奈医誌. (J. Nara Med. Ass.) 48, 141~150, 1997

PHARMACOLOGY WITH A CUP OF COFFEE: DOUBLE-BLIND ANALYSIS FOR ARITHMETIC SKILL AND HEMODYNAMIC EFFECTS

HIROYASU SATOH, TAKESHI TANAKA and TOSHIKATSU NAKASHIMA
Department of Pharmacology, Nara Medical University

Received March 26, 1997

Abstract: Effects of caffeine on arithmetic skill test and hemodynamic effects were investigated after drinking caffeine-free or caffeine-containing (180 mg) coffee. The development of tolerance in habitual coffee-drinkers was also examined. A double-blind study of 417 healthy medical university students (22.4±2.1 years old) was carried out. Ten rounds of the arithmetic tests were performed; the first four rounds before, and the subsequent six rounds after a coffee-break. Each round was composed of three 1-min arithmetic tests and two 1-min rests between the tests. The arithmetic skill per round was averaged. Simultaneously, heart rate and blood pressure were also measured once a round. The mean value of arithmetic skill at the 4th round before coffee-break was 86.7±3.9/min (n=417). The heart rate and mean blood pressure were 73.7±3.3 beats/min and 85.7±3.8 mmHg. As compared with caffeine-free group (n=205), caffeine-containing groups had a significant enhancement of arithmetic skill at 45 to 60 min later. The mean blood pressure increased at 15 to 90 min, but the heart rate was unaffected. Simultaneously, the serum concentration of caffeine increased from 0.9±0.3 to 4.3±1.6 μg/ml (n=12, $P<0.01$) at 45-60 min after coffee (180 mg caffeine)-drinking. Habitual coffee-drinkers reduced the stimulations of arithmetic skill and mean blood pressure, as compared with non-habitual coffee-drinkers. These results indicate that a cup of coffee (containing 180 mg caffeine) enhances the arithmetic skill and modulates the hemodynamic actions, presumably resulting from stimulation of the central and sympathomimetic nervous systems. A tolerance to caffeine's actions also develops in habitual coffee-drinkers.

Index Terms

caffeine (coffee), arithmetic skill, hemodynamic actions, tolerance

INTRODUCTION

Caffeine (1, 3, 7-trimethylxanthine) contained in coffee, cocoa or tea has been consumed for the past 5-6 centuries and more. Epidemiologic studies for the public health consequences of caffeine intake have already been performed. In general, a cup of coffee contains approximately 100-120 mg of caffeine[1)]. After oral consumption, almost 90% of caffeine is absorbed from the stomach[2)] and the peak plasma concentration is observed at approximately 30 to 60 min[3-5)]. The effective plasma concentration is in a range of 5 to 10 μg/ml. Caffeine stimulates the central nervous system, and too much coffee disturbs sleep and heart rhythms. Caffeine (75 to 100 mg) also causes auditory and visual evoked responses[6,7)], and increases reading speed and speech rate[8-10)].

In isolated hearts, caffeine modulates the ionic currents and action potentials, which affects the developmental tension and the sinus rhythm[11-15]. In canine Purkinje fibers and rabbit sino-atrial (SA) nodal cells, caffeine (0.5-10 mM) caused initially positive inotropic and chronotropic effects, and subsequently negative inotropic and chronotropic effects. The initial positive effects resulted from enhancements of Ca^{2+} release from sarcoplasmic reticulum (SR) and the L-type Ca^{2+} current due to accumulation of cAMP by phosphodiesterase (PDE) inhibition. The subsequent negative effects were presumably due to production of cellular calcium overload by re-uptake blockade of Ca^{2+} into SR by caffeine itself. This is strongly supported by the evidence that a dysrhythmia induced by triggered activities, such a delayed and early afterdepolarizations (DAD and EAD) or transient inward and inward tail currents, occurred under the depressant conditions[11,12,16,17].

The aim of the present study is to examine, using a double blind method, whether a change in arithmetic skill is produced after coffee-drinking (caffeine-free or caffeine-containing), and also whether habitual coffee-drinkers develop a tolerance for caffeine's effects. Simultaneously, we sought to examine the hemodynamic effects on heart rate (HR) and blood pressure (BP).

