Habitual protein intake, protein distribution patterns and dietary sources in Irish adults with stratification by sex and age

ABSTRACT

Background: Given the importance of habitual dietary protein intake, distribution patterns and dietary sources in the etiology of age-related declines of muscle mass and function, this study examined these factors as a function of sex and age in Irish adults aged 18-90 years comprising The National Adult Nutrition Survey (NANS).

Methods: *n*=1051 (males, *n*=523; females, *n*=528) undertook a four-day semi-weighed food diary. Total, body mass relative intake, and percentage contribution to total energy intake of dietary protein were determined in addition to protein distribution scores (PDS), and contribution of food groups, animal- and plant-based foods to total protein intake.

Results: Total and relative protein intake were highest in those aged 18-35 y (96±3g per day, 1.32±0.40g/kg/d), with lower protein intakes with increasing age i.e. in adults aged ≥65 y (82±22g, 1.15±0.34g/kg/d, $P<0.001$ for both). Differences in protein intake between age groups were more pronounced in males compared to females. Protein distribution followed a skewed pattern for all age groups (breakfast, 15±10g; lunch, 30±15g; dinner, 44±17g). Animal-based foods were the dominant protein source within the diet (63±11% vs. 37±11% plant protein, $P<0.001$).

Conclusions: Protein intake and the number of meals reaching the purported threshold for maximising postprandial anabolism were highest in young, and lower with increasing age. For main meals, breakfast provided the lowest quantity of protein across all age categories, and may represent an opportunity for improving protein distribution, whereas in older adults, increasing the number of meals reaching the anabolic threshold regardless of distribution pattern may be more appropriate.

KEYWORDS

ageing; animal; diet record; nutrition survey; plant;

INTRODUCTION

The age-related decline in skeletal muscle mass and function (e.g. gait speed, strength) is a fundamental threat to healthy ageing, and maintaining independence and functional capacity in later life [(1)]. Lifestyle factors including physical activity and diet are important determinants of the rate of decline, which for the latter has recently focussed on habitual protein intake, pattern and sources [(2-5)]. The European Food Safety Authority (EFSA) population reference intake (PRI) for dietary protein is currently set at 0.83 g of protein per kg body mass per day (g/kg/d) [(6)]. This is a recommended intake based around the criterion of nutrition adequacy, but re-evaluation of these guidelines now suggests as much ~1.2 g/kg protein per day [(7)]. This is similar to recommendations for protein intake in older adults for the maintenance of lean body mass (LBM) and physical function [(8)].

Ingestion of dietary protein acts as an anabolic stimulus by resulting in the stimulation of muscle protein synthesis (MPS) [(9-11)]. The control of skeletal muscle mass explained by temporal fluctuations in MPS and muscle protein breakdown (MPB) in response to nutrient intake, exercise and inactivity ultimately dictating the net gain or loss of human muscle protein [(12)]. If each eating occasion (EO) is considered as an opportunity to increase rates of MPS, the cumulative magnitude and duration of postprandial MPS after the ingestion of each protein-containing meal determines the daily and long-term likelihood of a positive net protein balance [(2-5)]. Therefore, targeting 'per meal' protein recommendations is emerging as a potential strategy to favour maintenance or accretion of skeletal muscle mass.

Protein ingestion of 0.24g/kg and 0.4g/kg body mass in young and old, respectively, maximises MPS after a single meal [(9,10,13,14)]. These doses are considered the threshold to be met or exceeded in order to maximally stimulate anabolism in skeletal muscle, and therefore often translated as a recommendation of an "anabolic threshold" of 20 to 30g protein per EO. However, daily protein intake in Western societies usually follows a 'skewed' pattern of distribution in which protein intake is highest at dinner, but lower, and often below the anabolic threshold, at other meals and snacks [(15-19)]. The larger protein dose at dinner is unlikely to provide an additional anabolic response in skeletal muscle above that achieved by 20 to 30g of high quality protein [(9,10)], and the resultant lower protein intakes at breakfast, lunch and snack times are likely to be suboptimal protein intakes for maximising MPS [(9,10,13,14)]. Importantly, a skewed protein distribution pattern has been associated with an increase in incidence of frailty [(20)], while the daily consumption of one or two main meals over the 30g protein per meal threshold is positively associated with greater LBM and strength in older adults [(21)].

Whether such observations are due to the protein distribution pattern, or the number of EOs in a day that exceed an anabolic threshold regardless of distribution pattern, remains to be fully established. For example, modulating protein distribution to provide an even protein intake at each meal, for example ≥20g high quality protein ingested at three main meals, results in higher rates of

MPS over 12 h in young men and women [22,23]. This effect would, in theory, result in a greater cumulative anabolic response over an extended period of time compared to a skewed pattern of protein intake. However, when examined in older adults, rates of MPS do not differ between even compared skewed patterns of protein distribution [24-26]. One potential explanation for discrepant findings between young and old is that an even distribution of protein intake for older adults means that each EO then fails to reach the higher anabolic threshold that is required in this population [4,27].

The source of dietary protein is also of relevance, since animal proteins produce a greater postprandial anabolic response per equivalent dose of protein when compared to plant-based proteins [28-32]. The greater anabolic response is most likely due to the higher essential amino acid (EAA) content, and in particular leucine, of animal-based protein sources [33,34].

Habitual dietary protein intake, protein distribution patterns and dietary sources in Irish adults remains underexplored. The National Adult Nutrition Survey (NANS) investigated habitual food and beverage consumption, lifestyle and health indicators in 1500 adults aged 18-90 years in the Republic of Ireland between 2008 and 2010. The series of interrelated databases, which has been compiled from the data collected in this survey, provide the most complete and up-to-date collection of food consumption data available for adults in Ireland, therefore offering valuable information about the protein intake pattern across sex and ages. The aim of this current study was to examine habitual dietary protein intakes, the patterns of protein distribution and dietary sources of protein in Irish adults and explore whether these habits are influenced by age and sex.

