



Trabajo Original

Obesidad y síndrome metabólico

Effects of a functional yogurt enriched with soluble dietary fiber or vegetable proteins on appetite profile. An acute randomized controlled clinical trial

Efectos de un yogur funcional enriquecido con fibra dietética soluble o proteína vegetal sobre el perfil del apetito. Ensayo clínico controlado, aleatorizado y de corta duración

Denisse Chu-Montiel¹, Samara Palma-Milla^{1,2}, Bricia López-Plaza¹, Marlhyn Valero-Pérez¹, Carmen Gómez-Candela^{1,2}, Laura M. Bermejo³

¹Nutrition Research Group. Instituto de Investigación Hospital Universitario La Paz (IdiPAZ). Madrid, Spain. ²Dietetics and Clinical Nutrition Department. Hospital Universitario La Paz. Madrid, Spain. ³Department of Nutrition and Food Science. Faculty of Pharmacy. Universidad Complutense de Madrid. VALORNUT Research Group. Universidad Complutense de Madrid. Madrid, Spain. Fundación para la Investigación Biomédica del Hospital Clínico San Carlos (IdISSC). Madrid, Spain

Abstract

Introduction: designing functional foods to control appetite could be a useful strategy for managing overweight and obesity. Fiber and proteins could be interesting ingredients to consider.

Objectives: to evaluate the appetite profile of two experimental yogurts (fiber-enriched [FEY] and vegetable protein-enriched [PEY]) versus a control yogurt (CY) in a group of overweight/obesity people.

Material and methods: an acute, randomized, double-blind, crossover clinical trial was carried out in a group of twelve healthy overweight/obesity type I people; randomized to consume 3 yogurts in a different order for 3 acute study days. The appetite profile (1. hunger, 2. satiety, 3. fullness, 4. prospective food consumption, 5. desire to eat something fatty, salty, sweet or savoury) was assessed using a Visual Analog Scale (ranging from 0 "not at all" to 10 "extremely") at 12 moments in each acute study. Additionally, total food consumption in an ad libitum lunch was assessed.

Results: FEY produce a significantly lower desire to consume any food at 30 (1.50 ± 0.42) and 60 minutes (2.78 ± 0.42) after consumption compared to PEY (3.46 ± 0.53 ; 4.33 ± 0.54) and CY (3.27 ± 0.69 ; 4.0 ± 0.78) respectively ($p < 0.016$). Also, FEY consumption produced a higher satiety and fullness and a lower desire to ingest something fatty, salty or savory after 90 minutes consumption compared to the other products, but the difference was not significance.

Conclusion: FEY might be a good functional food prototype to control appetite in overweight and obese people.

Keywords:

Obesity. Appetite. Energy intake. Satiety. High fiber foods. Protein.

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Correspondence:

Laura M Bermejo. Department of Nutrition and Food Science. Faculty of Pharmacy. Universidad Complutense de Madrid. Avda. Complutense, s/n. 28040 Madrid
e-mail: mbermej@ucm.es

Resumen

Introducción: el diseño de alimentos funcionales para controlar el apetito podría ser una estrategia útil para controlar el sobrepeso y la obesidad. La fibra y algunas proteínas podrían ser ingredientes interesantes a tener en cuenta.

Objetivos: evaluar el perfil del apetito de dos yogures experimentales (enriquecido en fibra [YEF] y enriquecido en proteínas vegetales [YEP]) frente a un yogur de control (YC) en un grupo de personas sanas.

Material y métodos: se llevó a cabo un ensayo clínico agudo, aleatorizado, doble ciego y cruzado en un grupo de doce personas sanas con sobrepeso/obesidad de tipo I; aleatorizadas para consumir los 3 yogures en un orden diferente. El perfil del apetito (1. hambre, 2. saciedad, 3. plenitud, 4. consumo prospectivo de alimentos, 5. deseo de comer algo graso, salado, dulce o salado) se evaluó mediante una escala visual analógica (de 0 "nada" a 12 "extremadamente" puntos) en 12 momentos del estudio agudo. Además se evaluó el consumo total de alimentos en un almuerzo ad libitum.

Resultados: el consumo de YEF produjo un menor deseo de ingerir algún alimento a los 30 ($1,50 \pm 0,42$) y 60 minutos ($2,78 \pm 0,42$) después de su consumo, comparado con el YEP ($3,46 \pm 0,53$; $4,33 \pm 0,54$) y el YC ($3,27 \pm 0,69$; $4,0 \pm 0,78$), respectivamente ($p < 0,016$). Además, con el consumo de YEF se produjo una mayor saciedad y plenitud y un menor deseo de ingerir algo graso, salado o sabroso desde los 90 minutos posteriores a consumir el yogur en comparación con el YEP y el YC, aunque las diferencias no fueron significativas.

