HIGH FAT VS HIGH PROTEIN AND APPETITE HORMONES

NCT # 04518930

Document Date: 03/06/2024

Study protocol

Study design and participants

The study design utilized a parallel-arm intervention, employing a non-blind randomized clinical trial format with two distinct treatment groups. This study enrolled fifty-two healthy Jordanian women from a private nutrition clinic in Amman through randomization.

This study was conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki. The research procedures involving study participants received approval by the Institutional Research Board committee at the University of Jordan (No. 19/2021/180). Furthermore, the study was registered on ClinicalTrials.gov (No. NCT 04518930). Written informed consent was obtained from all individuals who participated in the study.

Inclusion and exclusion criteria

The study's inclusion criteria encompassed women aged between 30 and 40 years, with a body mass index (BMI) ranging from 25 to 35 kg/m². The included age group comprises predominantly overweight and obese individuals attending the nutrition clinic, demonstrating high adherence to the study protocol. These individuals engaged in light exercise 1-3 times per week (activity factor 1.375) and adhered to a routine of consuming three regular meals, along with two daily snacks. They maintained a water intake of eight cups and ensured a sleep duration of 8 hours. Additionally, participants were required to have no known allergies, particularly to nuts, especially peanuts and yogurt.

Conversely, women were ineligible for the study if they met any of the following conditions: age below 30 years or above 40 years, BMI below 25 kg/m² or above 35 kg/m², engaged in little or no exercise or engaged in moderate to heavy exercise , were pregnant or lactating mothers, had allergies to nuts, peanuts, yogurt, milk, or milk products, were

using medications, hormonal therapy, supplements, oral contraceptives, or herbal/botanical products claimed to suppress appetite. Furthermore, exclusion criteria encompassed women with hormonal disturbances, including those in a menopausal state, and those within their menstrual cycle days or within one week prior, commonly experiencing hyperphagia due to elevated progesterone levels. Additionally, individuals with chronic diseases and metabolic disorders such as cardiovascular diseases, diabetes, hypertension, kidney diseases, thyroid disorders, gastrointestinal diseases, polycystic ovary syndrome, or androgen disorders were excluded, following a weight-reducing diet, experiencing sleep disorders, or sleeping less than 8 hours per day, and consuming less than 8 cups of water per day were also excluded from the study.

Sample size determination

The required sample size for the study was determined using the formula:

$n = 2 * (Z\alpha + Z\beta)^2 * SD^2 \div \delta^2$, where n represents the required number of participants in both the treatment and placebo groups. $Z\alpha$ and $Z\beta$ are the values from the standard normal distribution corresponding to specific confidence levels of 95% and a two-sided α of 0.05 ($Z\alpha = 1.96$) and a power of 80% ($Z\beta = 0.84$). SD refers to the standard deviation (pooled), while δ represents the estimated difference between the treatment and placebo groups.

Based on the above equation, the sample size calculation indicated that approximately 20 subjects were needed for each arm of the trial to detect a change of 1.76 μU/mL in insulin levels between the treatment and placebo groups, with 80% power and 5% significance. The standard deviation

(SD) was assumed to be 2.1. Therefore, $n = 2 * (1.96 + 0.84)^2 * (2.1)^2 \div (1.76)^2 \approx 22$ participants per group.

To account for potential participant dropouts and to enhance the statistical power of the analysis, the number of participants was increased to 26 women per group.

Overview of the study design and procedures

The study employed a two-arm parallel design, consisting of 52 participants who were randomized into two groups. The Greek yogurt group (n = 26) received one serving (380 g, 200 kcal) of zero-fat Greek yogurt, and the peanut group (n = 26) received one serving (35 g, 200 kcal) of unsalted peanuts. Due to one participant from each group dropping out and not attending the second visit, only 25 subjects were included in the study analysis in each group.

