Daily water regime and sample sampling affect Blood and Urine parameter value change in healthy individuals

Snezana M Jovicic (✉ sneza90bg@hotmail.com)

Faculty of Biology, University of Belgrade

Research

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Abstract

Background: Blood and Urine biomarkers are indicators of the subject health status. Understanding the effect of pre-analytical factors is important for the data quality of bio-specimens. Water as a necessary element for the normal functioning of living beings and sampling frequency influences the homeostatic range of parameters. The study examines the effect of 9-days fluid intake and 2-time sampling on concentration changes of 7-Urine and 17-Blood variables.

Material and Method: SPSS software v23.0 applies to data processing. The group of 23 healthy subjects divide based on water intake and gender.

Results: A statistically significant difference($p<0.01$) between 1st/2nd sampling is confirmed for Freezing point depression, Sodium, Potassium, Creatinine Urea and Urate in Urine, Urea, Urate, Glucose, Hematocrit, Thrombocyte in Blood. The difference between water intake after 1st sampling is confirmed ($p<0.01$) for Freezing point depression, Sodium, Urate and($p<0.05$) for Potassium($p<0.05$), Chloride($p<0.05$), Creatinine($p<0.05$), Urate, Urea in Urine and Potassium($p<0.01$) and Chloride($p<0.05$) in Blood. Difference between gender exists for Urea($p<0.05$) in Urine after 2nd sampling and Urate($P<0.01$), Glucose($p<0.01/0.05$), Ht($p<0.01/0.05$) after 1st and 2nd sampling and MCHC($p<0.01$) after 2nd sampling in Blood samples.

Conclusion: Water intake increases Blood and Urine biomarker range after sampling.

Background

Homeostasis improves and maintains organism functional systems^[1]. The nervous system regulates organism homeostasis in the inward environment by answering external stimulus^[1]. The vegetative and autonomous nerve system regulate the function of the internal organs^[1]. Enable the transport of hormones, vitamins, minerals and metabolism products through the Blood to distant tissues and organ systems^[1]. Fluid in the inner environment contains nutritional material and oxygen, enabling the continuous renewal of molecules at the capillary^[1]. Body fluid consists of cellular (in cells) and extracellular (in the Blood) water^[2]. The Water amount in an organism depends on body mass, nutrition, age and gender^[3]. The cellular fluid has a changeable lining and depends on tissue and organ^[3]. Water has a protective (part of immunoglobulin, proteins) or regulator (par of hormones or enzymes) role in the body^[2]. Water is an essential molecule for all living organisms and the most common compound on earth^[2]. It is the ideal solvent and forms a useful environment for biochemical processes in a temperature range of 0-100°C^[2]. Water forms hydrogen bonds with proteins, DNA and RNA molecules^[2]. Water intake and hydration status are associated with disease prevalence, disease development and exercise performance^[3].

The homeostatic mechanism precisely maintains Blood plasma and extracellular fluid content [3]. The **Blood** regulates cardiac function, integrity and elasticity of blood vessels, communicates with cells, tissues and organs [4]. Blood has a respiratory, nutritive, excretory, regulatory, transport and defensive role in an organism [4]. Water is quantitatively the most significant blood constituent. Blood contains fluid (45%, plasma, serum) and constitutive (55%, shaped elements like Erythrocytes, Leucocytes, Platelets) component [4]. *The serum* is plasma without fibrinogen. *Plasma* contains organic and inorganic matter, like water (92%), proteins (7%) and other ingredients (1-2%) [5]. Plasma contains blood cells of whole blood, proteins (albumin, globulin and fibrinogen), glucose, clotting factors, electrolytes, hormones and carbon dioxide. The most indispensable cation ions are Na^+ , K^+ , Ca^{2+} , Mg^{2+} and anion ions like Cl^- , P^{2+} , PO_3^- , SO_4^- , NaH_2PO_4 , Na_2HPO_4 and microelements like Fe^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , $\text{J}^{6,17}$. The most common blood salts, NaCl and NaCO_3 , regulates osmotic pressure and chemical reactions. [7].

