Daily Caffeine Intake and the Effect of Caffeine on Pilots' Performance After Extended Wakefulness

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INTRODUCTION: Fatigue is a major contributor to aviation accidents. Sufficient sleep may be difficult to achieve under operational conditions in military aviation. Countermeasures include caffeine, however, studies evaluating its effects often do not represent daily practice with regular caffeine consumption. This study aims to establish the effect of caffeine on psychomotor performance in a realistic scenario (i.e., after a limited period of extended wakefulness).

METHODS: This randomized, double-blind, crossover, placebo-controlled trial included 30 aeromedically fit subjects. On trial days, subjects followed their normal routine till 17:00, after which caffeine intake was stopped. At midnight, subjects were given 300 mg of caffeine or placebo and performed the Psychomotor Vigilance Test, Vigilance and Tracking Test, and the Stanford Sleepiness Scale hourly up to 04:00 and again at 06:00 and 08:00. Four blood samples were collected. Statistical analyses included repeated-measures ANOVA or Friedman tests, marginal models, and Wilcoxon Signed Rank tests.

RESULTS: Median time awake at midnight was 17 h (IQR 16.5–17.5 h). Performance decreased significantly less during the night in the caffeine condition versus placebo. Neither habitual intake nor daytime caffeine consumption affected this. No statistically significant correlation was identified between blood concentrations of caffeine and performance.

DISCUSSION: A single dose of 300 mg of caffeine has beneficial effects on performance during the night in a realistic scenario for military aviation. Daytime caffeine consumption does not affect the effects of caffeine at night. These findings could be relevant for all industries in which optimal performance is required during nighttime after a limited period of extended wakefulness.

KEYWORDS: aviation, fatigue, shift work, sleep, wakefulness-promoting agents, performance enhancement, caffeine.

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In 2008, Air Traffic Control lost contact with Mesa Airlines Canadair Flight YV-1002, which flew 26 mi past the destination airport. Luckily, communications with the flight crew were restored and the airplane landed safely at the designated airport. According to the National Transportation Safety Board, the probable cause of this incident was the captain and first officer inadvertently falling asleep during the flight.[30] The captain's undiagnosed obstructive sleep apnea and the flight crew's recent work schedules (with consecutive days of early-morning start times) were reported to be contributing factors in this incident.

This incident might have easily become an accident if there had been a shortage of fuel or if the pilots had remained asleep longer. Fatigue contributed to 21–24% of major aviation accidents in the past two decades, but the significance of fatigue in aviation is probably even more paramount because, like in this incident, not all occurrences of fatigue lead to accidents.[30,44]

As stated in the International Civil Aviation Organization's definition of fatigue, fatigue can impair one's performance: "a physiological state of reduced mental or physical performance capability resulting from sleep loss, extended wakefulness,

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circadian phase, and/or workload (mental and/or physical activity) that can impair a person's alertness and ability to perform safety related operational duties."[19]

This definition identifies several possible causes of fatigue and potential areas for prevention or mitigation. The best way to prevent fatigue is to get sufficient sleep. However, as illustrated by the incident with flight YV-1002, this is often difficult to achieve because of flight crews' demanding work schedules.[30,44] It is even more difficult during military deployments as it may be tactically necessary to perform military operations at night, thereby disrupting the normal sleep pattern. This, combined with poorer sleep quality at deployment, may lead to irregular sleep during deployment, which may cause fatigue.[21] Also, when performing nighttime operations, pilots might be forced to fly during circadian phases dedicated for sleep, like the Window of Circadian Low (WOCL), when levels of attention are at their lowest, additionally increasing the chance of incidents.[39]

One possible option for counteracting the effects of fatigue is to prescribe stimulants, i.e., medications that increase vigilance and reduce fatigue. Caffeine is a widely available and well-known stimulant that has shown its beneficial effects on vigilance in different populations, such as students, but also military aviators.[12,26,37] It is a nonprescription substance that stimulates the central nervous system by blocking adenosine receptors.[6] Absorption of caffeine through the small intestine (i.e., after drinking a cup of coffee or energy drink or taking caffeine pills) is quick (15–40 min) and its effects are noticeable within 15–20 min.[4] When using caffeine chewing gum, the absorption rate is higher, with effects observed after 3–5 min.[44] Its half-life is 4–6 h, and it has beneficial effects in vigilance tasks for as long as 8 h after administration which can increase after repeated administration.[23,36] Coffee is one of the most widely used stimulants worldwide, and its consumption has been promoted as an optimal method to temporarily sustain the alertness of personnel with a limited level of medical oversight.[3] A survey of naval aviation candidates found that 86% drank coffee daily, consistent with the percentage of the general population.[33] Side-effects are dose- and user-dependent, and include agitation, irritability, tremor, dysrhythmia, and gastrointestinal complaints.[6] Consumption of caffeine at low dosages (<200 mg, equal to approximately two cups of coffee) is generally regarded as safe, with few or no side-effects reported.[27] Higher dosages may lead to side-effects such as nausea, jitteriness, and nervousness.[24] Additionally, individuals consuming higher daily quantities may experience withdrawal symptoms, such as headaches and muscle tremors, when caffeine intake is halted. Relative contraindications for caffeine use are hypertension, hyperthyroidism, epilepsy, mania, schizophrenia, and gastric and duodenal ulcers.[6]

The Royal Netherlands Air Force (RNLAF) allows the use of 300 mg caffeine tablets as an in-flight fatigue countermeasure.[28] Unfortunately, some aircrew members report that caffeine tablets are not sufficiently effective for reducing fatigue. This may be due to individual differences in caffeine metabolism or tolerance development. The metabolism of caffeine is primarily based on the action of CYP1A2.[31] CYP1A2 activity may vary by 5–6-fold between individuals due to environmental and (epi)genetic factors.[17] For example, 23% of the Caucasian population has a genetic CYP1A2 variant that increases tolerance to caffeine.[45] Additionally, caffeine clearance is increased by smoking and decreased by oral contraceptives.[17]