All participants attended two visits to the clinic. During the initial visit, participants were instructed to arrive fasting for 10-12 hours, during which anthropometry and body composition analysis were conducted. They also received education and instructions to follow a three-day low-calorie diet totaling 1200 kcal, comprising breakfast, snack 1, lunch, snack 2, and dinner, prior to the interventions. On the second visit (post-diet visit), participants received and consumed their snacks, completed the visual analog scale (VAS) questionnaire and blood samples were withdrawn.

Treatment/intervention and randomization

Intervention

Two distinct interventional snacks were provided to the participants: plain Greek yogurt and peanuts. In the Plain Greek yogurt group (n=25), each participant consumed 380g of plain Greek

yogurt, equating to approximately 200 calories. In the peanut group, (n=25) each participant consumed 35g of roasted, unsalted peanuts, providing approximately 200 calories.

The plain Greek yogurt (non-flavored /30g protein/13.5g carbohydrate/0g fat) was procured from the supermarket and stored in the refrigerator until serving. The peanuts (roasted, unsalted, with skin/ 8.4g protein/7.6g carbohydrate/17.5g fat) were purchased one day prior to the serving date to preserve their attributes. They were meticulously prepared in a safe and sterile manner, portioned into small transparent pouches, each containing 35g. These packed peanuts were then stored in a dry, room-temperature environment until the serving date. Table 1 shows the macro and micronutrients composition of the two interventional snacks.

The intervention was provided to each participant upon arrival at the clinic based on pre randomization and they were asked to consume it at 1:00 pm.

Randomization

The participants were assigned to two groups in sequentially, following a predetermined randomization list. “Peanut” and “Greek yogurt” were each written clearly in Arabic on 26 folded papers. The list was created by randomly selecting folded papers from a basket, each containing either the label "peanut," or "plain Greek yogurt". The groups were then identified as the peanut group and the Greek yogurt Group based on the random selection of each participant .

All participants adhered to a 1200 kcal diet plan, including breakfast, snack 1, lunch, snack 2, and dinner. This diet plan remained consistent for all participants over three consecutive days, except during the second visit, where specific snacks were provided based on the randomization list.

Data collection and assessment

Pre-diet visit

During the initial visit, participants were provided with a three-day low-calorie diet menu totaling 1200 kcal. This menu included breakfast, snack 1, lunch, snack 2, and dinner, along with a follow-up sheet. They were instructed to adhere to the foods listed in the diet menu and to refrain from consuming peanuts or any peanut-based products, as well as Greek yogurt, during snack 1 and snack 2. Additionally, they were briefed on the necessity of daily follow-up via phone to monitor their compliance with the given instructions. Body composition analysis was performed using bioelectrical impedance analysis (Inbody 770). To categorize participants into groups, they were randomly allocated based on a pre-determined randomization list.

Anthropometric and body composition analysis

Before the body composition analysis test, each participant visited the clinic after overnight fasting for a period of 10-12 hours. The analysis was conducted using bioelectrical impedance technology, which automatically recorded several parameters, including weight, height, BMI, skeletal muscle mass, soft lean mass, fat mass, and abdominal circumferences. To ensure precise readings, participants were advised to wear lightweight clothing and remove any metal items, such as accessories, belts, money, and socks before the test.

Post-diet visit

On the second visit, scheduled after three days of adhering to the 1200 Kcal diet, all participants arrived at the clinic at 1:00 pm. Prior to their arrival, they had breakfast at home at 9:00 am. Upon arrival, a trained healthcare professional collected baseline blood samples from each participant.

At 1:00 pm, participants in the plain Greek yogurt and peanut groups consumed their assigned snacks. Following snack consumption, a validated VAS questionnaire was distributed to assess sensations and appetite levels at 0 minutes (1:00 pm), 30 minutes (1:30 pm), and 60 minutes (2:00

pm). Additionally, one hour after their arrival, at 2:00 pm, a second blood sample was collected from all participants. Throughout this period, participants were situated in a stress-free environment designed for relaxation and comfort, with soft music played to enhance their experience. No adverse events were reported by any of the participants following snack consumption.

