



UNIVERSITY OF LEEDS

This is a repository copy of *Dietary Protein Intake and Overall Diet Quality in Adults with Cystic Fibrosis Following Elexacaftor/Tezacaftor/Ivacaftor Therapy*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/228900/>

Version: Accepted Version

---

**Article:**

Morgan, P.T., Ellis, T.-J., Smeuninx, B. et al. (6 more authors) (2025) Dietary Protein Intake and Overall Diet Quality in Adults with Cystic Fibrosis Following Elexacaftor/Tezacaftor/Ivacaftor Therapy. British Journal Of Nutrition. ISSN 0007-1145 (In Press)

<https://doi.org/10.1017/s0007114525103760>

---

This article has been published in a revised form in <https://doi.org/10.1017/S0007114525103760>. This version is free to view and download for private research and study only. Not for re-distribution or re-use. © copyright holder.

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.



[eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk)  
<https://eprints.whiterose.ac.uk/>

## Original research article

### Dietary Protein Intake and Overall Diet Quality in Adults with Cystic Fibrosis Following Elexacaftor/Tezacaftor/Ivacaftor Therapy

Paul T. Morgan<sup>1\*</sup>, Tanith-Jade Ellis<sup>1</sup>, Benoit Smeuninx<sup>2,3</sup>, Leigh Breen<sup>3</sup>, Laura Kinsey<sup>4</sup>, Owen W. Tomlinson<sup>5</sup>, Helen White<sup>6</sup>, Laura R. Caley<sup>7,8</sup> and Daniel G. Peckham<sup>7,8</sup>

<sup>1</sup> Department of Sport and Exercise Sciences, Institute of Sport, Manchester Metropolitan University, 99 Oxford Road, Manchester, UK

<sup>2</sup> Monash Institute of Pharmacological Sciences, Monash University, Parkville, VIC, AU

<sup>3</sup> School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, UK

<sup>4</sup> Manchester Adult Cystic Fibrosis Centre, Wythenshawe Hospital, Manchester University Hospitals NHS Foundation Trust, UK

<sup>5</sup> University of Exeter Medical School, St Luke's Campus, Exeter, UK

<sup>6</sup> Leeds Beckett University, Nutrition, Health & Environment, Leeds, UK

<sup>7</sup> Leeds Institute of Medical Research, University of Leeds, UK

<sup>8</sup> Department of Respiratory Medicine, Leeds Teaching Hospitals NHS Trust, Leeds, UK

**\*Correspondence author:** Paul Morgan, Ph.D. Institute of Sport, Manchester Metropolitan University, 99 Oxford Road, School of Sport and Exercise Sciences, Manchester, M1 7EL, United Kingdom. Tel: 0161 247 5100; E-mail: p.morgan@mmu.ac.uk

**Journal:** British Journal of Nutrition [Cambridge University Press]

**Short title:** Protein and Diet Quality in Cystic Fibrosis

**Key words:** Ageing; CFTR Modulators; Cystic Fibrosis; Dietary Protein; Diet Quality.



This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI

10.1017/S0007114525103760

The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

**List of abbreviations:** AA, amino acid; BL, baseline; CFTR, cystic fibrosis transmembrane conductance regulator; pwCF, people with cystic fibrosis; awCF, adults with cystic fibrosis; CON, control; DIAAS, Digestible Indispensable Amino Acid Score; EAA, essential amino acid; ETI, Elexacaftor/Tezacaftor/Ivacaftor; FUP, follow up; HEI, Heating Eating Index; MPS, muscle protein synthesis; RDA, recommended dietary allowance.

## ABSTRACT

The RDA for dietary protein is likely to be insufficient for individuals with cystic fibrosis (CF), whereas a higher daily intake of  $\geq 1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  may be more appropriate in view of the increased risk of sarcopenia and reduced muscle quality. This study sought to characterise protein intake and diet quality in adults with cystic fibrosis (awCF), before and after ellexacaftor/tezacaftor/ivacaftor (ETI) therapy, compared to healthy controls. Dietary intake was assessed by diet diary in awCF at baseline (BL,  $n=40$ ) and at follow-up  $>3$  months post ETI therapy (FUP,  $n=40$ ), and in age-matched healthy controls (CON,  $n=80$ ) free from known disease at a single time point. Protein intake dose and daily distribution, protein quality, protein source and overall diet quality was calculated for each participant. Both CON ( $1.39 \pm 0.47 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) and CF (BL:  $1.44 \pm 0.52 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ , FUP:  $1.12 \pm 0.32 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) had a higher mean daily protein intake than the protein RDA of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . There was a significant reduction in daily protein intake in the CF group at FUP ( $P=0.0003$ ,  $d=0.73$ ), with levels below the alternative suggested dietary intake of  $\geq 1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . There were no sex differences or noticeable effects on protein quality or source following the commencement of ETI therapy when compared to CON (all  $P>0.05$ ), although overall diet quality decreased between time points ( $P=0.027$ ,  $d=0.57$ ). The observed reduction in daily protein intake in the present cohort emphasises the importance of ensuring appropriate dietary protein intake to promote healthy ageing in adults with CF. More research is needed to evidence base dietary protein requirements in this at-risk population.

## INTRODUCTION

Current estimates suggest ~150,000 people worldwide are diagnosed with Cystic fibrosis (CF) <sup>(1)</sup>. CF is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This results in the absence or dysfunction of CFTR, a protein which functions as an anion channel, conducting chloride and bicarbonate, and regulating sodium transport. The ionic imbalance in CF is associated with dehydrated and acidic airway surface liquid, a predisposition to lung infections, innate inflammation, and tenacious secretions in both respiratory and digestive systems <sup>(2)</sup>.

Aberrations in normal physiology also impair pulmonary and digestive function, nutrient absorption, and predispose individuals to diabetes, osteoporosis, liver disease, colorectal cancer and skeletal muscle dysfunction <sup>(2-5)</sup>. Historically, treatment for CF has been based on frequent and intense antibiotic therapies, airway clearance, exercise and nutritional regimens, which often included a high fat and low fibre diet <sup>(6-9)</sup>. However, this emphasis on a high calorie intake at any cost has recently changed with the introduction of CFTR modulators; a new class of drug targeting the underlying defect rather than disease complications.

Elexacaftor / tezacaftor / ivacaftor (ETI) is the newest combination to be licensed, being effective for ~85% of people with CF (pwCF) <sup>(10)</sup>. Treatment with ETI results in improved quality of life, lung function and weight gain, as well as reduced exacerbations, even in those with advanced pulmonary disease <sup>(11-15)</sup>. Alongside significant improvements in quality of life, pwCF who are treated with highly effective modulator therapy have a projected median survival of >71 years, with children born today having a relatively normal life expectancy <sup>(16)</sup>. Indeed, in 2014 life expectancy for pwCF was as low as 40 years <sup>(17)</sup>. These transformative changes are leading to an ageing CF population. A hallmark of non-CF ageing is progressive and accelerated loss of skeletal muscle mass, quality, and function, termed sarcopenia <sup>(18)</sup>. These changes contribute to loss of functional health and increased morbidity, highlighting the importance of maintaining skeletal muscle health in ageing <sup>(18)</sup>. Reduced muscle mass and function is also present in pwCF, reflecting a complex milieu of malnutrition, infection, inflammation and dysregulated calcium homeostasis in skeletal muscle <sup>(19,20)</sup>. Importantly the reported increase in weight, post ETI, may be more reflective of alterations in the fat compartment rather than muscle mass <sup>(15,21)</sup>. Reduced muscle mass, quality, and function are also independently associated with disease progression <sup>(5,22,23)</sup>, but until now, counteracting symptoms of accelerated ageing has not been a clinical priority in pwCF.

