Effect of beer consumption on methylation and redox metabolism

Title page:

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Short title: Effect of beer consumption on selected parameters.

Summary:

Aim: To investigate the influence of beer consumption on levels of homocysteine (HCY), vitamin B_6, B_{12}, folic acid (FA), dimethylglycine (DMG), betaine (BET) and other selected markers.

Methods: One hundred and sixteen male volunteers were enrolled in the study. A one-month period of alcohol abstinence was followed by a one month when participants drank 830 mL of alcoholic beer every day. After that phase, one month of alcohol abstinence followed. At the beginning and after every phase blood samples were taken and analysed.

Results: Ninety-three participants completed the study. After the phase of alcohol consumption, uric acid (UA) (**p<0.0001**), antioxidative capacity (AOC) (**p=0.02**), superoxide dismutase (SOD) (**0.025**), glutathione reductase (GRH) (**0.0001**), total cholesterol (**p<0.0001**), HDL-cholesterol (**p<0.0001**), Apolipoprotein-AI (ApoAI) (**p<0.0001**), LDL-cholesterol (**p<0.039**) and Apolipoprotein B (ApoB) (**p<0.009**) increased, while vitamin B_{12} (**p=0.0001**) and fibrinogen (**p<0.0001**) decreased. Other tested parameters (DMG, BET, vitamin B_6 and FA) did not show any significant changes. UA changes and changes in AOC were statistically significantly correlated (**r=0.52, p<0.0001**).

Conclusion: HCY, DMG and BET levels did not show any statistically significant changes after beer consumption, whereas some markers of redox metabolism increased (UA, AOC, SOD and GRH). A statistically significant correlation denotes the dependence of UA and AOC changes in connection with beer consumption.

Keywords: alcohol drinking, beer, homocysteine, antioxidative capacity, B-vitamins

1. Introduction:

The association between atherosclerosis and cardiovascular complications is well known. The influence of high concentrations of homocysteine (HCY) as an independent risk factor of cardiovascular disease has also been published [1]. However, the metabolism of HCY is complex and HCY-methionine (MET) metabolism is connected to the metabolism of vitamins B_6, B_{12}, folic acid (FA), betaine (BET) and other substances, e.g. dimethylglycine (DMG) [2,3]. Concentrations of HCY and other substances involved in the metabolism of HCY in association with alcohol abuse have been widely studied [4–6]. Ethanol influences several enzymes participating in HCY metabolism, however, its influence is complex [7]. In some studies, ethanol increased HCY levels and caused fatty liver disease by increasing lipotropic effects [8]. Moreover, HCY degradation via the transulphuration pathway influenced antioxidative metabolism, for example by lowering glutathione availability (GSH), one of the most important intracellular antioxidants [2]. Alcohol abuse is a medical and social problem in many countries and is associated with a higher risk of cardiovascular and other complications [9]. On the other hand, some beers contain potentially beneficial substances (polyphenols, BET, vitamins B_6, B_{12} and folic acid).

The goal of our study was to investigate the influence of beer consumption in healthy middle-aged men on levels of HCY, vitamin B_6, B_{12}, FA, DMG, BET and selected markers of oxidative stress, as well as the lipid profile.

2. Materials and Methods

2.1. Study subjects

One hundred and sixteen middle-aged male volunteers were enrolled in the study. Twenty-one subjects were excluded because of pathological findings in the laboratory assessment before the study or because of increased blood pressure (BP). Two participants were excluded because of a newly acquired diagnosis of chronic disease. Ninety-three healthy men completed the entire study. All participants gave informed consent about participation in the study. The study was approved by Ethical Commission of University Hospital and Faculty of Medicine in Pilsen. Details about the study cohort and the characteristics of the beer administered to participants in the study are listed in **Table 1**.

