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Effects of caffeine with repeated dosing

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Summary. We have recently demonstrated dose-dependency of caffeine metabolism under multiple dosing conditions. Whether there are persistent pharmacodynamic actions of caffeine under such circumstances is the focus of this report. Nine healthy subjects were given, in randomized 5 day blocks, placebo, 4.2 (low) and 12 (high) $mg \cdot kg^{-1} \cdot day^{-1}$ of caffeine in 6 divided doses.

After 5 days, complete tolerance developed to the effects of caffeine on blood pressure, heart rate and plasma glucose concentrations. The 24-h area under the curve (AUC) for plasma norepinephrine and the AUC for the total sum of free fatty acids (FFA) both demonstrated a trend to increase with the high dose caffeine treatment. When the AUC for norepinephrine was split into 12 h time periods, a significant difference between the placebo and the high dose treatment block was seen.

We conclude that regular consumption of 12 $mg \cdot kg^{-1}$ of caffeine per day (equivalent to approximately 6 to 11 cups of coffee per day) may produce pharmacodynamic effects not completely compensated for by the development of tolerance. Mechanisms of tolerance may be overwhelmed by the nonlinear accumulation of caffeine and other methylxanthines in the body when caffeine metabolism becomes saturable.

Key words: Caffeine, tolerance; catecholamines, free fatty acids, dose-dependency

For the past 20 years coffee consumption has been implicated as a risk factor for the development of ischemic heart disease. While there have been a number of negative studies and some of the early positive studies suffered from the problems of confounding variables and selection bias, recent better designed case control studies and prospective trials have found an association between myocardial infarction and coffee consumption [1–4]. There is also an association between coffee consumption and raised cholesterol concentrations and, while this association has been reported to occur only in people who drink boiled (rather than filtered) coffee [5, 6], other studies in populations where boiled coffee is rarely consumed have demonstrated this association as well [7, 8]. Some of these epidemiological studies have suggested that the risk of coronary heart disease increases disproportionately with increasing levels of coffee consumption [2, 3, 7].

Caffeine is an important pharmacologically active component of coffee. The mechanism by which caffeine might aggravate the risk of ischemic heart disease is unknown. In caffeine-naive subjects, caffeine in single doses raises blood pressure [9], increases plasma concentrations of catecholamines [9, 10] and free fatty acids [11, 12], and increases plasma renin activity [9]. Caffeine may also initiate arrhythmias [13, 14]. These effects of caffeine, which could possibly promote the development of or aggravate coronary heart disease, result primarily from antagonism of the actions of adenosine via receptor block-

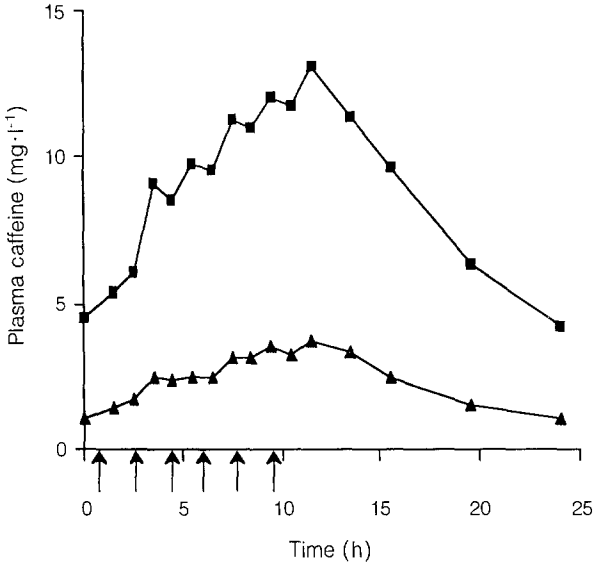

Fig. 1. Mean plasma concentrations of caffeine measured over 24 h during low and high caffeine consumption conditions (▲ low ■ high)

