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Does the diet style affect the creatinine excretion?

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ABSTRACT

There is a growing interest in alternative diets such as vegan or lacto-ovo vegetarian diet. The choice is made mainly for ethical, health reasons or both. Creatinine is a waste product of creatine phosphate metabolism in muscles, and the excretion rate is relatively constant. Forty-nine participants were tested in total: 5 lacto-ovo vegeterians, 14 vegans, and 30 omnivores. To compare the groups, all participants had a similar diet consisting of bread, bran flakes and wheat bran. The creatinine level was measured before the initiation of the diet in the first morning void, and in the sample of 24-h urine. The results confirmed slightly higher mean creatinine levels in males (1139 ± 517 mg/L), compared to females (901 ± 539 mg/L). Regular consumption of different diets did not show statistically significant differences in datasets on either first void or 24-h samples. The highest median result after 24-h was determined in lacto-ovo vegetarians (2175 mg/L), followed by omnivores (1328 mg/L), while vegans had the lowest median (1235 mg/L). Based on these results, there is no evidence that the vegetarian or vegan lifestyle influences the creatinine excretion rate.

Introduction

In the last few decades, there is a growing trend in consuming high-energy density food; mostly added sugars or food rich in animal fat and proteins. At the same time, scientists have found correlation with a number of diseases and meat consumption (Abete et al., 2014). Nutritionists have come up with scientific knowledge that a well-balanced vegetarian diet based on plant foods improves health, when compared with the diet based on meat (Sabaté, 2003). Some of the reasons for being vegetarian, vegan or for avoiding meat are moral and ethical concerns about animal wellbeing, disliking of farming conditions, fear of hormones and “chemicals” in meat, health concerns and the price of meat (Beardsworth and Keil, 1991). There are many studies that link consumption of meat with lower life expectancy (Singh et al., 2003; Key et al., 1999; Pan et al., 2012). Aubertin-Leheudre and Adlercreutz (2009) concluded that women consuming animal protein had more muscle mass than vegetarian women with the same amount of plant protein intake.
Creatinine is an organic compound created from creatine phosphate metabolism by using ATP (adenosine triphosphate) in the muscle, and it is filtered and secreted by kidneys. Since muscular mass is relatively constant, the creatinine production is almost constant on a daily basis. It is the most widely used marker for assessing excretion due to the fact that muscles produce it on a relatively constant rate, is freely filtered in the glomerular membrane and actively secreted in the tubules of the kidneys. When the renal function is decreased, tubular secretion of creatinine is increased (Payne, 1986; Purde et al., 2015). In the muscle tissue 98% of the

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total body creatine pool can be found in the form of creatine and phosphocreatine. Conversion of creatine to creatinine occurs at a rate of 1.1% per day, while conversion from the phosphocreatine is at a rate of 2.6% per day. Because the urinary creatinine emerged from both forms, differences in a turnover rate between individuals are caused by different creatine to phosphocreatine ratio (Heymsfield et al., 1983). Urinary creatinine concentration is also used to correct human exposures to chemicals (drugs, mycotoxins, occupational chemical exposure etc.) due to water dilution (Arndt, 2009; Warth et al., 2013; Barr et al., 2005). When observing metabolism, distribution and excretion, a 24-h urine sample is ideal for the analysis. To confirm that samples are collected correctly, they have to be compared to a creatinine excretion concentration, because a 24-h creatinine excretion rate varies less than a 24-h urine volume (Jackson, 1966). To determine analyte concentration in the urine sample, the dilution of urine has been adjusted by dividing the analyte (µg/L urine) with the creatinine concentration (mg creatinine/L urine), which provides information about the weight of analyte (µg) per mg of creatinine (Barr et al., 2005). Women, in general, have lower muscle mass than men. Cohort study, in which 45000 urine samples were tested, proved correlation with the creatinine excretion and sex. Mean values for women were 723 mg/L, measured in a clinical chemistry lab, and 921 mg/L in a toxicological chemistry lab, while mean values for men were 975 mg/L and 1395 mg/L, respectively (Arndt, 2009). In the study performed by Johnston et al. (2004), test subjects were divided in two low-fat, energy restricted groups. During a 6-week trial, one group consumed high-protein and the other high-carbohydrate food, and despite the fact that meat is a source of exogenous creatinine, there was no change in creatinine excretion.

