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Daily water regime and sample sampling affect blood and urine parameter value change in healthy individuals

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Abstract

Background: Homeostasis is a mechanism for maintaining a stable inner environment in healthy participants. Blood and urine biomarkers are indicators of the subject health status. Biomarkers apply for early diagnosis, prognosis, and treatment. Understanding the effect of pre-analytical factors on biomarker range is significant for the data quality of bio-specimens, reproducibility, and minimizing potential results errors. Water as a necessary element for the normal functioning of living beings and sampling frequency as pre-analytical factors influences the homeostatic range of parameters. The purpose of the study is to examine the effect of 9-day fluid intake and 2-time sampling on concentration changes of 7-urine (freezing point depression, potassium, sodium, chloride, urea, creatinine, urate) and 17-blood (urea, creatinine, urate, glucose, C-reactive protein, leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume, thrombocytes, potassium, sodium, and chloride) variables.

Methods: Parametric and non-parametric test apply to data processing with SPSS software v23.0.

Results: The group of 23 healthy subjects was divide based on water intake (test: more than 1800 ml; control: less than 2000 ml) and gender (female: 10(43%); male: 13(57%)). The mean value of the amount of fluid consumed for the test group is 2183.33 ml, while the control is 1395.83 ml. Investigated parameters, freezing point depression, sodium, potassium, creatinine urea and urate in urine, urea, urate, glucose, hematocrit, and thrombocyte in blood show a significant difference ($P < 0.01$) between the first and second sampling. The difference between water intake after first sampling ($P < 0.01$) observes 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 second sampling and urate ($P < 0.01$), glucose ($P < 0.01/0.05$), hematocrit ($P < 0.01/0.05$) after the first and second sampling, and mean corpuscular hemoglobin concentration ($P < 0.01$) after secondary sampling in blood samples.

Conclusion: Water intake increases the blood and urine biomarker range after the first and second sampling. Further studies should include a larger number of participants to get precise reference limits in a healthy population. Compare health and disease states to draw practical clinical conclusions.

Keywords: Pre-analytical factors, Water intake, Sample sampling, Blood biomarkers, Urine biomarkers, Healthy participants

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Background

Homeostasis improves and maintains organism functional systems (Ramachandran, 2012). The nervous system regulates organism homeostasis in the inward environment by answering external stimulus (Ramachandran, 2012). The vegetative and autonomous nerve system regulates the function of the internal organs (Ramachandran, 2012). Enable the transport of hormones, vitamins, minerals, and metabolism products through the blood to distant tissues and organ systems (Ramachandran, 2012). Fluid in the inner environment contains nutritional material and oxygen, enabling the continuous renewal of molecules at the capillary (Ramachandran, 2012). Body fluid consists of cellular (in cells) and extracellular (in the blood) water (Levy & Onuchic, 2006). The water amount in an organism depends on body mass, nutrition, age, and gender (Popkin, D'Anci, & Rosenberg, 2010). The cellular fluid has a changeable lining and depends on tissue and organ (Popkin et al., 2010). Water has a protective (part of immunoglobulin, proteins) or regulator (par of hormones or enzymes) role in the body (Levy & Onuchic, 2006). Water is an essential molecule for all living organisms and the most common compound on earth (Levy & Onuchic, 2006). It is the ideal solvent and forms a useful environment for biochemical processes in a temperature range of 0–100 °C (Levy & Onuchic, 2006). Water forms hydrogen bonds with proteins, DNA, and RNA molecules (Levy & Onuchic, 2006). Water intake and hydration status are associated with disease prevalence, disease development, and exercise performance (Popkin et al., 2010).

