Received: 17 June 2024 | Revised: 23 September 2024 | Accepted: 1 October 2024

DOI: 10.1111/dom.16013

ORIGINAL ARTICLE

WILEY

Low-calorie, high-protein diets, regardless of protein source, improve glucose metabolism and cardiometabolic profiles in subjects with prediabetes or type 2 diabetes and overweight or obesity

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Funding information
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Abstract

Aim: The aim was to study the effect of two low-calorie, high-protein (HP) diets, with most of the protein coming from animal or plant sources, on glycaemic and other cardiometabolic outcomes in subjects with overweight or obesity and glucose metabolism disorders.

Materials and Methods: A total of 117 participants aged >18 years with body mass index over 27.5 kg/m^2 and prediabetes or type 2 diabetes mellitus (T2DM) were randomized to one of two HP low-calorie diets (35% of total calories from protein), in which 75% of the protein was from either plant-based sources (HPP) or animal sources (HPA). For both diets, 30% and 35% of the total calories were from fat and carbohydrates, respectively. The dietary intervention lasted 6 months.

Results: Both diets improved body composition to a similar extent, including weight loss (-8.05 ± 5.12 kg for the HPA diet and -7.70 ± 5.47 kg for the HPP diet at 6 months) and fat mass, mainly visceral fat. Both diets had a similar beneficial effect on glucose metabolism, including fasting glucose, insulin, homeostasis model assessment of insulin resistance index and glycated haemoglobin. Other biochemical parameters, including lipid profiles, liver enzymes, adipokines and inflammatory biomarkers,

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similarly improved in both groups. Fasting incretins, mainly glucagon-like peptide 1, decreased significantly in both groups, and this effect correlated with weight loss.

Conclusions: Low-calorie HP diets improved body composition, glucose metabolism and other cardiometabolic outcomes, regardless of protein source (either animal or plant sources), in outpatients with prediabetes or T2DM.

Clinical Trial Registration: The clinical trial was registered in ClinicalTrials.gov (identifier: NCT05456347) https://clinicaltrials.gov/study/NCT05456347?term=NCT05456347&rank=1.

KEYWORDS

animal protein, diet, obesity, plant protein, type 2 diabetes

1 | INTRODUCTION

The worldwide prevalence of diabetes in adults is estimated to reach 643 million in 2030 and 783 million by 2045.[1] Obesity is the key pathophysiological driver of type 2 diabetes mellitus (T2DM), and weight loss is considered the primary means of prevention and treatment.[2,3] Reducing adiposity can improve not only micro- and macrovascular complications but also other comorbidities like non-alcoholic steatohepatitis, and can even result in T2DM remission.[3] Thus, leading diabetes organizations highlight weight loss as a priority and urge the exploration of strategies that promote substantial and durable weight loss to curb the progression of this pandemic.[4]

Increasing the protein content of low-calorie diets has been demonstrated to better maintain fat-free mass (FFM), which is crucial for people with T2DM, who have a high prevalence of sarcopenia.[5] Low skeletal muscle mass is an independent risk factor for all-cause mortality in subjects with T2DM.[6] In addition, preserving muscular mass after weight loss leads to high resting energy expenditure, which helps maintain weight loss.[7,8] Animal proteins, especially branched chain amino acids (BCAA), promote lean mass maintenance, primarily through mTORC1 stimulation.[9,10] This effect is highly dependent on protein quality, which is determined by amino acid composition and bioavailability. High-quality protein from plant-based sources has also been demonstrated to favour lean mass and muscle strength.[9,11]

Proteins and amino acids play an essential role in glucose homeostasis, and high-protein (HP) diets have been shown to improve glycaemic parameters, regardless of weight loss.[7,12,13] Although the mechanisms are not fully understood, this effect is mostly attributed to the insulinotropic activity of amino acids.[12] Cationic amino acids (e.g. arginine and lysine) enhance β-cell depolarization and favour insulin exocytosis, and mTORC1 activation by BCAAs leads to insulin synthesis by β-cells.[14,15] Several studies have demonstrated that protein intake attenuates postprandial glycaemia and increases insulin and incretin release after carbohydrate consumption, with different effects observed in response to the consumption of different proteins and amino acids.[16]

However, little is known about the effects of low-calorie HP diets comprising different protein sources on glycaemic and other cardiometabolic improvements in prediabetes and T2DM. Thus, the aim of this study was to determine the effect of a 6-month dietary intervention involving two energy-restricted HP diets (one in which 75% of the total protein content was from animal sources and one in which 75% of the total protein content was from plant sources) on glycaemic and other cardiometabolic outcomes in subjects with overweight or obesity and prediabetes or T2DM.

2 | MATERIALS AND METHODS

2.1 | Participants

Eligible volunteers were women and men aged >18 years with a body mass index (BMI) ranging from 27.5 to 40 kg/m^2 who had maintained a steady weight (±5%) in the previous 3 months. We included subjects with the diagnosis of prediabetes or T2DM according to the American Diabetes Association guidelines: (a) fasting glucose concentration ≥100 mg/dL for prediabetes or ≥126 mg/dL for T2DM and/or glycated haemoglobin (HbA1c) ≥5.7% for prediabetes or ≥6.5% for T2DM, and not taking antidiabetic drugs; (b) previous diagnosis of prediabetes or T2DM on stable dose of metformin, regardless of glucose and/or HbA1c levels.[17] Exclusion criteria included HbA1c ≥8%; hypoglycaemic drug use in the past 2 months, except for metformin (if taken consistently for ≥6 months); regular intake of nutraceuticals that could interfere with lipid and/or glycaemic metabolism; any vitamin supplement intake, use of weight loss medications and the presence of any other disease or condition that could interfere with study outcomes and dietary intervention compliance (e.g. uncontrolled endocrine disorders or pregnancy and breastfeeding). We also excluded women and men who consumed over 20 and 30 g per day of ethanol, respectively. The study protocol was approved by the local ethics committee (Comité de Ética e Investigación Clínica de Aragón, PI19/485), and all procedures were carried out in accordance with the ethical standards of that committee and with the principles embodied in the Declaration of Helsinki. All participants provided written informed consent to participate in the study. This clinical trial was registered in ClinicalTrials.gov (identifier: NCT05456347).

