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Dose-Dependent Effects of Caffeine on Cognitive Performance, Hormonal Regulation, and Gut Microbiota across Adulthood

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ABSTRACT

Caffeine is a psychoactive drug that has a wide range of uses, including being consumed to increase alertness and cognitive function. Recently, there has also been some evidence that caffeine can influence biochemical markers and gut microbiota composition, which also requires further research. **Objectives:** To assess the dose-dependent effects of caffeine on reaction time, alertness, and cognitive performance in various demographic groups and to investigate its biochemical and microbiological effects. **Methods:** This study used a double-masked, quasi-experimental design. Participants were given caffeine in low (50 mg), moderate (100 mg), or high (200 mg) doses in separate sessions with a 48-hour washout period and placebo control. Reaction time, self-reported alertness, and cognitive task performance were measured. The blood and stool samples were taken before and after caffeine consumption to assess cortisol, adrenaline, and gut microbiota profiles using 16S rRNA sequencing. **Results:** No statistically significant differences in reaction time and alertness were observed between any of the caffeine doses ($p>0.050$). A significant reduction in cortisol levels was observed at the higher dose ($p=0.048$), but no change in adrenaline levels was seen. No significant differences in the relative abundance of bacterial phyla were found, except for Firmicutes, which was moderately up-regulated by caffeine ($p=0.049$). Performance improvements showed trends favoring younger adults, though these were not statistically significant. **Conclusions:** This hypothesis-generating study demonstrates trends toward dose-dependent hormonal and microbial modulation (notably a reduction in cortisol at 200 mg and an increase in Firmicutes at 100 mg). However, no statistically significant cognitive enhancements were observed. These preliminary findings warrant confirmation in larger, adequately powered studies before clinical recommendations can be made.

INTRODUCTION

Caffeine is one of the most common psychoactive compounds in the world and is mainly used as a stimulant for the central nervous system (CNS) [1]. It occurs naturally in coffee, tea, energy drinks, and chocolate. Its main action is to block adenosine receptors, causing an increase in the activity of nerve cells and the release of excitatory neurotransmitters like dopamine and noradrenaline [1, 2]. These neurochemical changes are linked to increased alertness, reduced fatigue, and better psychomotor and cognitive performance [3, 4]. Many studies have validated the ability of caffeine to improve reaction time, vigilance, and memory, especially in the moderate dose range of 100-200 mg [5, 6]. These effects are thought to be secondary to its effect on adenosine pathways and subsequent activation of regions of the brain associated with attention and cognition [7, 8]. These effects, however, differ in intensity and length of time among people. These are affected by several physiological and demographic factors

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such as age, sex, caffeine habit, and genetic differences in caffeine metabolism [9]. The cognitive improvements seen in younger adults are generally larger than those observed in older adults, possibly because of age-related variations in receptor sensitivity and hepatic metabolism [9, 10]. Some studies indicate that there are differences in the sensitivity and metabolism of caffeine between genders, but the results are not consistent [11]. Caffeine also activates the hypothalamic-pituitary-adrenal (HPA) axis, which triggers a release of cortisol and adrenaline, hormones that play a role in the body's stress and arousal response [12, 13]. Transient increases in these hormones can improve alertness and preparedness in the mind, but a prolonged or high level of these hormones can harm cardiovascular and metabolic health [13, 14]. Caffeine has also been shown to affect the gut microbiome, which is an important part of the gut-brain axis. Recent studies have investigated the mechanisms that link caffeine to the microbiome, such as the regulation of microbial metabolites like short-chain fatty acids (SCFAs), changes in bile acid metabolism, and modifications to intestinal permeability. Caffeine and its metabolites, including paraxanthine, theobromine and theophylline, can have prebiotic effects by stimulating the growth of beneficial microorganisms like Bifidobacterium and Lactobacillus, and by inhibiting microorganisms like pathogens due to their antimicrobial properties. Furthermore, the impact of caffeine on the gut microbiota can be indirect, impacting cognitive function through neural, endocrine, and immune pathways within the so-called 'gut-brain axis'. High doses (above 200 mg/dose) have, on the other hand, been linked to disturbance in microbial balance and stimulation of opportunistic and pathogenic bacteria in animal models [15, 16]. They could be caused by a pH change in the intestine, bile acid secretion, and direct antimicrobial action on beneficial species in the intestine after caffeine intake. The clinical relevance and the threshold for such effects in humans remain to be studied. Combining the information obtained from the cognitive, hormonal, and microbial data, this study offers a multidimensional approach to the health benefits of caffeine consumption and reinforces the need for personalized guidance on caffeine consumption for the various population groups.