The supply of essential amino acids (EAA) is necessary for a positive protein balance, the stimulation of muscle protein synthesis (MPS), and the prevention of skeletal muscle mass

loss<sup>(24)</sup>, which pwCF are at risk of<sup>(25)</sup>. In the postprandial period, dietary protein robustly stimulates MPS contributing to net muscle protein accretion<sup>(26)</sup>. However, an impaired muscle anabolic response to the ingestion of lower doses of protein in older individuals, termed skeletal muscle ‘anabolic resistance’, is thought to be a critical factor in age-related muscle deterioration<sup>(27)</sup>. In addition, the Recommended Dietary Allowance (RDA) for protein (UK: 0.75 g·kg<sup>-1</sup>·day<sup>-1</sup>) is thought to be insufficient for repeated, robust stimulation of MPS and, hence maintenance of muscle in older adults without CF, the critically ill and those with chronic respiratory disease<sup>(28,29)</sup>. Indeed, higher protein intakes of >1.2 g·kg<sup>-1</sup>·day<sup>-1</sup> are associated with increased muscle mass, quality, and function in older individuals<sup>(29–32)</sup>. In addition to the dose, the quality of dietary protein is an important determinant of postprandial MPS stimulation and skeletal muscle remodelling. Protein quality is defined by a number of factors including the EAA content, profile and bioavailability, combined with protein and/or amino acid (AA) needs, and the digestion kinetics and delivery of AA to biological tissues for protein synthesis<sup>(29)</sup>. In a typical Western diet, protein consumption primarily originates from animal products, which have an EAA profile closely matching bodily requirements<sup>(29,33,34)</sup>. Notwithstanding, there have been calls to increase the intake of plant-based proteins, in part owed to increased health, environmental and ethical concerns associated with animal-based foods<sup>(35)</sup>. This is despite significant impairments in nutrient absorption and reduced muscle mass, quality, and function in pwCF<sup>(5,22,23,36)</sup>. We envisage that an ageing CF population will be at greater risks for sarcopenia due to persistent infections, inflammation, gastrointestinal abnormalities, and a catabolic state. Therefore, combined with the anticipated improvements in lifespan, a better understanding of dietary protein intake in CF is urgently required.

In the current study, our primary aim was to comprehensively characterise protein intake and overall diet quality in this population of awCF pre- and post-ETI therapy, comparing against current UK recommendations for non-CF adults and a healthy control group free from known disease.

## METHODS

### *Study design and Ethical approval*

A portion of the data presented herein have previously been published elsewhere<sup>(15,37)</sup>.

Briefly, this study was part of a prospective observational cohort project conducted across four UK Adult CF Care Centres (Leeds, Royal Papworth, Birmingham, Manchester)<sup>(15)</sup>, with control participants being recruited as part of a separate study in Birmingham, UK<sup>(37)</sup>. A total of 40 pancreatic insufficient awCF were recruited for this part of the CF cohort study (Age at

baseline [BL]:  $35.6 \pm 9.8$  years; Body Mass Index at BL:  $23.3 \pm 2.8 \text{ kg}\cdot\text{m}^{-2}$ ). At baseline, 15 awCF (38%) were on double therapy, which is less clinically effective than ETI, and not associated with such significant changes in BMI<sup>(15)</sup>. Exclusion criteria for CF participants comprised lung transplant recipients, prognosis <6 months, pregnant or having another significant gastrointestinal pathology. Favourable ethical opinion was received from London Bromley Research Ethics Committee (REF: 18/LO/2241). Healthy controls (CON) were age-matched to the CF group (n=80; Age:  $37.7 \pm 14.6$  years; Body Mass Index:  $25.0 \pm 5.0 \text{ kg}\cdot\text{m}^{-2}$ ) and eligible if free from disease and deemed ostensibly healthy based on a general health questionnaire. Control participants were recruited from the Birmingham area (West Midlands, UK) and ethical approval was obtained through the University of Birmingham Research Ethics Committee (REF: 13-1475A). Finally, this study was approved by the Science and Engineering Ethics Committee of Manchester Metropolitan University (Ref No. EthOS 52086). Across the 120 participants, both sexes were recruited near-evenly (male: 52%, female 48%). Voluntary, written, informed consent was received from all CF and CON participants. This study was conducted according to the guidelines laid down in the Declaration of Helsinki.

#### *Dietary data collection*

For the CF group, the study had two time points: baseline (BL), and follow-up (FUP), originally scheduled six months apart as part of a wider study<sup>(15)</sup>. However, the study paused from March until December 2020 owing to the Covid-19 pandemic. The pause resulted in a >6-month gap between time points for those awCF partway through data collection. During this time, ETI modulator therapy became more widely available in the UK, being licensed in mid-2020<sup>(38)</sup>. This significant clinical development was incorporated into the CF observational study with FUP data being collected  $\geq 3$  months after commencing ETI therapy for these individuals. For participants with CF, the most recent clinical weight measurement was recorded for each time point. For the healthy control group, dietary intake was assessed at a single time point. All participants recorded all food, fluid, and any oral nutritional supplements or enteral nutrition for 3–4 days (two or three weekdays and one weekend day) by diet diary. For more detailed information on the study design(s), including details relating to dietary recording, see Caley et al. (2023)<sup>(15)</sup> and Smeuninx et al., (2020)<sup>(37)</sup>.

#### *Analytical methods: calculating protein intake and diet quality*

Initially, daily mean nutritional intake at each time point was calculated for each participant. Thereafter, protein intake dose (relative to bodyweight in kilograms), protein intake

distribution throughout the day (relative to bodyweight in kilograms), protein quality, relative (%) dietary protein source intake and overall diet quality were calculated for each participant. Protein intake dose was calculated by dividing total daily protein intake by the corresponding participant's bodyweight in kilograms, with improved relevance for recommendations for skeletal muscle anabolism. Relative protein intakes were also compared with the current RDA for dietary protein consumption in the UK of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . Similarly, protein intake distribution (or 'meal-specific protein intake') was calculated by dividing total daily protein intake at each meal opportunity by the participant's corresponding bodyweight, and separated into breakfast, lunch, dinner, and snacks. Relative protein intake at each meal was compared against  $0.24 \text{ g} \cdot \text{kg}^{-1}$  and  $0.40 \text{ g} \cdot \text{kg}^{-1}$  thresholds for maximal MPS stimulation for young (based on 18-35 years-olds) and older (based on >60-year olds) individuals, respectively, and used to assess the proportion of meals that reached these respective thresholds <sup>(39)</sup>. The number of individuals reaching the RDA for protein of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  and higher  $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  recommendation within each group (based on the notion of  $3 \times 0.40 \text{ g} \cdot \text{kg}^{-1}$  and following recent calls for increases in the current protein RDA <sup>(40)</sup>) were expressed as a percentage of the total group.

To compare protein quality across groups and time points, we multiplied the protein dose by the corresponding Digestible Indispensable Amino Acid Score (or 'DIAAS') score as reported by Adhikari et al., (2022) <sup>(41)</sup>, incorporating ileal digestibility, to provide a score that reflected a combination of the quality and total intake (in grams) of protein, whereby a higher score reflects higher quality. Whilst we acknowledge that the DIAAS might be considered a somewhat crude assessment of protein quality for human nutrition purposes <sup>(42)</sup>, there are no existing universally agreed means to quantitatively assess dietary protein quality that would address the limitations of the current systems that are available and DIAAS represents the most accurate means to routinely give a single protein quality value for a stand-alone food <sup>(42)</sup>. Therefore, as an additional marker of protein quality, we also calculated protein intake relative to total caloric intake. The relative intake of dietary protein sources was presented by separating protein foods by the following categories: a) Meat & Poultry; b) Fish; c) Dairy/Eggs; d) Cereal, Grain & Bread; e) Fruit/Vegetable; f) Other Animal; g) Other Plant; h) Other, and expressed as a percentage of total protein intake. Finally, overall diet quality was assessed according to the Healthy Eating Index (HEI), whereby a higher value (out of 100) corresponds to higher dietary quality based on the consumption of the following food groups: Total Fruits, Whole Fruits, Total Vegetables, Greens and Beans, Whole Grains, Dairy, Total



Protein Foods, Seafood & Plant Proteins, Fatty Acids<sup>(43)</sup>. In addition, the HEI accounts for moderation of the following: Refined Grains, Sodium, Added Sugars, Saturated Fats<sup>(43)</sup>.

#### *Primary outcome measures*

For analysis, the primary outcomes were daily protein intake dose, protein intake distribution throughout the day (i.e., meal-specific protein intakes), protein quality, and overall diet quality. For comparative purposes, the aims of this study were three-fold, to assess: 1) the change in protein intake and diet quality in awCF following initiation of ETI; 2) the differences in protein intake and diet quality between awCF not on ETI modulator therapy and a control healthy population 3) the differences in protein intake and diet quality between awCF following initiation of ETI modulator therapy and a control healthy population.