Tolerance development can be explained in two ways. Firstly, chronic intake of caffeine upregulates adenosine receptors in the brain. Over time, a larger amount of caffeine is required to attain the same stimulation as before.[38] Secondly, chronic intake of caffeine can induce CYP1A2, which increases the clearance rate and thus reduces and shortens the stimulatory effect of caffeine.[5,7] However, the influence of tolerance on the behavioral effects of caffeine is disputed and probably varies between individuals.[15,31,41]

Even so, differences in CYP1A2 variants and tolerance development may influence the effect of caffeine administration on performance. Additionally, genetic determinants may influence one's susceptibility to sleep deprivation and caffeine intake.[9,11] However, most research studying the efficacy of caffeine administration only included low-to-moderate caffeine users and/or instructed subjects to abstain from caffeine consumption for 48 h or longer prior to the start of the study.[8,24,42] This is impossible and/or impracticable in operational conditions; therefore, it is necessary to know more about the effect of daily caffeine consumption on the effects of caffeine during periods of sleep deprivation. This knowledge may help to personalize stimulant use in pilots and increase flight safety.

This study is part of a larger randomized controlled trial, which investigated several aspects of implementation of modafinil and caffeine as countermeasures for fatigue in a scenario realistic to military aviation. In a previously published manuscript about this trial, we concluded that both modafinil and caffeine significantly decrease the effects of an extended period of continuous wakefulness on vigilance compared with placebo.[43] The present study intended to determine the influence of previous caffeine consumption on the effect of caffeine (300 mg) administration on performance during a limited period of sleep deprivation. The period of continuous wakefulness was 24 h, and caffeine consumption was monitored through journals. In addition, caffeine blood levels were determined. We expected higher previous caffeine consumption to negatively affect the efficacy of caffeine administration.

METHODS

Subjects

The randomized controlled trial that this study is part of was conducted at the Center for Man in Aviation, RNLAF (Soesterberg, Netherlands), and adhered to the principles of the Declaration of Helsinki, the International Conference on Harmonization, and the Good Clinical Practice guidelines. The protocol was approved by the Medical Ethical Committee Brabant (reference: NL62145.028.17/P1749) and the Surgeon

General of the Ministry of Defense. The trial was registered in the Dutch Trial Register (No. NTR6922) and EU Clinical Trials Register (No. 2017-002,288-16).

Healthy employees of the RNLAF aged between 18–60 yr were eligible for inclusion. Eligible subjects were fit to fly according to the RNLAF Military Aviation Regulations or European Aviation Regulations.[13,29] Exclusion criteria were mainly based on possible side-effects or any of the following: interactions of one or both medicines (e.g., pregnancy or breastfeeding); the use of medication that is metabolized through CYP3A4/5, CYP2C19, or CYP2C9; and/or a history of psychiatric illness, including sleep disorders.

After being informed, both verbally and in writing, about the aims, consequences, and constraints of the trial, subjects gave written consent. This informed consent was voluntary and could be retracted at any time without any consequences. According to international privacy regulations, no study data were included in the medical files of the subjects.

The trial included 32 subjects, 2 of whom only completed the placebo trial day due to operational reasons. Their test results were excluded from the analysis of the present study because analysis of treatment effects according to a cross-over design could not be performed. The 30 remaining subjects were aged between 25–59 yr (median: 30.4 yr, IQR: 28.8–34.2 yr). Of the 30 subjects, 5 (17%) were women and 21 (70%) were pilots. None of the subjects smoked during the trial days. There were three (10%) subjects who used oral contraceptives during the study. On the caffeine trial day, the median waking time of the subjects was 07:00, meaning that at caffeine administration, the subjects had a median period of wakefulness of 17 h (range: 15.5–19.25 h, IQR: 16.5–17.5 h). Similarly, on the placebo trial day, the median waking time was 07:00 and the median period of wakefulness was 17 h (range: 16–19.5 h, IQR: 16.9–17.9 h).

Materials

On the trial days, several parameters were measured seven times: baseline measurement at 6 h (T-6) before administering the investigational product (T0) and at 1, 2, 3, 4, 6, and 8 h after T0 (T1, T2, T3, T4, T6, and T8, respectively). Between these measurements, subjects were free to choose what activity to take part in, except sleeping or napping.

The Vigilance and Tracking test (VigTrack) is a dual task that measures vigilance performance under the continuous load of a compensatory tracking task. The test has been used in various studies and is sensitive for measuring vigilance and alertness.[35,40] During the tracking task, subjects had to steer a blue dot, using a joystick, such that it remained below a red dot in the center of the display. The blue dot is programmed to move continuously from the center of the display. While tracking, subjects had to perform an additional vigilance task. Inside the red dot, a black square alternated with a diamond once per second. At random intervals, a hexagon was presented. When this occurred, subjects had to press an additional key on the joystick. The duration of this test was 10 min, and primary endpoints included root mean square tracking error, percentage omissions, and mean reaction time. At the start of every trial day, three familiarization sessions of 5 min of the VigTrack were scheduled for all subjects to avoid practice bias during the actual measurements.

The psychomotor vigilance task (PVT) measures the speed with which subjects respond to a red stimulus and is used to assess the vigilance of subjects.[1] The interstimulus interval, defined as the period between the last response and the appearance of the next stimulus, varies randomly from 2–10 s. The duration of this test was 10 min, and primary endpoints included 1/mean reaction time and lapses. Lapses (errors of omission) were defined as reaction times ≥500 ms. At the start of every trial day, a familiarization session of 5 min of the PVT was scheduled for all subjects to avoid practice bias during the actual measurements.

The Stanford Sleepiness Scale (SSS) was used to subjectively assess the degree of sleepiness in subjects during the trial days.[18] This subjective rating scale is sensitive to any significant increase in sleepiness or fatigue and is highly correlated with flying performance and the threshold of information-processing speed during periods of intense fatigue.[32]

Blood samples were taken four times throughout the night to determine caffeine blood levels (at T0, T3, T6, and T8). These samples were taken by qualified medical personnel in concordance with Dutch quality and safety standards and were analyzed by an external, qualified diagnostic laboratory.