2.2. Study design

All participants included in the study underwent four visits to our workplace. During the first visit, laboratory screening testing, BP examination and completion of the alcohol consumption questionnaire were performed. The second visit including the blood draw was after one month of

alcohol abstinence (sample A). Between the second and the third visit (sample A and B), there was a one-month period when all participants drank 830 mL of beer every day. The third visit (sample B) was followed by a one-month period of abstinence, after which sample C was collected. During each visit, adherence to the study protocol was checked by questionnaire, BP and body weight were measured and blood samples were taken. The questionnaire included a diary with records of unusual activities, excesses in food or beverages intake and participants filled a food frequency questionnaire, frequencies were compared to ensure comparable food intakes. Venous blood samples were drawn with safety vacuum tubes system (Vacuette, Greiner) into tubes without anticoagulant and with lithium heparin. Lithium heparin plasma was used for most analyses, serum was used for AOPP and AGE measurement. All collected samples were centrifuged and processed within 2 hours after blood collection and stored at -80°C until analysis.

2.3. Materials and methods

In samples A, B and C, concentrations of aspartate aminotransferase (**AST**, Dialab, Vienna, Austria), alanine aminotransferase (**ALT**, Dialab, Vienna, Austria)**,** uric acid (**UA**, Dialab, Vienna, Austria), gamma-glutamyltransferase (**GGT**, Human, Wiesbaden, Germany), total bilirubin (**TB**, BLW Diagnostics, Prague, Czech Republic), hypersensitive C-reactive protein (**hsCRP**; K-ASSAY Kamiya Biomedical Company, Seattle, USA), **TG** (Human, Wiesbaden, Germany), total cholesterol (**TC**, Human, Wiesbaden, Germany), HDL-cholesterol (**HDL**, Roche Diagnostics, Mannheim, Germany), apolipoprotein AI and apolipoprotein B (**ApoAI and ApoB**, Tina-quant, Roche Diagnostics, Mannheim, Germany), **fibrinogen** (CA-1500 analyser, Sysmex, Japan), **HCY** (enzymatic method from Carolina, Brea, CA, USA), fructosamine (**FRUCT**, Roche Diagnostics, Mannheim, Germany), advanced glycation end products (**AGE**, analysed using Fluorescence Spectrophotometer, Varian Crompack International BV, Netherlands), **vitamin B_{12}** and **FA** (Abbott, Chicago, IL, USA), reduced glutathione (**GSH**, Bioxytech GSH-400, OXIS, Westlake Village, California, USA), thiobarbituric acid reactive substances (**TBARS**, analysis according to the method of Jentzsch [10]), advanced oxidation protein products (**AOPP**, spectrophotometric method of Witko-Sarsat [11]), superoxide dismutase (**SOD**, Randox, Crumlin, United Kingdom), glutathione peroxidase (**GPx**, Randox, Crumlin, United Kingdom), glutathione reductase (**GRH**, Bioxytech, GR-340, OXIS, California, USA), paraoxonoase (**PONS**, activity of paraoxonase 1 was measured as the amount of hydrolysed paraoxone by monitoring the increase of absorbance at 410 nm and 37 °C [12]) and total antioxidative capacity (**AOC**, Randox, Crumlin, United Kingdom) were measured. In samples A and B, concentrations of pyridoxal phosphate and pyridoxal (**PLP** and **PL**, HPLC method with fluorimetric detection of Talwar), **BET** and **DMG** (HPLC method with UV detection of Laryea) were measured [13]. Analyses in all samples were performed batch-wise. Values of LDL-cholesterol (**LDL**) were calculated using the Friedwald equation [14]. Renal functions

were assessed using the CDK-EPI formula based on creatinine levels [15]. BP was measured on the left hand after 5 minutes at rest while the participant was in a sitting position (automatic device Omron M5-I).

