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ORIGINAL CONTRIBUTION

# Effect of the intake of dietary protein on insulin resistance in subjects with obesity: a randomized controlled clinical trial

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## Abstract

**Purpose** We compared the effect of diets with different amounts and sources of dietary protein on insulin sensitivity (IS) in subjects with obesity and insulin resistance (IR).

**Methods** Eighty subjects with obesity (BMI ≥ 30 kg/m$^2$) and IR (Matsuda index < 4.3 and HOMA-IR ≥ 2.5) over 18 years old were randomized to four groups for a one-month period: a normal protein diet (< 20%) with a predominance of animal protein (Animal NP) or vegetable protein (Vegetable NP) and a high-protein diet (25–30%) with a predominance of animal protein (Animal HP) or vegetable protein (Vegetable HP). Baseline and final measurements of body weight, body composition, biochemical parameters, blood pressure (BP), resting energy expenditure and plasma amino acid profiles were performed.

**Results** Body weight, BMI and waist circumference decreased in all groups. Interestingly, the IS improved more in the Animal HP (Matsuda index; 1.39 vs 2.58, $P = 0.003$) and in the Vegetable HP groups (Matsuda index; 1.44 vs 3.14, $P < 0.0001$) after one month. The fat mass, triglyceride levels, C-reactive protein levels and the leptin/adiponectin index decreased; while, the skeletal muscle mass increased in the Animal and Vegetable HP groups. The BP decreased in all groups except the Animal NP group.

**Conclusion** Our study demonstrates that a high-protein hypocaloric diets improves IS by 60–90% after one month in subjects with obesity and IR, regardless of weight loss and the source of protein, either animal or vegetable.

**Trial registration** The trial is registered at clinicaltrials.gov (NCT03627104), August 13, 2018.

**Keywords** Dietary protein · Insulin resistance · Obesity · Weight loss · Branched-chain amino acids

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## Introduction

One of the most common metabolic complications of obesity is insulin resistance (IR), which is considered a major risk factor for the development of type 2 diabetes and cardiovascular disease (CVD) [1]. IR is characterized by decreased insulin sensitivity (IS) in peripheral tissues, affecting insulin-mediated glucose absorption, particularly in skeletal muscle tissue, and ultimately generating a state of hyperinsulinemia [2, 3]. The cornerstone of treatment in subjects with obesity is weight loss through dietary energy restriction, since it improves or even normalizes IS and the related comorbidities [4, 5]. However, the optimal recommendation related to the distribution of macronutrients in these diets has been controversial in recent years, mainly regarding the protein composition. It is often recommended to increase protein intake to increase the resting energy expenditure (REE) by preserving the fat-free mass and to increase satiety to decrease energy intake [6]. However, several studies have observed that consumption of high-protein diets (25–30% of energy or > 1.2 g/kg/day) decreases IS and increases gluconeogenesis and hepatic glucose output [7–10]. Some systematic reviews and meta-analyses, however, indicate that the effects of protein intake per se on IS are controversial as a result of different sources of protein origin (animal or vegetable protein) [11–13].

Diets high in vegetable proteins, such as soy protein, improve IS in the presence of a diet high in saturated fats in animal models [14], while diets high in animal proteins are associated with a decrease in IS and an increased risk of developing type 2 diabetes [7, 15, 16]. This finding is probably due to the concentrations and types of amino acids contained in the dietary protein [17]. Protein of vegetable origin has been reported to contain lower concentrations of the branched-chain amino acids (BCAAs) leucine, isoleucine and valine compared to animal protein [17, 18]. On the other hand, it has been shown that the plasma concentration of BCAAs is increased in subjects with obesity and is associated with up to a fivefold increase in the risk of developing type 2 diabetes [19, 20]; that is, the elevation in the plasma concentration of BCAAs is considered a marker of decreased IS [20, 21].

One way to restore BCAA concentrations is through modifications in the amounts and sources of protein in the diet [17]. Therefore, our hypothesis is that a diet high in vegetable protein will decrease IR in subjects with obesity following one month of treatment. Our primary objective was to evaluate the effects of the amount and source of protein in the hypocaloric diet on the improvement of IS over a 1-month period. As a secondary objective, we evaluated the effect on anthropometric parameters, body composition, blood pressure, biochemical parameters related to obesity and plasma amino acid concentrations.

## Research design and methods

### Participants

This study was conducted at the Department of Physiology of Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán (INCMNSZ) in Mexico City from August 2018 to September 2019. Mestizo Mexican subjects aged, 18–60 years with BMI ≥ 30 and ≤ 60 kg/m$^2$ and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR ≥ 2.5) and insulin sensitivity index proposed by Matsuda and DeFronzo (Matsuda index < 4.3) were included [22, 23]. Exclusion criteria were patients with a diagnosis of diabetes, high blood pressure, a history of cardiovascular events, acquired diseases leading to obesity and secondary diabetes, weight loss > 3 kg in the last 3 months, cancer, estimated glomerular filtration rate (eGFR; CKD-EPI) [24] < 60 mL/min/1.73 m$^2$, liver disease, pregnancy, smoking, substance abuse, alcohol consumption, or who were taking any hypolipemic, antihypertensive, hypoglycemic, steroid, chemotherapeutic, immunosuppressive, radiotherapeutic, or anorexic drugs during the 6 months prior to the dietary intervention. This study was conducted according to the guidelines in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the INCMNSZ (REF 2373). All participants were informed about the scope and procedures of the study, and prior to any procedures, written informed consent was formally obtained. The study was registered on ClinicalTrials.gov as NCT03627104.

### Study design

The study consisted of an open-label randomized controlled clinical trial. Subjects were invited to participate through open advertising in hospital centers and social networks. Subjects who met the selection criteria were randomly assigned to one of the groups, which consisted of a hypocaloric dietary intervention. The distribution of carbohydrates and fats was based on the Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) [25]. The study had four intervention groups: group 1, normal protein diet with a predominance of animal origin protein (Animal NP); group 2, normal protein diet with a predominance of vegetable origin protein (Vegetable NP); group 3, high-protein diet with a predominance of animal origin protein (Animal HP); and group 4, high-protein diet with a predominance of vegetable origin protein (Vegetable HP).

The study consisted of 5 weekly visits during one month of follow-up. At the first visit, the medical history was evaluated and dietary intervention groups were assigned. In the first and final visits, a 24-h diet recall, a physical activity questionnaire, and determinations of anthropometric measurements, body composition and biochemical parameters in the serum and plasma were performed. In addition, a 2-h oral glucose tolerance test (OGTT) was performed to assess the area under the glucose and insulin curves (AUC) (Fig. 1).

## Diet

Each participant received a menu with a hypocaloric diet of 1800 kcal. The diets contained the following distribution of macronutrients with respect to total caloric value: (1) Animal NP: protein 19%, with 60% of protein of animal origin, carbohydrates 57% and fats 24%; (2) Vegetable NP: protein 19%, with 60% vegetable protein, carbohydrates 58%, and fats 23%; (3) Animal HP: protein 29.5%, with 60% animal protein, carbohydrates 50.6%, and fats 19.9%; and (4) Vegetable HP: protein 29%, with 60% vegetable protein; carbohydrates 50%; and fats 21%. Diets in all groups contained < 7% saturated fat and < 200 mg of cholesterol (Online Resource 1).

