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How animal and plant-based proteins affect energy metabolism during the postprandial phase in overweight and obese men: a cross-over design study

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Abstract

Background Animal proteins (APs) and plant proteins (PPs) seem to exhibit different thermic and metabolic effects, which may be attributed to differences in amino acid profiles, bioavailability, and digestibility.

Objectives In this study, we aimed to investigate and compare the postprandial effects of AP and PP meals on energy metabolism parameters, including resting energy expenditure (REE) and substrate oxidation (SO), in overweight and obese men.

Methods This acute randomized crossover clinical trial involved forty-eight overweight and obese men, with a mean age of 33.48 ± 8.35 years and an average BMI of 29.15 ± 2.33 kg/m^2. Participants consumed two high-protein test meals with different protein sources (AP and PP) on separate days, with a washout period of 7 to 10 days between them. On each test day, energy metabolism parameters were measured in both the fasting state and postprandial phase using indirect calorimetry. Statistical analysis was conducted using SPSS version 25 and R programs, evaluating the effects of carry-over, treatment, time, and treatment × time interaction through generalized estimating equations (GEE) analysis.

Results After controlling for baseline values, there was a significant effect of time ($P < 0.05$), protein source ($P < 0.05$), and protein source × time ($P < 0.05$) on REE, TEF, and carbohydrate oxidation. REE showed an increase following the consumption of both meals; however, the rise observed after AP (14.2%) was greater than that of PP (9.55%). The trends in TEF changes were similar to those of REE. The mean carbohydrate oxidation after consuming PP remained relatively stable throughout the test, whereas the AP meal gradually increased, reaching its peak at the 180th minute. The decline in carbohydrate oxidation was more pronounced following the AP meal than the PP meal by the end of the test.

Conclusion This clinical trial demonstrates that animal-based protein results in higher energy expenditure and carbohydrate oxidation than plant-based protein.

Keywords Animal Protein, Plant protein, Resting energy expenditure, Substrate oxidation, Postprandial

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Introduction

The metabolism of protein in humans is closely linked to energy metabolism. This connection stems from various processes, including amino acid transport, intracellular protein turnover, ammonia detoxification, purine and pyrimidine formation, renal reabsorption of amino acids, and the excretion of nitrogenous metabolites, all of which require energy [1]. Compared to carbohydrates and fats, protein has a higher thermogenic effect, as the energy cost of processes such as digestion, absorption, and metabolism in protein (23%) exceeds that of fat (3%) and carbohydrates (6%) [2–4]. In this regard, it has been suggested that high-protein diets can lead to more significant weight loss, help maintain muscle mass, and lower the chances of weight regain [5, 6].

Not all dietary sources of protein exhibit identical thermic and metabolic effects due to variations in amino acid profiles, bioavailability, and digestibility. The metabolic response to protein largely depends on the availability of essential amino acids, particularly branched-chain amino acids. Since different protein sources vary in their amino acid composition and their effects on protein synthesis, one can expect them to have distinct impacts on energy metabolism [7]. Research on the postprandial effects of animal versus plant proteins on energy metabolism is limited, and the findings in existing studies are often conflicting. An animal protein diet (pork meat) has been shown to increase 24-h energy expenditure more than a soy protein diet [8]. In the realm of energy metabolism research, beyond merely examining energy expenditure, it is crucial to consider substrate oxidation (SO). This pertains to whether ingested nutrients are oxidized for energy, stored in their original form, or converted for storage in a different manner [9]. Thus, achieving an energy balance involves not just matching energy intake with energy expenditure, but also accounting for the overall balance of carbohydrates, proteins, and fats. The regulation of body protein stores is influenced by various factors, indicating that an imbalance in protein levels alone cannot be directly attributed as a cause of obesity; however, protein intake could potentially impact fat oxidation [10]. Limited studies have examined the effects of various dietary protein sources on SO. Findings from a study by Tan et al. revealed a significant difference in protein oxidation depending on the dietary protein consumed, with beef exhibiting a greater increase in protein oxidation compared to soy protein [11]. As mentioned earlier, few studies have investigated the acute effects of dietary protein source on energy metabolism, and those have yielded inconsistent results. The question of whether the quality and source of dietary protein influence these metabolic responses remains unresolved. Moreover, prior research has predominantly focused on the postprandial effects of isolated dairy proteins, such as whey, often administered in commercial beverages or formulas. Fewer studies have explored the postprandial effects of animal and plant proteins consumed within combined, typical daily meals. Variations in structure, amino acid composition, digestibility, and absorption between animal and plant protein sources suggest the potential for differing effects on energy and macronutrient metabolism during the postprandial phase. Thus, the present study aimed to investigate and compare the postprandial effects of AP and PP meals on energy metabolism parameters (including REE and SO) in overweight and obese men.

Methods

This study was approved by the medical ethical committee of Mashhad University of Medical Sciences (ethical code: IR.MUMS.MEDICAL.REC.1400.399) and registered at the Iranian Registry of Clinical Trials; code: IRCT20211230053570N1 (the direct access link to the resource: https://irct.behdasht.gov.ir/trial/61001). The study was performed from November 2022 to May 2023 in the Persian study research center at Imam Reza Hospital, affiliated with the Mashhad University of Medical Sciences, located in the North-East of Iran. The protocol for this study was published in June 2023 [12]. In the main study, we investigated the effects of dietary protein sources (AP and PP) on multiple postprandial responses, including energy metabolism, glycemic and lipemic responses, and hemodynamic parameters. In the present study, we focus exclusively on reporting the results related to energy metabolism.

