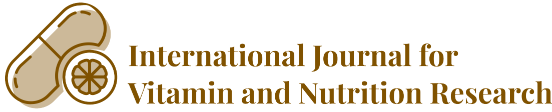

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Original Communication

Short-Term Turkish Coffee Consumption Elevates Cardiovascular Risk Markers, Decreases Leptin Levels, and Impairs Sleep Quality in Healthy Young Women: A Pilot Randomized Controlled Trial

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Abstract

Background: Unfiltered Turkish coffee (UTC) is a traditional drink with high levels of bioactive compounds, but evidence of the associated specific physiological effects is inconclusive, and few studies have examined coffee in general. This pilot study aimed to investigate the short-term effects of daily UTC consumption on cardiovascular parameters, lipid profile, appetite-regulating hormones (leptin, ghrelin), glucose metabolism, inflammatory markers, and sleep quality in healthy young women. **Methods**: This is a pilot randomized controlled trial that randomly assigned 40 healthy young women (aged 18–25 years) to intervention and control groups at a 1:1 ratio after 3 weeks of caffeine washout. The intervention group consumed three 40 mL cups of traditional-brewed UTC daily for 4 weeks, whereas the controls maintained abstinence from caffeine. The primary outcomes were cardiovascular (blood pressure, heart rate), lipid parameters, and the secondary ones were appetite hormones (leptin, ghrelin), glucose metabolism (markers), inflammatory biomarkers, and sleep quality, which were evaluated at baseline and at week 4. **Results**: UTC consumption produced significant between-group differences (time × group interactions) compared to controls: systolic blood pressure (+3.0 mmHg; p = 0.025), heart rate (+10.6 bpm; p = 0.007), and insomnia severity scores (+4.05 points intervention vs. –1.00 points control; $p \leq 0.001$), while significantly decreasing leptin levels (–0.04 ng/mL; p = 0.014). Significant changes in low-density lipoprotein (LDL) cholesterol were found (p = 0.002), although high-density lipoprotein (HDL) changes were no longer found significant on baseline correction (p = 0.385). Body composition parameters (body mass index (BMI), body fat mass, fat-free mass, skeletal muscle mass) remained unchanged throughout the intervention (all $p > 0.05$). No significant effects were observed for fasting blood glucose, glycated hemoglobin (HbA1c), inflammatory markers (C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α)), or ghrelin (all $p > 0.05$). **Conclusions**: Four weeks of UTC intake in this pilot trial were associated with variations in several cardiometabolic variables: interventions in systolic blood pressure (SBP) (+3.0 mmHg) and heart rate (HR) (+11.9 bpm) resulted in higher LDL levels, reduced leptin levels, and poor sleep quality, independent of body composition alterations. However, since the p-values are nominal and not multiplied by a correction, hypothesis-generating results require verification through properly powered studies. These exploratory findings should be considered by individuals with prior cardiovascular risk factors or sleep disorders when considering the intake of unfiltered coffee. **Trial registration**: This trial was registered at ClinicalTrials.gov (NCT07133373, https://clinicaltrials.gov/study/NCT07133373). Retrospectively registered on 13 August 2025.

Keywords: unfiltered Turkish coffee (UTC); female; caffeine; cardiovascular health; inflammation; insomnia; appetite

1. Introduction

Coffee is also among the most popular beverages in the world that has approximately 2.25 billion cups of coffee being taken by people of various cultures like nationalities [1,2]. Besides the social worth, coffee is an important source of food bioactive molecules like caffeine, chlorogenic acids, diterpenes and polyphenols with metabolic, cardiovascular and neurological effects [3]. Unfiltered Turkish coffee (UTC) has recently gained a new following, especially with younger generations in Middle Eastern, Mediterranean, and urban academia, among the classic techniques of brewing coffee [4–6]. In regions such as Turkey, Cyprus, and much of the Middle East, UTC remains deeply embedded in cultural traditions: finely ground coffee is boiled in a cezve without filtration, creating a beverage consumed with suspended grounds [7]. This unfiltered method retains substantially higher concentrations of certain bioactive compounds, especially the diterpenes and kahweol that are mostly eliminated by filtration using paper in other brewing processes [8]. The quantitative variations in the bioactive compounds lead to different physiological responses of cardiovascular and metabolic health to filtered coffee [8,9]. Coffee has bioactive compounds that are present in all the brewing processes and therefore have complicated and even contradictory health effects [10]. Having been found in all types of coffee, chlorogenic acids slow down the absorption of glucose in the intestine and improve insulin sensitivity by regulating the glucose-6-phosphatase activity [11], which can help people with glycemic regulation [10,12]. These polyphenolic compounds have comprehensive antioxidant and metabolic advantages irrespective

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of the means of preparation [10,12]. The primary distinction between unfiltered and filtered coffee is the existence of diterpenes: cafestol and kahweol are found 4–6 times more in UTC than in the filtered ones due to the absence of paper filtration [8,13]. Although these diterpenes have antioxidant and hepatoprotective effects [13] they are also linked with high levels of low-density lipoprotein (LDL) cholesterol as a result of hepatic cholesterol metabolism alteration which are possible cardiovascular risk factors at the higher concentrations obtained in unfiltered coffee [14,15]. It has been proved in human trials that consumption of unfiltered coffee can increase total cholesterol and homocysteine levels, which are cardiovascular disease risk factors [16] but the actual effect of Turkish coffee (TC) has been inconsistently demonstrated [17]. Certain reports have found an increase in homocysteine and poor lipid modifications, whereas others have found a decrease in endothelial functioning and decrease in arterial rigidity, with results varying depending on the mode of preparation, dosage, timing, and inter-individual variation [17]. Extensive literature indicates moderate levels of coffee are linked to reduced risk of cardiovascular disease, but these effects seem to be nonlinear in nature and depend upon timing, amount, and genetic polymorphisms to process caffeine, specifically the Cytochrome P450 Family 1 Subfamily A Member 2 (CYP1A2) polymorphs [18,19]. Besides the cardiovascular effect, the caffeine in TC can control leptin and ghrelin hormones, which form the centre of the counter of appetite regulation. Caffeine suppresses the appetite and increases the energy expenditure by activation of the sympathetic nervous system [18,19]. However, most of the investigations have involved overall coffee preparation or individual caffeine, and little is known regarding TC, particularly among women [19]. Additionally, caffeine as an adenosine receptor antagonist, may disrupt sleep architecture by delaying the onset and reducing total sleep duration [20,21]. Timing seems to be the key: caffeine in the evening has a significant negative effect on the quality of sleep [21], but in the morning, it might produce the least disruptive effect and some cardiovascular effect due to circadian regulation [22].

Special attention is justified by these effects in young women, as they have different caffeine pharmacokinetics, in which the enzymatic activity of CYP1A2 is slower, with the result that caffeine half-life is prolonged, and there is prolonged physiological exposure [23,24]. The menstrual cycle hormonal changes further modify the caffeine metabolism and recent evidence suggests that women may have a greater response to the identical dose of caffeine in terms of the cardiovascular effect [23,24]. In spite of the fact that women constitute the majority of UTC consumers in Middle Eastern societies [25], in the intervention trials, it is mostly the male or mixed-gender cohort that is enrolled without sex-stratified analyses, which is a knowledge gap. The newer consumer trends in Jordan suggest that cultural biases were high towards medium dark roasts blends that are made in the traditional Turkish fashion with the average 40 mL portions having around 6–7 g of coffee [25], which underscores the need to determine the health outcomes of the practice using culturally representative preparation techniques in populations where UTC is regularly used.

Nevertheless, the absence of strict scientific studies examining its specific physiological effect on the human body despite the cultural significance and the fact that it has popularized among people is rather surprising. Most coffee research extrapolates throughout the brewing procedure without taking into account the fact that bioactive substances, particularly diterpenes, are of high concentration and represent the characteristics of unfiltered preparations. Chlorogenic acids and caffeine are common in any kind of coffee [10,12], UTC's unfiltered method results in diterpene concentrations (cafestol, kahweol) 4–6 times higher than filtered coffee [8,14], fundamentally altering the balance of cardiovascular and metabolic effects [8,9]. With the increased attention to conventional preparation methods among young adults who care about their health, specific studies are needed to provide evidence-based advice. Although comparative studies on brewing techniques would provide useful mechanistic data, developing a baseline understanding of the physiological action of UTC versus caffeine withdrawal is an essential preliminary measure, as there are virtually no controlled studies directly examining UTC. It is here that coffee research methodology has established a precedent [5,7].

Therefore, this pilot randomized controlled trial investigated the short-term effects of four consecutive weeks of standardized daily UTC consumption on health-related parameters in healthy young women. Cardiovascular parameters systolic blood pressure (SBP), diastolic blood pressure (DBP), resting heart rate (HR), and lipid profile [high-density lipoprotein (HDL), LDL cholesterol, total cholesterol, triglycerides]) were assessed as primary outcomes. Secondary outcomes included appetite-regulating hormones (leptin, ghrelin), glucose metabolism markers (fasting blood glucose, glycated hemoglobin (HbA1c)), inflammatory biomarkers (C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α)), and sleep quality indicators. We hypothesized that four weeks of UTC consumption would produce: (1) elevated cardiovascular parameters (increase SBP, DBP, HR); (2) unfavorable lipid alterations (increased LDL, total cholesterol, triglycerides; decreased HDL); (3) reduced leptin and elevated ghrelin concentrations; (4) potential changes in glucose metabolism or inflammatory markers; and (5) impaired sleep quality compared to caffeine abstinence. This gendered study focuses on the fact that women have been underrepresented in coffee studies and assesses those physiological parameters previously unmeasured which are particular to traditional UTC preparation, useful in understanding the impact of the unfiltered caffeine sources on cardiovascular activ-

ity, metabolic regulation, hormonal regulation, and sleep in the main group of consumers.

