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Black bean (*Phaseolus vulgaris* L.) protein hydrolysates reduce acute postprandial glucose levels in adults with prediabetes and normal glucose tolerance

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ABSTRACT

This study evaluated the acute effect of black bean protein hydrolysate (BPH) on postprandial glucose and insulin levels in adults with normal glucose tolerance (NGT) or prediabetes. First, an *in silico* predictive digestion was performed on twenty peptides identified in BPH. Then, 28 adults with NGT or prediabetes were randomized to a parallel double-blind, placebo-controlled trial. Following consent, participants were randomly assigned to one of two groups, placebo, or 5 g of BPH. To evaluate the acute effects of BPH, an oral glucose tolerance test was administered, and blood samples were collected at 60, 120, and 150 min. In the 5 g BPH group, the area under the curve for glucose, and glucose levels at 120 min were statistically lower compared to the placebo group ($p = 0.04$, $p = 0.02$). In this study, BPH had an acute glucose lowering effect after an oral glucose tolerance test in subjects with NGT or prediabetes.

1. Introduction

Type 2 diabetes (T2D) is a chronic disease often preceded by a prediabetes state characterized by impaired fasting glucose, glucose tolerance and/or glycated hemoglobin (Rahim et al., 2023; ADA, 2018). Monitoring glucose blood levels and improving lifestyle habits, such as diet, can slow the progression from prediabetes to type two diabetes (Rahim et al., 2023).

In recent years, functional food ingredients have been designed from natural sources and shown promising results in the blood glucose levels in normal glucose tolerance (NGT) subjects as well as in T2D patients (Reed, Bain, & Kanamarlapudi, 2021; Rendell, 2021). Proteins from black beans (Phaseolus vulgaris L.) have been studied as a source of bioactive peptides. Small bioactive peptides (<1,000–200 Da) present important characteristics such as high biological potential, low toxicity, low or null allergenicity, high structural diversity, and small size (relative to antibodies) (Agyei, Ongkudon, Wei, Chan, & Danquah, 2016). These properties allow bioactive peptides to be applied as therapeutic agents against several diseases, including prediabetes and T2D (Sarmadi & Ismail, 2010).

Enzymatic hydrolysis is the most popular method to generate bioactive peptides from common beans and other pulses' parental protein sequences (Luna-Vital, Mojica, González de Mejía, Mendoza, & Loarca-Piña, 2015; Mojica, Chen, & de Mejía, 2015; Mojica & de Mejía, 2016; Mojica, Luna-Vital, & E. Gonzalez de Mejia, 2018; Oseguera-Toledo, de Mejia, & Amaya-Llano, 2015; Valencia-Mejía, Batista, Fernández, & Fernandes, 2019).

Black bean protein hydrolysates BPH generated with Alcalase® inhibited the *in vitro* activity of dipeptidyl peptidase-IV (DPP-IV), whose biological function is to inactivate glucagon-like peptide-1 (GLP-1) (Mojica & de Mejía, 2016). Also, it inhibited the enzymes α-amylase and α-glucosidase, which are involved in the starch breaking-down process (Mojica, Chen, & de Mejía, 2015; Mojica & de Mejía, 2016; Mojica, Luna-Vital, & González de Mejía, 2017; Nuñez-Aragón et al., 2019). Moreover, it has been reported that BPH could reduce the expression and translocation of glucose transporters in the plasmatic membrane, such as glucose transporter-2 (GLUT2) and the sodium-dependent glucose cotransporter (SGLT1) (Mojica, Gonzalez de Mejia, Granados-Silvestre, & Menjivar, 2017). Besides, *in vivo* experiments have reported the efficacy of BPH reducing postprandial glucose in non-

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hyperglycemic (16.9–24.5 %) and hyperglycemic (22.7–47.7 %) Wistar rats (Mojica, Gonzalez de Mejia, Granados-Silvestre, & Menjivar, 2017). The results of *in vivo* and *in vitro* experimental models reported the potential of BPH to inhibit digestive enzymes and block gastrointestinal glucose transporters (involved in regulating postprandial blood glucose). All these effects are related to potential beneficial effects in hyperglycemic states, especially diminishing postprandial glucose levels.

As the evidence shows, bioactive peptides from BPH may exert antidiabetes effects through several mechanisms. All the studies showing the antidiabetes potential of BPH and bioactive peptides have been performed using *in silico*, biochemical assays, *in vitro*, and *in vivo* studies (Mojica & de Mejía, 2016; Mojica, Luna-Vital, & González de Mejía, 2017; Nuñez-Aragón et al., 2019). However, clinical trials are needed to validate the hypoglycemic effect of black bean protein hydrolysates. In addition, a thorough understanding of the peptide composition of BPH is essential to provide insight into their biological activity. Therefore, the aim of this study was to identify BPH peptides with potential biological properties through *in silico* predictive digestion and to investigate in a clinical trial the acute effects of BPH on postprandial glucose and insulin levels in adults with normal glucose tolerance or prediabetes.

2. Experimental section

2.1. Materials

Black beans (*Phaseolus vulgaris* L.) "San Luis" cultivar was harvested and acquired in Zacatecas, Mexico (season 2018). HT Proteolytic kit was purchased from ENMEX, S.A. de C.V. (Tlalnepantla, Mexico); glucose-LQ (GOD-POD Liquid), cholesterol-LQ (CHOD-POD Liquid), triglycerides-LQ (GPO-POD Liquid), and HDL-cholesterol P (Precipitating reagent) were supplied by Spinreact (Girona, Spain), the insulin ELISA kit (80-INSHU-E01.1) was supplied by ALPCO (Salem, NH, USA) and the dextrosol 75 g (anhydrous glucose liquid solution 75 g) was supplied by Hycel (Zapopan, Mex); alcalase (EC 3.4.21.62), NaOH, HCl, and other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Black bean protein hydrolysates production

Black bean protein hydrolysates were generated according to patent request No. MX/a/2021/011985 entitled "Proceso a nivel planta piloto para la generación y purificación de hidrolizados de proteína de frijol común (*Phaseolus vulgaris* L.) con potencial hipoglucemiante". Briefly, dehusked black bean seeds were ground in a 1:10 dilution (ground beans: water), and the pH was adjusted to 9.0 with NaOH. The protein was extracted and then centrifuged at 4,000 rpm, and the supernatant was recovered. The pH was modified to 3.8 using HCl and centrifuged at 4,000 rpm at 2 °C. The precipitated protein was recovered and stored at −20 °C. Protein was solubilized in 0.05 N NaOH in a 1:20 ratio and autoclaved to denature proteins. Then, the sample was cooled, and a commercial protease (HT proteolytic) was added in a 1:10 (v/v) ratio according to the protein content of the solution. Protein hydrolysis was performed, and the undigested protein was separated by centrifugation. The supernatant containing the BPH was recovered.

