URINARY BIOCHEMICAL PARAMETERS MODIFIED DURING PERIODIC FASTING

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Abstract: *The paper is a prospective parallel study on the values of urinary biochemical parameters in 29 healthy men and women who were divided into two groups: the PF (periodic fasting) group (19 volunteers) who provided urine samples at baseline and after 7 weeks of religious fasting and the NPF (non-periodic fasting) group (10 volunteers) with omnivorous diet. Urine analysis shows a significant difference between calcium levels (0.10 ± 0.08 g/L vs 0.26±0.21 g/L, p <0.05), uric acid (0.11 ± 0.03 g/L versus 0.49 ± 0.38 g/L, p <0.05) and urea (17.21 ± 5.06 g/L versus 25.04 ± 4.28 g/L, p <0.05) in PF and NPF groups at the end of the trial, while the difference is not significant for ascorbic acid and creatinine values.*

INTRODUCTION

Dieting in the modern world is caused by several factors: factors related to the individual's health (dietary measures in dyslipidemia, obesity, diabetes, etc.) or personal reasons (aesthetic criteria – restrictive diets in nutrients intake or related to religious faith - the religious post).

The Greek or Orthodox religious fasting resembles the intake of kcal and nutrients with a vegetarian diet. There are two types of fasting periods: intermittent fasting that involve a limitation of caloric intake and nutrients of animal origin over a period of 16-48 hours, 2 days a week or every other day and periodic fasting (PF) where the intake of nutrients is limited for 2 to 21 days.(1)

Caloric restrictions have proven clinical trial benefits - changing the metabolism of various energy substrates (glucagon-related switch from glucose utilization as the main cellular energy carrier to lipolysis and fatty acid metabolism by β-oxidation) (2), modifying cellular apoptosis, activation of cellular stress mediators (3), DNA repair, stem cell regeneration.(1)

Nowadays, 12% of the world's population is over 65 years and the percentage is rising. Studies show that caloric restriction and intermittent fasting increase lifespan and delay the onset of diseases such as cancer or diabetes.(4)

However, a study by *Ajabnoor GM et al*. in 2017 shows that during Ramadan fasting, sleep disturbances occur, suppression of circadian biorhythm regulating cortisol secretion with concomitant increase in basal levels, increase in serum levels of some insulin-releasing adipokines (5) and babies born to mothers who followed Ramadan during the first months of pregnancy had a lower birth weight.(6)

During the PF, the caloric intake generally decreases because the consumption of food of animal origin is reduced and the intake of trophins of vegetable origin increases, their absorption being influenced by the presence of food fibers, pectins and other substances that are partially digestible or not digestible in man. In PF as well as in vegetarian diet by lowering saturated fat intake results in lower total cholesterol and LDL fraction but without changes in serum triglyceride levels (7), while protein and amino acid intake especially essential aminoacids but nonproteinogenic amino acids such as creatine or β-alanine is reduced.(8) Carbohydrate intake is not modified or it is even increased, animal products are often replaced in the daily meals by carbohydrate, especially those with high glycemic index.(9)

PURPOSE

The purpose of the study is to identify urinary biochemical parameters modified by restrictive diet in PF in young volunteers without known chronic pathologies by simple determinations from the first urine. The values obtained at the end of the PF are compared with the values obtained for the same person before the PF onset and with a NPF group.

MATERIALS AND METHODS

Study design. This is a prospective parallel study, which took place in Tîrgu Mureş for a period of 7 weeks (27.02.2017-16.04.2017). The study was approved by the Ethics Committee for Scientific Research of the University of Medicine and Pharmacy Tîrgu Mureş.

Study participants. In this study, 29 students from the Faculty of Nutrition (University of Medicine and Pharmacy) and members of their families were divided into two groups on a voluntary basis after the informed consent was obtained: 19 volunteers were assigned to the group PF (periodic fasting) following a restrictive dietary intake diet during the 7-week religious fasting and 10 healthy participants of age and gender similar to the PF group were allocated to the non-periodic posting (NPF) group who did not follow restrictive / periodic fasting.

Urine sample. A sample first urine of urine was collected at the time 0 (before the start of the PF and at the end of the PF, 7 weeks later); the urine sample was frozen and handed over to the analyst.

Subject selection. Determinations using the Medi-test Uryxxon Stick 10 strips allowed the identification and quantification of blood, urobilinogen, bilirubin, proteins, nitrite, ketone bodies, pH, urine density and leukocytes in the urine by

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introducing the strip into the urine for about 1 second and comparing color changes with the color chart on the tube after 30-60 seconds (for leukocytes after 60-120 seconds). Only healthy individuals were selected - the parameters determined (the presence of blood, urobilinogen, bilirubin, proteins, nitrites and leucocytes) are characteristic for a pathological urine and secondary identification of ketone bodies in urine. The volunteers verified the presence of ketone bodies in the urine several times during the 7 weeks of PF (ketosis and ketonuria are characteristic in individuals following an unbalanced diet / fasting in carbohydrate intake). No ketone bodies were identified in any of the samples, because low-carbohydrate intake and high glycemic index of fruits and vegetables prevented the body from functioning on ketosis.

