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Macronutrients effects on satiety and food intake in older and younger adults: A randomised controlled trial

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ABSTRACT

Older adults are advised to increase their protein intake to maintain their muscle mass. However, protein is considered the most satiating macronutrient and this recommendation may cause a decrease in total energy intake. To date, satiety studies comparing all three macronutrients have been undertaken in young adults, and it is unclear if the same response is seen in older adults. The objective of this study was to compare the effect of preloads high in protein, fat, and carbohydrate but equal in energy (~300 kcal) and volume (250 ml) on energy intake, perceived appetite, and gastric emptying in younger and older adults. Twenty older and 20 younger adults completed a single-blinded randomised crossover trial involving three study visits. Participants consumed a standard breakfast, followed by a preload milkshake high in either carbohydrate, fat, or protein. Three hours after the preload, participants were offered an *ad libitum* meal to assess food intake. Visual analogue scales were used to measure perceived appetite and gastric emptying was measured via the ^{13}C-octanoic acid breath test. There was no significant effect of preload type or age on energy intake either at the *ad libitum* meal, self-recorded food intake for the rest of the test day or subjective appetite ratings. There was a significant effect of preload type on gastric emptying latency phase and ascension time, and an effect of age on gastric emptying latency and lag phase such that older adults had faster emptying. In conclusion, energy intake, and perceived appetite were not affected by macronutrient content of the preloads in both younger and older adults, but gastric emptying times differed.

1. Introduction

The ageing population is increasing across the world, and it is predicted that around 25% of the UK's population will be 65 years and over by 2038 (Public Health England, 2021). With ageing, many physical and physiological changes occur in the body, which can decrease appetite and reduce food intake (Lorenzo Maria Donini et al., 2013; Morley, 2001; Payette, Gray-Donald, Cyr, & Boutier, 1995; van der Meij et al., 2017). Consequently, it has been reported that older adults have lower appetite and energy intake compared to younger adults (Giezenaar et al., 2016). This reduction in appetite, which was first named by John Morley as "anorexia of ageing" (Morley & Silver, 1988), occurs in 15–30% of older people (Malafarina, Uriz-Otano, Gil-Guerrero, & Iniesta, 2013). Anorexia of ageing may be caused by physiological factors such as delayed gastric emptying, changes in appetite related hormones, and changes to the senses of taste and smell (Ahmed & Haboubi, 2010); pathological factors such as chronic diseases or depression (Cabrera, Mesas, Garcia, & de Andrade, 2007; Lorenzo M Donini, Savina, & Cannella, 2003) and social factors such as loneliness and widowhood (Ramic et al., 2011). Such a decrease in appetite with aging makes it difficult for older adults to meet nutritional requirements (Ahmed & Haboubi, 2010; Leslie & Hankey, 2015). Although maintaining a good nutritional status has a very important place in increasing the quality of life of this population by reducing the risk of disease

Abbreviations: BMI, Body Mass Index; CNAQ, Council on Nutrition Appetite Questionnaire; TFEQ, Three Factor Eating Questionnaire; DEBQ, Dutch Eating 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; RM-ANOVA, Two-way Repeated Measures Analysis of Variance; SD, Standard Deviation; CHO, Carbohydrate; δVPDB, Delta Vienna Pee-Dee Belemnite.

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(Jones, Duffy, Coull, & Wilkinson, 2009), it has been shown that free-living older adults over the age of 75 often do not meet their estimated energy requirements (Roberts et al., 2018). This can lead to a range of adverse health outcomes such as malnutrition, sarcopenia, frailty, functional deterioration, morbidity, and mortality (Landi et al., 2016; Morley, 2012; Wysokiński, Sobów, Kłoszewska, & Kostka, 2015).

In the UK, older adults' dietary recommendations for protein, fat, and carbohydrate are the same as for the general adult population (Dorrington, Fallaize, Hobbs, Weech, & Lovegrove, 2020). Although it is known that protein can be useful in ageing to maintain muscle mass, and prevent sarcopenia, frailty, osteoporosis, impaired immune response, and associated comorbidities in later life (Bonjour, 2011; Bradlee, Mustafa, Singer, & Moore, 2018; Chernoff, 2004; Wolfe, 2012), the Reference Nutrient Intake (RNI) for protein for all adults in the UK is 0.75 g/kg/per day (Department of Health, 1991). However, authors have recommended that protein intake is increased for healthy older adults to 1–1.2 g/kg/day, and for older adults who are malnourished or at risk of malnutrition to 1.2–1.5 g/kg/day (Bauer et al., 2013; Deutz et al., 2014; Dorrington et al., 2020).

To date, the effect of different macronutrients (carbohydrate, protein, and fat) on appetite and satiety have been extensively explored and it is widely believed that macronutrients with the same caloric content have different impacts on satiation and satiety (Bludell, Lawton, Cotton, & Macdiarmid, 1996; Holt, Brand Miller, Petocz, & Farmakalidis, 1995). However, the satiety value of different macronutrients remains an on-going topic of debate and discussion within the scientific community. While protein is commonly assumed to be the most satiating of all macronutrients per kJ (Hill & Blundell, 1986; Latner & Schwartz, 1999; Rolls, Hetherington, & Burley, 1988; J. Stubbs, Ferres, & Horgan, 2000), concerns have been raised that the recommendation to increase protein intake in older adults, who already have diminished appetite, may result in a decrease in energy consumption. A recent meta-analysis emphasized that protein supplementation had a positive effect on energy intake in the included acute studies (Ben-Harchache, Roche, Corish, & Horner, 2021). However, it is worth noting that most of these studies comparing protein and other nutrients were not isovolumetric and equicaloric. Therefore, the satiety values of macronutrients in ageing and how it is comparable to younger adults remain uncertain.

Therefore, the aims of this study were.

(i) to determine if increasing protein intake in older adults leads to a compensatory decrease in energy intake due to protein's satiating effect;
(ii) to compare the effect of preloads high in protein, fat, and carbohydrate but equal in energy and volume on perceived appetite, gastric emptying, and subsequent energy intake in younger and older adults.

2. Materials and methods

2.1. Participant characteristics

Twenty healthy younger (22–34 years) and 20 healthy older (65–79 years) adults participated in the study. The research protocol was given a favourable opinion for conduct by the University of Reading Research Ethics Committee (study number UREC19/25) and the study was conducted at the Hugh Sinclair Unit of Human Nutrition, a part of the School of Chemistry, Food and Pharmacy at the University of Reading (Clinical Trials Database Registration ID NCT04623450). Exclusion criteria were as follows: being <18 years or between 41 and 64 years (inclusive); having a disease and using a medication that can impact on appetite in the past three months; having an allergy to any of the test foods; disliking or cannot eat any of the test foods; being pregnant or breastfeeding; being obese (Body mass index (BMI) > 30 kg/m^2); being on a weight loss diet; smoking more than 10 cigarettes a day.

2.2. Study design

The study was a three-way, crossover, randomized, single-blind controlled study and included one pre-test day and three test days.

- **Pre-test day**

On the pre-test day, participants were asked to come to the Hugh Sinclair Unit of Human Nutrition. All study details were explained to the participants and then informed consent was obtained. Afterwards, participants completed a series of questionnaires pertaining to health and eating habits (Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messick, 1985), Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien, Frijters, Bergers, & Defares, 1986), Council on Nutrition Appetite Questionnaire (CNAQ) (Wilson et al., 2005) and General Practice Physical Activity Questionnaire (Department of Health, 2009)). Additionally, their anthropometric measurements (height, weight, and waist and hip circumference) were taken, and body composition was estimated by bioelectrical impedance scale (Tanita; BC – 418 MA; Tokyo, Japan). If the participants fell within the exclusion criteria at this stage, they were not asked to continue the study and those who were eligible were invited to the test days.

- **Test day**

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

On the evening prior to a test, participant were asked to avoid the consumption of caffeine, alcohol and nicotine, to avoid unusual, strenous exercise and to fast for 12h (water was allowed). Participants were also asked to record their food intake for the day before the first trial and repeat it prior to subsequent trials to ensure that their food intake was similar prior to all three trials. This was checked at the beginning of each test day and no differences in recorded in take where observed.