2.4. Statistical analysis

Unless stated otherwise, descriptive statistics are expressed as median (1^{st}–3^{rd} quartile) values. Statistical analyses were performed using R software (version R 1.9.1, R Development Core Team, R Foundation, Vienna, Austria) and MedCalc software (MedCalc Software, version 16.4.1, MedCalc Software, Ostend, Belgium). Differences between samples A, B and C were calculated using ANOVA analysis with repeated measures to detect the effect of beer and the persistence of the effect. In the case of positive results via ANOVA analysis, a post-hoc test was used for the pairwise comparison of subgroups. The Wilcoxon test (paired version) was used for the comparison of differences between groups A and B, when sample C was not measured (PL, PLP, DMG and BET). The Spearman correlation coefficient was used to find correlation between selected parameters. The level of statistical significance was set at $\alpha = 0.05$.

3. Results

We observed statistically significant increase in systolic BP after beer consumption and these changes were persistent in sample C. Regarding diastolic BP, we found significant increase only in sample C in comparison with sample A. We found statistically significant increase in AST, GGT, UA, TC, HDL, LDL, apoAI, apoB after the period of beer consumption and these changes were persistent in sample C only in case of apoAI and HDL. On the contrary, we found statistically significant decrease in fibrinogen levels after the period of beer consumption and these changes were persistent in sample C. We observed statistically significant changes in vitamin B_{12} levels after beer consumption, however these changes were not persistent in sample C. Changes in HCY levels and parameters related to its metabolism were not statistically significant. Results of all mentioned parameters are summarised in **Table 2 and 3**. We observed statistically significant increase in AOC, SOD and GRH levels after beer consumption and these changes were persistent in sample C only in case of GRH. Changes of parameters involved in oxidative stress are summarised in **Table 3**. Boxplots of selected parameters are shown in **Figure 1**.

Correlations of selected parameters are summarised in **Table 4**. There was a strong correlation between changes in UA levels and changes in AOC after beer consumption. Correlation graph is plotted in **Figure 2**.

4. Discussion

Our intervention study provides the unique metabolic characteristics of the influence of beer drinking in middle-aged healthy men. We measured a complex of methylation and redox metabolism markers. Our results suggest that the complex of vitamins in the beer partly prevents the expected disruption of methylation pathways by ethanol and somehow improves the blood lipid profile. However, we provide some evidence that beer drinking causes an oxidative stress challenge.

We observed statistically significant increase in systolic BP after phase of alcohol consumption; these changes were persistent until the end of the next period of abstinence (sample C). Regarding diastolic BP, we only observed statistically significant increase after one month of alcohol abstinence (sample C in comparison with sample A). However, the median change of diastolic BP was 3 mm Hg, so these changes are beyond the border of clinical significance. Most authors published no changes in systolic and diastolic BP in connection with beer intake, particularly in short-term studies [16, 17]. Nishiwaki reported that beer intake in low amounts can have a positive influence on arterial stiffness [18], while Xin published the positive influence of alcohol reduction on BP, especially in heavy drinkers [19]. Not only heavy drinking [20] but also low to moderate drinking can have increasing effect to BP; however, the pattern of drinking is also important [21]. The volume of 830 mL of beer is not a negligible amount, so we cannot fully eliminate the influence of liquid intake. On the other hand, in the study of Chiva-Blanch, with participants who drank 900 ml of non-alcoholic beer every day, the authors observed a positive influence on lowering systolic BP in comparison with drinking 600 mL of alcoholic beer [17]. Clearly, there are a lot of factors which influence BP and the influence of beer intake on BP should be evaluated carefully.

We observed an increase in TC, HDL and LDL cholesterol, and ApoAI and ApoB levels. Interestingly, HDL cholesterol and ApoAI levels did not show a tendency to decrease after the second period of abstinence. Although the observed increase in sample C in comparison with sample A in ApoAI and HDL cholesterol concentrations were statistically significant, absolute changes in measured concentrations were subtle and did not achieve clinical significance. Published studies describing changes in the lipid profile are not completely uniform. Some authors have reported that short-term alcohol consumption positively influences HDL cholesterol or ApoAI levels but does not influence LDL cholesterol, ApoB or TC levels [16, 17, 22]. On the contrary, Baer published an increase in HDL cholesterol and ApoAI, but a decrease in LDL cholesterol and TG concentrations in the population of postmenopausal women, even though changes in LDL and TG concentrations showed borderline statistical significance ($p=0.05$ and $p=0.04$, respectively) [23]. Padro, in study on an overweight population, did not observe any changes in lipid profile, including HDL cholesterol [24]. One possible explanation for these discrepant results in the literature is that very small changes in HDL and ApoAI

can be masked by uncertainty of measurement and only a proper research design (with batch-wise measurement) can reveal such tiny changes.