Menus with preparation instructions for 15 days were given according to the participant's assigned group, along with a recipe booklet with instructions for the meals suggested in the menus and videos with the steps of each recipe contained in the menu. In addition, a weekly food pantry with 80% of the food items on the menus was provided for better compliance with the diet (Online Resource 2). Social networks (WhatsApp and private groups on Facebook) were used to resolve participants' doubts. Participants were blinded to their assigned group using colors set in each menu and recipe book. They saw the foods, but did not know in which treatment group they were randomized, and also did not know the menus of the other treatment groups, due to the color difference of each recipe book.

## Random assignment

The assignments were completed using blocked randomization. The participants were divided into four groups using fixed blocks of four cells supported by a table of random numbers. Once the number was assigned for each block, the treatment combinations were used. This randomization was carried out by a person outside the study. This person kept the randomization in a locked cabinet.

## Blinding mechanisms

The study was open to the researcher who indicated the diet and provided the menu along with the recipe book. The researchers who carried out the determinations of

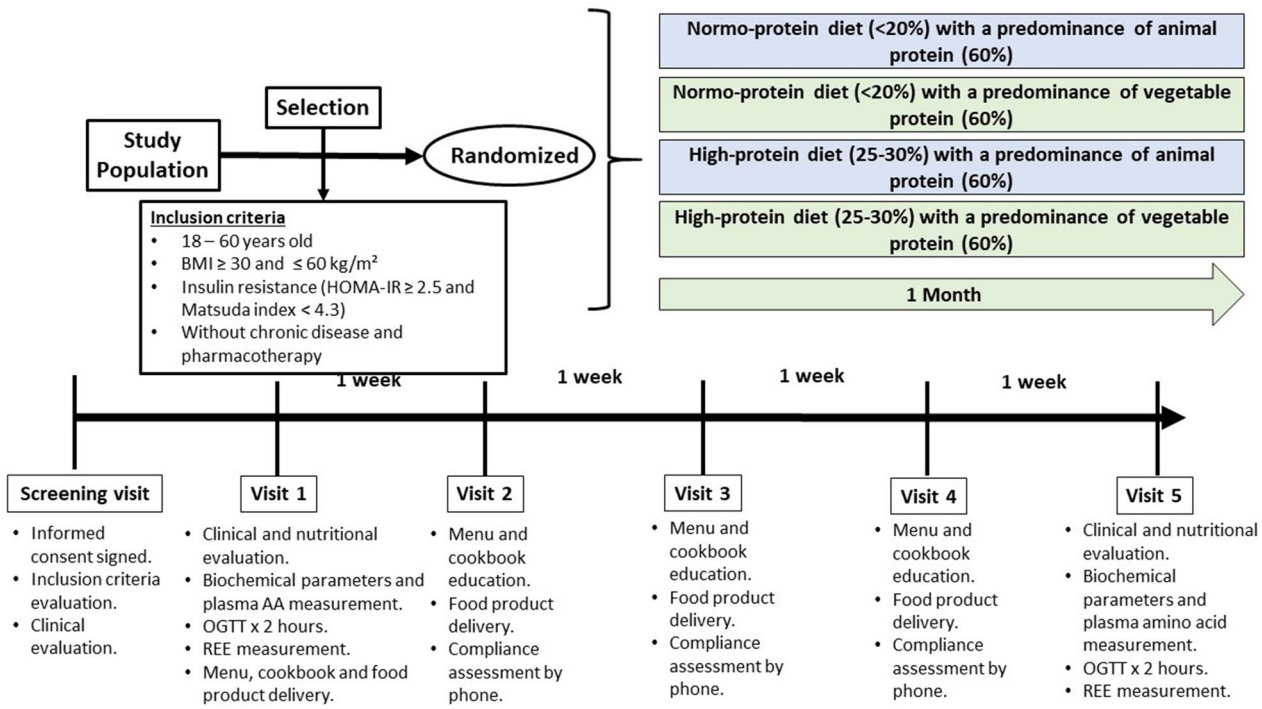


**Fig. 1** Methodology of the study process over time. *BMI* body mass index, *AA* amino acids, *OGTT* oral glucose tolerance test, *REE* resting energy expenditure

anthropometric parameters, body composition, blood pressure, calorimetry and biochemical parameters were blinded to the treatment. The menus and recipe books were designed to be identical in appearance; only the researcher knew the assignment by means of the color of each recipe book. The participants did not know which group they were assigned to. Additionally, the statistical analysis was blinded and performed by a researcher outside of the study.

### Compliance with the diet

Compliance with the diet was monitored through a 24-h reminder carried out in person each week and by twice weekly telephone calls with nutritionists. Participants recorded what they consumed in food logs 3 days a week (2 days during the week and 1 day over the weekend). The data were processed and analyzed in grams using Food Processor software (Version 11.6.522 2018, ESHA Research, USA).

### Biochemical and clinical parameters

In the initial and final visits, the Matsuda index was calculated as a marker for IR along with the fasting serum glucose (mmol/L) and insulin (mUI/L) levels as well as the average concentrations of glucose and insulin obtained during the OGTT [26]. The OGTT was performed after 10 h of fasting, where the serum glucose and insulin concentrations were determined at minutes 0, 15, 30, 45, 60, 90 and 120 after consuming 75 g of glucose, after which, the AUC of glucose and insulin was obtained. The HOMA-IR index was determined using the equation of fasting serum glucose (mmol/L) × plasma insulin (mIU/mL)/22.5 [27].

BP was measured at the initial and final visits of the intervention with a digital sphygmomanometer (Omron, HEM-781INT, China), while the participants were sitting with their right arm uncovered. Four measurements were taken at three-min intervals, eliminating the first measurement and averaging the last three measurements to determine the systolic and diastolic BP. The blood samples were obtained after a 10–12-h fast. The sample was centrifuged at 3000 rpm for 10 min, then the serum or plasma was stored at a temperature of − 70 °C until analysis. The levels of glucose, total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, C-reactive protein (CPR), creatinine and serum urea were determined by the enzymatic colorimetric method using the Cobas integra analyzer, Roche Diagnostics. Indianapolis. IN. Serum leptin (EZHL-80SK Millipore), adiponectin (80-ADPHU-E01 ALPCO) and insulin (80-INSHU-E01 ALPCO) concentrations were determined using different ELISA kits.

### Anthropometric measurements and body composition

Body weight and body composition, including fat-free mass, skeletal muscle mass (SMM) and fat mass (FM) were determined prior to the REE measurement by a trained nutritionist using a standard calibrated electronic scale and multi-frequency BIA, the Inbody 720 (Biospace Co.). Measurements were taken with subjects in light clothing and without shoes. The height (HT) was measured in centimeters using the stadiometer BSM 370 (Biospace Co. Ltd., Seoul, Republic of Korea) to the nearest mm. BMI was calculated using the weight in kilograms divided by the square of the height in meters. Three waist circumference (WC) measurements were made using a flexible tape measure (SECA, Germany), recording the average obtained from the three measurements. Weight, HT, and WC were obtained according to the Lohman method [28].

### Measurement of REE

REE was measured using the calorimeter Quark PFT device (Cosmed, Roma, Italy). Measurements were obtained in a thermoneutral (20–25 °C), humidity-controlled (45–55%), quiet environment. The calorimeter was calibrated by a trained nutritionist prior to each testing session using gas mixtures with concentrations of 16% $O_2$ and 1% $CO_2$, according to the manufacturer's instructions. All the measures were performed in the morning (between 0700 and 0930) following 8–12 h of fasting. Participants were instructed to refrain from exercise for at least 12 h (vigorous resistance exercise for 24 h) prior to their laboratory visit and to abstain from drinking alcohol or consuming caffeine at least while fasting. REE was determined according to Weir's equation [29] without using urinary urea nitrogen level [30].