Test days commnenced between 8 and 9am depending on the participants preferences, and this start time was repeated at subsequent visitis. Upon arrival at the Unit, participants consumed an entire standardised breakfast meal consisting of muesli (Sainsbury's, Reading, UK), ground almonds (ASDA, Reading, UK), and whole milk (Co-op, Reading, UK), which represented 20% of their estimated calorie intake for a normal day, within 15 min, calculated from the data obtained from the pre-screening day (height, weight, age, physical activity level - assessed with the General Health Physical Activity Questionnaire) (Harris & Benedict, 1918). Participants then rested for 3 h. They had access to water which was allowed *ad libitum* on the first test day and their intake was recorded, and were given the same amount of water to consume at subsequent sessions. During this time, the participants were allowed to do sedentary work, read, watch films, etc. Participants were closely monitored during the test period to prevent any activities such as watching or reading content that could potentially influence their appetite or desire to eat. Three hours later, participants were given a preload which was consist of strawbery milkshake that was either high in protein (low fat strawbery yogurt (ASDA, Reading, UK), dry pure whey protein (Bulk Powders, UK) and strawberry natural food flavouring (Foodie Flavours, Hertfordshire, UK)), fat (low fat strawberry yogurt (ASDA, Reading, UK) and double cream (Elmlea, Co-op, Reading, UK)) or carbohydrate (low fat strawberry yogurt (ASDA, Reading, UK) and dry maltodextrin (Bulk Powders, UK)). The preload milkshake on each test day were equicaloric and isovolumetric (Table 1). They were served in a colored glass (350 ml) so that the small differences between the colors of the milkshakes were not noticeable. After the first sip and after consuming the whole preload milkshake (after 10 min), participants were asked to complete 100 mm visual analogue scale (VAS) on paper to

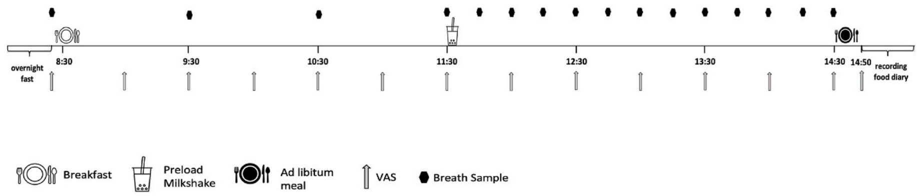

Fig. 1. Timeline of the test days.

Table 1
Energy and macronutrient composition of the preload milkshakes.

	High Fat	High Carbohydrate	High Protein
Energy (kcal)	339	328	339
Volume (ml)	250	250	250
Protein (g)	5.7	5.3	48.2
Carbohydrate (g)	20.4	71.3	22.4
Fat (g)	26.0	2.4	6.3
Protein (% of energy)	6.7	6.6	57.0
Carbohydrate (% of energy)	24.1	87.0	26.4
Fat (% of energy)	69.2	6.4	16.6
Ingredients (g)			
Yoghurt	151	160	155
Cream	47	-	-
Maltodextrin	-	51	-
Whey Protein Isolate	-	-	56
Strawberry Flavouring	-	-	Approx. 15 drops

assess appearance, aroma, flavour, pleasantness, and texture of the preload milkshake, to confirm that the products were perceived as similar. Three hours after the preload milkshake, participants were offered an *ad libitum* buffet meal of pasta (Tesco, Reading, UK) with tomato sauce (ASDA, Reading, UK), green grapes (Co-op, Reading, UK), and chocolate digestive biscuits (Tesco, Reading, UK), and asked to consume it until they were comfortably full. The time to consume the *ad libitum* meal was 20 min, but they were allowed to stop eating if they felt full before 20 min. All meals and preload milkshakes were freshly prepared on the study days and participants consumed their meal alone with no distractions, using isolated sensory booths. Participants repeated the test day for all three macronutrient preloads with at least 2 days and no more than 4 weeks between test days. Young females were tested at the same phase of the menstrual cycle when progesterone is lowest, which is the first 14 days after the start of menstruation (Dye & Blundell, 1997).

Outcome measures

Each participant was offered a pre-weighed *ad libitum* meal and food consumption at the *ad libitum* meal was measured by weighing the leftover food. Additionally, participants were asked to record their food intake using weighed food diary of what they have eaten for the rest of the day.

Four subjective feelings (hunger, fullness, desire to eat, and prospective consumption) of appetite were measured using 100 mm VAS anchored with the terms 'not at all' and 'extremely'. Before breakfast and every 30 min throughout the test day, participants were asked to rate 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 (A. Flint, Raben, Blundell, & Astrup, 2000).

Before breakfast and every hour until the preload milkshake and every 15 min for 3 h after the preload, breath samples for measurement of gastric emptying were taken by blowing into a small glass tube (Exetainer, Labco, Ceredigion, UK) through a straw while wearing a nose clip. One hundred mg of 1–^{13}C octanoic acid (Eurisotop, Saint-Aubin, France) was added to the preload drinks, which is a safe, reliable and valid method for measuring gastric emptying (Davies, 2020; von Gerichten et al., 2022). ^{13}C labelled octanoate is rapidly absorbed in the duodenum and emerges in the breath as completely oxidized ^{13}C labelled carbon dioxide (CO_2) (von Gerichten et al., 2022).

An isotope ratio mass spectrometer (ABCA, Sercon LTD, Cheshire, UK) was used to determine the ratio of $^{13}CO_2$ recovered in the breath sample, relative to a single point calibration (Werner & Brandt 2001) cylinder gas (5% CO_2 95% N_2, -37.17 ± 0.04 Delta Vienna Pee-Dee Belemnite (δVPDB) against NBS-19; n = 15, Iso-analytical, Crewe, UK). Abundance in δVPDB units was converted to atom fraction and used to calculate gastric emptying. Data were displayed as percentage of $^{13}CO_2$ dose recovered per hour and cumulative percentage $^{13}CO_2$ dose recovered over time. Carbon dioxide production was assumed to be 300 mmol/m^2 body surface area per hour (Shreeve, Cerasi, & Luft, 1970). To calculate the participants' body surface area, a validated weight-height formula was used (Haycock, Schwartz, & Wisotsky, 1978) and then the findings fitted into a gastric emptying model developed by Ghoos et al. (1993). After that, the formulae in the gastric emptying model was used to calculate the lag phase *(T_{lag})*, which is time taken to maximal rate of $^{13}CO_2$ excretion, the half time *(T_{half})*, which is the time it takes for 50% of the ^{13}C dose to be excreted, the 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 (Jackson, Bluck, & Coward, 2004; Schommartz, Ziegler, & Schadewaldt, 1998).

2.3. Statistical analysis

The hypotheses were outlined before the data were collected and the analytic plan was pre-specified. In total 40 (20 older and 20 younger) participants were recruited to this study based on the appetite methodological review paper by Blundell et al. (2010). Depending on the specific VAS scale, power level (0.8 or 0.9), and paired or unpaired design, 8–35 individuals would be needed to detect a 10 mm (10%) difference, which is accepted as a 'reasonable and realistic difference', mean appetite ratings for two foods in 4.5 h (A. Flint et al., 2000). This is consistent with evidence from other research that, in favourable experimental circumstances, 20–25 volunteers are typically enough to detect a 10% difference in mean or area under the curve (AUC) appetite ratings between foods (Blundell et al., 2010).

Statistical analysis was performed using the SPSS (version 27; Chicago, Illinois, United States) and Excel (version 14.0.); Arlington, United States). All data were firstly tested for normal distribution. The participants' characteristics except age were compared by using a two-tailed independent sample T-test. Age was not normally distributed; therefore, a Mann Whitney *U* test was performed. Energy intake at the *ad libitum* meal was calculated using manufacturers details on Excel and energy intake for the rest of the test day was calculated using the Nutritics (Nutrition Analysis Software for Professionals; Dublin, Ireland) program. The change from baseline in VAS scores of perceived appetite was calculated in Excel and the total area AUC from baseline to 360 min and AUC from 180 min (after preload consumption) to 360 min for each variable was calculated using the trapezoidal rule. Two-way repeated measures analysis of variance (RM-ANOVA) test with pair-wise comparisons (Bonferroni corrected) was used to determine the effects of different preload milkshakes on subsequent energy intake, the VAS scores including the palatability of preloads, and gastric emptying in younger and older adults and to compare any differences between the groups. P-value <0.05 was accepted as significant in all analyses.