Appetite perceptions

Appetite sensation was evaluated using a validated VAS questionnaire, designed to measure subjective ratings of hunger, fullness, satiety, prospective food consumption, and desire to eat. The VAS questionnaire included questions assessing "how strong is your feeling of" hunger, fullness, and desire to eat or "how much food can you eat right now" with anchors of "not (much) at all" to "extremely/an extreme amount".

Participants were instructed to indicate their sensations on the scale at various time points during the study: upon arrival, 30 minutes, and 60 minutes after consuming the snack. This allowed us to discern the impact of consuming Greek yogurt and peanuts on appetite perceptions over time. The decision to assess at 30 and 60 minutes was based on the rapid increase in gut hormones following food intake, with their secretion peaking around 1-2 hours later. The VAS questionnaire was self-administered, ensuring a convenient and straightforward assessment for the participants.

Biochemical analysis

The collected blood samples (baseline and after 60 minutes of intervention) were labeled with a unique code to ensure proper identification before being transported to the medical laboratory.

The blood samples were drawn into plain tubes containing separation gel and left undisturbed until coagulation occurred. After coagulation, serum was separated from the blood cells through centrifugation at 882 *xg* for 20 minutes. The resulting serum was then carefully transferred into Eppendorf tubes and stored at -20 °C to preserve the integrity of the peptides for subsequent analysis. These storage procedures adhered to the recommendations outlined in studies conducted by Hallworth and Lobely.

All biochemical blood assays were conducted in duplicate, and the average of these duplicate measurements was utilized in the statistical analysis.

CKK, PYY, GLP-1 and insulin analysis

The blood samples were sent to the laboratories of Aurum Biotechnology Company, where the researcher conducted the analysis following the instructions provided in the respective manuals. The analysis utilized the following ELISA kits: Human PYY ELISA kit (Lot No: 9680012101) (catalog no. RK02174; ABclonal, USA), Human GLP-1 ELISA kit (Lot No: 9680012107) (catalog no. RK09098; ABclonal, USA), Human CCK ELISA kit (Lot No: 1210528141) (catalog no. MBS770851; MyBioSourceA) and Human Insulin ELISA kit (lot No: 9680004240821) (Catalog. no. RK 00302; ABclonal, USA).

Ghrelin analysis

The analysis of GHRL (ghrelin) was carried out using the Human GHRL ELISA kit (Lot No: 38401422) (catalogue no. MBS2602099; MYBioSource, USA). This kit utilizes the Competitive-ELISA method. The well plates used for the analysis were pre-coated with GHRL. In the reaction, the GHRL present in the sample or standard competed with a fixed amount of GHRL on the solid phase supporter for binding sites on the Biotinylated Detection Antibody specific to

GHRL.

Statistical analysis

The statistical analysis was conducted using SPSS version 25.0 (IBM SPSS Statistics for Windows, IBM Corporation). Before analysis, a nonparametric Kolmogorov-Smirnov test was employed to assess the normal distribution of all continuous variables. Descriptive statistics were used to assess the frequencies of categorical variables, while means ± standard deviations (SD) were calculated for continuous variables.

To determine differences between the means of normally distributed continuous variables in both the plain Greek yogurt and peanut groups, a Student t-test for independent samples was employed. Additionally, a paired sample t-test was used to assess variations in means of normally distributed variables before and after snack consumption in both groups. In instances where variables displayed significantly skewed distributions, medians were compared using the Mann-Whitney U test for independent samples, while paired samples were compared using the Wilcoxon Signed Rank test. A two-way repeated measures ANOVA was performed to test the main and interaction effects of Greek yogurt, peanuts, and time on appetite and insulin hormones. Additionally, the repeated measures procedure of the General Linear Model (GLM) was utilized to analyze the impact of plain Greek yogurt and peanuts on appetite perceptions over time.

Correlations between gut hormones, insulin, and anthropometric measurements were assessed using the Pearson two-tailed bivariate test for continuous variables. All p-values are two-tailed, and a significance level of $p \leq 0.05$ was considered.