MATERIALS AND METHODS

Measurement of arithmetic performance

The arithmetic skill test of 417 medical university students was investigated, according to the methods developed by Sakuma[18]. The test was done in the afternoon. Students (22.4±2.1 years old) were told not to take caffeine-containing beverages at lunch time before the test. A double-blind study was carried out, in which caffeine-free or caffeine (180 mg)-containing coffee was taken after the 4th round test. Each group was 205 students, and seven students did not have coffee during the tests. Each round every 15 min was composed of three arithmetic tests for 1 min and two rests for 1 min, as shown in Fig. 1. An arithmetic skill test for 1 min was simply an addition using an Uchida-Kraepelin's test (Japan Psychotech. Ins. Co. Tokyo,

PROCEDURE

−45 min	1st	Kraepelin test
−30 min	2nd	Kraepelin test
−15 min	3rd	Kraepelin test
0 min	4th	Kraepelin test
	Coffee break	
15 min	5th	Kraepelin test
30 min	6th	Kraepelin test
45 min	7th	Kraepelin test
60 min	8th	Kraepelin test
75 min	9th	Kraepelin test
90 min	10th	Kraepelin test

Fig. 1. Procedure of Kraepelin tests. The tests were carried out four times (−45 to 0 min) before, and six times (15 to 90 min) after coffee-drinking. One round of test was composed of three times-Kraepelin tests for 1 min.

Japan). The average value of three arithmetic tests in a round was represented. The first (−45 min) to fourth (0 min) round tests were carried out for the subjects to become skilled in the arithmetic test. After coffee-drinking, the arithmetic tests were repeated 6 rounds more every 15 min. The values are normalized by taking those at the 4th round as 100%, because the values of all the students, and also of the other 7 students without coffee-drinking during the tests, became almost steady at the 4th round. The significance of differences was assessed with ANOVA and Student's *t*-test for paired data. Values are presented as mean±S.E.M.

Measurements of heart rate and blood pressure

Heart rate (HR) and blood pressure (BP) were measured using a digital pressometer (Ei and Dei, UA-732, Tokyo, Japan) once a round (after three arithmetic skill tests). The mean blood pressure (MBP) was calculated by an equation; diastolic pressure + (systolic pressure-diastolic pressure)×1/3.

Serum concentration of caffeine

Blood was taken from 24 students before and at 45-60 min after coffee-drinking and from 3 students who had no coffee during the tests. The serum sampling procedure and a sensitive high-performance liquid chromatography method were used for the determination of caffeine and other xanthines, according to previous reports[19,20]. Caffeine and other xanthines working standards (50μl), and serum sample containing internal standard (25μl) and β-hydroxyethyl-theophyllne (2 μg/ml) were mixed with 100 μl of 1 M borate buffer at pH 9.0 in a 15 ml conical centrifuge tube. Then, 1 ml of chloroform and ethanol (82.5 : 17.5) was added and centrifuged. The organic layer was evaporated to dryness and suspended in 50 μl of mobile phase for depuration on an Altex Ultrasphere C 18 column (5 μm particle size, 150×2.0 mm i.d., San Ramon, CA). The eluate from the column was monitored at 273 nm absorbance on a liquid chromatograph (Shimazu, LC-6 A, Kyoto, Japan). The mobile phase was methanol and 0.05% acetic acid (1 : 4), and 28 mM sodium acetate buffer, 1.4 mM tetrabutylammonium phosphate (at pH 2.15) and 40% phosphoric acid (12 : 52 : 36). The flow rate was set at 0.3 ml/min or operated at a pressure of 103 bar (1500 p.s.i.). Caffeine was eluated by 35% methanol.

Coffee-making

Caffeine-free instant coffee (Nescafe red-label, Nestle Co., Tokyo) was used. A cup of coffee (150 ml) was regularly made with a full spoon of the coffee (1.7-1.8 g), one of sugar and one of creaming powder (Creama, Yukijirushi Co., Tokyo). But the instant coffee contained 0.88 mg/g of caffeine from our analysis. In the caffeine-containing coffee, caffeine (180 mg) was added into a half of 410 cups at random. The cups were numbered by a controller, and it was impossible to distinguish between caffeine-containing or caffeine-free coffee.

RESULTS

Effects on arithmetic skill

The arithmetic skill test was administered before and after drinking caffeine-free coffee for 205 students or caffeine (180 mg)-containing coffee for 205 students. Before coffee-drinking at -45 to 0 min, arithmetic skills in both groups increased round by round. The average value of all the students (n=417) was 86.7±3.9 per min. Figure 2 shows the time-dependent changes