Conclusión: el YEF podría ser un buen prototipo de alimento funcional para controlar el apetito en personas con sobrepeso y obesidad.

Palabras clave:

Obesidad. Apetito. Ingesta energética. Saciedad. Alimentos ricos en fibra. Proteínas.

INTRODUCTION

Overweight and obesity have become one of the most relevant public health problems worldwide. Excess weight is a risk factor for numerous pathologies, highlighting metabolic syndrome, type 2 diabetes *mellitus* and cardiovascular diseases. For this reason, it is important to seek for strategies that help reduce body weight. It is a fact that the accumulation of body fat that defines obesity is fundamentally a reflection of positive energy balance, where energy consumed as food intake exceeds that energy expended (1). Regulation of appetite and control of satiety to reduce the food intake could be a nutritional strategy aiming to prevent obesity development (2).

The intensity and duration of the hunger and satiety effects are determined by different factors, including the nutrient composition of foods and beverages (2). Among the nutritional components with "satiating power" fiber or protein are typically a good choice for promoting satiety (3). Dietary fiber could promote weight loss mainly through three mechanisms: by promoting a decrease in energy intake through increased satiety, and/or reducing efficiency in the absorption of energy nutrients, and/or improving glucose tolerance and decreasing insulin levels (4). Moreover, diets with a high fiber content require more chewing time, which slows down swallowing speed. Also, in the stomach, soluble fibers, as a consequence of their viscosity, slow down gastric emptying and increase gastric distention, providing longer periods of satiety sensation (5) Finally, high fiber intake may affect gut hormone secretion, independent of glycemic response, and has been associated with lower BMI and consequently a lower risk and progression of type 2 diabetes, cardiovascular disease, and cancer (6).

Furthermore, different studies have documented that protein intake could have greater satiety potential and lead to greater weight loss compared to other macronutrients. Different studies have described that protein produces an increase in satiety hormones and activate metabolic signals that reduce appetite (7,8).

Because of this, carrying out strategies that increase the consumption of food components such as fiber and protein being harnessed to interact with the physiology to naturally limit calorie

intake could be of great interest to control the mechanisms of satiety and appetite and consequently achieve benefits in controlling body weight (1,2).

According to this, designing functional foods enriched in these nutrients could be very useful as a strategy to promote satiety and control weight (9). Different studies have evaluated the satiating power of natural foods or functional foods designed to optimize their satiating effectiveness, improve long-term satiety, and facilitate body weight control (10-12). Such functional foods include dairy products (yogurts), cereals, ready-to-eat meals, and even snack foods (chocolate bars). Among these, dairy products and specially yogurt do not provide a high caloric load and are easy to consume "between meals" (mid-morning, afternoon snack) for most of the population, so they are usually a food of choice to be consumed at those times of the day (11). Moreover, yogurt naturally has a high protein content and also possesses rheological and organoleptic properties that could contribute to reinforce its potential to positively influence satiety (8,11). In addition, different studies have described the health benefits of yogurt and fermented milks due their high nutritional value, leading to an ideal vehicle for functional foods development (11,13).

Currently to date there are few clinical trials that have evaluated the satiating effect of functional foods of dairy products, particularly yogurts enriched with fiber or protein intended to have satiating effects.

Based on these facts, an acute study was conducted to evaluate the satiating properties of two functional yogurts (yogurt with inulin, wholegrain oat flour, rye bran, and poppy seeds added); yogurt with pea protein) using a hunger and satiating Visual Analog Scale in a group of healthy overweight subjects.

MATERIALS AND METHODS

STUDY DESIGN

An acute, randomized, double-blind and crossover clinical trial was designed to evaluate the satiating properties of two experimental yogurt prototypes (an enriched-in-fiber yogurt and a pro-

tein-enriched yogurt) versus a control yogurt in a group of healthy overweight people.

Subjects were randomized by sex and were assigned to one of the three products of the study (fiber-enriched yogurt [FEY], protein enriched yogurt, [PEY] and control yogurt [CY]). All the yogurts were already commercialized products made from cow milk with the following characteristics: FEY (natural yogurt with inulin, wholegrain oat flour, rye bran and poppy seeds added) was selected because of a European approved nutritional claim of “high fiber content” (contains at least 6 g of fiber per 100 g); PEY (Greek yogurt with pea protein) was selected because pea protein was an alternative, sustainable plant-based high quality protein with low allergenicity (14) and potential satiating power (15), and because of a European approved nutritional claim of “source of protein” (at least 12 % of the energy value of the food is provided by protein); and CY (natural yogurt).

Each subject underwent 3 experimental phases, attending 3 visits, with a washout period of 7 days between them, in which they took the assigned yogurt.

The nutritional composition of each yogurt is specified in table I. All study products (experimental and placebo) were supplied by the company DELAFRUIT SLU (formerly known as Go Fruselva SL) in a “yogurt” container of 125 grams.

