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Effects of Acute Caffeine Intake on Insulin-Like Growth Factor-1 Responses to Total Sleep Deprivation: Interactions with *COMT* Polymorphism – A Randomized, Crossover Study

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Keywords

Total sleep deprivation · Caffeine · Hormones · Genetics

Abstract

Introduction: Genes encoding *catechol-O-methyl-transferase* (*COMT*) and *adenosine A2A receptor* (*ADORA2A*) have been shown to influence cognitive performances and responses to caffeine intake during prolonged wakefulness. The rs4680 single-nucleotide polymorphism (SNP) of *COMT* differentiates on memory score and circulating levels of the neurotrophic factor IGF-1. This study aimed to determine the kinetics of IGF-1, testosterone, and cortisol concentrations during prolonged wakefulness under caffeine or placebo intake in 37 healthy participants, and to analyze whether the responses are dependent on *COMT* rs4680 or *ADORA2A* rs5751876 SNPs. **Methods:** In caffeine (2.5 mg/kg, twice over 24 h) or placebo-controlled condition, blood sampling was performed at 1 h (08:00, baseline), 11 h, 13 h, 25 h (08:00 next day), 35 h, and 37 h of prolonged wakefulness, and at 08:00 after one night of recovery sleep, to assess hormonal

concentrations. Genotyping was performed on blood cells. **Results:** Results indicated a significant increase in IGF-1 levels after 25, 35, and 37 h of prolonged wakefulness in the placebo condition, in subjects carrying the homozygous *COMT* A/A genotype only (expressed in absolute values [\pm SEM]: 118 \pm 8, 121 \pm 10, and 121 \pm 10 vs. 105 \pm 7 ng/mL for A/A, 127 \pm 11, 128 \pm 12, and 129 \pm 13 vs. 120 \pm 11 ng/mL for G/G, and 106 \pm 9, 110 \pm 10, and 106 \pm 10 vs. 101 \pm 8 ng/mL for G/A, after 25, 35, and 37 h of wakefulness versus 1 h; $p < 0.05$, condition X time X SNP). Acute caffeine intake exerted a *COMT* genotype-dependent reducing effect on IGF-1 kinetic response (104 \pm 26, 107 \pm 27, and 106 \pm 26 vs. 100 \pm 25 ng/mL for A/A genotype, at 25, 35, and 37 h of wakefulness vs. 1 h; $p < 0.05$ condition X time X SNP), plus on resting levels after overnight recovery (102 \pm 5 vs. 113 \pm 6 ng/mL) ($p < 0.05$, condition X SNP). Testosterone and cortisol concentrations decreased during wakefulness, and caffeine alleviated the testosterone reduction, unrelated to the *COMT* polymorphism. No significant main effect of the *ADORA2A* SNP was shown regardless of hormonal responses. **Conclusion:** Our results indicated that the *COMT*

polymorphism interaction is important in determining the IGF-1 neurotrophic response to sleep deprivation with caffeine intake (NCT03859882).

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Introduction

Caffeine (CAF) is one of the most commonly used psychoactive stimulants to improve alertness and alleviate cognitive performance deficits and fatigue in sleep deprivation protocols in the laboratory, or during prolonged military operations, or in the medical departments of emergency or security services [1–3]. Pharmacodynamics of CAF has been ascribed mainly to its nonselective antagonism of adenosine receptors, and most of its effects at low levels appear to be dependent on interactions with A1 and A2A receptors [4]. Adenosine is a modulator of central nervous system neurotransmission, and its modulation of dopamine transmission through A2A receptors has been implicated in the effects of CAF, because of the colocalization of adenosine A2A receptor (*ADORA2A*) and dopamine D2 receptor in striatal neurons [4]. The neurotransmitter dopamine contributes to the regulation of different brain functions, including sustained attention and EEG oscillations during wakefulness [5]. On the other hand, several single-nucleotide polymorphisms (SNPs) in dopamine genes are reported to affect cognitive phenotypes [6] during sleep deprivation particularly [7, 8], and robust functional effects were found for the *catechol-O-methyltransferase* (*COMT*) gene (*Val158Met* polymorphism or rs4680) [6, 9]. This enzyme breaks down dopamine mostly in the part of the brain responsible for higher cognitive and executive function (prefrontal cortex). In human, the rs4680_ *COMT* markedly affected enzyme activity, protein abundance, and protein stability [10]. This functional polymorphism modulates the activity of *COMT* and was shown a promising candidate gene for both cognitive function and emotion [11, 12]. The A/A (methionine [Met]/Met) genotype results in decrease of *COMT* enzyme activity and high dopamine in prefrontal cortex, and the G/G (valine [Val]/Val) genotype results in increase of *COMT* enzyme activity and lower dopamine. With respect to *ADORA2A*, several polymorphisms in the gene, the rs5751876 in particular, were found to contribute to trait-like interindividual differences in psychomotor vigilance in the rested and sleep-deprived state, without interaction with acute CAF intake [3, 13].

