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# Habitual dietary collagen intake is lower in women and older Irish adults compared to younger men

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**Running title:** Collagen intake in the Irish adult population

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**Keywords:** glycine, proline, hydroxyproline, vitamin C, protein

## Abbreviations

ANCOVA, Analysis of Covariance; COFID, Composition of Foods Integrated Dataset; ECM, Extracellular Matrix; EPAQ2, European Prospective Investigation into Cancer Physical Activity Questionnaire; HC, Hydrolyzed Collagen; IUNA, Irish Universities Nutrition Alliance; MDI, Mean Daily Intake; MET, Metabolic Equivalent of Task; NANS, National Adult Nutrition Survey; NHANES, National Health and Nutrition Examination Survey; PA, Physical Activity; RDA, Recommended Dietary Allowance; TGF β1, growth factor beta 1; $\eta p^2$, partial eta squared, WISP, Weighed Intake Software Program.

# Abstract

**Background:** Collagen ingestion is purported to benefit connective tissues, such as skin, bone, muscle, tendon, and ligament. However, the quantity of collagen intake in the diet of European adults is unknown.

**Objective:** To investigate collagen intake in the habitual diets of Irish adults, and whether it differed according to sex and/or age.

**Methods:** We conducted secondary analysis of the Irish National Adult Nutrition Survey, which assessed typical dietary intake using a four-day food diary in 1,500 adults, aged 18-90 years. We categorized participants into three age groups: young (18-39 years, n=630), middle-aged (40-64 years, n=644), and older (≥65 years, n=226) adults. Collagen composition of each individual food item in the database was determined by applying a percentage collagen value from analytical sources, allowing computation of collagen mean daily intake (MDI), collagen MDI relative to body mass, and collagen/total protein MDI. Differences in intakes between age groups and sexes were evaluated using physical activity level as a covariate.

**Results:** Collagen MDI for the entire population was 3.2±2.0 $g \cdot day^{-1}$, representing 3.6±1.9% total protein intake. Men had higher absolute and relative collagen MDI than women, regardless of age (4.0±2.1 $g \cdot day^{-1}$ vs. 2.3±1.4 $g \cdot day^{-1}$, $p<0.001$), while older adults had lower absolute collagen MDI than middle-aged adults (2.9±1.8 $g \cdot day^{-1}$ vs. 3.3±2.0$g \cdot day^{-1}$, $p=0.021$).

**Conclusions:** Collagen intake in the Irish adult population was considered low (relative to total protein intake and to dose-response studies), particularly in women and older individuals. Increasing daily collagen intake may therefore be warranted to optimise the health of collagen-rich tissues.

# Introduction

Collagen is the most abundant protein in the human body, found in the extracellular matrix (ECM) of connective tissue (e.g., in skin, bones, tendon, ligament and cartilage). Collagen is synthesized by fibroblasts through a process that involves the transcription of collagen genes, translation into pre-procollagen, and extensive post-translational modifications, with vitamin C being a crucial co-factor for proper folding into a triple-helix structure (1). Collagen synthesis decreases with age, while collagen degradation increases simultaneously, leading to reduced dermal volume and elasticity, which can be observed as increased skin wrinkles and slower cutaneous wound healing (2, 3). Moreover, collagen loss with age is associated with bone loss and increased tendon compliance, which are both risk factors for higher fracture rates and fall injuries in older adults (4, 5).

Dietary collagen is rich in glycine, proline, and hydroxyproline, with the latter being exclusive to collagen-containing foods. While collagen can be synthesized endogenously, dietary intake is required to maintain sufficiently high levels of these amino acids, particularly glycine, which may be conditionally essential in humans (6, 7). For ingested collagen to contribute to systemic collagen turnover, it must undergo enzymatic hydrolysis in the gastrointestinal tract, being digested into peptides and free amino acids prior to being absorbed into the blood stream (8). In recent years, there has been significant interest on the effects of collagen intake, with and without exercise, on skin appearance, wound healing, joint pain, exercise recovery, body composition, sleep quality, and muscle and tendon function (9-12). Medium to long-term supplementation with 2.5 to 12 g hydrolyzed collagen (HC) appears to have positive effects on skin ageing by increasing elasticity and hydration, and reducing the appearance of wrinkles in adult women aged 20 – 70 years (13). Additionally, collagen ingestion combined with acute skipping or resistance exercise has been associated with increased markers of collagen synthesis, with doses ranging between 5 and 30 g collagen in young healthy, recreationally trained men and women (14-16). Moreover, 15 to 30 g daily HC supplementation in conjunction with chronic exercise is purported to improve fat-free mass, tendon morphology, and markers of strength in both athletic and untrained adults (17).

These findings indicate positive effects of increasing dietary collagen intake, however, there is no established recommended daily allowance for collagen, and we cannot assume the dietary collagen requirements of adults are the same across the age range and between sexes. Firstly, this is because ageing is associated with collagen loss, especially after 40 years of age (18, 19). Secondly, connective tissues comprising mostly collagen, such as ligament, tendon and bone, all contain estrogen receptors (20-22), resulting in sex-specific collagen turnover (23). Thus, before making recommendations on increasing collagen intake (either via dietary food or supplementation), the amount of collagen ingested in the habitual diets of both men and women across the age range must be documented.

Despite the clear interest in collagen supplements in areas of dermatology, musculoskeletal health, ageing, and sports performance, only one study, to our knowledge, has attempted to estimate collagen intake in habitual diets. Paul et al. (24) estimated that the average daily collagen protein consumption in the 'standard American diet' to be either 3 or 23 $g \cdot day^{-1}$ (based on whether individuals were low or high consumers of processed meat), which was derived from the National Health and Nutrition Examination Survey (NHANES) 2001-2004. However, estimates of collagen in food items were unjustifiably averaged across a limited number of food items (e.g., beef, pork, veal, lamb, and game), which likely confounded the estimations of collagen intake, thus potentially providing erroneous conclusions.

The National Adult Nutrition Survey (NANS) investigated habitual food and beverage consumption in 1,500 adults in Ireland between 2008 and 2010. By conducting secondary analysis of NANS, we determined collagen composition of each individual food item in the database by applying a percentage collagen value from analytical sources, allowing computation of collagen mean daily intake (MDI), relative MDI ($g\cdot kg^{-1}$), and collagen/total protein MDI (%). We categorized participants into young, middle-aged and older adults, and we compared men with women. Thus, the objective of our study was to determine the habitual dietary collagen intake of Irish adults, stratified by age group and sex, using detailed collagen content data from specific food items. In addition, due to vitamin C being an essential component for collagen synthesis (1), we investigated vitamin C intake according to age and sex. Finally, as increased physical activity is associated with a higher metabolic rate and greater energy intake (25), we used habitual physical activity as a covariate in our dietary analyses. Additionally, habitual physical activity (PA) is positively associated with protein intake in older adults across multiple populations (26), suggesting a potential link between PA levels and collagen intake, given that collagen is a specific source of dietary protein. Based on the higher habitual protein intake in younger men versus older men and women (27), we hypothesized that absolute and relative collagen MDI would be lower in older and middle-aged individuals compared to young adults, and lower in women compared to men.