The homeostatic mechanism precisely maintains blood plasma and extracellular fluid content (Popkin et al., 2010). The blood regulates cardiac function, integrity, and elasticity of blood vessels and communicates with cells, tissues, and organs (Jacob, Chappell, & Becker, 2016). Blood has a respiratory, nutritive, excretory, regulatory, transport, and defensive role in an organism (Jacob et al., 2016). Water is quantitatively the most significant blood constituent. Blood contains fluid (45%, plasma, serum) and constitutive (55%, shaped elements like erythrocytes, leucocytes, platelets) component (Jacob et al., 2016). The serum is plasma without fibrinogen. Plasma *c*ontains organic and inorganic matter, like water (92%), proteins (7%) and other ingredients (1–2%) (Mathew, Sankar, & Varacallo, 2020). 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+}, $P0_3^-$, $S0_4^-$, NaH_2PO_4, Na_2HPO_4, and microelements like Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, and J^- (Armstrong, Johnson, McKenzie, & Munoz, 2013; Wilkerson, Horvath, Gutin, Molnar, & Diaz, 1982). The most common blood salts, NaCl and $NaC0_3$, regulate osmotic pressure and chemical reactions (Ashton, 2013).

Urine is a renal product of organic and inorganic material, a bright liquid with a light yellow color, specific smell, and salt-bitter taste (Putnam, 2021). The founded state of metabolism and nutrition causes changes in urine constituent in the physiological and non-physiological range. The color 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-} (Putnam, 2021). Organic components can be nitrogen and non-nitrogen (Putnam, 2021). Nitrogen materials are urea, creatinine_ Cr, uric acid, amino acid, while non-nitrogen materials belong to phenol, oxalate, glucuronic acid, and milky acid (Putnam, 2021). A small number of ferments, hormones, and vitamins are present (Putnam, 2021). Intercellular fluids are relatively constant fluids, while tissue fluid and blood plasma are inconstant to change (Ramachandran, 2012). Maintaining organism homeostasis is significant for permanent composition and volume of body fluid (Ramachandran, 2012). Human organism contains small water supplies. During the day, 1.5–2 l of water evaporates through urine, feces, lung sweat, and the skin (Puga, Lopez-Oliva, Trives, Partearroyo, & Varela-Moreiras, 2019). 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 (Narayanan, 2000). Pre-analytic variables include three 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 (Narayanan, 2000). 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 (Yin, Lehmann, & Xu, 2015). Studies analyzed conditions like storage time, temperature, freezing-thawing cycle on biomarker range, and reproducibility (Narayanan, 2000). 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 (Narayanan, 2000). Critical aspects of biomarker results, stability during clinical planning, sample collection, training, selection of sample preservation, buffers, shipping, logistics, and method analysis are known for the most utilized bio-specimens (Narayanan, 2000). Urine and blood samples are the most commonly used in clinical practice reflecting state of metabolome and metabolic end product (Yin et al., 2015). 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 three semesters is estimated (McKenzie et al., 2017). Observational studies recorded and assessed the amount of firm food/beverage and urine parameters (osmolarity, volume, gravity, and color) in healthy children and elderly individuals for 24 h (Athanasatou, Kandyliari, Malisova, & Kapsokefalou, 2019; Kavouras et al., 2017). A cross-sectional study on healthy male college students in China assed hydration status, fluid intake, and urine biomarker (osmolarity, specific gravity, pH, the concentration of K^+, Na^+, and Cl^{2-}) for 24 h (Zhang et al., 2017). Moreover, urine hydration biomarker ($P0_4^-$, uric acid, urea, Cr, K^+, Na^+, Mg^{2+}, Ca^{2+}) for 24 h is analyzed (Perrier et al., 2013). Change in blood biomarkers (whole blood cells, erythrocyte_ER, hemoglobin_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 2 weeks later are determine (Hyun-Kyung, Kim, & Ryu, 2017). Urea concentrations in blood samples after freezing at − 80° is analyzed (Armstrong et al., 2013). 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 (Hansson et al., 2018). Daily circadian rhythm influence physiological processes and diurnal dynamic (Ournier et al., 2017). 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 (Ournier et al., 2017).

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, urate, 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.