Although systemic effects of caffeine have gained interest, there is limited research that has explored the comprehensive effect of caffeine on neurocognition, biochemistry, and microbiology at varying doses and across different demographics. The lack of literature in this area restricts the scope of recommendations that can be made for optimal caffeine intake in various populations based on the evidence. Thus, this study aimed to check the dose-dependent effect of caffeine on reaction time, alertness, and cognitive functioning of various age groups and genders, to investigate biochemical changes such as cortisol and adrenaline levels after a caffeine dose, and to measure the changes in the composition of gut bacteria by using 16S rRNA sequencing to check the impact of caffeine on gut microbiota.

METHODS

This study used a double-masked, quasi-experimental design with each participant completing four experimental sessions for the placebo and each caffeine dose (50 mg, 100 mg, 200 mg). The study was approved by the Institutional Review Board (IRB) of the University of Lahore (IREC-2023-57 dated 27 November 2023). The study duration was from January 2024 to June 2024. Written informed consent was obtained from all participants. The study was carried out in compliance with the Declaration of Helsinki and national and international ethical guidelines. Institutional ethical guidelines. A 48-h washout period was allowed between sessions to prevent possible carryover effects. One hundred fifty healthy adults aged 18 to 70 were recruited, divided into 5 age groups of 50 each: 18-30, 31-40, 41-50, 51-60, and 61-70. There were 60 males (40%) and 90 females (60%), with 20 males and 30 females in each age group. Participants were included in the study if they were between 18 and 70 years of age, had a normal cognitive function (Mini-Mental State Examination score ≥ 24), had no neurological, gastrointestinal, endocrine, or psychiatric disorder, consumed < 300 mg/day caffeine, and were willing to refrain from caffeine for 24 hours before sessions. Medications that may affect cognition, hormones, or gut microbiota, antibiotic use within the past 3 months, chronic diseases, pregnancy or lactation, history of drug abuse and caffeine intolerance, and enrollment in another clinical trial within 30 days were exclusion criteria. The threshold of <300 mg/day was chosen because literature indicated that regular daily intake ≥300 mg/day could result in tolerance and changes in physiological response, thus complicating the dose-response assessment, which is equivalent to about 3 cups of coffee daily and is considered moderate caffeine intake by the EFSA. The capsules were taken by mouth after 4 hours of fasting to ensure the uniform absorption of the drugs and reduce interference of food with the drug. Baseline and 60 minutes after the dose, cognitive assessments were conducted, as the pharmacokinetic data suggested peak plasma concentration of caffeine should be reached around 30-120 minutes post-oral dose. Reaction time was measured in milliseconds in both simple and choice reaction time tasks, performed using a computer-based, validated paradigm (Cogstate or E-Prime). Subjective

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alertness was measured by the Stanford Sleepiness Scale (SSS) and Karolinska Sleepiness Scale (KSS) (1-9 scale), with lower scores representing increased alertness. For cognitive performance, short-term memory, sustained attention, and task switching tests were used, and results were reported in percentage accuracy and response latency. Blood samples were taken before and 60 minutes after consumption of the caffeine, and plasma cortisol (ng/mL) and plasma adrenaline (pg/mL) were determined in duplicate using Abcam ELISA (cortisol: ab108665; adrenaline: ab238274) performed on a BioTek ELx808 microplate reader, with inter-assay and intra-assay CVs <8% and <7%, respectively. Stool samples were collected before and 24 hours post-administration to capture acute shifts in the gut environment. We acknowledge that this timeframe may not fully capture long-term microbial remodeling; however, it aligns with previous acute dietary intervention studies assessing early changes in relative phyla abundance. The V3–V4 region of the 16S gene was amplified and sequenced on the MiSeq Illumina platform (2×300 bp) and quality filtered in QIIME2 (Phred score ≥20, read length ≥200 bp and at least 10 bp overlap). The depth of sequencing was adjusted to 10000 reads per sample, taxonomic classification was done with the SILVA database (v138), and the relative abundance was compared among the phyla of Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. Shannon index (alpha diversity) and Bray-Curtis dissimilarity (beta diversity) were used to assess microbial diversity. A priori power analysis was performed with the program G*Power (version 3.1.9.7) for repeated-measures ANOVA with within-between interaction with an assumed effect size (f = 0.25) the analysis showed a total sample size of 36 participants. Anticipated dropout rate is expected to be 20%, yielding a target of 45 participants. However, to ensure adequate power for the five age-group subgroup analyses and to account for multiple comparisons, we oversampled and recruited 250 participants (50 per age group). Assumptions of repeated-measures ANOVA were tested systematically before data analysis. Data normality was tested on each dose group for each dependent variable with Shapiro-Wilk tests and visually with QQ plots. The homogeneity of variance within groups was determined with Levene's test. Mauchly's test of sphericity was used to evaluate sphericity. If sphericity was not met ($p<0.050$), the Greenhouse-Geisser correction was used to correct the degrees of freedom, and the corrected p values are presented. Non-parametric alternatives (Kruskal–Wallis tests) were used for variables that did not meet normality assumptions (Shapiro-Wilk $p<0.050$).

