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ORIGINAL RESEARCH

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# Impact of a daily legume-based meal on blood and anthropometric parameters in a group of omnivorous adults: A pilot study

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## Abstract

This pilot study aimed to assess the impact of substituting a traditional lunch for a vegetarian legume-based meal on blood and anthropometric parameters in a group of omnivorous adults. A one-group comparison, quasi-experimental dietary intervention was designed. A vegetarian legume-based meal was offered for 8 consecutive weeks (weekdays) to non-vegetarian individuals ($n=26$), (28 years [$P_{25}=20.0$, $P_{75}=35.5$]; 21.9 kg/m$^2$ [$P_{25}=21.3$, $P_{75}=24.8$]). Sociodemographic data, health status and lifestyle-related information were recorded. Three-day food records were used to collect food intake at baseline and at the end of the intervention. Anthropometric parameters were recorded and fasting blood analyses were performed following standard procedures. Wilcoxon signed-rank test was used for statistical comparisons. A $p$-value <0.05 was considered statistically significant. Participants showed a median intake of 79.8 g of cooked legumes per meal, meaning 13 (50.0%) subjects met the Portuguese daily legume intake recommendations during the intervention days. There were no statistically significant differences in anthropometric parameters. Transferrin concentration increased after 8 weeks (+12.5 mg/dL; $p=0.001$). Total cholesterol concentration reduced after 8 weeks (–6 mg/dL; $p=0.041$), as well as low-density lipoprotein (LDL) cholesterol (–7 mg/dL; $p=0.003$). Triglycerides (+9 mg/dL; $p=0.046$), fasting glucose (+2 mg/dL; $p=0.037$) and HbA1c (+0.1 mg/dL; $p=0.010$) concentration increased after the 2-month legume-based trial. Results suggest a cholesterol-lowering potential of legume-rich diets. However, unfavourable results regarding the impact on glucose metabolism-related biomarkers and triglyceride levels were observed. The study's limitations in design and sample size emphasise the importance of conducting further research with larger cohorts to attain more conclusive findings.

**KEYWORDS**
blood biomarkers, body composition, legumes, plant-based, vegetarian

# INTRODUCTION

The current food and environmental crises have pushed the scientific community and made world policymakers reflect on new ways to feed present and future generations without compromising both populational health needs and the planet's natural resources (Zurek et al., 2022). Meeting such demands has become one of the most significant challenges of the 21st century in food and nutrition research fields. The publication of the EAT-Lancet commission featuring new food sustainability guidelines has brought to discussion the need for a global transition towards more plant-based dietary patterns (Willett et al., 2019). This diet shift is believed to ensure the subsistence of the world's population both in relation to health and the environment. Specifically, it will be required that traditionally omnivorous individuals increase their intake of alternatives to animal-based protein food sources, such as legumes (Alae-Carew et al., 2022).

Legumes are edible seeds or grains harvested from pods of plants belonging to the *Leguminosae* family (Vasconcelos et al., 2020). They can include different types of beans, chickpeas, peas and lentils. Besides the high-protein content, legumes are low in fat and saturated fat, high in fibre and convey several vitamins (e.g., B complex vitamins) and minerals (e.g., iron), as well as numerous bioactive compounds (e.g., phenolic acids) that may be health-promoting (Singh et al., 2017). Indeed, for centuries the health and environmental advantages of legumes have been empirically appreciated by mankind (Gomes & Vasconcelos, 2014) and legumes have long featured in official food guides of several countries around the world (Hughes et al., 2022). Also, scientific evidence has been growing surrounding the potential health benefits of legume-rich diets. Data from prospective cohort studies and a systematic review of controlled trials support the implementation of diets containing legumes for cardiometabolic risk management (Ferreira et al., 2020). In detail, evidence suggests that legume consumption is associated with a positive modulation of blood lipid profile, glycaemic control, inflammatory status, oxidative stress, as well as gut microbiota (Ferreira et al., 2022). Of note, some of these biological outcomes were improved in one study in 'healthy' individuals (Fernando et al., 2010) and, in another intervention trial, independently of weight loss (Saraf-Bank et al., 2015).

However, several methodological concerns arise in relation to studies currently published in the literature, which would make it challenging to draw robust conclusions and establish intake recommendations (Ferreira et al., 2020). Firstly, the study design differs significantly between publications, mainly featuring populations with pre-established health conditions (Ferreira et al., 2020). This impairs the extrapolation of results to the everyday consumer and the actual desirable target for the global dietary transition. Also, only two publications were found, reporting the results from a study that addressed the health impact of replacing animal-based foods with legumes (Hosseinpour-Niazi, Mirmiran, Fallah-Ghohroudi, & Azizi, 2015; Hosseinpour-Niazi, Mirmiran, Hedayati, & Azizi, 2015). For instance, the substitution of red meat with non-soy legumes (3 cups/week or ≈ one serving/day; cooked) for 8 weeks, within the *Therapeutic Lifestyle Change Diet*, in a group of 31 patients with overweight and type 2 diabetes significantly decreased fasting blood glucose (FBG), fasting insulin, triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) (Hosseinpour-Niazi, Mirmiran, Hedayati, & Azizi, 2015). Furthermore, there was a reduction of inflammatory biomarkers, namely, C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), compared to the control legume-free diet (Hosseinpour-Niazi, Mirmiran, Fallah-Ghohroudi, & Azizi, 2015).

Unsurprisingly, a comprehensive meta-analysis of prospective studies still shows that the strength of the scientific evidence regarding the health benefits of legume consumption remains relatively weak (Viguiliouk et al., 2019). Also, the metabolic impact of legumes used as 'meat alternatives' remains underexplored in traditionally omnivorous healthy individuals.