2.2 | Study design and intervention

This study consisted of a 6-month dietary intervention and was carried out between February 2020 and December 2022 at the Miguel Servet University Hospital (Zaragoza, Spain). Participants were randomly assigned to one of two calorie-restricted HP diets: (a) 35% total caloric value (TCV) from protein, mainly from animal sources (75% of total protein) (HPA); or (b) 35% TCV from protein, mainly from plant sources (75% of total protein) (HPP). The remaining macronutrient distribution was the same for both diets: 30% lipids (of which <7% were saturated fatty acids, 15%–20% monounsaturated fatty acids and 5%–7% polyunsaturated fatty acids) and 35% carbohydrates (of which <10% were simple carbohydrates), including ≈25 g per day of fibre.

Randomization was not stratified and was performed by nutritionists using an online software programme (Random.org) to generate a sequence of numbers for each arm. The researchers who carried out the laboratory analyses and processed the data were blinded to the group assignments. Blinding of the nutritionists and participants was not feasible due to the study design. Both diets represented a 30% calorie restriction, applied based on total energy expenditure as calculated by Harris–Benedict. A sample daily menu for each diet is provided in Table S1. Dieticians performed individual consultations every 2 weeks to reinforce the intervention and motivate the participants to lose weight. The main study outcomes were assessed at baseline and after 3 and 6 months of the dietary intervention.

2.3 | Anthropometric and clinical parameters

Body composition was assessed using dual-energy X-ray absorptiometry (software version 8.1, Lunar Prodigy, General Electrics, Chicago, IL, USA). Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 813 and Seca 217, Seca Deutschland, Hamburg, Deutschland). Waist circumference was measured using an anthropometric tape midway between the lowest rib and the iliac crest (Seca 201, Seca Deutschland). Blood pressure was measured using a validated semiautomatic oscillometer (Omron HEM-907-E, OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).

2.4 | Biochemical parameters and metabolic biomarkers

Blood samples were taken by venipuncture after 12 h of fasting, and spot urine samples were also collected. The following metabolic biomarkers were assessed: gastric inhibitory peptide (GIP), total glucagon-like peptide 1 (GLP-1), leptin, adiponectin, resistin, monocyte chemoattractant protein-1, tumour necrosis factor α, plasminogen activator inhibitor-1, interleukin-1, interleukin-6, interleukin-8, interleukin-10 and vascular endothelial growth factor A. The methodologies used to assess the levels of the biochemical and metabolic biomarkers are provided in Table S2.

2.5 | Lifestyle parameters

Participants were asked to complete a 3-day (including 2 working days and 1 day off) weighed food record before each visit. Dietary analysis was performed using EasyDiet (Biocentury, S.L.U., Barcelona, Spain), which is based on Spanish food composition tables. Adherence to the diet that was prescribed to the participants was assessed by calculating the percentage of protein consumed, based on total calories, at each study visit. In addition, the ratio of animal to plant protein consumption was calculated to determine if the target ratio was met. We calculated the ratio of animal to plant protein intake by dividing the percentage of the TCV of protein from animal sources by the percentage of the TCV of protein from plant sources. The ratio was assessed only within each diet group (not between the two diet groups).

Physical activity was measured objectively using accelerometry (ActiGraph wGT3X-BT, Pensacola, FL, USA). The participants were asked to wear the device at their waist for seven consecutive 24-h periods and to remove it only when engaging in water-based activities. Data were collected every 10 s in epoch intervals and were analysed using the manufacturer's software. Data from participants who did not wear the accelerometer for at least three complete days were not analysed. Activity was defined as light, moderate, vigorous or very vigorous based on cut-off points established by Freedson et al.[18] Sedentary time was estimated as the amount of time below 100 counts per minute.

2.6 | Statistical analysis

HbA1c concentration was the main outcome, and its variance was estimated as 1%. We hypothesized that there would be no difference between diets that could lead to a decrease of 0.40 [−0.65–(−0.10)]% (median and interquartile range) in HbA1c concentration. We previously showed that a decrease of this magnitude occurred in participants with prediabetes or T2DM and overweight or obesity who consumed a low-calorie diet containing 35% protein for 6 months.[19] For the current non-inferiority trial, a sample size calculation based on 80% power (Z_β unilateral = 0.842) and a confidence interval ($1-\alpha$) of 90% (Z_α unilateral = 1.645) yielded a total sample size of 56 subjects per group. All subjects who completed the study were included in the data analysis, independent of reported dietary compliance or weight loss, according to intention-to-treat analysis. Continuous variables are expressed as mean ± standard deviation or median (25th percentile−75th percentile), as applicable, and categorical variables are reported as percentages. Differences between independent variables were assessed using *t* test or Mann–Whitney test, whereas categorical variables were compared using the χ^2 test. Differences that occurred during the study within each diet group were evaluated using Friedman test or repeated-measures analysis of variance (ANOVA) for analysing non-parametric and parametric variables, respectively. We calculated the differences between the diet groups (group × time interaction) using a generalized linear model (GLM), including log-transformed data. We performed two adjusted GLM tests by including (a) age, sex and BMI and (b) age, sex, BMI and animal-to-plant protein intake ratio. Correlation was assessed using Spearman test. To control for the increased risk of type I errors due to multiple comparisons, a Bonferroni correction was applied for the GLM analysis of primary variables of interest (glucose, HbA1c, insulin, homeostasis model assessment-estimated insulin resistance, BMI, fat mass, FFM and visceral fat change). This method adjusts the significance level by dividing 0.05 by the number of tests conducted. All statistical analyses were performed using R, version 3.5.0, and significance was set at $p < 0.05$.

3 | RESULTS

3.1 | Study participants

Out of 163 subjects who participated in screening visits, 117 were ultimately included in the study and randomized to one of two low-calorie HP diets, and 102 completed the entire 6-month intervention (Figure S1). Seven participants (four from the HPA group and three from the HPP group) withdrew from the study during the first 3 months, and eight (three from the HPA group and five from the HPP group) withdrew during the next 3 months.

There were no differences in baseline clinical and biochemical characteristics between the two groups, except for alanine aminotransferase (ALT) (Table 1): the subjects randomized to the HPA group had higher ALT levels than those randomized to the HPP diet. Sex distribution was homogeneous between the groups, and the participants were mostly middle aged (57.3 ± 7.96 years), with a mean BMI of 35.6 ± 17.8 kg/m^2. The amount of visceral fat was higher in the HPA group, which may be attributed to the higher concentrations of ALT observed in this group, but the difference was not significantly different. Metformin use did not differ between the groups at the beginning of the study or change during the study.

