Effect of caffeinated and decaffeinated coffee on serum uric acid and uric acid clearance, a randomised, within-subject, experimental study

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Abstract

Objective

The effect of coffee on serum uric acid (SUA) has shown conflicting results. This study was to determine the effects of caffeinated coffee (CC) and decaffeinated coffee (DC) on SUA, serum xanthine oxidase activity (sXOA) and urine uric acid clearance (UAC).

Methods

This was a prospective randomised within-subject experimental study design in 51 healthy male participants. Each study period consistsed of 3 periods, including a control, an intervention, and washout period for 1, 3 and 1 week, respectively. During the intervention period, the participants received 2, 4 or 6 gram/day of coffee, either CC or DC.

Results

For DC groups, SUA significantly decreased by 6.5 (±1.1) mg/dL to 6.2 (±1.1) mg/dL during the intervention period (p=0.014). sXOA significantly increased by 0.05 (±0.07) nmol/min/mL to 0.20 (±0.38) nmol/min/mL during the intervention period (p=0.010) of CC. For UAC, there was no significant change with CC or DC. In hyperuricaemic participants, SUA significantly decreased by 7.7 (±0.7) mg/dL to 7.2 (±0.7) mg/dL during the intervention period (p=0.028) of DC. For non-hyperuricaemic, CC significantly increased SUA by 5.9 (±0.7) mg/dL to 6.2 (±0.9) mg/dL during the intervention period (p=0.008) and significantly decreased SUA to 6.0 (±0.8) mg/dL (p=0.049) during the withdrawal period. A significant increase of sXOA according with SUA in CC groups from 0.05 (±0.07) nmol/min/mL to 0.25 (±0.44) nmol/min/mL during the intervention period (p=0.040) was presented in non-hyperuricaemic participants.

Conclusion

DC had a significant decrease of SUA during the intervention period. However, in non-HUS participants, SUA significantly increased in CC.

Key words

coffee, serum uric acid, xanthine oxidase, uric acid clearance, caffeine

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Introduction

Serum uric acid (SUA) level in the body is determined by the balance between its production either from purine intake in diet or endogenous production and its excretion mainly by the kidney (1). Various food and substances, including meat, alcohol, milk and coffee have been associated with SUA levels (2-5).

Coffee is the major source of caffeine that is a purine alkaloid in the diets of adults (6). The major complex of chemical compounds, including caffeine (1, 3, 7-trimethyl-xanthine) and 5-*O*- caffeoylquinic acids (chlorogenic acid, 5-CQA) were found in coffee and had many biological effects (6). Paraxanthine, theobromine, and theophylline that are performed by the cytochrome P450 enzyme are the metabolites of caffeine. Finally, they are oxidised to 3-methyuric acid by xanthine oxidase (7, 8). Although caffeine is the major chemical component in coffee, one study has shown that other unknown substances, other than caffeine, might have an inverse association with SUA (9).

The relationship between coffee and SUA has been presented in many studies (4, 9-15). An inverse association between coffee consumption and SUA level was significantly observed in Japanese men and women (10, 11). According to the third National Health and Nutrition Examination Survey in the US (9) and the Nutrition and Health Survey in Taiwan (13) increasing coffee intake significantly decreased SUA, and the frequency of consumption of coffee was negatively associated with hyperuricaemia (HUS), respectively. In contrast, a study that was conducted among participants from the Singapore Chinese Health Study found that there was no significant association between consumption of coffee and SUA levels (14). Furthermore, there was no statistical change of SUA levels for coffee after adjustment for potential confounders among men and women (15). Finally, the association between coffee and SUA in a few recent meta-analysis studies was conflicting (2-4).

Since there have been conflicting results for hypouricaemic effects with both caffeinated coffee (CC) and decaffeinated coffee (DC), this study was designed to evaluate the hypouricaemic effects, serum xanthine oxidase activity (sXOA), and uric acid clearance (UAC) of CC and DC in healthy male participants.

Materials and methods

Participants

This is a controlled randomised within-subject study. Volunteers were healthy men, recruited from the health care staff of Naresuan University Hospital. The inclusion criteria were healthy men, age 20–60 years old, having a normal estimated glomerular filtration rate (eGFR) (≥60 mL/min/1.73m^2), normal serum aspartate aminotransferase (AST) (≤40 unit/L), serum alanine transaminase (ALT) (≤41unit/L), and normal serum albumin (≥3.5 mg/dL). The exclusion criteria were eGFR of less than 60 mL/min/1.73m^2, abnormal AST and/or ALT, or use of a nutritional supplement that can interfere with SUA, such as vitamin C. In addition, if participants who were included and took coffee tablets had any serious side effects, including severe disturbed normal sleep pattern, severe headache, blood pressure ≥140/90 mmHg, and heart rate ≥100 beat/min or arrhythmia; the participants were withdrawn from this protocol. Complete blood count and EKG were evaluated. All participants gave their written informed consent prior to entering the study. Hyperuricaemia was defined as SUA higher than 6.8 mg/dL (16).