2. Materials and Methods

2.1 Study Design and Participants

This pilot randomized controlled trial examined the physiological effects of four-week UTC consumption in healthy young women aged 18–25 years. The study received ethical approval from the Institutional Review Board (IRB) at the University of Petra (UOP) under protocol number E/H/5/10/2024. The trial was registered post factum by the Open Science Framework (OSF, DOI: 10.17605/OSF.IO/2V96N; registered 13 May 2025) and ClinicalTrials.gov (NCT07133373; registered 13 August 2025) when the data collection had been finalized but the final manuscript was being prepared. Transparency statement: The registration was done retrospectively to comply with the existing reporting requirements, but the study protocol, primary outcomes (cardiovascular parameters and lipid profile), secondary outcomes, and the plan of statistical analysis had been pre-determined before the participants were recruited, and no changes were made after data inspection. This assertion agrees with the retrospective registration time and protocol integrity [26]. All study procedures were conducted in accordance with the Declaration of Helsinki ethical principles and adhered to CONSORT 2010 guidelines for transparent reporting of randomized controlled trials [27]. Primary outcomes were prespecified as cardiovascular parameters: SBP, DBP, HR, and lipid profile components (HDL, LDL, total cholesterol, triglycerides). Appetite- regulating hormones (leptin, ghrelin), glucose metabolism (fasting blood glucose, HbA1c), inflammatory (C-reactive protein (CRP), TNF-α), sleep (Severity Index of Insomnia), and body composition parameters were the secondary outcomes. All subjects signed informed consent following a thorough explanation of research processes, risks, and rights.

Fifty healthy young women were recruited from the University of Petra and surrounding Amman community (Jordan) via digital advertisements and social media. All participants were of Middle Eastern ethnicity, representing the primary UTC consumer demographic in this cultural region. Eligibility was assessed through structured face-to-face interviews collecting demographic and health data. Inclusion criteria: age 18–25 years, Body Mass Index (BMI) 18.5–24.9 kg/m^2, non-smokers, minimal caffeine intake ($\leq$1 cup/week). Female-only enrollment was implemented based on: (1) women comprising the primary UTC consumer demographic in Middle Eastern cultures [6,25]; and (2) addressing systematic underrepresentation of women in coffee intervention trials. The pharmacokinetic rationale for sex-specific responses is discussed in relation to our findings (Eren and Besler, 2019 [5]; Tabrizi e*t al*., 2019 [28]). Participants self-reported regular menstrual cycles during screening (irregular cycles excluded). Oral contraceptive use was not systematically documented. Menstrual cycle phase was not biochemically verified or used as a stratification variable, consistent with methodological approaches in prior coffee intervention trials [5,29,30]. Exclusion criteria included: chronic illnesses (diabetes, thyroid disorders, cardiovascular/renal conditions), medications or supplements affecting appetite/metabolism/sleep, therapeutic diets, or concurrent clinical trial enrollment [5,30]. To introduce a caffeine washout period of three weeks to regulate the residual caffeine effects and harmonize the baseline conditions [5,29,30]. Subjects were not allowed to use any products that had caffeine (coffee, tea, chocolate, soft drinks, energy drinks, supplements) and compliance was monitored by self-report logs (daily) and unscheduled follow-ups. Three participants were excluded for non-compliance with caffeine abstinence during washout. Following successful washout, 47 participants underwent stratified block randomization (by BMI) into intervention (TC consumption) or control (continued caffeine abstinence) groups [29,30]. Prior to intervention commencement, seven participants withdrew voluntarily due to personal reasons or scheduling conflicts. Complete participant flow is detailed in Fig. 1 per CONSORT guidelines [27]. The randomization sequence was generated via Randomization.com and administered by an independent researcher to maintain allocation concealment and reduce selection bias [29].

2.2 Intervention Protocol and Coffee Samples

Following randomization, intervention participants consumed UTC daily for four weeks while control participants-maintained caffeine abstinence and usual dietary habits. Due to TC's distinct sensory characteristics (foam, suspended grounds, aroma, flavor), participant blinding was not feasible; this was an open-label trial. Biochemical analysis outcome assessors were also blinded to the group assignment and anthropometric measurements were automated to reduce group recognition at this point. All the participants were given the uniform guidelines in order to maintain constant dietary patterns during the research. A standardized batch of finely ground Brazilian Arabica coffee (medium-dark roast blend: 50% medium + 50% dark roast) was purchased from a single supplier. Each 10-gram serving (5 g medium + 5 g dark roast) represents typical Jordanian UTC preparation [25]. This roast combination balances chlorogenic acid preservation (medium roast) with diterpene availability (dark roast) [31–33]. Coffee was distributed to participants with standardized storage, preparation, and consumption instructions. UTC was prepared traditionally by steeping 5 grams finely ground coffee in 55 mL cold water in a Rakwa (traditional Turkish coffee pot), heated slowly over low heat to form surface foam. Final serving volume was 40 mL/cup per Jordanian consumption standards. This preparation method preserved bioactive compounds including chlorogenic acids

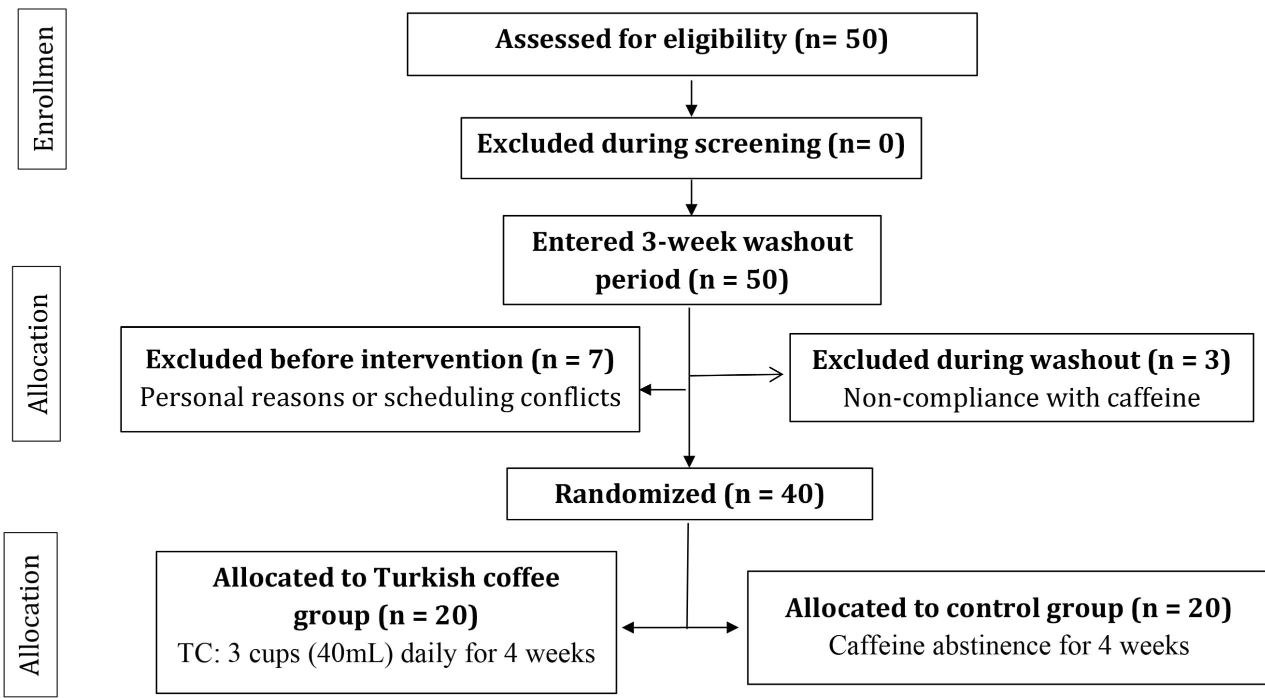

Fig. 1. CONSORT flow diagram showing participant recruitment, screening, randomization, and allocation. The diagram illustrates the complete participant flow through the study from initial recruitment (n = 50) through final analysis (n = 40). Following initial screening, 47 participants completed a mandatory three-week caffeine washout period. Three participants were excluded during washout due to non-compliance with caffeine abstinence requirements. The remaining 47 eligible participants underwent stratified block randomization (stratified by BMI) into intervention (Turkish coffee consumption) or control (caffeine abstinence) groups. Seven participants withdrew voluntarily before intervention commencement due to personal reasons or scheduling conflicts. The final analyzed cohort comprised 40 participants: 20 in the intervention group (receiving three 40 mL cups of standardized Turkish coffee daily for four weeks) and 20 in the control group (maintaining caffeine abstinence for four weeks). All 40 participants who commenced the intervention completed the four-week protocol and were included in the final analysis with no dropouts or loss to follow-up. Abbreviations: TC, Turkish coffee; BMI, body mass index.

and diterpenes. Participants consumed three 40-mL cups daily at standardized times: 7–9 AM, 12–2 PM, and 3–4 PM, with evening consumption prohibited to minimize sleep disruption [21]. Participants were monitored with regards to adherence to the protocols by daily logs and phone calls. UTC was consumed without any additives, except that habitual sugar users did not reduce their sugar amount. Coffee was standardized with pre-weighed utensils and preparation guidelines to follow. Caffeine content was quantified via high-performance liquid chromatography with diode array detection and mass spectrometry HPLC-DAD-MS (LCMS-2020 system with photodiode array detector, Shimadzu Corporation, Kyoto, Japan (at 1.77 mg/mL (71 mg/40-mL cup). Diterpenes (cafestol, kahweol) and chlorogenic acids were not directly measured in our UTC samples. Mechanistic discussions referencing these compounds rely on literature-derived estimates from chemically similar UTC preparations (Brazilian Arabica, medium-dark roast, unfiltered brewing) suggesting typical concentrations of cafestol 4.4–6.2 mg/cup, kahweol 3.2–4.8 mg/cup [31,32], and chlorogenic acids 70–115 mg/150 mL [33]. These estimates provide context for hypothesis-generating mechanistic interpretations but do not establish causation, and substantial batch-to-batch variability may exist. The lack of direct bioactive compound quantification represents a major limitation discussed below. Single 24-hour dietary recalls at baseline and post-intervention assessed dietary intake, with total macronutrient intake included as a covariate in statistical analyses to control for dietary confounding.