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 were obtained 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%) was numerous compared 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 lover 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 are 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 Tables 1 and 2. Proposed values of 7 urine (FPD, K, Na, Cl, urea) and 17 blood (urea, Cr, urate, 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, and TR in the blood. Wilcoxon t test shows the absence of a statistically significant difference ($P > 0.05$, $\alpha = 0.05$, 95% CI) for CRP in the blood. Results

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

Participant urine parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
Freezing point depression (mK)_1st sampling	Female	852.75	591.82	479.40	240.33
	Male	1072.00	455.10	334.44	103.97
Freezing point depression (mK)_2nd sampling	Female	752.50	287.25	858.40	380.45
	Male	1000.20	362.34	1283.44	579.44
K (mmol/L)_1st sampling	Female	37.50	19.23	27.40	13.72
	Male	59.20	28.58	17.56	9.61
K (mmol/L)_2nd sampling	Female	23.75	8.46	38.20	18.09
	Male	51.40	19.45	39.00	17.20
Na (mmol/L)_1st sampling	Female	82.25	65.51	38.40	15.42
	Male	99.80	48.02	23.89	8.67
Na (mmol/L)_2nd sampling	Female	103.50	57.74	68.00	25.09
	Male	101.00	61.56	84.56	47.67
Cl (mmol/L)_1st sampling	Female	77.50	64.92	49.40	18.69
	Male	140.80	73.65	33.56	13.00
Cl (mmol/L)_2nd sampling	Female	82.25	50.41	52.20	19.92
	Male	115.60	77.35	78.89	61.76
Urea (mmol/L)_1st sampling	Female	188.50	145.42	111.40	70.96
	Male	210.80	85.51	80.11	44.90
Urea (mmol/L)_2nd sampling	Female	144.50	58.03	216.00	126.16
	Male	212.00	70.25	370.22	188.24
Cr (mmol/L)_1st sampling	Female	13.08	13.43	4.26	4.23
	Male	12.06	9.09	2.84	1.68
Cr (mmol/L)_2nd sampling	Female	8.25	1.63	9.68	5.54
	Male	8.88	4.40	15.07	8.59
Urate (mmol/L)_1st sampling	Female	2.18	1.71	0.96	0.55
	Male	2.02	0.86	0.78	0.32
Urate (mmol/L)_2nd 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

Participant blood parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
Urea (mmolL)_1st sampling	Female	3.65	1.68	3.74	0.86
	Male	4.26	1.40	3.77	1.13
Urea (mmolL)_2nd sampling	Female	3.60	0.82	3.82	0.68
	Male	4.32	1.43	4.51	1.67
Cr (μmolL)_1st sampling	Female	72.25	9.54	61.80	4.60
	Male	67.00	6.08	72.44	10.50
Cr (μmolL)_2nd sampling	Female	71.25	7.85	63.80	4.38
	Male	64.80	5.45	74.11	10.40
Urate (μmolL)_1st sampling	Female	245.50	18.63	263.60	32.53
	Male	329.80	38.23	280.00	21.17
Urate (μmolL)_2nd sampling	Female	259.00	14.70	287.20	20.19
	Male	332.20	58.98	310.22	42.42
Glucose (mmolL)_1st sampling	Female	4.05	0.26	4.20	0.16
	Male	4.84	0.79	4.62	0.48
Glucose (mmolL)_2nd sampling	Female	4.25	0.24	4.46	0.30
	Male	4.92	0.57	4.67	0.45
CRP (mg/L)_1st sampling	Female	1.00	0.00	0.80	0.84
	Male	2.80	2.59	0.67	0.50
CRP (mg/L)_2nd sampling	Female	1	1	1	2
	Male	3	3	1	1
LE (10^9/L)_1st sampling	Female	6.45	0.62	6.00	0.97
	Male	5.86	1.71	6.86	1.70
LE (10^9/L)_2nd sampling	Female	6.8	1.1	6.6	2.1
	Male	6.0	1.4	6.6	1.3
ER (10^9/L)_1st sampling	Female	4.48	0.36	4.64	0.18
	Male	4.90	0.32	4.80	0.34
ER (10^9/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

