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EFFECTS OF LOW-COST PROTEIN BARS RICH IN POLYPHENOLS ON DIET QUALITY AND ANTHROPOMETRIC, BIOCHEMICAL, AND OXIDATIVE STRESS PARAMETERS IN SUBJECTS WITH OBESITY

EFEITOS DE BARRAS PROTEICAS DE BAIXO CUSTO RICAS EM POLIFENÓIS NA QUALIDADE DA DIETA E EM PARÂMETROS ANTROPOMÉTRICOS, BIOQUÍMICOS E DE ESTRESSE OXIDATIVO EM INDIVÍDUOS COM OBESIDADE

EFECTOS DE BARRAS PROTEICAS DE BAJO COSTO RICAS EN POLIFENOLES EN LA CALIDAD DE LA DIETA Y EN PARÁMETROS ANTROPOMÉTRICOS, BIOQUÍMICOS Y DE ESTRÉS OXIDATIVO EN SUJETOS CON OBESIDAD

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ABSTRACT

Previous studies have supported the association between intake of phenolic compounds and improvement of obesity parameters. However, most of them are in vitro studies and/or with isolated compounds. This study aimed to assess the feasibility of using low-cost protein bars enriched with phenolic compounds on anthropometric, biochemical, and oxidative stress parameters of individuals with obesity. This was a pilot, controlled intervention study with a parallel, placebo-controlled, double-

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blind design. Participants were allocated into two groups, control (n = 9) and treatment (n = 12). Outcomes were assessed at baseline and after the 30-day intervention. No significant changes in the outcomes evaluated were noted between groups. The trial showed acceptable retention rates and adherence to protocol. These results may be useful in the design and implementation of long-term, large-scale studies, contributing to the existing evidence on the beneficial impact of diets with food sources of bioactive compounds on health outcomes.

KEYWORDS: Polyphenols. Functional Food. Oxidative stress. Antioxidant response. Metabolic disorders. Obesity.

RESUMO
Estudos prévios têm demonstrado associação entre a ingestão de compostos fenólicos e a melhora de parâmetros relacionados à obesidade; entretanto, a maioria dessas evidências é proveniente de estudos *in vitro* e/ou com compostos isolados. O presente estudo teve como objetivo avaliar a viabilidade de uma intervenção utilizando barras proteicas de baixo custo enriquecidas com compostos fenólicos sobre parâmetros antropométricos, bioquímicos e de estresse oxidativo em indivíduos com obesidade. Este foi um estudo piloto de intervenção controlado, com delineamento paralelo, duplo-cego e controlado por placebo. Os participantes foram alocados em dois grupos: controle (n = 9) e tratamento (n = 12). As avaliações foram realizadas no momento basal e após 30 dias de intervenção. Não foram observadas diferenças estatisticamente significativas entre os grupos para os desfechos avaliados. O ensaio apresentou taxas adequadas de retenção e boa adesão ao protocolo. Esses resultados podem contribuir para o planejamento e a implementação de estudos de maior duração e escala, fortalecendo as evidências sobre os potenciais benefícios de dietas contendo fontes alimentares de compostos bioativos sobre desfechos em saúde.

PALAVRAS-CHAVE: Polifenóis. Alimento funcional. Estresse oxidativo. Resposta antioxidante. Doenças metabólicas. Obesidade.

RESUMEN
Estudios previos han demostrado una asociación entre la ingesta de compuestos fenólicos y la mejora de parámetros relacionados con la obesidad; sin embargo, la mayoría de estas evidencias proviene de estudios in vitro y/o con compuestos aislados. El objetivo del presente estudio fue evaluar la viabilidad de una intervención basada en el consumo de barras proteicas de bajo costo enriquecidas con compuestos fenólicos sobre parámetros antropométricos, bioquímicos y de estrés oxidativo en sujetos con obesidad. Este fue un estudio piloto de intervención controlado, con diseño paralelo, doble ciego y controlado con placebo. Los participantes fueron asignados a dos grupos: control (n = 9) y tratamiento (n = 12). Las evaluaciones se realizaron al inicio del estudio y después de 30 días de intervención. No se observaron diferencias estadísticamente significativas entre los grupos en los desenlaces evaluados. El ensayo presentó tasas aceptables de retención y adecuada adherencia al protocolo. Estos resultados pueden ser útiles para el diseño y la implementación de estudios de mayor duración y escala, contribuyendo a la evidencia existente sobre los beneficios potenciales de dietas que incluyen fuentes alimentarias de compuestos bioactivos en la salud.

PALABRAS CLAVE: Polifenoles. Alimento funcional. Estrés oxidativo. Respuesta antioxidante. Enfermedades metabólicas. Obesidad.

1. INTRODUCTION

Obesity causes expansion of white adipose tissue through increased lipid deposition, leading to adipocyte hypertrophy, hypoxia, and increased cell death. This contributes to the induction of low-grade chronic inflammation, which occurs in a physiological environment free of infection or clinically detectable tissue injury (Boccellino; D'Angelo, 2020; Taylor, 2021). In addition to the onset of chronic inflammation, obesity also predisposes individuals to increased production of reactive oxygen and nitrogen species with high reactive potential and the ability to alter the permeability, fluidity, and integrity of cell membranes, predisposing them to cell damage and/or death (Pérez-Torres *et al.,* 2021; Sandoval *et al.,* 2020).