The study protocol was approved by the Naresuan University Institutional Review Board and Ethics Committee (IRB no. 1098/60) and registered with the Thai Clinical Trials Registry (TCTR). The TCTR identification number is TCTR20190304003.

Protocol

The study was designed to use commercial CC in the first period and then use commercial DC in the second period in the same healthy male participants. The study consisted of 3 periods including control, intervention, and washout period for a duration of 9 weeks. The first week was the control period in which participants underwent diet control and received no other intervention. If participants had 15% or more change in

SUA during the control period (day 0 to day 7), they were excluded. The following 3 weeks were the interventional period in which participants consumed 2, 4 or 6 g/day of CC, according to their assigned experimental groups by simple randomisation. The last week was the washout period after CC was discontinued, and this period was also the control period of DC. After the washout period, the same participants repeated the protocol but consumed 2, 4 or 6 g/day of DC instead of CC and at the same dosage. History taking, physical examination, EKG and blood examination for AST, ALT, serum creatinine, eGFR, SUA, UAC, and sXOA were performed at the beginning and end of each study period.

The participants were instructed to consume coffee 2 times daily after morning and lunch meals. The simple randomisation was applied using lots to make a draw for self-allocation by each participant. Twenty labels of each experimental coffee dosage were prepared in a closed envelope. The participants opened 1:1 by themselves during randomisation starting from CC and DC, respectively, following the protocol (Supplementary Fig. S1).

During the study, the participants were intensively followed-up by phone about compliance to consume the coffee tablets three times per week. Also, they were asked to record their daily food consumption, reminded about maintaining constant dietary intake, and told to avoid consuming foods that might affect the SUA level, including all types of tea and coffee products, alcoholic drinks, and food supplements. The participants were also required to return the coffee bottle after the interventional period to ensure their adherence to protocol.

The primary outcome of the study was a change in the SUA level after either CC or DC consumption. Secondary outcomes included changes in sXOA and UAC as well as adverse events related to coffee consumption, and optimal dosage of coffee in lowering the SUA level.

Coffee preparation

Tablets containing 500 mg of CC and DC of the same commercial brand were prepared by AT. The quality of the tablets complied with the requirement of the British Pharmacopoiea 2010 (17) and were proved to be stable at least for 30 days at room temperature (Supplementary Table S1). All participants received coffee tablets of the same quality throughout the study, and these tablets were checked for the exact number.

Serum xanthine oxidase activity assay

Blood was collected from each subject following the protocol. The serum was obtained and stored at -20°C for the sXOA assay. The assay was performed according to the instructions of the commercial assay kit (Xanthine Oxidase Activity Assay Kit, MAK078, Sigma-Aldrich.co, USA).

Blood chemistry determination

SUA, serum creatinine, eGFR, AST, ALT and 24 hours urine creatinine and urine uric acid (for UAC) that were immediately analysed after blood or urine collection in the morning at Naresuan University hospital was measured using an automate analyser, COBAS C 501® (Roche Diagnostic Company, Switzerland). As for the reliability of the machine, the standard operating procedure of the laboratory conformed to ISO 15189:2012 (18).

Statistical analysis

For descriptive statistics, data are presented as mean and SD for continuous variables and frequency (percentage) for categorical variables. Baseline characteristics were compared using one-way ANOVA and Chi-square statistics for quantitative and qualitative variables, respectively. Comparisons between each period of intervention and withdrawal in the CC and DC groups were performed using the Wilcoxon signed rank test. Differences were considered statistically significant at p-value <0.05 with two-tailed tests. All analyses were carried out using SPSS statistics 17.0 for Windows (SPSS Inc., Chicago, IL).

Results

Baseline participant characteristics

Of the 75 participants enrolled in this study, twenty participants were excluded by the study criteria (Suppl. Fig. S1).

Table I. Data characteristics.

Characteristics	Total (n=51)	Amount of caffeinated and decaffeinated coffee			p-value*
		2 gram coffee group (n=16)	4 gram coffee group (n=17)	6 gram coffee group (n=18)	
Age, years, mean (SD)	32.51 (8.20)	31.63 (9.17)	34.18 (7.60)	31.72 (8.05)	0.599
Systolic blood pressure, mmHg, mean (SD)	121 (10)	116 (12)	121 (8)	126 (7)	0.010
Pulse rate, beat/min, mean (SD)	80 (10)	82 (12)	81 (8)	77 (8)	0.387
Mean arterial blood pressure, mean (SD)	88.90 (7.77)	85.19 (8.89)	89.47 (5.42)	91.67 (7.65)	0.042
Body mass index, kg/m^2, mean (SD)	24.05 (3.08)	24.40 (2.85)	24.05 (3.66)	23.76 (2.80)	0.836
Estimated glomerular filtration rate, ml/min/1.73m^2, mean (SD)	87.39 (15.85)	101.84 (15.56)	96.28 (15.50)	93.59 (15.96)	0.226
Serum uric acid, mg/dL, mean (SD)	6.42 (1.05)	6.40 (1.27)	6.44 (0.90)	6.42 (1.02)	0.994
Hyperuricaemia§, n (%)	15 (29.4)	5 (31.3)	5 (29.4)	5 (27.8)	0.976
24 hour urine uric acid, mg/24 hours, mean (SD)	512.63 (163.85)	413.50 (156.45)	498.15 (128.48)	614.42 (146.29)	0.001
24 hour urine creatinine, g/24 hours, mean (SD)	1.40 (0.42)	1.12 (0.40)	1.44 (0.41)	1.63 (0.31)	0.001