3.2 | Changes in anthropometric and clinical parameters in response to dietary interventions

Table 2 and Table S3 indicate that both diets led to improvements in anthropometric and clinical parameters, with no significant differences found between the two groups. The HPA and HPP diets induced similar decreases in body weight (−6.18 ± 3.98 and −8.05 ± 5.12 kg at 3 and 6 months, respectively, for the HPA group; −6.15 ± 4.26 and −7.70 ± 5.47 kg at 3 and 6 months for the HPP group), fat mass (−7.02 ± 5.79 and −10.0 ± 8.70% at 3 and 6 months, respectively, for the HPA group; −6.64 ± 7.94 and −10.0 ± 11.7% at 3 and 6 months, respectively, for the HPP group), abdominal subcutaneous fat and visceral fat. The decrease in lean mass was similar in both groups, with no statistical difference between them ($p = 0.725$). There was still no significant difference when the GLM was adjusted for age, sex, BMI and ratio of animal-to-plant protein intake. Interestingly, changes in fat mass and lean mass were significantly correlated for participants in both groups. The HPA and HPP diets induced comparable improvements in diastolic blood pressure and systolic blood pressure.

3.3 | Changes in glucose metabolism and other biochemical parameters in response to dietary interventions

Both diets enhanced all glucose parameters tested throughout the study, with no significant differences between the groups (Table 3; Table S4). Both diets resulted in significant and similar improvements in triglyceride, high-density lipoprotein cholesterol and apolipoprotein A1 levels, and there were no significant differences between the two groups (Table 3). Although there were differences in the changes in total and low-density lipoprotein cholesterol, apolipoprotein B, C-reactive protein and uric acid levels between the two diet groups, none of these differences were significant. Liver enzymes improved to the same extent in both diet groups.

Urine microalbumin levels declined in both groups, and urea concentrations changed to different extents, although the differences between the groups were not significant. Generally, urea excretion was slightly reduced, because the participants' total protein intake was decreased on the low-calorie diet compared with their baseline diets. Given that urea excretion is related to protein intake, the lack of significant differences between the two diets suggests that the protein intake of the participants in both groups was similar.[20]

3.4 | Changes in metabolic biomarkers in response to dietary interventions

The two HP diets improved metabolic biomarkers, mainly related to glucose homeostasis, satiety and inflammation, to a similar extent (Table 4; Table S5). Fasting incretin levels decreased in response to dietary intervention in both groups, with no significant difference between the two groups. The changes in incretin concentration did not correlate with variations in glucose parameters; however, a significant correlation was observed between weight loss and decreased GLP-1 levels at 3 months ($R = 0.255$, $p = 0.008$), though not at 6 months ($R = 0.143$, $p = 0.163$). Among the adipokines assessed, we observed a significant decrease in leptin levels in response to the dietary interventions, as well as an increase in adiponectin levels, with no significant differences between the two groups. Most inflammatory markers improved throughout the study, with no marked differences between the diets.

3.5 | Changes in dietary and lifestyle parameters in response to dietary interventions

Dietary habit analysis revealed a decrease in energy intake, in accordance with the dietary interventions (Table 5; Table S6). Participants demonstrated an increased percentage of protein consumption

TABLE 1 Baseline clinical, anthropometric and biochemical characteristics with comparison between both randomized groups.[a]

		Animal HP diet (HPA) ($N = 59$)	Plant HP diet (HPP) ($N = 58$)	p[b]
Age (years)		58.3 ± 7.38	56.4 ± 8.47	0.209
Men, *n* (%)		35 (59.3%)	26 (44.8%)	0.166
Body weight (kg)		94.2 ± 13.8	89.9 ± 12.0	0.075
BMI (kg/m^2)		33.8 ± 4.34	32.7 ± 2.84	0.115
Fat mass (%)		41.8 (36.5–46.9)	43.1 (37.5–48.2)	0.621
Abdominal visceral fat (kg)		2.18 (1.44–2.84)	1.64 (1.39–2.35)	0.058
Abdominal subcutaneous fat (kg)		2.41 (1.72–3.14)	2.21 (1.72–3.08)	0.559
Lean mass (kg)		53.5 (46.0–60.0)	48.5 (41.7–57.5)	0.477
Smoking status, *n* (%)	Former	30 (50.8)	23 (41.1)	0.522
	Current	9 (15.3)	12 (21.4)	
	Non-smoked	20 (33.9)	21 (37.5)	
Type 2 diabetes, *n* (%)		29 (49.2)	25 (43.1)	0.638
Prediabetes, *n* (%)		30 (50.8)	33 (55.9)	
Hyperlipidaemia, *n* (%)		21 (35.6)	22 (37.9)	0.944
Hypertension, *n* (%)		25 (42.4)	20 (34.5)	0.492
Cardiovascular disease, *n* (%)		4 (6.78)	4 (6.90)	1.000
Metformin, *n* (%)		16 (27.6)	19 (33.3)	0.641
Lipid-lowering medication, *n* (%)		17 (28.8)	20 (35.7)	0.554
Hypertension medication, *n* (%)		21 (35.6)	16 (27.6)	0.464
Antiaggregant medication, *n* (%)		4 (6.78)	1 (1.72)	0.371
Glucose (mg/dL)		118 (109–137)	116 (105–134)	0.536
HbA1c (%)		5.95 (5.60–6.40)	5.75 (5.50–6.20)	0.216
HbA1c (mmol/mol)		42 (38–46)	39 (37–44)	
Insulin (μU/L)		12.7 (9.60–18.5)	12.5 (9.68–17.7)	0.819
HOMA-IR index		3.93 (2.92–5.74)	3.57 (2.59–5.36)	0.677
Triglycerides (mg/dL)		112 (93.5–142)	114 (96.3–163)	0.445
Total cholesterol (mg/dL)		209 ± 39.6	211 ± 45.3	0.795
LDL cholesterol (mg/dL)		134 ± 36.3	134 ± 38.8	0.995
HDL cholesterol (mg/dL)		52.0 (46.0–60.0)	54.0 (46.0–59.0)	0.998
Uric acid (mg/dL)		6.00 ± 1.28	5.76 ± 1.28	0.307
GGT (U/L)		30.0 (22.0–45.5)	27.0 (18.0–41.0)	0.108
ALT (U/L)		27.0 (20.0–37.0)	23.0 (17.3–32.8)	0.037
AST (U/L)		24.0 (20.0–29.0)	22.0 (18.3–27.0)	0.131

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; GGT, gamma glutamil transferase; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HP, high protein; HPA, high protein mainly from animal sources; HPP, high protein mainly from plant sources; LDL, low-density lipoprotein.

