PHARMACOKINETICS AND THE EFFECTS ON PSYCHOMOTOR PERFORMANCE OF CAFFEINE IN ENERGY DRINKS IN THAI HEALTHY VOLUNTEERS

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Caffeine is a widely consumed psychoactive substance found in a variety of beverages, foods, and medicines. In Thailand, the effects of caffeine on the general health of laborers have been considered as a health problem. However, the safe limit of consumption per day is not generally known. The purpose of this study was to determine the pharmacokinetic parameters of caffeine in popular energy drinks and its effects on psychomotor performance. The pharmacokinetic studies in 12 healthy male subjects indicated C_{max} = 5.45±0.57 μg/ml, T_{max} = 0.92±0.39 hr, $t_{1/2}$ = 4.57±0.37 hr. AUC_{0-8} and $AUC_{0-\alpha}$ values were 26.04±4.28 and 38.94±8.41 μg.hr/ml. Vd was 0.60±0.12 L/kg and CL was 1.53±0.31 ml/min.kg. It was suggested that caffeine is rapidly absorbed and distributed throughout the body. It is also rapidly discharged from the body. To investigate the effects of caffeine on psychomotor performance by determining changes over the pre-dose reaction time (RT) of the simple reaction time (SRT) and choice reaction time (CRT), another 12 subjects received placebo, 200 mg and 400 mg of caffeine in a crossover study. It was found that the administration of low dose of caffeine (200 mg) improved performance as the increment over the pre-dose values of SRT, 3CRT, 6CRT and 9CRT were significantly different from placebo ($p<0.05$). High dose of caffeine (400 mg) produced less performance enhancement than that of the lower dose. The peak performance was observed around 1.5-2 hr with the lower dose, whereas the peak was generally observed between 0.5-1 hr with the higher dose of caffeine. About 65 % of the subjects in this study complained of palpitation, nervousness and restlessness after taking 400 mg of caffeine. However, none of the subjects taking 200 mg of caffeine reported any adverse effects. The overall findings of the present study did not implicate the adverse effects of a single dose administration of 200 mg of caffeine containing in the popular energy drinks in the Thai healthy male subjects. It is reasonable to assume that the consumption of caffeine-containing beverages not exceeding 200 mg of caffeine per day should not pose a serious health risk to the Thai population at large. However, long term intake of caffeine should be rigorously assessed using well-controlled studies in order to determine the long-term effects of caffeine consumption on human health.

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ดำรงศักดิ์ เป็กทอง : เภสัชจลนศาสตร์และการศึกษาผลต่อสมรรถภาพของระบบประสาทการเคลื่อนไหวของแคฟเฟอีนในเครื่องดื่มชูกำลังในอาสาสมัครไทยสุขภาพดี (PHARMACOKINETICS AND THE EFFECTS ON PSYCHOMOTOR PERFORMANCE OF CAFFEINE IN ENERGY DRINKS IN THAI HEALTHY VOLUNTEERS) คณะกรรมการควบคุมวิทยานิพนธ์; กำพล ศรีวัฒนกุล พ.บ., ปร.ด. กรองทอง ยุวถาวร ภ.บ.,ปร.ด., กิตติมา ศรีวัฒนกุล Ph.D. 128 หน้า. ISBN 974-664-472-6

แคฟเฟอีนเป็นสารออกฤทธิ์ต่อจิตประสาทซึ่งถูกใช้อย่างแพร่หลาย ในประเทศไทยปัญหาของการบริโภคแคฟเฟอีนต่อสุขภาพของกลุ่มผู้ใช้แรงงานถูกมองว่าเป็นปัญหาสำคัญทางสาธารณสุขเรื่องหนึ่ง อย่างไรก็ตาม ปริมาณสูงสุดที่สามารถบริโภคได้อย่างปลอดภัยในแต่ละวันยังไม่เป็นที่ทราบชัด วัตถุประสงค์ของการวิจัยนี้คือการศึกษาข้อมูลทางเภสัชจลศาสตร์ของแคฟเฟอีนในเครื่องดื่มที่เชื่อกันว่าให้พลังงาน และศึกษาผลของแคฟเฟอีนต่อสมรรถภาพของระบบประสาทการเคลื่อนไหว (Psychomotor performance)

ผลจากการศึกษาค่าเภสัชจลนศาสตร์ของอาสาสมัครชายไทยสุขภาพดี 12 คน พบว่า ระดับความเข้มข้นสูงสุดในพลาสมา (C_{max}) เท่ากับ 5.45±0.57 ไมโครกรัมต่อมิลลิลิตร เวลาที่ระดับความเข้มข้นสูงสุดในพลาสมา (T_{max}) เท่ากับ 0.92±0.39 ชั่วโมง ค่าครึ่งชีวิตของการกำจัดยา ($t_{1/2}$) เท่ากับ 4.57±0.37 ชั่วโมง และพื้นที่ใต้กราฟระหว่างความเข้มข้นของแคฟเฟอีนในพลาสมากับเวลาที่พบแคฟเฟอีน จาก 0 ถึง 8 ชั่วโมง (AUC_{0-8}) และจาก 0 ถึงอินฟินิตี้ ($AUC_{0-\infty}$) คือ 26.04±4.28 และ 38.94±8.41 ไมโครกรัม-ชั่วโมงต่อมิลลิลิตรตามลำดับ ค่าปริมาตรการกระจายตัวของยา (Vd) เท่ากับ 0.60±0.12 ลิตรต่อกิโลกรัม และค่าการกำจัดยา (CL) เท่ากับ 1.53±0.31 มิลลิลิตรต่อนาที-กิโลกรัมจากข้อมูลนี้บ่งบอกว่าแคฟเฟอีนถูกดูดซึมได้เร็วและกระจายตัวไปในส่วนต่าง ๆ ของร่างกายได้ดี

เพื่อเป็นการศึกษาผลของแคฟเฟอีนต่อสมรรถภาพของระบบประสาทการเคลื่อนไหวการวิจัยนี้จึงได้เปรียบเทียบการเปลี่ยนแปลงของเวลาที่ตอบสนองต่อสิ่งเร้า (Reaction time) ก่อนและหลังได้รับแคฟเฟอีนโดยวัดความแตกต่างของเวลาของการตอบสนองแบบง่าย (Simple reaction time) และแบบมีตัวเลือก (Choice reaction time) ในอาสาสมัครสุขภาพดี 12 คนซึ่งทุกคนได้รับเครื่องดื่มที่ประกอบด้วยสารแคฟเฟอีน 200 และ 400 มิลลิกรัมผสมอยู่และยาหลอก (placebo) ในช่วงเวลาที่แตกต่างกันผลการทดลองแสดงให้เห็นว่า การได้รับแคฟเฟอีนขนาดต่ำ (200 มิลลิกรัม) เพิ่มสมรรถภาพของระบบประสาทการเคลื่อนไหว โดยมีความเร็วของการตอบสนองแบบง่ายและแบบมีตัวเลือกต่างจากการได้รับยาหลอกอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ส่วนผลของแคฟเฟอีนในขนาดสูง (400 มิลลิกรัม) มีผลเพิ่มสมรรถภาพของระบบประสาทการเคลื่อนไหวได้ต่ำกว่าเมื่อเทียบกับการได้รับคาเฟอีนในขนาดต่ำ ผลกระตุ้นสูงสุดจะเกิดขึ้นในช่วงระยะเวลา 1.5-2 ชั่วโมงเมื่อได้รับแคฟเฟอีนขนาดต่ำ และในเวลา 0.5-1 ชั่วโมง เมื่อได้รับแคฟเฟอีนขนาดสูง ประมาณ 65%ของอาสาสมัครระบุว่ามีอาการใจสั่น หงุดหงิด และกระสับกระส่ายหลังดื่มเครื่องดื่มที่มีแคฟเฟอีนผสมอยู่ 400 มิลลิกรัม ในขณะที่ไม่มีอาสาสมัครรายใดที่ได้รับคาเฟอีนขนาด 200 มิลลิกรัมระบุว่ามีผลข้างเคียง ผลการวิจัยในภาพรวมไม่แสดงให้เห็นว่าแคฟเฟอีนขนาด 200 มิลลิกรัมที่ผสมในเครื่องดื่มที่ได้รับความนิยมสูง ทำให้เกิดอาการอันไม่พึงประสงค์ในอาสาสมัครชายไทยสุขภาพดีดังนั้นจึงอาจประเมินได้ว่าการบริโภคเครื่องดื่มที่มีแคฟเฟอีนผสมในขนาดไม่เกินวันละ200 มิลลิกรัมไม่น่าที่จะก่อให้เกิดปัญหาต่อสุขภาพแก่ประชากรไทยส่วนใหญ่ อย่างไรก็ตามควรมีการศึกษาวิจัยอย่างจริงจังถึงผลของการบริโภคสารแคฟเฟอีนในระยะยาวโดยใช้การวิจัยที่มีการควบคุมอย่างดีเพื่อประเมินผลระยะยาวต่อสุขภาพของมนุษย์ที่บริโภคสารแคฟเฟอีน

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LIST OF ABBREVIATIONS

%	= percentage
%CV	= percent of coefficient variation
°C	= degree Celsius
3CRT	= 3 choice reaction time
6CRT	= 6 choice reaction time
9CRT	= 9 choice reaction time
a.m.	= ante meridian
AFMU	= 5-acetylamino-6-formylamino-3-methyluracil
ANOVA	= analysis of variance
AUC_{0-8}	= the area under the plasma concentration-time curve from time zero to eight hours
$AUC_{0-\alpha}$	= the area under the plasma concentration-time curve from time zero to infinity
BA	= bioavailability
BP	= blood pressure
cAMP	= cyclic-adenosine-5-monophosphate
Ca^{2+}	= calcium ion
C_{max}	= peak plasma concentration
CL	= clearance
cm^2	= square centimeter
CNS	= central nervous system
CO_2	= carbon dioxide

LIST OF ABBREVIATIONS (cont)

Conc	= concentration
CSF	= cerebrospinal fluid
CYP	= cytochrome P-450
e.g.	= exampli gratia (for example, for instant)
et al.	= et alli (coworker)
EEG	= electroencephalogram
EKG	= electrocardiogram
F	= relative bioavailability
FDA	= Food and Drug Administration
g	= gram
GI	= gastrointestinal
HPLC	= high-performance liquid chromatography
HR	= heart rate
hr	= hour
Hz	= hertz, cycles per second
i.e.	= id est (that is)
i.p.	= intraperitoneal
i.v.	= intravenous
IS	= internal standard
K^+	= potassium ion
ke	= eliminate rate constant
kg	= kilogram

LIST OF ABBREVIATIONS (cont)

Kpsi	= kilopascal per square inches
L	= liter
mg	= milligram
min	= minute
ml	= milliliter
mM	= millimolar
msec	= millisecond
MW	= molecular weight
nm	= nanometer
pH	= the negative logarithm of the hydrogen ion concentration
pKa	= the negative logarithm of the dissociation constant
p.m.	= post meridian
p.o.	= per os (orally)
r	= correlation
rpm	= revolution per minute
SRT	= simple reaction time
SD	= standard deviation
SEM	= standard error of mean
T_{max}	= time to reach peak plasma concentration
$t_{1/2}$	= half-life
μg	= microgram

LIST OF ABBREVIATIONS (cont)

μl	= microliter
μM	= micromolar
UV	= ultraviolet
Vd	= the volume of distribution
yr	= year
w/v	= weight by volume

CHAPTER I

INTRODUCTION

Caffeine (1, 3, 7-trimethylxanthine) is a widely consumed psychoactive substance found in a variety of beverages, foods, and medicines (1, 2). Approximately 90% of all caffeine consumed are from coffee and tea (3) and mean daily caffeine intake is about 3 mg/kg for all adults in the general population and approximately 4 mg/kg for consumers of caffeine (3). More than 100 plant species are known to contain methylxanthines, particularly caffeine, however, human beings have chosen to use only a few, intrigued by their taste. Consumption of caffeine-containing foods and beverages spread systematically, despite recurring efforts to restrict or eliminate their use (4). The basis for its popularity is the stimulatory effects on human mental activities and its propensity to reduce physical fatigue (5).

The kinetic behavior of caffeine is well characterized (6-10). After oral administration, it is rapidly and completely absorbed. Plasma concentration reaches its peak from 15 to 120 min depending on variation in gastric emptying and presentation of dietary components (7, 8). After absorption, it is uniformly distributed into body fluid. The volume of distribution (Vd) of caffeine in man ranges from 0.5 to 0.8 L/kg and mean plasma half-life ($t_{1/2}$) of 2.5 to 7.5 hr are observed in adults (7-11). Metabolism by demethylation occurs almost exclusively in the liver (12, 13) and approximately 2% is excreted unchanged in the urine. Caffeine is eliminated by apparently first-order kinetics, described by a one-compartment open model system in

human (8, 56, 85). Many factors such as race, genetic composition, body size and life-style may have significant effects on caffeine kinetic behavior (7-10, 14, 15).

Caffeine and other related methylxanthines share several pharmacological actions of therapeutic interest. They relax smooth muscle, notably bronchial muscle, stimulate central nervous system (CNS), stimulate cardiac muscle, and act on the kidney to produce diuresis (16, 17).

Caffeine is well known for its psychoactive effects, including stimulation of mood and psychomotor performance (18-21). Dose-dependent effect of single doses of caffeine have been found in animal models and in human subjects, with positive stimulatory effects at low and intermediate doses and more aversive effects at high doses. The maximum stimulatory effects of caffeine on activity in rodents are found at low to intermediate doses and intermediate plasma and brain caffeine concentrations (10-20 μg/ml or μg/g, respectively) (22, 23).

In human, low to intermediate doses of caffeine also have stimulatory and euphoric effects and increase arousal, alertness, concentration, sense of well being, and performance on cognitive and motor tasks. High doses cause nervousness and adverse somatic effects such as restlessness, agitation, chills, tremors, nausea, and diuresis, and disrupt performance (18-21, 23). Subjective effects of caffeine have been correlated with quantitative pharmacodynamic measures, such as the electroencephalogram (EEG), cognitive and motor performance (20, 21, 24). From these previous studies, it appears that a nonlinear relationship exists between caffeine dose and concentrations and stimulatory effects.

Nowadays, caffeine-containing beverages have become social acceptable drinks by many people. The widespread use of caffeine has led some to suspect that it

might have detrimental effects on health. It is the responsibility for regulatory agencies in every country to set a maximum level of caffeine consumption per day. For instance, the health authority of Canada has set up the upper limit of daily consumption of caffeine not to exceed 400-450 mg (25).

In Thailand, caffeine-containing drinks are widely consumed by hard-working laborers and truck drivers. These drinks contain caffeine about 50 mg/bottle. Other ingredients are sugar, amino acid, nutrients and vitamins. The Thai Food and Drug Administration has recommended the public not to consume these beverages more than 2 bottles a day, which is equivalent to 100 mg caffeine. However, heavy consumers usually take more than 2 bottles per day with the maximum of 4 bottles per day (26). Since the data on pharmacokinetics of caffeine in Thai subjects are very limited (27-30) and the caffeine-containing beverages are widely consumed, it is essential to perform the pharmacokinetic studies of caffeine in these products in the Thai populations. This might be useful to correlate plasma caffeine concentrations from these beverages with its pharmacological effects.

CHAPTER II

OBJECTIVES

The purposes of this investigation were as follows:

1. To study the pharmacokinetic profile of a single oral dose of 200 mg caffeine in energy drinks in Thai healthy volunteers.
2. To study the effects of 200 and 400 mg caffeine in energy drinks on psychomotor performance in Thai healthy volunteers.

CHAPTER III

LITERATURE REVIEW

PART 1 CAFFEINE

1 Chemical and physical data

1.1 Chemical name and synonyms

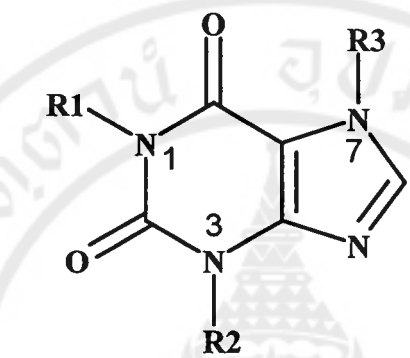
Chemical name: 3, 7-Dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione

Synonyms: Anhydrous caffeine; coffeine; coffeinum; guaranine; methyltheobromine; 7-methyltheophylline; thein; theine; 1, 3, 7-trimethyl-2, 6-dioxopurine; 1, 3, 7-trimethylxanthine

1.2 Chemical and physical properties

Caffeine is a compound belonging to the methylxanthine group. It is a methylated purine derivative and may also be classified as alkaloids that include the closely related compounds, theobromine and theophylline (31).

From its structure, caffeine can be identified as 1, 3, 7-trimethylxanthine with chemical formula $C_8H_{10}N_4O_2$ and molecular weight of 194.19, whereas theobromine is 3, 7-dimethylxanthine and theophylline is 1, 3-dimethylxanthine. Caffeine is composed of C 49.48%, H 5.19%, N 28.85%, and O 16.48% (32). Chemically, these alkaloids closely resemble metabolically important compounds such as purines, xanthines, and uric acid. The structure formulae of xanthines and caffeine are shown in Figure 1.



R1=R2=R3=H	Xanthine
R1=R2=R3=CH_3	Caffeine
R1=H, R2=R3=CH_3	Theobromine
R1=R2=CH_3, R3=H	Theophylline
R1=R3=CH_3, R2=H	Paraxanthine

Figure 1. Structures of xanthine, caffeine, and dimethylxanthines

Caffeine is a weak base with pK_a of 1 and 14 (33). The solubility of caffeine is low and much enhanced by the complex formation such as caffeine with sodium benzoate. These complexes dissociate to yield caffeine when dissolved in aqueous solution (34). In boiling water, caffeine is very soluble from which it crystallizes as a monohydrate with one molecule of water whereas from organic solvents it crystallizes as an anhydrous material (35). It melts at 234 to 239 °C and sublimes at 178 to 180 °C (36). Pure caffeine is white, odorless, and slight bitter taste, with a taste threshold in water of 0.2-1.8 mM. The maximum ultraviolet (UV) absorption in aqueous acid is 273 nm. It is stable at temperature, pH, and salt concentration normally encountered in food processing.

2 Production, use, and occurrence

2.1 Production

Caffeine is produced commercially by both extraction and synthetic procedures. Extraction procedures involve three methods, direct decaffeination of green coffee beans with solvents; extraction from tea dusts and wastes, and fragments of tea leaves; and extraction from cola nuts (37). Caffeine has been obtained as a by-product from the manufacture of caffeine-free coffee (22). The presence of water in the decaffeination process is necessary to dissolve the caffeine-potassium chlorogenate complex. In addition to water, the solvents and absorption materials used in decaffeination are dichloromethane (38), ethylacetate (39), fats and oils, carbon dioxide (40), and acid-treated active carbon, either as such or charged with sucrose. Refining processes are needed to provide the pure caffeine for commerce.

Pressurized carbon dioxide is employed to remove caffeine from tea in the production of decaffeinated tea (38). The extraction yields in the production of natural caffeine have declined significantly in recent years, following the increasing use of water-based as opposed to direct solvent-based extraction procedures.

Synthetic production of caffeine involves the methylation of various xanthines (primarily theobromine) (37) and theophylline (41) or the reaction of theophylline with carbon monoxide and methanol (42); total synthesis can be achieved with dimethyl carbamide and malonic acid (43).

2.2 Use

Approximately 80-90% of caffeine extracted from green coffee is used in beverage industry and most of the remainder and synthetic caffeine are used in pharmaceutical applications (40). Caffeine is permitted in the U.S.A. in nonalcoholic carbonated cola-type beverages at a content of up to 0.02% by weight of the finished product (43). It may be used as a flavor enhancer at levels of up to 200 ppm (0.02%) and as a flavoring agent in baked goods, frozen dairy desserts, mixes, gelatins, puddings, fillings and soft candy at levels of up to 400 ppm (43).

Caffeine is an ingredient in many prescriptions and nonprescription drugs including stimulant tablets, headache and cold remedies, tablets for the relief of menstrual pain, weight control aids and diuretics (44). About 1,000 prescription drugs and 2,000 "over-the-counter" drugs available in the U.S.A. contain caffeine (3). Caffeine is widely used in oral drug preparation, often in combination with analgesics (45) such as aspirin, paracetamol and propoxyphene for the relief of headaches or menstrual tension, with ergotamine tartrate for the treatment of migraine and in combination with some antihistamines to overcome their soporitic effects (34).

Caffeine, usually as caffeine citrate, has been used intravenously in the treatment of neonatal apnea (34), to control asthmatic symptoms and to relieve bronchial spasm (2). Injection of caffeine and sodium benzoate has been used for the symptomatic relief of headache following spinal puncture (34). Caffeine has also been used in combination with cisplatin (45) and cytarabine in phase I-II chemotherapy of advanced pancreatic cancer (46).

The contents of caffeine are 100-200 mg/tablet in stimulants, 15-65 mg/tablet in analgesic combination (47), 15-33 mg/tablet in cold and allergy relief formulations, 66-200 mg/capsule in weight control aids, 16-200 mg/tablet in diuretic (44) and 33-65 mg/tablet in menstrual relief product (47). Caffeine levels in over-the-counter drugs vary widely but in generally are 15-200 mg/tablet or capsule, depending on both of the dosage form and the brand name (3).

2.3 Occurrence

a) Natural occurrence

Caffeine occurs in more than 60 plant species throughout the world (3). Dry green beans of arabica and robusta coffees contain caffeine at levels of 0.9-1.4% and 1.5-2.6%, respectively (48). Darkly roasted coffee beans may contain about 20% more caffeine by weight than green beans (49). The level of caffeine in tea (*Camellia sinensis*) is affected by a wide variety of parameters, including seasonal variations, genetic origin and use of nitrogen in fertilizers; thus, only average values can be estimated. The caffeine content of tea can be as high as 5% (4) but it is usually around 3.5% (50). The weight average caffeine level in tea sold in the U.S.A. is approximately 3.0% (4), whereas those sold in the U.K. range from 2.7 to 3.2% (51).