§Hyperuricaemia was defined as SUA higher than 6.8 mg/dL.
*p-value was compared in 2, 4, and 6 grams of coffee.

Fifty-five participants were randomised into groups based on grams of coffee. Two participants each in the 2- and 4-gram groups discontinued due to loss of contact. Two and one participant(s) in the 2- and 4-gram groups, respectively, were withdrawn due to abnormal liver enzymes and their unwillingness to participate in follow up investigations. A participant was withdrawn from the 6-gram group because he was just diagnosed with type 2 diabetes mellitus in between an intervention period. Therefore, a total of 51 participants, including 15, 16 and 17 participants in the 2,- 4 -and 6-gram groups for both CC and DC, respectively, participated in the study as per protocol. The baseline characteristic data are presented in Table I.

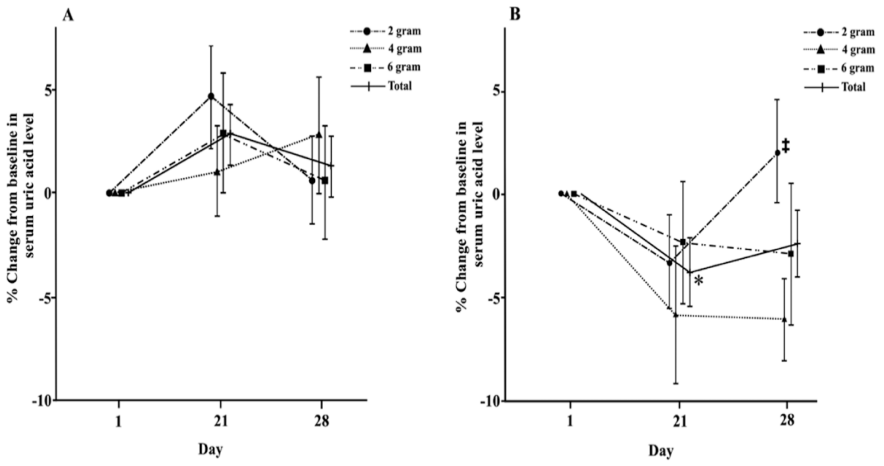

Fig. 1. Changes in serum uric acid during the study period overall. The analysis was compared with the previous value. Day 1, 21 and 28 represents the baseline, the intervention period, and the washout period, respectively.
A: Caffeinated coffee overall. **B**: Decaffeinated coffee overall. *p<0.05 for all participants in decaffeinated coffee. ‡ p<0.05 for 2 grams in the withdrawal period of decaffeinated coffee.

The primary end point
– Serum uric acid level

Overall, there was no significant change of SUA between baseline date of the protocol that was before administration of CC and DC (p=0.411) and the final date of CC and DC administration (p=0.058), respectively. Therefore, it could be assumed that throughout the study period, all participants had a constant dietary intake. *For the CC groups*, irrespective of the amount of CC, SUA increased approximately 3% within the 3 weeks and decreased to baseline during the withdrawal, but there were no significant differences. Regarding the amount of CC, although SUA had increased in each dosage during the intervention period of CC, no significant change was found. *In the subgroup analysis*, for non-HUS, irrespective of the amount of CC (n=36), SUA had significantly increased (p=0.008) and decreased (p=0.049) in the intervention period and withdrawal period, respectively. However, SUA had a marginally significant increase (p=0.052) in the intervention period of the 2-gram groups. In HUS participants, the effects of CC on SUA are shown in Figure 2 and Table II.