The containers of the 3 yogurts were only distinguished by an identification number established by the company (yogurt 1, 2 and 3), without the research team knowing at any time which one was which, in order to guarantee the double blind approach.

Once the study was completed, unblinding was performed, non-compliance with the protocol was verified, and the data was reviewed and analyzed.

SUBJECT SELECTION AND ALLOCATION

A total of 12 healthy overweight and obesity type 1 volunteers (6 men and 6 women), were recruited through the Clinical Nutrition and Dietetics Unit of La Paz University Hospital (HULP) in Madrid, Spain. The inclusion criteria were: ages between 18 and 50,

BMI between ≥ 25 and < 35 kg/m², with an education level sufficient to understand the study. All of the subjects signed an informed consent. The exclusion criteria were: BMI < 25 or ≥ 35 kg/m²; subjects who followed a vegetarian diet pattern or had a fiber intake ≥ 30 g/day; individuals with diabetes *mellitus*, dyslipidemia or arterial hypertension under drug treatment; smoking or alcohol use > 2 -3 servings/day in the case of men, and > 1 serving/day in women; having lost or gained more than 4 kg or adhered to weight loss diets in the last 6 months; subjects with gastrointestinal diseases affecting the digestion or absorption of nutrients; and pregnant or breastfeeding women. Finally, subjects with intense physical activity, allergies or lactose intolerance, celiac disease or gluten intolerance, and those who rejected the consumption of the foods included in the study (yogurt, potato omelette and bread) were not included.

All 12 volunteers were randomized into one of the three study sequences based on their sex (Fig. 1). The randomization procedure was carried out by the HULP Biostatistics Unit by assigning an “identification number” according to a randomization table to consume the 3 study yogurts in a different order (Y1-Y2-Y3: $n = 4$; Y3-Y1-Y2: $n = 4$; Y2-Y3-Y1: $n = 4$).

The research protocol was approved by the HULP Clinical Research Ethics Committee (code 5007) under the regulations described in the Declaration of Helsinki (16).

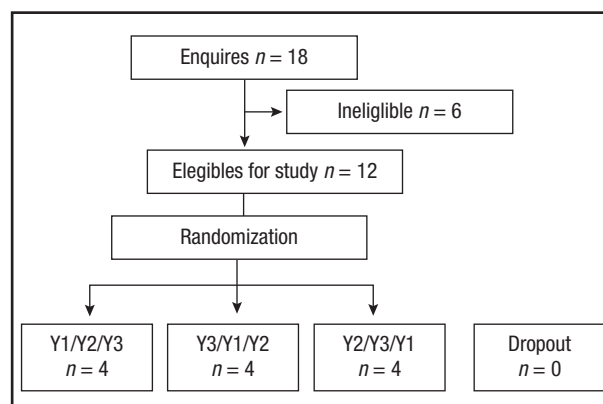


Figure 1.

Consort flow diagram of the recruitment, enrollment, and random assignment processes.

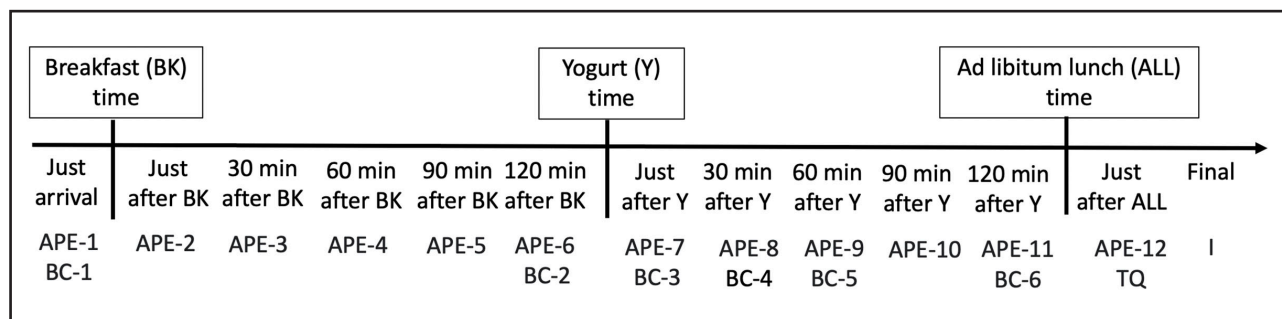
Table I. Experimental and control composition of yogurt per 100 g

		FEY	PEY	CY
Energy	kcal	103	101	67
Total fat	g	1.1	2.9	1.8
Saturated fat	g	0.7	1.9	1.2
Protein	g	1.3	3.7	2.1
Total carbohydrate	g	11	14	10
Sugars	g	8.0	8.7	7.8
Fiber	g	8.6	1.8	1.0
Sodium	g	0.03	0.084	0.1

CONDUCTION OF THE STUDY

This study was carried out according to European Food Safety Authority requirements (17). All the participants delivered the signed informed consent to participate and were scheduled to attend to 3 visits in the HULP clinical Research and clinical trials Unit. In each visit, the following protocol was followed (Fig. 2).