Taking all this into account, a pilot study was carried out to propose an 8-week vegetarian dietary approach which aimed to replace habitually consumed weekday omnivorous (lunch) meals with meals based on legumes and assess their impact on the modulation of metabolic health biomarkers, including anthropometric parameters and biochemical blood profile, in young healthy Portuguese omnivorous adults. We posited two hypotheses for investigation: the first hypothesis was that replacing omnivorous lunch meals with legume-based meals would not induce adverse outcomes, particularly concerning iron and B12 status; the second hypothesis was that such a dietary substitution would yield benefits in terms of lipid and glucose metabolism, as suggested by the existing literature, with discernible impacts on biochemical markers.

# METHODS

## Subjects and the study design

The present pilot study was a one-group comparison, quasi-experimental dietary intervention conducted at the Faculty of Biotechnology of the Catholic University of Portugal (FB-CUP). All study procedures were carried out in full compliance with the Declaration of Helsinki on ethical principles for medical research involving humans and were validated by the Institute of Bioethics of the Catholic University of Portugal (Ethics Screening Report 11/2017). Non-vegetarian individuals

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were recruited at FB-UCP and at the university campus neighbouring areas. The aim was to recruit 40 subjects to align with previous studies. Subjects were screened according to specific eligibility criteria: (1) male or female individuals between 18 and 45 years old; (2) having a daily intake of animal-protein food sources, as part of both lunch and dinner meals; (3) intake of legumes lower than 25 g (dry weight) per day; (4) willingness to follow the intervention diet. Exclusion criteria included the following: (1) intake of grain legumes above 25 g (dry weight) per day; (2) suffering from severe food allergies or food intolerances; (3) suffering from severe chronic inflammatory, infectious, endocrine, or metabolic diseases, including gastrointestinal disorders; (4) intake of antibiotic drugs or probiotic foods/supplements within 2 weeks before intervention; and (5) pregnancy or breastfeeding. Volunteers who met the inclusion criteria signed an informed consent form.

## Demographic, health and lifestyle-related information

At the beginning of the study, information regarding sociodemographic data (e.g., gender, age, occupation, education level) was gathered, as well as general health and lifestyle-related information about the participants, namely, food (e.g., dietary changes, habitual legume intake, food restrictions and supplement use), smoking status and physical activity habits. To assess physical activity levels, a validated questionnaire was applied, developed for the Portuguese population (Camões et al., 2010). Participants were then stratified into two groups according to their exercise habits, namely, 'Exercise' if they practised regular moderate-intensity physical activity (defined as 3.0–5.9 metabolic equivalents [METs] (Yang, 2019)), at least 45–60 min, at least twice a week versus 'No exercise' if their exercise habits did not match this definition (i.e., if they did not exercise regularly). The presence of any health conditions and the use of any medication were recorded. Dietary restrictions (allergies/intolerances) and intake of nutritional supplements were also noted.

## Legume-based dietary intervention

Due to logistical constraints, the dietary intervention was planned to run in three phases. Volunteers were asked to replace their typical omnivorous lunch meal with an ovo-lacto-vegetarian legume-based meal provided, for 8 consecutive weeks, five times per week (Monday to Friday). No other restrictions were made on habitual food intake. Intervention meals were provided by the catering company EUREST and form part of their standard canteen menus. The intervention meals were made available ad libitum and included four basic elements: (1) vegetable soup, (2) the main course, (3) seasonal fruit as dessert and (4) water as the beverage. Each main course contained one of five legumes replacing animal-based protein food sources: chickpea (dry), bean (dry; black, red, white), lentil (dry; brown), pea (frozen; green), or soybean (granulated) (see online Supplementary file 1 for a sample menu). Meal nutritional composition was based on reference values for the general Portuguese adult population (Rodrigues et al., 2006), as well as the Dietary Reference Values (DRVs) of the European Food Safety Authority (EFSA) (2017). Legume intake was calculated by weighing the main dish's components before and after the meal sessions. Participants were provided with identical legume-based main dishes as frozen meals during expected holidays, for example Easter holidays (first group [$n=20$]: 5 days [84 frozen meals]; second group [$n=8$]: 1 day [8 frozen meals]). In case of unpredicted diet interruptions (e.g., unexpected absences), participants were instructed to prepare and consume a legume-based vegetarian meal outside the intervention setting and send to the research team a detailed description of the foods eaten (first group [$n=20$]: no self-made meals); second group ([$n=8$]: 7 subjects consumed self-made meals [on one single occasion per person]).

## Daily food and nutrient intake estimates

Participants' daily food and nutrient intake were assessed through 3-day food records (Willett, 1998) (3 non-consecutive days: 2 weekdays and 1 weekend day), self-completed at baseline and at the end of the food intervention, using weights, household measures and/or units. A detailed analysis of the type of lunch meal was also performed, considering just omnivorous lunches reported in baseline food records and legume-based lunches reported in the final food records (excluding weekend days). More details on the methods used for food and nutrient estimates can be found in a previous publication (Ferreira et al., 2023).