Cacao is a major source of theobromine and contains only small amounts of caffeine; significant differences in the caffeine content of dried unfermented and fermented cotyledons have been found, as well as in the bark, beans, leaves, roots and pods of *Theobroma cacao*. The bean is the main caffeine storage site, and there are only traces in the leaves and pods (52).

Caffeine also occurs in the *Ilex paraguariensis* plant from which the South American beverage mate is prepared and in other plants of the holly species. Caffeine levels in mate vary from 0.9 to 2.2%. The age of the leaf is an important determinant of the caffeine content; young, growing leaves, 2.0-2.2%; adult, one-year old leaves, 1.6%, two-year old leaves, 0.68% (53).

b) Food and beverages

The monographs on coffee, tea and mate contain extensive information on the methylxanthine content of these beverages (2-4). One or more caffeine-containing foods or beverages are consumed by most adults and children, although 90% of caffeine consumed is in the form of coffee or tea (50). The caffeine content of natural products varies according to the plant species, growing conditions, the amount used and the method of brewing (e.g., brewing time) and preparation (3). Many early values were determined using a variety of analytical methods, often undocumented, and different volumes or "cup" size (3). The caffeine contents of a variety of food products are given in Table 1.

Data on human consumption of caffeine are generally based on overall product usage or on a relatively small number of dietary consumption surveys. Mean daily caffeine intake is approximately 3 mg/kg for all adults in the general population and approximately 4 mg/kg for consumers of caffeine (3, 55). Among 10% of adults who

consumed the highest amount of caffeine, mean daily intake is approximately 7 mg/kg (3).

c) Caffeine drink in Thailand

Caffeine drink has been produced and marketed in Japan for more than 25 yrs. It has been registered as both food and drug. It was imported from Japan to Thailand and at first was classified as drug. After that it has been classified as one kind of food products. At present, in Thailand there are about 80 brand names of this beverage registered as specific controlled food according to Ministry of Public Health Notification Number 62 (B.E. 2524) under Food Act (B.E. 2522).

Since 1978, caffeine, one of ingredients in this beverage has been limited in the amount of not more than 80 mg/bottle, which is equal to the amount of caffeine in a cup of coffee. In 1991, in order to protect consumer's health, NGOs and mass media requested Ministry of Public Health (MOPH) to reconsider the situation of this product. As the result, MOPH has established criteria for this type of beverage as follow:-

1) The amount of caffeine in this beverage must not exceed 50 mg/bottle.
2) The manufacturers must indicate warnings on the label as follows.
   - "Should not be taken more than 2 bottles per day because it may cause tachycardia and insomnia."
   - "Should not be drunk by children and pregnant women."
   - "Patients should consult their physicians before consumption."
3) The product cannot be claimed as an "energy drink" especially on the label.

Table 1. Caffeine content in various beverages and food products (54)

Product	Volume or weight	Caffeine content (mg)	
		Range	Average
Roasted, ground coffee (percolated)	5 oz	40-170	80
Instant coffee	5 oz	30-120	65
Roasted, ground coffee (decaffeinated)	5 oz	2-5	3
Instant coffee (decaffeinated)	5 oz	1-5	2
Roasted, ground coffee (drip)	5 oz	60-180	115
Instant, percolated and Drip coffees	5 oz	29-176	-
Tea			
Major US brands	5 oz	20-90	40
Imported brands	5 oz	25-110	60
Bagged tea	5 oz	28-44	-
Iced tea	12 oz	67-76	70
Leaf tea	5 oz	30-48	41
Instant tea	5 oz	25-50	30
Cocoa			
African	5 oz	-	6
South American	5 oz	-	42
Cocoa	5 oz	2-20	4
Chocolate bar	30 g	-	20
Milk chocolate	1 oz	1-15	6
Sweet chocolate	1 oz	5-36	20
Dark chocolate, semi-sweet	1 oz	5-35	20
Chocolate milk	8 oz	2-7	5
Baking chocolate	1 oz	-	35
Chocolate-flavored syrup	1 oz	-	4
Soft drinks			
Regular cola	6 oz	-	18
Decaffeinated cola	6 oz	trace	-
Diet cola	6 oz	1-29	-
Decaffeinated diet cola	6 oz	0-trace	-

4) The advertisements of this product have to follow the criteria recommended by Food and Drug Administration (FDA).

The other ingredients in this beverage are sugar, amino acids, nutrients and vitamins. The summary of the formula is shown in Table 2.

3 Pharmacokinetics of caffeine

The metabolic pathways of caffeine may be responsible for its pharmacological or toxicological effects. Caffeine has pKa of 1 and 14 (33) and a lipid partition coefficient of 0.85. As a consequence, the molecule exists largely as an undissociated weak electrolyte in the gastric fluid (pH 2-3). Caffeine can be detected in all body fluids and it passes through all biological membranes, including the blood-brain and placental barrier (10). No specific accumulation of caffeine or its metabolites has been observed in any organs or tissues. Its metabolic profile in plasma is characterized by the predominance of paraxanthine, only 0.5-2% of an ingested caffeine dose is excreted unchanged in the urine (12, 13). Twenty-eight metabolites have been identified in the urine of animals and humans. Species differences are important in the quantitative and qualitative pathways. In human, chronic ingestion or restriction of caffeine intake does not modify its metabolism. Genetic expression plays a role in caffeine elimination and metabolism in animals and in humans. However, the polymorphism of acetylator phenotype determined from urinary metabolite concentrations has no effect on caffeine clearance. Several other urinary concentration ratios constitute indexes of hepatic enzyme activities. This is the case of xanthine oxidase and the caffeine metabolic ratio that correlates with caffeine 3-demethylation, caffeine plasma clearance, and cytochrome P-450IA2 activity(10).

Table 2. Composition of ingredients containing in caffeine drink in Thailand

Composition	Range (mg / bottle)
1. Sweetener	
Sucrose	6,000 – 40,500
Glucose	400 – 12,000
Honey	2,000 – 3,000
2. Caffeine	40 – 50
3. Glucuronolactone	300 – 600
4. Taurine	0.1 – 1.15
5. Inositol	25.0 – 112.5
6. Lysine	50
7. Threnonine	1.4
8. Vitamins	
Thiamine (B1)	0.5 – 15.0
Riboflavin (B2)	1.0 – 6.5
Pyridoxine (B6)	1.0 – 7.0
Cyanocobalamin (B12)	2.5 – 42.0*
Panthothenate	2.5 – 6.6
Nicotinamide	10.0 – 50.0
Vitamin C	130 – 145

* = micrograms

From: Food Controls Division, Food and Drug Administration, Ministry of Public Health, Thailand

3.1 Absorption

Caffeine absorption from the gastrointestinal tract is rapid and complete in humans, with 99% of the administered dose absorbed in about 45 min (range 15 to 120 min, depending on variations in gastric emptying and the presence of dietary constituent) (7, 8, 56, 57). Orally administered caffeine is mainly absorbed from the small intestine although 20% has been reported to be absorbed from the stomach (58). Complete absorption has been demonstrated in animals using radiolabelled caffeine (59, 60), with the exception of horses where the apparent bioavailability of an oral dose was only 39% (61).

Pharmacokinetics of caffeine are independent on the route of administration. After oral or intravenous doses, plasma concentration curves were superimposable, suggesting that there is no important hepatic first-pass effect in human (62) or animals (63). The efficacy of percutaneous caffeine absorption has been demonstrated *in vitro* (64), in animals (65), and in premature infants treated for neonatal apnea. Sweating does not play a significant role in the flux of caffeine (66).

3.2 Distribution

Caffeine is sufficiently hydrophobic to pass through all biological membranes. In contrast to its dimethylxanthine metabolites (70-72), no blood-brain barrier has been observed for caffeine in the adult or fetal animal (67-69). A brain-to-plasma ratio of 0.80 for caffeine is found in animal models (73). The concentration ratio between blood and semen is 1, and the decline after administration is similar (74, 75). Caffeine can also be detected in umbilical cord blood (76, 77), bile (70, 78), and saliva (79-84). Since caffeine equilibrates rapidly with serum, an average milk-to-serum concentration ratio of 0.52 was found. A more recent study reported a ratio of 0.81 for

the right and left breasts. Mean distribution volume was 0.7 L/kg (0.5-0.8 L/kg) in newborn infants, adult, and aged subjects (10, 12). In various animal species, a similar distribution volume of 0.8 L/kg has been reported (85). These values are in agreement with the distribution of caffeine into the intracellular tissue water (86).

3.3 Metabolism

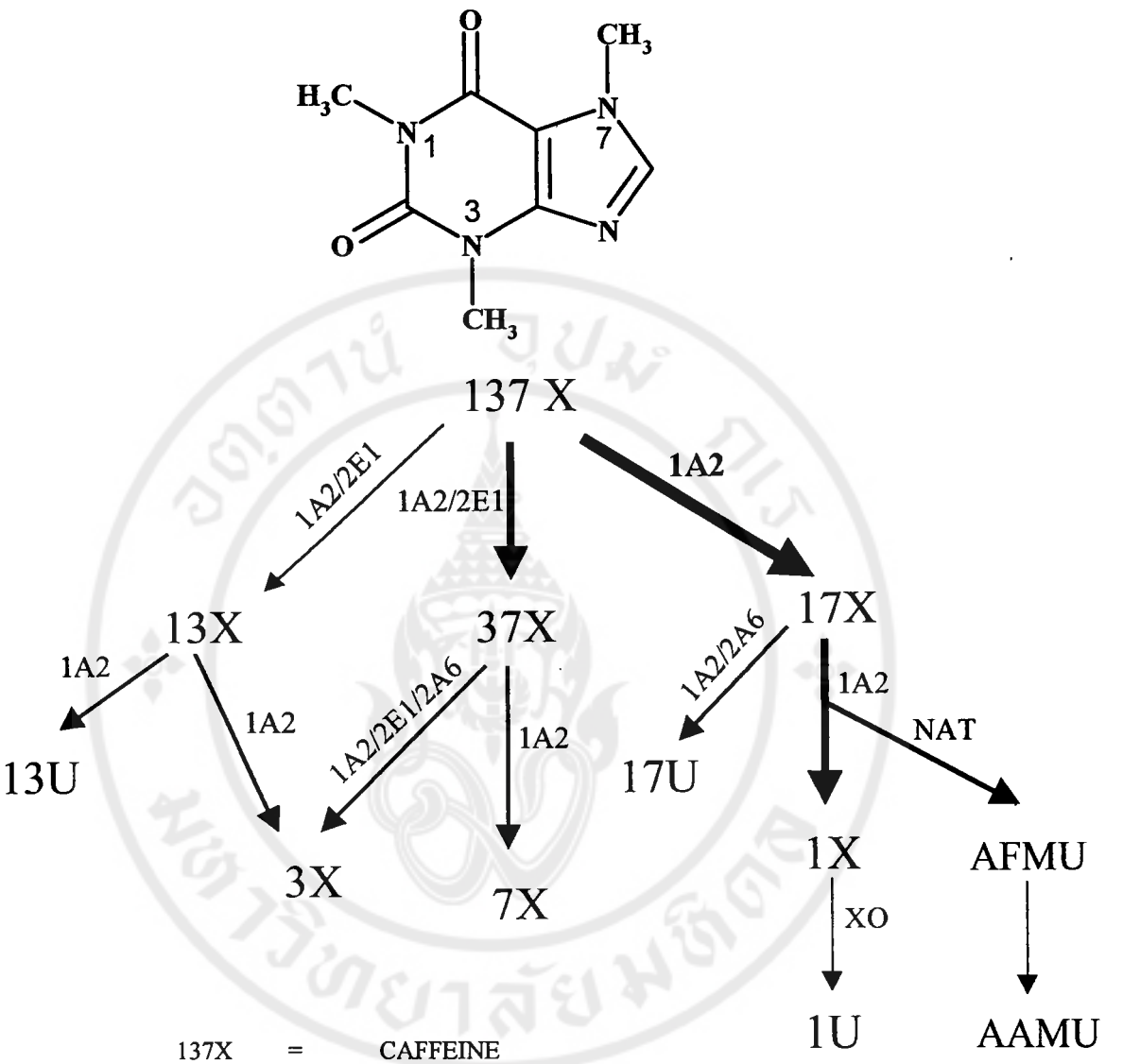
The metabolism of caffeine is the rate-limiting factor for its plasma clearance (57). It is transformed by hepatic microsomal enzyme (13), and no significant metabolism occurs in other organs (57). The metabolism of caffeine deals primarily with the 3-, 1-, and 7-demethylations that lead to the production of 1, 7-dimethylxanthine (17X, paraxanthine), 3, 7-dimethylxanthine (37X, theobromine), and 1, 3-dimethylxanthine (13X, theophylline), respectively (87).

Caffeine 3-demethylation (17X formation) is the most prominent reaction and accounts for 83.9±5.4% of demethylation reactions; the caffeine 1-demethylation accounts for 12.1±4.1% and the 7-demethylation for 4.1±1.4% in human subjects (88). It has long been known that an inducible enzyme, a member of which is now called the CYPIA family, catalyzes caffeine metabolism (89) and the 3-demethylation, in particular (90). Butler *et al.* (91) in 1989 specified isozyme to be CYPIA2, and this was confirmed 2 years later by Berthou *et al.* (92). Complementary deoxyribonucleic acid (cDNA) produced human CYPIA2 and five other P450s (IIA6, IIB6, IIE1, IIIA4, and IIIA5) were tested for their ability to catalyze the biotransformation of caffeine and caffeine metabolites. CYPIA2 is the only enzyme that catalyzes the 3-demethylation of caffeine (93), confirming the data of Butler *et al.* (91). However, CYPIA2 also catalyzes the 1- and 7-demethylations of caffeine, with rates representing 13.3% and 6.6%, respectively, of the rate of caffeine 3-demethylations

(88). It means that CYPIA2 activity accounts for most of the systemic caffeine clearance in most subjects as shown in Figure 2. This observation is supported by the *in vivo* inhibition of caffeine metabolism by furafylline, a specific and potent inhibitor of human CYPIA2 (94, 95). Paraxanthine subsequently undergoes two transformations, one of which is 8-hydroxylation (by CYPIIA 6 and CYPIA2) to form 1, 7-dimethylurate (17U); the other metabolic route involves 7-demethylation (by CYPIA2) and acetylation (by N-acetyltransferase) to generate either the acetylated ring split product, 5-acetylamino-6-formylamino-3-methyluracil, or 7-demethylation to form 1-methylxanthine (1X) with subsequent 8-hydroxylation (by xanthine oxidase) to form 1-methylurate (1U) (93, 96, 97). The major pathway of theophylline metabolism involves 8-hydroxylation, resulting in the formation and excretion of 1, 3-dimethyluric acid (13U). Theobromine (37X) is excreted as 3X and 7X. The 7X formation is entirely catalyzed by CYPIA2 (93).

3.4 Elimination

Caffeine and its metabolites are excreted mainly by the kidney (98). Little of ingested caffeine is excreted as such in the urine. The high lipid solubility of caffeine would mean that urinary excretion would be very slow unless there were an active transport mechanism, which there is not. The great majority of ingested caffeine is metabolized, some to CO_2 and water, but much to polar metabolites that are then rapidly excreted, so that the concentrations of metabolites that circulated are mostly small. The metabolites are of low biological activity, and this combined with the low concentrations that exist means that they are not of consequence to health (99).



137X	=	CAFFEINE
17X	=	PARAXANTHINE
13X	=	THEOPHYLLINE
37X	=	THEOBROMINE
13U	=	1,3-DIMETHYLURATE
17U	=	1,7-DIMETHYLURATE
1X	=	1-METHYLXANTHINE
3X	=	3-METHYLXANTHINE
7X	=	7-METHYLXANTHINE
1U	=	1-METHYLURATE
AFMU	=	5-ACETYLAMINO-6-FORMYLAMINO-3-METHYLURACIL
AAMU	=	5-ACETYLAMINO-6-AMINO-3-METHYLURACIL
NAT	=	N-ACETYLTRANSFERASE
XO	=	XANTHINE OXIDASE

Figure 2. The metabolism of caffeine

Caffeine is eliminated by first-order kinetics in human (8, 56, 85). The plasma $t_{1/2}$ of caffeine in human is generally 2.5 to 7.5 hr (12, 100, 101). Systematic studies have shown that $t_{1/2}$ (hr) values vary with species: mouse, 0.7; rat, 0.8; rabbit, 1.6; and monkey, 3.2 (12). The $t_{1/2}$ of caffeine decreases gradually after birth and reaches adult values at about the age of six months (102, 103). A serum clearance of 31.5 ml/hr.kg in 1-2.5 month-old infants increases to a mean maximum value of 331.7 ml/hr.kg in 5-6 month-old infants (104). No significant difference in the elimination of caffeine in young and elderly subjects has been found, although a slight decrease in plasma caffeine binding was observed in the older group (7). The metabolic disposition or volume of distribution of caffeine does not differ between men and women (57, 84). In women, the use of oral contraceptives is shown to double the $t_{1/2}$ of caffeine (84). A gradual prolongation of the $t_{1/2}$ is also shown during pregnancy (105), and caffeine clearance is increased by more than 3-folds at 2-12 weeks postpartum (106). A small percentage (0.5-4%) of an ingested dose of caffeine is excreted unchanged in urine. Caffeine is also excreted in bile (57) and is found in saliva (107), semen (74, 75) and breast milk (108); it was also detected in umbilical cord blood (109). As salivary concentrations correspond to 65-85% of plasma concentrations, they can be used to predict serum concentrations (27, 28, 80-84).

3.5 Blood-brain transfer

Kinetic studies in rodents have emphasized that plasma caffeine and metabolite levels closely reflect brain concentrations. The series of work by Kaplan and his coworkers (22, 23, 110) showed that the slope of the regression line obtained by plotting brain versus plasma concentrations was 1.0, 0.4, 0.3, and 0.5 for caffeine, theophylline, paraxanthine, and theobromine, respectively, during a 1- to 4-hr period

after 40 mg/kg, i.p. Zhi and Levy (111), using a continuous i.v. infusion paradigm, observed that the regression line, when plotting brain (or CSF) to serum concentrations of caffeine over a range of approximately 0.5 to 40 μg/ml, was linear and had a slope of 0.6, suggesting that caffeine, within the plasma concentration ranges used, moves into the brain by simple diffusion. McCall *et al.* (73), using the Oldendorf rat carotid-brain clearance model (movement into brain following direct intracarotid injection), found that caffeine appeared to enter the brain by a simple diffusion process and by a low-affinity (Km =5.4 mM), saturable transport (73).

The movement of caffeine into the brain following direct intracarotid injection is rapid and reflects uptakes that are similar to free water distribution (73), with nearly complete following a single passage. Consistent with this rapid movement, significant brain levels are observed within 5 min, even after oral administration of caffeine, with peak levels being observed within 30 min (112).

With regard to clearance from brain, at a low oral dose (1 mg/kg, p.o.), the $t_{1/2}$ of caffeine in brain and plasma are identical (130 min), whereas at a higher dose (10 mg/kg, p.o.) the $t_{1/2}$ in brain increases significantly (189 versus 81 min, respectively) (112). After i.p. injection, peak plasma and brain levels are observed at the earliest times examined (5 min) and are approximately 30 μg/ml and 40 μg/ml, respectively. The $t_{1/2}$ in brain and plasma do not differ statistically (1.3 and 1.6 hr, respectively) (22, 23, 110).

In human adult, there are no data regarding the redistribution of caffeine into brain or CSF. However, there is no reason to believe that human differs from other animals in this regard. Moreover, caffeine has been shown to achieve significant CSF

to blood ratios in newborns, indicating that, as in animals, this substance gains significant and rapid access to CNS sites (113).

4 Factors governing caffeine disposition

In addition to species differences, a number of factors appear to contribute to the absorption and kinetics of caffeine.

4.1 Age

Rates of caffeine clearance are similar in older and younger adults (7). In neonates, there is evidence that the metabolic clearance of caffeine occurs more slowly because of the lack of development of the mixed-function oxidase systems. Thus, the $t_{1/2}$ was reported to be 65 to 103 hr in preterm infants, 82 hr in term infants, 14 hr in 3- to 4-month-old infants, and 2.6 hr in 5- to 6-month-old infants; the latter $t_{1/2}$ corresponds to values reported in adults, generally 3 to 5 hr (62).

The maturation of the different pathways of caffeine metabolism during infancy was studied in four premature newborn infants and 10 older infants receiving caffeine citrate solution (114). It was found that total demethylation and N3- and N7-demethylation increased exponentially with postnatal age and the plateau was reached by 120 days, whereas N1-demethylation showed no variation with postnatal age. It was suggested that N3-demethylation was more important in young infants than in adults and that maturation of N1-demethylation occurred later than 19 months of age. 8-Hydroxylation was mature as early as 1 month of age and might be higher in infants than in adults. Acetylation was not mature before at least 1 yr of age and the differences in maturation rate of acetylation might be related, in part, to genetic acetylator status (114).

4.2 Genetics

Caffeine metabolism is subject to the polymorphism associated with N-acetyltransferase, i.e., it exhibits both fast and slow acetylation rates. Ethnic groups display significant differences in the proportion of the population regarded as slow acetylators, as well as in the generation of other metabolite (101). The frequency distribution of N-acetylation of caffeine was determined in 140 unrelated healthy Japanese subjects by measuring the amount of two main metabolites of caffeine, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X), in urine after an oral dose of caffeine (115). N-acetylation capacity for caffeine appeared to be polymorph in Japanese population, 10.7% of the Japanese subjects were phenotype as slow acetylators, whereas 89.3% were phenotype as rapid ones (115). The frequency of slow acetylators in this study was similar to that reported previously for isoniazid (116) and dapsone (117) and polymorphism in Japanese population. The determination of caffeine metabolite ratios can serve as a probe for acetylation phenotyping (118).

4.3 Smoking/enzyme inducers

Smoking stimulates caffeine clearance, such that the $t_{1/2}$ may be reduced by as much as one-half (119). Half-lives of caffeine in the smokers and heavy caffeine users were shorter (3.2 and 4.1 hr, respectively) than those in the nonsmokers and non-caffeine users (5.1 and 5.3 hr, respectively) (120). After stop smoking for only 3 or 4 days, the rate of caffeine metabolism is substantially slower (121). The mechanism underlying this effect is likely to be involved in drug metabolism, reflecting the induction of hepatic enzymes such as cytochrome P450IA2 by smoking (122). Polycyclic aromatic hydrocarbons, such as polychlorinated or polybrominated

biphenyls, or rifampicin similarly increase the rate of caffeine demethylation in human (90).