2.3 Blood Collection and Biomarker Assessment

Blood samples were collected at baseline and week 4 after $\geq$10-hour overnight fasting. Venous blood was drawn from the antecubital vein by a certified phlebotomist using standardized sterile protocols. Samples were immediately centrifuged (3000 rpm, 10 min, 4 °C) using a bench-top centrifuge (LC-8 Series, Benchmark Scientific, Amman, Jordan) to separate serum and plasma, then stored at –80 °C until analysis.

2.4 Metabolic and Glycemic Biomarkers

Fasting blood glucose was measured using the TRUE METRIX® Meter (TRUE METRIX, Trividia Health, Inc., Fort Lauderdale, FL, USA) (electrochemical biosensor technology; 0.5 µL sample volume). HbA1c was assessed via automated immunoassay analyzer (HP-AFS/1-PLUS, Shijiazhuang Hipro Biotechnology Co., Ltd., Shijiazhuang, Hebei, China) using integrated nephelometry, turbidimetry, and immunofluorescence methods.

2.5 Cardiovascular Risk Indicators and Inflammation Biomarkers

Total cholesterol, triglycerides, LDL, and HDL were analyzed using an automated immunoassay analyzer using nephelometry, turbidimetric immunoassay, and fluorescence immunoassay according to the manufacturer's instructions. The analyzer was calibrated to international reference standards with output in mmol/L, which is reported throughout this manuscript. Lipid measurements were performed by certified laboratory technicians following standardized protocols. Resting SBP, DBP, and HR were measured at baseline and week 4 using an automated upper-arm oscillometric monitor, M7 Intelli IT (HEM-7361T-EBK; Omron Healthcare Co., Ltd., Kyoto, Japan). Measurements were conducted between 8:00–10:00 AM in a quiet environment after $\geq$10-hour overnight fasting and $\geq$30-minute post-arrival rest. Participants sat with back supported, feet flat, and right arm at heart level. After 5-minute rest, three consecutive right-arm measurements at 1-minute intervals were averaged to minimize intra-individual variability [34].

2.6 Assessment of Appetite Biomarkers

Appetite regulatory mechanisms were studied by measuring serum levels of leptin and ghrelin at intervention baseline and postintervention. Quantification of these hormones was performed with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (EH0216, EH0544, Wuhan Fine Biotech Co., Ltd. Wuhan, Hubei, China) that was specific to humans. The two assays were conducted as per the instructions of the manufacturer in the standardized procedures. The absorbance was measured by a BioTek Epoch™ 2 Microplate Spectrophotometer (EPOCH2NSC, Agilent Technologies, Santa Clara, CA, USA) in Gen 5™ software (version 3,Agilent Technologies, Santa Clara, CA, USA). Each sample was subjected to a duplicate analysis, and a statistical analysis was done considering the mean values.

2.7 Dietary Intake

Single 24-hour dietary recalls were used to evaluate dietary intake at the time of intervention. The rationale of the choice of this approach was feasibility and minimizing possible confounding by energy and macronutrient differences that could affect the cardiovascular, metabolic, and hormonal outcomes [29,30,35]. All the food and beverage products that had been consumed during the last 24 hours were described by a trained researcher including the quantity of the consumed food, the method of preparation, and the time taken. ESHA Food Processor software (version 8.6, ESHA Research, Salem, OR, USA) was used to analyze the intake of macronutrients and micronutrients. All statistical analyses had covariates on total energy and macronutrient intake (carbohydrates, proteins, fats) as a potential dietary factor confounding cardiovascular, metabolic and hormonal results [29,30,35]. Although single 24-hour recalls lacked day-to-day dietary variability, such a method is the usual methodology of short-term coffee intervention trials in terms of feasibility to statistical control [29,30].

2.8 Insomnia Assessment

The Arabic version of the Insomnia Severity Index (ISI) was subjected to validation and included the evaluation of insomnia symptoms at baseline and week 4 [36]. This version demonstrated acceptable internal consistency (Cronbach's α = 0.84) and high convergent validity with pittsburgh sleep quality index (PSQI) (r = 0.76, $p < 0.001$) [36]. The ISI is a 7-item self-administered questionnaire assessing sleep difficulty severity and daytime functional impact. Each item is scored 0–4, yielding total scores of 0–28, categorized as: 0–7 (no insomnia), 8–14 (subthreshold), 15–21 (moderate), 22–28 (severe).

2.9 Body Composition Assessment

Baseline anthropometric parameters were assessed during initial screening to determine eligibility. Body composition parameters (fat mass, fat-free mass, total body water, skeletal muscle mass) were measured using InBody 970 Body Composition Analyzer (MC-780 MA, Tanita Co., Seoul, Korea) via multifrequency bioelectrical impedance analysis. The device was regularly calibrated according to the requirements of the manufacturer. All testing took place in the morning (a single and uniform time slot for all participants) at least 10 hours after overnight fasting before any study procedures. The participants were advised not to drink alcohol and engage in vigorous activity in the 24 hours before the experiment, to keep themselves well-hydrated, remove any metal objects, and the clothes were standardized. Where feasible, measurements were done at the follicular phase to reduce the effects of hormones on bioelectrical impedance [23,24]. Parameters of body composition were used to affirm baseline homogeneity and ensure high BMI eligibility (18.5–24.9 kg/m^2), as well as to check how the parameters might change with the 4-week intervention [29,37]. The 40 participants (20 assayed in thereof) went through the 4-week protocol with 100% retention. Protocol adherence (verified via daily logs and random phone calls) was $\geq$95% for prescribed coffee consumption, and no adverse events observed.

Table 1. Baseline demographic, anthropometric, and body composition characteristics of study participants (n = 40).

Characteristic	Intervention group (n = 20)	Control group (n = 20)	p-value
Age (years)	20.55 ± 1.75	20.43 ± 1.85	0.83
Baseline caffeine intake (cups/week)	0.65 ± 0.30	0.70 ± 0.35	0.62
BMI (kg/m^2)	22.71 ± 2.57	21.75 ± 2.41	0.23
Weight (kg)	57.98 ± 6.71	57.72 ± 9.44	0.92
TBW (L)	28.00 ± 2.59	29.82 ± 5.74	0.19
ICW (L)	17.35 ± 1.66	18.51 ± 3.65	0.19
ECW (L)	10.65 ± 0.94	11.31 ± 2.09	0.18
BFM (kg)	19.67 ± 4.76	17.00 ± 4.22	0.07
FFM (kg)	38.31 ± 3.56	40.72 ± 7.77	0.22
SMM (kg)	20.64 ± 2.16	22.12 ± 4.77	0.21
PBF (%)	33.60 ± 5.44	29.43 ± 5.85	0.02
BMR (kcal)	1197.3 ± 76.99	1249.6 ± 167.88	0.21

Data are mean ± SD. Between-group comparisons: independent t-test; $p < 0.05$ considered significant. Participant eligibility: All participants were healthy young women who met inclusion criteria (age 18–25 years, BMI 18.5–24.9 kg/m^2, non-smokers, minimal baseline caffeine intake ≤1 cup/week, medication-free) and had no chronic illnesses per exclusion criteria. Body composition measurement: All parameters measured via InBody 970 Body Composition Analyzer using multifrequency bioelectrical impedance analysis. Abbreviations: BMI, body mass index; TBW, total body water; ICW, intracellular water; ECW, extracellular water; BFM, body fat mass; FFM, fat-free mass; SMM, skeletal muscle mass; PBF, percent body fat; BMR, basal metabolic rate. All participants: Amman, Jordan (Middle Eastern ethnicity).

2.10 Statistical Analysis

Sample size was calculated a priori using G*Power (version 3.1.9.4, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) for repeated-measures ANOVA with two groups and two time points (baseline, week 4). Assumptions: medium effect size (f = 0.25), $\alpha = 0.05$, power = 0.80, correlation = 0.5, nonsphericity correction $\varepsilon = 1$. Minimum required sample was n = 34 (17/group); our final sample (n = 40; 20/group) exceeded this requirement (observed power >0.80 for primary outcomes). As a pilot hypothesis-generating trial [27,38], no corrections for multiple comparisons were applied; all p-values are nominal and findings are exploratory. Normality was assessed using Shapiro-Wilk tests. Non-normal variables underwent log transformation and were re-evaluated before parametric testing. Repeated-measures ANOVA was applied when normality assumptions were satisfied. Two-way repeated-measures ANOVA examined time effects (baseline vs. week 4; within-subject factor), group effects (intervention vs. control; between-subject factor), and Time × Group interactions. Total macronutrient intake (from 24-hour dietary recall) was included as a covariate to control for dietary confounding. Statistical analyses were performed using IBM SPSS Statistics (Version 25.0, IBM Corp., Armonk, NY, USA). Effect sizes (partial η^2) serve as the primary magnitude estimates for Time × Group interactions in repeated-measures ANOVA, providing standardized metrics independent of sample size [38,39]. Analysis of covariance (ANCOVA) with adjusted mean differences with 95% confidence levels was done to offer unbiased values of effects in the case of outcomes with large baseline imbalances [25]. For HDL, outlier diagnostics were conducted using standardized z-scores (threshold: $|z| > 2.5$) and the interquartile range method (threshold: values >Q3 + 1.5 × IQR or <Q1 − 1.5 × IQR) to assess whether elevated baseline values reflected extreme observations [40]. There was no dropout of any data; all the 40 participants had assessments in both time points. The intention-to-treat analysis and per-protocol were the same and all subjects were considered randomized. In case of results with substantial results in an analysis of results with high baseline differences, ANCOVA was used as the main analysis with post-intervention results as the dependent variable, group as the independent variable, and baseline results as covariates. This approach provides unbiased treatment effect estimates by controlling for pre-existing differences and regression to the mean [25].