#### *Statistical analyses*

Paired t-tests were used to compare the effects of ETI modulator therapy on daily protein intake, protein quality and overall diet quality in CF (i.e., BL compared with FUP). Independent t-tests were employed to assess differences between CF at baseline (i.e., BL) and CON, and differences between CF at follow up (i.e., FUP) and CON in daily protein dose, protein quality and overall diet quality. Mixed model ANOVAs were employed to assess the effects of ETI therapy on protein distribution throughout the day, as well as differences between CF at baseline (i.e., BL) and CON, and between CF at follow up (i.e., FUP) and CON in protein distribution. Where the ANOVA revealed a significant effect, post hoc analysis was conducted, using a Bonferroni correction, to isolate specific between-group differences. For all tests, to assess any differences in or influence of sex, analyses were repeated, separating by biological sex at birth (male, female). Cohen's *d* was used to calculate the effect size for t-tests and post-hoc comparisons, where *d*=0.2, 0.5, and 0.8 indicate small, medium, and large effects, respectively. Where sphericity was violated, a Greenhouse-Geisser correction factor was used. Normal distribution was assessed using the Shapiro-Wilk test. Where appropriate, non-normally distributed variables were logarithmically transformed. For all the tests, results were considered statistically significant when  $P < 0.05$ . Data are presented as mean  $\pm$  standard deviation or standard error of the mean, unless otherwise indicated. All statistical analyses were conducted using IBM SPSS Statistics version 28.

## **RESULTS**

For more detailed demographics, clinical characteristics and macronutrient intake of CF participants, see Caley et al. (2023)<sup>(15)</sup>. More detailed information from CON can be seen in Smeuninx et al. (2020)<sup>(37)</sup>. For context of disease severity, in the CF cohort, average ppFEV1 (percentage predicted forced expiratory volume in one second) was 46.8% (Interquartile

range [IQR] 34.8, 65.8) at BL and 56.5 (IQR 43.5, 72.6) at FUP. CF-related diabetes and CF-related liver disease were diagnosed in 38% and 30% (¼ of whom had cirrhosis of the liver) of the cohort, respectively.

#### *Daily Protein Intake Dose*

An overview of daily dietary protein intake for CF at BL and FUP and for CON can be viewed in **Figure 1** and **Table 1**. Average daily protein intake was above the protein RDA of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  for all groups and at all time points (**Figure 1**). There was no difference between daily protein intakes in the CF group at BL compared with CON (BL:  $1.44 \pm 0.52 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ; CON:  $1.39 \pm 0.47 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ,  $P=0.63$ ,  $d=0.09$ , **Figure 1**). However, daily protein intakes were 28% and 24% lower at FUP ( $1.12 \pm 0.32 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) compared with BL ( $P=0.0003$ ,  $d=0.73$ ) and CON ( $P=0.001$ ,  $d=0.67$ ), respectively. There were no differences in relative protein intake (i.e., % contribution of total caloric intake) between groups or any effects of sex on daily protein intake (all  $P>0.05$ , **Table 1**).

93% and 98% of participants met the current RDA for protein intake of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  for awCF (at BL) and CON respectively, which reduced to 90% in CF at FUP. However, only 72% of CF participants at BL and 64% of CON group met the RDA on all measurement days. In CF participants at FUP, this reduced to 55% meeting the RDA on all recorded measurement days. When compared to the alternative higher protein recommendation of  $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ , a greater proportion of CON (60%) reached this protein intake on all 3 individual measurement days compared to the CF group both at BL (30%) and FUP with a further reduction (10%).

#### *Dietary Protein Distribution*

Meal-specific protein intakes are presented in **Figure 2** and **Table 1**. Daily dietary protein intake was distributed unevenly across meals with ~18, 29, 41, and 12% of protein in the CF group at BL and ~15, 30, 42 and 13% of protein in the CON group being consumed at breakfast, lunch, dinner, and as snacks, respectively (**Figure 2**). Similarly, in the CF group at FUP, protein intake remained unevenly distributed across meals (~18, 26, 45, 11% for breakfast, lunch, dinner, and snacks, respectively).

Significant main effects for time and group were found for protein intake distribution for the CF group at FUP compared with the CON group and then for CF participants at FUP compared with BL (all  $P<0.0001$ ), but not between CF participants at BL compared with CON (all  $P>0.65$ ). However, no significant interaction effects were found (all  $P>0.63$ ).

Whilst no differences were observed between CON group and CF at BL (BL:  $0.41 \pm 0.19 \text{ g} \cdot \text{kg}^{-1}$ , CON:  $0.42 \pm 0.21 \text{ g} \cdot \text{kg}^{-1}$ ,  $P=0.81$ ,  $d=0.05$ ), **Figure 2**), protein intake at lunch

significantly reduced at FUP in CF participants compared to both CON and CF at BL (FUP:  $0.29 \pm 0.12 \text{ g} \cdot \text{kg}^{-1}$ , both  $P < 0.05$ ,  $d > 0.76$ , **Figure 2**). There was no statistically significant differences between CF at BL, CF at FUP and CON for protein intakes at breakfast (BL:  $0.26 \pm 0.19 \text{ g} \cdot \text{kg}^{-1}$ , FUP:  $0.20 \pm 0.11 \text{ g} \cdot \text{kg}^{-1}$ , CON:  $0.21 \pm 0.13 \text{ g} \cdot \text{kg}^{-1}$ , both  $P > 0.10$ ,  $d < 0.35$ ), dinner (BL:  $0.60 \pm 0.29 \text{ g} \cdot \text{kg}^{-1}$ , FUP:  $0.51 \pm 0.19 \text{ g} \cdot \text{kg}^{-1}$ , CON:  $0.58 \pm 0.24 \text{ g} \cdot \text{kg}^{-1}$ , both  $P > 0.08$ ,  $d < 0.36$ ) or with snacks (BL:  $0.17 \pm 0.14 \text{ g} \cdot \text{kg}^{-1}$ , FUP:  $0.12 \pm 0.11 \text{ g} \cdot \text{kg}^{-1}$ , CON:  $0.18 \pm 0.19 \text{ g} \cdot \text{kg}^{-1}$ , both  $P > 0.07$ ,  $d < 0.39$ ). Across all groups, protein intakes were higher at dinner compared with breakfast and lunch, and higher at lunch compared with breakfast (all  $P < 0.001$ ).

On a meal-to-meal basis, the proposed dietary protein threshold for maximal MPS in younger individuals ( $0.24 \text{ g} \cdot \text{kg}^{-1}$ ) was only met on all recorded days by 10, 25, and 63% of CF individuals at BL and 10, 15, and 58% of CF individuals at FUP for breakfast, lunch, and dinner, respectively. When expressed relative to the maximum threshold for older individuals ( $0.40 \text{ g} \cdot \text{kg}^{-1}$ ), the threshold was met on all 3 recorded days by 0, 8, and 33% of CF individuals at BL and 0, 0, and 18% of CF individuals at FUP for breakfast, lunch, and dinner, respectively. Snacks were often not consumed as a single meal; therefore, this was not included analysis. There was no effect of sex, nor any differences in meal-specific protein intake between males and females (all  $P > 0.05$ , **Table 1**).

#### *Overall Diet and Protein Quality*

Using HEI as a marker of overall diet quality, it was found that diet quality was significantly higher in CF participants at BL compared with CON (BL:  $60.8 \pm 5.6 \text{ au}$ , CON:  $57.4 \pm 7.5 \text{ au}$ ,  $P < 0.01$ ,  $d = 0.51$ ). However, diet quality was significantly reduced in the CF group at FUP compared with BL (FUP:  $57.5 \pm 5.9 \text{ au}$ ,  $P = 0.027$ ,  $d = 0.57$ ). There was no difference in diet quality between CF participants at FUP and CON (**Figure 3A**,  $P = 0.79$ ,  $d = 0.07$ ). The most common source of protein intake across all groups was meat and poultry, with ~76%, ~75%, and ~75% of protein intake of animal origin in CF at BL, CF at FUP and CON, respectively, consisting largely of meat and poultry (~40-45% across all groups), fish (~4-5% across all groups), and dairy/eggs (~28-31% across all groups). To facilitate a comparison of protein quality, we used a method for determining protein quality (the 'DIAAS') for a single source combined with the total consumption of each protein, as well as protein intake relative to total caloric intake. However, our analysis revealed no differences in protein quality between any groups using either marker of protein quality (all  $P > 0.05$ , **Figure 3B**). There was no effect of sex, nor any differences in overall diet quality, protein quality or protein source between males and females (all  $P > 0.05$ ).