## Anthropometric assessments

Anthropometric assessment, including body composition (by bioelectrical impedance), was accomplished at baseline and week 8 (Table 1). The evaluation processes followed standard procedures (Stewart et al., 2011; WHO, 2008), as well as the user's manual of the body composition analyser used (InBody® 720) (Biospace, 2004). All parameters were measured after overnight fasting (minimum 8 h), wearing indispensable clothing and without shoes. Also, before the body composition evaluation, participants were advised not to perform physical exercise, have an empty bladder

**TABLE 1** Anthropometric assessments and blood parameters.

| $n = 26$ | Baseline Median | Baseline $P_{25}$ | Baseline $P_{75}$ | Week 8 Median | Week 8 $P_{25}$ | Week 8 $P_{75}$ | Baseline vs. 8 weeks[†] | Reference values (SI units) |
|---|---|---|---|---|---|---|---|---|
| *Anthropometry* | | | | | | | | |
| Weight (kg) | 57.8 | 54.3 | 66.2 | 58.2 | 53.1 | 65.6 | 0.943 | – |
| BMI (kg/m$^2$) | 21.9 | 21.3 | 24.8 | 22.1 | 21.1 | 24.7 | 0.848 | 18.50–24.99 kg/m$^2$[a] |
| Fat mass (%) | 30.7 | 25.6 | 33.7 | 30.6 | 24.7 | 33.7 | 0.269 | M: 10.0%–20.0%[b]<br>F: 18.0–28.0%[b] |
| Waist circumference (cm) | 71.4 | 66.2 | 75.1 | 71.0 | 66.2 | 75.0 | 0.542 | M: <94 cm[c]<br>W: <80 cm[c] |
| Waist: Hip ratio | 0.87 | 0.85 | 0.91 | 0.88 | 0.85 | 0.92 | 0.254 | M: <0.90[c]<br>W: <0.85[c] |
| *Blood parameters* | | | | | | | | |
| Haemoglobin (g/L) | 136 | 131 | 141 | 136 | 130 | 141 | 0.968 | Men: 130–180 g/L[d]<br>Women: 120–160 g/L[d] |
| Iron (μg/dL) | 108.0 | 92.0 | 129.8 | 100.5 | 89.3 | 143.0 | 0.879 | 49–151 μg/dL[d] |
| Ferritin (μg/L) | 46.0 | 32.6 | 83.6 | 40.3 | 32.7 | 84.5 | 0.058 | 20–250 μg/L[d] |
| Transferrin (g/L) | 2.76 | 2.48 | 3.155 | 2.955 | 2.52 | 3.25 | 0.001* | 2.0–3.6 g/L[d] |
| Transferrin saturation (%) | 28.0 | 21.8 | 34.0 | 27.0 | 19.5 | 34.3 | 0.299 | 20%–50%[d] |
| Folic acid (ng/mL) | 5.6 | 4.9 | 7.2 | 5.2 | 4.1 | 7.6 | 0.432 | 2.2–17.5 ng/mL[d] |
| Vitamin B12 (pg/mL) | 315.0 | 263.3 | 458.0 | 290.0 | 231.8 | 484.3 | 0.052 | 187–883 pg/mL[d] |
| Total cholesterol (mmol/L) | 4.825 | 4.0 | 5.176 | 4.475 | 3.937 | 4.977 | 0.041* | <5.18 mmol/L[d] |
| HDL (mmol/L) | 1.525 | 1.308 | 1.733 | 1.47 | 1.315 | 1.655 | 0.257 | >1.55 mmol/L[d] |
| LDL calculated (mmol/L) | 2.533 | 2.328 | 2.987 | 2.372 | 2.145 | 2.770 | 0.003* | <3.38 mmol/L[d] |
| Triglycerides (mmol/L) | 1.0 | 0.563 | 1.273 | 1.106 | 0.489 | 1.311 | 0.046* | <1.70 mmol/L[d] |
| TC/HDL | 3.0 | 2.7 | 3.2 | 2.9 | 2.6 | 3.2 | 0.118 | ≤3.3[e] |
| LDL/HDL | 1.6 | 1.5 | 1.9 | 1.6 | 1.4 | 1.9 | 0.015* | ≤2.3[e] |
| TG/HDL | 1.4 | 1.2 | 1.8 | 1.5 | 1.0 | 2.1 | 0.052 | ≤2.5[e] |
| Insulin (μU/mL) | 8.0 | 5.0 | 11.9 | 9.2 | 6.5 | 12.4 | 0.054 | 2.6–24.9 μU/mL[d] |
| Glucose (mmol/L) | 4.722 | 4.444 | 4.961 | 4.889 | 4.489 | 5.111 | 0.037* | 4.0–6.0 mmol/L[d] |
| HbA1c (%) | 5.1 | 4.8 | 5.2 | 5.2 | 4.9 | 5.4 | 0.010* | <2%[f] |
| HOMA-IR | 1.68 | 1.06 | 2.41 | 1.91 | 1.32 | 2.62 | 0.058 | <3.0[d] |
| CRP (mg/L) | 1.9 | 0.8 | 3.9 | 1.9 | 0.9 | 3.0 | 0.686 | <3.0 mg/L[d] |

Abbreviations: BMI, body mass index; CRP, C reactive protein; HbA1c, glycated haemoglobin A1c; HDL, high density lipoprotein cholesterol; HOMA-IR, Homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; M, man; TC, total cholesterol; TG, triglycerides; W, women.

[a]WHO (1995, 2000).

[b]Biospace (2004).

[c]WHO (2011).

[d]Laboratory reference values.

[e]Tian and Fu (2010).

[f]Gayoso-Diz et al. (2013).

*$p < 0.05$.