Study population

Forthy eight males aged 18–60 years, with a BMI of 25–35 kg/m^2, were recruited from students and staff of Mashhad University of Medical Sciences, Mashhad, Iran. We used flyers, social media advertisements, and personal invitations for recruitment. Participants were excluded from the study if they met any of the following criteria: being professional athletes; current smokers; having a history of cardiovascular diseases, hypertension, diabetes mellitus, hyperlipidemia, neurological or neuropsychological disorders, or renal issues; using medications or supplements that could impact energy and protein metabolism (such as thyroid medications, supplements containing L-carnitine, ephedrine, caffeine, or antidepressants); consuming protein supplements or any supplements aimed at weight loss or weight gain; using drugs or supplements that influence appetite; experiencing a significant weight change (> 10%) in the past 6 months; having irregular breakfast habits (less than 5 times per week); following dietary restrictions; exhibiting

trypanophobia (fear of needles); being unable to participate in the intervention due to dietary intolerances or preferences; or being diagnosed with hypertension, diabetes mellitus, or hyperlipidemia based on their biochemical and blood pressure profiles after enrollment in the study.

In this study, 120 men were initially evaluated for eligibility to participate. Among them, 53 individuals consented to join the study. However, two individuals were excluded due to a diagnosis of hypertension, and two others withdrew from the study on the first day. Consequently, 49 individuals completed the study on day one. On the following day, four participants did not attend the intervention, but following the intention-to-treat approach, they were still included in the analysis. After completing the laboratory tests towards the end of the study, one participant was diagnosed with diabetes and was subsequently excluded from the final analysis. Ultimately, the final analysis included 48 participants (Fig. 1).

Study design

This study was designed as an acute randomized crossover clinical trial of two protein-based meals with different protein sources (AP and PP) where participants received both test meals on two different days with a 7–10-day washout period in between (Fig. 2). Randomization in this study was conducted solely to initiate the intervention, with randomly allocated AP and PP conditions based on a 1:1 ratio (simple randomization) using a random number table. Participants were instructed to maintain regular eating habits and physical activity levels throughout the study; have consistent meals the evening before two test days to minimize the impact of recent food intake on measured factors; fast for 10–12 h before the test day; Get sufficient rest the night preceding the survey; prevent intense physical activity or exercise two days before the study; stay hydrated and avoid thirst by consuming an adequate amount of fluids; avoid fasting diets during the study period.

On the 2 test days, the participants arrived fasted (10–12 h) at the Persian cohort research center using a taxi (with minimum physical activity) before 08:00 for data collection. Upon arrival, individuals entered the facility, completed the informed consent form, provided demographic information, and underwent measurements of anthropometric parameters and body composition using a bioelectrical impedance analysis device (InBody 770-Waynesboro YMCA). The level of physical activity was assessed at the study's outset using the International Physical Activity Questionnaire (IPAQ) [13]. Then an intravenous catheter was inserted into an antecubital arm vein for blood sampling (the results of blood biomarkers are not reported in the present study).

The measurements were conducted in two phases (Fasting and postprandial Phases). During the fasting phase, before receiving test meals, energy metabolism parameters including resting metabolic rate (RMR), diet-induced thermogenesis (DIT), and substrate oxidation (SO) were measured using indirect calorimetry (IC). Then, participants were given 15 min to eat the test meal under the researcher's supervision until the test meal was wholly consumed.

During the postprandial phase, after consuming the test meal, calorimetry measurements were taken at specific time intervals over 5.5 h, corresponding to the typical gap between breakfast and lunch. Calorimetry was done at 60, 180, and 300 min post-meal. Detailed information regarding the study design is outlined in the study protocol [12].

Diets

Two high protein test meals based on [1]animal (Specialized-Olivier salad) and [2]plant protein (Specialized-Soy food) were designed following Iranian culture and taste preferences and consumed as a breakfast meal. Participants were instructed to consume the meals entirely within 15 min. To ensure complete consumption of the test meals, the research team monitored the participants at the time of meal delivery. The designed meals provided 20% of individuals' daily energy needs and contained 30% protein, 40% carbohydrates, and 30% fats. The Harris-Benedict formula was used to estimate individuals' daily energy requirements [14].

The composition of the experimental meals is presented in Table 1. The primary protein sources in the AP meal were chicken breast, egg, and yogurt, while the PP meal included textured soy protein. The composition of the test meals was determined based on the nutritional components table and using the N4 software. Before the study began, the various food combinations were tested, and the optimal combination in terms of sensory characteristics, nutritional content, and fiber content was selected by experts and the research team.

Both test meals were matched regarding energy content, macronutrients, and fiber, and meticulous care was taken in harmonizing the meals. The amount of seasonings and spices used in both meals was also identical. Consistent raw materials were utilized for all meals.

The study researcher prepared all test meals the evening before the test day with great care in the Nutrition Department kitchen at Mashhad University of Medical Sciences, Mashhad, Iran.