METHODS

Study population

This study is based on secondary analysis of National Adult Survey (NANS), a cross-sectional food consumption survey in Irish adults. The surveys were carried out by the Irish Universities Nutrition Alliance (IUNA; www.iuna.net) in a sample of 1500 free-living adults aged 18-90 years (males, *n*=740; females, *n*=760), in the Republic of Ireland between 2008 and 2010. Ethical approval was obtained from University College Cork Clinical Research Ethics Committee of the Cork Teaching Hospitals and the Human Ethics Research Committee of University College Dublin [ECM 3 (p) 4 September 2008]. Written consent was obtained from all participants in accordance with the *Declaration of Helsinki.* Respondents were randomly selected from a database of names and address from Data Ireland (An Post). Exclusion criteria include pregnancy, lactation and inability to complete the survey due to disability. The final survey response rate was 59.6%. The final sample was representative of the Irish population with respect to sex, age, location, social class and geographical location when compared to the most recent Irish census at that time (2007).

Primary anthropometric measures and dietary assessment

Anthropometric measurements were carried out by the researcher in the respondent’s home. Body mass (kg), fat-free mass (kg), fat mass (kg) and percentage body fat were assessed by bioelectrical impedance analysis using a Tanita BC-420MA Body Composition Analyzer (Tanita Corporation, Tokyo, Japan), with measurements made in adherence with the manufacturer’s guidelines at a measurement frequency of 50 Hz. Body composition estimates are derived from total body water based on segmental resistance index, and making use of proprietary equations developed by the manufacturer. Height was assessed using a Leicester portable height measure to the nearest 0.1cm. Body Mass Index (BMI) was calculated as body mass (kg) divided by height (m^2). A four-day semi-weighed food diary, at brand level where possible, was used to collect food, beverage and supplement intake. Participants were asked to report at least one weekend day. The researchers made three visits to the respondent’s homes during the four days: A visit to demonstrate how to use a food weighing scales and log the food diary; a second visit to review the diary 24-36 hours into the recording process; and a final visit 1-2 days after the recording period to review the last recording days and collect the diary. Food and beverage consumption was quantified using a food weighing scales (46%), a photographic food atlas (16%), a food portion size guide (11%), household measurements such as teaspoons, tablespoons, etc. (11%), manufacturers weights (10%), IUNA weight guide (4%) and an estimate made by the researcher (2%). Food and beverage intake was assessed using WISP version 3.0 (Tinuviel Software, Anglesey, UK). This analysis was based on data from the McCance and Widdowson’s, The Composition of Foods, 5th and 6th Editions, as well as nine supplementary volumes. Modifications to the food composition database was also performed to include commonly consumed Irish foods. The anthropometric and dietary assessment carried out is described in further detail elsewhere [35-37]. The final food database comprised of 133,068 rows of data, with each row representing each food or beverage item at every EO throughout the four days of recording.

Secondary data analysis

This secondary analysis was carried out using SPSS (IBM SPSS Statistics Version 24). Respondents who reported an energy intake <1.1 of basal metabolic rate (BMR) [38] were determined as under-reporters (*n*=449) and were excluded from the present analysis. The final sample size was *n*=1051 (males, *n*=523; females, *n*=528). New variables were computed to determine relative macronutrient intake on a gram per kg basis (g/kg). Protein Distribution Scores (PDS) [39] were calculated for the following: PDS^{20}, PDS^{30}, $\text{PDS}^{0.24g/kg}$, $\text{PDS}^{0.3g/kg}$, $\text{PDS}^{0.4g/kg}$. PDS^{20} and PDS^{30} represents the number of EOs per day containing greater than 20g and 30g of protein, respectively, averaged across the four days. $\text{PDS}^{0.24g/kg}$, $\text{PDS}^{0.3g/kg}$, $\text{PDS}^{0.4g/kg}$ represents the number of EOs per day containing greater than 0.24g/kg, 0.3g/kg and 0.4g/kg body mass of protein, averaged over the 4 days. PDS is a scoring system adapted from MacKenzie et al. (2015)[39] with the 0.24g/kg, 0.3g/kg and

0.4g/kg values being representative of the recommended per meal protein target to maximise MPS in young [9,10] and old [13,14]. 2,048 pre-existing food codes were aggregated into 16 food groups based on foods of similar type and protein content. The percentage contribution of these food groups to total protein intake was determined. These foods codes were further aggregated into two broad groupings described as either animal- or plant-based foods based on observation of the principal contributing protein source. The total and percentage contribution of animal- and plant-based foods to total protein intake, as well as per meal protein intake, was determined.

In general, the distribution of the data approximated normality, or was transformed as appropriate to approximate normality to allow detection of significant differences between groups using a two-way ANOVA allowing for main and interaction effects of sex*age, in which four age groups were created (18-35 y, 36-50 y, 51-64 y and ≥65 y). When main effects were indicated, post-hoc analysis using multiple comparisons with Bonferroni's adjustment was used to assess the differences between age groups. Statistical significance was accepted at $P<0.05$.

RESULTS

Anthropometric measures

Anthropometric measures for the total population, each age group 18-35 y, 36-50 y, 51-64 y and ≥65 y, and males and females are presented in Supplemental Table 1. There was a sex*age interaction for body fat, body fat percentage and waist-to-hip ratio (WTHR) ($P<0.01$ for all), in which there were greater differences between ages 18-35 y and 36-50 y in males, compared to females, who had smaller differences between the age ranges. There were main effects for sex for all anthropometric measures ($P<0.01$ for all). Males tended to have higher measures in height, body mass, BMI, WTHR and fat-free mass, compared to females, while females tended to have higher values than males for body fat percentage and fat mass (Supplemental Table 1).

There were main effects for age for differences in all anthropometric measures ($P<0.001$ for all). Body mass, BMI, WTHR, body fat percentage, fat mass tended to be greater with increasing age. However, there was a lower body mass in adults aged ≥65 y compared to 51-64 y ($P<0.01$). Height and fat-free mass tended to be lower with increasing age. However, for height, fat mass and fat-free mass, there was no significant difference between adults aged 35-50 y and 51-64 y (Supplemental Table 1).