### Amino acid profile

The amino acid profile was determined through the use of high performance liquid chromatography (HPLC). An aliquot of plasma was thawed, and 150 µL of plasma was added to 38 µL of 10% sulfosalicylic acid to deproteinize the sample. The samples were incubated for 30 min under refrigeration and centrifuged at 14,000 rpm for 10 min at 4 °C. Next, 100 µL was taken from the supernatant and added to 1 µL of the internal standard (norvaline; 15 mM); the sample was then derivatized and injected. The procedure was performed using a sampling device (Agilent; G1367F) coupled to an HPLC (Agilent 1260 Infiniti) and a fluorescence detector (Agilent; G1321B). A ZORBAX Eclipse AAA column was

used and maintained at 40 °C. Chromatographic conditions were maintained according to the column's technical instructions.

### Physical activity assessment

The international long-term physical activity questionnaire (IPAQ) was administered in the initial and final visits to obtain results in MET (min x week).

### Sample size

The sample size was estimated according to the primary objective of the percentage change in the Matsuda index following one month of dietary intervention based on previous studies [31] with a power of 80% and an α error of 0.05. We obtained an estimate of 15 participants per group plus 20% loss to follow-up, for a total of 18 participants per group.

### Statistical analysis

Continuous variables were expressed as the mean ± standard deviation (SD) or 95% confidence intervals (CI) when normally distributed or as median [25th percentile–75th percentile] otherwise. Dichotomous variables were expressed as frequencies and percentages. The distribution of variables was assessed using the Kolmogorov–Smirnov test. All subjects who entered the study were analyzed according to the principles of intention-to-treat (ITT) and per-protocol. The AUC of glucose and insulin was calculated using zero as the baseline, using the trapezoid rule [32]. To analyze whether there were differences in the baseline variables between the intervention groups, we used the one-way ANOVA test or the Kruskal–Wallis statistic for quantitative data and the chi-square statistic for categorical data. The baseline and final anthropometric, biochemical, clinical, REE and amino acid parameters were compared among groups using ANOVA for repeated measures with percentage weight change as a covariate; non-normally distributed variables were transformed logarithmically before the analysis. To analyze the differences within groups between baseline and study end, a paired *t* was used. The significant value of *P* was set at $< 0.05$. The data were analyzed using SPSS for Windows (version 24.00, SPSS Inc.). The figures were carried out using GraphPad Prism version 7 software.

## Results

### Participants

Of a total of 372 subjects, 80 participants met the inclusion criteria and were randomized by drawing assignments to one of the 4 dietary intervention groups. A total of 75 participants completed the study (Animal NP, $n = 18$; Vegetable NP, $n = 18$; Animal HP, $n = 19$, Vegetable HP, $n = 20$). Of the participants who did not complete the study, 2 were due to change of address, 1 was due to an accident and 2 were due to family reasons; there were no reported adverse effects (Fig. 2). The baseline characteristics of energy, macronutrient and amino acid intake of the participants were similar between the groups (Online Resource 3). The anthropometric, biochemical and clinical baseline characteristics of the participants were similar between the groups (Online Resource 4). The percentage of compliance to dietary treatment was 96.5% in the Animal NP, 97.1% Vegetable NP, 94% Animal HP and 95.7% in the Vegetable HP group ($P = 0.373$).

### Primary outcome: insulin resistance

We assessed the improvement in the IS using the Matsuda index. In the analysis by protocol, we observed an increase in IS in all groups: Animal NP 23.9% (95% CI 10.3, 85.1), Vegetable NP 30.4% (95% CI 4.64, 94.3), Animal HP 92.7% (95% CI: 52.9, 142), and Vegetable HP 60.4% (95% CI 38.2, 117). When comparing before and after the intervention, the change was significant in all groups ($P < 0.05$) with the exception of the Vegetable NP group ($P = 0.068$). When the time–treatment interaction analysis was performed, we observed a significant difference ($P = 0.004$) (Fig. 3a); the same result was observed in the analysis by ITT (Fig. 3b). In the analysis by protocol, we observed a decrease in the HOMA-IR index in all groups: Animal NP − 18.3% (95% CI − 37.5, 0.80), Vegetable NP − 11.9% (95% CI − 41.7, 17.8), Animal HP − 34.4% (95% CI − 53.9, − 14.8), and Vegetable HP − 29.9% (95% CI − 48.9, − 11.0) ($P = 0.416$). When comparing before and after the intervention, the results replicated what was found in the Matsuda index and were not significant in the Vegetable NP group ($P = 0.058$) (Fig. 4). The AUC of glucose in the OGTT decreased significantly in the Animal NP ($P = 0.038$), Vegetable NP ($P = 0.015$), and Vegetable HP ($P = 0.024$) groups but not in the Animal HP group ($P = 0.055$) (Fig. 5), while insulin AUC decreased significantly in the Animal NP ($P = 0.014$), Animal HP ($P = 0.001$), and Vegetable HP ($P = 0.003$) groups but not the Vegetable NP group ($P = 0.064$) (Fig. 6).

### Secondary outcomes

#### Anthropometric parameters, body composition and blood pressure

A significant decrease in body weight ($P < 0.0001$), BMI ($P < 0.0001$) and WC ($P < 0.0001$) was observed in all groups (Online Resource 5a–c). FM decreased significantly

