Pharmacology of ephedra alkaloids and caffeine after single-dose dietary supplement use

Objective: **Serious cardiovascular toxicity has been reported in people taking dietary supplements that contain ma huang *(Ephedra)* and guarana (caffeine). We assessed the pharmacokinetics and pharmacodynamics of a dietary supplement that contains these herbal stimulants.**

Methods: **Eight healthy adults received a single oral dose of a thermogenic dietary supplement labeled to contain 20 mg ephedrine alkaloids and 200 mg caffeine after an overnight fast. Serial plasma and urine samples were analyzed by use of liquid chromatography–tandem mass spectrometry for ephedrine alkaloid and caffeine concentrations, and heart rate and blood pressure were monitored for 14 hours.**

Results: **Plasma clearance and elimination half-lives for ephedrine, pseudoephedrine, and caffeine were comparable to published values reported for drug formulations. A prolonged half-life of ephedrine and pseudoephedrine was observed in 1 subject with the highest urine pH. Mean systolic blood pressure increased significantly to a maximum of 14 mm Hg above baseline at 90 minutes after ingestion ($P < .001$). There was a lag in the mean heart rate response that reached a maximum change of 15 beats/min above baseline at 6 hours after ingestion ($P < .001$). Diastolic blood pressure changes were insignificant. Two subjects who were taking oral contraceptives had longer caffeine half-lives (15.5 ± 0.3 hours versus 5.6 ± 1.7 hours) and lower values for oral clearance (0.34 ± 0.01 mL/min · kg versus 0.99 ± 0.41 mL/min · kg) than subjects who were not taking oral contraceptives.**

Conclusions: **Botanical stimulants have disposition characteristics similar to their pharmaceutical counterparts, and they can produce significant cardiovascular responses after a single dose. (Clin Pharmacol Ther 2002;71:421-32.)**

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A number of reports of serious cardiovascular toxicity have been reported in persons taking thermogenic dietary supplements that contain ma huang *(Ephedra sinica)*, a botanical source of ephedrine alkaloids, and guarana *(Paullinia cupana)*, a botanical source of caffeine. Adverse events have included psychosis, ischemic and hemorrhagic stroke, seizures, acute myocardial infarction, myocarditis, and sudden death.[1-6] In June 1997, the US Food and Drug Administration (FDA) proposed restrictions on dietary supplements that contained ephedrine alkaloids[7] but withdrew this proposal in April of 2000 after the US General Accounting Office concluded that there was insufficient evidence to support the proposed restrictions. Today, in the wake of several highly publicized deaths possibly linked to thermogenic supplement use, the FDA is again being called on to enact stricter regulation of ephedrine and caffeine-containing supplements.[8,9] Although the combination of ephedrine and caffeine has been investigated for its efficacy as an anorectic and ergogenic agent,[10-12] the pharmacokinetics and pharmacodynamics of the ephedra alkaloids and caffeine contained in dietary supplements have not been well characterized in humans.

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The Chinese herb ma huang is derived from the dried above-ground parts of *Ephedra* sp, most commonly *E sinica*. *E sinica* is a natural source of several ephedrine alkaloids, including ephedrine, pseudoephedrine, norephedrine, methylephedrine, norpseudoephedrine, and methylpseudoephedrine. The total alkaloid content of *E sinica* is approximately 1% to 2%, with ephedrine being the most abundant alkaloid.[13] Although the content can vary from sample to sample, ephedrine and pseudoephedrine together generally constitute more than 80% of the alkaloid content of the dried herb.[14] Norephedrine is structurally identical to phenylpropanolamine, a synthetic over-the-counter drug previously available for weight loss and nasal decongestion until a study that showed an increased risk of stroke led to the voluntary withdrawal of all phenylpropanolamine products in November 2000.[15] Phenylpropanolamine is a racemic mixture of norephedrine, but ephedra contains only the (–)-isomer.

Most dietary supplements that contain ephedra are marketed for weight loss or athletic enhancement and contain, in addition to ephedra, a number of other botanical ingredients intended to enhance the thermogenic and ergogenic effects of ephedrine. These products often include a botanical source of caffeine, either guarana *(P cupana)*, kola nut *(Cola nitida)*, or yerba mate, with a typical caffeine dose of 40 to 200 mg per serving. Guarana contains 3% to 5% caffeine and lesser amounts of the other methylxanthines, theophylline and theobromine.[16] Caffeine and theophylline are known to be subject to a number of clinically significant pharmacokinetic and pharmacodynamic drug-drug interactions. Caffeine and phenylpropanolamine appear to have important interactive pharmacodynamic effects,[17] and the FDA banned this drug combination in 1983 because of numerous reports of adverse events. Whether there are important herb-herb interactions in combination dietary supplements that contain ephedrine alkaloids and caffeine remains to be investigated.

Two studies have reported that the pharmacokinetics of ephedrine in the form of ephedra extracts are similar to those of synthetic ephedrine.[18,19] To date, the pharmacokinetics of the other ephedrine alkaloids and methylxanthines contained in dietary supplements have not been described. The purpose of this study was to characterize the pharmacokinetics and pharmacodynamics of the various ephedrine alkaloids and caffeine after oral ingestion of a commercial dietary supplement that contains botanical sources of these chemicals.

METHODS

Subjects

Eight healthy volunteers (5 women and 3 men) from 25 to 38 years old participated in the study. All volunteers gave written informed consent before study enrollment. The study protocol and consent form were approved by the Committee on Human Research of the University of California, San Francisco. Eligibility for the study was determined on the basis of medical history, brief physical examination, and screening laboratory tests that included complete blood count, serum chemistry values to assess liver and renal function, urine toxicology testing for drugs of abuse, and a urine pregnancy test for women. Exclusion criteria included any person with obesity (body mass index >30), or a history of heart, thyroid, liver, kidney, or psychiatric disease, diabetes, central nervous system disorders, prostate hypertrophy, narrow angle glaucoma, or pregnancy or lactation. Any person with recent use (previous 1 month) of any product that contained ephedrine alkaloids or with a history of illicit substance use within the previous year was excluded. Smokers and heavy users of caffeine (>4 cups of coffee per day) were excluded. Participants were not taking any medications that would cause changes in heart rate or blood pressure.