Oxidative stress induces the transcription of factors that are sensitive to the redox state in immune cells, such as nuclear factor kappa B (NFκB), which increases the transcription of proinflammatory cytokines (Dludla *et al.,* 2018; Pérez-Torres *et al.,* 2021). Furthermore, the maintenance of high levels of these cytokines also increases the production of reactive species, thus establishing a cycle in which the temporal sequence of the processes is unknown (Leyva-López *et al.,* 2016; Tabrizi *et al.,* 2020).

Several studies have been conducted to investigate the relationship between diet and obesity. The medical and nutritional societies in different countries indicate that control and prevention of obesity can be achieved with a healthy diet, based on the daily consumption of fruits, vegetables, and whole grains (Bagetta *et al.,* 2020; Moosavian *et al.,* 2020; Muñoz-Pérez *et al.,* 2023). Thus, it is plausible to state that foods that are sources of fiber and bioactive compounds are the main components of the diet because they are associated with lower levels of inflammatory markers, higher antioxidant activity, and a lower risk of comorbidities, such as type II diabetes and cardiovascular diseases (Aloo *et al.,* 2023; Moosavian *et al.,* 2020; Muñoz-Pérez *et al.,* 2023; Ramírez-Moreno *et al.,* 2022).

Bioactive compounds are widely distributed in animal and plant products. The latter group includes several compounds such as carotenoids, phytosterols, and phenolic compounds (Dludla *et al.,* 2018; Pérez-Torres *et al.,* 2021). Phenolic compounds, owing to their chemical structure, carboxyl functional groups, and aromatic rings in simple or polymer forms, play an antioxidant function, characterized by the ability of these substances to react with free radicals, such as peroxyl radicals, preventing the continuation of the reactions through the donation of electrons or hydrogen to these radicals (Dludla *et al.,* 2018; Mattera *et al.,* 2017; Shi *et al.,* 2022).

Studies have detailed the ability of polyphenols to modulate the expression or activity of the transcription factor NF-κB, the nuclear transcription factor erythroid 2p45, (NF-E2)-related factor 2 (Nrf2), the cytochrome P450 isoenzyme, and cyclooxygenase enzymes, and to increase cytosolic cyclic AMP, which in turn favors an increase in fatty acid oxidation and mitochondrial respiration, in addition to proinflammatory molecule suppression, indicating the action of these compounds on lipid profile, oxidative stress, and inflammation (Mello *et al.,* 2023; Pérez-Torres *et al.,* 2021; Tabrizi *et al.,* 2020).

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Despite the identification of the molecular effects already described by polyphenols, most are in vitro studies and/or with isolated compounds; that is, many studies have investigated metabolism separately from the physiological effects in vivo, especially in humans (Del Bo' *et al.,* 2019). Furthermore, in humans, the biological effects are dependent on metabolism, gut microbiota, and genetic variants, depending on the interindividual variation of responders or non-responders to the compounds studied (Bento-Silva *et al.,* 2020; Fraga *et al.,* 2019; Tomás-Barberán; Espín, 2019).

Over the last few decades, the spread of food products and supplements, most of which are expensive and marketed under strong marketing appeal and health claims regarding the bioactive properties of antioxidant-rich compounds, has increased (Cowan *et al.,* 2018). Besides the questionable effects due to the unclear bioavailability and stability of their components, these products have limited access to groups with socioeconomic disadvantages (Di Lorenzo *et al.,* 2021; Dima *et al.,* 2020).

Previously, our research group demonstrated the feasibility of developing sensorially accepted, low-cost food bars as sources of proteins and antioxidants (Mendes *et al.,* 2022). Previous studies have investigated the inclusion of polyphenols within Mediterranean dietary patterns or through fortified beverages to evaluate their effects in individuals with obesity and related comorbidities (Mullan *et al.,* 2016; Zelicha *et al.,* 2022). By utilizing a food product formulated with naturally occurring bioactive compounds at non-supplemental doses, this approach addresses a relevant gap in the literature, particularly considering that many studies investigate supplemented doses substantially higher than those naturally present in foods (Jayarathne *et al.,* 2017; Rodríguez-Pérez *et al.,* 2019). Building upon previous evidence, the present study evaluates a low-cost whole-food protein bar with antioxidant properties as an intervention strategy for obesity. While prior research has examined polyphenol supplementation or fortified products, fewer studies have focused on incorporating naturally occurring bioactive compounds into affordable food matrices at physiologically relevant doses. In this context, we preliminarily assessed the feasibility of this protein bar, a source of phenolic compounds, diet quality and anthropometric, biochemical, and oxidative stress parameters. By proposing an accessible food-based approach rather than high-dose supplementation, this intervention may represent a more practical strategy with broader public health applicability. These findings provide a basis for larger clinical trials investigating cost-conscious functional food interventions in obesity management.

2. METHODS

2.1. Study design and subjects

The present study was conducted as an intervention study with a parallel, placebo-controlled, double-blind design to evaluate health outcomes. The study was approved by the Human

Research Ethics Committee (Protocol 3.424.891) of the Federal University of Lavras. After clarification of the study objectives and detailed explanation of the potential risks and benefits, as well as participants' rights, individuals were enrolled upon providing written informed consent.