Materials and methods

Subjects

For the survey, 49 volunteers were divided into several categories: sex, age, BMI, smokers/non-smokers, vegetarians/vegans/omnivores, monthly income per household member, education. In the age category, there were 3 subgroups: 22 to 30 years of age with 21 respondents, 31 to 40 years with 24 respondents and 41 to 52 with 5 respondents. There were 19 male and 30 female respondents. The BMI category consisted of 4 subgroups. There were 4 malnourished subjects (BMI<18.50), 34 subjects with a normal body weight (BMI=18.51-24.99), 5 of them with an excessive body weight (BMI=25.00-29.99), and there were 5 obese subjects (BMI>30.00). Fifteen respondents were smokers and 34 non-smokers. Based on the type of a diet, the subjects were divided into 3 subgroups: 5 lacto-ovo vegetarians, 30 omnivores and 14 vegans. In the education category, 11 respondents had a high school degree, 32 a bachelor’s degree, and 6 a master’s degree. Subjects were all healthy adults that did not use any medicine during the experiment. All participants volunteered and were informed about the study. Research was conducted with the permission of the ethical committee of the Faculty of Food Technology Osijek.

Collection of samples

All participants consumed the same food based on bread, bran, and bran flakes. They could consume milk and dairy products, meat, eggs, fruits and vegetables and all non-alcoholic and alcoholic beverages other than beer and should exclude all other cereals. The urine collection began in the morning after waking up. First morning urine was collected in a separate container. During the day, participants had to collect all the urine in a separate container. The urine had to be kept closed and in the refrigerator. The last sample of the first morning urine was collected the next day to complete a 24-h urine collection.

Samples preparation

After collection of urine, total volume was measured and 15 mL was separated, frozen and stored at -20 °C until measurement. Each sample was thawed at room temperature, mixed, and 1 mL was transferred to a 2 mL Eppendorf tube followed by a centrifugation for 3 min at 5600×g at room temperature. From the supernatant, 100 µL aliquots was mixed with 900 µL of distilled water, and this dilution step was repeated giving in total 1:10000 dilution of the urine sample. The diluted samples were transferred to vials before injecting to a LC-MS/MS system.

Creatinine analysis

Urinary creatinine levels were determined on AB Sciex 2000 LC-MS/MS (Foster City, CA) with a electro-spray ionization (ESI) and using method described in the experiment (Warth et al., 2012). Five µL were injected and separated isocratically on an Ascentis Expreess (2.1 mm×30 mm, Supelco, St. Louis, MO, USA). Particle size of column was 2.7 µm and a system had a C18 security guard cartridge. Eluent A was water and eluent B was ACN; 0.1% of acetic acid was added in both. Eluation with 95% of eluent A was performed during 1.5 min. Two transitions were monitored for additional conformation via ion ratio. The protonated molecule $[M H]^+$ was observed at *m/z* 114, as well as two product ions *m/z* 44.2 and 86.0.

Statistical analysis

Normality of the data distribution was tested using Shapiro-Wilk W test. Variance homogeneity was tested by the Levene's test. The differences between subgroup medians were tested by a pairwise Mann–Whitney U-test or Kruskal-Wallis ANOVA (depending on the number of variables). A *p* value <0.05 was considered statistically significant. All analyses were performed using Dell Statistica 12.0 (StatSoft, Tulsa, OK, USA) and Microsoft Office Excel 2016 (Microsoft, Redmond, WA, USA).