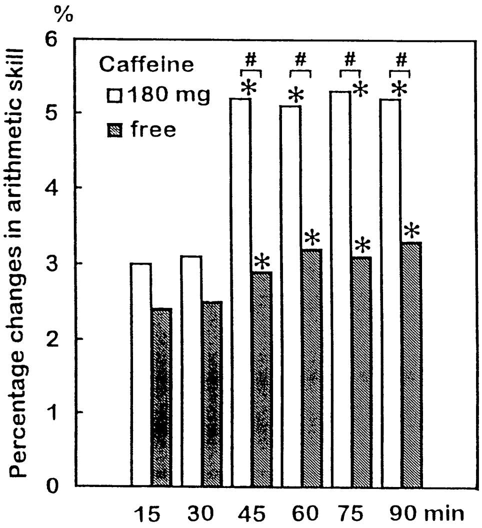

Fig. 2. Effect of caffeine on arithmetic skill before and after a coffee-drinking. Time-dependent changes in the arithmetic skill were shown. The averaged scores for three times-Kraepelin tests in one round are plotted. Data in caffeine-free and caffeine-containing groups were calculated by taking fourth test (0 min) as a base. Columns used are 180 mg caffeine (open, n=205) and caffeine-free (shadow, n=205). * : $P<0.05$, with respect to control values. # : $P<0.05$, in comparison between both groups.

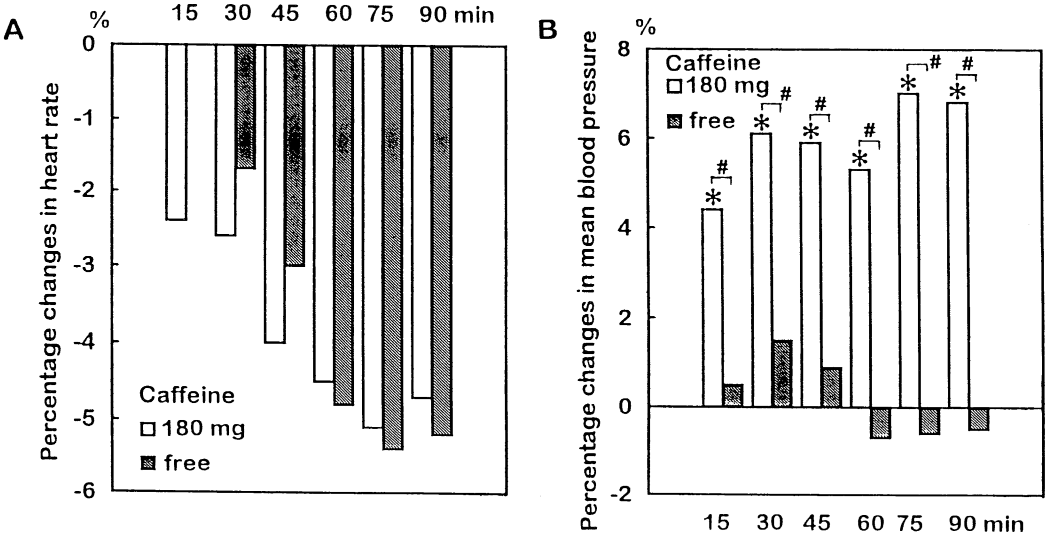

Fig. 3. Hemodynamic actions of caffeine before and after a coffee-drinking. Data in caffeine-free and caffeine (180 mg)-containing groups were taken the fourth test (0 min) as a bese. A : Time-dependent changes in the heart rate (HR). B : Time-dependent changes in the mean blood pressure (MBP). Columns used are 180 mg caffeine (open, n=205) and caffeine-free (shadow, n=205). * : $P<0.05$, with respect to control values. # : $P<0.05$, in comparison between both groups.

in arithmetic skill in the caffeine-free and -containing groups. The values are calculated by taking those at the 4th round as a control. After coffee-drinking, skills in the caffeine-containing group and even in the caffeine-free group were significantly increased at 45-90 min later. In comparison between both groups, significant effective responses were also observed at 45 to 90 min in the caffeine-containing group ($P<0.05$). No students in the caffeine and non-caffeine groups complained of any symptons.

The arithmetic errors were shown as a ratio; a number of errors/total mumber of arithmetic skill, in 3 tests for a round. The mean ratio at the 4th round in all the students was 0.025 ±0.002%(n=417). The ratios in both groups decreased round by round, but not significantly.

No change in the factors occurred in the seven students who did not have any drinks (or coffee) during the tests.

Effect on heart rate

The heart rate (HR) decreased time-dependently before and after coffee-drinking, independent of coffees with and without caffeine. The HR reached an almost steady state at the 4th round, and the average value was 73.7±3.3 beats/min (n=417). After drinking coffee, the HR decreased further in both groups, bot not significantly. Figure 3A shows the summarized values. Seven students who had no coffee did not show any changes. The difference between both groups was not significant.