4.4 Drugs

A variety of co-administered drugs are reported to lead to an impairment of caffeine elimination, often by competition at the enzymatic level. These include oral contraceptives (84), cimetidine (119), disulfiram (123), alcohol (124), and idrocilamide (125).

4.5 Exercise

When given a single oral dose of 250 mg caffeine to human, moderate exercise increased the peak plasma concentration and reduced the $t_{1/2}$ (126). This reduced $t_{1/2}$ (4.0 to 2.3 hr) appears paradoxical because exercise reduces hepatic blood flow and caffeine is largely metabolized in the liver, but such changes in metabolism have also been described for antipyrine, which is also cleared in a manner similar to caffeine (127).

4.6 Pregnancy

A prolongation of the $t_{1/2}$ of caffeine (2.5 to 7 times) occurs during the second and third trimester (128). There is no placental barrier to the passage of caffeine from the mother to the fetus (129). Therefore, caffeine crosses the placenta and enters the fetal circulation by passive diffusion (130).

4.7 Disease

Caffeine is metabolized primarily by liver enzyme (92). It is, therefore, not surprising that liver diseases (cirrhosis, viral hepatitis, and alcohol-induced fatty liver) can decrease the rate of caffeine demethylation in human (101).

5 Pharmacological effects of caffeine

5.1 Overview of caffeine and methylxanthine actions

Caffeine has pharmacological effects on the function of cardiovascular, respiratory, renal, and nervous systems (16, 17). It appears that blockade of adenosine receptors by caffeine has a significant role in pharmacology, but effects on calcium storage, phosphodiesterases, and perhaps other, undefined targets cannot be excluded (17). In particular, the effects of caffeine on behavior are complex and are not readily explained by blockade of A1-and/or A2-adenosine receptors alone (131). Caffeine appears to affect function of norepinephrine, dopamine, serotonin, acetylcholine, GABA, and glutamate systems in brain. The relevance of alterations in function of such systems and of adenosine systems to the tolerance that develops during chronic caffeine treatment requires further study, as does the basis for the withdrawal syndrome after chronic caffeine treatment. Adenosine receptors are upregulated during chronic treatment with caffeine, but this alone does not fully explain the tolerance to a receptor antagonist (132).

The mechanism (s) underlying the pharmacological effects of caffeine and theophylline have been and remain controversial. Three hypotheses have been advanced: (a) mobilization of calcium; (b) inhibition of phosphodiesterases; and, most recently, (c) antagonism of adenosine receptors. At present, it is generally conceded that antagonism of adenosine receptors (132), at least in part, underlies the pharmacological effects of low doses of caffeine and theophylline, while inhibition of phosphodiesterases and mobilization of calcium become more significant at higher doses. Wide ranges of pharmacological effects of caffeine/theophylline are presented in Table 3. In many instances adenosine or adenosine analogs have pharmacological

effects opposite to those of the methylxanthines (Table 3) (131). Much further research is nevertheless needed to clarify our understanding of how caffeine affects human beings. The rapid development of tolerance to most *in vivo* effects of caffeine in humans and animals remains an enigma.

5.2 Adenosine receptor antagonism

Purine receptors are classified as nucleoside (P1) or nucleotide (P2) receptor (133). Adenosine (P1 purine) sites are further classified as A1 or A2 receptors. The original classification of adenosine receptors was based on respective effects on the adenylate cyclase system: lower doses of adenosine inhibited adenylate cyclase and decreased cAMP levels via an A1 receptor, and higher doses of adenosine stimulated adenylate cyclase and enhanced cAMP levels via an A2 receptor (134).

Adenosine has widespread effects in the body, including regulation of coronary blood flow and effects on the kidney and the immune system. In the brain, there are A2 receptors in the striatum, olfactory tubercle, and nucleus accumbens. Adenosine is a CNS depressant, and its actions are antagonized by caffeine and theophylline (135).

Effects of adenosine on the cAMP transduction system are mediated via nucleotide-binding proteins (G-proteins) (136). These effects are not, however, the only mechanism for signal transduction by adenosine. Adenosine modulates K^+- and Ca^{2+}-channel activity in neural and nonneural tissues (137). In many cases, these actions of adenosine are mediated via G-proteins, without the involvement of cAMP (137, 138). Adenosine also modulates the effects of histamine on inositol phospholipid metabolism, i.e., inhibition in rat (139) and augmentation in guinea pig (140). Such effects may be secondary to alterations in intracellular Ca^{2+} fluxes (139).

Table 3. Comparison of pharmacological effects of methylxanthines and adenosine analogs (131)

System	Effect of caffeine/theophylline	Effect of adenosine analog
Cardiovascular		
Heart	Positive ionotropic/chronotropic	Negative iotropic/chronotropic
Vasculature		
Coronary	Dilation	Dilation
Renal	Dilation	Constriction
Peripheral	Dilation	Dilation
Central	Constriction	Dilation
Respiratory	Bronchodilation	Bronchodilation or bronchoconstriction
	Stimulation of respiration	Inhibition of respiration
Renal	Diuresis	Antidiuresis
	Stimulation of renin release	Inhibition of renin release
Gastrointestinal	Stimulation of gastric secretion	Inhibition of gastric secretion
Smooth muscle	Relaxation	Relaxation
Adipose	Stimulation of lipolysis	Inhibition of lipolysis
Platelet	Inhibition of aggregation	Inhibition of aggregation
Central nervous	Stimulation	Depression

Methylxanthines, such as caffeine and theophylline, behave like competitive adenosine antagonists in ligand binding studies (132), in experimental animal studies (141) and in human *in vivo* situation (142). An antagonism between adenosine and caffeine in human vascular beds might have clinical importance. This may be especially true because endogenous adenosine may play a role in several physiologic processes of the cardiovascular system, such as postischemic hyperemia (143), exercise-induced vasodilatation (144), and the regulation of coronary blood flow (145).

6 Physiological effects of caffeine

Caffeine, theophylline, and theobromine share in common several physiological effects. They relax smooth muscle, notably bronchial muscle, stimulate the CNS, stimulate cardiac muscle, and acts on the kidney to produce diuresis (16, 17).

6.1 Central nervous system effects

Caffeine's popularity is, in part, due to its stimulatory effects on the CNS. At low doses (up to 2 μg/ml in blood), caffeine stimulates the CNS, and this effects is perceived by many caffeine users as beneficial. High blood concentrations (10-30 μg/ml) of caffeine may produce restlessness, excitement, tremor, tinnitus, headache, and insomnia (17). Caffeine may affect psychomotor coordination, sleep patterns, concentration, mood, and behaviour (18-21). Caffeine also increases psychomotor coordination (19). These results in decreased motor time and increased vigilance (19, 21). The ingestion of 85 to 250 mg of caffeine, the amount contained in 1 to 3 cups of coffee, produces an increased capacity for sustained intellectual effort and decreases reaction time, however, tasks involving delicate muscular coordination and accurate

timing or arithmetic skills may be adversely affected (57). Caffeine's stimulatory effect on quality and quantity of sleep exhibits wide individual variation. It may be dose-dependent, and may be subject to development of tolerance. When caffeine is given to non-caffeine drinkers, it cause a delayed sleep onset (146), greater increase in sleep latency, decreased sleep time, and lower sleep quality than the chronic users. Caffeine has been found to increase Stage 2 sleep and decrease Stages 3 and 4 sleep and to cause increased awakenings and arousability from Stage 2 sleep (18).

Variation of caffeine's effects on mood may be due to the development of tolerance or to caffeine intolerance. Effects range from increased alertness in chronic caffeine consumers to anxiety in non-caffeine consumers (147). Caffeine's effects may cause problems in the diagnosis and treatment of psychiatric disorders. Schizophrenic and mentally handicapped aggressive inpatients showed improvement when caffeine intake was decreased. Psychiatric patients may consume large amounts of caffeine because of thirst induced by psychotropic drugs. This could cause symptoms of high caffeine intake, including agitation and anxiety, possibly compounding an existing problem. Coffee and tea have been found to form an insoluble precipitate with a number of antipsychotic drugs, which may render them unavailable for absorption and, therefore, ineffective in the treatment of psychiatic disorders.

Dimpfel *et al.* (148) studied the effects of caffeine on the CNS under resting conditions and under conditions of mental load in healthy males by quantitative EEG analysis. Single oral doses of 200 and 400 mg caffeine induced a distinct decrease in spectral power density, especially in the alpha and theta ranges. The decrease in the delta range was seen clearly with 400 mg caffeine. The caffeine-dependent decrease

in theta and delta power observed after 400 mg caffeine were not found during the concentration performance test in the presence of caffeine. These findings indicated the influence of caffeine on the brain to be generalized. This study also showed the influence of caffeine during cognitive load to be expressed differently than under resting conditions.

6.2 Cardiovascular effects

The cardiovascular effects of caffeine concomitant with other life habits have long been questioned. Caffeine has been said to raise, lower, or not affect blood pressure (BP) and heart rate (HR) and to raise or not affect levels of epinephrine and norepinephrine (147). Low concentrations of caffeine may produce a small decrease in HR; high concentrations may produce tachycardia (147). Izzo and coworker (149) observed that caffeine (250 mg) produced a significant increase in both systolic and diastolic BP in caffeine-naïve subjects compared with individuals who regularly ingested caffeine. The increase in BP showed a significant positive correlation with age. HR declined slightly by 2 to 3 beats/min in both groups. Caffeine also produced a significant increase in plasma epinephrine concentration in the caffeine-naïve subgroup. This effect was more likely to occur in younger subjects. On the other hand, there were no significant changes in norepinephrine concentration, plasma renin activity, or vasopressin concentration in comparison with baseline values.

The acute pressor effect of caffeine in caffeine-naïve subjects has also been confirmed (150). After a period of abstaintion from caffeine-containing foods, a transient increase in BP was observed. Daily caffeine ingestion produced a small increase in BP for several days, with levels falling thereafter and remaining at baseline for the remainder of the four-week period of continued caffeine use (150).

Administration of caffeine after a minimum 24 hr abstinence period to various groups of subjects ranging from total caffeine abstainers to heavy coffee drinkers caused a slight increase in BP and a decrease in HR. Previous caffeine use had little effect on the cardiovascular responses, suggesting that some of caffeine's tolerance may dissipate within 24 hr (120).

Smits *et al.* (151) also reported a small increase in BP (5/7 mmHg) in young healthy subjects who had abstained from caffeine for one to two days. Despite the relatively small changes in BP, there was a significant negative correlation between basal plasma caffeine concentrations and a rise in systolic BP after an acute dosing with caffeine, whereas patients who had low basal caffeine concentrations exhibited the greatest increase in BP. However, their data suggested that few regular consumers of caffeine-containing beverages would have had a sufficiently low basal caffeine concentration to show a pressor response to caffeine ingested as part of their daily routine. Charney *et al.* (152) found no significant changes in BP in 11 normal coffee drinking subjects after a rather high dose (10 mg/kg) of caffeine in comparison with placebo.

From large epidemiologic studies in Algeria, both tea and coffee drinkers exhibited a slightly higher (2 to 3 mmHg) diastolic BP than did abstainers, with the systolic BP being similar for both groups. The small difference in diastolic BP was partly accounted for by age but remained statistically significant after multivariate analysis (153). In a similar survey in Paris, no difference in diastolic BP between coffee users and abstainers was found. However, systolic BP was significantly correlated with coffee consumption, although the overall increase in BP before adjustment for other variables was only 3.5 mmHg (154).

Astrup *et al.* (155) reported that only small and insignificant changes could be detected in systolic and diastolic BP after 100 and 200 mg caffeine and the changes did not differ from those observed after placebo. In contrast, 400 mg caffeine increased both systolic and diastolic blood pressure within 60 min after the intake. There was a positive correlation between caffeine dose and integrated increase in systolic BP whereas the relation between serum caffeine concentration and systolic BP was not statistically significant. Analysis showed a linear, positive correlation between the integrated responses of systolic and diastolic BP. The HR increased, on average, 4 beats/min after placebo. After all three doses of caffeine, a diphasic response was observed. Initially, HR decreased by 3-4 beats/min within 30-90 min after the intake and subsequently increased at 90-180 min. Only after 400 mg caffeine was the final increase significantly above baseline.

The acute hemodynamic effects of Italian coffee and 200 mg purified caffeine were investigated in healthy non-coffee drinkers. It was found that both coffee and caffeine significantly decreased rest flow and increased peripheral resistance. Systolic BP increased by 10% and diastolic BP increased by 5% for at least 2 hr. No variation in HR or cardiac contractility was found. Therefore, it has been suggested that Italian coffee and caffeine increased blood pressure via vasoconstriction (156).

In summary, acute administration of caffeine in coffee-naïve subjects results in a modest pressor effect, with biphasic changes in heart rate and significant increase in plasma catecholamines and sympathetic nerve activity. Inhibition of baroreflex activity plays an important role in these effects. This inhibition was clarified in normotensive subjects, in whom acute caffeine administration significantly reduced the reflex bradycardia evoked by a vasopressor agent. The exact mechanism of these

hemodynamic effects is likely to involve an antagonism of adenosine receptors within the CNS. Whereas adenosine infusion in conscious humans increases blood pressure, its administration in the CNS results in hypotention and bradycardia. More relevent, administration of minute doses of caffeine or related methylxanthines in the brainstem causes marked inhibition of baroreflex activation.

6.3 Gastrointestinal effects

Man is relatively sensitive to the effects of methylxanthines on gastric secretion. Moderate oral or parenteral doses of caffeine cause secretion of both acid and pepsin (157), perhaps by inhibition of phosphodiesterase activity in the gastric mucosal cells. Coffee and decaffeinated coffee stimulate gastric acid and pepsin secretion more than caffeine alone, indicating that other compounds found in coffee may also exert a significant effect on the gastrointestinal tract. The increase in gastric secretion could contribute to the development of ulcers, although this relationship remains unknown and has not been adequately studied (147).

6.4 Renal effects

The effects of caffeine and theophylline on renal function, namely, diuresis, increased blood flow, and stimulation of renin release, appear to be linked to blockade of adenosine receptors. Adenosine analogs have the opposite effects to xanthine (158). Xanthines, including theophylline and caffeine, reverse adenosine-mediated reduction in glomerular filtration, vasoconstriction, and inhibition of renin release (159-161). Enprofylline, a xanthine with low activity as an adenosine antagonist, has no diuretic activity.

There is some evidence indicating that generation of prostaglandins may be involved in xanthine-elicited diuresis. Indomethacin reduces theophylline-induced diuresis and excretion of prostaglandin E (162, 163). Theophylline > caffeine = theobromine, all stimulate prostaglandin production. Theophylline increases prostaglandin synthesis in rat kidney slices (164). The role of prostaglandins in the actions of xanthines in other biological systems has received little attention. Caffeine and theophylline were proposed to act as prostaglandin antagonists due to inhibition of phosphodiesterases in mesenteric arteries (165).

6.5 Respiratory System

In the respiratory system, it remains uncertain to what extent caffeine and theophylline exert anti-asthmatic effects through blockade of adenosine receptors, and to what extent through phosphodiesterase inhibition. With respect to bronchodilation, inhibition of phosphodiesterase may prove to be prime importance in view of the high activity of enprofylline, which is a potent phosphodiesterase inhibitor and a weak adenosine antagonist (166). As to the inflammatory component to asthma, adenosine-receptor mediated stimulation of release of histamine and serotonin from mast cells is relatively insensitive to blockade by xanthines (167, 168). Theophylline, at a concentration of 10 μM, stimulates eosinophils as does 8-phenyltheophylline, a xanthine with little activity as a phosphodiesterase activator (169). Adenosine has the opposite effect. Thus, with regard to the role of eosinophils in inflammatory responses, theophylline may act via blockade of adenosine receptors. Bronchospasm can be elicited by adenosine analogs via a pathway apparently involving the postganglionic cholinergic vagal nerve (170). It was not determined whether the

response was sensitive to blockade by xanthines, but presumably it would be. In trachea, adenosine analogs can cause contraction via A1 receptors, but the major effect in constricted muscles is to cause a relaxation (171-174). Relaxation is mediated both by a xanthine-sensitive A2-adenosine receptor and a xanthine-insensitive adenosine receptor (175). Xanthines, including caffeine, antagonize both adenosine-elicited contraction and cause relaxation in smooth muscle (170, 171). However, in one study the relaxation of guinea pig trachea by N^6-R-phenylisopropyladenosine was antagonized by theophylline and 8-phenyltheophylline, while the contraction was not (174).

6.6 Teratogenic effects

The effects of caffeine are not restricted to adults. Caffeine crosses the placenta and enters the fetal circulation and, therefore, may harm the fetus (130). During fetal and postnatal development, there is a lack of enzymes needed to demethylate caffeine. The plasma caffeine $t_{1/2}$ in newborns ranges from 32 to 149 hr. The adult pattern of elimination of partially demethylated metabolites does not develop until approximately 7 to 9 months after birth (114).

Caffeine produces several effects that may have important influences on fetal development. It decreases DNA polymerase activity, shortens DNA replicating units, and increases cAMP and cGMP levels (130). The use of caffeine during pregnancy appears to stimulate the fetal heart (176). A study of the effects of caffeine on the isolated fetal heart showed an age- and concentration-dependent increase in the spontaneous contraction rates of the fetal hearts.

The possible effects of caffeine use during pregnancy include skeletal abnormalities, a decrease in intrauterine fetal growth, and a lower birth weight. High caffeine intake has been associated with increased fetal loss and an increased frequency of congenital malformations in human (130, 177). In 1980, the U.S. FDA advised pregnant women to avoid or use sparingly caffeine-containing foods and beverages (147).

6.7 Carcinogenic effects

A possible effect of coffee drinking on the risk of cancer in human has been a matter of debate in the literature for many years (178). Recently, an IARC working group evaluated the risk of coffee and caffeine for cancer in human (179). On the basis of epidemiological studies, they concluded that there is limited evidence in human that coffee drinking is related to cancer of the urinary bladder, and that more research are needed in order to come to a definite conclusion.

7 Caffeine dependence and withdrawal

Chronic administration of caffeine and other methylxanthines leads to tolerance to the psychomotor and physiological actions of that agent. Removal of the agent for a period, which permits its clearance (3 to 24 hr), results in a reduced locomotor function (180), disruption of operant behavior (181) in animals and human, irritability, muscle twitching, and headaches (182). Subjects undergoing voluntary cessation of caffeine intake display a transient, but significant, incidence of headache frequency during the next 2 to 3 days (183). These observations have led to the speculation that under a number of conditions, such as following surgical procedures

when normal food consumption is restricted (184) or in the case of agent abuse when the subject may abuse large quantities of caffeine (185), there may be a hypersensitive state that would be treatable with a caffeine-containing medication.

Although chronic caffeine intake results in a modest upregulation of adenosine receptors, this does not appear to account for tolerance to caffeine (186, 187). Caffeine withdrawal has been shown to increase intracranial blood volume (188), a phenomena apparently reversed by caffeine treatment. Such a mechanism has been speculatively offered for the ability of caffeine to diminish headaches accompanied by low CSF pressures.

It appears likely that several symptoms may well be involved in the discomfort associated with caffeine withdrawal, such as (a) caffeine withdrawal-associated fatigue and mental state could occur in the absence of reported headache and (b) fatigue could precede the report of headache. It should be emphasized that relatively few studies of the biochemistry of the caffeine withdrawal state have been accomplished (189).

PART 2 PSYCHOMOTOR PERFORMANCE

Psychomotor performance can be defined as the ability of an individual to process and react to specific external information. To elicit significant improvements in psychomotor performance, less fit subjects, young subjects, exercise training for longer periods of time, a more complex task may be required (190). Psychomotor speed is a rubrict describing the speed with which an individual can perform a task which involves reacting motorically to an environment stimulus. It is dependent upon

efficient synaptic transmission in the mobilization of elaborate neuronal networks in many areas of the brain (191).

In general, the tasks for psychomotor performance can be classified into four categories: tasks for measuring time and speed, tasks for measuring accuracy, tasks for measuring extent of performance and secondary tasks. Two of the most important variables of motor behavior are speed of reaction and speed of movement. Since psychomotor speed is the speed with which an individual can perform a task which involves reacting motorically to an environmental stimulus. Then, tasks used to assess psychomotor speed are tasks such as simple and choice reaction time, tapping test, and movement time and response time. These tasks require fast motor organization, integration, and excretion (191).

1 The reaction time

The definition of the reaction time (RT) is that, it is the time between the occurrence of a particular stimulus and response to that stimulus. During the reaction response the neural impulses are transmitted via sensory pathways to the brain, processed there, then impulses transmitted via motor pathways to muscles, and movement is started. Elsass (192) suggested that reaction time had psychological as well as physiological aspects. A great number of reaction time experiments investigated the relation between the perceptual and motor components of simple human performance (193, 194). The interference of neuronal pathway involved at any points will usually prolong the RT (195).

1.1 Reaction time components

Fractionation of reaction time response by electromyographically (EMG) recording the initiation of muscle action potentials in the agonist musculature has separated into 2 components of the reaction time: the premotor time (PMT), a central processing component and the contractile time (CT) or the motor time (MT), a muscle contractile component (196).

The premotor time (PMT) is defined as the time from stimulus onset to the appearance of action potentials at the involved muscle. The PMT consists of two subsequent parts.

a) Reception time: the period between the moments where the stimulus is given and perceived.

b) Decision period: the time necessary for the response to perceived stimulus.

