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CLINICAL TRIALS

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Effect of three caffeine doses on plasma catecholamines and alertness during prolonged wakefulness

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Abstract *Objective*: Determine the relationship between caffeine, catecholamines, and alertness during prolonged wakefulness.
Methods: Following 49 h of prolonged wakefulness, each of 50 healthy males (18–32 years) orally ingested either a placebo or one of three doses of caffeine, 2.1 (low), 4.3 (medium), or 8.6 mg kg^{-1} body weight (high), in a randomized double-blind design. Wakefulness continued for an additional 12 h during which venous blood samples were collected for catecholamine and caffeine analysis [determined using high-performance liquid chromatography (HPLC)]. A sleep latency test, the Stanford sleepiness scale, and a choice reaction time test were administered periodically during the post-dosing period and served as measures of alertness (physiological, subjective, and behavioral, respectively).
Results: Caffeine had no significant effect on noradrenaline, but adrenaline was significantly increased between 1 h and 4 h post-dosing in the high dose group compared with a placebo group. Following caffeine administration, responses to sleep latency, sleepiness scores, and reaction time scores showed dose-related changes that were exhibited by significant correlation coefficients.
Conclusion: The results indicate that high doses of caffeine have a significant and beneficial effect on alertness during prolonged wakefulness.

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Introduction

The demands of modern work and life style frequently require individuals to sacrifice their regular sleep schedules resulting in the fragmentation or loss of significant amounts of sleep. Decrements in cognitive performance, mood, and alertness associated with prolonged wakefulness have been previously documented [1–3]. Pharmacologic agents, including D-amphetamine and caffeine, are often used to alleviate these performance decrements. Although D-amphetamine can effectively counteract the performance deficits associated with prolonged wakefulness, its highly addictive nature and negative physiological side effects render it unacceptable for general use [4]. In contrast, caffeine is the most widely used over the counter stimulant in the world and is commonly found in soft drinks, chocolate, and in combination with a number of other pharmacological agents [3, 5, 6]. Caffeine has been shown to stimulate the release of the catecholamines, adrenaline, and noradrenaline, which are associated with physiological arousal and stress [7].

Although a number of studies have examined the relationship between caffeine, catecholamines, and alertness, this is the first study to focus on these relationships during an extended period of prolonged wakefulness. At the present time, we have been unable to identify any published studies which have focused on the use of high doses of caffeine (greater than 300 mg) in conjunction with prolonged wakefulness. We hypothesized that caffeine would stimulate specific components of the sympathetic nervous system in a dose-dependent fashion, thereby altering the noradrenaline or adrenaline response, and concomitantly increase alertness as measured using sleep latency (SL), a self-rating sleepiness scale, and a reaction time test.

Materials and methods

Subjects

Fifty healthy, nonsmoking, males, 18–32 years (mean 23.6 years), volunteered to participate in this study and signed an informed consent. All subjects were within acceptable weight limits for their height, not currently taking any medication, did not normally consume in excess of 300 mg of caffeine per day, and had regular sleep patterns (6–8 h per night without difficulty falling asleep). Subjects were randomly assigned in a double blind design to one of four drug groups: placebo, low (2.1 mg kg^{-1}), medium (4.3 mg kg^{-1}), or high (8.6 mg kg^{-1}). These doses correspond to 150, 300, and 600 mg per 70 kg body weight. Characteristics of the four groups are presented in Table 1.

This research was conducted in conformity with AR 70–25, United States Army Medical Research and Development Command Reg. 70–25 on the use of human volunteers in research, and the "Guiding Principles for Research Involving Animals and Human Beings". Human volunteers participated after giving free and informed consent.

Procedure

Subjects arrived in the laboratory in groups of three or four the evening prior to the initiation of the sleep deprivation period having refrained from the use of caffeine, alcohol, or any pharmacologic agents during the previous 24 h. Blood and urine samples were collected and assayed to ensure compliance with these restrictions.