Effects on blood pressure

Blood pressure (BP) also fell during the three tests before coffee-drinking. The average values of systolic and diastolic BPs were 118.2±4.5 and 69.2±3.5 mmHg (n=410) at the

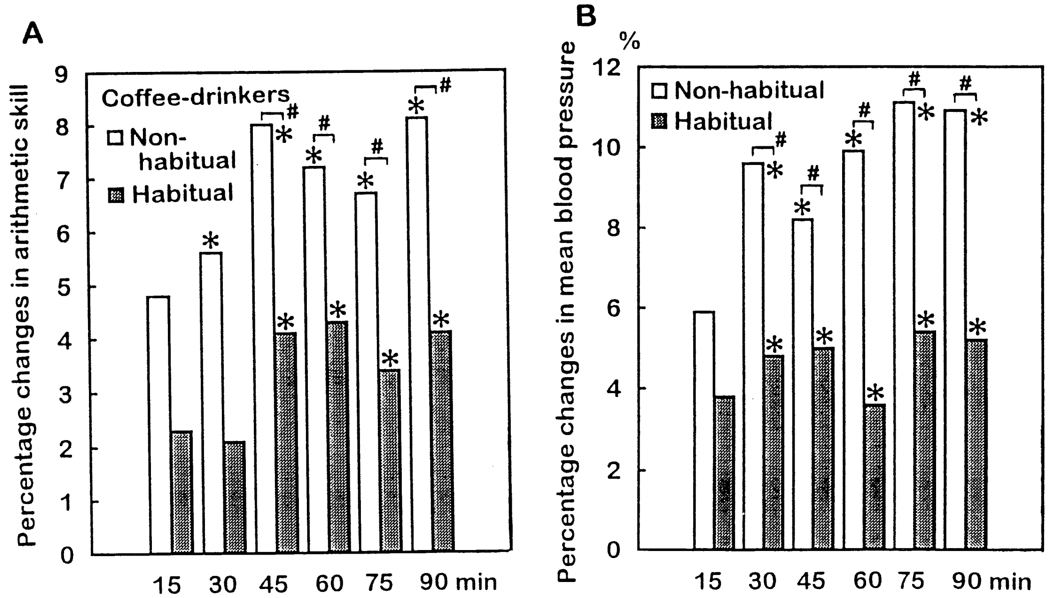

Fig. 4. Development of tolerance for caffeine's actions. Data in caffeine-free and caffeine (180 mg)-containing groups were taken the fourth test (0 min) as a base. A : Arithmetic skill. B : Mean blood pressure. Columns used are non-habitual coffee-drinkers (open, n=146) and habitual coffee-drrinkers (shadow, n=59). * : $P<0.05$, with respect to control values. #: $P<0.05$, in comparison between both groups.

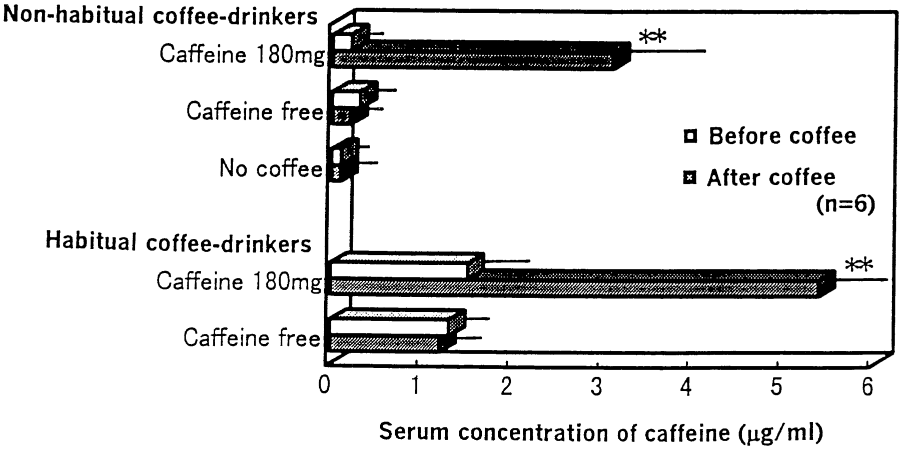

Fig. 5. Serum concentrations of caffeine before and after a coffee-drinking. Columns are before coffee (white) and after coffee-drinking (shadow). Values are represented as mean±SEM (n= 6). **$P<0.01$, with respective to control value. Blood was drawn before and 40 min after coffee-drinking.

fourth round, and the MBP was 85.7±3.8 mmHg. Caffeine increased both the systolic and diastolic BPs. As shown in Fig. 3B, a significant increase in the MBP was observed after 15-90 min in the caffeine-containing group, but not at all the rounds in the caffeine-free group. Seven students without coffee had no effect. The difference between both groups was significant at all times (15-90 min)($P<0.05$).