Data were analyzed using SPSS version 25.0 and R version 4.3.1. Based on a priori power analysis with G*Power, a sample size of 150 participants (50 per group) would have a power of 80% at $\alpha = 0.05$ for detecting medium effects ($f = 0.25$) in repeated measures ANOVA. Tests for normality (Shapiro-Wilk), homogeneity (Levene's), and sphericity (Mauchly's) were conducted and corrected for sphericity violations ($p<0.050$) using the Greenhouse- Geisser correction. For the main effects of caffeine dose on reaction time, alertness, cognitive performance, biochemical markers, and microbial phyla, repeated-measures ANOVAs were used, and Bonferroni-corrected post hoc comparisons were performed when significant main effects were found. For subgroup analyses, the 5 original age groups were collapsed into 3 broader categories (18–30, 31–50, 51–70 years) to maintain statistical power for interaction testing. This consolidation is explicitly justified in the statistical analysis section. and by gender. For all comparisons of means, Cohen's d effect sizes were calculated, and partial eta^2 was reported for all ANOVA results. The Kruskal-Wallis tests with Dunn's post hoc tests were used for microbiota data that did not satisfy the normality assumptions. Microbiome analyses were performed using alpha and beta diversity metrics calculated with QIIME2, with Benjamini-Hochberg FDR correction done for multiple testing. A p-value of <0.050 was deemed to be statistically significant.

RESULTS

Consumption of caffeine had non-significant trends of improving reaction time and alertness across all doses as compared to placebo; greatest improvements were seen at the moderate (100 mg) and high (200 mg) doses in the 18–30 age group. For younger adults, the 200 mg dose showed a trend toward improved reaction time ($\Delta = -25$ ms, 95% CI: −54.2 to 4.2, $p=0.083$, $d = 0.46$, 95% CI: 0.20–0.72), while older adults showed minimal change ($\Delta = -6$ ms, 95% CI: −18.3 to 6.3, $p=0.612$, $d = 0.11$, 95% CI: −0.08–0.30). There was no gender difference or gender × dose interaction (partial $\eta^2 < 0.02$; 95% CI for gender effect: −5.3 to 6.7 ms) (Table 1).

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Table 1: Dose-Dependent Effects of Caffeine on Cognitive, Biochemical, and Microbiome Parameters

Variables	Unit	Placebo (Mean ± SD)	50 mg (Mean ± SD)	100 mg (Mean ± SD)	200 mg (Mean ± SD)	p-value	d	95% CI for d	95% CI for Mean Difference (200 mg vs Placebo)
Reaction Time	ms	450.2 ± 159.8	447.5 ± 161.9	445.6 ± 142.7	430.8 ± 136.6	0.830	0.18	-0.14, 0.50	-19.4 (-45.2, 6.4)
Alertness Score	1-10	5.82 ± 2.88	5.77 ± 2.91	5.85 ± 2.81	6.14 ± 2.51	0.779	0.11	-0.21, 0.43	0.32 (-0.18, 0.82)
Cognitive Task Accuracy	%	82.1 ± 8.4	82.5 ± 8.6	84.0 ± 8.7	82.9 ± 7.9	0.642	0.14	-0.18, 0.46	0.8 (-1.6, 3.2)
Cortisol	ng/mL	16.23 ± 5.82	15.81 ± 5.71	15.34 ± 5.96	13.17 ± 5.09	0.048	0.52	0.20, 0.84	-3.06 (-5.18, -0.94)
Adrenaline	pg/mL	29.45 ± 11.52	29.84 ± 11.79	29.17 ± 12.85	30.06 ± 10.71	0.960	0.09	-0.23, 0.41	0.61 (-2.28, 3.50)
Bacteroidetes	%	34.82 ± 9.15	35.00 ± 9.08	37.42 ± 7.73	36.22 ± 9.81	0.393	0.25	-0.07, 0.57	1.40 (-1.14, 3.94)
Firmicutes	%	42.01 ± 9.61	42.14 ± 9.53	45.53 ± 8.82	46.26 ± 8.19	0.049	0.48	0.16, 0.80	4.25 (1.83, 6.67)
Actinobacteria	%	5.28 ± 2.58	5.33 ± 2.64	5.91 ± 2.49	5.70 ± 2.84	0.507	0.22	-0.10, 0.54	0.42 (-0.24, 1.08)
Proteobacteria	%	3.12 ± 1.02	3.08 ± 1.05	2.70 ± 1.01	3.03 ± 1.17	0.161	0.17	-0.15, 0.49	-0.09 (-0.35, 0.17)