Table 2 Blood parameter value during 1st and 2nd sampling *(Continued)*

Participant blood parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
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)_1st sampling	Female	239.75	26.70	251.60	51.57
	Male	261.80	31.07	264.00	52.69
TR (10^9/L)_2nd sampling	Female	259	38	269	50
	Male	255	36	275	39
K (mmolL)_1st sampling	Male	4.4	0.2	4.0	0.2
K (mmolL)_2nd sampling	Male	4.2	0.2	4.1	0.2
Na (μmolL)_1st sampling	Male	137	2	134	5
Na (μmolL)_2nd sampling	Male	137	2	136	5
Cl (μmolL)_1st sampling	Male	104	1	100	4
Cl (μmolL)_2nd sampling	Male	102	1	101	4

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 the 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 the 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 (Levy & Onuchic, 2006; Popkin et al., 2010). 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 1.3–4 l/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, color, volume, osmolarity, specific gravity, phosphate, uric acid, urea, Cr, K^+, Na^+, Cl^{2-}, Mg^{2+}, and citrate in urine samples were assessed (Athanasatou et al., 2019; McKenzie et al., 2017; Perrier et al., 2013; Zhang et al., 2017). Literature results from 24-h 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 color, osmolarity, specific gravity, and solute concentration (Kavouras et al., 2017). The cross-sectional analysis showed the association of urine volume with osmolarity, gravity and osmolarity, osmolarity and color, and color and gravity (Athanasatou et al., 2019; Zhang et al., 2017). 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

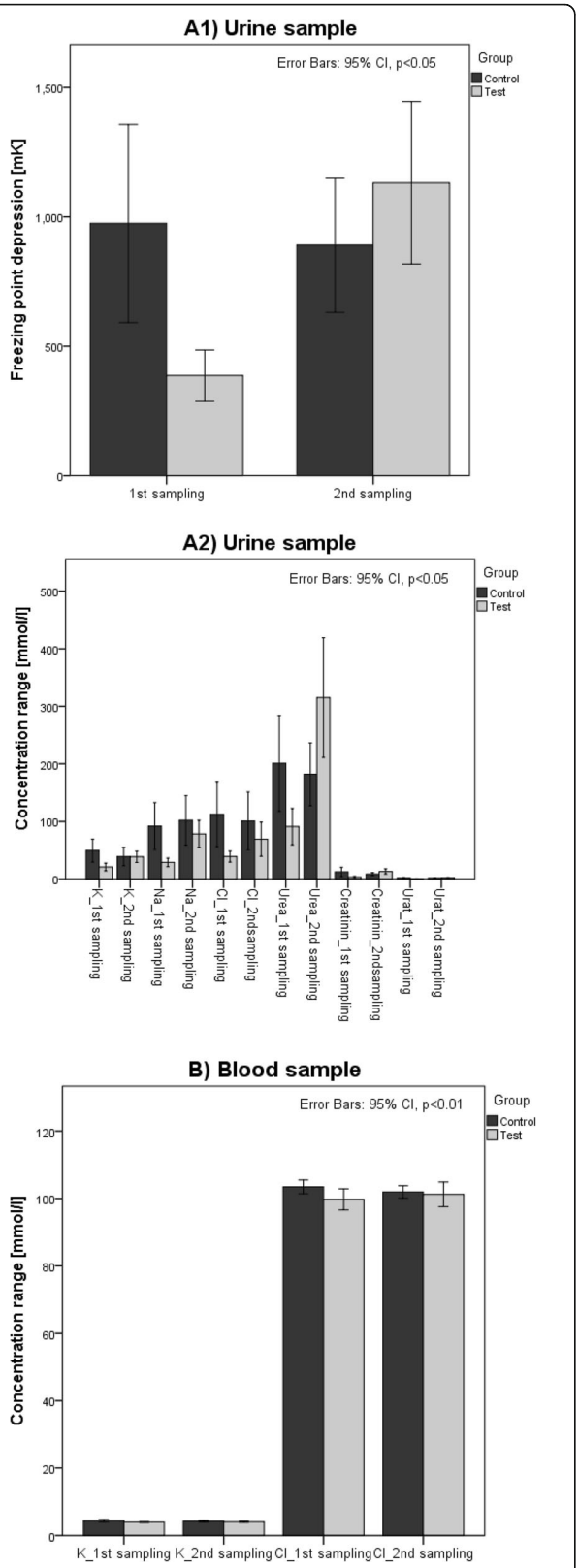

Fig. 1 Differences between test and control group in urine (**A1**, **A2**) and blood (**B**) sample

