DIETARY FACTORS MAY BE ASSOCIATED WITH MEASURES OF ULTRASOUND-DERIVED SKELETAL MUSCLE ECHO INTENSITY

Joe Page[1], Georgia A Scott[1], James N Aggett[1], Georgina K Stebbings[2], Liam P Kilduff[1,4], Caoileann H Murphy[3], Mark Waldron[1,4,5], Shane M Heffernan[1]

[1]*Applied Sports, Technology, Exercise and Medicine Research Centre (A-STEM), Faculty of Science and Engineering, Swansea University, Swansea University, United Kingdom;* [2]*Manchester Metropolitan University Institute of Sport, Department of Sport and Exercise Sciences, Manchester Metropolitan University, Manchester, United Kingdom;* [3]*Agrifood Business and Spatial Analysis, Teagasc Food Research Centre, Ashtown, Dublin, 15, Ireland;* [4]*Welsh Institute of Performance Science, Swansea University, Swansea, United Kingdom;* [5]*School of Health and Behavioural Sciences, University of the Sunshine Coast, Queensland, Australia*

**Address for correspondence:*

Joe Page

Applied Sports, Technology, Exercise and Medicine Research Centre

Swansea University, Bay Campus

Swansea, SA1 8EN, United Kingdom

Email: omniplantresearch@swansea.ac.uk

ORCID: 0000-0002-8582-6519

Key words: skeletal muscle, muscle quality, intramuscular fat, habitual diet, sarcopenia, ageing

Acknowledgements

Author contributions were as follows: conceptualisation (JP, SMH); data curation (JP, JNA); formal analysis (JP, GAS, JNA); investigation (JP, JNA); methodology (JP, SMH, CHM); project administration (JP, SMH); supervision (SMH, MW); writing- review and editing (JP, SMH, MW, LPK, GKS, CHM, GAS).

Funding

No external funding was obtained for this study.

Conflict of interest

No potential conflict of interest was reported by the author(s).

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data availability

Data generated or analysed during this study are available from the corresponding author upon reasonable request.

Abstract

Skeletal muscle echo intensity (EI) is affected by ageing and physical activity; however, the effects of nutrition are less understood. The aim of this study was to explore whether habitual nutrient intake may be associated with ultrasound-derived EI. Partial least squares regression (PLSR) models were trained on an initial sample (*n*=100, M=45; F=55; 38±15 years) to predict EI of two quadriceps muscles from 19 variables, using the '*jack-knife*' function within the '*pls*' package (RStudio), which was then tested in an additional dataset (*n*= 30, M=13; F=17; 38±16 years). EI was determined using B-mode ultrasonography of the rectus femoris (RF) and vastus lateralis (VL) and nutritional intake determined via three-day weighed food diaries. Mean daily intake of specific nutrients were included as predictor variables with age, sex and self-reported physical activity. PLSR training model 1 explained ~52% and model 2 ~46% of the variance in RF and VL EI, respectively. Model 1 also explained ~35% and model 2 ~30% of the variance in RF and VL EI in the additional testing dataset. Age and biological sex were associated with EI in both models (*P*<0.025). Dietary protein (RF: β=-7.617,VL: β=-7.480), and selenium (RF: β=-7.144,VL: β=-4.775) were associated with EI in both muscles (*P*<0.05), whereas fibre intake (RF: β=-5.215) was associated with RF EI only and omega-3 fatty acids (n-3/ω-3 FAs, RF: β=3.145) with VL EI only (*P*<0.05). Therefore, absolute protein, selenium, fibre and n-3 FAs may be associated with skeletal muscle EI, although further mechanistic work is required before claiming causal inference.

Introduction

The composition of skeletal muscle is widely accepted as a contributing factor to muscle quality (Correa-de-Araujo et al., 2017), now defined as the macro- and microscopic aspects of muscle architecture and composition (Cruz-Jentoft et al., 2019). These properties are associated with skeletal muscle functional performance, particularly in older populations with some data supporting similar relationships in younger people (Garrett, 2020). Discrepancies in the rate of decline in muscle mass and strength with age have previously been reported, which could be partly attributed to variability in muscle quality (Delmonico et al., 2009, Goodpaster et al., 2001). This has led to the recent incorporation of muscle quality into clinical definitions for age-related conditions, such as sarcopenia (Cruz-Jentoft et al., 2019).