# Methods

**Population**

The current study is a secondary analysis of a cross-sectional food consumption survey among Irish adults (described in detail elsewhere (27)). The original survey was the Irish NANS, conducted by the Irish Universities Nutrition Alliance (IUNA; www.iuna.net). This survey included 1,500 free-living adults aged 18–90 years (740 men and 760 women) residing in the Republic of Ireland between 2008 and 2010. Ethical approval was granted by the University College Cork Clinical Research Ethics Committee of the Cork Teaching Hospitals and the Human Ethics Research Committee of University College Dublin. All participants provided written consent in line with the *Declaration of Helsinki*. Participants were randomly chosen from a database of names and addresses provided by Data Ireland (National Postal Service). An invitation letter and participant information sheet were sent to the homes of potential participants. Participants were excluded if they were pregnant or lactating or were unable to complete the survey due to disability. The survey achieved a response rate of 59.6 %, and the final sample was demographically representative of the Irish population in terms of sex, age, location, social class, and geographic distribution according to the 2006 Irish census. The sample size has previously been demonstrated to be sufficient for detecting dietary intake differences by sex and age in recent secondary analyses of this dataset (27).

**Dietary assessment**

A four-day, food diary (detailed at the product brand level where possible) was employed to record food, beverage, and supplement intake. Participants were required to include at least one weekend day in their recordings. Researchers visited participants' homes three times during the four-day period: the first visit demonstrated how to use a food weighing scales and maintain the food diary; the second visit, 24-36 hours into the recording process, reviewed the diary entries; and the final visit, 1-2 days after the recording period, reviewed the last entries and collected the diary. Food and beverage consumption was quantified using food weighing scales where possible. However, for items that were not weighed, portion sizes were estimated using a photographic food atlas, a food portion size guide, household measurements, manufacturer weights, the IUNA weight guide, and researcher estimates. Nutrient intakes were estimated using WISP, version 3.0 (Tinuviel Software, Anglesey, UK), based on data from McCance and Widdowson’s "The Composition of Foods," 5th and 6th editions and their associated supplementary volumes (28-30). Dietary intake was averaged across the four days to provide mean daily intakes (MDI) for all nutrients of interest.

**Calculation of collagen composition**

The collagen content of food items was determined by applying a percentage weighting from estimated typical values. A database (SPSS v. 29, IBM, Armonk, NY, USA) was created containing all of the 2552 NANS foods consumed, including recipes. Secondly, this database was examined on a food code-by-food code basis, and each food code was assigned a collagen concentration based on analytical data and other published data sources, which are presented in Supplementary Table 1 (31-44). In total, 736 foods were identified to contain collagen. If a food item contained a meat mixture, i.e. more than one meat cut or meat source, then the total collagen for that item was calculated using the following equation, which was modifiable to include additional ingredients as required (31):

$$\frac{(A\times a)+(B\times b)+(C\times c)\ldots}{100}$$

where A, B, C, etc., represent the percentage of each meat cut present, and a, b, c, etc., represent the percentage of collagen in each meat cut. For example, an item containing 15% beef brisket lean (2.56% collagen), and 3% beef fat (5.76% collagen) would be calculated as:

$$\frac{(15\ \times\ 2.56)+(3\ \times\ 5.76)}{100}=\frac{38.4+17.28}{100}=0.56\%\ collagen$$

Collagen values for foods that were prepared whole but contain inedible portions such as bone were then multiplied by their edible conversion factor from the composition of foods integrated dataset (COFID) (45). For database items where the food label was not available, or the item was a meal/multi-ingredient item, e.g. beef lasagna, or chicken korma, the percentage of foods containing collagen was determined by dividing the mass (g) of each respective food containing collagen in the recipe by the sum of the mass (g) of all items in the recipe expressed as a percentage of total mass. Where mixed foods were sandwiches without precise quantities of the constituents, the recipe from the University of London survey of commercial sandwiches was used for all calculations (46). Finally, where collagen content of meat was presented as a percentage of total protein content, rather than a percentage of total weight (e.g. anchovies, bovine liver), the known total protein was multiplied by the collagen percentage to determine the collagen value for that food item.

**Physical activity levels**

Participants completed a validated physical activity questionnaire (EPIC Physical Activity Questionnaire (EPAQ2)) (47) to estimate habitual levels of physical activity. The questionnaire comprised three sections: activity at (i) home, (ii) work and (iii) recreation. To estimate participants' metabolic equivalent of the task (MET) values, we used the EPAQ2 responses to calculate the average MET hours spent per week. MET values were assigned to various activities based on established MET values for each type of activity. The total weekly MET hours were then calculated by summing the MET hours from all three activity domains (home, work, and recreation). Participants were subsequently categorized into three activity levels based on their total weekly MET hours (48): low activity: $< 7.5$ MET hours per week; moderate activity: $\geq 7.5$ to 15 MET hours per week; high activity: $> 15$ MET hours per week.

**Secondary data analysis**

Data were analyzed using the Statistical Package for the Social Sciences (SPSS v. 29, IBM, Armonk, NY, USA) and reported as mean ± standard deviation, with significance accepted at $P < 0.05$. The following new variables were computed and used for the analysis of collagen intake: absolute collagen mean daily intake (MDI) in grams; relative collagen MDI in g/kg (collagen MDI relative to body mass); and collagen/total protein MDI (%) (collagen MDI expressed as a percentage of total protein MDI).

To examine the effects of age-group and sex on collagen intake, participants were assigned to one of two categories for sex (male or female), and one of three categories for age: (i) young (18–39 years, male, n = 331; female, n = 299); (ii) middle-aged (40–64 years, male, n = 303; female, n = 341); and (iii) older (≥ 65 years, male, n = 106; female, n = 120). Data were assessed for normal distribution using visual inspection of Q-Q plots. Most nutrient intake variables approximated normality despite slight tail deviations in Q-Q plots (Supplementary Figure 1), typical of dietary data, and did not require transformation. However, protein intake, and correspondingly, collagen intake, exhibited slightly greater tail deviations in their respective Q-Q plots (Supplementary Figure 1). To ensure robustness, protein and collagen intake data were log-transformed, and the analyses were repeated. The results of these transformed analyses were consistent with those using the non-transformed data, suggesting that the observed effects were not influenced by these deviations. Chi-Square tests of independence were performed to examine the association between age group, sex and physical activity levels. Differences in nutritional intake (i.e. energy, protein, carbohydrate, fat, collagen, vitamin C) between age-group and sex were evaluated using one-way analysis of covariance (ANCOVA), with physical activity (PA) level category (low, moderate, high) incorporated as a covariate. Bonferroni adjustment was used for post-hoc comparisons. Partial eta squared ($\eta_p^2$) was reported as an estimate of effect size for ANCOVA main effects and interaction effects. The thresholds of $\eta_p^2$ are defined as small ($\eta_p^2$ = 0.01) medium ($\eta_p^2$ = 0.06) and large ($\eta_p^2$ = 0.14) (49).

# Results

## Collagen sources dataset

The IUNA dataset contained 2552 unique food codes, of which 28.8% (n = 736) were manually identified by the current investigators (CN) to contain collagen. Food codes contained both individual food items and complete recipes. Excluding food codes that did not contain collagen protein, the collagen composition ($g.100g^{-1}$) of food codes in this database ranged from 0.06 $g.100g^{-1}$ (i.e. soup, chicken, no vegetables) to 5.9 $g.100g^{-1}$ (i.e. bratwurst).