Urine is a renal product of organic and inorganic material, a bright liquid with a light yellow colour, specific smell and salt-bitter taste [8]. The founded state of metabolism and nutrition causes changes in Urine constituent in the physiological and non-physiological range. The colour of Urine depends on constituent concentration, pigments and pathological content. Urine contains 95-97% water and 3-5% diluted material. Inorganic constituents are Na^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4 and anion Cl^- , P^{2+} , HCO_3^- , SO_4^{2-} [8]. Organic components can be nitrogen and non-nitrogen [8]. Nitrogen materials are urea, creatinine_Cr, uric acid, amino acid, while non-nitrogen materials belong to phenol, oxalate, glucuronic acid and milky acid [8]. A small number of ferments, hormones and vitamins are present [8]. Intercellular fluids are relatively constant fluids, while tissue fluid and blood plasma are inconstant to change [1]. Maintaining organism homeostasis is significant for permanent composition and volume of body fluid (1). Human organism contains small water supplies. During the day, 1.5-2l of water evaporates through Urine, faeces, lung sweat and skin [20]. Knowledge about biological and chemical processes affects establishing reference range, disease diagnosis, prognosis and follow-up.

Sustainable effort must obtain for sensitivity, specificity, robustness and reproducibility of data. Pre-analytical factors influence sample quality; reproducibility, stability and false-positive results. [9]. Pre-analytic variables include 3. categories, physiologic (age, gender, sex, time, season, altitude, menstruation, pregnancy, lifestyle like diet, caffeine, ethanol, smoking), specimens collection (overnight fasting, time of specimen collection, posture during sampling, exercise, water intake, anticoagulants-blood ratio, specimen handling and processing, added additives with anticoagulants), and influence or interference factors (drug metabolites, laboratory tests, collection tube) affecting variable range [9]. Information about water intake and sampling enable appropriate diagnostic and therapeutic strategy for human disease monitoring and treatment. Water intake, a pre-analytical variable, contributes to biochemical processes and affects establishing reference range, disease diagnosis, prognosis and follow-up [10]. Studies analyzed conditions like storage time, temperature, freezing-thawing cycle on biomarker range and reproducibility [9]. Despite increasing sensitivity of methodology for determining biomarker range, discrepancies among variables exist in the literature due to sampling frequency, sample and pre-

analytical factor interaction complexity^[9]. Critical aspects of biomarker results, stability during clinical planning, sample collection, training, selection of sample preservation, buffers, shipping, logistics and method analysis is known for the most utilized bio-specimens^[9]. **Urine and Blood** samples are the most commonly used in clinical practice reflecting state of metabolome and metabolic end product^[10]. Few researchers correlated Urine and Blood parameters with water intake in healthy participants. The relationship between hydration, Urine/Blood biomarker and total fluid intake in pregnant and lactating women **during 3 semesters is estimated**^[11]. Observational studies recorded and assessed the amount of firm food/ beverage and **Urine parameters** (osmolarity, volume, gravity and colour) in healthy children and elderly individuals for 24h^[12,13]. A cross-sectional study on healthy Male college students in China assessed hydration status, fluid intake and Urine biomarker (osmolarity, specific gravity, pH, the concentration of K⁺, Na⁺ and Cl²⁻) for 24 hours^[14]. Moreover, Urine hydration biomarker (PO₄⁻, Uric acid, Urea, Cr, K⁺, Na⁺, Mg²⁺, Ca²⁺) for 24 hours is analyzed^[15]. Change in Blood biomarkers (Whole Blood cells, Erythrocyte_ER, Haemoglobin_Hg, Hematocrite_Ht, Mean Corpuscular Volume_MCV, Mean Corpuscular Hemoglobin_MCH, Mean Corpuscular Hemoglobin Concentration_MCHC, Mean Platelet Volume_MPV, Trombocytes_TR) in the control_C and test_T group on 1st day and two weeks later is determine^[16]. Urea concentrations in Blood samples after freezing at -80 degree is analyzed^[17]. Fluid intake habits and sample sampling effect on Urine and Blood biomarkers of healthy participants lack in literature. Sample sampling can lead to results bias. Protocol for sample collection frequency, handling, and storage ensure reliable analysis of disease in routine practice and clinical trials^[18]. Daily circadian rhythm influence physiological processes and diurnal dynamic^[19]. Time-of-day-dependent oscillations in Blood level molecules could be a potential cause of variability in laboratory results, making sampling time an important consideration^[19].