Energy and macronutrient intakes

Average total daily energy, protein, carbohydrates and fat; and percentage of total energy intake for each macronutrient are presented in Supplemental Table 2, with energy and macronutrient intake relative to body mass, and expressed in gram/kilogram body mass per day (g/kg), presented in Table 1. There was a sex*age interaction for total energy, protein (g/d) and relative protein intake

(g/kg)(*P*<0.01 for all). Energy intake was greater in males between ages 35-50 y compared to 51-64 y, while intake in females in these age categories were similar. Total and relative protein intake in males was greatest in young, and was lower with increasing age, while protein intake was similar between age groups for females. There were main effects for sex for total and relative calorie and macronutrient intakes (*P*<0.001 for all). There was a tendency for males to have higher intakes compared to females for all total and relative energy and macronutrient intakes.

There were main effects for age for differences in all total and relative calorie and macronutrient intakes (*P*<0.001 for all). Total energy, protein, carbohydrate and fat intakes were greatest in young, and tended to be lower with increasing age. However, there was no significant difference between intakes for total energy and macronutrient intakes in adults aged 35-50 y and 51-64 y. Relative protein, carbohydrate and fat intake, were greater in adults aged 18-35 y compared to 36-50 y, 51-64 y and ≥65 y (*P*<0.01 for all) (Table 1). Total protein intake in adults aged ≥65 y was 81.6±22.3g/d, which was significantly lower than that of adults aged 18-35 y (96.1±32.4g/d, *P*<0.001) (Supplemental Table 2). Relative protein intake in adults aged ≥65 y was 1.15±0.34g/k/d, which was significantly lower than intakes in adults aged 18-35 y (1.32±0.40g/kg/d, *P*<0.001) (Table 1).

Protein distribution scores

Protein distribution scores PDS^{20}, PDS^{30}, $PDS^{0.24g/kg}$, $PDS^{0.3g/kg}$, $PDS^{0.4g/kg}$ representing the number of EOs per day containing, respectively, over 20g, 30g, 0.24g/kg, 0.3g/kg and 0.4g/kg of protein, are presented in Table 2. There was a sex*age interaction for PDS^{20}, PDS^{30} and $PDS^{0.3g/kg}$ (*P*<0.05), in which there were greater differences between age groups for males, compared to females. There were main effects for sex for PDS^{20}, PDS^{30}, $PDS^{0.24g/kg}$ $PDS^{0.3g/kg}$ and $PDS^{0.4g/kg}$ (*P*<0.05 for all) in which males had higher scores than females for all PDS scores.

There were main effects for age in PDS^{20}, PDS^{30}, and $PDS^{0.4g/kg}$ and $PDS^{0.3g/kg}$ (*P*<0.01 for all). This revealed that the daily number of meals reaching these thresholds was lower with increasing age. However, for PDS^{30}, $PDS^{0.3g/kg}$ and $PDS^{0.4g/kg}$ there was no difference between ages 51-64 y and ≥65 y (Table 3).

Protein distribution represented by the average per meal protein intake, and the relative contribution of animal- and plant-based protein to total per meal protein intake are presented in Figure 1. Protein distribution followed a skewed pattern for all age groups (breakfast, 15±10g; lunch, 30±15g; dinner, 44±17g).

Contribution of food source to total calorie and protein intake

The contribution of animal- and plant-based proteins to overall protein and energy intake are presented in Table 3. There was a sex*age interaction for animal protein only (*P*<0.001) in which there were greater difference between age groups for males, compared to females. There were main effects for sex for all variables in Table 3 (*P*<0.05 for all), in which females tended to have a higher

percentage of protein and energy from plant-based foods compared to males, while males had a higher percentage of protein from animal-based foods compared to females. There were main effects for age for all variables in Table 3 ($P<0.05$ for all), in which total animal- and plant-based protein intake showed lower intakes with increasing age.

Percentage protein intake from animal-based protein was significantly greater in adults aged ≥65 y, while percentage protein intake from plant-based protein was significantly lower in adults aged ≥65 y (Table 3). The percentage contribution of sixteen food groups to total protein intake across each age group is presented in Figure 2. The composition of these food groups is described in Supplemental Table 3. In each age group, meat, dairy and breads were the predominant protein sources, accounting for 40±15%, 15±9% and 12±6% in the total population, respectively.

DISCUSSION

The daily intake, per meal dose, distribution pattern and food source of dietary protein are critical determinants of the anabolic response to protein-containing meals, and potentially influence skeletal muscle health across the life course. The present study identifies that, in this Irish adult cohort, total protein intake, and protein intake relative to body mass are generally lower with increasing age, with males typically consuming more protein than females, and greater difference apparent between age groups for males. The average number of meals per day reaching purported per meal protein thresholds to maximise MPS is typically lower with increasing age, but with males achieving a higher PDS score compared to females across age groups. Protein distribution across the day follows a skewed pattern across each age group, in which dinner represents the highest per meal protein intake, followed by lunch and breakfast. Animal-based foods are the dominant source of protein with meat and dairy having the largest contribution to total protein intake in all age groups, but plant-based protein sources are the predominant protein source at breakfast.

The National Adult Nutrition Survey (NANS) from which the present data set is derived was a cross-sectional food survey completed between 2008 and 2010 by the Irish Universities Nutrition Alliance. The sample was representative of the Irish population with respect to sex, age, urban–rural continuum, and social class as per the 2006 Irish census. Previous outputs have focussed on dietary factors such as vitamin D, folate, vitamin B12 and whole grain intakes across the population [35-37], whereas the present study focussed on the various aspects of habitual protein intake patterns.