[a] Quantitative variables are expressed as mean ± standard deviation, except for variables not following normal distribution that are expressed as medians (interquartile ranges). Qualitative variables are expressed as percentage.

[b] The *p*-value was calculated using *t* test, Mann–Whitney *U* test or χ^2 test, as appropriate.

compared to baseline. However, participants following neither the HPA diet nor the HPP diet achieved the target level of protein consumption, which was 35% of the TCV. The protein consumption percentage was slightly higher in participants on the HPA diet (24.6 (22.4–28.3)% and 23.8 (21.3–26.9)% of the TCV at the 3- and 6-month visits, respectively) than in participants on the HPP diet (22.0 (19.9–24.4)% and 21.6 (19.1–23.8)% of the TCV at the 3- and 6-month visits, respectively), although no significant differences were observed between the two groups. Subjects randomized to the HPA diet met the target ratio of animal to plant protein consumption, demonstrating 77.6% and 77.2% animal protein intake at 3 and 6 months, respectively. Participants following the HPP diet reported the consumption of 55.2% and 57.1% plant protein at 3 and 6 months, respectively. This suggests that it could be difficult to achieve a high intake of protein from plant sources, possibly due to their high fibre content. Indeed, fibre consumption was significantly higher in the

TABLE 2 Clinical and anthropometric characteristics change after 3 and 6 months of dietary intervention according to diet group.[a]

	Animal HP diet (HPA)			Plant HP diet (HPP)			
	3 months ($N = 55$)	6 months ($N = 52$)	p[c]	3 months ($N = 55$)	6 months ($N = 50$)	p[c]	p[b]
BMI (kg/m^2)	−2.21 ± 1.38	−2.90 (−3.95–(−1.67))	<0.001	−2.28 ± 1.49	−3.05 (−4.49–(−1.47))	<0.001	0.991[d]
Body weight (kg)	−6.18 ± 3.98	−8.05 ± 5.12	<0.001	−6.15 ± 4.27	−7.70 ± 5.47	<0.001	0.924
Fat mass (%)	−2.74 ± 2.02	−3.20 (−6.05–(−1.87))	<0.001	−2.10 (−4.05–(−1.22))	−2.85 (−6.7–(−1.58))	<0.001	0.801[d]
Abdominal visceral fat (kg)	−0.33 (−0.62–(−0.17))	−0.42 (−0.73–(−0.17))	<0.001	−0.31 (−0.58–(−0.1))	−0.47 ± 0.41	<0.001	0.450[d]
Abdominal subcutaneous fat (kg)	−0.41 (−0.67–(−0.12))	−0.63 ± 0.48	<0.001	−0.43 ± 0.39	−0.38 (−0.82–(−0.26))	<0.001	0.702
Lean mass (kg)	−1.42 ± 1.71	−1.60 ± 1.88	<0.001	−1.36 ± 2.02	−1.73 (−2.59–(−0.11))	<0.001	0.725[d]
Waist circumference (cm)	−5.00 (−8.50–(−1.88))	−6.50 (−11.8–(−2.50))	<0.001	−5.10 (−10.0–(−2.00))	−6.50 (−10.5–(−3.00))	0.006	0.229
Diastolic blood pressure (mmHg)	−8.00 (−13.0–(−0.50))	−8.45 ± 10.5	<0.001	−5.14 ± 8.70	−3.58 ± 9.93	0.013	0.219
Systolic blood pressure (mmHg)	−12.2 ± 14.2	−11.5 ± 14.9	<0.001	−10.0 (−17.25–(−0.25))	−9.50 (−17.5–(−2.50))	<0.001	0.681

Abbreviations: BMI, body mass index; HP, high protein; HPA, high protein mainly from animal sources; HPP, high protein mainly from plant sources.

[a] Quantitative variables are expressed as mean ± standard deviation, except for variables not following normal distribution that are expressed as medians (interquartile ranges).

[b] p-Value refers to overall differences between diet groups (group × time interaction) and was calculated using the generalized linear model (GLM), including log-transformed data and adjusting by age, sex and BMI.

[c] p-Value refers to differences along the study within the diet group and was calculated using Friedman test or repeated-measures analysis of variance (ANOVA) for analysing non-parametric and parametric variables, respectively.

[d] Bonferroni correction was applied to the GLM analysis of primary variables of interest (glucose, glycated haemoglobin, insulin, homeostasis model assessment-estimated insulin resistance, BMI, fat mass, fat-free mass and visceral fat change), so $p = 0.007$ should be considered as the significance level.

HPP group than in the HPA group, with reported intakes of 32.2 and 32.1 g per day at 3 and 6 months, respectively ($p < 0.001$). Fat and carbohydrate intakes differed significantly between the groups, with subjects in the HPA group reporting greater fat consumption, whereas higher carbohydrate intake was reported by those participants following the HPP diet. This is related to the different types of HP foods recommended for each group. The HPA diet involved foods like meat, oily fish or eggs, which have a somewhat high fat content, whereas the HPP diet included foods like oat, nuts or legumes, which have a high carbohydrate content.

There were no significant changes in physical activity or sleep habits between the two groups (Table S6). It is important to highlight that no specific exercise recommendations were made; the participants were simply advised to increase their daily physical activity from their baseline habits.

4 | DISCUSSION

To the best of our knowledge, this is one of the few studies investigating the effect of two low-calorie HP diets with different protein sources on glucose homeostasis and other health outcomes in outpatients with prediabetes or T2DM and overweight or obesity. The main finding is that both energy-restricted HP diets led to an improvement in body composition, glucose metabolism and other cardiometabolic parameters (e.g. adipokines and inflammatory markers), regardless of whether the protein was from animal or plant sources.