3. Results

3.1. Participants' characteristics

Of 67 volunteers pre-screened (37 younger and 30 older), 21 (14 younger and 7 older) volunteers were excluded from the study as they did not meet the inclusion criteria. Forty-six volunteers (23 older, 23 younger) were included in the study. After randomisation, six participants (3 older adults and 3 younger adults) were excluded or withdrew from the study, of which 2 younger participants were excluded as they could not finish the breakfast and the other withdrew without reason. Two older participants had health issues that arose during the test day, one older participant had memory issues, the second older participant became unwell, and the third could not complete the third test day within one month (Fig. 2).

The participants' characteristics are shown in Table 2. There was no significant difference in average height, weight, and hip circumference between younger and older adults. Average age, BMI, body fat percentage, body fat mass, and waist circumference of the older group were significantly higher than the younger group ($p < 0.05$). The CNAQ was used to assess older people's appetite. It has been found that 95% of older participants were not at risk of anorexia and 5% of them need a frequent reassessment. Additionally, the TFEQ and the DEBQ were used to measure restrained eating. This concluded that 50% of older adults and 20% of younger adults were restrained eaters in the present study. There was no difference in the mean eating restraint score based on the TFEQ between older and younger adults. However, the eating restraint score based on the DEBQ was significantly higher in older adults than in younger adults (**Table 2**). In appetite studies, participants who are restrained eaters are often excluded from the study, however, since restrained eating behaviour is highly prevalent older adults, we did not exclude participants based on their dietary restraint (Flint et al., 2008).

Table 2
Participants' characteristics.

	Younger Group ($n = 20$)	Older Group ($n = 20$)	Significance (p-value)
Age (years)	27.7 ± 3.7	70.2 ± 3.8	<0.001
Male/female, n	10/10	10/10	
Height (cm)	169.4 ± 9.52	167.5 ± 10.97	0.550
Weight (kg)	64.6 ± 10.97	71.0 ± 15.30	0.140
BMI (kg/m^2)	22.4 ± 2.6	25.1 ± 3.0	0.005
Body Fat Percentage (%)[a]	22.3 ± 6.52	29.2 ± 7.30	0.003
Body Fat Mass (kg)[a]	14.2 ± 4.10	20.4 ± 6.55	<0.001
Fat Free Mass (kg)[a]	50.5 ± 10.73	49.7 ± 12.43	0.850
Waist Circumference (cm)	80.4 ± 10.30	92.8 ± 14.28	0.003
Hip Circumference (cm)	96.8 ± 8.58	101.7 ± 7.81	0.067
CNAQ	30.5 ± 4.05	30.6 ± 2.15	0.810
DEBQ	2.0 ± 0.75	2.6 ± 0.86	0.020
TFEQ	6.9 ± 4.95	8.8 ± 3.93	0.190

BMI Body mass index; *CNAQ* Council on Nutrition Appetite Questionnaire; *DEBQ* Dutch Eating Behaviour Questionnaire; *TFEQ* Three-Factor Eating Questionnaire. Values are means ± SD.

[a] Statistical analysis has been done for 19 older participants due to missing bioelectrical impedance data.

3.2. Palatability of preload milkshakes

The palatability questionnaire given to the participants on two occasions (after the first sip and after consuming the whole preload

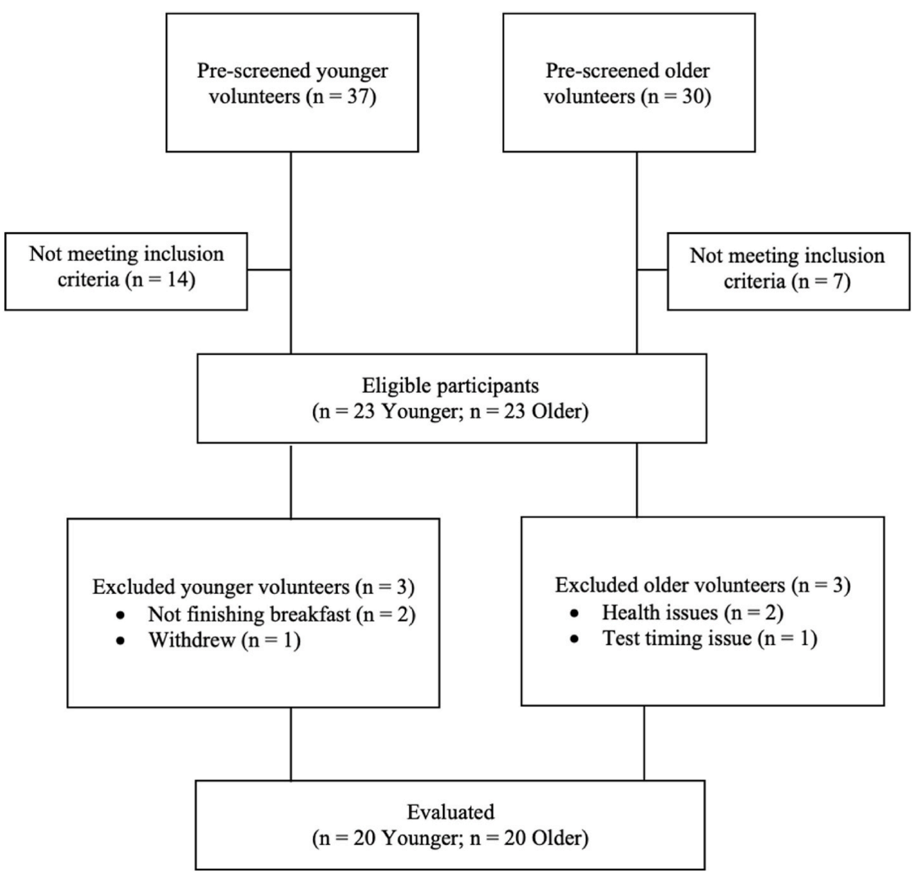

Fig. 2. A flow diagram of the participant recruitment.

milkshake) showed no difference in appearance liking ratings between the preload milkshakes after the first sip ($p > 0.05$). After the full preload consumption, there was a significant effect of different preloads on the appearance liking ratings ($F (2,76) = 4.8$, $p = 0.01$, $n_p^2 = 0.11$) where participants liked the appearance of the high fat milkshake more than the high protein and high carbohydrate milkshakes ($p = 0.019$, $p = 0.046$, respectively). After the first sip and full consumption of the milkshakes, participants liked the aroma ($F (2,76) = 6.4$, $p = 0.003$, $n_p^2 = 0.14$; $F (2,76) = 10.3$, $p < 0.001$, $n_p^2 = 0.21$, respectively), flavour ($F (2,76) = 9.1$, $p < 0.001$, $n_p^2 = 0.19$; $F (2,76) = 12.9$, $p < 0.001$, $n_p^2 = 0.25$, respectively), and texture ($F (2,76) = 10.1$, $p < 0.001$, $n_p^2 = 0.21$; $F (2,76) = 12.5$, $p < 0.001$, $n_p^2 = 0.25$, respectively) of the high fat milkshake more than the high protein milkshake ($p < 0.05$) and the high protein milkshake was found to be the least pleasant ($F (2,76) = 11.2$, $p < 0.001$, $n_p^2 = 0.23$; $F (2,76) = 16.3$, $p < 0.001$, $n_p^2 = 0.3$, respectively) compared to the other two milkshakes ($p < 0.05$).