To date, the effect of CAF on anabolic and catabolic hormones in healthy sleep-deprived subject has been

little investigated. Acute ingestion of moderate and high CAF increases adrenaline levels (a catabolic hormone) and alertness following 49 h of prolonged wakefulness (corresponding to two nights of sleep deprivation) in a dose-related fashion [14]. CAF supplementation was also found to increase testosterone concentration post-aerobic exercise in sleep-deprived subjects only (24 h without sleep) [15]. However, there are no data on the effects of CAF intake on the kinetic responses of the anabolic growth factor IGF-1 and the anabolic hormone testosterone during 38 h of continuous wakefulness. Studies have shown that experimental total sleep deprivation (TSD) affects circulating IGF-1 concentrations, with somewhat different results depending on the time of blood collection or sample size (i.e., no change on a single sample after 24 h of continuous wakefulness [16] or in kinetics up to 33 h of maximum continuous wakefulness [17], or even an increase when expressing the area under the curve over 24 h of continuous wakefulness [18], while the decrease in testosterone level seems more reproducible [17, 19]). What is interesting about the potential effects of CAF on the IGF-1 growth factor is that low doses of CAF, close to physiological concentrations, have been shown to increase longevity of the *Caenorhabditis elegans* through modulation of the growth hormone (GH)/IGF-1 system, which is an evolutionarily conserved pathway, and that this is dependent on adenosine signaling [20]. We can therefore hypothesize that the effect of CAF on IGF-1 may potentially involve adenosine/dopamine complex signaling, especially since some authors have described an association between baseline serum IGF-1 levels and the *COMT* rs4680 polymorphism; i.e., Val/Val (or G/G) carriers had significantly lower levels than Met (A/A or G/A) carriers in healthy elderly subjects [21]. In addition, basic memory scores were lower in Val/Val (or G/G) carriers [21]. Our previous results also indicated a greater cognitive vulnerability to sleep deprivation, as well as sensitivity to CAF intake, in healthy subjects carrying the *COMT* G/G genotype compared to G/A and A/A genotypes [3, 8]. Taken together, these results suggest that there may be *COMT* genotype-dependent interindividual variability in IGF-1 levels in response to an experimental condition such as sleep deprivation under CAF administration.

The aim of the current study was to evaluate the influence of two adenosine and dopamine polymorphisms (*COMT* and *ADORA2A*) on the kinetic response of the anabolic factor IGF-1 during 38 h of prolonged wakefulness with acute CAF intake, in thirty-seven

healthy subjects. The anabolic and catabolic hormones, testosterone and cortisol, were also evaluated. The duration of 38 h of prolonged wakefulness was chosen because (i) the greatest decrease in cognitive performance is observed after approximately 26 h (sleep pressure) [22], and (ii) the significant low-grade increase in circulating TNF- α is observed after 34 and 37 h [23].

Materials and Methods

Participants

42 participants, aged between 18 and 55 years, were initially included. The study received the agreement of the Cochin – CPP Ile de France IV (Paris) Ethics Committee and of the French National Agency for Medicines and Health Products Safety (ANSM) (Ile de France IV) and was conducted according to the principles expressed in the Declaration of Helsinki of 1975, as revised in 2001. All of the participants gave their informed written consent. This study is a part of the PERCAF clinical trial (NCT03859882). In this trial, the sample size was calculated based on the primary endpoint which was sustained attention performance (expression of speed) in the psychomotor vigilance test (with an expected difference of 30% due to sleep deprivation, as observed in our previous study [22]).

Participants were free from medical, psychiatric, and sleep disorders. Other exclusion criteria included physical or mental health troubles based on (I) Hospital Anxiety and Depression (HAD) scale, HAD ≥ 16 , (II) significant medical history, (III) Epworth Sleepiness Scale (ESS), ESS > 10 , (IV) Pittsburg sleep quality index > 8 , (V) morningness-eveningness questionnaire, < 31 or > 69 , (VI) habitual time in bed per night < 6 h [3, 8]. We also excluded subjects younger than 18 years and older than 55 years, with a BMI greater than 30 kg/m², working at night or in shift work or jet lag (> 3 time zones) in the previous month, drinking more than 1 glass of alcohol per day, and abusing tobacco. Habitual coffee consumption (number of cups per day), smoking habits, weekly consumption of alcohol, and physical activity time were assessed with a questionnaire. Participants were asked to complete a sleep/wake schedule and maintain their sleep and CAF consumption patterns for the week prior to the study.

Study Design and Testing Conditions

This randomized crossover study has been conducted in the sleep laboratory of the Armed Forces Biomedical Research Institute (IRBA), Brétigny-sur-Orge, France. Ambient temperature was controlled and maintained at 22°C ($\pm 1^\circ\text{C}$) during all the experiment. The brightness of the lighting has been maintained between 150 and 200 lux during the awaking periods, and lights were off during sleep periods. Meals and caloric intake were standardized for a caloric intake not exceeding 2,600 kcal/day. The meal time was fixed (08:15–8:45 for breakfast, 12:15–13:00 for lunch, and 19:00–20:00 for dinner).

Participants remained inside the laboratory for 3 consecutive days. The experimental protocol included (I) a habituation/training day (D0), (II) a baseline day (D1) beginning at 07:15 until 00:00, (III) a TSD day beginning on D2 00:00 until 21:00 (i.e., 38 h of continuous wakefulness), and (IV) a recovery night

until the end of the study (09:00 on D3). Participants were welcomed in groups of 4 around 16:00 on D0 day. During the experimental protocol, blood sampling was performed at 1 h (08:00, corresponding to baseline), 11 h and 13 h (in the D1 day), and 25 h (08:00), 35 h, and 37 h of wakefulness (in the D2 day), and at 08:00 on D3 after one night of recovery sleep.

The current study is part of a multi-aimed project. We previously reported results on the influence of genetic polymorphisms on neurobehavioral impairments related to TSD under CAF intake [3, 8]. During this laboratory protocol, participants were always under visual surveillance of research staff members. Our two previous studies did not evaluate changes in circulating concentrations of biomarkers.

When participants were not engaged in testing, meals, or sleep periods, they were not allowed to exercise and use tobacco, alcohol, or other psychoactive substances. However, they were allowed to read, to watch videos, or to speak with other participants or staff members, and play games, following a pre-established program. In addition, participants wore a wrist actigraphy to check that they stayed awake during the 38-h continuous wakefulness period.

