

to be
can be
ounds in
due to
in the

E.,
, van

CYTOCHROME P450 1A2 ACTIVITY IN MAN MEASURED BY CAFFEINE METABOLISM: EFFECT OF SMOKING, BROCCOLI AND EXERCISE

K. Vistisen, S. Loft and H.E. Poulsen

Department of Pharmacology, University of Copenhagen
Juliane Mariesvej 20, DK-2100 Copenhagen, Denmark

Caffeine is sequentially metabolized by cytochrome P4501A2, N-acetyl transferase (NAT) and/or xanthine oxidase (XO). After ingestion of caffeine, equivalent to the content of a cup of coffee, the activity of these three enzymes can be estimated from the ratios of the formed metabolites excreted into the urine (Grant, D.M. et al., 1983; Grant, D.M. et al., 1986; Campbell, M.E. et al., 1987; Figure 1). Cytochrome P4501A2 and NAT are considered the most important enzymes in the metabolic activation of foreign compound, such as arylamines, into carcinogens (Butler, M.A. et al., 1989; Hein, D.W., 1988), whereas XO may be important in tissue damage after ischaemia and in relation to infections (Simpson, P.J. et al., 1987; Oda, T. et al., 1989).

In the present study we investigated factors influencing these enzyme activities measured by the metabolite ratios of dietary caffeine.

MATERIALS AND METHODS

In one, cross-sectional, experiment spot urine samples were collected from 335 healthy volunteers who gave informations regarding age, height, weight and the consumption of tobacco, alcohol, coffee, tea, coca cola, protein and cruciferous vegetables during the preceding two weeks. 171 of the subjects were women, twelve of whom were pregnant and 28 used oral contraceptives.

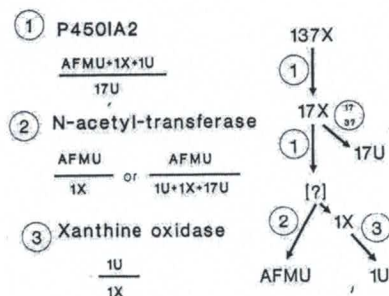


Figure 1. Metabolic ratios of caffeine

In a second, longitudinal, experiment spot urine samples were collected from 23 healthy men before and after 30 days with vigorous exercise, 8 hr per day.

In a third, longitudinal, experiment spot urine samples were collected from 9 healthy subjects after two 10 day periods with a diet supplemented with 500 g green beans or 500 g broccoli in random order.

In all three experiments sampling of urine was preceded by ingestion of at least one cup of coffee, or equivalent, within 2-6 hr. The samples were acidified with HCl to pH 3.5 and stored at 20°C for subsequent HPLC analysis (Campbell, M.E. et al., 1987).

RESULTS

In 331 subjects the AFMU/IX ratio measuring NAT activity showed a typically bimodal distribution with 47% fast acetylators and 53% slow acetylators divided by an antimode of 0.5 (Figure 2). This is in agreement with previous reports on acetylator frequency in Denmark by the use of conventional probes (Hein, D.W., 1988).

The ratio reflecting P450IA2 activity (IA2) was normally distributed (Figure 2). In male and female subjects smoking 10 cigarettes/day or more the IA2 ratio was 66% and 70% higher than in the corresponding non-smoking groups, demonstrating the expected induction of P450IA by tobacco, $p < 0.05$, but minimal sex-related differences (Campbell, M.E. et al., 1987; Figure 3). In 12 non-smoking pregnant women and in 28 smoking and non-smoking women using oral contraceptives the average IA2-ratio was reduced by 29% and 20% compared to the appropriate control groups ($p < 0.05$; Figure 3), respectively, demonstrating the expected inhibition of P450IA2 (Campbell, M.E. et al., 1987; Knutti, R. et al., 1981).

The ratio reflecting XO-activity was normally distributed (Figure 2). Amalgamating the male and female groups, but excluding pregnant women and oral contraceptives user, the XO-ratio was 1.04 ± 0.53 in the 191 non-smoking subjects compared to 1.26 ± 0.61 ($p < 0.05$) and 1.29 ± 0.61 ($p < 0.05$) in the 48 and 56 subjects smoking 1-9 and 10 or more cigarettes/day, respectively. This suggests that even light smoking increases XO activity.

Thirty days of vigorous exercise increased the IA2-ratio by 58%, i.e. from 5.2 ± 2.0 to 8.2 ± 2.2 ($p < 0.05$, $n=23$), the XO-ratio increased from 0.73 ± 0.3 to 1.53 ± 0.64 ($p < 0.05$, $n=23$), whereas the NAT-ratio was unchanged. An inducing effect of physical activity on cytochrome P450 activity has previously been demonstrated with antipyrine and aminopyrine as model compounds (Boel, J. et al., 1984).