Accumulation of fibrous and intramuscular adipose tissue (IMAT) reduces the proportion of contractile tissue within the muscle and alters architectural parameters, such as fascicle pennation angle (Addison et al., 2014a). Subsequently, IMAT accumulation has been associated with reduced maximal strength (Goodpaster et al., 2001, Manini et al., 2007, Pinel et al., 2021) and neuromuscular activation in both young and older adults (Yoshida et al., 2012, Lanza et al., 2020) as well as measures of reduced functional capacity such as gait speed, hand grip strength (Therkelsen et al., 2016), poor balance (Addison et al., 2014b) and increased risk of falls (Vitale et al., 2021) in ageing populations. This highlights the importance of establishing non-invasive techniques for assessing IMAT accumulation, particularly in populations at greater risk of age-related musculoskeletal conditions such as sarcopenia.

Ultrasound-derived echo intensity (EI) has been gaining interest as an easily accessible and low-cost measure of skeletal muscle quality, with growing discussions of the potential clinical applications (Isaka et al., 2019, Nagae et al., 2021, Akazawa et al., 2023). EI is the appearance of non-contractile material, such as adipose and fibrous tissue, in muscle ultrasound images that contribute to varying levels of echogenicity, quantified as mean gray-scale pixel intensity within a defined region of interest (Stock and Thompson, 2021). It is well established that EI is impacted by age, as muscle quality deteriorates across time due to fibrous and IMAT accumulation (Pillen et al., 2009). This has been demonstrated in both older men and women across various muscle groups in the upper-limbs (Fukumoto et al., 2015, Kobayashi et al., 2023), lower-limbs (Arts et al., 2010, Fukumoto et al., 2015, Strasser et al., 2013, Palmer and Thompson, 2017, Paris et al., 2020) and the trunk (Fukumoto et al., 2015, Ota et al., 2020). Similar to IMAT accumulation, EI inversely correlates with maximal muscle strength (Kuschel et al., 2022) and functional measures, such as sit-stand and gait speed tests (Rech et al., 2014, Wu et al., 2022, Paris et al., 2022). However, regular physical activity has been reported to help reduce skeletal muscle EI in both aged (Fukumoto et al., 2018) and clinical populations (Okura et al., 2022). Multiple intervention studies have found that six-months of resistance training can reduce muscle EI, thereby improving muscle quality (Radaelli et al., 2013, Radaelli et al., 2014, Wilhelm et al., 2014, Yoshiko et al., 2017). These findings have established that exercise-induced mechanical stress can positively impact EI, but little is known about the effects of nutrition.

The effects of dietary intake on skeletal muscle mass and strength/function are well established (Cruz-Jentoft et al., 2020). While not all studies agree, greater dietary protein intake has been associated with greater muscle mass and maximal strength, particularly in older populations (Sahni et al., 2015). Studies have shown that postmenopausal women consuming ≥ 1.2 g/kg/d of protein exhibited greater maximal strength and superior muscle quality, assessed via maximal quadriceps strength normalised to muscle mass, compared with individuals consuming 0.8 g/kg/d (Lemieux et al., 2014). Meta-analytical work has also shown that interventions increasing habitual fat intake may result in greater IMAT accumulation across multiple lower-limb muscles, including the vastus lateralis (VL), tibialis anterior and soleus (Ahmed et al., 2018). These studies provide an early insight into the potential influence of nutritional intake on skeletal muscle quality. It is also clear, however, that currently there is not enough existing evidence for researchers and clinicians to provide nutritional recommendations relating to preservation of muscle quality in clinical populations, such as older individuals (≥ 65 years of age). Identification of specific nutrients that can elicit beneficial effects on skeletal muscle quality could inform nutritional interventions to target the prevention and management of age-related skeletal muscle diseases such as sarcopenia.

Given that nutritional interventions are a common and feasible method for improving overall health, including skeletal muscle adaptation to exercise and ageing, it is important to determine whether parameters of, non-invasively measured, EI-derived muscle quality may be influenced by habitual dietary intake, which is yet to be investigated. Indeed, any dietary factors associated with skeletal muscle EI could provide a feasible alternative strategy to prevent declines in muscle mass, quality and function in clinical populations, particularly in cases where regular resistance exercise may be challenging. This study aspired to provide the basis for future research bridging a significant gap in the existing literature that could lead to the development of more refined and effective nutritional guidelines related to clinical populations (such as in sarcopenia) and for the exploration of potential targeted nutritional interventions for preservation of skeletal muscle quality in older populations. Therefore, the aim of this study was to explore whether habitual intake of specific dietary nutrients may be associated with skeletal muscle EI as a marker of muscle quality.

