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Article

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Differences in appetite, food intake, and gastric emptying responses to protein intake by older adults varying in level of physical activity: A randomised controlled trial

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Abbreviations: BMI Body Mass Index; CNAQ Council on Nutrition Appetite Questionnaire; TFEQ Three Factor Eating Questionnaire; DEBQ Dutch Eating Questionnaire; GPPAQ General Practice Physical Activity Questionnaire; VAS Visual Analogue Scale; CO_2 Carbon dioxide; T_{lag} Lag Phase; T_{half} Half Time; T_{lat} Latency Phase; T_{asc} Ascension Time; AUC Area Under the Curve; ANOVA Analysis of Variance; SD Standard Deviation; δVPDB Delta Vienna Pee-Dee Belemnite.

Abstract

Older adults are encouraged to increase their protein intake and engage in more physical activity to preserve muscle mass. However, since protein is considered the most satiating macronutrient, this advice might lead to a decrease in overall energy consumption. Physical activity is also recommended to older adults to enhance appetite, as it has been shown to help regulate appetite in younger adults, yet there is limited evidence to support this in older populations. The objective of this study was to investigate the impact of physical activity and protein on food intake, perceived appetite, and gastric emptying in older adults. Nineteen active and 19 less active older adults completed a single-blind, randomised, crossover trial involving two test days at home. Participants received a standard breakfast, followed by an isovolumetric (250 ml) and isocaloric (~300 kcal) high- or low-protein preload milkshake (57% versus 17% energy as protein) matched for sensory properties. Three hours after the preload, participants were offered an *ad libitum* meal. Food intake was weighed, perceived appetite was measured by 100 mm visual analogue scales, and gastric emptying via the ^{13}C-octanoic acid breath test. Higher protein intake did not affect subsequent energy intake or appetite ratings in both active and less active groups. Gastric emptying half time was longer following the high-protein milkshake compared to the low-protein milkshake. The active group had a lower perceived appetite, but faster gastric emptying time compared to the less active group. In conclusion, while higher protein intake slows gastric emptying, it did not reduce appetite or subsequent food intake in older adults, regardless of physical activity level. Additionally, being physically active suppresses perceived appetite and accelerates gastric emptying without affecting food intake.

Keywords: Protein, Physical Activity, Appetite, Energy intake, Older Adults

1. Introduction

Ageing brings about various changes at the cellular, organ, and whole-body levels, which are known to contribute to a decrease in appetite and a reduction in the intake of energy and nutrients (Dericioglu et al., 2024). These changes are linked to a decline in muscle mass, an increased risk of developing malnutrition, poorer healthcare outcomes, and most importantly, higher mortality rates (Brownie, 2006; Morley & Silver, 1988; Pilgrim et al., 2015; Wilson et al., 2005). Preserving muscle mass and function is vital for maintaining functional independence and optimal health among older adults (Wolfe, 2012). Protein has been consistently identified by numerous studies as a crucial nutrient for supporting muscle health in this age group (Baum et al., 2016). Notably, older adults have a diminished anabolic stimulus response to lower doses of amino acids compared to younger adults. (Katsanos et al., 2006). As a result, they require a higher intake of amino acids to effectively stimulate muscle protein synthesis (Moore et al., 2015; Wolfe, 2012). Therefore, it is recommended that older adults increase their protein intake to address this issue and maintain muscle mass and function (Jürgen Bauer et al., 2013; Deutz et al., 2014).

There is a widespread belief that protein is the most satiating macronutrient (Paddon-Jones et al., 2008), suggesting that increasing protein intake in older adults could potentially lead to a further reduction in appetite, a common issue with ageing (Dericioglu et al., 2024). Therefore, when considering an increase in protein intake for older adults, it is also important to consider their total energy intake (Baum et al., 2016). While a recent meta-analysis suggested that protein supplementation may be a viable solution to increase protein intake in healthy older adults without adversely affecting total energy intake due to appetite suppression (Ben-Harchache et al., 2021), it did not examine responses in individuals with different levels of physical activity, leaving a gap in understanding whether physical activity modulates these effects. Thus, further research is needed to identify the optimal balance between protein and energy intake in older adults with varying physical activity levels.

Along with recommendations to increase protein intake to maintain muscle mass with ageing, physical activity and exercise remain essential for preserving muscle mass and function (Deer & Volpi, 2015). Extensive evidence supports the notion that physical activity stimulates muscle protein synthesis (Deutz et al., 2014) and is recognised as another modifiable factor associated with better health outcomes, including improvements in muscle strength and function, reduced frailty, and lower mortality in older adults (Arem et al., 2015; Chou et al., 2012; Marzetti et al., 2017). Furthermore, physical activity may not only be effective in preserving muscle mass in older adults but also potentially regulate appetite by influencing the satiety signaling system, affecting food choices and macronutrient preferences, and altering the hedonic response to foods (Blundell et al., 2003). Consequently, various professional organisations, including the NHS and Age UK, recommend increasing physical activity to maintain or increase appetite in older adults (Age UK, 2017; NHS, 2018). However, the regulation of energy intake and appetite involves a complex interplay of multiple systems (Gregersen et al., 2011). While a systematic review has shown that habitual physical activity improves appetite control in younger individuals (Beaulieu et al., 2016), its effects in older adults are less clear (Crabtree et al., 2023). In fact, due to a lack of conclusive evidence, it remains uncertain whether physical activity effectively influences appetite control and food intake in older adults. Some have suggested that current guidelines recommending increased physical activity to enhance the appetite in older population lack sufficient supporting evidence (Clegg & Godfrey, 2018).

While it is generally accepted that younger individuals with higher physical activity levels exhibit better meal-induced satiety, as they can more effectively adjust energy intake after consuming preloads varying in energy content (Blundell, 2011; Donnelly et al., 2009), findings are not always consistent. For example, some studies have found no significant differences in hunger and satiety ratings following preloads of varying energy content, whether assessed in randomised controlled trials (Long et al., 2002) or after an exercise intervention program (Martins et al., 2013). Similarly, another study reported no differences in energy intake between high and low physical activity groups after consuming high-fat or high-carbohydrate preloads (Beaulieu et al., 2017). Despite these mixed findings, research exploring the effects of physical activity on appetite and food intake in older adults remains limited (Apolzan et al., 2009; de Jong et al., 2000; Shahar et al., 2009; Van Walleghen et al., 2007). Furthermore, no studies have specifically investigated how older adults with differing habitual physical activity levels respond to preloads high and low in protein, leaving an important gap in the literature.

Therefore, the aims of this study are:

(i) to investigate food intake, appetite, and gastric emptying between active and less active older adults (≥ 65 years),

(ii) to compare the effect of meals with high- or low-protein level, which are equal in energy and volume, on food intake, appetite, and gastric emptying in both active or less active older adults (≥ 65 years).

Based on these aims, we hypothesise that (i) active older adults will have a higher food intake, and consequently, a higher protein intake, increased appetite, and faster gastric emptying compared to less active older adults, and (ii) high-protein meals will lead to longer gastric emptying times but will only lead to reduced food intake and appetite in older adults where physical activity is low.

2. Material and Methods

2.1. Study design and participant criteria

The study was a two-way, crossover, randomised, single-blind controlled trial consisting of two test days. The research protocol was approved by the University of Reading Research Ethics Committee (study number UREC 21/40; Clinical Trials Database Registration ID NCT05507801), and the study was conducted at participants' homes due to the COVID-19 restrictions.