2.3. LC-MS/MS analysis and peptide sequencing

Selected protein hydrolysates were analyzed at IPICyT laboratory (Instituto Potosino de Investigación Científica y Tecnológica, A.C.). Nanoscale LC separation of BPH was performed with a nano-ACQUITY UPLC System (Waters, Milford, USA) equipped with a Symmetry C18 precolumn (100 Å, 5 µm × 20 mm, Waters) and a BEH130 C18 (130 Å, 1.7 µm, 75 mm × 100 µm, Waters) analytical column. Peptides were eluted at 35 °C with a linear gradient of 3 % to 50 % mobile phase B (acetonitrile with 0.1 % formic acid) over 30 min at a flow rate of 600 nL/min, followed by a 3 min rinse with 85 % of mobile phase B. The column was re-equilibrated at initial conditions for 15 min with mobile phase A (water with 0.1 % formic acid). The lock mass compound, [Glu1]-Fibrinopeptide B (Sigma Aldrich, Saint Luis, USA), was delivered by the auxiliary pump of the nano-ACQUITY UPLC System at 200 nL/min at a concentration of 100 fmol/mL to the reference sprayer of the Nano-Lock-Spray source of the mass spectrometer. Mass spectrometric analysis (LC-MS/MS) was conducted in an SYNAPT HDMS Q-TOF (Waters). The spectrometer was operated in V-mode, and analyses were performed in positive mode ESI. The TOF analyzer was externally calibrated with [Glu1]-Fibrinopeptide B from 50 to 2,422 *m/z*. The radiofrequency applied to the quadrupole was adjusted to efficiently transmit ions from 50 to 2,000 *m/z*. Accurate mass data were collected in an alternating Data-Independent Acquisition mode. In low-energy mode, data were collected at collision energy of 3 eV. In the elevated energy mode, the collision energy was ramped from 15 to 45 eV during 8 s of integration. The data were post-acquisition lock mass corrected using the doubly protonated monoisotopic ion of [Glu1]-Fibrinopeptide B (Sigma Aldrich, Saint Luis, USA). The reference sprayer was sampled every 30 s. The obtained MS/MS data were analyzed for peptide identification using the NCBIprot databases in the Viridiplantae taxonomy and MASCOT v2.3.02 (Matrix Science, London, UK), available at https://www.matrixscience.com. The following search parameters were used for the enzyme: pepsin A; fixed modifications: carbamidomethyl (C); variable modifications: oxidation (M); mass values: monoisotopic; protein mass: unrestricted; peptide mass tolerance: ±20 ppm; fragment mass tolerance: ±0.1 Da; max missed cleavages: 1; instrument type: ESI-QUAD-TOF; number of queries: 1,936. Identifications were considered successful when significant MASCOT scores were obtained, indicating the identity or extensive homology at $p < 0.05$.

2.4. In silico peptide digestion, physicochemical and biological properties prediction

Peptide physicochemical properties were predicted using PepDraw tool (https://www.tulane.edu/?biochem/WW/PepDraw/). *In silico* enzymatic digestions were performed to predict the release of short peptides with biological potential. The predicted digested peptide sequences and their biological potential were performed using BIOPEP database (https://www.uwm.edu.pl/biochemia/index.php/pl/biopep) with the enzymes pepsin (pH 1.3)-EC 3.4.23.1, trypsin-EC 3.4.21.4 and Chymotrypsin A-EC 3.4.21.1/Chymotrypsin C-EC 3.4.21.2 (Minkiewicz, Iwaniak, & Darewicz, 2019).

2.5. Randomized clinical trial

2.5.1. Study design

A parallel double-blind placebo-controlled randomized clinical trial was carried out in 28 adults to evaluate the acute effect of 5 g of BPH on postprandial glucose and insulin levels after an oral glucose tolerance test. All the procedures were performed at the research facilities of the Department of Medical Sciences of the University of Guanajuato, Mexico. The Institutional Ethics Committee from the University of Guanajuato approved this protocol (CIBIUG-P53-2018), and all subjects signed the informed consent before undergoing any procedure.

2.5.2. Participants

Participants in this study were recruited directly from local health fairs in León, Guanajuato, Mexico. As part of the screening process to determine eligibility for participation, capillary glucose, weight, height, and blood pressure measurements were taken. As part of this study, adults between the ages of 25 and 50 with overweight or obesity, according to their body mass index (BMI) (25–34.9 kg/m^2) were eligible to enroll. The study did not include participants who reported diabetes, cancer, cardiovascular disease, or other chronic diseases by self-report. Pregnant or breastfeeding women were not included. Following eligibility determination, participants were referred to the Department of Medical Sciences for clinical, anthropometric, and metabolic assessments. Two study visits were scheduled for participants, the first for the initial assessments and the second for the intervention. The period for each visit was at least one week and maximum four weeks apart.

On the day of the initial assessments, participants attended the clinical laboratory of the Department of Medical Sciences after an overnight fast. The initial metabolic assessment included a lipid profile, and an oral glucose tolerance test (OGTT) with 250 mL of dextrosol 75 g (an anhydrous glucose liquid solution, 75 g) with blood samples at 0 and 120 min. The participants' metabolic status (normal glucose tolerance (NGT) or prediabetes) was defined as followed: Normal glucose tolerance as fasting glucose levels < 100 mg/dL and postprandial (2 h) glucose levels < 140 mg/dL, and prediabetes as fasting glucose levels of 100–125 mg/dL, postprandial (2 h) glucose levels of 140–199 mg/dL, or both. After determining the participants' metabolic status, the subjects were classified as either with NGT or with prediabetes.

2.5.3. *Treatment*

The acute effect of BPH on postprandial glucose and insulin levels was assessed using either 5 g of powdered BPH dissolved in 120 mL of a red-colored commercial non-caloric beverage or a placebo consisting of 120 mL of the red-colored non-caloric commercial beverage. Participants could not distinguish which treatment they were receiving due to the red color of the commercial beverage.

2.5.4. *Study protocol*

Once the participants were included and classified by metabolic status, they were randomly assigned into either the group receiving BPH treatment (5 g) or the placebo group. The randomization plan for treatment assignment was done by a staff member who had no contact with participants using stratification by metabolic status and the online randomization software https://www.graphpad.com/quickcalcs/randomize1/, a similar number of participants with NGT and prediabetes were included in each treatment group (BPH or placebo).

Once participants were randomly assigned to their treatment group, they underwent a second oral glucose tolerance test to evaluate the acute postprandial effect of BPH. On the day of the intervention, participants attended the clinical laboratory of the Department of Medical Sciences after an overnight fast for at least 8 h.

After, a blood draw (0 min) participants drank either the dissolved BPH or the placebo according to their assigned treatment group, followed by 250 mL of the dextrosol 75 g. Successive blood samples were collected at 60, 120, and 150 min to determine glucose and insulin levels.

2.5.5. *Clinical evaluation and anthropometric assessment*

In this study, trained professionals administered a questionnaire to collect clinical data. Blood pressure was measured using a semi-automatic digital blood pressure monitor (Omron HEM-7200) on the participant's right arm in a sitting position and after resting for at least 10 min.

Body weight and height were measured with the subjects barefoot and wearing light clothing using a digital scale (Seca 769) and a stadiometer (Seca 213-I). Waist circumference was measured at the intermediate point between the last rib and the iliac crest using metal tape (Lufkin W606PM). Body mass index (BMI) was calculated as body weight (kg) divided by the squared height (m) according to WHO (Reinahuer, Home, Kanagasabapathy, & Heuck, 2005).