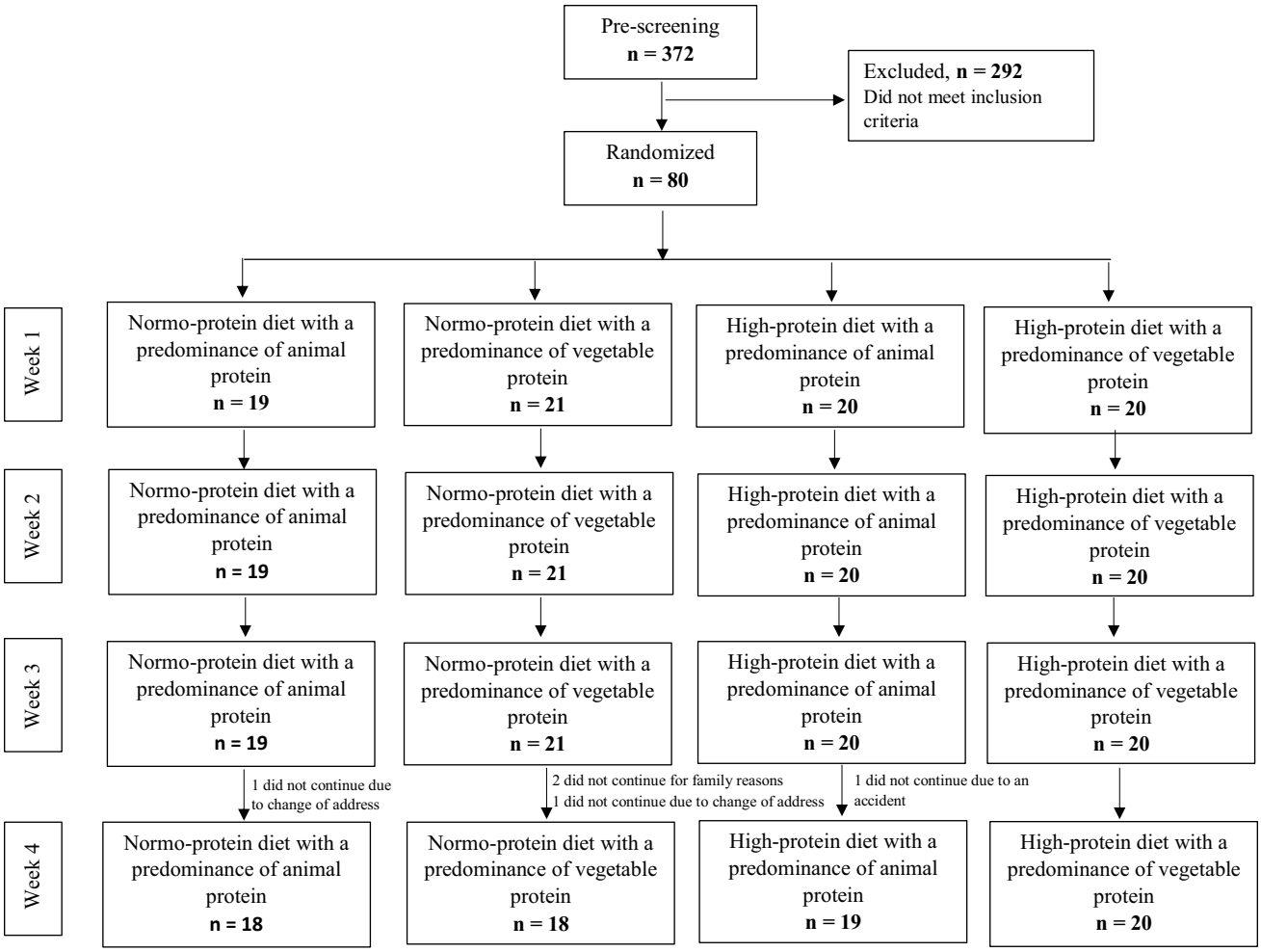


**Fig. 2** Consort flow diagram of the study

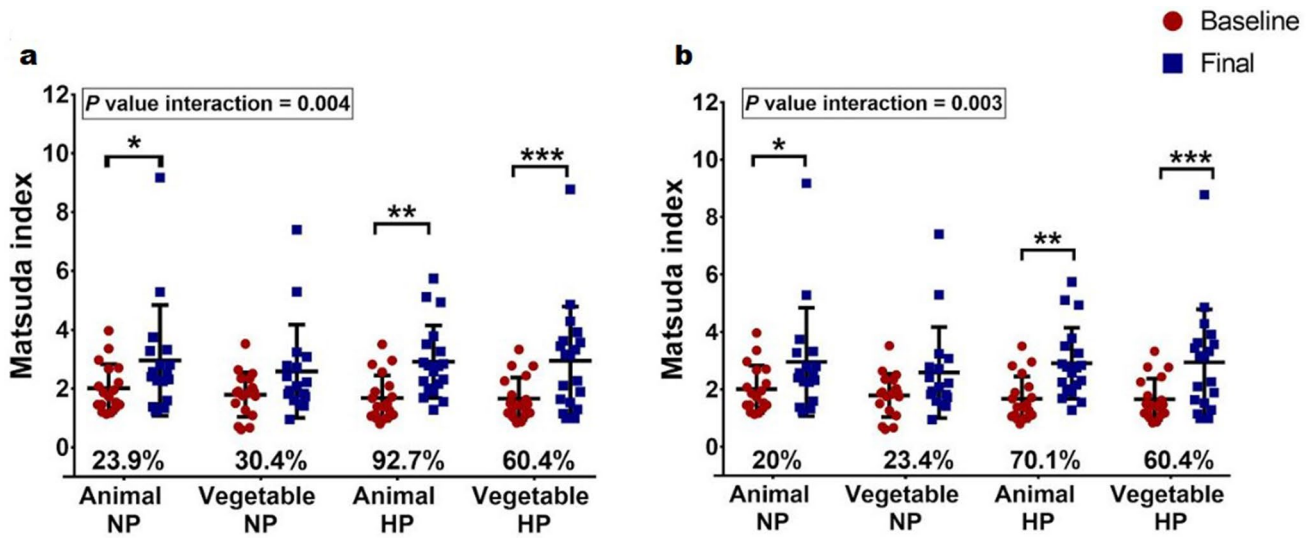


**Fig. 3** Changes in insulin sensitivity as measured by the Matsuda index stratified by intervention group. **a** Analysis by protocol. **b** Analysis by intention to treat. Statistical analysis was ANOVA for repeated measures adjusted for percentage weight change and to analyze only the differences within groups between baseline and endpoint, a paired *t* test was used, where $*P<0.05$, $**P<0.01$, and $***P<0.001$. %, median percentage change from baseline for each group; NP, normal protein; HP, high protein

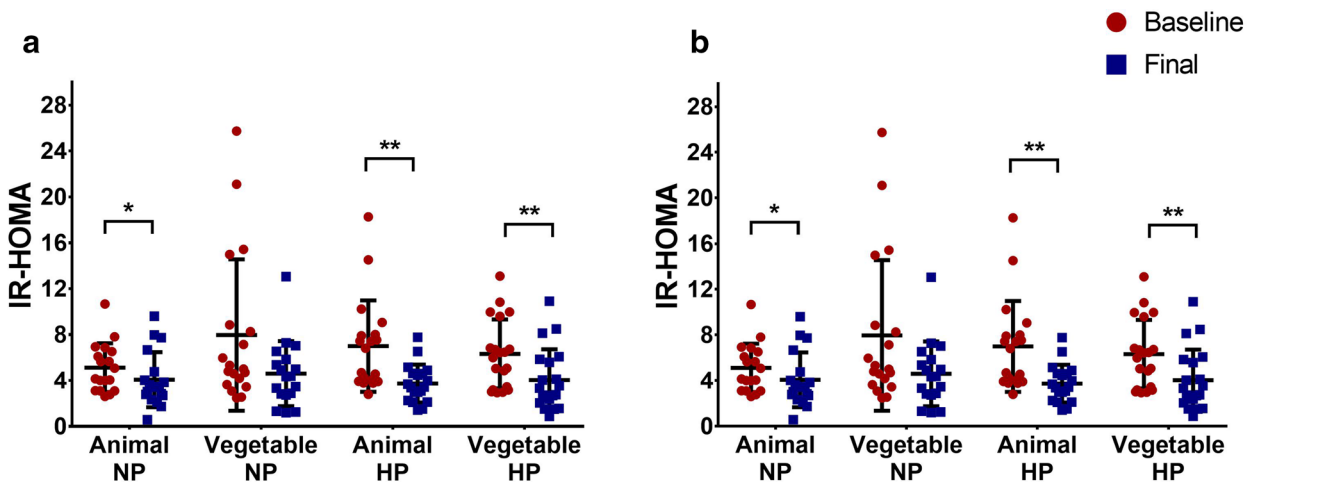


**Fig. 4** Changes in the IR-HOMA by intervention group. **a** Analysis by protocol. **b** Analysis by intention to treat. Statistical analysis to differences within groups between baseline and endpoint, was a paired *t* test, where $*P<0.05$, $**P<0.01$, and $***P<0.001$. *NP* normal protein, *HP* high protein