We observed a statistically significant decrease in fibrinogen levels after the period of drinking and the decrease was persistent after one month of alcohol abstinence, although there was tendency to increase back to the previous values. Studies describing changes in fibrinogen levels in association with alcohol intake are usually in accordance with our results. For example, Chiva-Blanch described a decrease in fibrinogen levels after an alcohol phase, which involved drinking beer or gin for 4 weeks, as did Sierksma after 3 weeks of drinking beer in comparison with non-alcoholic beer [17, 25]. However, limited data exist about long-term studies in large numbers of participants. For example, Okwuosa performed an observational study which analysed the data of 2520 men and women during a 13-year period and reported that levels of fibrinogen rose in people who became drinkers or remained drinkers, but the increase was less than in participants who quit drinking [26].

We found no changes in concentrations of HCY, DMG, BET, FA, PLP and PL levels and a decrease in the concentrations of vitamin B_{12} after beer intake in our study. To the best of our knowledge, the influence of beer consumption on DMG and BET levels in association with HCY metabolism has not yet been published. In our previous study, BET and FA were shown to be capable of maintaining lower HCY concentrations during alcohol consumption in comparison with the group without FA and BET supplementation, and BET was also shown to have a positive influence on DMG levels [5]. Some authors published an inverse association between HCY concentration and alcohol intake [4, 17]. Beulens observed a decrease in vitamin B_{12} levels and no changes in HCY levels after 3 weeks of beer consumption [27]. Van der Gaag observed no influence of moderate drinking of beer containing 40 g of alcohol on HCY levels and theorised that this is caused by the protective effect of vitamin B_6 contained in beer [28]. In comparison with our results, Beulens observed an increase in vitamin B_6 and pyridoxal-5-phosphate levels similar to van der Gaag [27, 28]. Even though beer contains vitamin B_6 and an increase of vitamin B_6 was described after beer consumption according to some authors, the rise in concentration could be caused by the effect of alcohol, because an increase in vitamin B_6 was not only described after beer consumption but also after the consumption of spirits, which do not contain vitamin B_6 [28]. The volume of beer used in our study (830 mL) contains more than one fifth of the recommended daily dose (RDA) of FA, approximately one tenth of the RDA of B_6 and 3.0% of the RDA of B_{12}. BET is usually referred to as another healthy substance present in beer. Alcohol-free types of beer do not contain ethanol, but these types of beer contain the lowest concentrations of FA. On the contrary, there are several types of alcoholic beer with relatively large amounts of FA. We can hypothesise that the influence of different types of beer in published studies can differ

according to the number of vitamins and ethanol contained within those beers [29]. Additionally, data describing the amounts of vitamins in different types of beer are lacking.