[†]Wilcoxon test.

and take off all personal metal objects in contact with the body (e.g., jewellery). Height was determined using a stadiometer (Seca® 213) with an accuracy of 0.1 cm, while participants were standing in a relaxed position with their heads positioned in the Frankfurt plane. Waist circumference was measured by placing a measuring tape at the narrowest zone of the abdomen ('natural waistline'), at the end of a normal expiration, to the nearest 0.1 cm (DGS, 2013). Bodyweight and body composition data (e.g., body fat percentage) were assessed using a Body Composition Analyser InBody 720 (InBody® 720). Body mass index (BMI) was calculated according to the Quetelet Index formula, dividing weight (kg) by height squared (m$^2$). The WHO cut-offs (WHO, 1995, 2000) were used to classify the BMI as follows: <18.5 underweight; 18.5 to 24.9 normal weight; 25 to 29.9 overweight or pre-obesity; and ≥30 kg/m$^2$ obesity. Also, the cut-offs for waist circumference, namely, <94 cm for men and <80 cm for women and the waist/hip ratio, namely, <0.90 for men and <0.85 for women, were used to assess cardiometabolic risk (WHO, 2011). The percentage of body fat was classified according to the user's manual of the body composition analyser, namely, 10.0% to 20.0% for men and 18.0% to 28.0% for women (Biospace, 2004).

## Blood collection and analytical parameters

Participants were asked to provide blood samples at weeks 0 (baseline) and 8. Samples were collected after overnight fasting (12 h). Blood collection occurred at the Faculty of Biotechnology of the Catholic University of Portugal (FB-CUP), inside a room prepared explicitly for this purpose. Volunteers were conformably placed and blood samples were obtained from peripheral veins by a skilled nurse, following standard safety procedures. Vacuum tubes containing gel (BD Vacutainer® SST II Advance) and anticoagulant tripotassium ethylenediaminetetraacetic acid (BD Vacutainer® EDTA) were used for serum and plasma collection, respectively. After collection, both tubes were placed in an ice box and delivered to the University Hospital Clinical Pathology Core Laboratory (University Centre of São João).

Whole-blood haemoglobin was measured using an automated blood counter Sysmex® XE-5000 (Sysmex® Europe GmbH, Norderstedt, Germany, 2012). Blood samples were centrifuged at 3500 rpm for 10 min and with the resulting serum the following biomarkers were determined. Serum glucose, iron, ferritin, transferrin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TG and CRP were measured using an automated clinical chemistry analyser Olympus® AU5400 (Beckman-Coulter®, Olympus, USA, 2012), by colourimetric and turbidimetric methods. Serum folic acid and vitamin $B_{12}$ were measured by way of a chemiluminescent microparticle immunoassay using the Architect i2000® automated analyser (Abbott® Diagnostics, Lake Forest, IL, USA, 2005). Fasting insulin was measured by way of an electrochemiluminescence immunoassay in Elecsys® Cobas e411 (Roche® Diagnostics GmgH, Mannheim, Germany, 2010). Glycated haemoglobin (HbA1c) was determined by an ion-exchange HPLC system with a D-10™ Bio-Rad® analyser (Bio-Rad® Laboratories, Inc., Portugal, 2006).

LDL-C was calculated according to Friedewald's equation (Friedewald et al., 1972). The Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated (fasting insulin (μU/L) × fasting glucose (mmoL/L)/22.5) and a cut-off value ≥2 identified insulin resistant individuals (Gayoso-Diz et al., 2013). Lipid ratios were calculated as well namely, TC/HDL, LDL/HDL and TG/HDL and values of 3.3, 2.3 and 2.5 were used as the upper limits, respectively (Tian & Fu, 2010).

## Statistical analyses

Statistical analysis was performed using *Statistical Package for the Social Sciences* version 27.0 (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to assess variable normality. Only transferrin, TC and fasting glucose concentration were normally distributed both at baseline and week 8, so non-parametric tests were applied to all variable comparisons, also considering the low sample size. Variables were described as medians and respective percentiles ($P_{25}$ and $P_{75}$). Accordingly, the Wilcoxon signed-rank test was used to compare median differences between paired observations. For independent observations, the Mann–Whitney test was used for median statistical comparisons. A $p$-value lower than 0.05 was considered statistically significant. A post-hoc stratified analysis of data comparing the 'exercise' and 'no exercise' groups was performed based on outputs of an exploratory analysis of our data which supports previous evidence that regular exercise can improve cardiometabolic risk factors (Amaro-Gahete et al., 2019).

# RESULTS

## Subjects' baseline characterisation

A total of 92 volunteers were screened for eligibility. The dietary intervention was planned to run in three phases. However, with the emergence of the COVID-19 pandemic and subsequent periods of lockdown, the third intervention phase was halted at the fourth week, rendering it unsuitable for inclusion in the present study and excluding 19 participants. As a result, the final number of participants did not reach the initial target of 40. Twenty-eight participants were initially enrolled in this study. The first phase took place from March to May 2018 ($n = 20$) and the second phase took place from October to December 2018 ($n = 8$). One participant dropped out and a second subject was excluded due to the lack of endpoint assessments required for the present analysis, including the final blood collection. Hence, only data from 26 individuals, namely one man and 25 women, were considered. Comparative analysis of food and nutrient intake estimates was only possible for 19 individuals because seven participants failed to deliver the final 3-day food record. Participants' median age at entry into the study was 28.0 ($P_{25} = 20.0$; $P_{75} = 35.5$) years. Only selected baseline minor health conditions were admitted for inclusion, such as mild depressive disorders ($n = 1$), allergic skin disorders ($n = 1$), allergic respiratory tract disorders ($n = 4$) and controlled thyroid disorders ($n = 2$). Regarding usual medication, the most prevalent drug was oral contraceptives ($n = 16$), yet some chronic drug therapies were present, including antidepressants ($n = 1$), thyroid hormone replacement ($n=2$) and asthma medicine ($n=1$). Additionally, five (19.2%) participants reported a baseline use of vitamin and/or mineral supplements. Most participants were non-smokers ($n=21$; 80.8%) and 12 (46.2%) reported practising moderate-intensity physical activity (3.0–5.9 METs) (Yang, 2019), at least 45–60 min, at least twice per week.