After a bean and broccoli supplemented diet the IA2 ratio was 3.7 ± 1.1 and 4.4 ± 1.6 ($p < 0.05$, $n=9$), corresponding to a 19% induction of P450IA2 activity by broccoli. This is in accordance with the expected effect of cruciferous vegetables as previously demonstrated with phenacetin and antipyrine as probes (Pantuck, E.J. et al., 1979). The NAT- and XO-ratios were not significantly changed by the diets.

CONCLUSION

The ratios of metabolites from dietary caffeine in spot urine offer simple estimates of P450IA2, NAT and XO activity. In the present study the reliability of these indices was demonstrated by the expected distribution of the NAT-ratio and effects of smoking, pregnancy, oral contraceptive use, exercise and a diet rich in cruciferous vegetables on the IA2-ratio.

The inducing effects of smoking and exercise on XO activity are not yet explained but may be of importance in ischaemic tissue damage related to smoking and physical strain, respectively.

rom 23

from 9
green

at least
th HCl
et al.,

ypically
d by an
stylator

gure 2).
as 66%
ing the
erences
d in 28
tio was
Figure
M.E. et

ure 2).
and oral
ubjects
subjects
en light

m $5.2 \pm$
 3 ± 0.64
physical
ed with

and $4.4 \pm$
proccoli.
eviously
, 1979).

: simple
bility of
atio and
t rich in

not yet
king and

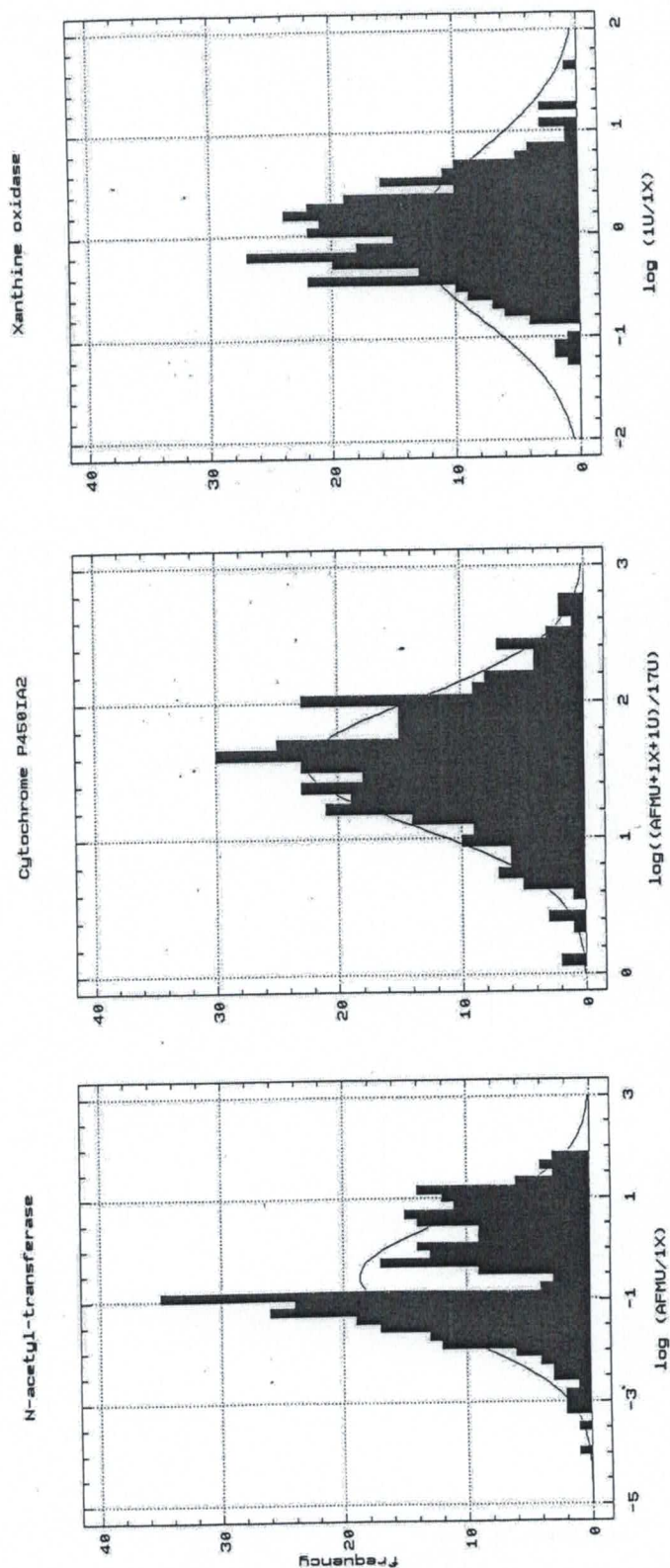


Figure 2. Distribution of the metabolic ratios of caffeine in 335 healthy subjects.