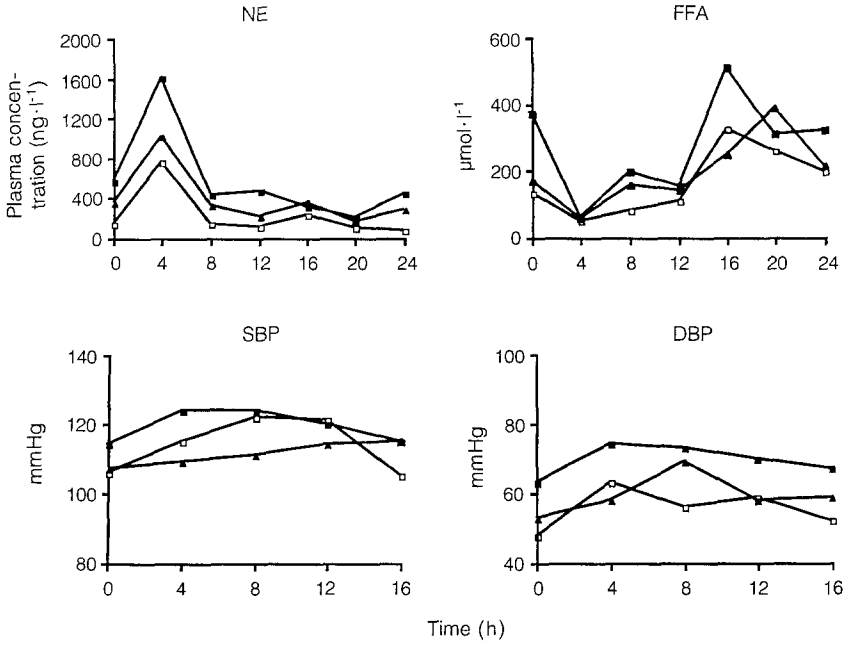

Fig. 2. Blood pressure, plasma norepinephrine, and total free fatty acid concentrations measured on Day 5 during the three different caffeine dosing conditions for a subject who did not demonstrate complete tolerance to effects of caffeine. SBP = systolic blood pressure, DBP = diastolic blood pressure, plasma norepinephrine (NE) in $ng \cdot l^{-1}$, FFA in $\mu mol \cdot l^{-1}$ □ Placebo ▲ low ■ high

ade [15]. In contrast to single dose investigations, the few chronic dosing studies published suggest that tolerance develops to the effects of caffeine within a few days [16, 17]. But it is unclear whether this tolerance is complete and whether some cardiovascular and metabolic effects persist with chronic consumption of caffeine.

We have recently reported the dose-dependency of caffeine metabolism under multiple dosing conditions [18]. In the same study we assessed whether some cardiovascular and metabolic actions of caffeine persisted in subjects regularly consuming caffeine at doses in the range where caffeine metabolism is dose-dependent, which is the subject of this report. We postulated that as caffeine metabolism becomes saturable the ensuing high concentrations of caffeine and its active primary metabolites (dimethylxanthines) overcome tolerance mechanisms. This could explain the nonlinearity in the dose-response relationship between the level of coffee consumption and the suspected adverse health effects seen in some of the epidemiological studies.

Subjects and methods

Subjects

Nine healthy nonsmokers who were habitual consumers of coffee (4 or more cups per day) were admitted to the General Clinical Research Center at San Francisco General Hospital Medical Center for 16 days. There were 7 m and 2 f and their ages ranged from 19 to 55 (mean 37) y. Subjects were healthy on the basis of history, physical examination, electrocardiogram and screening blood chemistry. The women had negative pregnancy tests before entry into the study. No subjects were taking medications (including oral contraceptive pills) and all were instructed to abstain from methylxanthine consumption for 1 week before admission. Written, informed consent was obtained from each subject and the study was approved by the University of California, San Francisco, Committee on Human Research.

Experimental protocol

The study was conducted in 3 treatment blocks, each lasting 5 days. The sequence of treatment blocks was ordered using latin squares. Each patient participated in all 3 blocks. The blocks consisted of placebo, low or high dose caffeine given in coffee to subjects every 2 h between 09.00 and 19.00 h every day (total 6 cups per day; daily caffeine dose 0, 4.2 or 12 $mg \cdot kg^{-1} \cdot day^{-1}$). The coffee was prepared by adding anhydrous caffeine or nothing to decaffeinated instant coffee (Taster's Choice; 1 mg caffeine per serving) mixed with a constant volume of water. The diet was a standard hospital diet with all methylxanthine-containing beverages and foods as well as alcohol prohibited. On the fifth day of every block the same diet was given to all of the subjects. Blood pressure and pulse were recorded every 4 h during the waking hours of each day.