Blood and Urine biomarker change during the regime of 9 consecutive days of water intake in healthy participant lack. There is a lack of literature about the effect of water intake regime and sample sampling as a pre-analytical factor influencing Blood and Urine biomarker range in healthy subjects. A current study was done as a part of the PhD thesis aim to examine the effect of 7 and 9-day regime water intake in Test (T) and Control (C) group on 7-Blood (Freezing point depression_FPD, Potassium_K⁺, Sodium_Na⁺, Chloride_Cl⁻, Urea, Creatinine_Cr, Urate) and 17-Urine (Urea, Cr, Urat, Glucose_Glu, C reactive protein_CRP, Leucocyte_LE, Erythrocyte_ER, Hemoglobine_Hg, Hematocrit_Ht, Mean Corpuscular Volume_MCV, Mean Corpuscular Hemoglobin_MCH, Mean Corpuscular Hemoglobin Concentration_MCHC, MPV_Mean Platelet Volume, Trombocyte_TR, K⁺, Na⁺, Cl⁻) parameters in healthy subjects, during 2 sample collection, depending on gender and water intake level. Hypothesis postulate that water intake affects the reference range of Urine and Blood biomarkers.

Material And Methods

Study group and Human biological material dataset

Experiments were performed according to ethical standards and with the written consent of the Blood donors. Permission from the National Medical Ethics Committee, number 82/07/14, is given. The human

Blood and Urine information dataset obtain from the collaborative institution.

Participants differ by Gender (Male_M; Female_F), the amount of drinking water, Blood and Urine parameter values. The total number of participants consists of 23 healthy participants (F=9, M=14). M (60.9%) were numerous in comparing to F (39.1%) participants. Mean water intake for 5 days, is recorded. An upward trend in the T group and downward in the C group exists. The T group drank more than 1800 ml with a mean water intake value of 2055 ml. The C group consumed less than 2000 ml, with a mean water intake value of 1846 ml. The number of M/F participants in the T/C group varies. The 14 subjects in the T group [M:(9/23); F:(5/23)] represent 39.12% M and 21.74% F of the total participant. Meanwhile, 9 subjects in the C group [M:(5/23); F:(4/23)] represent 21.74% M and 17.4 % F-number of the total participant.

The regime of water intake for 23 healthy subjects included 5-days of controlled water consumption, 2-days of arbitrary higher water intake in the T group before 1st sampling (7th-day of water intake), 2-days of desired lower water intake in the T group before 2nd sampling (9th-day of water intake), while C group drank the same level of water during 1st and 2nd sampling. Of the total M/F participants, 7-Urine and 14 Blood (Urea, Creatinine, Urate, Glucose, CRP, LE, ER, Hg, Ht, MCV, MCH, MCHC, MPV, TR) variables tested for a change. On 11 male participants, 3 additional electrolytes (Na⁺, K⁺, CL⁻) test for a change.

Statistical analysis

IBM-SPSS software v23.0 applies for statistical data analysis. Descriptive statistics, Shapiro-Wilk normality test, parametric and non-parametric statistical tests such as Paired and Independent T-test, Wilcoxon and Mann-Whitney/Kruskal-Wallis test is employed. Results display **mean (SD)** and **p-value**. A statistically significant correlation was assumed when **P<0.05**.

Results

Participant information

Urine and Blood characteristics are present in Table 1 and Table 2. Proposed values of **7 Urine (FPD, K, Na, Cl, Urea) and 17 Blood (Urea, Cr, Urat, Glu, CRP, Le, ER, Hg, Ht, MCV, MCH, MCHC, MPV, TR, Na, K, Cl) biomarker are subject to change during 1st and 2nd sampling**. Shapiro-Wilk test indicated the presence of normality ($P>0.05$) for FPD, K⁺, Na⁺, Cl⁻, Cr and Urate during 2nd sampling and Urea during 1st sampling in Urine samples and for Glucose, MPV during 2nd sampling, Urea, Urate, Ht, MPV during 1st sampling and K⁺ during 1st and 2nd sampling in Blood samples. All other variables showed an absence of normality ($P<0.05$). All non-parametric data transform to normality using log transformation, except for CRP and additional electrolytes in the Blood. Based on this notion, the parametric and non-parametric test is employed.