The sensory evaluation of the test meals was conducted using the Visual Analogue Scale (VAS). Initially, before the study commenced, the sensory evaluation was performed by a nutritional panel (a group of professors and

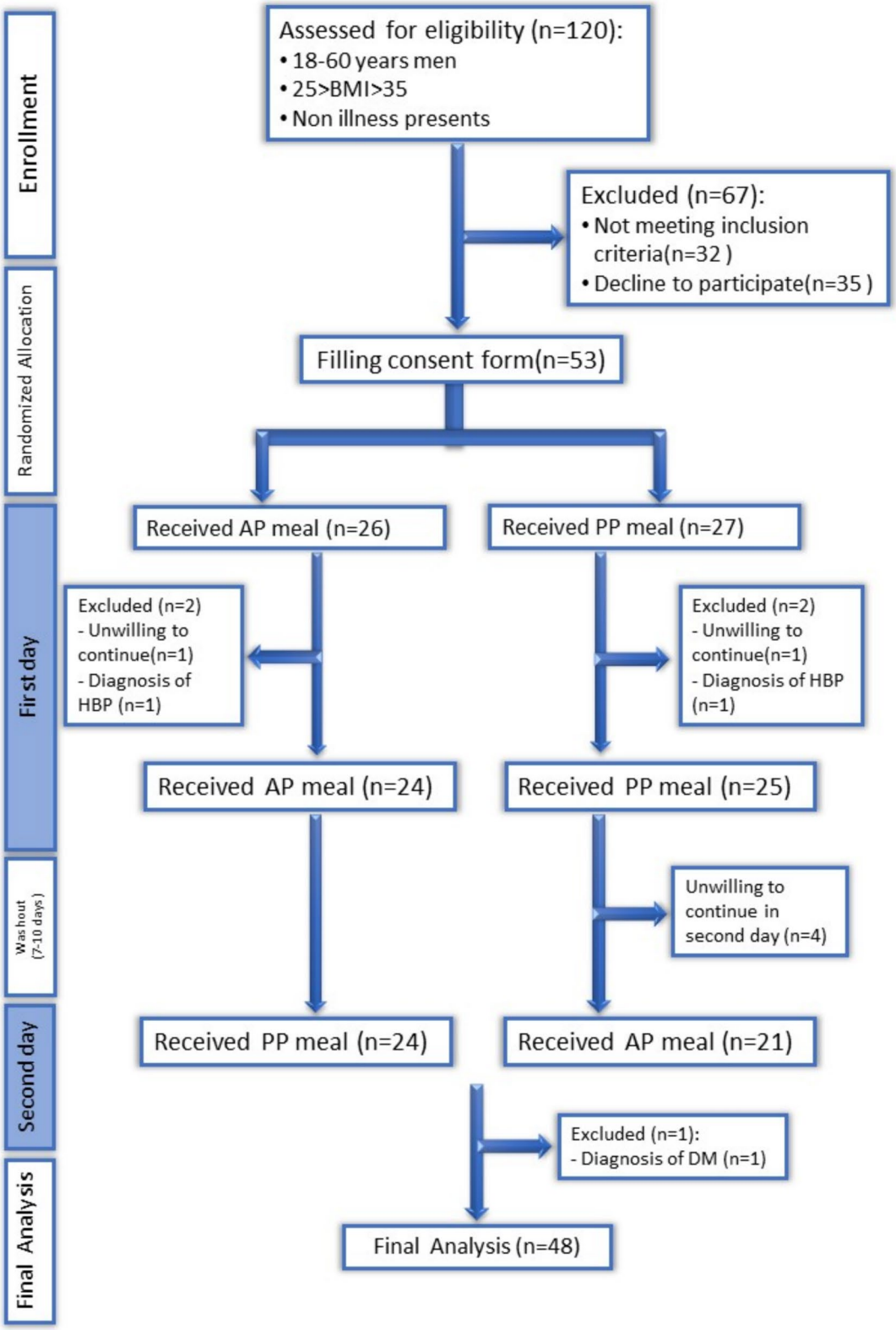


Fig. 1 Study enrollment and analysis flowchart

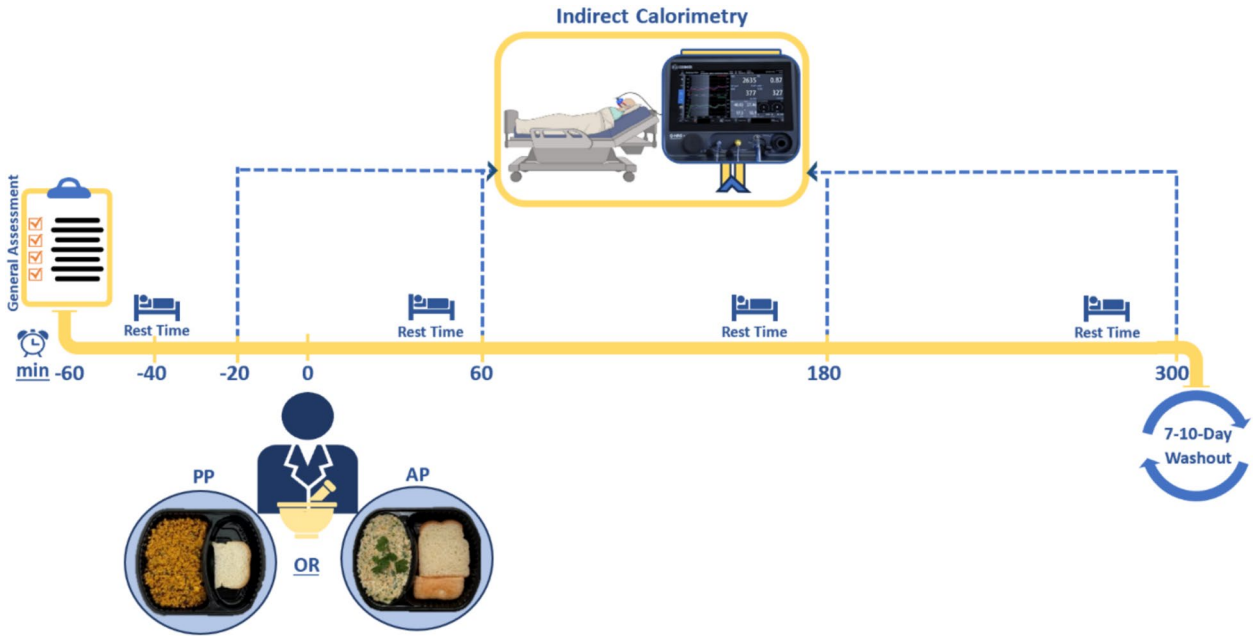


Fig. 2 Study diagram of interventions and assessments

Table 1 The composition of the test meals

Meal	AP	PP
Protein source	Chicken breast, egg, yogurt	Textured soy protein (made from soy flour)
Other ingredients	White toast, potato, carrot, pickles, parsley, sunflower oil	White toast, mushroom, bell pepper, onion, tomato paste, sunflower oil

students from the Nutrition Department at Mashhad University of Medical Sciences). Throughout the study, the participants themselves also carried out this evaluation. To ensure consistency in energy content, macronutrients, and fiber of the two test meals, a sample of each meal was prepared, and the macronutrient and fiber content of the samples were measured using standard methods at the nutrition laboratory of Mashhad University of Medical Sciences, Mashhad, Iran. The measured values of macronutrients and fiber for the test meals are presented in Table 2.

Indirect calorimetry

Indirect calorimetry was performed using the MetaLyzer® 3B device (CORTEX Biophysik GmbH, Germany). The MetaLyzer® 3B, as a breath-by-breath respiration system, continuously measures the volume and simultaneously determines the concentrations of CO_2 and O_2. The output of CO_2 and input of O_2 are calculated during each breath, and then the data is transferred to a personal computer for real-time display. The respiratory quotient (RQ) is the ratio of VCO_2 to VO_2 (VCO_2/VO_2), which reflects the rate of SO. TEF is the production of heat related to SO during energy uptake. TEF varies according to the quantity and type of oxidized substrate (i.e., carbohydrates, proteins, and fat). Participants were guided to assume a relaxed, motionless posture while awake, and air samples were gathered using a mask. Conducted in a tranquil environment with regulated temperature and lighting, the IC assessments necessitated that participants refrain from physical activity, and psychological stress, and maintain a fasting period of at least six hours beforehand. In preparation for calorimetry, participants reclined in a supine position for more than 20 min, with room temperature set between 23 and 25°C. The IC equipment underwent proper calibration before the evaluations.