CAF Administration

In this double-blind, crossover study, participants followed two conditions (i.e., CAF or placebo [PBO]), administered twice on D1 and D2), with a 2-week washout period between the two conditions during which they returned to their off-protocol lifestyle. Each participant received for the CAF condition, 2.5 mg/kg body weight of CAF powder mixed in decaffeinated beverage. PBO was a decaffeinated beverage with the same bitterness, smell, and taste. The CAF powders were premeasured by the project supervisor. This amount of CAF powder was chosen for its enhancing properties on attention in sleep-deprived conditions (2.5–8 mg/kg of CAF) [24]. The beverage was administered at 08:30 and 14:30 (i.e., at 1.5 and 7.5 h of prolonged wakefulness) on D1, and at 08:30 and 14:30 (i.e., at 25.5 and 31.5 h of prolonged wakefulness) on D2.

Genotyping

Genotyping for the *COMT* rs4680 and *ADORA2A* rs5751876 was performed on blood cells using the LAMP-MC method [25]. This method has been applied to complex biological matrices, such as whole blood and saliva, without prior DNA extraction.

Blood cell samples were collected on EDTA and were aliquoted in sterile microtubes and stored at -20°C until LAMP-MC analysis. The LAMP-MC genotyping assays were realized by use of the customized *COMT* rs4680 and *ADORA2A* rs5751876 kit (Cat#LC-SD-LP-24, LaCAR MDX, Liège, Belgium). A positive control and a negative control were supplied. LAMP-MC consists in a lysis of cells followed by the amplification of the target sequence at a constant temperature around 65°C using simultaneously three sets of primers, a polymerase with high-strand displacement activity in addition to a replication activity and a fluorophore-labeled probe. Detection of homozygous wild, heterozygous, and homozygous mutant genotype is performed by melting curve analysis after amplification.

For this experiment, the genotyping call rates were 100%. We compared the expected distribution under the Hardy-Weinberg hypothesis with the observed distribution for the rs4680 and rs5751876 SNPs in the studied population. Genotype frequencies

of both SNPs conformed to Hardy-Weinberg equilibrium ($p > 0.49$). The minor allele frequency was 0.49 for *COMT* rs4680 (G) and 0.34 for *ADORA2A* rs751876 (T).

Blood Sampling and Analyses

Blood was collected from each participant into EDTA plastic tubes for plasma (K2EDTA tubes), using a standard phlebotomy technique. Blood samples were immediately centrifuged at 1,900 g at 4°C, and aliquots were frozen and stored at -80°C until assays were performed. Hormonal concentrations were determined using commercially available enzyme-linked immunosorbent assay kits: IGF-1 (R&D Systems, DG100), cortisol (IBL, RE52061), and testosterone (IBL, RE52151). Sensitivity, intra-assay and inter-assay coefficients of variation were as follows: 2.9%; IGF-1 0.026 ng/mL, 3.5%, 7%; cortisol 11 nmol/L, 2.6%, 4.9%; testosterone 0.41 nmol/L, 5.4%, 5.5%. All measurements were above the sensitivity values and therefore detectable.

Statistics

Statistical analyses were performed using Statistica® (StatSoft Inc, Oklahoma, OK, USA). Values were expressed as mean \pm SEM. All data are normally distributed according to the Shapiro-Wilk test. Blood parameters were analyzed using a three-way mixed-effect analysis of variance (ANOVA) including awakening time or day (repeated measures), condition (PBO or caffeinated, repeated measures), and fixed effects for genetic polymorphism (non-repeated measures). Because there is an association between *COMT* polymorphism and estrogen levels in men [26], which themselves have a subsequent stimulatory effect on GH, thus potentially on IGF-1 levels, we adjusted for sex for all three biomarkers, IGF-1, testosterone, and cortisol in the ANOVA. Effect size was evaluated using the Eta squared (η^2): $\eta^2 \geq 0.01$ indicates a small effect, $\eta^2 \geq 0.06$ indicates a medium effect, and $\eta^2 \geq 0.14$ indicates a large effect. When the ANOVA revealed significant main effects with interactions, a Tukey post hoc test was used to identify differences without correction for multiple comparisons. The kinetics of changes in biological parameters were modeled using a 3-order polynomial regression. The fit quality was checked by evaluating the r^2 and its p value.

Results

Participants

42 participants were initially included in this study. We excluded one participant because of an important adverse effect after CAF intake and four participants due to failure to carry out their second participation. Finally, a total of 37 healthy participants (33.5 ± 1.3 years) fully followed the protocol, including 56.8% ($N = 21$) of women and 43.2% ($N = 16$) of men.

Participants' average daily CAF consumption was 250 ± 32 mg (mean \pm SEM). 27 participants were non smokers, and the remaining 10 smoked less than 12 cigarettes per day. The mean weekly alcohol consumption was 1.9 ± 0.2 glasses. The mean BMI was 23.0 ± 0.6 kg/m² for women and 23.5 ± 0.6 kg/m² for men. The mean

weekly exercise duration was 3.1 ± 0.4 h. The mean daily total sleep time was 7.2 ± 0.2 h, and the sleepiness score was 6.8 ± 0.6 . Chronotype was distributed this way: 40.5% had morning chronotype, 2.7% evening chronotype, and 56.8% middle tier.