Thirty-eight older adults (≥ 65 years) (19 active and 19 less active) participated in the study. Inclusion criteria were as follows: being healthy and living independently (free from diabetes or any disease likely to influence physical activity or appetite), the ability to walk independently; the capacity to understand and undertake the study procedures; a Body mass index (BMI) < 30 kg/m^2; not using any medication that could impact on appetite, food intake, or body weight in the past three months; no changes in diet and exercise, and no unexpected or voluntary weight loss in the past three months; not smoking more than ten cigarettes a day; no allergies to any of the test foods; and meeting the cut-off points criteria based on the accelerometer data from a previous study. Low activity was defined as ≤ 108.3 min/per day of moderate and vigorous activity for women, and ≤ 97.0 min/per day for men. High activity was defined as ≥ 162.0 min/per day for women ≥ 133.3 min/per day for men (Dericioglu, Methven, et al., 2023).

- **Pre-screening**

Prior to starting the study, participants were provided with an information sheet and asked to complete a two-stage pre-screening process. Firstly, they completed a health and lifestyle questionnaire online to determine their health status. Participants who met the inclusion criteria were then contacted with further information about the study, and informed consent was obtained online.

Afterwards, participants were delivered a study box containing a tape measure, a bioelectrical impedance scale (OMRON VIVA Smart Scale and Body Composition Monitor - HBF-2222T-EBK; UK), an accelerometer (AX3, 3-Axis Logging Accelerometer; Newcastle, UK), and a series of self-administered questionnaires (in paper format). The box included clear written instructions, and participants also received a video demonstration on how to use the equipment and complete the questionnaires. Further assistance was also provided via email, phone, or video chat as needed. After four days, one of the researchers collected the study boxes from the participants' homes.

On one morning during the screening period, participants were asked to measure their height, waist, and hip circumference in cm using the provided tape measure. For waist circumference, participants were instructed to measure at the narrowest part of the torso, typically just above the navel, and for hip circumference, at the widest part of the hips, following standard anthropometric procedures. For height, participants were instructed to stand straight against a wall with heels together and head level, measuring from the floor to the top of their head. Additionally, there were instructed to weigh themselves using the bioelectrical impedance scale for measurements of body weight in kg, percentage of body fat and muscle mass, and visceral fat, while fasted (before having breakfast and consuming water) and after using the toilet. They also completed questionnaires including the Council on Nutrition Appetite Questionnaire (CNAQ) to assess appetite (with scores of 8-16 indicating at risk for anorexia and the need for nutrition counselling, 17-28 indicating the need for frequent reassessment to due to the risk of anorexia, and scores >28 indicating not currently at risk) (Wilson et al., 2005). Additionally, the Dutch Eating Questionnaire (DEBQ) (Van Strien et al., 1986) and the Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messick, 1985) were used to identify restrained eating (with scores > 2.5 and >10, respectively indicating restrained eating behaviours), along with the General Practice Physical Activity Questionnaire (GPPAQ) (Department of Health, 2009).

Lastly, they were instructed to wear the accelerometer in an elastic waterproof waistband on their non-dominant wrist 24 hours a day for four consecutive days. Participants could wear it on either weekdays and weekends, as the study population consisted of retired older adults significant differences in activity levels between days were not expected. While a 7-day wear period is common in studies assessing physical activity, we opted for 4 days to reduce participant burden, particularly given the older adult population, while ensuring sufficient data for accurate group classification. The accelerometers were set up using the OMGUI software to record raw, triaxial acceleration at a rate of 100 Hz and a dynamic range of ± 8 g; they measured the minutes per day spent in activities of four different intensities: sedentary (<1.5 METS), light (≥1.5 METS, <4 METS), moderate (≥4 METS, <7 METS), and vigorous (≥7 METS) using a 60-second epoch length (Jackson, 2023). Data were extracted using the same software, and participants' moderate and vigorous intensity activity time (minutes/per day) was summed up. If at this stage participants did not meet the inclusion criteria for physical activity level based on the accelerometer cut-off points aligned with data from our previous study (Dericioglu, Methven, et al., 2023), they were excluded from continuing. Those classified as moderately active were excluded from participating in the study, while those participants categorised with a low or high activity level were included. Participants meeting the inclusion criteria were assigned to either the active or less active group. Each eligible participant was then called again and reminded of the procedures to follow before and on the test day, and test days were scheduled.

- **Test days**

Each participant undertook two test days in a randomised order **(Fig. 1).** Prior to recruitment, an online research randomiser was used to allocate eligible participants into predetermined preload groups (Randomizer, 2023). The allocation was done sequentially based the participants’ entry into the study.

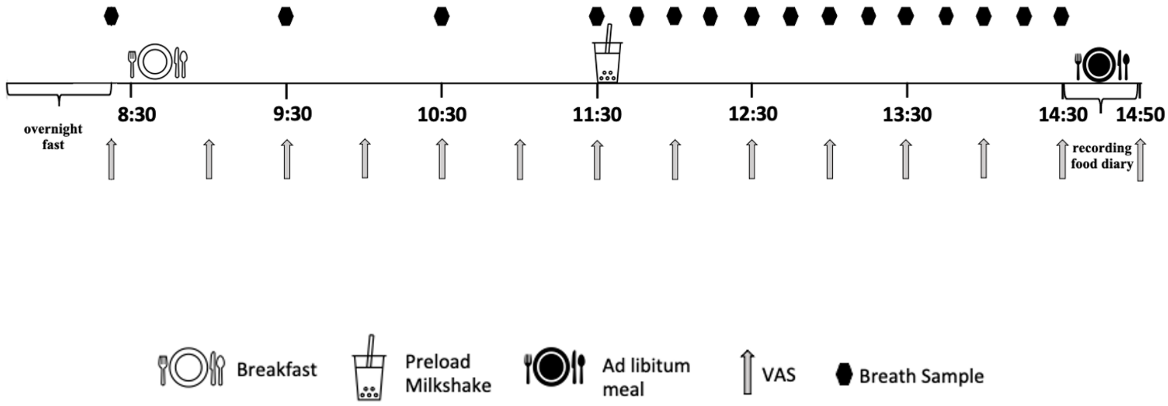

Fig. 1 Timeline of the test days

The day before the test days, one of the researchers delivered a test-day box to each participants' home. This box contained a breakfast, a test meal (preload milkshake), an *ad libitum* buffet meal, two bottles of water (one to consume until the *ad libitum* meal and one with the *ad libitum* meal), breath sample tubes (Exetainer, Labco, Ceredigion, UK) (with a nose clip and a straw), food diary sheets, a paper version of appetite rating and palatability scales (100 mm VAS), information on food storage conditions, a clear written instruction sheet, and a timetable tick list. Prior to delivery, participants received a video instruction detailing all stages of the study, including how to store food, collect breath samples, and complete the appetite and palatability scales. Additionally, when the test box was delivered, participants were asked to demonstrate how to collect their breath samples outside their house (following COVID-19 social distancing rules) to ensure they were doing it correctly.

On the evening prior to the test days, participants were asked to avoid the consumption of caffeine, alcohol, and nicotine, to avoid unusual, strenuous exercise, and to fast for 12h (overnight) (they were only allowed to consume water). Participants were also asked to record their food intake for the day before the first test day and to repeat it prior to the second test day to ensure consistency. The food diary record sheet for the day before the first test day was emailed or posted to the participants, and a digital kitchen scale was also delivered to those who did not have one at home.

On the test day, participants were called before the pre-agreed start time to ensure that they were ready to begin and were then reminded of the procedures via calls or texts at regular intervals throughout the day. They were also asked to follow the timetable sheet listing the required activities and to check off each activity as it was completed.