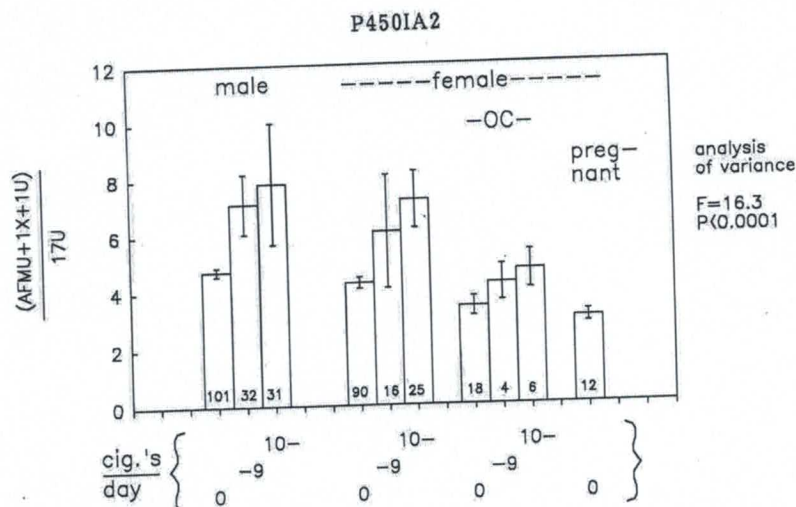


Figure 3. Effect of sex, smoking, pregnancy and oral contraceptive use on the IA2 metabolic ratio of caffeine.

The enzyme activities measurable from caffeine metabolism are especially relevant for the bioactivation of potentially toxic compounds and has retrospectively been linked to a variety of disease states, particularly cancer (Butler, M.A. et al., 1989; Hein, D.W., 1988; Guengerich, F.P., 1988). The assessment of caffeine metabolism by means of spot urine ratios of metabolites from dietarily ingested caffeine is applicable in large scale epidemiological studies. Thus, prospective testing of such relations between the enzyme activities and the development of disease is technically and practically feasible.

REFERENCES

- Boel, J., Andersen, L.B., Rasmussen, B. and Hansen, S.H., Dossing M. (1984). Hepatic drug metabolism and physical fitness. In *Pharmacol. Ther.* **36**, 121-126.
- Butler, M.A., Iwasaki, M., Guengerich, F.P. and Kadlubar, F.F. (1989) Human cytochrome P-450PA (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci.* **86**, 7696-7700.
- Campbell, M.E., Spielberg, S.P. and Kalow, W. (1987). A urinary metabolite ratio that reflects systemic caffeine clearance. *Clin. Pharmacol. Ther.* **42**, 157-165.
- Grant, D.M., Tang, B.K. and Kalow, W. (1983). Variability in caffeine metabolism. *Clin. Pharmacol. Ther.* **33**, 591-602.
- Grant, D.M., Tang, B.K., Campbell, M.E. and Kalow, W. (1986). Effect of allo, purinol on caffeine disposition in man. *Br. J. Clin. Pharmacol.* **21**, 454-458.
- Guengerich, F.P. (1988). Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res.* **48**, 2946-2954.
- Hein, D.W. (1988). Acetylator genotype and arylamine-induced carcinogenesis. *Biochem. et Biophysica. Acta.* **948**, 37-66.
- Knutti, R., Rothweiler, H. and Selatter, C.H. (1981). Effect of pregnancy on the pharmacokinetics of caffeine. *Eur. J. Clin. Pharmacol.* **21**, 121-126.
- Oda, T., Akaike, T., Hamamoto, T., Suzuki, F., Hirano, T. and Maeda, H. (1989). Oxygen radicals in influenza-induced pathogenesis and treatment with pyran polymer-conjugated SOD. *Science* **244**, 974-976.
- Pantuck, E.J., Pantuck, C.B., Garland, W.A., Min, B.H., Wattenberg, L.W., Anderson,

- K.E. and Kappas, A. (1979). Stimulatory effect of brussel sprouts and cabbage on human drug metabolism. *Clin. Pharmacol. Ther.* 88-95.
- Simpson, P.J., Lucchesi, B.R. (1987). Free radicals and myocardial ischemia and reperfusion injury. *J. Lab. Clin. Med.* 110, 13-30.

cially
tively
t al.,
feine
ested
esting
ase is

atic

narily
on of

that

l.

inol

pyran

ion,