Genotype Distribution

Participant genotypes and their corresponding characteristics are summarized in Table 1. The *COMT* rs4680 G > A polymorphism causes an amino acid change from Val to Met at codon 158 of the *COMT* gene. The enzymatic activity of *COMT* is altered by this SNP, resulting in a trimodal distribution (high activity in the G/G (Val/Val) genotype, intermediate activity in G/A (Val/Met), and low activity in A/A (Met/Met)). The participant frequency was similar to the 1000 Genomes Project data on the GRCh38 reference assembly. There was no significant difference in habitual CAF consumption between the three *COMT* genotype carriers. Genetic variation for the rs751876 *ADORA2A* is C > T; as the mutated homozygous alleles were extremely rare, mutated homozygous and heterozygous alleles were combined. Habitual CAF consumption was statistically higher in participants carrying the T allele (C/T-T/T) than in those carrying the C/C genotype.

Kinetic Responses of Circulating Hormonal Concentrations (i.e., after 1, 11, 13, 25, 35, and 37 h of Wakefulness in D1 and D2) in PBO and CAF Conditions

Regarding *COMT* polymorphism (SNP), the ANOVA indicated a significant Time (T) main effect for all hormonal parameters, without significant Condition (C) or SNP (3 genotypes) effects (Table 2). For IGF-1, data were analyzed as normalized to the 1 h of wakefulness (baseline) (i.e., Δ) to facilitate visualization of the response. The kinetic response of IGF-1 in absolute values is shown in online supplementary Figure 1 (for all online suppl. material, see <https://doi.org/10.1159/000529897>). There are significant interactions between C and time for all parameters (Table 2). For Δ -IGF-1 and absolute IGF-1 levels, there were higher levels after 25, 35, and 37 h compared to 1 h of wakefulness in the PBO condition and after 35 h of wakefulness in the CAF condition (Fig. 1a, and online suppl. Fig. 1A). These levels are significantly lower in the CAF compared to PBO condition after 25, 35, and 37 h of wakefulness (Fig. 1a, and online suppl. Fig. 1A). In addition, for Δ -IGF-1 and absolute IGF-1 levels, there are significant interactions between C and T and *COMT* SNP (Table 2). This is illustrated in Figures 1b and c for

Table 1. Genetic polymorphism distribution and individual characteristics in the studied population compared to 1000 Genomes international database

Polymorphisms	Genotypes	N (%)	1000 Genomes ^a , %	Age, years	Gender (♀, %)	Habitual CAF consumption, mg/d	Body mass index (BMI), kg/m ²
rs4680_COMT	G/G (ancestral)	11 (29.7)	26.4	32.7±1.7	72.7	249±69	23.1±0.8
	G/A	14 (37.8)	47.1	37.4±2.1	35.7	312±47	24.0±0.7
	A/A	12 (32.4)	26.5	30.0±2.5	66.2	178±48	22.4±0.7
rs5751876_ADORA2A	C/C (ancestral)	14 (37.8)	37.4	32.9±1.8	57.1	168±53	22.6±0.5
	C/T – T/T	23 (62.2)	62.6	33.9±1.8	56.5	300±36*	23.6±0.6

Values are mean ± SEM. * $p < 0.05$: significant difference between genotypes (for ADORA2A, C/T and T/T were grouped together due to the low number of T/T carriers [$n = 2$]). ^aExpected based on 1000 Genomes Project data on the GRCh38 reference assembly (<http://www.internationalgenome.org/>).

Δ -IGF-1 (the illustration with IGF-1 in absolute values is in online suppl. Fig. 1B, C). In the PBO condition, Δ -IGF-1 and absolute IGF-1 levels increased at 25, 35, and 37 h of wakefulness in subjects carrying the homozygous COMT A/A genotype and were not increased in carriers of G/G and G/A (Fig. 1b, and online suppl. Fig. 1B), whereas no genotype-related differences are observed in the CAF condition (Fig. 1c, and online suppl. Fig. 1C).

For cortisol and testosterone, there are the T main effect and interaction between C and T (Table 2). Compared to 1 h of wakefulness, cortisol and testosterone levels were lower at 11, 13, 25, 35, and 37 h of wakefulness in the PBO condition (Fig. 2a, b). In the CAF condition, cortisol level was lower at 35 h compared to 1 h of wakefulness, and testosterone levels were lower at 11, 13, 35, and 37 h (Fig. 2a, b). Cortisol levels were also higher at 35 and 37 h in the CAF compared to PBO condition, and testosterone levels were higher at 25 h for testosterone. For the ADORA2A SNP, no significant effect was observed on all hormonal parameters (data not shown).

Responses of Circulating Hormonal Concentrations at 08:00 before the Night of Sleep Deprivation (D1) and at Recovery after (D3) in PBO and CAF Conditions

Regarding COMT polymorphism (SNP), ANOVA indicated a significant main effect of day (D) (2 days, D1 and D3) on absolute IGF-1 levels (and not in delta, because of the 2 days statistically considered) and a significant interaction between condition (C) and

polymorphism (SNP) (Table 3). The post hoc analysis showed lower IGF-1 levels in the CAF condition in subjects carrying the homozygous COMT A/A genotype (102 ± 5 vs. 113 ± 6 ng/mL, $p < 0.05$). A significant interaction is present between C and D for testosterone (Table 3). The post hoc analysis showed that testosterone levels were lower at D3 compared to D1 in the PBO condition (7.6 ± 1.2 vs. 8.2 ± 1.4 nmol/L), and levels were higher at D3 in the CAF compared to PBO condition (8.2 ± 1.3 vs. 7.6 ± 1.2 nmol/L). For the ADORA2A SNP, no significant effect was observed on all hormonal parameters (data not shown).