Firstly, participants were asked to consume a standardised breakfast meal consisting of muesli, ground almonds, and milk within 15 minutes, representing 20% of their estimated daily calorie intake (50% carbohydrate, 20% protein, 30% fat). This was calculated from the data obtained during the pre-screening stage (height, weight, age, physical activity level – assessed by the GPPAQ (Roza & Shizgal, 1984)). They then rested for three hours without any more food, but they had access to water. On the first test day, water was allowed *ad libitum*, and on the second test day, they were given the same amount of water to consume. During these three hours, participants were permitted to read, watch TV, or do sedentary work but were not allowed to be physically active or leave their houses during the test period.

Three hours later, participants were asked to consume their preload, which consisted of a strawberry milkshake that was either high in protein or low in protein. The preload milkshakes were equicaloric and isovolumetric on both test days **(Table 1).** Additionally, the colour of the milkshakes was not noticeably different; based on the colour analysis, both the low protein and high protein milkshakes were a pink hue (mean a* values of 14.3 and 14.7, $p = 0.13$ and low mean b* values of 2.6 and 2.8, $p = 0.28$) and light in colour (mean L* 70.2 and 72.2, $p = 0.05$).

Table 1. Energy and macronutrient composition of the test meal

	Low Protein Milkshake	High Protein Milkshake
Energy (kcal)	331	337
Volume (ml)	250	250
Protein (g)	12.7	46.6
Carbohydrate (g)	47.2	22.2
Fat (g)	6.4	6.0
Protein (% of energy)	17.1	56.6
Carbohydrate (% of energy)	63.6	26.9
Fat (% of energy)	19.3	16.5
Ingredients		
Strawberry Yoghurt (g)	150	130
Whey Protein Isolate (g)	5	50
Whole milk (g)	50	70
Strawberry Nesquik (g)	25	-
Double Cream (g)	20	-
Sweetener (g)	-	4
Strawberry Flavouring	-	Approx. 15 drops
Food Colouring (g)	-	0.2

Three hours after consuming the preload, participants were given up to 20 minutes to consume an *ad libitum* buffet meal until they were comfortably full. Before the first test day, participants were asked to choose two sandwiches from a menu of eight equicaloric options (egg mayonnaise, cheese and tomato, tuna mayonnaise, chicken salad, cheese and pickle, hummus and salad, ham and cheese, or roast beef and tomato) (Clegg & Thondre, 2014). They were provided with two of each sandwich (4 sandwiches in total-8 slices of bread) along with snacks (grapes (~250 g), flapjack (~100 g), and mini cheddars (~70 g)) for the *ad libitum* buffet meal (~2700 kcal; 48% carbohydrate, 12% protein, 40% fat) **(Supplementary Table 1)**. All meals were freshly prepared the day before the test day, and participants were asked to consume their meal alone with no distractions. After the meal, one of the researchers collected the leftovers from participants' homes and weighed them. Finally, participants were asked to keep a weighed food diary of everything they ate or drank for the rest of the day. The test day was repeated for two different preloads, with at least 3 days and no more than 4 weeks between test days.

2.2. Outcome measures

Participants were delivered a pre-weighed *ad libitum* meal, and food consumption at this meal was measured by weighing the leftover food. They were also asked to record their food and drink intake for the rest of the day using weighed food diary sheets.

Four subjective feelings of appetite (hunger, fullness, desire to eat, and prospective consumption) were assessed using 100 mm VAS fixed with the terms 'not at all' and 'extremely'. Before breakfast and every 30 minutes throughout the test day, participants were asked to mark on this scale how hungry they felt, how full they felt, how strong their desire to eat was, and how much food they thought they could eat. Additionally, participants were asked to rate the preload milkshakes for appearance, aroma, flavour, pleasantness, and texture liking on a VAS after the first sip and after consuming the entire preload to test whether the preloads were perceived as similar.

Before breakfast and every hour until the test meal and every 15 minutes for 3 hours after the test meal, participants collected exhaled breath samples for measurement of gastric emptying by blowing into a small glass tube through a straw (with a nose clip worn to prevent possible nasal exhalation). One hundred mg of 1-^{13}C octanoic acid (CK Isotopes, Leicestershire, UK) was added to the preload milkshakes, which is a safe, reliable and valid method for measuring gastric emptying (Davies, 2020; Ghoos et al., 1993). Octanoic acid, rapidly absorbed in the duodenum and transported to the liver via the portal venous system, appears in the breath as completely oxidized ^{13}C labelled Carbon dioxide (CO_2) (Schwabe et al., 1964).

An isotope ratio mass spectrometer (ABCA, Sercon LTD, Cheshire, UK) was used to determine the ratio of $^{13}CO_2$ /$^{12}CO_2$ recovered in the breath sample, relative to a single point calibration (Werner & Brandt 2001) cylinder gas (5% CO_2 95% He, -37.17±0.04 Delta Vienna Pee-Dee Belemnite (δVPDB) which was commercially calibrated against NBS-19 (n=15, Iso-analytical, Crewe, UK). Abundance in δVPDB units was converted to atom fraction and used to calculate gastric emptying. The following assumptions were used for CO_2 production: CO_2 production assumed to be 300 mmol/m^2 body surface area per hour (Shreeve et al., 1970). Participants' body surface area was calculated from height and weight according to Haycock et al. (Haycock et al., 1978). Data were displayed as percentage of ^{13}C dose recovered per hour and fitted into a gastric emptying model (Ghoos et al., 1993). Lag phase *(*T_{lag}*),* which is time taken to maximal rate of $^{13}CO_2$ excretion, and the half time *(*T_{half}*),* which is the time it takes for 50% of the ^{13}C dose to be excreted were calculated. Latency phase *(*T_{lat}*),* which is the point of intersection of the tangent at the inflection point of the $^{13}CO_2$-excretion curve representing an initial delay in the excretion curve, and the ascension time *(*T_{asc}*)*, which is the time course between the T_{lat} and T_{half}, representing a period of high $^{13}CO_2$-excretion rates were also calculated (Jackson et al., 2004; Schommartz et al., 1998).

2.3. Statistical analysis

Thirty-eight healthy older adults (≥ 65 years) completed the study. This number was powered according to a previous paper that tested the effects of randomised whey-protein loads on energy intake and appetite in older people (Giezenaar et al., 2017). Based on a significant difference in energy intake of 80 kcal between groups (control and 30 g protein preload) and a standard deviation of 76 kcal including a power of 0.9 and a $\alpha = 0.05$ a total of 38 participants were required consisting of 2 groups (active and less active) of 19.

Statistical analysis was performed using the SPSS (version 27; Chicago, Illinois, United States) and Excel (version 14.0; Arlington, United States). All data were initially tested for normal distribution by the Shapiro-Wilk test and expressed as means and standard deviations (SD). Participants' characteristics were compared using an independent sample T-test. Energy intake at the *ad libitum* meal was calculated using an Excel file based on the manufacturer's declared nutritional composition and the rest of the test day's intake was calculated using the Nutritics (Nutrition Analysis Software for Professionals; Dublin, Ireland) program. The change from baseline in VAS scores for perceived appetite was calculated in Excel, and the total area under the curve (AUC) from baseline (0 min) to 360 min and AUC from 180 min (post-preload intake) to 360 min for each variable were determined using the trapezoidal rule. Main effects of different preload milkshakes on subsequent energy intake, VAS scores including the palatability of test meals, and gastric emptying in active and less active older adults were assessed using a repeated measures mixed-ANOVA, with preload as the within-subject factor and physical activity level as the between-subject factor. Additionally, the interaction effects of preloads and activity groups on subsequent energy intake, appetite and gastric emptying were determined using a paired-sample t-test. P-value < 0.05 was accepted as the cut-off for significance in all analyses.