Bolded values are statistically significant ($p < 0.050$). Effect sizes indicate moderate practical relevance even for non-significant trends

Two-way repeated-measures ANOVA revealed no statistically significant age × dose or gender × dose interactions across any of the outcome measures (all $p>0.050$). Although a marginal trend was observed for the age × dose interaction on cortisol levels ($p=0.112$, partial $\eta^2 = 0.035$), it did not reach the conventional significance threshold. Collectively, these findings suggest that the dose-dependent effects of caffeine on cognitive, hormonal, and microbial parameters were largely uniform across age groups and genders in this study (Table 2).

Table 2: Complete ANOVA Statistics for Age × Dose and Gender × Dose Interactions

Variables	Age × Dose Interaction				Gender × Dose Interaction			
	F	df1, df2	p-value	Partial η^2	F	df1, df2	p-value	Partial η^2
Reaction Time	1.42	6, 294	0.208	0.028	0.87	2, 294	0.421	0.006
Alertness Score	0.98	6, 294	0.441	0.020	0.53	2, 294	0.589	0.004
Cognitive Task Accuracy	1.15	6, 294	0.334	0.023	0.41	2, 294	0.664	0.003
Cortisol	1.76	6, 294	0.112	0.035	0.64	2, 294	0.529	0.004
Adrenaline	0.83	6, 294	0.547	0.017	1.02	2, 294	0.362	0.007
Bacteroidetes	0.71	6, 294	0.642	0.014	0.88	2, 294	0.415	0.006
Firmicutes	1.18	6, 294	0.318	0.023	0.92	2, 294	0.398	0.006
Actinobacteria	0.56	6, 294	0.762	0.011	0.45	2, 294	0.638	0.003
Proteobacteria	0.63	6, 294	0.705	0.013	0.61	2, 294	0.544	0.004

The 200 mg dose of caffeine significantly lowered cortisol levels ($p=0.048$), and had a moderate effect size in younger adults (Cohen's $d = 0.52$), but the age × dose interaction was not significant ($p=0.112$). Moderate caffeine (100 mg) was significantly associated with an increase in Firmicutes abundance (+3.4%, $p=0.049$), especially in the age group of 31-50 years. There were no significant differences among any dose or age group ($p>0.050$) for any of the other bacterial phyla, including levels of the hormone cortisol, adrenaline, Bacteroidetes, Actinobacteria, or Proteobacteria. At moderate doses, there was a non-significant tendency to an increase in alpha diversity ($p=0.093$), and in older adults, high doses had little effect on the diversity of microorganisms. (A) Mean cortisol levels (µg/mL) across three caffeine dosage groups (50 mg, 100 mg, 200 mg), showing a dose-dependent variation with higher standard deviations at lower doses. (B) Mean adrenaline levels (pg/mL) in the same groups, indicating a slight increase at higher caffeine doses, with overlapping error margins. Error bars represent standard deviation (Figure 1).

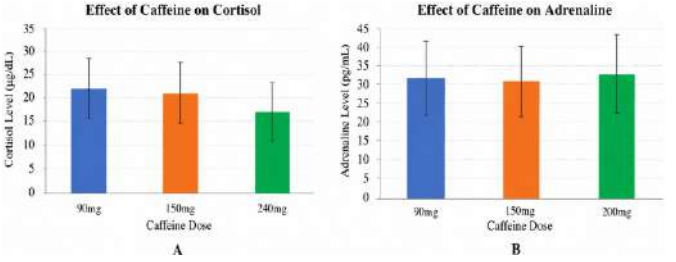

Figure 1: Biochemical Response to Varying Doses of Caffeine

(A) Bacteroides abundance showed a modest increase from 50 mg to 100 mg caffeine, stabilizing at 200 mg; (B) Firmicutes levels increased progressively with caffeine dose, with the highest abundance at 200 mg; (C) Actinobacteria levels remained relatively stable across all doses, with minor variation and larger error margins; (D) Proteobacteria abundance decreased slightly at 100 mg but returned to baseline levels at 200 mg. All data represent mean relative abundance (%) ± standard deviation (Figure 2).