Assessing the significance of mean difference (Paired and Independent T-test, Wilcoxon, Mann Whitney and Kruskal-Wallis test)

Paired T-test revealed a statistically significant difference ($P < 0.01$, 95% CI) between the 1st and 2nd sampling for FPD, Na^+ , K^+ , Cr, Urea, Urate in Urine and Urea, Urate, Glu, Ht, TR in Blood. Wilcoxon T-test shows the absence of a statistically significant difference ($P > 0.05$, $\alpha = 0.05$, 95% CI) for CRP in Blood. Results match with Mann-Whitney and Kruskal-Wallis test. The difference between the T/C group confirms independent T-test for FPD ($P < 0.01$), K^+ ($P < 0.05$), Na^+ ($P < 0.01$), Cl^- ($P < 0.05$), Cr ($P < 0.05$), Urate ($P < 0.01$) and Urea ($P < 0.05$) in Urine after 1st sampling and K^+ ($P < 0.01$) and Cl^- ($P < 0.01$) after 1st sampling in Blood. Figure 1. shows the difference between test and control group for 1st and 2nd sampling in Blood and Urine. Gender difference confirmed for Urea ($P < 0.05$) variable after 2nd sampling in Urine and for Urate ($P < 0.01/0.05$), Glu ($P < 0.01/0.05$) and Ht ($P < 0.01/0.05$) after 1st and 2nd sampling and MCHC ($P < 0.05$) during 2nd sampling in Blood.

Discussion

Understanding physiological changes enhance the quality interpretation of subject information. Investigating water intake regimes and days of sampling contribute to understanding the physiological response. Proper hydration can have a beneficial effect on human health [2, 3]. The literature contains insufficient information regarding the optimal amount of water intake for disease prevention, linkage of water amount and health outcome. Epidemiological data failed to provide appropriate health evaluation. The effects of water consumption on Blood and Urine samples are analyzed. Usually, studies examined the influence of 1.3-4l/per day water intake for 1 and 3 days, 1st and 3rd gestation week in healthy female/male participants, aged 18-83 years. The analysis contains a generation gap between 40-60 years of age.

Parameter changes of pH, colour, volume, osmolarity, specific gravity, phosphate, uric acid, Urea, Cr, K^+ , Na^+ , Cl^{2-} , Mg^{2+} and citrate in Urine samples were assessed [11, 12, 14, 15]. Literature results from 24h Urine sample analysis indicated the presence of discrepancy in correlated data and that different average water intake amount and proposed methodology affect parameter relation. The study reveals the association of total fluid intake with Urine colour, osmolarity, specific gravity and solute concentration [13]. The cross-sectional analysis showed the association of Urine volume with osmolarity, gravity and osmolarity, osmolarity and colour, colour and gravity [12, 14]. There are no publications regarding the influence of 5 days water intake combined with 2 days of arbitrary consumption of large water amount till 1st sampling and lower water amount till 2nd sampling in T group and continuous water intake in C group during 1st and 2nd sampling on Blood and Urine parameters in healthy individuals, as this is the case with this study.

Descriptive statistic details regarding subjects contribute to characteristics of Blood and Urine parameter change and correctness of the report by data collection and analysis. A subject profile, who took part in the survey, was developed. Age and Gender variables are present. Duration of water intake, sample sampling and influence is assessed. Quality control implements for data selection, analysis and interpretation. Reliability and validity are high, confirmed by the accuracy of measurements and

correlation value. This study has high standardization of the pre-analytical factors. Describes, justifies and discuss the research topic. Normally distributed data as a structural model of the Shapiro Wilk test is utilized in the analysis. Non-normal data converts to normal. Results are quantified. Preliminary results established research validity with statistical significance ($P < 0.01, 0.05$). Basic statistical methodology compares the test and control group of participant data. Results identified the correlation between multiple covariates in healthy participants indicating the importance of knowledge of appropriate statistics. The study has a short term follow up, 7 and 9 days considered in the analysis.