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 are 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. It 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 are 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 (Liska et al., 2019; Puga et al., 2019). 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 rate (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 (Halperin Kuhns & Woodward, 2020; Lam et al., 2017; Paller et al., 2012; Zhang et al., 2002). 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 (Halperin Kuhns & Woodward, 2020; Zhang et al., 2002). Male sex hormones (Testosterone) affect MCHC and Ht level through the increase of erythropoietin, reduction of ferritin and hepcidin (Paller et al., 2012). Testosterone influence protein metabolism and the Urea cycle (Lam et al., 2017).

Drinking more water improves kidney function and clearance of toxins by glomerular filtration, tubular secretion, and activation of various degradative metabolic pathways (Negoianu & Goldfarb, 2008). 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%), urate (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 osmolarity depends on cations, Na+, K+, NH4+, anions, and urea, whereas FPD enables estimation of urine osmolality (Perrier et al., 2015; Youhanna et al., 2017). Freezing point depression as a colligative property depends on the molality of the solute (Feher, 2017). Renal Cr excretion level depends on the glomerular filtration rate, proximal tubular secretion and OCT-2 transporter (Ciarimboli et al., 2012; Musso & Oreopoulos, 2011). Higher water intake after 3 days causes a decrease of uric acid, upregulation of GLUT9 and URAT1, and downregulation of ABCG2 and OAT1, while after 7 days affect NPT1 downregulation in hyperuricemia mice (Wen et al., 2020). Urate level depends on transport proteins (URAT1 and GLUT9), uricase inactivation, and possible change of the intestinal microbiota (Wen et al., 2020). Production of concentrated urine requires interactions among the nephron segments and vasculature in the kidney medulla (Guelinckx, Vecchio, Perrier, & Lemetais, 2016). 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 (Guelinckx et al., 2016). Vasopressin regulates urea transport acutely by increasing UT-A1 phosphorylation and the apical plasma-membrane accumulation of UT-A1 through two cAMP-dependent pathways (Sands, Blount, & Klein, 2011). Glut9 plays a role in urate homeostasis by its dual role in urate handling in the kidney and uptake in the liver (Preitner et al., 2009). Small water intake can lead to dehydration, activation of the renin-angiotensin system (RAS) through angiotensin receptors, and subsequent activation of signaling molecules, protein kinase C, reactive oxygen species, and MAP kinase pathway mediated with angiotensin (Coble, Grobe, Johnson, & Sigmund, 2015). Prolactin, aldosterone, and antidiuretic hormone influences water metabolism and electrolyte balance (Alamer, 2011; Andersson, Leksell, & Rundgren, 1982; Burstyn, 1978; Falconer & Rowe, 1977; Kenyon, Saccoccio, & Morris, 1984; Kulczycki & Robertson, 1988; Lorenz, 2008; Scott, Menouar, & Dunn, 2020; Work, Galla, Booker, Schafer, & Luke, 1985; Young, Pan, & Guyton, 1977).

Results show higher concentration percentage increase in variables from urine [FDP (68%), Na+ (62.96%), K+ (21.88%), Cr (65.2%), urate (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 (Andersson et al., 1982). 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 (Benozzi, Unger, Campion, & Pennacchiotti, 2018; Hyun-Kyung et al., 2017; Unal, 2017).

Ex vivo cellular injury, disintegration, cellular granule release, and protease activation causes alterations of cell release in vitro after sampling (Kang et al., 2013). Hydration biomarkers in 24-h urine correlate with daily total fluid intake volume in sedentary adults in free-living conditions (Perrier et al., 2013). 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). Changes in the blood and urine biomarkers during normal physiological conditions can be a guiding stone for the clinical outcome. 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 study hypothesis justifies concepts from the previous studies.