There was also a significant effect of age on the appearance, aroma, flavour, and pleasantness ratings after the first sip and the full consumption of the preloads. Older adults rated the preloads higher in liking of the appearance ($F (1,38) = 8.1$, $p = 0.007$, $n_p^2 = 0.18$; $F (1,38) = 6.6$, $p = 0.014$, $n_p^2 = 0.15$, respectively), aroma ($F (1,38) = 7.2$, $p = 0.011$, $n_p^2 = 0.16$; $F (1,38) = 11$, $p = 0.002$, $n_p^2 = 0.22$, respectively), flavour ($F (1,38) = 6.6$, $p = 0.014$, $n_p^2 = 0.15$; $F (1,38) = 7.1$, $p = 0.011$, $n_p^2 = 0.16$, respectively) and pleasantness ($F (1,38) = 7$, $p = 0.012$, $n_p^2 = 0.16$; $F (1,38) = 6.4$, $p = 0.016$, $n_p^2 = 0.14$, respectively) compared to younger adults. Being younger or older had no significant effect on texture rating after first sip and after the full consumption of the preloads ($p > 0.05$). There was also not a significant interaction between the effects of preload type and age ($p > 0.05$).

3.3. Subsequent energy, macronutrient, and fibre intake

There was no significant effect of preload type and age on energy, fat, carbohydrate, protein, or fibre intake neither at the *ad libitum* meal, nor on the evening of the test days, nor on the sum of the *ad libitum* meal and the remainder of the day ($p > 0.05$) (Table 3). There was a tendency for a preload by age interaction for energy intake for the rest of the day ($F (2,68) = 2.87$, $p = 0.073$, $n_p^2 = 0.08$), although without a clear direction of effect. Additionally, there was a significant preload by age interaction for fat intake for the rest of the day ($F (2,68) = 3.63$, $p = 0.032$, $n_p^2 = 0.1$) and for the sum of the *ad libitum* meal and the rest of the day ($F (2,68) = 4.19$, $p = 0.019$, $n_p^2 = 0.11$), however, again there was no clear direction of these effects.

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

The baseline hunger, fullness, desire to eat food and prospective consumption scores were not significantly different between the preloads and between the groups. There was no significant effect of consumption of different preload milkshakes (high in carbohydrate, fat, or protein) or age on the total (0–360 min) or 180–360 min AUC values of the subjective appetite rating scores ($p > 0.05$) (Figs. 3 and 4).

Table 3
Subsequent Energy, Macronutrient and Fibre Intake at the *ad libitum* meal, the rest of the day and the sum of the *ad libitum* meal and the rest of the day after consuming different preload milkshakes in younger and older adults.

	Younger Group (*n* = 20)			Older Group (*n* = 20)			Significance (*p*-value) (Between preloads)	Significance (*p*-value) (Between groups)	Significance (*p*-value) (Preload*group)
Preload milkshake	High Fat	High CHO	High Protein	High Fat	High CHO	High Protein			
***Ad libitum* meal**									
Energy (kcal)	1469 ± 612	1463 ± 647	1437 ± 700	1255 ± 497	1281 ± 419	1242 ± 480	0.809	0.254	0.952
Fat (g)	15.1 ± 10.2	16.6 ± 10	16.3 ± 11.5	16.0 ± 9.1	16.01 ± 8.6	15.5 ± 8.0	0.592	0.975	0.472
Carbohydrate (g)	268.4 ± 121.7	281.1 ± 121.8	272.8 ± 131.4	236.8 ± 92.5	242.1 ± 78.7	234.8 ± 91.1	0.541	0.274	0.903
Protein (g)	39.0 ± 18.0	40.4 ± 18.2	39.5 ± 19.7	33.8 ± 13.6	34.6 ± 11.6	33.7 ± 13.9	0.673	0.254	0.955
Fibre (g)	17.4 ± 7.7	17.9 ± 7.7	17.3 ± 8.2	15.8 ± 5.9	15.3 ± 5.0	14.8 ± 5.7	0.561	0.285	0.661
Rest of the day[a]									
Energy (kcal)	699 ± 453	590 ± 337	842 ± 480	742 ± 320	643 ± 274	630 ± 322	0.132	0.697	**0.073**
Fat (g)	28.0 ± 25.7	25.1 ± 20.8	38.9 ± 24.6	33.5 ± 17.9	29.3 ± 16.7	25.9 ± 17.3	0.384	0.838	**0.032**
Carbohydrate (g)	78.7 ± 48.9	69.0 ± 37.8	90.8 ± 58.1	69.6 ± 29.7	57.7 ± 35.6	62.9 ± 37.2	0.183	0.145	0.387
Protein (g)	30.3 ± 27.7	22.0 ± 14.7	29.1 ± 21.0	26.0 ± 9.7	29.6 ± 16.5	28.7 ± 17.4	0.559	0.844	0.130
Fibre (g)	8.7 ± 8.2	6.3 ± 3.9	9.5 ± 7.4	8.2 ± 4.7	8.5 ± 6.6	8.0 ± 6.0	0.410	0.961	0.226
***Ad libitum* meal + Rest of the day**[a]									
Energy (kcal)	2140 ± 733	2090 ± 723	2345 ± 760	1997 ± 607	1924 ± 417	1872 ± 461	0.427	0.167	0.168
Fat (g)	42.6 ± 26.2	41.7 ± 25.0	55.6 ± 23.8	49.5 ± 20.7	45.4 ± 16.5	41.4 ± 16.4	0.461	0.837	**0.019**
Carbohydrate (g)	306.4 ± 127.6	358.2 ± 127.2	376.7 ± 129.7	306.4 ± 104.0	299.9 ± 85.5	297.7 ± 94	0.785	0.078	0.473
Protein (g)	59.8 ± 16.	63.9 ± 22.1	70.7 ± 29.0	59.8 ± 16.1	64.2 ± 20.2	62.3 ± 16.0	0.814	0.314	0.308
Fibre (g)	26.9 ± 11.6	25.3 ± 8.4	26.8 ± 11.2	24.0 ± 8.0	23.8 ± 8.5	22.9 ± 8.5	0.799	0.310	0.682

Values are means ± SD. *CHO* Carbohydrate.

[a] Statistical analysis for rest of the day for younger group has been done for 16 younger participants due to participants not returning completed food diaries.

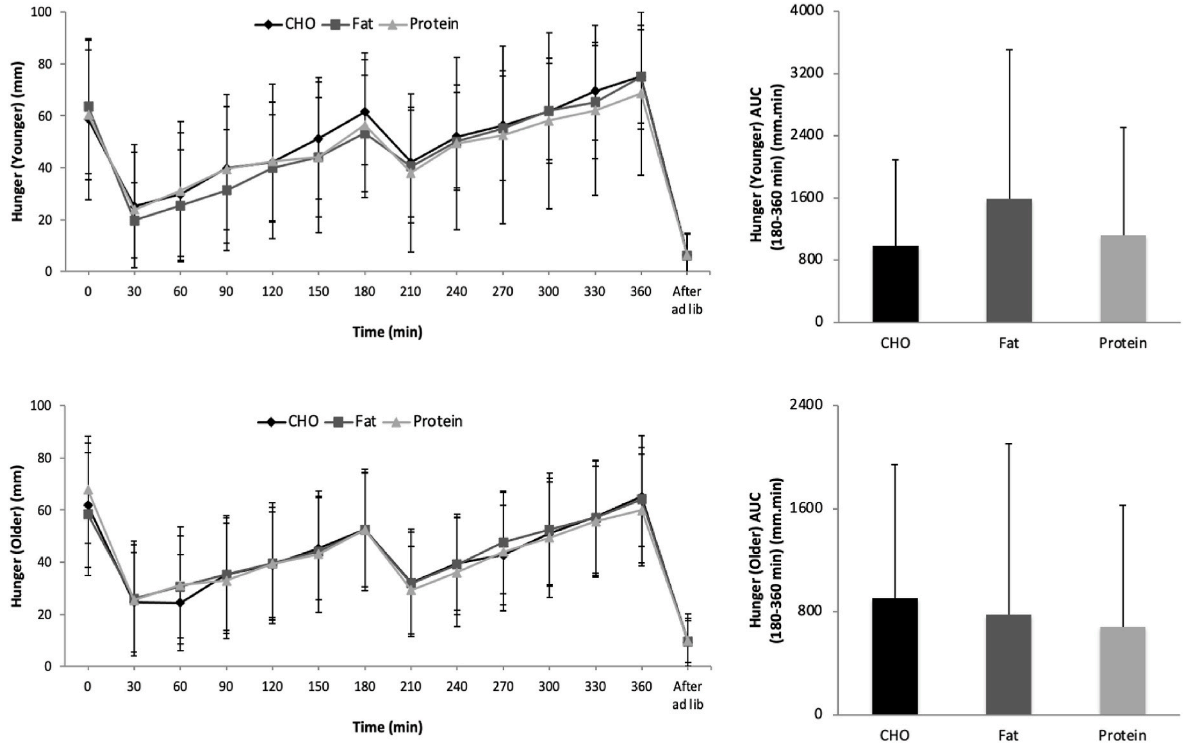

Fig. 3a. The VAS score of hunger during the test days in younger and older adults, as well as the AUC values of the hunger scores after consuming different preload milkshakes (180–360 min) for the two groups. Values are means ± SD represented by vertical bars. *CHO* Carbohydrate.