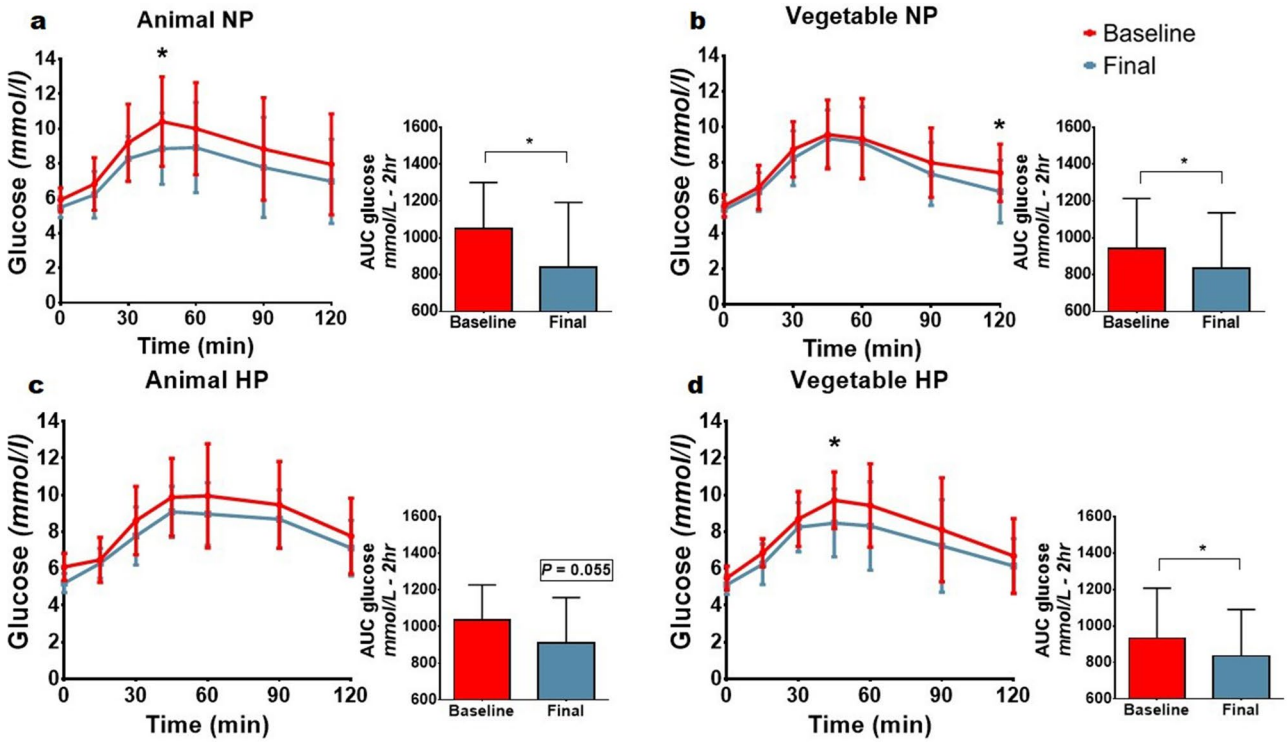


**Fig. 5** Changes in the area under the curve of glucose measured by oral glucose tolerance test. Statistical analysis was a paired *t* test, where $*P<0.05$. *NP* normal protein, *HP* high protein

in the Animal NP ($P=0.027$), Animal HP ($P=0.001$), and Vegetable HP ($P=0.001$) groups, while there was no significant change in the Vegetable NP group ($P=0.44$) (Fig. 7a). In contrast, an increase in the SMM was observed in the Animal NP ($P=0.030$), Animal HP ($P=0.011$), and Vegetable HP ($P=0.004$) groups, while no change was observed in the Vegetable NP group ($P=0.670$) (Fig. 7b). On the other hand, no significant changes in muscle strength were observed (Online Resource 5d). Regarding systolic BP, significant decreases of − 6.67 mmHg in the Vegetable NP (95% CI − 11.8, − 1.54, $P=0.014$), − 6.63 mmHg in the Animal HP (95% CI − 10.5, − 2.70, $P=0.002$), and − 5.67 mmHg

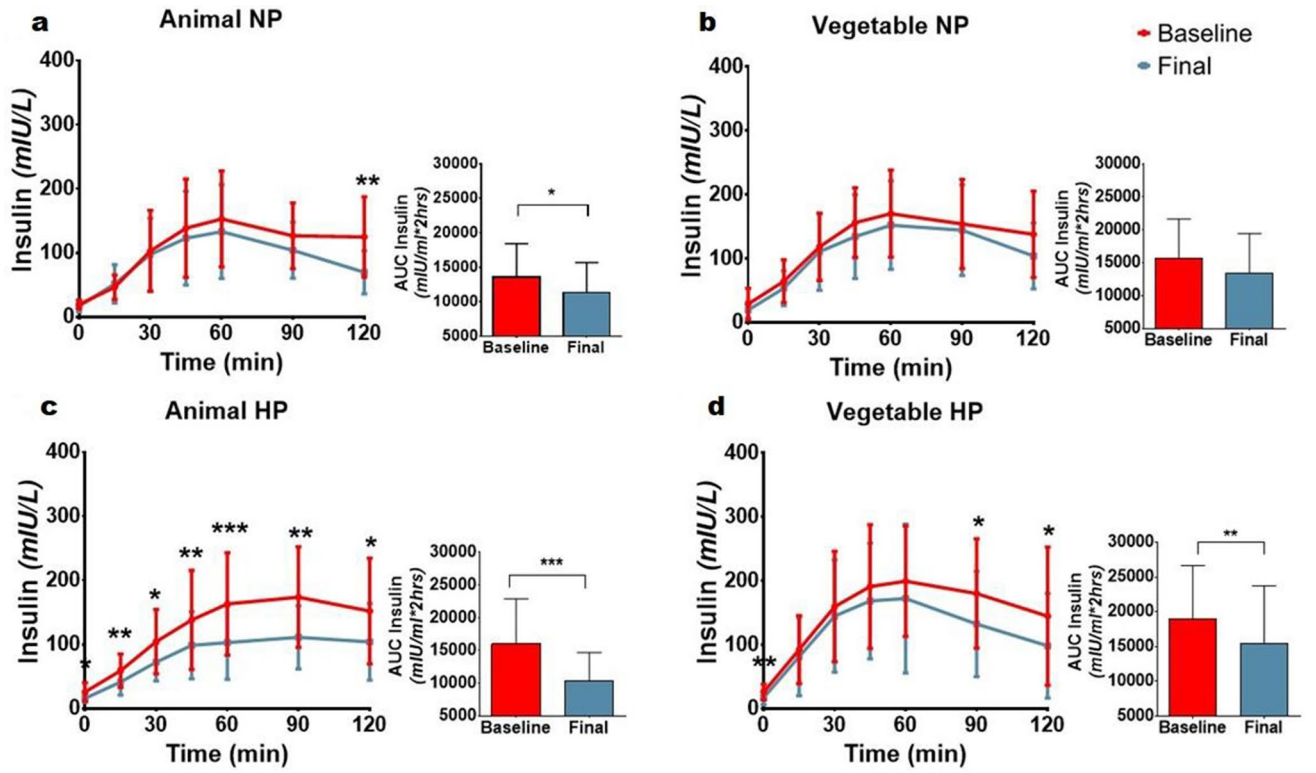


**Fig. 6** Changes in the area under the curve of insulin measured by oral glucose tolerance test. Statistical analysis was a paired *t* test, where $*P<0.05$, $**P<0.01$, and $***P<0.001$. *NP* normal protein, *HP* high protein

in the Vegetable HP (95% CI −9.98, −1.35, $P=0.013$) groups were observed. Significant decreases in diastolic BP of −4.67 mmHg and −5.16 mmHg were observed in the Vegetable NP (95% CI −8.68, −0.65, $P=0.025$) and, the Animal HP (95% CI −7.80, −2.52, $P=0.001$) groups, respectively (Online Resource 6a, b). There was no significant difference between groups in physical activity between the initial and final visits of the intervention ($P=0.732$) (Online Resource 7).