Design

This study was part of a larger, randomized, double-blind, crossover, active- and placebo-controlled clinical trial, in which the effects of modafinil and caffeine administration on vigilance were compared with those of placebo.[43] This trial had a within-subjects 3×7 design: treatment (modafinil, caffeine, placebo) x time (T-6, T0, T1, T2, T3, T4, T6, T8). It consisted of three nonconsecutive trial days for every participant during which modafinil, caffeine, or placebo capsules were each administered once just after midnight (see **Table I**). For the present study, only the results of the trial days on which caffeine and placebo were given were included, resulting in a 2×7 design. The dose of caffeine (300 mg) was the usual dose administered to RNLAF aircrew; it is considered a medium-range but effective dose, comparable to 3–4 cups of coffee.[4,25]

A wash-out period of at least 7 d was implemented to ensure that the investigational products were completely eliminated and would not interfere on subsequent trial days. The trial was double-blinded so that both the subjects and investigators were unaware of the treatment given on trial days. The order of the treatments for each individual subject (modafinil, placebo, or caffeine) was based on a computer-generated randomization schedule organized and monitored by an external statistician. Randomization was performed using six possible treatment sequences to ensure balance for carryover effects, i.e., improving skills or learning bias on the test battery. In the current study, even though the modafinil administration was excluded, the six possible treatment sequences were equally distributed across the population, maintaining a balanced cross-over design.

Table I. Overview of Study Design and Data Collection.*

TIMING	ACTIVITY
The 3 d before every trial day	Sleep diary Caffeine log
16:30	Vital parameters Stanford Sleepiness Scale Familiarization with PVT and VigTrack
17:00	Subject ceased caffeine consumption
18:00	Baseline block (T-6) Stanford Sleepiness scale Assessment of VigTrack and PVT
00:00	Second baseline block (T0) Vital parameters Stanford Sleepiness scale Assessment of VigTrack and PVT Blood samples Investigational product administration
01:00	First test block (T1) Stanford Sleepiness scale Assessment of VigTrack and PVT
02:00	Second test block (T2) Vital parameters Stanford Sleepiness scale Assessment of VigTrack and PVT
03:00	Third test block (T3) Stanford Sleepiness scale Assessment of VigTrack and PVT Blood samples
04:00	Fourth test block (T4) Stanford Sleepiness scale Assessment of VigTrack and PVT
06:00	Fifth test block (T6) Stanford Sleepiness scale Assessment of VigTrack and PVT Blood samples
08:00	Sixth test block (T8) Vital parameters Stanford Sleepiness scale Assessment of VigTrack and PVT Blood samples
Outtake	Sleep questionnaires

*All trial days were identical, the only difference being the investigational product administered.

For every trial day, the researchers received a treatment kit from the pharmacist. The treatment kits were labeled with the subject number and the trial day and contained identical capsules.

Procedure

For 1 wk prior to the start of the trial days, subjects remained within the time zone of the research center (GMT +1, daylight saving GMT +2) to prevent jetlag, which might confound the test results. During the trial days, no strenuous physical exercise (including sports) or sleeping was allowed, and subjects kept a log of their activities.

On three consecutive days before the trial day and on the trial day itself, the subjects recorded their caffeine intake in a journal. On the trial day, subjects were instructed to consume their normal amount of caffeine-based products until 17:00 and cease their consumption of caffeine products thereafter.

Vital signs (temperature, blood pressure, and pulse) were collected four times during each trial day, two times prior to investigational product administration, and 2 and 8 h after administration (see Table I). Additionally, on every trial day, female subjects were tested for pregnancy and all subjects were asked if they had taken any concomitant medication or unauthorized medications during the past 3 d. Subjects were asked about potential adverse events multiple times during the trial days. Any adverse events were recorded throughout the trial and at every visit after screening.

Statistical Analysis

Sample size calculations were performed with G*Power.[14] The assumed means and standard deviations of VigTrack were used to obtain the effect size (d) for sample size analysis.[23] Two-way testing of treatment effect using a repeated-measures analysis of variance (ANOVA) within subjects, with $\alpha = 0.05$, $\beta = 0.8$, and the aforementioned effect size (d), required a minimum of $N = 18$ to show the effects of caffeine. However, to compensate for dropouts and sample failures, at least 30 subjects were included.

Statistical analyses were performed using SPSS Statistics for Windows (IBM Corp.; Armonk, NY, USA: 2020, version 27.0). A factorial repeated-measures ANOVA was conducted to analyze the main and interaction effects of time and treatment on the VigTrack and PVT parameters. SSS scores were analyzed by nonparametric tests (Friedman test for repeated measures). Mauchly's test was performed to test if the assumption of sphericity had been violated for the different parameters. If this was the case, the degrees of freedom were corrected using Huynh–Feldt or Greenhouse-Geisser estimates of sphericity where appropriate.[16] A P-value of <0.05 was considered statistically significant.

The relationship between caffeine intake and blood concentration of caffeine was analyzed using the Wilcoxon Signed Rank test. The relation between the aforementioned parameters and both caffeine intake and caffeine blood concentrations were analyzed using marginal models (generalized estimating equations), with time of measurement during the night as a cofactor. A P-value of <0.05 was considered statistically significant.

RESULTS

The trial ended according to the protocol. No adverse events were encountered during the trial, and the subjects' vital signs were unaffected by drug administration. The results of the comparison between the effects of modafinil and caffeine with placebo on nighttime vigilance are published elsewhere.[43]

After checking for outliers in the data with boxplots, two subjects were removed from the analysis of the VigTrack parameters. These subjects showed extreme values for all the VigTrack parameters, likely because they may have not understood the task properly. No outliers were identified when analyzing other parameters.

Caffeine vs. Placebo

The VigTrack and PVT parameters in the caffeine and placebo conditions were analyzed using a two-way repeated-measures ANOVA. In all instances, Mauchly's test indicated a violation of the sphericity assumption; therefore, Greenhouse-Geisser results were analyzed. The results of Mauchly's test and subsequent correction of the degrees of freedom are provided in **Table II**. SSS scores were analyzed using the Friedman test. For all indices, performance degraded significantly less in the caffeine than in the placebo condition across the night. Test results for all primary endpoints are displayed in **Fig. 1** and Table II.