3. Results

3.1. Participants' characteristics

Of the 59 volunteers from Reading and the surrounding area in the UK who were pre-screened and wore the accelerometer, 19 volunteers were excluded from the study. Of these, 18 were excluded as their physical activity level was classified as moderately active, while one was excluded as they started a new medication. Additionally, two volunteers withdrew from the study, citing insufficient time to complete their participation. Consequently, a total of 38 older adults, with 19 classified as active and 19 as less active, participated in the study between January 2022 and August 2022 **(Fig. 2)**.

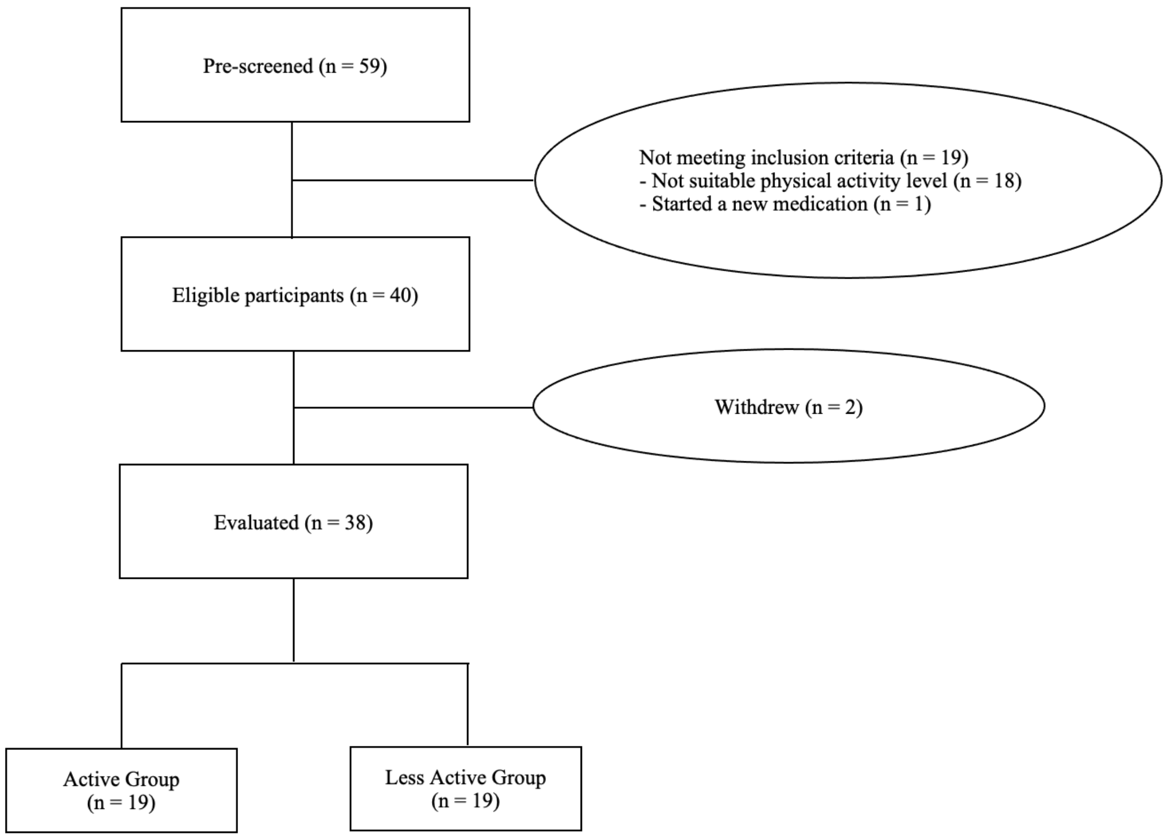

Fig. 2 A flow diagram of the participant recruitment

Participants' ages ranged from 65 to 85 years and **Table 2** displays the baseline characteristics of all the participants. There were no significant differences between the two groups in terms of mean age, height, body muscle mass, and waist circumference. The less active group had significantly higher mean values for weight, BMI, body fat mass, visceral fat, and hip circumference compared to the active group. No differences were found in the mean scores of the CNAQ, DEBQ, and the TFEQ between the groups. According to the CNAQ used to determine participants' appetite, 16 % of older adults in each group were found to require a frequent reassessment due to the risk of anorexia. There were no participants at the risk of anorexia (no scores of 8-16). Although the DEBQ and TFEQ were used to identify restrained eaters for exclusion, it is worth noting that dietary restraint tends to be higher among older adults (Flint et al., 2008). As a result, in this particular study, participants were not excluded based on their dietary restraint. Additionally, no participants in this study reported smoking, and thus, smoking was not considered a variable influencing appetite or energy intake in the analyses.

Table 2. Participants' characteristics

	Overall (*n* = 38)	Active Group (*n* = 19)	Less Active Group (*n* = 19)	Significance (*p*-value)[#]
Age (years)	71 ± 4	71 ± 5	70 ± 3	0.421
Male/female, n	16 / 22	8 / 11	8 / 11	
Height (cm)	168.1 ± 11.0	165.6 ± 11.45	170.5 ± 10.21	0.171
Weight (kg)	69.0 ± 14.1	64.0 ± 12.96	73.9 ± 13.73	0.029
BMI (kg/m^2)	24.2 ± 2.8	23.2 ± 2.95	25.2 ± 2.39	0.028
Body Fat Mass (kg)	20.7 ± 6.3 (30%)	18.4 ± 6.6 (29%)	22.9 ± 5.9 (31%)	0.035
Visceral Fat	7.8 ± 2.7	7.0 ± 2.57	8.7 ± 2.51	0.037
Body Muscle Mass (kg)	19.7 ± 5.56 (26%)	20.0 ± 5.20 (26%)	19.4 ± 5.92 (26%)	0.178
Waist Circumference (cm)	89.1 ± 11.8	86.1 ± 12.12	92.1 ± 10.84	0.113
Hip Circumference (cm)	99.6 ± 7.3	96.5 ± 5.89	102.6 ± 7.30	0.007
CNAQ	30.7 ± 2.1	30.7 ± 1.93	30.6 ± 2.17	0.814
Score (17-28) (%)	16	16	16	
DEBQ	2.5 ± 0.6	2.5 ± 0.71	2.6 ± 0.53	0.414
Restraint (Score >2.5) (%)	53	42	63	
TFEQ	9.1 ± 4.0	9.4 ± 4.10	8.7 ± 3.91	0.630
Restraint (Score >10) (%)	40	37	42	

PA levels measured by accelerometer (min/per day)	135 ± 40	204 ± 55	66 ± 25	< 0.001

[#]Data were analysed between the two activity groups by independent sample t test. *BMI* Body mass index; *CNAQ* Council on Nutrition Appetite Questionnaire; *DEBQ* Dutch Eating Behaviour Questionnaire; *TFEQ* Three-Factor Eating Questionnaire *PA* Physical Activity. Values are means ± SD.

3.2. Palatability of preload milkshakes

After the first sip, neither the type of preload consumed nor physical activity level had a significant effect on ratings of liking for appearance, aroma, flavour, pleasantness, or texture ($p > 0.05$). However, after consuming the entire milkshake, participants rated the low-protein milkshake as more appealing in terms of appearance ($F (1,36) = 6.9$, $p = 0.01$, $n_p^2 = 0.16$), aroma ($F (1,36) = 5.2$, $p = 0.03$, $n_p^2 = 0.13$), flavour ($F (1,36) = 8.9$, $p = 0.005$, $n_p^2 = 0.20$), and the texture ($F (1,36) = 8.7$, $p = 0.006$, $n_p^2 = 0.19$), and found it more pleasant ($F (1,36) = 7.4$, $p = 0.01$, $n_p^2 = 0.17$) compared to the high-protein milkshake. There was no significant effect of being in the active or less active groups on ratings of appearance, aroma, pleasantness, and texture rating after consuming the entire milkshake ($p > 0.05$). However, the scores for flavour liking were significantly higher in the active group compared to the less active group after consuming the entire preload milkshake ($F (1,36) = 6.4$, $p = 0.016$, $n_p^2 = 0.15$) **(Table 3)**.