2.5.6. *Biochemical assays*

In order to perform the biochemical assays, blood samples were collected after fasting with at least 8 h. For glucose (Spinreact, Girona, Spain) and lipid profile (total cholesterol, TG, and HDL-c) (Spinreact, Girona, Spain), enzymatic colorimetric assays were used. LDL-cholesterol was calculated using the Friedewald equation (Krishnaveni & Gowda, 2015). Glucose and lipid profile levels (triglycerides, total cholesterol, HDL-cholesterol) in serum were determined by internationally standardized methods at the clinical laboratory of the Department of Medical Sciences using a semi-automatic biochemistry analyzer SPINLAB (Spinreact, Girona, Spain).

In addition, insulin levels were performed by a serum sandwich format enzyme-linked immunosorbent assay (ELISA) using an ELISA kit (ALPCO, Inc., Salem, NH, USA). As indices of insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) and the Matsuda Index were calculated (Gastaldelli, 2022). Areas under the curves for glucose (AUC gluc) were calculated based on the trapezoidal rule.

2.5.7. *Ethical statement*

Ethical approval for the involvement of human subjects in this study was granted by the Institutional Ethics Committee from the University of Guanajuato (CIBIUG-P53-2018), and all subjects signed the informed consent before undergoing any procedure. The clinical trial was registered at ClinicalTrials.gov with the following identifier: NCT05869344.

2.5.8. *Statistical analysis*

Numerical continuous variables were expressed as mean and standard deviation or median and interquartile range according to their distribution, and categorical variables were summarized by counts and percentages. The outcomes for this study were postprandial glucose and insulin levels after BPH or placebo treatment: glucose 60 min (mg/dL); glucose 120 min (mg/dL); glucose 150 min (mg/dL); AUC gluc (mg.h/dL); insulin 60 min (μIU/mL); insulin 120 min (μIU/mL); insulin 150 min (μIU/mL).

Comparison between groups was performed using independent Student's *t*-test. In addition, considering that fasting glucose and postprandial 2-h glucose at baseline were used to classify participant's metabolic status, they were used as covariates for an analysis of covariance (ANCOVA). Thus, ANCOVA models were used to compare outcome variables between treatments, and the results were reported as mean differences of the placebo from the 5 g BPH treatment. Bonferroni adjustment was used to account for multiple comparisons. Another confounding variables were also considered for the ANCOVA models such as age, sex, BMI, waist circumference or metabolic status (NGT of Prediabetes). However, they did not have statistical significance and were not include in the ANCOVA models.

A statistical significance of $p < 0.05$ was accepted, and the statistical program SPSS 28.0 for Windows was used.

3. Results and discussion

3.1. *Black bean hydrolysate peptide identification*

It was possible to identify 20 peptides from 11 to 18 amino acids length (1,259.54–1,959.43 Da) in the black bean protein hydrolysate. Peptide sequences origin was identified by comparison with parental proteins from *Phaseolus vulgaris* cultivars (Table 1). Eighteen peptide sequences presented a similarity of 90–100 % to phaseolin β-type, the main storage protein (globulin) in this seed. Seven peptides had a similarity of 80 % to PvD1defensin, a metabolic protein synthesized for antimicrobial purposes by several pulses, and other plants. Other metabolic parental proteins identified in at least one sequenced peptide were plastocyanin, tyrosine-tRNA ligase, photosystem I-N subunit, α-amylase inhibitor complex, bean lectin-like inhibitor, L-asparaginase and purple phosphatase complex (similarity about 80 %).

Sequenced bioactive peptides isoelectric point (pI) ranged from 3.69 to 9.93. ELSKDDVFVIPAA and LKDGLSVISPKW, peptide sequences were the most acid and alkaline protein fractions, respectively. The net charge of peptides ranged from −2 to 1, the 15 of 20 of the sequenced peptides presented a negative charge (-2 = 2 of 20 peptides; −1 = 13 of 20 peptides), 3 of 20 peptides had a neutral charge, and 2 of 20 peptides

Table 1
Bioactive peptide sequences identified in the BPH.

Peptide Sequence	Protein name	Parental protein (similarity)	Amino acid length	Mass (Da)	pI	Net Charge	Hydrophobicity (Kcal/mol)
TSDNPIFSDHQ	Cupin type-1 domain-containing protein (fragment)	Phaseolin β-type (100 %)	11	1,259.54	3.91	−2	+17.61
TSDNPIFSDHQK			12	1,387.635	5.14	−1	+20.41
LVNPDPKEDLRI		Phaseolin β-type (100 %) or PvD1 defensin (80 %)	12	1,407.77	4.27	−1	+20.47
NPDPKEDLRIIQ			12	1,436.76	4.27	−1	+21.83
ELSKDDVFVIPAA			13	1,402.732	3.69	−2	+18.21
VNPDPKEDLRIIQ			13	1,535.828	4.27	−1	+21.37
YLVNPDPKEDLRIIQ			15	1,811.975	4.27	−1	+19.41
LVNPDPKEDLRIIQL			15	1,761.996	4.27	−1	+18.87
FYLVNPDPKEDLRIIQ			16	1,959.043	4.27	−1	+17.70
MPVNNPQIHEF	Cupin type-1 domain-containing protein	Phaseolin β-type (100 %)	11	1,324.622	5.06	−1	+12.65
AMPVNNPQIHEF			12	1,395.659	5.06	−1	+13.15
LVNPDPKEDLRI		Phaseolin β-type (100 %) or PvD1 defensin (80 %)	12	1,407.77	4.27	−1	+20.47
FSDNQKIPAGTIFY		Plastocyanin (100 %) or phaseolyn (92.86 %)	14	1,599.791	6.52	0	+12.09
QGDNPIFSDNQKIPAGTI		Tyrosine-tRNA ligase (100 %) of phaseolin β-type (93.75 %)	18	1,913.945	3.93	−1	+19.94
LKDGLSVISPKW	Uncharacterized protein	Photosystem I-N subunit (80 %), α-amylase inhibitor complex (80 %) or bean lectin-like inhibitor (80 %)	12	1,341.763	9.93	1	+13.18
YSGVWFPQPAPKD	Formate dehydrogenase/ mitochondrial	Non-significant similarity	13	1,490.7172	6.48	0	+12.67
SIGRALDGKDVLG	Uncharacterized protein	Phaseolin β-type (100 %), L-asparaginase (100 %) or purple phosphatase complex (80 %)	13	1,299.7125	6.67	0	+20.12
AMPVNNPQIHD	Cupin type-1 domain-containing protein (fragment)	Phaseolin β-type (90.91 %)	11	1,234.5747	4.98	−1	+14.87
LVNPDPKEDLRIIQ		Phaseolin β-type (100 %)	14	1,648.912	4.27	−1	+20.12
MDKPNGPVWRISG	Peptidase A1 domain containing protein	Phaseolin β-type (100 %)	13	1,455.727	9.91	1	+15.70

pI, isoelectric point. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophane; Y, tyrosine.

had a net charge of 1. The hydrophobicity of peptides ranged from + 12.09 (FSDNQKIPAGTIFY) to + 21.87 kcal/mol (NPDPKEDLRIIQ).

