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ORIGINAL PAPER

INFLUENCE OF GENDER ON THE PLASMA CONCENTRATION OF CAFFEINE AND ITS METABOLITES AFTER BODY WEIGHT-DEPENDENT DOSING

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Summary

Background. Caffeine is a widely consumed central nervous system stimulant, but its optimal dosing remains uncertain. This study examines gender-specific plasma caffeine and metabolite levels following weight-adjusted dosing.

Material and methods. The study included 38 women and 19 men (age 26.2±3.2; BMI 23.9±4.5). Participants received 6 mg of caffeine per kilogram of body weight, resulting in mean absolute doses of 398.8±91.9 mg for women and 485.8±86.2 mg for men. Blood samples were collected 60 minutes post-caffeine ingestion, and plasma concentrations of caffeine and its metabolites were analyzed using high-performance liquid chromatography.

Results. A Mann-Whitney U test revealed statistically significant differences in caffeine dosage between the study groups (U=144, $p<0.001$, r=0.49). The analysis of the caffeine concentration variable showed statistically significant differences between the study groups (U=244.5, $p=0.049$, r=0.26).

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Conclusions. Although women received significantly smaller total doses of caffeine, they exhibited significantly higher plasma concentrations of caffeine than men. No significant differences were observed in the concentrations of paraxanthine or theobromine, while theophylline was undetectable in any of the samples. These findings underscore gender-specific differences in caffeine metabolism and highlight the potential need for tailored caffeine dosing based on gender.

Keywords: paraxanthine, theophylline, theobromine, optimization, gender differences

Introduction

Caffeine is widely recognized as an effective psychostimulant with minimal adverse effects; however, improper dosing has been associated with side effects and health risks [1]. Despite a per capita consumption of only 4.7 kg, ranking the United States. 66th globally in coffee consumption (World Population Review, 2024), survey data highlights the substantial prevalence of caffeine intake in the United States. Approximately 75% of adults report daily coffee consumption, over 90% consume coffee at least once weekly, and 36% report drinking three to five cups per day [2]. A single cup of coffee contains between 51 mg and 322 mg of caffeine [3]. With a daily consumption of three to five cups, the daily consumption translates to an estimated caffeine intake of 153 mg to 1610 mg from coffee alone. Beyond coffee, caffeine is also consumed through energy drinks, medications, fat-burning and sports supplements, as well as other plant-based products. For instance, a typical serving of black tea contains approximately 40-45 mg of caffeine, while green tea provides around 25-35 mg. Cocoa-based products, such as chocolate, contain lower amounts, ranging from 5 to 35 mg per serving depending on the cocoa content. Guarana seeds are particularly rich in caffeine, containing between 3.6% and 5.8% by weight, significantly more than coffee beans. Kola nuts,

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traditionally used in the production of soft drinks, contain approximately 1.5-2.5% caffeine. Among commercial beverages, energy drinks typically contain about 32 mg of caffeine per 100 ml, whereas colas and other caffeinated soft drinks provide approximately 10-15 mg per 100 ml [4]. The widespread consumption of caffeine-rich products increases the risk of overdose, particularly among heavy energy drink and coffee consumers, athletes and users of fat-burning supplements [5]. The Food and Drug Administration (FDA) requires that the caffeine concentration in packaged beverages not exceed 20 mg per serving. However, powdered caffeine, often marketed as a dietary supplement for its fat-burning and ergogenic properties, is not subject to the same regulations. Given that daily caffeine consumption of 2000 mg can pose significant health risks and that the lethal dose is between 5 and 10 g per day, there is an urgent need to understand the factors that influence caffeine metabolism, especially as global caffeine consumption continues to rise in response to the increasing pace of life and near-constant demand for quick bursts of energy, as well as the consumption of caffeine-containing pharmaceuticals, fat-burning drugs and pre-workout supplements [6].