**Habitual collagen intake in Irish adults**

Absolute and relative collagen MDI are displayed in **Figure 1**. The collagen MDI for the entire sample (n = 1338) was 3.2 ± 2.0 $g·day^{-1}$. There was a main effect of sex on collagen MDI ($F_{1, 1331} = 217.042$, $P < 0.001$, $\eta_p^2 = 0.140$), where intake for males (4.0 ± 2.1 $g·day^{-1}$; n = 657) was higher than for females (2.4 ± 1.4 $g·day^{-1}$; mean difference = 1.6 $g·day^{-1}$; $p < 0.001$). There was also a main effect of age on collagen MDI ($F_{2, 1331} = 3.914$, $p = 0.020$, $\eta_p^2 = 0.006$). Collagen MDI was 3.2 ± 2.0 $g·day^{-1}$, 3.3 ± 2.0 $g·day^{-1}$, and 2.9 ± 1.8 $g·day^{-1}$ for young, middle-aged and older adults, respectively. Post-hoc comparisons revealed a mean difference of 0.4 $g·day^{-1}$ between middle-aged and older adults (p = .021), but there were no differences between young and middle-aged (p = 1.000), or between young and older adults (p = .053). There was no interaction between sex and age on collagen MDI ($F_{2, 1331} = 1.021$, $p = 0.360$, $\eta_p^2 = 0.002$), and physical activity level had no influence on the statistical model ($F_{1, 1331} = 0.161$, $P = .689$, $\eta_p^2 = .000$).

With regards to relative collagen MDI (collagen intake relative to body mass), the intake for the entire sample (n = 1338) was 0.05 ± 0.03 $g·kg^{-1}·day^{-1}$. There was a main effect of sex on collagen MDI ($F_{1, 1331} = 70.873$, $p < 0.001$, $\eta_p^2 = 0.052$), with males' intake being higher than for females. Specifically, the males' intake was 0.05 ± 0.03 $g·kg^{-1}·day^{-1}$, while the females' intake was 0.03 ± 0.02 $g·kg^{-1}·day^{-1}$ (mean difference = 0.01 $g·kg^{-1}·day^{-1}$; $p < 0.001$). There was no main effect of age on relative collagen MDI ($F_{2, 1283} = 2.224$, $p = 0.108$, $\eta_p^2 = 0.003$). Intake in young adults was 0.04 ± 0.03 $g·kg^{-1}·day^{-1}$, while intake in both middle-aged and older adults was 0.04 ± 0.02 $g·kg^{-1}·day^{-1}$. There was no interaction between sex and age on relative collagen MDI ($F_{2, 1283} = 0.97$, $p = 0.379$, $\eta_p^2 = 0.002$). Additionally, physical activity level did not influence the model ($F_{1, 1283} = 0.01$, $p = 0.91$, $\eta_p^2 = 0.000$).

FIGURE 1 NEAR HERE

Collagen MDI as a percentage of total daily protein intake is displayed in **Figure 2**. There was no main effect of age ($F_{1,1331} = 1.185$, $p = 0.306$, $\eta_p^2 = 0.001$), however, males consumed a greater amount of collagen (4.0 ± 1.9 %) as a proportion of total protein intake compared to females (3.4 ± 1.8 %; $F_{1,1331} = 41.359$, $p < 0.001$, $\eta_p^2 = 0.030$), but there was no age × sex interaction ($F_{1,1331} = 0.470$, $p = 0.625$, $\eta_p^2 = 0.001$). These findings persisted when collagen intake and total protein intake were normalized to body mass (age group: $F_{1,1331} = 0.977$, $p = 0.377$, $\eta_p^2 = 0.002$; sex: $F_{1,1331} = 40.189$, $p < 0.001$), $\eta_p^2 = 0.030$; age × sex interaction: $F_{2,1283} = 0.669$, $p = 0.513$, $\eta_p^2 = 0.001$).

FIGURE 2 NEAR HERE

**Energy and macronutrient intake of Irish adults**

Mean daily energy and macronutrient intake are detailed in **Table 1**. The mean daily energy intake for the entire sample (n = 1,338) was 2,006 ± 639 kcal·day$^{-1}$. There were main effects of age ($F_{2, 1492} = 28.703$, $p < 0.001$, $\eta_p^2 = 0.032$) and sex ($F_{1,1492} = 367.756$, $p <0.001$, $\eta_p^2 = 0.208$) on daily energy intake, and there was an interaction between age and sex ($F_{2, 1492} = 4.747$, $p = 0.004$, $\eta_p^2 = 0.005$). With regards to absolute intake, males had a higher mean energy intake compared to females across all age groups. Regarding age, energy intake was lower in older adults compared to both young adults and middle-aged adults. No differences were observed in energy intake between young adults and middle-aged adults for either sex. The interaction was likely a result of the sex difference in middle-aged adults, being smaller than the sex differences in both young and older adults.

When normalized to body mass, the daily energy intake for the entire sample was 26.6 ± 8.7 kcal·kg$^{-1}$·day$^{-1}$. There were main effects of age group and sex on normalized energy intake ($F_{1,1492}$ = 31.143, $p < 0.001$, $\eta_p^2$ = 0.022; $F_{1,1492}$ = 29.501, $p < 0.001$, $\eta_p^2$ = 0.022) but no age × sex interaction ($F_{2, 1492}$ = 2.751, p = 0.93, $\eta_p^2$ = 0.005). Normalized energy intake was lower in young compared to middle-aged adults, but there was no difference between middle-aged and older adults. Females had lower normalized energy intake compared with males in all age groups.

Regarding macronutrient intake, there were main effects of age, and sex on protein, carbohydrate and fat intake, respectively ($p < 0.01$). However, there was only an interaction between age and sex on absolute and normalized protein intake ($F_{2, 1331}$ = 9.968, $p < 0.001$, $\eta_p^2$ = 0.015; $F_{2, 1283}$ = 8.383, $p < 0.001$, $\eta_p^2$ = 0.013), and not regarding any other macronutrient intake normalized to body mass. Absolute and normalized protein intake was lower in females ($p < 0.001$) but was not different across age groups. Absolute protein intake in males, however, tended to be lower with age (young vs. middle-aged, p = 0.006, young vs. old, $p < 0.001$, middle-aged vs. old. p = 0.064). Normalized protein intake in males was lower in middle-aged and older compared to young (both groups vs. young, $p < 0.001$), but there was no difference between middle-aged and old (p = 1.000). While protein intake was generally consistent across most groups, young males had a notably higher intake compared to their female counterparts and other age groups.

Normalized carbohydrate intake was lower in females, and regardless of sex, intake in young adults was higher than in both middle-aged and older adults, and intake in middle-aged adults was higher than in older adults. There was a main effect of age ($F_{1,1331}$ = 13.482, $p < 0.001$, $\eta_p^2$ =.020) and sex ($F_{1,1331}$ = 13.482, $p < 0.001$, $\eta_p^2$ =.127) on fat intake, but no sex × age interaction. ($F_{2, 1331}$ = 1.122, p = 0.127), which was similar for normalized fat intake (age: $p < 0.001$, $\eta_p^2$ = .025, sex: $p < 0.001$, $\eta_p^2$ = .001, sex × age: p = 0.445, $\eta_p^2$ = .001). Physical activity was included as a covariate in the ANCOVA model to account for potential confounding effects. However, this variable did not influence the model for any nutrient apart from fat intake. Physical activity was associated with mean daily fat intake ($F_{1,1331} = 4.20$, p =.041, $\eta_p^2$ =.003) and normalized fat intake (F1,1283 =4.65, p = .031, $\eta_p^2$ =.004), explaining 0.3-0.4% of the variance. This indicates that, while physical activity levels were statistically related to fat intake, its influence on the model was minimal. Importantly, even after adjusting for physical activity, main effects of sex and age on fat intake were observed.