Amino acid composition, sequence of amino acids, and chain length are some of the main parameters influencing protein hydrolysis during enzymatic treatments. However, net charge, and hydrophobicity are key indicators of peptide digestibility (Ahmed, Sun, & Udenigwe, 2022; Swaney, Wenger, & Coon, 2010). Most of the peptide sequences found in BPH show negative net charge (15 of 20 peptides), which means that these sequences are more unstable at intestinal pH (Ahmed, Sun, & Udenigwe, 2022). Additionally, most of these peptides do not present C-terminal Leu, present relatively high molecular weight (>1,000 Da), and are mostly hydrophobic (>+12.0 kcal/mol) (Ahmed, Sun, & Udenigwe, 2022). These properties are decisive factors in the production of small peptides during gastrointestinal digestion. Therefore, their subsequent bioaccessibility, bioavailability, and bioactivity depend on these key factors (Ahmed, Sun, & Udenigwe, 2022; Amigo & Hernández-Ledesma, 2020).

3.2. *In silico prediction of peptide digestion*

Biological potential of peptides produced by *in silico* digestive prediction from common beans protein and protein hydrolysates have been previously reported (Luna-Vital, Mojica, González de Mejía, Mendoza, & Loarca-Piña, 2015; Mojica, Gonzalez de Mejia, Granados-Silvestre, & Menjivar, 2015; Mojica, Luna-Vital, & González de Mejía, 2015; Mojica & de Mejía, 2015; Mojica & de Mejía, 2016). *In silico* digestive assays predict the enzymatic breakdown of polypeptides to shorter peptides (<1 kDa) by mimicking enzymatic digestive conditions of the human body (Coscueta, Batista, Gomes, da Silva, & Pintado, 2022; Marseglia et al., 2019). Bioaccessibility, bioavailability, and bioactivity relationships of black bean digested peptides (BDP) have been directly related to their chemical structure. The small size of generated peptides could facilitate their interaction with enzyme active sites, biological markers in the bloodstream, cell receptors, hormones, etc. BDP could participate in numerous metabolic and physiological processes (Coscueta, Batista, Gomes, da Silva, & Pintado, 2022; Indrati, 2021; Marseglia et al., 2019; Yan, Zhao, Yang, & Zhao, 2019).

In silico prediction revealed that 20 BDP sequences from BPH presented at least one di- or tripeptide with antidiabetes and antihypertensive potential (Table 2). Pepsin and chymotrypsin are endopeptidases that break peptide bonds in hydrophobic and aromatic amino acids. At the same time, trypsin cleaves the peptide bond between the carboxyl group of arginine or the carboxyl group of lysine and the amino group of the adjacent amino acid (Boschin, Scigliuolo, Resta, & Arnoldi, 2014; Simpson, 2006). A combination of pepsin/trypsin/chymotrypsin is a better representation of the enzymatic process during the gastrointestinal digestion of protein substrates. It produces more bioactive BDP compared to digestions using two of these enzymes (pepsin-trypsin, pepsin-chymotrypsin, or trypsin-chymotrypsin). This could be due to the sequence of proteases cleaving proteins, and the resulting peptides were then cleaved by another protease that could not hydrolyze the original protein (Boschin, Scigliuolo, Resta, & Arnoldi, 2014; Maillet, 2019; Simpson, 2006).

Several of the released di- or tripeptides are reported to exert antidiabetes potential, such as α-amylase, α-glucosidase, and dipeptidyl peptidase-IV (DPP-IV) inhibition, as well as GLP-1 receptor agonists (Yan, Zhao, Yang, & Zhao, 2019).

Twenty-three BDP (22 dipeptides and 1 tripeptide ranging in 160–441 Da) from BPH were related to DPP-IV inhibitory activity (Table 2). DPP-IV is an enzyme targeted for the treatment of T2D (Neumiller, Wood, & Campbell, 2010). DPP-IV inactivates two incretin hormones that participate in insulin secretion (GLP-1 and glucose-

Table 2
Predicted biological potential of di- and tri-peptides generated by *in silico* digestive prediction of BPH peptide sequences.

Peptide Sequence	Bioactive Di/Tri-Peptides (MW)	Bioactivity
TSDNPIFSDHQ	IF (278 Da)	ACE inhibitory
TSDNPIFSDHQK	IF (278 Da), QK (274 kDa)	ACE inhibitory
LVNPDPKEDLRI	VN (231 Da), DP (230 Da), PK (243 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
	LR (287 Da)	ACE inhibitory, DPP III inhibitory, renin inhibitory
NPDPKEDLRIIQ	NP (229 Da), DP (230 Da), PK (243 Da), IQ (259 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
ELSKDDVFVIPAA	EL (260 Da)	Antioxidant
	SK (233 Da)	DPP IV inhibitory
	VF (264 Da), IP (228 Da), IPA (299 Da)	ACE inhibitory, DPP IV inhibitory
	AA (160 Da)	ACE inhibitory, DPP IV inhibitory, hypotensive
VNPDPKEDLRIIQ	VN (231 Da), DP (230 Da), PK (243 Da), IQ (259 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
YLVNPDPKEDLRIIQ	YL (294 Da)	ACE inhibitory, anxiolytic, DPP III inhibitory, DPP IV inhibitory
	VN (231 Da), DP (230 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
LVNPDPKEDLRIIQL	DP (230 Da), PK (243 Da), LQ (259 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
	LR (287 Da)	ACE inhibitory, DPP III inhibitory, renin inhibitory
FYLVNPDPKEDLRIIQ	FY (328)	ACE inhibitory
	VN (231 Da), DP (230 Da), PK (243 Da), IQ (259 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
MPVNNPQIHEF	MP (264 Da), VN (231 Da)	DPP IV inhibitory
	PQ (243 Da)	ACE inhibitory, DPP IV inhibitory
	IH (268 Da)	DPP III inhibitory, DPP IV inhibitory
	EF (294 Da)	ACE inhibitory, CaMPDE inhibitory, hypolipidemic, renin inhibitory
AMPVNNPQIHEF	VN (231 Da)	DPP IV inhibitory
	PQ (243 Da)	ACE inhibitory, DPP IV inhibitory
	EF (294 Da)	ACE inhibitory, CaMPDE inhibitory, hypolipidemic, renin inhibitory
LVNPDPKEDLRI	VN (231 Da), DP (230 Da), PK (243 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
	LR (287 Da)	ACE inhibitory, DPP III inhibitory, renin inhibitory
FSDNQKIPAGTIFY	QK (274 Da), IFY (441 Da)	ACE inhibitory
	IP (228 Da), IPA (299 Da)	ACE inhibitory, DPP IV inhibitory

Table 2 (*continued*)