Study design

A single-arm, open-label pilot study was designed to determine the pharmacokinetics and pharmacodynamics of ephedrine alkaloids and caffeine contained in a commercial dietary supplement administered as a single oral dose to healthy volunteers. Subjects were asked to abstain from caffeine consumption for 24 hours before the study. After an overnight fast, subjects were admitted to the General Clinical Research Center at San Francisco General Hospital and given 2 capsules of Metabolift Thermogenic Diet Formula (a weight loss aid manufactured by Twin Laboratories Inc, Ronkonkoma, NY) with water at 8 AM. Subjects continued to fast for an additional 2 hours but were allowed water, and then they were given caffeine-free meals. There was no change in the activity level of the subjects during the course of the study. Heart rate and blood pressure were recorded just before each sample collection with use of automated sphygmomanometry. An intravenous catheter was placed in a forearm vein of each subject and 7 mL blood was withdrawn into heparinized tubes just before dosing and at 0.5, 1, 1.5, 2, 4, 6, 8, 11, and 14 hours after ingestion. Samples were centrifuged and the plasma was collected and frozen at −20°C for later analysis of ephedrine alkaloid and

caffeine concentrations. Subjects completed 2 questionnaires that rated their moods, emotions, and physical symptoms before dosing and at 1 hour and 5 hours after ingestion. Subjects were discharged from the General Clinical Research Center the following morning.

Product analysis

Two capsules of Metabolift (lot No. 9C0335) were analyzed for concentrations of ephedrine, pseudoephedrine, norephedrine, methylephedrine, norpseudoephedrine, methylpseudoephedrine, caffeine, theophylline, and theobromine. The material from the capsules was extracted by a method similar to that described by Gurley et al.[20] The material from the capsules was ground with a mortar and pestle, weighed, and transferred to a glass culture tube (13 × 100 mm). To the tube was added 10 mL of 0.15 mol/L ammonium acetate buffer, pH 4, in 94:6 water/methanol (vol/vol). The tube was heated at 80°C for 30 minutes and then cooled and centrifuged. The supernate was diluted to 50 mL with the above acetate buffer, and an aliquot of this was diluted 1:500 with HPLC-grade water and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) as described for plasma and urine.

Plasma-urine analysis

The analytical methods are described in detail in the Appendix.

Pharmacokinetic and statistical analysis

Plasma concentration data were used to calculate pharmacokinetic parameters for ephedrine, pseudoephedrine, norpseudoephedrine, norephedrine, and caffeine by use of a noncompartmental model with the WinNonlin computer program (Professional Version 3.1, Pharsight, Mountain View, Calif). The area under the plasma concentration versus time curve (AUC) was calculated with use of the linear trapezoidal rule. The clearance divided by bioavailability (CL/F) was determined with the following equation:

$$\text{CL/F (L/h} \cdot \text{kg)} = \text{Dose (mg)}/\{[\text{AUC (mg} \cdot \text{h/L)} \times \text{subject weight (kg)}]\}$$

The other ephedrine alkaloids and methylxanthines were present in insufficient amounts for pharmacokinetic analysis. Concentrations of the ephedrine alkaloids and caffeine were measured in the total urine collected from time zero to study completion at 14 hours after dosing [Ae(0-14h)]. We obtained the renal clearance (CL_R) by dividing Ae(0-14h) by the plasma AUC for the same time period:

$$CL_R = \text{Ae(0-14h)/AUC(0-14h)}$$

Differences in mean values of heart rate and blood pressure from baseline were analyzed by paired Student *t* tests without correction for multiple comparisons with use of Statistica, version 5.5 (StatSoft Inc, Tulsa, Okla). The Wilcoxon signed-rank test was used to identify significant differences in reports of subjective mood, emotion, and physical symptom changes. In all analyses, statistical significance was defined as $P < .05$.

RESULTS

Product analysis

Analysis of 2 capsules of Metabolift (labeled to contain 10 mg ephedra alkaloids from ma huang extract, 100 mg caffeine from guarana seed extract, 50 mg L-carnitine, and 100 μg chromium picolinate per capsule) revealed an average of 8.65 mg ephedrine, 2.64 mg pseudoephedrine, 0.21 mg norpseudoephedrine, 0.25 mg methylephedrine, and 0.10 mg norephedrine, with a total ephedrine alkaloid content of 11.85 mg per capsule (119% of declared amount). Ephedrine alkaloid concentrations of the 2 analyzed capsules were nearly identical. The average caffeine content was 87.5 mg per capsule (87.5% of declared amount). No significant amount of theophylline or theobromine was detected. Therefore the standard oral dose of 2 capsules given in this study consisted of a total of 23.7 mg ephedrine alkaloids that consisted of 17.3 mg ephedrine, 5.3 mg pseudoephedrine, and insignificant amounts of the other alkaloids. This quantity of ephedrine is comparable to typical amounts contained in drug decongestant formulations. The total oral dose of caffeine was 175 mg.

Pharmacokinetics

Plasma concentration–time profiles for ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, and caffeine are shown in Fig 1. The time to maximum plasma concentration (t_{max}) was the same for ephedrine and pseudoephedrine (2.4 hours) and longer than the t_{max} for caffeine (1.5 hours). Pharmacokinetic parameters for ephedrine, pseudoephedrine, and caffeine are listed in Tables I, II, and III, respectively. The ratios of the observed plasma AUC values of metabolite to parent drugs for norephedrine and norpseudoephedrine, the primary metabolites of ephedrine and pseudoephedrine, along with the metabolite CL_R values, are shown in Table IV.