The amount of water intake, diseases, usage of drugs and profession type can lead to electrolyte misbalance resulting in quality and interpretation, further prognosis, diagnosis and patient follow-up [20, 21]. In this study for 5 days, F drank a higher water amount comparing to M. In this study, the percentage difference between genders exists for Urea (52.22%) after 2nd sampling in Urine. In Blood for Urate (1st:15.16%; 2nd:14.5%), Glu (1st:13.63%; 2nd:8.7%), Ht (1st and 2nd:7.23%) during 1st and 2nd sampling and MCHC (2.1%) during 2nd sampling. Males have higher values in comparing to females. Sex hormones affect gender differences [22-25]. Female sex hormones (Estrogen) regulate the activity of glucose and urate transporters (ABCG2 and SLC2A9), having different transporter expression (transcription, post-translational modification), localization and activity [22, 23]. Male sex hormones (Testosterone) affect MCHC and Ht level through the increase of erythropoietin, reduction of ferritin and hepcidin [24]. Testosterone influence protein metabolism and the Urea cycle [25].

Drinking more water improves kidney function and clearance of toxins by glomerular filtration, tubular secretion, and activation of various degradative metabolic pathways [26]. Results indicate that water intake influence Urine [FPD (86.5%), K⁺ (81.7%), Na⁺ (104.1%), Cl⁻ (97.37%), Urea (75.34%), Cr (116.45%), Urat (89.65%)] and Blood [K⁺ (9.5%), Cl⁻ (3.92%)] during 1st sampling. There is a link between water intake and homeostatic mechanisms to maintain water balance and health outcomes. Urine osmolality depends on cations, Na⁺, K⁺, NH₄⁺, anions and Urea, whereas FPD enables estimation of Urine osmolality [27, 28]. Freezing point depression as a colligative property depends on the molality of the solute [29]. Renal Cr excretion level depends on the glomerular filtration rate, proximal tubular secretion and OCT-2 transporter [30, 31]. Higher water intake after 3. days causes a decrease of uric acid, up-regulation of GLUT9 and URAT1 and down-regulation of ABCG2 and OAT1, while after 7. days affect NPT1 down-regulation in hyperuricemia mice [32]. Urate level depends on transport proteins (URAT1 and GLUT9), uricase inactivation and possible change of the intestinal microbiota [32]. Production of concentrated Urine requires interactions among the nephron segments and vasculature in the kidney medulla [33]. Arginine vasopressin (AVP) is a crucial molecule in water homeostasis. Increase water intake, decrease AVP, reduce risk of renal and metabolic diseases and improve health outcome [33]. Vasopressin regulates Urea transport acutely by increasing UT-A1 phosphorylation and the apical plasma-membrane accumulation of UT-A1 through two cAMP-dependent pathways [34]. Glut9 plays a role in urate homeostasis by its dual role in urate handling in the kidney and uptake in the liver [35]. Small water intake can lead to dehydration, activation of the renin-angiotensin system (RAS) through angiotensin receptors and subsequent

activation of signalling molecules, protein kinase C, reactive oxygen species, MAP kinase pathway mediated with angiotensin ^[36]. Prolactin, aldosterone and antidiuretic hormone influences water metabolism and electrolyte balance ^[37-46].

Results show higher concentration percentage increase in variables from Urine [FDP (68%), Na⁺ (62.96%), K⁺ (21.88%), Cr (65.2%), Urat (69.2%), Urea (96.27%)] in comparing to Blood [Urea (10.5%), Urate (7.1%), Glu (2.2%), Ht (2.43%), TR (3.9%)] between 1st and 2nd sampling of 7th and 9th day of water intake. Studies indicate that daily water intake in healthy Japanese adults' decrease blood pressure, Ht, Urine gravity and a rise in body temperature ^[39]. Lower and steady daily water intake increase Cr, Cortisol, Urea, Uric acid, Na⁺, Hg; decrease CRP, and have no change in Ht, active rennin, aldosterone and plasma osmolality ^[16, 47, 48].

Ex vivo cellular injury, disintegration, cellular granule release and protease activation causes alterations of cell release in vitro after sampling ^[49]. Hydration biomarkers in 24h Urine correlate with daily total fluid intake volume in sedentary adults in free-living conditions ^[15]. Literature findings follow the results of the study. Explain differences in concentration changes of healthy participants due to water intake and sample sampling.

Conclusions

Information about the water regime during 7-9 days and gender in healthy participants positively impact further clinical studies on disease patients. Water intake and sample sampling change Blood and Urine biomarker concentration. Future work should emphasize disadvantages by comparing health/disease states to draw clinically applicable conclusions. **Advantage** of the study is clinical reproducibility, applicability, fast and precise insight into physiological changes. Descriptive and preliminary results can be a reference point for protocol standardization and quality control check. Lead the improvement of healthcare service. **The study disadvantage** is the number of participants and the absence of additional information (habits, demography and genetic analysis). Change of Blood and Urine parameters represents the clinical outcome. Demographic characteristics are not analyzed. The comparison between the water intake group, sample sampling and gender are analyzed. Methodology for determining the range of Blood and Urine parameter is missing. Subjects who are missing data regarding water intake do not participate in the study. The hypothesis justifies concepts from the previous studies.

