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1. Background

Obesity is one of the global public health problems in spite of all efforts of the scientific community and public health strategies. In the most recent World Health Organization data, 39% and 13% of adults aged 18 years and over were reported as overweight and obese respectively (W.H.O., 2016). Obesity has been also shown to be associated with a range of health-related problems, including hypertension, dyslipidaemia, type 2 diabetes, cardiovascular disease, and cancer (Guh, et al., 2009). Recently, low-glycaemic index (GI) diets have been recommended as an alternative dietary approach for managing obesity and its associated comorbidities, because they may be more effective than traditional energy-restricted low-fat diets at reducing body weight and controlling glucose and insulin metabolism (Juanola-Falgarona, et al., 2014). In addition to improved glycaemic control, increased satiety or decreased food intake have been shown after consuming low-GI diets in short-term studies (Lennerz, et al., 2013; Zafar, Kabir, & Ghazaii, 2011). However, obesity has been linked to low levels of physical activity and Western-style diets, enriched in high-GI foods such as highly refined carbohydrates, sugary beverages, etc. (Varlamov, 2017). Thus, strict adherence to low-GI diets may be difficult for obese people who consumed the Western-style diets for a long time.

On the other hand, recent studies have suggested that gut microbiota may be a novel factor involved in body weight management, and play a role by the regulation of energy harvest, fat storage and appetite (Firouzi, 2018; Pimenta, et al., 2018). Therefore, the gut microbiota modulation using pre- or probiotics has led to much interest in its therapeutic potential. Dietary intervention studies have unveiled that probiotics may help to lose weight by providing appetite control (Belguesmia, et al., 2016), and reducing food intake (Zoumpopoulou, Pot, Tsakalidou, & Papadimitriou, 2017). However, it has been yet unknown the potential effects of probiotics combined with low- or high-GI meals/diets on satiety or voluntary food intake.

2. Objective

The aim of this study was to determine whether kefir, a natural probiotic, would have provided any beneficial effect on appetite sensations and subsequent food intake, when consumed with low- or high-GI meals.

3. Materials and methods

3.1. Participants

Twenty four healthy, normal-weight (BMI 18.5–25 kg/m2) females, aged 21–24 years, were recruited from Erciyes University and the surrounding community. Exclusion criteria were following an energy-restricted diet during the last three months, change in body weight >5 kg during the last three months, lack of appetite, physician-diagnosed conditions or medications that influence metabolism, having any chronic diseases such as diabetes, hypertension etc., smoking, practicing endurance sports, having difficulties with swallowing/eating, hypersensitivity for the food products under study, being a vegan, and pregnant or lactating. Furthermore, one enrolled participant withdrew from the trial and the other one was not

included to the analysis of data because of influenza. Finally, leaving a total of 22 participants were assessed for the main outcomes (Fig. 1).