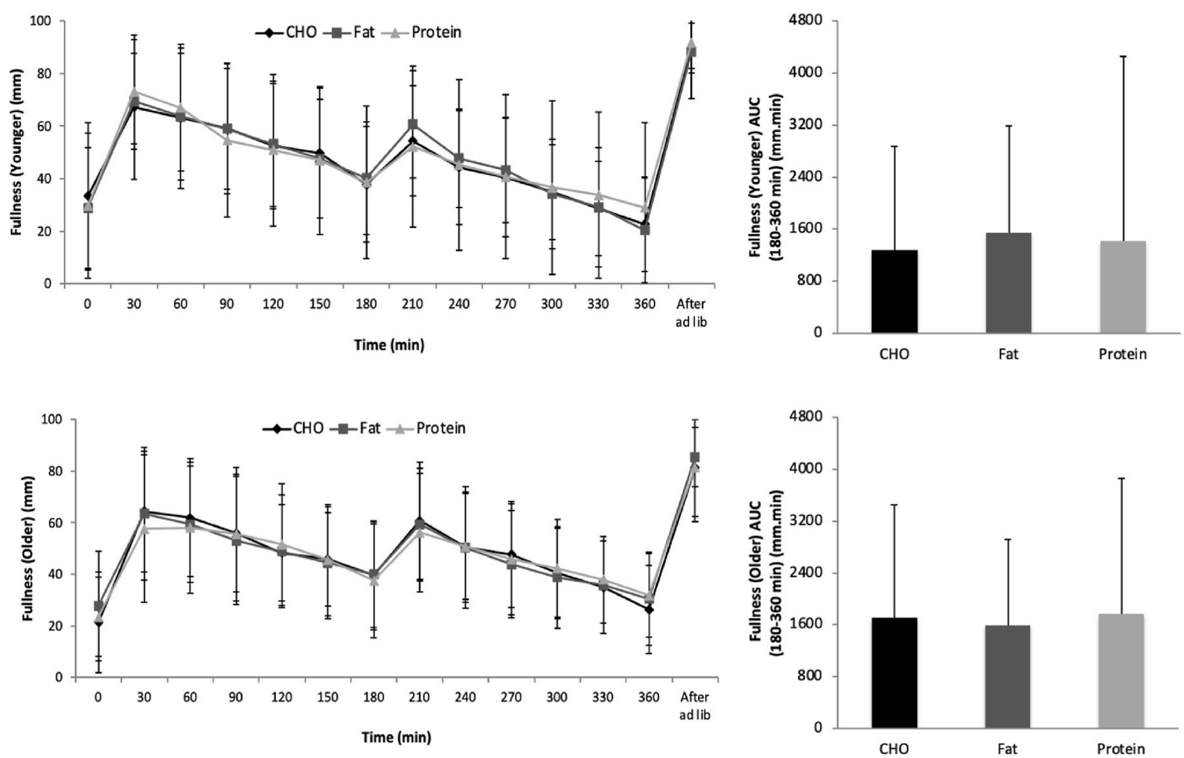

Fig. 3b. The VAS score of fullness during the test days in younger and older adults, as well as the AUC values of the fullness scores after consuming different preload milkshakes (180–360 min) for the two groups. Values are means ± SD represented by vertical bars. *CHO* Carbohydrate.

3.5. Gastric emptying

There was no significant effect of consumption of different preload milkshakes or age on gastric emptying T_{half} (Table 4). Gastric emptying T_{lag} and T_{lat} were significantly longer in younger adults compared to older adults (F (1,38) = 4.35, p = 0.044, $n_p^2 = 0.1$, F (1,38) = 5.4, p < 0.026, $n_p^2 = 0.$, respectively). There was a significant effect of consumption different preloads on gastric emptying T_{lat} (F (2,76) = 54.5, p

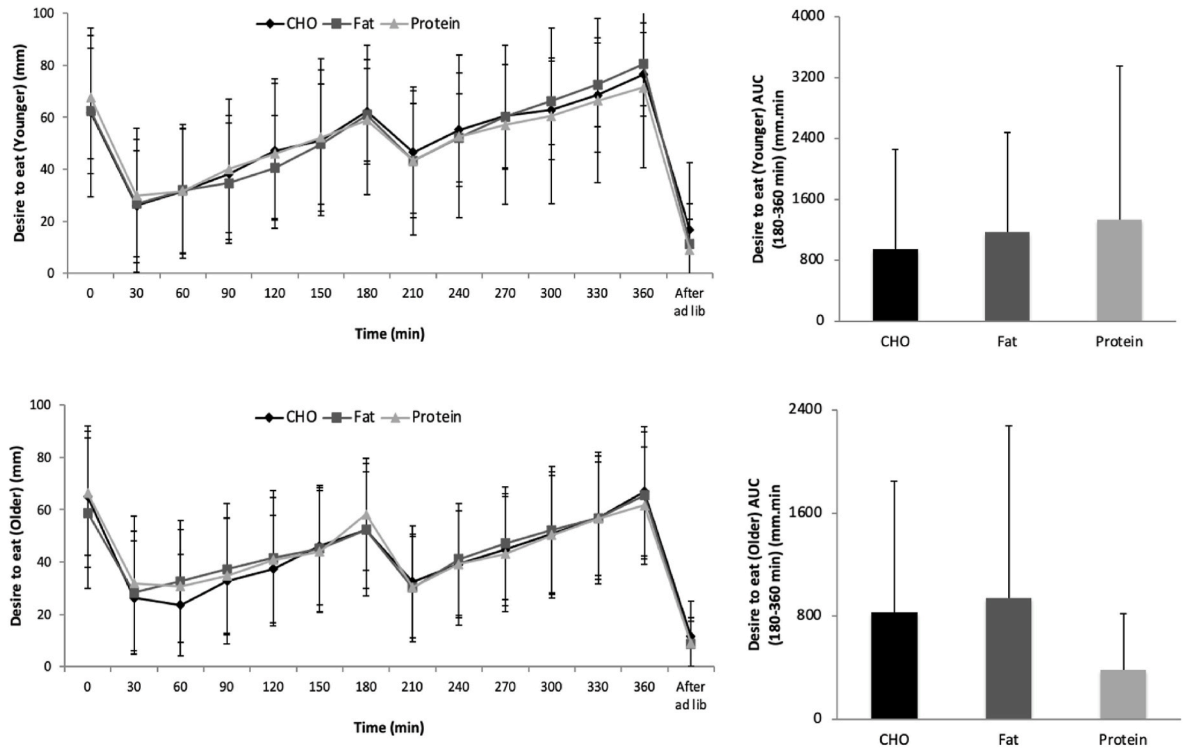

Fig. 3c. The VAS score of desire to eating during the test days in younger and older adults, as well as the AUC values of the desire to eating scores after consuming different preload milkshakes (180–360 min) for the two groups. Values are means ± SD represented by vertical bars. *CHO* Carbohydrate.