Discussion

In the current study, we evidenced that COMT polymorphism influences the kinetic responses of circulating IGF-1 during 38 h of prolonged wakefulness with acute CAF intake, in thirty-seven healthy participants. IGF-1 levels were significantly increased after 25, 35, and 37 h of prolonged wakefulness compared with 1 h in the condition of acute PBO intake, and this is only observed in subjects carrying the COMT homozygous A/A genotype (i.e., the polymorphism is in significant ANOVA interaction with condition (i.e., PBO and CAF) plus time of wakefulness. The IGF-1 levels are also increased in the CAF condition but after 35 h of prolonged wakefulness only, and levels were significantly lower than in the PBO condition at 25, 35, and 37 h of wakefulness, erasing the difference between COMT genotypes. There was no

Table 2. ANOVA for biomarker concentrations (2 conditions, 6 awaking times, 3 genotypes)

	F	p value	η^2
IGF-1			
Condition (F _{1, 31})	0.92	0.35	<0.01
Time (F _{1, 155})	10.23	0.001	0.25
Condition × time (F _{5, 155})	2.66	0.03	0.08
SNP (F _{2, 31})	0.20	0.82	<0.01
Condition × SNP (F _{2, 31})	2.28	0.12	0.02
Time × SNP (F _{10, 155})	0.79	0.64	<0.01
C × T × SNP (F _{10, 155})	1.96	0.02	0.18
Δ IGF-1			
Condition (F _{1, 31})	1.92	0.18	0.01
Time (F _{1, 155})	10.23	0.001	0.25
Condition × time (F _{5, 155})	2.66	0.02	0.09
SNP (F _{2, 31})	0.28	0.76	<0.01
Condition × SNP (F _{2, 31})	2.54	0.09	0.02
Time × SNP (F _{10, 155})	0.79	0.64	<0.01
C × T × SNP (F _{10, 155})	1.96	0.02	0.09
Cortisol			
Condition (F _{1, 31})	1.24	0.27	<0.01
Time (F _{1, 155})	45.6	0.001	0.32
Condition × time (F _{5, 155})	5.35	0.01	0.14
SNP (F _{2, 31})	0.10	0.91	<0.01
Condition × SNP (F _{2, 31})	0.20	0.82	<0.01
Time × SNP (F _{10, 155})	1.10	0.36	<0.01
C × T × SNP (F _{10, 155})	1.85	0.06	0.07
Testosterone			
Condition (F _{1, 31})	0.05	0.82	<0.01
Time (F _{1, 155})	33.5	0.001	0.28
Condition × time (F _{5, 155})	2.84	0.01	0.14
SNP (F _{2, 31})	0.06	0.94	<0.01
Condition × SNP (F _{2, 31})	1.32	0.28	<0.01
Time × SNP (F _{10, 155})	1.56	0.12	0.02
C × T × SNP (F _{10, 155})	0.84	0.59	<0.01

Note: C, T, and SNP correspond, respectively, to condition, awaking time, and rs4680 *COMT* genotype effects. ANOVA effect: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, gender adjusted. η^2 is effect size value: $\eta^2 \geq 0.01$ indicates a small effect, $\eta^2 \geq 0.06$ indicates a medium effect, and $\eta^2 \geq 0.14$ indicates a large effect.

COMT main effect or interactions on testosterone and cortisol levels that decrease as a function of wakefulness and circadian effect.

Our results show first and before analysis of the genetic influence that circulating IGF-1 levels increased after 25, 35, and 37 h compared to 1 h of wakefulness (i.e., the 08:00 level) and remained higher after one night of sleep recovery (i.e., the 08:00 level at D3 day) (day main effect without condition effect). Previous results have described no change in IGF-1 levels expressed on a single blood sample at 8:00 a.m. after a night of sleep deprivation compared with the previous day at the same time, or expressed as area under

the curve [16, 17], or also an increase in IGF-1 levels expressed as area under the curve during 24 h of sleep deprivation [18]. In these studies, the number of participants is limited ($n = 13$ or less), with a different blood sampling protocol (single or serial sampling [16, 17], or serial sampling throughout a 24-h period of sleep deprivation but after an eccentric exercise-induced muscle injury protocol [18]), thus different primary endpoints, which limits the comparison. Furthermore, the acute CAF intake significantly attenuates the IGF-1 response to prolonged wakefulness, and this is shown in healthy subjects for the first time to our knowledge. We have previously described the beneficial effect of CAF on the alteration of neuro-behavioral responses with wakefulness, apart from the polymorphism effect [3]. Despite the paucity of data on the effects of CAF on IGF-1 levels, acute CAF intake (at 6 mg/kg, almost 2.5 times higher than in our study) has been shown to decrease the response of GH (the primary stimulator of IGF-1 production in the blood) to a single resistance exercise [27].

In this study, we asked the question of the potential relationship between polymorphisms of *COMT* or *ADORA2A* and the kinetics of hormonal responses to prolonged wakefulness under acute PBO or CAF intake, because we have previously analyzed their relationship with cognitive responses [3, 8]. We assessed IGF-1 because accumulating evidence reveals that circulating levels significantly affect brain cognitive function, and it has been suggested that better long-term cognitive function is associated with optimal serum levels of IGF-1 [28, 29]. In addition, we assessed testosterone and cortisol, the two hormonal responses representative of the anabolic/catabolic ratio, which are sensitive to sleep deprivation [19]. We previously showed that participants carrying the ancestral G/G genotype of *COMT* rs4680 or the T allele of *ADORA2A* rs5751876 were more cognitively impaired (compared with carriers of the A allele (A/A and G/A) for *COMT*, and C/C for *ADORA2A*), with a beneficial effect of acute CAF intake for carriers of the A allele for *COMT* [3, 8].