Anthropometric measurements

Height was measured using a wall-mounted stadiometer, accurate to 0.1 cm, with subjects barefoot and stretched against the measuring rod, ensuring hips and shoulders were in contact. Body composition analysis was conducted using bioelectrical impedance analysis (InBody

Table 2 The macronutrient and fiber composition of the test meals

Variable		Meal based on animal protein (100 g)[a]	Meal based on plant protein (100 g)
Humidity(%)		75	71
Dry matter (gram)		25	29
Protein	Gram[b]	9/1	10/73
	Percent of energy	30/3	30/76
Carbohydrate	Gram[b]	11/87	13/57
	Percent of energy	39/5	38/9
Fat	Gram[b]	4/03	4/7
	Percent of energy	30/18	30/3
Energy		120/15	139/5
Fiber(gram)		1/33	1/5
Overall acceptance (mm)		72/33 ± 14/32	71/33 ± 14/19

[a] The composition of macronutrients and fiber has been measured in 100 g of sample meals

[b] The amount of macronutrients and fiber is reported as "grams in dry matter" (dry matter in 100 g of a meal based on vegetable protein is more than a meal based on animal protein)

770-Waynesboro YMCA) on the fasting morning of the test day. Body weight was measured with an accuracy of 0.1 kg using the InBody770 scale. Body mass index was calculated by dividing weight in kilograms by the square of height in meters.

Statistical analysis

Statistical analysis was conducted using SPSS software (SPSS Inc., Chicago IL, Ver. 25) and R (R Core Team, 2022, ver 4.3.2). The normality of continuous data was examined using the Kolmogorov–Smirnov test. Descriptive statistics were presented in mean ± standard deviation (SD) or standard error (SE). Independent samples t-test was used to compare quantitative variables at the beginning of the test and between groups at each time point. The intervention effect (AP vs. PP) on energy metabolism parameters, considering the crossover and longitudinal nature of the study, was analyzed using Generalized Estimating Equations (GEE) within the framework of a Linear Mixed Model, taking the subjects as a random effect and considering the intervention × time interaction effect, using R software (geeglm package). The carry-over effect was also investigated by modeling the intervention order's impact on baseline values. In all analyses, the baseline values were adjusted as a confounding factor. A significance level of 0.05 was considered for all tests.