2.2. Polyphenol- and protein-enriched bars

The development and optimization of protein bars rich in phenolic compounds used in the present pilot clinical trial were performed as described in a previous study (Mendes *et al.,* 2022). The developed bar was compared in the present study with a control bar, with a concentration of phenolic compounds approximately 70% lower than that in the former, based on the data obtained from the Brazilian Table of Food Composition (NEPA, 2011) and the United States Department of Agriculture Food Data Central (USDA, 2024), as described in Table 1.

Polyphenol- and protein-enriched bars were prepared with the following ingredients: dehydrated black plum, whey protein concentrate - 80%, roasted peanuts, raisins, skimmed milk powder, fine oat flakes, water, brown flax seed, dry dates without pits, and cocoa powder. Control bars were prepared with corn breakfast cereal with and without added sugar, banana (Prata variety) at a 5-7 ripening stage, based on the classification used by Rinaldi *et al.,* (2010), whey protein concentrate - 80%, roasted peanuts, skimmed milk powder, and brown food colouring.

The bulk obtained from the homogenization of these ingredients was used to prepare the bars, which were molded and cut into portions of approximately 10 × 2 cm (34 g), wrapped in aluminum foil, coded, and frozen until the date of delivery to the patients. During food handling, all sanitary measures established in the Technical Regulation of Good Practices for Food Services (Brasil, 2004) were followed.

2.3. Subjects

An initial recruitment questionnaire was disseminated in different media to identify participants interested in participating in the study. The information disclosed included the following inclusion criteria: adults (between 18 and 60 years old) of both sexes, residing or able to visit in the municipality of Lavras, Minas Gerais, MG, Brazil, with body mass index (BMI) > 30 kg/m². Participants were selected based on the exclusion criteria after 498 participants were recruited.

ISSN: 2675-6218 - RECIMA21

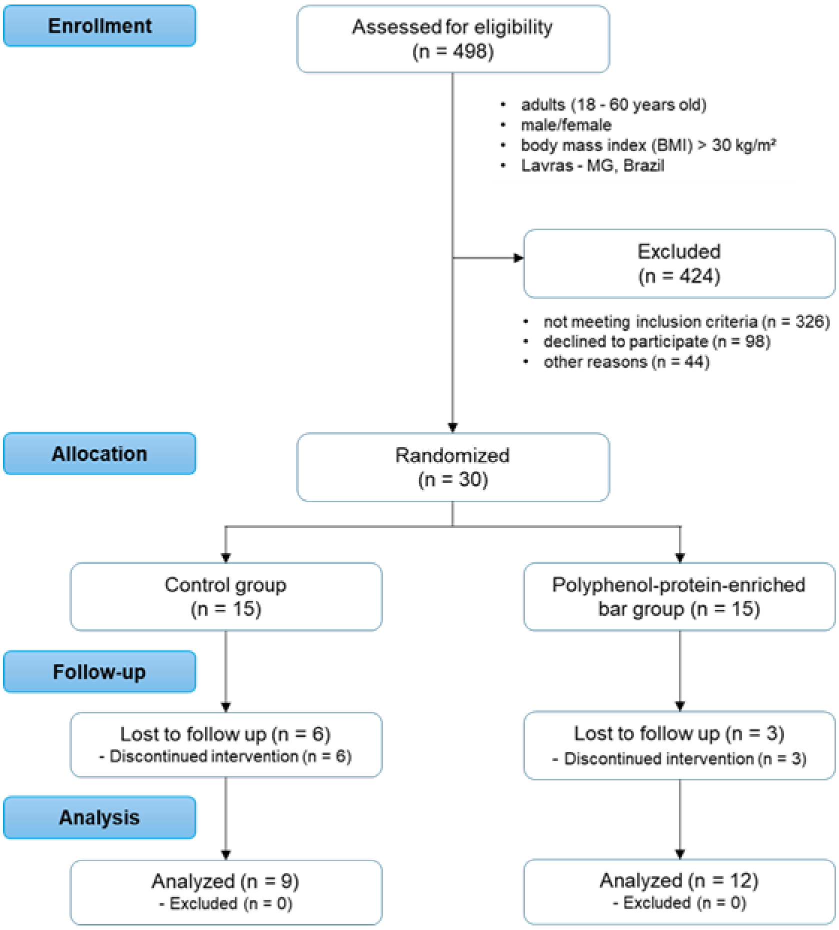
Table 1. Nutritional composition of polyphenol- and protein-enriched and control bars used in the intervention study

Nutritional composition	Control bar		Polyphenol- and protein-enriched bar	
	100g	34g (portion)	100g	34g (portion)
Moisture (%)	17.50	5.95	30.09	10.23
Energy value (kcal)	339.34	115.38	354.38	120.49
Protein (g)	21.68	7.37	22.70	7.72
Lipids (g)	4.58	1.56	10.69	3.63
Cholesterol (mg)	2.75	0.94	2.75	0.94
Carbohydrate (g)	54.25	18.45	45.42	15.44
Fiber (g)	1.76	0.60	5.94	2.02
Ashes (g)	1.58	0.54	1.67	0.57
Calcium (mg)	266.37	90.57	270.46	91.96
Magnesium (mg)	34.21	11.63	56.93	19.36
Manganese (mg)	0.44	0.15	0.43	0.14
Phosphorus (mg)	264.79	90.03	264.61	89.97
Iron (mg)	1.01	0.34	1.84	0.63
Sodium (mg)	236.44	80.39	128.88	43.82
Potassium (mg)	308.08	104.75	636.25	216.33
Copper (mg)	0.11	0.04	0.17	0.06
Zinc (mg)	0.90	0.31	1.02	0.35
Retinol (µg)	32.89	11.18	560.03	190.41
RE (µg)[a]	6.40	2.18	53.03	18.03
ERA (µg)[b]	3.20	1.09	0.00	0.00
Thiamine (mg)	0.50	0.17	0.12	0.04
Riboflavin (mg)	0.33	0.11	0.20	0.07
Pyridoxine (mg)	0.13	0.04	0.03	0.01
Niacin (mg)	1.89	0.64	1.57	0.53
Vitamin C (mg)	7.17	2.44	1.03	0.35
Total phenolics (mg)	6.90	2.34	58.09	19.75

[a] RE: Retinol Equivalent; [b] ERA: Retinol Activity Equivalent.