Electrodes were attached to the scalp and face, using the international 10–20 system of electrode placement, for collection of the multiple sleep latency test (MSLT) data [8]. Electroencephalograms (EEG), electrooculogram (EOG), and sub-mental electromyograms (EMG) were recorded continuously using an eight-channel Oxford Medilog ambulatory cassette recorder. Subjects received a standardized diet throughout the study and water was available ad libitum.

Subjects retired at 2300 hours on day 0 and were allowed to sleep until 0700 hours the next morning (day 1). They were kept awake for the following 62 h except for brief periods during administration of the MSLT (described below). Measures of mood and cognitive function were also administered at various times throughout the study and the results have been reported elsewhere [3, 9].

Following 48 h of prolonged wakefulness (0700 hours, day 3) a Teflon catheter was inserted into a forearm vein and maintained with a heparin lock (heparin sodium, 20 U cc^{-1}). At 0800 hours (time 0) control samples were collected immediately prior to the administration of placebo or one of the three doses of caffeine (caffeine anhydrous, USP, City Chemical Corporation, NY). Caffeine or placebo was administered orally in 250 ml of an artificially sweetened lemon juice drink in a double-blind design. Blood samples were then collected 12 times post-administration, namely at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h.

Catecholamines and caffeine concentrations

Samples were collected into chilled, heparinized Vacutainers containing glutathione and ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), centrifuged at 2000 *g* for 15 min, the plasma separated into polyethylene tubes, and stored at −70 °C until analysis. Plasma noradrenaline and adrenaline were isolated using alumina extraction as previously described [10] and high-performance liquid chromatography (HPLC) with electrochemical detection to determine the plasma catecholamine concentrations. The sensitivity of these assays was 10 pg ml^{-1} with a signal-to-noise ratio of 4:1. The within-day (interday) variation was less than 1% and the between-day (intraday) variation was less than 3% for standards in the range 5.0–5000 pg ml^{-1}. Plasma caffeine concentrations were also determined using HPLC on samples collected during the same period; the methodology and pharmacokinetics have been previously reported [6].

Alertness measures

On eight occasions post-dosing (beginning at 1, 2, 3, 4, 6, 8, 10, and 12 h), a series of tests were given. Three alertness measures from this test battery are reported here, namely the Stanford sleepiness scale (SSS), a choice reaction time test (CRT), and the MSLT. The SSS represents a subjective self-rating measure, the CRT a performance-based measure of alertness, and the MSLT a physiological measure.

Stanford sleepiness scale

The SSS [11] is a quick-to-administer scale in which subjects self-rate their current state of alertness by choosing one of seven descriptive statements ranging from 1 ("feeling active and vital; alert; wide awake") to 7 ("almost in reverie; sleep onset soon; lost struggle to remain awake"). Over days 1 and 2, this scale was administered periodically on 22 occasions, then given as the first part of the test battery at the eight times indicated above on day 3.

Choice reaction time test

This computer-based, single-choice reaction time test (a component of the Walter Reed Performance Assessment Battery [12]) consisted of the subject matching a digit (from 0 to 9) that appeared on the monitor by pressing as soon as possible the appropriate numbered keyboard key. In each administration of this test of the battery, 50 numbers were presented. Dependent variables available from this test were speed (the reciprocal of mean response time), accuracy (percentage of correct matches), and throughput (the speed–accuracy product, a measure of useful work output [13]). This latter measure was used for analysis in the present study. Periodically during day 1 and day 2, this test was administered 22 times, then given on the post-dosing schedule as indicated above.

Multiple sleep latency test

For each MSLT, subjects were asked to lie on a bed in a darkened, sound-attenuated room and were instructed to close their eyes, relax, and allow themselves to fall asleep. EEG, EOG, and EMG were displayed on a Grass Electroencephalograph (Model 8-10D) for on-line scoring. Subjects were awakened by an investigator following the identification of 30 s of stage-2 sleep or the onset of rapid eye movement (REM) sleep (per standards in Rechtschaffen and Kales [8]). If sleep did not occur within 20 min according to

Table 1 Subject descriptors by dose group (ranges)