Results and discussion

Results indicate that there were no statistically significant differences in creatinine concentration (mg/L) when a first morning void and a 24-h urine sample are compared with different categories, as shown in Fig. 1. When observing median of creatinine concentrations in 24-h urine of lacto-ovo vegetarians (2175 mg/L), vegans (1235 mg/L) and omnivores (1328 mg/L), there is also no statistically significant difference, which is in correlation with the Johnston et al. (2004) study.

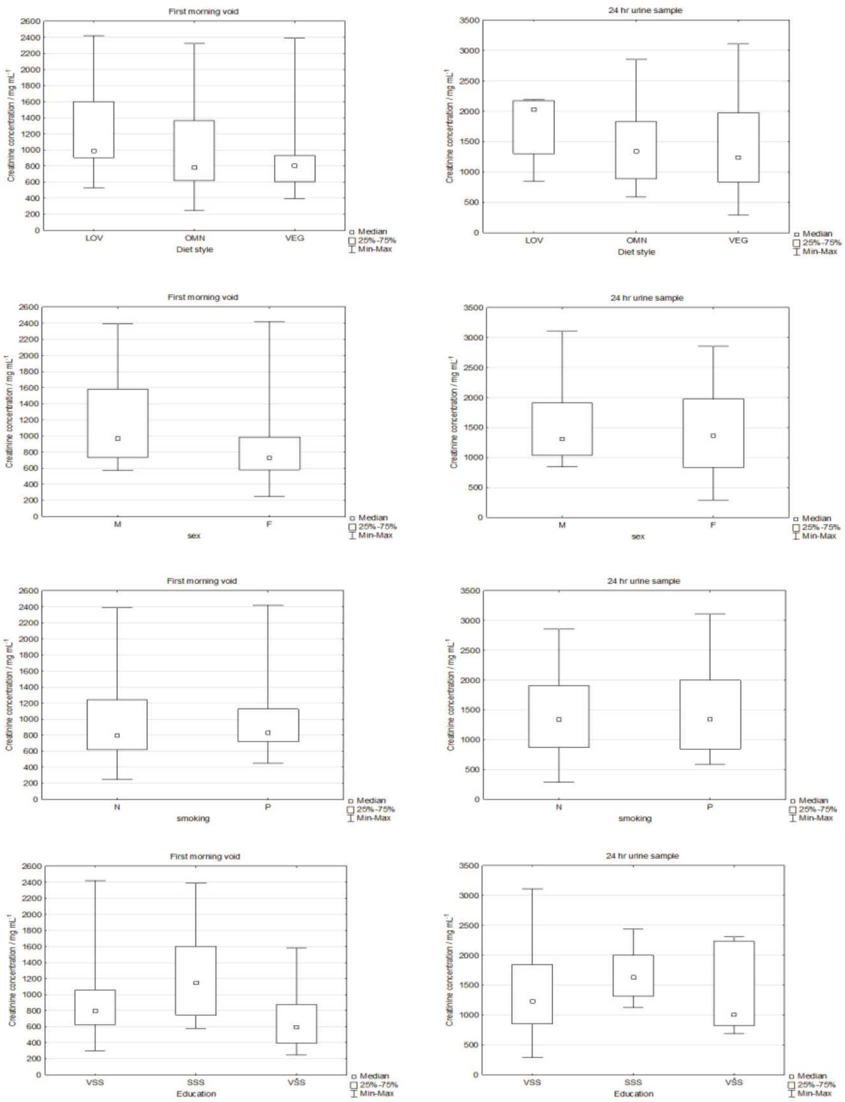

Fig. 1. Statistical analysis of the obtained data – first morning void and 24-h urine samples compared with the different categories of subjects