Methods

Participants

One hundred and thirty participants (*n* = 58 males; *n* = 72 females; 93 % Caucasian, 3 % Asian, 2 % Mixed White and Asian, 2 % Other) were randomly selected from the existing sample recruited as part of the ongoing Omnivorous and Non-meat eater Integrative Physiology and Nutrition (OMNIPLaNT) study. The data presented in the current study were collated as part of a wider cross-sectional observational study investigating the effects of dietary patterns on a number of physiological markers of skeletal muscle, bone and vascular health. Random selection bared a sample comprised of individuals following a range of dietary patterns (*n* = 130, omnivores = 48, vegetarians = 18, vegans = 49, pescatarians = 5, flexitarians = 10). Participants were eligible to take part in the study providing they had no history of chronic disease, were not using prescribed medication and had not sustained a lower-limb injury in the preceding six-months. To assess 'real-world' habitual diet, all dietary supplements were permitted and recorded during the study duration. Further, exclusion criteria included a history of smoking (including vaping), excessive alcohol/drug use, or alterations to habitual dietary pattern in the two-years prior to recruitment. All participants provided written informed consent prior to taking part in the study, which was approved by the Faculty of Science and Engineering Research Ethics Committee, Swansea University (Approval Number: JP_24-06-21b). This study complied with the declaration of Helsinki 2013, apart from pre-registration.

Design

Following an initial screening telephone interview to establish inclusion/exclusion criteria, all participants attended the Swansea University Applied Sports, Technology, Exercise and Medicine (A-STEM) laboratory on two occasions. For their first visit, participants avoided exercise for 24 h prior, in line with previous research indicating that muscle EI values return to baseline ~24 h post-resistance exercise (Yitzchaki et al., 2020). They were asked to abstain from water consumption from waking until their arrival in the lab but were permitted to drink small amounts of water once they had arrived. Muscle quality was measured as B-mode ultrasound-derived EI in the rectus femoris (RF) and VL, with physical activity levels self-reported via the Baecke physical activity questionnaire (Baecke et al., 1982). Participants were provided with a three-day food diary and a standardised set of weighing scales to record exact quantities of all food and drink consumed during this period. The diaries were returned upon their second visit to the laboratory and participants underwent an interview with a member of the research team to discuss and clarify all entries.

Skeletal muscle echo intensity

Participants lay in the supine position with the right leg fully extended for assessment of skeletal muscle EI. B-mode ultrasound images (4-15 MHz linear array transducer, MyLab9, Esaote, Genoa, Italy) were taken of the RF with the transducer held in a transverse orientation. The image site was standardised at the mid-thigh, defined as 60% of the manually measured distance between the anterior superior iliac spine and the superior border of the patella, which were identified via palpation. For VL EI, panoramic B-mode images were taken at 50% of the muscle length, determined as the distance between the muscle origin at the greater trochanter and the insertion at the patellar tendon, identified using ultrasound imaging. Ultrasound parameters such as the gain (50%, 20 dB), dynamic range (12, 62 dB) and time-gain-compensation, were standardised between scans and participants. Probe tilt also remained constant throughout the study, whilst ensuring minimal skin pressure. Image depth and focal position were altered when required to achieve the optimal image. The same experienced sonographer, with >5 years of experience, performed all ultrasound assessments and subsequent data processing. Test-retest reliability of both RF and VL EI were determined from repeat scans of eight participants and coefficient of variation (CV) calculated as (SD*1.96)/mean*100 (Reeves et al., 2004). The CV for RF EI was 7.55% and for VL EI was 7.27%, similar to previous studies (Caresio et al., 2015).

Each ultrasound image was initially processed using ImageJ software (NIH ImageJ, version 1.53a, National Institutes of Health, Bethesda, USA). Analysis was performed using the polygon function and a large region of interest (ROI) was drawn within the muscle belly, with no encroachment of the aponeurosis (Figure 1). Raw EI was determined using the histogram function, ranging between 0 and 255 A.U. (black = 0, white = 255) and EI was taken from the mean of three images. Subcutaneous fat thickness (cm) was calculated as the distance between the lower border of the skin layer and the upper border of the aponeurosis, using the straight-line function in ImageJ (Figure 1). Mean subcutaneous fat thickness was calculated from measurements at three sites in each image (left, right and centre) and EI correction was performed using a previously published correction factor equation (Young et al., 2015):

$$corrected\ EI = raw\ EI + (subcutaneous\ fat\ thickness\ [cm]) \times 40.5278$$

Insert Figure 1 here.

Habitual dietary intake

Three-day weighed food diaries were used to determine habitual nutrient intake. All participants were shown an instructional video and verbal demonstration during visit one, in which they were asked to weigh and record all food and drink consumption across two weekdays and one weekend day (in addition to viewing in the lab, all participants were also given 24 h access to the instructional video). Participants were clearly instructed to follow their habitual diet and were specifically reminded not to deviate from their usual food choices. Details within each food diary were fully discussed with a member of the research team upon their return to ensure accuracy for later analysis.