- Just after arrival: blood pressure and heart rate measured, anthropometric study, dietary study, physical activity study, 1st blood collection (BC) and 1st appetite profile evaluation (APE).

**Figure 2.**

Diagrammatic representation of the experimental protocol in each visit (BC: blood collection; APE: appetite profile evaluation; TQ: tolerance questionnaire; I: instructions for exit).

- Breakfast time: including milk (200 ml), white bread (60 g), grated tomato (30 g), extra virgin olive oil (10 g) and orange juice (200 ml). Participants had a maximum of 15 minutes for consumption.
- Just after breakfast: 2nd APE.
- 30 min after breakfast: 3rd APE.
- 60 min after breakfast: 4th APE.
- 90 min after breakfast: 5th APE.
- 120 min after breakfast: 2nd BC and 6th APE.
- Yogurt time (P1, P2 o P3). Participants had a maximum of 10 minutes to eat their assigned yogurt according to the randomization scheme.
- Just after the yogurt: 3rd BC, 7th APE and a product sensory perception evaluation.
- 30 min after the yogurt: 4th BC and 8th APE.
- 60 min after yogurt: 5th BC and 9th APE.
- 90 min after yogurt: 10th APE.
- 120 min after yogurt: 6th BC and 11th APE.
- Ad libitum lunch time: including potato omelette and bread. Participants were able to eat as much of the food offered as they wanted and 500 ml of water in a maximum time of 20 minutes.
- Just after lunch: 12th APE and a tolerance questionnaire to the product consumed was collected.

As shown in figure 1, appetite sensation ratings were obtained at 12 specific time-points throughout the entirety of the testing visit. During the visit, no eating or drinking was allowed during the 4 hours of the intervention; reading, studying, talking or listening to music was allowed, but they were not allowed to sleep. It is important to highlight that the formulation of the yogurt consumed as a snack was the only methodological characteristic that was different between each visit.

Before finishing the visit, the researchers gave them a 24-h record to complete with all the foods they consumed throughout the day of the intervention in order to control the overall food consumption during the acute study day.

Finally, the participants were instructed to maintain their usual activity and food consumption pattern until the next visit. Also, to control this aspect, researchers gave them a 72-h food record, a food frequency questionnaire and the “International Physical Activity Questionnaire” short form (18) to be completed 3 days prior to the next visit 7 days later.

OUTCOMES

For the present article the primary outcome was the appetite profile assessment using the validated Visual Analog Scale (VAS) and the secondary outcome was the total food consumption in the ad libitum lunch offered in each visit. Further variables will be explored in future articles.

APPETITE PROFILE EVALUATION

The appetite profile was assessed using a validated VAS ratings on sensation of hunger, satiety, fullness, prospective food consumption, desire to eat something fatty, salty, sweet or savoury, and palatability of the meals (Raben et al., 1995).

Subjects rated appetite profile sensations using a 10-cm scale ranging from 0 (“not at all”) to 10 (“extremely”) (19,20). This questionnaire was completed in twelve moments according to the previous described protocol.

The appetite was also controlled assessing the total food consumption in the ad libitum lunch offered in each visit such as detailed in the previous protocol (Fig. 1) using a weighed record. Researcher served a weighed homogeneous ad libitum lunch and subjects were given a self-served ad libitum lunch at each visit on the study day. The lunch consisted of bread and a Spanish omelette made with potatoes, onions and eggs, cooked in olive oil. Subjects were instructed to eat until pleasantly satiated. The staff at the study site weighed any remaining food, and total food consumption in the ad libitum lunch (g) was calculated.

Finally, two scores related with appetite profile was calculated: the composite appetite score (CAS = [satiety + fullness + (100 - prospective food consumption) + (100 - hunger)] / 4) as a global measure of satiety (19), and the appetite score (AS = [desire to eat + hunger + (100 - fullness) + prospective food consumption] / 4) as a global measure of the motivation-to-eat (21).

COMPLIANCE VARIABLES

Participants were instructed to maintain their usual physical activity and food consumption pattern between visits. To control

this aspect, researchers given them a 72-h food record, a food frequency questionnaire and the “International Physical Activity Questionnaire” in the short format (18) to be completed 3 days prior to the next visit 7 days later. These questionnaires were given to the subjects at the end of the screening visit and at the end of visit 1 and visit 2 of the intervention study. In addition, blood pressure and heart rate measures and anthropometric variables (weight, height and waist circumference) also were controlled during the study. Weight was measured on a digital scale for clinical use (capacity, 0-150 kg) and height using a millimeter-precision stadiometer. Finally, the waist circumference was determined at the narrowest point between the last rib and the iliac crest, with the tape close to the skin, without compression.