The primary mechanism of action of caffeine is its antagonistic effect on A1 and A2 adenosine receptors [7]. Caffeine binding to adenosine receptors stimulates the sympathetic nervous system, reducing fatigue and increasing concentration [8]. At low doses of CAF, the fundamental mode of action in the central nervous system is binding to adenosine receptors. At higher doses of caffeine, however, many other molecular targets can also play an important role [9]. After ingestion, caffeine readily crosses biological membranes, and maximum plasma concentrations are reached after about 45 to 60 minutes [10], although studies suggest that peak plasma caffeine levels are reached after about 30 minutes [11]. The elimination half-life of caffeine in the blood usually is 2 to 6 hours [12] and is generally shorter in cigarette smokers and longer at higher caffeine doses or in people with liver metabolism disorders [13]. More than 90% of caffeine is metabolized in the liver, although small amounts can also be

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metabolized in the brain, skeletal and cardiac muscle and fatty tissue. Caffeine is mainly metabolized by the enzyme CYP1A2 and leads to three main metabolites: paraxanthine (about 80% of total caffeine metabolism in the liver), theophylline (16%) and theobromine (4%) [14]. Caffeine clearance typically occurs at approximately 2 mg per kg per minute. However, this clearance rate decreases significantly at higher doses due to the saturable biotransformation process of paraxanthine and its subsequent reduced excretion [12].

The European Food Safety Authority (EFSA) and FDA state that up to 400 mg of caffeine per day, which they estimate is equivalent to about four to five cups of coffee, is safe for healthy adults without caffeine hypersensitivity [15]. However, these recommendations do not account for body weight. Due to caffeine's ability to readily cross biological membranes, individuals with lower body weight will likely exhibit higher concentrations of caffeine and its metabolites when consuming the absolute dose of 400 mg per day recommended by the EFSA or FDA. Women generally have a lower body weight than men, so they are more susceptible to higher concentrations of circulating caffeine at the same caffeine intake [16,17]. Since the concentrations of caffeine and its metabolites are directly linked to its effects, this discrepancy may result in positive and negative outcomes, particularly as reports indicate that these recommended doses are often exceeded by both sexes [18].

A more effective approach to caffeine dosing considers body weight, a method commonly applied in supplementation [1,5]. Recommended relative doses are categorized as low (~3 mg/kg body weight), moderate (5-6 mg/kg) and high (~9 mg/kg), primarily based on studies involving male athletes [9]. However, due to growing concerns over absolute dosing, weight-based dosing (mg/kg) is gaining popularity across all groups. Nevertheless, strategies derived from male athlete studies may not accurately reflect the needs or responses of women or non-athletes, given differences in overall metabolism, adaptation to ergogenic supplements, and body composition [9].

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Studies by Skinner [19] and Domaszewski [1,20] suggest that relative body fat percentage may influence caffeine metabolism and its ergogenic or adverse effects. It suggested the hypothesis that caffeine and its metabolites may differ between obese and non-obese individuals. Given that women typically have a higher relative fat mass than men, this could also contribute to gender differences in caffeine metabolism. Surprisingly, the FDA and EFSA caffeine dosing recommendations do not account for any additional factors affecting caffeine metabolism [1].

Aim of the work

This study investigates how gender influences caffeine metabolism 60 minutes after consuming 6 mg per kg of body weight. The authors hypothesized that gender significantly affects plasma concentrations of caffeine and its metabolites. Confirmation of these hypotheses could lead to more precise caffeine dosing strategies, enhancing the consistency of its optimal ergogenic effects and reducing caffeine-induced health issues.

Material and methods

Study participants

68 participants were initially included in the study, but 9 did not fulfil the inclusion criteria, and 2 withdrew. The final group included 57 participants: 19 men (mean age 26.2±3.2 years, weight 81.3±14.4 kg, Body Mass Index (BMI) 24.1±3.5) and 38 women (mean age 25.1±2.7 years, weight 66.5±15.3 kg, BMI 23.8±5.0). The characteristics of the groups are shown in Table 1, and the flow diagram of the study design is presented in Figure 1.