In the DC groups, a significant decrease of approximately 5% of SUA within 3 weeks (p=0.014) was presented irrespective of the amount of DC; there was but no significant increase to the baseline during the withdrawal

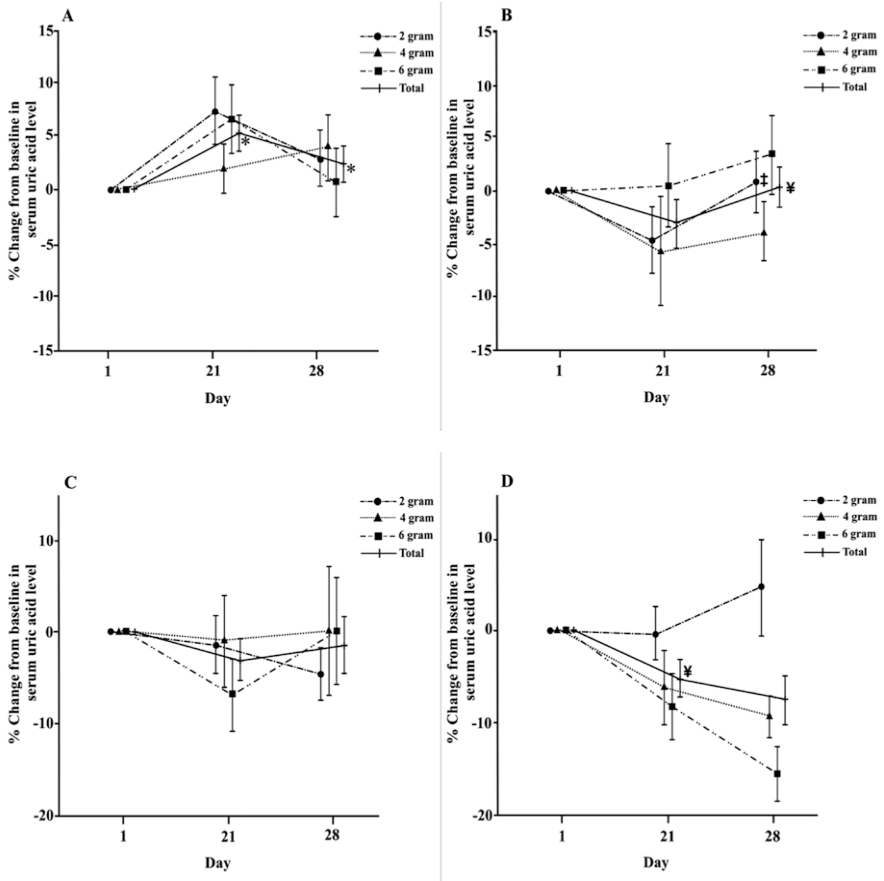

Fig. 2. Changes in serum uric acid during the study period in non-hyperuricaemic and hyperuricaemic participants. The analysis was compared with the previous value. **A** and **C**, Caffeinated coffee; **B** and **D**, Decaffeinated coffee.
Day 1, 21, and 28 represents the baseline, the intervention period, and the washout period, respectively.
A: Caffeinated coffee in non-hyperuricaemic participants. *p<0.05 for all non-hyperuricaemic participants.
B: Decaffeinated coffee in non-hyperuricaemic participants. *p<0.05 for all non-hyperuricaemic participants, ¥p<0.05 for all non-hyperuricaemic participants, ‡p<0.05 in withdrawal period of 2 grams of decaffeinated coffee in non-hyperuricaemic participants, ¥p<0.05 in intervention period for all hyperuricaemic participants.

Table II. The effect of caffeinated and decaffeinated coffee to serum uric acid level, serum xanthine oxidase activity, and uric acid clearance.

