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Urinary metabolites of caffeine in pregnant women

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In a study of caffeine and its metabolites in pregnant and non-pregnant women, on average 56.8% of the administered caffeine dose was recovered in the urine in both groups. However, compared to the controls, the pregnant subjects produced smaller amounts of 1-methylxanthine and 1-methyluric acid, whereas the excretion of most of the other metabolites, particularly 3,7-dimethylxanthine and 3-methylxanthine tended to be greater. It is suggested that hormonal influences on the hepatic caffeine metabolising enzymes might be implicated.

Keywords caffeine pregnancy metabolism

Introduction

Studies on caffeine in pregnancy have consistently shown a substantial and progressive decrease in its clearance and increase in elimination half-life values during the second and third trimesters (Aldridge *et al.*, 1981; Knutti *et al.*, 1982). As similar findings were also reported in women taking oral contraceptives (Patwardhan *et al.*, 1980) and, furthermore, the volume of distribution and protein binding of caffeine were found unchanged in pregnancy (Knutti *et al.*, 1982), the reason for this impaired clearance of caffeine is generally thought to be of hormonal origin. In women taking oral contraceptives, prolonged plasma half-life of caffeine was associated with reduced plasma concentration of 1,7-dimethylxanthine. The composition of the urinary metabolites in these subjects was not, however, significantly different from that of a normal male or ovulating female group.

In the present study we investigated the metabolism of caffeine in pregnancy, by comparing urinary metabolite profiles of a group of pregnant women, who received a controlled dietary intake of caffeine, with those of a group of non-pregnant healthy female volunteers.

Methods

A group of 15 pregnant women of between 34–36 weeks gestation and a control group consisting of nine female volunteers not receiving oral contraceptives were enrolled after careful explanation of the study. Each subject was instructed to consume a series of pre-weighed amounts of coffee, of known caffeine content, equivalent to their normal daily consumption of caffeinated beverages. They were requested to refrain from consuming any other form of caffeinated beverage, chocolate and caffeine containing analgesics for the duration of the study. Using this protocol the 24 h caffeine intake for the pregnancy group and the non-pregnant control group ranged from between 123–369 mg and 300–750 mg, respectively. Following a 24 h period of controlled caffeine intake, participants were requested to collect urine samples for a 24 h period whilst maintaining the same caffeine intake. The urine volumes were recorded and aliquots were stored at −20° C until analysis.

Methylxanthine metabolite concentrations were measured using reverse phase high performance liquid chromatography (h.p.l.c.) as described previously (Scott *et al.*, 1986). The overall sensitivity of the procedure was 0.6 mg l^{-1} with values ranging from 0.3 mg l^{-1} for caffeine to 1.4 mg l^{-1} for theophylline. Respective mean overall coefficient of variation values for within batch and between batch precision were 8.12% and 8.04%.

Results

The urinary recoveries of caffeine metabolites obtained in the two groups of volunteers are shown in Table 1. The total 24 h urine volumes were similar for both groups. Despite a variation in the 24 h caffeine intake, the % recovery of the administered dose in 48 h for the pregnancy group and the non-pregnant control group was similar, the mean ± s.d. values being 56.8 (± 8.9) and 56.8 (± 8.6), respectively.

Compared with the non-pregnant subjects, the pregnancy group showed a marked decrease (mean ± s.d.) in the excretion of 1-methylxanthine (11.4% ± 1.9 to 7.3% ± 3.4) and 1-methyluric acid (19.5% ± 5.3 to 9.4% ± 3.7) with a general increase (mean ± s.d.) in the recoveries of the remaining metabolites, in particular those of 3,7-dimethylxanthine (1.4% ± 0.6 to 4.3% ± 3.4) and 3-methylxanthine (2.6% ± 0.7 to 5.6% ± 3.2).

Discussion

The metabolite recoveries for the non-pregnant control group in this study are similar to those found previously following a single oral dose of caffeine (Callahan *et al.*, 1983). The majority of administered caffeine undergoes *N*-demethylation at the 7 and 3 positions producing 1-methylxanthine and its further metabolite 1-methyluric acid. The other quantitatively important metabolites are 7-methylxanthine, 1,7-dimethylxanthine and 1,7-dimethyluric acid (Figure 1).

The significant decrease in the production of 1-methyl metabolites, in the pregnancy group, with relatively normal values of 1,7-dimethyl metabolites, suggests that during pregnancy the further metabolism of 1,7-dimethylxanthine may be impaired. This could be the result of an impairment in 7-*N* demethylation with respect to 1,7-dimethylxanthine as a substrate. Alternatively the regulation may be located at the level of 3-*N* demethylation of caffeine. It is of significance that the clearance of theophylline (1,3-dimethylxanthine), which involves 3-*N* demethylation, is not altered during pregnancy (Sutton *et al.*, 1978). The increased recoveries of metabolites possessing a 3-methyl group suggests that, during pregnancy, the route of caffeine metabolism is altered in favour of an initial 1-*N* demethylation of caffeine to theobromine followed by 7-*N* demethylation to 3-methylxanthine. To a lesser extent, the initial 7-*N* demethylation of caffeine to form theophylline may also be increased during pregnancy, since the recoveries of 1,3-dimethyluric acid and theophylline are both significantly increased.

