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Comparison of the urinary metabolite profile of caffeine in young and elderly males

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1 The urinary metabolite profile of caffeine was compared in a group of seven healthy young men aged 18–29 years and in a group of five healthy elderly men aged 66–71 years. All subjects were given 5 mg/kg doses of caffeine as an aqueous oral solution or an intravenous infusion on two separate occasions in a randomized crossover design. Urine samples were collected for 24 h after dosing and analysed for caffeine and eleven of its metabolites by high-performance liquid chromatography.

2 The effects of age, route of administration, and order of administration by route on the metabolite profile of caffeine were examined. The route of administration and the order of administration by the two routes were found not to influence the urinary metabolite pattern significantly. The urinary metabolite profile did not vary substantially with age except for the observation that significantly greater amounts of 1-methyluric acid, 7-methyluric acid and 1,7-dimethyluric acid were excreted by the elderly subjects.

Keywords caffeine metabolites young elderly

Introduction

Recently there has been renewed interest in elucidating any possible pharmacokinetic differences in the elderly relative to younger populations, with a view toward improving their drug therapy (Crooks *et al.*, 1976; Vestal, 1978). Caffeine would appear to be a particularly useful model compound for such studies. It is found in a variety of common foods and drinks as well as in prescription and non-prescription medication and is thus widely consumed by all segments of the human population, including the elderly. Its metabolism proceeds primarily via successive *N*-demethylations to form di- then mono-methylated xanthines, some of which are then oxidized to form uric acids. These metabolic conversions occur primarily in the liver and involve the cytochrome P-450 mono-oxygenase system, with available data suggesting that caffeine is a particularly good substrate for cytochrome P-448 (Wietholtz *et al.*, 1981; Bonati *et al.*, 1980, 1984). The slow maturation of these enzyme systems affects the metabolite pattern of caffeine in the early period of life in both humans (Aldridge *et al.*, 1979; Aranda *et al.*, 1981) and animals (Aldridge & Neims, 1980; Warszawski *et al.*, 1981, 1982), and it is of interest to ask whether advanced age might cause a similar alteration of any of these metabolic pathways. In this study we have investigated whether age and/or the route of administration can affect the urinary metabolite profile of caffeine in young and elderly men.

Methods

Subjects

Two groups of human male subjects were

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studied. The young group consisted of seven Caucasians, aged 18–29 years (22.2 ± 4.0; mean ± s.d.), ranging in weight from 64 to 106 kg (78.9 ± 13.4).The elderly group consisted of five Caucasians, aged 66–71 years (69.6 ± 2.3), who ranged in weight from 58.5 to 88 kg (70.7 ± 11.4). All subjects were volunteers recruited from the local (Edinburgh) population who led active, normal lives. The subjects were assessed as being 'healthy' on the basis of a history, physical examination, and electrocardiogram. In addition, the subjects were within the normal limits for the following laboratory tests: plasma urea, electrolytes, creatinine, bilirubin, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, and creatinine clearance and complete blood count. Written informed consent was obtained from all subjects.

None of the subjects was taking any prescription or non-prescription medication at the time of the study. One of the young subjects smoked about 10 cigarettes daily and one of the elderly men smoked 6–8 cigarettes per week, while two of the elderly were light pipe smokers (2 oz per week). The remainder of the subjects had not smoked for 5 years or more. One of the young and one of the elderly subjects were non-drinkers while the remainder admitted modest consumption of ethanol. Their use of caffeinated beverages consisted mainly of tea and coffee (with tea predominating) and ranged from < 3 cups per day in one young subject to as much as 5–6 cups per day in another young subject. The remaining subjects consumed an average of 3 to 5 cups per day.