TABLE 1 NEAR HERE

**Habitual vitamin C intake of Irish adults**

Vitamin C MDI is presented in **Table 1**. There was no main effect of age on vitamin C MDI ($F_{2, 1331} = 0.661$, p = 0.516), and no age × sex interaction ($F_{2, 1331} = 0.554$, p = 0.575). However, there was a main effect of sex ($F_{2, 1331} = 5.448$, p = 0.020, $\eta_p^2$= 0.004), where females' intake (141 ± 291 mg·day$^{-1}$) was higher than for males (115 ± 151 mg·day$^{-1}$) (p < 0.001). Physical activity had no influence on the ANCOVA model ($F_{1, 1331} = 0.239$, p = 0.625).

**Habitual physical activity levels of Irish adults**

Although the NANS included 1,500 respondents in its dataset, 162 cases were excluded from this study due to incomplete physical activity questionnaires, leaving a final 1,338 valid cases which were used for analysis. From the final analyzed sample, 1,135 cases were in the 'low' PA category, with 534 (47.0%) males and 601 (53.0%) females. The age distribution of this PA sub-group was as follows: 475 (41.9%) young (231 males, 244 females), 495 (43.6%) middle-aged (226 males, 269 females), and 165 (14.5%) older (77 males, 88 females). There was no significant association between age group and sex in the 'low' PA category ($\chi^2$(2, n = 1,135) = 0.958, p = 0.619).

Similarly, there was no significant association between age group and sex in the 'moderate' PA category ($\chi^2(2, n = 184) = 2.614, p = 0.271$). Of the 184 cases in the 'moderate' PA category, 109 (59.2%) were males and 75 (40.8%) were females. The age distribution was 108 (58.7%) young (69 males, 39 females), 75 (40.8%) middle-aged (40 males, 35 females), and 1 (0.5%) older (0 males, 1 female).

In contrast, the analysis for the 'high' PA category revealed a significant association between age group and sex ($\chi^2(2, n = 19) = 8.051, p = 0.018$). Specifically, there was a higher proportion of young adults engaged in 'high' PA compared to middle-aged and older adults. Additionally, the sex distribution within the 'high' PA category differed notably, with males being more represented among the younger age group compared to females. For the 19 cases in this category, there were 14 (73.7%) males and 5 (26.3%) females. The age distribution was 12 (63.1%) young (12 males, 1 female), 5 (26.3%) middle-aged (2 males, 3 females), and 1 (5.3%) older (0 males, 1 female). Finally, the aggregate data across all physical activity categories revealed no overall association between age group and sex ($\chi^2(2, n = 1{,}338) = 4.534, p = 0.104$).

# Discussion

The main objective of this study was to provide the first estimate of collagen intake in a European adult population, based on data from the National Adult Nutrition Survey (NANS). The main findings were that the collagen mean daily intake (MDI) for the entire study population was ~ 3 g per day, which represented just ~ 4 % of all protein consumed daily. This intake is considerably lower than the doses necessary to enhance collagen synthesis in intervention studies. Specifically, in young men, 15 g but not 5 g of gelatin increased whole-body collagen synthesis following skipping exercise (16), while 30 g, but not 15 g of collagen hydrolysate was required to enhance collagen synthesis following resistance exercise (15). This suggests that the levels of collagen habitually consumed in the Irish diet are likely insufficient to elicit a meaningful collagen synthesis response. Interestingly, in our study, men had greater absolute intakes of collagen than women regardless of age, and this sex difference remained when intakes were adjusted for body mass. Furthermore, older adults consumed less collagen than middle-aged adults in absolute terms (with no difference between middle-aged and young adults) but this age difference disappeared when collagen intake was normalized to body mass. Notably, habitual collagen MDI was not influenced by habitual physical activity levels, i.e. more physically active individuals did not ingest more collagen.

The collagen MDI for the total population was remarkably low, thus the availability of exogenous glycine, proline, and hydroxyproline (the highly abundant amino acids in collagen known to stimulate collagen synthesis (50, 51)) is also limited. Glycine is not an essential amino acid, as it can be synthesized from other amino acids (predominantly serine, but threonine, choline and glyoxylate may make minor contributions) (6, 52). However, habitual protein/amino acid intake plays a role in supplying glycine, proline, and hydroxyproline to support connective tissue turnover in the skin, heart, blood vessels, and musculoskeletal tissue, although it has been shown that dietary glycine intake between 1.5 and 3 $g \cdot day^{-1}$ falls short of the amount required for collagen synthesis in metabolism (6). Although glycine may be available from some food sources, such as soy or legumes, the amount of glycine available from the ~ 3 g MDI collagen in the Irish diet, is likely to be as low as 1 $g \cdot day^{-1}$ since glycine comprises 1/3 of collagen (53).

Moreover, collagen is the only dietary source of hydroxyproline. It has been demonstrated in human dermal fibroblasts, that hydroxyproline stimulates collagen synthesis in two ways: 1. by increasing growth factor beta 1 (TGF β1) levels; and 2. by directly stimulating the protein kinase B (AKT) and mammalian target of rapamycin (mTOR) signaling pathways (54). Given this crucial role in supporting connective tissue turnover, the unique presence of hydroxyproline in collagen further emphasizes the importance of including collagen in the diet.

Despite this importance, the only previously reported habitual intakes of collagen were the 3 to 23 g·day$^{-1}$ in 'the standard American diet' reported by Paul, Leser and Oesser (24). There are several reasons for the discrepancies between our data and those of Paul, Leser and Oesser (24), where our data are much closer to the lower end of collagen MDI estimates in the study by Paul et al. (2019). There are crucial differences in methodology, where Paul, Leser and Oesser (24) estimated collagen MDI by averaging collagen content (% dry weight) across multiple food groups, and expressed this as a percentage of mean male and female intake at population level, as calculated in the National Health and Nutrition Examination Survey (NHANES). In contrast, our study applied specific collagen content values to 736 food items on an individual basis and integrated these into the food diaries of each participant in NANS. For example, Paul, Leser and Oesser (24) grouped beef, pork, veal, lamb and game, and assigned these foods a collagen content of 5.15 % of product dry weight, however the collagen content of pork cuts alone can vary from ~1 to ~22 % (31). Moreover, any of these foods could be included in a food mixture, which is not accounted for in these analyses by Paul, Leser and Oesser (24). Finally, the higher end of collagen MDI range reported in the NHANES may be attributed to differences in regional food regulation, as European Union Regulation (EU) No. 1169/2011 sets out maximum connective tissue content (measured as collagen content) for ingredients designated by the term 'meat' at 25 % (31). In stark contrast, Paul, Leser and Oesser (24) reports the collagen content of frankfurters, sausages and luncheon meat in to be ~ 55% in the United States of America (USA), highlighting the lack of applicability of these findings across different jurisdictions, and the likelihood that collagen intake is lower in Europe compared to the USA.

We found that dietary collagen intake was lower in Irish female adults, both in absolute terms and relative to both body mass and total protein intake. Using the same dataset as used in this study, Hone et al. (27) recently reported that animal based foods contributed to a larger proportion of total energy and were the dominant source of protein intake in the Irish adult diet. However, women obtained a higher proportion of protein from plant sources compared to men (27), and since collagen is exclusively found in animal products, this may explain the lower relative collagen intake in women, despite similar relative protein intake. Although the mean difference in total collagen intake between men and women appears modest (1.6 g·day$^{-1}$), the large effect size ($\eta p^2 = 0.140$) suggests that future research should explore sex-specific recommendations for increasing collagen intake, with the goal of improving connective tissue health, especially in women.