Our data suggest well-correlated change in UA levels with AOC and an increase in the levels of SOD and GRH after beer intake. Alcohol is mainly metabolised by alcohol dehydrogenase and cytochrome P450 enzymes in liver tissue. Cytochrome P450 enzymes are stimulated by chronic alcohol abuse, and this pathway generates reactive oxygen species. Redox metabolism of ethanol in the liver produces NADH and lowers NAD^+, thus increasing the NADH/NAD^+ ratio. NAD^+ is part of the redox reactions and chronic alcohol abuse inhibits several reactions [7]. NADH inhibits pyruvate dehydrogenase and the conversion of pyruvate to acetyl-CoA and increases the conversion to lactic acid. Xanthine oxidase, an important enzyme involved in the transformation of hypoxanthine to UA, is also involved in the metabolism of ethanol. The intake of alcohol can be associated with higher UA levels [30]. While ethanol is metabolised, the production of superoxide stimulates SOD and subsequently increases the production of hydrogen peroxide. This production leads to an increase in GRH, which helps to maintain sufficient levels of reduced glutathione needed for the appropriate function of GPX. Finally, an increase in antioxidant capacity can be explained by the stimulation of protective mechanisms against reactive oxygen species, including the mechanisms mentioned above [16]. However, even though the amount of ethanol in beer is lower in comparison with spirits, some authors advocate the higher increase of UA after beer intake due to the increased amounts of purines in beer, especially guanosine [31]. According to our correlation analysis, the changes in AOC and UA levels after beer intake are connected and the AOC increase is probably at least partially mediated by UA increase. For this reason, AOC increases after beer drinking should be cautiously interpreted.

The main strength of our study was that we performed an interventional study with a cross-over design of the influence of alcoholic beer on reasonable amounts of participants in a real-life setting. Furthermore, we involved several important parameters including parameters of the methylation cycle or oxidative stress. Our data include the influence of beer intake on relatively poorly investigated parameters such as DMG and BET. Different kinds of beer contain different amounts of vitamins, so our results cannot be generally applicable to all beers. The limitation of our study is that dietary intake was controlled by questionnaire only. Additionally, we did not investigate genetic factors (e.g., MTHFR mutations) influencing methylation metabolism.

5. Conclusion

The parameters of methylation metabolism (HCY, DMG and BET) did not show statistically significant changes after beer consumption, whereas some markers of redox metabolism increased (UA, AOC,

SOD and GRH). According to our correlation analysis, the changes in AOC and UA levels after beer intake are connected and the AOC increase can be mediated by UA increase. However, AOC changes can be influenced by multiple factors and should be cautiously interpreted.

6. Conflict of Interest

There is no conflict of interest.

7. Acknowledgements

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Tables:

Table 1 Characteristics of population included in the study and beer used in the study.
Data describing population presented as median (1^{st}–3^{rd} quartile). **BMI** – body mass index, **BP** – blood pressure, **CKD-EPI** – The Chronic Kidney Disease Epidemiology Collaboration equation, **eGFR** – estimated glomerular filtration rate.
Characteristics of the beer used in the study and amount (%) of **RDA** (recommended daily allowance) of selected substances in 830 mL of the beer consumed during the experiment.

Characteristics of population included in the study	
N	93
Age [years]	42.0 (32.0–53.0)
BMI [$kg.m^{-2}$]	25.6 (24.0–28.0)
Systolic BP [mm Hg]	126 (24.0–28.1)
Diastolic BP [mm Hg]	79.0 (73.0–85.0)
eGFR (CKD-EPI creatinine) [$mL.s^{-1}$]	1.3 (1.25–1.48)
Education (elementary/secondary/tertiary)	8 (7%)/30 (26%)/78 (67%)
Average alcohol intake [g/day]	21 (13–36)
Proportion of different alcoholic beverages before the study (beer, wine, spirits) [%]	77, 14, 9
Characteristics of beer used in the study	
Alcohol Content [% of Weight]	4.1
Polyphenolic compounds	172
pH	4.61
Carbon dioxide [%]	0.50
Sulphur dioxide [mg/L]	8.7
Vitamin B_6 [µg/100 g]	53 (20% RDA)
Vitamin B_{12} [ng/L]	85 (3% RDA)
Folic Acid [µg/L]	57 (24% RDA)

Table 2 Changes of BP, eGFR and selected biochemical parameters. Data presented as median (1st–3rd quartile), p values of changes between samples A-B and A-C included. **ApoAI** – Apolipoprotein-AI, **ApoB** - Apolipoprotein B, **ALT** – alanine aminotransferase, **AST** – aspartate aminotransferase, **BP** – blood pressure, **eGFR** – estimated glomerular filtration rate, **GGT** – gamma-glutamyltransferase, **HDL** – HDL-cholesterol, **hsCRP** – hypersensitive C-reactive protein, **LDL** – LDL-cholesterol, **TB** – total bilirubin, **TC** – total cholesterol, **TG** – triglycerides, **UA** – uric acid.