	No. in group	Age (years)	Height (cm)	Weight (kg)
Placebo	12	22.1 (19–26)	177.3 (170.2–186.7)	73.8 (64.1–79.5)
Low	13	23.5 (21–31)	180.3 (165.1–203.2)	77.8 (54.1–95.5)
Medium	12	25.2 (20–29)	173.9 (162.6–182.9)	71.7 (61.4–78.2)
High	13	23.3 (18–32)	175.5 (165.1–186.7)	72.8 (64.5–89.1)

procedures developed by Carskadon and Dement [14, 15], the test was terminated. The MSLT measure is defined as the elapsed time from initiation of the test to awakening or termination by an investigator at 20 min. Twelve tests were conducted intermittently during the initial 48 h of prolonged wakefulness. The last baseline MSLT was administered at the 37-h mark (day 2, 2230 hours). Drug (or placebo) was administered at time 0 (0800 hours, day 3) and additional MSLTs were administered as the last part of the test battery during the eight offerings which began at the times indicated above.

Statistical analysis

To scrutinize the time course of the catecholamines, two sets of analyses of variance were performed on each of the adrenaline- and noradrenaline-dependent variables. The first analysis was run on the blood samples taken at 0800 hours (day 3) to see whether there were any differences among the four dose groups just before drug administration. The second analysis looked at group differences and the effects of the sleep-deprivation period after dosing. The key analysis of variance term of interest was the interaction effect. To guard against a potential positive bias of the F ratio, which can occur in terms involving a repeated measure, adjustment of degrees of freedom procedures were adopted [16]. A simple main-effects analysis was run on each of the 12 time points following a significant interaction, and, if this analysis was significant, a comparison of the four group means against each other was made using the Newman–Keuls multiple comparison procedure [16].

As a follow-up to analyses of variance on the catecholamine data, Pearson correlation coefficients were run on the catecholamine and the caffeine serum concentration values (thus the coefficients were run on only the three dose groups). Data pairs were the matched individual values at each of the observation points. One correlation involved 12 data pairs per subject (corresponding to all the 12 post-drug occasions of measurement). A second correlation focused on the time points 15 min through 90 min post-dosing (early phase), a third on time points 120 min through 240 min (middle phase), and the final set on 360 min through 720 min (late phase). These time blocks were chosen because they most closely corresponded to the pharmacokinetic profile of absorption, distribution, and elimination for caffeine. Each of the three focused correlations involved four data pairs per subject.

To assess changes in the three alertness measures (MSLT, SSS, and CRT test), each was correlated with the caffeine, adrenaline, and noradrenaline concentration levels, and also correlated with each other. Pearson correlations were used for all comparisons. Correlations were only performed on data from the three groups that received caffeine. Data pairs were the matched individual values at each of the observation points. An overall correlation involved 12 data pairs per subject (that is, all the 12 post-drug occasions of measurement). Three additional correlations were run that closely corresponded respectively to the pharmacokinetic profile of absorption, distribution, and elimination of caffeine: one focused on the time points 15 min through 90 min post-dosing (early phase), another on time points 120 min through 240 min (middle phase), and one on 360 min through 720 min (late phase). Each of these three correlations involved four data pairs per subject. A P value less than 0.05 was accepted as significant for all statistical tests.

Results

Caffeine concentrations

It was previously shown by Kamimori et al. [6] that the three doses used in this study were in fact divergent enough throughout the time course to cause dose-dependent differences in caffeine pharmacokinetics in these severely sleep-deprived subjects. Mean serum concentrations for each group as a function of time post-dosing are presented in Table 2.

Catecholamine concentrations

Figure 1 shows the mean epinephrine concentration for each group just prior to and for 12 h following drug administration. At 49 h of prolonged wakefulness (time 0), there was no significant difference in epinephrine between placebo and any of the three drug groups. The overall analysis of variance for the 12-h dosing period showed a significant dose group-by-time interaction. Simple main effects were significant for the six time points between 60 min and 240 min post-dosing. With the exception of the 150-min and 180-min time points, the ordering of the four dose groups showed a dose–response pattern. A consistent finding for the six time points from the multiple comparison tests was that the high dose group was significantly higher than the placebo group; also the high dose group's epinephrine was higher than that of the low dose group for time points 60 min through 150 min.