Peptide Sequence	Bioactive Di/Tri-Peptides (MW)	Bioactivity
QGDNPIFSDNQKIPAGTI	QG (203 Da), IF (278 Da), QK (274 Da)	ACE inhibitory
	IP (228 Da), IPA (299 Da)	ACE inhibitory, DPP IV inhibitory
LKDGLSVISPKW	LK (259 Da)	Antioxidant
	KW (332 Da)	ACE inhibitory, DPP IV inhibitory
YSGVWFPQPAPKD	VW (303 Da)	α-glucosidase inhibitory, ACE inhibitory, antioxidant, DPP IV inhibitory
	PQ (243 Da), AP (186 Da), KD (261 Da)	ACE inhibitory, DPP IV inhibitory
	PA (186 Da), PK (243 Da)	DPP IV inhibitory
SIGRALDGKDVLG	IG (188 Da)	ACE inhibitory
	RA (245 Da)	ACE inhibitory, DPP IV inhibitory, ubiquitin-mediator proteolysis activator
AMPVNNPQIHD	VN (231 Da)	DPP IV inhibitory
	PQ (243 Da)	ACE inhibitory, DPP IV inhibitory
	IH (268 Da)	ACE inhibitory, DPP III inhibitory, DPP IV inhibitory
LVNPDPKEDLRIIQ	VN (231 Da), DP (230 Da), PK (243 Da), IQ (259 Da)	DPP IV inhibitory
	KE (275 Da)	ACE inhibitory, DPP IV inhibitory
	LR (287 Da)	ACE inhibitory, DPP III inhibitory
MDKPNGPVWRISG	PN (229 Da)	DPP IV inhibitory
	GP (172 Da)	ACE inhibitory, DPP IV inhibitory, stomach mucosal activity regulator, antithrombotic & prolyl endopeptidase inhibitor
	VW (303 Da)	α-glucosidase inhibitory, ACE inhibitory, antioxidant, DPP IV inhibitory

A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophane; Y, tyrosine. ACE, angiotensin-converting enzyme; DPP IV, dipeptidyl peptidase-4; DPP III, dipeptidyl peptidase-3; CaMPDE, calmodulin-dependent cyclic nucleotide phosphodiesterase.

dependent insulinotropic peptide (GIP)) (Yan, Zhao, Yang, & Zhao, 2019). BDP could interact with the active site of the DPP-IV enzyme and interrupt the degradation of incretin hormones, which could enhance insulin secretion to decrease the blood glucose levels in T2D patients (Estrada-Salas, Montero-Morán, Martínez-Cuevas, González, & Barba de la Rosa, 2014; Indrati, 2021; Neumiller, Wood, & Campbell, 2010; Saleh et al., 2018; Yan, Zhao, Yang, & Zhao, 2019).

Several BDP with DPP-IV inhibitory potential also have been found in legume and cereal protein hydrolysates, such as soybean (DP, IP, AA, MP, AP, PA, NP, VN), pea (VN, DP, NP, IQ, SK, AA, PQ), lupin (PK), chickpea (YL, VF), rice bran (GP, IP, RA, AA, PQ, VW) and whey protein (IPA) (Bollati et al., 2022; Lammi et al., 2016; Pooja, Rani, & Prakash, 2017; Tulipano, Sibilia, Caroli, & Cocchi, 2011). However, BPH presents the highest diversity of short peptides with DPP-IV inhibitory potential compared to other seed protein hydrolysates.

Twenty-two BDP (20 dipeptides and 2 tripeptides with molecular weight ranging from 160 to 441 Da) presented angiotensin-converting enzyme (ACE) inhibitory potential (Table 2). ACE inhibition is related to hypotensive effects, retarding nephropathies, and cardiovascular disease progression in patients with prediabetes and T2D (Velarde-Salcedo et al., 2013), additionally in animal models of metabolic syndrome improves insulin sensitivity (Loloi et al., 2018). During digestion, endopeptidases (pepsin, trypsin, and chymotrypsin) can produce short peptides from polypeptides having a hydrophobic and/or aromatic amino acid as a terminal group (mainly proline, tryptophan, phenylalanine, and tyrosine) (Vermeirssen, Van Camp, & Verstraete, 2004). These terminal groups are common in the BDP predicted by *in silico* digestion of BPH (Table 2). The 20 BDP could interact with the ACE catalytic site, permitting its inhibition (Boschin, Scigliuolo, Resta, & Arnoldi, 2014; Vermeirssen, Van Camp, & Verstraete, 2004). Negatively charged BDP may bind to ACE, especially interacting with amino acid residues at *C*- end and/or *N*- end (Daskaya-Dikmen, Yucetepe, Karbancioglu-Guler, Daskaya, & Ozcelik, 2017).

Several short-chain peptides from common beans and other pulses have shown potential to block ACE and interfere with the renin-angiotensin system (Fountain et al., 2023). Moreover, dipeptides found in BPH have also been reported with ACE inhibitory activity in other pulses protein hydrolysates such as chickpea (YL, VF, LR), soy (IP, AA, AP), pea (IF, AA, LQ, PQ), as well as rice bran (VF, GP, IP, RA, AA, QG, QK, PQ, IF, VW) (Arámburo-Gálvez et al., 2022; Bollati et al., 2022; Velarde-Salcedo et al., 2013). However, BPH presents higher diversity of these biomolecules.

Most BDP could be released from more than one peptide sequence from the BPH, and some may exhibit more than one biological potential. Dipeptides such as AA, VW, and GP have been reported in other plant protein sources; their biological potential is also related to ACE and DPP-IV inhibition (Bollati et al., 2022; Pooja, Rani, & Prakash, 2017).

BDP presented other bioactivities that could be related to promoting a glucose-lowering effect in diabetic and prediabetic patients (antioxidant, ubiquitin-mediator proteolysis activation, renin, α-glucosidase, and CaMPDE inhibitory activities) (Daskaya-Dikmen, Yucetepe, Karbancioglu-Guler, Daskaya, & Ozcelik, 2017; Hadi & Suwaidi, 2007; Tonyan et al., 2022; Ying, Agyei, Udenigwe, Adhikari, & Wang, 2021).

Other dipeptides (160–303 Da) showing different biological potentials (DPP-III, anxiolytic, hypotensive, etc.) were identified after *in silico* digestion of BPH.

Characterizing peptides in common bean protein hydrolysates helps to understand the relationship between peptide structure and biological potential. Using *in silico* assays is important to compare results with available published data. The information in the *in silico* assays helps theoretically hypostatize biological potential related to the identified peptide structures. Together, they contribute to a comprehensive understanding of the role of bioactive peptides and proteins in *in vivo* or clinical assays.

Moreover, through peptide identification and *in silico* assays, more potent or interesting peptide sequences could be purified or synthesized to evaluate their individual biological potential.

3.3. Randomized clinical trial

In this study, 52 subjects were assessed for eligibility in the Department of Medical Sciences, 22 adults failed to meet the inclusion criteria, and 2 adults retired their consent before randomization. Thus, 28 adults, (65 % women) (14 subjects with normal glucose tolerance and 14 with prediabetes), were randomly assigned by stratification to one of the two treatments: 5 g BPH ($n = 14$) or placebo ($n = 14$). Hence, 7 participants with NGT and 7 with prediabetes were included in each treatment group (Fig. 1).