## DISCUSSION

To our knowledge, this is the first study comprehensively assessing dietary habits in awCF with an explicit focus on the amount, pattern, quality, and source of dietary protein intake and overall diet quality, prior to and (>3-months) following the commencement of ETI therapy, and compared with a healthy age-matched control group. We found that daily protein intake for awCF was higher than the RDA at both time points, with no differences between CON and CF at BL. However, in line with declines in total energy intake<sup>(15)</sup>, protein intake significantly reduced in CF at FUP, with a statistically significant effect on protein intake at lunch. Diet quality also significantly reduced at FUP in awCF compared with BL, to a level comparable to CON.

### *Daily Protein Intake*

Despite impaired nutrient absorption and reduced muscle mass and function in CF<sup>(5,22,23,36)</sup>, specific protein guidelines for pwCF are lacking, a notable omission noted within CF management guidelines<sup>(44)</sup>. This is perhaps unsurprising given the historic poor prognosis of CF, and the use of a high-fat, high-calorie diet to maintain weight and minimise lung function decline and declines in overall health. The improvement in clinical stability following ETI therapy provides an opportunity for dietary modification with a focus a healthy and balanced diet, including adequate protein to counteract CF and age-related complications such as the deteriorating skeletal muscle mass and function<sup>(18)</sup>.

Within the current study, the UK RDA for protein of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ , which is based on the consumption of high-quality protein in order to satisfy daily EAA requirements, was met by the majority of CF and CON individuals. However, 70% and 90% of individuals with CF did not reach the higher recommended daily protein intake of  $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  on all measurement days at BL and FUP, respectively, compared with 40% in CON. These higher recommended levels have been proposed for older age<sup>(39,40)</sup>, as well respiratory diseases at risk of comorbid sarcopenia, such as COPD<sup>(45)</sup>, and are largely based on observations of attenuated muscle loss in those >60-years-old and on the notion of consuming 3 meals daily containing  $\sim 0.40 \text{ g} \cdot \text{kg}^{-1}$  of protein, achieving a near maximal MPS response at each meal opportunity<sup>(39,40)</sup>. However, an uneven pattern of dietary protein intake was observed across meals for all groups, which was likely insufficient to reach the proposed threshold for maximal MPS stimulation at each meal and exacerbated by the lower total daily intake of protein in awCF at FUP.

The meal-specific distribution pattern of daily protein intake has been proposed as a factor in maximising stimulation of MPS across the day, with an evenly spread protein intake thought

to enhance daily net postprandial muscle anabolism <sup>(46)</sup>. The majority of individuals in the present study failed to reach maximal MPS stimulation thresholds at all meal opportunities, particularly at breakfast, lunch, and in participants on ETI therapy. In addition to a decline in overall food intake, this may in part reflect the requirement of consuming fat containing foods with ETI administration, which may have influenced breakfast or even lunch choice, if medication was delayed. The proposed dietary protein threshold for maximal MPS in young ( $0.24 \text{ g} \cdot \text{kg}^{-1}$ ) was only met on all recorded days by 10, 25, and 63% of CF individuals at BL and 10, 15, and 58% of CF individuals at FUP for breakfast, lunch, and dinner, respectively. When expressed relative to the higher MPS threshold for older individuals ( $0.40 \text{ g} \cdot \text{kg}^{-1}$ ), the threshold was met on all recorded days each by 0, 8, and 33% of CF individuals at BL and 0, 0, and 18% of CF individuals at FUP for breakfast, lunch, and dinner, respectively.

Therefore, it is plausible that the dietary protein habits of our CF cohort are insufficient to support skeletal muscle mass, and even more so in older age, due to a failure to maximally, or even robustly, stimulate MPS with every meal. Importantly, even though we did not assess protein intake in an older CF population, *per se* (18–28 years old  $n=9$ ; 29–39 years old  $n=18$ ; 40–50 years old  $n=11$ ; 51+ years  $n=2$ ), yet still compared our data to protein thresholds for older age in CF, we would expect protein intake to continue to decline with increasing age, as has been demonstrated numerous times before <sup>(37)</sup>. Indeed, our data of the skewed distribution of protein intake are consistent with observations in other cohorts of older individuals of varying health status <sup>(37,47–49)</sup>. However, our observations should be interpreted with caution as whilst data with isolated protein sources in an acute laboratory setting are encouraging, clear confirmatory data of the relative significance of this concept of per meal protein distribution across the day remained to be reported, particularly with whole-food studies which are more representative of habitual dietary patterns. It may, therefore, be more prudent based on current available evidence to focus on how many eating occasions an individual hits a proposed “*threshold*”, rather than the distribution of protein, *per se*.

#### *Protein and Overall Diet Quality*

The availability of sufficient EAAs within the diet is important for a robust increase in MPS and to support skeletal muscle remodelling <sup>(29,50)</sup>. Higher-quality proteins, reflected by superior digestible indispensable amino acid scores, have a greater protein density, greater EAA-to-Non-Essential AA ratio, and a favourable EAA profile which closely matches the bodily needs <sup>(29,50)</sup>. Based on these characteristics, animal, rather than plant-based proteins, are generally considered to be higher quality <sup>(33,34,48)</sup>. This is particularly pertinent to note in a cohort such as pwCF with impaired nutrient absorption and higher nutritional needs,

characteristics also observed with ageing<sup>(51)</sup>. Indeed, given their pre-existing nutrient absorption issues, consumption of a higher proportion of lower-quality plant-based proteins which are less digestible and bioavailable<sup>(33,34)</sup>, might exacerbate nutrient status and malnutrition in CF. However, despite significant impairments in nutrient absorption and reduced muscle mass, quality, and function in pwCF to suggest a benefit for higher protein intakes for skeletal muscle regulation<sup>(5,22,23,36)</sup>, no guidelines exist for protein requirements in this population other than a general recommendation to increase food intake, perhaps owing in part to the poor historical prognosis of CF<sup>(44)</sup>. In the present study, no notable differences were observed between groups in protein sources consumed at each meal, nor protein quality (Figure 3B). Nevertheless, substituting lower- for higher-quality proteins, particularly at lunch, may represent one viable easy-to-implement dietary approach to help to increase EAA delivery and support skeletal muscle maintenance in an ageing CF population. However, it is also worthy of note that breakfast is typically considered a particularly low protein dense meal, and has been identified as an important opportunity to raise daily protein intake to combat age-related muscle deterioration<sup>(46,52,53)</sup>. This was supported by data presented herein across all groups (Figure 2, Table 1), and therefore may benefit from the provision of an increase of higher quality proteins at this meal.

In contrast with our observations of no differences in dietary protein quality, overall diet quality was significantly higher in CF participants at BL, but this was significantly reduced at FUP to a level which was similar to our control group. Whilst a small change (5.5% reduction in diet quality) over a short time period, this may represent a reduced focus on the quantity and quality of food consumption in pwCF on ETI therapy, potentially owing to the improved clinical stability and significant weight gain of pwCF, which was previously difficult to achieve<sup>(15)</sup>. By contrast, that diet quality was higher at baseline in pwCF compared with CON may reflect an increased individual and clinical focus on the quality of food intake to help counteract significant digestive and nutrient absorption issues associated with CF pre-commencement of ETI therapy. A focus on diet quality may therefore be needed as a means to also to improve the quality of dietary protein and support metabolic and muscle health.