Lastly, there was a significant interaction between preload milkshake type and physical activity level for the appearance and aroma of the milkshakes after the entire milkshakes were consumed ($F (1,36) = 7.4$, $p = 0.01$, $n_p^2 = 0.17$; $F (1,36) = 5.1$, $p = 0.03$, $n_p^2 = 0.12$, respectively). Comparisons revealed that participants in the active group liked the aroma and appearance of the low-protein milkshake more than the high- protein milkshake ($t (18) = -3.0$, $p = 0.008$; $t (18) = -3.5$, $p = 0.03$, respectively) **(Table 3)**.

Table 3. Palatability of the preload milkshakes

	Active Group (*n* = 19)		Less Active Group (*n* = 19)		Sig (*p*-value) (Between preloads)[#]	Sig (*p*-value) (Between groups)[#]	Sig (*p*-value) (Preload Group)[#]
	Low Protein	High Protein	Low Protein	High Protein			
After the first sip							
Appearance	75.5 ± 14.5	69.0 ± 22.9	64.1 ± 26.6	65.2 ± 19.4	0.314	0.244	0.158
Aroma	63.3 ± 20.2	53.2 ± 25.1	61.7 ± 23.8	60.1 ± 22.1	0.108	0.688	0.241
Flavour	53.4 ± 22.9	49.3 ± 28.1	41.5 ± 28.4	36.4 ± 28.1	0.222	0.125	0.893
Pleasantness	53.9 ± 23.0	51.6 ± 27.2	41.2 ± 27.1	40.5 ± 25.8	0.678	0.121	0.827
Texture	64.7 ± 24.7	57.5 ± 27.1	56.0 ± 26.4	54.3 ± 26.4	0.175	0.469	0.444
After the entire preload							
Appearance	76.3 ± 13.4	62.0 ± 24.7	64.0 ± 27.4	64.2 ± 23.3	0.013	0.467	0.010
Aroma	65.4 ± 18.0	48.1 ± 27.2	52.8 ± 28.1	52.7 ± 28.0	0.029	0.595	0.031
Flavour	56.4 ± 23.6	38.4 ± 28.6	33.0 ± 31.8	24.5 ± 20.9	0.005	0.016	0.294
Pleasantness	53.2 ± 25.7	35.9 ± 28.8	35.2 ± 34.4	27.1 ± 20.8	0.010	0.093	0.329
Texture	62.3 ± 26.5	48.1 ± 27.3	54.9 ± 32.6	51.5 ± 24.4	0.006	0.816	0.082

[#]Data were analysed by repeated measures mixed-ANOVA test. Values are means ± SD.

3.3. Subsequent energy, macronutrient, and fibre intake

There was no main effect of preload type on subsequent energy intake at the *ad-libitum* meal. However, there was a main effect of preload type on carbohydrate and protein intake at the *ad-libitum* meal (F (1,36) = 4.67, p = 0.038, n_p^2 = 0.12; F (1,36) = 5.15, p = 0.029, n_p^2 = 0.13, respectively), with higher carbohydrate and protein intake following consumption of low-protein milkshake compared to the high-protein milkshake. Fat and fibre intake at the *ad libitum* meal after consumption of low-protein preload were close to being significantly higher (p = 0.057; p = 0.061, respectively). However, the consumption of different preload milkshakes did not have a significant effect on energy, macronutrient, or fibre intake for the rest of the day. Additionally, there was no significant main effect of being active or less active on energy, macronutrient, or fibre intake at the *ad libitum* meal or for the rest of the day **(Table 4)**.

There was a significant preload by physical activity level interaction for fibre intake for the rest of the day (F (1,36) = 12.94, $p < 0.001$, $n_p^2 = 0.26$) and for total intake (the sum of the breakfast, the *ad libitum* meal and the rest of the day) (F (1,36) = 12.31, p = 0.001, $n_p^2 = 0.26$). Participants in the less active group consumed significantly more fibre after consumption of the low-protein compared to the high-protein milkshake for both the rest of the day (*t* (18) = -3.03, p = 0.007) and the total intake (*t* (18) = -3.35, p = 0.004). There was, however, no significant difference between the fibre intake after consumption of different preloads in the active group for the rest of the day (*t* (18) = 1.94, p = 0.068) and for the total intake (*t* (18) = 1.36, p = 0.19). Additionally, there was a significant preload by physical activity level interaction for carbohydrate intake for the rest of the day (F (1,36) = 5.63, p = 0.023, n_p^2 = 0.14); however, no significant differences were found in carbohydrate intake following the consumption of high- or low-protein milkshake in either the active group (*t* (18) = 1.79, p = 0.09) or the less active group (*t* (18) = -1.65, p = 0.117). The same significant preload by physical activity level interaction for carbohydrate intake were also seen for total intake (the sum of the breakfast, the *ad libitum* meal and the rest of the day) (F (1,36) = 4.552, p = 0.04, n_p^2 = 0.11). While participants in the less active group had more carbohydrate after the consumption of the low-protein compared to high-protein milkshake (*t* (18) = -2.21, p = 0.04), participants in the high active group did not have different carbohydrate consumption after consumption of different preloads (*t* (18) = 0.61, p = 0.553) **(Table 4).**

Table 4. Subsequent energy, macronutrient and fibre intake at the *ad-libitum* meal, the rest of the day and the sum of the breakfast, *ad libitum* meal and the rest of the day after consuming different preload milkshakes in active and less active groups.

	Active Group (n = 19)		Less Active Group (n = 19)		Sig (p-value) (Between preloads)[#]	Sig (p-value) (Between groups)[#]	Sig (p-value) (Preload*group)[#]
Preload milkshake	Low Protein	High Protein	Low Protein	High Protein			
Breakfast							
Energy (kcal)	422 ± 91		444 ± 101			0.424	
Fat (g)	14.1 ± 3.0		14.8 ± 3.4			0.422	
Carbohydrate (g)	52.7 ± 11.4		55.5 ± 12.7			0.424	
Protein (g)	21.1 ± 4.6		22.2 ± 5.1			0.424	
Fibre (g)	4.9 ± 1.1		5.1 ± 1.2			0.427	
***Ad libitum* meal**							
Energy (kcal)	1064 ± 391	1041 ± 350	1049 ± 391	961 ± 338	0.102	0.673	0.339
Fat (g)	46.8 ± 17.3 (39%)	44.6 ± 15.9 (39%)	44.8 ± 14.2 (39%)	41.5 ± 15.0 (40%)	0.057	0.614	0.689
Carbohydrate (g)	124.1 ± 44.0 (47%)	118.8 ± 40.1 (47%)	118.9 ± 41.1 (47%)	108.7 ± 38.8 (45%)	0.038	0.555	0.509
Protein (g)	37.8 ± 13.6 (14%)	35.8 ± 12.7 (14%)	37.1 ± 12.1 (14%)	33.7 ± 12.5 (15%)	0.029	0.730	0.576
Fibre (g)	7.4 ± 2.7	7.1 ± 2.7	7.0 ± 3.2	6.4 ± 2.8	0.061	0.576	0.394
Rest of the day							
Energy (kcal)	539 ± 190	624 ± 304	614 ± 325	557 ± 339	0.769	0.138	0.962
Fat (g)	24.8 ±14.2	30.3 ± 20.0	26.1 ± 19.6	25.8 ± 21.8	0.388	0.770	0.330
Carbohydrate (g)	52.7 ± 25.3	63.0 ± 29.3	65.5 ± 35.2	51.7 ± 27.5	0.732	0.929	0.023
Protein (g)	20.3 ± 13.0	23.2 ± 15.7	21.2 ±12.3	21.7 ± 17.1	0.462	0.936	0.598
Fibre (g)	6.3 ± 4.5	7.6 ± 4.9	8.0 ± 3.7	5.0 ± 3.7	0.165	0.694	< 0.001
All meals							
Energy (kcal)	2024 ± 435	2086 ± 433	2107 ± 451	1962 ± 424	0.531	0.870	0.122
Fat (g)	85.7 ± 22.1	89.0 ± 25.3	85.7 ± 22.5	82.1 ± 24.1	0.961	0.631	0.295
Carbohydrate (g)	229.5 ± 43.4	234.5 ± 42.9	239.9 ± 54.3	215.9 ± 45.2	0.168	0.763	0.040
Protein (g)	79.2 ± 19.0	80.1 ± 20.5	80.4 ± 16.2	77.6 ± 22.2	0.730	0.916	0.529
Fibre (g)	18.6 ± 4.1	19.6 ± 4.5	20.1 ± 3.9	16.5 ± 4.7	0.065	0.553	0.001