Abbreviations

AVP: Arginine vasopressin; Cl^-: Chloride; C: Control; Cr: Creatinine; CRP: C-reactive protein; ER: Erythrocyte; F: Female; FPD: Freezing point depression; Glu: Glucose; Ht: Hematocrit; Hg: Hemoglobine; LE: Leucocyte; M: Male; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MPV: Mean platelet volume; K^+: Potassium; Na^+: Sodium; T: Test; TR: Trombocyte

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Author's contributions

Author (JS, PhD student at the Faculty of Biology, University of Belgrade, Serbia) originated data collection, analysis, interpretation, and writing. The author JS made all the effort, accuracy, integrity, and quality. The author JS have read and approved the manuscript and ensures that this is the case. The author JS confirms that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

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Availability of data and materials

Data are available in Tables 1 and 2.

Declarations

Ethics 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 interests

The author, a PhD student, Snežana Jovičić, declares no competing interests.

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References

Alamer, M. (2011). The role of prolactin in thermoregulation and water balance during heat stress in domestic ruminants. *Asian Journal of Animal and Veterinary Advances.*, *6*(12), 1153–1169.

Andersson, B., Leksell, L. G., & Rundgren, M. (1982). Regulation of water intake. *Annual Review of Nutrition*, *2*, 73–89.

Armstrong, L. E., Johnson, E. C., McKenzie, A. L., & Munoz, C. X. (2013). Interpreting common hydration biomarkers on the basis of solute and water excretion. *European Journal of Clinical Nutrition*, *67*(3), 249–253.

Ashton, N. (2013). Physiology of red and white blood cells. *Anaesthesia & Intensive Care Medicine*, *14*(6), 261–266.

Athanasatou, A., Kandyliari, A., Malisova, O., & Kapsokefalou, M. (2019). Fluctuation of water intake and of hydration indices during the day in a sample of healthy Greek adults. *Nutrients.*, *11*(4), 793.

Benozzi, S. F., Unger, G., Campion, A., & Pennacchiotti, G. L. (2018). Fasting conditions: Influence of water intake on clinical chemistry analytes. *Biochemia Medica (Zagreb).*, *28*(1), 010702.

Burstyn, P. G. (1978). Sodium and water metabolism under the influence of prolactin, aldosterone, and antidiuretic hormone. *The Journal of Physiology*, *275*, 39–50.

Ciarimboli, G., Lancaster, C. S., Schlatter, E., Franke, R. M., Sprowl, J. A., Pavenstädt, H., … Sparreboom, A. (2012). Proximal tubular secretion of creatinine by organic cation transporter OCT2 in cancer patients. *Clinical Cancer Research*, *18*(4), 1101–1108.

Coble, J. P., Grobe, J. L., Johnson, A. K., & Sigmund, C. D. (2015). Mechanisms of brain renin angiotensin system-induced drinking and blood pressure: importance of the subfornical organ. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *308*(4), R238–R249.

Falconer, I. R., & Rowe, J. M. (1977). Effect of prolactin on sodium and potassium concentrations in mammary alveolar tissue. *Endocrinology.*, *101*(1), 181–186.

Feher J. (2017). Quantitative human physiology. An introduction. In. Osmosis and Osmotic pressure. 2nd edition. Elsevier: 2017:182-198.

Guelinckx, I., Vecchio, M., Perrier, E. T., & Lemetais, G. (2016). Fluid intake and vasopressin: connecting the dots. *Annals of Nutrition & Metabolism*, *68*(Suppl 2), 6–11.

Halperin Kuhns, V. L., & Woodward, O. M. (2020). Sex differences in urate handling. *International Journal of Molecular Sciences*, *21*(12), 4269.

Hansson, O., Mikulskis, A., Fagan, A. M., Teunissen, C., Zetterberg, H., & Vanderstichele, H. (2018). The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: A review. *Alzheimer's & Dementia*, *14*, 1313–1333.

Hyun-Kyung, K., Kim, S. H., & Ryu, J. K. (2017). Changes in the blood components caused by water intake. *Korean J of Clinical and Laboratory Science.*, *49*(3), 227–232.

Jacob, M., Chappell, D., & Becker, B. F. (2016). Regulation of blood flow and volume exchange across the microcirculation. *Critical Care*, *20*(1), 319.