#### *Experimental Considerations and Future Directions*

It is important to acknowledge several experimental considerations of our work. First, a paucity of studies studying nutrient absorption and protein metabolism in pwCF make it difficult to formulate CF-specific MPS stimulatory thresholds and dietary protein recommendations<sup>(15)</sup>. Given the improvement in prognosis pwCF, this represents an important avenue for future research. Our findings support calls for future studies to



investigate first, whether anabolic resistance to protein provision is present in pwCF and subsequently whether increasing or redistributing per-meal protein intakes in CF, targeting breakfast and lunch, could maintain skeletal muscle health, particularly in an ageing CF population. Whether this is best achieved through protein supplementation, fortifying commonly consumed foods with protein/EAA, or altering meal macronutrient composition in favour of protein, also remains to be elucidated. Further, given the high infection risk and inflammation observed in pwCF, which may also independently affect protein turnover <sup>(54)</sup>, assessing protein turnover at the whole-body level, combining the use of stable isotope tracers with non-invasive approaches (e.g., breath and urine to determine exogenous AA oxidation and retention, and 3-methylhistidine enrichments, respectively) may represent a particularly promising avenue for future work in CF.

In our study, it is also important to acknowledge that we did not comprehensively assess the physical activity status or body composition changes of our CF participants. This is important to highlight as physical activity status may be an significant determinant of muscle anabolic responsiveness <sup>(55)</sup>. Specifically, physical activity/exercise act in synergy with dietary protein ingestion to further enhance MPS, and can therefore improve muscle anabolic responsiveness in older individuals regularly failing to consume adequate daily protein amounts <sup>(56–58)</sup>. It is widely accepted that combining dietary protein strategies with regular physical activity, particularly in the form of structured resistance exercise training, offers the most potent non-pharmacological means of maintaining or improving muscle mass, strength and function in older age, and represents an important consideration in an ageing CF population <sup>(59–61)</sup>.

Knowledge of body composition changes in this study would also have been valuable, as a reduction in lean/muscle mass at FUP would have provided further strength to our suggestion of an increased consumption of dietary protein in CF to mitigate against muscle loss, and, excess adiposity impairs metabolic health and can contribute to anabolic resistance to protein provision <sup>(55,62)</sup>. Whilst we acknowledge that our CF cohort is more representative of a younger-to-middle-aged population with specific reference to chronological age, we felt it was important to assess the implications for healthy ageing if protein intakes reduce at FUP and continue to decline thereafter, as observed in healthy adults <sup>(37)</sup>. In addition, as CF is associated with reduced muscle mass and function, and a short life expectancy, this condition represents a model of accelerated ageing, thereby justifying the comparison with MPS thresholds for older adults, and declines in age-related muscle mass are actually generally apparent from middle-age onwards <sup>(63)</sup>.

It is also prudent to acknowledge some of the valid limitations of the method of data collection primarily used for the data presented herein, including underreporting of food intake and over reporting of food quality (in accordance with social desirability) that may skew some of our observations<sup>(64,65)</sup>. However, high validity and precision has been reported for dietary records, particularly in clinical practice when used following adequate procedures and considering sufficient number of days<sup>(64–66)</sup>. In addition, diet diaries are regularly completed by this population as part of their clinical support programme and the use of a well-trained facilitator was employed in the current study, thereby reducing some of the concerns typically associated with dietary records<sup>(64–66)</sup>. A notable limitation of our data though is the relatively short follow-up period (3-months), which may not be sufficient to capture long-term dietary or metabolic adaptations following ETI therapy in adults with CF and requires further investigation. Our data also lack supporting behavioural/qualitative data and future work should incorporate assessments of dietary preferences or eating behaviours to substantiate some of our interpretations. Similar to our control group, we also observed inherent variability across our CF participants, highlighting some of the limitations with interpretation of our data but that a personalised approach is likely essential when devising protein recommendations for pwCF, particularly as there may also be individuals where higher protein intakes are clinically contraindicated (e.g. significant renal impairment). Finally, given the anticipated improvements in life expectancy in CF as well as observations of weight gain, significant research in CF is warranted as we begin to observe an older, obesogenic, CF population for the first time. Indeed, ageing, obesity and periods of energy deficit are also associated with impaired protein turnover/anabolic resistance, a higher need for dietary protein and accelerated skeletal muscle deterioration<sup>(55,62,67–69)</sup>. Future work may wish to explore the consequences of changes in macronutrient distribution in CF, given that adiposity<sup>(55)</sup>, energy deficit (as a result of reducing adiposity)<sup>(69)</sup> and essential fatty acid consumption (low levels of which are associated with energy restriction)<sup>(70)</sup> are associated with alterations to protein turnover that may negatively affect muscle mass.

### *Conclusion*

Daily protein intake was higher in CF participants than the current protein RDA of  $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . However, protein intake significantly reduced following initiation of ETI therapy. Meal-specific protein distribution was uneven and inadequate to repeatedly reach the proposed threshold for maximal MPS stimulation, with the majority of individuals not meeting the proposed higher protein recommendation of  $>1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  which might be expected for a condition associated with severe suppurative lung disease, exaggerated innate



inflammation, malabsorption and a recognised risk of sarcopenia and reduced muscle quality, even in younger pwCF. No differences in protein quality or protein source were observed, however, diet quality was significantly reduced at follow up in awCF, after commencing ETI therapy. Increasing the total intake and quality of dietary protein, particularly at breakfast and lunch, in combination with regular physical activity and exercise in CF could potentially help mitigate muscle loss to support an increasingly ageing CF population in. However, these changes need to form part of an overall more balanced diet to minimise longer-term age-related co-morbidities and complications.

### **CRedit authorship contribution statement**

P.T. Morgan: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Project administration, Resources, Funding acquisition, Supervision, Project administration, Writing – original draft; T.J. Ellis: Formal analysis; B. Smeuninx: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Project administration, Writing – review & editing; L. Breen: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Project administration, Writing – review & editing; L. Kinsey: Investigation, Writing – review & editing; O.W. Tomlinson: Conceptualization, Writing – review & editing; H. White: Conceptualization, Methodology, Funding acquisition, Supervision, Writing – review & editing; L.R. Caley: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Project administration, Writing – review & editing; D.G. Peckham: Conceptualization, Methodology, Resources, Funding acquisition, Supervision, Project administration, Writing – review & editing. All authors gave their final approval of the version of the article to be published.

### **Authorship Statement**

Conforming to the International Committee of Medical Journal Editors definition of authorship, we can confirm that all authors:

1. Made substantial contributions to conception and design, data acquisition, analysis and/or interpretation;
2. Contributed to drafting the article and/or revising it critically for important intellectual content;
3. Approved the latest version; and
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Acknowledgments**

The authors would like to extend their thanks to authors of Caley et al. (2023) [PMID: 37422432] and Smeuninx et al. (2020) [PMID: 32232047] who contributed to previous work associated with data presented herein. Myfood24 was developed through Medical Research Council funding, grant G110235. Myfood24 is now being supported by spinout company Dietary Assessment Ltd. Requests to use Myfood24 should be made to [enquiries@myfood24.org](mailto:enquiries@myfood24.org).

**Disclosure of funding**

This research was sponsored by a summer studentship from the CF Trust awarded to P.T.M (Grant reference SS24/01). The original work (PMID: 37422432) was supported by the UK CF Trust (SRC 012) and the College of Life and Environmental Sciences, University of Birmingham (PMID: 32232047).

**Competing interests**

LRC has previously received a speaker fee from Vertex pharmaceuticals (Elexacaftor-tezacaftor-ivacaftor is a prescription treatment for cystic fibrosis developed by Vertex Pharmaceuticals). DGP: speaker/board honoraria from Vertex Pharmaceuticals. OWT: speaker fees from Vertex Pharmaceuticals. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## REFERENCES