The lower daily protein intake observed in older adults aligns with findings in other Western European jurisdictions (55-57). It is striking, however, that there was an age × sex interaction regarding protein, and not collagen, intake relative to body mass. There are known effects of age and sex on collagen turnover in healthy humans, especially in type I collagen, the main extracellular matrix component of bone, tendon, and ligament (58-60). Biomarkers of type I collagen synthesis decline from young adulthood until middle age before levelling off in both men and women (59, 60). Moreover, young and middle-aged women display lower levels of collagen synthesis than men, with lowest levels reported in middle-aged, pre-menopausal women, and an increase in the post-menopausal years (59). These observational data suggest a role for hormonal status on collagen turnover. Consequently, middle-aged and older Irish women in particular, who also have the lowest intake of collagen according to our data, may have different dietary collagen requirements to young adults. Additionally, study designs that seek to measure the effects of dietary collagen should avoid grouping male and female participants, as differences in collagen turnover and hormonal status, even in age-matched participants, are likely to lead to erroneous conclusions.

We estimated habitual vitamin C intake in the Irish adult population, as it is essential for the hydroxylation of proline and lysine, a crucial step in the synthesis of collagen (1). Although vitamin C intake was ~23 % greater in women than men, our sex-specific values were similar to the recommended dietary allowance (RDA) of 95 and 110 $mg \cdot day^{-1}$ for men and women, respectively (61). However, it remains unclear whether the timing of vitamin C ingestion (for example co-ingestion with collagen rich food or supplements) is essential to optimize collagen synthesis in response to feeding. Despite adequate daily intake, the inability of humans to store vitamin C (62), suggests that any discontinuity between intake of vitamin C and collagen could potentially limit the level of endogenous collagen production.

**Physical activity levels**

We used habitual physical activity as a covariate when analyzing differences in collagen intake across age groups due to the recent interest in the interaction between collagen supplementation and various types of exercise (9, 17). Although not the primary outcome of our study, it is concerning that 85 % of all participants were in the low activity category, with low levels of moderate physical activity in middle-age, and indeed that only one older adult could be classified as highly active. This aligns with international data indicating lower physical activity levels among older populations (63, 64). A recent meta-analysis suggested that chronic exercise with collagen ingestion can improve fat-free mass, tendon and muscle morphology, maximal strength and recovery from damaging exercise bouts (17). Since the current study is a secondary analysis of questionnaire based physical activity, we were not able determine whether moderate or highly active participants were engaging in resistance exercise, endurance exercise, or other activities. The observation that habitual physical activity was lower in middle-aged adults compared to young, and none of the older adults were highly active, supports the notion that the combined effects of increased collagen ingestion and exercise may have the greatest benefit in terms of stimulating collagen production for maintaining or improving connective tissue health in middle-aged and older populations.

## Strengths and Limitations

A key strength of this study is the application of specific collagen content values to individual food items, which allowed for a more precise estimation of collagen intake compared to a previous population-level assessment that relied on broad food group averages. Additionally, these data are derived from a demographically representative sample, allowing generalizability to the Irish adult population. Our analysis is from the Irish NANS, conducted between 2008 and 2010. Dietary habits and supplement use may have changed since then, given the growth in global sales of collagen supplements and increased research in active and athletic groups. It could be suggested that collagen intake may be increasing or will increase in future, surpassing the most recent data available for the Irish adult population. The study achieved a response rate of 60 %, which is relatively high but still leaves room for potential non-response bias. The sample was demographically representative, but specific sub-groups (e.g., highly active middle-aged and older adults) were small, limiting the generalizability of findings to these populations.

## Conclusion

Habitual intake of collagen protein was remarkably low in the diet of Irish adults and may fall short of that required for optimal collagen turnover to maintain healthy connective tissues. Since collagen ingestion with exercise may improve musculoskeletal health and function, increasing collagen ingestion may be an effective strategy for maintaining connective tissue health. Achieving effective doses between 5 and 30 g through diet alone may be challenging, therefore, supplementation may be warranted. This may be especially important for women and older adults, who typically consume less collagen than men and younger adults, according to our data.

**Acknowledgements**

The authors would like to thank the Irish University Nutrition Alliance for providing the original data. CDN and RME designed the study; JW contributed to the execution of the study and provided expert advice throughout; CDN and RME analyzed the data; CDN drafted the manuscript; and all authors read and approved the final manuscript.

**Data availability**

Data described in the manuscript, code book, and analytic code will be made available upon reasonable request (e.g., application and approval, payment, other).

**Funding**

The NANS project and preliminary analysis were supported by funding from the Irish Government, Department of Agriculture, Food and the Marine under the 'Food for Health Research Initiative' 2007–2012 and Project 13 F 542 - National Nutritional Databases for Public Health and New Product Development. There was no additional funding for the secondary analysis described in this manuscript.

# References

1. **Canty EG, and Kadler KE**. Procollagen trafficking, processing and fibrillogenesis. *Journal of Cell Science* 118: 1341-1353, 2005.
2. **Calleja-Agius J, Brincat M, and Borg M**. Skin connective tissue and ageing. *Best Practice & Research Clinical Obstetrics & Gynaecology* 27: 727-740, 2013.
3. **Jun JI, and Lau LF**. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat Cell Biol* 12: 676-685, 2010.
4. **Tomlinson DJ, Erskine RM, Morse CI, and Onambélé GL**. Body Fat Percentage, Body Mass Index, Fat Mass Index and the Ageing Bone: Their Singular and Combined Roles Linked to Physical Activity and Diet. *Nutrients* 11: 2019.