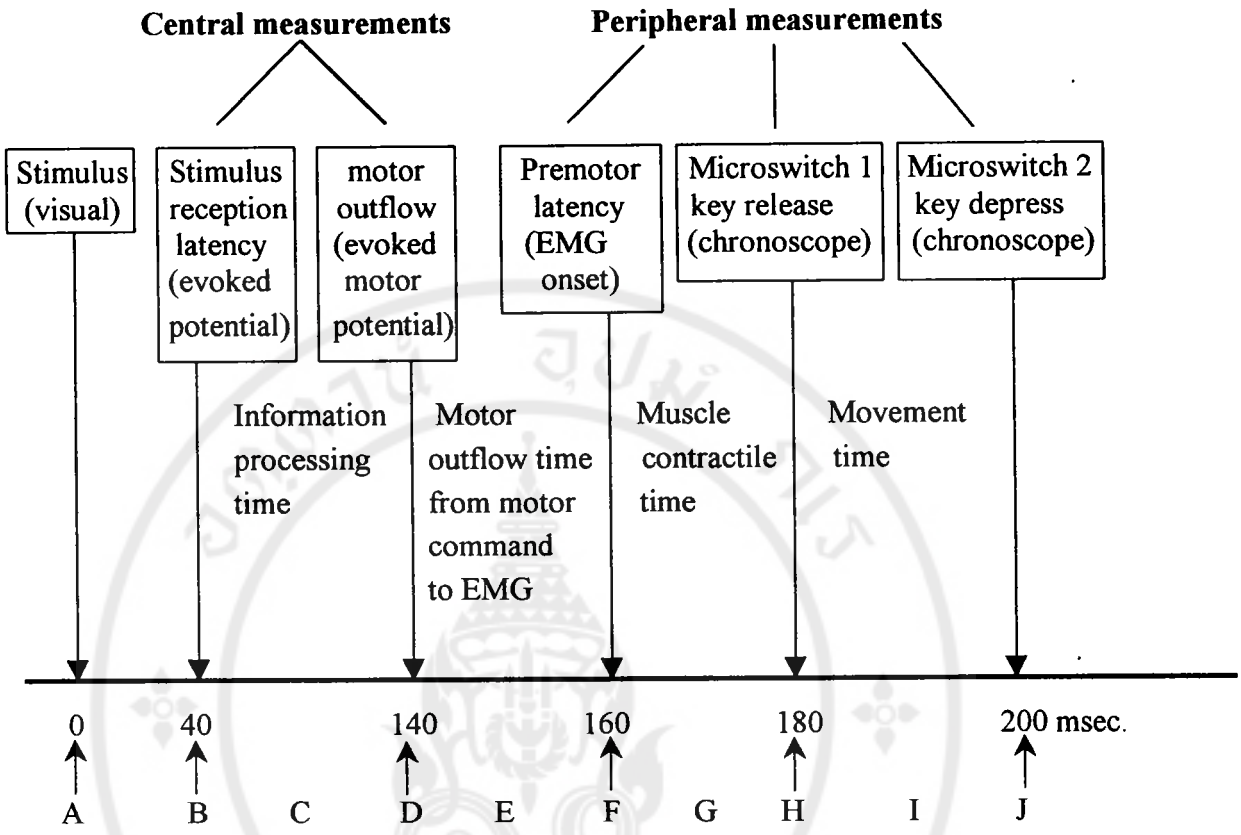
Motor time (MT) is defined as the time from the appearance of the muscle action potential (EMG) to the movement of the limb off or upon a sensitive microswitch.

Spirduso (191) suggested that PMT and MT should be identified as the central and peripheral components, respectively (Figure 3).

1.2 Classification of reaction time

Reaction time can be classified into two different types:

a) The simple reaction time (SRT) is the time from stimulation (using various forms of stimuli) of an appropriate sense organ to the quickest voluntary response of a given effector organ (197). There is only one stimulus and one simple response.



A = Visual stimulus initiated; chronoscopes initiated
B = Evoked potential in occipital cortex
C = D-B; Dependent on task complexity
E = F-D; Nerve conduction time, including an unknown but relatively small number of synaptic transmission times supraspinally and at the spinal motor pool
F = Onset of electromyographical interference pattern
G = H-F; Dependent on mechanical and electronic characteristics of microswitch circuitry
H = Reaction time; Release of microswitch and termination of first chronoscope
I = J-H; Dependent on distance between microswitchs
J = Depression of microswitch and termination of second chronoscope

Figure 3. Fractionation of a reaction time task into central and peripheral components

This is a conceptual scheme of the process; differences in methodology and interpretations of components exist among investigators.

Donder (198) believed that SRT was a baseline that took into account factors (such as total speed of nerve conduction) that were components of more complex reaction times. Repperger *et al.* (199) defined that this reaction time can be considered as the sum of six components: activation of sensor, afferent signals to brain, interpretation of signals, selection of response, efferent signals to muscles, and activation of muscular response.

b) The choice reaction time (CRT) is the time involving the mental operations of stimulus identification and response selection. There is more than one stimulus and more than one response. Each stimulus has its own unique response. CRT is considered to be a primary indicator of overall cerebral function (200).

The SRT can be either warned or unwarned reaction time.

a) Warned reaction time is obtained from a subject who has been warned a few seconds before an actual stimulation; therefore subject is mentally more prepared to response to the actual stimulus.

b) Unwarned reaction time is obtained from a subject who has not been warned before an actual stimulation; therefore the subject is mentally less prepared to respond to the given stimulus (199).

The warned SRT is therefore shorter than the unwarned SRT because less time is taken in the "decision-making" in the brain during the stimulus-response events.

2 Factors affecting reaction time

Elsass (192) suggested that the duration of the RT was influenced by a function of three variables:

2.1 Stimulus characteristics

a) Stimulus modality: RT has been investigated for its dependence on stimulus modality, such as, e.g., visual, auditory and tactile stimulation. The visual RT from eye stimulation to response of a given effector organ seems to be consistently longer than the auditory RT from ear stimulation to the response of the same effector (197). The RT norms vary from one study to another depending on the stimulus presentation and the response device (202).

b) Stimulus pattern complexity: The SRT is shorter than the CRT. A simple stimulus, which requires little cerebral processing, will result in a faster RT than a complex stimulus which requires discrimination (202). CRT has a proportionately larger percentage of central, premotor activity than does SRT (203).

c) Stimulus intensity: Hasbroug *et al.* (204) showed that tactile RT is shorter for the more intense stimulus than for the less intense. In addition, other investigators studied the effects of loudness on the latency of evoked potentials and on SRT and found that both RT and the evoked-potential latency increased with decreasing stimulus intensity.

2.2 Organism characteristics

a) Subject's age: The RT is longer in very young children. The RT in older adults (50-80 yr) is longer than in younger adults (20-45 yr) (209). In addition, the recent data in 1994 on cross-sectional and longitudinal analysis of age and RT showed of simple RT across decades and increased variability with age in subjects who ranged in age from 17 to 96 (210). Investigations employing the fractionated RT technique have generally corroborated the idea that differences in total reaction time between young and old individuals that cause of age related lengthening of RT are due

primarily to a lengthening of the premotor or CNS component rather than the motor time of muscular component (200).

b) Sex: A slight difference in reaction and movement time has been shown between sex, with men slightly faster (207). The other study by Welford (201) showed a small but significant difference on the basis of large materials: Women react more slowly than men do in occurrence both in older age and adolescence groups (208, 209).

c) Adaptability: Adaptability can also be shown to affect RT, which results from the extent of learning the task (199). With accumulated practice, RT and decision time might be significantly reduced by decreasing the time necessary to make a decision and improving on precision of movement (210, 211).

d) Physical activity level: The individual differences in speed of reaction and speed of movement are, to a considerable extent, dependent on neuromuscular coordination abilities. It has been found that athletes possess better RT than the non-athletes (212).

2.3. Response characteristics

a) Speed and accuracy of response: Fitts (213) suggested that the greater the accuracy of movement, and the greater its extent, the more time it takes to perform the movement. These cause the movement time being linear with the information measure of the task.

b) Response condition: Bjorklund (214) measured reaction time in two conditions: a key-press and a key-release. The main finding for the perceptual phase was the more rapid RT in the key-press condition compared to the release condition.

It is assumed that the key-press condition requires only minor sensory feedback while the key-release condition requires more sensorimotor coordination.

CHAPTER IV

MATERIALS AND METHODS

PHARMACOKINETIC STUDY

1 Chemicals

The following agents were obtained commercially in the highest purity available and were used without further purification: caffeine anhydrous and internal standard, 8-chlorotheophylline from Sigma Chemical Co.; methanol (HPLC grade), zinc sulfate, potassium phosphate (monobasic) and sodium phosphate (dibasic) from J. T. Baker Chemical. Deionized distilled water (grade A) was obtained from Department of Biochemistry, Faculty of Science, Mahidol University. The energy drink with the trade name "LIPOVITAN-D", produced by Osothsapha (Tek Heng Yoo), Ltd., Thailand, was obtained from local store.

2 Subjects

Twelve healthy Thai males participated in the study. Their ages were in the range of 22 to 34 yr and their body weights between 48 and 72 kg. Physical examination, routine blood chemistry screening (liver function tests, kidney function tests, complete blood count) and urinalysis revealed that the subjects were healthy. The data of usual daily intake of caffeine were obtained by retrospective questionnaires. The study protocol was explained to and the written informed consent was obtained from all participants. They had to abstain from caffeine-containing beverages and food at least 24 hr prior to the investigation and during the study.

3 Study design

The study aimed to determine the pharmacokinetics of a single oral dose (200 mg) of caffeine containing in 4 bottles of energy drinks. After an overnight fast, the subjects drank 4 bottles of the energy drink (400 ml) within 2 min. Fluid was withheld for the next 2 hr and no food was allowed for 4 hr after dosing.

4 Methods

After an overnight fast, control blood samples (5 ml) were collected from a forearm vein through a heparinized indwelling cannula at 7.00 a.m. (time 0). After that, the subjects drank 4 bottles (400 ml) of the energy drink containing 50 mg of caffeine per bottle. Venous blood samples (5 ml) were collected at 15, 30, 45, 60, 90 min, 2, 3, 4, 6, 8 and 24 hr after dosing. Lunch and dinner (caffeine-free meals) were provided at 11 a.m. and 5 p.m. All blood samples in heparinized tubes were centrifuged at 2,000 rpm (Speedfuge HSC 10K, Savant Instrument. Inc, NY) for 5 min, and the plasma was then separated and frozen at -20°C until analysis.

5 HPLC Apparatus

A model 126 Solvent Modules pump was used to deliver the mobile phase and model 7725 injector fitted with 20 µl sample loop was used for injection of samples. A symmetry column (particle size 5 µm, C_8, 3.9 mm x 150 mm I.D.) with Sentry guard column (particle size 10 µm, C_8, 3.9 mm x 20 mm) were used for the analysis. The eluent was monitored with UV spectrophotometric detector at 273 nm. (Model 168 Detector Module). The absorbance was recorded by an integration recorder.

6 Analytical procedures

Plasma caffeine concentrations were determined by using a reverse-phase HPLC method.

6.1 Preparation of mobile phase and reagents

a) Mobile phase

Buffer: 10 mM of potassium phosphate, adjusted to pH 7.0 with glacial acetic acid, was prepared and filtered through a 47 mm x 0.45 μm pore size filter (Nylon Milipore®), then degassed in an ultrasonic bath for 30 min.

Methanol: This solvent was filtered through a 47 mm x 0.45 μm pore size filter and degassed in an ultrasonic bath for 30 min.

Mobile phase used in this study was buffer:methanol in a ratio of 76:24 by volume.

b) Zinc sulfate (10% w/v)

Zinc sulfate (10 g) was dissolved in 100 ml deionized distilled water in a volumetric flask.

6.2 Preparation of standard and internal standard solutions

a) Standard caffeine solution

Caffeine anhydrous (0.1 g) was dissolved in 100 ml distilled water to give a stock solution of 1000 μg/ml.

A series dilution of standard caffeine was prepared from the stock solution (1000 μg/ml), by diluting with distilled water to give the final concentrations of 0.5, 1, 2, 4, 6, 8, 10 and 15 μg/10 μl.

b) Internal standard (IS) solution

8-Chlorotheophylline (0.1 g) was prepared in 500 ml of methanol to give a stock solution of 200 μg/ml. The working IS was prepared from stock solution to give a final concentration of 30 μg/ml.

7 Preparation of plasma samples

Zinc sulfate (100 μl) and methanol containing 30 μg/ml of 8-chlorotheophylline (250 μl) were added to 500 μl of plasma. Each sample was mixed thoroughly by vortex mixer (Labinco B.V. Model 525) for 30 sec, then centrifuged for 5 min at 4,000 rpm (Speedfuge HSC 10K, Savant Instrument. Inc, NY). The supernatant was filtered through a 0.2 μm pore size filter (NY Linda Manufacturing Corp.) and 60 μl of this filtrate, by completely loading the loop (at least 3 times the volume of the loop), was injected into the HPLC system.

8 Determination of plasma caffeine concentration

8.1 HPLC operating conditions

The HPLC system was operated at ambient temperature. A mobile phase (buffer:methanol 76:24) was used at the flow rate of 1.0 ml/min to allow the operating pressure of 2.0-2.4 Kpsi. The eluent was monitored at 273 nm, which corresponded to the maximum wavelength for caffeine. This condition was run within approximately 8 min.

8.2 Standard curve construction and calculation of caffeine concentration

Various concentrations of standard caffeine solutions (5µl) were added to each caffeine-free human plasma (495 µl) to give the final concentrations of caffeine in plasma of 0.5-15 µg/ml. These plasma samples were stored in the refrigerator.

Standard curve was constructed by determining caffeine and 8-chlorotheophylline peak area ratio in duplicate and plotted against the respective caffeine concentrations. The standard curve data were subjected to least square linear regression analyses, and the resulting equation was utilized to calculate caffeine concentrations in the unknown samples.

9 Validation of analytical method

9.1 Accuracy

To evaluate the accuracy of the method, peak area ratio of caffeine / 8-chlorotheophylline in plasma was determined and compared to that obtained following the injection of aqueous caffeine solution as percentage of the spiked concentration and then expressed as analytical recovery.

9.2 Precision

Precision was expressed as the coefficient of variation (CV). Within-batch and between-batch precision analyses were established on caffeine-free plasma. They were performed by repeated analysis of spiked plasma samples. In order to determine within-batch precision, caffeine concentrations of 1.5 µg/ml (N=6), 5 µg/ml (N = 6) and 9 µg/ml (N = 6) were spiked into caffeine-free plasma. In the case of between-batch precision analyses, concentrations of 1.5 µg/ml (N = 6), 5 µg/ml (N = 6) and 9 µg/ml (N = 6) were spiked into caffeine-free plasma.

9.3 Specificity

To ensure the specificity, standard caffeine solution was monitored by UV absorbance at different detection wavelengths. The optimal wavelength at 273 nm gave the highest specificity for caffeine.

9.3 Reproducibility

Caffeine-free plasma samples containing 0.5-15 μg/ml caffeine were repeatedly chromatograph occasionally during the analysis. Peak area ratio was determined and compared with the results obtained from the other days.

9.4 Sensitivity and linearity

Using the sample preparation procedure described above, standard caffeine-free plasma spiked with standard caffeine solution at the concentrations of 0.02, 0.04, 0.05, 0.1, 0.2 and 0.3 μg/ml were analyzed to determine the lowest detected limit.

The same procedures were carried out up to the concentration of 30 μg/ml to determine the linear range of the standard calibration curve.

9.6 Lower limit of detection

This is defined as the smallest single result which with stated probability (commonly 95%), can be distinguished from a suitable blank. The limit may be a concentration or an amount and defined the point at which the analysis becomes just feasible. The detection limit is related to precision and also depends on the amplitude of blank readings. A more quantitative definition is that the detection limit is equal to 3 times the standard deviation of the blank, or is located 3 standard deviations above the measured average blank.

10 Pharmacokinetics analysis

Plasma concentration-time data of caffeine was fitted to an appropriate pharmacokinetics model. The plasma concentration-time curves were constructed on a semi-logarithmic paper.

The following pharmacokinetic parameters of caffeine in plasma using a non-compartment model were determined:

i) C_{max} (maximum plasma concentration) and T_{max} (time to reach peak plasma concentration) were obtained from visual inspection of the data.

ii) Elimination rate constant (ke) was obtained from the slope of straight line representing the terminal exponential phase (from 2-8 hr after dosing).

iii) Half-life ($t_{½}$) was calculated from the following formula;

$$t_{½} = \frac{0.693}{ke} \quad (hr)$$

iv) Area under the curve (AUC) was calculated by trapezoidal rule from time 0 to time t plus addition of the area in the tail, which was calculated by the following formula.

$$AUC_{tail} = C_{last} / ke \quad (\mu g \cdot hr/ml) \quad \text{and then}$$

$$\boxed{AUC_{0 \rightarrow \alpha} = AUC_{(0-t)} + AUC_{tail}}$$

in which C_{last} = the last measured plasma concentration of the drug

If a nonzero value was measured for the zero time plasma sample, the contribution of this residual amount of caffeine in the plasma to the AUC was

calculated according to the same formula as AUC_{tail} and the subtracted from the $AUC_{0\rightarrow\alpha}$ as follows:

$$AUC_{0\rightarrow\alpha} = AUC_{(0-t)} + AUC_{tail} - AUC_0 \qquad (\mu g \cdot hr/ml)$$

in which $$AUC_0 = \frac{C_0}{ke}$$

Where C_0 = plasma caffeine concentration at time 0

v) Clearance (CL) was calculated by using this formula:

$$CL = \frac{F \times Dose}{AUC_{0\rightarrow\alpha}} \qquad (ml/min \cdot kg)$$

in which F is the oral bioavailability (assumed to be 1)

vi) Apparent volume of distribution (Vd) was calculated from the following formula:

$$Vd = \frac{Dose}{ke \cdot AUC_{0\rightarrow\alpha}}$$

or $$= \frac{CL}{ke} \qquad (L/kg)$$

11 Statistical analysis

The results were expressed as mean $\pm$ SD. Linear regression analysis was used to set up the equation for a standard curve. This standard curve was used to calculated the caffeine level in the plasma.

PSYCHOMOTOR PERFORMANCE

1 Subjects

Twelve healthy Thai males participated in the study. Their ages were in the range of 18 to 26 yr, height between 160 to 175 cm and weighed between 55 to 70 kg. Subjects were requested not to take any medication, food or beverage containing caffeine at least 24 hr before and throughout the study. The study procedure was explained to all subjects and written informed content was obtained from each participant.

2 Equipments

2.1 Stop watch 1/10 second reading

2.2 Reaction apparatus (Constructed by Jaroonsuck S, Mahidol University, Thailand) containing:

a) 1/1000 second digital timer with start, stop and reset switches.

b) Stimulus of 1000 Hz, 40 dB sound to a loud speaker, microswitch for touching, red light and digital light with delayed timer and display linked to the start and stop circuit of the timer.

c) Stop switch, and 1-9 choices stop switches linked to the stop circuit of the timer.

3 Method

This study was a double blind, three-way crossover design, with 3-day intervals. The subjects were divided into a group of six and were tested individually. On each test day, they were given either placebo (400 ml of electrolyte solution) or

200 mg of caffeine solution (from 4 bottles of the energy drink) or 400 mg of caffeine solution (from 4 bottles of the energy drink plus 200 mg of caffeine anhydrous), using a randomized design. Twenty-four hours prior to each test day, subjects were asked to abstain from caffeine and alcohol. Subjects remained fasting until 3 hr after administration, after which they resumed their normal diet (without caffeine-containing food and beverages). The study medication was administered at 9:00 a.m. A psychomotor speed test of unwarned simple reaction time (SRT) and choice reaction time (CRT) were performed ten times prior to medication and at 30, 60, 90, 120 min, 3, 4, 6 and 8 hr after dosing. The ten score of pre-dose baseline of SRT, 3CRT, 6CRT, and 9CRT were averaged and all post-dosage RT were expressed as an increment or decrement relative to the mean pre-dose value. High value means an improvement in psychomotor performance (shorten RT).

4 Psychomotor speed tests

Subjects were instructed to sit in a comfortable position on a chair in front of the RT apparatus. The non-dominant hand rested on the table or on the subject's lap. Instructions were then given on the procedures of the test.

4.1 Simple reaction time (SRT)

To start each trial, subjects lightly pressed the microswitch key with the index finger of the dominant hand and waited for the presentation of a light stimulus. Following presentation of the stimulus, subjects pushed the key as fast as possible. The light stimulus appeared at a randomly generated time interval between 1 and 5 sec. The scores of ten trials were recorded for each stimulus-effector organ measurement.

4.2 Choice reaction time (CRT)

Subjects received numeric light warning signal of 2 sec before the presentation of the stimulus. Stimuli were presented in a random order within 3 choices (number 1, 2 or 3) for 3CRT test, 6 choices (number 1 to 6) for 6CRT test and 9 choices (number 1 to 9) for 9CRT test. Subject used the 1-9 buttons due to limited choices of memory demands, that ten scores were used for calculation of the mean of all correct responses and plotted RT changes over baseline after administration of 3 doses at various times.

5 Statistical analysis

The results were expressed as mean ± SEM. SPSS 7.5 for Window at a probability of 0.05 was selected as the criterion for statistical significance. From normally distributed population, the distribution of the sample mean and variance would be processed in one-way ANOVA (with Turky multiple comparison method).

CHAPTER V

RESULTS

The demographic data of subjects in the pharmacokinetic study, actual dose of caffeine, and history of cigarette smoking and caffeine consumption are summarized in Table 4. The means ± SD of age and weight of the subjects were 29.1±4.1 yr and 58.7±7.3 kg, respectively. Two subjects were non-coffee drinkers while another two were habitual coffee drinkers. The remaining eight subjects consumed coffee irregularly. None of them regularly consumed other caffeine-containing beverages. Ten of them were non-smokers, the remainders smoked 2-4 cigarettes/day. All drank alcohol only occasionally. Table 5 illustrates the data of hematological and biochemical examinations whereas Table 6 shows the results from urinalysis. All subjects were healthy. The use of male subjects ensured that there would be no sex-linked variations in the pharmacokinetics of caffeine.

