis of variance and paired *t* test were used to compare means. Statistical significance was set at *P* < 0.05.

\*Values are expressed as number of food units from each group.

<sup>†</sup>Statistical significant differences between baseline and 4 wk of KD.

<sup>‡</sup>Statistical significant differences between baseline and 12 wk of KD.

<sup>§</sup>Statistical significant differences between 4 and 12 wk of KD.

### Intake of minerals and their serum levels

To evaluate the risk for micronutrient deficiencies, intakes of selected minerals were calculated from 3-d food records at baseline, at week 4, and at the end of week 12 (Table 3). During the intervention period, intakes of all investigated minerals were significantly lower compared with the baseline. The intake of sodium at baseline was higher than recommended and during intervention decreased closer to appropriate levels. The intake of calcium, magnesium, potassium, and iron were less than the recommended daily values both at week 4 and 12. For these four minerals the intakes in women decreased at week 4 and then remained stable, whereas in men they decreased further at week 12. The intake of phosphorus in both genders progressively decreased and was less than the recommended value at the end of the intervention.

The decreases in intake did not reflect in the decreased serum levels (Table 3), except for calcium, which dropped from 2.52 ± 0.10 mmol/L at baseline to 2.36 ± 0.07 mmol/L after 12 wk (*P* < 0.001). Phosphate serum levels increased significantly from 1.02 ± 0.23 mmol/L at baseline to 1.26 ± 0.16 mmol/L at week 4 (*P* < 0.001) and 1.30 ± 0.23 mmol/L at week 12 (*P* < 0.001). Despite these changes, the levels of calcium and phosphate remained within reference ranges at all times. The changes were similar in men and women.

### Fatty acids intake and the associations with calcium serum levels

The absorption of calcium may depend on the diet's fatty acid composition. The analysis of total fat, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid [5], and ω-3 and ω-6 fatty acids intake (Table 4) and the calculation of associations (Table 5) were therefore performed. Although the total percentage of fat intake was kept at 75% of EI during the whole intervention, the absolute intake of fats in grams per day only increased at week 4 (baseline: 105 ± 41 g/d; week 4: 137 ± 30 g/d, *P* < 0.001) and because of the decrease in EI returned to baseline levels (91 ± 33 g/d) at week 12 [5]. The same holds for saturated fatty acid (baseline: 34 ± 14 g/d; week 4: 42 ± 14 g/d, *P* = 0.023; week 12: 30 ± 13 g/d) and monounsaturated fatty acid (baseline: 14 ± 7 g/d; week 4: 27 ± 11 g/d, *P* < 0.001; week 12: 16 ± 3 g/d), whereas the absolute intakes of polyunsaturated fatty acid significantly decreased at week 12 (baseline: 9.5 ± 4.5 g/d; week 4: 8.6 ± 4.2 g/d; week 12: 6.3 ± 3.2 g/d, *P* = 0.004). At that time, the decrease was notable for ω-6 and even more drastic for ω-3 (Table 4). Consequently, the ratio of ω-3 to ω-6 decreased significantly from 1.0:5.6 at baseline to 1.0:7.2 (*P* = 0.018) at week 12.

The association of relative differences in serum calcium and relative differences in the intake of fatty acids were then calculated (Table 5). No association with saturated and monounsaturated fatty acids was found. However, after the adjustment for relative EI, the moderate negative association with polyunsaturated fatty acids was significant (*r* = −0.561, *P* = 0.019). When ω-3 and ω-6 were considered separately, the association was only significant with ω-6 (*r* = −0.411, *P* = 0.048) but not with ω-3 (*r* = −0.046, *P* = 0.862).

### Intake of vitamins and total serum antioxidative potential

During the intervention, the intakes of several vitamins were less than the reference daily intakes (Table 6). The intakes of riboflavin, niacin, vitamin B6, pantothenic acid, and biotin were reduced already at week 4. Apart from niacin, all decreased further at week 12 in men but not in women. In addition to these, the intakes of vitamin C in men, and thiamin and vitamin B12 in both genders were reduced at week 12. The intakes of vitamin A and vitamin D were too low already at baseline but did not decrease further during the intervention. Folic acid intake at week 4 decreased in men and at week 12 in both men and women. Intakes of vitamin E and K were suitable throughout the intervention.