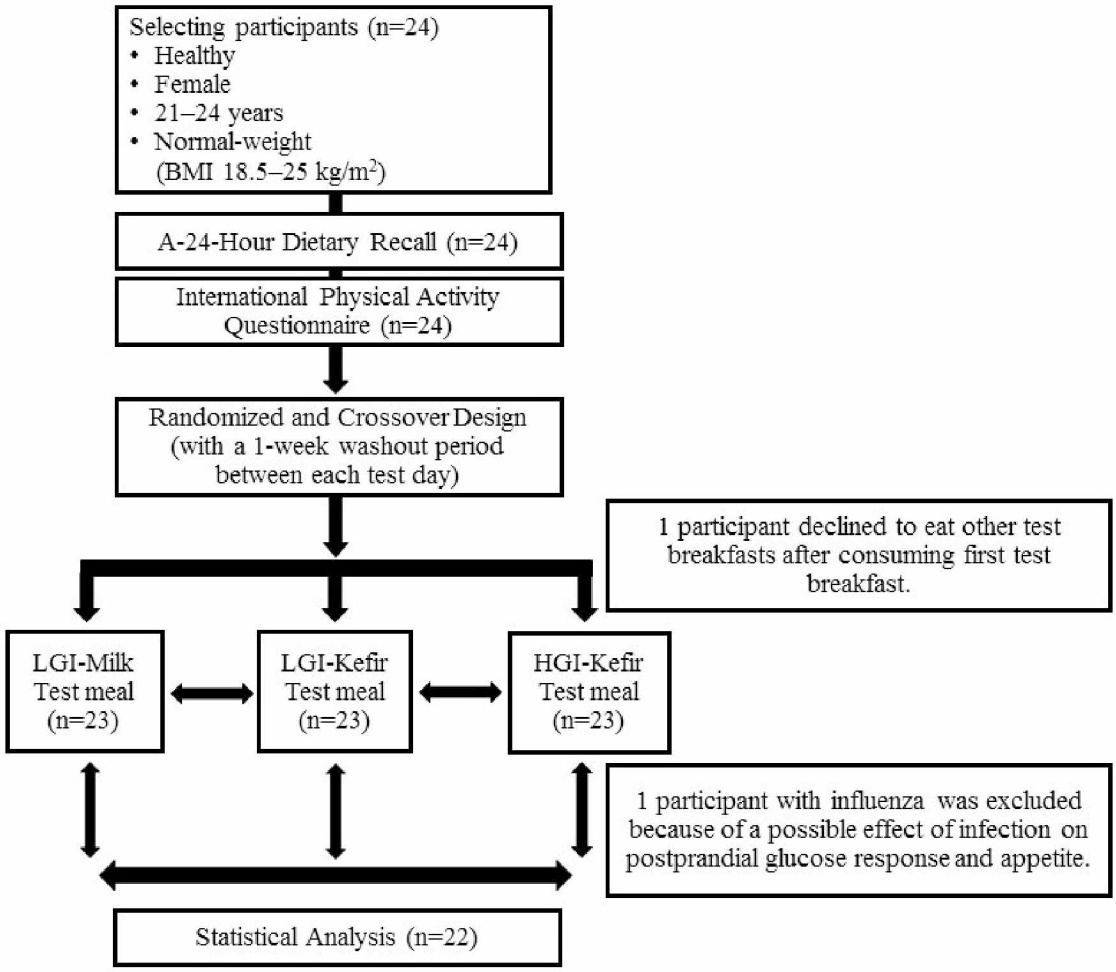

Figure 1. Participant recruitment flow diagram

3.2. Nutritional evaluation

Body weight and height of participants were obtained, and BMI was calculated. Waist and hip circumference were measured using a non-elastic tape with the participants standing, with the face directed towards, shoulders relaxed, and the tape was positioned at a level parallel to the floor.

Participants' dietary intakes were assessed by the a-24-hour dietary recall using a photographic atlas of food portion sizes to quantify the data in the beginning of study and on the day of each test meal. Diet composition was analysed by the BeBiS Nutrition Information System software version 7.2.

Physical activity level was evaluated simultaneously with dietary assessment by the International Physical Activity Questionnaire (IPAQ) short form, a validated survey instrument (Saglam, et al., 2010). The 7-item IPAQ records self-reported physical activity in the last seven

days. Responses were converted to Metabolic Equivalent Task (MET) minutes per week according to the IPAQ scoring protocol.

3.3. Study design

This was a randomized, single-blind, 3-intervention crossover trial conducted on 3 separate days, with a 1-week washout period between each study day. All participants were randomly submitted to three different test meals with the following different GI amounts and milk or kefir: a low-GI and milk content (LGI-Milk), a low-GI and kefir content (LGI-Kefir), and a high-GI and kefir content (HGI-Kefir). The order of the test meals was determined by using a computer-generated randomization sequence before recruitment. The primary outcomes were appetite sensations and subsequent food intake. Desire for specific food types and palatability of test meals were the secondary outcomes.

Participants received each test meal in a randomly assigned order on three different mornings separated by a washout period of 1-week when they were asked to maintain their usual diet and physical activity. On the day before each test meal, participants were instructed to eat a standard evening meal at 21:00 h and to refrain from eating and/or drinking (except for water) and/or doing any physical activity beyond that of their typical daily activities. Moreover, participants were tested within the follicular phase of their menstrual cycle (3–10 d after onset of menses) to avoid possible influences of menstrual cycle phase on hormonal changes and appetite (Chowdhury, Richardson, Tsintzas, Thompson, & Betts, 2015).

On the each testing day, participants arrived in the testing room at 08.30 h following a 12-h fast and anthropometric measurements were completed before eating the test meal. Also, first appetite scores were measured for baseline measurements (time zero). At 09:00 h participants received the test meal blinded to its nutritional characteristics and were asked to consume within 15 min. During the postprandial period, participants remained at rest in the testing room and appetite scale was applied at 15, 30, 60, 90, 120, 150 and 180 min. Moreover, participants were asked to assess the palatability of test meal at 15 min (immediately after consuming test meal). No food or drink other than water was allowed following consumption of the test meal until an ad libitum lunch. Water was available ad libitum throughout the first trial; however, the volume consumed was measured and the participants drank the same volume during the second and third trial. Participants were permitted to watch movies, read, or play with electronic devices (laptop computer, mobile phone etc.) or undertake other similar sedentary activities throughout each test day but were not allowed to sleep.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures were approved by the Clinical Research Ethics Committee of the Erciyes University (2016/547) on 21 October 2016, and all participants gave written informed consent. In addition, it was registered at ClinicalTrials.gov as NCT03636217.

3.4.Test meals composition

All test meals were matched for energy, LGI-Milk and LGI-Kefir meals also for macronutrients and GI, but HGI-Kefir meal had a nearly 2-fold difference in GI (Table 1). Furthermore, both milk and kefir drinks contained 120mg/100ml calcium, and kefir drink also had 107 CFU/g

probiotic bacteria (Lactobacillus spp. and Streptococcus spp.). The energy content of test meals (~445 kcal) were estimated as corresponding to about 25% daily energy needs of a sedentary female (Chowdhury, et al., 2015), and the daily energy needs were also calculated by the Schofield equation, taking into account: gender, age, weight and a physical activity level of 1.3.