In this Irish adult cohort, daily total and relative protein intakes are lower in older adults compared to younger adults, by an average of ~13% in terms of relative protein intake. For example, adults aged 18-35 y typically consume 96±3g (or 1.32±0.40g/kg) per day, while adults aged ≥65 y consume less at 82±22g (or 1.15±0.34g/kg). These averages exceed the EFSA PRI for dietary protein of 0.83 g/kg/d [6], a recommended intake based on nutrition adequacy, but are closer to intakes ~50%

higher per day at ~1.2 g/kg/d of protein proposed by authors focussed on optimal intakes for healthy ageing [7,8]. Lower daily protein intake with increasing age has been reported elsewhere in Western populations [15,40,41]. For example, in the USA, the National Health and Nutrition Examination Survey (NHANES) 2003-3004 reported daily protein intake in young adults aged 19-30 years was 91±22g/d (or 1.3g±0.4g/kg; *n*=874), and in older adults aged >71 years was 66±17g/d (or 1.0±0.3g/kg; *n*=818) [40]. Despite the lower total and relative protein intake with increasing age, because energy intake was lower with each age group increment, the percentage contribution of protein to total energy intake was higher with increasing age. Energy intake is positively correlated with protein intake in older adults, and therefore daily energy intake is an important determinant of habitual protein intake [15]. The decline in energy intake and increase in percentage contribution of protein have both been previously observed in a European population [41]. Several factors are likely to contribute to reduction in energy intake in older adults including a decrease in appetite with age, the higher cost of more nutrient-dense foods, difficulty chewing fibrous foods, perceived food intolerances and fear of eating excessive fat and cholesterol in foods [42-44]. However, the absence of difference between intakes for total energy, carbohydrate, protein and fat in adults aged 35-50 y and 51-64 y suggests that adults in these middle age groups tend to eat a similar overall energy and macronutrient profile.

In comparing by sex, Irish male adults typically consume more protein, by an average of ~11% in terms of relative protein intake, than females across the whole population (105±27g and 1.31±0.36g/kg vs. 76±19g and 1.16±0.33g/kg, respectively). This sex effect is similar to the Italian national food consumption survey, which reported that males aged 18-65 y consumed 93±25 protein per day, and females consumed 76±20 protein per day [45]. However, in the present study, there was sex*age interaction effect for total and relative protein intakes in which males had lower protein intakes with each increment in age category, while there was no difference in protein intake between females aged 35-50 y and 51-64 y. This lack of difference may be related to an increase in dairy intake, which is often promoted by healthcare practitioners as females approach peri-menopausal age as a prophylactic for osteoporosis [46].

The protein distribution pattern over the course of a typical day is a key determinant of cumulative rates of MPS [2-5]. Furthermore, there is an increased recognition of importance of per meal protein recommendations, with 20 to 30g of high quality protein, or 0.24-0.40g/kg protein per meal, proposed as the dose needed to maximise MPS from young to old [9,10,13,14]. The general pattern of protein intake across this Irish adult cohort was skewed as evidenced by intakes of 15±10g, 30±15 and 44±17g at breakfast, lunch and dinner, respectively. A skewed pattern of protein intake has been widely-reported elsewhere in Western societies [15-21]. For example, in the adults aged ≥65 y, breakfast, lunch and dinner accounted for 15%, 29% and 37% of average daily protein intake, which is similar to previous findings in a similar aged community-dwelling cohort, in which these meals

accounted for 16%, 31% and 38% of total protein intake respectively [15]. While similar in protein distribution, per meal protein intake in the present study is more optimal, as both lunch and dinner potentially meet the 20 to 30g threshold (15±7g, 29±14g and 37±16g at breakfast, lunch and dinner, respectively), due to a higher overall protein intake (82±22 vs. 71±18g/d) compared to the previous work [15]. However, inevitably due to the large variability in protein intake for lunch and dinner, there are people who still fell below these protein thresholds for those meal times. Regardless, breakfast in the present study was representative of a suboptimal per meal protein intake, which is a worthy consideration when designing strategies to minimise the decline in muscle mass with age. Indeed, a nutrition intervention targeting supplemental dairy protein intake at breakfast and lunch (0.17g/kg protein/~12g protein per meal) was successful in increasing in LBM over 24 weeks in Irish adults aged 50-70 y even in the absence of exercise training [47].

While the distribution of protein across the day provides a useful perspective, the PDS has been proposed as an objective measure to quantify protein distribution based on per meal thresholds [39]. The PDS describes the number of EOs per day where protein intake meets or exceeds a pre-defined threshold, primarily with a focus on maximising the anabolic response in skeletal muscle to an EO. PDS^{20} and PDS^{30} (the number of EOs ≥20g or ≥30g per day, respectively) were generally lower with increasing age. For example, adults aged 18-35 y had 1.73±0.60 EOs containing 20g of protein, whereas adults aged ≥65 y had less EOs providing ≥20g protein (1.45±0.51). Mirroring the trend for daily protein intake, there were greater differences in PDS^{20}, PDS^{30} and $PDS^{0.3g/kg}$ between age groups for males compared to females, who showed smaller differences between age groups. Given that protein intake relative to body mass is the most appropriate metric for age-related comparisons [14], PDS was determined on per meal protein intake based on EOs reaching 0.24g/kg, 0.3g/kg and 0.4g/kg. There was no difference in the number of EOs reaching 0.24g/kg protein across age groups, which averaged ~1.8 EOs per day in the whole cohort. At the threshold of 0.4g/kg per EO, young adults aged 18-35 y had a greater number of EOs (1.19±0.51) than all other age groups. In fact, for $PDS^{0.4g/kg}$ many adults aged ≥65 y failed to meet or exceed this threshold even once on a daily basis as evidenced by a group average of 0.98±0.43 EOs. In contrast to the present findings, in community-dwelling adults aged >70 y in United Kingdom, adults meeting the 0.4g/kg protein per meal threshold for eating EO 1, 2 and 3 was 3%, 42% and 68% [48]. These are different protein distribution trends to that found in the present analysis. However, that work [48] assessed a cohort of *n*=38, while the present analysis of ≥65 y was n=144, and was a nationally representative survey, such that the dietary intakes reported therein are less likely to be representative of intakes for the entire older population.

In any case, the patterns of protein distribution and/or the numbers of meals reaching an age-appropriate anabolic threshold in this Irish cohort are representative of patterns that are suboptimal

compared to recent guidelines for healthy ageing [2-5]. The potential importance of an even protein distribution as an influence on skeletal muscle size and function has been demonstrated. For example, in older adults while there was no difference frailty status (frail, pre-frail and non-frail) for total protein intake, frail participants tended to have a more uneven protein distribution, with a lower intake at breakfast and higher at lunch (11.9% and 61.4% of total protein consumed at breakfast and lunch in frail), whereas more evenly distributed pattern of protein intake was prevalent in the non-frail older adults [20]. Furthermore, there is a positive correlation between per meal protein with muscle size and function [21]. Based on data from NHANES 1999-2001, middle-to-older aged respondents who consumed one or two main meals over the 30g protein per meal threshold had greater LBM and strength. Indeed, there was a positive dose-response relationship between protein meal thresholds (15g/meal, 20g/meal, 25g/meal) and leg LBM and strength, with this association plateauing at 30g/meal when two meals met this threshold and plateauing at 45g/meal when one meal met this threshold [21]. These data support the notion that a more evenly-distributed protein intake, one in which per meal anabolic thresholds are met, could be more favourable for supporting healthy ageing by augmenting or maintaining LBM and strength.