As a pilot hypothesis-generating trial [27,38], no formal corrections for multiple testing were applied. All p-values are nominal and findings should be interpreted exploratorily, prioritizing primary outcomes (cardiovascular parameters, lipid profile) over secondary outcomes.

3. Results

3.1 Protocol Adherence and Retention

All 40 randomized participants (20 per group) completed the 4-week intervention with 100% retention (Fig. 1). Mean coffee consumption in the intervention group was 20.8 ± 0.6 cups/week (98.6% of prescribed 21 cups/week), with all participants achieving ≥95% adherence verified via daily logs and phone monitoring. Control participants-maintained caffeine abstinence, confirmed through logs and phone calls. No adverse events or protocol deviations occurred.

Table 2. Effects of four weeks of Turkish coffee consumption on body composition parameters in healthy young female adults (case vs control), (n = 40).

Measure	Intervention group (n = 20)		Control group (n = 20)		F (Time Effect)	p value (Time Effect)	F (Time × Group)	p value (Time × Group)	Partial Eta Squared
	Baseline	Post-Intervention	Baseline	Post-Intervention					
Weight (kg)	57.98 ± 6.71	58.50 ± 6.89	57.72 ± 9.44	57.92 ± 9.45	1.19	0.28	0.23	0.64	0.03
BMI (kg/m^2)	22.71 ± 2.57	22.80 ± 2.58	21.75 ± 2.41	21.83 ± 2.41	0.33	0.57	≤0.001	0.96	0.01
TBW (L)	28.00 ± 2.59	27.93 ± 3.11	29.82 ± 5.74	29.81 ± 5.81	0.08	0.79	0.04	0.84	0.00
ICW (L)	17.35 ± 1.66	17.30 ± 1.66	18.51 ± 3.65	18.51 ± 3.71	0.08	0.78	0.08	0.77	0.00
ECW (L)	10.65 ± 0.94	10.63 ± 1.13	11.31 ± 2.09	11.30 ± 2.13	0.06	0.81	0.01	0.94	0.00
BFM (kg)	19.67 ± 4.76	20.30 ± 4.93	17.00 ± 4.22	17.20 ± 4.46	1.95	0.17	0.50	0.48	0.05
FFM (kg)	38.31 ± 3.56	38.20 ± 4.26	40.72 ± 7.77	40.72 ± 7.90	0.07	0.80	0.07	0.80	0.00
SMM (kg)	20.64 ± 2.16	20.57 ± 2.60	22.12 ± 4.77	22.12 ± 4.84	0.07	0.80	0.07	0.80	0.00
PBF (%)	33.60 ± 5.44	34.44 ± 5.89	29.43 ± 5.85	29.70 ± 6.42	2.18	0.15	0.59	0.45	0.05
BMR (kcal)	1197.3 ± 76.99	1194.8 ± 92.01	1249.6 ± 167.88	1249.4 ± 170.46	0.09	0.77	0.06	0.80	0.00

Data are mean ± SD; p-values from repeated-measures ANOVA, α = 0.05. Units and measurement method: All body composition parameters measured via InBody 970 Body Composition Analyzer using multifrequency bioelectrical impedance analysis. BMI in kg/m^2; TBW (total body water), ICW (intracellular water), ECW (extracellular water) in liters (L); BFM (body fat mass), FFM (fat-free mass), SMM (skeletal muscle mass) in kilograms (kg); PBF (percent body fat) in %; BMR (basal metabolic rate) in kilocalories (kcal).

Table 3. Cardiometabolic parameters after four weeks of Turkish coffee in healthy young women (case–control; n = 40).

Measure	Intervention group (n = 20)		Control group (n = 20)		F (Time Effect)	p-value (Time Effect)	F (Time × Group)	p-value (Time × Group)	Partial Eta Squared
	Baseline	Post-Intervention	Baseline	Post-Intervention					
SBP (mmHg)	108.30 ± 6.78	112.3 ± 8.71	110.95 ± 9.47	111.95 ± 8.24	1.37	0.25	5.459	0.025	0.126
DBP (mmHg)	81.90 ± 7.11	81.85 ± 6.70	78.55 ± 5.49	79.20 ± 7.40	0.21	0.65	0.245	0.624	0.006
HR (bpm)	82.45 ± 8.40	93.00 ± 7.26	82.70 ± 10.59	81.45 ± 9.38	5.02	0.03	8.08	0.007	0.175
HDL (mmol/L)	2.45 ± 0.82	1.26 ± 0.17	1.81 ± 0.42	1.43 ± 0.32	10.19	<0.001	0.772	0.385	0.019
Cholesterol (mmol/L)	4.36 ± 0.80	5.14 ± 0.85	4.05 ± 0.87	4.84 ± 0.73	14.63	<0.001	0.001	0.978	0.000
TG (mmol/L)	2.86 ± 0.82	2.91 ± 0.49	2.97 ± 0.78	3.10 ± 0.16	0.47	0.49	0.103	0.75	0.003
LDL (mmol/L)	0.60 ± 0.36	2.56 ± 0.96	1.37 ± 1.15	1.77 ± 0.83	26.43	<0.001	11.492	0.002	0.232
FBS (mg/dL)	92.40 ± 5.51	91.45 ± 5.52	90.80 ± 4.51	89.25 ± 5.51	2.20	0.15	0.127	0.724	0.003
HbA1c	4.60 ± 1.20	4.16 ± 1.78	3.78 ± 1.62	4.56 ± 1.67	0.23	0.64	2.905	0.096	0.071

Data are mean ± SD. Statistics: repeated-measures ANOVA with Time (baseline, post) as within-subject factor and Group (intervention, control) as between-subject factor; α = 0.05, two-sided. Effect sizes: partial η^2. Units and assay methods: SBP/DBP measured in mmHg via automated oscillometric sphygmomanometer (Omron M7 Intelli IT); HR in beats per minute (bpm); lipids (HDL, LDL, total cholesterol, triglycerides) in mmol/L via HP-AFS/1-PLUS analyzer using nephelometry/turbidimetry; FBS in mg/dL via electrochemical biosensor (TRUE METRIX®); HbA1c in % via HP-AFS/1-PLUS using immunofluorescence. All p-values are nominal without adjustment for multiple comparisons, consistent with the exploratory pilot design. Effect sizes (partial η^2) provide standardized magnitude estimates for Time × Group interactions, interpreted as: 0.01 = small, 0.06 = medium, 0.14 = large [38]. Descriptive change scores with standard deviations are reported in Results text to enhance interpretability. Due to significant baseline imbalance (p = 0.02), HDL Time × Group statistics reflect ANCOVA controlling for baseline values rather than repeated-measures ANOVA interaction. ANCOVA results: adjusted mean difference –0.17 mmol/L (95% CI: –0.56 to 0.22), F = 0.772, p = 0.385, Hedges' g = –0.28. HDL findings are exploratory only. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FBS, fasting blood glucose; HbA1c, glycated hemoglobin.

Table 4. Effects of four weeks of Turkish coffee consumption on leptin and ghrelin across time points by group in healthy young female adults (case vs control), (n = 40).

Measure	Intervention group (n = 20)		Control group (n = 20)		F (Time Effect)	*p* value (Time Effect)	F (Time × Group)	*p* value (Time × Group)	Partial Eta Squared
	Baseline	Post-Intervention	Baseline	Post-Intervention					
Leptin (ng/mL)	0.218 ± 0.189	0.180 ± 0.15947	0.302 ± 0.066	0.304 ± 0.067	6.67	0.01	8.65	0.01	0.19
Ghrelin (pg/mL)	1237.22 ± 624.83	1314.15 ± 650.96	1628.83 ± 379.59	1615.38 ± 536.39	0.18	0.68	0.36	0.56	0.01
CRP (mg/L)	2.61 ± 2.73	1.85 ± 2.55	2.83 ± 2.81	2.53 ± 4.81	0.49	0.49	0.09	0.77	0.00
TNF-α (pg/mL)	160.76 ± 218.8	147.30 ± 120.16	103.0 ± 77.9	132.16 ± 123.4	0.14	0.71	1.03	0.32	0.03

Data are mean ± SD; *p*-values from repeated-measures ANOVA, α = 0.05. Units and assay methods: Leptin in ng/mL and ghrelin in pg/mL measured via enzyme-linked immunosorbent assay (ELISA) with absorbance readings obtained using BioTek Epoch™ 2 Microplate Spectrophotometer; CRP in mg/L measured via high-sensitivity immunoturbidimetric assay (HP-AFS/1-PLUS analyzer); TNF-α in pg/mL measured via high-sensitivity ELISA kit with detection range 15.63–1000 pg/mL. Abbreviations: CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha.