### Biochemical variables

When performing the before and after analysis for each intervention group, we observed that in the Animal NP group, the TC decreased from 4.39 ± 0.58 to 4.00 ± 0.67 mmol/L ($P<0.0001$), LDL-C from 3.12 ± 0.75 to 2.71 ± 0.56 mmol/L ($P=0.004$), and CRP from 4.84 [2.31, 7.58] to 3.86 [1.65, 5.23] mg/L ($P=0.002$). In the Animal HP group, TC decreased significantly from 4.84 ± 0.88 to 4.22 ± 0.83 mmol/L ($P<0.0001$), LDL-C from 3.70 ± 0.88 to 2.82 ± 0.90 mmol/L ($P<0.0001$), ALT from 32.8 ± 13.8 to 27.8 ± 10.6 U/L ($P=0.041$), albumin from 41.2 [39.2, 42.8] to 39.8 [37.5, 41.8] g/L ($P=0.005$), and insulin from 25.7 ± 14.6 to 15.9 ± 6.17 mU/L ($P=0.014$). In the Vegetable HP group, there was a significant decrease in TC from 4.68 ± 0.91 to 4.01 ± 0.67 mmol/L ($P=0.031$), LDL-C from 3.26 ± 0.75 to 2.82 ± 0.71 mmol/L ($P=0.003$), AST from 29.6 ± 14.8 to 24.2 ± 7.67 U/L ($P=0.007$), CPR from 4.55 [2.28, 5.92] to 2.44 [0.56, 3.53] mg/L ($P=0.014$), and adiponectin from 7.06 ± 3.84 to 6.23 ± 2.87 µg/mL ($P=0.050$). A significant decrease in TG was observed in the Animal ($P=0.001$) and Vegetable ($P=0.033$) HP groups (Fig. 7c). In all 4 groups there was a significant decrease in glucose levels, leptin levels and the leptin/adiponectin index. When performing the time x treatment interaction analysis per protocol, a significant difference was observed in glucose ($P=0.004$), TC ($P=0.018$), LDL-C ($P=0.002$), leptin ($P=0.006$), the leptin/adiponectin index ($P=0.001$) (Fig. 7d–h) and albumin ($P=0.005$). When performing the interaction time × treatment analysis by ITT only, a significant difference in CRP was added ($P=0.021$) (Online Resource 8a, b), while the significance in glucose was lost ($P=0.053$).

### REE measurement

No time x treatment interaction was observed in the analysis of REE, $VO_2$, and respiratory quotient between the groups (Online Resource 9).

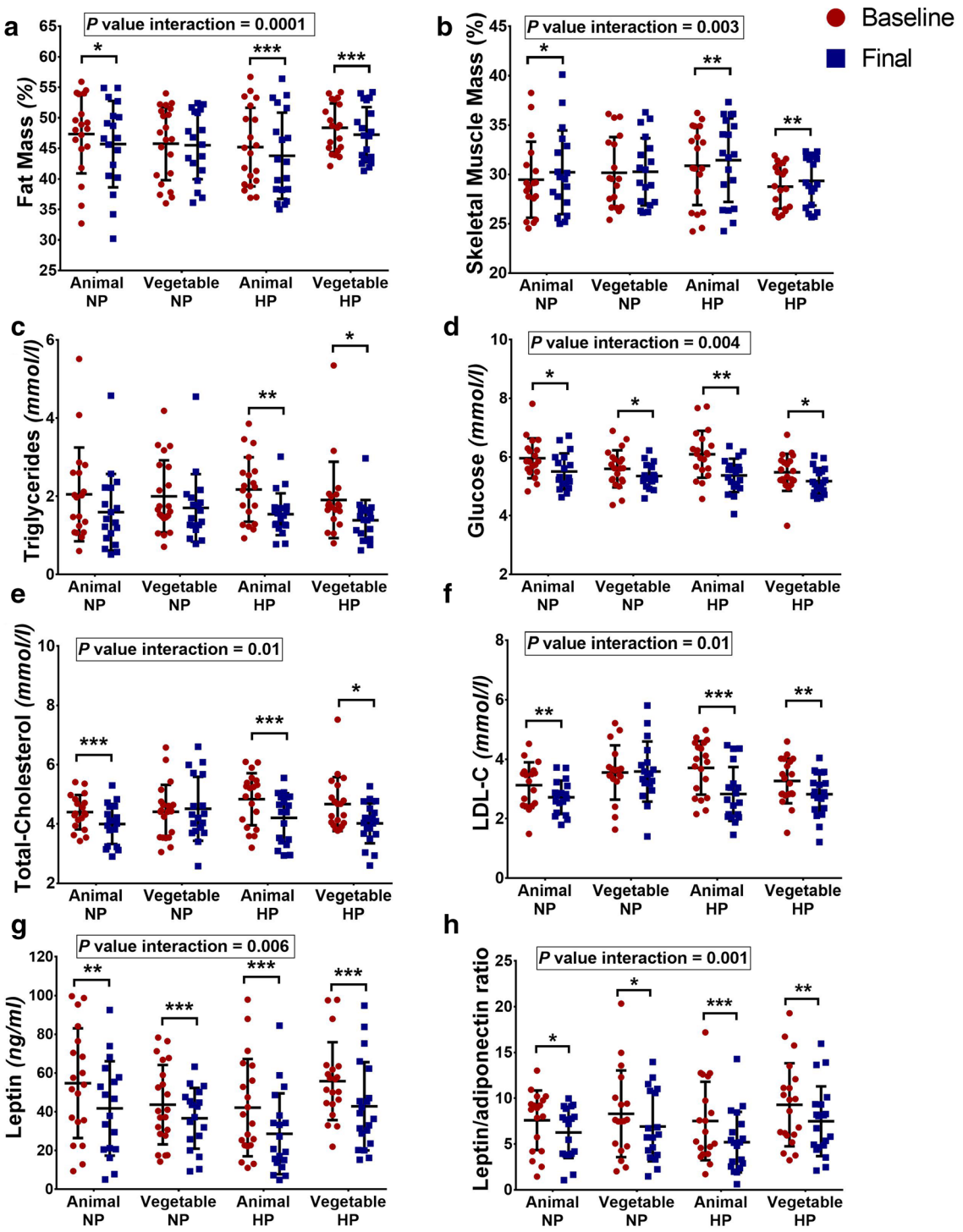


**Fig. 7** Changes in body composition and biochemical parameters after the intervention. **a** Percentage of fat mass; **b** percentage of skeletal muscle mass; **c** triglycerides; **d** glucose; **e** total cholesterol; **f** LDL cholesterol; **g** leptin; **h** leptin/adiponectin ratio. The statistical analysis was ANOVA for repeated measures adjusted for percentage weight change and to analyze only the differences within groups between baseline and endpoint, a paired *t* test was used, where $*P<0.05$, $**P<0.01$, and $***P<0.001$. *NP* normal protein, *HP* high protein

### Amino acid profile

The results showed that consumption of an Animal NP diet minimally modified the concentrations of circulating amino acids, only affecting tyrosine ($P=0.036$) and glutamate ($P=0.04$). However, the consumption of a Vegetable NP diet led to a decrease in ten circulating amino acids; isoleucine, leucine, tyrosine, tryptophan, aspartate, glutamate, alanine, methionine, lysine and threonine, five of which were indispensable amino acids, drawing attention to the decrease in the total sum of BCAAs ($P=0.029$) and aromatic amino acids ($P=0.009$). The consumption of a Vegetable HP diet prevented the decrease in circulating amino acids compared to the Vegetable NP diet, while the Animal HP diet produced a decrease of 9 amino acids; valine, phenylalanine, tryptophan, asparagine, glutamine, glycine, alanine, proline and threonine, four of which were indispensable amino acids. It is noteworthy that in all dietary treatments, the concentration of circulating alanine decreased ($P<0.05$) (Table 1).