The source of protein (animal vs. plant) was the final focus of the present study, since animal-based proteins have been reported to elicit a greater postprandial increase in MPS [28-32]. In the present analysis of Irish adults intakes, dietary protein is derived predominantly from animal sources (animal protein, 63.1±10.8% vs. plant protein, 36.9±10.8%). Meat (39.8±14.5%) and dairy (15.0±8.5%) had the largest percentage contribution to total protein intake across both sexes and all age groups. This is similar to findings in an older Dutch population, in which 60% of dietary protein consumed originated from animal sources, with meat and dairy as dominant sources [15], and ~70% protein intake from animal protein and ~30% from plant protein, in adults aged 29-86 y in the USA [49].

When assessed on a per meal basis, lunch (63±19%) and dinner (72±16%) had the greatest animal protein contribution, whereas breakfast was predominantly plant protein (57±22%). The divergent sources at morning versus afternoon and evening EOs have been reported in both Dutch elderly and athletes [15,19], and were quantitatively similar to the present study. Breakfast, therefore, is likely to contain a lower EAA content compared to lunch and dinner [33,34], and combined with the lower total protein intake at breakfast described above, indicates a clear opportunity for improvement. Emphasising adequate, high quality protein at breakfast is a necessary consideration when applying the per meal protein targets in practice across the life course [33,34]. That said, protein sources may differentially affect LBM compared to strength as in cross-sectional data, animal protein intake in associated with the former and plant protein intake is associated with the latter [49]. However, consideration must also be given to the ever-growing ecological debate around sustainable diets, and in particular whether the emphasis on animal sources of protein will present greater environmental

impacts compared to plant sources of protein. Exploring alternative protein sources and transitioning towards greater contribution of plant protein may be encouraged on the assumption that these approaches have less environmental impact, are more sustainable, and satisfy nutrition requirements in relevant populations [2,34]. That said, in older adults a priority could be placed on animal-based protein sources because when equal but small portions of animal or plant-based proteins sources are consumed, animal sources on average contain more protein per calorie than a similar portion of a plant source of protein [33,34].

Moreover, recommendations to increase protein intake at breakfast should not be taken out of context, and applied in a manner that may compromise the overall health of an individual's diet. For example, the consumption of highly processed meats, which are associated with an increased risk of heart disease, stroke, diabetes mellitus and cancers [50,51], should continue to be discouraged. Similarly, consideration of the age-appropriateness of strategies is warranted. Unlike in young adults [22,23], an even distribution of protein does not increase short-term MPS in older adults when compared to a skewed distribution [24-26]. Because of the generally lower daily intake of protein, and higher anabolic threshold in older adults, prescribing an even distribution of protein may paradoxically result in less EOs per day reaching the anabolic threshold for this population [4,27]. The aforementioned data indicate that the average number of EOs that meet or exceed the 0.4 g/kg threshold is <1 in those aged >65 y in this Irish cohort. Advising this age group to increase the frequency of meals that exceed this threshold may be more appropriate than focussing on achieving an even distribution pattern throughout the day. Therefore, a more evenly-distributed protein intake may be a favourable strategy for the maintenance of LBM and strength in early-to-middle age, which in turn would confer benefits to skeletal muscle health across the life course, but may not be optimal as an intervention strategy in older age. The feasibility of these potential strategies in older adults needs to be assessed, in particular whether higher protein intakes at certain eating occasions will influence subsequent food intake, and whether higher protein diets are feasible because of reduced appetite, dislike of certain protein-dense foods, or the inability to masticate protein-rich foods such as meat [4,27].

In conclusion, this study is the first to report habitual protein intake patterns in Irish adults using data from the national food survey, NANS. The trends for daily protein intake, protein distribution pattern and dietary sources of protein are broadly similar to those reported by European and north American food surveys. While daily protein intake is adequate to satisfy population reference intake guidelines, the number of EOs reaching purported threshold for maximising postprandial anabolism is lower with increasing age, and given the commensurate distribution pattern, is likely to be suboptimal for healthy ageing for skeletal muscle health. For main meals, breakfast provided the lowest quantity of protein across all age categories, and is the lowest animal protein-containing main meal. Since both protein dose and protein source strongly dictate the postprandial

anabolic response to a meal, breakfast may represent an opportunity for improving overall protein intake, and protein distribution. Alternatively, in older adults increasing the number of meals reaching an age-appropriate anabolic threshold, regardless of distribution pattern, may be a more efficacious strategy. These divergent, albeit related, approaches are important considerations both for public health strategies that would target age-associated declines in skeletal muscle mass and function, and for future research in the same paradigm.

TRANSPARENCY DECLARATION

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted.

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Table 1. Overview of energy and macronutrient intake relative to body mass (grams per kilogram body mass) for all participants, across sex and age groups.