At week 12 the intakes of antioxidant vitamins A and C were in men less than one-third of the recommended values (30.3% and 26.4%, respectively) and in women less than two-thirds (62.5% and 53.7%, respectively). This could reflect in lowered serum antioxidative potential. To check these potential changes, Photochem apparatus was used. Both oxygen radical absorbance capacity and total antioxidative potential of serum samples at the baseline and the end of intervention remained unchanged (Table 7).

### Discussion

During the 12-wk KD in healthy obese adults, the intakes of calcium, potassium, magnesium, iron, chloride, vitamins C, B6, B12, thiamin, riboflavin, niacin, pantothenic acid, biotin, and folic acid were significantly reduced and were less than the values recommended in the national guidelines [25]. Additionally, vitamins A and D already did not meet the recommendations at baseline but did not change further because of the intervention. Reduced levels of micronutrients could be a result of the omission of whole food groups or the reduction in daily EI alone [24]. In the first 2 wk of the intervention, the participants were prescribed a caloric restriction, the rules of the diet were thoroughly explained by the dietitian, and they were given a list of suitable foods and some typical menus to ensure the synthesis of ketone bodies. After this initial period, they had to maintain the consumption of fat to at least 75% of EI and limit the consumption of carbohydrates to the maximum of 10% of EI throughout the intervention, but their EI was *ad libitum*. However, already at weeks 2 and 4 the participants reported an increase in the feeling of fullness and further decreased the EI from recommended 1200 to 1500 kcal to 1115 ± 357 kcal [5].

Additional reason for inadequate micronutrient supply could be the small variability in the choice of foods. Although the participants were instructed to keep the macronutrients in the prescribed percentages, they were allowed to choose foods according to their preference. Repeated consumption of a few items was reported in most participants. Olive oil, eggs, nuts, avocado, broccoli, cauliflower, and sardines were regularly selected but not salmon, liver, mussels, brussels sprouts, spinach, or kale, which were included in the list of suggested foods and could be a source of relevant micronutrients. Consumption of berries was discouraged during the initial 2 wk, but they were also not consumed later.

**Table 3**  
Dietary intakes and serum levels of microelements at baseline, after 4 wk, and after 12 wk of KD for all participants and separated by sex