1. Guo J, Garratt A, & Hill A (2022) Worldwide rates of diagnosis and effective treatment for cystic fibrosis. *J Cyst Fibros* **21**, 456–462.
2. Ooi CY & Durie PR (2016) Cystic fibrosis from the gastroenterologist's perspective. *Nat Rev Gastroenterol Hepatol* **13**, 175–185.
3. Coderre L, Debieche L, Plourde J, Rabasa-Lhoret R, & Lesage S (2021) The Potential Causes of Cystic Fibrosis-Related Diabetes. *Front Endocrinol (Lausanne)* **12**. doi:10.3389/fendo.2021.702823.
4. Birch RJ, Peckham D, Wood HM, Quirke P, Konstant-Hambling R, Brownlee K *et al.* (2023) The risk of colorectal cancer in individuals with mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene: An English population-based study. *J Cyst Fibros* **22**, 499–504.
5. Gruet M, Troosters T, & Verges S (2017) Peripheral muscle abnormalities in cystic fibrosis: Etiology, clinical implications and response to therapeutic interventions. *J Cyst Fibros* **16**, 538–552.
6. Frantzen T, Barsky S, LaVecchia G, Marowitz M, & Wang J (2023) Evolving Nutritional Needs in Cystic Fibrosis. *Life* **13**, 1431.
7. Tomas J, Mulet C, Saffarian A, Cavin J-B, Ducroc R, Regnault B *et al.* (2016) High-fat diet modifies the PPAR- $\gamma$  pathway leading to disruption of microbial and physiological ecosystem in murine small intestine. *Proc Natl Acad Sci* **113**. doi:10.1073/pnas.1612559113.
8. Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, & Vandamme P (2011) Cross-Sectional and Longitudinal Comparisons of the Predominant Fecal Microbiota Compositions of a Group of Pediatric Patients with Cystic Fibrosis and Their Healthy Siblings. *Appl Environ Microbiol* **77**, 8015–8024.
9. Sutherland R, Katz T, Liu V, Quintano J, Brunner R, Tong CW *et al.* (2018) Dietary intake of energy-dense, nutrient-poor and nutrient-dense food sources in children with cystic fibrosis. *J Cyst Fibros* **17**, 804–810.
10. Nichols DP, Paynter AC, Heltshe SL, Donaldson SH, Frederick CA, Freedman SD *et al.* (2022) Clinical Effectiveness of Elexacaftor/Tezacaftor/Ivacaftor in People with Cystic Fibrosis: A Clinical Trial. *Am J Respir Crit Care Med* **205**, 529–539.
11. Gj C, S M, Tc L, N S, Ss S, T M *et al.* (2025) Real-world impact of Elexacaftor-Tezacaftor-Ivacaftor treatment in young people with Cystic Fibrosis: A longitudinal study. *Respir Med* **236**, 107882.
12. Burgel P-R, Durieu I, Chiron R, Ramel S, Danner-Boucher I, Prevotat A *et al.* (2021) Rapid Improvement after Starting Elexacaftor–Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis and Advanced Pulmonary Disease. *Am J Respir Crit Care Med* **204**, 64–73.
13. Barry PJ, Mall MA, Álvarez A, Colombo C, de Winter-de Groot KM, Fajac I *et al.* (2021) Triple Therapy for Cystic Fibrosis Phe508del –Gating and –Residual Function Genotypes. *N Engl J Med* **385**, 815–825.
14. Griesse M, Costa S, Linnemann RW, Mall MA, McKone EF, Polineni D *et al.* (2021) Safety and Efficacy of Elexacaftor/Tezacaftor/Ivacaftor for 24 Weeks or Longer in

- People with Cystic Fibrosis and One or More F508del Alleles: Interim Results of an Open-Label Phase 3 Clinical Trial. *Am J Respir Crit Care Med* **203**, 381–385.
15. Caley LR, Jarosz-Griffiths HH, Smith L, Gale L, Barrett J, Kinsey L *et al.* (2023) Body mass index and nutritional intake following Elexacaftor/Tezacaftor/Ivacaftor modulator therapy in adults with cystic fibrosis. *J Cyst Fibros* **22**, 1002–1009.
  16. Lopez A, Daly C, Vega-Hernandez G, MacGregor G, & Rubin JL (2023) Elexacaftor/tezacaftor/ivacaftor projected survival and long-term health outcomes in people with cystic fibrosis homozygous for F508del. *J Cyst Fibros* **22**, 607–614.
  17. Keogh RH, Szczesniak R, Taylor-Robinson D, & Bilton D (2018) Up-to-date and projected estimates of survival for people with cystic fibrosis using baseline characteristics: A longitudinal study using UK patient registry data. *J Cyst Fibros* **17**, 218–227.
  18. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz A V. *et al.* (2006) The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. *Journals Gerontol Ser A Biol Sci Med Sci* **61**, 1059–1064.
  19. Lamhonwah A, Bear CE, Huan LJ, Chiaw PK, Ackerley CA, & Tein I (2010) Cystic fibrosis transmembrane conductance regulator in human muscle: Dysfunction causes abnormal metabolic recovery in exercise. *Ann Neurol* **67**, 802–808.
  20. Divangahi M, Balghi H, Danialou G, Comtois AS, Demoule A, Ernest S *et al.* (2009) Lack of CFTR in Skeletal Muscle Predisposes to Muscle Wasting and Diaphragm Muscle Pump Failure in Cystic Fibrosis Mice. *PLoS Genet* **5**, e1000586.
  21. Navas-Moreno V, Sebastian-Valles F, Rodríguez-Laval V, Knott-Torcal C, Marazuela M, de la Blanca NS *et al.* (2024) Impact of CFTR modulator therapy on body composition as assessed by thoracic computed tomography: A follow-up study. *Nutrition* **123**, 112425.
  22. Tomlinson OW, Barker AR, Fulford J, Wilson P, Shelley J, Oades PJ *et al.* (2023) Skeletal muscle contributions to reduced fitness in cystic fibrosis youth. *Front Pediatr* **11**, 1211547.
  23. Shei R-J, Mackintosh KA, Peabody Lever JE, McNarry MA, & Krick S (2019) Exercise Physiology Across the Lifespan in Cystic Fibrosis. *Front Physiol* **10**, 1382.
  24. Biolo G, Fleming RY, Maggi SP, & Wolfe RR (1995) Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am J Physiol Metab* **268**, E75–E84.
  25. Tipton K (1999) Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J Nutr Biochem* **10**, 89–95.
  26. Wolfe RR (2002) Regulation of Muscle Protein by Amino Acids. *J Nutr* **132**, 3219S–3224S.
  27. Shad BJ, Thompson JL, & Breen L (2016) Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am J Physiol Metab* **311**, E803–E817.
  28. Morgan PT, Witard OC, Højfeldt G, Church DD, & Breen L (2023) Dietary protein recommendations to support healthy muscle ageing in the 21 st Century and beyond: considerations and future directions. *Proc Nutr Soc* 1–32.

29. Morgan PT, Harris DO, Marshall RN, Quinlan JI, Edwards SJ, Allen SL *et al.* (2021) Protein Source and Quality for Skeletal Muscle Anabolism in Young and Older Adults: A Systematic Review and Meta-Analysis. *J Nutr* **151**, 1901–1920.
30. Nunes EA, Colenso-Semple L, McKellar SR, Yau T, Ali MU, Fitzpatrick-Lewis D *et al.* (2022) Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults. *J Cachexia Sarcopenia Muscle* **13**, 795–810.
31. Carbone JW & Pasiakos SM (2019) Dietary Protein and Muscle Mass: Translating Science to Application and Health Benefit. *Nutrients* **11**, 1136.
32. Campbell WW, Deutz NEP, Volpi E, & Apovian CM (2023) Nutritional Interventions: Dietary Protein Needs and Influences on Skeletal Muscle of Older Adults. *Journals Gerontol Ser A* **78**, 67–72.
33. van Vliet S, Burd NA, & van Loon LJ (2015) The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. *J Nutr* **145**, 1981–1991.
34. Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB *et al.* (2018) Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids* **50**, 1685–1695.
35. van der Heijden I, Monteyne AJ, Stephens FB, & Wall BT (2023) Alternative dietary protein sources to support healthy and active skeletal muscle aging. *Nutr Rev* **81**, 206–230.
36. Dodge JA & Turck D (2006) Cystic fibrosis: Nutritional consequences and management. *Best Pract Res Clin Gastroenterol* **20**, 531–546.
37. Smeuninx B, Greig CA, & Breen L (2020) Amount, Source and Pattern of Dietary Protein Intake Across the Adult Lifespan: A Cross-Sectional Study. *Front Nutr* **7**, 1–9.
38. Tomlinson OW, Barker AR, Denford S, & Williams CA (2024) Adapting, restarting, and terminating a randomised control trial for people with cystic fibrosis: Reflections on the impact of the COVID-19 pandemic upon research in a clinical population. *Contemp Clin Trials Commun* **39**, 101294.
39. Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD *et al.* (2015) Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men. *Journals Gerontol Ser A Biol Sci Med Sci* **70**, 57–62.
40. Phillips SM, Chevalier S, & Leidy HJ (2016) Protein “requirements” beyond the RDA: implications for optimizing health. *Appl Physiol Nutr Metab* **41**, 565–572.
41. Adhikari S, Schop M, de Boer IJM, & Huppertz T (2022) Protein Quality in Perspective: A Review of Protein Quality Metrics and Their Applications. *Nutrients* **14**, 947.
42. Moughan PJ & Lim WXJ (2024) Digestible indispensable amino acid score (DIAAS): 10 years on. *Front Nutr* **11**. doi:10.3389/fnut.2024.1389719.
43. Kennedy ET, Ohls J, Carlson S, & Fleming K (1995) The Healthy Eating Index. *J Am Diet Assoc* **95**, 1103–1108.
44. (2016) Nutritional Management of Cystic Fibrosis (2nd ed.). Cyst. Fibros. Trust. [https://www.cysticfibrosis.org.uk/sites/default/files/2020-12/Nutritional Management of cystic fibrosis Sep 16.pdf](https://www.cysticfibrosis.org.uk/sites/default/files/2020-12/Nutritional%20Management%20of%20cystic%20fibrosis%20Sep%2016.pdf) (accessed January 2025).
45. Collins PF, Yang IA, Chang Y-C, & Vaughan A (2019) Nutritional support in chronic