1 HPLC analysis of plasma caffeine

Under the chromatographic condition used in this study, good separation and detectability of caffeine in plasma could be achieved. Excellent resolution of caffeine and IS was achieved in less than 8 min, with the retention times of IS and caffeine of 3.6 and 5.7 min, respectively. No interfering components were found in the drug-free plasma as illustrated in Figures 4 and 5. The validity of this HPLC method was established. Within-batch and between-batch precision were examined following the

Table 4. Demographic data of 12 male subjects for pharmacokinetic study

Subject No.	Age (yr)	Height (cm)	Weight (kg)	Caffeine dosage (mg/kg)	Coffee (cup/day)	Smoking (cigarettes/ day)
1	22	171	61	3.28	IR	NONE
2	33	153	48	4.17	IR	NONE
3	34	165	58	3.45	IR	NONE
4	26	155	51	3.92	IR	NONE
5	30	165	58	3.45	NONE	NONE
6	31	170	72	2.78	IR	NONE
7	34	160	58	3.45	NONE	NONE
8	33	169	61	3.28	IR	2-4
9	30	168	62	3.22	IR	NONE
10	26	162	55	3.64	IR	NONE
11	26	170	50	4.00	1-2	2-4
12	24	175	70	2.86	>2	NONE
Max	34	175	72	4.17		
Min	22	153	48	2.78		
$\bar{X}$	29.1	165.2	58.7	3.46		
SD	4.1	6.7	7.3	0.42		

IR = Irregular consumption of caffeine

Table 5. The results of hematological and biochemical examinations of 12 healthy subjects

Test	Normal Range	Results											
		Subject No.											
		1	2	3	4	5	6	7	8	9	10	11	12
Complete Blood Count													
White Blood Count	(1.5-10.7)x10/µl	8.2	6.5	10.1	5.7	9.2	9.6	7.5	7.6	5.5	6.2	4.8	7.2
Hematocrit	40-55%	46	52	47	43	49	45	43	45	48	47	46	43
Platelet Count	(150-400) x10/µl	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq	Adeq
Neutrophil	40.0-74.5%	57	65	63	49	70	53	68	58	47	62	55	61
Lymphocyte	16.3-40%	42	26	27	32	40	31	28	19	22	17	32	21
Monocyte	0-7.8%	6	2	3	5	-	6	3	-	2	-	6	5
Eosinophil	0-9%	5	2	7	-	1	10	5	-	7	6	-	1
Basophil	0-1.2%	-	1	-	-	2	-	-	-	-	-	-	-
Liver Function													
Total Protein	6.0-8.0 gm%	7.4	7.2	7.6	7.3	8.0	7.0	7.1	7.7	7.2	7.5	7.5	7.7
Albumin	3.5-5.5 gm%	3.7	4.2	3.6	3.7	3.5	3.9	4.0	3.9	3.6	4.0	3.5	3.7
Globulin	2.5-3.5 gm%	2.9	3.4	3.3	4.1	3.7	3.5	3.1	3.1	2.9	3.4	3.4	2.9
Total Bilirubin	0.0-1.5 mg%	0.9	1.2	1.4	0.7	0.4	0.7	0.8	1.0	1.2	0.7	1.6	0.7
Direct Bilirubin	0.0-0.5 mg%	0.2	0.4	0.4	0.1	0.1	0.3	0.2	0.3	0.3	0.1	0.5	0.2
Indirect Bilirubin	0.5-1.0 mg%	0.7	0.8	1.0	0.6	0.3	0.4	0.6	0.7	0.9	0.6	1.1	0.5
AST*	10-40 U/L	23	31	19	32	30	40	33	41	36	37	29	30
ALT**	5-35 U/L	19	23	31	40	27	22	25	30	27	19	30	26
Alk Phos***	50-250 U/L	150	132	161	139	177	158	164	230	180	130	197	172
Kidney Function													
Blood Urea Nitrogen	7-20 mg%	16	17	14	17	18	15	21	16	19	18	17	19
Creatinine	0.8-1.5 mg%	1.4	1.2	1.3	1.5	1.3	1.1	1.3	1.5	1.0	1.0	1.3	1.1

AST* = Aspartate aminotransferase ALT** = Alanine aminotransferase
Alk Phos*** = Alkaline phosphatase Adeq = Adequate

Table 6. The results of urinalysis of 12 healthy subjects

Test	Normal Range	Results											
		Subject No.											
		1	2	3	4	5	6	7	8	9	10	11	12
Urinalysis													
Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Pale Y	Yellow	Yellow	Yellow	Yellow	Yellow
Appearance	Clear	C	C	SC	C	C	C	C	C	C	C	C	C
pH	5-8	6	6.5	7	7.5	6	6	5.5	6	6.5	7	7.5	6.5
Specific Gravity	1.01-1.03	1.004	1.025	1.022	1.018	1.019	1.022	1.027	1.015	1.006	1.015	1.020	1.014
Protein	Negative	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Sugar	Negative	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Acetone	Negative	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Occult Blood	Negative	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Epithelial Cells		-	-	0-1	-	-	-	-	-	-	-	-	-
White Blood Cells		-	-	0-1	-	0-1	-	-	-	-	-	-	-
Red Blood Cells		-	-	-	-	-	-	0.1	-	-	-	-	-
Bacteria		-	few	-	-	-	-	few	-	-	-	-	-

SC = Slightly Clear, C = Clear, Pale Y = Pale Yellow
Neg = Negative

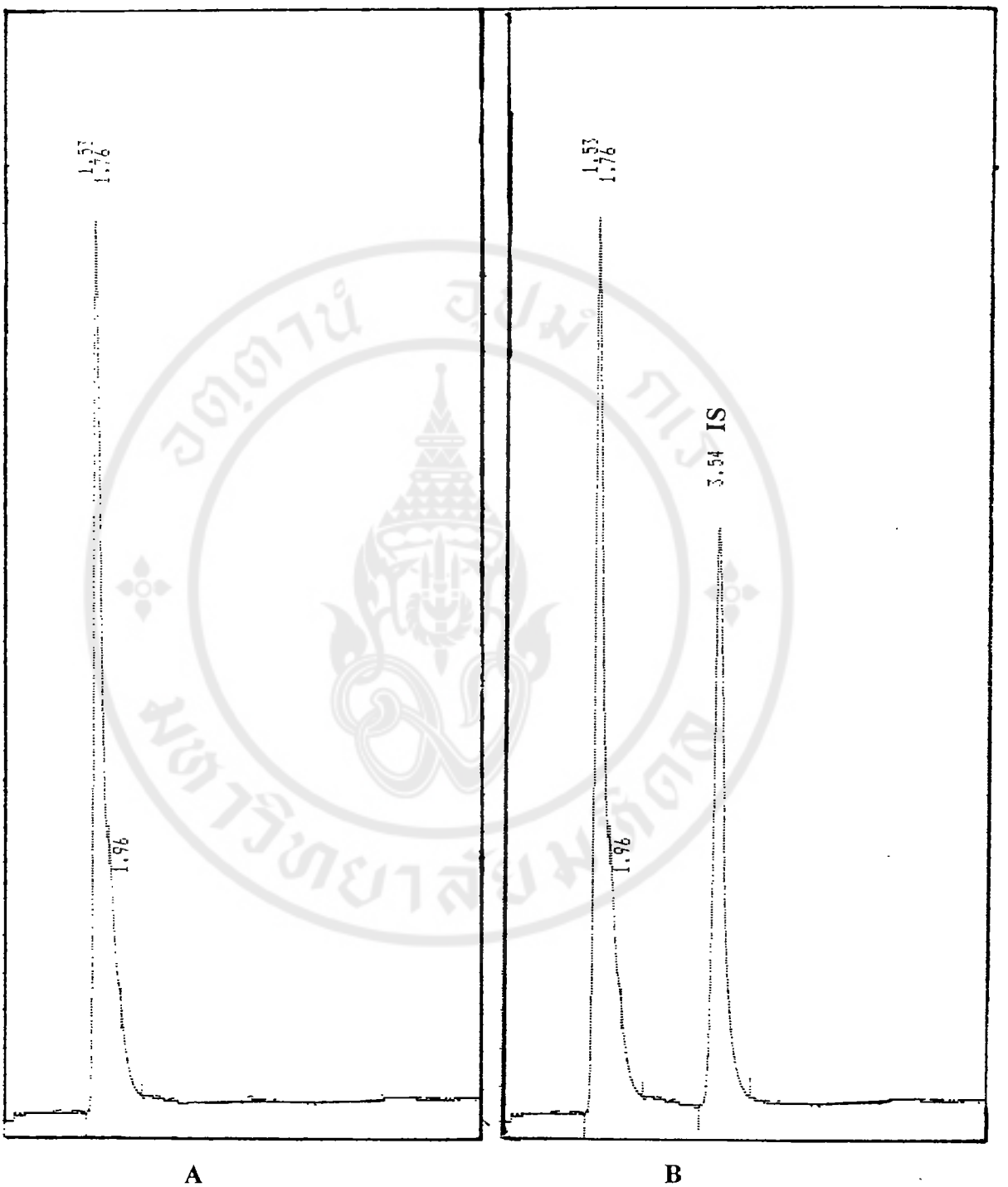


Figure 4. HPLC chromatograms of

A. Drug-free plasma.

B. Plasma spiked with internal standard (IS).

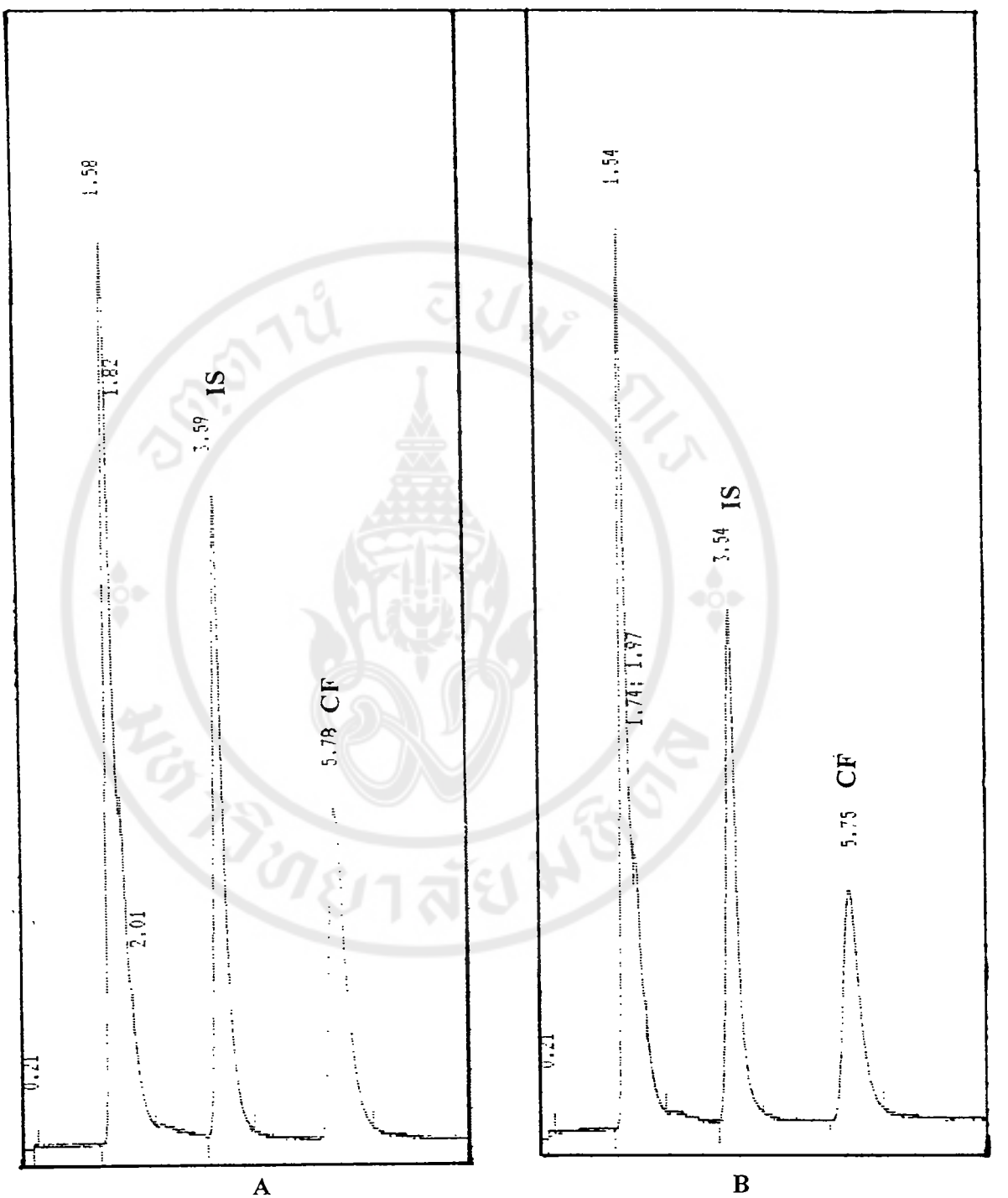


Figure 5. HPLC chromatograms of

A. Plasma spiked with caffeine (CF) and internal standard (IS).

B. Plasma sample collected 45 min after a single oral administration of 200 mg caffeine in energy drinks.

above mentioned procedure. The coefficients of variation in Table 7 were not more than 10%; i.e. within-batch precision were 5.44, 2.15 and 1.89%, respectively and between-batch precision were 2.07, 1.99 and 0.90%, respectively. The precision of the method was satisfactory within the acceptable criteria. To determine the accuracy of the method, the analytical recovery was performed using various concentrations of caffeine in distilled water and drug-free plasma. The mean percentage of recovery was 100.9±2.5% in aqueous solutions and 101.2±3.8% in plasma samples (Table 8).

The standard curve was linear over caffeine concentration ranges of 0.5-15 μg/ml with the correlation coefficient (r) of 0.9992. The equation described this straight line was Y = 0.1864X - 0.0087; where Y is peak area ratio and X is the concentration of caffeine in μg/ml (Figure 6). The linearity was still obtained up to 30 μg/ml of caffeine solution (data not shown). The detection limit was 0.1 μg/ml.

Standard drug-free plasma samples containing 0.5-15 μg/ml caffeine were repeatedly chromatographed. The reproducibility of the analytical method was clearly demonstrated since the determined levels of caffeine were similar in all repeated determinations. Caffeine concentrations in freshly prepared samples were assumed to be 100 %. The percentages remaining of caffeine was 98.9 %after a 8 week storage time (Table 9)

2 Pharmacokinetic analysis of caffeine in healthy subjects

The pharmacokinetic parameters of caffeine after a single oral administration of 200 mg caffeine (4 bottles of energy drink) in all subjects are summarized in Tables 10 and 11. The caffeine concentration-time curve is demonstrated in Figure 7. The mean peak caffeine concentration (C_{max}) was 5.45±0.57 μg/ml. The time to reach peak concentration (T_{max}) of caffeine was 0.92±0.39 hr. The $t_{½}$ and ke of caffeine

Table 7. Precision of synthetic caffeine analysis by HPLC

Caffeine added (μg/ml)	Within-batch		Between-batch	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
1.5	1.47±0.08	5.44	1.48±0.04	2.70
5	5.12±0.11	2.15	5.03±0.10	1.99
9	9.04±0.17	1.89	8.90±0.08	0.90

Number of samples = 6

Table 8. Analytical recovery of caffeine in aqueous solution and plasma samples

Conc. added (μg/ml)	Caffeine in aqueous solution		Caffeine in plasma samples	
	Conc. determined* (μg/ml)	Recovery (%)	Conc. determined* (μg/ml)	Recovery (%)
0.5	0.52	104.0	0.55	110.0
1	1.05	105.0	1.02	102.0
2	1.98	99.0	2.01	100.5
4	4.05	101.2	3.97	99.2
6	5.90	98.3	5.99	99.8
8	8.00	100.0	8.04	100.5
10	10.10	101.0	9.80	98.0
15	14.80	98.7	14.88	99.2
	Mean ± SD	100.9±2.5	Mean ± SD	101.2±3.8

* The value is an average from duplicate determinations.

Table 9. Reproducibility of caffeine in plasma samples

Storage time (week)	% Concentration remaining of 2 μg/ml of caffeine in plasma (N=3)
0	100.0
1	99.8
2	99.5
4	99.7
8	98.9

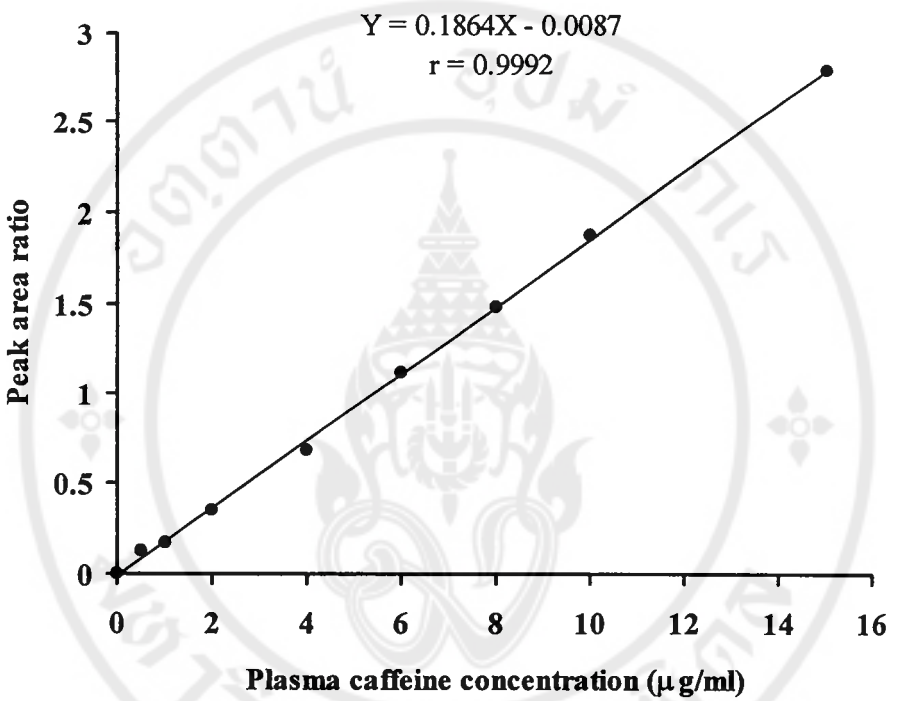


Figure 6. Standard curve of caffeine prepared in the plasma versus peak area ratio

Table 10. Plasma caffeine concentrations in 12 healthy subjects after an oral administration of a single dose of 200 mg caffeine in energy drinks.

Subject No.	Plasma caffeine concentration (μg/ml) Time after dosing (hr)											
	0	**0.25**	**0.5**	**0.75**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**24**
1	0	0.76	2.64	3.63	5.27	4.99	5.10	3.36	2.76	2.34	1.77	0.75
2	0	1.23	3.12	4.13	5.70	5.92	5.73	5.00	4.32	3.79	3.27	1.76
3	0	0.10	1.30	2.12	3.17	4.69	4.55	3.94	3.79	2.69	1.82	1.09
4	0	0.54	1.94	2.58	2.75	4.92	3.34	2.66	2.60	1.89	1.42	0.86
5	0	2.04	6.13	6.00	5.72	5.31	5.01	4.50	3.90	3.22	2.45	0.61
6	0	1.90	3.92	5.71	5.50	5.21	4.51	3.92	3.37	2.80	1.93	0.53
7	0	1.17	3.01	5.51	5.31	5.10	4.72	4.43	3.21	2.53	1.76	0.21
8	0	0.16	2.05	5.80	5.40	4.82	4.62	4.21	3.07	2.47	2.09	0.42
9	0	3.17	5.61	5.52	4.95	4.66	4.13	3.57	3.02	2.11	1.52	0.09
10	0	1.87	6.05	5.67	5.32	5.17	4.80	4.32	3.62	3.19	2.21	1.27
11	0	2.22	3.68	4.78	5.46	4.80	4.69	4.10	3.54	2.23	1.80	1.10
12	0	3.34	3.92	4.27	4.02	3.57	3.30	2.83	2.17	1.79	1.19	0.76
$\bar{X}$	**0**	**1.54**	**3.61**	**4.64**	**4.88**	**4.93**	**4.54**	**3.90**	**3.28**	**2.59**	**1.94**	**0.79**
SD	0	1.08	1.61	1.31	1.00	0.55	0.69	0.69	0.60	0.59	0.54	0.47

Table 11. Pharmacokinetic parameters in 12 healthy volunteers after an oral administration of 200 mg caffeine in energy drinks.