Study protocol

Each subject received 5 mg/kg doses of caffeine, both orally and intravenously, on separate occasions about 1 week apart, in a randomized crossover fashion. The subjects were asked to abstain from caffeine-containing foods and beverages, ethanol and smoking for 72 h before and during each oral and intravenous study. They were fasted from at least 10 h before until 2 h after the oral dose, but were allowed a normal breakfast (except for the absence of caffeine) on the morning of their intravenous dose. Each subject received his caffeine dose at approximately 09.00 h. The appropriate quantity of caffeine (caffeine and sodium benzoate injection, U.S.P, Eli Lilly and Co., Indianapolis, IN 46206) was diluted with sufficient isotonic saline (sodium chloride solution, B.P., Travenol Laboratories, Ltd, Thetford, Norfolk, England) to produce 50 ml of infusion fluid, 30 ml of which was administered into an arm vein via an indwelling heparinized cannula at a constant rate of 1 ml/min using a previously calibrated infusion pump (Braun Unita 1, Braun, Melsungen AG, West Germany). The oral dose consisted of caffeine (Caffeine, Baker grade, J. T. Baker Chemical Co., Phillipsburg, NJ 08865) dissolved in 200 ml of distilled water which was ingested rapidly, following which the container and the subject's mouth were rinsed with two 50 ml portions of distilled water to ensure that the entire dose was administered. This procedure took 1 min or less to complete and zero time was recorded as the mid-point of the dosing interval.

A 'blank' urine sample was voided immediately before each dose of caffeine, and each subject's total urine was collected into tightly-sealed 3 l glass containers over the 24 h period after dosing. The volumes of the blank and 24 h urine samples were recorded and aliquots were stored at –20°C for subsequent assay.

Analytical procedures

The intravenous and oral solutions administered to each subject were assayed using the procedure of Blanchard *et al.* (1981) in order to establish the actual doses used. The concentration of caffeine and eleven of its reported metabolites in both blank and 24 h urines were determined using the h.p.l.c. procedure of Tang-Liu & Riegelman (1982). The lower limit of quantitation with this procedure was 1 μg/ml for each compound assayed.

Sources of the xanthine and uric acid derivatives used in chromatographic analysis were as follows: xanthine (ICN Pharmaceuticals, Irvine, California); 1-methylxanthine (1-MX), 1-methyluric acid (1-MU), 3-methylxanthine (3-MX), 3-methyluric acid (3-MU), 7-methylxanthine (7-MX), 7-methyluric acid (7-MU), 1,3-dimethyluric acid (1,3-MU), 3,7-dimethyluric acid (3,7-MU), 1,7-dimethylxanthine (1,7-MX), 1,7-dimethyluric acid (1,7-MU), and 1,3,7-trimethyluric acid (1,3,7-MU) (Adams Chemical, Round Lake, Illinois); theophylline (1,3-dimethylxanthine, 1,3-MX), and β-hydroxyethyltheophylline (Sigma Chemical, St Louis, Missouri); theobromine (3,7-dimethylxanthine, 3,7-MX) (Mallinckrodt Chemical Works, St. Louis, Missouri), caffeine (1,3,7-trimethylxanthine, 1,3,7-MX), and uric acid (Eastman Organic Chemical, Rochester, New York).

Treatment of data

Compliance of the subjects with the caffeine-free dietary regimen was verified by assaying the 'blank' urine samples. If more than a trace

of any metabolite was found in a subject's blank urine sample, the data from that dose were excluded from the analysis. This criterion plus the breakage of some sample tubes resulted in our using data from five elderly men and seven young men instead of our original plan to use eight elderly and ten young subjects.

The urinary metabolite concentrations were determined from calibration curves prepared simultaneously which thus corrected for the varying extraction efficiencies of each metabolite. The concentration of each metabolite was then expressed as a molar percentage of the caffeine dose. In the case of intravenous administration, the caffeine dose administered was used in these calculations while for the oral doses a correction for the bioavailability of that dose in a given subject was made using procedures described earlier (Blanchard & Sawers, 1983).

The urinary concentrations of caffeine and the eleven metabolites measured as well as the total % recovery, were compared using a repeated measures design analysis of variance (ANOVA) procedure to see if significant differences in any of these values existed with respect to the subject's age, the route of administration, and the order of administration of caffeine by the two routes. In all statistical comparisons tested $P < 0.05$ was taken as the minimum level of significance.

Results

The urinary excretion pattern of caffeine and eleven of its metabolites following oral and intravenous doses to young and elderly men is shown in Table 1. The values found in the smokers were typical of those found in the other subjects.