Results

Baseline characteristics

Table 3 presents the demographic data and physical characteristics of the study population. The participants had a mean ± SD age of 33.48 ± 8.35 years. The average BMI was 29.15 ± 2.33 kg/m^2, and the average waist circumference was 105.43 ± 6.71 cm. Regarding physical activity levels within the studied population, 25% exhibited low physical activity, 39.6% engaged in moderate physical activity, and 35.4% reported high physical activity. Sensory characteristics of experimental meals are presented in Table 4. The average sensory characteristics scores between the two meals did not show a significant difference ($p < 0.05$), except for the appearance score of the food. Regarding this, there was a significant difference between the two meals ($p = 0.002$), such that the meal based on animal protein (76.56 ± 14.41) received a higher average appearance score compared to the meal based on plant protein (66.56 ± 15.44) from the participants.

Table 3 The baseline characteristics of the study population

Variable ($n = 48$)	Mean ± SD	
Age (year)	33/48 ± 8/35	
Body weight (kg)	92/58 ± 10/34	
BMI (kg/m^2)	29/15 ± 2/33	
Fat mass (kg)	26/67 ± 5/93	
Free fat mass (kg)	65/91 ± 8/02	
WC (cm)	105/43 ± 6/71	
Physical activity	**Low**	12 (25)
	Moderate	19 (39/6)
	High	17 (35/4)

Energy metabolism parameters

Table 5 displays the energy metabolism parameters at different time points following the consumption of two AP and PP test meals. There were no significant differences

Table 4 Mean and standard deviation of sensory attribute scores for experimental meals

Valuable	Animal Protein based meal	Plant Protein based meal	*P*-value
Appearance (mm)	76/56 ± 14/41	66/56 ± 15/44	0/002
Texture (mm)	70/89 ± 15/6	66/56 ± 15/66	0/19
Color (mm)	79 ± 12/5	73/11 ± 17/55	0/07
Taste (mm)	65/11 ± 20/07	68/33 ± 18/34	0/43
Salinity level (mm)	74/78 ± 16/98	75/22 ± 18/67	0/9
Odor (mm)	75 ± 16/61	73/67 ± 13/24	0/67
Overall Acceptability (mm)	72/33 ± 14/32	71/33 ± 14/19	0/74

Data are reported as mean ± standard deviation

Quantitative data were compared between two meal consumption phases using an independent t-test

in the mean energy metabolism parameters during the fasting state between the two phases ($p < 0.05$). However, comsumpting AP meal resulted in significantly higher REE and TEF 60 min after the meal than the PP meal recievers ($p < 0.001$).

Regarding SO, carbohydrate oxidation 300 min post-meal was significantly greater in the PP meal eaters than in the AP meal eaters ($p = 0.031$). Conversely, fat oxidation at the same time point was significantly lower following consumption of the PP meal compared to the AP meal ($p = 0.019$). Protein oxidation was significantly higher 60 min post-meal following consumption of the AP meal than the PP meal ($p < 0.001$). The average RQ 180 min after the meal following the AP meal was significantly higher than the PP ($p = 0.024$); conversely, 300 min post-meal, the RQ was significantly higher following consumption of the PP meal than the AP ($p = 0.046$).

The pattern of changes in REE and TEF

After controlling for baseline values, there was a significant effect of time ($P < 0.05$), protein source ($P < 0.05$), and protein source × time ($P < 0.05$) on REE and TEF. Figure 3 displays changes in REE and TEF during the postprandial phase. REE showed a rise following the consumption of both meals; Notably, after consumption of the AP meal, it peaked 60 min post-meal, whereas following consumption of the PP meal, it peaked later at 180 min post-meal, followed by a decline. The peak in REE observed after AP indicated a 14.2% increase from baseline, whereas after PP, it showed a 9.55% increase from baseline. By the end of the test (300 min), REE across consumption of both meals returned to similar levels (Fig. 3-A). The trends of changes in TEF were identical to those of REE (Fig. 3-B).

The pattern of changes in substrate oxidation

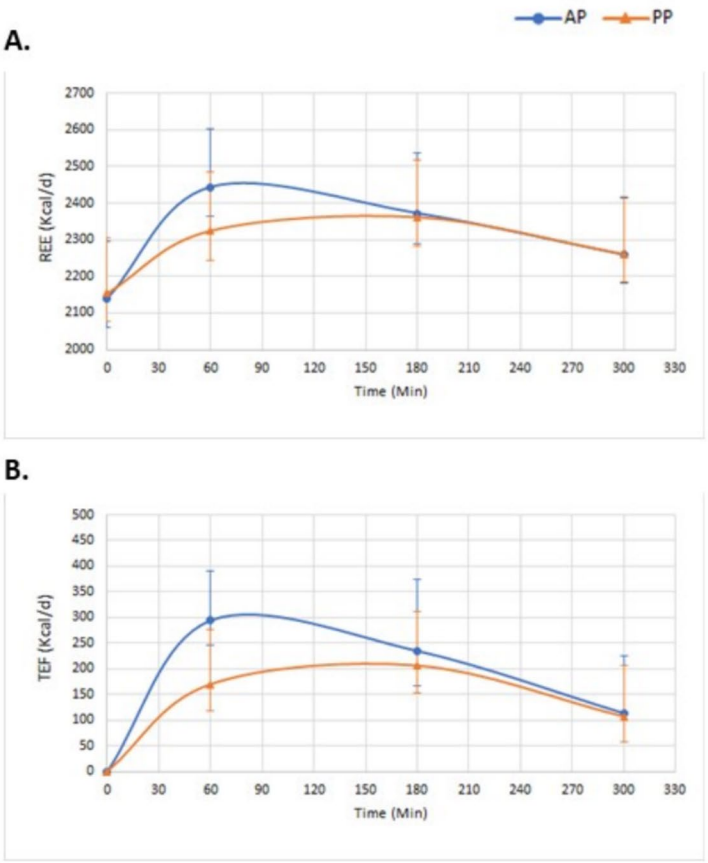
After controlling for baseline values, there was a significant effect of time ($P < 0.05$), protein source ($P < 0.05$),