## Characterisation of the legume-based lunches

A total of 959 ovo-lacto-vegetarian legume-based lunch meals were delivered, containing one of the following five types of legumes: chickpea ($n=286$), bean ($n=234$), lentil ($n=199$), pea ($n=190$) or soybean ($n=50$). Thirty different recipes were applied throughout the 8 weeks and grain legumes alone represented 30.4% ($P_{25}=23.2$; $P_{75}=37.2$) of the main dishes' cooked weight, which accounted for 55.1% ($P_{25}=31.9$; $P_{75}=63.3$) of the total protein content. Over the 8-week trial, the participants consumed 38.0 ($P_{25}=35.0$; $P_{75}=39.3$) meals per subject, resulting in a median intake of 79.8 g ($P_{25}=75.0$; $P_{75}=92.5$) of cooked grain legumes per meal. At the individual level, on intervention days, 13 subjects (50.0%) showed an average intake of cooked grain legumes that met the Portuguese guidelines (Rodrigues et al., 2006), whereas 4 (15.4%) reached the recommendations suggested by Marinangeli et al. (Marinangeli et al., 2017), that is ≥80 g and ≥100 g per day, respectively. Despite additionally providing fruit and vegetables as part of the provided lunches, there were no statistically significant variations in the daily consumption of vegetables and fruits between the baseline and the legume-based intervention period (Ferreira et al., 2023).

Compared to lunches habitually consumed at baseline, intervention meals offered lower amounts of total fat (−4.3 g/meal [$P_{25}=-15.2$; $P_{75}=1.5$]; $p=0.049$), cholesterol (−61.7 mg/meal [$P_{25}=-115.2$, $P_{75}=29.2$]; $p=0.015$), riboflavin (−0.1 mg/meal [$P_{25}=-0.2$; $P_{75}=0.0$]; $p=0.035$), niacin (−5.2 mg/meal [$P_{25}=-10.1$; $P_{75}=-1.9$]; $p=0.001$), vitamin $B_{12}$ (−0.6 µg/meal [$P_{25}=-2.5$; $P_{75}=-0.1$]; $p=0.001$), phosphorous (−136.8 mg/meal [$P_{25}=-203.0$; $P_{75}=3.00$]; $p=0.011$) and zinc (−0.7 mg/meal [$P_{25}=-1.2$; $P_{75}=0.0$]; $p=0.019$). In contrast, higher quantities were found for total dietary fibre (+2.9 g/meal [$P_{25}=1.1$; $P_{75}=7.3$]; $p=0.002$).

## Daily nutrient intake

Data regarding the participants' daily nutritional intake has been published previously (Ferreira et al., 2023). In summary, baseline intake was as follows (median [$P_{25}$; $P_{75}$]): energy (kcal), 1730.7 (1607.8; 1971.3); total fat (% daily energy), 32.2 (28.1; 33.3); saturated fatty acids (% daily energy), 9.8 (8.9; 11.2); polyunsaturated fatty acids (% daily energy), 5.8 (4.3; 7.0); *trans* fatty acids (% daily energy), 0.6 (0.4; 0.8); cholesterol (mg), 258.5 (171.4; 299.1); total carbohydrates (% daily energy), 47.0 (41.7; 52.2); total sugars (g), 92.9 (62.5; 119.3); total dietary fibre (g), 20.5 (15.4; 22.4); total protein (% daily energy), 17.7 (14.9; 22.0); vitamin $B_{12}$ (µg), 3.0 (2.2; 7.2); folate (µg), 195.5 (167.5; 215.6); iron (mg), 10.0 (7.9; 12.0). Daily nutrient intake at the end of the food intervention was not statistically different ($p>0.05$) from the baseline assessment. However, the prevalence of inadequate vitamin $B_{12}$ intake (defined as the percentage of participants with intakes below the reference value of 4 µg/day (Adequate intake) (EFSA, 2017)) significantly increased after the 8-week legume-based meal challenge, namely from 52.6% (95% CI: 28.9–75.6) to 78.9% (95% CI: 54.4–94.0).

## Anthropometry and blood parameters

Anthropometric assessments and blood results are summarised in Table 1. The 26 individuals presented a median BMI and a median waist circumference within the recommended ranges at both assessment time points (baseline BMI: 21.9 kg/m$^2$ [$P_{25}=21.3$, $P_{75}=24.8$]; baseline waist circumference 71.4 [$P_{25}=66.2$, $P_{75}=75.1$], see Table 1). In contrast, the percentage of total body fat and the waist: hip ratio median values fell above the desirable ranges, according to reference values (see Table 1). There was no significant variation in body composition parameters measured at baseline and the end of the 2 months (see Table 1).