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Table 1. Characteristics of the participants

Characteristics	Men (n=19) (X±SD)	Women (n=38) (X±SD)
Age [years]	26.2±3.2	25.1±2.7
Height [cm]	182.0±5.1	166.8±6.4
Weight [kg]	81.0±14.4	66.5±15.3
BMI [kg/m^2]	24.1±3.5	23.8±5.0

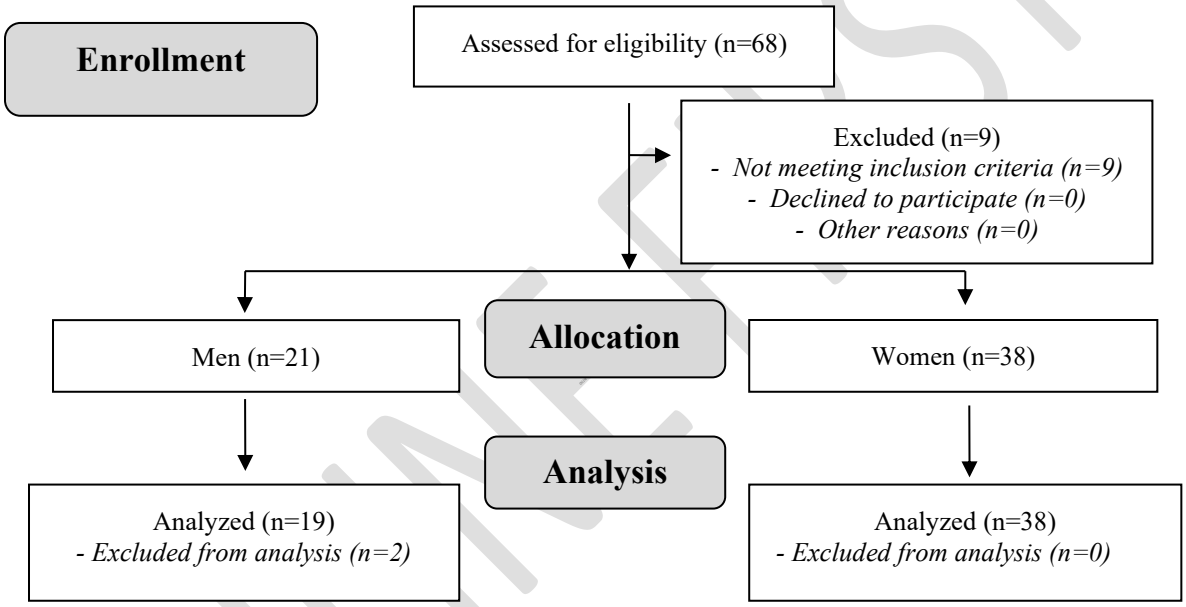

Figure 1. Flow diagram of the study design

Inclusion criteria

The participants had to meet the following requirements: a) provided informed consent and agreed to comply with all study guidelines; b) no known hypersensitivity to caffeine, demonstrated by tolerance to coffee, caffeine supplements or energy drinks; c) young adults aged 18 to 31 years; d) non-smokers; e) not reliant on electronic life-support systems (e.g.

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pacemakers or active prosthetic devices); f) not taking any medications that could interfere with caffeine metabolism (e.g. beta-blockers, theophylline, oral contraceptives); g) in generally good health with no medical contraindications (e.g. cardiovascular disease, uncontrolled hypertension); h) adhered to dietary restrictions 48 hours prior to the study, including avoiding caffeine, alcohol and cruciferous vegetables; i) not pregnant or breastfeeding; j) not suffering from chronic conditions that could impact caffeine metabolism or affect study outcomes (e.g. diabetes, liver or kidney disease).

Experiment design

Both groups received 6 mg of caffeine per kg of body weight, administered in transparent cellulose capsules and with water. As caffeine is usually consumed in the morning and early afternoon, all measurements were taken between 7:00 and 12:00. Participants were provided with written instructions to abstain from caffeine-containing products, including coffee, tea, soda-type beverages, energy drinks and medications containing caffeine, for at least 48 hours prior to the study. They were also instructed to avoid any medications that could interfere with caffeine metabolism (e.g. beta-blockers, hormonal contraceptives and others). Additionally, participants were asked to refrain from intense physical activity during the 48 hours prior to testing and to consume a light, easily digestible meal no later than 2 hours before the experiment. Blood samples were collected in 1.3 mL plasma tubes and allowed to clot. Subsequently, the samples were centrifuged at 5500 × g and 4°C for 10 minutes. The resulting plasma was carefully extracted and aliquoted into 0.6 mL storage tubes. Each plasma sample was then frozen at −80°C until further analysis.

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Chemicals

The chemicals used in the study were caffeine (CAF), theobromine (TB), theophylline (TP), paraxanthine (PX) and caffeine – d9 (internal standard, IS). Additional reagents included acetonitrile, methanol, water and formic acid of LS-MS grade, all purchased from Merck (Poznań, Poland).