Table 5. Effects of four weeks of Turkish coffee consumption on insomnia score in healthy young female adults (case vs control), (n = 40).

Measure	Intervention group (n = 20)		Control group (n = 20)		F (Time Effect)	*p* value (Time Effect)	F (Time × Group)	*p* value (Time × Group)	Partial Eta Squared
	Baseline	Post-Intervention	Baseline	Post-Intervention					
Insomnia	8.95 ± 2.50	13.00 ± 4.03	11.00 ± 4.38	10.00 ± 3.31	5.25	0.03	14.40	≤0.001	0.28

Data are mean ± SD; *p*-values from repeated-measures ANOVA, α = 0.05. Measurement: Insomnia Severity Index (ISI) total score (range 0–28 points) via validated Arabic version [36]; higher scores indicate greater insomnia severity.

3.2 Demographic Characteristics

The baseline characteristics were fairly balanced between the groups (Table 1), and no significant differences could be found in most demographic, anthropometric, and body composition variables (all $p \geq 0.05$) indicating that randomization was successful and the level of baseline confounding was reduced.

3.3 Effects of the Intervention on the Body Composition over Four Weeks

Body composition parameters were assessed at baseline and week 4 to monitor potential changes during the intervention period (Table 2). Body weight showed minimal changes in both groups, with the intervention group increasing by 0.52 kg (57.98 ± 6.71 to 58.50 ± 6.89 kg) and the control group increasing by 0.02 kg (57.72 ± 9.44 to 57.92 ± 9.45 kg), with no significant Time × Group interaction (p = 0.638). BMI remained stable in both the intervention group (22.71 ± 2.57 to 22.80 ± 2.58 kg/m^2) and control group (21.75 ± 2.41 to 21.83 ± 2.41 kg/m^2), with no significant time or interaction effects (p = 0.959). Total body water, intracellular water, and extracellular water volumes showed no significant changes in either group (all $p \geq 0.05$). Body fat mass increased slightly in the intervention group (19.67 ± 4.76 to 20.30 ± 4.93 kg) and control group (17.00 ± 4.22 to 17.20 ± 4.46 kg), but these changes were not statistically significant (time effect p = 0.171; Time × Group interaction p = 0.482). Fat-free mass and skeletal muscle mass remained stable in both groups throughout the intervention period (all $p > 0.05$). Overall, no significant alterations in body composition parameters were observed over the four-week intervention period. The stability of body weight and composition across both groups provides indirect support that major dietary or energy balance changes did not occur during the intervention period.

3.4 Effects of the Intervention on the Cardiometabolic Parameters over Four Weeks

Baseline Comparison and Adjusted Analysis for HDL: Independent samples *t*-tests comparing baseline values between groups revealed that HDL was the only outcome with significant baseline imbalance (intervention: 2.45 ± 0.82 mmol/L vs. control: 1.81 ± 0.42 mmol/L; t = –2.39, df = 41, p = 0.02, mean difference = –0.64 mmol/L [95% CI: –0.95 to –0.08]). All other outcomes showed no significant baseline differences (all $p \geq 0.05$). Outlier assessment for baseline HDL in the intervention group (range: 1.12–4.15 mmol/L) revealed no extreme values: all standardized z-scores were <2.5, and the highest value (4.15 mmol/L) remained below the IQR-based outlier threshold (Q3 + 1.5 × IQR = 4.28 mmol/L), confirming that the elevated group mean reflected overall distribution variability rather than outlier leverage [40]. Given this imbalance, ANCOVA was conducted with post-intervention HDL as the dependent variable and baseline HDL as covariate. Results showed no significant group effect (F = 0.772, p = 0.385, partial η^2 = 0.019), with adjusted between-group mean difference of –0.17 mmol/L (95% CI: –0.56 to 0.22, Hedges' g = –0.28). ANCOVA with baseline as covariate inherently adjusts for individual baseline variability, providing robustness to any influential observations [25]. The Time × Group interaction from repeated-measures ANOVA (original p = 0.003) was driven by regression to the mean rather than a treatment effect. Therefore, HDL findings should be considered exploratory and hypothesis-generating only. SBP increased in the intervention group (108.30 ± 6.78 to 112.30 ± 8.71 mmHg; change: +4.0 ± 7.2 mmHg) with minimal change in controls (110.95 ± 9.47 to 111.95 ± 8.24 mmHg; change: +1.0 ± 6.8 mmHg). The Time × Group interaction for SBP was significant (p = 0.025, F = 5.459, partial η^2 = 0.126), confirming a between-group difference in change scores of +3.0 mmHg (intervention increased 4.0 mmHg while control increased 1.0 mmHg; Table 3, Ref. [38]). DBP remained stable in both groups (intervention: 81.90 to 81.85 mmHg; control: 78.55 to 79.20 mmHg), with no significant time or interaction effects (p = 0.624). HR increased significantly in the intervention group (82.45 ± 8.40 to 93.00 ± 7.26 bpm; change: +10.6 ± 9.8 bpm) while remaining stable in controls (82.70 ± 10.59 to 81.45 ± 9.38 bpm; change: –1.25 ± 8.9 bpm), with significant time (p = 0.031, F = 5.019, partial η^2 = 0.117) and Time × Group interaction effects (p = 0.007, F = 8.080, partial η^2 = 0.175), indicating a net between-group difference of approximately +11.9 bpm. Between-group differences in change scores were: SBP +3.0 mmHg (95% CI: 0.4–5.6, p = 0.025), DBP –0.70 mmHg (95% CI: –3.8 to 2.4, p = 0.624), and HR +11.90 bpm (95% CI: 3.5–20.3, p = 0.007). The HDL reduced in both the intervention (2.45 ± 0.82 to 1.26 ± 0.17 mmol/L) and the control (1.81 ± 0.42 to 1.43 ± 0.32 mmol/L), and the time effect was significant ($p < 0.001$, F = 63.264, partial 0.625). Nonetheless, an ANCOVA of the adjusted baseline showed no statistically significant treatment effect (p = 0.385) so the apparent Time × Group interaction was due to regression to the mean of the imbalance at baseline and not an actual effect of the treatment Total cholesterol increased in both groups (intervention: 4.36 to 5.14 mmol/L; control: 4.05 to 4.84 mmol/L) with significant time effect ($p < 0.001$, F = 14.626, partial η^2 = 0.278) but no significant interaction (p = 0.978).

Triglycerides showed minimal non-significant changes (intervention: 2.86 to 2.91 mmol/L; control: 2.97 to 3.10 mmol/L; p = 0.495). LDL increased in both groups (intervention: 0.60 to 2.56 mmol/L; control: 1.37 to 1.77 mmol/L) with significant time ($p < 0.001$, F = 26.428, partial η^2 = 0.410) and Time × Group interaction effects (p = 0.002, F = 11.492, partial η^2 = 0.232), indicating greater elevation in the intervention group.

Fasting blood glucose showed minimal non-significant changes (intervention: 92.40 ± 5.51 to 91.45 ± 5.52 mg/dL; control: 90.80 ± 4.51 to 89.25 ±

5.51 mg/dL), with no significant time (p = 0.146) or Time × Group interaction effects (p = 0.724, partial η^2 = 0.003). HbA1c also showed no significant changes (intervention: 4.60 ± 1.20 to 4.16 ± 1.78%; control: 3.78 ± 1.62 to 4.56 ± 1.67%), with no significant time (p = 0.638) or interaction effects (p = 0.096, partial η^2 = 0.071).

3.5 Effects of the Intervention on the Appetite-Regulatory and Inflammatory Biomarkers over Four Weeks

Leptin decreased in the intervention group (0.218 to 0.180 ng/mL) while remaining stable in controls (0.302 to 0.304 ng/mL), with significant time (p = 0.014, F = 6.673, partial η^2 = 0.185) and Time × Group interaction effects (p = 0.006, partial η^2 = 0.185; Table 4). Ghrelin showed minimal non-significant changes (intervention: 1237.22 to 1314.15 pg/mL; control: 1628.83 to 1615.38 pg/mL; all $p > 0.05$). CRP and TNF-α showed no significant changes. CRP: intervention (2.61 to 1.85 mg/L), control (2.83 to 2.83 mg/L); time effect p = 0.489, interaction p = 0.768, partial η^2 = 0.002. TNF-α: intervention (160 to 147 pg/mL), control (103 to 132 pg/mL); time effect p = 0.710, interaction p = 0.316, partial η^2 = 0.026.

3.6 Effects of the Intervention on Insomnia Scores

Insomnia severity increased significantly in the intervention group (8.95 ± 2.50 to 13.00 ± 4.03) while decreasing in controls (11.00 ± 4.38 to 10.00 ± 3.31), with significant time (p = 0.028, F = 5.25) and Time × Group interaction effects ($p \leq 0.001$, F = 14.401, partial η^2 = 0.275; Table 5, Ref. [36]).

4. Discussion

Despite TC's widespread consumption in Middle Eastern cultures, rigorous research examining its specific physiological effects remains limited, with most coffee studies generalizing across brewing methods without accounting for unfiltered preparations' distinct biochemical properties [8,9]. The present study aimed to evaluate the effects of four weeks of UTC consumption on key health parameters among young females. The effects of four-week of UTC consumption on cardiovascular, metabolic, hormonal, and sleep parameters among healthy young women were explored in this pilot trial. Novel contributions include: (1) first controlled trial examining UTC in young women from the Middle Eastern region—the primary consumer demographic—using culturally authentic preparation methods reflecting local consumption practices [41]; (2) simultaneous multi-system assessment (cardiovascular, metabolic, hormonal, sleep) providing integrated physiological insight; (3) first documentation of significant leptin reduction without ghrelin changes in coffee trials, suggesting selective appetite-regulatory mechanisms; (4) validated insomnia assessment (ISI) rather than self-reported sleep duration [20,21]; and (5) rapid lipid profile changes (HDL decrease, LDL increase) within four weeks, faster than previously recognized [41,42].

