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Effect of Tea and Coffee Consumption on Serum Uric Acid Levels by Liquid-Chromatographic and Uricase Methods

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Caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine) are ubiquitous in human plasma due to the almost universal dietary intake of coffee, tea and many soft drinks (Becker et al. 1984). Caffeine in humans can be metabolized to methylated substances such as theophylline that can convert to DMUA(1,3-dimethyluric acid) and TMUA(1,3,7-trimethyluric acid). These two compounds are very similar in structure to uric acid. Therefore, if uric acid levels are assessed using the uricase assay method, it is important to be aware of the potential interference effect of tea and coffee consumption. When a subject consumes caffeine or theophylline prior to uricase assay, quinoneimine is catalyzed by xathine oxidase and forms DMUA and TMUA. The presence of these two compounds inflates uric acid levels and results in false positive hyperuricemia. Buchanan (1945) discovered that using the phosphotungstate method to analyze serum levels in uric acid, interference from caffeine and theophylline uptake causes false positive hyperuricemia. However, when Buchanan analyzed methylpurine metabolization, he concluded that the uricase assay method of analysis was not affected by metabolites of theophylline. Further research is needed to clarify the role (if any) that tea and coffee consumption plays in producing false positive hyperuricemia.

Uric acid is an oxidative product of xanthine that is derived from purine nucleotide. The metabolic enzyme responsible for this conversion, xanthine oxidase, is mainly restricted to the liver and intestines in humans and other primates. Abnormal metabolism of purines leads to deposition of sodium urate crystals in joints and causes an extremely painful inflammation known as gout (Wallace et al. 1997). Patients with serum uric acid concentrations exceeding 7.0 mg/dl are diagnosed with hyperuricemia (Wolfe 1991; Zimmet et al. 1978). This condition is frequently caused by a combination of several factors including: increased uric acid biosynthesis, decreased kidney-mediated uric acid discharge, alcohol consumption, obesity, fever or heat stroke. Since hyperuricemia is considered to be a major cause of gout, it is not surprising that incidence rates of both ailments are currently rising in Taiwan based on community epidemiological surveys. The severity of this trend is alarming and demands immediate attention. (Chou 1987; Soong1993; Chou and Lai 1998).

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Uric acid samples taken from hyperuricemia patients and analyzed in serum using uricase assay method consistently revealed higher uric acid levels compared to samples from gout patients (Wolfe. 1991). Since these hyperuricemia patients did not show any symptoms of gout, this trend prompted the authors to suspect that chemical interference (Chou and Lai. 1998; Fossati et al. 1980) by uric acid derivatives such as 1,3-dimethyluric acid (DMUA) and 1,3,7-trimethyluric acid (TMUA) when using the uricase method may have led to inaccurate results (Leakey. 1990). The objective of this study was to compare uric acid values from both uricase assay and HPLC and to determine whether the difference could be contributed to the presence of TMUA or DMUA.

MATERIALS AND METHODS

All subjects in this study were selected from a hospital in central Taiwan. All patients' verbal consent was obtained prior to the commencement of the study. Subjects were divided into three groups: normal uric acid concentration (<= 7 mg/dl)(n=32); Hyperuricemic without gout (>7 mg/dl)(n=32). Hyperuricemic with gout (>7mg/dl) (n=32). Gout patients were diagnosed by a physician based on three criteria: the simultaneous presence of hyperuricemia, acute or chronic arthritis, and monosodium urate crystal precipitates (Levinson and Becker. 1992). Demographic data was collected using a questionnaire. Subjects were asked how frequently they consumed tea and coffee (often over three times a week), sometimes (once a week), seldom (once a month), none). Because there were not many subjects, for the purposes of analysis, the 'often' and 'sometimes' groups, and the 'seldom' and 'none' groups were combined to form the 'yes' and the 'no' group, respectively. There was no significant difference among the three groups with regard to tea/coffee consumption, age and gender.

Subjects fasted before the blood draw and blood samples were stored before analysis. The serum specimens were divided into two samples for analysis by the two methods (HPLC and uricase assay). The uricase method was performed based on procedures used by Fossati (1980). Quinoneimine was measured by spectrophotometry with a wavelength of 520 nm. The chemical reactions are summarized below:

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{uricase}} \text{allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHBS} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + H_2O$$

One hundred µl serum samples were added to a 1.5-ml mixed solution of 2-propanol and dichloromethane in a ratio of 1:9 (v:v). Extracts were placed in a block heater set below 37 °C and evaporated to dryness under a gentle stream of oxygen-free nitrogen. The dried extracts were reconstituted in 100 ml of mobile phase A (0.01% THF in 10 mM acetate, pH 4.0). After 5 min in a refrigerated centrifuge set at 10,000 rpm, 20 µl of samples were injected into a HPLC with a 100 RP-18 column (250 × 4.5 mm, 5 µm) and analyzed as described above