Determination of ascorbic acid in urine was performed as follows: 3 ml of distilled water, 1 ml of 2% HCl, 2.5 ml of 1% KI, 2 ml of 0.5% starch and 1 ml of diluted urine. The mixture was stirred and titrated with KIO_3 N/1000 until a blue color appears. A control sample containing 1 ml of distilled water in exchange for the biological product was also made.

Determination of urea by enzymatic method. Urea is decomposed quantitatively into NH_3 and CO_2 by urease, and the released ammonia is determined by the Berthelot reaction with phenol and sodium hypochlorite in alkaline medium. 0.02 ml of urine is incubated with 0.2 ml urate for 15 minutes, 1 ml of phenol and 1 ml of hypochlorite reagent are added and the absorbance of the test sample and standard sample at 630 nm is determined.

Determination of uric acid by colorimetric method. 4 ml of urine was added 0.2 ml of phosphotungstic reagent and 1.8 ml of 22% Na_2CO_3. After 10 minutes the absorbance was determined at 610 nm against a control which contained distilled water instead of urine.

Determination of calcium by complexonometric method. To 2 ml of urine 0.2 ml of 9N NaOH was added and triturate with complexion III N/100 until purple staining occurs in the presence of the murexide indicator.

Determination of creatinine. 0.5 ml of urine is treated with 1.5 ml picric acid, 0.1 ml 10% NaOH is added and the absorbance of the sample is determined at $\lambda = 530$ nm.

RESULTS

The results obtained in urine samples analysis at the beginning of the PF period for the studied parameters in the NPF and PF lots are shown in table no. 1.

Table no. 1. Urine values of the analyzed parameters for NPF group at the beginning of the study (NPF_i) and for the PF group (PF_i)

Parameter	NPF_i (10 volunteers) Value ± SD (limits min,max)	PF_i (19 volunteers) Value ± SD (limits min,max)	P value
Creatinine (g/L)	1.37 ± 0.97 (0.20-1.76)	1.25 ±0.52 (0.27-1.71)	NS
Uric acid (g/L)	0.58 ± 0.35 (0.22-0.83)	0.47 ± 0.26 (0.25-0.89)	NS
Urea (g/L)	26.72 ±12.19 (18.00-35.44)	24.81 ± 10.07 (19.80-29.82)	NS
Ascorbic acid (g/L)	9.68 ± 10.53 (0- 26.40)	7.82 ±9.68 (0-35.20)	NS
Ca^{2+}(g/L)	0.23±0.18 (0.18-0.63)	0.27 ± 0.25 (0.14-0.40)	NS

The results obtained in urine samples analysis at the end of the PF period (7 weeks) for the studied parameters in the NPF and PF lots are shown in table no. 2.

Table no. 2. Urine values of the analyzed parameters for NPF (NPF_f) and for the PF (PF_f) group at the end of the study

Parameter	NP_f (10 volunteers) Value ± SD (limits min,max)	P_f (19 volunteers) Value ± SD (limits min,max)	P value
Creatinine (g/L)	1.21 ± 0.76 (0.69-1.53)	1.15 ±0.63 (0.53-1.26)	NS
Uric acid (g/L)	0.49 ± 0.38 (0.34-0.97)	0.11 ± 0.03 (0.09-0.41)	p<0.05
Urea (g/L)	25.04 ± 4.28 (21.98-29.11)	17.21 ± 5.06 (14.69-19.73)	p<0.05
Ascorbic acid (g/L)	10.23 ± 7.21 (0- 11.55)	9.69 ± 8.89 (0-22.95)	NS
Ca^{2+}(g/L)	0.26±0.21 (0.15-0.57)	0.10 ± 0.08 (0.06-0.15)	p<0.05

All results are expressed as mean ± SD. Data obtained from the PF and NPF groups were analyzed at the beginning and at the end of the FP and depending on the results obtained in the normality test (Kolomonov-Smirnov), the ANOVA one way test or its equivalent for non-parametric variations Kruskal Wallis test was used. For multiple comparisons as a post-hoc test, Tukey-Kramer test (in case of normal distribution) and Dunn's Multiple Comparison test (for nonparametric distribution) were used.