Caffeine Intake

Median caffeine intake on the caffeine trial day was 260.0 mg (range: 0.0–765.0 mg, IQR: 172.5–347.5 mg), which was nearly identical to the median habitual caffeine intake of 260.0 mg (range: 0–770 mg, IQR: 173.1–340.0 mg). The results of statistical analyses of habitual caffeine consumption were similar to those of statistical analyses of caffeine intake on the trial day and were therefore not included in this study. Median caffeine intake on the placebo trial day was slightly lower at 247.2 mg (range: 0.0–632.0 mg, IQR: 102.3–340.0 mg).

Table III shows the results of the marginal model for caffeine intake on the caffeine trial day. For all primary endpoints (PVT, SSS and VigTrack parameters), marginal models did not show a statistically significant effect of the amount of caffeine intake on the trial day. Time of assessment was associated with a statistically significant lower performance on all parameters, with the exception of the VigTrack mean tracking error ($P = 0.083$). **Fig. 2** displays these results visually, in which the trendlines show the relation between caffeine intake on the trial day and the performance at 00:00, 03:00, 06:00, and 08:00, respectively. An analysis of the subjects with the upper and lower 25% of caffeine intake revealed no statistically significant difference compared to the other subjects.

Caffeine Blood Concentrations

The mean caffeine concentration in blood at 00:00 on the caffeine trial day was 1.4 μg · ml^{-1} (range: < 0.1–12.5 μg · ml^{-1}, IQR: 0.6–3.7 μg · ml^{-1}). This was similar to the mean caffeine concentration in blood at 00:00 on the placebo trial day, which was 1.3 μg · ml^{-1} (range: <0.1–11.0 μg · ml^{-1}, IQR: 0.6–2.5 μg · ml^{-1}). After 00:00, the caffeine concentrations in blood showed different patterns in the two conditions (**Fig. 3**). Mauchly's test indicated that sphericity was met [$\chi^2(5) = 4.820$, $P = 0.438$]. In the marginal model, the caffeine concentration was significantly higher in the caffeine condition than in the placebo condition [$F(3, 87) = 56.662$, $P < 0.0001$] and peaked at 3 h after administering caffeine tablets.

The Wilcoxon Signed Rank test showed that caffeine consumption during the trial days had no statistically significant effect on the blood concentration at 00:00 ($P < 0.001$). This was also the case for habitual caffeine consumption and blood caffeine concentrations ($P < 0.001$).

Table II. Results of the Statistical Tests of the Main Effects Per Parameter.*

TEST	PVT–1/MEAN REACTION TIME	PVT–NUMBER OF LAPSES	SSS	VIGTRACK–MEAN TRACKING ERROR	VIGTRACK–MEAN PERCENTAGE OMISSIONS	VIGTRACK–MEAN REACTION TIME
Mauchly's test Correction	$\chi^2(27) = 57.020$, $P = 0.001$ ε = 0.632 (GG)	$\chi^2(27) = 67.234$, $P < 0.001$ ε = 0.616 (GG)	NA	$\chi^2(27) = 224.153$, $P < 0.001$ ε = 0.143 (GG)	$\chi^2(27) = 274.794$, $P < 0.001$ ε = 0.143 (GG)	$\chi^2(27) = 89.600$, $P < 0.001$ ε = 0.445 (GG)
ANOVA caffeine group	$F(3.593, 104.199) = 19.438$, $P < 0.001$, $\eta^2 = 0.401$	$F(3.387, 98.230) = 15.022$, $P = 0.001$, $\eta^2 = 0.341$	NA	$F(1.454, 42.179) = 2.025$, $P = 0.156$, $\eta^2 = 0.065$	$F(1.385, 40.154) = 1.643$, $P = 0.210$, $\eta^2 = 0.054$	$F(2.309, 61.903) = 3.913$, $P = 0.023$, $\eta^2 = 0.119$
ANOVA placebo group	$F(3.914, 121.347) = 49.705$, $P < 0.001$, $\eta^2 = 0.616$	$F(4.060, 125.845) = 31.597$, $P < 0.001$, $\eta^2 = 0.505$	NA	$F(2.371, 73.509) = 10.539$, $P < 0.001$, $\eta^2 = 0.254$	$F(1.640, 50.852) = 13.609$, $P = 0.001$, $\eta^2 = 0.305$	$F(3.739, 115.919) = 36.159$, $P < 0.001$, $\eta^2 = 0.538$
Friedman test	NA	NA	$\chi^2(1) = 148.324$, $P < 0.001$	NA	NA	NA
Interpretation	Performance after caffeine administration degraded less across the night than after placebo administration	Performance after caffeine administration degraded less across the night than after placebo administration	Subjective sleepiness across the night is less affected after caffeine administration than after placebo administration	Performance after caffeine administration degraded less across the night than after placebo administration	Performance after caffeine administration degraded less across the night than after placebo administration	Performance after caffeine administration degraded less across than after placebo administration

*After Mauchly's test indicated a violation of the sphericity assumption, the degrees of freedom were corrected using Greenhouse-Geisser (GG) estimates. Afterwards, separate ANOVAs were conducted to test the effects of the test condition on each parameter. *P*-values lower than 0.05 indicated statistically significant results. For the Stanford Sleepiness Scale (SSS) a Friedman test was conducted due to the nonparametric nature of the data. PVT: Psychomotor Vigilance Test; SSS: Stanford Sleepiness Scale; VigTrack: Vigilance and Tracking Test.