STATISTICS METHODS

This being a pilot study, the sample size was chosen taking into account the study by Hess et al. 2011 (22).

The qualitative results have been expressed as absolute frequencies and percentages and the quantitative data as mean and standard deviation or median and quartiles. Before any statistical analyses, all variables were checked for normality using the Shapiro-Wilk test. To analyze the crossover study of the data, the area for each subject per treatment is calculated. In order to determine this, the tracking curve is parameterized with polygons and the total area is calculated as the sum of all the partial areas. To study areas and temporal evolution and rule out a cross effect or treatment period effect, a mixed variance model was fitted that included sequence, treatment and period. Post hoc multiple comparisons were performed to detect changes due to treatment at each time point and the Bonferroni correction was applied. Friedman’s non-parametric analysis of variance was carried out for the study of the influence of time in each of the treatments. For the study of the influence of treatment, the Wilcoxon signed rank test were evaluated at each time. In both cases the Bonferroni correction is applied. All statistical tests were considered

bilateral and, as significant values, those *p* less than 0.05. Data was analyzed with the statistical program SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and SPSS 25.

RESULTS

A diagram based on the selection and crossover random assignment of the participants who were involved in the study is shown in figure 2. A total of 18 people were interested in participating; 6 people did not meet the selection criteria, a total of 12 met the selection criteria and were randomized to carry out the study. Throughout the clinical trial, no individual dropped out of the study. All participants completed testing according to protocol.

Baseline demographic, anthropometric characteristics of the healthy adult volunteers are shown in table II. No significant differences were found among the subjects included in each sequence of the yogurt consumption. Moreover, the participants maintained their dietary and physical activity patterns during the week prior to each visit without significant changes observed in the results obtained in the questionnaires used (data not shown).

The mean age of the population was 33.6 ± 10.7 years and the sample was homogeneously distributed, being 50 % female and 50 % male in each sequence. The mean BMI was 30.02 ± 1.5 kg/m², that is, the mean of the population presented grade I obesity and mean waist circumference was 92.2 ± 21.6 cm, indicating a slightly elevated cardiovascular risk (CCi > 90 cm). The mean blood pressure was normal, being within the normal range for the population (120/80 mmHg). According to the physical activity, 50 % of the participants performed low physical activity (< 60 min/day). All study volunteers also reported sitting for 6-7 hours per day.

Figure 3 presents mean values of appetite profile at specific time-points during the testing visit using the VAS questionnaire. After analyzing the results in the primary outcome, significant differences were observed for some items included in the appetite

Table II. Baseline demographic, anthropometric characteristics of the healthy adult volunteers according to randomization (XSD)

Characteristic	Total	Sequence 1 FEY/CY/PEY	Sequence 2 CY/PEY/FEY	Sequence 3* PEY/FEY/CY
Gender M/F (%)	6 (50)/6 (50)	2 (50)/2 (50)	2 (50)/2 (50)	2 (50)/2 (50)
Age (years)	33.6 ± 10.7	33.75 ± 12	29.7 ± 11.3	37.25 ± 10.4
BMI (kg/m ²)	30.02 ± 1.5	29.5 ± 1.14	30.5 ± 1.7	30.0 ± 1.8
Waist circumference (cm)	92.2 ± 21.6	96.2 ± 4.3	80.3 ± 34.5	100.1 ± 14.5
Blood pressure (mmHg)				
Diastolic pressure	68.42 ± 8.1	72.5 ± 11.3	69.2 ± 7.3	63.5 ± 2.1
Systolic pressure	103.4 ± 9.7	106.7 ± 11.1	104.7 ± 11.5	98.7 ± 6.4
Dietary energy intake (kcal/day)	1971.2 ± 473.7	1951.7 ± 405.1	1940.5 ± 603.8	2021.5 ± 473.7

*There were no significant differences among sequences in baseline characteristics.