- obstructive pulmonary disease (COPD): an evidence update. *J Thorac Dis* **11**, S2230–S2237.
46. Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore M *et al.* (2014) Dietary Protein Distribution Positively Influences 24-h Muscle Protein Synthesis in Healthy Adults. *J Nutr* **144**, 876–880.
  47. Cardon-Thomas DK, Riviere T, Tiegues Z, & Greig CA (2017) Dietary protein in older adults: Adequate daily intake but potential for improved distribution. *Nutrients* **9**, 1–10.
  48. Tieland M, Borgonjen-Van den Berg K, Van Loon L, & De Groot L (2015) Dietary Protein Intake in Dutch Elderly People: A Focus on Protein Sources. *Nutrients* **7**, 9697–9706.
  49. Paddon-Jones D, Campbell WW, Jacques PF, Kritchevsky SB, Moore LL, Rodriguez NR *et al.* (2015) Protein and healthy aging. *Am J Clin Nutr* **101**, 1339S-1345S.
  50. Phillips SM (2016) The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab (Lond)* **13**, 64.
  51. Fujita S & Volpi E (2006) Amino Acids and Muscle Loss with Aging. *J Nutr* **136**, 277S-280S.
  52. Layman DK, Anthony TG, Rasmussen BB, Adams SH, Lynch CJ, Brinkworth GD *et al.* (2015) Defining meal requirements for protein to optimize metabolic roles of amino acids. *Am J Clin Nutr* **101**, 1330S-1338S.
  53. Khaing I-K, Tahara Y, Chimed-Ochir O, Shibata S, & Kubo T (2024) Effect of breakfast protein intake on muscle mass and strength in adults: a scoping review. *Nutr Rev.* doi:10.1093/nutrit/nuad167.
  54. Costamagna D, Costelli P, Sampaolesi M, & Penna F (2015) Role of Inflammation in Muscle Homeostasis and Myogenesis. *Mediators Inflamm* **2015**, 805172.
  55. Smeuninx B, Mckendry J, Wilson D, Martin U, & Breen L (2017) Age-Related Anabolic Resistance of Myofibrillar Protein Synthesis Is Exacerbated in Obese Inactive Individuals. *J Clin Endocrinol Metab* **102**, 3535–3545.
  56. Pennings B, Koopman R, Beelen M, Senden JM, Saris WH, & van Loon LJ (2011) Exercising before protein intake allows for greater use of dietary protein-derived amino acids for de novo muscle protein synthesis in both young and elderly men. *Am J Clin Nutr* **93**, 322–331.
  57. Tieland M, Dirks ML, van der Zwaluw N, Verdijk LB, van de Rest O, de Groot LCPGM *et al.* (2012) Protein Supplementation Increases Muscle Mass Gain During Prolonged Resistance-Type Exercise Training in Frail Elderly People: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Am Med Dir Assoc* **13**, 713–719.
  58. Thomas DK, Quinn MA, Saunders DH, & Greig CA (2016) Protein Supplementation Does Not Significantly Augment the Effects of Resistance Exercise Training in Older Adults: A Systematic Review. *J Am Med Dir Assoc* **17**, 959.e1-959.e9.
  59. Cermak NM, Res PT, de Groot LC, Saris WH, & van Loon LJ (2012) Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr* **96**, 1454–1464.
  60. Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E *et al.* (2018) A systematic review, meta-analysis and meta-regression of the effect of



- protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med* **52**, 376–384.
61. McGlory C, Devries MC, & Phillips SM (2017) Skeletal muscle and resistance exercise training; the role of protein synthesis in recovery and remodeling. *J Appl Physiol* **122**, 541–548.
  62. Morgan PT, Smeuninx B, & Breen L (2020) Exploring the Impact of Obesity on Skeletal Muscle Function in Older Age. *Front Nutr* **7**. doi:10.3389/fnut.2020.569904.
  63. Volpi E, Nazemi R, & Fujita S (2004) Muscle tissue changes with aging. *Curr Opin Clin Nutr Metab Care* **7**, 405–10.
  64. Ortega RM, Pérez-Rodrigo C, & López-Sobaler AM (2015) Dietary assessment methods: dietary records. *Nutr Hosp* **31 Suppl 3**, 38–45.
  65. Greaney C, Bohan K, Tecklenborg S, Casserly B, Green J, Van de Ven P *et al.* (2022) Diet quality in cystic fibrosis - associations with patient reported outcome measures and enablers and barriers to eating a healthy diet: A protocol paper for a mixed methods study. *HRB open Res* **5**, 33.
  66. Grimm P (2010) Social Desirability Bias. In *Wiley International Encyclopedia of Marketing*. Wiley.
  67. Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BBL, Verdijk LB *et al.* (2015) Aging Is Accompanied by a Blunted Muscle Protein Synthetic Response to Protein Ingestion. *PLoS One* **10**, e0140903.
  68. Paulussen KJM, McKenna CF, Beals JW, Wilund KR, Salvador AF, & Burd NA (2021) Anabolic Resistance of Muscle Protein Turnover Comes in Various Shapes and Sizes. *Front Nutr* **8**. doi:10.3389/fnut.2021.615849.
  69. Gwin JA, Church DD, Wolfe RR, Ferrando AA, & Pasiakos SM (2020) Muscle Protein Synthesis and Whole-Body Protein Turnover Responses to Ingesting Essential Amino Acids, Intact Protein, and Protein-Containing Mixed Meals with Considerations for Energy Deficit. *Nutrients* **12**, 2457.
  70. McGlory C, Calder PC, & Nunes EA (2019) The Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Turnover in Health, Disuse, and Disease. *Front Nutr* **6**. doi:10.3389/fnut.2019.00144.