[#]Data were analysed by repeated measures mixed-ANOVA test. Values are means ± SD.

3.4. The effect of different preloads on perceived appetite based on VAS scores

The baseline subjective appetite rating scores were not significantly different between the active and less active groups. Considering the total AUC appetite values (0-360 min), there were no significant effects of preload type nor activity level on hunger, fullness, desire to eat, and prospective consumption ($p > 0.05$). However, when considering specifically the AUC values post-preload (180-360 min), hunger, desire to eat food, and prospective consumption values were lower in the active group compared to the less active group ($F(1,36) = 8.2$, $p = 0.007$, $n_p^2 = 0.19$; $F(1,36) = 6.2$, $p = 0.017$, $n_p^2 = 0.15$; $F(1,36) = 12.3$, $p = 0.001$, $n_p^2 = 0.25$, respectively), while fullness was lower in the less active group compared to the active group ($F(1,36) = 4.6$, $p = 0.038$, $n_p^2 = 0.12$) **(Fig. 3)**. Additionally, there were no significant preload by activity group interaction for either the total AUC or AUC (180-360 min) of the subjective appetite rating scores ($p > 0.05$).

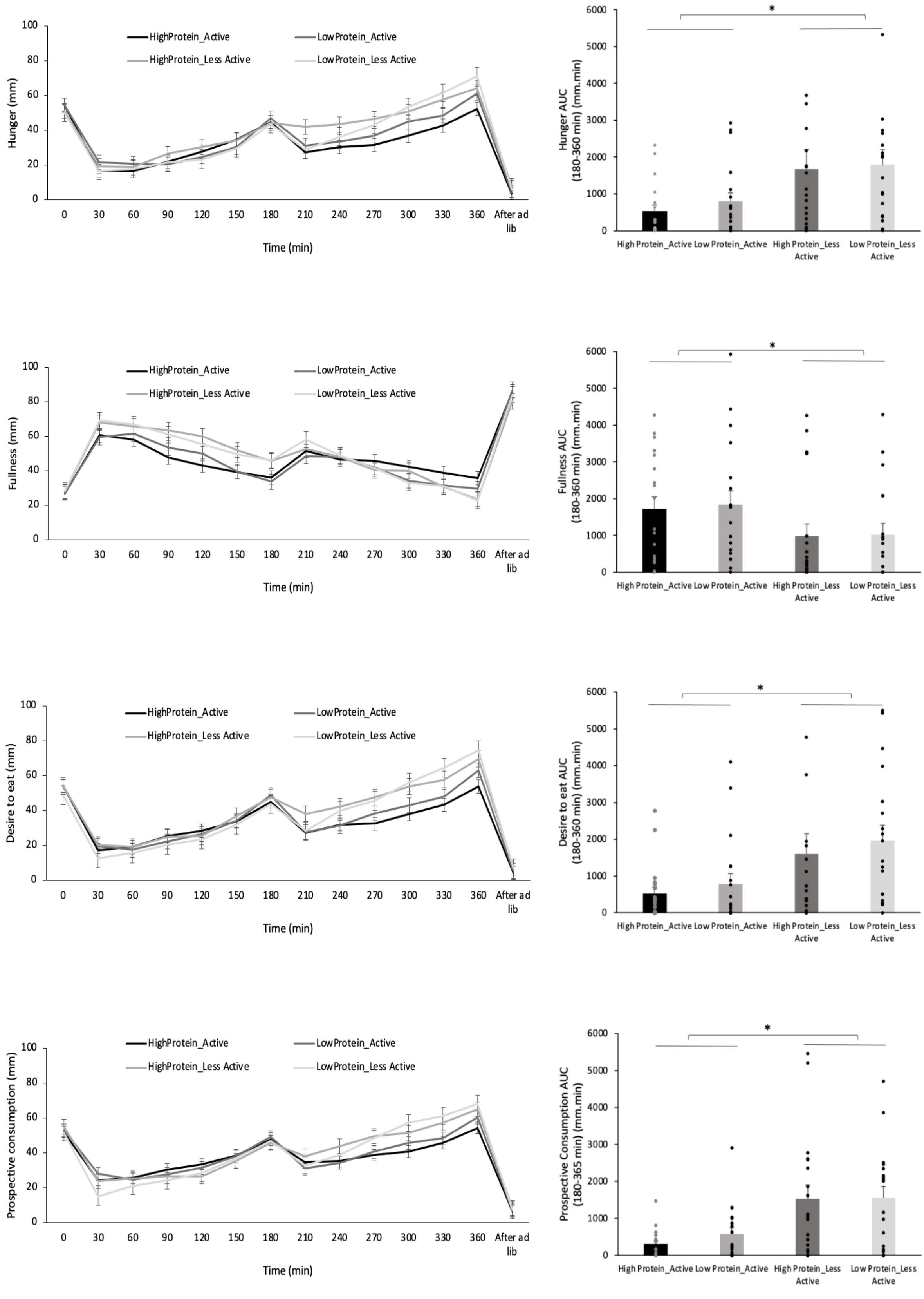

Fig. 3 The VAS score of hunger, fullness, desire to eat and prospective consumption during the test day in high and low active group, as well as the AUC values of the hunger after consuming high and low protein milkshakes (180-360 min) for the two groups. Values are means, with standard error represented by vertical bars. * $p < 0.05$

3.5. Gastric emptying

The high-protein milkshake significantly delayed gastric emptying compared to low-protein preload, as measured by all four parameters **(Table 5)**: Gastric emptying T_{half} (F (1,35) = 30.0, p < 0.001, n_p^2 = 0.46), T_{lag} (F (1,35) = 27.6, p < 0.001, n_p^2 = 0.44), T_{lat} (F (1,35) = 7.0, p = 0.012, n_p^2 = 0.17), and T_{asc} (F (1,35) = 33.7, p < 0.001, n_p^2 = 0.49). Similarly, being less active delayed gastric emptying compared to being active: Gastric emptying T_{half} (F (1,35) = 8.0, p = 0.008, n_p^2 = 0.19), T_{lag} (F (1,35) = 9.8, p = 0.004, n_p^2 = 0.22), T_{lat} (F (1,35) = 7.0, p = 0.012, n_p^2 = 0.17), and T_{asc} (F (1,35) = 6.3, p = 0.017, n_p^2 = 0.15). There was a significant interaction between preload type and physical activity level for gastric emptying T_{half} (F (1,35) = 4.8, p = 0.035, n_p^2 = 0.12) and T_{asc} (F (1,35) = 4.6, p = 0.039, n_p^2 = 0.12), indicating that high-protein preload was more effective at delaying gastric emptying in the less active group. Comparisons revealed that participants in both the active and less active groups had significantly longer gastric emptying T_{half} after consumption of high-protein milkshake compared to the low-protein milkshake (*t* (17) = 5.60, p < 0.001; *t* (18) = 4.11, p < 0.001, respectively). Similarly, participants in both groups had significantly longer gastric emptying T_{asc} after high-protein preload compared to the low-protein preload (*t* (17) = 5.70, p < 0.001; *t* (18) = 4.31, p < 0.001, respectively) **(Table 5)**.