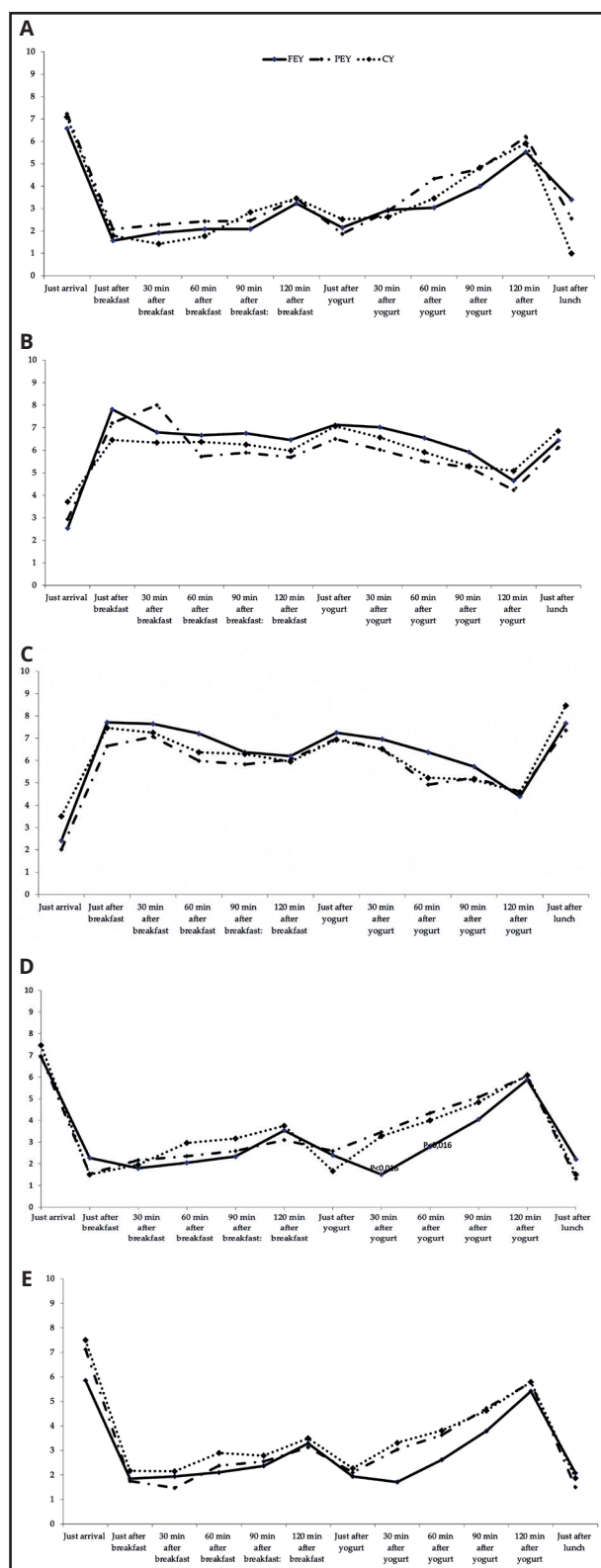


Figure 3.

VAS scores (0-10) for sensation of hunger (A), satiety (B), fullness (C), prospective food consumption (D) and desire to eat something fatty, salty, sweet or savoury, and palatability of the meals (E) after consumption of FEY, PEY and CY in different moments in the acute study.

profile evaluation between the consumption of the different study products using the VAS questionnaire.

Concerning the hunger sensation, FEY consumption produced a lower hunger sensation than PEY and CY both at 30, 60 and 90 minutes after yogurt consumption. However, no significant differences were observed (Fig. 3A). Also, no significant differences were observed related to the sensation of satiety or fullness, however, FEY produced a greater sensation of satiety (Fig. 3B) and fullness (Fig. 3C) than PEY and CY both at time 30, 60 and 90 min after yogurt consumption.

Regarding question four of the VAS (prospective food consumption) (Fig. 3D), the participants presented a statistically significant lower desire to consume any food when they consumed FEY versus PEY and CY, both at 30 minutes after yogurt consumption (FEY: 1.50 ± 1.53 ; PEY: 3.46 ± 1.92 ; CY 3.27 ± 2.48 , $p < 0.016$), and 60 minutes after yogurt consumption (FEY: 2.78 ± 1.50 ; PEY: 4.33 ± 1.95 ; CY: 4.00 ± 2.80 , $p < 0.016$). The desire to consume any food was also lower at 90 minutes after FEY consumption versus PEY and CY, but in this moment, the difference was not significant.

Figure 3E presents the results of the VAS regarding desire to eat something fatty, salty, sweet or savoury. The consumption of the FEY showed a lower desire to eat something fatty, salty, sweet or savory at 30, 60, 90, and 120 minutes after yogurt consumption, compared to PEY and CY. Despite of this, the differences are not statistically significant.

According to the total food (Spanish omelette and bread) consumed ad libitum in the acute study visit, it was higher when participants consumed CY (313.67 ± 121.35 g) versus FEY (281.5 ± 92.40 g) and PEY (250.5 ± 100.86 g). However, these differences were not significant. Moreover, the correlation values of CAS just before ad libitum lunch time with total food ad libitum was inverse, both for the total sample and for each treatment (total sample: -0.09 ; FEY: -0.19 ; PEY: -0.06 ; CY: -0.22 , non-significant). Therefore, higher satiety (higher CAS) corresponds to lower food consumption in the ad libitum meal. Regarding the association of AS just before ad libitum lunch time with ad libitum food intake, positive correlations were observed (total sample: 0.13 ; FEY: 0.31 ; PEY: 0.15 ; CY: 0.30 , non-significant), meaning that higher motivation-to-eat leads to a greater amount of food consumed ad libitum. However, none of the correlations were significant.