LC-MS/MS analytical procedure

The analyses were conducted using a liquid chromatography system connected to a microOTOF-Q II™ mass spectrometer (Bruker Daltonics, Germany). Separation was achieved with an Acquity UPLC® C18 column (2.1 mm × 50 mm, 1.7 μm particle size) from Waters Corporation (Ireland), maintained at 30ºC with a low rate of 0.3 mL/min. The mobile phase consisted of ultrapure water with 0.2% formic acid (A) and methanol (B) in a 60:40 ratio, running isocratically. The total run time was 6 minutes. The instrumental parameters were capillary voltage: 4.5 kV, nebulizer gas flow (N_2): 1.2 bar; desolvation line temperature: 300°C; drying gas flow (N_2): 8 L/min; and collision-induced dissociation gas pressure (Ar): 230 kPa. The analyses were carried out in multiple reaction monitoring (MRM) mode. The mass spectrometer monitored the following transition: CAF (m/z 195.1 → 138.0, RT 4.5 min), CAF-d9 (m/z 195.1 → 138.0, RT 4.5 min), CAF-d9 (m/z 204.14 → 144.1, RT 4.4 min), PX (181.1 → 124.1, RT 3.6 min), TP (181.1 → 123.9, RT 3.6 min) and TB (181.01 → 138.0, RT 3.1 min). Data processing was performed using DataAnalysis Version 4.0 SP 5 software (Bruker Daltonics).

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Method validation

Stock solutions of CAF, PX, TB and TP were individually prepared at 100 ug/ml in a $1{:}1_{v/v}$ mixture of methanol and water and stored at 4°C. The internal standard (IS) stock solution, with 1 mg/ml concentration, was prepared in methanol. Calibration standards were prepared by spiking 50 µl of blank plasma with appropriate volumes of the working solutions, resulting in six calibration points for each analyte. Two calibration curves were developed for caffeine, one covering the 0.1-10 µg/ml range and another for 10-25 µg/ml. The calibration range for PX, TB and TP was 0.1-7 µg/ml. Selectivity was verified by comparing chromatograms of blank serum to confirm no interfering peaks at the retention times for CAF, PX, TB and TP. Linearity was evaluated by plotting the analyte/IS peak area ratios against their corresponding concentrations using a weighted (1/x2) least-squares regression. Precision was assessed and reported as the relative standard deviation (RSD). For analysis, frozen plasma samples were thawed, and 50 µl of plasma was spiked with 200 µl of CAF-d9 internal standard solution. The mixture was vortexed and centrifuged at 13000 rpm for 5 min, and the resulting supernatant was transferred to a sample vial for LC-MS analysis.

Statistical analysis

The parameters of the caffeine variables were statistically analyzed as follows:

- first, the Shapiro-Wilk test was performed to assess the normality of the data;
- a Mann-Whitney U test for independent samples was then performed to analyze the differences between the groups. Data analysis was performed using Jamovi 2.4.14;

– the effect size of the analyzed variable was also calculated using the GPower program. The sample size of 57 participants in 2 groups was considered sufficiently sensitive enough to detect effect size d=0.86 power 80% and a 5% (two-sided) significance level.

Results

The concentrations of caffeine, paraxanthine, theobromine and theophylline in the study group, measured 60 minutes after ingesting 6 mg/kg of caffeine, are summarized in Table 2.

Table 2. Descriptive statistics and statistical significance of differences in individual variables

Variable			Descriptive statistics		Test statistics		95% confidence interval		Effect size
Outcome	**Predictor**	**Group**	**X±SD**	**Median**	**U**	***p***	**Lower limit**	**Upper limit**	**r**
CAF dosage [mg]	**Gender**	**Women**	398.8±91.9	383	144	<0.001	51	126	0.6
		Men	485.8±86.2	463					
CAF concentration [µg/ml]	**Gender**	**Women**	7.87±5.43	7.31	244.5	0.049	-5.99	0	0.32
		Men	4.89±4.35	3.54					
PX concentration [µg/ml]	**Gender**	**Women**	1.73±1.38	1.44	306.5	0.361	-0.84	0.34	0.15
		Men	1.41±1.17	1.15					
TB concentration [µg/ml]	**Gender**	**Women**	0.45±0.45	0.31	357.5	0.959	-0.17	0.16	0.01
		Men	0.46±0.43	0.31					

Notes: CAF – caffeine, PX – paraxanthine, TB – theobromine.