These data illustrate that the metabolic profile of caffeine in both subject groups was not affected significantly by the route of administration. The one exception to this was the observation that significantly ($P = 0.042$) greater amounts of the metabolite 3,7-MX (theobromine) were excreted following the oral doses within a given age group. In addition, the analysis of variance indicated that the order in which the subjects received their oral and intravenous doses did not have a significant effect on any of the parameters tested and that none of the interaction terms involving the order of administration term was affected significantly. A significant age by route interaction ($P = 0.041$) in the amounts of 1,3,7-MU excreted was observed. Only three of the eleven caffeine metabolites measured (i.e., 1-

Table 1 Urinary excretion pattern of caffeine and its metabolites in young and elderly males

	Elderly (n = 5)		*Young* (n = 7)		*Probability*[b]		
Compound	*Oral*	*Intravenous*	*Oral*	*Intravenous*	*Age*	*Route*	*Age-by-route*
1,3-MX	0.48 ± 0.31[a]	0.41 ± 0.26	0.77 ± 0.69	0.21 ± 0.32			
3,7-MX	1.22 ± 0.79	0.76 ± 0.86	2.04 ± 1.96	1.42 ± 1.55		$P = 0.042$	
1,7-MX	3.37 ± 1.47	3.22 ± 1.63	3.49 ± 1.87	3.25 ± 0.73			
1-MX	8.90 ± 5.43	8.44 ± 5.20	9.48 ± 3.70	8.04 ± 1.52			
3-MX	0.94 ± 0.51	1.06 ± 0.68	1.43 ± 0.91	1.27 ± 1.62			
7-MX	2.32 ± 1.18	2.38 ± 2.05	2.40 ± 1.45	2.98 ± 3.15			
1-MU	38.12 ± 14.23	29.16 ± 9.76	22.05 ± 4.69	23.29 ± 6.43	$P = 0.028$		
7-MU	9.30 ± 3.12	9.55 ± 3.24	6.09 ± 1.96	6.85 ± 1.86	$P = 0.028$		
1,3-MU	3.37 ± 0.89	3.07 ± 1.14	2.73 ± 0.86	2.71 ± 0.87			
1,7-MU	12.56 ± 1.99	11.85 ± 2.80	7.81 ± 3.36	9.18 ± 1.74	$P = 0.049$		
1,3,7-MU	2.57 ± 0.83	2.77 ± 1.11	2.93 ± 1.05	1.98 ± 1.00			$P = 0.041$
1,3,7-MX	1.93 ± 0.57	1.36 ± 0.91	2.35 ± 2.05	1.37 ± 0.92			
% Recovery	81.79 ± 25.93	74.03 ± 22.26	63.58 ± 13.70	62.54 ± 10.97			

[a] Values represent the mean ± s.d. of each compound expressed as a molar percentage of the caffeine dose
[b] Probabilities not listed are not significant ($P > 0.05$)

MU, 7-MU, and 1,7-MU) differed significanty in the percentage of the dose excreted between the two age groups. All of the other metabolite levels measured did not differ significantly with age.

Discussion

Our observation that the urinary metabolite profile of caffeine in both young and elderly men is not affected by the route of administration, nor by the order in which the subjects received their oral and intravenous doses of caffeine indicates that the previous suggestion (Tang-Liu *et al.*, 1982) that methylxanthines could be degraded when exposed to the bacterial flora of the gastrointestinal tract is improbable. Our results are consistent with the work of Monks *et al.* (1979) who noted that the pattern of urinary metabolites for the related methylxanthine derivative, theophylline, was nearly identical after oral and intravenous doses. This finding is also consistent with the observation that caffeine is absorbed rapidly following oral administration (Blanchard & Sawers, 1983) which would not allow much time for contact of the drug with the gastrointestinal contents. Furthermore, the present study indicates that there was relatively little intra-individual variation in the metabolism of caffeine on the two occasions of dosing. Callahan *et al.* (1982) made a similar observation concerning two oral doses given 14 days apart to males, aged 21–39 years. The one apparent exception to above generalizations was the excretion of significantly greater amounts of theobromine (3,7-MX) following the oral doses. Although the theobromine pathway is reportedly of much lesser importance than the paraxanthine pathway (Arnaud *et al.*, 1980; Arnaud & Welsch, 1980; Brazier *et al.*, 1980; Cornish & Christman, 1957) and unlikely to have a great impact on the overall metabolism of caffeine, it would nevertheless be interesting to determine the significance of this finding as well as its cause.