5. **Reeves ND, Narici MV, and Maganaris CN**. Myotendinous plasticity to ageing and resistance exercise in humans. *Exp Physiol* 91: 483-498, 2006.
6. **Meléndez-Hevia E, de Paz-Lugo P, Cornish-Bowden A, and Cárdenas ML**. A weak link in metabolism: the metabolic capacity for glycine biosynthesis does not satisfy the need for collagen synthesis. *Journal of Biosciences* 34: 853-872, 2009.
7. **Li P, and Wu G**. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino Acids* 50: 29-38, 2018.
8. **Virgilio N, Schön C, Mödinger Y, van der Steen B, Vleminckx S, van Holthoon FL, Kleinnijenhuis AJ, Silva CIF, and Prawitt J**. Absorption of bioactive peptides following collagen hydrolysate intake: a randomized, double-blind crossover study in healthy individuals. *Frontiers in Nutrition* 11: 2024.
9. **Khatri M, Naughton RJ, Clifford T, Harper LD, and Corr L**. The effects of collagen peptide supplementation on body composition, collagen synthesis, and recovery from joint injury and exercise: a systematic review. *Amino Acids* 53: 1493-1506, 2021.
10. **Holwerda AM, and van Loon LJC**. The impact of collagen protein ingestion on musculoskeletal connective tissue remodeling: a narrative review. *Nutrition Reviews* 80: 1497-1514, 2022.
11. **Thomas C, Kingshott RN, Allott KM, Tang JCY, Dunn R, Fraser WD, Thorley J, Virgilio N, Prawitt J, Hogervorst E, Škarabot J, and Clifford T**. Collagen peptide supplementation before bedtime reduces sleep fragmentation and improves cognitive function in physically active males with sleep complaints. *European Journal of Nutrition* 63: 323-335, 2024.
12. **Miyab KB, Alipoor E, Vaghardoost R, Isfeedvajani MS, Yaseri M, Djafarian K, and Hosseinzadeh-Attar MJ**. The effect of a hydrolyzed collagen-based supplement on wound healing in patients with burn: A randomized double-blind pilot clinical trial. *Burns* 46: 156-163, 2020.
13. **de Miranda RB, Weimer P, and Rossi RC**. Effects of hydrolyzed collagen supplementation on skin aging: a systematic review and meta-analysis. *International journal of dermatology* 60: 1449-1461, 2021.
14. **Lee J, Tang JCY, Dutton J, Dunn R, Fraser WD, Enright K, Clark DR, Stewart CE, and Erskine RM**. Effects of resistance exercise, collagen ingestion and circulating oestrogen concentration on collagen synthesis in a female athlete: A case report. *Experimental Physiology* n/a: 2024.
15. **Lee J, Tang JCY, Dutton J, Dunn R, Fraser WD, Enright K, Clark DR, Stewart CE, and Erskine RM**. The Collagen Synthesis Response to an Acute Bout of Resistance Exercise Is Greater when Ingesting 30 g Hydrolyzed Collagen Compared with 15 g and 0 g in Resistance-Trained Young Men. *J Nutr* 2023.
16. **Shaw G, Lee-Barthel A, Ross ML, Wang B, and Baar K**. Vitamin C–enriched gelatin supplementation before intermittent activity augments collagen synthesis. *The American journal of clinical nutrition* 105: 136-143, 2017.
17. **Bischof K, Moitzi AM, Stafilidis S, and König D**. Impact of Collagen Peptide Supplementation in Combination with Long-Term Physical Training on Strength, Musculotendinous Remodeling, Functional Recovery, and Body Composition in Healthy Adults: A Systematic Review with Meta-analysis. *Sports Medicine* 2024.
18. **Baumann L**. Skin ageing and its treatment. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland* 211: 241-251, 2007.
19. **Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ, and Voorhees JJ**. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. *The American journal of pathology* 168: 1861-1868, 2006.
20. **Hart DA, Archambault JM, Kydd A, Reno C, Frank CB, and Herzog W**. Gender and neurogenic variables in tendon biology and repetitive motion disorders. *Clinical Orthopaedics and Related Research®* 351: 44-56, 1998.

21. **Liu SH, Al-Shaikh RA, Panossian V, Finerman GA, and Lane JM**. Estrogen affects the cellular metabolism of the anterior cruciate ligament: a potential explanation for female athletic injury. *The American journal of sports medicine* 25: 704-709, 1997.
22. **Lee KC, and Lanyon LE**. Mechanical loading influences bone mass through estrogen receptor α. *Exercise and sport sciences reviews* 32: 64-68, 2004.
23. **Miller BF, Hansen M, Olesen JL, Schwarz P, Babraj JA, Smith K, Rennie MJ, and Kjaer M**. Tendon collagen synthesis at rest and after exercise in women. *Journal of Applied Physiology* 102: 541-546, 2007.
24. **Paul C, Leser S, and Oesser S**. Significant Amounts of Functional Collagen Peptides Can Be Incorporated in the Diet While Maintaining Indispensable Amino Acid Balance. *Nutrients* 11: 2019.
25. **Beaulieu K, Hopkins M, Blundell J, and Finlayson G**. Does habitual physical activity increase the sensitivity of the appetite control system? A systematic review. *Sports Medicine* 46: 1897-1919, 2016.
26. **Lourida I, Boer JMA, Teh R, Kerse N, Mendonça N, Rolleston A, Sette S, Tapanainen H, Turrini A, Virtanen SM, Visser M, and Jagger C**. Association of Daily Physical Activity and Sedentary Behaviour with Protein Intake Patterns in Older Adults: A Multi-Study Analysis across Five Countries. *Nutrients* 13: 2574, 2021.
27. **Hone M, Nugent A, Walton J, McNulty B, and Egan B**. Habitual protein intake, protein distribution patterns and dietary sources in Irish adults with stratification by sex and age. *Journal of Human Nutrition and Dietetics* 33: 465-476, 2020.
28. **Holland B, Unwin I, and Buss DH**. *Cereals and cereal products. Third supplement to McCance and Widdowson's The Composition of Foods*. 1988.
29. **Holland B, Unwin I, and Buss D**. *Milk products and eggs. Fourth supplement to McCance and Widdowson's the composition of foods*. 1989.
30. **McCance RA, and Widdowson EM**. *McCance and Widdowson's the Composition of Foods*. Royal Society of Chemistry, 2014.
31. **FSAI**. Guidance Note 17: Meat Labelling 2018.
32. **Olley J**. Structure and proteins of fish and shellfish. 1. 1981.
33. **Combes S, Lepetit J, Darche B, and Lebas F**. Effect of cooking temperature and cooking time on Warner–Bratzler tenderness measurement and collagen content in rabbit meat. *Meat Science* 66: 91-96, 2004.
34. **Daszkiewicz T, and Janiszewski P**. The effect of sex on the quality of meat from farmed pheasants (Phasianus colchicus). *Animal Science Journal* 91: e13404, 2020.
35. **Nuckles R, Smith D, and Merkel R**. Meat by-product protein composition and functional properties in model systems. *Journal of Food Science* 55: 640-643, 1990.
36. **El S**. Evaluating protein quality of meats using collagen content. *Food chemistry* 53: 209-210, 1995.
37. **Nishimura S, Sagara A, Teshima A, and Tabata S**. The comparison of the collagen fiber contents and hepatic stellate cell distribution in male and female chicken livers. *Animal science journal* 82: 759-763, 2011.
38. **Park CH, Lee JH, Kang KT, Park JW, and Kim J-S**. Characterization of acid-soluble collagen from Alaska pollock surimi processing by-products (refiner discharge). *Food science and biotechnology* 16: 549-556, 2007.
39. **Teixeira A, Almeida S, Pereira E, Mangachaia F, and Rodrigues S**. Physicochemical characteristics of sheep and goat pâtés. differences between fat sources and proportions. *Heliyon* 5: 2019.
40. **Stevenson J, Seman D, and Littlejohn R**. Seasonal variation in venison quality of mature, farmed red deer stags in New Zealand. *Journal of Animal Science* 70: 1389-1396, 1992.
41. **Danos N, Fisch N, and Gemballa S**. The musculotendinous system of an anguilliform swimmer: muscles, myosepta, dermis, and their interconnections in Anguilla rostrata. *Journal of Morphology* 269: 29-44, 2008.
42. **McBride J, MacLeod R, and Idler D**. Seasonal variation in the collagen content of Pacific herring tissues. *Journal of the Fisheries Board of Canada* 17: 913-918, 1960.