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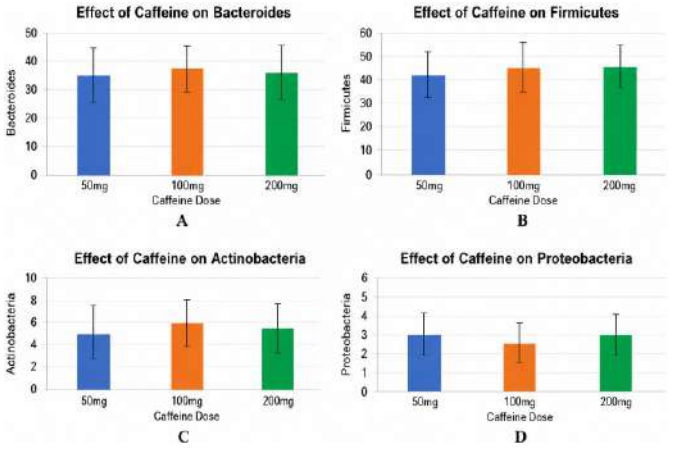

Figure 2: Effect of Caffeine Dosage on Gut Microbial Composition

DISCUSSION

The present study aimed to assess the effects of various doses of caffeine on cognitive function, physiological parameters, and gut microbiota composition in different age groups. While no statistically significant cognitive improvements were observed across any dose or age group, non-significant trends toward improved reaction time and alertness were noted at the 100 mg and 200 mg doses, particularly among younger adults (18-30 years). The Although the 200-mg dose showed a non-significant trend toward improved reaction time in younger adults (p=0.083), the effect size (Cohen's d = 0.46) falls within the small-to-moderate range. Given that the 95% confidence interval crosses zero (0.20 to 0.72), this finding should be interpreted cautiously as a signal for future research, not as evidence of practical relevance. An overall decrease in reaction time, alertness, or cognitive task performance was not found; however, trends towards improvement were found at 100mg and 200mg, particularly among younger adults (18-30 years). There are moderate effect sizes (Cohen's d = 0.46) for this subgroup, which might have practical significance, but these results are hypothesis-generating, not confirmatory. Currently, there is no strong evidence from this study to support these mechanistic explanations. Physiologically, the current study found that cortisol was significantly decreased after a high dose of caffeine (200 mg), but this did not occur with adrenaline. The current results contradict previous reports linking caffeine to higher HPA axis activity [12, 14]. A delayed suppression of the feedback or variation in stress hormone regulation with age may be one of the potential explanations [17-19]. The effects of caffeine on cortisol were seen to be greater in younger adults, suggesting that there may be age-related differences in metabolic response to caffeine. Microbiome analysis found significantly more Firmicutes with moderate caffeine consumption (100 mg) and a non-significant trend to increased microbial diversity. The middle-aged group (31–50 years) showed the greatest changes. However, high doses of caffeine (200mg) were linked to a reduction in alpha diversity in the older adults, indicating that caffeine could have a threshold for disrupting gut microbiota. These results corroborate the growing body of literature that explores the gut-brain axis and the effect of diet on microbiota [12, 20] but require further investigation with larger sample sizes and metagenomic characterization. Overall, our results are consistent with the idea that moderate consumption of caffeine can have selective cognitive and microbial benefits, particularly in younger and middle-aged adults. But high amounts of caffeine may have negative effects on gut health and may not necessarily improve cognition in proportion. Moderate effect sizes for some of these outcomes indicate that the results are important but should not only be judged as statistically significant, but also from a practical.

The magnitude of the effects observed in younger adults is suggestive of practical importance but needs to be confirmed by larger and better-powered studies. With the small number of cases involved and the limited scope and significance of the results, clinical or personalized recommendations for caffeine use should be made with some caution. Future studies with more participants, extended intervention periods, and functional metagenomic analysis are required to confirm the clinical relevance of microbial changes induced by caffeine, especially in older adults and those with baseline gut dysbiosis.

CONCLUSIONS

This study provides evidence of dose-dependent effects in both hormonal regulation and gut microbiota, showing potentially beneficial effects of moderate caffeine intake on the modulation of gut microbiota, specifically in Firmicutes abundance, but with limited evidence for cognitive outcomes.

Authors' Contribution

Conceptualization: SH
Methodology: SH, FN, AM, MFS, MI
Formal analysis: SH, UR, MI
Writing and Drafting: SH, FN, AM, UR, MFS, MI
Review and Editing: SH, FN, AM, UR, MFS, MI

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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