The correlation of epinephrine with caffeine over all time points was 0.36 (Table 3); for the early phase (time points 15–90) the value was 0.49; for the middle phase (time points 120–240), 0.28, and for the late phase (time points 360–720), 0.11. All but the last correlation were statistically significant.

The noradrenaline data are illustrated in Fig. 2. Although plasma levels appeared to increase with caffeine, the dose group-by-time interaction was not significant, and no post-hoc analysis of variance procedures were performed.

Alertness measures: descriptive statistics and correlations

The SL measures for the four dose groups closely followed each other during the first two days of the study.

Table 2 Serum caffeine concentrations (μg ml^{-1}) as a function of dose group and time post-dosing. Data from Kamimori et al. [6]

Dose group	Time post-dosing (min)											
	15	30	60	90	120	150	180	240	360	480	600	720
Low	1.20	2.22	2.60	2.64	2.49	2.36	2.25	2.09	1.69	1.20	0.83	0.66
Medium	2.12	3.78	5.38	5.90	5.46	4.98	4.92	4.52	3.52	2.82	2.27	1.59
High	5.53	8.34	11.75	12.29	11.96	11.57	10.82	9.92	8.72	7.00	5.48	4.21

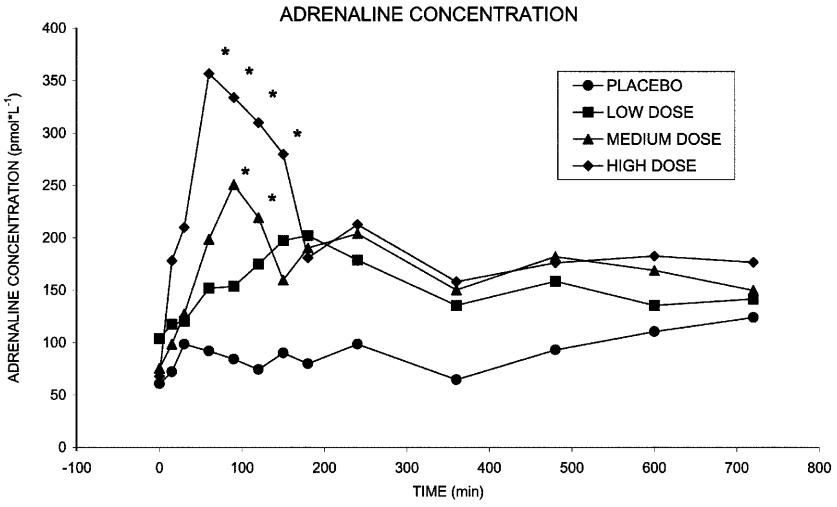

Fig. 1 Mean adrenaline concentration just prior to and for 12 h following drug administration. *Significantly different from placebo, $P \leq 0.05$

Table 3 Correlations of catecholamines with caffeine serum concentrations and with each other. All correlations involve three dose groups only (placebo group did not receive caffeine). *Early phase* 15–90 min; *middle phase* 120–240 min; *late phase* 360–720 min; (number of data pairs per correlation); *CAF* caffeine; *ADR* adrenaline; *NORADR* noradrenaline

	ADR				NORADR			
	All points (409–421)	Early phase (138–144)	Middle phase (135–141)	Late phase (136–143)	All points (409–421)	Early phase (138–144)	Middle phase (135–141)	Late phase (136–143)
CAF	0.36*	0.49*	0.28*	0.11	−0.06	−0.05	−0.14	0.03
ADR	–	–	–	–	−0.02	−0.06	−0.04	−0.12