Abbreviations

Arginine vasopressin (AVP), Chloride_Cl⁻, Control_C, Creatinine_Cr, C reactive protein_CRP, Erythrocyte_ER, Female_F, Freezing point depression_FPD, Glucose_Glu, Hematocrit_Ht, Hemoglobine_Hg, Leucocyte_LE, Male_M, Mean Corpuscular Volume_MCV, Mean Corpuscular Hemoglobin_MCH, Mean Corpuscular Hemoglobin Concentration_MCHC, MPV_Mean Platelet Volume, Potassium_K⁺, Sodium_Na⁺, Test_T, Trombocyte_TR,

Declarations

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Availability of data materials

Data are available in Table 1 and 2.

Ethical approval and consent to participate

Experiments were performed according to ethical standards and with the written consent of the sample donors. Permission from the National Medical Ethics Committee, number 82/07/14, is given.

Consent for publication

"Not applicable"

Competing interest

The author, a PhD student, Snežana Jovičić, declare no competing interest.

Author's contribution

Author (Snežana Jovičić, PhD student at the Faculty of Biology, University of Belgrade, Serbia), originated data collection, analysis, interpretation and writing. The author made all the effort, accuracy, integrity and quality. The author approves the final version of the presented manuscript for submission. The author confirms that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Author's information

Snežana Jovičić, a PhD student, is born on 17.10.1990 in Belgrade, Serbia. Academic qualifications: enrolment of PhD studies, Genetics (2014). Snežana Jovičić finished M.Sc. Human Molecular biology, (2014) and B.Sc. Molecular Biology and Physiology (2013).

Author Details

¹ Department of Genetics, Faculty of Biology, University of Belgrade, Belgrade, Serbia

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Tables

Table 1. Urine parameter value during 1st and 2nd sampling

Table 1. Participant Urine parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
Freezing point depression (mK)_1 st sampling	Female	852.75	591.82	479.40	240.33
	Male	1072.00	455.10	334.44	103.97
Freezing point depression (mK)_2 nd sampling	Female	752.50	287.25	858.40	380.45
	Male	1000.20	362.34	1283.44	579.44
K (mmol/L)_1 st sampling	Female	37.50	19.23	27.40	13.72
	Male	59.20	28.58	17.56	9.61
K (mmol/L)_2 nd sampling	Female	23.75	8.46	38.20	18.09
	Male	51.40	19.45	39.00	17.20
Na (mmol/L)_1 st sampling	Female	82.25	65.51	38.40	15.42
	Male	99.80	48.02	23.89	8.67
Na (mmol/L)_2 nd sampling	Female	103.50	57.74	68.00	25.09
	Male	101.00	61.56	84.56	47.67
Cl (mmol/L)_1 st sampling	Female	77.50	64.92	49.40	18.69
	Male	140.80	73.65	33.56	13.00
Cl (mmol/L)_2 nd sampling	Female	82.25	50.41	52.20	19.92
	Male	115.60	77.35	78.89	61.76
Urea (mmol/L)_1 st sampling	Female	188.50	145.42	111.40	70.96
	Male	210.80	85.51	80.11	44.90
Urea (mmol/L)_2 nd sampling	Female	144.50	58.03	216.00	126.16
	Male	212.00	70.25	370.22	188.24
Cr (mmol/L)_1 st sampling	Female	13.08	13.43	4.26	4.23
	Male	12.06	9.09	2.84	1.68
Cr (mmol/L)_2 nd sampling	Female	8.25	1.63	9.68	5.54
	Male	8.88	4.40	15.07	8.59
Urat (mmol/L)_1 st sampling	Female	2.18	1.71	0.96	0.55
	Male	2.02	0.86	0.78	0.32