In this phase, patients who experienced any of the following conditions were excluded: chronic inflammatory diseases such as rheumatoid arthritis, cardiovascular disease, multiple sclerosis, cancer, asthma and allergies, smoking, severe hypertension, alternative diets (vegan and macrobiotic, among others), daily consumption of eight or more servings of fruits and/or eight or more servings of fresh vegetables, intake of alcoholic beverages in excess (more than 30 g of ethanol per day), daily physical activity, consumption of more than 1 L of coffee per day, pregnancy, daily use of anti-inflammatory drugs such as aspirin and/or mineral and vitamin supplements, use of medications for depression and appetite suppressants, and incomplete elementary education. Finally, 30 volunteers who met the established criteria were selected to participate in this study (Figure 1).

Figure 1. Participant recruitment, screening, and allocation flow diagram



2.4. Intervention

Participants were randomly allocated (1:1) to two parallel groups using a computer-generated random sequence created by an independent researcher not involved in participant recruitment, data collection, or statistical analysis. The allocation sequence was generated using a dedicated randomization software and stored in a password-protected digital file accessible only to the independent researcher.

The treatment group ingested two units per day of the polyphenol- and protein-enriched bar, while the control group consumed two units per day of the control bar, lasting 30 days. The intervention was preceded by a ten-day washout period to eliminate the residual effects of the participants' previous diet. The volunteers were instructed to eat the bars between the main meals of the day (breakfast, if the participant used to have it, lunch, and dinner).

Participants were instructed to maintain their eating habits and routine activities throughout the experiment, in addition to guidance regarding the consumption of foods rich in phenolic compounds. Adherence to the study was confirmed by monitoring volunteers every 10 days through face-to-face meetings. During the washout period, the first blood collection was performed for biochemical analyses, and visits were made to collect anthropometric measurements and dietary data and to perform randomization. After 10 days (t = 0), food bars were consumed by the respective groups, and new data collection was performed. At the end of 30 days of consumption (t = 30), the last blood sample, anthropometric measurements, and dietary data were collected through 24-hour food recall.

2.5. Measures

2.5.1. Anthropometric assessment

The anthropometric assessment was conducted at the Laboratory of Nutritional Assessment of the Department of Nutrition, Federal University of Lavras, by duly trained evaluators.

Body composition was analyzed using bioelectrical impedance (InBody230®) following the body composition assessment protocol proposed by the manufacturer, and information on body fat percentage (% BF) and skeletal muscle mass (SMM) was collected. Waist (WC) and abdominal (AC) circumferences were measured according to the World Health Organization's protocol (WHO, 1995). Calf circumference (CC) was measured at the point of maximum calf perimeter using a non-elastic measuring tape, with the participant standing and the leg relaxed. The mean of two measurements was used for analysis (WHO, 1995). Mid-upper arm circumference (MUAC) was also measured, and neck circumference (NC) was measured at the base of the neck at the cricothyroid cartilage height level based on a study by Ben-Noun *et al.,* (2001). Waist-to-height ratio (WHtR) was calculated by dividing waist circumference (cm) by height (cm). A WHtR ≥ 0.50 was considered indicative of increased cardiometabolic risk (Ashwell; Hsieh, 2005).

2.5.2. Evaluation of food intake

To assess food intake, data were obtained from a 24-hour dietary recall in which the volunteers reported the food consumed and the respective home measurements used by duly trained interviewers. To obtain the diet quality index, the method proposed by Previdelli *et al.,* (2011) and Fisberg *et al.,* (2004) was adopted, which uses a spreadsheet containing data to estimate food intake and the values of macronutrients and micronutrients. The Diet Quality Index for the Brazilian Population - Revised (DQI-R) was calculated based on the methodology proposed by Previdelli *et al.*, (2011).

2.6. Biochemical analysis

2.6.1. Blood collection and blood glucose measurement

Blood samples were collected and blood glucose was evaluated by the Santa Lúcia Laboratory of Clinical Analyses, located in the city of Lavras, MG, on the first day of each evaluation period (t = 0 and t = 30) after a 12-hour fasting period. Subsequently, plasma and serum were separated and stored at -20°C until analysis. Blood glucose levels were measured using a colorimetric enzymatic method and were expressed in mg/dL.

2.6.2. Lipid profile and liver enzymes

The serum concentrations of total cholesterol (TC), HDL cholesterol, and triacylglycerols (TG) were measured by enzymatic colorimetric methods using commercial kits (Liquiform®) following the protocols described by the manufacturer (Labtest Diagnóstica®, Lagoa Santa, Brasil), and their concentrations were expressed in mg/dL. LDL-c cholesterol was obtained using the Friedewald formula: i.e. LDL-c = TC – HDL-c – TG/5, where TG/5 is an estimate of VLDL-c; all concentrations are expressed in mg/dL (Hotta *et al.,* 2017).