The data in Table 1 illustrate how significantly greater amounts of 1-MU, 7-MU and 1,7-MU were excreted in the elderly subjects. A comparison of our findings in young subjects following oral dosing with those of other recent studies involving this age group and route of administration indicates the good overall agreement of our data with these previous studies (Callahan *et al.*, 1982; Bonati *et al.*, 1982; Tang-Liu *et al.*, 1983). While the percentage of 1-MU found varied considerably

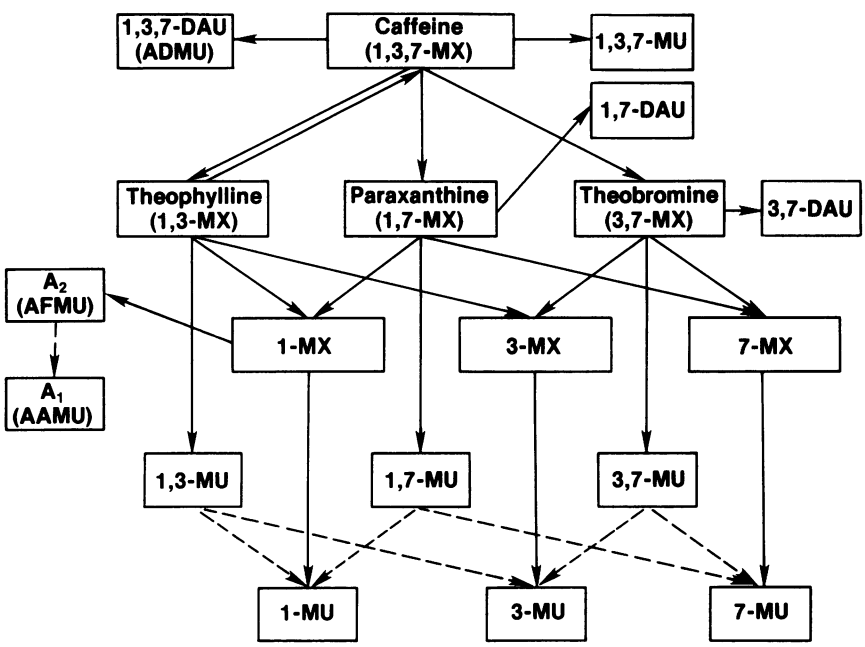

Figure 1 Metabolic pathways for caffeine in man based upon urinary excretion data. Dashed lines indicate unestablished pathways. Recent reports (Birkett *et al.*, 1983; Arnaud, 1984) indicate that the dimethyluric acids are not demethylated to produce monomethylurates. 1,3,7-DAU(ADMU) = 6-amino-5-[*N*-formylmethylamino]-1,3-dimethyluracil; 3,7-DAU = 6-amino-5-[*N*-formylmethylamino]-1-methyluracil; 1,7-DAU = 6-amino-5-[*N*-formylmethylamino]-3-methyluracil.

between studies, it seems clear that it is the major urinary metabolite of caffeine in adult men.

A diagram illustrating the proposed metabolic pathways for caffeine in man which have been reported to date is shown in Figure 1. The amounts of each metabolite are not shown since the percentages of metabolites formed by some of these pathways have not yet been established unequivocally. This figure is intended primarily to demonstrate the complexity of caffeine's metabolism. The methylation of theophylline to caffeine has been reported to occur in premature infants (Bada *et al.*, 1979; Boutroy *et al.*, 1979) but was believed to be absent or to occur to a much lesser extent in children and adults until Tang-Liu & Riegelman (1981) reported its presence in adults. Tserng *et al.* (1983) postulated that theophylline methylation occurs to the same degree in adults and premature infants and that the absence, or presence of only negligible amounts of caffeine following ingestion of theophylline is due to the fact that caffeine is immediately converted to its metabolites by the mature cytochrome P 450 mono-oxygenase system present in adults.

The presence of substantial amounts of urinary 7-MU was a significant finding in this study since the assay used by previous investigators apparently could not measure it (Arnaud & Welsch, 1980; Bonati *et al.*, 1982; Callahan *et al.*, 1982). To our knowledge the only previous study to report substantial quantities of this metabolite in the urine was the recent work of Tang-Liu *et al.* (1983). The findings of that study and the present one differ with the report of Callahan *et al.* (1983) who found only about 1% of 7-MU in the urine following a 5 mg/kg caffeine dose to young (aged 18–30 years) male and female subjects. No reasons for this difference are apparent, but on the basis of our observations it is recommended that this metabolite be monitored in future studies, since suitable assay methodology is available (Tang-Liu & Riegelman, 1982). The results also indicated that significantly ($P = 0.028$) greater amounts of this metabolite are present in the urine of elderly subjects.