**TABLE 1**

**Table 1.** Comprehensive summary of daily dietary protein intake for CF at BL and CF at FUP and for CON.

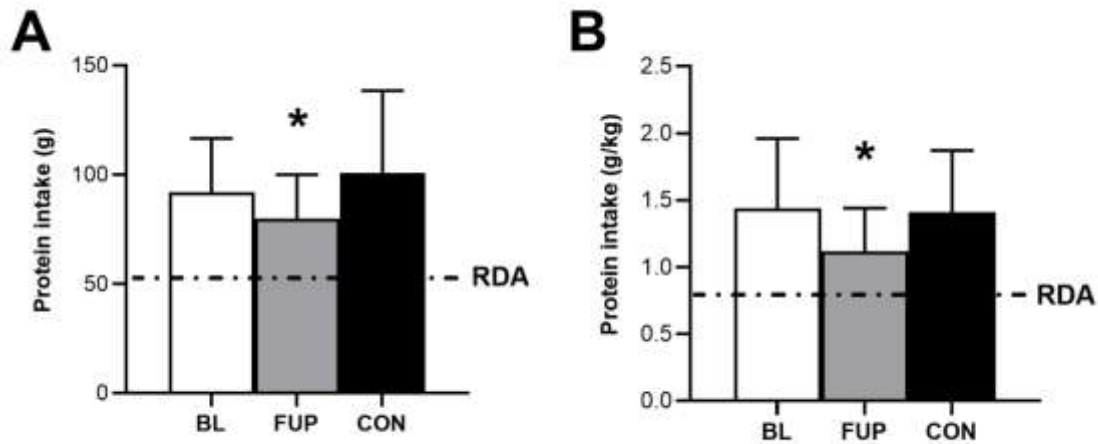
	<b>CF at BL</b> (n=40)	<b>CF at FUP</b> (n=40)	<b>CON</b> (n=80)
<b>Total daily protein intake (g·day<sup>-1</sup>)</b>	92.0 ± 24.6	79.9 ± 20.1 * †	100.6 ± 37.8
<i>Male</i>	97.7 ± 24.9	85.6 ± 20.2 * †	115.2 ± 42.8
<i>Female</i>	87.8 ± 23.8	71.4 ± 17.6 * †	87.3 ± 26.8
<b>Total daily protein intake (g·kg<sup>-1</sup>·day<sup>-1</sup>)</b>	1.44 ± 0.52	1.12 ± 0.32 * †	1.41 ± 0.46
<i>Male</i>	1.36 ± 0.47	1.10 ± 0.27 * †	1.48 ± 0.51
<i>Female</i>	1.53 ± 0.57	1.14 ± 0.37 * †	1.35 ± 0.42
<i>Meal-specific protein intake</i>			
<b>Breakfast Protein intake (g)</b>	16.5 ± 10.5	14.2 ± 7.4	15.0 ± 10.1
<i>Male</i>	15.3 ± 6.6	15.3 ± 8.7	17.9 ± 12.1
<i>Female</i>	17.8 ± 13.6	13.0 ± 5.6	12.4 ± 7.1
<b>Lunch Protein intake (g)</b>	27.0 ± 10.9	20.8 ± 9.2 * †	30.8 ± 16.9
<i>Male</i>	29.3 ± 9.5	24.1 ± 10.1 * †	37.5 ± 21.0
<i>Female</i>	24.5 ± 12.1	17.2 ± 6.5 * †	24.7 ± 8.7
<b>Dinner Protein intake (g)</b>	38.5 ± 15.2	35.5 ± 11.8	42.0 ± 17.8



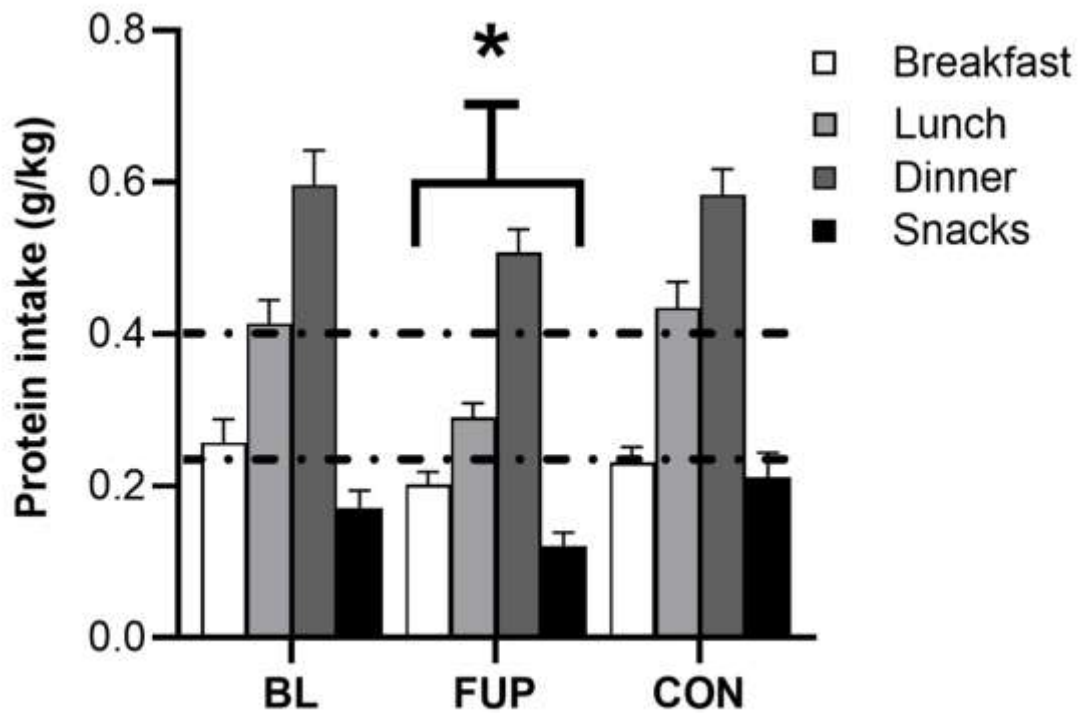
	<i>Male</i>	40.9 ± 17.1	35.9 ± 12.4	43.4 ± 18.6
	<i>Female</i>	35.8 ± 12.6	35.0 ± 11.4	40.7 ± 17.2
<b>Snacks Protein intake (g)</b>		11.0 ± 8.1	8.4 ± 7.2	12.8 ± 14.0
	<i>Male</i>	12.2 ± 8.4	10.3 ± 7.4	16.3 ± 16.6
	<i>Female</i>	9.6 ± 7.9	6.2 ± 6.5	9.5 ± 10.3

CF, Cystic Fibrosis; BL, Baseline; FUP, Follow-up; CON, Control. \* denotes significant difference from CON (P<0.05). † denotes significant difference from BL (P<0.05).

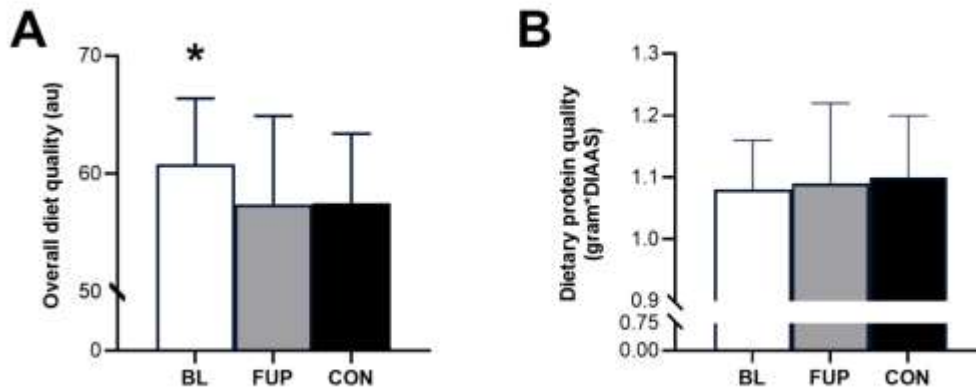
## FIGURE LEGENDS



**Figure 1.** Daily protein intake for baseline (BL, clear bar), follow up (FUP, grey bar) and healthy controls (CON, black bar) are shown in Panel A. Daily protein intake relative to bodyweight (in kilograms) is shown in Panel B. The dashed line represents the current RDA for protein in the UK ( $0.75 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ). The dashed line in Panel A represents a typical 70 kg individual. Values are presented as means  $\pm$  SD. Significance was set at  $P < 0.05$ . \* Significantly different to BL and CON.



**Figure 2.** Meal-specific protein intake relative to bodyweight (in kilograms) for baseline (BL), follow up (FUP) and healthy controls (CON) at breakfast (clear bars), lunch (light grey bars), dinner (dark grey bars) and as snacks (black bars). The dashed lines represent protein intake required for near maximal stimulation of muscle protein synthesis for younger ( $\sim 0.24 \text{ g} \cdot \text{kg}^{-1}$ ) and older ( $\sim 0.40 \text{ g} \cdot \text{kg}^{-1}$ ) individuals, respectively, taken from Moore et al., (2015) <sup>(39)</sup>. Values are presented as means  $\pm$  SD. Significance was set at  $P < 0.05$ . \* Significantly different to BL and CON.



**Figure 3.** Overall diet quality and dietary protein quality for baseline (BL, clear bar), follow up (FUP, grey bar) and healthy controls (CON, black bar) are shown in Panels A and B, respectively. Overall diet quality was assessed using the Healthy Eating Index (Panel A). Dietary protein quality was assessed by multiplying protein intake by the respective Digestible Indispensable Amino Acid Score (DIAAS), factoring in ileal digestibility for a single protein source (Panel B). Values are presented as means  $\pm$  SD. Significance was set at  $P < 0.05$ . \* Significantly different to FUP and CON.