Regarding blood parameters, both baseline and final median endpoints were within reference intervals, except for HDL-C concentration, which was lower than desirable (see Table 1). Compared to the baseline, seven blood parameters expressed variations with statistical significance during the 8-week dietary intervention. Transferrin concentration significantly increased after 8 weeks (+0.125 g/L [$P_{25}=4.3$, $P_{75}=21.5$]; $p=0.001$). TC concentration significantly reduced after 8 weeks (−0.492 mmol/L [$P_{25}=-19.0$, $P_{75}=3.3$]; $p=0.041$), as well as LDL-C (−0.492 mmol/L [$P_{25}=-19.0$, $P_{75}=0.3$]; $p=0.003$). The TG concentration significantly increased at week 8 (+0.102 mmol/L [$P_{25}=-4.0$, $P_{75}=22.0$]; $p=0.046$). Concerning lipid ratios, the LDL/HDL ratio was slightly reduced at the end of the trial (−0.1 [$P_{25}=-0.3$, $P_{75}=0.0$]; $p=0.015$). Both FBG (+0.111 mmol/L [$P_{25}=-1.0$, $P_{75}=6.3$]; $p=0.037$) and HbA1c (+0.1 [$P_{25}=0.0$, $P_{75}=0.3$]; $p=0.010$) concentration significantly increased after the 2-month legume-based trial (see Table 1).

The analysis stratified by exercise revealed that the reduction in LDL-C blood concentration and LDL/HDL ratio was statistically significant ($p=0.016$) compared to baseline in the case of individuals who did not

**TABLE 2** Blood lipid profile according to the physical activity level.

| | Baseline | | | Week 8 | | | Baseline vs. 8 weeks[†] |
|---|---|---|---|---|---|---|---|
| | Median | $P_{25}$ | $P_{75}$ | Median | $P_{25}$ | $P_{75}$ | |
| *'No exercise' (n = 14)* | | | | | | | |
| Total cholesterol (mmol/L) | 4.997 | 3.872 | 5.174 | 4.718 | 3.884 | 5.202 | 0.177 |
| HDL (mmol/L) | 1.508 | 1.293 | 1.976 | 1.503 | 1.264 | 1.998 | 0.806 |
| LDL calculated (mmol/L) | 2.570 | 2.126 | 2.982 | 2.387 | 2.121 | 2.714 | 0.016* |
| Triglycerides (mmol/L) | 1.041 | 0.617 | 1.240 | 1.104 | 0.590 | 1.274 | 0.116 |
| TC/HDL | 2.9 | 2.6 | 3.3 | 2.9 | 2.4 | 3.3 | 0.074 |
| LDL/HDL | 1.6 | 1.4 | 2.0 | 1.6 | 1.2 | 1.9 | 0.009* |
| TG/HDL | 1.4 | 1.1 | 1.8 | 1.4 | 1.0 | 2.0 | 0.198 |
| *'Exercise' (n = 12)* | | | | | | | |
| Total cholesterol (mmol/L) | 4.437 | 4.081 | 5.228 | 4.150 | 3.992 | 4.972 | 0.136 |
| HDL (mmol/L) | 1.525 | 1.384 | 1.724 | 1.482 | 1.333 | 1.635 | 0.145 |
| LDL calculated (mmol/L) | 2.442 | 2.362 | 3.122 | 2.357 | 2.279 | 2.768 | 0.077 |
| Triglycerides (mmol/L) | 0.907 | 0.746 | 1.361 | 0.985 | 0.642 | 1.542 | 0.209 |
| TC/HDL | 3.0 | 2.8 | 3.3 | 3.0 | 2.8 | 3.1 | 0.583 |
| LDL/HDL | 1.7 | 1.6 | 2.0 | 1.7 | 1.5 | 1.8 | 0.388 |
| TG/HDL | 1.4 | 1.2 | 1.9 | 1.5 | 0.9 | 2.3 | 0.182 |

*Note*: 'Exercise' was defined as practising moderate-intensity physical activity (3.0–5.9 metabolic equivalents (METs) (Yang, 2019)), at least 45–60 min, at least twice per week. 'No exercise' was defined as having exercise habits that did not meet the 'exercise' definition.

*$p < 0.05$.

[†]Wilcoxon test.

exercise regularly but not in the group who exercised regularly (see Table 2). The baseline lipid profile of both groups was not significantly different ($p > 0.05$; data not shown).

## DISCUSSION

This pilot study assessed the impact of the provision of legume-based weekday lunch meals on body composition and blood parameters in omnivorous adults. A significant reduction in TC and LDL-C concentrations was observed, without significantly impairing iron status and no changes in body composition parameters were detected. However, our findings also revealed significant (slight) increases in triglycerides, fasting glucose, HbA1c and a difference in HOMA-IR that approached significance.

The absence of statistically significant variations in all anthropometric measurements (Table 1) was expected and desirable. On one hand, there were no differences in the daily energy intake during the dietary intervention. On the other hand, trial meals were carefully planned to meet the average Portuguese population's daily calorie and nutrient needs, minimising the influence that body composition shifts could exert over biochemical parameters. Nevertheless, the literature reports that metabolic improvements can be seen in weight maintenance conditions, supporting the current research hypothesis (Hermsdorff et al., 2011; Saraf-Bank et al., 2015; Zhang et al., 2010).