Female participants exhibited significantly higher caffeine concentration levels (Mdn=7.31) than male participants (Mdn=3.54) despite the inverse proportionality in total caffeine dosage due to gender differences in body weight. The Mann-Whitney U test indicated this difference was statistically significant (U=244.5, p=0.049, r=0.32). However, no

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statistically significant differences were observed between groups for the PX and TB variables. Additionally, theophylline was not detected in either group.

Discussion

The findings of this study demonstrate that, despite women receiving significantly lower total doses of caffeine than men, they exhibited higher plasma caffeine concentrations. No significant differences were observed in the plasma concentrations of paraxanthine or theobromine; theophylline was undetectable in all samples. These results underscore gender-specific variations in caffeine metabolism and raise concerns regarding the appropriateness of current caffeine dosing strategies.

Gender-specific differences in caffeine metabolism have significant public health implications, particularly given the pervasive consumption of caffeine through beverages, supplements and medications. The prevailing one-size-fits-all guidelines—such as the FDA's recommendation of 400 mg/day, appear to inadequately address individual variability, remarkably variability related to body weight. Although weight-based dosing (e.g. 3 or 6 mg/kg) provides a more individualized approach, our study suggests that failing to account for gender may undermine the effectiveness of these strategies, leading to significantly higher plasma caffeine concentrations in women. Elevated plasma caffeine levels may increase the risk of adverse effects, including anxiety, gastrointestinal discomfort and cardiovascular complications [1]. While these increased caffeine concentrations do not appear to reduce the performance-enhancing benefits of caffeine, they may directly contribute to the onset of caffeine-induced side effects [19]. Despite both genders experiencing comparable ergogenic benefits, such as enhanced endurance and cognitive performance, women are more prone to reporting adverse effects at equivalent caffeine doses compared to men [21].

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Although recent studies suggest that gender may not significantly influence the enzymatic pathways involved in caffeine metabolism or the plasma concentrations of caffeine and its metabolites [22], the results of our study highlight the importance of considering gender-specific responses when designing caffeine dosing regimens. Pharmacokinetics and resultant plasma concentrations can differ substantially between men and women, even at equivalent doses relative to body weight. Furthermore, our findings underscore the need for further research to deepen our understanding of gender-based metabolic differences and their implications for clinical practice.

The conflicting reports regarding gender-specific differences in the effects of caffeine and its metabolism can be attributed to several factors. First, women generally have lower body weight and blood volume than men, which can result in misleading conclusions when comparing caffeine responses between sexes—mainly when doses are administered in absolute amounts without adjusting body weight (e.g. 200 mg per dose) [16]. Second, hormonal fluctuations in women, particularly those associated with the menstrual cycle, pregnancy and contraceptive use, may influence caffeine metabolism [23]. However, the influence of hormones on caffeine metabolism is not yet fully understood, with limited characterization and inconsistent findings across existing studies [24]. Third, women typically have a higher relative fat mass than men [25], and some studies suggest that the greater ratio of adipose tissue to lean body mass may influence caffeine's effects and contribute to elevated plasma caffeine concentrations [19,25].

Additionally, gender-specific differences in the regulation of the sympathetic nervous system may contribute to variability in caffeine responses, although these differences are more related to physiological effects than serum caffeine levels or metabolite concentrations. Research indicates that women exhibit lower sympatho-adrenal activation and more significant inhibitory responses than men. Furthermore, women demonstrate higher baroreflex sensitivity,

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enabling more efficient blood pressure regulation [19]. These factors may influence the body's response to caffeine's stimulatory effects, offering valuable insights into the observed gender differences in caffeine-induced outcomes [26].

Genetic polymorphisms in the CYP1A2 and ADORA2A genes involved in caffeine metabolism do not exhibit significant gender-based differences [27]. However, these polymorphisms may still affect the overall metabolism and physiological responses to caffeine. It is important to note that relatively few studies have specifically investigated the dynamics of plasma caffeine levels and its metabolites. Most existing research has concentrated on caffeine's physiological effects, often utilizing diverse dosing strategies rather than directly measuring plasma concentrations of caffeine and its metabolites [13,22,28].