Starting at 08.00 h on the fifth day of each block, blood samples were drawn for measurement of caffeine, catecholamines, free fatty acids and glucose. Sampling times for caffeine were 0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20 and 24 h. For catecholamines, free fatty acids and glucose sampling times were 0, 4, 8, 12, 16, 20 and 24 h. Urine was collected over this 24 h time period for measurement of catecholamines. Blood was collected in tubes containing sodium heparin (or sodium fluoride for glucose) and the plasma frozen until analysis.

Table 1. Plasma norepinephrine AUCs during different caffeine dosing conditions for individual subjects

Subject	Placebo	Low	High
1	5440	5240	4230
2	5390	4950	5260
3	7220	4590	9550
4	3240	4560	7580
5	5850	9680	14190
6	5210	2690	3380
7	5170	4170	10600
8	5610	8680	7350
9	5210	6010	5904
Mean[a]	5370	5620	7560
	(1020)	(2220)	(3430)

Results expressed in $ng \cdot l^{-1} \cdot h$; standard deviation in parentheses
[a] P value = 0.07

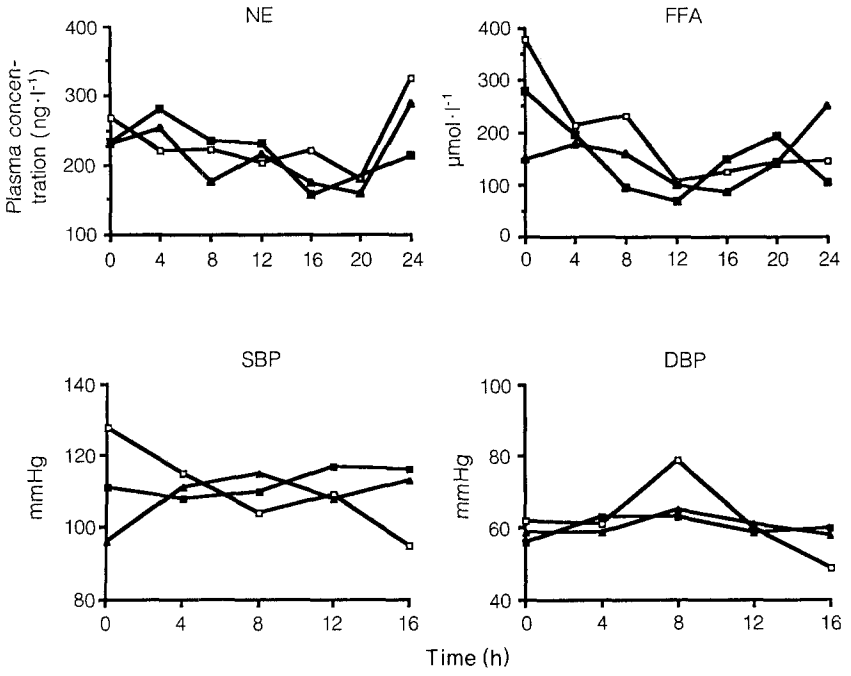

Fig. 3. Blood pressure, plasma norepinephrine, and total free fatty acid concentrations measured on Day 5 during the three different caffeine dosing conditions for a subject who demonstrated complete tolerance to effects of caffeine. Symbols and units as in Fig. 2 □ Placebo ▲ low ■ high

Samples collected for analysis of catecholamines and free fatty acids were immediately placed in ice after blood collection.

Analytical methods

Plasma caffeine was measured by HPLC using a modification of the method of Tang-Liu et al. [19]. The coefficient of variation was 8.9% at 100 $\mu g \cdot l^{-1}$. The plasma catecholamine assay utilized the method of Hjemdahl et al. [20], but used alpha methyl norepinephrine as the internal standard. Coefficient of variation for norepinephrine was 6.2% at 150 $ng \cdot l^{-1}$ and for epinephrine it was 11.5% at 25 $ng \cdot l^{-1}$. The urine catecholamine assay was based on the method of Higa et al. with some minor modifications [21]. A number of different free fatty acids were measured in plasma: myristic acid, palmitoleic acid, palmitic acid, stearic acid and the 18 carbon unsaturated fatty acids (oleic, linoleic and linolenic acids – which could not be individually separated in our assay). FFA extraction was carried out by the method of Belfrage and Vaughan [22] modified by using heptadecanoic acid as the internal standard. The FFA were then esterified to methyl esters using pentafluoropropionic anhydride and methanol and assayed by gas chromatograpy using a 12 m fused silica capillary column coated with cross-linked methylsilicone. Coefficient of variation did not exceed 5%. Plasma glucose was measured enzymatically using YSI Model 27 Industrial glucose analyzer.