	Sample A	**Sample B**	**P (A-B)**	**Sample C**	**P (A-C)**
Systolic BP	115.0 (110.0–125.0)	128.0 (120.0–136.3)	**<0.0001**	131.0 (123.0–140.1)	**<0.0001**
Diastolic BP	77.0 (70.0–81.25)	78.0 (73.0–86.3)	0.47	80.0 (75.0–85.3)	**0.008**
eGFR [CKD–EPI]	1.34 (1.25–1.46)	1.37 (1.24–1.5)	1.0	1.36 (1.26–1.47)	1.0
TB [µmol/L]	12.6 (10.6–16.8)	12.6 (9.8–15.8)	1.0	12.3 (9.9–16.3)	0.91
AST [µkat/L]	0.40 (0.36–0.47)	0.43 (0.37–0.50)	**0.005**	0.41 (0.36–0.49)	0.76
ALT [µkat/L]	0.47 (0.36–0.57)	0.49 (0.38–0.62)	0.15	0.46 (0.37–0.59)	0.66
GGT [µkat/L]	0.42 (0.29–0.52)	0.47 (0.32–0.57)	**<0.0001**	0.42 (0.32–0.52)	0.11
hsCRP [µg/L]	0.56 (0.26–0.94)	0.61 (0.37–0.99)	0.34	0.60 (0.35–0.93)	0.36
UA [µmol/L]	292.0 (256.8–325.8)	303.0 (274.0–352.3)	**<0.0001**	298.0 (261.0–334.0)	1.0
TG [mmol/L]	1.24 (0.93–1.63)	1.31 (0.93–1.74)	0.17	1.18 (0.93–1.65)	1.0
TC [mmol/L]	5.49 (5.03–6.38)	5.75 (5.38–6.44)	**<0.0001**	5.65 (5.03–6.29)	1.0
HDL [mmol/L]	1.44 (1.29–1.60)	1.52 (1.35–1.66)	**<0.0001**	1.46 (1.33–1.60)	**0.019**
LDL [mmol/L]	3.08 (2.58–3.80)	3.12 (2.76–3.76)	**0.039**	3.12 (2.52–3.78)	1.0
apoAI [g/L]	1.24 (1.13–1.33)	1.27 (1.18–1.39)	**<0.0001**	1.23 (1.16–1.33)	**0.0096**
apoB [g/L]	0.94 (0.81–1.11)	0.95 (0.96–1.14)	**0.009**	0.94 (0.82–1.09)	1.0
Fibrinogen [g/L]	2.92 (2.63–3.28)	2.66 (2.34–3.04)	**<0.0001**	2.86 (2.49–3.15)	**0.021**

Table 3 Changes in parameters related to the metabolism of HCY and parameters connected with oxidative stress. Data presented as median (1^{st}–3^{rd} quartile), p values of changes between samples A-B and A-C included.
AGE – advanced glycation end products, **AOC** – antioxidative capacity, **AOPP** – advanced oxidation protein products, **B_{12}** – vitamin B_{12}, **BET** – betaine, **DMG** – dimethylglycine, **FA** – folic acid, **FRUCT** – fructosamine, **GPx** – glutathione peroxidase, **GRH** – glutathione reductase, **GSH** – reduced glutathione, **HCY** – homocysteine, **PL** – pyridoxal, **PLP** – pyridoxal phosphate, **PONS** – paraoxonoase, **SOD** – superoxide dismutase, **TBARS** – thiobarbituric acid reactive substances.