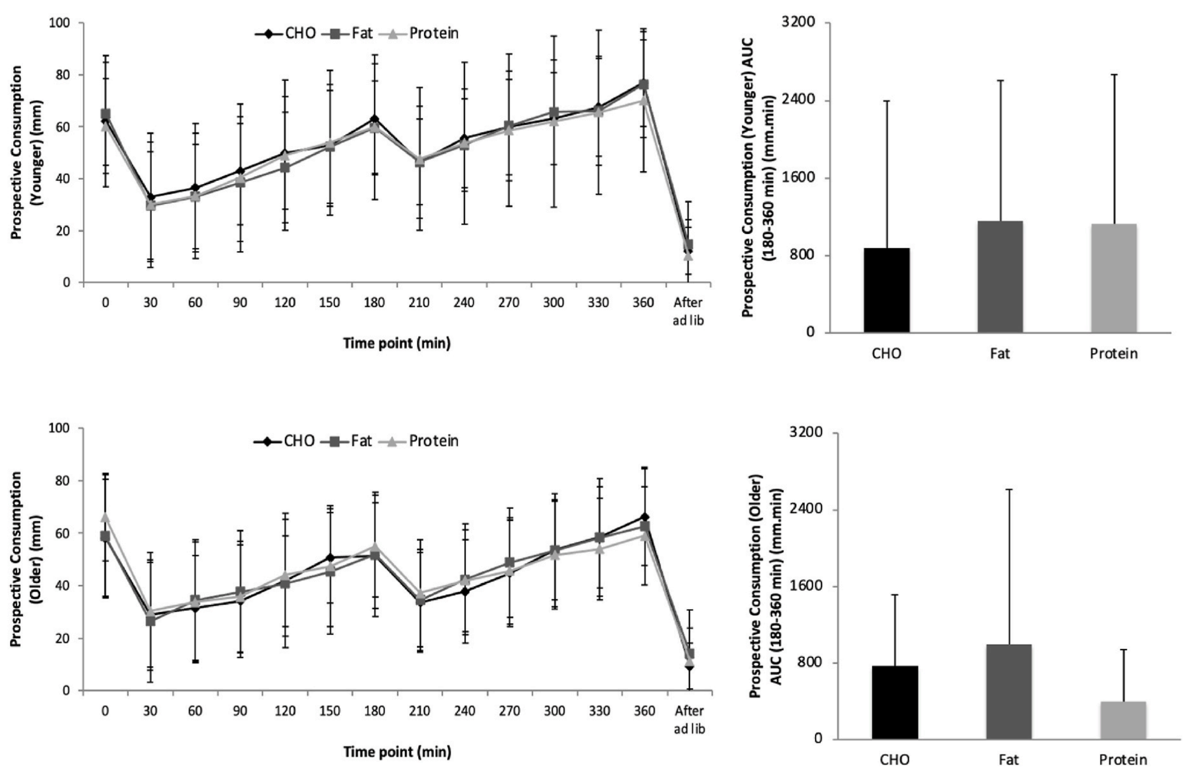

Fig. 3d. The VAS score of prospective consumption during the test days in younger and older adults, as well as the AUC values of the prospective consumption scores after consuming different preload milkshakes (180–360 min) for the two groups. Values are means ± SD represented by vertical bars. *CHO* Carbohydrate.

< 0.001, $n_p^2 = 0.7$), which was shortest after consumption of high protein preload compared to the other preloads. However gastric emptying T_{asc} was significantly longer after consumption of high protein preload compared to high fat preload ($F(2,76) = 6.9$, $p = 0.003$, $n_p^2 = 0.3$).

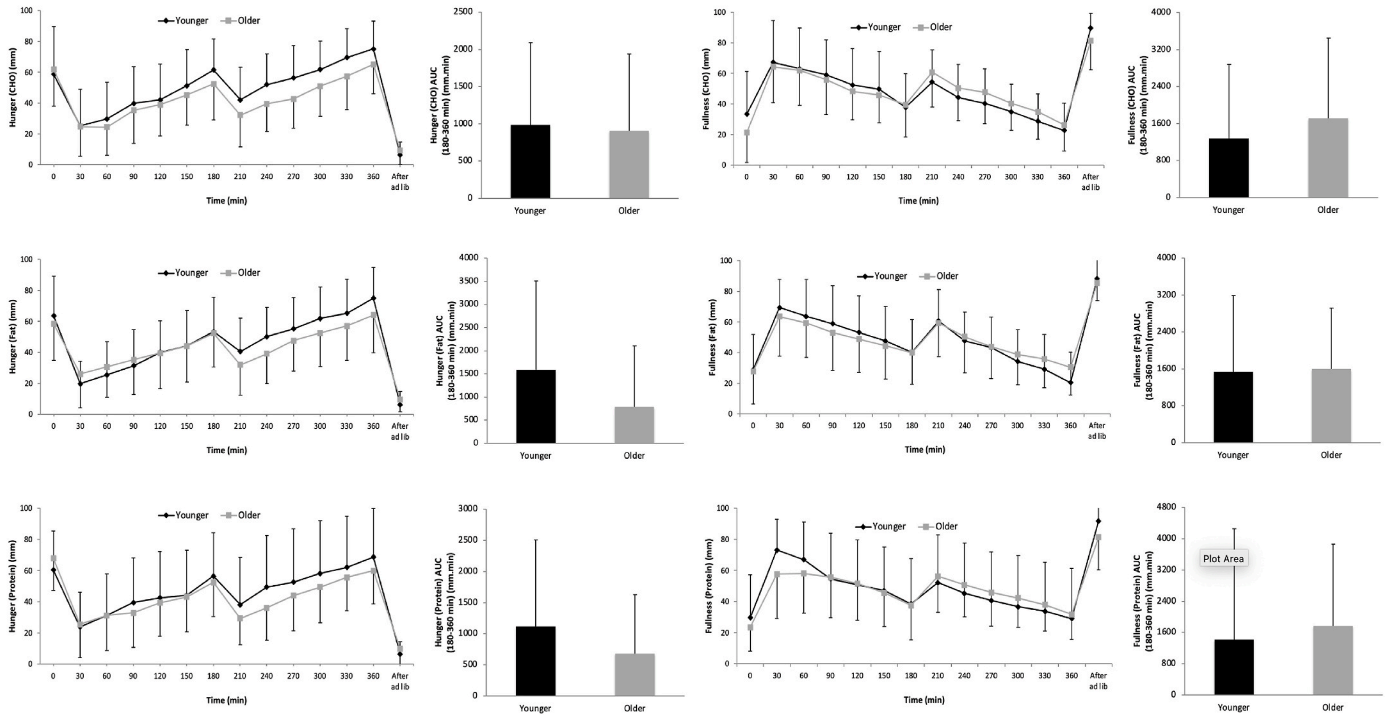

Fig. 4a. Comparison the VAS scores of hunger and fullness during the test days between younger and older adults following the different preload milkshakes (high in carbohydrate-top graph, fat-middle graph and protein-bottom graph), as well as the AUC values of the hunger and fullness scores after consuming preload milkshakes (180–360 min) for the two groups. Values are means ± SD represented by vertical bars. *CHO* Carbohydrate.