The plasma concentrations of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase were measured using an enzymatic colorimetric method with a commercial kit (Bioclin®) following the manufacturer's protocol. The concentrations of AST and ALT are expressed in units (U)/mL, and the concentration of alkaline phosphatase is expressed in units (U)/L.

2.7. Markers of oxidative stress and inflammation

2.7.1. Protein dosage

The results were normalized to the protein concentration, as determined using the Bradford (Bradford, 1976). The concentration was calculated based on a known protein standard curve (bovine serum albumin), and the value was multiplied by the sample dilution factor to obtain the protein concentration in µg/mL.

2.7.2. Thiobarbituric acid reactive substances – TBARS

The TBARS test was conducted following the protocol suggested by Buege & Aust (1978) with adaptations regarding the sample used, which consisted of serum rather than tissue homogenate, as originally suggested by the authors. For this purpose, 500 µL of a solution containing trichloroacetic acid (TCA, 15%), thiobarbituric acid (TBA, 0.0375%), and hydrochloric acid (0.25 N HCl) was added to 250 µL of serum. The tubes were protected from light and placed in a boiling water bath for 15 minutes. After cooling the tubes in cold running water, 0.75 mL of butanol was immediately added, and the samples were homogenized and centrifuged at 6000 rpm for 10 min. In a 96-well microplate, 200 μL of the supernatant was added per well in triplicate; readings were performed at a wavelength of 550 nm, and the results were expressed as nmol MDA/mg protein. To calculate the MDA concentration, the equation obtained from the standard curve of absorbance generated by known concentrations of the standards was used. The results were normalized to the protein concentration, as determined by the Bradford method (Bradford, 1976).

2.7.3. Hydroperoxides

Hydroperoxide concentration was measured as described by Banerjee *et al.,* (2002). A total of 40 μL of serum, followed by FOX-2 reagent, was pipetted into a microplate. The samples were kept at room temperature and protected from light for 30 min, after which the absorbance was measured at 560 nm. Subsequently, the hydroperoxides were reduced with triphenylphosphine (TPP) by adding 10 μL TPP solution in methanol (10 mM TPP) to 30 μL serum. Next, FOX-2 reagent was added, and the absorbance was read at 550 nm. Hydroperoxide was quantified by subtracting the measurements without TPP from those with TPP, and the results were normalized to the protein concentration of each sample.

2.7.4. Catalase

Catalase activity was measured in triplicate according to the method described by Aebi (1984). Serum (50 μL) was added to a cuvette containing 2000 μL phosphate buffer (50 mM; pH 7.0) and 25 μL diluted hydrogen peroxide (0.3 M). Spectrophotometer readings were performed at zero, 15 s, and 60 s at a wavelength of 240 nm. The enzymatic activity was calculated by subtracting the absorbance values obtained at zero and 60s, divided by the sample volume (mL), and corrected by the dilution factor used. The results were normalized to the protein concentration and expressed as ∆E/min/mg of protein.

2.7.5. Superoxide dismutase (SOD)

The quantification of superoxide dismutase (SOD) followed the method adapted by Marklund & Marklund (1974). In the assay, a microplate was loaded with the blank, that is, 144 μL of phosphate buffer (50 mM) + 6 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (1.25 mM); the standard, that is, 129 μL of phosphate buffer (50 mM) + 6 μL of MTT (1.25 Mm) + 15 μL of pyrogallol (100 mM); and the sample, that is, 99 μL of phosphate buffer (50 mM) + 30 μL of sample + 6 μL of MTT (1.25 mM) + 15 μL of pyrogallol (100 mM). After this step, the plate was protected from light with aluminum foil and incubated at 37 °C for 5 min. Next, 150 μL of dimethyl sulfoxide (DMSO) was added to all wells to stop the reaction. The plate was then read at a wavelength of 550 nm. The final values were normalized to protein concentration and expressed as U of SOD/mg of protein.

2.7.6. Ferric reducing antioxidant power (FRAP)

The FRAP reagent was prepared at the time of analysis by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM TPTZ in 40 mM hydrochloric acid - HCl), and 2.5 ml of 20 mM ferric chloride (FeCl3) in aqueous solution. A 100 µL aliquot of serum was added to 3 mL of FRAP reagent, and the mixture was incubated at 37 °C in a water bath for 30 min. Absorbance was measured at 593 nm after the incubation period, and the spectrophotometer was zeroed with the FRAP solution (Benzie & Strain, 1996). A calibration curve was obtained with ferrous sulfate (100-2000 µM), and the results were expressed in µmol Fe2+/mL.

2.7.7. Cytokine analysis

Tumor necrosis factor (TNF) -α (Catalog no. E0082HU, BT-Bioassay Technology Laboratory, Shanghai, China) and Interleukin (IL) -6 (Catalog no. 88-7066-22, INVITROGEN®, California, USA) were determined by enzyme immunoassay kit according to the respective manufacturer's instructions. C-reactive protein (CRP) was measured using a colorimetric method with plasma serum using commercial kits (EliKine™ Human CRP ELISA kit®) following the protocols described by the manufacturer.