The significant methodological limitation needs to be mentioned first. Although we directly determined caffeine content (71 mg/cup), diterpenes (cafestol, kahweol) and chlorogenic acids were not measured in our UTC samples. Mechanistic interpretations using these compounds are hypothesis-generating inferences using literature estimates of chemically related preparations [31–33], not definitive causal attributions. The observed physiological changes (cardiovascular, lipid, hormonal, sleep) are empirical facts; the biochemical mechanisms underlying these changes remain speculative pending direct bioactive compound analysis. We prioritize discussion of measured outcomes while contextualizing findings within established coffee pharmacology literature.

Our findings confirmed the hypothesized effects of UTC consumption on cardiovascular function, lipid metabolism, leptin regulation, and sleep quality. Body composition parameters (weight, BMI, body fat, fat-free mass, skeletal muscle mass) remained unchanged over four weeks, consistent with prior studies showing that short-term coffee consumption does not alter body composition without concurrent exercise or dietary modification [29,35,43]. The 4-week duration was insufficient for body composition remodeling, which typically requires 8–12 weeks [37]. The stability of body composition despite major cardiovascular and metabolic changes (reduction in HDL, rise in LDL, hypertension, fall in leptin) is plausibly due to direct effects of pharmacological actions and not the effect of adipose. This association reinforces the fact that bioactive compounds of TC, especially diterpenes and caffeine, have direct hepatic and cardiovascular effects even without considering the changes in energy balance or fat mass [5,29,37].

UTC consumption significantly increased SBP (+4.0 mmHg intervention vs. +1.0 mmHg control; Time × Group p = 0.025) without affecting DBP (p = 0.624). The 4 mmHg SBP elevation exceeds meta-analytic estimates for caffeine (2.0–2.4 mmHg) [34,44], potentially reflecting unfiltered TC's higher diterpene content (cafestol, kahweol) compared to filtered preparations [8,45,46]. Diterpenes elevate cholesterol and stimulate aldosterone production, contributing to BP increases [8]. The clinical significance of the 3.0 mmHg increase in SBP in healthy normotensive young women requires cautious interpretation. However, this magnitude has limited immediate clinical relevance for metabolically healthy individuals [34], [45]. Population-level meta-analyses suggest sustained 2–5 mmHg elevations contribute to long-term cardiovascular risk over decades, though extrapolating 4-week changes to chronic outcomes requires caution [34]. Clinical importance may be greater for individuals with borderline hypertension or additional cardiovascular risk factors [45]. The 4 mmHg SBP elevation combined with increased HR (10.6 bpm increase) and stable body composition is consis-

tent with adenosine receptor blockade from the measured caffeine content (71 mg/cup), which produces sympathetic nervous system activation and vasoconstriction [47,48]. Whether diterpenes contributed to cardiovascular effects through aldosterone modulation [8] could not be determined without direct compound measurement, though the concurrent LDL elevation suggests bioactive compounds beyond caffeine were physiologically active. Caffeine blocks adenosine receptors (A1 and A2A), producing sympathetic nervous system activation and vasoconstriction [47], while increasing catecholamine release and peripheral vascular resistance [48]. The different effects between the SBP and DBP measurements reported in our study support physiological norms. The acute vascular tone regulation caused by adenosine receptor blockade and catecholamine discharge from caffeine influences SBP primarily by increasing heart output and peripheral resistance [49]. DBP seems less sensitive to these transient effects among healthy young females with well-functioning vasculature because it measures peripheral resistance during cardiac relaxation [50].

HR increased significantly in the intervention group (82.45 to 93.00 bpm; approximately 13% increase) with significant time (p = 0.031) and Time × Group interaction effects (p = 0.007). This finding aligns with Papakonstantinou *et al*. (2016) [51], who reported similar HR increases following Greek coffee consumption, and Buscemi *et al*. (2010) [52], who documented caffeine-induced cardiovascular activation via adenosine receptor blockade. Beyond adenosine receptor antagonism, caffeine inhibits phosphodiesterase enzymes, increasing cyclic adenosine monophosphate (cAMP) and enhancing cardiac contractility [53]. TC's unfiltered preparation retains higher bioactive compound concentrations (chlorogenic acids, diterpenes) compared to filtered coffee, potentially intensifying cardiovascular effects [8]. The HR increase that was seen (+10.6 bpm intervention versus –1.25 bpm control; net difference –11.9 bpm) is probably a result of several factors. Caffeine-naive participants had undergone three weeks of mandatory washout, and therefore, there was no tolerance adaptation, as is common with chronic consumption [54,55]. Additionally, HR measurements occurred 1–3 hours post-consumption when plasma caffeine peaks [56], potentially capturing maximal cardiovascular activation. The open-label design may have introduced arousal or expectancy effects, though standardized protocols (≥30-minute rest, quiet environment, triplicate measurements) were implemented to minimize such influences. One should take these contextual factors into consideration when the observed cardiovascular responses are being interpreted. While Grosso *et al*. (2017) [54] documented attenuated cardiovascular responses with chronic coffee consumption, our 4-week intervention may have been insufficient for metabolic adaptation to develop. The magnitude of cardiovascular responses observed in our female cohort warrants consideration of sex-specific caffeine pharmacokinetics. Women exhibit slower CYP1A2 enzyme activity compared to men, resulting in prolonged caffeine half-life (approximately 20–25% longer) and extended physiological exposure to equivalent doses [23,24]. This metabolic difference may amplify acute cardiovascular responses—particularly sympathetic nervous system activation—in female populations. Our female-only design addresses a critical research gap, as most coffee intervention trials have enrolled predominantly male or mixed-gender cohorts without sex-stratified analyses [23,24]. The pronounced HR elevation (+10.6 bpm) and SBP increase (+4.0 mmHg) observed in our study align with emerging evidence suggesting women may experience more pronounced cardiovascular responses to caffeine than men [23,24], though direct comparative trials remain limited. This underscores the importance of sex-specific coffee research and suggests our findings may not be directly generalizable to male populations. Leptin decreased significantly in the intervention group (0.218 to 0.180 ng/mL) while remaining stable in controls (0.302 to 0.304 ng/mL), with significant time (p = 0.014) and Time × Group interaction effects (p = 0.006). Critically, this reduction occurred despite stable body fat mass (p = 0.171), indicating that UTC affects leptin secretion independent of adiposity changes—a mechanistic distinction from weight loss interventions where leptin decreases proportionally to fat mass [57]. This dissociation suggests that chlorogenic acids or other UTC polyphenols may directly modulate adipocyte leptin secretion or gene expression rather than acting indirectly through energy balance [58], consistent with polyphenol-induced leptin reductions observed in other dietary interventions [59]. While leptin serves as a key satiety signal [57], its reduction in the context of stable body composition may reflect improved leptin sensitivity rather than disrupted satiety regulation, as observed with coffee polyphenols in preclinical models [60]. Bioactive compounds including chlorogenic acids may modulate adipocyte function and leptin secretion pathways [58], though the functional implications of these changes in healthy individuals with normal baseline leptin levels require further investigation. In contrast to leptin, ghrelin showed no significant changes (intervention: 1237.22 to 1314.15 pg/mL; control: 1628.83 to 1615.38 pg/mL; p = 0.555). This differential response—significant leptin reduction without ghrelin changes—suggests TC's bioactive compounds may selectively affect adipocyte-derived hormones without equivalent effects on gastric hormone secretion [61]. This selectivity emphasizes the complexity of the process of regulating appetite hormones and it shows that the action of TC is not uniform throughout all the mechanisms of regulation. Our a priori hypothesis predicted ghrelin elevation based on caffeine's documented appetite-regulatory effects [19]; however, ghrelin remained unchanged (p = 0.555). Potential explanations include: (1) insufficient interven-