*Correlation is significantly different from zero at the 0.05 level or less

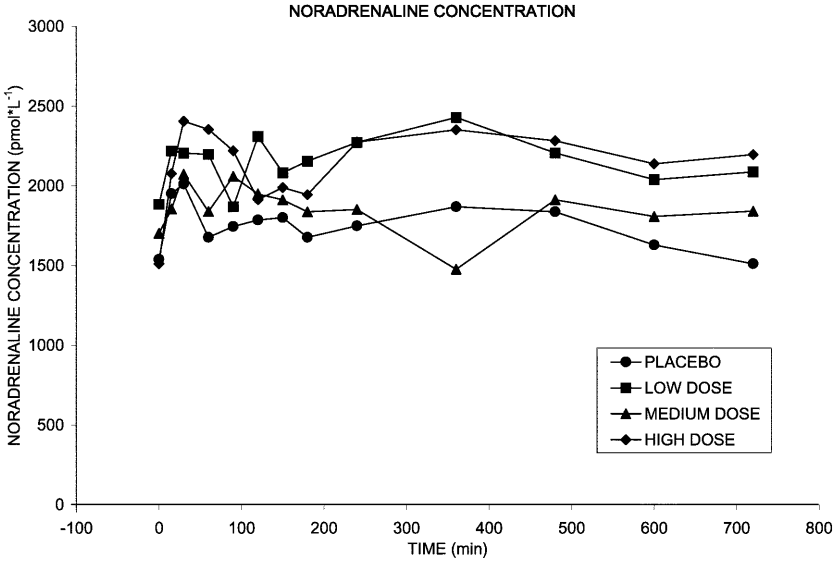

Fig. 2 Mean noradrenaline concentration just prior to and for 12 h following drug administration

On day 1, averaged values across all subjects (i.e., over the four dose groups) ranged from 16.3 min to 19.9 min, then dropped on day 2 to a range of 5.6–7 min (the last overall average before dosing, given at 2030 hours on day 2 was 7.0). After dosing, as shown in Table 4, the times to reach stage-2 sleep showed differences as a function of dose group and time post-dosing, and indicate a dose–response relationship, especially during the first 4 h post-dosing. During this interval, placebo and low dose group latencies continued the decline from days 1 and 2, whereas the latencies for the medium and high groups showed a differential increase; the latencies for the high group were 2.0–3.4 times higher than those for placebo, and medium group latencies were some 1.3–2.6 times higher than placebo.

Table 4 Average alertness measure values (±SD) by dose group and time post-dosing. The number of values per average was either 12 or 13. *Low dose* 2.1 mg kg^{-1}; *medium dose* 4.3 mg kg^{-1}; *high dose* 8.6 mg kg^{-1}; *Stanford sleepiness scale* rating from 1 "feeling alert, wide awake" to 7 "almost in reverie"; *choice reaction time* throughput – a measure of both accuracy and speed

Dose group	Time post-dosing (min)							
	60	120	180	240	360	480	600	720
Sleep latency (min)								
Placebo	5.0 ± 3.8	4.0 ± 2.4	3.9 ± 2.3	3.0 ± 1.6	4.5 ± 4.6	4.6 ± 3.4	4.0 ± 2.5	7.6 ± 4.8
Low	4.8 ± 4.7	5.0 ± 2.8	4.1 ± 2.1	3.6 ± 1.6	3.6 ± 1.6	3.4 ± 2.1	3.9 ± 2.4	4.6 ± 3.8
Medium	6.6 ± 3.0	7.4 ± 4.4	8.3 ± 5.1	7.9 ± 4.6	5.8 ± 3.1	5.8 ± 3.8	6.1 ± 3.8	9.3 ± 5.9
High	10.2 ± 6.9	10.0 ± 6.2	9.9 ± 6.6	10.2 ± 6.2	5.9 ± 3.9	4.7 ± 2.5	6.6 ± 3.6	8.3 ± 6.0
Stanford sleepiness scale								
Placebo	3.5 ± 1.6	3.8 ± 1.3	3.7 ± 1.2	3.2 ± 1.5	2.9 ± 0.7	2.8 ± 0.8	2.9 ± 0.9	2.8 ± 0.8
Low	2.2 ± 1.2	2.2 ± 1.4	2.5 ± 1.1	2.8 ± 1.3	3.0 ± 1.2	3.3 ± 1.4	3.5 ± 1.1	3.1 ± 1.0
Medium	2.4 ± 1.4	2.7 ± 1.5	2.4 ± 1.4	3.2 ± 1.7	3.3 ± 1.5	3.1 ± 1.5	3.0 ± 1.3	3.3 ± 1.6
High	1.9 ± 0.8	2.2 ± 0.9	2.5 ± 1.2	2.5 ± 1.1	2.5 ± 1.0	2.8 ± 1.2	2.6 ± 0.8	2.3 ± 0.8
Choice reaction time								
Placebo	63.1 ± 14.0	65.3 ± 26.1	68.0 ± 22.0	63.1 ± 21.7	70.6 ± 22.3	64.3 ± 22.1	68.6 ± 24.2	75.9 ± 22.6
Low	81.2 ± 24.0	84.4 ± 25.8	84.3 ± 19.2	78.9 ± 22.5	78.2 ± 23.7	69.4 ± 22.1	72.4 ± 24.7	79.9 ± 19.6
Medium	77.5 ± 19.3	82.5 ± 18.2	80.4 ± 19.4	78.3 ± 22.5	75.9 ± 24.1	75.4 ± 19.3	81.8 ± 16.6	78.1 ± 17.1
High	88.5 ± 17.0	87.7 ± 18.6	90.7 ± 17.6	89.5 ± 13.1	87.3 ± 16.0	85.1 ± 16.3	85.2 ± 17.1	87.1 ± 16.0