	Sample A	**Sample B**	**P (A-B)**	**Sample C**	**P (A-C)**
HCY [µmol/L]	10.7 (9.4–12.3)	10.7 (9.6–12.3)	0.19	10.7 (9.5–12.2)	1.0
B_{12} [ng/L]	284.4 (231.4–367.0)	266.3 (215.2–322.1)	**0.0001**	286.6 (237.2–246.6)	1.0
FA [µg/L]	5.86 (4.76–7.29)	5.62 (4.78–7.09)	0.22	6.01 (4.81–7.61)	1.0
PLP [nmol/L]	40.3 (29.8–59.3)	49.8 (36.1–66.8)	0.13	–	–
PL [nmol/L]	17.8 (14.0–23.0)	21.8 (18.9–36.3)	0.73	–	–
BET [nmol/L]	25.8 (20.7–34.3)	27.4 (18.9–36.3)	0.17	–	–
DMG [nmol/L]	4.95 (3.65–8.05)	4.80 (3.78–6.65)	0.70	–	–
AOC [mmol/L]	1.44 (1.38–1.50)	1.45 (1.40–1.51)	**0.02**	1.44 (1.38–1.48)	1.0
SOD [U/g Hb]	1492.4 (1382.4–1651.1)	1532.1 (1450.1–1698.5)	**0.025**	1534.3 (1390.1–1657.4)	0.54
GPx [U/g Hb]	63.5 (54.1–71.4)	64.0 (56.9–71.5)	1.0	62.7 (55.5–72.9)	1.0
GRH [U/g Hb]	11.5 (10.6–12.9)	12.2 (11.5–13.5)	**0.0001**	12.3 (11.4–13.3)	**0.002**
GSH [mmol/L]	1.84 (1.68–1.98)	1.85 (1.62–2.08)	0.62	1.84 (1.64–2.05)	1.0
TBARS [µmol/L]	2.16 (1.95–2.5)	2.15 (1.81–2.59)	1.0	2.06 (1.77–2.37)	0.12
AGE [FU/g protein]	4.76 (4.22–5.10)	4.78 (4.29–5.36)	0.68	4.66 (4.17–5.13)	1.0
AOPP [mmol/L]	34.2 (28.7–41.2)	35.7 (31.4–42.9)	0.084	33.7 (28.58–43.2)	1.0
PONS [U/L]	237.0 (105.0–362.3)	246.0 (105.5–377.8)	0.92	230.0 (100.3–338.8)	0.91
FRUCT [µmol/L]	336.5 (323.1–347.8)	331.4 (317.8–349.8)	0.32	331.4 (317.5–348.8)	0.58

Table 4 Spearman correlation coefficients of changes in selected parameters. P values included. **ALT** – alanine aminotransferase, **apoAI** – Apolipoprotein-AI, **apoB** – Apolipoprotein B, **AOC** – antioxidative capacity, **AST** – aspartate aminotransferase, **GGT** – gamma-glutamyltransferase, **HDL** – HDL-cholesterol, **TC** – total cholesterol, **UA** – uric acid.

Selected parameters	r	P
AST – HDL	**0.25**	**0.018**
AST – TC	**0.30**	**0.003**
AST – apoAI	**0.31**	**0.002**
AST – apoB	**0.27**	**0.009**
GGT – HDL	0.17	0.10
GGT – TC	**0.31**	**0.002**
GGT – apoAI	**0.21**	**0.04**
GGT – apoB	**0.29**	**0.004**
UA – AOC	**0.45**	**<0.0001**
HDL – AOC	**0.25**	**0.018**
apoAI – AOC	**0.35**	**0.0007**

Figure 1 Boxplots of changes in concentrations of uric acid (**p<0.0001**), fibrinogen (**p<0.0001**), vitamin B_{12} (**p=0.0001**), AOC (**p=0.02**), SOD (**p=0.025**) and GRH (**p=0.0001**).
AOC – antioxidative capacity, **GRH** – glutathione reductase, **SOD** – superoxide dismutase