Data analysis

Areas under the concentration-time curves (AUC) were calculated by the linear trapezoidal rule. Most of the data were analyzed using repeated measures analysis of variance (ANOVA). The Tukey post-test was used for multiple comparisons.

Results

With 6 cups of coffee given at regular intervals, the concentrations of caffeine for the low and high dose treatment blocks progressively rose throughout the day (Fig. 1). The peak concentration occurred around 20.00 h (1 h after the last cup) and reached on average 13 $mg \cdot l^{-1}$ with the 12 $mg \cdot kg^{-1} \cdot day^{-1}$ dose of caffeine. Blood pressure, pulse rate and glucose measured on Day 5 were on average similar in all treatment blocks, though some individuals had an elevation of systolic and diastolic blood pressures during the high caffeine dose period. Individual variation in response to blood pressure, plasma norepinephrine and free fatty acid concentrations is illustrated in Fig. 2 and 3. Fig. 4 depicts the plasma mean norepinephrine concentrations for the 3 treatment blocks on Day 5. (A median concentration time plot was similar.) The average plasma norepinephrine concentration was greater during the high dose caffeine treatment, especially in the first 12 h, compared with placebo.

The AUCs for individuals for plasma norepinephrine concentrations over 24 h are shown in Table 1. Six of the

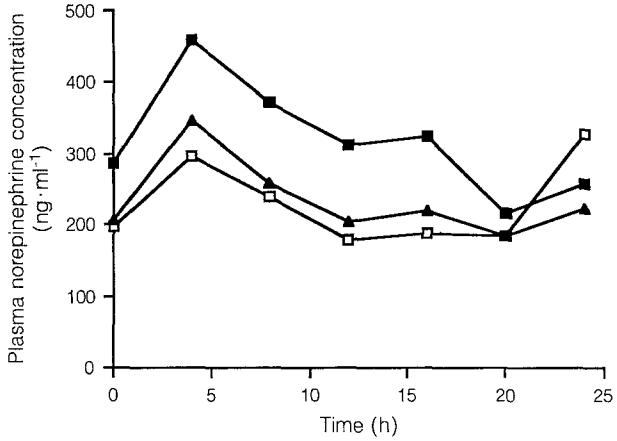

Fig. 4. Mean plasma concentrations of norepinephrine plotted over 24 h during three different caffeine dosing conditions □ Placebo ▲ low ■ high

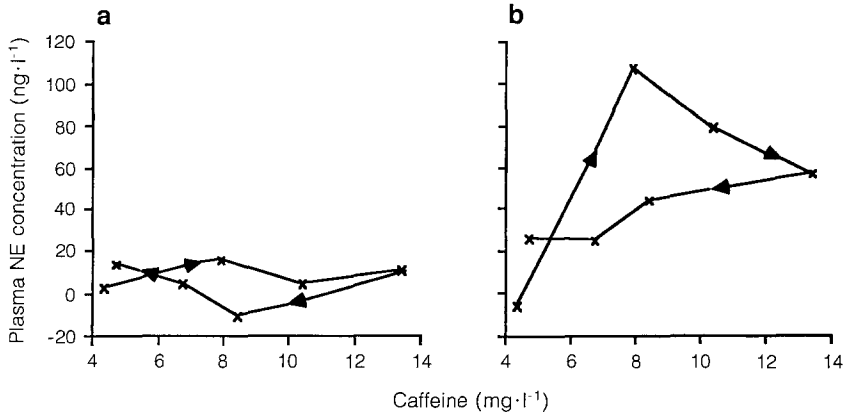

Fig. 5a, b. Concentration-response curves for median plasma norepinephrine (after correction for the norepinephrine measured during the placebo period) and median plasma caffeine concentrations measured during both low **(a)** and high **(b)** caffeine dosing conditions. The arrows indicate the order the samples were taken throughout Day 5