The SSS values likewise were similar for the four groups at each time point during day 1 and day 2. Averages over all subjects on day 1 ranged from 1.6 to 4, and on day 2 from 3.1 to 4.8. The last value pre-dosing, at 0600 hours, was 4.8. During the interval 60–240 min post-dosing, the averages ranged from 0.5 to 0.8 of the placebo groups.

Mean throughput values on the CRT tests at each time point were similar for the four groups during day 1 and day 2. On day 1, the means ranged from 77 to 84, and on day 2 from 72 to 85; the last average before dosing, gathered at 0600 hours on day 3, was 62. The post-dosing averages for each group are shown in Table 4. Within each of the eight administrations, the average value of throughput in each of the three dose groups is greater than the placebo value. The highest value at each time point is in the high-dose group; these values range from 1.1 to 1.4 times greater than placebo value.

Correlation coefficients were used as a convenient summary statistic to compare alertness measures both with each other as well as with caffeine and catecholamine serum concentration levels during the post-dosing period. The results are listed in Table 5 [1].

For the correlations involving caffeine over all the time points, all three alertness measures are significantly associated with this stimulant in the hypothesized direction, although SL and the performance-based reaction time measure appear to predict somewhat better than the subjective SSS measure. Techniques for statistically comparing two correlations for the present type of design are not well developed, and thus descriptive rather than analytical comparisons are made. When looked at as a function of early versus late phase, the association for CRT holds up over both phases, whereas SL's relationship is in the early phase only [2].

For the catecholamines, significant relationships are found with epinephrine correlating with SSS and SL; a significant association of SSS with adrenaline was found over both phases of the dosing period. No predictability is apparent with noradrenaline and the subjective SSS measure, but some relationship is seen with the physio-

Table 5 Correlations of alertness measures with catecholamines and caffeine serum concentrations. All correlations involve three dose groups only. A minus sign for a correlation of SSS with another variable implies that subjective alertness increased as serum concentration or sleep latency increased (values at the low end of the SSS indicate higher alertness). *CAF* caffeine; *ADR* adrenaline; *NORADR* noradrenaline; *SSS* Stanford sleep scale; *SL* sleep latency; *CRT* choice reaction time; *all time points* 60–720 min post-dosing; *early phase* 60–240 min post-dosing; *late phase* 360–720 min post-dosing

	All time points (275–304 data pairs/correlation)			Early phase (136–152 data pairs/correlation)			Late phase (139–152 data pairs/correlation)		
	SSS	SL	CRT	SSS	SL	CRT	SSS	SL	CRT
CAF	−0.18*	0.30*	0.30*	−0.08	0.34*	0.29*	−0.15	0.08	0.28*
ADR	−0.25*	0.18*	−0.01	−0.19*	0.16	0.02	−0.27*	0.09	−0.10
NORADR	0.05	0.13*	−0.12*	0.01	0.21*	−0.19*	0.06	0.05	−0.05
SSS	–	−0.15*	−0.29*	–	−0.11	−0.29*	–	−0.12	−0.26*
SL	–	–	−0.02	–	–	−0.07	–	–	0.02