2.8. Data analysis

The sample profile was characterized by absolute and proportional frequency counting. Outcome data are presented as mean ± standard deviation (SD) to estimate the pre/post-differences. Post-intervention results (t = 30) were evaluated against baseline (t = 0), obtaining the pre/post mean difference with a confidence interval of 95%. The primary outcome data were evaluated for equal variances and normality, and no major violations of the model assumptions were found. Comparisons of the baseline outcomes (t = 0) and the pre/post mean difference between the control and treatment groups were examined using an independent sample t-test. P < .05 was interpreted as statistically significant. We used the JASP software (version 0.95.1) (JASP Team, 2025) to conduct the analyses.

3. RESULTS

The study started with 30 subjects; however, throughout the experimental period, nine volunteers withdrew from the study, totalling 21 volunteers at the end of the trial, with nine subjects in the control group and 12 in the polyphenol- and protein-enriched bar group. The withdrawals were mainly due to difficulties in reconciling scheduled appointment times at the study center.

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Regarding the sample profile, 76.2% (n = 16) were female, the mean age was 36 (20-55) years, and the mean BMI was 35.81 ± 2.97 kg/m². The prevalence of sedentary individuals was 61.9% (n=13). Eight individuals (38.1%) reported the presence of pathology at the time of the interview, including polycystic ovary syndrome, fibromyalgia, hypertension, and gastroesophageal reflux.

Table 2 presents the main baseline characteristics of each group in terms of primary outcomes. There were no differences in the outcomes between the groups at the beginning of the trial, except for SOD and FRAP measures, which were slightly higher in the control group.

Table 2. Baseline characteristics, expressed as mean and standard deviation (SD), regarding the outcomes evaluated

Outcome[a]	**Control group (n = 9)**	**Polyphenol- and protein-enriched bar group (n = 12)**	**p-value [b]**
Anthropometric measures			
Body fat (%)	47.39 (4.72)	43.59 (8.33)	0.236
BMI (kg/m²)	36.82 (2.60)	35.06 (3.04)	0.178
WHtR	1.02 (0.07)	1.02 (0.05)	0.786
BMR (kcal)	1555.14 (232.16)	1534.58 (211.72)	0.883
SMM (kg)	30.59 (6.45)	30.28 (6.01)	0.917
CC (cm)	40.63 (3.78)	39.67 (3.54)	0.593
MUAC (cm)	38.57 (4.38)	37.33 (3.62)	0.521
WC (cm)	110.07 (7.87)	103.46 (5.64)	0.054
AC (cm)	116.43 (8.48)	114.32 (7.30)	0.582
Food intake			
DQI-R	55.70 (14.28)	62.64 (10.26)	0.247
Biochemical measures			
Blood glucose (mg/dL)	84.00 (6.91)	80.50 (8.25)	0.317
AST (U/mL)	58.10 (52.93)	45.21 (13.29)	0.424
ALT (U/mL)	17.28 (16.55)	10.68 (18.81)	0.415
Alkaline phosphatase (U/L)	55.55 (13.89)	54.42 (15.48)	0.865
Triacylglycerols (mg/dL)	173.43 (58.27)	204.68 (76.22)	0.319
Total cholesterol (mg/dL)	270.22 (98.96)	325.31 (169.77)	0.397
HDL-c (mg/dL)	41.06 (18.30)	56.55 (89.09)	0.616
LDL-c (mg/dL)	194.47 (93.10)	227.82 (123.68)	0.507
VLDL-c (mg/dL)	34.69 (11.65)	40.94 (15.24)	0.319
Markers of oxidative stress and inflammation			
TBARS (nmol MDA/mg of protein)	0.18 (0.12)	0.146 (0.12)	0.623
SOD (U/mg of protein)	0.13 (0.03)	0.10 (0.03)	0.036
Catalase (ΔE/min/mg of protein)	0.07 (0.08)	0.04 (0.03)	0.227
Hydroperoxides (µmol/mg of protein)	908.79 (731.75)	990.61 (1181.16)	0.857

FRAP c (µmol Fe2+/mL)	1566.11 (204.48)	1273.23 (183.22)	0.004
TNF-α (ng/L)	203.50 (85.39)	260.30 (143.52)	0.357
IL-6 (pg/mL)	0.07 (0.05)	0.05 (0.04)	0.205
C-reactive protein (pg/mL)	2673.09 (663.34)	2869.35 (652.14)	0.548

[a]: BMI, body mass index; WHtR, waist-to-height ratio; BMR, basal metabolic rate; SMM, skeletal muscle mass; CC, calf circumference; MUAC, Mid-upper arm circumference; WC, waist circumference; AC, abdominal circumference; DQI-R, Revised Diet Quality Index for the Brazilian Population; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; FRAP: Ferric reducing antioxidant power. TNF-α, Tumor necrosis factor-α ; IL-6, Interleukin-6

[b] Examined by independent samples t-test. P < .05 was interpreted as statistically significant.

[c] The assumption of equal variances was violated; therefore, Welch's t-test for independent samples was applied.