Table 5 Energy metabolism parameters at various time points following the consumption of two test meals

Variable	Meal	Time			
		Fasting	60 min	180 min	300 min
REE (kcal/day)	Animal protein	2139/2 ± 39/46	2443 ± 39/87	2371/6 ± 41/18	2258/2 ± 39/18
	Plant protein	2154/18 ± 38/1	2323/31 ± 40/06	2360/1 ± 39/4	2260/6 ± 37/99
	P-value	0/57	**< 0/001**	0/7	0/92
TEF (kcal/day)	Animal protein	-	304/93 ± 30/14	231/67 ± 30/84	114/2 ± 27/44
	Plant protein	-	170/01 ± 28/87	206/55 ± 27/64	107/08 ± 25/47
	P-value	-	**0/002**	0/54	0/85
RQ	Animal protein	0/873 ± 0/008	0/875 ± 0/007	0/883 ± 0/008	0/853 ± 0/007
	Plant protein	0/887 ± 0/007	0/872 ± 0/006	0/866 ± 0/007	0/868 ± 0/007
	P-value	0/065	0/57	**0/024**	**0/046**
Carbohydrate oxidation (g/d)	Animal protein	305/01 ± 13/73	343/42 ± 14/96	347/69 ± 16/15	279/55 ± 13/18
	Plant protein	323/49 ± 14/19	322/96 ± 13/69	319/23 ± 14/06	304/92 ± 14/55
	P-value	0/12	0/11	0/052	**0/031**
Fat oxidation(g/d)	Animal protein	81/72 ± 5/18	99/48 ± 5/71	90/61 ± 5/59	108/21 ± 4/8
	Plant protein	77/72 ± 5/18	96/06 ± 4/95	99/47 ± 5/49	96/48 ± 5/58
	P-value	0/4	0/51	0/11	**0/019**
Protein oxidation (g/d)	Animal protein	23/99 ± 0/44	27/42 ± 0/45	26/57 ± 0/45	25/5 ± 0/44
	Plant protein	24/02 ± 0/42	26/06 ± 0/44	26/4 ± 0/43	25/36 ± 0/41
	P-value	0/91	**< 0/001**	0/61	0/64

Data are reported as mean ± standard error

Quantitative data comparison between two meal consumption phases at each time was done using "independent t-test"



Variable (p value)	Protein source effect	Time effect	Protein source × time effect
REE	<0.001	<0.001	0/0015
TEF	<0.001	<0.001	0/0015

Fig. 3 The pattern of changes in REE (**A**) and TEF (**B**) during the postprandial phase

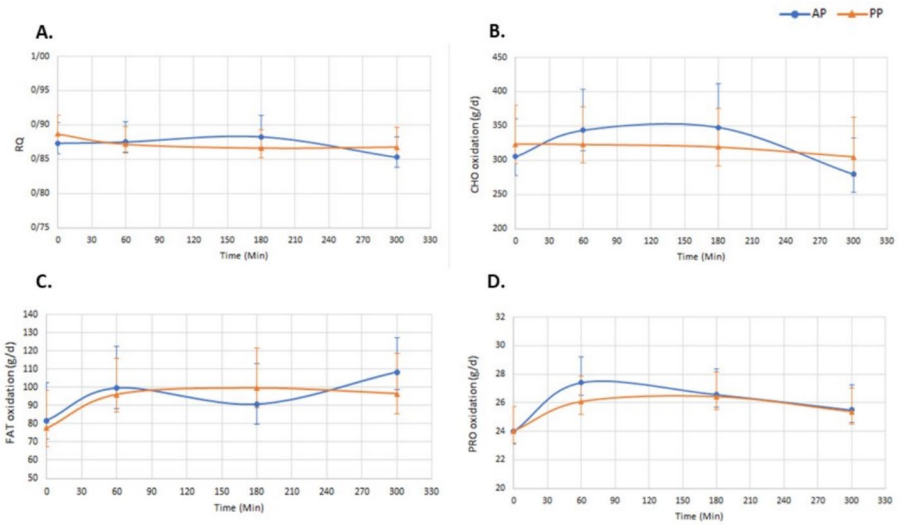
and protein source × time ($P < 0.05$) on carbohydrate oxidation. Regarding RQ, protein oxidation, and fat oxidation, there were no significant effects for a protein source or protein source × time ($p > 0.05$).

Figure 4 displays the pattern of changes in SO (carbohydrate, protein, and fat oxidation) and RQ during the postprandial phase. The mean RQ decreased slightly after the consumption of PP, reaching its lowest point at minute 180. In contrast, consumption of the AP meal showed a slight increase until minute 180, followed by a decrease, ultimately falling below baseline levels by the end of the test (Fig. 4-A). Nevertheless, there was no significant discrepancy between the consumers of two meals ($p > 0.05$).