43. **Sivakumar P, Suguna L, and Chandrakasan G**. Purification and partial characterization of a type V like collagen from the muscle of marine prawn, Penaeus indicus. *Journal of biosciences* 22: 131-141, 1997.
44. **Chemistry AMCotRSo**. FSA UK Guidelines on the Labelling and Declaration of Meat Content in Meat Products UK: Food Standards Agency, UK, 2002.
45. **PublicHealthEngland**. Composition of Foods Integrated Dataset (COFID) 2021.
46. **University of London School of Life Sciences MoA, Fisheries and Food**. Analysis of composition of commercial sandwiches. Ministry of Agriculture, Fisheries and Food, UK, 1997.
47. **Wareham NJ, Jakes RW, Rennie KL, Mitchell J, Hennings S, and Day NE**. Validity and repeatability of the EPIC-Norfolk physical activity questionnaire. *International journal of epidemiology* 31: 168-174, 2002.
48. **Bayles MP**. *ACSM's exercise testing and prescription*. Lippincott Williams & Wilkins, 2023.
49. **Cohen J**. *Statistical power analysis for the behavioral sciences*. routledge, 2013.
50. **de Paz-Lugo P, Lupiáñez JA, and Meléndez-Hevia E**. High glycine concentration increases collagen synthesis by articular chondrocytes in vitro: acute glycine deficiency could be an important cause of osteoarthritis. *Amino Acids* 50: 1357-1365, 2018.
51. **Szoka L, Karna E, Hlebowicz-Sarat K, Karaszewski J, and Palka JA**. Exogenous proline stimulates type I collagen and HIF-1α expression and the process is attenuated by glutamine in human skin fibroblasts. *Molecular and cellular biochemistry* 435: 197-206, 2017.
52. **Wang W, Wu Z, Dai Z, Yang Y, Wang J, and Wu G**. Glycine metabolism in animals and humans: implications for nutrition and health. *Amino acids* 45: 463-477, 2013.
53. **Ramshaw JA, Shah NK, and Brodsky B**. Gly-XY tripeptide frequencies in collagen: a context for host–guest triple-helical peptides. *Journal of structural biology* 122: 86-91, 1998.
54. **Surazynski A, Miltyk W, Prokop I, and Palka J**. Prolidase-dependent regulation of TGF c and TGF β receptor expressions in human skin fibroblasts. *European Journal of Pharmacology* 649: 115-119, 2010.
55. **Tieland M, Borgonjen-Van den Berg KJ, Van Loon LJ, and de Groot LC**. Dietary protein intake in Dutch elderly people: a focus on protein sources. *Nutrients* 7: 9697-9706, 2015.
56. **Ruiz E, Ávila JM, Valero T, Del Pozo S, Rodriguez P, Aranceta-Bartrina J, Gil Á, González-Gross M, Ortega RM, and Serra-Majem L**. Energy intake, profile, and dietary sources in the Spanish population: Findings of the ANIBES study. *Nutrients* 7: 4739-4762, 2015.
57. **Sette S, Le Donne C, Piccinelli R, Arcella D, Turrini A, Leclercq C, and Group I-SS**. The third Italian national food consumption survey, INRAN-SCAI 2005–06–part 1: nutrient intakes in Italy. *Nutrition, Metabolism and Cardiovascular Diseases* 21: 922-932, 2011.
58. **Kehlet SN, Willumsen N, Armbrecht G, Dietzel R, Brix S, Henriksen K, and Karsdal MA**. Age-related collagen turnover of the interstitial matrix and basement membrane: Implications of age- and sex-dependent remodeling of the extracellular matrix. *PLOS ONE* 13: e0194458, 2018.
59. **Glover SJ, Garnero P, Naylor K, Rogers A, and Eastell R**. Establishing a reference range for bone turnover markers in young, healthy women. *Bone* 42: 623-630, 2008.
60. **Morovat A, Catchpole A, Meurisse A, Carlisi A, Bekaert A-C, Rousselle O, Paddon M, James T, and Cavalier É**. IDS iSYS automated intact procollagen-1-N-terminus pro-peptide assay: method evaluation and reference intervals in adults and children. *Clinical Chemistry and Laboratory Medicine (CCLM)* 51: 2009-2018, 2013.
61. **EFSA Panel on Dietetic Products N, and Allergies**. Scientific opinion on dietary reference values for vitamin C. *EFSA Journal* 11: 3418, 2013.
62. **Li Y, and Schellhorn HE**. New Developments and Novel Therapeutic Perspectives for Vitamin C1,2. *The Journal of Nutrition* 137: 2171-2184, 2007.
63. **Posadzki P, Pieper D, Bajpai R, Makaruk H, Könsgen N, Neuhaus AL, and Semwal M**. Exercise/physical activity and health outcomes: an overview of Cochrane systematic reviews. *BMC Public Health* 20: 1724, 2020.

64. **Kettle VE, Madigan CD, Coombe A, Graham H, Thomas JJC, Chalkley AE, and Daley AJ**. Effectiveness of physical activity interventions delivered or prompted by health professionals in primary care settings: systematic review and meta-analysis of randomised controlled trials. *BMJ* 376: e068465, 2022.

**Table 1.** Habitual energy, protein, carbohydrate, fat, collagen, and vitamin C intake of Irish adults. Values are expressed as mean ± SD.

| **Nutrient** | | **Young adults (n = 596)** | **Middle-aged adults (n = 575)** | **Older adults (n = 167)** | **Effect of Sex** | **Effect of Age** | **Sex × Age Interaction** |
|---|---|---|---|---|---|---|---|
| **Energy Intake (kcal·day$^{-1}$)** | All | 2135 ± 692 | 1952 ± 576 | 1808 ± 555 | P < 0.001 | P < 0.001 | P = 0.004 |
| | Male | 2481 ± 656 | 2256 ± 578 | 2086 ± 585 | | | |
| | Female | 1755 ± 506 | 1689 ± 425 | 1564 ± 391 | | | |
| **Energy Intake (kcal·kg$^{-1}$·day$^{-1}$)** | All | 28.8 ± 9.3 | 25.2 ± 7.5 | 26.7 ± 8.7 | P < 0.001 | P < 0.001 | P = 0.93 |
| | Male | 30.5 ± 9.5 | 26.1 ± 7.6 | 25.5 ± 8.9 | | | |
| | Female | 26.8 ± 8.7 | 24.2 ± 7.6 | 23.8 ± 7.3 | | | |
| **PRO Intake (g·day$^{-1}$)** | All | 87 ± 31 | 84 ± 25 | 79 ± 24 | P = 0.005 | P < 0.001 | P < 0.001 |
| | Male | 104 ± 31 | 97 ± 24 | 91 ± 24 | | | |
| | Female | 69 ± 20 | 73 ± 20 | 69 ± 18 | | | |
| **PRO Intake (g·kg$^{-1}$·day$^{-1}$)** | All | 1.2 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.4 | P < 0.001 | P = 0.002 | P < 0.001 |
| | Male | 1.3 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.4 | | | |
| | Female | 1.0 ± 0.3 | 1.1 ± 0.3 | 1.0 ± 0.4 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **CHO Intake (g·day⁻¹)** | All | 243 ± 82 | 225 ± 76 | 214 ± 69 | P < 0.001 | P < 0.001 | P = 0.053 |
| | Male | 278 ± 84 | 257 ± 83 | 240 ± 74 | | | |
| | Female | 205 ± 60 | 197 ± 57 | 191 ± 54 | | | |
| **CHO Intake (g·kg⁻¹·day⁻¹)** | All | 3.3 ± 1.1 | 2.9 ± 1.0 | 2.9 ± 1.0 | P = 0.001 | P < 0.001 | P = 0.277 |
| | Male | 3.4 ± 1.2 | 3.0 ± 1.1 | 3.0 ± 1.0 | | | |
| | Female | 3.1 ± 1.0 | 2.9 ± 1.0 | 2.8 ± 1.0 | | | |
| **Fat Intake (g·day⁻¹)** | All | 81 ± 30 | 75 ± 27 | 70 ± 29 | P = 0.001 | P < 0.001 | P = 0.326 |
| | Male | 92 ± 30 | 86 ± 29 | 80 ± 33 | | | |
| | Female | 68 ± 24 | 66 ± 21 | 61 ± 20 | | | |
| **Fat Intake (g·kg⁻¹·day⁻¹)** | All | 1.3 ± 0.5 | 1.2 ± 0.5 | 1.1 ± 0.5 | P < 0.001 | P < 0.001 | P = 0.445 |
| | Male | 1.4 ± 0.5 | 1.3 ± 0.5 | 1.2 ± 0.6 | | | |
| | Female | 1.2 ± 0.5 | 1.1 ± 0.4 | 1.0 ± 0.5 | | | |
| **Vitamin C Intake (mg·day⁻¹)** | All | 134 ± 279 | 119 ± 175 | 132 ± 215 | P = 0.020 | P = 0.516 | P = 0.575 |
| | Male | 127 ± 167 | 104 ±130 | 104 ± 134 | | | |
| | Female | 141 ± 363 | 131 ± 205 | 141 ± 291 | | | |