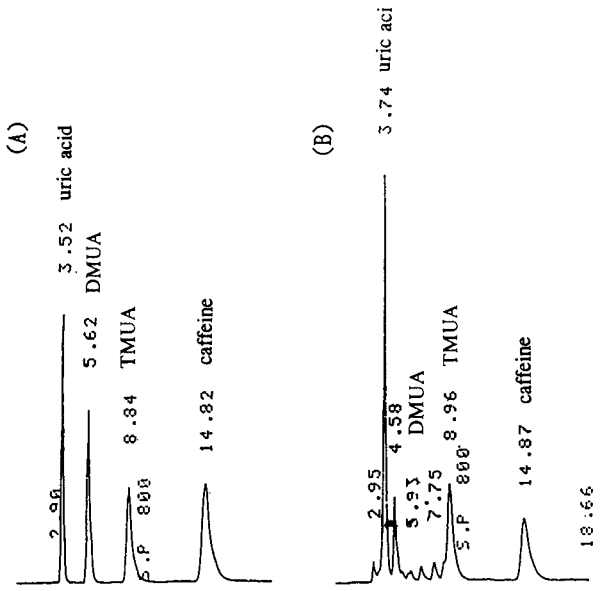

Figure 1. HPLC analysis of uric acids, DMUA, TMUA and Caffeine in standard solution (A) and serum sample from subjects (B)

(Leakey, 1990; Tanaka, 1992). Uric acid, DMUA, TMUA and caffeine were eluted from a linear gradient of 0% mobile phase B (A+B) to 42% mobile phase B (25% acetonitrile, 2% THF in 10 mM acetate, pH 4.0) with a gradient rate of 2.1% B/min. Elution flow rate was 0.8 ml/min. Uric acid (HUA), DMUA, TMUA and caffeine were measured individually by UV-absorbance at 270 nm. Results from standard solutions for the four chemicals (Fig. 1A) were consistent with those from HPLC analysis (Fig. 1B). Several interference peaks near the DMUA peak may have interfered with the results. Recovery rates for uric acid, DMUA, and TMUA were 71.2%, 82.3%, and 90.3%, respectively.

All data were analyzed using SAS for Windows 6.04 (1986) software. Statistical methods were chosen according to the parameters and purpose of the study and included: analysis of variance (ANOVA), post-comparison test with Scheffes method, correlation analysis, and multiple linear regression. The p value was 0.05.

RESULTS AND DISCUSSION

Uric acid results from the two methods differed significantly between the three groups (Table 1). Uric acid levels were highest among hyperuricemia patients and lowest among the normal group. Overall, uric acid levels from the uricase assay method were higher than levels determined using HPLC. Using HPLC, DMUA values were highest among hyperuricemia patients (0.42 mg/dl) and lowest among the normal group (0.18 mg/dl). Although results did not differ significantly, TMUA

Table 1. Comparison of uric acid, DMUA, and TMUA values (means ± SD)

	Normal (N=32)	Hyperuricemia (N=32)	Gout (N=32)	*P* value
UUA*	4.72 ± 1.19[a]	9.02 ± 1.34	8.91 ± 1.04	<0.01
HUA*	2.17 ± 1.22	4.89 ± 2.22	4.58 ± 2.21	<0.01
DMUA	0.18 ± 0.32	0.42 ± 0.44	0.22 ± 0.36	<0.05
TMUA	0.85 ± 1.00	1.01 ± 0.85	1.10 ± 1.01	NS
UUA-HUA	2.55 ± 1.24[a]	4.13 ± 1.59	4.33 ± 1.80	<0.01
DMUA+TMUA	1.03 ± 1.07	1.43 ± 1.05	1.33 ± 0.97	NS

*Values of serum uric acid determined by HPLC and uricase method and expressed as HUA and UUA [a] means± SD DMUA(1,3-dimethyluric acid) TMUA(1,3,7-trimethyluric acid).

using uricase assay and HPLC methods based on the three groups values were highest among gout patients (1.10 mg/dl) and lowest in the normal group (0.85 mg/dl). Results showed that gout patients had the highest UUA - HUA values (4.33 mg/dl) and the normal group had the lowest (2.55 mg/dl). Hyperuricemia patients had the highest DMUA + TMUA values while the normal group had the lowest. Ratios of UUA - HUA to DMUA + TMUA were significantly correlated among the three groups. The correlation coefficient was highest for the normal group ($r=0.75$, $P<0.01$) and lowest for the hyperuricemia patients ($r=0.57$, $P<0.01$). Loenen (1990) showed that theophylline and caffeine derivatives were present in human serum. Uric acid levels tend to be artificially inflated when analyzed using the uricase assay method. When the HPLC method is applied, theophylline and caffeine in serum can be separated and accurate results can be achieved. The current study produced results consistent with Ingebretsen's (1982) study that determined uric acid using reversed-phase liquid chromatography and compared this method to the uricase method. The results of the latter study showed that the HPLC method was precise with no interferences. Also, HPLC was found to be simple and can be used with a fully automatic system.