Element	Unit	Sex	At baseline	At week 4	At week 12	Recommended [25]
Dietary intake of microelements						
Na	mg/d	All	2341 ± 1374	932 ± 725*	1021 ± 788 <sup>†</sup>	
		Male	2990 ± 1945	957 ± 306*	1038 ± 519 <sup>†</sup>	550
		Female	2003 ± 819	920 ± 869*	1015 ± 881 <sup>†</sup>	550
Ca	mg/d	All	831 ± 355	536 ± 347*	388 ± 222 <sup>‡</sup>	
		Male	904 ± 374	641 ± 510*	298 ± 187 <sup>‡</sup>	1000
		Female	793 ± 346	484 ± 224*	422 ± 229 <sup>‡</sup>	1000
K	mg/d	All	2325 ± 626	1395 ± 431*	1215 ± 556 <sup>†</sup>	
		Male	2583 ± 783	1634 ± 462*	979 ± 213 <sup>‡</sup>	2000
		Female	2191 ± 492	1276 ± 369*	1305 ± 621 <sup>†</sup>	2000
Mg	mg/d	All	249 ± 73	159 ± 69*	143 ± 56 <sup>†</sup>	
		Male	275 ± 80	200 ± 82*	108 ± 51 <sup>‡</sup>	350
		Female	235 ± 67	139 ± 52*	157 ± 54 <sup>†</sup>	300
Fe	mg/d	All	11.4 ± 4.1	6.2 ± 3.0*	6.2 ± 3.9 <sup>†</sup>	
		Male	12.3 ± 2.9	6.5 ± 2.8*	3.7 ± 1.9 <sup>‡</sup>	10
		Female	10.9 ± 4.6	6.1 ± 3.2*	7.2 ± 4.1 <sup>†</sup>	10
Cl	mg/d	All	3687 ± 2090	1419 ± 1131*	1519 ± 1201 <sup>†</sup>	
		Male	4683 ± 2971	1424 ± 499*	1588 ± 795 <sup>†</sup>	830
		Female	3169 ± 1227	1416 ± 1351*	1492 ± 1341 <sup>†</sup>	830
P	mg/d	All	1147 ± 398	730 ± 305*	556 ± 235 <sup>‡</sup>	
		Male	1362 ± 471	897 ± 307*	407 ± 154 <sup>‡</sup>	700
		Female	1036 ± 309	656 ± 282*	612 ± 238 <sup>†</sup>	700
Serum levels of microelements						
Na	mmol/L	All	137 ± 4	138 ± 5	138 ± 2	Reference ranges
		Male	137 ± 4	138 ± 5	139 ± 3	135–145
		Female	136 ± 4	138 ± 5	137 ± 1	135–145
Ca	mmol/L	All	2.52 ± 0.10	2.59 ± 0.13	2.36 ± 0.07 <sup>‡</sup>	
		Male	2.55 ± 0.07	2.61 ± 0.14	2.40 ± 0.05 <sup>‡</sup>	2.11–2.55
		Female	2.51 ± 0.10	2.58 ± 0.13	2.34 ± 0.08 <sup>‡</sup>	2.11–2.55
K	mmol/L	All	4.4 ± 0.3	4.3 ± 0.3	4.4 ± 0.3	
		Male	4.3 ± 0.3	4.2 ± 0.3	4.4 ± 0.3	3.8–5.5
		Female	4.4 ± 0.4	4.3 ± 0.3	4.5 ± 0.3	3.8–5.5
Mg	mmol/L	All	0.85 ± 0.06	0.86 ± 0.07	0.87 ± 0.06	
		Male	0.85 ± 0.07	0.88 ± 0.10	0.89 ± 0.07	0.65–1.05
		Female	0.85 ± 0.06	0.86 ± 0.05	0.86 ± 0.05	0.65–1.05
Fe	mmol/L	All	15.1 ± 6.5	14.2 ± 6.3	14.5 ± 6.9	
		Male	15.9 ± 4.8	18.1 ± 7.3	16.8 ± 5.0	10.6–28.3
		Female	14.7 ± 7.3	12.1 ± 4.7	13.0 ± 7.7	6.6–26.0
Cl	mmol/L	All	101 ± 18	103 ± 4	104 ± 3	
		Male	103 ± 4	102 ± 5	104 ± 3	94–110
		Female	99 ± 22	104 ± 4	103 ± 2	94–110
PO <sub>4</sub> <sup>3-</sup> §	mmol/L	All	1.02 ± 0.23	1.26 ± 0.16*	1.30 ± 0.23 <sup>†</sup>	
		Male	1.01 ± 0.12	1.23 ± 0.19*	1.18 ± 0.10 <sup>†</sup>	0.87–1.45
		Female	1.03 ± 0.27	1.28 ± 0.13*	1.37 ± 0.26 <sup>†</sup>	0.87–1.45

KD, ketogenic diet.

Means ± SD are given, and repeated measures analysis of variance and paired *t* test were used to compare means. Statistical significance was set at *P* < 0.05.

\*Statistically significant differences between baseline and 4-wk of KD.

<sup>†</sup>Statistically significant differences between baseline and 12-wk of KD.

<sup>‡</sup>Statistically significant differences between 4 and 12-wk of KD.

§Recommended daily intake values are for P, serum reference values are for PO<sub>4</sub><sup>3-</sup>.

**Table 4**  
Dietary intakes at baseline, after 4 wk, and after 12 wk of KD

	Unit	At baseline	At week 4	At week 12
EI	kcal/d	1909 ± 549	1615 ± 304*	1115 ± 357 <sup>‡</sup>
ω-3	g/d	1.2 ± 1.1	1.3 ± 1.5	0.75 ± 0.68 <sup>‡</sup>
ω-3	% EI	0.57 ± 0.52	0.72 ± 1.28	0.61 ± 0.55
ω-6	g/d	6.8 ± 4.1	6.9 ± 3.4	5.5 ± 2.7 <sup>b,‡</sup>
ω-6	% EI	3.2 ± 1.9	3.8 ± 1.3	4.4 ± 2.2
ω-3/ω-6		1.0:5.6	1.0:5.3	1.0:7.2 <sup>‡</sup>

EI, energy intake; KD, ketogenic diet; ω-3, omega-3 unsaturated fatty acids; ω-6, omega-6 unsaturated fatty acids.

Means ± SD are given, and repeated measures analysis of variance and paired *t* test were used to compare means. Statistical significance was set at *P* < 0.05.

\*Statistically significant differences between baseline and 4 wk of KD.

<sup>†</sup>Statistically significant differences between baseline and 12 wk of KD.

<sup>‡</sup>Statistically significant differences between 4 and 12 wk of KD.