The influence of *COMT* on neurobehavioral vulnerability to sleep deprivation of healthy participants has been described repeatedly, and the studies point out that this is not observed when participants are well rested [7, 8, 30]. At the contrary, there are very few studies on the relationship between *COMT* polymorphism and IGF-1 levels in healthy adults, and to our knowledge, there is only the study by Witte et al. [21] that primarily investigates the link with cognitive performance and the effects of nutritional interventions in healthy elderly subjects. At baseline, lower IGF-1 levels and lower memory score were noted in Val/Val (G/G) genotype carriers compared to Met carriers, with a very

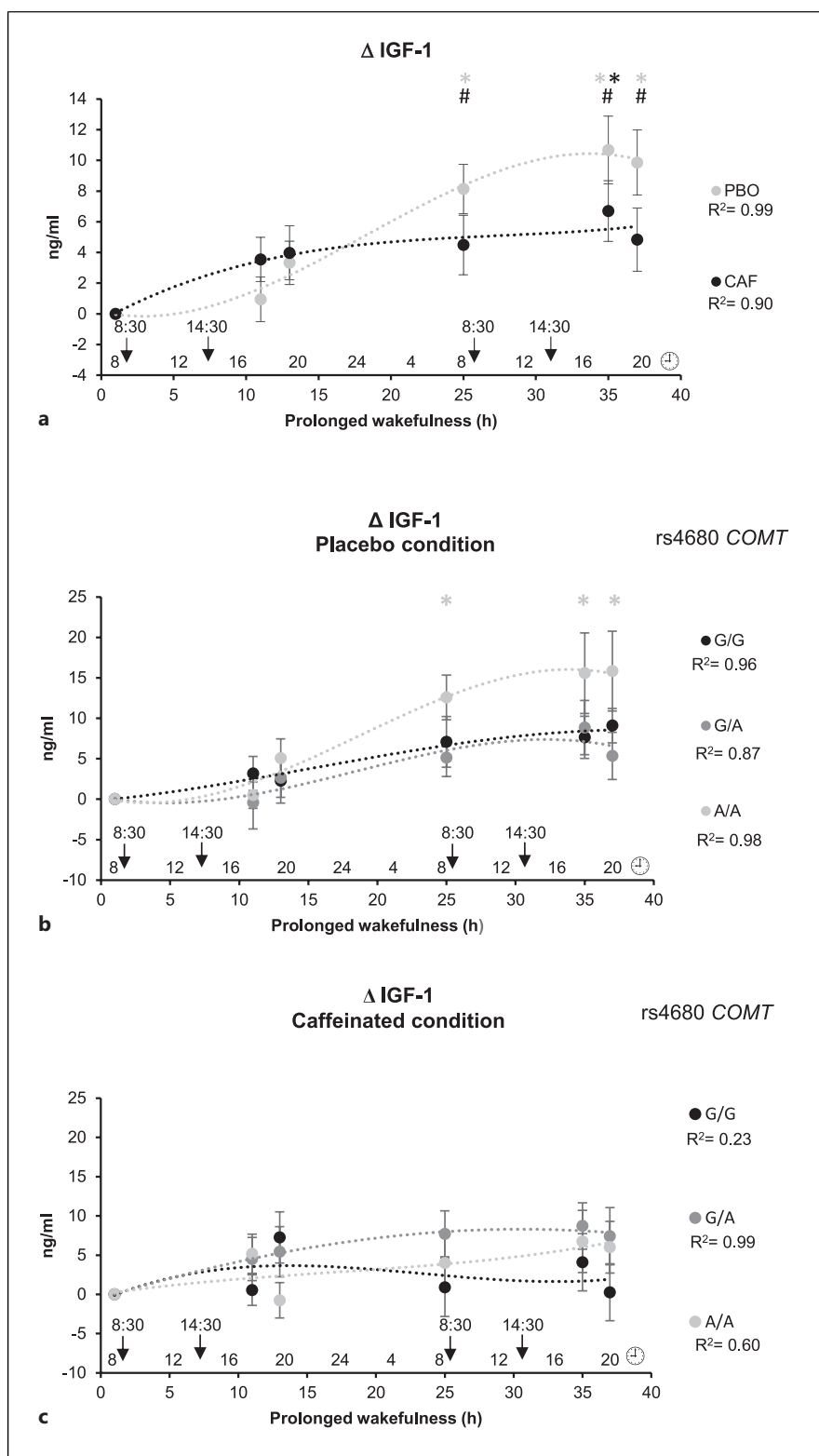
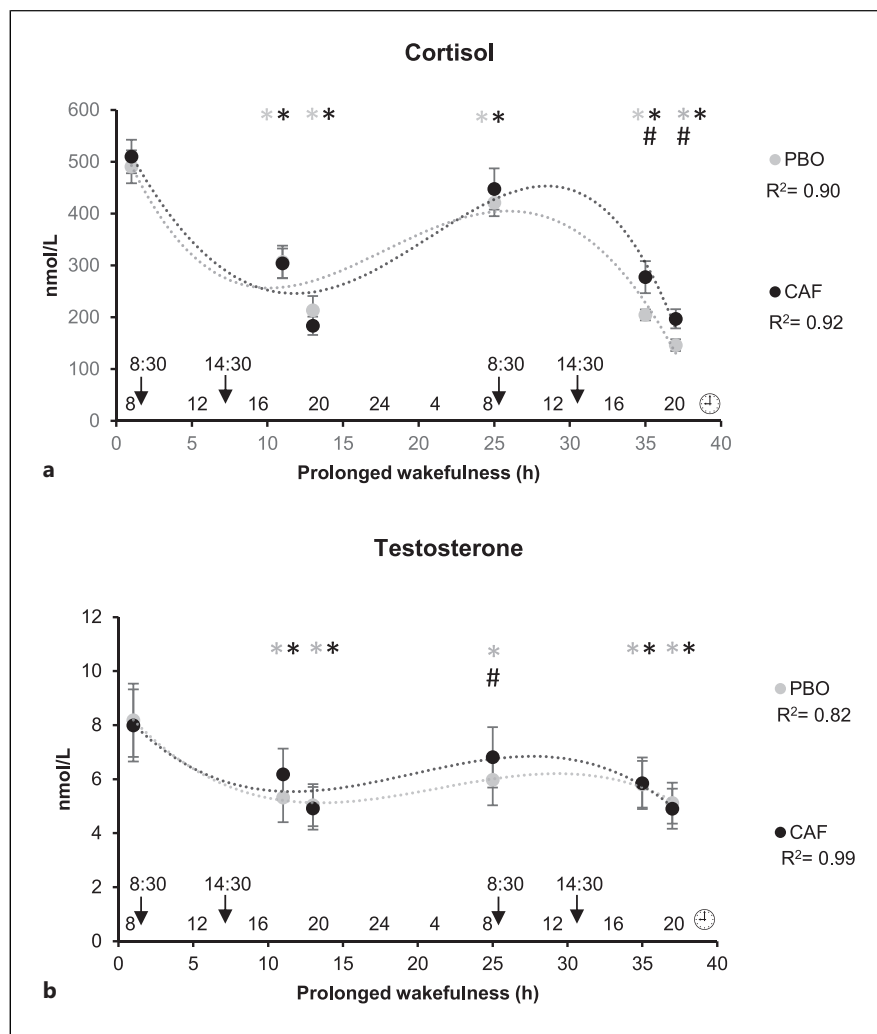