4. DISCUSSION

This study aimed to preliminarily evaluate the effects of low-cost protein food bars as a source of phenolic compounds ingested at a non-supplemental dose on diet quality and anthropometric, biochemical, and oxidative stress parameters in subjects with obesity. To ensure the supply of phenolic compounds, the main ingredients used in its composition were prune (rich in chlorogenic and neochlorogenic acid), raisins (rich in malvidin-3-glucoside, quercetin, and 3,4-dihydroxybenzoic acid), and brown dry dates (khajur) (rich in rutin, gallic, ferulic, and caffeic acids) (Bouhlali *et al.,* 2020; Fulgoni *et al.,* 2017). This resulted in a total phenolic concentration 69.74% higher than that of the control bars (Table 1). The incorporation of flaxseed seeds into the preparation ensures higher levels of α-linolenic fatty acids (Bagetta *et al.,* 2020; Meza-Miranda *et al.,* 2016). However, our results demonstrated no significant changes in the outcomes evaluated after the 30-day intervention period. However, the study showed acceptable retention rates and adherence to the protocol, without barriers related to product acceptability of the bars developed.

Table 3. Outcome changes, expressed as pre/post mean difference with a confidence interval of 95% [lower; upper; CI - 95%] for each treatment during the 30-day intervention

Outcome[a]	**Control group pre - post difference (n = 9)**	**Polyphenol- and protein-enriched bar group pre - post difference (n = 12)**	**p-value [b]**
	Anthropometric measures		
Body fat (%)	-6.37 [-19.90; 7.13]	-0.03 [-0.69; 0.62]	0.224
BMI (kg/m²)	-3.89 [-12.71; 4.93]	0.19 [-0.10; 0.49]	0.23
WHtR	0.01 [-0.01; 0.04]	0.00 [-0.02; 0.02]	0.304
BMR (kcal)	-1.78	13.67	0.422

	[-43.78; 40.22]	[-6.13; 33.47]	
SMM (kg)	0.01	0.38	0.45
	[-1.02; 1.04]	[-0.17; 0.94]	
CC (cm)	-4.52	1.93	0.22
	[-14.26; 5.22]	[-4.85; 8.71]	
MUAC (cm)	-5.62	0.61	0.208
	[-13.38; 2.14]	[-6.59; 7.81]	
WC (cm)	-15.18	7.55	0.102
	[-41.32; 10.97]	[-9.36; 24.46]	
AC (cm)	-12.60	-3.08	0.608
	[-40.50; 15.30]	[-31.52; 25.37]	
Food intake			
DQI-R	-11.17	-12.46	0.247
	[-43.51; 21.17]	[-35.52; 10,61]	
Biochemical measures			
Blood glucose (mg/dL)	-7.78	1.17	0.409
	[-34.90; 19.34]	[-4.93; 7.27]	
AST (U/mL)	6.69	17.89	0.343
	[-17.22; 30.60]	[4.04; 31.74]	
ALT (U/mL)	-7.15	21.98	0.195
	[-55.92; 41.62]	[-0.51; 44.48]	
Alkaline phosphatase (U)/L	-15.22	-5.31	0.359
	[-39.91; 9.48]	[-15.10; 4.49]	
Triacylglycerols (mg/dL)	-30.49	-12.30	0.574
	[-96.53; 35.55]	[-50.41; 25.81]	
Total cholesterol (mg/dL)	1.13	-3.29	0.648
	[-208.36; 21.62]	[-239.10; -15.26]	
HDL-c (mg/dL)	-93.37	-127.18	0.886
	[-23.00; 25.26]	[-58.31; 51.73]	
LDL-c (mg/dL)	-6.10	-2.46	0.573
	[-191.92; 15.11]	[-202.37; -40.50]	
VLDL-c (mg/dL)	-88.40	-121.43	0.574
	[-19.31; 7.11]	[-10.08; 5.16]	
Markers of oxidative stress and inflammation			
TBARS (nmol MDA/mg of protein)	0.11 [-0.10; 0.32]	0.19 [0.00; 0.39]	0.521
SOD (U/mg of protein)	-0.07	-0.03	0.086
	[-0.10; -0.03]	[-0.06; -0.01]	
Catalase (ΔE/min/mg of protein)	0.01 [-0.08; 0.02]	0.07 [0.00; 0.14]	0.179
Hydroperoxides (µmol/mg of protein)	-582.69 [-1179.83; 14.45]	-439.06 [-1108.03; 229.92]	0.735

FRAP c (μmol Fe2+/mL)	-461.12	-250.68	0.493
	[-1207.44; 285.21]	[-469.98; -31.39]	
TNF-α (ng/L)	-19.53	-33.50	0.469
	[-54.124; 15.06]	[-59.45; -7.56]	
IL-6 (pg/mL)	0.02	0.01	0.205
	[-0.03; 0.06]	[-0.02; 0.03]	
C-reactive protein (pg/mL)	-4.83	-225.05	0.618
	[-800.62; 790.96]	[-863.79; 413.68]	

[a]: BMI, body mass index; WHtR, waist-to-height ratio; BMR, basal metabolic rate; SMM, skeletal muscle mass; CC, calf circumference; MUAC, Mid-upper arm circumference; WC, waist circumference; AC, abdominal circumference; DQI-R, Revised Diet Quality Index for the Brazilian Population; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; FRAP: Ferric reducing antioxidant power. TNF-α, Tumor necrosis factor-α ; IL-6, Interleukin-6
[b] Examined by independent samples t-test. P < .05 was interpreted as statistically significant.
[c] Equal variance assumption was violated; therefore, the independent samples t-test with correction for unequal variances (Welch's t-test) was applied.