**Table 5**

Associations between relative differences in serum Ca and relative differences in dietary intake parameters before and after adjustments for relative differences in energy intake (EI)

	Δ serum Ca		Δ serum Ca (adjustment for Δ EI)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Dietary fatty acid intakes				
Δ Saturated fatty acids	0.013	0.958	0.060	0.820
Δ Monounsaturated fatty acids	-0.342	0.165	-0.406	0.106
Δ Polyunsaturated fatty acids	-0.392	0.107	<b>-0.561</b>	<b>0.019</b>
Δ ω-3	-0.076	0.756	-0.046	0.862
Δ ω-6	-0.371	0.129	<b>-0.411</b>	<b>0.048</b>

Relative differences (Δ): ([week 12 – baseline] / baseline).

Pearson's and Spearman's correlation analyses (crude and adjusted for EI) were performed to detect different associations between relative differences in serum Ca levels and relative differences in fatty acids intake.

**Table 6**

Intakes of vitamins at baseline, after 4 wk, and after 12 wk of KD for all participants and separated by sex

Vitamin	Unit	Sex	At baseline	At week 4	At week 12	Recommended [25]
Vitamin A	$\mu\text{g}/\text{d}$	All	578 $\pm$ 277	546 $\pm$ 325	446 $\pm$ 322	
		Male	540 $\pm$ 137	483 $\pm$ 325	303 $\pm$ 127	1000
		Female	648 $\pm$ 369	577 $\pm$ 328	500 $\pm$ 358	800
Vitamin D	$\mu\text{g}/\text{d}$	All	3.7 $\pm$ 4.6	4.1 $\pm$ 7.6	3.8 $\pm$ 3.6	
		Male	3.3 $\pm$ 2.4	6.1 $\pm$ 9.4	3.0 $\pm$ 3.8	20
		Female	3.6 $\pm$ 5.5	3.1 $\pm$ 6.5	4.0 $\pm$ 3.7	20
Vitamin E	$\text{mg}/\text{d}$	All	12 $\pm$ 7	16 $\pm$ 8	12 $\pm$ 8	
		Male	14 $\pm$ 9	16 $\pm$ 10	9 $\pm$ 5*	13
		Female	11 $\pm$ 6	16 $\pm$ 7	13 $\pm$ 8	12
Vitamin K	$\mu\text{g}/\text{d}$	All	144 $\pm$ 150	143 $\pm$ 216	118 $\pm$ 116	
		Male	94 $\pm$ 60	96 $\pm$ 54	83 $\pm$ 32	70
		Female	170 $\pm$ 160	167 $\pm$ 261	132 $\pm$ 130	60
Vitamin C	$\text{mg}/\text{d}$	All	68 $\pm$ 49	57 $\pm$ 42	45 $\pm$ 32 <sup>†</sup>	
		Male	70 $\pm$ 53	56 $\pm$ 47	29 $\pm$ 20 <sup>†</sup>	110
		Female	60 $\pm$ 32	58 $\pm$ 35	51 $\pm$ 34	95
Thiamin	$\text{mg}/\text{d}$	All	1.0 $\pm$ 0.4	0.8 $\pm$ 0.6	0.4 $\pm$ 0.2* <sup>†</sup>	
		Male	1.1 $\pm$ 0.5	1.3 $\pm$ 0.8	0.4 $\pm$ 0.2* <sup>†</sup>	1.2
		Female	0.9 $\pm$ 0.4	0.6 $\pm$ 0.3	0.4 $\pm$ 0.2* <sup>†</sup>	1.0
Riboflavin	$\text{mg}/\text{d}$	All	1.5 $\pm$ 0.6	0.8 $\pm$ 0.8 <sup>‡</sup>	0.7 $\pm$ 0.4 <sup>†</sup>	
		Male	1.8 $\pm$ 0.6	0.8 $\pm$ 0.3 <sup>‡</sup>	0.4 $\pm$ 0.2* <sup>†</sup>	1.3
		Female	1.3 $\pm$ 0.6	0.7 $\pm$ 0.9 <sup>‡</sup>	0.8 $\pm$ 0.4 <sup>†</sup>	1.0
Niacin	$\text{mg}/\text{d}$	All	21 $\pm$ 10	7 $\pm$ 6 <sup>‡</sup>	9 $\pm$ 7 <sup>†</sup>	
		Male	27 $\pm$ 14	10 $\pm$ 7 <sup>‡</sup>	6 $\pm$ 3 <sup>†</sup>	15
		Female	18 $\pm$ 6	5 $\pm$ 5 <sup>‡</sup>	10 $\pm$ 8 <sup>†</sup>	12
Vitamin B6	$\text{mg}/\text{d}$	All	1.4 $\pm$ 0.6	1.0 $\pm$ 0.5 <sup>‡</sup>	1.0 $\pm$ 0.8	
		Male	1.7 $\pm$ 0.8	1.4 $\pm$ 0.7	0.7 $\pm$ 0.2* <sup>†</sup>	1.5
		Female	1.3 $\pm$ 0.4	0.9 $\pm$ 0.4 <sup>‡</sup>	1.1 $\pm$ 0.9	1.2
Pantothenic acid	$\text{mg}/\text{d}$	All	4.7 $\pm$ 1.8	2.8 $\pm$ 1.9 <sup>‡</sup>	2.7 $\pm$ 1.6 <sup>†</sup>	
		Male	5.9 $\pm$ 1.8	3.1 $\pm$ 1.0 <sup>‡</sup>	1.6 $\pm$ 0.8* <sup>†</sup>	6
		Female	4.2 $\pm$ 1.6	2.7 $\pm$ 2.3 <sup>‡</sup>	3.2 $\pm$ 1.6	6
Biotin	$\mu\text{g}/\text{d}$	All	27 $\pm$ 13	19 $\pm$ 18 <sup>‡</sup>	19 $\pm$ 15 <sup>†</sup>	
		Male	27 $\pm$ 14	19 $\pm$ 17 <sup>‡</sup>	7 $\pm$ 5* <sup>†</sup>	30
		Female	26 $\pm$ 12	19 $\pm$ 18	21 $\pm$ 16	30
Folic acid	$\mu\text{g}/\text{d}$	All	233 $\pm$ 116	201 $\pm$ 181	129 $\pm$ 61* <sup>†</sup>	
		Male	231 $\pm$ 83	160 $\pm$ 90 <sup>†</sup>	86 $\pm$ 26* <sup>†</sup>	300
		Female	234 $\pm$ 131	221 $\pm$ 211	145 $\pm$ 63* <sup>†</sup>	300
Vitamin B12	$\mu\text{g}/\text{d}$	All	5.1 $\pm$ 5.1	5.3 $\pm$ 16.1	2.2 $\pm$ 1.4* <sup>†</sup>	
		Male	6.2 $\pm$ 4.2	3.6 $\pm$ 4.4	1.4 $\pm$ 1.2 <sup>b,‡</sup>	3.0
		Female	4.5 $\pm$ 5.5	6.2 $\pm$ 19.6	2.6 $\pm$ 1.3 <sup>b,‡</sup>	3.0