*Correlation is significantly different from zero at the 0.05 level or less

logical alertness measure SL in the early phase [3]. For the alertness measures correlated with each other, SL and CRT show a significant correlation with SSS over all the time points. The CRT association with SSS is subjectively greater than SL, and occurs in both phases.

Discussion

Many segments of society put in extended workdays. Whether the tasks involve such endeavors as driving, studying for exams, projects at the office, or in extreme cases performing long-term emergency disaster relief or military operations, a stimulant such as caffeine is often taken to maintain alertness. The results of this study demonstrate that following 49 h of prolonged wakefulness, ingestion of moderate and high doses of caffeine significantly increase plasma adrenaline and alertness in a dose-related fashion.

To our knowledge, this is the only study in which relatively high doses of caffeine (8.6 mg kg^{-1} or 600 mg 70 kg^{-1}) have been administered in conjunction with an extended period of prolonged wakefulness (49 h baseline plus 12 h post-dosing). The duration of prolonged wakefulness chosen for this study was based on our previous studies, in which we have reported significant decreases in both cognitive performance and alertness following 48 h of prolonged wakefulness [2–4]. The design allowed for both charting of the time course of the catecholamines plus correlating their values with our three separate alertness measures (subjective, behavioral, and physiological).

There were no significant differences between groups in the control concentrations (at the 49-h mark) of either noradrenaline or adrenaline, indicating that prolonged wakefulness per se had no significant affect on the basal circulating catecholamine levels. The caffeine administration, given as a bolus at the 49-h mark, resulted in dose-dependent serum levels (Table 2) and stimulated an adrenaline response (best defined during the 15- to 90-min block; Fig. 1, Table 3). As caffeine levels decreased in the late phase, their influence on adrenaline stimulation greatly decreased. Although mean noradrenaline levels appear to be increased in the caffeine groups, there was no significant difference in comparison with placebo concentrations. These results are consistent with other caffeine studies that have reported a significant increase in the resting levels of adrenaline, but observed no significant effect on noradrenaline [17–19]. These findings support the contention that caffeine acts predominantly at the adrenomedullary level rather than through undifferentiated sympathetic stimulation (e.g., postural changes).

The more meaningful associations were seen during the first 4 h post-dosing. For example, in the case of SL, the high dose resulted in a time-to-stage-2 SL twice that of the placebo group at the 60-min mark and maintained at least this difference throughout the 240-min mark. The highest value of 10.2 min, occurring in the high dose group, is similar to the mean SL value found by Walsh et al. [20] after approximately 20 h of wakefulness in subjects given 4.0 mg kg^{-1} caffeine (essentially our medium dose). In comparison with a study using the same design but D-amphetamine instead of caffeine, Newhouse et al. [4] were able to reverse the effects of prolonged wakefulness to almost 100% of that seen in rested conditions; these effects began 2 h post-dosing and significant differences in SL compared with control groups continued for an additional 5 h.

It is important to note the short SLs in the low-dose group. Although plasma adrenaline was increased in the low group (Fig. 1), the SLs suggest that the low dose had no effect on alertness. These data can be explained by the threshold effect, which has been previously associated with caffeine. In this type of a relationship, a minimum blood concentration of a drug is required to elicit a significant pharmacodynamic response, in this case from the adrenal medulla (i.e., adrenaline). A second possible explanation may be related to each individual's tolerance to caffeine. Subjects were screened for the consumption of 4.3 mg kg^{-1} of caffeine or less per day, so it is possible that participants habitually ingested more than 2.1 mg kg^{-1}. It is well established that chronic caffeine use will result in an increase in tolerance to both its physiological and psychological effects [21]. The ineffectiveness of the low dose on alertness could also be explained by a subject's habitual use of caffeine, which would lead to an increase in tolerance to the drug. Lastly, the high individual variability common with epinephrine response may also affect SL.