Since we did not attempt to collect and analyse fractions of the h.p.l.c. eluate corresponding to the peak for each metabolite it was possible that other metabolites of caffeine may have co-eluted with 7-MU or some of the other metabolites reported here and falsely elevated their levels. Unfortunately, at the time this study was performed we did not attempt to measure AFMU and AAMU since these compounds were not readily available. In addition, Callahan *et al.* (1982) had not yet established that caffeine was the primary source of AAMU. Furthermore, Tang *et al.* (1983) had not yet shown that AAMU was an artifact of the isolation procedure and that AFMU was a major metabolite of caffeine in man. However, subsequent to the completion of our study we were able to obtain authentic samples of AAMU (courtesy of Dr Wolfgang Pfleiderer, University of Konstanz, Konstanz, W. Germany) and AFMU (courtesy of D. M. Grant, Department of Pharmacology, University of Toronto, Canada). Upon chromatographing these compounds on our h.p.l.c. system we found that the retention times were 3.11 and 5.78 min for AAMU and AFMU, respectively. Thus these two compounds do not elute in the region of any of the metabolites reported here. These observations are consistent with those of Tang *et al.* (1983) who found that these two metabolites had the same relative retention order on their h.p.l.c. system, which utilized conditions similar to ours. In addition, this group (Grant *et al.*, 1983) also observed that AFMU eluted prior to any of the other caffeine metabolites of interest.

Our observation that about 8% of an oral dose was present as 1,7-MU in the urine of the young subjects is consistent with the findings of 5–7.5% in other studies involving young adults (Bonati *et al.*, 1982; Callahan *et al.*, 1982; Tang-Liu *et al.*, 1983). Our data indicate that significantly ($P = 0.049$) greater amounts of this metabolite are excreted by the elderly subjects. Figure 1 reveals how these two metabolites, i.e., 7-MU and 1,7-MU are believed to be derived primarily from the corresponding xanthines, i.e., 7-MX and 1,7-MX, respectively via a process of oxidation at C_8. In the case of theophylline, this process is reported to be mediated by a form of the cytochrome P 450 mono-oxygenase system different from the form responsible for *N*-demethylation processes (Grygiel & Birkett, 1980, 1981). This is supported by the recent observation that the demethylation pathways for caffeine appear to be controlled primarily by forms of the microsomal cytochrome P-450 system preferentially induced by polycyclic aromatic hydrocarbons, e.g., cytochrome P-448, in man (Wietholtz *et al.*, 1981), animals (Aldridge & Neims, 1979) and microsomal preparations (Bonati *et al.*, 1980, 1984). It is known that the prior intake of methylxanthines can alter their own disposition (Drouillard *et al.*, 1978; Monks *et al.*, 1979). However, it seems unlikely that differing intakes of the methylxanthines were responsible for the increased excretion of the uric acid derivatives in the elderly since other workers (Grant *et al.*, 1983; Mitoma *et al.*, 1969) have

indicated that any effects on metabolism due to normal intersubject differences in habitual caffeine consumption would likely be eliminated by the 3 day abstention period used in this study. The finding of zero to trace levels of these metabolites in the blank urine samples of all subjects reported is further proof that differences in methylxanthine intake were not an important factor.

Grant *et al.* (1983) recently reported that the combined excretion of 1-MX + 1-MU was greater in slow acetylators than in fast acetylators. This raises the possibility that the greater excretion of 1-MU by our elderly subjects may have been due to the presence of more slow acetylators in that group. While this possibility cannot be ruled out entirely since we did not determine the acetylator phenotype of our subjects, we think it is unlikely for several reasons. First, Grant *et al.* (1983) found no statistically significant differences between acetylator phenotypes with respect to age. Furthermore, it seems highly improbable that sufficient numbers of our elderly and young subjects were slow and rapid acetylators, respectively to have caused the observed difference in 1-MU excretion between the two age groups since all of the subjects were selected randomly.