Urat (mmol/L)_2 nd sampling	Female	1.78	0.75	1.74	0.78
	Male	1.82	0.53	2.74	1.24

Table 2. Blood parameter value during 1st and 2nd sampling

Table 2. Participant blood parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
Urea (mmol/L)_1 st sampling	Female	3.65	1.68	3.74	0.86
	Male	4.26	1.40	3.77	1.13
Urea (mmol/L)_2 nd sampling	Female	3.60	0.82	3.82	0.68
	Male	4.32	1.43	4.51	1.67
Cr (μmol/L)_1 st sampling	Female	72.25	9.54	61.80	4.60
	Male	67.00	6.08	72.44	10.50
Cr (μmol/L)_2 nd sampling	Female	71.25	7.85	63.80	4.38
	Male	64.80	5.45	74.11	10.40
Urat (μmol/L)_1 st sampling	Female	245.50	18.63	263.60	32.53
	Male	329.80	38.23	280.00	21.17
Urat (μmol/L)_2 nd sampling	Female	259.00	14.70	287.20	20.19
	Male	332.20	58.98	310.22	42.42
Glucose (mmol/L)_1 st sampling	Female	4.05	0.26	4.20	0.16
	Male	4.84	0.79	4.62	0.48
Glucose (mmol/L)_2 nd sampling	Female	4.25	0.24	4.46	0.30
	Male	4.92	0.57	4.67	0.45
CRP (mg/L)_1 st sampling	Female	1.00	0.00	0.80	0.84
	Male	2.80	2.59	0.67	0.50
CRP (mg/L)_2 nd sampling	Female	1	1	1	2
	Male	3	3	1	1
LE (10 ⁹ /L)_1 st sampling	Female	6.45	0.62	6.00	0.97
	Male	5.86	1.71	6.86	1.70
LE (10 ⁹ /L)_2 nd sampling	Female	6.8	1.1	6.6	2.1
	Male	6.0	1.4	6.6	1.3

ER (10⁹/L)_1st sampling	Female	4.48	0.36	4.64	0.18
	Male	4.90	0.32	4.80	0.34
ER (10⁹/L)_2nd sampling	Female	4.5	0.4	4.7	0.3
	Male	4.9	0.4	4.8	0.4
Hg (g/L)_1st sampling	Female	132.00	6.78	135.20	8.14
	Male	144.20	14.97	141.56	11.06
Hg (g/L)_2nd sampling	Female	130	8	138	9
	Male	143	14	139	12
Ht_1st sampling	Female	0.39	0.01	0.40	0.02
	Male	0.43	0.04	0.42	0.03
Ht_2nd sampling	Female	0.39	0.02	0.41	0.02
	Male	0.43	0.03	0.42	0.03
MCV (fl)_1st sampling	Female	88.25	6.18	86.80	3.03
	Male	88.00	6.20	88.33	4.95
MCV (fl)_2nd sampling	Female	88	5	87	3
	Male	89	7	88	5
MCH (pg)_1st sampling	Female	29.50	2.08	29.00	1.00
	Male	29.60	2.51	29.56	2.24
MCH (pg)_2nd sampling	Female	30	2	30	1
	Male	29	2	29	2
MCHC (g/L)_1st sampling	Female	335.00	8.21	337.20	6.02
	Male	334.40	5.90	334.11	8.10
MCHC (g/L)_2nd sampling	Female	333	9	341	5
	Male	328	8	331	10
MPV (fl)_1st sampling	Female	8.75	.50	8.40	0.89
	Male	9.00	1.22	7.78	0.97
MPV (fl)_2nd sampling	Female	9	1	8	1
	Male	9	1	8	1

TR (10 ⁹ /L)_1 st sampling	Female	239.75	26.70	251.60	51.57
	Male	261.80	31.07	264.00	52.69
TR (10 ⁹ /L)_2 nd sampling	Female	259	38	269	50
	Male	255	36	275	39
K (mmol/L)_1 st sampling	Male	4.4	0.2	4.0	0.2
K (mmol/L)_2 nd sampling	Male	4.2	0.2	4.1	0.2
Na (μmol/L)_1 st sampling	Male	137	2	134	5
Na (μmol/L)_2 nd sampling	Male	137	2	136	5
Cl (μmol/L)_1 st sampling	Male	104	1	100	4
Cl (μmol/L)_2 nd sampling	Male	102	1	101	4

Figures

Figure 1

Differences between Test and Control group in Urine (A1, A2) and Blood (B) sample