Table 3 shows the general baseline characteristics by metabolic status, normal glucose tolerance (NGT), and prediabetes. Anthropometric and biochemical variables (age, weight, BMI, waist circumference, systolic/diastolic blood pressure, cholesterol values, and triglycerides) did not show significant differences at baseline ($p > 0.05$). As expected by the selection criteria, only fasting glucose (NGT = 94.8 mg/dL, prediabetes = 109.9 mg/dL) and postprandial glucose (2 h) (NGT = 99.5, mg/dL, prediabetes = 124.8 mg/dL) had significant differences ($p < 0.01$).

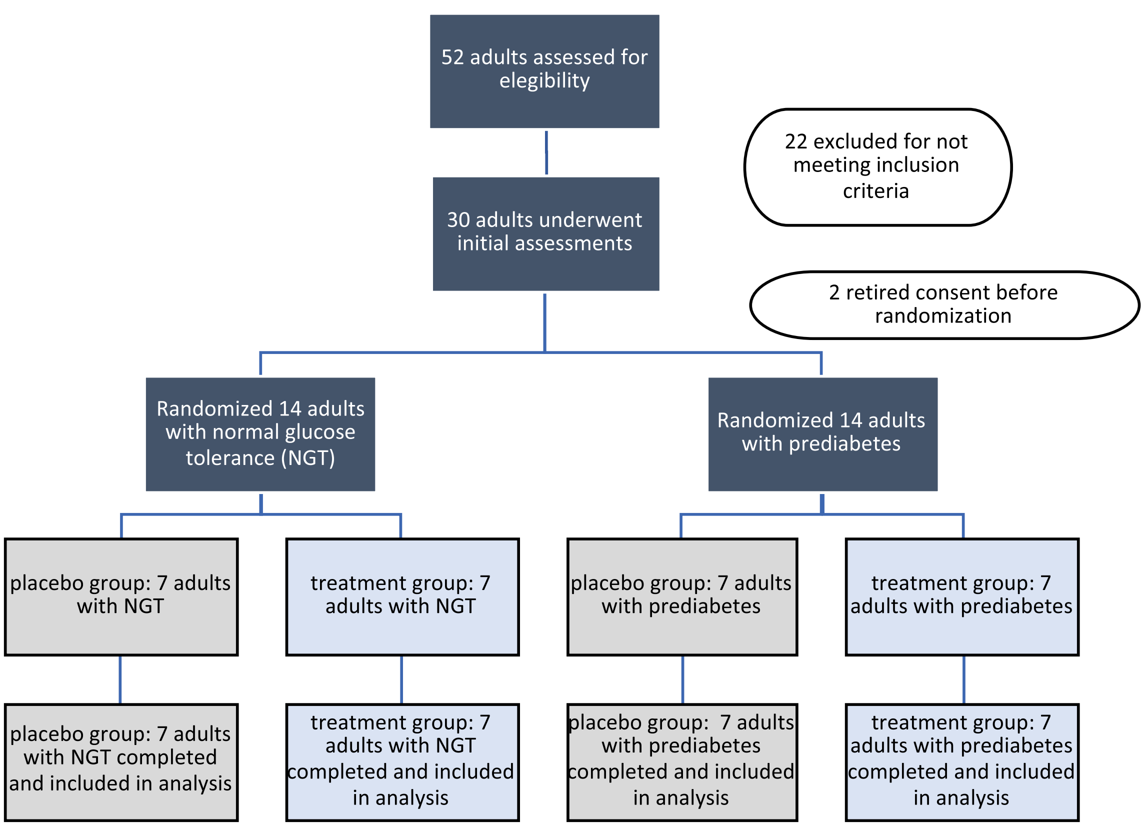

Fig. 1. Participants flow chart.

Table 3
Baseline general characteristics by metabolic status (normal glucose tolerance and prediabetes).

Clinical and Metabolic Variables	NGT (n = 14)	Prediabetes (n = 14)	p-value
Age (years)	40.5 ± 8.3	38.3 ± 8.8	0.5
Weight (kg)	79.8 ± 10.0	78.7 ± 9.1	0.8
Body mass index (kg/m^2)	29.3 ± 2.1	30.5 ± 3.5	0.3
Waist circumference (cm)	98.5 ± 7.2	100.0 ± 9.1	0.6
Systolic blood pressure (mmHg)	113.6 ± 12.4	115.1 ± 12.3	0.8
Diastolic blood pressure (mmHg)	73.6 ± 7.0	73.5 ± 9.6	0.9
Fasting glucose (mg/dL)	94.8 ± 3.7	109.9 ± 8.2	<0.01*
Postprandial glucose (mg/dL)	99.5 ± 15.7	124.8 ± 27.5	<0.01*
Total cholesterol (mg/dL)	194.2 ± 39.9	183.6 ± 46.9	0.5
C-HDL (mg/dL)	41.2 ± 7.4	44.1 ± 8.5	0.3
C-LDL (mg/dL)	123.5 ± 39.4	119.4 ± 34.1	0.8
Triglycerides (mg/dL)	125.9 ± 42.3	100.6 ± 47.3	0.1

All variables are presented as mean ± Standard Deviation. The difference between groups was evaluated with a Student's *t*-test for independent variables. *$P < 0.05$. NGT: Normal glucose tolerance; C-HDL: high-density lipoprotein; LDL-C: low-density lipoprotein

In Table 4, the general baseline characteristics are shown for both treatment groups (5 g BPH and placebo), without regard to metabolic status. Anthropometric and biochemical variables (age, weight, BMI, waist circumference, systolic/diastolic blood pressure, cholesterol, triglycerides, and HOMA-IR values) did not show significant differences at baseline ($p > 0.05$). Thus, randomization resulted in similar groups.

In Table 5 and Fig. 2, the unadjusted outcome variables are shown during the intervention. Postprandial glucose levels were lower in the 5 g BPH group, but only at 120 min were statistically significant ($p = 0.02$) (93.1 ± 21.6 mg/dL) when compared with the placebo (116.2 ± 27.2 mg/dL) (Fig. 2 **A**). Similarly, insulin levels were lower in the 5 g BPH group, however, the results were not statistically significant ($p > 0.05$) (Fig. 2**B**).

To account for the participants metabolic status ANCOVA models were built to include as covariates fasting glucose and 2-h postprandial glucose levels at baseline. The adjusted results for outcome variables are presented in Table 6. Indeed, glucose levels at 120 min presented a significant mean adjusted difference of 18.1 mg/dL with 95 % IC (2.8 to 33.3 mg/dL) ($p = 0.03$) when comparing placebo to the 5 g BPH group. Insulin levels remained not statistically different between groups.

Table 4
Baseline general characteristics by intervention group.