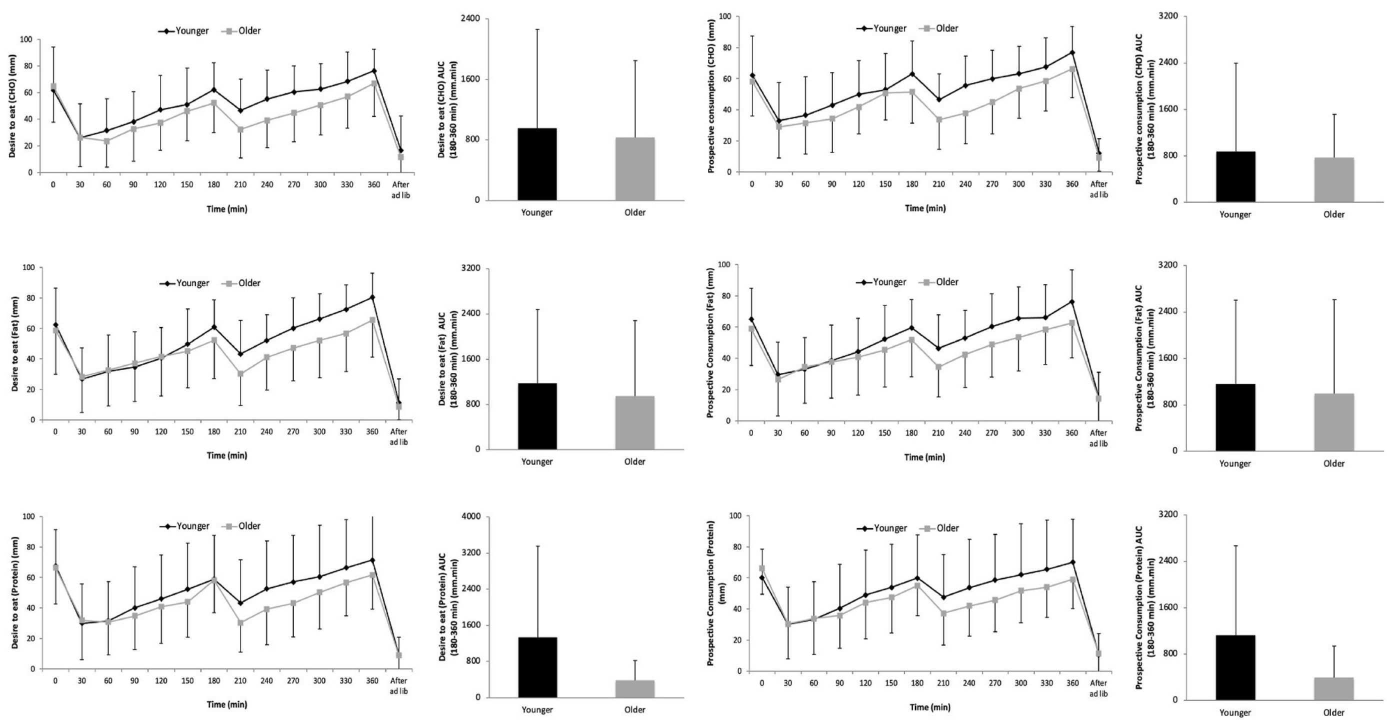

Fig. 4b. Comparison the VAS scores of desire to eat and prospective consumption during the test days between younger and older adults following the different preload milkshakes (high in carbohydrate-top graph, fat-middle graph and protein-bottom graph), as well as the AUC values of the desire to eat and prospective consumption scores after consuming preload milkshakes (180–360 min) for the two groups. Values are means ± SD represented by vertical bars. *CHO* Carbohydrate.

4. Discussion

To our knowledge, this is the first paper to directly compare the effect of different macronutrients (carbohydrate, fat, and protein) on subsequent food intake, perceived appetite, and gastric emptying in older compared to younger adults. The findings of the current study showed that consuming different equicaloric and isovolumetric preload milkshakes that were either high in carbohydrate, fat, or protein had no

Table 4
Gastric emptying times following the high fat, high carbohydrate (CHO), and high protein preload milkshakes.

	Younger Group ($n = 20$)			Older Group ($n = 20$)			Significance (p-value) (Between preloads)	Significance (p-value) (Between groups)	Significance (p-value) (Preload* group)
Time (min)	High Fat	High CHO	High Protein	High Fat	High CHO	High Protein			
T_{half}	73 ± 51	89 ± 103	89 ± 43	52 ± 16	55 ± 21	73 ± 46	0.123	0.068	0.675
T_{lag}	30 ± 16	26 ± 26	28 ± 18	20 ± 8	16 ± 11	19 ± 18	0.378	0.044	0.998
T_{lat}	35 ± 10	26 ± 12	22 ± 9	30 ± 4	23 ± 6	17 ± 6	<0.001	0.026	0.612
T_{asc}	113 ± 49	140 ± 105	143 ± 45	95 ± 16	105 ± 22	131 ± 49	0.003	0.091	0.598

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

significant effects on subsequent energy intake and appetite, in younger or older adults. In relation to the primary objective of the study, increasing protein intake in older adults did not result in a reduction in their subsequent food intake. Although T_{half} was not different between older and younger adults, older adults had faster gastric emptying T_{lag} and T_{lat}. Additionally, while high protein preload consumption delayed gastric emptying T_{asc}, it accelerated gastric emptying T_{lat}.

Numerous studies to date have supported the idea of a hierarchy in macronutrients' satiety effect (Hermsdorff, Volp, & Bressan, 2007; Paddon-Jones et al., 2008; Westerterp-Plantenga, 2003) and high protein intake has been shown to have a major impact on subsequent energy intake (Fallaize, Wilson, Gray, Morgan, & Griffin, 2013; Poppitt, McCormack, & Buffenstein, 1998). However, some studies in the literature were also in agreement with our findings and showed no difference in the satiating efficiencies of protein, fat, and carbohydrate (de Graaf, Hulshof, Weststrate, & Jas, 1992; Raben, Agerholm-Larsen, Flint, Holst, & Astrup, 2003; R. Stubbs, Van Wyk, Johnstone, & Harbron, 1996; van der Klaauw et al., 2013). For instance, in a study of healthy adults using isovolumetric and isoenergetic fluid preloads but had different macronutrient compositions, no difference was found in the acute *ad libitum* energy intake after different preloads (Dougkas & Östman, 2016). There are also other studies in the literature similar to our study that examined whether there is a difference in subsequent macronutrient consumption, as well as energy intake, after consuming a preload with different macronutrient content. The results were consistent with our findings and no difference was found in subsequent macronutrient intake (de Graaf et al., 1992; Vozzo et al., 2003).

In addition to studying the effect of preloads with differing macronutrient contents on subsequent energy and macronutrient intakes, research has also examined macronutrient impacts on subjective appetite, as in our study. Findings shown that protein has a significant short-term satiating effect, resulting in decreased hunger and increased satiety compared to other macronutrients (Fallaize et al., 2013; Johnstone, Stubbs, & Harbron, 1996; Poppitt et al., 1998). Nevertheless, in agreement with our results, other studies have found no significant differences in subjective feelings of appetite after protein intake when compared with carbohydrate and fat of equal energy content (de Graaf et al., 1992; Raben et al., 2003). For instance, in a study examining the effects of preload breakfasts consumptions with different macronutrient contents on energy intake and appetite in *ad libitum* lunch, it was found that a high protein preload did not suppress short-term hunger when compared with other macronutrients. However, in this study, it has also been found that a high protein preload suppress the long term (over 24 h) hunger to a greater extent than the other preloads (R. Stubbs et al., 1996). Since we did not measure appetite for the rest of the test days in our study, we cannot say what effect the preloads have on long-term appetite.

Studies revealing the hierarchy of satiety effects among macronutrients have generally used solid foods as a protein preload, such as eggs on toast in Fallaize et al. (2013) and chicken breast in Marmonier, Chapelot, and Louis-Sylvestre (2000). While both studies reported that protein preloads suppressed hunger much more than fat and carbohydrate preloads, it is important to note that preloads used in these studies were different in terms of density, texture, and flavour and this may have affected the result. It has been claimed that when protein is consumed as a beverage, its reported greater satiety effect than carbohydrate or fat is reduced or lost (Carreiro et al., 2016). However, there are studies suggest that protein's satiating effect may not be limited to solid food and can extend to liquid preloads as well. For example, in a study, participants consumed preloads in liquid form, but with the same appearance, texture, and flavour, protein was found to reduce hunger much more than carbohydrates and fat (Latner & Schwartz, 1999). Similarly, in another study, participants were given dairy fruit drinks which were either protein- or carbohydrate-enriched as a preload and it has been found that significantly less energy was consumed at the *ad libitum* lunch after the protein preload compared to the carbohydrate preload (Bertenshaw, Lluch, & Yeomans, 2008). On the other hand, in Potier's study, after consumption of hot chocolates composed of carbohydrate, protein, or fat, protein did not have a greater satiety effect than carbohydrates (2010). Our study support these findings and we did not find any difference in appetite and food intake after protein preload consumption compared to other macronutrient preloads although the preloads were liquid.