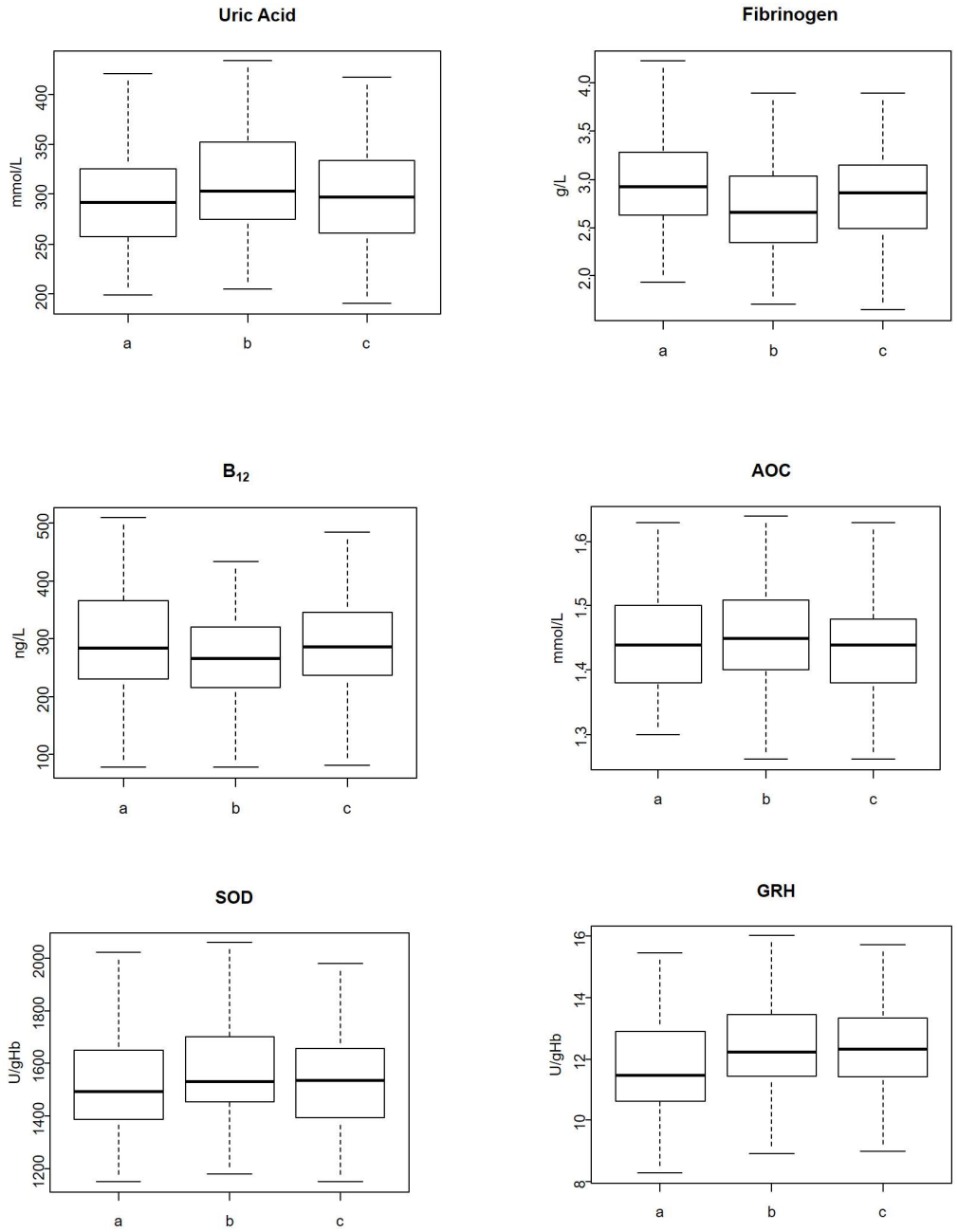

Figure 2 Graph of correlation of changes between **AOC** (antioxidative capacity) and **UA** (uric acid) after one month of beer consumption, **r=0.45**, **p<0.0001**.