It might also be asked whether or not the observed differences in the excretion of the three urates might be due to differences in renal function rather than liver metabolic capacity since renal function is known to decline with ageing. Although the glomerular filtration rate (GFR) of our elderly subjects, as assessed by creatinine clearance, was significantly reduced relative to that of our young subjects, we do not feel that differences in renal function are responsible for the observed differences in the excretion of the three urates for the following reasons. Tang-Liu *et al.* (1982) reported that the renal clearances of 3-MX, 1-MU and 1,3-MU all exceeded the GFR following theophylline administration, indicating that they are actively secreted. They also showed that the renal clearance of each metabolite was much greater than that of theophylline and concluded that 'the urinary excretion rates of the metabolites are rate limited by, as well as a measure of, their formation rates.' They also demonstrated that the maximal formation clearance of these metabolites was much smaller than their renal clearance, which again supports the contention that their elimination is much more rapid than their formation. Thus, the urates formed from caffeine can reasonably be expected to also be eliminated via active renal secretion processes which exceed the GFR. Thus any age-related differences in active secretion would likely have no effect on the renal elimination of these metabolites since the GFR alone would be much larger than the formation clearance and therefore their elimination would still be formation rate-limited.

We also observed a significant ($P = 0.041$) age by route interaction in the percentage of 1,3,7-MU excreted. Essentially this means that differences in the amount of this metabolite excreted due to the route of administration were different in the two age groups, i.e., there is a significant difference in 1,3,7-MU excretion due to route of administration in the young but not in the elderly subjects. This was the result of the low percentage excreted by the young subjects following their intravenous dose. There is no apparent reason why this occurred. In any case, the percentages of 1,3,7-MU found in the urine in this study are consistent with other reported studies (Callahan *et al.*, 1982; Bonati *et al.*, 1982; Tang-Liu *et al.*, 1983) and with the value of 2% reported by Arnaud *et al.* (1980), and indicate that this is a relatively minor urinary metabolite of caffeine in man.

The overall urinary recoveries shown in Table 1 compare quite favourably with previous reports (Callahan *et al.*, 1982; Bonati *et al.*, 1982; Tang-Liu *et al.*, 1983), which in some instances utilized longer collection periods. The data of Callahan *et al.* (1982) illustrate how the vast majority of caffeine's metabolites are recovered within the first 24 h after dosing. Of the human metabolites of caffeine verified at the time our study was performed the only two which we could not detect in our subject's urines, even in trace amounts, were 3-MU and 3,7-MU. These two metabolites have not been quantitated in most previous studies as well, possibly because they represent very minor fractions of the total quantity of metabolites excreted. This is supported by the observation of Callahan *et al.* (1982) that 'little, if any, 3,7-dimethyluric acid is produced in humans' and their subsequent measurement of only about 0.5% of a dose in the form of 3-MU in young male and female subjects (Callahan *et al.*, 1983). The faecal excretion of caffeine metabolites has for some time now been considered to be an unimportant pathway (Burg & Stein, 1972). This is supported by Callahan *et al.* (1982) who reported that the faeces contained radioactivity equivalent to only 2–5% of an administered dose of 2-^{14}C-labelled caffeine.

Thus, we have accounted for all of the major reported metabolites of caffeine in man except for A_1 (AAMU; 5-acetylamino-6-amino-3-methyluracil) and A_2 (AFMU; 5-acetylamino-6-formylamino-3-methyluracil). While A_2 can

be converted irreversibly to A_1 by methanol or mild basic conditions *in vitro*, the reverse process is unlikely (Tang *et al.*, 1983). Arnaud & Welsch (1981) indicate that 1-MX is a precursor of A_1 and that at least one intermediate (such as A_2) is present in the pathway. However, others (Grant *et al.*, 1983; Tang *et al.*, 1983) have indicated that A_2 is the primary uracilic metabolite formed upon caffeine administration and that the presence of A_1 in the urine may arise from non-caffeine sources and/or the method of extraction used to isolate it from the urine sample. Regardless of their actual formation mechanism, A_1 and A_2 combined comprise, on the average, about 18% of a dose of caffeine excreted in the urine (Callahan *et al.*, 1982). Thus, if these metabolites plus the 2–5% of faecal metabolites are considered, it can be seen from Table 1 that our urinary recovery figures would approach complete recovery, indicating that the percentages of the metabolites reported here are accurate.

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