The results showed that both tea and coffee drinkers had significantly lower levels of uric acid using HPLC method (Table 2). Tea drinkers had higher levels of DMUA (0.38 mg/dl) than non- tea drinkers (0.16 mg/dl). DMUA+TMUA levels were higher among tea drinkers compared to non-tea drinkers, but this was not significant. Coffee drinkers had higher levels of TMUA, UUA-HUA and DMUA+TMUA compared to non-coffee drinkers. Uricase method results were consistently higher (i.e. HUA < UUA) for each tea and coffee group analyzed. Since HPLC can also detect DMUA and TMUA, HPLC is the more informative and accurate method for measuring uric acid levels. The current study investigated the positive relationship between UUA-HUA and

Table 2. Comparison of uric acids levels (means ± SD) UUA, HUA, DMUA and TMUA based on consumption of tea and coffee

	Tea group		Coffee group	
	Yes (N=50)	No (N=46)	Yes (N=26)	No (N=70)
UUA[a]	6.84 ± 2.57	8.32 ± 1.76**	7.89 ± 2.26	7.42 ± 2.35
HUA[a]	3.36 ± 1.96	4.84 ± 1.87**	3.38 ± 1.66	4.33 ± 1.66*
DMUA	0.38 ± 0.33	0.16 ± 0.18**	0.40 ± 0.41	0.23 ± 0.22
TMUA	1.04 ± 0.87	0.97 ± 1.02	1.71 ± 1.11	0.67 ± 0.66**
UUA-HUA	3.48 ± 1.42	3.48 ± 1.93	4.52 ± 2.09	3.09 ± 1.32**
DMUA+TMUA	1.40 ± 1.04	1.12 ± 1.04	2.21 ± 1.77	0.92 ± 0.73**

*$p<0.05$ ** $p<0.01$ [a]Values of serum uric acid determined by HPLC and uricase method and expressed as HUA and UUA.
DMUA(1,3-dimethyluric acid) TMUA(1,3,7-trimethyluric acid).

DMUA+TMUA The authors postulate that UUA-HUA is a constant value based on TMUA+DMUA, so that when one value increases, there is a concomitant decrease in the other. If both DMUA and TMUA are present in the serum, when measured by uricase assay method, a false positive hyperuricemia may result.

Both tea and coffee consumption was negatively correlated to uric acid levels (Table 3) based on multiple linear regression. Hyperuricemia and gout patients had significantly higher uric acid levels compared to the normal group. DMUA and TMUA levels were positively correlated to tea and coffee consumption. There was a positive correlation between UUA-HUA levels and coffee drinkers (compared to non-coffee drinkers) and gout patients (compared to normal subjects). The current study showed that DMUA, TMUA and UUA-HUA were significantly correlated with tea and coffee consumption. However, tea was negatively correlated with UUA and HUA values. Morita (1984) demonstrated that there were elevated levels of serum uric acid in among male asthmatics receiving theophylline compared to male control subjects not taking theophylline. The authors suggest that the phosphotungstate method for uric acid measurement has been demonstrated to be affected by theophylline metabolites, and the ingestion of theophylline has been shown to cause falsely elevated uric acid levels in urine when this test was used for uric acid determination. Thus it may be possible to speculate that theophylline causes hyperuricemia by stimulating the release of adrenal catecholamines. Further studies are needed to elucidate the exact mechanisms of theophylline-induced hyperuricemia,

In conclusion, the uricase assay method consistently measured higher uric acid values than the HPLC method. The consumption of tea, coffee or other caffeinated items produced higher DMUA and TMUA levels when using the uricase assay and showed false positive hyperuricemia. The authors suggest that the HPLC method be used when determining uric acid values in order to ensure more accurate results. If the uricase assay method is applied, any artificially inflated results may be due to the presence of theophylline and caffeine-derived methyl uric acids.

Table 3. Multiple linear regression model to explain variance of uric acids UUA, HUA, DMUA and TMUA

	UUA β (SE)	HUA β (SE)	DMUA β (SE)	TMUA β (SE)	UUA - HUA β (SE)
Group					
Hyperuricemia (normal=1)	3.80(0.35)**	3.25(0.34)**	0.25(0.06)**	0.02(0.20)	0.55(0.39)
Gout (normal=1)	3.86(0.38)**	1.80(0.36)**	0.16(0.06)*	0.37(0.21)	2.07(0.42)**
Gender (male=1)	0.39(0.31)	0.17(0.29)	0.05(0.05)	0.26(0.18)	0.23(0.34)
Tea consumed (yes=1)	0.61(0.31)*	-1.07(0.30)**	0.26(0.05)**	0.17(0.18)	0.46(0.34)
Coffee consumed (yes=1)	0.39(0.32)	-1.33(0.31)**	0.15(0.06)**	1.17(0.19)**	1.73(0.36)**
R^2	0.69	0.61	0.36	0.33	0.35

*p<0.05 ** p<0.01 [a]Values of serum uric acid determined by HPLC and uricase method and expressed as HUA and UUA.
DMUA(1,3-dimethyluric acid) TMUA(1,3,7-trimethyluric acid).

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