Fig. 2. Mean cortisol (a) and testosterone (b) concentrations over 38 of prolonged wakefulness in the placebo (PBO) and caffeine (CAF) conditions. Data are the mean \pm SE ($n = 37$). Down arrows: CAF or PBO intake. *Significant differences in comparison with the 08:00 concentrations in PBO and *CAF conditions. #Significant differences between PBO and CAF conditions.



limited sample size ($n = 6$ carrying the G/G genotype vs. $n = 29$ carrying the A allele). Our study thus provided additional information on the influence of *COMT* on the IGF-1 response to prolonged wakefulness in younger subjects than in Witte et al. [21] (33.5 vs. 61.3 years). On the other hand, we do not observe a *COMT* main effect or an interaction with day concerning IGF-1 levels, when we consider them to the 08:00 blood sampling at D1 and after the night of recovery (D3). We could thus suggest that the *COMT* polymorphism influences the response of IGF-1 beyond 25 h prolonged wakefulness, and not resting baseline levels.

Our results showed that homozygous carriers of the *COMT* A allele, resulting in high dopamine levels due to low *COMT* enzymatic activity, had increased levels of IGF-I in response to prolonged wakefulness beyond 25 h in the PBO condition, whereas IGF-I levels were

not increased in carriers of the G allele (either G/A or G/G) resulting in low dopamine levels due to high *COMT* enzymatic activity. This appears to be consistent with poorer cognitive performance (in the executive go-no-go inhibition task) during prolonged wakefulness in subjects carrying the G/G genotype compared to carriers of the A allele (homozygous and heterozygous), as we have previously shown [8]. We can thus suggest that IGF-I may be involved in sustaining proper cognition during prolonged wakefulness, but that this response would be *COMT* genotype dependent favoring the A allele carriers, as this polymorphism strongly modulates dopaminergic activity. This can be paralleled by the meta-analysis showing a significant association between *COMT* genotype and prefrontal activation without evidence of publication bias, and that executive cognition paradigms favor Met

Table 3. ANOVA for biomarker concentrations (2 conditions, 2 days, 3 genotypes)

	F	p value	η^2
IGF-1			
Condition (F _{1, 35})	2.01	0.16	0.01
Day (F _{1, 35})	20.44	0.001	0.22
Condition × day (F _{1, 35})	0.45	0.51	<0.01
SNP (F _{2, 34})	0.90	0.42	<0.01
Condition × SNP (F _{2, 34})	3.75	0.03	0.12
Day × SNP (F _{2, 34})	0.92	0.41	<0.01
C × D × SNP (F _{2, 34})	1.55	0.46	<0.01
Cortisol			
Condition (F _{1, 35})	1.86	0.18	<0.01
Day (F _{1, 35})	0.00	0.96	<0.01
Condition × day (F _{1, 35})	0.03	0.87	<0.01
SNP (F _{2, 34})	0.11	0.89	<0.01
Condition × SNP (F _{2, 34})	1.47	0.25	<0.01
Day × SNP (F _{2, 34})	0.76	0.48	<0.01
C × D × SNP (F _{2, 34})	1.75	0.19	<0.01
Testosterone			
Condition (F _{1, 35})	1.25	0.29	<0.01
Day (F _{1, 35})	0.92	0.34	<0.01
Condition × day (F _{1, 35})	5.80	0.02	0.15
SNP (F _{2, 34})	1.80	0.18	<0.01
Condition × SNP (F _{2, 34})	2.70	0.08	0.01
Day × SNP (F _{2, 34})	0.78	0.47	<0.01
C × D × SNP (F _{2, 34})	3.69	0.06	0.06

Note: C, D, and SNP correspond, respectively, to condition, day, and rs4680 *COMT* genotype effects. ANOVA effect: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, gender adjusted. η^2 is effect size value: $\eta^2 \geq 0.01$ indicates a small effect, $\eta^2 \geq 0.06$ indicates a medium effect, and $\eta^2 \geq 0.14$ indicates a large effect.