		All Ages	18-35 y	36-50 y	51-64 y	≥65 y	*P* Value		
		mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	Sex	Age	S*A
Energy intake (kcal/kg/day)	All	30.7±7.6	34.0±8.1[bcd]	29.8±6.3[ad]	28.8±6.2[a]	27.0±7.6[ab]	<0.001	<0.001	0.571
	Males	32.6±7.6	35.8±7.7	31.4±6.1	30.0±6.2	28.6±7.7			
	Females	28.9±7.3	31.8±8.0	28.3±6.1	27.7±6.1	25.8±7.3			
Protein (g/kg/day)	All	1.23±0.35	1.32±0.40[bcd]	1.21±0.31[a]	1.18±0.30[a]	1.15±0.34[a]	<0.001	<0.001	0.01
	Males	1.31±0.36	1.43±0.42	1.28±0.27	1.20±0.30	1.16±0.32			
	Females	1.16±0.33	1.18±0.33	1.15±0.33	1.15±0.31	1.13±0.35			
Carbohydrate (g/kg/day)	All	3.49±0.98	3.78±1.01[bcd]	3.39±0.92[a]	3.35±0.92[a]	3.16±0.94[a]	<0.001	<0.001	0.86
	Males	3.65±1.03	3.93±1.03	3.53±1.01	3.46±0.93	3.29±1.00			
	Females	3.34±0.91	3.59±0.95	3.27±0.81	3.25±0.91	3.06±0.89			
Fat (g/kg/day)	All	1.17±0.37	1.28±0.37[bcd]	1.14±0.32[a]	1.09±0.34[a]	1.06±0.43[a]	0.001	<0.001	0.944
	Males	1.22±0.39	1.31±0.38	1.19±0.34	1.13±0.36	1.12±0.47			
	Females	1.13±0.35	1.24±0.37	1.10±0.31	1.06±0.31	1.01±0.39			

Values are expressed as mean±standard deviation. *n* =964 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=356; 279;185;144]; males, *n*=475 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=195; 128; 88; 64]; females, *n*=489 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=161; 151; 97; 80]. A two-way ANOVA was performed (Sex*Age). When $P<0.05$, post-hoc analysis with Bonferroni's adjustment was used to determine where differences existed between age groups. Difference existing between age groups is indicated by: [a] $P<0.05$ vs. 18-35 years; by [b] $P<0.05$ vs. 36-50 years; [c] $P<0.05$ vs. 51-64 years; [d] $P<0.05$ vs. ≥65 years.

Table 2: Protein Distribution Scores (PDS) for all participants, across sex and age groups.

		All Ages	18-35 y	36-50 y	51-64 y	≥65 y		*P* Value	
		mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	Sex	Age	S*A
PDS (20g)*	All	1.64±0.57	1.73±0.60[cd]	1.64±0.57[d]	1.61±0.51[ad]	1.45±0.51[abc]	<0.001	<0.001	0.003
	Males	1.88±0.56	2.01±0.55	1.91±0.54	1.79±0.51	1.62±0.56			
	Females	1.39±0.47	1.40±0.48	1.41±0.49	1.45±0.46	1.30±0.41			
PDS (30g)*	All	1.08±0.51	1.21±0.57[bcd]	1.05±0.51[a]	0.98±0.43[a]	0.96±0.42[a]	<0.001	<0.001	0.002
	Males	1.34±0.49	1.48±0.52	1.35±0.47	1.21±0.38	1.13±0.45			
	Females	0.82±0.39	0.87±0.42	0.79±0.38	0.78±0.37	0.81±0.33			
PDS (0.24g/kg)**	All	1.81±0.56	1.86±0.55	1.81±0.54	1.76±0.56	1.73±0.60	0.02	0.105	0.057
	Males	1.89±0.54	1.97±0.55	1.91±0.50	1.75±0.50	1.77±0.59			
	Females	1.73±0.57	1.74±0.53	1.72±0.56	1.78±0.61	1.70±0.61			
PDS (0.3g/kg)**	All	1.47±0.52	1.55±0.53[cd]	1.45±0.50	1.42±0.49[a]	1.39±0.53[a]	<0.001	0.005	0.015
	Males	1.56±0.51	1.67±0.52	1.55±0.46	1.42±0.44	1.41±0.53			
	Females	1.39±0.51	1.41±0.49	1.37±0.51	1.41±0.53	1.37±0.52			
PDS (0.4g/kg)**	All	1.07±0.47	1.19±0.51[bcd]	1.02±0.44[a]	0.98±0.41[a]	0.98±0.43[a]	<0.001	<0.001	0.493
	Males	1.17±0.46	1.28±0.52	1.15±0.40	1.05±0.39	1.05±0.41			
	Females	0.97±0.45	1.07±0.47	0.91±0.45	0.92±0.42	0.93±0.43			

Values are expressed as mean±standard deviation. PDS^{20} and PDS^{30} represent the number of eating occasions per day containing over 20g and 30g of protein, averaged across the 4 days. $PDS^{0.24g/kg}$, $PDS^{0.3g/kg}$, $PDS^{0.4g/kg}$ represent the number of eating occasions per day containing over the 0.24g/kg, 0.3g/kg and 0.4g/kg body mass of protein, averaged over the 4 days. **n* values, total populations, *n* =1051 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=377; 308; 204; 162]; males, *n*=523 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=207; 143; 98; 75]; females, *n*=528 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=170; 165; 106; 87]. ***n* values, total populations, *n* =964 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=356; 279;185;144]; males, *n*=475 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=195; 128; 88; 64]; females, *n*=489 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=161; 151; 97; 80]. A two-way ANOVA was performed (Sex*Age). When $P<0.05$, post-hoc analysis with Bonferroni's adjustment was used to determine where differences existed between age groups. Difference existing between age groups is indicated by: [a] $P<0.05$ vs. 18-35 years; by [b] $P<0.05$ vs. 36-50 years; [c] $P<0.05$ vs. 51-64 years; [d] $P<0.05$ vs. ≥65 years.

Table 3: Percentage (%) and total (g) contribution of animal and plant protein to total protein intake, and percentage contribution (%) of animal and plant foods to total energy intake, for all participants, across sex and age groups.