The CL/F for ephedrine averaged 0.35 ± 0.07 L/h · kg. The CL_R averaged 0.21 ± 0.07 L/h · kg, which represented 60% of the total CL/F. The half-life ($t_{\frac{1}{2}}$) averaged 6.1 ± 1.3 hours for ephedrine. Variation in the extent of metabolism of ephedrine to norephedrine

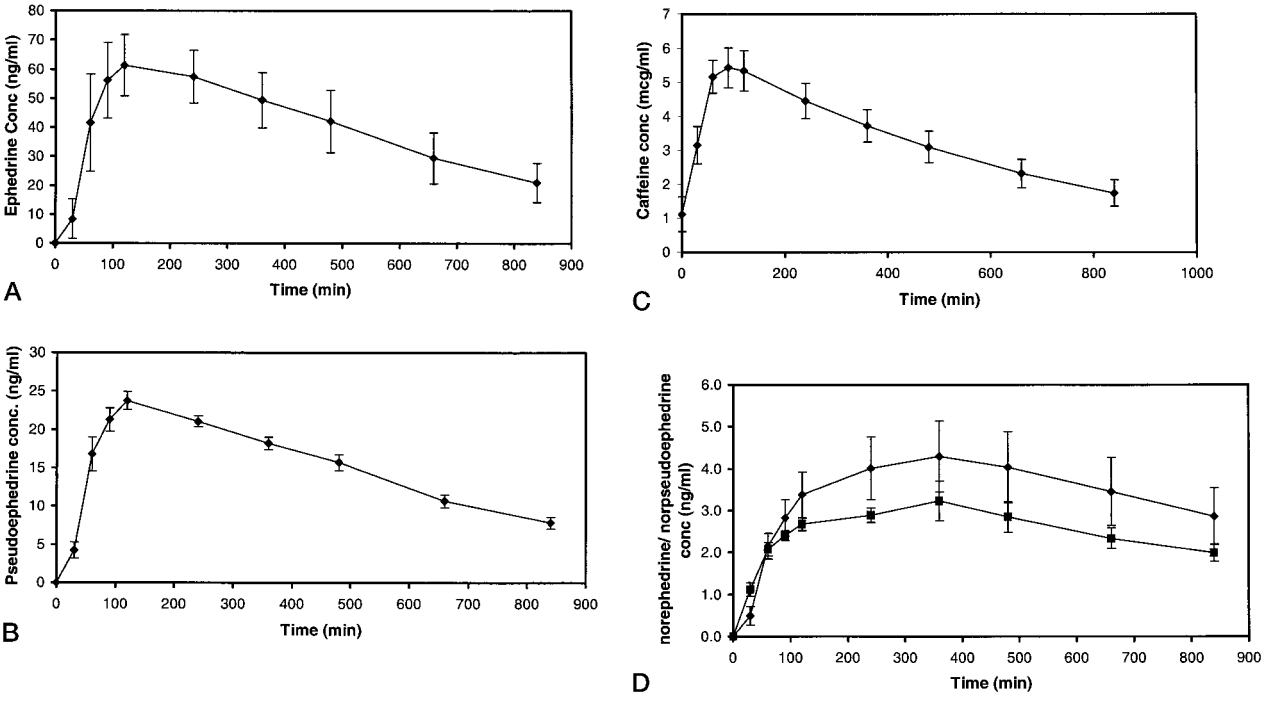

Fig 1. Plasma concentrations (conc) versus time for ephedrine **(A),** pseudoephedrine **(B),** caffeine **(C),** and norephedrine (**D;** *diamonds*) and norpseudoephedrine (**D;** *squares*). *Data points* represent mean values ± SEM (n = 8).

Table I. Ephedrine pharmacokinetic parameters (dose of 17.3 mg)

Subject No.	*Body weight (kg)*	t_{max} *(min)*	C_{max} *(ng/mL)*	$t_{\frac{1}{2}}$ *(h)*	*AUC (ng · h/mL)*	*CL/F (L/h · kg)*	*V/F (L/kg)*	CL_R *(L/h · kg)*
1	78.6	120	49.5	4.65	537.9	0.41	2.14	0.14
2	68.1	120	69.0	7.25	947.9	0.27	2.18	0.20
3	52.0	240	63.4	8.18	1000.4	0.33	3.06	0.19
4	76.2	240	58.1	5.62	748.7	0.30	1.92	0.12
5	56.8	120	77.2	4.90	727.2	0.42	2.31	0.33
6	72.7	120	51.6	5.32	517.9	0.46	2.75	0.20
7	54.6	90	80.0	5.51	950.3	0.33	2.07	0.25
8	88.9	90	59.5	7.0	645.0	0.30	2.38	0.23
Mean	—	142.5	63.5	6.06	759.4	0.35	2.35	0.21
SD	—	61.6	11.2	1.26	189.6	0.07	0.38	0.07

t_{max}, Time after dosing to maximum plasma concentration; C_{max}, maximum plasma concentration achieved after a single oral dose; $t_{\frac{1}{2}}$, elimination half-life; AUC, area under the plasma concentration versus time curve; CL/F, clearance divided by bioavailability; V/F, apparent volume of distribution; CL_R, renal clearance.

was observed, with the ratio of plasma AUC values ranging from 0.02 to 0.33 and the amount of norephedrine eliminated in the urine ranging from 0.29 mg to 2.34 mg. The amount of norephedrine eliminated divided by the dose of ephedrine averaged 0.063 ± 0.038, which represented the fraction of ephedrine metabolized to norephedrine in 14 hours, assuming total renal elimination of the metabolite. The CL_R of norephedrine averaged 0.33 L/h · kg, which was 57% higher than the average ephedrine CL_R.

For pseudoephedrine, CL/F averaged 0.28 ± 0.05 L/h · kg and CL_R averaged 0.22 ± 0.06 L/h · kg, which represented 79% of the total CL/F. The $t_{\frac{1}{2}}$ averaged 6.3 ± 1.6 hours for pseudoephedrine. Metabolism of pseudoephedrine to norpseudoephedrine also showed significant interindividual variation, with plasma AUC

Table II. Pseudoephedrine pharmacokinetic parameters (dose of 5.3 mg)

Subject No.	t_{max} *(min)*	C_{max} *(ng/mL)*	$t_{\frac{1}{2}}$ *(h)*	*AUC (ng · h/mL)*	*CL/F (L/h · kg)*	*V/F (L/kg)*	CL_R *(L/h · kg)*
1	240	19.4	5.87	245.2	0.28	2.33	0.16
2	120	24.1	6.88	305.5	0.26	2.53	0.23
3	120	23.1	9.83	398.7	0.26	3.62	0.19
4	240	22.1	6.43	323.0	0.22	2.00	0.14
5	120	29.3	4.55	254.8	0.37	2.40	0.32
6	120	22.1	6.00	228.6	0.32	2.76	0.20
7	90	29.1	5.38	318.6	0.31	2.37	0.30
8	120	23.7	5.15	223.8	0.27	1.98	0.25
Mean	146.3	24.1	6.26	287.3	0.28	2.50	0.22
SD	58.8	3.5	1.62	60.1	0.05	0.52	0.06