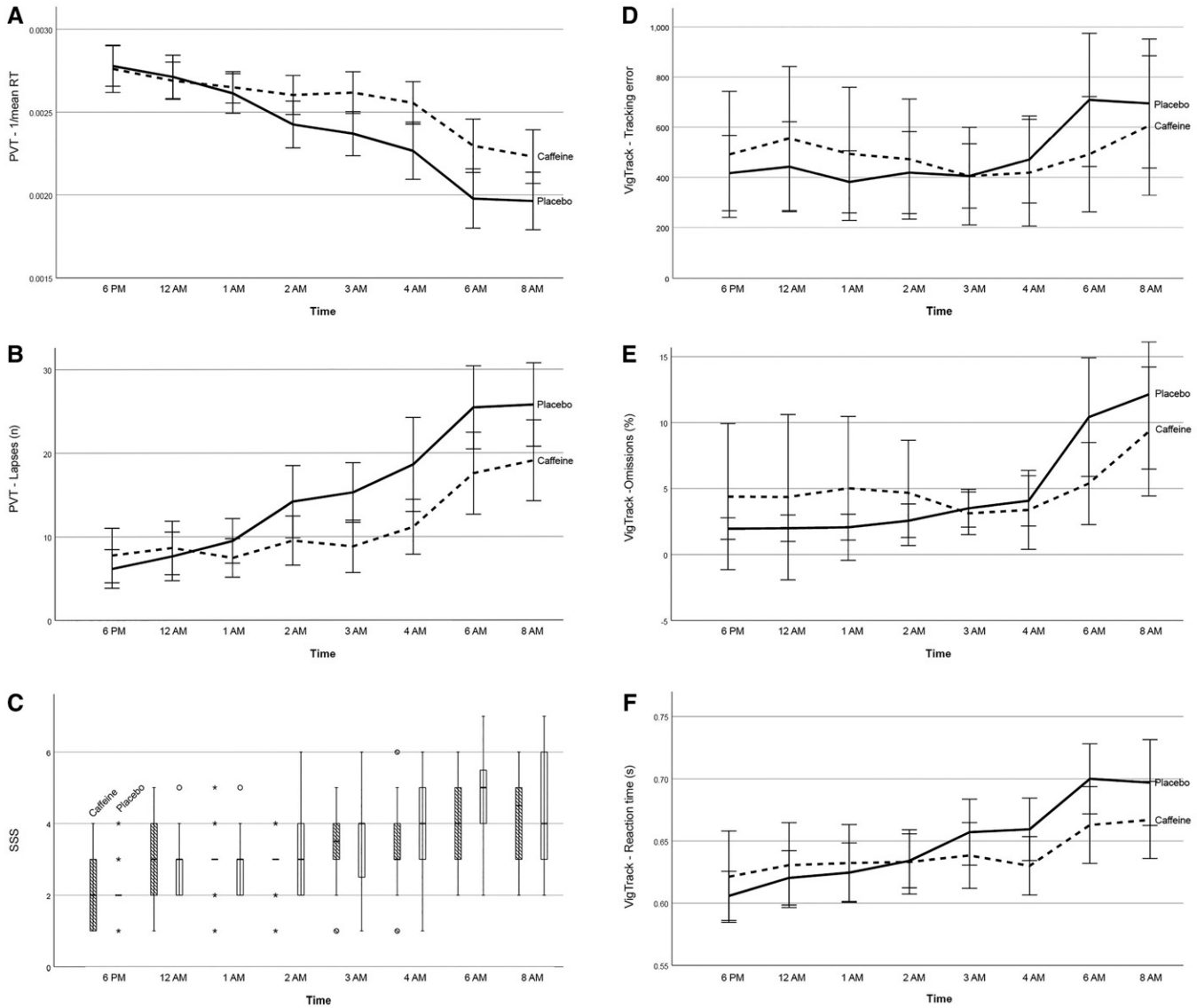

Fig. 1. Performance on caffeine trial day vs. placebo trial day. A) PVT–1/mean reaction time; B) PVT–number of lapses; C) SSS; D) VigTrack–mean tracking error; E) VigTrack–mean percentage omissions; and F) VigTrack–mean reaction time. Dashed line = caffeine, solid line = placebo. A lower score is a lower performance, except for PVT—1/mean reaction time.

Similar to caffeine intake, no statistically significant relationship was found between caffeine blood concentrations and performance on any primary endpoints (PVT, SSS, and VigTrack parameters), using a marginal model. **Table IV** shows the results of this marginal model. Time of assessment was associated with a statistically significantly lower performance on all parameters. **Fig. 4** displays these results visually, and the trendlines show the relationship between caffeine blood concentration on the trial days and the performance at 00:00, 03:00, 06:00 and 08:00, respectively.

Table III. Results of the Marginal Model; Performance vs. Trial Day Caffeine Intake.

COVARIATE	PVT–1/MEAN REACTION TIME	PVT–NUMBER OF LAPSES	SSS	VIGTRACK–MEAN TRACKING ERROR	VIGTRACK–MEAN PERCENTAGE OMISSIONS	VIGTRACK–MEAN REACTION TIME
Intercept	0.003	4.817	2.601	317.867	−1.364	0.598
P-value	<0.001*	0.011*	<0.001*	<0.001*	0.472	<0.001*
Assessment	$-7.07.10^{-5}$	1.874	0.213	18.373	1.133	0.008
P-value	<0.001*	<0.001*	<0.001*	0.083	0.003*	0.001*
Caffeine	$-6.39.10^{-8}$	0.001	$-7.24.10^{-5}$	−0.069	0.002	$2.82.10^{-5}$
P-value	0.742	0.873	0.933	0.750	0.763	0.767

*Statistically significant results ($P < 0.05$) from the Wald Chi-squared test. PVT: Psychomotor Vigilance Test; SSS: Stanford Sleepiness Scale; VigTrack: Vigilance and Tracking Test.