In a previous study where urinary metabolites of caffeine from normal ovulating females were compared with those obtained from women receiving oral contraceptives (Callahan *et al.*, 1983), it was concluded that the patterns of excretion were not dissimilar. But with regard to the individual metabolites, oral contraceptives appeared to diminish the excretion of 1-methylxanthine, 1-methyluric acid and to increase 1,7-dimethyluric acid as seen during pregnancy in the present study. It would be of interest to measure the acetylated uracil derivatives, such as 5-acetylamino-6-amino-3-methyluracil; which probably represent a large part of the unaccounted metabolites in our two groups of subjects.

Although pregnancy in animals has been associated with a progressive decrease in hepatocellular cytochrome P-450 mediated oxidative drug metabolism, in man the effect appears to

Table 1 Urinary metabolites (mean ± s.d.) of caffeine in healthy subjects receiving a controlled 24 h dietary caffeine intake

Metabolite	*Recovery (% administered dose)* *Non-pregnant*	*Pregnant*	*Student's* t-*test* (P)
7-methyluric acid	1.4 ± 0.5	2.0 ± 0.6	< 0.05
7-methylxanthine	4.0 ± 0.3	5.0 ± 2.6	< 0.25
1-methyluric acid	19.5 ± 5.3	9.4 ± 3.7	< 0.001
3-methylxanthine	2.6 ± 0.7	5.6 ± 3.2	< 0.05
1-methylxanthine	11.4 ± 2.1	7.3 ± 3.4	< 0.02
1,3-dimethyluric acid	1.6 ± 0.5	2.6 ± 0.9	< 0.02
3,7-dimethylxanthine	1.4 ± 0.6	4.3 ± 3.4	< 0.05
1,7-dimethyluric acid	7.2 ± 2.1	9.3 ± 2.9	< 0.1
1,7-dimethylxanthine	4.7 ± 0.9	5.8 ± 1.1	< 0.05
1,3-dimethylxanthine	0.8 ± 0.4	1.6 ± 0.5	< 0.001
1,3,7-trimethyluric acid	0.7 ± 0.2	1.4 ± 0.6	< 0.01
1,3,7-trimethylxanthine	2.0 ± 1.1	3.3 ± 1.4	< 0.05

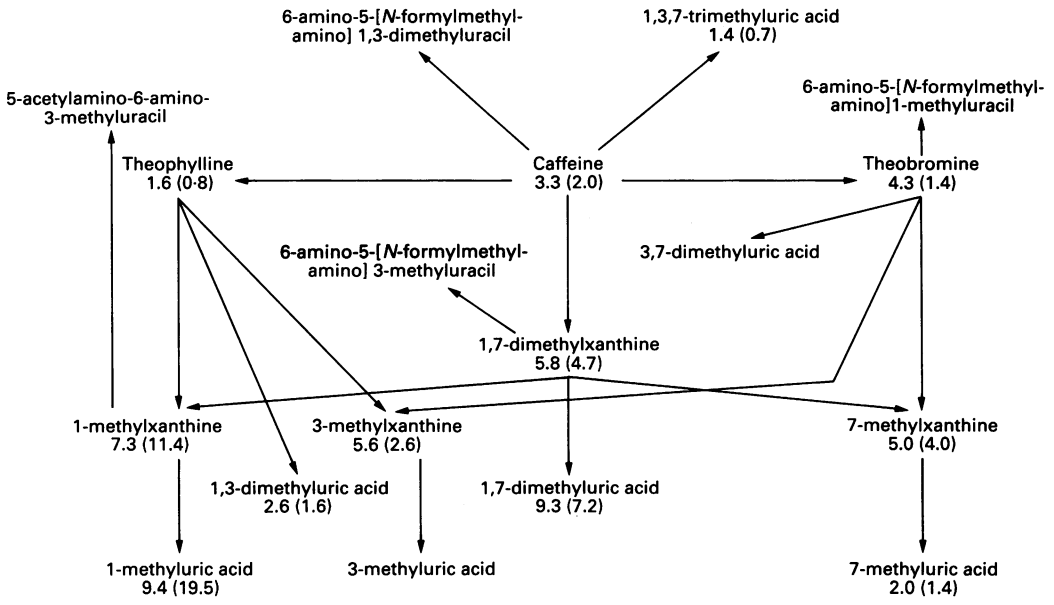

Figure 1 Quantitative metabolic pathways of caffeine. Numbers refer to 24 h urinary excretion as a % of dietary intake of caffeine. Values obtained from pregnant subjects are followed by those (in brackets) from non-pregnant subjects.

depend on the particular drug in question (Rudofsky *et al.*, 1966; Mygind *et al.*, 1976). The steroid hormones of pregnancy, progesterone, oestrogens and their metabolites, have been shown to induce, repress, or directly inhibit selective mono-oxygenase reactions (Feuer *et al.*, 1975; MacKinnan *et al.*, 1977; Field *et al.*, 1979). These modulations in hepatic metabolism in pregnancy have been suggested to be a consequence of changes in microsomal membrane phospholipids and/or the cytochrome P-450 spin state equilibrium (Symons *et al.*, 1982). Thus, for example, the apparent impairment of 7-*N* demethylation of 1,7-dimethylxanthine but not of 3,7-dimethylxanthine may reflect heterogeneity of the response of hepatic cytochrome P-450 haemoproteins to pregnancy hormones. Further understanding of such changes in enzyme characteristics can only come from metabolic clearance studies with selected intermediates of caffeine metabolism because, as indices of metabolic activity, urinary metabolites have considerable limitation.

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