Table III. Caffeine pharmacokinetic parameters (dose of 175 mg)

Subject No.	t_{max} *(min)*	C_{max} *(μg/mL)*	$t_{\frac{1}{2}}$ *(h)*	*AUC (mg · h/L)*	*CL/F (mL/min · kg)*	*V/F (L/kg)*
1	120	3.19	3.69	23.8	1.56	0.51
2	90	5.01	3.98	36.3	1.18	0.42
3*	120	8.47*	5.71	83.3	0.67*	0.34*
4	60	6.14	8.25	82.5	0.46	0.34
5	60	5.94	5.67	63.3	0.81	0.41
6	120	3.97	6.50	42.0	0.96	0.55
7	120	6.61	15.7	157.8	0.34	0.47
8	60	4.35	15.2	100.1	0.33	0.44
Mean	94	5.0	8.1	73.7	0.81	0.44
SD	29.7	1.26	4.76	43.0	0.46	0.07
No OCP			5.6 ± 1.7		0.99 ± 0.41	
OCP			15.5 ± 0.3		0.34 ± 0.01	

OCP, Oral contraceptive pills.
*Subject 3 data excluded from analysis of C_{max}, CL/F, and V/F because predose caffeine level was 3.8 μg/mL, indicative of a protocol violation.

ratios ranging from 0.18 to 0.70. The CL_R of norpseudoephedrine averaged 0.18 L/h · kg, which was 18% lower than the average CL_R of pseudoephedrine.

In subject 3, the $t_{\frac{1}{2}}$ for ephedrine was 8.2 hours, 1.7 times the standard deviation greater than the mean, and the $t_{\frac{1}{2}}$ for pseudoephedrine was 9.8 hours, 2.2 times the standard deviation greater than the mean. Subject 3 was noted to have the highest average urine pH among the study subjects. A trend of increased elimination $t_{\frac{1}{2}}$ values of the alkaloids with increased average urine pH is shown in Fig 2.

The mean t_{max} of caffeine was 94 ± 30 minutes. The mean CL/F for caffeine was 0.81 ± 0.46 mL/min · kg, and the mean elimination $t_{\frac{1}{2}}$ for caffeine was 8.1 hours. Subjects 7 and 8 had caffeine elimination $t_{\frac{1}{2}}$ values that were nearly 3 times longer than the other subjects (15.5 versus 5.6 hours). These subjects were women who reported they were taking oral contraceptives. One male subject was excluded from the calculation of mean pharmacokinetic values for caffeine because of a high baseline plasma caffeine concentration indicative of an apparent protocol violation.

Pharmacodynamics

Adverse effects during the study were limited to 2 complaints of palpitations. Fig 3 depicts the mean change in systolic and diastolic blood pressure and heart rate over time. The average baseline heart rate was 67 beats/min. The mean maximum heart rate was 82 beats/min, which occurred 6 hours after ingestion. The mean change in heart rate was statistically significant at 4 hours, 6 hours, and 11 hours compared with baseline. One subject had recorded tachycardia at 6 and 11 hours after ingestion, with a maximal heart rate of 108 beats/min. The greatest individual heart rate change occurred in a subject with a baseline heart rate of 53 beats/min and a peak heart rate of 90 beats/min at 6 to 8 hours after ingestion.

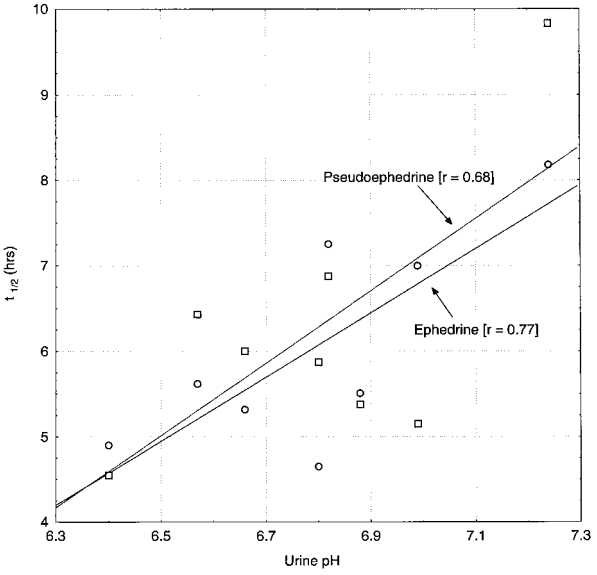

Fig 2. Elimination half-life ($t_{\frac{1}{2}}$) of each subject shown as a function of average urine pH for ephedrine (*circles;* $r = 0.77$; $P < .05$) and pseudoephedrine (*squares;* $r = 0.68$; $P > .05$).

Table IV. Metabolite/parent drug plasma AUC ratios and metabolite CL_R values for ephedrine alkaloids

Subject No.	*Plasma NE/ ephedrine AUC ratio*	*NE CL_R (L/h · kg)*	*Plasma NPE/ pseudoephedrine AUC ratio*	*NPE CL_R (L/h · kg)*
1	0.33	0.33	0.70	0.16
2	0.17	0.26	0.20	0.18
3	0.19	0.39	0.26	0.18
4	0.29	0.23	0.33	0.11
5	0.05	0.40	0.28	0.24
6	0.17	0.32	0.24	0.22
7	0.02	0.43	0.18	0.15
8	0.13	0.31	0.27	0.22
Mean	0.17	0.33	0.31	0.18
SD	0.11	0.07	0.17	0.04

NE, Norephedrine; NPE, norpseudoephedrine.