Research comparing the effect of different macronutrients on appetite and energy intake has mostly focused on younger adults, with limited research carried out in older adults to explore the potential appetite-suppressing effect of protein. Therefore, it remains unclear whether the satiating effect of protein observed in younger adults extends to older populations. A recent meta-analysis in which 7 acute and 11 longitudinal studies were included showed that although acute protein intake suppressed appetite and energy intake in *ad libitum* meal, it increased total energy intake. Additionally, it has been found that protein had no effect on appetite and total energy intake in longitudinal studies (Ben-Harchache et al., 2021). However, it is worth mentioning that the test meals used in most of the acute studies included in this study were not equal in volume and caloric content. Another recent study, which was in agreement with this meta-analysis, examined the impact of short-term protein supplementation on the appetite and energy intake of adults aged 50–75 years. The study found that 20 g of whey protein given outside of meal times had no significant effect on food intake and appetite (Tuttiett et al., 2021). Similarly, the findings from the present study were also promising for older adults to increase their protein intake without this impacting their energy intake, showing that ~50 g protein consumption had no effect on subsequent energy intake and perceived appetite.

In addition to this, there are a limited number of studies comparing the effect of protein intake on energy intake and appetite between older and younger adults. One such study comparing the effect of whey protein intake demonstrated that protein suppressed energy intake less in older adults compared to younger adults, and while appetite decreased in younger adults, it increased in older adults (Giezenaar et al., 2015). This may be due to the fact that, following the protein preload, gastric emptying was slower in older adults than in younger adults. However, it should be taken into account that the number of participants in this study was relatively small (8 older and 8 younger). In contrast, the present study had a larger sample size, and despite gastric emptying being faster in older adults, we did not observe any statistically significant differences in the energy intake of older and younger adults after the consumption of protein preload. It should be kept in mind that appetite and food intake are regulated by not only gastric emptying but also various other mechanisms, including hormones and neural mechanisms (Clyburn, Carson, Smith, Travagli, & Browning, 2023; Hameed, Dhillo, & Bloom, 2009; Moss, 2013). Therefore, it is possible that the absence of a significant difference in energy intake between older and younger adults in the present study may be attributed to the interplay of these other factors.

To date, studies examining the response of macronutrients to gastric emptying used different methods including breath tests and 3D-ultrasonography and have shown conflicting results. Moreover, most of these studies have focused on younger adults and have utilised a single type of macronutrient, particularly protein and fat (Cecil, Francis, & Read, 1999; Giezenaar et al., 2018; Goetze et al., 2007; Marciani et al., 2015). To the best of our knowledge, our study is the first to directly compare of three different macronutrients on gastric emptying in both older and younger adults. Additionally, in line with previous studies, whey protein was chosen as the type of protein in this study due to its faster digestion and ability to suppress hunger more quickly compared to other types of protein (Boirie et al., 1997; Pal & Ellis, 2010; Pal, Radavelli-Bagatini, Hagger, & Ellis, 2014).

Some studies in the literature showed that orally consumed fat or infusion of fat into the small intestine delays gastric emptying (Cecil et al., 1999; Read et al., 1984; IMSK Welch, Saunders, & Read, 1985; IM Welch, Sepple, & Read, 1988). Another research on younger adults measuring the effect of breakfasts with different macronutrient composition on gastric emptying, has also reported that gastric emptying T_{half} was delayed after consuming a high-fat preload compared to the low-fat preload (Clegg & Shafat, 2010). In contrast to these findings, a study of younger adults showed that gastric emptying T_{half} was slower after consuming a high-carbohydrate meal in comparison to a high-fat meal. However, it has to be noted that although these two meals were equally palatable, the carbohydrate meal had overall 18% higher calorie content, and this difference might have affected the gastric emptying T_{half} (Marciani et al., 2015). Similar to the methodology of our study, a study on young adults comparing the effects of three different macronutrients showed that the gastric emptying T_{half} of the protein solution was significantly longer than that of the fat and glucose solution (Goetze et al., 2007). Nevertheless, it should be noted that in this previous study, the participants did not consume these nutrients orally, but they were infused directly into their stomachs. These finding were further supported by research conducted by Giezenaar et al. (2018) on younger adult, which used by 3D-ultrasonography to examine the acute effects of whey-protein intake on gastric emptying. They found that gastric emptying time slowed down with increased protein intake.

In this present study, although we did not find any difference in gastric emptying T_{half} after consumption of different macronutrients, gastric emptying was found to be delayed in T_{lat} after consumption of fat preload compared to protein and carbohydrate preloads. Additionally, after consumption of protein preload, gastric emptying T_{asc} was found to be the longest while T_{lat} was the shortest compared to other macronutrients. It is worth emphasizing that our study was distinguished by the inclusion of both younger and older adults, whereas discussed previous studies included only younger adults. Compared to other studies, our test meals were also matched for energy and volume ensuring tighter control of the parameters being assessed, which is not the case for other studies such as Giezenaar et al. (2018). Furthermore, in our study, the preloads were consumed orally by the participants, whereas in some previous studies, they were directly infused into their stomachs. It should be highlighted that infusion experiments do not always foretell what will occur in vivo after oral food intake, as previously suggested by Cecil et al. (1999). Moreover, a different method (^{13}C-octanoic acid breath test) was used to assess gastric emptying in our study, which may have contributed to the variations in findings compared to other studies in the literature, such as Giezenaar et al. (2018). These methodological distinctions are important to consider when interpreting the results of this study and comparing them to those of previous study.

In addition to the studies in younger adults, there are small number of research that have examined the impact of aging on gastric emptying and it has been indicated that older adults have slower gastric emptying and gastrointestinal transit than younger adults (Clarkston et al., 1997; Horowitz et al., 1984). One study that compared the gastric emptying of whey protein in older and younger adults found that older individuals had slower gastric emptying after oral whey protein consumption compared to young adults (Giezenaar et al., 2015). Despite these findings, our study found no significant differences in gastric emptying T_{half} between younger and older adults after consumption of different macronutrients. However, gastric emptying was found to be delayed in T_{lag} and T_{lat} in younger adults compared to older adults. This may be explained by the fact that older participants had significantly higher fat mass than the younger ones, and adiposity may have masked any effects of macronutrients on gastric emptying (Clegg & Shafat, 2009).

The major strength of the present study was that is the first study to compare the effect the three different macronutrients on subsequent energy intake, appetite, and gastric emptying between younger and older adults. The sample size of the study was larger than the similar studies (e.g. (Giezenaar et al., 2015)) and test meals were matched for energy and volume. However, there are also a few limitations that need to be addressed. One important limitation that should be acknowledged is that we did not assess the malnutrition in our participants and our sample of older adults was unlikely to be experiencing malnutrition. Our observations indicate that the sample mainly consisted of older individuals with a favourable socio-economic status, who have a conscious approach towards maintaining a healthy life. These characteristics may have influenced the absence of significant study effects. Secondly, although the test meals were equicaloric and isovolumetric, the palatability of the test meal was found different by the participants. However, participants received the *ad libitum* meal after 3 h of consumption of test meal and repeated the tests with at least two days of space in between, therefore, we believe that this difference did not cause unnecessary bias on subsequent energy intake or perceived appetite. Furthermore, our analysis revealed no significant correlation between the palatability scores and perceived appetite or energy intake ($p > 0.05$). Lastly, we did not take blood samples to test appetite-related hormones in this study, and the addition of these parameters would provide significant insight into the absence of differences in energy intake and appetite. Therefore, future studies including blood samples are needed to compare appetite hormones and neural mechanisms when comparing the effect of different macronutrients in older adults.

5. Conclusion

This study showed subsequent energy intake and perceived appetite were not affected by consuming different macronutrient preloads with the same caloric content and volume in both younger and older adults. Our findings suggested that older people can increase their protein intake without this impacting their satiety and food intake.

Author contributions

Design of the study (MC, SO), implementation (DD, SO), analysis of breath samples (AS), data analysis (DD, MC, LM), 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. All participants provided written informed consent before participation.

Declaration of competing interest

None

Data availability

Data will be made available on request.

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