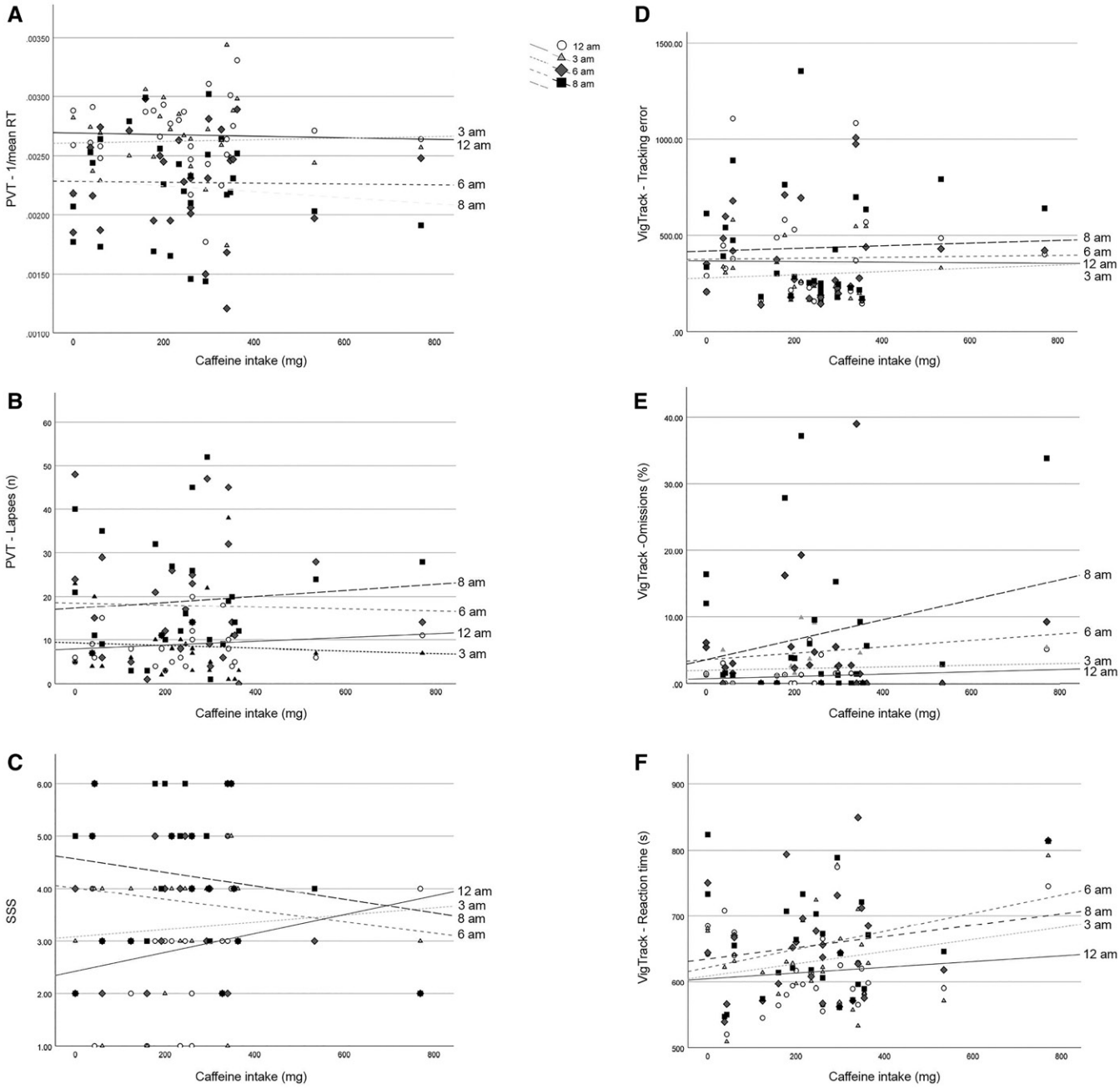

Fig. 2. Performance vs. trial day caffeine intake. A) PVT–1/mean reaction time; B) PVT–number of lapses; C) SSS; D) VigTrack–mean tracking error; E) VigTrack–mean percentage omissions; and F) VigTrack–mean reaction time. A lower score is a lower performance, except for PVT–1/mean reaction time.

DISCUSSION

This study demonstrates that previous caffeine consumption does not interfere with the effect of caffeine administration on performance during an extended period of continuous wakefulness (median 17 h). Additionally, although administration of caffeine improved performance compared with placebo, this study revealed no statistically significant relationship between the height of the caffeine blood concentration and the effect on performance. In addition, there was no statistically significant relationship between the blood caffeine concentration at midnight and caffeine intake on the trial day or habitual caffeine consumption.

Studies have reported mixed and inconclusive results regarding the effect of caffeine administration on vigilance during continuous wakefulness.[10] Several studies found no clear evidence of objective benefit, while pilots receiving caffeine tended to perceive their performance too optimistically, which might cause safety problems.[22,25] Other studies found that although caffeine does not improve subjectively assessed sleepiness, it increases vigilance and performance of sleep-deprived individuals, sometimes beyond baseline levels.[8,20] These conflicting

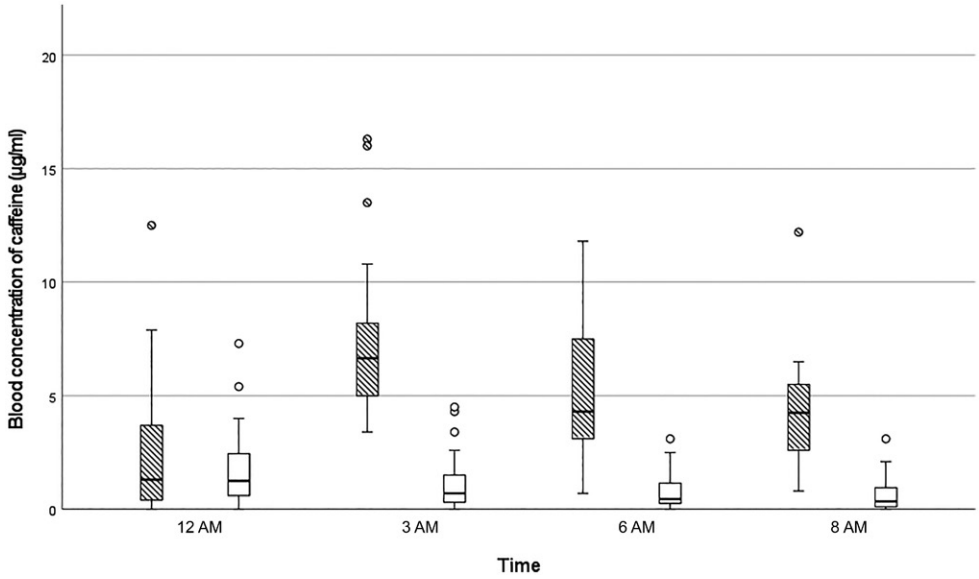

Fig. 3. Concentration-time curve of caffeine on the caffeine and placebo trial day. Striped = caffeine, blank = placebo.