Baseline Clinical and Metabolic Variables	Placebo (*n* = 14)	BPH (*n* = 14)	p-value
Age (years)	38.7 ± 9.3	40.2 ± 7.9	0.7
Weight (kg)	77.7 ± 7.7	80.6 ± 11.0	0.4
Body mass index (kg/m^2)	30.4 ± 2.8	29.5 ± 3.0	0.4
Waist circumference (cm)	98.7 ± 6.7	99.6 ± 9.6	0.6
Systolic blood pressure (mmHg)	112.1 ± 11.4	116.7 ± 12.9	0.3
Diastolic blood pressure (mmHg)	71.2 ± 6.8	76.1 ± 9.1	0.1
Fasting glucose (mg/dL)	102.9 ± 8.9	101.8 ± 11.2	0.8
Postprandial glucose (mg/dL)	116.2 ± 26.6	108.1 ± 24.5	0.4
Total cholesterol (mg/dL)	189.8 ± 50.0	188.1 ± 36.9	0.9
C-HDL (mg/dL)	40.9 ± 8.0	44.5 ± 8.0	0.2
C-LDL (mg/dL)	120.4 ± 44.1	122.6 ± 28.0	0.9
Triglycerides (mg/dL)	121.6 ± 42.0	104.9 ± 49.6	0.3
HOMA-IR	2.1 ± 0.9	1.9 ± 0.9	0.5

All variables are presented as mean ± Standard Deviation. The difference between groups was evaluated with a Student's *t*-test for independent variables. *, $P < 0.05$. C-HDL: high lipoprotein; LDL-C: low-density lipoprotein; HOMA-IR: Homeostatic model assessment.

Table 5
Metabolic variables during the intervention.

	Placebo (*n* = 14)	BPH (*n* = 14)	Mean difference	p-value
Glucose 0 min (mg/dL)	96.2 ± 13.4	93.0 ± 10.0	3.2 (-6.0 to 12.0)	0.5
Glucose 60 min (mg/dL)	142.9 ± 43.6	117.1 ± 32.5	25.8 (-4.0 to 55.7)	0.09
Glucose 120 min (mg/dL)	116.2 ± 27.2	93.1 ± 21.6	23.1 (4.0 to 42.3)	0.02*
Glucose 150 min (mg/dL)	91.4 ± 26.5	81.1 ± 16.6	10.3 (-7.0 to 27.4)	0.2
AUC for glucose (mg.h/dL)	300.9 ± 66.2	253.6 ± 48.1	47.3 (2.4 to 92.2)	0.04*
Insulin 0 min (μIU/mL)	9.0 ± 3.7	8.6 ± 3.9	0.4 (-2.5 to 3.3)	0.8
Insulin 60 min (μIU/mL)	114.9 ± 49.3	107.2 ± 53.7	7.7 (–32.4 to 47.7)	0.7
Insulin 120 min (μIU/mL)	77.2 (70.86)	32.61 (47.83)	25.7 (-3.2 to 54.5)	0.06
Insulin 150 min (μIU/mL)	24.47 (51.58)	13.17 (16.14)	15.8 (-5.7 to 37.3)	0.4
Matsuda Index	2.91 (2.48)	4.10 (4.01)	−0.8 (-3.5 to 1.9)	0.1

Variables are presented as mean ± Standard Deviation or median (interquartile range). Also mean difference (95 % CI) is presented, the difference between groups was evaluated with a Student's *t*-test for independent variables or the Mann-Whitney *U* test for nonparametric variables. *, $P < 0.05$.

Furthermore, the AUC for glucose was lower in the 5 g BPH group (260.4 ± 10.9 mg.h/dL) when compared with the placebo (294.2 ± 10.9 mg.h/dL) ($p = 0.04$) with a mean adjusted difference of 18.1 mg.h/dL with 95 % IC (1.7 to 65.9 mg.h/dL) (Table 6). These results suggest that BPH could have an acute effect on lowering postprandial glucose independent of the metabolic status.

Finally, HOMA-IR and Matsuda index did not show statistical difference ($p > 0.05$) between the 5 g BPH and placebo groups (Tables 4 and 5).

These results found in the clinical study may indicate that the postprandial hypoglycemic effect of BPH may occur at the small intestine level by reducing the glucose absorption (Geerts et al., 2011; Mojica, Chen, & de Mejía, 2015; Mojica, Luna-Vital, & Gonzalez de Mejia, 2018; Mojica & de Mejía, 2016; Oseguera-Toledo, de Mejia, & Amaya-Llano, 2015; Valencia-Mejía, Batista, Fernández, & Fernandes, 2019; Wu et al., 2020). A previous *in vitro* study indicated that small bioactive peptides delayed the breakdown of dietary carbohydrates and reduced glucose absorption from the small intestine. This suggests an inhibitory effect on postprandial glucose levels (Ibrahim et al., 2018).

Additionally, transepithelial transport of glucose could block the transduction signals for the expression of SGLT1 glucose transporter by BPH/BSH peptides in the intestine cell epithelium. Since this protein depends on glucose concentration in the intestine's apical site (Chan-Zapata, Sandoval-Castro, & Segura-Campos, 2022; Ina et al., 2020). BPH peptides could also act as antagonists of the sweet-taste receptor (T1R2/T1R3) and inhibit the expression of SGLT1; moreover, these peptides may retard the diffusion rate of glucose in the intestine (Ina et al., 2020; Rahmi & Arcot, 2023; Shirazi-Beechey, Moran, Batchelor, Daly, & Al-Rammahi, 2011). SGLT1 is responsible for the induction of membrane depolarization of Ca^{++}, which subsequently activates the protein kinase C II leading to the translocation of GLUT2 to the apical membrane from the cytoplasm by vesicle transport (Kellett & Brot-Laroche, 2005). GLUT2 is the main cellular glucose transporter at the intestine level; thus, by interrupting its activity, it is possible to modulate glucose absorption and its following incorporation into the bloodstream (Kellett &

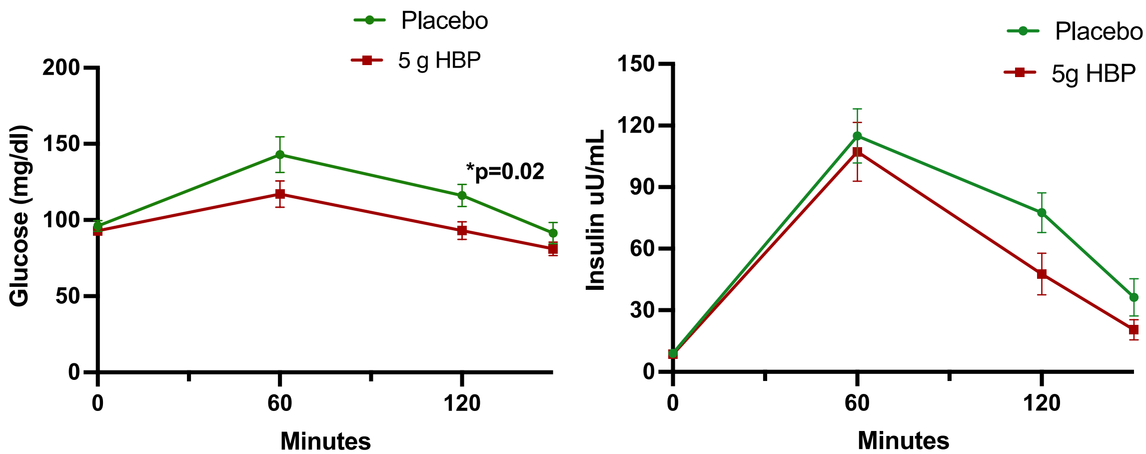

Fig. 2. (**A**) Glucose levels during the oral glucose tolerance test intervention. (**B**) Insulin levels during the oral glucose tolerance test intervention. The difference between the means was evaluated with a Student's *t*-test for independent variables.