**Table 1** Plasma amino acid profile at baseline and at the end of each dietary treatment

| Amino acids, nmol/mL | Animal NP | | Vegetable NP | | Animal HP | | Vegetable HP | | *P* value[b] |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline ($n=19$) | Final | Baseline ($n=21$) | Final | Baseline ($n=20$) | Final | Baseline ($n=20$) | Final | |
| Valine | 177 ± 37.0 | 17 1 ± 43.5 | 182 ± 35.0 | 168 ± 40.6 | 204 ± 27.7 | 189 ± 24.2* | 191 ± 34.0 | 182 ± 38.2 | 0.64 |
| Isoleucine | 50.2 ± 11.9 | 46.4 ± 12.6 | 55.1 ± 16.9 | 47.1 ± 12.0** | 60.7 ± 14.1 | 57.2 ± 13.8 | 57.3 ± 11.0 | 54.9 ± 14.1 | 0.73 |
| Leucine | 87.2 ± 18.7 | 82.4 ± 19.7 | 92.4 ± 24.1 | 83.2 ± 22.6* | 99.7 ± 15.4 | 93.2 ± 12.2 | 98.8 ± 20.6 | 89.4 ± 17.6 | 0.75 |
| Phenylalanine | 41.7 ± 6.55 | 40.5 ± 10.9 | 38.9 ± 7.48 | 35.4 ± 6.78 | 42.5 ± 4.93 | 38.5 ± 8.53* | 43.5 ± 8.74 | 38.9 ± 9.36 | 0.27 |
| Tyrosine | 57.0 ± 13.9 | 50.8 ± 11.5* | 58.0 ± 16.2 | 47.3 ± 10.3* | 62.3 ± 9.19 | 54.0 ± 9.87 | 58.7 ± 12.2 | 53.1 ± 11.4 | 0.76 |
| Tryptophan | 32.7 ± 8.31 | 32.3 ± 10.0 | 36.5 ± 10.8 | 31.0 ± 8.68* | 35.8 ± 5.84 | 32.5 ± 5.66* | 36.6 ± 7.27 | 34.5 ± 8.24 | 0.62 |
| Aspartate[a] | 4.83 (4.39, 6.90) | 4.61 (4.09, 5.42) | 5.31 (4.85, 6.05) | 4.58 (4.01, 6.26)* | 4.36 (3.78, 5.61) | 5.54 (4.29, 8.76) | 5.04 (4.18, 5.49) | 5.99 (5.64, 6.87) | 0.34 |
| Glutamate | 60.5 ± 22.2 | 46.0 ± 15.8* | 79.1 ± 30.7 | 63.0 ± 26.7* | 68.3 ± 48.1 | 76.2 ± 31.9 | 78.6 ± 40.8 | 62.8 ± 31.5 | 0.95 |
| Asparagine | 25.2 ± 6.31 | 23.5 ± 6.75 | 24.1 ± 8.09 | 21.5 ± 4.26 | 27.6 ± 6.66 | 23.6 ± 3.17* | 25.2 ± 6.11 | 24.8 ± 4.95 | 0.20 |
| Serine | 76.0 ± 18.1 | 79.8 ± 22.5 | 78.7 ± 21.6 | 76.9 ± 17.9 | 78.9 ± 15.0 | 86.6 ± 14.0 | 78.6 ± 15.9 | 91.2 ± 21.5** | 0.25 |
| Glutamine[a] | 309 (246, 413) | 293 (213, 362) | 299 (229, 329) | 276 (245, 305) | 361 (288, 4178) | 274 (222, 309)* | 271 (254, 419) | 254 (116, 318) | 0.46 |
| Histidine[a] | 26.0 (17.0, 39.0) | 15.0 (8.99, 31.0) | 26.0 (18.0, 32.0) | 23.0 (16.0, 28.0) | 35.0 (19.0, 43.0) | 22 (15.0, 42.0) | 26.0 (22.0, 37.0) | 87 (12.0, 146) | 0.30 |
| Glycine | 176 ± 78.3 | 149 ± 54.8 | 158 ± 61.4 | 140 ± 49.2 | 164 ± 72.3 | 121 ± 16.2* | 165 ± 61.4 | 154 ± 63.6 | 0.34 |
| Arginine[a] | 55.9 (48.8, 65.3) | 52.9 (47.9, 67.7) | 59.1 (47.4, 64.5) | 55.8 (42.3, 58.3) | 65.8 (57.7, 73.7) | 66.3 (58.9, 74.4) | 64.1 (49.7, 69.9) | 59.8 (49.3, 70.3) | 0.64 |
| Alanine | 330 ± 101 | 279 ± 70.0* | 340 ± 86.3 | 266 ± 57.9* | 343 ± 85.5 | 288 ± 39.8** | 349 ± 91.1 | 287 ± 62.6** | 0.74 |
| Cysteine | 229 ± 86.9 | 232 ± 84.8 | 239 ± 71.6 | 240 ± 76.2 | 199 ± 72.6 | 254 ± 74.6 | 222 ± 70.6 | 250 ± 75.6 | 0.88 |
| Methionine[a] | 6.80 (4.00, 17.0) | 6.70 (1.00, 13.0) | 13.2 (5.00, 18.0) | 3.10 (0.98, 7.00) * | 7.87 (4.55, 5.47) | 7.93 (3.94, 12.28) | 7.95 (4.65, 17.3) | 7.16 (1.44, 10.2) | 0.81 |
| Proline | 209 ± 82.5 | 203 ± 66.7 | 187 ± 68.5 | 162 ± 50.1 | 259 ± 85.2 | 182 ± 66.9** | 198 ± 90.4 | 193 ± 76.9 | 0.06 |
| Lysine | 122 ± 26.4 | 114 ± 25.6 | 123 ± 33.5 | 112 ± 28.4* | 126 ± 19.7 | 124 ± 26.9 | 124 ± 16.5 | 116 ± 18.1 | 0.88 |
| Threonine | 181 ± 55.3 | 151 ± 35.8 | 189 ± 33.0 | 165 ± 40.4* | 200 ± 25.0 | 175 ± 41.4* | 186 ± 22.7 | 159 ± 50.2* | 0.92 |
| ∑ BCAA | 314 ± 64.6 | 300 ± 72.6 | 329 ± 72.8 | 298 ± 72.3* | 365 ± 54.4 | 339 ± 46.6* | 347 ± 63.2 | 326 ± 66.3 | 0.73 |
| ∑ AAA | 133 ± 26.5 | 124 ± 30.3 | 136 ± 31.3 | 114 ± 21.4** | 141 ± 17.2 | 125 ± 17.6** | 137 ± 25.8 | 125 ± 24.8 | 0.81 |
| ∑ GAA | 1935 ± 376 | 1707 ± 284* | 1865 ± 334 | 1671 ± 265 | 2013 ± 292 | 1787 ± 178* | 1932 ± 323 | 1827 ± 301 | 0.83 |
| ∑ KAA | 210 ± 43.7 | 196 ± 43.8 | 221 ± 54.8 | 195 ± 49.3* | 226 ± 32.0 | 217 ± 33.1 | 223 ± 33.6 | 205 ± 33.4* | 0.83 |
| ∑ SAA | 340 ± 92.2 | 275 ± 125 | 335 ± 70.6 | 327 ± 99.5 | 300 ± 80.2 | 348 ± 91.4 | 332 ± 72.0 | 344 ± 93.0 | 0.22 |