		All Ages mean±SD	18-35 y mean±SD	36-50 y mean±SD	51-64 y mean±SD	65 y mean±SD	*P* Value Sex	 Age	 S*A
Percentage Protein Contribution	**Animal Protein (%)**								
	All	63±11	63±11[d]	63±11[d]	63±11[d]	66±9	0.013	0.012	0.303
	Males	64±10	64±11	65±10	63±10	65±8			
	Females	62±11	61±12	61±12	62±11	66±9			
	Plant Protein (%)								
	All	37±11	37±11[d]	37±11d	37±11[d]	34±9	0.013	0.012	0.303
	Males	36±10	36±11	35±10	37±10	35±8			
	Females	38±11	39±12	39±12	38±11	34±9			
Total Daily Protein Intake	**Animal Protein (g)**								
	All	57.9±23	61.2±27.1c[d]	57.2±22.2	56.0±18.7[a]	53.9±17.3[a]	<0.001	0.021	<0.001
	Males	68.3±24.5	73.2±28.1	68.8±23.2	64.0±18.9	59.5±19.3			
	Females	47.6±15.5	46.5±16.5	47.1±15.4	48.7±15.3	49.1±13.9			
	Plant Protein (g)								
	All	32.6±12.4	34.9±13.7[bd]	32.4±12.9[ad]	32.4±10.2[d]	27.7±9.2[abc]	<0.001	<0.001	0.042
	Males	36.8±11.9	39.8±12.4	36.2±11.5	36.0±10.9	31.0±10.0			
	Females	28.4±11.4	28.9±12.8	29.2±13.1	29.1±8.2	25.0±7.3			
Percentage Total Energy Contribution	**Animal-Based Foods (%)**								
	All	36±10	33±9[bcd]	36±10[ad]	36±10[ad]	41±10[abc]	0.008	<0.001	0.313
	Males	37±10	35±10	37±10	37±10	41±10			
	Females	35±10	32±8	34±10	35±10	42±10			
	Plant-Based Foods (%)								
	All	64±10	67±9[bcd]	64±10[ad]	64±10[ad]	59±10[abc]	0.008	<0.001	0.313
	Males	63±10	65±10	63±10	63±10	59±10			
	Females	65±10	68±8	66±10	65±10	58±10			

Values are expressed as mean±standard deviation. *n* =1051 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=377; 308; 204; 162]; males, *n*=523 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=207; 143; 98; 75]; females, *n*=528 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=170; 165; 106; 87]. A two-way ANOVA was performed (Sex*Age). When *P*<0.05, post-hoc analysis with Bonferroni's adjustment was used to determine where differences existed between age groups. Difference existing between age groups is indicated by: [a] *P*<0.05 vs. 18-35 years; by [b] *P*<0.05 vs. 36-50 years; [c] *P*<0.05 vs. 51-64 years; [d] *P*<0.05 vs. ≥65 years.

FIGURE LEGENDS

Figure 1. Total protein intake at each eating occasion in males and females aged 18-35 years (A), 36-50 years (B), 51-64 years (C) and ≥65 years (D). Values are expressed as mean±standard deviation.

Figure 2. Percentage contribution of food groups to total protein intake in males and females aged 18-35 years (A), 36-50 years (B), 51-64 years (C) and ≥65 years (D). Values are expressed as mean±standard deviation.

SUPPLEMENTARY DATA

Supplemental Table 1. Anthropometric measures for all participants, across sex and age groups.

		All Ages		**18-35 y**		**36-50 y**		**51-64 y**		**≥65 y**		**P Value**		
		mean±SD	*n*	mean±SD	*n*	mean±SD	*n*	mean±SD	*n*	mean±SD	*n*	Sex	Age	S*A
Height	All	1.69±0.10	*966*	1.73±0.10[bcd]	*357*	1.68±0.09[ad]	*279*	1.68±0.10[ad]	*186*	1.65±0.09[bc]	*144*	<0.001	<0.001	0.224
(m)	Male	1.76±0.07	*477*	1.79±0.07	*195*	1.75±0.07	*128*	1.75±0.08	*89*	1.72±0.07	*65*			
	Female	1.62±0.07	*489*	1.65±0.05	*162*	1.62±0.07	*151*	1.61±0.06	*97*	1.59±0.06	*79*			
Body mass	All	75.1±14.6	*964*	73.7±14.1[bc]	*356*	76.0±14.6[a]	*279*	78.1±16.4[ad]	*185*	72.9±12.2[c]	*144*	<0.001	<0.001	0.270
(kg)	Male	82.6±12.8	*475*	80.2±12.8	*195*	85.1±11.8	*128*	86.5±14.3	*88*	79.6±10.6	*64*			
	Female	67.8±12.2	*489*	65.9±11.4	*161*	68.2±12.1	*151*	70.5±14.4	*97*	67.6±10.7	*80*			
BMI	All	26.2±4.3	*905*	24.5±3.6[bcd]	*341*	26.8±4.2[ac]	*270*	27.9±5.1a[b]	*170*	26.9±3.6[a]	*124*	0.002	<0.001	0.273
(kg/m^2)	Male	26.6±3.9	*441*	24.8±3.4	*186*	27.7±3.6	*123*	28.4±4.2	*78*	27.1±3.3	*54*			
	Female	25.8±4.6	*464*	24.2±3.8	*155*	26.0±4.5	*147*	27.4±5.7	*92*	26.8±3.9	*70*			
WTHR	All	0.87±0.08	*856*	0.84±0.07[bcd]	*321*	0.88±0.08[ac]	*258*	0.91±0.08[ab]	*163*	0.90±0.08[a]	*114*	<0.001	<0.001	<0.001
	Male	0.91±0.08	*408*	0.86±0.07	*168*	0.93±0.07	*116*	0.96±0.07	*75*	0.95±0.07	*49*			
	Female	0.84±0.08	*448*	0.81±0.07	*153*	0.85±0.08	*142*	0.86±0.07	*88*	0.87±0.07	*65*			
Body fat	All	27.9±8.9	*870*	23.3±8.8[bcd]	*339*	29.9±7.8[ac]	*269*	31.7±7.8[ab]	*163*	31.8±6.8[a]	*99*	<0.001	<0.001	0.002
(%)	Male	22.4±7.3	*431*	17.6±5.8	*185*	25.0±6.4	*123*	26.8±6.4	*76*	27.3±4.7	*47*			
	Female	33.2±6.9	*439*	30.0±6.8	*154*	34.1±6.4	*146*	36.0±6.3	*87*	35.9±5.7	*52*			
Fat mass	All	21.3±9.4	*864*	17.2±7.9[bcd]	*336*	23.5±9.9[a]	*268*	25.1±10.0[a]	*161*	23.3±6.3[a]	*99*	<0.001	<0.001	0.003
(kg)	Male	19.5±10.0	*427*	14.6±6.8	*183*	22.8±11.2	*122*	24.6±11.1	*75*	22.5±5.8	*47*			
	Female	23.1±8.4	*437*	20.3±7.9	*153*	24.1±8.6	*146*	25.6±8.8	*86*	24.2±6.7	*52*			
Fat-free mass	All	51.1±10.7	*864*	53.2±11.1[bcd]	*336*	50.3±10.3[ad]	*268*	50.1±10.8[ad]	*161*	47.4±9.5[abc]	*99*	<0.001	<0.001	0.163
(kg)	Male	60.3±7.2	*427*	61.9±7.2	*183*	60.0±6.6	*122*	59.8±7.4	*75*	56.0±6.0	*47*			
	Female	42.1±3.7	*437*	42.9±3.2	*153*	42.3±3.8	*146*	41.7±3.9	*86*	39.7±3.5	*52*			