Regarding blood results, the intervention diet seems to have affected markers related to iron dynamics and both lipid and glucose metabolism. The observed increase in transferrin concentration across the 8 weeks might be an adaptation to the reduction in the daily intake of food sources containing more bioavailable iron forms, as a consequence of the plant-based dietary intervention (Anderson & Frazer, 2017). Transferrin is a protein responsible for iron transportation, so the lower iron supply could have upregulated transferrin mobilisation into the bloodstream as a coping mechanism to ensure iron homeostasis (Vogt et al., 2021). No other iron-related parameters expressed statistically significant variations. However, for blood iron, ferritin and transferrin saturation, there was a tendency for an overall decrease in all these markers at the end of the 8 weeks, especially regarding iron stores measured by ferritin status (46.0 vs. 40.3 µg/L; $p = 0.058$). This brings challenges concerning the longer-term nutritional impact of the present dietary intervention. In this context, it is important to note that blood vitamin $B_{12}$ concentrations could be affected as well over the long run because they were lower at the end of the trial with a borderline statistically significant reduction in the mean difference for individuals (–17 pg/mL [$P_{25} = -50.8$, $P_{75} = 5.5$]; $p = 0.052$). Food intake data from the present study had already revealed that the proposed dietary shift could increase the prevalence of inadequate intakes of $B_{12}$ (Ferreira et al., 2023), namely from 52.6% (95% CI: 28.9–75.6) to 78.9% (95% CI: 54.4–94.0) for B12. This is particularly important since the present intervention was carried out in a group dominated by young women. Such dietary challenges could compromise nutrients essential during pregnancy and lactation periods (Pellinen et al., 2022).

A recent Finnish 12-week randomised controlled trial testing the nutritional impact of replacing the daily intake of animal-source protein with plant-source protein (animal protein group—70% animal-source protein; '50/50 group'—50% animal-source protein, 50% plant-source protein; plant protein group—70% plant-source protein) found no differences in any of the blood biomarkers used to monitor iron status, namely plasma ferritin, plasma transferrin receptor and haemoglobin (Pellinen et al., 2022). However, the plant protein group had a higher intake of iron, which, together with high vitamin C intake, could have helped to overcome the potential negative impact of more vegetarian-like diets on non-heme iron bioavailability and maintained iron homeostasis (Pellinen et al., 2022). In turn, a dose-dependent inverse response was observed in the vitamin $B_{12}$ intake and its blood status when increasing the proportion of plant-based protein sources in the diet. The authors suggest that the smaller amount of animal-based foods in these diets may not be sufficient to secure an adequate and healthy vitamin $B_{12}$ status in well-nourished individuals (Pellinen et al., 2022). Also, legumes are not a source of vitamin $B_{12}$ (EU, 2011). This may also be true for the present group, especially since some nutritional inadequacies were already present at the beginning of the trial (Ferreira et al., 2023). Briefly, a prevalence of inadequate intake (below recommendations) above 50.0% was found for five micronutrients, both at baseline and week 8, namely, alpha-tocopherol, vitamin $B_{12}$, folate, potassium, calcium and magnesium. However, another recent work claims that nutrient intake can be sustained if less restrictive approaches were applied and only the daily intake of red and processed meat was partially replaced by legumes, without changing the consumption of other animal-based foods (Kaartinen et al., 2022).

The reduction of TC and LDL-C concentration after the 8-week trial goes in line with what has been previously published in the literature about the metabolic effects of legume-rich diets (Ferreira et al., 2020), including in individuals with cholesterol concentrations within the normal range at baseline (Winham et al., 2007). The reduction in LDL-C concentration naturally explains the significantly lower LDL/HDL ratio found at the end of the 2 months. The hypocholesterolemic effect of legumes could result from the higher intake of both soluble and insoluble fibres conveyed by these foods (Hervik & Svihus, 2019; Müller et al., 2018). Fibre acts as an obstacle to energy and nutrient uptake (e.g., fat and glucose) (Hervik & Svihus, 2019; Müller et al., 2018) and some fibre types are fermented by gut microbiota (Cronin et al., 2021). Both attributes have been proposed as mechanisms that may contribute towards positive lipid and glucose blood profile modulation (Veronese et al., 2018). The biochemical mechanisms have been further explored in a previous publication (Ferreira et al., 2020). Indeed, present data showed that the current legume-based lunches offered higher amounts of dietary fibre than lunches typically consumed by the subjects at baseline, even though it did not affect the total daily fibre intake, which did not differ significantly from baseline.

Previous research shows that sedentarism is a risk factor for impaired lipid metabolism (Crichton & Alkerwi, 2015). In the present study, the legume-based diet was able to improve LDL-C concentration in individuals who did not practice regular physical activity, perhaps minimising the negative influence of low exercise rates on metabolic health. It could be that for the more physically active subjects, the benefits of exercise overshadowed the impact of the dietary challenge on lipid profile.

Based on the above rationale, the increased blood concentrations found for TG, FBG and HbA1c after the legume-based dietary intervention were unexpected when compared to results from most previous trials (Ferreira et al., 2020), even though they are most likely outcomes with low clinical relevance. Still, such controversial findings regarding glucose metabolism are not unprecedented (Alizadeh et al., 2014; Hartman et al., 2010). Within the *Legume Inflammation Feeding Experiment* (Hartman et al., 2010), a 4-week legume-enriched (250 g or ≈ three servings per day of cooked beans) low-glycemic index (GI) diet significantly increased the FBG concentration (+1.8%; $p=0.016$), among 64 middle-aged men, characterised for colorectal adenomas and insulin resistance status (Hartman et al., 2010). Similarly, a 6-week hypocaloric diet enriched in non-soy legumes (one cup or two servings per day; cooked) in a group of 34 women with central obesity significantly increased fasting insulin concentration and HOMA-IR after 3 weeks (fasting insulin: +31%, $p=0.039$; HOMA-IR: +35%, $p=0.002$), but their significant effects disappeared after 6 weeks (Alizadeh et al., 2014). The authors do not point out potential biochemical-based explanations for these biological responses. Yet, they have acknowledged that glucose-related metabolic improvements are more likely to occur in individuals with diabetes or insulin-resistant individuals than in healthy individuals (Alizadeh et al., 2014).