The average systolic blood pressure at baseline was 115 mm Hg, and the mean diastolic blood pressure was 72 mm Hg. The maximum change in mean systolic blood pressure was an increase of 14 mm Hg above baseline, which occurred 90 minutes after ingestion. Compared with baseline, the mean systolic blood pressure change was statistically significant ($P < .05$) at 60, 90, and 120 minutes after ingestion. The maximum mean change in diastolic blood pressure was 6 mm Hg,

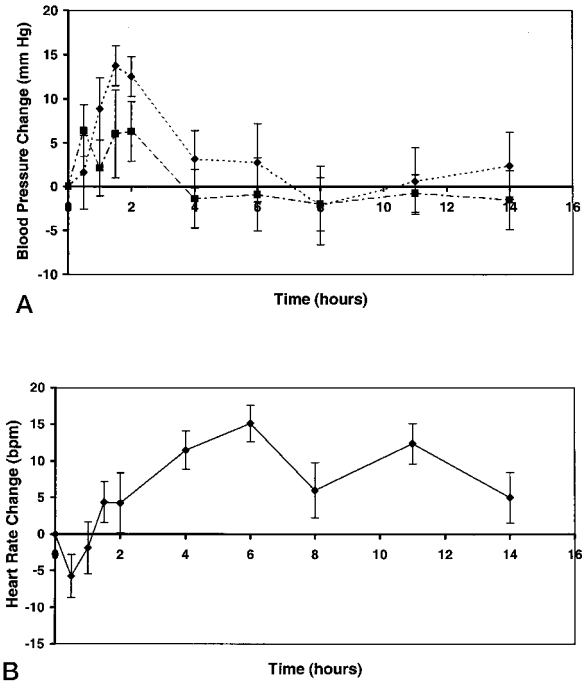

Fig 3. Changes in blood pressure **(A)** and heart rate **(B)** plotted over time. *Data points* represent the mean values ± SEM (n = 8) for changes in systolic *(***A;** *solid diamonds)* and diastolic *(***A;** *solid squares)* blood pressure and heart rate *(***B**; *solid diamonds)* compared with baseline measurements.

which occurred at 30, 90, and 120 minutes after ingestion. Compared with baseline, this change approached statistical significance ($P = .06$) at 30 minutes. Three subjects had at least 1 recorded episode of mild clinical hypertension during the study: 2 with diastolic blood pressure measurements of 90 mm Hg and 1 with a systolic blood pressure measurement of 143 mm Hg.

Fig 4, *A*, shows the systolic blood pressure change and Fig 4, *B*, shows the mean heart rate change as a function of mean plasma ephedrine concentration. The heart rate and blood pressure changes are shown in relation to caffeine concentrations in Fig 5, *A* and *B*. The clockwise direction of the hysteresis curve in Fig 4, *A*, suggests that tolerance develops to the pressor effects of ephedrine. The reverse directionality in the heart rate response seen in Figs 4, *B*, and 5, *B*, indicates that there is a lag-time in the heart rate response.

Subjective effects

The responses from the subject questionnaires on mood, emotional feelings, and adverse physical symptoms at 1 and 5 hours after dosing are shown in Tables V and VI, respectively. At baseline, 99% of the symp-

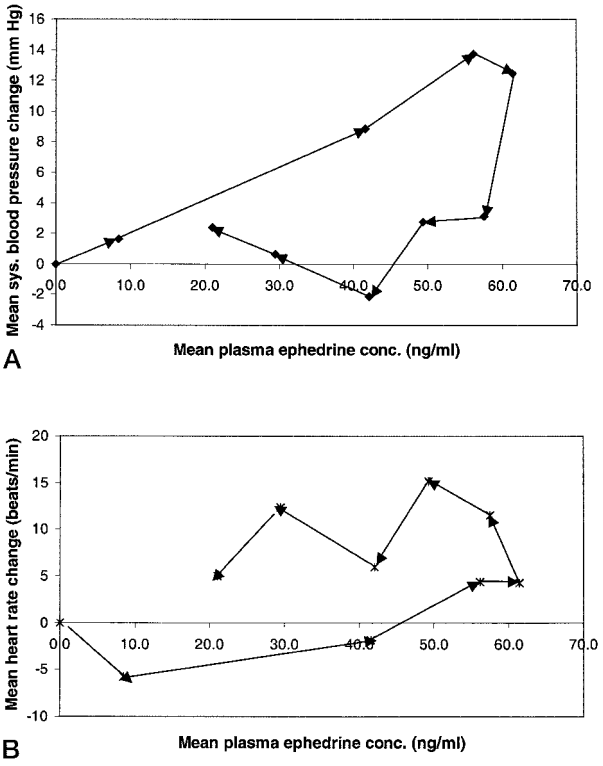

Fig 4. Changes in systolic blood pressure **(A)** and heart rate **(B)** are plotted versus plasma ephedrine concentration. *Data points* represent mean values (n = 8) and *arrows* represent the order in which concentrations and measurements were taken.

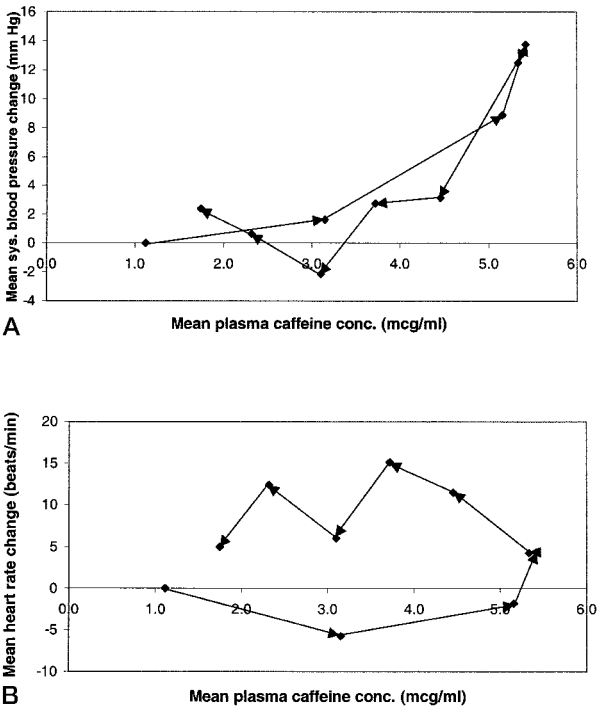

Fig 5. Changes in systolic blood pressure **(A)** and heart rate **(B)** versus plasma caffeine concentration. *Data points* represent mean values (n = 8) and *arrows* represent the order in which concentrations and measurements were taken.

tom responses and 54% of the mood responses were "None." A total of 14 physical symptoms were recorded at 1 hour and 16 symptoms at 5 hours after dosing. The most common adverse effects reported were restlessness, heart pounding, and shakiness. Three symptoms were reported to be of strong intensity at 1 hour: shakiness (n = 2), heart pounding (n = 1), and restlessness (n = 2); 1 symptom was reported to be of strong intensity at 5 hours: shakiness (n = 1). Compared with baseline responses, shakiness at 5 hours was the only statistically significant physical symptom with use of the Wilcoxon matched-pairs test.