It is well known that protein consumption plays a key role in glucose homeostasis.[12,21] Prior studies have demonstrated that HP diets can ameliorate glucose metabolism, mainly by promoting insulin secretion, although they are less effective in individuals with impaired glucose metabolism.[7,12,13,22] Variation in dietary protein sources could explain the inconsistent findings from different studies.[7,13,23,24] Some studies have found that soy and whey proteins have comparable effects on modulating the postprandial glucose response after carbohydrate intake.[11,25] However, another study found that whey protein has a more beneficial effect on the postprandial glycaemic response than protein from rice or potatoes, which is of lower quality.[16] Thus, protein quality may matter much more than total protein amount or protein source in mid- and long-term interventions.[24] The present study involved HP diets that included high-quality proteins, including those from plant sources, such as soy, quinoa, oats, legumes and nuts. Our findings reveal that both low-calorie HP diets enhanced glucose metabolism regardless of protein source, whether animal or plant. A similar study was conducted by González-Salazar et al. in patients with obesity and insulin resistance.[26] The participants were randomly assigned to one of four diets for a 1-month period: a normal-protein diet with a higher amount of animal protein or protein from plant sources and a HP diet including predominantly animal protein or predominantly protein from plant sources. They found that insulin sensitivity improved in all participants who followed HP diets, regardless of the protein source. However, they did not investigate HbA1c because of the short duration of the study.

Multiple mechanisms seem to be responsible for the improved glucose regulation conferred by HP diets, most of which are related to

TABLE 3 Biochemical characteristics change after 3 and 6 months of dietary intervention according to diet group.[a]

	Animal HP diet (HPA)			Plant HP diet (HPP)			
	3 months (*N* = 55)	**6 months (*N* = 52)**	***p*[b]**	**3 months (*N* = 55)**	**6 months (*N* = 50)**	***p*[b]**	***p*[c]**
Glucose (mg/dL)	−14.5 (−21.3–(-2.75)	−10.0 (−20.5 to −2.5)	<0.001	−9.00 (−17.5 to −1.00)	−9.50 (−20.5 to −1.25)	<0.001	0.992[d]
Insulin (µUI/mL)	−3.25 (−6.33 to −1.48)	−4.99 ± 5.33	<0.001	−3.50 (−6.50 to −0.40)	−4.40 (−7.72 to −1.49)	<0.001	0.856[d]
HOMA-IR index	−1.40 (−2.61 to −0.54)	−1.45 (−2.53 to −0.64)	<0.001	−1.02 (−2.38 to −0.28)	−1.44 (−2.83 to −0.53)	<0.001	0.873[d]
HbA1c (%)	−0.20 (−0.40–0.00)	−0.20 (−0.48–0.00)	<0.001	−0.20 (−0.40 to −0.10)	−0.28 ± 0.33	<0.001	0.513[d]
HbA1c (mmol/mol)	−2.00 (−4.75 to −0.25)	−2 (−4.25–0.00)		−1.50 (−4.00–0.00)	−2.00 (−4.25–0.00)		
Triglycerides (mg/dL)	−9.00 (−32.3–9.75)	−13.0 (−28.5–7.00)	0.043	−8.00 (−41.5–9.50)	−24.0 (−40.5 to −5.50)	<0.001	0.364
Total cholesterol (mg/dL)	−8.50 (−25.3–1.50)	−2.73 ± 31.2	0.375	−15.0 (−38.0–7.00)	−13.8 ± 36.3	0.008	0.393
LDL cholesterol (mg/dL)	−8.16 ± 22.7	−3.55 ± 27.0	0.223	−9.00 (−29.5–7.50)	−15.0 (−26.0–9.00)	0.015	0.546
HDL cholesterol (mg/dL)	−1.00 (−5.00–2.00)	2.61 ± 7.53	0.001	−1.00 (−6.00–2.00)	0.42 ± 7.45	0.011	0.534
Apolipoprotein A1 (mg/dL)	−10.1 ± 17.6	0.17 ± 20.2	0.002	−8.06 ± 20.9	−3.20 ± 19.2	0.017	0.527
Apolipoprotein B (mg/dL)	−10.7 ± 19.1	−3.85 ± 26.1	0.158	−9.81 ± 23.2	−7.18 ± 24.5	0.028	0.503
CRP (mg/dL)	−0.02 (−0.14–0.02)	−0.03 (−0.13–0.04)	0.094	−0.06 (−0.18–0.00)	−0.07 (−0.23–0.00)	<0.001	0.768
Iron (µg/dL)	−5.36 ± 34.9	−8.08 ± 34.0	0.066	−0.89 ± 35.4	−1.76 ± 35.0	0.879	0.210
Ferritin (ng/dL)	−0.95 (−30.2–13.82)	−2.70 (−30.5–18.2)	0.633	−6.15 (−26.8–14.4)	−3.20 (−25.1–12.7)	0.600	0.966
Vitamin B12 (pg/dL)	2.50 (−39.0–31.0)	12.5 (−38.3–60.0)	0.385	−39.5 ± 72.6	−17.4 ± 83.2	0.074	0.337
Folic acid (ng/dL)	0.76 ± 3.01	0.85 (−0.72–3.22)	0.020	2.27 ± 2.88	2.51 ± 3.06	<0.001	0.436
Creatinine (mg/dL)	0.01 (−0.03–0.07)	0.01 (−0.05–0.06)	0.459	0.00 ± 0.08	−0.01 ± 0.10	0.525	0.926
Uric acid (mg/dL)	−0.23 (−0.53–0.20)	−0.23 ± 0.63	0.006	−0.06 ± 0.64	−0.16 ± 0.71	0.077	0.953
Albumin (g/dL)	0 (−0.1–0.1)	0.01 ± 0.25	0.479	0.11 ± 0.17	0.08 ± 0.27	0.002	0.196
GFR (mL/min × 1.73^2)	−0.63 (−3.71–2.6)	−0.47 (−4.06–3.57)	0.377	−0.28 ± 8.57	−0.10 ± 9.90	0.513	0.453
GGT (U/L)	−5.00 (−11.0 to −0.75)	−4.00 (−11.0 to −1.00)	<0.001	−4.00 (−11.0 to −1.50)	−7.00 (−11.8 to −2.00)	<0.001	0.722
ALT (U/L)	−3.00 (−11.0–1.00)	−4.00 (−14.0 to −0.50)	0.139	−4.00 (−8.50 to −0.50)	−4.00 (−9.75 to −1.00)	0.008	0.703
AST (U/L)	−1.50 (−5.00–1.00)	−1.50 (−5.00–1.00)	<0.001	−2.00 (−4.00–1.00)	−1.06 ± 5.07	<0.001	0.869
Urine							
Microalbumin (mg/dL)	−0.20 (−0.40–0.00)	−0.15 (−0.49–0.02)	<0.001	−0.10 (−0.30–0.03)	−0.10 (−0.30–0.00)	<0.001	0.903
Creatinine (mg/dL)	−29.5 ± 55.0	−15.8 ± 77.1	0.261	−27.4 (−56.2 to −3.61)	−22.0 ± 58.5	0.011	0.994
Microalbumin–creatinine ratio	−0.7 (−2.06–0.35)	−0.08 (−1.99–1.19)	0.017	0.25 (−0.96–0.93)	0.23 (−0.92–1.4)	0.376	0.763
Urea (mg/dL)	2.55 ± 6.13	3.53 ± 7.84	0.014	1.45 ± 7.60	1.42 ± 9.07	0.014	0.813

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; GFR, glomerular filtration rate; GGT, gamma glutamil transferase; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HP, high protein; HPA, high protein mainly from animal sources; HPP, high protein mainly from plant sources; LDL, low-density lipoprotein.