PRO, protein; CHO, carbohydrate; young adults were 18-39 years-old; middle-aged adults were 40–64 years-old; older adults were >65 years-old.

**Figure Legends**

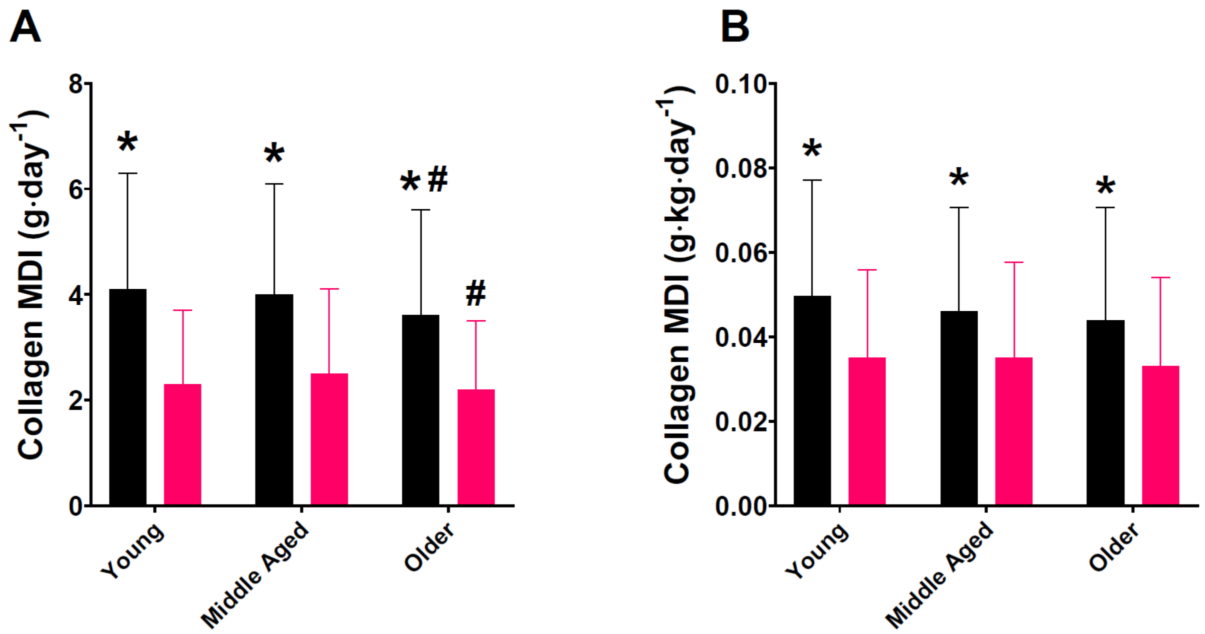


**Figure 1. A.** Absolute and **B.** normalized (to body mass) mean daily intake (MDI) of collagen in young, middle-aged, and older males (black bars) and females (pink bars). * Higher than females ($p < 0.001$), # lower than middle-aged ($p = 0.021$).

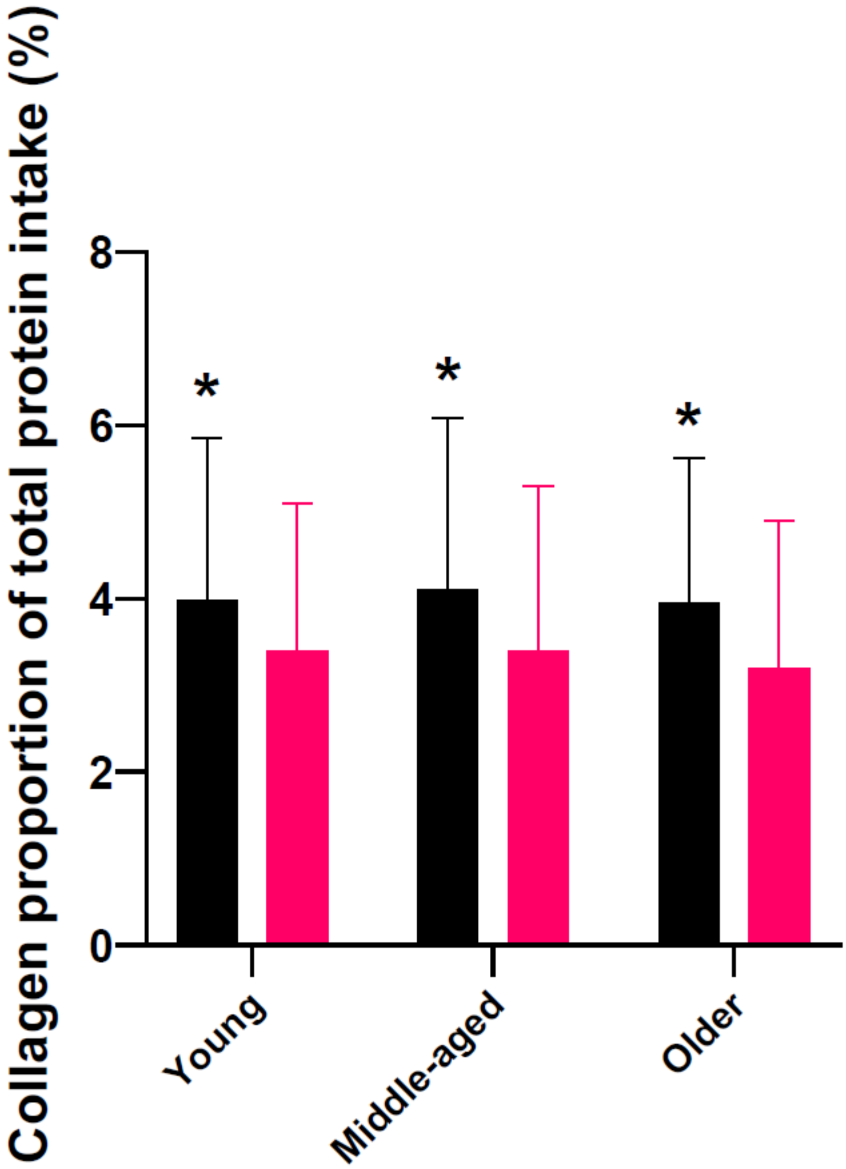


**Figure 2.** Mean daily intake (MDI) of collagen expressed as a percentage of total daily protein intake in young, middle-aged, and older males (black bars) and females (pink bars). * Higher than females ($p < 0.001$).