The mean carbohydrate oxidation after consuming PP remained relatively stable throughout the test, with only a minor decrease observed towards the end of the test, dropping below the baseline levels. In contrast, consumption of the AP meal showed a gradual increase in carbohydrate oxidation, reaching its peak at the 180th minute before declining and eventually dropping below the baseline values by the end of the test. The decline in carbohydrate oxidation was more prominent following consumption of the AP meal than the PP meal by the end of the test (Fig. 4-B).

Regarding fat oxidation, initially, fat oxidation increased at 60 min post-meal following consumption of both meals. Subsequently, following consumption of the PP meal, the fat oxidation trend remained relatively stable, experiencing a slight decrease towards the end of the test. Conversely, following consumption of the AP meal, there was a quicker decline in fat oxidation at 180 min post-meal, followed by a subsequent increase towards the end of the test (Fig. 4-C). Nevertheless, there was no significant discrepancy between the two meal consumers ($p > 0.05$).

The pattern of changes in protein oxidation revealed an increase after each meal, peaking at different time points for each. Following consumption of the AP meal, the peak occurred 60 min post-meal, whereas, after the PP meal, it was observed at 180 min post-meal (with a delay). Subsequently, there was a decline in protein oxidation after consumption of both meals. Following the consumption of AP, the peak of protein oxidation (a 14.3% increase compared to baseline) was higher than that observed with PP (a 9.9% increase compared to baseline)



Variable (p-value)	Protein source effect	Time effect	Protein source × time effect
RQ	0/97	0/74	0/97
CHO oxidation	**0/012**	**<0/001**	**0/03**
Fat oxidation	0/41	0/16	0/53
PRO oxidation	0/1	**<0/001**	0/45

Fig. 4 The pattern of changes in Respiratory rate (**A**), Carbohydrate oxidation (**B**), Fat oxidation (**C**), and protein oxidation (**D**) during the postprandial phase

(Fig. 4-D). Nevertheless, there was no significant discrepancy between the two meal consumers ($p > 0.05$).

Discussion

The present study tested the hypothesis that APs and PPs have different effects on energy metabolism parameters. Our findings demonstrate a more significant thermogenic effect of AP compared to PP, as AP consumption resulted in a more pronounced and rapid increase in REE and TEF. As we previously reported in a review study, there are limited studies examining the impact of dietary protein sources on energy metabolism that have produced varying and inconsistent results [15]. Some studies suggest varying thermogenic effects of dietary proteins in the postprandial phase. In the study by Mikkelsen et al., a significant increase in EE and TEF was observed after animal protein (pork) compared to plant protein (soy) [8]. Similarly, Acheson et al. reported a higher thermogenic effect of animal protein (whey) compared to plant protein (soy) [16]. On the other hand, some studies indicate similar effects of different protein sources on EE. Studies by Hawley et al. and Melson et al. demonstrated comparable effects of different protein sources (whey and soy proteins) on EE and TEF [17, 18]. The variations in results across different studies could be attributed to differences in protein doses, types of proteins used, and the composition of the provided meals.

The higher thermogenic response following the consumption of APs is maybe due to their potential impact on protein synthesis in the body; the rate of protein synthesis after dietary protein intake depends on the alignment between the composition of essential amino acids in the protein and the body's requirements for optimal protein synthesis [19, 20]. Therefore, a balanced amino acid mixture, such as that found in APs, generates a greater thermogenic response than PPs [21]. Previous studies have shown that protein synthesis following the consumption of plant protein (such as soy) is lower compared to that of animal proteins (like casein and beef) [22, 23].An animal trial comparing the effects of various protein sources (pork, chicken breast, and soy proteins) on intracellular transcription, translation, and translocation factors revealed a more significant induction of protein synthesis following meat protein consumption. This study also indicated a higher level of thyroid hormones after consuming pork, suggesting a greater thermogenic response to AP [24]. Long-term studies have also demonstrated the beneficial effects of animal proteins on lean mass and muscle strength compared to plant proteins; a recent systematic review and meta-analysis of thirty randomized controlled trials (RCTs) revealed that, in comparison to animal protein, plant protein resulted in lower muscle mass following the intervention, with more pronounced effects observed in younger individuals [25]. Another potential mechanism for the enhanced thermogenic effect of APs is their superior digestion and absorption rates compared to PPs; thus, consuming animal sources may lead to a rapid release of bioactive amino acids, resulting in a quicker induction of protein synthesis and turnover. This, in turn, can lead to a more immediate and substantial increase in EE in the body. In addition to the observed effects on thermogenesis, AP and PP may influence SO differentially. Fat oxidation increased equally one hour after consuming