Undeniably, the magnitude of the lipid-related benefits observed in other publications was greater (Abete et al., 2009; Abeysekara et al., 2012; Alizadeh et al., 2014; Crujeiras et al., 2007; Duane, 1997; Hermsdorff et al., 2011; Hosseinpour-Niazi, Mirmiran, Hedayati, & Azizi, 2015; Jenkins et al., 2012; Pittaway

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et al., 2008; Tonstad et al., 2014; Trinidad et al., 2010; Winham et al., 2007; Zhang et al., 2010). Two main aspects may explain this. Firstly, several positive outcomes have been found among more controlled dietary settings, frequently associated with calorie-restricted diets leading to other metabolic changes, such as weight loss, that could per se underly the biochemical improvements observed (Abete et al., 2009; Crujeiras et al., 2007). Secondly, most of the previously published papers have investigated the benefits of legumes on health-impaired populations, which do not allow direct extrapolation of the outcomes to the present group of subjects under study (Ferreira et al., 2020). Consequently, the variety of methodological approaches reported in the literature (e.g., different dietary challenge duration, different kinds and amounts of legumes, different types of control diets, different assessment time points and different target populations) greatly hinders the comparison of the present results with those of previous publications. Considering the present work, one possible explanation for the lack of more pronounced results may be attributed to subtle compensatory dietary changes, which could potentially offset the positive effects of a plant-based diet. In our detailed analysis of dietary intake, as published in Ferreira et al., 2023, despite not achieving statistical significance, we observed that while overall meat consumption decreased, the consumption of red meat doubled. At the same time, there was a significant decrease in fish consumption. Consequently, we can speculate that these dietary choices might have reduced the actual positive impact of introducing legumes into the blood profile of the participants.

As strengths of this work, we emphasise the long-term nature of the present legume-based dietary intervention, where the research team weighed the meals, encouraging meal compliance. Also, the quasi-experimental study design minimised confounders of biological outcomes since participants were their own controls and we applied pairwise statistical analysis. Finally, a single highly qualified nutrition technician performed the anthropometric assessments, minimising measurement errors. Some limitations of this study should be discussed as well. Firstly, we acknowledge that a power calculation was not performed and the sample size was determined based on previous publications and influenced by study resources. The final sample size was also smaller than we had planned. Secondly, the relatively small sample of highly educated subjects, the vast majority of whom were women, poses a challenge in generalising the findings to the broader society, emphasising the pilot nature of the work presented. Also, food records were missing for several participants which affected the strength of the analysis of food intake data. Moreover, data collected in two separate time frames was combined. In fact, the intervention was implemented in distinct timeframes due to logistical constraints, since it was unfeasible to enrol all participants simultaneously. Nevertheless, this approach may not have exerted a great adverse influence on the outcomes presented, as both intervention periods spanned the milder seasons of fall and spring. These seasons typically have a less pronounced impact on daily dietary habits compared to the more extreme seasons. It is worth noting that the trial menus remained consistent across both interventions. Consequently, we did not consider this a significant limitation and, in fact, the joint analysis proved beneficial in enhancing the statistical power of our study. Still, the influence of seasonal variation in dietary intake cannot be ruled out since there is no published data available confirming this for the Portuguese population. Moreover, recognising the potential influence of variables like BMI, waist circumference and blood lipid levels at baseline as confounding factors, it is crucial to note that, despite the option to incorporate them into the inclusion/exclusion criteria, the limited participant pool compelled a pragmatic approach. The study design, therefore, represents the most thoughtful consideration possible within the confines of these limitations. Additionally, the stratified comparison was not pre-specified, but during the data overview, we explored additional post-hoc analyses considering insights from the literature. Moreover, even though the individuals acted as their own controls, controlling for between-subject variation, in the present study design, we cannot exclude the possible effects of external non-specific factors on our results. Also, since the lunch meals were supplied to the participants, there is no guarantee that the same choices would happen outside the study setting. Still, unpublished data revealed that the pulse-based lunches were highly appreciated by our volunteers which could predict a higher receptivity to these types of meals should they be more available on menus in the out-of-home setting (e.g., restaurants, canteens, takeaways). Finally, the bias naturally associated with self-completed food records is acknowledged. Nonetheless, all food data was coded and analysed by highly skilled nutrition researchers supporting the quality of the data presented.

## CONCLUSION

This work assessed the impact of the transition to more sustainable plant-based diets on the metabolic health of a group of omnivorous adults. To the authors' knowledge, this is the first study on this topic carried out in apparently healthy individuals, assessing the effect of substituting animal-based foods for legumes on blood and anthropometric parameters. Indeed, results support the cholesterol-lowering potential of legume-rich diets. However, potentially adverse results are reported regarding the impact on vitamin B12, triglycerides and glucose metabolism-related biomarkers. The study's limitations in design and sample size emphasise the importance of conducting further research with larger cohorts to attain more conclusive findings.

## AUTHOR CONTRIBUTIONS

The authors' responsibilities are as follows: HF, MWV, EP and AMG designed the dietary intervention study; HF performed data collection; JS performed anthropometric assessments; PA was responsible for defining the methodology for blood collection; SM, MA and JTG were responsible for the laboratory analysis of the blood samples and its interpretation; HF performed data processing and statistical analysis; HF, MA, MWV and EP drafted the original manuscript and have primary responsibility for the final content; MWV, EP and AMG supervised the dietary intervention study execution, validated data outputs and provided essential intellectual inputs; and all authors revised and approved the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

## 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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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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