Comparison between habitual and non-habitual drinkers of coffee

Among all the students, we examined whether a tolerance for caffeine's actions develops in habitual coffee-drinkers. The 146 habitual coffee-drinkers in the caffeine-containing coffee group (n=205) usually have 3.5±1.1 cups of coffee per day. Three students have 5 cups a day. In non-habitual (n=59) drinkers, both arithmetic skill and MBP significantly increased at 30-90 min (Fig. 4). Habitual drinkers (n=146) also significantly enhanced the arithmetic skill at 45-90 min and the MBP at 30-90 min. In comparison between habitual and non-habitual coffee-drinkers, significant differences occurred at 45-90 min in arithmetic skill and at 30-90 min in MBP. On the other hand, the HR in both groups was unaffected (by about 4-8%, $P>0.05$).

The serum concentration of caffeine 40-50 min after drinking a cup of coffee (180 mg caffeine) increased from 0.2±0.1 to 3.1±1.1 μg/ml (n=6, $P<0.01$) in non-habitual coffee-drinkers, and from 1.5±0.6 to 5.4±1.3 μg/ml (n=6, $P<0.01$) in habitual coffee-drinkers. The values are summarized in Fig. 5. The concentrations of twelve students in caffeine-free drinkers and three of seven students who had no coffee were unaffected.

DISCUSSION

Caffeine in one of the pharmacologically active chemicals. Caffeine in coffee or tea has been in widespread use for a long time. The present experiments by means of a double-blind study for 410 university students (total 417 students) showed that caffeine at 180 mg (after coffee-drinking) enhanced arithmetic skill and increased MBP. Caffeine is water-soluble, and is completely absorbed from caffeinated beverages. The caffeine concentration in blood reaches a peak at 30-60 min later[3-5,9,21]. The half-life ($T_{1/2}$) is 3-5 hr[22-25]. In this study, the average value at 45-60 min later increased to approximately 4 μg/ml. These pharmacological characteristics are reflected in the data in the present studies. The significant differences for arithmetic skill and MBP between both groups lasted for 30 to 90 min after coffee-drinking.

Arithmetic skill

In general, caffeine at relatively higher concentrations increases sleep latency and decreases total sleep time. It is also known that caffeine is primarily a stimulant, increasing flow of thought and vigilance, reducing fatigue, and shortening motor reaction time[26,27]. In the present experiments, the arithmetic skills in both groups increased round by round, irrespective of coffee (with and without caffeine)-drinking. The increase would probably be due to habituation and learning by the repeated tests, as would be the decrease in arithmetic errors (but not significant). However, caffeine at 180 mg enhanced arithmetic skill significantly, as compared with caffeine-free coffee-drinkers. The enhancement of arithmetic skill was consistent with the results of Sakuma[18] and Horiuchi et al.[28]. This would result from a stimulatory action on the central nervous system due to an inhibition of adenosine receptor. Furthermore, the accumulation of cAMP by its phosphodiesterase (PDE) inhibitory action would cause vasodilation in brain and other tissues. The vasodilation as well as the BP increase might indirectly contribute to the elevation of brain activity. Benowitz[21] has reported that alcohol inhibits caffeine metabolism, whereas tobacco smoke accelerates it. The $T_{1/2}$ may be reduced by as much as one-half[29,30]. The mechanism underlying this effect is likely metabolic, reflecting the induction of hepatic enzymes such as cytochrome P450 1A2 by smoking[31-33]. In this experiment, smokers (84 students) had approximately twenty cigarettes a day. A comparison between smokers and non-smokers was also examined, because the nicotine level in blood for smokers before the start of the tests might be kept relatively high. The smokers significantly decreased the arithmetic skill and MBP, as compared with non-smokers (unpublished data). However, the effective duration of caffeine was still unclear.

Hemodynamic effects

In the present study, the HR in all the groups decreased round by round even before coffee-drinking, but not significantly. This is probably due to the subjects becoming relaxed mentally. After coffee-drinking, the HR decreased further. This would be induced via baroreceptor-mediated reflexes against the increase in MBP. In addition, the decrease might result from the mental refreshment of drinking a cup of coffee (that is, a coffee-break). On the other hand, a significant increase in the BP occurred at 15 to 90 min later. The amount of caffeine is equivalent to approximately two cups of coffee. It has already been shown that caffeine (two cups of coffee) increases blood pressure (by 4 to 6 mmHg), decreases HR slightly, and causes systemic release of epinephrine, norepinephrine and renin[34,35]. Therefore, the pressor effect might be due to an increase in cardiac output (via the PDE inhibition by caffeine) and peripheral vasoconstriction (via the adenosine antagonism).