Subject No.	C_{max} (μg/ml)	T_{max} (hr)	ke (hr^{-1})	t ½ (hr)	AUC $_{0-8}$ (μg. hr/ml)	AUC $_{0-\infty}$ (μg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
1	5.27	1.0	0.164	4.23	24.00	34.80	0.57	1.57
2	5.92	1.5	0.135	5.13	33.85	58.07	0.53	1.19
3	4.69	1.5	0.156	4.43	24.64	36.31	0.61	1.58
4	4.92	1.5	0.140	4.95	19.02	29.16	0.96	2.24
5	6.13	0.5	0.145	4.77	30.87	47.77	0.50	1.20
6	5.71	0.75	0.142	4.88	27.44	41.03	0.48	1.13
7	5.51	0.75	0.175	3.96	26.57	36.63	0.54	1.57
8	5.80	0.75	0.150	4.62	25.75	39.68	0.55	1.38
9	5.61	0.5	0.151	4.59	24.70	34.77	0.61	1.55
10	6.05	0.5	0.143	4.85	30.00	45.45	0.56	1.33
11	5.46	1.0	0.174	3.98	26.08	36.42	0.63	1.83
12	4.27	0.75	0.156	4.43	19.53	27.16	0.67	1.75
Max	6.13	1.5	0.175	5.13	33.85	58.07	0.96	2.24
Min	4.27	0.5	0.135	3.96	19.02	27.16	0.48	1.13
$\bar{X}$	**5.45**	**0.92**	**0.152**	**4.57**	**26.04**	**38.94**	**0.60**	**1.53**
SD	0.57	0.39	0.013	0.37	4.28	8.41	0.13	0.32

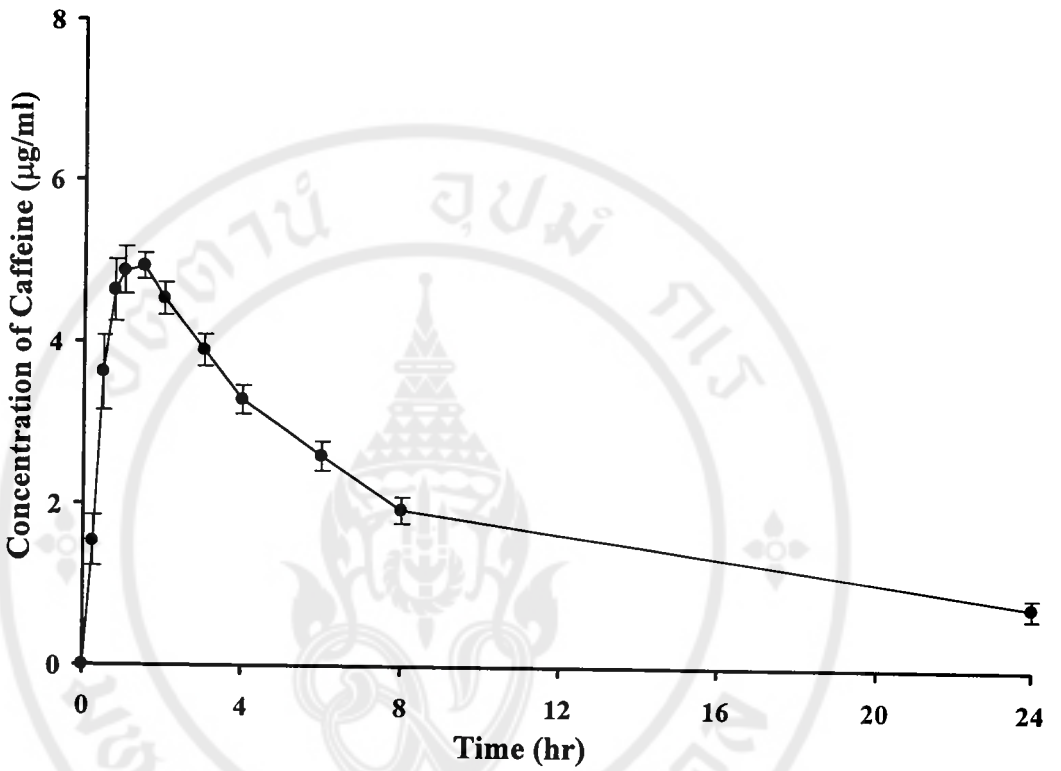


Figure 7. Plasma caffeine concentration-time profile after an oral administration of 200 mg of caffeine in energy drink

Each point represents mean values ± SD

were 4.57±0.37 hr and 0.152±0.013 hr^{-1}, respectively. $AUC_{0\rightarrow 8}$ and $AUC_{0\rightarrow \alpha}$ were 26.04±4.28 and 38.94±8.41 μg.hr/ml, respectively. Vd was 0.60±0.12 L/kg and CL was 1.52±0.31 ml/min.kg.

3 Effect of caffeine on psychomotor performance

Another group of 12 healthy volunteers was recruited in the pharmacodynamic study of caffeine. The demographic data of subjects are summarized in Table 12. The mean ± SD of age and weight of the subjects were 22.3±2.5 yr and 63.3±5.6 kg, respectively. All subjects were assessed as being healthy on the basis of history and physical examination. Their normal coffee intake ranged between 0 and 2 cups/day (about 0- 120 mg of caffeine).

All subjects taking 200 mg of caffeine produced no significant adverse effects. However, the subjects taking 400 mg of caffeine reported some adverse effects as listed in Table 13. The effects believed to be due to caffeine were palpitation, nervousness, restlessness, tremor, and nausea.

Tables 14-17 show the mean ± SEM of the RT changes over baseline (pre-dose RT, at time 0) after receiving caffeine at various times were expressed in msec. The subjects were categorized according to the treatment received, i.e., placebo, 200 mg and 400 mg caffeine (repeated measures design). High value of the sum of RT differences indicated improvement in psychomotor performance (shorter RT). The data were analyzed in two ways: RT changes over baseline were first analyzed on a time series basis, followed by a comparison between 3 groups by using one-way ANOVA.

Table 12. Demographic data of 12 subjects for psychomotor performance

Subject No.	Age (yr)	Height (cm)	Weight (kg)	Caffeine Dosage (mg/kg)		Coffee (cup/day)	Smoking (cigarettes/day)
				200 mg	400 mg		
1	20	170	63	3.17	6.35	IR	NONE
2	23	170	55	3.64	7.27	IR	NONE
3	18	160	58	3.45	6.90	NONE	NONE
4	26	167	60	3.33	6.66	1-2	NONE
5	25	165	70	2.86	5.72	IR	NONE
6	22	173	70	2.86	5.72	IR	NONE
7	24	160	65	3.08	6.15	1-2	NONE
8	20	169	60	3.33	6.66	IR	NONE
9	24	167	65	3.08	6.15	IR	NONE
10	20	165	55	3.64	7.27	IR	NONE
11	21	175	68	2.94	5.88	1-2	NONE
12	25	170	70	2.86	5.72	NONE	NONE
Max	26	175	70	3.64	7.27		
Min	18	160	55	2.86	5.72		
X	22.3	168.0	63.3	3.19	6.37		
S.D.	2.5	5.0	5.6	0.29	0.58		

IR = Irregular consumption of caffeine

Table 13. Side effects reported by subjects taking 400 mg of caffeine

Side effects	Number of subjects reported*
Palpitation	8/12 (67%)
Restlessness	8/12 (67%)
Tremors	5/12 (42%)
Nausea	4/12 (33%)

* Total number of subject =12

Table 14. Average of simple reaction time changes over baseline after an oral administration of 0, 200 and 400 mg of caffeine in energy drinks at various times

Dose of caffeine (mg)	Average simple reaction time (SRT, msec) Time after dosing (hr)								Sum of RT difference
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	
0 (Placebo)	1.0±2.8	6.4±1.9	6.6±1.7	8.7±0.9	4.9±2.2	6.2±1.4	5.6±1.0	1.4±1.6	40.8
200	2.2±2.6	19.8±3.2*	19.3±2.6*	23.5±2.0*	20.6±2.2*	13.8±1.7*	10.6±1.7	8.4±1.3*	118.2*
400	5.1±2.7	13.7±1.6	13.1±1.6	11.6±2.9#	9.2±1.6#	4.5±1.9#	3.4±1.7#	4.4±0.7	65.0#

Each value represents mean ± SEM of 12 subjects

* $p<0.05$ (from Placebo)

# $p<0.05$ (from 200 mg group)

Table 15. Average of 3 choice reaction time changes over baseline after an oral administration of 0, 200 and 400 mg of caffeine in energy drinks at various times

Dose of caffeine (mg)	Average 3 choice reaction time (3CRT, msec)								Sum of RT difference
	Time after dosing (hr)								
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	
0(Placebo)	7.3±6.8	16.2±3.5	16.7±3.7	20.1±3.2	13.4±3.9	10.8±3.7	12.0±1.9	8.7±2.6	105.2
200	33.1±13.2	45.9±8.6*	51.1±8.2*	47.2±8.0*	34.1±4.1*	34.1±6.0*	37.6±4.8*	30.0±3.5*	313.1*
400	36.1±4.4	24.3±4.1#	13.1±1.6	19.0±5.6#	12.9±5.4#	23.6±3.9	7.4±4.7#	14.8±2.8#	169.9#

Each value represents mean ± SEM of 12 subjects

* $p<0.05$ (from Placebo)

# $p<0.05$ (from 200 mg group)

Table 16. Average of 6 choice reaction time changes over baseline after an oral administration of 0, 200 and 400 mg of caffeine in energy drinks at various times

Dose of caffeine (mg)	Average 6 choice reaction time (6CRT, msec)								Sum of RT difference
	Time after dosing (hr)								
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	
0(Placebo)	-1.33±4.5	2.3±1.9	5.4±2.5	9.4±2.4	8.2±2.8	3.6±4.1	9.8±3.3	7.7±1.5	45.1
200	16.7±11.0	23.8±15.7	26.1±7.2*	31.6±2.7*	25.0±3.3*	23.6±2.6*	29.1±4.5*	23.4±3.5*	199.3*
400	22.7±5.3	17.2±4.6	14.1±2.6	12.3±2.6#	14.3±3.0#	10.5±2.1#	14.0±3.2#	15.2±2.2	120.3* #

Each value represents mean ± SEM of 12 subjects

* $p<0.05$ (from Placebo)

# $p<0.05$ (from 200 mg group)

Table 17. Average of 9 choice reaction time changes over baseline after an oral administration of 0, 200 and 400 mg of caffeine in energy drinks at various times

Dose of caffeine (mg)	Average 9 choice reaction time (9CRT, msec)								Sum of RT difference
	Time after dosing (hr)								
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	
0(Placebo)	15.8±3.7	19.2±3.6	12.2±4.1	14.0±2.9	14.3±2.9	8.6±2.3	14.0±2.7	8.6±2.9	106.7
200	56.1±11.2*	58.0±6.4*	56.3±12.4*	54.0±6.4*	52.8±6.4*	41.4±4.4*	37.3±3.7*	25.5±3.3*	381.4*
400	44.9±6.3*	39.4±4.4* #	39.1±3.7	27.0±4.6#	19.3±4.8#	16.6±3.2#	17.7±2.6#	9.9±1.5#	234.0* #

Each value represents mean ± SEM of 12 subjects

* $p<0.05$ (from Placebo)

# $p<0.05$ (from 200 mg group)

4 Effect of caffeine on SRT

The averages of SRT changes over baseline after an oral administration of 0, 200 and 400 mg of caffeine at various times are given in Table 14. The pattern of changes in these values are illustrated in Figure 8 in which SRT (changes over baseline) has been plotted against time series. In the placebo group, the performance was slightly increased (shorter RT) during the period tested. At the dose of 200 mg caffeine, the RT changes over baseline increased significantly in performance at 1, 1.5, 2, 3, 4 and 8 hr after dosing compared to placebo group. At early time point (0.5-1.5 hr) after administration of 400 mg caffeine, the RT change over baseline was slightly increased. The significant difference from those receiving 200 mg of caffeine was observed at time 2, 3, 4 and 6 hr.

The sum of RT differences indicated statistically significant difference between placebo and 200 mg caffeine ($p < 0.05$). However, no significant difference between placebo and 400 mg caffeine was observed.

5 Effects of caffeine on CRT

Tables 15-17 show the averages of CRT changes over baseline after an oral administration of 0, 200 and 400 mg of caffeine at 3 days apart and these values are illustrated in Figures 9-11. In the placebo group, the performances were slightly increased during the period tested. It is clearly demonstrated that at low dose, caffeine produced significantly improved performance on the CRT changes over baseline compared to placebo, whereas high dose of caffeine produced less performance enhancement than the lower one. The peak performance was observed around 1.5-2 hr

with the lower dose of caffeine, whereas the peak was generally observed between 0.5-1 hr with the higher dose. The CRT values remained different from baseline values throughout the 8 hr observation periods. The ANOVA performed as in the sum of RT differences and the sum of 3CRT differences values indicated statistically significant difference between placebo and 200 mg caffeine ($p < 0.05$) and significant difference between 200 and 400 mg caffeine ($p<0.05$) but no significant difference between placebo and 400 mg caffeine. The sums of 6CRT and 9CRT differences values indicated a significant difference among all three groups of caffeine.

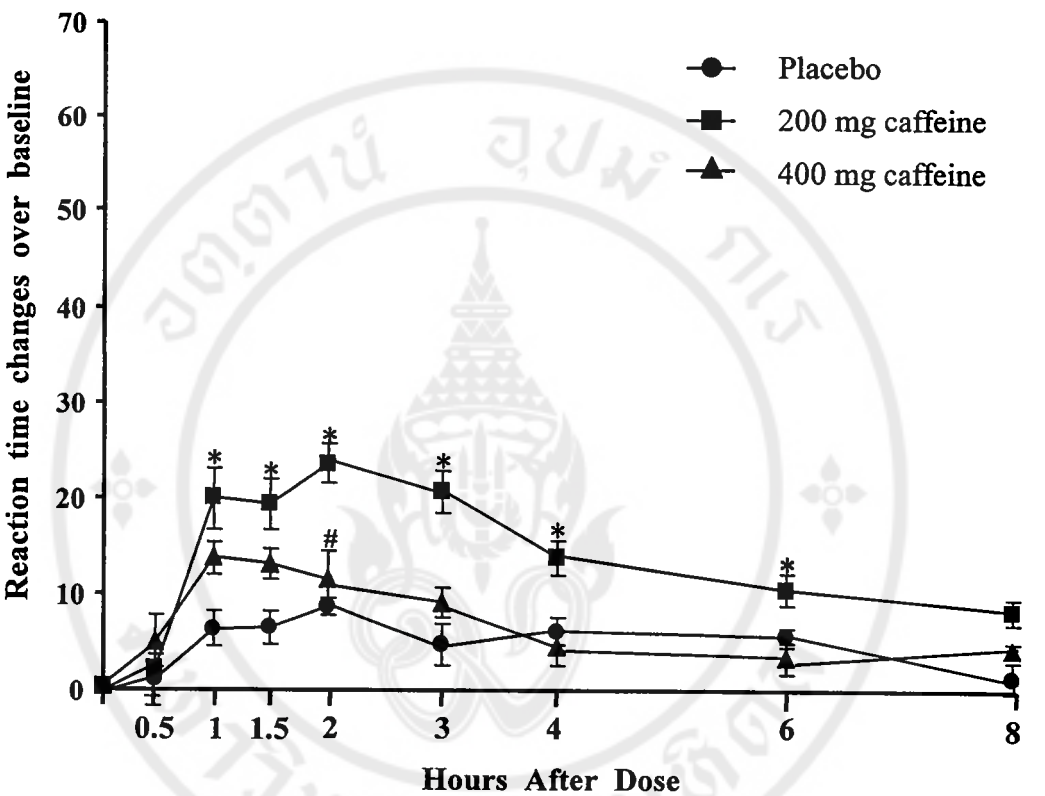


Figure 8. The simple reaction time changes over baseline at different doses of 0, 200 and 400 mg caffeine at various time

Each point represent mean ± SEM of 12 subjects

* Significant different from placebo at $p<0.05$

Significant different from 200 mg caffeine at $p<0.05$

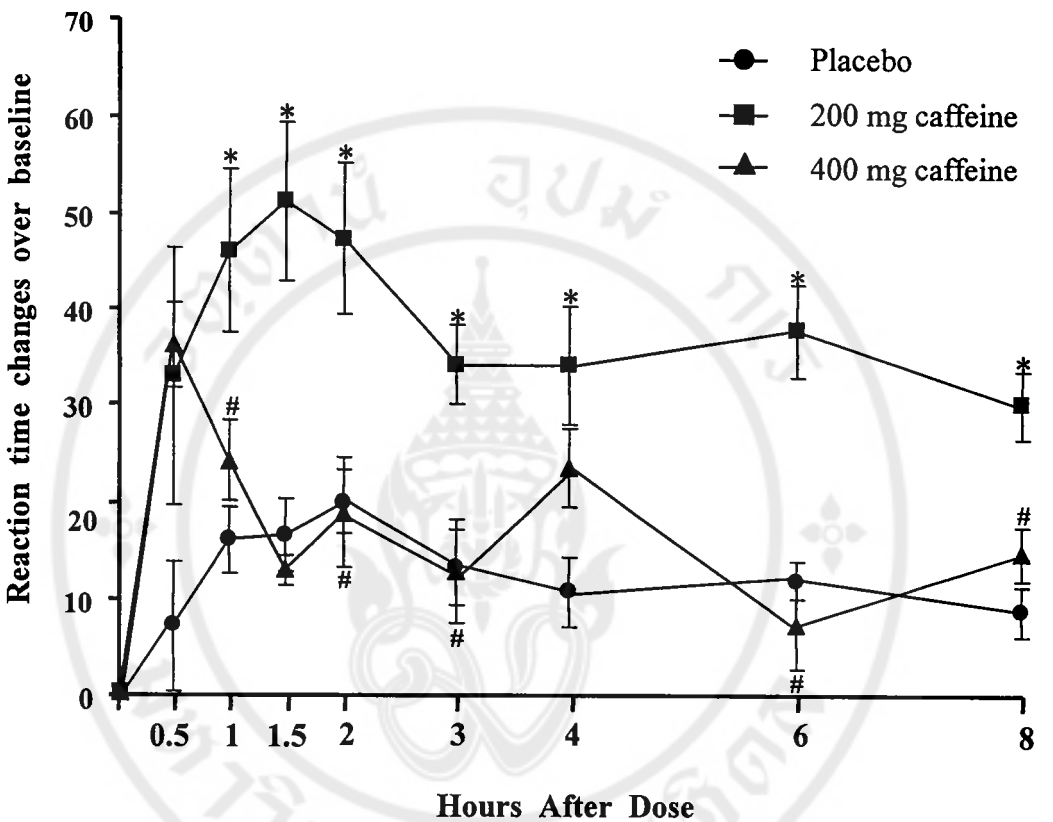


Figure 9. The 3 choices reaction time changes over baseline at different doses of 0, 200 and 400 mg caffeine at various time

Each point represent mean ± SEM of 12 subjects

* Significant different from placebo at $p<0.05$

Significant different from 200 mg caffeine at $p<0.05$

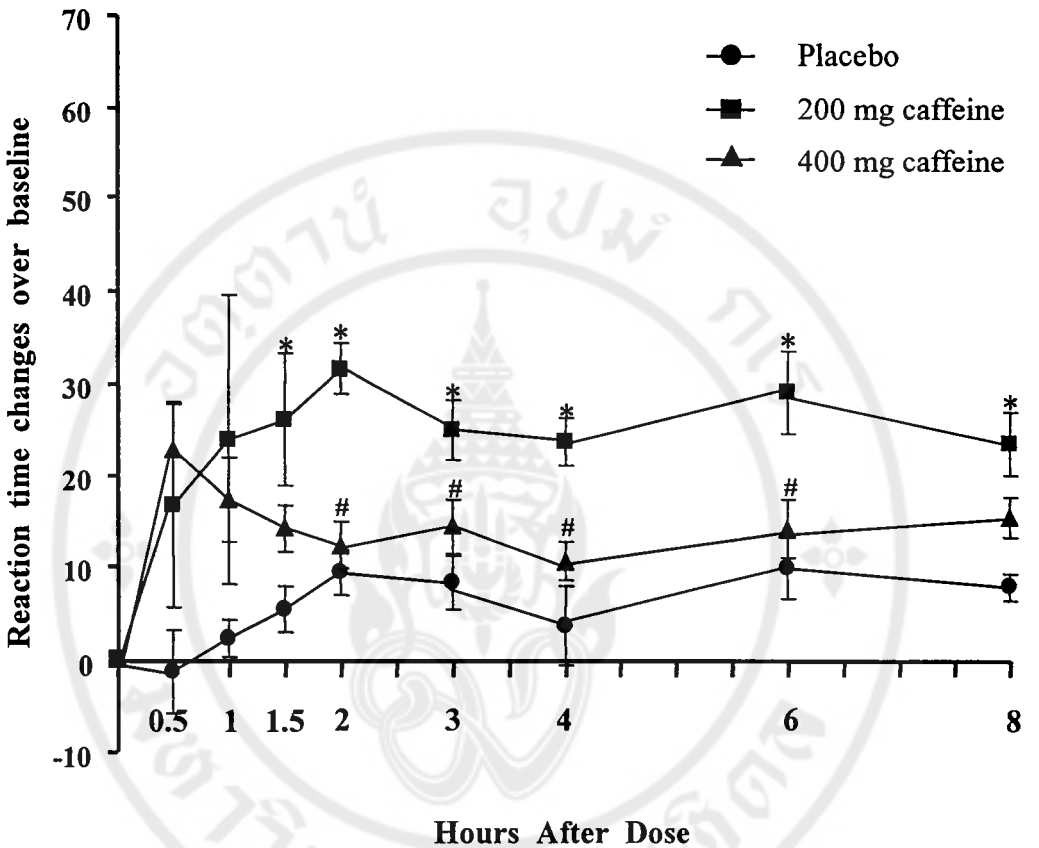


Figure 10. The 6 choices reaction time changes over baseline at different doses of 0, 200 and 400 mg caffeine at various time

Each point represent mean ± SEM of 12 subjects

* Significant different from placebo at $p<0.05$

Significant different from 200 mg caffeine at $p<0.05$

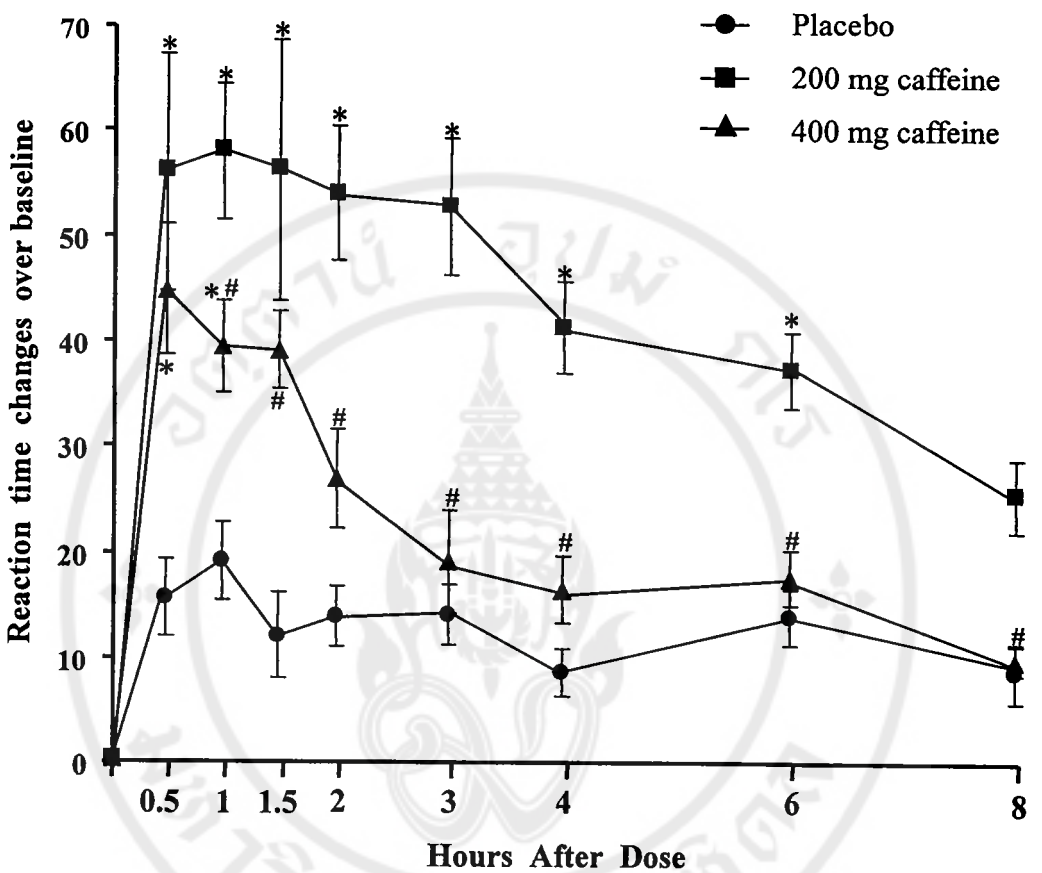


Figure 11. The 9 choices reaction time changes over baseline at different doses of 0, 200 and 400 mg caffeine at various time

Each point represent mean ± SEM of 12 subjects

* Significant different from placebo at $p<0.05$

Significant different from 200 mg caffeine at $p<0.05$

CHAPTER VI

DISCUSSION

The dietary intake of caffeine has a long history, spanning a diversity of cultures. The basis of its widespread use can be attributed to central stimulant properties (5, 16, 17). It has also received criticism from both scientists and general public for its possible ill effects on health. The safe limit of consumption per day is not generally well accepted worldwide. Energy drink has been the favorite beverage of people in Thailand both for males and females among all occupations. According to marketing promotion of the products as the energy drink, the consumption of this product has been increased rapidly during the last 10 years (26). It is, therefore, necessary to perform the pharmacokinetic studies of caffeine on this type of product and correlate with its pharmacodynamic effects in Thai healthy subjects as being done in this study. The data obtained may thus be used to assess the safe limit of consumption in Thai populations.