KD, ketogenic diet.

Means  $\pm$  SD are given, and repeated measures analysis of variance and paired *t* test were used to compare means. Statistical significance was set at  $P < 0.05$ .

\*Statistically significant differences between 4 and 12 wk of KD.

†Statistically significant differences between baseline and 12 wk of KD.

‡Statistically significant differences between baseline and 4 wk of KD.

**Table 7**

Serum antioxidative properties at baseline and at the end of intervention

	At baseline	At week 12
Total ORAC ( $\mu\text{mol TE}$ [trolox equivalents])	3759 $\pm$ 2889	3180 $\pm$ 3438
TAC (nmol/ $\mu\text{L}$ )	9.38 $\pm$ 4.86	10.08 $\pm$ 2.58

ORAC, oxygen radical absorbance potential; TAC, total antioxidative potential.

Means  $\pm$  SD are given, and paired *t* test was used to compare means. Statistical significance was set at  $P < 0.05$ .

Changes for several micronutrients in women were more prominent at the beginning of the intervention and later reached a plateau, whereas in men the changes progressed gradually. In the same intervention, a similar trend was identified for other parameters, such as body mass and fat-free mass reduction [5].

Importantly, the reduced intakes of minerals did not reflect in the reduced serum concentrations. In fact, all measured values were within the reference ranges even at week 12. However, a significant decrease in the concentration of calcium was detected, which could be due to the omission of legumes and a major reduction in the consumption of milk and dairy products. High fat consumption alone could add to the decrease in serum calcium levels and availability for tissues because lower calcium digestibility was

reported in an animal model for different high-fat diets [26]. In KD this was proved to be due to high fat consumption and not the restriction of carbohydrates alone [27]. Notably, in our study the decrease in serum calcium was accompanied by the increase in serum phosphate level despite its reduced intake. This may be due to the interaction of calcium ions with fatty acids, which is why in the intestines less calcium is available to bond with phosphorous ions and the bioavailability of phosphorus consequently increases [26]. We also cannot exclude the possibility that the increase in phosphate level is a sign of the resorption of calcium from bone [28]. To prevent this process, in such dietary protocols calcium should be carefully monitored, especially when the diet is to be consumed for a longer period.