DISCUSSION

The present study shows that the design of functional yogurts enriched with fiber, might have a positive effect on the modulation of appetite and specifically on prospective food consumption, aiming this as a strategy to induce a negative energy balance and therefore prevent obesity development and its consequences.

In a recent systematic review of 136 intervention studies (107 acute, 29 long-term) evaluating the effects of various fiber interventions on appetite (23), it has been found that these effects can differ according to the type of fiber used. Specifically, this

review concludes that only alginate and guar gum (viscous soluble fibers), as well as oat fiber (one of the fibers included in the FEY prototype of our study), predominantly showed positive results in improving appetite profiles in acute studies. This review also mentions that dextrin intake had positive effects on appetite control in chronic studies.

Several mechanisms have been described on how dietary fiber contributes to increasing the sensation of satiety and reduces energy intake (24). For viscous soluble dietary fibers (such as the β -glucan present in the oats of FEY), their physiological effects are primarily due to their water-holding capacity. This property directly increases the fiber's volume and viscosity, which may cause delayed gastric emptying, nutrient absorption and increased volume of gastrointestinal contents, thereby subsequently influence satiety (24,25).

Moreover, soluble fiber is fermented by bacteria in the colon producing short-chain fatty acids (SCFAs) (26), and altering the secretion of gut hormones to enhance satiety (27). These SCFAs play a crucial role in regulating appetite and satiety by stimulating specific receptors in the enteroendocrine cells of the intestine, triggering the release of appetite-regulating peptides such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), thus enhancing the sensation of satiety (28).

In the case of the fiber in our FEY prototype, it is a mixed fiber. Therefore, our study design does not enable the assessment of the individual effects of each fiber type; instead, the observed effects are due to the combined action of all the fibers.

Regarding, fiber doses in which benefits are observed, Mah et al. (23) mention that positive effects on appetite profiles are seen with a dose of 2 g of alginate, > 3 g of guar gum, and 5 g of oat fiber. In our clinical trial, the FEY included a total of 10.75 g per serving (8.6 g/100 g) from the addition of inulin, whole oats, whole rye bran, and poppy seeds. Hence, FEY is a mixture of soluble and insoluble fibers.

On the other hand, Mah et al. (23) reported that most acute studies assess appetite using VAS within a range of 2 to 4 hours post-consumption of the product. In our study, we evaluated the appetite profile during the 2 hours following the consumption of the assigned prototype, making the design suitable for our purposes. Nonetheless, including a longer evaluation period could be of interest to assess the effect over a longer-term.

In regards to the considerable variability found, Mah et al. conclude that, unfortunately, the current evidence to date is not consolidated, and is highly varied due to the many differences in methodology employed for evaluating the effects on appetite (type of fiber, dose used, and evaluation times) (23). Consequently, the effects of individual fibers on satiety may not be predictable and requires testing (29).

Our study contributes to enhance the knowledge on the effects of prototype that include fiber mixtures on appetite profile. In fact, when evaluating prospective food consumption, a decrease in VAS ratings was observed in the participants who consumed FEY, being this a statistically significant difference, although this was not accompanied by a significant reduction in the ad libitum lunch. Moreover, associations between higher global satiety

score with lower food consumption in the ad libitum meal and a higher global motivation-to-eat score leads to a greater amount of food consumed ad libitum were observed in total sample, and with FEY, PEY and CY; however, these effects were not accompanied by a significant reduction or increase in food intake at the ad libitum lunch respectively. This often occurs because food cannot be expected to act like a drug (30). Indeed, other authors have described that increased ratings of satiety and fullness were not accompanied by a decrease in subsequent energy intake. These findings were likely because of other physiologic effects as well as psychological and environmental factors that influence food intake (17).

Related to the protein intake, in a recent systematic review and meta-analysis of 68 clinical trials (49 acute studies and 19 long-term studies) conducted by Kohanmoo et al., found that acute interventions, in which appetite was assessed during the hours following protein consumption, showed a decrease in appetite, a reduction in ghrelin, and an increase in CCK and GLP-1. However, there are still numerous limitations for long-term effects (31).

Acute trials assessed the effect of proteins on appetite shortly after consumption (< 5.5 hours, with a minimum evaluation time mostly at 3 hours), whereas in long-term trials, the intervention period ranged from 3 days to 9 months. The protein supplementation range used in the studies was within 8.5-130 g per day. Finally, regarding the type of protein, Kohanmoo et al. also found significant variability, with whey protein being the most studied among other animal-based proteins (casein, milk, yogurt, beef, turkey, and egg) or plant-based proteins (soy, wheat, gluten, pea), or products containing protein blends (31).