Kang, H. J., Jeon, S. Y., Park, J. S., Yun, J. Y., Kil, H. N., Hong, W. K., … Han, B. G. (2013). Identification of clinical biomarkers for pre-analytical quality control of blood samples. *Biopreservation and Biobanking*, *11*(2), 94–100.

Kavouras, S. A., Bougatsas, D., Johnson, E. C., Arnaoutis, G., Tsipouridi, S., & Panagiotakos, D. B. (2017). Water intake and urinary hydration biomarkers in children. *European Journal of Clinical Nutrition*, *71*(4), 530–535.

Kenyon, C. J., Saccoccio, N. A., & Morris, D. J. (1984). Aldosterone effects on water and electrolyte metabolism. *The Journal of Endocrinology*, *100*(1), 93–100.

Kulczycki, L. L., & Robertson, M. T. (1988). The sweat chloride concentration and prolactin activity in cystic fibrosis. *Scandinavian Journal of Gastroenterology. Supplement*, *143*, 28–30.

Lam, T., Poljak, A., McLean, M., Bahl, N., Ho, K. K. Y., & Birzniece, V. (2017). Testosterone prevents protein loss via the hepatic urea cycle in human. *European Journal of Endocrinology*, *176*(4), 489–496.

Levy, Y., & Onuchic, J. N. (2006). Water mediation in protein folding and molecular recognition. *Annual Review of Biophysics and Biomolecular Structure*, *35*, 389–415.

Liska, D., Mah, E., Brisbois, T., Barrios, P. L., Baker, L. B., & Spriet, L. L. (2019). Narrative review of hydration and selected health outcomes in the general population. *Nutrients.*, *11*(1), 70.

Lorenz, J. M. (2008). Chapter 3 - Potassium Metabolism. In W. Oh, J.-P. Guignard, S. Baumgart, & A. Richard (Eds.), *Polin, nephrology and fluid/electrolyte physiology: neonatology questions and controversies*, (pp. 54–65). W.B. Saunders.

Mathew, J., Sankar, P., Varacallo, M. (2020). Physiology, blood plasma. Resource document. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK531504/. Accessed 17 Nov 2020.

McKenzie, A. L., Perrier, E. T., Guelinckx, I., Kavouras, S. A., Aerni, G., Lee, E. C., et al. (2017). Relationships between hydration biomarkers and total fluid intake in pregnant and lactating women. *European Journal of Nutrition*, *56*(6), 2161–2170.

Musso, C. G., & Oreopoulos, D. G. (2011). Aging and physiological changes of the kidneys including changes in glomerular filtration rate. *Nephron Physiology*, *119*(1), 1–5.

Narayanan, S. (2000). The preanalytic phase. An important component of laboratory medicine. *American Journal of Clinical Pathology*, *113*(3), 429–452.

Negoianu, D., & Goldfarb, S. (2008). Just add water. *Journal of the American Society of Nephrology*, *19*(6), 1041–1043.

Ournier, S., Iten, L., Marques-Vidal, P., Boulat, O., Bardy, D., & Beggah, A. (2017). Circadian rhythm of blood cardiac troponin T concentration. *Clinical Research in Cardiology*, *106*, 1026–1032.

Paller, C. J., Shiels, M. S., Rohrmann, S., Menke, A., Rifai, N., Nelson, W. G., … Dobs, A. S. (2012). Association between sex steroid hormones and hematocrit in a nationally representative sample of men. *Journal of Andrology*, *33*(6), 1332–1341.

Perrier, E., Rondeau, P., Poupin, M., Le Bellego, L., Armstrong, L. E., Lang, F., et al. (2013). Relation between urinary hydration biomarkers and total fluid intake in healthy adults. *European Journal of Clinical Nutrition*, *67*(9), 939–943.