Although several methods have been used for the detection and quantification of caffeine in biological materials including UV spectrophotometry, liquid chromatography, thin-layer chromatography, immunoassays and gas chromatography (62, 216), HPLC method is well accepted for its precision and accuracy (27, 28, 217). The HPLC method used in this study is modified from those described by Phavichitr *et al.* (27) and Holland *et al.* (215). The method is very simple and rapid. It requires only a deproteinization step by an addition of 10% $ZnSO_4$ for sample preparation. In addition, the time taken for each sample is about 8 min, as shown in Figures 4 and

5. The results obtained after validation of the method indicated that this HPLC method has sufficient selectivity, sensitivity, precision and accuracy to be suitable for pharmacokinetic studies of caffeine. Therefore, this method could be recommended as a routine procedure for analysis of caffeine.

It has been demonstrated previously that caffeine is rapidly and completely absorbed after oral administration (7, 8, 56, 57). The excellent absorption of caffeine is attributable to its physicochemical characteristics as being undissociated weak electrolyte at physiological pH, with pKa 1 and 14 and partition coefficient of 0.85. In this study, the results confirmed its rapid absorption with T_{max} of 0.92±0.39 hr and C_{max} of 5.45±0.57 μg/ml (Figure 7). The results of the present study are in good agreement with those from previous reports in which T_{max} and C_{max} were in the range of 0.5-1.0 hr and 5-6.7 μg/ml, respectively (5, 7, 29, 30). However, different absorption rates among these studies may be due to the large volume of the energy drinks (400 ml) being taken.

Regarding the distribution, caffeine is rapidly distributed, and pass through all biological membranes. A mean Vd of 0.60±0.13 L/kg was reported in this study (Table 11) leading to the conclusion that caffeine does distribute into total body fluids. Animal and human studies have shown that no physiological barriers limit the passage of caffeine through tissue, so that easy and rapid equilibrium is reached between mother and fetus and between blood and all tissues (5, 8-10).

The $t_{1/2}$ and CL of caffeine in energy drink were 4.57±0.37 hr and 1.53±0.32 ml/min.kg, respectively (Table 11). These results were similar to previous reports by Wittayalertpanya *et al.* (28), and Tapna (30) who have shown that the $t_{1/2}$ of caffeine

in normal Thai subjects were 6.9±2.6 and 5.63±0.86 hr respectively, and CL were 0.97±0.30 and 1.40±0.18 ml/min.kg, respectively. However, several pharmacokinetic parameters including $t_{1/2}$ and CL of caffeine in the Caucasians are greater than those found in the Thais (6-11). The apparent difference in $t_{1/2}$ and CL may be due to the differences in age, body size, genetic constitution, nutritional factors, as well as xenobiotic exposure (6-11).

Caffeine is usually eliminated by apparent first-order kinetics, described by a one–compartment open model system, over different ranges of doses (11, 56). It is metabolized extensively via hepatic enzyme system, initially by demethylation to dimethylxanthines (12, 16, 87). The dimethylxanthines are pharmacologically active and may contribute to the effects of caffeine in people. Sequential metabolic steps include acetylation, 8-hydroxylation, and metabolism by xanthine oxidase. Ratio of different metabolites in the urine may be used to assess an individual's genetically determined acetylation rate or to assess the influences of environmental exposures on CYP450-mediated metabolism (14, 87, 118).

Caffeine has also been reported to exhibit dose-dependent kinetics in rat at doses higher than 10 mg/kg (8). It appeared that caffeine kinetics in man were linear up to 10 mg/kg. In the present study, a dose of 200 mg caffeine (the average dose of 3.5 mg/kg) in energy drink was chosen. This level of intake is equivalent to drinking 4 bottles of energy drink or 3-6 cups of coffee, which is higher than the average consumption of caffeine in Thai people (1, 3).

Undoubtedly, caffeine's stimulatory effects on human mental activities are the reason for its used in various forms of beverages by millions of people. Generally, at the dose normally ingested from food sources, caffeine is expected to produce a

variety of biological effects. A dose of 1-2 mg/kg that produced peak plasma concentration of 1 to 2 μg/ml, can increase alertness and lessen fatigue (21, 23). Slight increase in the dose to 3-5 mg/kg could bring the peak plasma levels up to 5 μg/ml, which could induce mild anxiety, respiratory stimulation, cardiovascular effects, diuretic, tremor, headache, irritability and GI disturbances (21, 23). In this study, the dose of 200 mg of caffeine which was equivalent to around 3.5 mg/kg produced peak plasma concentration of 5.45±0.57 μg/ml. This caffeine level should exert certain pharmacological effects in Thai healthy subjects and did not exceed the plasma concentration of 10-30 μg/ml that could induce serious acute toxicity. The psychomotor performance was also improved after taking 200 mg of caffeine. When caffeine dose was increased to 400 mg or equivalent to 7 mg/kg, the estimated caffeine concentration should be above 10 μg/ml. Although we did not obtain the plasma concentration of caffeine, the development of certain adverse effects such as palpitation, nervousness and restlessness indicated the toxic levels of caffeine concentration (Table 13). The decrease in psychomotor performance was also shown in Figures 8-11.

In experimental animals, the stimulant actions of caffeine are manifested as enhanced spontaneous locomotor activity, enhanced electrical activity in brain regions and effects on coordination paradigms requiring vigilance. In humans, caffeine increases vigilance, decreases psychomotor reaction time and increases sleep latency and waking time. Caffeine may also influence intellectual performance if boredom or tiredness compromises this. The precise mechanisms underlying the stimulant actions of caffeine remain poorly defined.

Dose-dependent effects of a single dose of caffeine have been found in animal models and in human subjects, with positive stimulant effects at low and intermediate doses and more aversive effects at high doses (22, 23). The maximal stimulatory effects of caffeine on the activity in rodents have been found at low to intermediate doses and intermediate plasma and brain caffeine concentrations (10-20 μg/ml). Similarly, the rewarding effects of caffeine were demonstrated at low doses (3 mg/kg) in rats as shown by place preferences for environmental stimuli associated with the drug (22, 23).

In this study, psychomotor performance testing indicated enhanced RT performance at several time points with low and high doses of caffeine compared to placebo. However, high dose of caffeine produced less performance enhancement than the lower one (Tables 14-17). The peak performance was observed around 1.5-2 hr with the lower dose of caffeine, whereas the peak was generally observed between 0.5-1 hr with the higher dose. The more rapid peak performance with the high dose of caffeine is likely to be due to the quicker rise of plasma concentrations up to the stimulating levels of caffeine. There was an indication that the stimulating effect of caffeine on psychomotor performance had a positive correlation with the change in caffeine concentration at different times. It is not practical to measure plasma caffeine concentrations in this group of subjects, since it would interfere with the reaction time testing procedures. However, the pharmacokinetic data obtained from similar group of Thai healthy males could also be used to provide information on blood levels of caffeine.

The SRT was shorter than CRT. This is due to the fact that a simple stimulus, which requires little cerebral processing, will result in a faster RT than a

complex stimulus, which requires discrimination. The SRT and CRT scores remained different from baseline values throughout the 8 hr observation period. Elimination of caffeine was mirrored by formation of three metabolites. Since both caffeine and its metabolites can bind to adenosine receptors and elicit similar stimulant properties, these metabolites, therefore, contribute to the net stimulant effects of caffeine.

Up until now, caffeine's effects on RT have hardly been consistent. Estler (218) as well as James (219) came to the conclusion that a series of intervening conditions might determine the outcome of the measurements. Among these conditions, the sensory modality involved, the type of motor response required, the levels of attention and arousal, and stimulus intensity might all be similarly important. With respect to the substance *per se*, dosage and habituation represent additional factors, as it has often been suggested that medium doses might produce improvements and high doses could exert impairments or no effects (220). In addition, it should be considered that "optimal dose" levels might be different between high and low users of caffeine.

In the previous study performed by Tapna (30), caffeine at the dose of 200 mg produced small changes in EEG when compared to placebo. Reduced total, beta and alpha amplitudes compared to placebo were observed. However, the EEG consequences of caffeine administration in humans have not been completely elucidated. Despite the clear dose-dependent effects of caffeine on mood and performance, caffeine produced only small change in the EEG, which did not vary with doses (21, 30). The overall findings suggest that analysis of the EEG does not provide data useful for tracking the central pharmacodynamic effects of caffeine.

About 65% of subjects, participated in this study, complained about palpitation, nervousness and restlessness after taking 400 mg of caffeine. In human, low to intermediate doses of caffeine (generally <250 mg) have stimulant effects and increase arousal, alertness, and performance on cognitive and motor tasks. High doses (>500 mg) cause nervousness and adverse somatic effects such as restlessness, agitation, chills, tremors, nausea, and disrupt performance (16).

A range of acute adverse effects can occur following ingestion of high dose of caffeine or usual dose in sensitive individuals. The present study did not implicate the adverse effects of a single dose administration of 200 mg of caffeine containing in the popular energy drink in the Thai healthy male subjects. A less favorable response, however, was observed with the dose of 400 mg caffeine.

The typical caffeine contents in various beverages, i.e., 50 mg per one bottle of energy drink, 85-115 mg per cup of brewed coffee, 60-65 mg per cup of instant coffee, 40-50 mg per cup of tea, and 10-20 mg per a bottle of soft drink (1, 54) should not pose a serious health risk to the Thai population at large. It is reasonable to assume that intake of a single oral dose of caffeine in Thai healthy individuals should not exceed 200 mg. However, a much higher dose than regularly obtained from dietary sources should be discouraged.

Long term intake of caffeine has been constantly implicated in various adverse effects on human health. Controversies still exist in the published literature regarding the long-term effects of caffeine consumption. The better-controlled studies are needed to substantiate the long-term effects of this widely used CNS stimulant.

CHAPTER VII

CONCLUSION

1. In this study, the HPLC method for quantitative analysis of caffeine in plasma exhibits acceptable specificity and sensitivity. This method showed linearity up to caffeine concentration of 30 μg/ml. Good analytical recovery was observed both in aqueous solutions and in plasma samples. The CV of within-batch and between-batch precision of caffeine were not more than 10 %. These results were satisfactory within the acceptable criteria for analytical assays of plasma caffeine.

2. The pharmacokinetic parameters of caffeine after oral administration of 200 mg caffeine in energy drink were determined in 12 healthy volunteers. The peak plasma concentration of caffeine levels reached the mean value of 5.45±0.57 μg/ml at T_{max} value of 0.92±0.39 hr. The average $t_{1/2}$ was 4.57±0.37 hr. The other pharmacokinetic parameters include $AUC_{0\rightarrow 8}$ and $AUC_{0\rightarrow \alpha}$ values of 26.04±4.28 and 38.94±8.41 μg.hr/ml, respectively, Vd value of 0.60±0.12 L/kg and CL value of 1.53±0.31 ml/min.kg.

3. In the pharmacodynamic studies of caffeine, another 12 subjects received oral placebo, 200 mg and 400 mg of caffeine in a randomized, crossover study with 3-day intervals. Psychomotor performance testing indicated enhanced SRT and CRT. The lower dose of caffeine (200 mg) improved performance as the increment over the

pre-dose baseline scores compared to placebo ($p<0.05$), whereas high dose of caffeine (400 mg) produced less effect.

4. The ANOVA performed as in the sum of RT differences, the sum of SRT and 3CRT differences indicated statistically significant differences between the placebo and 200 mg caffeine and between 200 mg and 400 mg caffeine ($p<0.05$) but showed no significant difference between placebo and 400 mg caffeine. In the case of 6CRT and 9CRT the sum of differences indicated statistically significant differences among the three doses of caffeine.

5. The peak performance was observed around 1.5-2 hr with the lower dose of caffeine, whereas the peak was generally observed between 0.5-1 hr with the higher dose.

6. The SRT and CRT scores remained significantly different from baseline values throughout the 8 hr observation period.

7. The overall results indicated that caffeine at the dose of 200 mg produces performance-enhancing stimulant effect while caffeine at the dose of 400 mg produces less enhancement compared to the placebo.

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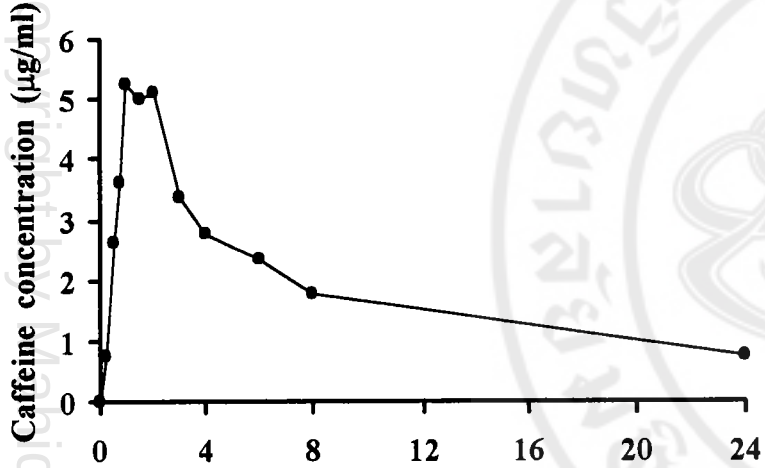
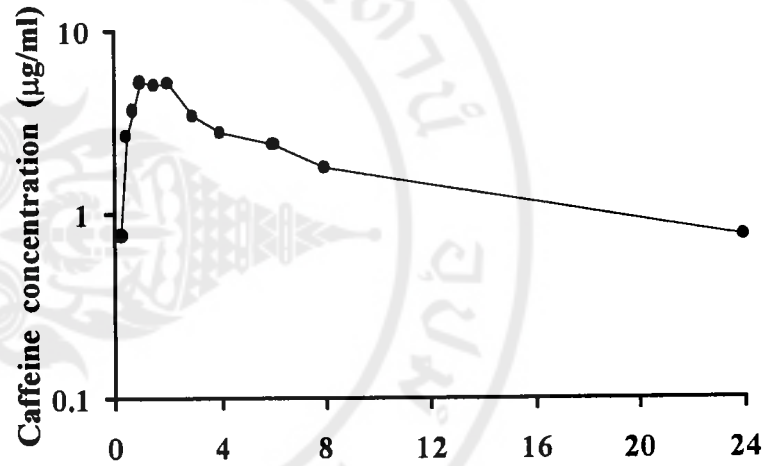
Appendix

The individual plasma caffeine concentration-time profile and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink

SUBJECT No. 1

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.



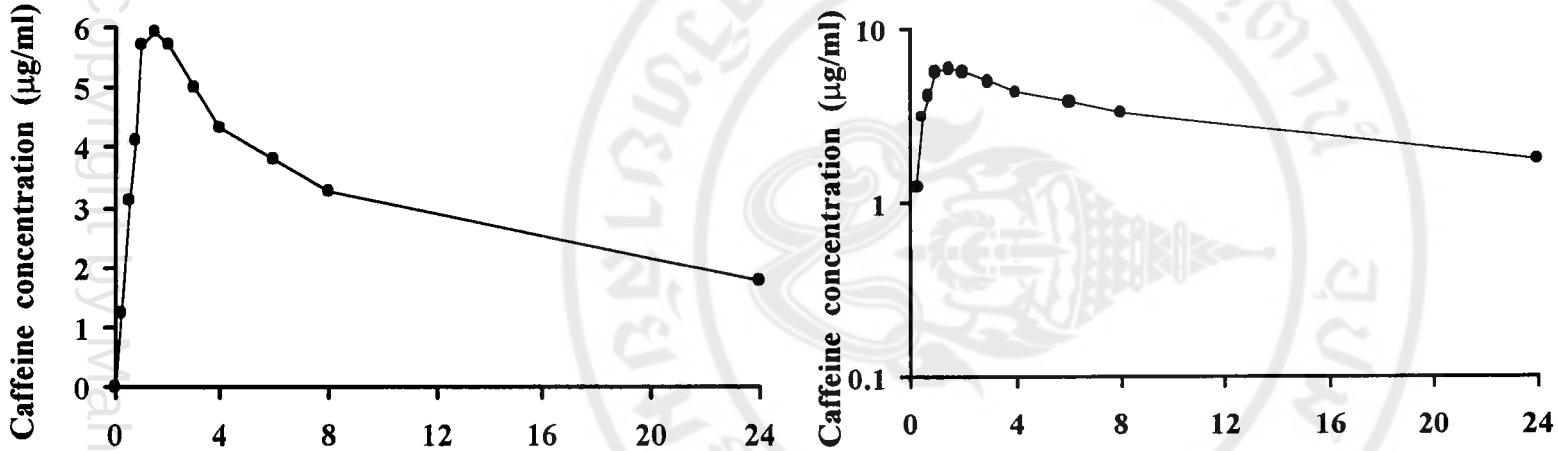


Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
5.27	1	0.164	4.23	24	34.8	0.57	1.57

SUBJECT No. 2

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.

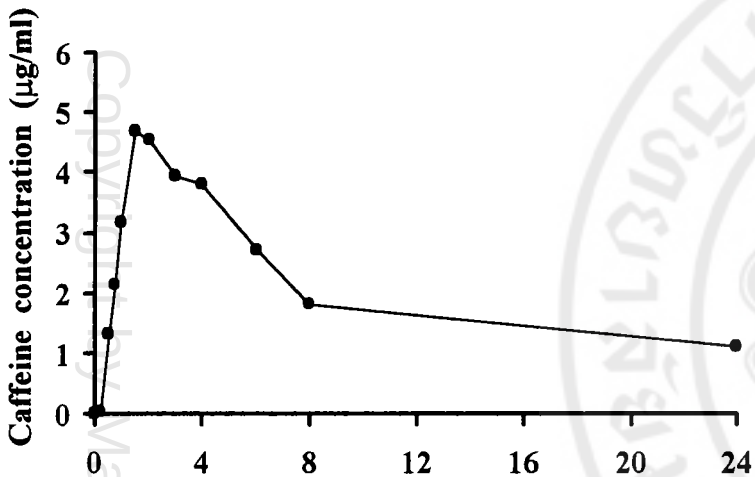


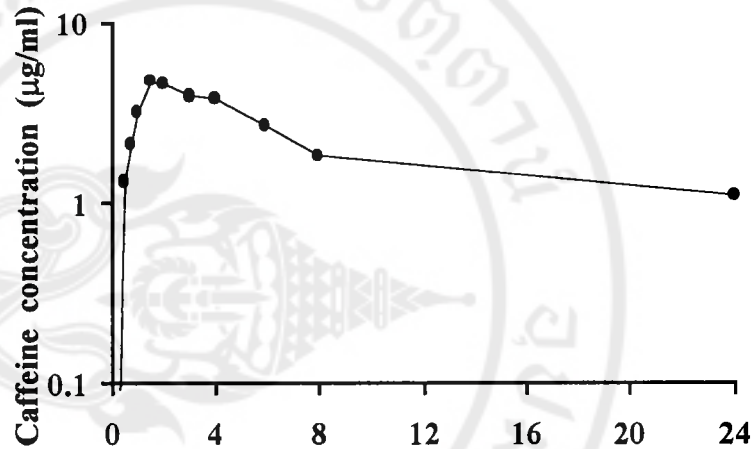
Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
5.92	1.5	0.135	5.13	33.85	58.07	0.53	1.19

SUBJECT No. 3

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.