BMI, body mass index; WTHR, waist-to-hip ratio. Values are expressed as mean±standard deviation. A two-way ANOVA was performed (Sex*Age). When $P<0.05$, post-hoc analysis with Bonferroni's adjustment was used to determine where differences existed between age groups. Difference existing between age groups is indicated by: **[a]** $P<0.05$ vs. 18-35 years; by **[b]** $P<0.05$ vs. 36-50 years; **[c]** $P<0.05$ vs. 51-64 years; **[d]** $P<0.05$ vs. ≥65 years.

Supplemental Table 2. Average energy intake (kcal) and macronutrient intake (g and % of total energy intake) for all participants, across sex and age groups

		All Ages	18-35 y	36-50 y	51-64 y	≥65 y	*P* Value		
		mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	Sex	Age	S*A
Energy intake (kcal/day)	All	2234±623	2457±663^{bcd}	2190±565^{ad}	2157±523^{ad}	1899±555^{abc}	<0.001	<0.001	0.006
	Males	2586±594	2816±556	2552±533	2475±510	2162±625			
	Females	1886±423	2020±501	1876±371	1862±327	1673±359			
Protein (g/day)	All	90.5±27.7	96.1±32.4^{bcd}	89.6±26.2^{ad}	88.4±21.4^{ad}	81.6±22.3^{abc}	<0.001	<0.001	<0.001
	Males	105.2±27.4	113.1±30.5	105.0±24.6	99.9±19.9	90.4±24.7			
	Females	75.9±18.8	75.4±20.2	76.3±19.3	77.8±16.9	74.1±16.7			
Protein intake (% Energy Intake)	All	16.4±3.4	15.7±3.6^{bcd}	16.6±3.4^{ad}	16.7±3.0^{a}	17.6±3.2^{ab}	0.953	<0.001	0.08
	Males	16.5±3.5	16.2±4.0	16.7±3.1	16.4±2.7	17.2±3.5			
	Females	16.4±3.3	15.1±3.0	16.5±3.6	16.9±3.2	17.9±2.9			
Carbohydrate (g/day)	All	252.9±76.6	272.5±80.5^{bcd}	248.1±73.0^{ad}	249.1±71.2^{ad}	221.1±67.5^{abc}	<0.001	<0.001	0.079
	Males	287.9±79.4	308.3±76.5	283.7±78.7	282.6±75.3	246.6±76.5			
	Females	218.2±54.9	228.9±61.6	217.3±50.6	218.1±50.5	199.2±49.3			
Carbohydrate (% Energy Intake)	All	45.6±7.3	44.7±7.3^{d}	45.5±7.4	46±7.0	46.9±7.2^{a}	0.001	0.01	0.963
	Males	44.7±7.9	44.0±7.8	44.5±8.3	46±7.3	46.0±8.0			
	Females	46.4±6.5	45.6±6.5	46.4±6.5	47±6.7	47.7±6.4			
Fat (g)	All	84.9±28.5	91.7±28.3^{bcd}	84.0±27.7^{ad}	82.2±26.2^{ad}	74.3±29.3^{abc}	<0.001	<0.001	0.739
	Males	96.6±30.3	102.4±27.9	96.8±30.1	93.4±28.7	84.5±35.1			
	Females	73.3±20.9	78.7±22.9	72.8±19.5	71.9±18.6	65.5±19.5			
Fat (% Energy Intake)	All	34.2±6.3	33.8±6.1	34.4±6.0	34±6.4	34.9±6.9	0.04	0.367	0.291
	Males	33.5±6.4	32.7±6.2	33.8±5.8	34±6.8	34.7±7.3			
	Females	34.9±6.1	35.0±5.7	34.9±6.1	35±6.1	35.1±6.6			

Values are expressed as mean±standard deviation. N values, total populations, *n* =1051 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=377; 308; 204; 162]; males, *n*=523 [18-35 y; 36-50 y; 51-64 y; ≥65 y, *n*=207; 143; 98; 75]; females, *n*=528 [18-35 y; 36-50 y; 51-64 y; ≥65 y, n=170; 165; 106; 87]. A two-way ANOVA was performed (Sex*Age). When $P<0.05$, post-hoc analysis with Bonferroni's adjustment was used to determine where differences existed between age groups. Difference existing between age groups is indicated by: ^{a} $P<0.05$ vs. 18-35 years; by ^{b} $P<0.05$ vs. 36-50 years; ^{c} $P<0.05$ vs. 51-64 years; ^{d} $P<0.05$ vs. ≥65 years.

Supplementary Table 3. Food groups titles and their composition

Beverages	Beverages
Biscuits, Cakes and Sweets	Biscuits, cakes, sweets, pastries, sugars, confectionary, preserves and savoury snacks
Breads	Breads and rolls
Cereals	Breakfast cereals
Dairy	Milk, yoghurt, cheese, butter, spreading fats, oils, cream, ice-cream and desserts
Eggs	Eggs and egg dishes
Fish	Fish and fish dishes
Fruit and Vegetables	Vegetables, vegetable dishes, fruit, fruit dishes, potatoes and potato dishes
Grains	Grains, rice, pasta and savouries
Meat	Meat and meat products
Other	Soups, sauces, nuts, seeds herbs, spices and miscellaneous foods
Supplements	Nutritional supplements