Data are presented as the mean ± SD

*NP* normal protein, *HP* high protein, *BCAA* branched-chain amino acids, *AAA* aromatic amino acids, *GAA* gluconeogenic amino acids, *KAA* ketogenic amino acids, *SAA* sulfur amino acids

[a]Median (25th, 75th percentile). These data were log-transformed before statistical analyses

[b]Statistical analysis was ANOVA for repeated measures adjusted for percentage weight change and to analyze only the differences within groups between baseline and final, a paired *t* test was used, where $*P<0.05$, $**P<0.01$, and $***P<0.001$

## Discussion

The metabolic abnormalities associated with the development of obesity have promoted the development of several dietary strategies. Most of the studies to date have emphasized the percentages of carbohydrates and fats for the treatment of these patients. However, the amounts and sources of protein in diets for the treatment of patients with obesity and IR has been less frequently studied. Some concerns have emerged, particularly regarding the increased concentrations of circulating BCAAs associated with the development IR, suggesting limiting the amount of dietary protein in the diet. However, some clinical studies have shown contradictory results, particularly regarding the source of dietary protein, which has not previously been well established [33–35].

Our study clearly showed that the 1-month consumption of a hypocaloric normal protein or high-protein diet in subjects with obesity and IR modestly improved some anthropometric parameters, such as decreasing body weight and body fat. The most interesting aspect of our study is that regardless of protein source, a high-protein hypocaloric diets improves IS in these patients. These results are consistent with the previous studies that have demonstrated that consumption of high-protein diets improved IS [33–35].

In other studies, only with diets rich in animal protein it has been observed a decrease in the IS, response that can be modified by diverse factors such as the presence of processed meat or the method of preparation of food of animal origin [35]. However, in our study, both vegetable and animal protein-rich diets improved IS, indicating that possibly other components of the protein sources are responsible for the decrease in IS. On the other hand, the mechanism by which these high-protein hypocaloric diets can improve IS is not well established. Some studies have shown that high levels of BCCA and methionine, contained mainly in animal protein diets, can activate the mTOR pathway, and therefore promote IR by phosphorylating the insulin receptor substrate in serine residues [34]. Likewise, arginine may improve IS by increasing microvascular function via nitric oxide production, whereas the asparagine shows tight links with glucose homeostasis [34]. However, in our study, we do not observe differences in circulating BCCA concentration between the different diets, neither other amino acids that could be associated with improvement of IS. Therefore, the beneficial effect of both high-protein hypocaloric diets in IS, could possibly be explained by other components related to lower carbohydrate intake and FFM preservation [12]. On the other hand, the decrease in FM can improve the functionality of adipose tissue, and recent data have shown that adipocytes play an important role in BCAA catabolism [36].

Furthermore, in our study, high-protein hypocaloric diets decreased blood pressure, and this effect was observed mainly in subjects consuming the Animal HP diet. In addition, subjects consuming Vegetable HP diets showed a decrease in circulating CRP levels. The effect on inflammatory factors can be influenced by several factors; although in recent years, it has been suggested that fiber and gut microbiota may affect these levels through metabolic endotoxemia [37]. There is important evidence that the source and amount of protein can affect the gut microbiota [38, 39], which may influence the concentration of CRP [40]. However, more studies are needed to clarify this potential effect.

It seems as if the Vegetable NP diet stands out as having a smaller effect. However, it has to be considered that this was also the group that showed the smallest and a non-significant change in FM. In addition, the baseline and final changes in the Vegetable NP diet are presented as not statistically significant but were for the most part borderline ($P > 0.05$ but $< 0.06$). This clearly must be considered.

Our study has some limitations, including the use of the HOMA-IR and Matsuda index instead of the reference standard, the hyperinsulinemic–euglycemic clamp to measure IS; however, the Matsuda index shows a good correlation with the reference standard ($r = 0.73$, $P < 0.0001$) [24]. Additionally, when obtained directly from an OGTT, the Matsuda index is considered a good indicator of IS in peripheral skeletal muscle [41].

Another factor to consider in this study is that when the protein content of the diet is increased, the concentration of other macronutrients is also modified, which may partly influence the beneficial effects observed. In such a way that it is difficult to know if the effects observed in IS are a consequence of the increase amount of protein diets or due to change in distribution of other macronutrients. In addition, it is important to mention that in this study, the high-protein diets were similar in the content of other nutrients, resulting in less than 7% saturated fat and less than 200 mg of cholesterol. One of the characteristics of real life is that a diet rich in foods of animal origin is the high content of saturated fats and cholesterol; therefore, this effect is not observed in our study.

On the other hand, we study the additive effect of a diet with energy restriction and different amount and source of protein. Hence, we still need to study to effect of the change in amount and source of protein without energy restriction, and this could lead to future studies.

However, the biggest strength of our study was the very good compliance of the patients to the consumption of each of the dietary treatments, strengthening our conclusion. More studies are needed to test our recommendation in a different population. It is also necessary to study the effect of this type of diet on the gut microbiota and to determine the long-term effects of these diets.

In conclusion, our study demonstrated that the consumption of a high-protein hypocaloric diet could be used as an important dietary strategy to improve IS in patients with obesity, regardless of amount and source of protein.

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**Author contributions** MGC, ART, LGNL conceived of and designed the study. BPG, AVM, and MGC developed the menus and recipe booklets. LEGS, PKHGC, AVM, MGC, and BPG developed and handled the logistics of the food pantries. LEGS, EPO, AVM, KGHG, LAS, AESZ, GCL, CZL, RGH, and MGC assisted with participant inclusion and follow-up. LEGS, KGHG, PKHGC, AVM, MGC, and AAN determined the biochemical parameters. OGP, RGH, AFL determined the AA concentrations by HPLC. IMV performed the statistical analyses. ART, LEGS, AESZ, BPG, IMV, NT, and MGC completed the data interpretation. ART, LEGS, IMV, JGRG, and MGC wrote the draft manuscript. All authors have seen and approved the final version of the manuscript.

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**Data availability** Data are available upon reasonable request. Data are available upon request to the correspondent author. Open access.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethics approval** The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This protocol was approved by the ethics committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (reference 2373). Written informed consent was obtained from all patients prior to their inclusion in the study.

**Consent to participate** All participants signed the consent statement prior to their inclusion in the study.

**Consent for publication** This manuscript is not being simultaneously submitted elsewhere and no portion of the data has been published elsewhere.

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