Perrier, E. T., Buendia-Jimenez, I., Vecchio, M., Armstrong, L. E., Tack, I., & Klein, A. (2015). Twenty-four-hour urine osmolality as a physiological index of adequate water intake. *Disease Markers*, *2015*, 231063.
Popkin, B. M., D'Anci, K. E., & Rosenberg, I. H. (2010). Water, hydration, and health. *Nutrition Reviews*, *68*(8), 439–458.
Preitner, F., Bonny, O., Laverrière, A., Rotman, S., Firsov, D., Da Costa, A., … Thorens, B. (2009). Glut9 is a major regulator of urate homeostasis and its genetic inactivation induces hyperuricosuria and urate nephropathy. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(36), 15501–15506.
Puga, A. M., Lopez-Oliva, S., Trives, C., Partearroyo, T., & Varela-Moreiras, G. (2019). Effects of drugs and excipients on hydration status. *Nutrients.*, *11*(3), 669.
Putnam, DF. (2021). Composition and concentrative properties of human urine. Resource document. NASA. https://ntrs.nasa.gov/api/citations/19710023044/downloads/19710023044.pdf. Accessed 23 Feb 2021.
Ramachandran, V. S. (2012). Encyclopedia of Human Behaviour. In L. Asarian, V. Gloy, & N. Geary (Eds.), *Homeostasis. Reference module in Neuroscience and Behavioural psychology*, (2nd ed., pp. 324–333). Elsevier/Academic Press.
Sands, J. M., Blount, M. A., & Klein, J. D. (2011). Regulation of renal urea transport by vasopressin. *Transactions of the American Clinical and Climatological Association*, *122*, 82–92.
Scott, J. H., Menouar, M. A., & Dunn, R. J. (2020). *Physiology, aldosterone. Resource document*. StatPearls https://www.ncbi.nlm.nih.gov/books/NBK470339/. Accessed 17 Nov 2020.
Unal, K. (2017). The effect of daily water intake on blood biochemical markers in healthy individuals. *Turkish Journal of Occupational/Environmental Medicine and Safety*, *1*(3), 230–237.
Wen, S., Wang, D., Yu, H., Liu, M., Chen, Q., Bao, R., … Wang, T. (2020). The time-feature of uric acid excretion in hyperuricemia mice induced by potassium oxonate and adenine. *International Journal of Molecular Sciences*, *21*(15), 5178.
Wilkerson, J. E., Horvath, S. M., Gutin, B., Molnar, S., & Diaz, F. J. (1982). Plasma electrolyte content and concentration during treadmill exercise in humans. *Journal of Applied Physiology*, *53*(6), 1529–1539.
Work, J., Galla, J. H., Booker, B. B., Schafer, J. A., & Luke, R. G. (1985). Effect of ADH on chloride reabsorption in the loop of Henle of the Brattleboro rat. *The American Journal of Physiology*, *249*(5 Pt 2), F698–F703.
Yin, P., Lehmann, R., & Xu, G. (2015). Effects of pre-analytical processes on blood samples used in metabolomics studies. *Analytical and Bioanalytical Chemistry*, *407*(17), 4879–4892.
Youhanna, S., Bankir, L., Jungers, P., Porteous, D., Polasek, O., Bochud, M., … Devuyst, O. (2017). Validation of surrogates of urine osmolality in population studies. *American Journal of Nephrology*, *46*(1), 26–36.
Young, D. B., Pan, Y. J., & Guyton, A. C. (1977). Control of extracellular sodium concentration by antidiuretic hormone-thirst feedback mechanism. *The American Journal of Physiology*, *232*(5), R145–R149.
Zhang, N., Du, S., Tang, Z., Zheng, M., Yan, R., Zhu, Y., et al. (2017). Hydration, fluid intake, and related urine biomarkers among male college students in Cangzhou, China: a cross-sectional study-applications for assessing fluid intake and adequate water intake. *International Journal of Environmental Research and Public Health*, *14*(5), 513.
Zhang, Y., Howard, B. V., Cowan, L. D., Yeh, J., Schaefer, C. F., Wild, R. A., … Lee, E. T. (2002). The effect of estrogen use on levels of glucose and insulin and the risk of type 2 diabetes in american Indian postmenopausal women: the strong heart study. *Diabetes Care*, *25*(3), 500–504.

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