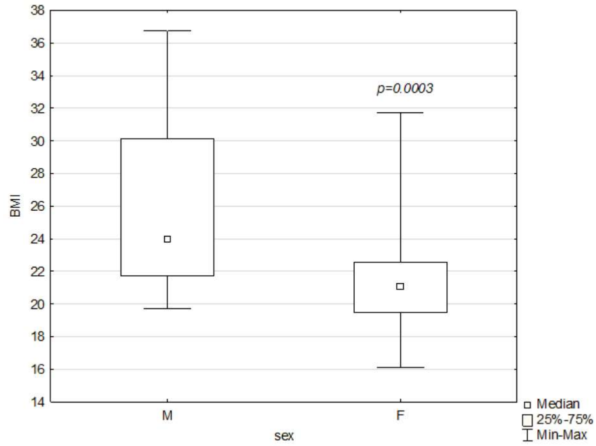

Fig. 2. Comparison of BMI and gender

The difference is that their subjects were divided in two energy restricted groups: high-protein and a high-carbohydrate diet, while in this study the subjects' diet was based on bread, bran and bran flakes, which are rich in carbohydrates. However they could eat their favourite food, with little restrictions, as long as they eat all the food that was delivered to them, including meat in the omnivore group, which is a source of proteins and *exogenous* creatinine. Another study (Mayersohn et al.,1983) reported an average of 13% increase in 24-h urinary creatinine excretion after feeding the subjects with boiled beef. Our omnivore subjects had a small increase in urinary creatinine, but since they could choose what to eat, aside from the food they were supposed to eat, they could eat less meat during intervention day than subjects from the Mayersohn study. Highest median of creatinine concentrations in 24-h urine was found in lacto-ovo vegetarians (2175 mg/L), but in that category there were only 5 subjects, so the results may not be representative, or some other factors such as sex, age, muscle mass and health status must be taken into consideration. Omnivore group had lower creatinine concentration median (1328 mg/L) and vegans had the lowest median (1235 mg/L). We could assume that omnivores had more exogenous creatinine from meat consumption, but generally, vegans take more care of their health, body and exercise (Appleby and Key, 2016), so they have more muscle mass and that could explain why the results are statistically insignificant. Males had higher creatinine concentrations in the first morning void (median 970 mg/L, ranging from 575 to 2394 mg/L) than females (median 732 mg/L, ranging from 249 to 2423 mg/L), but they also have higher percentage of muscle mass than females (Arndt, 2009), so when both categories (males and females) had majority of meals containing bread, bran and bran flakes on intervention day, there are no significant differences. Creatinine excretion in 24-h urine samples increased only slightly and median for males was 1314 mg/L (min 840, max 3113 mg/L), and for females 1359 mg/L (min 291, max 2859 mg/L). Third pair of graphs in Fig. 1 shows that there is also no significant difference in the creatinine excretion from a 24-h urine sample between smokers (median 1347 mg/L) and non-smokers (1336 mg/L). When 24-h urinary excretion of creatinine is compared with education, the highest results were obtained for subjects with a high school degree SSS (median 1630, min 1123, max 2443 mg/L), lower in urine of subjects with a bachelor's degree (median 1230, min 291, max 3113 mg/L), and the lowest in a subgroup of subjects with a master's degree (median 1003, min 686, max 2314). Although the results are not statistically significant, we can notice that the higher the education level, the lower the creatinine excretion, suggesting that educated people are more concerned with their dietary habits and have lower intake of red meat, and therefore exogenous creatinine (Daniel et al., 2011).

Only statistically significant differences can be noticed in BMI between females (21.11) and males (25.53) and are shown in Fig. 2. We have expected such results, as there are differences in the body structure between the genders. The difference can also arise from the expectations that women must have a slim, feminine figure leading to a more careful choice of food and meat avoidance (Jackson et al., 1987).

Conclusions

When the diet was normalised, the differences between different dietary styles and urinary creatinine excretion were not statistically significant. There were only statistically significant differences in BMI between females and males. This is a small scale study and to confirm the preliminary results, the study with more samples, homogenous subject groups with the same BMI, muscle mass and the same ratio of male/female gender and smokers/non-smokers should be repeated.

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