Variables	Total (n=51)					2 grams of coffee (n=16)					4 grams of coffee (n=17)					6 grams of coffee (n=18)				
	Baseline	Intervention	Washout	p_1 value	p_2 value	Baseline	Intervention	Washout	p_1 value	p_2 value	Baseline	Intervention	Washout	p_1 value	p_2 value	Baseline	Intervention	Washout	p_1 value	p_2 value
	Day[+]1 Mean (SD)	Day[+]21 Mean (SD)	Day[+]28 Mean (SD)			Day[+]1 Mean (SD)	Day[+]21 Mean (SD)	Day[+]28 Mean (SD)			Day[+]1 Mean (SD)	Day[+]21 Mean (SD)	Day[+]28 Mean (SD)			Day[+]1 Mean (SD)	Day[+]21 Mean (SD)	Day[+]28 Mean (SD)		
Overall																				
Caffeine																				
SUA, mg/dL	6.4 (1.0)	6.6 (1.0)	6.5 (1.1)	0.168	0.206	6.4 (1.3)	6.6 (1.1)	6.4 (1.1)	0.191	0.086	6.4 (0.9)	6.5 (1.1)	6.6 (1.1)	0.776	0.740	6.4 (1.0)	6.6 (1.0)	6.5 (1.2)	0.485	0.452
sXOA, nmol/min/mL	0.05 (0.07)	0.20 (0.38)	0.17 (0.17)	*0.010*	0.261	0.05 (0.07)	0.19 (0.48)	0.22 (0.23)	0.959	0.148	0.04 (0.06)	0.13 (0.21)	0.16 (0.16)	*0.022*	0.435	0.06 (0.08)	0.28 (0.42)	0.13 (0.12)	*0.020*	0.913
UAC, mg/24 hours	512.63 (163.85)	496.47 (242.53)	540.06 (238.65)	0.183	0.056	413.50 (156.45)	383.17 (176.38)	496.26 (236.32)	0.438	*0.007*	498.15 (128.48)	580.07 (313.29)	599.79 (242.56)	0.554	0.163	614.42 (146.29)	518.22 (183.31)	522.57 (239.07)	*0.035*	0.711
Decaffeinate																				
SUA, mg/dL	6.5 (1.1)	6.2 (1.1)	6.3 (1.0)	*0.014*	0.375	6.4 (1.1)	6.2 (1.3)	6.5 (1.3)	0.196	*0.024*	6.6 (1.1)	6.2 (1.2)	6.1 (0.8)	0.083	0.856	6.5 (1.2)	6.2 (1.0)	6.2 (1.0)	0.227	0.861
sXOA, nmol/min/mL	0.17 (0.17)	0.16 (0.24)	0.09 (0.08)	0.200	0.829	0.22 (0.23)	0.11 (0.18)	0.07 (0.04)	*0.035*	0.679	0.16 (0.16)	0.25 (0.30)	0.09 (0.06)	0.795	0.113	0.13 (0.12)	0.12 (0.20)	0.12 (0.11)	0.687	0.306
UAC, mg/24 hours	540.06 (238.65)	471.92 (217.97)	486.32 (211.82)	0.068	0.603	496.26 (236.32)	439.09 (231.96)	397.31 (176.02)	0.535	0.187	599.79 (242.56)	462.91 (250.20)	530.20 (233.91)	*0.022*	0.287	522.57 (239.07)	509.61 (175.34)	523.99 (205.53)	0.948	0.616
Non HUS[$]																				
Caffeine	(n=36)					(n=11)					(n=12)					(n=13)				
SUA, mg/dL	5.9 (0.7)	6.2 (0.9)	6.0 (0.8)	*0.008*	*0.049*	5.8 (0.9)	6.2 (1.0)	5.9 (0.9)	0.052	0.137	6.0 (0.6)	6.1 (0.9)	6.2 (0.8)	0.475	0.753	5.9 (0.4)	6.3 (0.9)	5.9 (0.8)	0.074	0.061
sXOA, nmol/min/mL	0.05 (0.07)	0.25 (0.44)	0.16 (0.18)	*0.040*	0.925	0.06 (0.08)	0.24 (0.58)	0.21 (0.25)	0.929	0.594	0.02 (0.02)	0.13 (0.21)	0.15 (0.16)	0.158	0.530	0.07 (0.09)	0.36 (0.47)	0.12 (0.12)	0.064	0.345
UAC, mg/24 hours	500.78 (151.07)	489.82 (197.95)	584.99 (247.92)	0.405	*0.012*	427.51 (160.95)	391.66 (212.20)	533.13 (265.13)	0.477	*0.010*	481.68 (136.64)	500.74 (173.13)	633.65 (255.57)	0.754	*0.041*	580.42 (125.32)	562.78 (185.92)	583.95 (236.61)	0.221	0.972
Decaffeinate	(n=33)					(n=11)					(n=10)					(n=12)				
SUA, mg/dL	5.8 (0.7)	5.6 (0.9)	5.8 (0.8)	0.173	*0.032*	5.9 (0.8)	5.6 (0.9)	5.9 (0.9)	0.138	*0.044*	5.9 (0.6)	5.5 (1.0)	5.6 (0.5)	0.292	0.646	5.8 (0.7)	5.8 (0.8)	5.9 (0.9)	0.969	0.127
sXOA, nmol/min/mL	0.17 (0.18)	0.14 (0.20)	0.08 (0.05)	0.360	0.768	0.23 (0.24)	0.08 (0.16)	0.07 (0.05)	0.139	0.328	0.17 (0.18)	0.25 (0.25)	0.09 (0.07)	0.646	0.114	0.12 (0.12)	0.11 (0.18)	0.08 (0.04)	0.790	0.638
UAC, mg/24 hours	535.85 (232.41)	476.15 (238.29)	476.39 (197.36)	0.195	0.816	504.83 (253.69)	463.68 (265.63)	403.15 (186.35)	0.657	0.120	560.09 (224.04)	434.03 (257.15)	476.14 (168.13)	0.169	0.508	544.07 (236.61)	522.67 (206.89)	543.75 (219.79)	0.754	0.480
HUS[$]																				
Caffeine	(n=15)					(n=5)					(n=5)					(n=5)				
SUA, mg/dL	7.7 (0.7)	7.4 (0.8)	7.5 (1.0)	0.146	0.528	7.8 (0.7)	7.6 (0.6)	7.4 (0.7)	0.498	0.461	7.5 (0.6)	7.4 (0.9)	7.5 (1.1)	0.683	0.892	7.8 (0.8)	7.2 (0.9)	7.8 (1.2)	0.138	0.104
sXOA, nmol/min/mL	0.05 (0.06)	0.09 (0.14)	0.21 (0.15)	0.140	*0.017*	0.04 (0.03)	0.06 (0.09)	0.26 (0.18)	0.686	*0.043*	0.07 (0.10)	0.14 (0.23)	0.18 (0.16)	0.080	0.686	0.04 (0.01)	0.07 (0.07)	0.17 (0.11)	0.225	0.080
UAC, mg/24 hours	541.06 (193.91)	512.44 (334.25)	432.23 (179.14)	0.281	0.820	382.68 (159.06)	364.49 (58.76)	415.15 (147.43)	0.893	0.500	537.69 (109.16)	770.47 (495.95)	518.53 (209.83)	0.686	0.686	702.82 (174.11)	402.35 (126.01)	363.00 (175.76)	0.080	0.500
Decaffeinate	(N=18)					(n=5)					(n=7)					(n=6)				
SUA, mg/dL	7.7 (0.7)	7.2 (0.7)	7.1 (0.9)	*0.028*	0.381	7.5 (0.7)	7.5 (0.8)	7.9 (1.0)	0.999	0.279	7.6 (0.7)	7.1 (0.8)	6.9 (0.5)	0.128	0.461	7.8 (0.8)	7.2 (0.5)	6.6 (1.0)	0.080	0.173
sXOA, nmol/min/mL	0.17 (0.15)	0.19 (0.30)	0.12 (0.11)	0.306	0.983	0.21 (0.21)	0.16 (0.23)	0.06 (0.03)	*0.043*	0.500	0.15 (0.14)	0.25 (0.39)	0.09 (0.04)	0.866	0.612	0.16 (0.12)	0.13 (0.26)	0.20 (0.15)	0.753	0.345
UAC, mg/24 hours	547.78 (256.39)	464.17 (181.00)	504.52 (241.03)	0.184	0.647	477.40 (218.84)	385.00 (141.63)	384.48 (170.52)	0.686	0.893	656.50 (274.27)	504.16 (253.72)	607.43 (302.78)	*0.043*	0.499	479.58 (260.45)	483.48 (95.58)	484.49 (185.84)	0.753	0.753