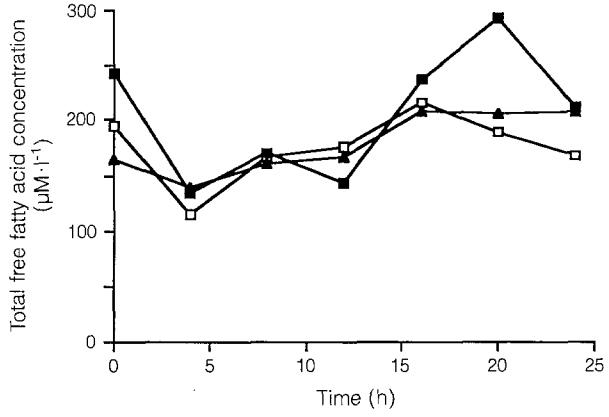

Fig. 6. Mean plasma concentrations of total free fatty acids over 24 h during three different caffeine dosing conditions □ Placebo ▲ low ■ high

9 subjects demonstrated an increase in norepinephrine AUC during the high dose treatment period compared to placebo. The trend for the increase in norepinephrine AUC for high dose caffeine did not quite reach statistical significance ($P = 0.07$). However, when splitting the AUC into 0–12 and 12–24 h time periods, the difference in AUC between the two 12 h time periods during the high dose caffeine block was significantly greater than the difference between the two 12 h time periods during the placebo treatment block ($P < 0.025$). In Fig. 5 the median plasma norepinephrine concentrations (after subtraction of plasma norepinephrine concentrations measured during the placebo treatment period) for the low and high dose treatment blocks are plotted against the median plasma caffeine concentrations sampled at the same time. A clockwise hysteresis curve can be seen for the high dose period. Table 2 shows the mean results for plasma and urinary catecholamines.

The mean sum of the plasma fatty acid concentrations measured throughout Day 5 are shown in Fig. 6. (A median concentration time plot was similar.) Elevation in total free fatty acid concentration can be seen for the high caffeine dose over the last 8 h (overnight). This was also seen in the individual plots of stearic acid, palmitic acid, palmitoleic acid, 18 carbon fatty acids and, to a lesser extent, myristic acid. Table 3 shows the AUC over 24 h for the fatty acids measured. A trend for an increased AUC during the high caffeine dose can be seen. While there was a statistical difference for palmitic acid between low and high caffeine doses, the total sum of fatty acids just failed to reach statistical significance ($P = 0.07$).

Discussion

Tolerance to the effects of caffeine has been noted in many studies. However, whether complete tolerance develops to effects of caffeine is unknown. In some subjects considerable sympathetic activation was observed after 5 days of caffeine consumption (demonstrated in Fig. 2), whereas in others there was no evidence of a caffeine effect after 5 days (Fig. 3). Data suggest that on average dosing of caffeine for 5 days produces persistent activation of the sympathetic nervous system. We found a trend for increased plasma norepinephrine concentrations for the high caffeine dose especially for the first 12 h of the day. There was a 41% increase in the AUC of norepinephrine for high dose caffeine compared to placebo treatment. When the AUC was split into 12 h time periods, a significant difference in the interaction term of the ANOVA was seen. The difference between 0–12 and 12–24 h AUCs for the high caffeine dose block was significantly greater than the change that occurred between these two AUC time

Table 2. Plasma and urine catecholamines during different caffeine dosing conditions

	Placebo	Low	High
Plasma AUC			
Epinephrine	942 (279)	1140 (243)	1150 (209)
Dopamine	969 (244)	1040 (378)	1060 (380)
Urine excretion			
Norepinephrine	42 (16)	48 (24)	51 (25)
Epinephrine	4 (2)	5 (2)	5 (2)
Dopamine	254 (104)	285 (117)	279 (96)

Results expressed as mean (standard deviation); plasma AUC in $ng \cdot l^{-1} \cdot h$; urine excretion in $\mu g \cdot 24\ h^{-1}$. Plasma norepinephrine data are shown in table 1.

Table 3. AUC of plasma free fatty acid concentrations during different caffeine dosing conditions

	Myristic	Palmitoleic	Palmitic[a]	C18	Stearic	Total[b]
Placebo	159	134	1650	1550	1150	4190
	(60)	(59)	(453)	(496)	(1290)	(973)
Low	152	143	1610	1620	745	4270
	(48)	(79)	(330)	(344)	(171)	(808)
High	167	169	1810	1800	810	4780
	(69)	(90)	(474)	(513)	(174)	(1250)

Results expressed in μmol h/l; mean (standard deviation)
[a] Significantly different between Low and High dose only ($P < 0.05$)
[b] P value = 0.07

periods for the placebo treatment block. As seen in Fig. 5, the concentration-response relationship between plasma caffeine and plasma norepinephrine concentrations and the clockwise nature of the hysteresis loop is consistent with the development of tolerance during the day. Probably the overnight caffeine abstinence allowed tolerance to abate somewhat so that individuals became sensitive to the catecholamine-releasing effects of caffeine by the next morning. As tolerance to caffeine developed during the day, norepinephrine levels returned to levels similar to those observed during the placebo treatment.