Caffeine exerts multiple influences on different tissues. Application of caffeine causes relaxation of smooth muscle, stimulation of skeletal muscle, and diuretic action[36]. Satoh and colleagues have already shown that in isolated cardiac muscles, caffeine (0.5-10 mM) caused positive inotropic and chronotropic effects, and subsequently negative inotropic and chronotropic effects[11-15]. As a result, caffeine induced cellular calcium overload. Then, a dysrhythmia occurred ; DAD and/or EAD[16,17]. Clinical reports reflected the present results. Mean serum caffeine concentration in 600 medical outpatients was 2.1 μg/ml (ranging from 0.2 to 13.1 μg/ml)[37]. In this study, the concentration of caffeine in habitual coffee-drinkers was lower, 1.5 μg/ml before coffee-drinking, and elevated to 5.4 μg/ml by a cup of coffee (180 mg caffeine). The lower level of caffeine might result from the anouncement of no coffee before the test. However, since caffeine increases Ca^{2+} sensitive to cardiac muscles[38], the changes in concentration might not be necessary for the responses.

The high plasma content of caffeine in heavy coffee-drinkers may induce calcium overload. For one or two cups of coffee a day, the relative risk is 1.3, and for over five cups per day, is 2.5, as compared with no coffee-drinking[39,40]. In clinical use, caffeine as a drug is utilized for patients with headache and postprandial orthostatic hypotension[41].

Tolerance

The response to caffeine is dependent on the sensitivity of individuals. The importance of tolerance or adaptation to the caffeine actions must be considered. It has been reported that when even one or two cups of coffee are consumed during a day, a tolerance develops to the caffeine actions within a few days[20,42]. The tolerance is associated with an increased number of adenosine receptors in the brain[43,44]. Adenosine is a competitive antagonist of caffeine among xanthine derivatives.

In this study, the habitual coffee-drinkers showed a tolerance for arithmetic skill and MBP, as compared with the values of non-habitual coffee-drinkers. Even in habitual coffee-drinkers developing the tolerance, however, the serum concentration of caffeine was elevated, and caffeine significantly stimulated both arithmetic skill and MBP. Most students (about 80%) usually had 3-4 cups of coffee a day. Therefore, we concluded that habitual coffee-drinkers develop a caffeine tolerance. This is consistent with several reports that the tolerance to pressor effect develops within a short period of time with repeated dosing of caffeine[20,21,42,45-47]. Thus, the caffeine actions were strongly influenced by the developed tolerance.

Conclusion

Caffeine (180 mg) in a cup of coffee produced significant actions on the arithmetic skill and the MBP of the university students. These results strongly show the beneficial effect of several cups of coffee for work, but a tolerance develops in habitual coffee-drinkers. Caffeine may also have dangerous risks. Heavy coffee-drinkers needs to be careful about their health, and especially for its aggravating effects on cardiac diseases.

REFERENCES

1) **Lelo, A., Miners, J. O., Robson, R.** and **Birkett, D. J.**: Assessment of caffeine exposure : Caffeine content of beverages, caffeine intake, and plasma concentrations of methylxanthines. Clin. Pharmacol. Ther. **39** : 54-59, 1986.

2) **Chvasta, T. E.** and **Cook, A. R.** : Emptying and absorption of caffeine from the human stomach. Gastroenterology **61** : 838-843, 1971.

3) **Grab, F. L.** and **Reinstein, J. A.**: Determination of caffeine in plasma by gas chromatography. J. Pharm.

Sci. **57** : 1703-1706, 1968.

4) **Bonati, M., Latini, R., Galetti, F., Young, J. F., Tognoni, G.** and **Garattini, S.** : Caffeine disposition after oral doses. Clin. Pharmacol. Ther. **32** : 98-106, 1982.

5) **Blanchard, J.** and **Sawers, S. J. A.** : Comparative pharmacokinetics of caffeine in young and elderly men. J. Pharmacokinet. Biopharm. **11** : 109-126, 1983.

6) **Clubley, M., Bye, C. E., Henson, T. A., Peck, A. W.** and **Riddington, C. J.** : Effects of caffeine and cyclizine alone and in combination on human performance, subjective effects and EEG activity. Br. J. Clin. Pharmacol. **7** : 157-163, 1979.