[a] Quantitative variables are expressed as mean ± standard deviation, except for variables not following normal distribution that are expressed as medians (interquartile ranges).

[b] *p*-Value refers to differences along the study within diet group and was calculated using Friedman test or repeated-measures analysis of variance (ANOVA) for analysing non-parametric and parametric variables, respectively.

[c] *p*-Value refers to overall differences between diet groups (group × time interaction) and was calculated using the generalized linear model (GLM) including log-transformed data and adjusting by age, sex and BMI.

[d] Bonferroni correction was applied to the GLM analysis of primary variables of interest (glucose, HbA1c, insulin, homeostasis model assessment-estimated insulin resistance, BMI, fat mass, fat-free mass and visceral fat change), so $p = 0.007$ should be considered as the significance level.

the insulinotropic effect of proteins.[12] As previously stated, some amino acids (e.g. lysine or proline) enhance β-cell depolarization and favour insulin exocytosis, whereas others (e.g. BCAAs or arginine) are key ligands for mTORC1, which supports insulin synthesis in β-cells.[15,27] The beneficial effect of BCAAs (leucine, isoleucine and valine) on glucose homeostasis remains controversial. Some studies

TABLE 4 Metabolic biomarkers change after 3 and 6 months of dietary intervention according to diet group.[a]

	Animal HP diet (HPA)			Plant HP diet (HPP)			
	3 months ($N = 55$)	6 months ($N = 52$)	p[b]	3 months ($N = 55$)	6 months ($N = 50$)	p[b]	p[c]
GIP (pg/mL)	−5.95 (−23.0–7.89)	−5.87 (−27.3–10.6)	0.086	−7.17 (−22.1–7.98)	−7.15 (−19.18–3.80)	0.007	0.719
GLP-1 (pg/mL)	−50.4 ± 83.7	−56.3 (−92.9 to −15.6)	<0.001	−46.9 ± 64.1	−68.7 ± 57.0	<0.001	0.871
Leptin (pg/mL)	−4492 (−9448 to −2138)	−4236 (−9002 to −2459)	<0.001	−6196 ± 6799	−5630 (−10 377 to −1623)	<0.001	0.939
Adiponectin (pg/mL)	439 (−10 422–13 823)	7938 ± 35 248	0.083	4144 (−5598–15 956)	10 275 (−2399–38 263)	0.034	0.348
Resistin (pg/mL)	−3.80 (−10.3–12.8)	−1.51 (−13.1–9.45)	0.860	2.56 (−11.6–20.9)	−0.80 (−17.5–13.2)	0.354	0.708
MCP-1 (pg/mL)	−24.0 ± 65.6	−21.5 (−64.0–10.9)	0.004	−19.7 ± 77.5	−32.7 ± 78.8	0.041	0.975
TNF-α (pg/mL)	−1.26 (−4.03–0.3)	−2.68 (−4.42 to −1.64)	<0.001	−1.00 (−2.92–0.49)	−2.54 ± 2.73	<0.001	0.606
PAI1 (pg/mL)	−80.8 ± 142	−153 ± 160	<0.001	−50.7 ± 177	−130 ± 193	0.002	0.507
IL-6 (pg/mL)	−0.07 (−1.04–0.45)	−0.64 (−1.99–0.68)	0.005	0.00 (−0.66–0.69)	−0.64 (−1.17 to −0.01)	<0.001	0.905
IL-1β (pg/mL)	0.00 (−1.8–1.85)	−0.05 (−2.14–1.1)	0.589	−0.32 (−1.58–1.3)	−0.28 (−1.84–0.8)	0.048	0.828
IL-8 (pg/mL)	−1.27 (−3.43–0.69)	−0.99 (−3.64–1.13)	0.032	−0.16 ± 2.91	0.25 ± 3.42	0.583	0.191
IL-10 (pg/mL)	−0.74 (−1.92–0.5)	−0.13 (−2.7–1.19)	0.290	−0.06 (−2.35–0.85)	−1.28 (−1.98–0.49)	0.048	0.358
VEGF-A (pg/mL)	−40.7 (−129–19.9)	−41.8 (−136–21.4)	0.005	−27.5 ± 163	−19.3 (−127–52.2)	0.206	0.518

Abbreviations: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; HP, high protein; HPA, high protein mainly from animal sources; HPP, high protein mainly from plant sources; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; PAI1, plasminogen activator inhibitor-1; TNF-α, tumour necrosis factor α; VEGF-A, vascular endothelial growth factor A.

[a] Quantitative variables are expressed as medians (interquartile ranges). GIP denotes glucose-dependent insulinotropic polypeptide.

[b] *p*-Value refers to differences along the study within diet group and was calculated using Friedman test or repeated-measures analysis of variance (ANOVA) for analysing non-parametric and parametric variables, respectively.