SUA: Serum uric acid, sXOA: Serum xanthine oxidase activity, UAC: Uric acid clearance, HUS: Hyperuricaemia

p_1 value, the statistical analysis between the baseline and the intervention period of the protocol in caffeinated *or* decaffeinated coffee.

p_2 value, the statistical analysis between the intervention and the washout period of the protocol in caffeinated *or* decaffeinated coffee.

[+] Date of the protocol of each coffee type.

[$] Hyperuricaemia (HUS) was defined as serum uric acid higher than 6.8 mg/dL.

period. Regarding the dosage of DC, SUA had no significant decrease in each dosage during the intervention period. However, SUA significantly increased (p=0.024) after the 2 grams withdrawal of DC. *In the subgroup analysis* for non-HUS, irrespective of the amount of DC (n=33), although SUA had no significant decrease during the intervention period, there was a significant increase (p=0.032) during the withdrawal period. In the withdrawal period of 2 grams in non-HUS, SUA significantly increased (p=0.044). For HUS, during the intervention period, the significant decrease of SUA (p=0.028) was demonstrated irrespective of the amount of DC (n=18). In HUS participants, the dependency on the dosage of DC on SUA is shown in Figure 2 and Table II.

The secondary end points

– Serum xanthine oxidase activity

Among the CC groups, irrespective of the amount of CC, the sXOA significantly increased in the intervention period of CC (p=0.010). The sXOA including in the intervention and withdrawal period was concordant with SUA. Respective to the amount of CC, the sXOA significantly increased in the intervention period of the 4-gram (p=0.022) and the 6-gram (p=0.020) groups. Except in the withdrawal period of 2 grams of CC, the sXOA was concordant with SUA. *In the subgroup analysis*, for non-HUS participants, irrespective of the amount of CC, the sXOA significantly increased (p=0.040) in the intervention period and decreased, but not significantly, in the withdrawal period of CC. Depending on the dosage of CC in non-HUS, the results are presented in Table II. Regardless of the amount of CC and all the amounts of CC in the both periods of CC, the sXOA was concordant with SUA. In HUS participants, irrespective of the amount of CC, sXOA that accorded with SUA had a significant increase during the withdrawal period (p=0.017). The other results are presented in Table II.

Among the DC groups, irrespective of the amount of DC, there was no significant change of the sXOA in the both periods of DC. The sXOA was concordant with SUA only during the intervention period of DC. Depending on the dosage for DC, a significant decrease of sXOA that was concordant with SUA was demonstrated between the intervention period of the 2-gram (p=0.035) groups. Most of sXOA was concordant with SUA in the DC groups. *In the subgroup analysis*, for non-HUS participants, irrespective of the amount of DC, sXOA that accorded with SUA in the intervention period but had no significant decrease. With respective to the amount of DC, there was no significant change. In HUS participants, sXOA is presented in Table II.