both high-protein meals; however, after the first hour, a comparison of the trends indicated a rapid decrease in fat oxidation following the intake of AP (not statistically different). In contrast, following consuming the PP meal, participants experienced a subtle increase in fat oxidation that continued up to three hours after the meal. The faster reduction in fat oxidation after AP is likely due to the antilipolytic effects of insulin, as previous studies have reported a higher insulin response from animal proteins compared to plant sources [26–30]. Glucose and insulin are potential stimulators of carbohydrate oxidation; however, research indicates that insulin plays a more significant role in stimulating this process due to its antilipolytic effect [31]. On the other hand, both glucose and insulin reduce fat oxidation by regulating the amount of fatty acid that enters the mitochondria [32]. Additionally, the availability of free fatty acids in the serum also influences carbohydrate and fat oxidation, promoting fat oxidation while inhibiting carbohydrate oxidation [33]. Considering insulin's role in stimulating carbohydrate oxidation, it was anticipated that carbohydrate oxidation following consumption of the AP meal would rise for up to three hours post-meal. In contrast, consumption of the PP meal exhibited a relatively stable trend, with a slight decrease in carbohydrate oxidation over time. The influence of dietary protein sources on fat and carbohydrate oxidation has been investigated in relatively few studies. Lorenzen et al. discovered that casein protein intake led to a more substantial increase in fat oxidation compared to whey protein [34]. Conversely, Acheson et al. reported that while dietary protein sources did not significantly affect fat oxidation overall, whey protein led to more increase in fat oxidation than soy protein [16]. Additionally, another study indicated a lower respiratory rate for whey than soy protein, suggesting enhanced fat oxidation [28]. However, the increased fat oxidation observed after consuming animal protein (such as whey) versus soy protein, as noted in two previous studies, contradicts our findings and the established antilipolytic effects of insulin. These conflicting results suggest that other mechanisms may be involved, underscoring the necessity for further research in this area. Research has shown that protein oxidation tends to increase after a high-protein meal [35]. This increase is likely influenced by the availability of substrates and the elevated concentrations of amino acids that follow such meals [36]. In the study by Labayen et al., participants who consumed a high-protein meal experienced a notable increase in overall protein oxidation, along with heightened leucine oxidation, specifically in obese individuals [37]. Consistent with these findings, the current study also observed a significant increase in protein oxidation following both protein-rich meals.

Amino acids serve as critical mediators of protein synthesis, hydrolysis, and oxidation, meaning that the diverse profiles of amino acids present after a meal can influence protein metabolism [38]. Consequently, it is anticipated that various protein sources will produce distinct effects on protein oxidation during the postprandial phase. In the present study, while the dietary protein source did not significantly impact overall protein oxidation, our comparisons indicated a greater and more rapid increase in protein oxidation after the intake of AP. In the study by Tan et al., which examined the postprandial effects of meat and soy protein on protein oxidation, the findings were contrary to those of the present study, as they reported a decrease in protein oxidation following meat consumption compared to soy protein [11]. Similarly, Acheson et al. found no significant effect of dietary protein sources on protein oxidation [16]. Another study investigated the postprandial effects of whey and casein proteins—representing dietary proteins with fast and slow digestion—on protein synthesis and oxidation. This research employed labeled leucine to evaluate both exogenous and endogenous oxidation. The findings indicated that the rate of protein digestion and amino acid absorption significantly impacts protein metabolism in the postprandial phase. Specifically, whey protein intake led to a rapid, pronounced, and transient increase in plasma amino acids, which was linked to enhanced synthesis and oxidation of total protein and exogenous leucine [38]. Therefore, the increased protein oxidation observed after AP consumption in the current study may be attributed to a quicker digestion and absorption rate, resulting in a more rapid release of amino acids. Further research is required to clarify the postprandial effects of various dietary proteins on protein oxidation. Additionally, the changes observed in SO in this study are not conclusively associated with dietary proteins and fats compared to endogenous proteins and fats. To resolve this issue, food labeling will be essential for improved diagnosis.

Strengths and limitations

The primary strength of this study lies in its well-structured randomized crossover clinical trial design, where all participants experienced both interventions on separate days. Additionally, the study showcased high compliance among participants, reflected in a minimal dropout rate. Moreover, we monitored participants' responses for up to 5.5 h post-meal consumption, an optimal timeframe for examining the acute phase. This duration allows for evaluating responses at the onset of the fasting phase.

Another notable strength of this study is that the meals were prepared as mixed dishes tailored to Iranian tastes, using commonly utilized essential food items. This

approach enhances the generalizability of the results to a complete protein diet based on either animal or vegetable protein.

Nevertheless, it is crucial to recognize certain limitations of the study. Throughout the research, participants were restricted from any physical activity and were primarily required to lie down or sit. This limitation led to some individuals feeling drowsy and bored, which may have influenced their metabolic responses. The monitoring and oversight of participants regarding important pre-study requirements, such as avoiding intense physical activity or consuming the same meals for the last meal of the day before the study, were conducted through phone calls and self-reports. While this method may have been accurate in some cases, it may lack the necessary precision in others. Also, we could not complete the biochemical tests because of financial limitations. However, after obtaining permission from the participants, the samples are frozen in a −80-degree freezer to be used if there are future possibilities to complete the tests. Another limitation is that, although the meals in this study were matched for macronutrient and fiber content, it was not feasible to precisely match the types of fats and carbohydrates used, nor the volume of the experimental meals. These discrepancies may have affected the results.

Conclusion

In conclusion, this study demonstrated that consuming a meal rich in animal protein results in higher energy expenditure and carbohydrate oxidation than a meal high in plant protein. Future research should explore the mechanisms underlying the metabolic effects of animal and plant protein sources.

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Authors' contributions
The authors' responsibilities were as follows—ZD, MS, RR, and MSh designed research; ZD, ShS, and MN: conducted research; ZD, AJ, and FH: performed statistical analysis; ZD, HB, and ShS: wrote paper; MS: had primary responsibility for final content; and all authors: read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the medical ethical committee of Mashhad University of Medical Sciences (ethical code: IR.MUMS.MEDICAL.REC.1400.399) and registered at the Iranian Registry of Clinical Trials; code: IRCT20211230053570N1 (the direct access link to the resource: https://irct.behdasht.gov.ir/trial/61001).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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