[c] *p*-Value refers to overall differences between diet groups (group × time interaction) and was calculated using the generalized linear model including log-transformed data and adjusting by age, sex and BMI.

have demonstrated that these amino acids enhance insulin secretion and reduce postprandial glucose concentrations, whereas other studies have shown that high plasma BCAA levels in humans are related to insulin resistance and T2DM development, though the underlying reason is unknown.[28–30]

Peptides and amino acids are recognized by nutrient sensors in enteroendocrine cells like L and K cells that release GIP and GLP-1.[31] These peptide hormones are known as incretin hormones; they are released postprandially and promote insulin secretion, satiety and β-cell proliferation and survival.[31] This effect is mainly mediated by CaSR, an L-amino acid sensor that is expressed in the gut.[31] Although CaSR binds to a wide range of amino acids, it has a strong affinity for aromatic amino acids like L-phenylalanine and L-tryptophan.[32] Several studies have shown that protein-enriched meals increase the release of incretins, mainly GLP-1, which improves the postprandial response to carbohydrate intake.[33,34] However, changes in fasting incretin concentrations after weight loss have been explored only in a few studies that drew different conclusions. We found that fasting GIP, and particularly fasting GLP-1, levels decreased in response to both dietary interventions, with no differential effects noted based on protein source. de Luis et al. found that GLP-1 decreased by 6% in outpatients with obesity who lost weight (4.8 ± 1.6 kg) in response to a low-calorie dietary intervention.[35] It has been suggested that this reduction could be related to the increase in the production of free fatty acids observed after weight loss, as they could interfere with GLP-1 release from enteroendocrine cells.[36] Another hypothesis suggests that the reduction in GLP-1 levels may reflect a metabolic adaptation to starvation, because GLP-1 favours satiety[31,37]; this would represent a response to a negative energy balance maintained during mid- and long-term dietary interventions. We observed 29.9% and 35.1% decreases in fasting GLP-1 concentrations in the HPA and HPP groups, respectively, at the end of the study. These figures are much higher than those reported by de Luis et al., whose study participants exhibited less weight loss than those involved in our trial.[35] We also discovered a direct and significant correlation between weight loss and a decrease in fasting GLP-1, and found that the reduction was larger in the first period of the study, when the highest rate of weight loss was observed. These findings are in line with the hypothesis that the decline in fasting GLP-1 could be associated with a neuroendocrine response to starvation in the mid-term. As previously stated, it is important to highlight that the main physiological effect of incretins on glycaemic control occurs postprandially.

A decrease in muscle mass after weight loss could compromise the benefits of weight loss, which is especially important in subjects with T2DM, who exhibit a high prevalence of sarcopenia.[5,6,38,39] The evidence suggests that lean mass decreases by 2%–10% in subjects with obesity after losing 8%–10% of their body weight.[39] Different strategies have been proposed to prevent this, mainly focused on exercise training and increasing protein intake within low-calorie diets. The effectiveness of HP diets in preserving FFM is still controversial.[39] Conflicting results may be related to variations in diet composition (both quantity and types of protein), diverse study designs and

TABLE 5 Dietary characteristics at baseline and after 3 and 6 months of dietary intervention according to diet group.[a]

	Animal HP diet (HPA)				Plant HP diet (HPP)				
Daily intake	**Baseline ($N = 59$)**	**3 months ($N = 55$)**	**6 months ($N = 52$)**	p[c]	**Baseline ($N = 58$)**	**3 months ($N = 55$)**	**6 months ($N = 50$)**	p[c]	p[b]
Energy (kcal)	2010 (1665–2257)	1460 (1349–1634)	1443 (1303–1650)	<0.001	1994 (1673–2222)	1611 (1412–1765)	1659 (1502–1916)	<0.001	0.065
Protein, % TCV	19.1 (17.7–21.7)	24.6 (22.4–28.3)	23.8 (21.3–26.9)	<0.001	17.9 (16.5–20.2)	22.0 (19.9–24.4)	21.6 (19.1–23.8)	<0.001	0.674
Animal protein, % total protein	71.8 (66.7–78.3)	77.6 (71.4–82.5)	77.2 (72.1–82.6)	0.002	70.2 (62.8–75.4)	37.1 (29.7–46.4)	39.7 (31.8–52.1)	<0.001	<0.001
Vegetal protein, % total protein	26.4 (21.5–32.7)	20.8 (17.3–30.0)	22.8 (17.0–27.3)	0.002	29.8 (24.4–35.0)	55.2 (46.0–66.2)	57.1 (46.6–63.0)	<0.001	<0.001
Fat, % TCV	46.7 (43.0–52.2)	43.4 (39.9–47.4)	44.9 (40.8–49.9)	0.016	45.6 (42.8–49.4)	36.9 (34.0–41.2)	38.9 (33.4–43.0)	<0.001	0.009
SFA, % TCV	13.0 (10.7–14.4)	10.1 (8.69–11.5)	10.3 (9.08–11.6)	<0.001	12.6 (11.3–14.3)	6.58 (5.83–8.10)	7.19 (5.92–9.58)	<0.001	0.006
MUFA, % TCV	23.5 (20.3–25.6)	22.5 (20.0–24.1)	23.6 (21.3–25.4)	0.744	22.0 (20.5–24.0)	16.4 (15.3–19.1)	18.3 (14.7–20.4)	<0.001	<0.001
PUFA, % TCV	6.10 (5.15–7.04)	6.22 (4.43–7.28)	6.10 (5.33–6.99)	0.274	5.73 (5.09–7.35)	6.62 (5.90–7.98)	6.56 (5.20–7.65)	0.223	0.253
Dietary cholesterol (mg)	380 (258–420)	313 (250–387)	327 (287–415)	0.529	353 (264–498)	191 (129–304)	179 (128–296)	<0.001	<0.001
Carbohydrates, % TCV	31.4 (25.4–35.5)	30.4 (24.8–34.2)	30.3 (25.5–34.7)	0.441	34.2 (31.2–36.8)	37.8 (33.3–42.4)	38.9 (34.3–45.34)	0.002	<0.001
Fibre (g)	19.2 (13.6–25.3)	19.9 (14.3–32.4)	17.9 (13.0–20.4)	0.012	20.6 (15.7–24.5)	32.2 (25.3–35.9)	32.1 (26.1–37.8)	<0.001	<0.001
Sodium (mg)	2700 (1817–3382)	1991 (1493–2497)	2331 (1435–2714)	<0.001	2391 (1640–2967)	1803 (1151–2560)	1960 (1489–2513)	0.551	0.369
Potassium (mg)	3225 (2645–3873)	3442 (3002–3999)	3272 (2931–3796)	0.307	3141 (2643–3804)	3289 (2948–3698)	3490 (3085–4001)	0.109	0.737

Abbreviations: HP, high protein; HPA, high protein mainly from animal sources; HPP, high protein mainly from plant sources; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TCV, total caloric value.

[a] Quantitative variables are expressed as mean ± standard deviation, except for variables not following normal distribution that are expressed as medians (interquartile ranges).

[b] *p*-Value refers to overall differences between diet groups (group × time interaction) and was calculated using the generalized linear model (GLM) including log-transformed data and adjusting by age and sex.