Statistically significant ($P < .05$) differences in reported mood and emotions 1 hour after dosing compared with baseline by use of the Wilcoxon matched-pairs test were feeling "energetic" (n = 6), "contented" (n = 4), and "relaxed" (n = 3). At 5 hours after dosing, subjects most commonly reported feeling "contented" (n = 6), "energetic" (n = 5), "relaxed" (n = 5), "happy/elated" (n = 4), and "irritable" (n = 4), but these responses were not statistically significant.

Subjects were also asked to rate on a 10-cm visual analog scale how well given words or phrases matched their feelings at baseline and at 1 hour and 5 hours after dosing. Questions included feeling "high," "any drug effect," "good drug effect," "bad drug effect," "drug liking," "talkative," "closeness to others," "energetic," "insightful," "anxious," "friendly," and "confident." Baseline values were zero for all subjects. Subjective responses were statistically significant ($P < .05$) at 1 hour compared with baseline for feeling "high," "any drug effect," "good drug effect," "drug liking," and "energetic" based on the Wilcoxon matched-pairs test. At 5 hours after dosing, "any drug effect" was the only statistically significant response compared with baseline.

DISCUSSION

This study provides novel data on the plasma concentrations and pharmacokinetic profiles of several ephedrine alkaloids and caffeine, as well as on the relationship between pharmacodynamic responses and plasma levels. It also provides novel information on

Table V. Mood, emotion, and physical symptom responses 1 hour after administration (n = 8)

	No. of subjects reporting physical symptoms			*No. of subjects reporting mood and emotional feelings*		
	None	*Slight*	*Strong*	*None*	*Slight*	*Strong*
Headache	8	0	0	—	—	—
Stomach upset	8	0	0	—	—	—
Chest pain	7	1	0	—	—	—
Shakiness	5	1	2	—	—	—
Heart pounding	4	3	1	—	—	—
Flushed feeling	6	2	0	—	—	—
Short of breath	8	0	0	—	—	—
Dizziness	7	1	0	—	—	—
Numbness/tingling	7	1	0	—	—	—
Restlessness	4	2	2	—	—	—
Sweating	7	1	0	—	—	—
Tired	—	—	—	6	1	1
Energetic*	—	—	—	2	3	3
Happy/elated	—	—	—	3	4	1
Irritable	—	—	—	4	2	2
Worried/nervous	—	—	—	6	1	1
Concentrating	—	—	—	5	3	0
Relaxed*	—	—	—	5	1	2
Tense	—	—	—	5	1	2
Contented*	—	—	—	4	3	1
Sad/depressed	—	—	—	7	1	0

*$P < .05$.

Table VI. Mood, emotion, and physical symptom responses 5 hours after administration (n = 8)

	No. of subjects reporting physical symptoms			*No. of subjects reporting mood and emotional feelings*		
	None	*Slight*	*Strong*	*None*	*Slight*	*Strong*
Headache	8	0	0	—	—	—
Stomach upset	8	0	0	—	—	—
Chest pain	8	0	0	—	—	—
Shakiness*	3	4	1	—	—	—
Heart pounding	5	3	0	—	—	—
Flushed feeling	7	1	0	—	—	—
Short of breath	8	0	0	—	—	—
Dizziness	6	2	0	—	—	—
Numbness/tingling	7	1	0	—	—	—
Restlessness	5	3	0	—	—	—
Sweating	7	1	0	—	—	—
Tired	—	—	—	4	4	0
Energetic	—	—	—	3	4	1
Happy/elated	—	—	—	4	4	0
Irritable	—	—	—	4	3	1
Worried/nervous	—	—	—	7	1	0
Concentrating	—	—	—	4	4	0
Relaxed	—	—	—	3	4	1
Tense	—	—	—	6	2	0
Contented	—	—	—	2	4	2
Sad/depressed	—	—	—	8	0	0

*$P < .05$.

metabolite formation from the major alkaloids contained in *Ephedra*.

The pharmacokinetic parameters for ephedrine, pseudoephedrine, and caffeine determined in this study are in close agreement with values reported in previous studies. For pharmaceutical ephedrine hydrochloride, previously reported pharmacokinetic values include a $t_{\frac{1}{2}}$ of 6.75 hours and a CL/F of 23.3 L/h in 10 subjects,[21] as well as a $t_{\frac{1}{2}}$ of 5.37 hours and a CL/F of 28.5 L/h in another study of 10 subjects.[18] Two studies have investigated the pharmacokinetics of ephedrine taken as ephedra and reported $t_{\frac{1}{2}}$ values that ranged from 4.9 to 6.5 hours and CL/F values that ranged from 24.3 to 34.1 L/h.[18,19] These results are comparable to the parameters shown in Table I. One of these studies[19] showed a significantly slower rate of absorption and longer t_{max} of ephedrine from powdered ma huang compared with ephedrine hydrochloride and ma huang extract. This may be a result of differences in absorption of ephedrine from powdered ma huang compared with ephedrine obtained as a chemical extract of the plant. Metabolift, like most currently available dietary supplements, contains ephedra alkaloids in the form of extracts of ma huang. The mean apparent volume of distribution (V/F) of 2.4 L/kg in this study was smaller than some previously reported values that were in the range of 3.0 to 3.5 L/kg.[18,21]

The pharmacokinetic data on pseudoephedrine obtained in this study are the first data to be reported for this alkaloid derived from ephedra. Although the dose of botanical pseudoephedrine in the dietary supplement studied was small compared with the amount present in decongestant preparations, the kinetic parameters are comparable to results obtained in previous investigations of the drug formulation of pseudoephedrine.[22-24] Like ephedrine, the V/F of 2.5 L/kg determined in this study for pseudoephedrine was smaller than the previously reported values that ranged from 2.6 to 3.5 L/kg.[22]

For caffeine, the mean $t_{\frac{1}{2}}$ in subjects not taking oral contraceptives agreed closely with previously reported values of 6.8 hours[25] and 6.0 hours[26] in nonsmokers. CL/F values of caffeine in subjects who did not use oral contraceptives were comparable as well: 0.94 mL/min · kg reported by Campbell et al[25] and 1.14 mL/min · kg reported by Cheng et al.[26] The pharmacokinetic results

reported in our study indicate that ephedrine alkaloids and caffeine derived from plants have disposition characteristics similar to their pharmaceutical counterparts.