tion duration, as gastric hormone adaptation may require longer than 4 weeks [35,61]; (2) moderate caffeine dose (213 mg/day) below the threshold for ghrelin modulation documented in studies using acute doses ≥400 mg [19]; (3) substantial intra-individual ghrelin variability influenced by meal timing, sleep, and stress in free-living conditions [61]; and (4) possible sex-specific ghrelin regulation, as most prior studies enrolled predominantly male participants [19,35]. The leptin-ghrelin discordance highlights the complexity of appetite regulation and suggests TC's effects are pathway-specific rather than uniform. HDL decreased in both groups. There was however, great baseline imbalance which resulted in the need to use baseline-adjusted analysis. ANCOVA adjusting for baseline HDL found no treatment effect and showed that the observed Time × Group interaction in repeated-measures ANOVA was due to the baseline effect of regressing to the mean value, but not a true UTC effect. This result highlights the paramount essence of the necessity of baseline-adjusted analysis when randomization results in unbalanced groups and is to be regarded as exploratory, before replication in studies with balanced lipid profiles in the baseline. The non-significant HDL finding (after baseline adjustment) precludes mechanistic interpretation for this outcome. For completeness, prior studies of unfiltered coffee with measured diterpene content documented HDL suppression through hepatic ATP-binding cassette transporter A1 (ABCA1) transporter inhibition [41] though we cannot assess whether similar mechanisms operated in our study without direct compound quantification. Based on prior analyses of chemically identical UTC (Brazilian Arabica, medium-dark roast, unfiltered preparation), typical concentrations are: cafestol 4.4–6.2 mg/cup, kahweol 3.2–4.8 mg/cup [31,32], and chlorogenic acids 70–115 mg/150 mL [33]. These bioactive levels affect hepatic lipid metabolism and reverse cholesterol transport [31,33], though confirmation through direct measurement in future studies is warranted. LDL increased more in the intervention group (0.60 to 2.56 mg/dL) than controls (1.37 to 1.77 mg/dL; Time × Group p = 0.002), consistent with meta-analytic evidence that unfiltered coffee elevates LDL [41,42]. This increase was despite the statistical adjustment of the macronutrient intake and the absence of changes in body composition. Although we cannot conclusively pin this on the effect of certain bioactive compounds unless we directly measure them, the trend follows that observed due to diterpene-mediated effects of cholesterol metabolism in controlled feeding studies where cafestol and kahweol were directly determined [41,62]. Future studies should directly measure diterpene content and correlate concentrations with individual lipid responses to establish dose-response relationships. Total cholesterol increased similarly in both groups (intervention: 4.36 to 5.14 mg/dL; control: 4.05 to 4.84 mg/dL) with no significant between-group difference (p = 0.978), suggesting factors beyond UTC consumption influenced this outcome. These findings align with Urgert and Katan (1997) [63], who reported 0.5 mmol/L total cholesterol increases with 4-week unfiltered coffee consumption. Triglycerides remained stable in both groups (intervention: 2.86 to 2.91 mg/dL; p = 0.495), consistent with prior findings in healthy adults consuming moderate coffee amounts [61]. Lipid responses to coffee depend on preparation method, dosage, and genetic factors, particularly CYP1A2 polymorphisms affecting caffeine metabolism [64]. Our findings of HDL reduction and LDL elevation differ from epidemiological studies suggesting cardiovascular benefits of moderate coffee consumption [65], likely reflecting unfiltered TC's distinct diterpene profile compared to filtered preparations predominantly studied in those analyses. These lipid alterations warrant caution for individuals with dyslipidemia or cardiovascular risk factors. Future trials should consider genetic polymorphisms cytochrome P450 1A2 (CYP1A2), adenosine A2A receptor (ADORA2A) that modulate individual responses to caffeine, potentially explaining inter-individual variability in lipid, hormonal, and cardiovascular outcomes [66,67]. Fasting blood glucose showed minimal non-significant changes in both groups (intervention: 92.40 ± 5.51 to 91.45 ± 5.52 mg/dL; p = 0.146), indicating that 4-week UTC consumption does not affect fasting glucose in healthy young women. This aligns with Reis *et al*. (2018) [68], who reported unchanged fasting glucose following moderate coffee consumption in healthy adults with intact glucose homeostasis. HbA1c showed no significant changes (intervention: 4.60 ± 1.20 to 4.16 ± 1.78%; control: 3.78 ± 1.62 to 4.56 ± 1.67%; Time × Group p = 0.096, partial η^2 = 0.071). The absence of significant HbA1c or fasting glucose effects suggests that 4-week UTC consumption does not substantially alter glycemic control in healthy young women with normal baseline glucose metabolism. While bioactive compounds in TC including chlorogenic acids and polyphenols can improve insulin sensitivity by reducing hepatic glucose-6-phosphatase activity [69,70] and increasing adiponectin [71], these effects may require longer intervention periods or be more pronounced in individuals with impaired glucose metabolism. The small, non-significant effect size (partial η^2 = 0.071) may reflect several factors: (1) short intervention duration (4 weeks) insufficient for pronounced metabolic adaptation; (2) healthy baseline glycemic status of participants, as coffee's glycemic benefits are more pronounced in individuals with impaired glucose metabolism [72,73]; and (3) inter-individual variability in bioactive compound responses. While TC's bioactive compounds can modulate Glucose transporter type 4 (GLUT4) translocation and cellular glucose uptake [74], these effects may manifest more clearly in longer trials or populations with metabolic impairment.

CRP and TNF-α showed no significant changes over 4 weeks (CRP intervention: 2.61 to 1.85 mg/L, p = 0.489; TNF-α intervention: 160.76 to 147.30 pg/mL, p =

0.710). These null findings align with prior short-term coffee trials reporting unchanged inflammatory markers despite improvements in other metabolic parameters [29]. The absence of inflammatory marker changes may reflect: (1) insufficient duration, as anti-inflammatory effects of polyphenols typically emerge after 8–12 weeks of sustained exposure [75]; (2) normal baseline inflammatory status in our healthy young participants, limiting potential for further reduction—elevated baseline markers show stronger polyphenol responses [76]; and (3) opposing effects of caffeine (pro-inflammatory via cortisol elevation) and chlorogenic acids (anti-inflammatory via NF-κB inhibition) potentially producing net-neutral effects [77,78]. TC's bioactive bioavailability and pharmacokinetics may also differ from filtered preparations previously studied. Our baseline inflammatory markers were within normal ranges for healthy young women (CRP: 2.61 mg/L; TNF-α: 160.76 pg/mL), consistent with the limited inflammatory response observed in metabolically healthy populations [76]. While coffee's phenolic acids and melanoidins can modulate NF-κB pathway activation and cytokine production [77,78], these anti-inflammatory effects may be most pronounced in individuals with elevated baseline inflammatory status. Insomnia severity increased significantly in the intervention group (8.95 $\pm$ 2.50 to 13.00 $\pm$ 4.03) while decreasing in controls (11.00 $\pm$ 4.38 to 10.00 $\pm$ 3.31), indicating substantial sleep impairment with daily UTC consumption. This finding aligns with established evidence that caffeine consumed 6–9 hours before bedtime disrupts sleep despite its 5–7 hour half-life in healthy adults [79]. The observed sleep impairment is particularly concerning given cultural practices favoring afternoon or evening UTC consumption in Middle Eastern societies. Our standardized consumption protocol (last cup 3–4 PM) minimized this risk, yet significant effects emerged (partial η^2 = 0.275), indicating UTC consumption explained 27.5% of variance in insomnia scores—a substantial effect. Drake *et al*. (2013) [21] demonstrated dose-dependent sleep disruption with afternoon/evening caffeine consumption, underscoring the importance of consumption timing. Mechanistically, caffeine antagonizes adenosine receptors, preventing the accumulation of sleep pressure during waking hours [80]. Even caffeine consumed 6 hours pre-bedtime can disrupt sleep architecture and reduce total sleep duration by $>$1 hour in sensitive individuals [21]. TC's moderate-to-high caffeine content (71 mg/40 mL cup; 213 mg daily total) combined with additional bioactive compounds retained through unfiltered preparation [81] likely contributed to the pronounced sleep impairment observed. Individual variability in sleep responses may reflect CYP1A2 polymorphisms affecting caffeine metabolism, as evidenced by increased standard deviation at follow-up [56]. Unlike studies reporting tolerance to caffeine's sleep-disrupting effects in habitual coffee drinkers [55], our participants underwent caffeine washout before intervention, potentially explaining their heightened sensitivity. For individuals experiencing sleep disturbances, limiting UTC consumption or restricting intake to morning hours warrants consideration.

5. Strength and Limitations

This pilot trial offers several important contributions to coffee intervention research. We provide the first controlled investigation of the unfiltered Turkish coffee's physiological effects specifically in young women—the primary consumer demographic—using culturally authentic preparation methods and addressing systematic underrepresentation of women in coffee research [8,23,24]. The rigor of the study is well established (pre-registered protocol OSF DOI: 10.17605/OSF.IO/2V96N), three-week caffeine washout control of baseline standardization, coffee preparation which was required to be precisely controlled (quantified caffeine content by HPLC-DAD-MS), high retention of all study participants (100 percent completion rate), and validated measurement measures of all results. The simultaneous recording of cardiovascular, metabolic, hormonal and sleep variables provide composite physiological data, which is rarely elucidated in the study of the effects of coffee intervention. Our findings reveal novel mechanistic observations, such as the leptin loss without body composition changes and high rates of lipid changes in four weeks, to add significant preliminary information to be used in future confirmatory studies. Several limitations warrant acknowledgment. The caffeine abstinence control design, while appropriate for our primary research question and consistent with established coffee intervention methodology [29,30,68], limits conclusions about Turkish coffee's specificity compared to other brewing methods. Female-only enrollment enhances internal validity through reduced inter-individual variability but restricts generalizability to males and older populations, though this approach directly addresses sex-specific caffeine pharmacokinetics documented in the literature [23,24]. The small sample size (n = 20 each group), is an indication that this research was an exploratory pilot study and limits that research to population level representativeness, where a need to replicate these studies with larger and demographically varied populations is necessary before making generalizations. Only caffeine content was directly quantified (71 mg/cup via HPLC-DAD-MS); diterpenes (cafestol, kahweol) and chlorogenic acids were not measured in our UTC samples. All mechanistic discussions attributing effects to these compounds represent hypothesis-generating inferences based on literature estimates from chemically similar preparations [31–33], not empirically established causation. Substantial batch-to-batch variability in bioactive compound concentrations may exist depending on bean origin, roasting degree, grinding fineness, and brewing time [31]. Without direct measurement and dose-response analysis, we cannot definitively attribute observed physiological changes to specific bioactive compounds. Future trials