In our study, we did not observe statistically significant gender differences in paraxanthine and theobromine levels, likely due to the timing of blood sample collection. The 60-minute post-ingestion sampling interval probably captured the initial distribution phase of caffeine metabolism rather than the peak metabolic activity of these secondary metabolites. The absence of detectable theophylline levels across all groups can similarly be attributed to the short sampling interval following caffeine administration [11,12]. Our findings are consistent with those of Petrovic et al., who reported no significant differences in plasma and urinary concentrations of caffeine and its metabolites, including paraxanthine, theobromine and theophylline, between the sexes [29].

To the best of the authors' knowledge, this is the first study to investigate the impact of gender on plasma concentrations of caffeine, paraxanthine, theobromine and theophylline following administration of a 6 mg/kg body weight dose. Our findings demonstrate that when caffeine is dosed based on body weight, gender significantly influences plasma caffeine concentrations, potentially leading to disproportionately high levels in women. Women are more susceptible to physiological responses to caffeine, so the higher plasma concentrations

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observed in this group may increase their risk of caffeine-induced adverse effects. In light of the rising number of reported caffeine overdose cases, some of which have resulted in severe health complications and fatalities [30,31], there is an urgent need to reevaluate current caffeine dosing strategies. Updated recommendations from food and drug regulatory authorities are essential to ensure that dosing guidelines are safe and effective for all populations. As global caffeine consumption continues to increase, integrating these findings into public health policies and personalized medicine frameworks will be crucial to optimizing its use while minimizing adverse outcomes. Future research can further refine our understanding of caffeine metabolism and its implications across diverse populations by addressing the challenges and gaps identified. The findings suggest a shift from absolute dosing strategies, or milligrams per kilogram of body weight, toward more individualized approaches. This approach aligns with the emerging trends in precision medicine, which emphasize tailoring interventions to the unique characteristics of individuals. Optimized recommendations should aim to reduce caffeine doses for women to align with their unique pharmacokinetic profiles [28,30].

Limitations of the study

This study has several limitations that should be acknowledged. The relatively small sample size and the lack of genotyping to account for individual metabolic variability represent key constraints. Additionally, hormonal fluctuations may influence caffeine metabolism and warrant further investigation as potential confounding factors. The absence of a placebo group is another limitation; however, based on pilot testing and a thorough review of literature, we determined that a 48-hour caffeine withdrawal period is sufficient to render caffeine and its metabolites undetectable in the blood of non-sensitive individuals when using HPLC methods, thereby minimizing the necessity of a placebo control. Moreover, the study focused exclusively

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on young adults, limiting the generalizability of findings to broader age groups. Although participants adhered to pre-study restrictions, detailed dietary records were not collected, which could introduce residual confounding. Nonetheless, this research constitutes one of the most comprehensive studies to date on body weight-adjusted caffeine dosing and its gender-specific metabolic effects, addressing an important gap in existing literature.

Conclusions

Although women received significantly smaller total doses of caffeine than men, they exhibited significantly higher plasma concentrations of caffeine. No significant differences were observed in the concentrations of paraxanthine or theobromine, while theophylline was undetectable in any of the samples. These findings underscore gender-specific differences in caffeine metabolism and highlight the potential need for tailored caffeine dosing based on gender. Further research on caffeine dosing strategies for body composition and gender is needed to support the development of updated recommendations by food and drug authorities.

Disclosures and acknowledgements

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All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Declaration of Helsinki and its later amendments. The study received approval

from the Bioethics Committee of the Poznan University of Medical Sciences (Approval No. 108/22) prior to participant recruitment. All participants were thoroughly informed, both verbally and in writing, about the nature and objectives of the study, the methods of data collection (including the handling of sensitive personal information), the potential risks involved and the intended use of the collected data. Written informed consent was obtained from each participant before the start of any procedure. Biological sample collection was performed by qualified medical staff at a certified clinical facility, ensuring appropriate standards of professional competence and biosafety. The study was registered in The Australian New Zealand Clinical Trials Registry (No. 12622000823774) as part of a larger research project on caffeine metabolism.

Artificial intelligence (AI) was not used in the creation of the manuscript.

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