(A) allele carriers while emotional paradigms favor Val (G) allele carriers [12]. Furthermore, the lack of effect of *COMT* on testosterone and cortisol, two peripheral hormones also involved in cognition [31, 32], also emphasized that the *COMT* genotype dependence of cognitive responses relies on the efficacy of the dopamine/IGF-1 pattern. Several studies underlined that IGF-1 modulates synaptic strength by controlling the synthesis and release of diverse neurotransmitters such as acetylcholine or dopamine (reviewed by [33]). The link between IGF-1 and dopamine and cognition has been demonstrated in Parkinson's disease: it has been demonstrated that IGF-1 exerts a positive effect on dopaminergic neurons in both in vitro and in vivo models of the disease [34], and lower IGF-1 circulating levels in Parkinson's patients are associated with poor cognitive performance [35].

Our results also evidenced that the reducing effect of acute CAF intake on IGF-1 levels at the 25, 35, and 37 h of

wakefulness is related to participants carrying the A/A genotype of *COMT*, the high dopamine level-related genotype. In addition, the reducing effect of CAF specifically on the *COMT* A/A genotype is also observed on IGF-1 at rest (in D1 and D3, at 08:00 after sleep). CAF globally promotes wakefulness by unselectively antagonizing adenosine receptors, particularly A2A, in the brain regions, and helps restore cognitive function [3, 4]. There are also interactions between *ADORA2A* and dopamine D2 receptor in the brain, and an inhibition of A2A receptors by CAF would be expected to increase neurotransmission via dopamine at D2 receptors [4]. The acute CAF intake may have increased dopamine signaling in carriers of all three *COMT* genotypes via the A2A-D2 receptor interactions, leading to optimal dopamine signaling in A/A carriers who are themselves characterized by high dopamine levels, thereby limiting their physiological need to produce IGF-1 in response to prolonged wakefulness. This may be in line with our previous observation of a beneficial cognitive effect of acute CAF intake on carriers of the A allele of *COMT* at 20 h of wakefulness [3].

Finally, we did not observe a significant effect of *ADORA2A* polymorphism rs5751876 neither on the responses of IGF-1 levels nor on testosterone and cortisol to wakefulness under CAF or PBO intake. This result also highlights the importance of the *COMT* polymorphism that establishes a relationship between dopamine level and IGF-1 response to prolonged wakefulness, which would be less dependent on the dopaminergic activity controlled by the interaction of A2A and D2 receptors with CAF. In the literature, it is also established that the *ADORA2A* rs5751876 polymorphism (or HT4 haplotype including the T allele of rs5751876 SNP) is sensitive to prolonged wakefulness for performance of sustained attention (psychomotor vigilance test) but not to CAF intake [3, 13].

The strength of our study is that we analyzed hormonal responses during TSD (38 h of continuous wakefulness) in a group of healthy European subjects, and that we declined these responses according to the three *COMT* rs4680 genotypes. The sample size of 37 participants may seem small, but the number of subjects of each *COMT* genotype is greater than or equal to 11, which makes statistical analysis acceptable, especially since this type of laboratory protocol is very restrictive in terms of time (the same subjects must be evaluated in the 2 conditions, CAF and PBO, in a randomized way), dedicated staff, financial cost, etc. Nevertheless, some limitations must be taken into account when interpreting our results. Because we did not apply a correction for multiple comparisons in our ANOVA, the results of this study should be

confirmed in a larger population. In addition, the subjects' habitual CAF consumption may have affected hormonal concentrations, but this has been mainly described for high consumption at more than 400 mg per day [36], whereas our subjects were moderate consumers (the mean is 250 ± 32 mg (SEM)). On the other hand, we took into account the sex factor because there is a link between *COMT* genotype and estrogen levels [26], and prefrontal activity [37]. Finally, we did not assess other gene \times gene interactions, which could have exerted influence on dopaminergic neurotransmission, such as genes encoding the dopamine transporter or the dopamine D2 receptors [6]. This should be examined in future research. Regarding the genetic influence, it also would have been interesting to investigate the *CYP21A* polymorphism, in particular rs7624551 which affects the metabolism of CAF and cognitive performance [38], thus potentially the hormonal concentrations.

Conclusion

Our results describe the increased kinetic response of IGF-1 to prolonged wakefulness that can be reduced by acute CAF ingestion ($2.5 \text{ mg}\cdot\text{kg}^{-1}$). Carriers of the *COMT* AA genotype may be particularly responsive to this intervention, highlighting interindividual differences in the circulating IGF-1 response to sleep deprivation and CAF supplementation. Findings on the effects of CAF on the neurotrophic factor IGF-1, coupled with previous findings on cognition [3, 8], could help develop individualized therapies in future research.

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Statement of Ethics

The study received the agreement of the Cochin – CPP Ile de France IV (Paris) Ethics Committee and of the French National Agency for Medicines and Health Products Safety (ANSM) (Ile de France IV) (ID-RCB: 2017-A02793-50 (CPP IDF IV) and was conducted according to the principles expressed in the Declaration of Helsinki of 1975, as revised in 2001. Written informed consent for each subject was obtained for participation in this study.

Informed consent was obtained from all subjects involved in the study.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

F.S., C.D., M.E., and M.C. planned the study. F.S., C.D., M.E., and the investigators' group realized the experiment and collected the data. C.D., D.G.M., F.S., and M.E. conducted the analyses. D.G.M., C.D., and F.S. wrote the manuscript. D.L., C.T., and M.C. provided extensive feedback. All authors reviewed and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary materials. Further inquiries can be directed to the corresponding author.

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