Table 6
Outcome variables adjusted for metabolic status.

	Placebo (*n* = 14)	BPH (*n* = 14)	Mean adjusted difference	p-value
Glucose 60 min (mg/dL)	139.0 ± 8.3	120.9 ± 8.3	18.1 (-6.3 to 42.4)	0.09
Glucose 120 min (mg/dL)	113.7 ± 5.2	95.6 ± 5.2	18.1 (2.8 to 33.3)	0.03*
Glucose 150 min (mg/dL)	88.7 ± 4.0	83.7 ± 4.0	4.9 (-6.8 to 16.8)	0.4
AUC for glucose (mg.h/dL)	294.2 ± 10.9	260.4 ± 10.9	33.8 (1.7 to 65.9)	0.04*
Insulin 60 min (μIU/mL)	115.2 ± 14.4	106.8 ± 14.4	8.4 (–33.8 to 50.7)	0.7
Insulin 120 min (μIU/mL)	72.5 ± 10.2	48.6 ± 10.2	23.9 (-6.1 to 53.9)	0.1
Insulin 150 min (μIU/mL)	34.2 ± 6.6	22.5 ± 6.6	11.7 (-7.7 to 31.1)	0.2

Variables are presented as mean ± Standard error of mean, these results are adjusted for the covariates baseline fasting glucose and 2-h postprandial glucose. The mean adjusted difference (95 % CI) was calculated using ANCOVA models with baseline fasting glucose and 2-h postprandial glucose as covariates. *, $P < 0.05$.

Brot-Laroche, 2005). Mojica, Luna-Vital, and Gonzalez de Mejia (2018) found that acute expression and translocation of GLUT2 decreased 0.5-fold with an acute exposition of BPH and glucose to Caco-2 cells. Thus, derived peptides from BPH may induce acute hypoglycemic effects by interfering with glucose uptake by reducing the expression of SGLT1 and interrupting the expression and the translocation of GLUT2 from the cytoplasm.

Other studies have found similar hypoglycemic effects in small peptides (<400 Da) from rice albumin hydrolysates (Ina et al., 2020), germinated chickpea protein hydrolysate (Chandrasekaran & de Mejia, 2022), soy protein derivatives (Lu et al., 2012; Oliva et al., 2015; Lammi, Zanoni, & Arnoldi, 2015) and black bean peptides (Mojica & de Mejía, 2016; Mojica, Luna-Vital, & Gonzalez de Mejia, 2018). Also, in protein hydrolysates from defatted salmon backbones (Slizyte et al., 2016) and milk-protein hydrolysate in a chronic evaluation (Sartorius et al., 2019) using cell culture models or clinical trials.

Similar to our results in the AUC for glucose, peptide fractions from rice and common beans also showed lower glucose AUC levels in acute *in vivo* and human clinical trials, whose mechanisms of action could be related to the reduction in the expression of glucose transporters and inhibition of α-glucosidase activity at intestine level (Chan-Zapata, Sandoval-Castro, & Segura-Campos, 2022; Ibrahim et al., 2018; Mojica, Luna-Vital, & González de Mejía, 2017; Mojica & de Mejía, 2015; Mojica & de Mejía, 2016; Oseguera-Toledo, de Mejia, & Amaya-Llano, 2015; Valencia-Mejía, Batista, Fernández, & Fernandes, 2019).

Similar to our results, in other acute or chronic clinical assays, casein milk-protein, soy, and egg white hydrolysates did not present changes in HOMA-IR or Matsuda index values between placebo and experimental treatments (de Campos Zani, Wu, & Chan, 2018; Gutch, Kumar, Razi, Gupta, & Gupta, 2015; Sartorius et al., 2019).

The *in silico* assays showed that BPH presents a great diversity of short peptides with DPP-IV and ACE inhibitory activity as shown in Table 2. Since ACE inhibition has shown to decrease glucose levels and improve insulin sensitivity in long term studies the observed results in this clinical trial could not be attributed to ACE inhibition.

Furthermore, DDP-IV is identified as an important marker related to the hypoglycemic effect of black bean peptides. However, DPP-IV inhibition could not be the mechanism of action for the results observed in this study. This clinical trial only evaluated the acute effect of BPH (60 to 150 min), and since the peptides must be absorbed and enter circulation to block DDP-IV, 120 min is not enough time to promote insulin release and, in this way, to regulate blood glucose levels through DPP-IV inhibition. In addition, in this study participants did not show a significant increase in insulin secretion.

Mojica et al., 2017, 2018, described the potential mechanism of action of the bean peptides through the interaction with glucose transporters GLUT2 and SGLT1, decreasing their expression and translocation to the membrane. This interaction could be the main responsible for lowering postprandial glucose levels in this clinical trial.

The current study represents the first clinical trial evaluating the effects of black bean protein hydrolysates (*Phaseolus vulgaris* L.) on postprandial glucose and insulin levels along with the identification of BPH peptides with potential biological properties through *in silico* predictive digestion. A major strength of this study is its randomized placebo-controlled design. Furthermore, confounding variables, such as sex, age, body mass index, and metabolic status (normal glucose tolerance and prediabetes), were controlled by design or included as covariates in the statistical analysis.

Despite the strengths of the study, there are some limitations as well. First of all, we could not conduct a randomized crossover study, which would have been the ideal experimental design for our research question. Considering the disadvantages of crossover studies, which have a longer duration and higher dropout rates, we decided to evaluate the acute effect of BPH in a clinical setting using a parallel randomized trial. Moreover, the acute evaluation of HPF did not allow us to observe long-term glucose metabolism changes, and it did not show effect on postprandial insulin levels.

Nonetheless, our study showed that 5 g of HPF could be used acutely to reduce postprandial blood glucose levels in adults with NGT or prediabetes. Comprehensive pharmacologic and chemical studies are required to address the detailed molecular mechanism responsible for the acute glucose-lowering effect of the BPH observed in the study. In addition, long-term clinical trials are needed to evaluate if BPH could have a hypoglycemic effect in adults with NGT or prediabetes.

Our results indicate that the evaluated postprandial glucose inhibition in patients is influenced by the composition, sequence, and molecular weight of the derived peptides. The specific peptides responsible for inhibiting acute postprandial glucose levels are not precisely identified, and only certain combinations of peptides from BHP demonstrate inhibitory effects in adults with NGT or with prediabetes, which is also supported by *in silico* approaches.

4. Conclusion

This study shows a glucose lowering effect of BPH after an acute oral glucose tolerance test on adults with prediabetes and normal glucose tolerance. *In silico* digestive peptide prediction of BPH can infer the profile of small bioactive peptides with the ability to interact with cellular mechanisms to decrease glucose uptake at the small intestine level. BPH could assist in regulating glucose metabolism and maintaining blood glucose homeostasis to treat or prevent prediabetes. These results provide scientific evidence to validate the health benefits of common bean biofunctional ingredients.

CRediT authorship contribution statement

Armando Gómez-Ojeda: Methodology, Investigation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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