Table 1. Nutritional composition and glycaemic index of the component foods in test meals

	Portion size (g/ml)	Energy (kJ[kcal])	AvCHO* (g)	Protein (g)	Fat (g)	GI (%)
LGI-Milk						
Milk (full-fat)	200	524(125)	9.9	5.8	6.8	31
Cheddar cheese	25	424(101)	0.0	6.4	8.5	0
Apple	200	469(112)	24.9	0.9	0.9	36
Grain bread	50	435(105)	19.1	4.8	0.9	50
Toplam		**1852(443)**	**53.9**	**17.9**	**17.1**	**40**
LGI- Kefir						
Kefir (full-fat, plain)	200	526(126)	10.0	5.8	6.3	36
Cheddar cheese	25	424(101)	0.0	6.4	8.5	0
Apple	200	469(112)	24.9	0.9	0.9	36
Grain bread	50	435(105)	19.1	4.8	0.9	50
Toplam		**1854(444)**	**54.0**	**17.9**	**16.6**	**41**
HGI- Kefir						
Kefir (full-fat, plain)	200	526(126)	10.0	5.8	6.3	36
Raspberry jam	50	568(136)	31.8	1.7	0.0	78
White bread	75	773(185)	34.0	7.0	1.5	70
Toplam		**1868(447)**	**75.8**	**14.5**	**7.9**	**70**

*AvCHO, available carbohydrate including sugars and starch, excluding fiber.

GI of foods in test meals was estimated by using the GI tables, with glucose as the reference food (Bao, Atkinson, Petocz, Willett, & Brand-Miller, 2011). The average meal GI was calculated as follows:

$$\text{Meal GI} = \frac{\sum_{a=1}^{N} (GI_a \times AvCHO_a \times Frequency_a)}{\sum_{a=1}^{N} (AvCHO_a \times Frequency_a)}.$$

where n is the number of foods consumed, GIa is the GI for food a, AvCHOa is the available carbohydrate content per serving of food a, and Frequencya is the consumption frequency of one serving of food a during the meal.

3.5. At libitum lunch

At 180 min after the test breakfast, participants were presented with the ad libitum lunch following appetite measurements. Frequently consumed foods were used to prepare the lunch consisting of pasta with tomato sauce, yogurt drink and mandarin. Participants were asked to

consume whatever they wanted and to eat until they felt comfortably full. Foods were weighted or measured to the nearest 0.1 g before consumption, and any remaining food was reweighed to determine intake at lunch. Energy and macronutrient values were calculated using The National Food Composition Database (TurKomp), and manufacturer labelling.

3.6. Assessment of appetite sensations

Subjective assessment of appetite sensations was performed by using a visual analogue scale (VAS) composed of lines (of 100 mm in length) with words anchored at each end, expressing the most positive and the most negative rating. VAS was used to assess appetite scores (hunger, fullness, desire to eat, and prospective food consumption), desire for specific food types (sweet, salty, savoury, and fatty) and the palatability of test meals (visual appeal, smell, taste, after taste, and overall palatability) (Flint, Raben, Blundell, & Astrup, 2000). A series of VAS were administered using the paper-and-pen method at specific time-points during the examination period. Participants were asked to make a single vertical mark at the appropriate point between the 2 anchors on each scale corresponding to their feelings. A new VAS booklet was provided to participants for each rating time, and nobody could compare to his/her previous ratings when marking the VAS. Appetite scores were quantified by measuring the distance in millimetres between the left end of each line and the mark. Furthermore, the separate VAS components such as hunger, fullness, desire to eat and prospective food consumption were combined to produce an additional measure termed 'composite appetite score'. This validated average appetite score was calculated for each time point using the following equation: [(hunger + desire to eat + prospective food consumption + (100 - fullness)]/4 (Leidy, et al., 2015).

4. Statistical Analysis Plan

4.1. *Sample size*

A power-based sample size calculation based on previous research from Stevenson et al. (Stevenson, Astbury, Simpson, Taylor, & Macdonald, 2009) revealed that 21 participants were needed to provide 80% power to detect 5% difference between groups in primary outcomes. To allow discontinuation during the study, 24 participants (considering 15% losses) were recruited, and the study was concluded with 22 participants.

4.2. Data analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 22.0; IBM SPSS Statistics) software. Data were expressed as the mean ± SD unless otherwise indicated. Normality was assessed using the histogram and normal Q-Q plots, and also Shapiro-Wilk test. Furthermore, continuous variables were examined for skewness and kurtosis, and log-transformed before analysis and reported back-transformed geometric means (G) ± standard error (S.E) when required (Akin, et al., 2015). Postprandial appetite sensations were quantified as area under the curve (AUC) calculated according to the trapezoidal rule. One-way (1-factor) analysis of variance (ANOVA) for repeated measures was applied to determine statistical differences between groups. In addition, the data were analysed by using 2-factor (time x meal) repeated-measures ANOVA, and Bonferroni post hoc tests were applied to significant time x

meal interactions. For all statistical analyses, p values less than 0.05 were considered to have statistical significance.

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