7) **Terry, W. S.** and **Phifer, B.** : Caffeine and memory performance on the AVLT. J. Clin. Psychol. **42** : 860-863, 1986.

8) **Furlong, F. W.** : Possible psychiatric significance of excessive coffee consumption. Can. Psychiatr. Assoc. J. **20** : 577-583, 1975.

9) **Reimann, H. A.** : Caffenism. A cause of long-continued, low-grade fever. Jama **202** : 1105-1106, 1967.

10) **Stephenson, P. E.** : Physiologic and psychotropic effects of caffeine on man. A review. J. Am. Diet. Assoc. **71** : 240-247, 1977.

11) **Satoh, H.** : Caffeine depression in spontaneous activity in rabbit sino-atrial node cells. Gen. Pharmacol. **24** : 555-563, 1993.

12) **Satoh, H.** : Positive and negative effects of caffeine in spontaneously beating rabbit sino-atrial node cell. Gen. Pharmacol. **24** : 1223-1230, 1993.

13) **Satoh, H.** and **Vassalle, M.** : Reversal of caffeine-induced calcium overload in cardiac Purkinje fibers. J. Pharmacol. Exp. Ther. **234** : 172-179, 1985.

14) **Satoh, H.** and **Vassalle, M.** : The role of catecholamines on cellular calcium overload induced by caffeine in canine Purkinje fibers. Am. J. Physiol. **257** : H 226-H 237, 1989.

15) **Satoh, H.** and **Vassalle, M.** : Ca^{2+} -dependence of caffeine modulation of the rate-force relation in cannine cardiac Purkinje fibers. J. Pharmacol. Exp. Ther. **278** : 826-835, 1996.

16) **Hasegawa, J., Satoh, H.** and **Vassalle, M.** : Induction of the oscillatory current by low concentration of caffeine in sheep cardiac Purkinje fibers. Naunyn-Schmiedeberg's Arch. Pharmacol. **335** : 310-320, 1987.

17) **Satoh, H., Hasegawa, J.** and **Vassalle, M.** : On the characteristics of the inward current induced by calcium overload. J. Mol. Cell. Cardiol. **21** : 5-20, 1989.

18) **Sakuma, A.** : Coffee effect : try at student practice. Jpn. J. Clinc. Pharmacol. **3** : 314-316. 1972.

19) **Tang-Liu, D. D. S.** and **Riegelman, S.** : An automated HPLC assay for simultaneous quantitation of methylated xanthines and uric acids in urine. J. Chromatogr. Sci. **20** : 155-159, 1982.

20) **Shi,J., Benowitz, N. L., Denaro, C. P.** and **Sheiner, L. B.** : Pharmacokinetics and drug disposition ; Pharmacokinetic-pharmacodynamic modeling of caffeine : tolerance to pressor effects. Clin. Pharmacol. Ther. **53** : 6-14, 1993.

21) **Benowitz NL.** : Clinical pharmacology of caffeine. Annu. Rev. Med. **41** : 277-288, 1990.

22) **Arnaud, M. J.** : The pharmacology of caffeine. Prog. Drug Res. **31** : 273-313, 1987.

23) **Bonati, M., Latini, R.** and **Tognoni, G.** : Interspecies comparision of in vivo caffeine pharmacokinetics in man, monkey, rabbit, rat, and mouse. Drug Metab. Rev. **15** : 1355-1383, 1985.

24) **Kalow, W.** : Variability of caffeine metabolism in humans. Arzneim. Forsch. **35** : 319-324, 1985.

25) **Busto, U., Bendayan, R.** and **Sellers, E. M.** : Clinical pharmacokinetics of nonopiate abused drugs. Clin. Pharmacokinet. **16** : 1-26, 1989.

26) **Lieberman, H. R., Wurtman, R. J., Emde, G. G., Roberts, C.** and **Coviella, I. L. G.** : The effects of low doses of caffeine on human performance and mood. Psychopharmacology **92** : 308-312, 1987.

27) **Sawyer, D. A., Julia, H. L.** and **Turin, A. C.** : Caffeine and human behavior : Arousal, anxiety, and perfor-

mance effects. J. Behav. Med. **5** : 415-439, 1982.

28) **Horiuchi, K., Mushiake, H.** and **Endo, R.** : Effects of caffeine on arithmetic performance. Jpn. J. Pharmacol. **31** : 164 P, 1981.

29) **Parsons, W. D.** and **Neims, A. H.** : Effect of smoking on caffeine clearance. Clin. Pharmacol. Ther. **24** : 40 -45, 1978.