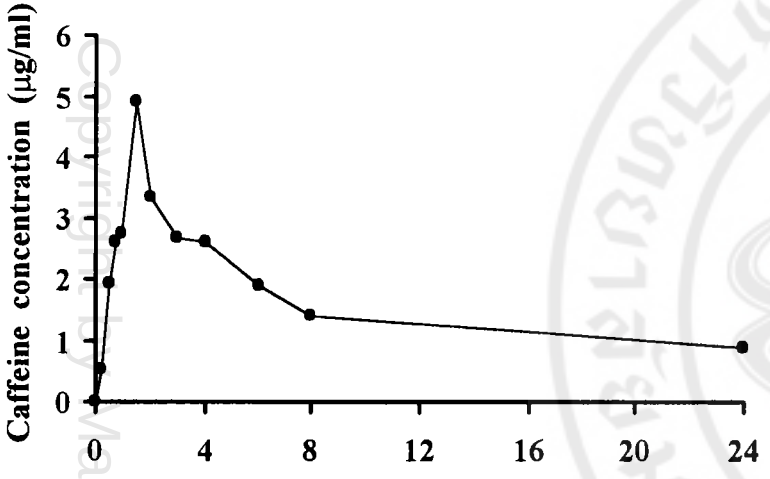


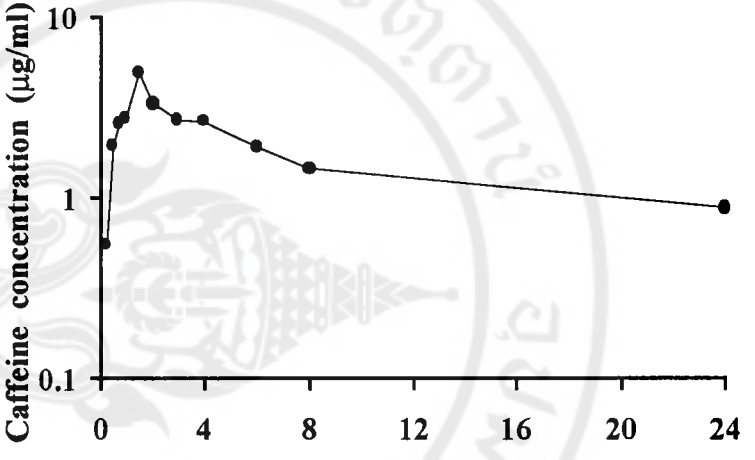
Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
4.69	1.5	0.156	4.43	24.64	36.31	0.61	1.58

SUBJECT No. 4

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.



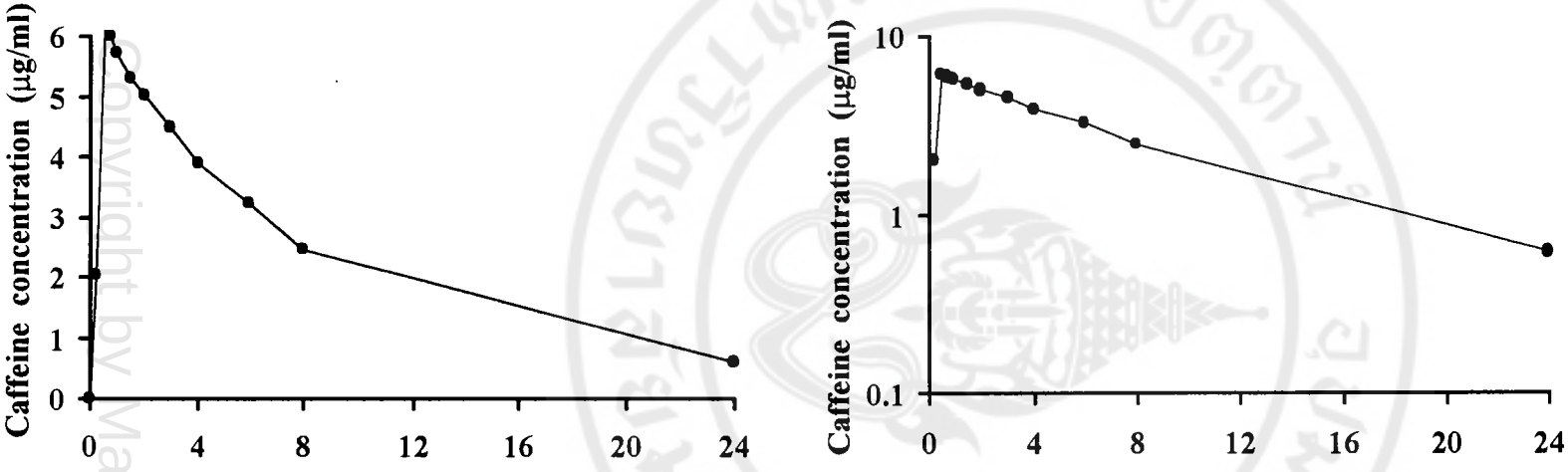


Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
4.92	1.5	0.14	4.95	19.02	29.16	0.96	2.24

SUBJECT No. 5

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.

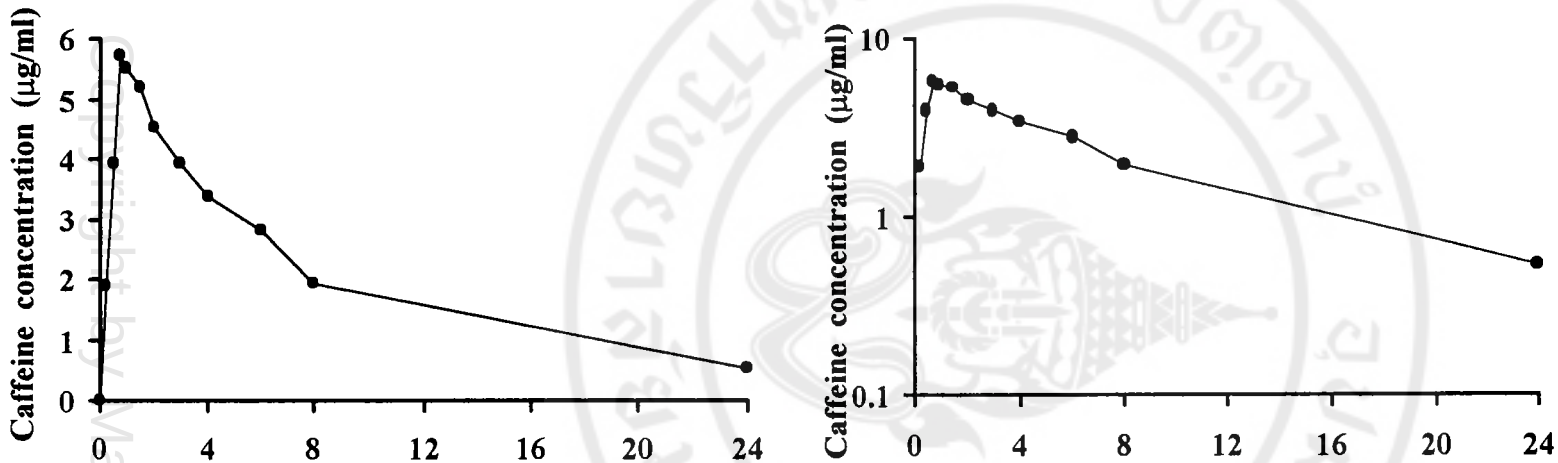


Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
6.13	0.5	0.145	4.77	30.87	47.77	0.5	1.2

SUBJECT No. 6

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.

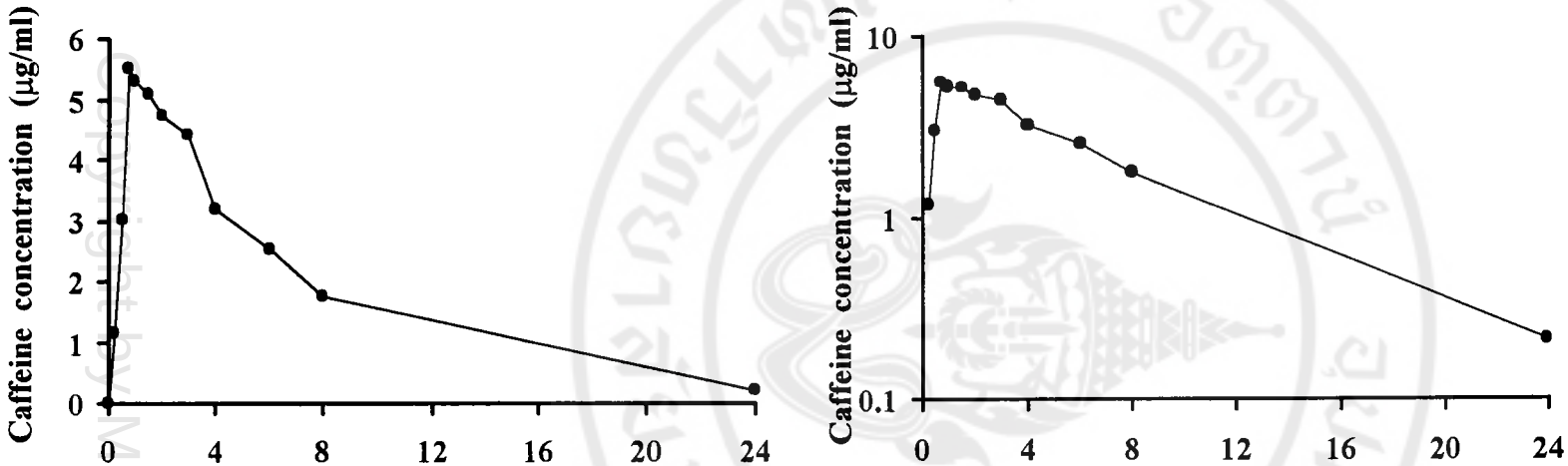


Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
5.71	0.75	0.142	4.88	27.44	41.03	0.48	1.13

SUBJECT No. 7

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.

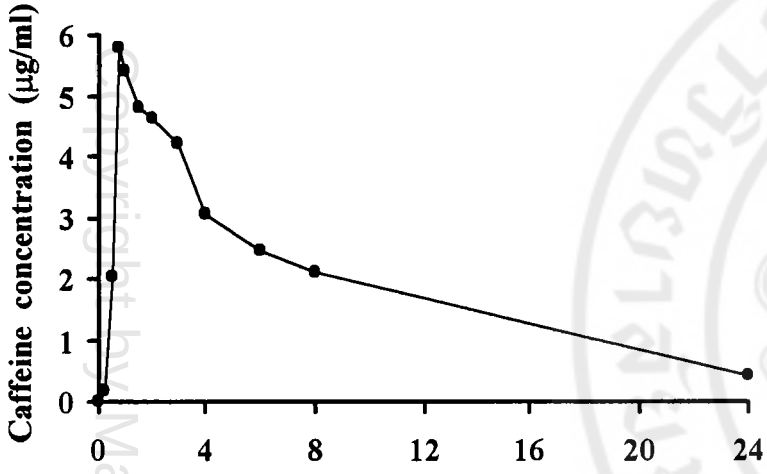


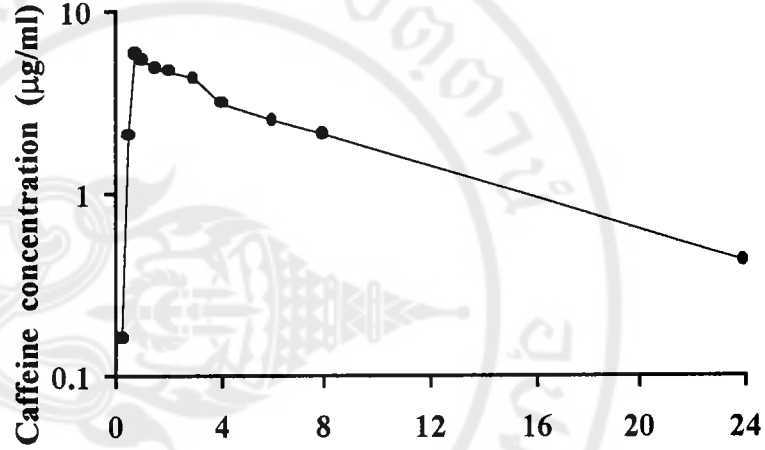
Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
5.51	0.75	0.175	3.96	26.57	36.63	0.54	1.57

SUBJECT No. 8

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.



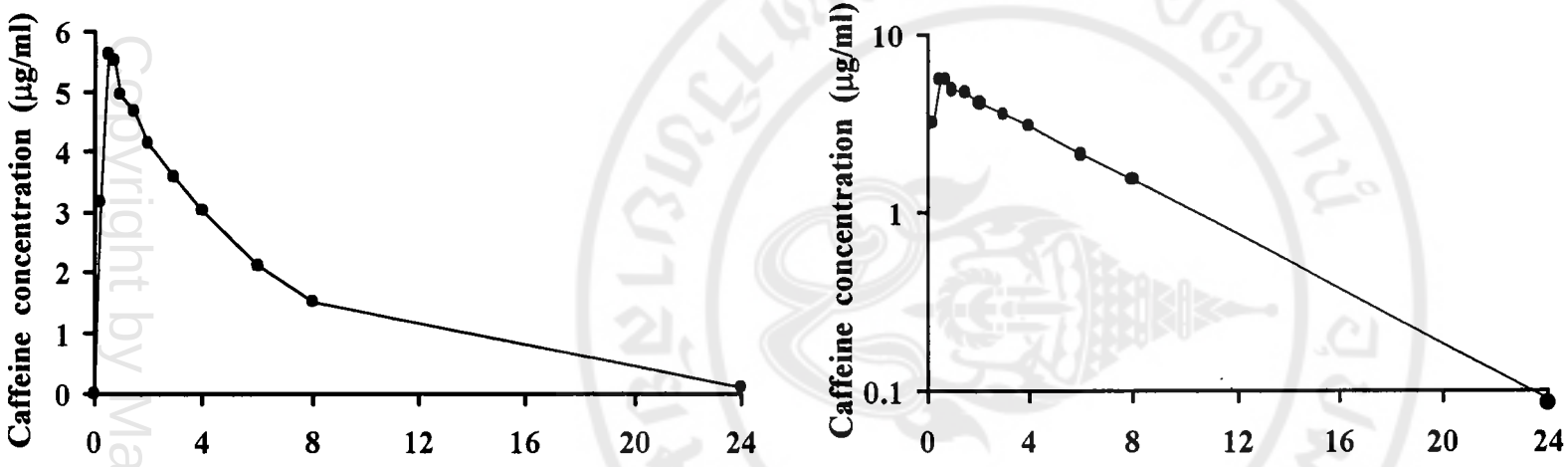


Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
5.8	0.75	0.15	4.62	25.75	39.68	0.55	1.38

SUBJECT No. 9

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.

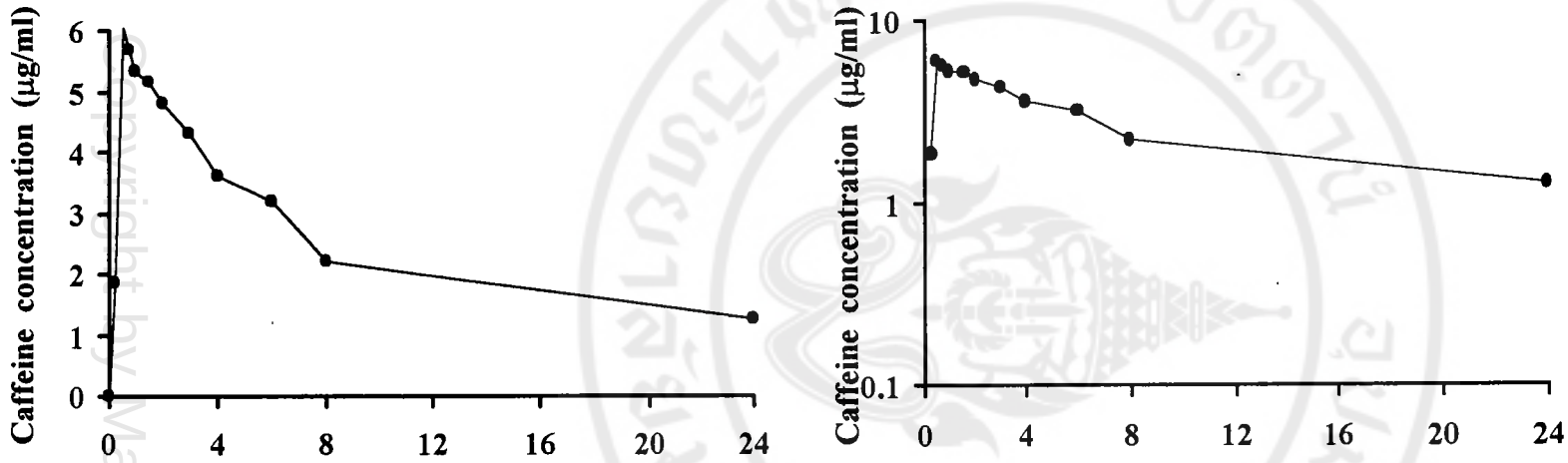


Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
5.61	0.5	0.151	4.59	24.7	34.77	0.61	1.55

SUBJECT No. 10

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.

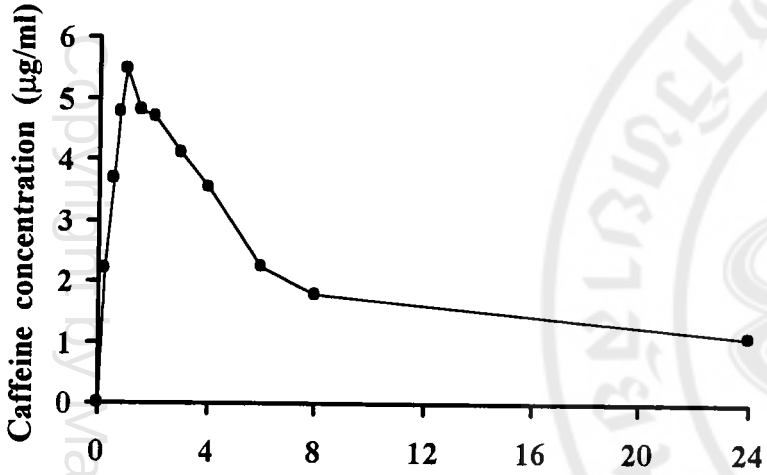


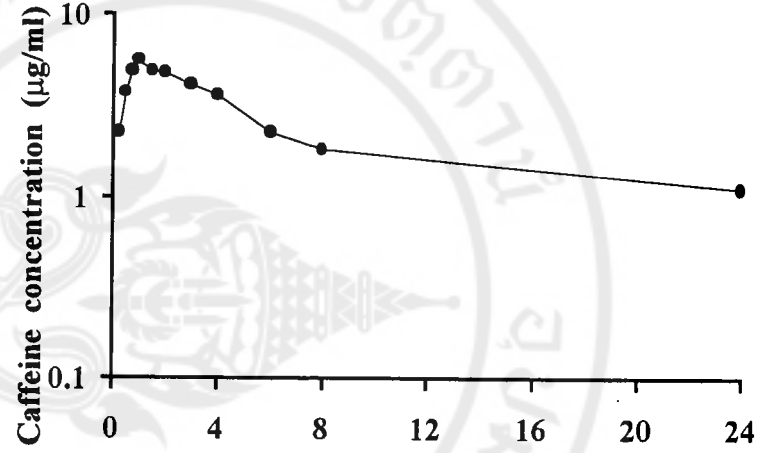
Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
6.05	0.5	0.143	4.85	30	45.45	0.56	1.33

SUBJECT No. 11

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.



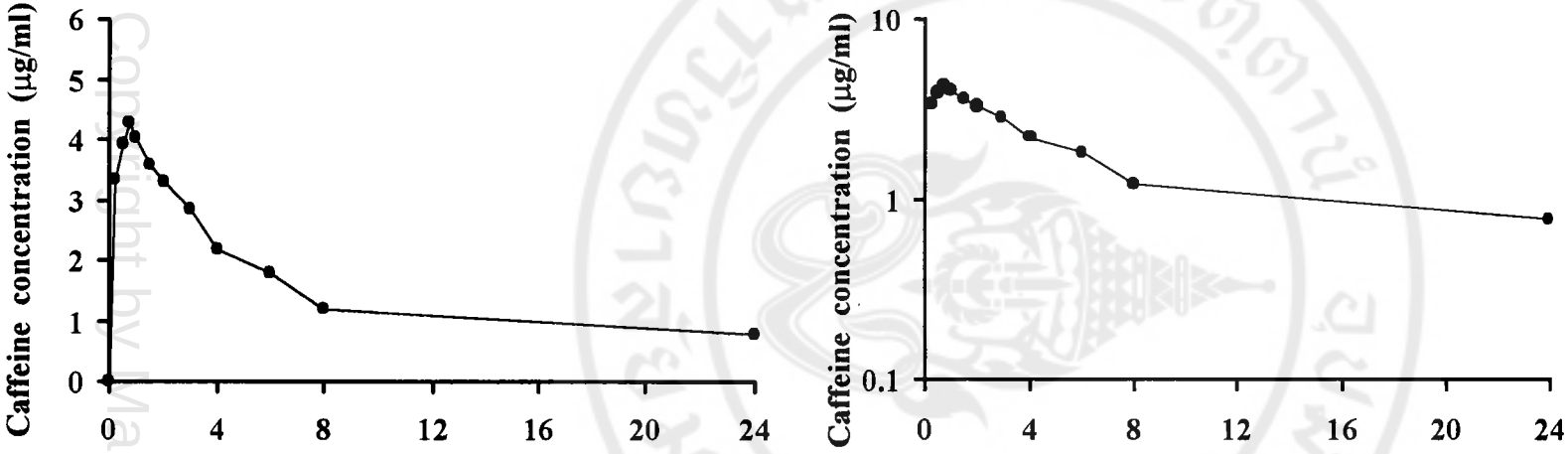


Pharmacokinetic parameters

C_{max}	T_{max}	ke	$t_{1/2}$	$AUC_{0\rightarrow 8}$	$AUC_{0\rightarrow \alpha}$	Vd	CL
(µg/ml)	(hr)	(hr^{-1})	(hr)	(µg. hr/ml)	(µg. hr/ml)	(L/kg)	(ml/min.kg)
5.46	1	0.174	3.98	26.08	36.42	0.63	1.83

SUBJECT No. 12

Plasma caffeine concentration-time curve and pharmacokinetic parameters after taking an oral dose of 200 mg caffeine in energy drink.



Pharmacokinetic parameters

C_{max} (µg/ml)	T_{max} (hr)	ke (hr^{-1})	$t_{1/2}$ (hr)	$AUC_{0\rightarrow 8}$ (µg. hr/ml)	$AUC_{0\rightarrow \alpha}$ (µg. hr/ml)	Vd (L/kg)	CL (ml/min.kg)
4.27	0.75	0.156	4.43	19.53	27.16	0.67	1.75

BIOGRAPHY

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Chiang Mai University, 1991-1996:

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Master of science (Pharmacology)