Uric acid clearance

For 24-hour urine creatinine, the minimal mean (SD) of CC and DC were 1.07 (0.51) and 1.05 (0.51), respectively. The results suggested that all participants completed collection for the 24-hour urine volume (Table II).

In the CC groups, regardless of the amount of CC, there was a decrease of UAC, but not significant, in intervention period. However, UAC marginally significantly increased in the withdrawal period (p=0.056). UAC was concordant with SUA, irrespective of the amount of CC in the both periods. With respective to the amount of CC, in the withdrawal period of 2 grams of CC, UAC significantly increased (p=0.007). For the 6-gram groups of the intervention period, UAC significantly decreased (p=0.035). Particularly in the 2-gram and 6-gram groups for the both periods of CC, UAC was concordant with SUA. *In the subgroup analysis*, irrespective of the amount of CC in non-HUS, UAC significantly increased in the withdrawal period (p=0.012). Moreover, UAC was concordant with SUA in both periods. With respective to the amount of CC in non-HUS, UAC significantly increased in the withdrawal period of the 2-gram (p=0.010) and 4-gram (p=0.041) groups. Particularly in the 2-gram and 6-gram groups of CC, UAC was concordant with SUA. For HUS, the results are showed in Table II.

Among the DC groups, irrespective of the amount of DC, there was no significant change for UAC. The significant decrease (p=0.022) was presented in the intervention period of the 4-gram groups. However, irrespective of the amount of DC, most UAC did not accord with SUA. *In the subgroup analysis*, for non-HUS, the results are shown in Table II. For HUS, UAC significantly decreased in the intervention period of 4 grams of DC (p=0.043).

Adverse events

All participants tolerated the side effects well. These side effects were less common in 2 grams of coffee. All these events were mild and did not require any medication (Table III). Blood pressure and pulse rate did not change significantly throughout the study. However, a mild elevation of AST or ALT was found in two participants who received 2 grams of coffee in the latest date of the intervention period of DC. The first par-

Table III. Adverse events of all participants.

Adverse events	Caffeinated coffee (n=53*)		Decaffeinated coffee (n=51)	
	Intervention period*, (n=53), n (%)	Withdrawal period, (n=51), n (%)	Intervention period, n (%)	Withdrawal period, n (%)
Palpitation	9 (17.0)	0 (0)	0 (0)	0 (0)
Insomnia	6 (11.3)	0 (0)	0 (0)	0 (0)
Constipation	4 (7.5)	0 (0)	0 (0)	0 (0)
Diuresis	2 (3.8)	0 (0)	0 (0)	0 (0)
Increase appetite	2 (3.8)	0 (0)	0 (0)	0 (0)
Dizziness	2 (3.8)	0 (0)	1 (2.0)	0 (0)
Light headache	1 (1.9)	4 (7.8)	0 (0)	2 (3.9)
Nausea	1 (1.9)	0 (0)	0 (0)	0 (0)
Drowsiness	0 (0)	6 (11.8)	3 (5.9)	5 (9.8)
Mild hepatitis	2 (3.8)	0 (0)	0 (0)	0 (0)

*In total, fifty-three participants were included for analysis during the intervention period of caffeinated coffee for adverse events. However, two participants who had abnormal liver function tests and refused follow up as to the cause were excluded from the study following the exclusion criteria of the protocol.

ticipant who had mildly elevated ALT administrated medications by himself including a high dosage of ibruprofen (2,400 mg/day) and sulfamethoxazole (800 mg)-trimethoprim (160 mg) since he had a toothache. His blood test improved after withdrawal of these medications. The second participant who had mildly elevated AST and ALT had no medication or supplementary food. The screening of viral hepatitis B and C, antinuclear antibody, prothrombin time, and serum ferritin was conducted and these results showed normality. AST and ALT returned to normal after the withdrawal of the DC.

The optimal dosage of coffee for lowering the SUA level

In our study, there was no association between the dosage of CC and DC and the change of SUA.

Discussion

This study has shown that DC had approximately a 5% hypouricaemic effect within 3 weeks. For subgroup analysis, CC had significantly increased SUA in non-HUS participants. In HUS participants, DC had a significant hypouricaemic effect within 3 weeks. These effects disappeared when coffee consumption was stopped. The mechanism of increasing SUA for CC was the increase of endogenous uric acid production by increasing sXOA and decreasing uric acid excretion. No associations between the dosage of these coffees and the effect to SUA were found in the study. Finally, the side effects of both coffees were mild and no medical treatment was required.

In adults, the average half-life of caffeine in plasma was between 2.5-5 hours (7). Therefore, the washout period that was 7 days before beginning DC in our study was adequate enough to wash out the effect of caffeine. Moreover, the uricase method that was used to determine serum and urine uric acid in our study had high specificity and catalysed only the oxidation of uric acid, not 3-methyuric acid that was the last metabolite of caffeine, to allantoin (19-21).