Significant interindividual variation was observed in the pharmacokinetic parameters of both the ephedrine alkaloids and caffeine. The elimination $t_{\frac{1}{2}}$ values of ephedrine and pseudoephedrine were inversely correlated with urine pH. One subject with an average urine pH in the slightly alkaline range had a longer $t_{\frac{1}{2}}$ and larger V/F for ephedrine and pseudoephedrine compared with the mean value. The pH dependence of renal elimination of the ephedrine alkaloids has been reported previously in drug studies that involved pseudoephedrine, ephedrine, and phenylpropanolamine.[22-24,27,28] This is the first report of the pH dependence of elimination $t_{\frac{1}{2}}$ of ephedrine alkaloids derived from ephedra. Persons with urine pH values that are in the alkaline range can be expected to have a prolonged duration of action of the ephedrine alkaloids which, with multiple daily doses, could lead to undesirable side effects such as insomnia and accumulation of ephedrine to potentially toxic levels.

Considerable variation in the extent of metabolism of the major ephedrine alkaloids was apparent, with coefficients of variation (standard deviation divided by the mean) of 65% for the plasma AUC ratio of norephedrine to ephedrine and 55% for the plasma AUC ratio of norpseudoephedrine to pseudoephedrine. Significant interindividual variation in the extent of metabolism and generation of active metabolites could explain some of the differences in cardiovascular responses observed among users of supplements that contain ephedra alkaloids.

Norephedrine has both α- and β_1-adrenergic receptor activity that causes vasoconstriction and increased blood pressure. Its racemic drug form, phenylpropanolamine, has been voluntarily recalled because of an increased risk of hemorrhagic stroke.[15] The fraction of ephedrine eliminated by *N*-demethylation to norephedrine has been reported to be in the range from 8% to 20% after an oral dose of 30 mg.[22] Norephedrine is largely excreted unchanged in the urine, with about 4% biotransformation to 4-hydroxynorephedrine and hippuric acid.[29]

Less than 1% of pseudoephedrine is transformed by hepatic metabolism to norpseudoephedrine.[22] This active metabolite has more potent central nervous system–stimulant effects than ephedrine and, because of its high abuse potential, (+)-norpseudoephedrine has been classified as a Schedule IV controlled substance.[18] (+)-Norpseudoephedrine, also known as cathine, is the major alkaloid constituent of khat *(Catha edulis),* a plant used as a stimulant in east Africa.[30] The main psychoactive alkaloid in khat is not cathine but cathinone, which is more lipophilic and more easily penetrates the central nervous system. Norpseudoephedrine and norephedrine are metabolic products of cathinone.

Two female subjects who were taking oral contraceptive pills had caffeine elimination $t_{\frac{1}{2}}$ values of approximately 15 hours, versus 5.6 hours for the other subjects. The mean $t_{\frac{1}{2}}$ of caffeine in oral contraceptive users was reported to be 10.7 hours in a previous study.[31] Caffeine clearance is inhibited by a number of drugs and conditions that affect cytochrome P4501A2 isozyme activity, including verapamil, oral contraceptives, disulfiram, cimetidine, alcohol, pregnancy, and liver disease.[32] Smoking stimulates caffeine clearance by induction of hepatic enzymes.[33] The markedly prolonged $t_{\frac{1}{2}}$ of botanically derived caffeine in the 2 users of oral contraceptive in this study suggests a clinically significant drug-herb interaction. Given the widespread consumption of caffeinated beverages and the high prevalence of oral contraceptive use, this interaction could be significant in young female adults who use thermogenic dietary supplements. Guarana-containing supplements frequently contain label instructions to consume the product 2 to 3 times per day, which could result in greatly elevated caffeine levels in oral contraceptive users and in persons taking other drugs that inhibit caffeine metabolism.

The stimulant effects of ephedra alkaloids would be expected to intensify the cardiovascular effects of caffeine in combination supplements. With modest doses, caffeine increases blood pressure, decreases heart rate slightly, and causes release of catecholamines and renin. Ephedrine has effects at α- and β-adrenergic receptors that cause increased catecholamine release, greater cardiac contractility, and increased heart rate and blood pressure.

A remarkable finding in our study was that the heart rate and systolic blood pressure responses differed substantially over time and in relationship to plasma levels of caffeine and ephedrine. The early increase to maximum systolic blood pressure change is most likely related to the time course of rising concentrations of caffeine. The increase in heart rate 4 hours after dosing may in part reflect the later rise to maximum plasma concentration of ephedrine and the diminution of the reflex heart rate slowing produced by caffeine.

The direction of the hysteresis in the blood pressure–concentration curves observed in Figs 4, *A,* and 5, *A,* is consistent with the known development of tolerance to the pressor effects of stimulant drugs such as caffeine.[33] The reverse directionality of the hysteresis curves in Figs 4, *B,* and 5, *B,* suggests that there is a lag-time in

heart rate response without apparent development of tolerance. The lag-time in heart rate response and the opposite direction of the hysteresis curves for blood pressure and heart rate suggest a pharmacodynamic interaction between the heart rate and blood pressure responses. We hypothesize that the baroreceptor-mediated slowing in heart rate in response to the acute vasoconstrictor effect of caffeine predominates early after dosing, masking any chronotropic effect of ephedrine. Later, as caffeine levels decline or tolerance develops to the pressor effects of caffeine, the chronotropic effects of ephedrine become apparent.

A limitation in interpreting these results is that the relative cardiovascular effects of the individual stimulants cannot be distinguished. Further studies are required to identify the concentration-response profiles of caffeine and ephedrine alone to further understand the responses of the drug combination.