results may be due to differences in subject characteristics, study procedures, sample sizes, and statistical power. In the previously published manuscript about this trial, it was concluded that both modafinil and caffeine significantly decrease the effects of an extended period of continuous wakefulness on vigilance compared with placebo.[43] In the present study, caffeine did not fully counteract the negative effect of extended wakefulness on performance because parameters were negatively affected in both conditions during the night. However, all performance parameters were affected less after caffeine administration than after placebo administration. Therefore, this study confirms that caffeine administration led to less impaired vigilance during an extended period of continuous wakefulness.

Although this, to our knowledge, is the first randomized, placebo-controlled trial to allow the use of caffeine products within 48 h of caffeine administration, we still imposed a 7 h caffeine-free period before caffeine administration. We introduced this period to mimic operational situations in which aircrews are advised to observe a similar period in order to maximize caffeine's beneficial effects. Caffeine's half-life is 4–6 h; therefore, caffeine blood concentrations would have decreased by 50–75% after 7 h.[23] This is congruent with the low median blood caffeine concentrations at 00:00 on the caffeine and placebo trial days of 1.35 and 1.30 $\mu g \cdot ml^{-1}$, respectively, which are regarded as low and harmless levels.[34] Allowing caffeine consumption until closer to investigational product administration likely would have increased caffeine blood concentrations at 00:00 and throughout the night. In the current study, caffeine blood concentrations did not exceed 20 $\mu g \cdot ml^{-1}$, which is considered an elevated, but nontoxic, concentration.[34] Additionally, shortening the caffeine-free period might have revealed a relationship between the amount of previous caffeine consumption and the effects of caffeine administration on performance because caffeine's beneficial effects can last up to 8 h.[23] Despite using several tests for statistical analyses, no statistically significant relationship was found between the height of blood caffeine concentrations and the effect on performance. This might be attributable to the limited number and timing of blood samples taken during the night. Samples were taken at 3, 6, and 8 h after caffeine administration. Given that caffeine's half-life is 4–6 h, caffeine concentrations would already have decreased significantly at 3 h after administration. For this reason, a statistical comparison between the area under the curve and performance parameters was not deemed advantageous. Although the sample size was sufficient to reject the null hypothesis in this study, increasing the number of blood samples would have allowed us to better establish caffeine blood concentration curves during the night and to correlate these

Table IV. Results of the Marginal Model; Performance vs. Caffeine Blood Concentrations.

COVARIATE	PVT–1/MEAN REACTION TIME	PVT–NUMBER OF LAPSES	SSS	VIGTRACK–MEAN TRACKING ERROR	VIGTRACK–MEAN PERCENTAGE OMISSIONS	VIGTRACK–MEAN REACTION TIME
Intercept	0.003	5.358	2.526	306.986	−0.0.870	0.616
P-value	<0.001*	0.029*	<0.001*	<0.001*	0.481	<0.001*
Assessment	$-8.08.10^{-5}$	1.901	0.207	19.037	1.136	0.009
P-value	<0.001*	<0.001*	<0.001*	0.035*	0.002*	<0.001*
Caffeine	$-2.96.10^{-7}$	−0.077	0.017	−2.064	−0.011	−0.003
P-value	0.986	0.873	0.622	0.842	0.960	0.297

*Statistically significant results ($P < 0.05$) from the Wald Chi-squared test. PVT: Psychomotor Vigilance Test; SSS: Stanford Sleepiness Scale; VigTrack: Vigilance and Tracking Test.