Plasma epinephrine has been shown in other studies to be more sensitive to caffeine effects than norepinephrine [9]. However, a number of the plasma epinephrine measurements were below the limits of quantification of our assay during repeated dosing of caffeine and calculation of the epinephrine AUC for all patients under all conditions was not possible. This lessened the power of the repeated measures ANOVA considerably.

Elevation in plasma FFA concentrations was seen primarily overnight during high dose caffeine treatment. There was a 14% increase in the 24 h AUC for total FFA for the high dose caffeine treatment compared with placebo. A significant difference in the AUC between low and high caffeine dose blocks for palmitic acid was seen and similar trends were seen for most of the individual FFAs (Table 3). While caffeine is well known to acutely elevate plasma FFA levels, FFA release is suppressed by insulin which is released after eating [23]. Presumably, the effects of meals on FFA concentrations during the day masked caffeine-mediated FFA release, while at night, when insulin levels are low, FFA release due to caffeine is observed.

The results for plasma norepinephrine and total FFA AUCs over 24 h just failed to reach the empirical P value of 0.05 accepted for statistical significance. This was due to the considerable wide interpatient variation in these measures and the relatively small numbers of subjects studied. However, the dose-related trends involving different response measures and the significant temporal patterns seen in our plasma norepinephrine analysis strongly suggest that the effects of repeated consumption of caffeine on the sympathetic nervous system are real. Persistent sympathetic activation and elevation of FFA over many years could explain accelerated atherosclerosis in coffee drinkers. The dose of 12 $mg \cdot kg^{-1} \cdot day^{-1}$ of caffeine that our subjects consumed would be equivalent to 6 to 11 cups of coffee per day depending on the body weight of the individual, the method in making the coffee and the size of the cup [24]. This calculation does not include caffeine from other sources such as tea, caffeinated soft drinks, chocolate and medications.

A potential criticism of our conclusion about persistent effects of caffeine is that tolerance may require longer than 5 days to develop. The data of Robertson et al. [16] suggest that tolerance develops over 3 days and indeed we saw complete tolerance to blood pressure elevation over a similar period of time. Studies of more prolonged caffeine exposure will be needed to clarify the issue.

The effect of regular caffeine consumption on catecholamines has been studied by Robertson et al. [16]. When compared to the response after a single dose of caffeine, substantial tolerance was clearly demonstrated. However, the investigators did not look to determine if complete tolerance developed. They measured catecholamines for only 4 h after the dose and subjects in their placebo group were different from those receiving caffeine. In light of the substantial intersubject variation seen in our study, their power to resolve differences would have been low.

Support for the notion that the extent of development of tolerance to effects of caffeine may be incomplete comes from several different types of research. The pressor effects of caffeine persist after 7 days of caffeine dosing in patients with autonomic failure [25]. A pressor response to caffeine taken in the morning can still be seen in regular coffee drinkers, particularly those with lower morning concentrations of caffeine [16, 26]. A rise in plasma free fatty acids and cortisol was demonstrated in chronic (greater than 2 cups/day) coffee users after a 12 h fasting period [12]. And an association between chronic coffee consumption and elevated serum cholesterol concentrations has been found in some of the epidemiological reports [5–8] and in long term-animal studies [27].

We have previously demonstrated that caffeine metabolism is saturable under multiple dosing conditions at both of the doses given to our subjects [18]. In that study there was evidence to suggest that the primary metabolites of caffeine, the dimethylxanthines, which may also have pharmacological actions, were subject to dose-dependent metabolism as well. We suggest that with high levels of consumption of caffeine, possibly related in part to saturable metabolism with the ensuing high concentrations of caffeine and dimethylxanthines, tolerance may not fully compensate for some of the pharmacological effects of caffeine and its metabolites. Both the dose-dependency of caffeine metabolism and changes in catecholamines and FFA could help to explain the link between coffee consumption and coronary artery disease and the non-linearity of this dose-response relationship noted in some epidemiological studies. Individual differences in the extent of development of tolerance to effects of caffeine may underlie individual differences in susceptibility to adverse effects of caffeine on health.

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