30) **May, D. C., Jarboe, C. H., Van Bakel, A. B.** and **Williams, W. M.** : Effects of cimetidine on caffeine disposition in smokers and nonsmokers. Clin. Pharmacol. Ther. **31** : 656-661, 1982.

31) **Kalow, W.** and **Tang, B. K.** : Caffeine as a metabolic probe : Exploration of the enzyme-inducing effect of cigarette smoking. Clin. Pharmacol. Ther. **49** : 44-48, 1991.

32) **Campbell, M. E., Grant, D. M., Inaba, T.** and **Kalow, W.** : Biotransformation of caffeine, paraxanthine, theophyline, and theobromine by polycyclic aromatic hydrocarbon-inducible cytochrome (s) P-450 in human liver microsomes. Drug Metab. Dispos. **15** : 237-249, 1987.

33) **Lambert, G. H., Schoeller, D. A., Humphrey, H. E., Kotake, A. N., Lietz, H., Campbell, M., Kalow, W., Spielberg, S. P.** and **Budo, M.** : The caffeine breath test and caffeine urinary metabolite ratios in the Michigan cohort exposed to polybrominated biphenyls : a preliminary study. Environ. Health Perspect. **89** : 175-181, 1990.

34) **Pincomb, G. A., Lovallo, W. R., Passey, R. B., Whitsett, T. L.** and **Silverstein, S. M.** : Effects of caffeine on vascular resistance, cardiac output and myocardial contractility in young men. Am. J. Cardiol. **56** : 119-122, 1985 1

35) **Robertson, D., Frolich, J. C., Carr, R. K., Watson, J. T.** and **Hollifield, J. W.** : Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N. Engl. J. Med. **298** : 181-186, 1978.

36) **Rall, T. W.** : Drugs used in the treatment of asthma. In : Goodman Gilman, A., Rall, T. W., Nies, A. S. and Taylor, P. (eds) The Pharmacological Basis of Therapeutics Chap. 25, Pergamon Press, New York, pp. 618-630, 1990.

37) **Smith, J. M., Pearson, S.** and **Marks, V.** : Plasma caffeine concentrations in outpatients. Lancet **2** : 985-986, 1982.

38) **Bonnet, V.** and **Leoty, C.** : Effects of caffeine and cyclopiazonic acid on contractile proteins of adult and newborn ferret cardiac fibres. J. Mol. Cell. Cardiol. **28** : 1287-1295, 1996.

39) **LaCroix, A. Z., Mead, L. A., Liang, K., Thomas, C. B.** and **Pearson, T. A.** : Coffee consumption and the incidence of coronary heart disease. N. Engl. J. Med. **315** : 977-982, 1986.

40) **Vandenbroucke, J. P., Kok, F. J., Van't Bosch, G.** and **Van Den Dungen, P. J. C.** : Coffee drinking and mortality in a 25-year follow-up. Am. J. Epidemiol. **123** : 359-361, 1986.

41) **Heseltine, D., Dakkak, M.** and **Woodhouse, K.** : The effect of caffeine on postprandial hypotension in the elderly. J. Am. Gerial. Soc. **39** : 160-164, 1991.

42) **Robertson, D., Hollister, A. S.** and **Kincaid, D.** : Caffeine and hypertension. Am. J. Med. **77** : 54-60, 1984.

43) **Boulenger, J. P., Patel, J., Post, R. M., Parona, A. M.** and **Marangos, P. J.** : Chronic caffeine consumption increases the number of brain adenosine receptors. Life Sci. **32** : 1135-1142, 1983.

44) **Green, R. M.** and **Stiles, G. L.** : Chronic caffeine consumption sensitizes the A_1 adenosine receptor-adenylate cyclase system in rat cerebral cortex. J. Clin. Invest. **77** : 222-227, 1986.

45) **Robertson, D., Wade, D., Workman, R., Woosley, R. L.** and **Oates, J. A.** : Tolerance to the humoral and hemodynamic effects of caffeine in man. J. Clin. Invest. **67** : 1111-1117, 1981.

46) **Ammon, H. P. T., Bieck, P. R., Mandalaz, D.** and **Verspohl, E. J.** : Adaptation of blood pressure to continuous heavy coffee drinking in young volunteers. Br. J. Clin. Pharmacol. **15** : 701-706, 1983.

47) **Izzo, J. L., Ghosal, A., Kwong, T., Freeman, R. B.** and **Jaenike, J. R.** : Age and prior caffeine use alter the cardiovascular and adrenomedullary responses to oral caffeine. Am. J. Cardiol. **52** : 769-773, 1983.