The association between coffee and SUA has been studied previously (3, 4, 9, 10, 12). Each study was different in study design, population and type or method of coffee. The result of our study accorded with the prospective controlled study of Strandhagen et al. which demonstrated that SUA had significantly decreased and increased when healthy participants abstained and consumed filtered coffee, respectively (12). Also, our result, only DC, agreed with Choi *et al*. which presented the association between decreasing SUA and DC intake (22). However, there was no experimental study in humans about the association between DC and SUA other than the effect of CC on SUA which was an experimental study in men that demonstrated an increase of SUA after ingestion of caffeine (23). However, particularly in CC, our result was different from previous studies (10, 11, 22). The difference in study design might be responsible for the differences in these results.

We hypothesised that firstly, the effect of CC to increase SUA, especially significantly in non-HUS, might be due to caffeine that was metabolited to theophylline (7, 8). The increasing SUA induced by theophylline that had an inhibitory effect on the organic anion transporter (OAT) family (24) has previously been demonstrated (25, 26). Secondly, all metabolites of caffeine were oxidised to 3-methyuric acid by xanthine oxidase (7, 8), accordingly with the change of SUA in CC and sXOA in our study; sXOA that was stimulated for the oxidation process increased activity in the body, and its effect also increased the purine metabolism which finally increased uric acid synthesis simultaneously. For DC, that had the significant hypouricaemic effects during the intervention period in our study particularly in all participants and HUS, the cause of this effect was unclear. However, we theorised that 5-CQA, an antioxidant compound found in coffee, played a vital role to decrease SUA (27) since 5-CQA has significant hypouricaemic effects, inhibiting xanthine oxidase activity which has been demonstreated in an animal model (27). However, roasting conditions, country, and commercial brand can affect the amount of 5-CQA (28). For instant coffee, caffeoylquinic acids including 5-CQA that was 37–152 mg/serving has been reported (28).

For UAC, there were associations with CC between UAC and SUA overall and particularly in non-HUS where there was a significant decrease and increase in SUA and UAC, respectively, in our study. These results demonstrated that CC increased the uric acid excretion in the kidney and resulted in a hypouricaemic effect. A previous study had demonstrated that many metabolites of caffeine including methyxanthines and methyuric acid were found in urine and their clearance was greater than their eGFR; the renal secretory mechanism was purposed in this study (29). The mechanism of uric acid excretion that used a specific transporter, particularly the OAT family in the kidney, was presented (30). Caffeine, theophylline, and metabolites of caffeine including xanthine and uric acid related compounds had a strong inhibitory effect on the transport activity of OAT (24). Therefore, the inhibitory effect of caffeine and its metabolites on transport activity of OAT was a cause of the decrease or increase of UAC in the intervention and withdrawal periods of CC and affected SUA in our study.

The major strength of our study was the use of a within-subject study design that reduced the individual difference effect between participants. The study had prescribed the quantity in grams of both coffees. Therefore, each participant received a certain dosage of coffee each day. Finally, tablets of CC and DC were prepared in our study. The other ingredients particularly any milk or fructose that have a hypouricaemic effect (2) were not added. Therefore, those confounding factors were controlled.

The limitations of this study should be recognised. The pure substances especially 5-CQA or others in coffee that have been reported as the hypouricaemic effect (27) were not evaluated. The effect of coffee in SUA was evaluated in only healthy male, not female, participants and was not evaluated in gouty patients. Although all participants were directly called to control the normal daily food pattern even the study, the types of food that directly affect SUA in each participant were different. Tim-

ing of the blood test after the last tablet of coffee intake did not be limited. Therefore, this study could not conclude about duration, including acute or long-term effect to SUA. Only the caffeine substance was purposed to be the cause for increasing SUA and sXOA in our study with a significant difference between CC and DC; however, other substances particularly 5-CQA was included in both types of coffee. Nevertheless, the proportion that had the effect on SUA including increasing and decreasing of caffeine and 5-CQA, respectively, was not tested in this study. Finally, although the sample size was calculated in our study, the small sample size especially in the subgroup analysis should be interpreted with caution in its results. These limitations should be considered in further research.

In conclusion, in healthy males, DC had a significant hypouricaemic effect within 3 weeks, particularly in HUS participants. Although CC had no significant increase of SUA overall, a significant increase of SUA was found, especially in non-HUS participants. These effects disappeared when CC and DC were stopped. Dose-independence of both coffees was found in our results. The mechanism of change in SUA for CC was the increase of endogenous uric acid production by increasing sXOA and decreasing UAC. For DC, 5-CQA may be a key substance for the hypouricaemic effect. Caffeine and 5-CQA are hypothesised to be responsible for the increase and decrease of SUA, respectively.

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