Table 5. Gastric emptying times following the high and low protein preload milkshakes.

	Active Group (*n* = 18)[*]		Less Active Group (*n* = 19)		Sig (*p*-value) (Between preloads)[#]	Sig (*p*-value) (Between groups)[#]	Sig (*p*-value) (Preload* Group)[#]
Time (min)	Low Protein	High Protein	Low Protein	High Protein			
T_{half}	36 ± 10	57 ± 23	50 ± 20	99 ± 63	< 0.001	0.008	0.035
T_{lag}	10 ± 8	16 ± 11	19 ± 13	33 ± 21	< 0.001	0.004	0.087
T_{lat}	25 ± 5	22 ± 5	30 ± 9	28 ± 9	0.012	0.012	0.830
T_{asc}	81 ± 10	107 ± 24	92 ± 18	149 ± 68	< 0.001	0.017	0.039

[#]Data were analysed by repeated measures mixed-ANOVA test.

T_{half} Half time; T_{lag} Lag phase; T_{lat} Latency time; T_{asc} Ascension time. Values are means ± SD.

[*]Missing data.

4. Discussion

This is the first paper to directly compare the acute effects of protein intake on subsequent food intake, perceived appetite, and gastric emptying in older adults with varying levels of physical activity. The results indicated that the consumption of a high-protein preload (~47 g protein) did not significantly affect food intake or appetite compared to a low protein preload (~ 13 g protein), regardless of physical activity level, while it was accompanied by a 45 % increase in gastric emptying time. There was a significant interaction between preload type and physical activity level for gastric emptying (T_{half} and T_{asc}), with the high-protein preload having a more pronounced effect, delaying gastric emptying in the less active group. Despite this, the effect of the high-protein preload on gastric emptying was still observed in both active and less active groups, indicating that protein intake consistently influenced gastric emptying across activity levels. Furthermore, while there were no significant differences in appetite ratings between preload types, the less active group had a significantly greater perceived appetite than the active group, despite experiencing a longer gastric emptying time.

Adequate protein intake is widely recommended for older adults as part of strategies to prevent age-related decline in muscle mass and function, alongside exercise (Jürgen Bauer et al., 2013; Deutz et al., 2014). However, since protein is considered the most satiating macronutrient (Paddon-Jones et al., 2008), increasing protein intake in older adult may also influence their total daily energy consumption by enhancing satiety. This is particularly important, as increased satiety could limit energy intake, especially in populations where maintaining adequate caloric intake is crucial (Boirie et al., 2014). Our findings provide exciting insights, suggesting that older adults can increase their protein intake without negatively affecting their energy intake. Specifically, the high-protein (~47 g) preload did not affect appetite ratings or energy intake at the *ad libitum* meal or throughout the rest of the day compared to the low-protein (~13 g) preload consumption. Additionally, the current results showed that older adults were able to meet their daily protein requirements (J. Bauer et al., 2013).

The literature on appetite and energy intake responses to protein in older adults remains limited and inconsistent. In our previous study, we found no significant differences in either appetite ratings or subsequent energy intake following whey protein preload consumption (~48 g) (Dericioglu, Oldham, et al., 2023), which aligns with the findings of Giezenaar et al., who also reported no significant effect on appetite and *ad libitum* meal intake following 70 g of whey protein consumption (alone or with added carbohydrate) (2018). Our current study further supports these findings, showing no significant changes in appetite ratings or energy intake. In contrast, other studies have reported a reduction in appetite in older adults following whey protein intake (Butterworth et al., 2019; Soenen et al., 2014). Soenen et al. has also reported a reduction in subsequent energy intake following intraduodenal infusion of high doses of whey protein (45 g) (2014), although their study found intraduodenal protein at low doses (8 g and 23 g) actually increased total energy intake. Additionally, a meta-analysis focused on older individuals, including some of the aforementioned studies, supports the general view that a protein preload suppresses appetite in older adults (Ben-Harchache et al., 2021). However, this meta-analysis also showed that in the acute studies included, while energy intake decreased following protein intake compared to a control, total/daily energy intake increased when the energy content of the preload was considered (Ben-Harchache et al., 2021). It is worth noting that the studies included in this meta-analysis encompassed various protein sources (essential amino acid gel, bar, gel), not limited to whey protein, and differed in administration methods from our study, where protein was introduced intraduodenally, directly into the duodenum. These differences in protein sources and administration methods may explain the discrepancies between our findings and those of the meta-analysis, highlighting the need for further research using consistent protocols.

Within the literature, a few studies have investigated the effects of protein on appetite, food intake, and gastric emptying in older adults, aligning with the objectives of our study. Although slower gastric emptying is typically linked to decreased appetite and reduced energy intake in younger adults (Halawi et al., 2017), studies in older adults have presented conflicting results. In our study, the consumption of approximately 47 g of whey protein resulted in significantly slower gastric emptying compared to the 13 g whey protein preload. Despite this, we observed no significant changes in appetite or *ad libitum* food intake. These findings are consistent with studies showing slower gastric emptying in older adults following protein consumption. For instance, a study that examined the effects of whey protein consumption at different doses (0g/~2kcal, 30g/120kcal, 70g/280 kcal) in both older (69-80 y) and younger (18-34 y) men found that protein intake led to slower gastric emptying in both age groups (Giezenaar et al., 2015). However, protein fortification also suppressed energy intake in both age groups in their study, albeit a blunted response in the older groups, which contrasts with our non-significant effect on intake. More similar to our findings, when the same study protocol was applied to older adults only (69-80 y, male and female), protein was found to slow gastric emptying without impacting *ad libitum* energy intake. In fact, when accounting for the caloric content of the preloads, protein consumption resulted in increased total energy intake (Giezenaar et al., 2017). However, it is important to consider that these studies used non-equicaloric preloads, and the authors acknowledged limitations, including underpowered samples size for analysing appetite and gastric emptying measures. In another study conducted by the same research group, the effects of whey protein were examined in two forms: 70 g whey protein alone and a mixed macronutrient preload (14 g whey protein + 28 g carbohydrate + 12.4 g fat) (Giezenaar et al., 2018). Their findings were also aligned with ours, showing that while protein consumption slowed gastric emptying, it did not suppress appetite or *ad libitum* energy intake compared to the control group. Interestingly, after accounting for the caloric content of the preloads, the mixed macronutrient preload with 70 g protein resulted in an increase in total energy intake (Giezenaar et al., 2018). In contrast to this study, our study used preloads with the same energy content and volume, minimising potential confounding effects from differences in preload consumption. Taken together, these findings suggest that the effect of slower gastric emptying on appetite and food intake in older adults may be less pronounced than in younger adults. In both our study and existing literature, the finding that appetite did not change or increase despite slower gastric emptying after protein consumption in older adults may be attributed to the decreased perception of gastric distension commonly observed in healthy older individuals (Rayner et al., 2000).