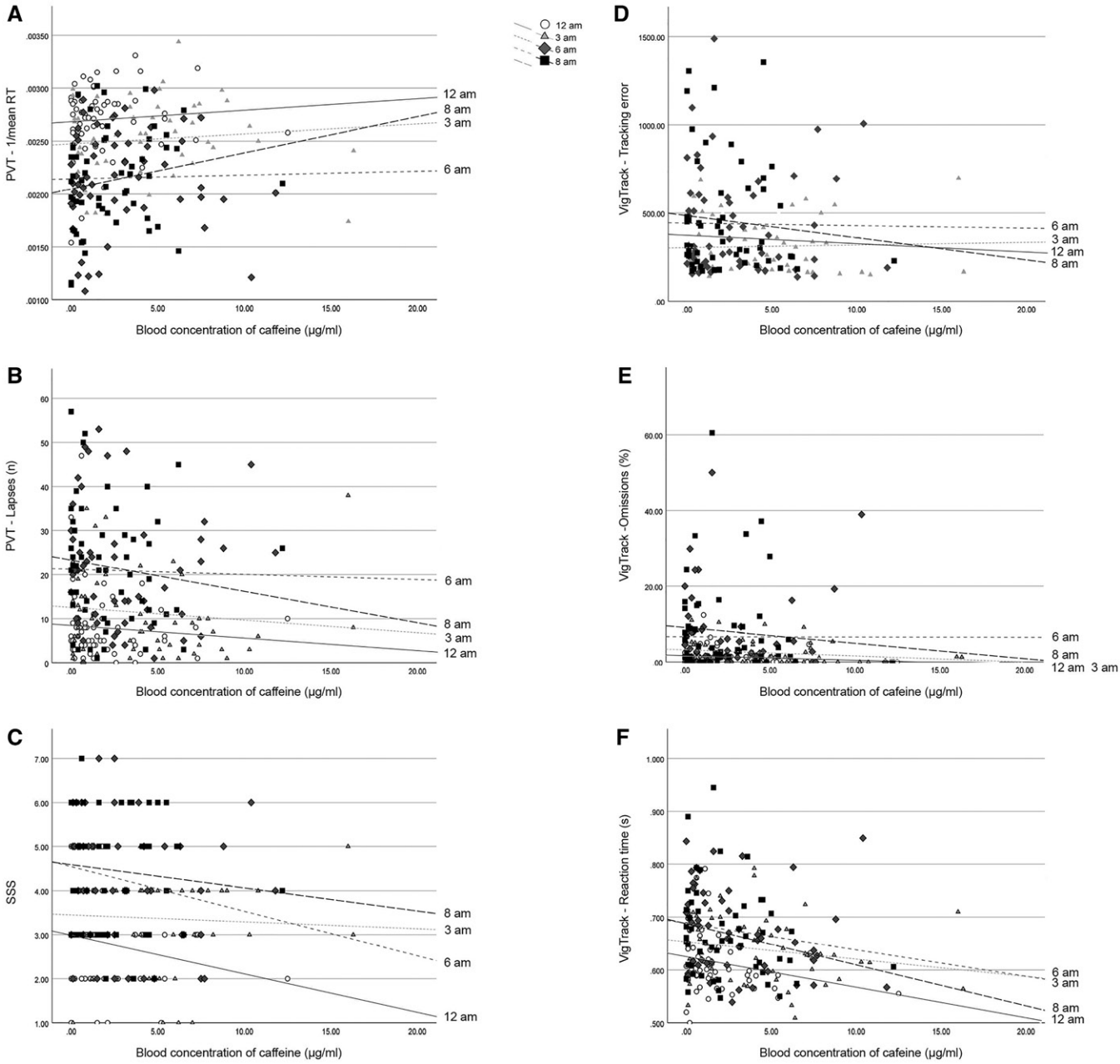

Fig. 4. Performance vs. caffeine blood concentrations. A) PVT–1/mean reaction time; B) PVT–number of lapses; C) SSS; D) VigTrack–mean tracking error; E) VigTrack–mean percentage omissions; and F) VigTrack–mean reaction time. A lower score is a lower performance, except for PVT–1/mean reaction time.

with the performance parameters. Moreover, the lack of a statistically significant relationship between blood caffeine levels and results of the psychomotor parameters, despite a sufficiently powered study, could mean there is a higher than earlier assumed interindividual response to the effects of caffeine. Alternatively, the conclusion might be that there is no clear relation between response and concentration in blood, as was suggested in previous literature.[2] Further studies are required to elucidate this.

Another possible limitation of our study is the reliability of the caffeine intake equations. The amount of caffeine in various caffeinated drinks varies and is not always reproduceable. It was impossible to account for this in the current study and therefore average amounts were used. Despite this possible incongruency, we do not believe this significantly influenced the results because the same equations were used for all subjects, and subjects consumed caffeine at the same site, meaning all subjects were affected similarly. Furthermore, calculated caffeine intake on the trial days was very similar to habitual caffeine consumption. This suggests that subjects did not change their caffeine consumption during the trial days. Both nicotine and oral contraceptives influence caffeine clearance.[17] None of the subjects smoked during the study, but 3 (10%) of the 30 subjects who completed the caffeine trial day took oral contraceptives.

Oral contraceptives decrease caffeine clearance; therefore, the caffeine blood concentrations of these subjects may have been higher than those of the other subjects. However, there was no statistically significant relationship between the caffeine blood concentration and performance; therefore, we believe the effects were limited. Additionally, 23% of the Caucasian population has a CYP1A2 variant that increases tolerance to caffeine.[45] There was no statistically significant relationship between the caffeine blood concentration and performance; therefore, we do not advise that the CYP1A2 variant be analyzed in aircrew members.

This trial was designed to resemble realistic operational situations (i.e., the period of wakefulness was limited to approximately 17 h). Additionally, to best reflect circumstances of operational military aviation, the subjects were not given specific bedtimes or waking times. Therefore, the time since the last sleeping period and the duration of that sleeping period differed between subjects. These differences may have caused variation in performance during the trial periods. However, due to its crossover design and the similar waking times of the subjects on the placebo and caffeine trial days, we do not believe this affected the results of our study. Additionally, we allowed all types of caffeine consumers to participate in this trial because the RNLAF aircrew comprises low-to-high caffeine users. Our study shows that previous caffeine consumption does not interfere with the effect of caffeine administration on performance. Additionally, we checked whether subjects with the upper and lower 25% of caffeine intake scored differently than the other subjects, but we did not detect a statistically significant difference. Thus, we conclude that aircrew can continue their habitual caffeine consumption as long as they abstain from caffeine for 7 h before caffeine administration. Future research should investigate whether a shorter caffeine-free period is sufficient to benefit from the effects of caffeine administration.

In conclusion, although administration of caffeine (300 mg) improved performance compared with placebo, neither previous caffeine consumption nor the caffeine blood concentration interfered with the effect of caffeine administration on performance during an extended period of continuous wakefulness (median 17 h). Stimulants may play an important role in military aviation, especially in situations where pilots are already fatigued but operational necessity requires them to continue their mission. Therefore, it is paramount to be able to properly advise aircrew about what to use and when. This study shows that a 7 h caffeine-free period seems to be sufficient to negate any interfering effects of previous caffeine consumption. Additionally, there was no difference between subjects with high caffeine intake and those with low caffeine intake, allowing aircrew to continue their habitual caffeine consumption. Future research should investigate whether a shorter caffeine-free period is sufficient to benefit from the effects of caffeine administration. Furthermore, future studies could include more blood samples to better establish blood caffeine concentration curves during the night and to correlate these with the performance parameters. The results of this study are not just relevant for military aviation, in which the use of caffeine tablets is already allowed, but for all industries in which peak performance is demanded during nighttime or after periods of continuous wakefulness (like civil aviation, healthcare, and logistics). Caffeine is widely available and therefore these findings can be used to determine when to have a cup of coffee, caffeine chewing gum, or any other caffeine-containing product, not just caffeine tablets.

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