DISCUSSIONS

Creatinine values are within the normal range (0.8 - 1.9 g/24 hours) of this method. There was no significant difference between the initial and final values of the studied groups, this parameter being characteristic of renal function, not significantly altered in the case of short-term diets. Creatine is a nonproteinogenic amino acid synthetically produced in humans from the amino acids glycine and arginine or obtained from muscle animal protein consumption where it is in the form of phosphoric acid ester - creatine phosphate. Lack of animal proteins in the diet for vegetarian diets significantly reduces the intake of creatine and precursors for synthesis and, therefore, decreases the amount of creatinine excreted in the urine.(10) A study by *Pilis W et al* shows that low intake of animal protein on the one hand reduces the risk of cardiovascular disease, body mass index, atherosclerosis, cancer, insulin resistance, but can increase the risk of protein malnutrition, anemia, hyperhomocysteinemia, reducing muscle creatine-phosphate with decreased muscle strength and effort capacity and even menstrual disorders in women.(11) Besides the well-known effects of creatine phosphate as cellular energy mediator in striated muscle, recent studies show its antioxidant and antiapoptotic role extremely important in neurology (in cerebral ischemia, hypoxic ischemic encephalopathy), cardiology as an adjuvant in heart surgery (valve replacement, coronary artery bypass) or in the acute myocardial infarction.(12)

Calcium urinary elimination in the PF group decreases significantly ($p < 0.05$) due to a diet rich in insoluble fiber (cellulose, phytic acid, oxalic acid) that binds calcium in a non-absorptive form and is eliminated by faeces. There are literature data suggesting vitamin D deficiency in vegetarian or restrictive diets as it is synthesized in the skin from provitamin - cholesterol of animal origin and ergosterol derived from vegetable oils.(8)

Urine ascorbic acid values are within the normal range for both the NPF group and the PF group proof of balanced nutrition, these values do not change significantly after 7 weeks of fasting. The pharmacokinetics of ascorbic acid is extremely complex involving active or passive transport processes between different compartments, the distribution being selective in different organs and tissues.(13) The results are according to literature data, *Lindblad M et al* demonstrate no significant

differences in a relatively short period of time, such as religious fasting, in the elimination of ascorbic acid in urine even in the case of increased intake of vegetal products.(14)

Urea is the non-toxic form of ammonia elimination in humans, produced by hepatic and renal ureogenesis. In PF group urea elimination decreases significantly ($p < 0.05$) after 7 weeks of fasting, this being explicable if we consider that the digestibility of vegetal proteins is lower than those of animals and in case of balanced caloric intake the use of amino acids as energy suplies and increased endogenous proteolysis is out of the question.(15) A 2015 study shows that muscle protein anabolism is reduced in case of high consumption of soy or wheat protein compared to animal proteins - this being due to the low content of essential amino acids in plant proteins, especially in leucine and other branch chain amino acids, but also on the presence of insoluble food fibers such as cellulose that are non-digestible in humans and in addition can adsorb on their surface the amino acids that become practically inabsorbable.(16) Carnitine is a nonproteinogenic amino acid important in the transfer of activated fatty acids through the mitochondrial membrane for β-oxidation obtained from diet - animal meat or from endogenous synthesis from trimethyl-lysine. Studies show, however, that a carnitine deficiency syndrome may occur in the case of a vegetarian diet, and clinical manifestations of carnitine deficiency only occur if the level in the body is extremely low.(17)

Uric acid values are within the normal range in the urine of the PF and NPF groups, with no significant differences between groups at baseline, but after the PF, urinary values decrease significantly ($p < 0.05$) in the PF group. Uric acid is a purine structure compound resulting from the catabolism of proteins in the nucleus. Serum uric acid levels are highly dependent on diet, and uricosuria levels depend on serum values - a large population study on 670 men and 1023 women shows that uric acid levels have decreased from omnivorus diet to vegetarians to fish consumers.(18) Data on the influence of PF on urine and serum levels of uric acid are contradictory. During fasting, the diet is similar to vegetarian over a certain period and then to the fish-vegetarian diet when fish products are allowed, which would suggest a decrease in serum uric acid levels during fasting, while a study conducted in 35 students during Ramadan post show a change in LDL/HDL cholesterol ratio but not a change in blood pressure, HbA1c or serum uric acid.(19) A meta analysis by Zhao G et al indicates that elevated serum uric acid levels are an independent predictive factor in all-cause mortality associated with cardiovascular mortality.(20,21)

CONCLUSIONS

The religious fasting is similar to a vegetarian diet to some extent, and this is also demonstrated by the values of urinary biochemical parameters. Changing the intake of trophins of animal origin in favor of those of vegetal origin is reflected in the value of some urinary parameters such as urea or uric acid suggesting a beneficial effect on short term but long-term studies show that undesirable changes in parameters such as calcium or urinary pH also occur.

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