[c] *p*-Value refers to differences along the study within diet group and was calculated using Friedman test or analysis of variance (ANOVA) for analysing non-parametric and parametric variables, respectively.

small study sample sizes.[9] We found that the FFM of participants following the HPA diet decreased by 2.69 %± 3.93%, whereas that of participants randomized to the HPP diet decreased by 2.43% ± 5.28%. Fat mass had decreased by 10% in both groups by the end of the study, showing that most of the weight loss was due to the loss of adipose tissue. The reduction in FFM that we observed is similar to that described in other studies exploring HP diets that induced a similar degree of weight loss.[40] A few studies have investigated the effect of different amino acids or protein sources on FFM loss. Some studies have concluded that animal protein is more beneficial for lean mass retention than plant-based protein, whereas others have not reported any differences in the effects of these two protein sources.[9] González-Salazar et al. concluded that low-calorie HP diets did not significantly change lean mass after a 1-month intervention in subjects with insulin resistance, regardless of whether the protein was from animals or plants, although their study participants lost less body weight than ours did.[26] However, they did describe a significant decrease in FFM in subjects who consumed the normal-protein diet, and most of the protein in their dietary intervention was from plant sources. Our findings reveal that the consumption of a low-calorie HP diet can lead to a significant reduction in total fat mass and visceral fat by preserving lean mass to a large extent, independent of protein source. Beyond protein quantity or source, this evidence suggests that protein quality plays a crucial role in preserving FFM in people with prediabetes or T2DM consuming a low-calorie diet.

When HP diets are prescribed, there are concerns about their possible nephrotoxicity or detrimental effects on glucose and other cardiometabolic parameters, which would be especially concerning in subjects with T2DM.[24,41] It has been hypothesized that the consumption of large quantities of protein could lead to mTORC1 hyperactivation and increase insulin resistance in the mid- or long term.[15,42] Our results contradict this hypothesis and demonstrate that insulin resistance improved in people with prediabetes and T2DM following a hypocaloric HP diet. In addition, we did not note any worsening of renal function. Further, microalbuminuria decreased in both diet groups, an effect that is associated with better renal function and less morbimortality in T2DM.[43] Some researchers postulate that uric acid levels could increase when following a long-term HP diet, especially in men.[44] In our study, uric acid concentrations improved significantly in both groups.

Although our dietary interventions included 35% of TCV from protein, participants in both groups reported a lower intake of this macronutrient. This finding is common in real-world trials involving HP diet prescriptions that are based on typically consumed foods and do not include supplements.[7,26] The dietary habits reported by the participants suggest that they found it difficult to adhere to a low-calorie diet, with 35% of TCV from protein without using protein supplements. Despite the lower protein intake observed in our study, the benefits to body composition and cardiometabolic health outcomes were similar to those reported in other studies involving higher protein consumption. It is important to note that a quite similar intake of high-quality protein was achieved with both animal and plant protein sources, leading to equally positive health outcomes.

Our study has some limitations that are worth mentioning. The participants randomized to the HPP diet did not achieve the prescribed ratio of animal-to-plant protein consumption (25/75% of total protein), which could have affected the findings. In addition, the mid-term duration of the intervention could have affected the findings, and confirming the results in a long-term study could help to more fully establish dietary recommendations for T2DM prevention and management. Furthermore, the relatively small sample size could have limited the significance of the effect of any diet on some outcomes. In this regard, it is important to note that the sample size calculation indicated the need for 56 participants per group, although only 52 and 50 participants in the HPA and HPP groups, respectively, completed the trial, which could have also affected the results. Dietary compliance was assessed using self-reported questionnaires, although we assessed urine nitrogen with spot samples, which is an established surrogate biomarker of protein consumption.[20] Nevertheless, 24-h urinary nitrogen determination would have provided a more reliable measurement of protein intake and each patient's nitrogen balance. Determination of creatinine clearance in 24-h urine samples would have also helped evaluate renal function and interpret the findings.

In conclusion, a low-calorie HP diet improved body composition, glucose metabolism (e.g. insulin resistance and glucose management) and other cardiometabolic outcomes, such as lipid profiles, adipokines and other inflammatory biomarkers, regardless of whether the protein was obtained mostly from animal or plant sources. These findings should be confirmed in future studies with larger sample sizes and longer durations. Our results strengthen the recommendation of HP diets as a weight loss approach for the prevention and management of prediabetes and T2DM and suggest that different protein sources confer the same benefits in this context. In summary, our findings support increasing dietary intake of protein from both plant and animal sources, thereby enhancing diet variety and promoting the consumption of protein from more sustainable sources.

AUTHOR CONTRIBUTIONS

Design: Rocío Mateo-Gallego and Itziar Lamiquiz-Moneo. Conduct/data collection: Carmen Rodrigo-Carbó, Loreto Madinaveitia-Nisarre, Irene Gracia-Rubio, Carlos Galindo-Lalana, Sofía Pérez-Calahorra, Rocío Mateo-Gallego and Itziar Lamiquiz-Moneo. Analysis: Carmen Rodrigo-Carbó, Alberto Cebollada, Rocío Mateo-Gallego and Itziar Lamiquiz-Moneo. Manuscript writing: all authors.

ACKNOWLEDGEMENTS

The authors thank the participants for their kind collaboration and participation in the study.

FUNDING INFORMATION

Carmen Rodrigo-Carbó received a predoctoral grant from Aragon government, and Irene Gracia-Rubio received a postdoctoral grant from Carlos III Health Institute. The research group within authors developed the study received funding from Centro de Investigación Biomédica en Red Enfermedades Cardiovaculares

(CIBERCV) (co-supported by the European Regional Development Fund [ERDF], which is allocated by the European Union, IIS16/0114) and Aragon government (B-14). This project received a grant in the call for 'Projects for Young Researchers' of the University of Zaragoza (2022).

CONFLICT OF INTEREST STATEMENT

The authors state that they do not have relevant conflicts of interest to disclose.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.16013.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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How to cite this article: Rodrigo-Carbó C, Madinaveitia-Nisarre L, Pérez-Calahorra S, et al. Low-calorie, high-protein diets, regardless of protein source, improve glucose metabolism and cardiometabolic profiles in subjects with prediabetes or type 2 diabetes and overweight or obesity. *Diabetes Obes Metab*. 2024;1-12. doi:10.1111/dom.16013