At the end of the study, most dose groups' scores suggested a slight increase in alertness (Table 4). This may have been partially caused by an end-spurt phenomenon, as subjects were aware of the ensuing release from the study.

Because of the time-locked nature of the individual biochemical and alertness measures over the 49-h post-dosing period, a correlation approach was used to examine the relationships. Those that were statistically significant formed the basis for the discussion above. It may also be asked whether the correlations are practically significant, that is, are the strengths of the relationships meaningful within the intended applied context of our research program. A useful concept that conveys practical significance is that of effect size, the degree to which the null hypothesis is false, or the degree to which the phenomenon is present in the population [22]. Cohen has established guidelines for categorizing the effects as small, medium, or large. For product-moment correlations, a value between 0.10 and 0.29 is considered a small effect; if between 0.30 and 0.49, medium, and if 0.50 or greater, large. Using this scheme, of the 21 statistically significant correlations presented in Table 3 and Table 5, the effect sizes of five are medium and the rest are small. It is noteworthy that trends did appear in spite of the within-subject and between-subject variability inherent in our six measures. Depending on the measure, the variability is a by-product of

pharmacokinetics, fatigue state (both physiological and perceived), time of day (diurnal rhythm effect), and motivation.

This study is the first phase of a research program to investigate caffeine's effects on alertness, and the relationships we did find are suggestive enough to help guide the course of study. One experiment is currently in progress and another is planned to look at an alternative means of administering caffeine, namely through caffeine-containing chewing gum as a distribution medium. We will be investigating different dosages, their time course, their initial alertness effect in sleep-deprived subjects, as well as the efficacy of ''maintenance dosing'', and will compare these measures with the effects from caffeine pills.

As caffeine is a stimulant, it is important to note the possible side effects associated with its use. The effects of moderate doses of caffeine (300–600 mg) on the cardiovascular system have been extensively examined. The administration of doses of 250–350 mg may produce a small decrease in heart rate and modest increases in both the systolic and diastolic blood pressure, but this response may not be seen in habitual caffeine users [23]. At higher concentrations (greater than 300 mg) caffeine will result in a significant tachycardia and sensitive individuals may experience other arrhythmias. However, in a study of 22 patients with a history of symptomatic nonsustained ventricular tachycardia, ventricular tachycardia, or ventricular fibrillation, Chelsky et al. [24] administered 275 mg caffeine and found no significant alteration in the inducibility or severity of arrhythmias. Newcombe et al. [25] administered approximately 500 mg to 34 normal subjects and found no increase in ventricular arrhythmias on a 24-h Holter recording. Myers and Harris [26] reported similar results in 35 patients who had had a myocardial infarction and received 450 mg caffeine. They reported a very low likelihood of caffeine being associated with an increase in ventricular ectopy. These studies suggest that although a rare patient may possess a particular susceptibility to caffeine, which could lead to the development of a cardiac arrhythmia, in general, the ingestion of a moderate amount of caffeine (about 500 mg) is unlikely to cause an arrhythmia. The administration of high doses of caffeine has also been associated with a number of side effects, including nausea, dizziness, muscle tremors, nervousness, restlessness, and insomnia [5]. The possibility that manual dexterity would be affected by an increase in muscle tremor did not appear to be a factor in this study as performance was improved, as opposed to degraded, with the administration of the high dose of caffeine.

In conclusion, this study quantifies the effects of caffeine upon three measures of alertness in severely sleep-deprived subjects. The high dose of caffeine (8.6 mg kg^{-1}) was effective in stimulating the release of adrenaline and improving alertness, as defined for example by the physiological measure of SL, whose values were 2.0–3.4 times that of a baseline condition (i.e., no caffeine) over the first 4 h post-dosing. Although these effects were not as effective as D-amphetamine, caffeine is still considered to be the stimulant of choice as it is a universally available, legal, socially accepted, and widely used stimulant with a low toxicity and abuse potential.

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