Besides increasing protein intake, increasing physical activity is also considered one of the most effective strategies for preserving muscle mass and increasing appetite in older adults (Blundell et al., 2003; Deer & Volpi, 2015). A study involving participants aged 20 to 60 years, which measured physical activity levels with a questionnaire, showed that individuals with high physical activity (defined as engaging in hard or moderate exercise several times a week or at least 4 hours weekly) had decreased satiety and increased hunger compared to those with low physical activity (light exercise or no exercise, less than 4 hours weekly) (Gregersen et al., 2011). Similarly, among the limited studies including older adults that assessed physical activity based on self-reported time spent in moderate and vigorous activities, it was found that active older adults (engaging in ≥150 minutes/week of moderate and/or vigorous physical activity for at least 2 years) consumed more energy than the inactive ones (Van Walleghen et al., 2007). Furthermore, a recent study examining the effect of physical activity and protein intake in older adults across five different countries reported that more active older adults had higher energy consumption compared to the inactive individuals (Lourida et al., 2021). However, variations in the definition of physical activity across studies limited comparability, and reliance on self-reported measures of physical activity and dietary intake may have introduced social desirability bias. Despite insufficient evidence specifically in older adults, substantial research supports the notion that physically active individuals tend to experience decreased appetite but can better compensate for high-energy preloads by reducing subsequent energy intake compared to inactive controls (Beaulieu et al., 2016; Blundell, 2011; Donnelly et al., 2009). In our study, we used accelerometers to determine physical activity levels, which is an objective measure of physical activity. Our findings aligned with previous observations, demonstrating that the high active group had lower appetite scores compared to the low active group. Despite this lower appetite scores in the high active group, there was no significant difference in energy intake at the *ad libitum* meal or for the rest of the day between the groups. Although we did not find a significant positive effect of physical activity on food intake and appetite in older adults, these results suggest that high levels of physical activity do not appear to suppress food intake. In addition to physical activity, body composition, particularly fat-free mass, is known to influence energy intake (Hopkins et al., 2023). There were differences in anthropometric measurements between the active and less active groups. As perhaps expected, the less active group were significantly higher in weight, BMI, body fat mass, visceral fat, and hip circumference. However, the two groups did not differ significantly in proportion of body muscle mass. Although we did not explore this factor further within this study, as it did not differ between activity groups and was not the primary focus of the study, future research could investigate this factor in older adults more directly.

It is well documented that satiety and energy intake are directly linked to gastric emptying (Clegg & Shafat, 2010), and the present study is the first to investigate the relationship between physical activity and gastric emptying in older adults. A previous study involving healthy men aged 18-55 years, where physical activity levels were assessed using accelerometers, demonstrated that active men had a faster gastric emptying times compared to inactive men (Horner et al., 2015). Consistent with these findings, our study revealed that physically active older adults had faster gastric emptying compared to those with lower levels of physical activity. This link between physical activity and faster gastric emptying may be explained by its impact on the sympathetic nervous system, as physical activity can reduce resting blood pressure and decrease sympathetic nerve activity, thereby accelerating gastrointestinal motility (Matsuzaki et al., 2016). Additionally, physical activity may influence hormonal regulation, as it has been associated with increased ghrelin levels (Davis et al., 2020), and elevated ghrelin can promote faster gastric emptying (Levin et al., 2006). Furthermore, when evaluating the effect of protein intake on gastric emptying in both active and less active individuals, we observed that high protein intake prolonged gastric emptying time compared to low protein intake in both groups. Although the active group had faster gastric emptying than the less active group, the less active group reported a higher level of perceived appetite. Surprisingly, these differences in appetite and gastric emptying did not result in significant differences in food intake between the two groups.

The major strength of the current study is that it is the first investigation to examine the impact of protein intake on subsequent energy intake, perceived appetite, and gastric emptying in older adults with varying levels of physical activity. However, there are also a few limitations that need to be addressed. Firstly, due to COVID-19 restrictions, the study was conducted in participants' homes, which prevented us from providing a consistent sensory environment during the *ad libitum* meal consumption. However, in order to minimize this variable, participants were instructed to consume their meals in the same location, alone, and without distractions such as television. Secondly, although regular contact was maintained with participants via phone or text throughout the test day to ensure compliance with study requirements, we relied on self-reported compliance due to the study's non-clinical setting. However, it is worth highlighting that conducting appetite studies in clinical settings is often criticised for not reflecting real-world conditions. On the contrary, this study provided a valuable opportunity to investigate appetite in a more habitual setting aligned with participants' normal eating environments. What might initially be perceived as a limitation actually emerges as an important advantage that increases the value of the study. Another potential limitation is the difference in palatability between the high- and low-protein milkshakes, which could have influenced participants' subsequent energy intake. Although initial sips showed no significant differences in liking, participants rated the high-protein milkshake as less appealing in appearance, aroma, flavor, texture, and overall pleasantness after consuming the entire preload. Differences in palatability could affect subsequent intake however no participants had any difficulty with finishing the preload. Sensory differences between the drinks could also lead to differences in sensory-specific satiety, and previous studies have demonstrated that satiety induced by high-caloric foods has been shown to transfer to other high-caloric foods (Qiu et al., 2023), however this should not have impacted this study to any great extent as the *ad libitum* buffet provided foods that were dissimilar to the preload milkshake. However, future studies should control for palatability to better isolate the effects of protein on energy intake.

We also acknowledge while accelerometers were used to measure physical activity and assign participants to groups, these devices have some limitations in accurately recording weight-bearing or arm movement activities. However, the accelerometer data provided an objective method for group classification, which is preferable to relying on self-reported questionnaires. Furthermore, despite the study being conducted during COVID-19 restrictions, the active group had notably high accelerometer readings, indicating that they remained relatively active throughout the study. This may reflect a self-selection bias, as individuals who were already more active or health-conscious may have been more likely to participate, which could limit the generalisability of our findings to the broader older adult population. Lastly, it is important to acknowledge that this study did not include blood sample analysis to examine appetite-related hormones. Appetite and food intake are regulated by a complex interplay of mechanisms, including not only gastric emptying but also hormonal and neural mechanisms. Therefore, future studies incorporating blood sample analysis are needed to compare appetite hormones and neural mechanisms when evaluating the impact of protein intake on appetite and food intake in older adults with varying activity levels.

Additionally, longer-term intervention studies are needed to determine the lasting effects of protein intake and physical activity on appetite and energy intake in this population.

5. Conclusion

In summary, this study demonstrates that increased protein intake does not suppresses food intake or appetite but does prolong gastric emptying in older adults, regardless of physical activity level. Additionally, regardless of protein intake, higher levels of physical activity in older adults were associated with accelerated gastric emptying and decreased appetite. Future well-controlled studies, including appetite-related hormones are required to establish a more conclusive understanding of the effect of physical activity and protein intake on appetite and food intake in older adults.

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Authors' contributions

Design of the study (DD, MC, LM), implementation (DD), analysis of breath samples (AS), data analysis (DD, MC), writing the manuscript (DD), editing and approval of the final manuscript (all authors).

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Ethical Statement

This study was performed in accordance with the Declaration of Helsinki, and it was approved by University of Reading Research Ethics Committee (study number UREC 21/40; Clinical Trials Database Registration ID NCT05507801). All participants provided written informed consent before participation.

Data availability

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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