We found no correlation of serum calcium with saturated fats, in contrast to the fact that more abundant soap formation of calcium with saturated compared with unsaturated fats has a negative impact on calcium absorption [29]. Interestingly, serum calcium concentration was negatively associated with the consumption of  $\omega$ -6 fatty acids but not with the consumption of  $\omega$ -3. Because the present study was conducted in a Slovenian coastal region where extra virgin olive oil is the preferred fat source and fish are regularly consumed, the overall consumption of saturated

fats was relatively low and the intake of  $\omega$ -3 was greater than recommended 0.5% EI [25]. However, during the intervention, the participants often (on daily basis) ate eggs, which contain  $\omega$ -3 and  $\omega$ -6 in the ratio from 1:10 to 1:40 [30]. With reduced EI they also reduced intake of  $\omega$ -3-containing foods and consequently the ratio of  $\omega$ -3 to  $\omega$ -6 significantly shifted in favor of  $\omega$ -6. Considering both the observed drop in serum calcium and a known beneficial role of higher  $\omega$ -3 to  $\omega$ -6 ratio for calcium absorption [31,32] and bone health [33,34], the selection of foods to improve this ratio would be essential. The fact that  $\omega$ -3 promotes calcium absorption especially when dietary calcium is low [32] further supports this notion.

Previous studies of KD in children also found reduced serum magnesium. This was true after consuming such diet for 1 year [13], whereas in our 12-wk long intervention the reduced intake of magnesium did not result in changed serum levels yet. It should be noted that the inability of serum levels to fully determine magnesium deficiency was recently pointed out [35]. In a recent study iron for women was found to be the only micronutrient that, even with careful food selection, would be difficult to provide in sufficient amounts [20]. Here we found that regardless of reduced intake, no changes in iron serum levels were detected. Possibly, low calcium and the absence of cereals and legumes, which contain inhibitors of iron absorption, may increase its bioavailability [36]. Further, intakes of sodium and chloride, crucial determinants of high blood pressure that in most parts of the world are too high [37], were improved with our intervention.

The intakes of water-soluble vitamins were lower than recommended. However, the insufficient intake of antioxidative vitamins did not cause a reduction in serum antioxidative properties. Levels of uric acid were increased during intervention and there was a slight increase in bilirubin serum levels [5]. We therefore propose that the production of endogenous antioxidants was enough to compensate for this shortage at least for such short period. Vitamin D intake, which was low at baseline and throughout the intervention, only seldomly meets the recommendations in any dietary plan. In KD this fat-soluble vitamin could be absorbed more efficiently, so even though not measured here, we do not expect a negative effect of the diet on serum levels. Because the study was initiated in early spring and ended at the beginning of summer when sun exposure and hence endogenous vitamin D production increases, a potential increase in serum level would in this single-arm study be impossible to ascribe to the diet.

A low-energy, low-carbohydrate, high-fat KD intervention had numerous beneficial effects in healthy obese adults, from substantial weight loss, decreased fat mass, and decreased insulin to improved cognitive and physical performance [5]. Yet among nutrition experts, because of a possible micronutrient deficiency, a hesitation remains. In the present study we found that although intakes of several micronutrients were low, after 12 wk of such diet this did not reflect in reduced serum levels or reduced antioxidative potential of serum. In addition, other weight loss diets with such extensive caloric restriction would likely show insufficient intakes, as shown for both vegetarian and non-vegetarian caloric restriction diet [38]. Nevertheless, careful monitoring, especially of calcium and water-soluble vitamins, should be advised if KD is to be consumed for longer periods. Nonexperts, such as participants in our study, were, with their own food choices, able to follow the prescribed macronutrient supply but to meet the needs for the micronutrients either more detailed instructions or a multivitamin and multimineral supplement should be given. As recently reported, careful formulation of meals could in KD be sufficient to achieve micronutrient repletion [20]. Moreover, as shown for a 4-wk KD before bariatric surgery [39], with appropriate supplementation of micronutrients the benefits of KD can be fully exploited with a concomitant preservation or even improvement of micronutrient status.

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## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.06.003.

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