Previous studies have shown a positive relationship between dietary profiles and metabolic complications. Dietary patterns with a high concentration of phenolic compounds are characterized by high consumption of fruits, vegetables, fish, poultry, and oils, as encouraged by the Mediterranean diet and Dietary Approaches to Stop Hypertension (DASH), which are associated with a lower prevalence of obesity, reduced levels of inflammatory markers, and a lower risk of associated comorbidities (Bagetta *et al.,* 2020; Moosavian *et al.,* 2020; Rodríguez-Pérez *et al.,* 2019). Currently, data on the isolated effects of phenolic compounds on obesity and its metabolic disturbances are not fully supported.

In a meta-analysis conducted by Kiyimba *et al.,* (2023) to evaluate the available evidence on the impact of dietary polyphenols on blood pressure, lipid profile, blood glucose, waist circumference, vascular function, and markers of inflammation, polyphenols demonstrated beneficial effects on most of the selected outcomes compared with placebo. However, no substantial effects on waist circumference or glucose homeostasis were observed. Besides these improvements, the authors highlighted the need to interpret these results cautiously because of the high heterogeneity and risk of bias among randomized controlled trials.

The consumption of polyphenols and their relationship with body weight have been the subject of research for some time because of the potential effect of polyphenols in reducing obesity. Some of the active actions currently investigated include suppression of fat absorption from the intestine, glucose uptake by skeletal muscles, suppression of anabolic pathways, stimulation of catabolic pathways in adipose tissues, liver, and other tissues, stimulation of apoptosis of mature adipocytes, and reduction of inflammation associated with adiposity (Castro-Barquero *et al.,* 2018; Finicelli *et al.,* 2019; Stohs; Badmaev, 2016). However, in humans, this relationship is still uncertain, probably due to differences in experimental designs, individual variations, chemical forms of dietary phenolic compounds, time, and confounding factors such as other weight-reducing agents or food and/or caloric restriction (Llaha; Zamora-Ros, 2020).

A review of randomized clinical trials evaluating polyphenol-rich supplements combined with traditional weight loss strategies (hypocaloric diets and/or physical activity) concluded that polyphenol supplementation is not yet supported as an effective complementary approach to enhance weight loss outcomes (Llaha; Zamora-Ros, 2020).

Previous evidence also supports an association between the intake of different polyphenols and the improvement of several obesity parameters. For example, a combination of EGCG and resveratrol was effective in downregulating pathways related to energy metabolism, oxidative stress, inflammation, and the immune system in overweight and obese participants (Qiu *et al.,* 2023). Moreover, human intervention studies have demonstrated the anti-obesity effects of several polyphenol-rich foods, including citrus fruits, green tea, berries, apples, and onions (Boccellino; D'Angelo, 2020). However, our findings do not support this evidence. Similarly, the study by Mullan *et al.*, (2016) did not demonstrate improvements in vascular function in obese individuals following 4 weeks of supplementation with a (poly)phenol-rich beverage. No significant changes were observed in plasma markers of adiposity, lipid profile, renal function, metabolic parameters, inflammation, or endothelial activation. In contrast, dietary modification appears to confer more pronounced benefits. Greater improvements were observed among subjects who combined adherence to a Mediterranean dietary pattern with polyphenol supplementation, with evidence indicating that an 18-month dietary intervention significantly reduced visceral adipose tissue in obese patients (Zelicha *et al.,* 2022).

Notably, in the present study, phenolic compounds were provided through the inclusion of a food bar in the volunteers' diet in a natural and non-concentrated form (extracts or capsules), a factor that directly contributed to the results obtained. Additionally, the absorption, metabolism, and transport of phenolic compounds are extremely important for the bioaccessibility of each phenolic compound. The interaction between phenolic compounds and intestinal transport systems can alter their absorption and metabolism and have a strong impact on their protective and/or therapeutic potential in target tissues (Redan *et al.,* 2016; Tabrizi *et al.,* 2020; Zhao *et al.,* 2017). To enhance these functional properties, the incorporation of phytochemicals into food matrices may promote structural interactions capable of improving compound stability during digestion and supporting probiotic survival. Such interactions may increase bile tolerance and resistance to gastric acidity, thereby contributing to improved modulation of intestinal microbiota composition and activity (Marcìa-Fuentes *et al.,* 2026). In this context, novel food sources represent a promising alternative for the development of next-generation functional foods aimed at enhancing microbiota balance, increasing the bioavailability of bioactive compounds, and improving overall nutritional quality (Marcìa-Fuentes *et al.,* 2026). From a public health perspective, interventions based on low-cost functional foods may expand access to bioactive compounds, particularly among individuals with obesity who often face socioeconomic barriers to dietary improvement.

The present study has some limitations: a) the lack of chemical analyses to determine the polyphenol content in the polyphenol- and protein-enriched bar and the plasma profile of individuals, b) the small sample size due to the current impossibility of repeating the study with a new sample group, and c) the use of multiple comparisons without a formal adjustment for type I error, which increases the likelihood of false-positive findings. Therefore, these results should be considered exploratory and require confirmation in future studies with greater statistical power.

5. CONCLUSION

Although no significant effects were observed in the secondary anthropometric, biochemical, or oxidative stress outcomes, this pilot trial demonstrated adequate feasibility, as reflected by satisfactory adherence and retention rates over 30 days. These findings support the refinement and design of adequately powered, longer-term randomized trials to more robustly evaluate the efficacy of the developed low-cost polyphenol– and protein-enriched bar. Future studies should incorporate extended intervention periods, clearly defined clinical endpoints, and detailed compositional and biochemical analyses to strengthen internal validity and causal inference.

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Conflict of interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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