must incorporate comprehensive phytochemical profiling (HPLC-MS quantification of diterpenes, chlorogenic acids, trigonelline, melanoidins) coupled with pharmacokinetic assessments to establish causal mechanisms underlying cardiovascular, lipid, and hormonal responses. Menstrual cycle phase was not biochemically verified or controlled as a stratification variable. While participants self-reported regular cycles and randomization should distribute cycle-phase variability equally between groups, hormonal fluctuations may have contributed to individual response variability. The 4-week intervention duration spans 1–2 complete menstrual cycles, potentially averaging hormonal effects across time points. Future trials would benefit from cycle-phase stratification to reduce unexplained variance [23,24]. These uncontrolled hormonal variations are inherent limitations to the study as a pilot study due to the small sample size (n = 40), though randomization will evenly allocate this variability across the groups. Genetic polymorphisms affecting caffeine metabolism (CYP1A2) and cardiovascular responses (ADORA2A adenosine receptors) were not assessed. The *1F and *1A alleles of CYP1A2 produce up to 40% differences in caffeine clearance rates between fast and slow metabolizers [66,67], while ADORA2A variants modulate blood pressure and anxiety responses to caffeine [64]. Our ethnically homogeneous Middle Eastern cohort may reduce inter-ethnic genetic variability compared to multiethnic samples, as allele frequencies differ substantially across populations [67]. While randomization should distribute genotypes equally between groups, genetic heterogeneity likely contributed to individual response variability (reflected in standard deviations). The robust group-level effects observed for cardiovascular, lipid, and sleep outcomes suggest treatment signals detectable despite genetic variability. Future trials would incorporate genotyping to enable stratified analyses and identify individuals at higher risk for adverse responses [64,66,67]. Single 24-hour dietary recalls, while feasible for pilot studies and statistically controlled through covariate adjustment, cannot capture day-to-day dietary variability. Dietary assessment using single 24-hour recalls at baseline and post-intervention, while consistent with established coffee trial methodology [29,30], does not capture day-to-day dietary variability or long-term nutritional patterns. Total macronutrient intake was statistically controlled as a covariate in all analyses, and the complete stability of body composition parameters (weight, BMI, body fat mass; all $p > 0.05$) provides indirect validation that major dietary changes did not occur. However, unmeasured variability in specific micronutrients (dietary cholesterol, saturated fat, fiber) may have contributed to individual differences in lipid responses. The significant LDL elevation occurred despite macronutrient adjustment and in the absence of body composition changes, supporting a direct pharmacological mechanism rather than dietary confounding. Future trials would benefit from multiple-day food records or controlled feeding to eliminate dietary variability as a potential confounder. The 3.0 mmHg SBP elevation, though statistically significant, approaches intra-individual measurement variability for oscillometric devices in healthy normotensive individuals [34]. As an exploratory pilot trial, no multiplicity corrections were applied; *p*-values are nominal and require confirmatory investigation in adequately powered trials [27,38]. Finally, the four-week intervention duration, while appropriate for pilot feasibility assessment, precludes evaluation of long-term metabolic adaptations or chronic health outcomes.

These research design limitations provide important future UTC research priorities. To begin with, it would increase the intervention time to 8–12 weeks to evaluate adenosine receptor tolerance formation, CYP1A2 metabolic accommodation [57], and long-term cardiovascular effects reported in long-term coffee studies [35,45,46]. Second, head-to-head comparative trials directly quantifying diterpene content across brewing methods (UTC vs. filtered preparations) with parallel cardiometabolic monitoring would definitively isolate cafestol/kahweol effects [8,13,42] from caffeine pharmacology [48,49], addressing our limitation of inferring mechanisms from literature-derived bioactive estimates [32–34]. Third, sex-stratified (male) cohort replication using CYP1A2 genotype would be used to ascertain whether our exaggerated response (SBP +4.0 mmHg, HR +10.6 bpm) is indicative of female-specific slower caffeine clearance (20–25% longer half-life [24,25] or are more generalizable UTC effects. Fourth, the trials that involve populations with cardiometabolic risk (dyslipidemia, prehypertension, metabolic syndrome) baseline would help determine clinical thresholds of contraindications versus acceptable risk [5,35]. Fifth, objective polysomnography coupled with systematic consumption timing manipulation would quantify circadian disruption beyond self-reported ISI scores [22,80], revealing optimal windows preserving alertness while minimizing sleep architecture impairment [21,81]. Lastly, genetic stratification (CYP1A2 -1A-1F alleles dictating metabolizer phenotype; ADORA2A variants dictating adenosine sensitivity [64,66,67]) should be included, which would allow precise definition of individuals at high risk, and change UTC guidance (population generalizations) to genetically sensitive, baseline health status-specific and consumption patterns change. All of these would help overcome existing limitations and progress mechanistic insight into complex physiological effects of UTC.

6. Clinical and Public Health Implications

The current results are relevant to the practice of UTC consumers and healthcare providers. The four weeks of moderate daily UTC (three 40-mL cups) resulted in statistically significant changes in a variety of parameters: SBP elevation (+4 mmHg), the increase in HR (+10.6 bpm), reduction of HDL, and increase of LDL levels, leptin decrease, and clinically significant sleep impairment (1.5-point rise

in the ISI score). Although the changes were observed in healthy young women, their combination and speed are worth being considered among people with risk factors in the cardiovascular system, dyslipidemia, or sleeping disorders. Counselors who discuss the use of coffee with a patient must separate the filtered and unfiltered coffee because the lipid effects of UTC and filtered coffee vary significantly [8,41,63]. It can be recommended that individuals who have pre-existing dyslipidemia, hypertension, or insomnia should limit the intake of UTC or use filtered preparations. The considerable sleep disruption observed even when the consumption ceased by 3–4 PM implies the need to be very mindful of the timing of consumption especially among the caffeine-sensitive individuals. On the other hand, no negative effects on glycemic or inflammatory in our healthy group imply that moderate UTC intake can be permissible among metabolically healthy people with no cardiovascular or sleep issues, however, it is better to consume it earlier in the day [21,22]. The findings offer evidence-based information on the population in which UTC is culturally relevant and recognize that different people would respond differently depending on genetic influences (CYP1A2 polymorphisms), the current health status, and patterns of consumption [64,66,67].

7. Conclusions

This pilot randomized controlled trial is the first to have conducted a full assessment of the short-term effects of UTC consumption on young women who used culturally sensitive preparation procedures. The nature of the pilot study design such as small sample size (n = 40), no control over the menstrual cycle, and 4 weeks of intervention limits the generalizability and accuracy of effect estimates. This nominal statistically significant (no multiplicity adjustment) hypothesis-generating study showed that four weeks of standardized daily UTC intake was associated with physiological alterations in various systems: cardiovascular (SBP +3.0 mmHg, HR +11.9 bpm), lipid profile (LDL increased; HDL none after baseline adjustment), hormonal (leptin reduced with no effect on ghrelin) and sleep quality (worsened) impairments, which all happened without a change in body composition. The 4-week intervention period provides initial mechanistic information but is not able to deal with longer period adaptations, tolerance formation, and chronic health results, and all the outcomes of the study are nominal without multiple testing correction. These results are to be construed as coming up with certain hypotheses to be validated in sufficiently powered trials, as opposed to conclusive evidence of causal associations. The rapidity and magnitude of these effects, especially lipid changes and sleeping disturbance, should be given clinical attention in giving advice to UTC consumers. Notably, neither body composition, glycemic, nor inflammatory changes were present despite the severe impact of the cardiovascular and lipid effects, which indicates direct pharmacological action of the bioactive compounds of UTC (diterpenes, caffeine, chlorogenic acids) rather than indirect metabolic pathways. These findings address critical knowledge gaps by: (1) establishing baseline physiological responses in the primary consumer demographic (young women), (2) demonstrating that unfiltered coffee's effects differ from general coffee literature, and (3) providing evidence for sex-specific research in coffee interventions. Although these preliminary results are pending validation, healthcare professionals can take them into account when advising patients who have already acquired a cardiovascular risk or dyslipidemia or sleep disorders, about UTC drinking habits. Much-power adequately studies to overcome these pilot study limitations such as stratification of the menstrual cycle, larger samples with greater population diversity, and longer intervention periods are necessary to determine clinical applicability and population-wide guidelines. Future research should examine dose-response relationships, conduct comparative trials between brewing methods, investigate sex differences, and evaluate longer-term adaptations to inform evidence-based public health guidance for populations where UTC holds cultural significance.

Abbreviations

BFM, body fat mass; BMI, body mass index; BMR, basal metabolic rate; CRP, C-reactive protein; DBP, diastolic blood pressure; ECW, extracellular water; ELISA, enzyme-linked immunosorbent assay; FBS, fasting blood glucose; FFM, fat-free mass; HDL, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; HPLC, high-performance liquid chromatography; HR, heart rate; ICW, intracellular water; IRB, institutional review board; ISI, Insomnia Severity Index; LDL, low-density lipoprotein cholesterol; MS, mass spectrometry; PBF, percent body fat; SBP, systolic blood pressure; SMM, skeletal muscle mass; TBW, total body water; UTC, unfiltered Turkish coffee; TG, triglycerides; TNF-α, tumor necrosis factor-alpha; η^2, partial eta squared (effect size measure).

Availability of Data and Materials

The data presented in this study are available upon request from the corresponding author for privacy, legal or ethical reasons.

Author Contributions

Conceptualization, NAE; Methodology, NAE and OAA; Formal analysis, NAE; Investigation, NAE and OAA; Writing—review & editing, NAE & OAA. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This research was conducted based on the ethical principles outlined in the Declaration of Helsinki guidelines. in

addition, the Research Ethics Committee of the Faculty of Pharmacy and Medical Sciences at the University of Petra, Amman, Jordan, granted approval before embarking on the research, with an ethical approval number of (Grand number: E/H/5/10/2024, August 2024). All participants gave their informed consent before joining the study. They were fully briefed on the research details, and we ensured that they met the study criteria before moving forward. Each participant signed a written consent form before we began.

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Conflict of Interest

The authors declare no conflict of interest.

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