The interpretation of the subjective results must be qualified by the lack of a placebo-control group in this study. It may be that the positive responses compared with baseline reflect decreased subject anxiety after the study procedures were completed (ie, physical examination and venipuncture). However, there is evidence from previous research[34,35] that subjective effects of ephedrine are similar to those of other sympathomimetic amines, including amphetamine and methamphetamine. The mostly positive subjective mood and emotion responses to Metabolift in this study suggest that this supplement has central nervous stimulant effects. Martin et al[34] found that the predominant effect of the centrally acting amines was feelings of relaxation, well-being, and contentment, which were observed in our study. Another study in humans showed that ephedrine use increased subject ratings of feeling "high" and increased their euphoria score but did not affect the ratings of "drug liking."[35] Repeated daily use of a supplement that produces a preponderance of pleasurable feelings could become habit-forming and lead to misuse, particularly in individuals with a history of drug abuse, addiction, or other psychiatric diseases. Case reports of ephedrine abuse in the medical literature show evidence of drug-seeking behavior and development of toxic psychosis.[34,36] A recent study of 36 female weight lifters found that 19% displayed signs of ephedrine dependence.[37] Prolonged use of dietary supplements that contain combinations of ephedrine and caffeine needs to be carefully scrutinized in the context of their potential to induce dependence.

Our small study shows that young healthy adults can have significant cardiovascular responses and central nervous system effects after a single dose of a supplement that contains relatively modest doses of ephedrine alkaloids and caffeine. The product we studied contained relatively few ingredients compared with some other thermogenic supplements, some of which list 15 to 20 herbal constituents. Concern about potential herb-herb interactions with these combination products warrants further clinical investigation. Future studies that use multiple-dosing regimens should be conducted to investigate the effects associated with long-term use of these supplements as currently marketed and consumed for weight loss and athletic enhancement.

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APPENDIX

Method of biologic specimen analysis for ephedrine alkaloids

One hundred microliters of a solution of the internal standards in 0.01 mol/L hydrochloric acid was added to 0.5 mL of plasma sample, standard, or control contained in glass culture tubes (13 × 100 mm). The internal standard solution contained 1 μg/mL of ephedrine-d_3, 1 μg/mL pseudoephedrine-d_3, 1 μg/mL of a mixture of norephedrine-d_5 and norpseudoephedrine-d_5, 1 μg/mL of a mixture of methylephedrine-d_5 and methylpseudoephedrine-d_5, and 1 μg of $^{13}C_3$-caffeine. Ephedrine-d_3 and pseudoephedrine-d_3 were purchased from Isotech (Miamisburg, Ohio), and $^{13}C_3$-caffeine was purchased from Cambridge Stable Isotopes (Cambridge, Mass). The norephedrine-d_5/norpseudoephedrine-d_5 and methylephedrine-d_5/methylpseudoephedrine-d_5 mixtures were synthesized in our laboratory. The tube was briefly vortex-mixed, and 100 μL of 30% perchloric acid was added to precipitate the protein. After vortex-mixing and centrifugation, the supernate was decanted to a new tube. One milliliter of 50% tripotassium phosphate (wt/

vol) and 3 mL pentane/ethyl acetate/isopropyl alcohol (80:15:5, vol/vol/vol) were added. The tube was vortex-mixed for 5 minutes, centrifuged, and placed in a dry ice–acetone mixture to freeze the aqueous layer. The organic layer was poured to a new tube, 100 μL of 10% hydrochloric acid in methanol was added, and the extract was evaporated to dryness in a centrifugal vacuum evaporator. Urine samples were extracted by use of the same extraction procedure used for plasma, except that 100 μL urine diluted to 0.5 mL with water was used. The dried extracts were reconstituted in 150 μL of 10 mmol/L ammonium formate/0.1% formic acid (vol/vol) in water and transferred to an autosampler vial for LC-MS/MS analysis.

Fifty-microliter aliquots of the extracts were injected into the LC-MS/MS system, which consisted of a Hewlett-Packard 1090 HPLC (Palo Alto, Calif) interfaced with a Finnigan TSQ 7000 triple-stage quadrupole mass spectrometer with an API2 ion source (Thermo-Finnigan, San Jose, Calif). The chromatography was carried out with use of a 4.6 mm × 50 mm Hypersil Phenyl BDS column fitted with a Hypersil Phenyl BDS guard column (Thermo-Hypersil-Keystone, Bellefonte, Pa). A binary gradient elution was used, starting with 100% 10 mmol/L ammonium formate/0.1% formic acid (vol/vol) in water, changing to 20% 10 mmol/L ammonium formate/0.1% formic acid (vol/vol) in methanol over 7 minutes, and then changing to 100% 10 mmol/L ammonium formate/0.1% formic acid (vol/vol) in methanol over 0.5 minutes. The flow rate was 0.70 mL/min. This resulted in baseline separation of all analytes.

The mass spectrometer was operated in the positive ion mode with use of atmospheric pressure chemical ionization. Quantitative analyses were carried out by use of selected reaction monitoring, with the collision gas (argon) pressure in the second quadrupole set at ~2.5 mm Hg. The selected reaction monitoring transitions monitored were as follows: ephedrine and pseudoephedrine, *m/z* 166 to *m/z* 148; norephedrine and norpseudoephedrine, *m/z* 152 to *m/z* 134; methylephedrine and methylpseudoephedrine, *m/z* 180 to *m/z* 162; caffeine, *m/z* 195 to *m/z* 138; ephedrine-d_3 and pseudoephedrine-d_3, *m/z* 169 to *m/z* 151; norephedrine-d_5 and norpseudoephedrine-d_5, *m/z* 157 to *m/z* 139; methylephedrine-d_5 and methylpseudoephedrine-d_5, *m/z* 185 to *m/z* 167; and $^{13}C_3$-caffeine, *m/z* 198 to *m/z* 140.

Calibration curves were constructed from peak area ratios of each analyte to its respective internal standard with use of linear regression. Standard curves were linear over the concentration ranges studied: 0.5 ng/mL to 1000 ng/mL for ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine; 1 ng/mL to 1000 ng/mL for methylephedrine and methylpseudoephedrine; and 25 ng/mL to 5000 ng/mL for caffeine. Precision and accuracy was evaluated by replicate analysis of spiked plasma samples at 3 concentrations that spanned the concentration ranges for the analytes in clinical study samples. Within-run precision (percentage of coefficient of variation; $n = 6$) ranged from 0.16% to 2.5%. Accuracy (percentage of expected values; $n = 6$) ranged from 97% to 105%.