In the present acute study, no differences were observed concerning the appetite profile after the consumption of PEY compared to the other prototypes. These findings could be due to several reasons: evaluation time of the appetite profile, protein doses, type of protein, food matrix, the characteristic of the study sample, among others. On one hand, the evaluation time of the appetite profile using the VAS after yogurt consumption in our study was 120 minutes, which is shorter than the minimum time used in most acute studies reviewed by Kohanmoo et al. (> 3 hours). It is possible that a longer time duration may be required to observe the effects of protein consumption. On the other hand, the protein dose consumed with the 125 g of PEY was 4.63 g/serving (3.7 g/100 g). This dose was lower than the range used in the trials included in the review by Kohanmoo et al. (8.5 to 130 g/day) (27). The dose used in PEY was low primarily because pea protein can cause changes in organoleptic perception, such as an increase in bitter and astringent flavors (32). In fact, the product was designed on a Greek yogurt base to improve the sensory perception of the product by the consumer. Despite this drawback, pea protein was chosen as functional ingredient for PEY because, in recent years, plant-based proteins have gained attention as a better option compared to animal proteins among consumers seeking for more sustainable foods and for health reasons. Moreover, it is a high-quality protein with easy availability and low allergenicity. Recent studies also highlight its solubility, water and oil retention capacity, emulsifying

abilities, gelling properties, and viscosity. Likewise, it has been noted to have a greater satiating power than other types of proteins (whey protein, maltodextrin) (33). The appetite-suppressing effects of peas may be related to high amounts of protein which may delay gastric emptying, attenuate glucose absorption and concentration and stimulate the release of appetite-regulating hormones (27,33). In this context, certain effects are related to smaller peptides formed during protein fermentation. These peptides are linked to the production of appetite-regulating hormones, as well as involved in slowing gastric emptying, thereby prolonging the sensation of fullness after meals. (14). Therefore, many companies seek to leverage these characteristics to design functional foods.

In the context of the food matrix, yogurt per se, is a satiating food that may favorably influence energy balance and body composition (34). Zemel et al, reported in a 1-year intervention, the most consistent evidence to demonstrate that yogurt can favorably influence weight control, during which, African American participants consumed 1 serving of yogurt per day showing an average loss of fat body weight of 4.9 kg (35). Chapelot and Payen studied the effects of isocaloric portions of liquid yogurt and chocolate bars on appetite sensations (36). Their results showed that yogurt consumption resulted in a more pronounced effect on hunger, the desire to eat, and feelings of fullness. Although, these appetite sensations were not accompanied by significant delays in requesting the next meal or a reduction in ad libitum energy intake at the subsequent meal (36). Additionally, the flexibility of yogurt structure allows it to accommodate supplementation of ingredients, for example, fibers, proteins, bacterias that also have the potential to promote negative energy balance (34). Therefore, using yogurt as a study source seems to be a very good option in satiety studies, but also a great snacking option for in between meals.

Consequently, more interventional studies are needed, but also future research in the line of satiating products with proteins should take into consideration to use higher doses than those used in this work.

The main strength of the present study is the well-conducted acute clinical trial with a good design based on the EFSA recommendations. However, a limitation that must be considered when interpreting the results of the present study is the sex of the participants. Although sex was considered in the randomization procedure, the study was not designed to determine sex differences. Nevertheless, other studies have reported differences in the biological response to meal ingestion or appetitive responses and food intake, due to the phase of the menstrual cycle. In the present study did not determine the menstrual phase of the female participants which also may have masked an effect on our outcome measures. Future studies that are powered to identify sex differences, that use carefully developed inclusion/exclusion criteria that are chosen with thought to their sex and gender impact, and control for the phase of the menstrual cycle, should be conducted to robustly determine sex gender effects of this type of intervention (37). Other limitation was that the fat content in PEY was higher than in the other yogurts. Pea protein its richness in amino acids with a variety of bioactivities that can enhance

saltiness, umami, and kokumi (38). Nonetheless, the practical application of pea protein and its hydrolysates in food industry are limited due to their poor sensory perception, such as undesirable green and beany flavor, or the bitterness and astringency (30). The company decided to use a Greek yogurt that contains a little more of fat to mask the taste. Another limitation is that in the present article hormonal parameters are not presented to confirm the effects on appetite control, however these results will be analyzed in a future article. Therefore, the results should be interpreted with caution.

CONCLUSIONS

The results of the present study suggest that a yogurt enriched in fiber might be a good prototype functional food to control appetite and reduce total consumption in overweight and obese people. Therefore, these foods might be integrated as an alternative functional food within a hypocaloric weight control diet. Despite this, it is necessary to carry out more studies that explore the effects on biochemical variables and carry out long-term interventions to be able to gain more insight into the impact of these products on body weight control and their function in the appetite profile.

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