Scales were accurate to 0.1 g (Superior mini–Digital Kitchen Scale, CHWARES, Guangzhou, China). Participants were further instructed to record all cooking methods and the mass of leftovers. In cases where bespoke recipes were curated, participants were asked to record the mass of each raw ingredient, along with the cooking method and then provide a final mass of the portion consumed from the recipe. All drinks and any food supplements were also recorded within the food diary.

Food diary analysis was conducted using an online dietary analysis software (Nutritics, Research Edition, v5.83, Dublin, Ireland). All foods were selected from one of three databases, the ‘UK McCance and Widdowson 2015’, ‘Nutritics-sourced Foods, Supplements and Additives’ or the ‘GS1 Brandbank Live Feed’. A hierarchical structure was developed and systematically followed for selection of food products (Figure 2). This protocol consisted of a primary preference for the UK McCance and Widdowson database (McCance and Widdowson, 2014), if food products were not retrievable from this database or nutrient data were incomplete, foods were then carefully selected from ‘Nutritics-sourced Foods, Supplements and Additives’ or finally from ‘GS1 Brandbank Live Feed’. Records were made and the protocol was followed consistently across all participants and throughout the study period. In cases where full nutrient data were not available in any database, details were requested from the manufacturer. Any data received were then combined with the closest matching food product (with full nutrient data available) from McCance and Widdowson and label data to create a new food. Once a new food product had been created within the software, it could then be re-used for consistency between participants. In cases where data were not available from the manufacturer, nutritional information from the closest matching food item in the McCance and Widdowson database were either combined with incomplete data from one of the secondary databases or product label data to form a new food item.

Insert Figure 2 here.

To assess the accuracy of dietary intake data, the Goldberg cut-off method was used to identify potential misreporters of total energy intake (Black, 2000). Participants were deemed to be under-, over- or plausible reporters based on the ratio of energy intake to estimates of basal metabolic rate (BMR). Schofield equations were used to estimate age- and biological sex-specific BMR using individual stature and body mass data. European Food Safety Authority (EFSA) recommended physical activity level (PAL) of 1.6 was used in the equations to represent a moderately active sample (European Food Safety Authority, 2013). Finally, Goldberg cut-offs were then estimated using following recommended equations:

$$\text{Lower cut-off: } Energy\ Intake{:}BMR > PAL \times \exp\left[SDmin \times \frac{S/100}{\sqrt{n}}\right]$$

$$\text{Upper cut-off: } Energy\ Intake{:}BMR < PAL \times \exp\left[SDmax \times \frac{S/100}{\sqrt{n}}\right]$$

where *SD* (standard deviation) is 2, *S* is the factor accounting for variation in energy intake, BMR and PAL, and *n* is the number of participants in the sample.

Individuals with an energy intake:BMR ratio outside of the cut-offs were deemed to be energy misreporters. Whilst it has been recommended that misreporters should not be removed from statistical analyses (European Food Safety Authority, 2013), subsequent sensitivity analyses of the statistical models were performed excluding these individuals to confirm their accuracy.

Statistical analysis

All statistical analyses were carried out using RStudio (version 12.0, 2022, RStudio, Inc. software, Boston, MA). Participant characteristics and habitual nutrient intakes, including model predictors, are presented as means, SDs and ranges in Table 1. Partial least squares regression (PLSR) models were performed using the '*pls*' package (R script provided in Supplementary file S1). Given that most predictor variables were mean daily macro- and micronutrient intakes, which increases the likelihood of co-linearity between variables, the use of a PLSR model was deemed appropriate, as this is not an assumption of this model. In brief, the orthogonal construction of new principal components that comprise different linear combinations of predictor variables reduces the threat of co-linearity to the regression model (Wold et al., 2001). In addition to overcoming co-linearity, PLSR is also capable of maintaining statistical power with relatively small sample sizes (Hair et al., 2021). The sample size (n = 130) was deemed appropriate for the analysis in line with the minimum sample size determined via the inverse square root approach (Hair et al., 2021). This was performed based on a conservative estimated path coefficient of 0.3, 80 % power and a Bonferroni adjusted alpha level of 0.025 which returned an estimated minimum sample size of n = 106. Each model was utilised to explain the variance (R^2) in the response variables (RF EI; model 1, and VL EI; model 2) from 19 predictors (Table 1). The number of components was based on minimisation of the root mean squared error of the prediction (RMSEP) and maximisation of the R^2 values following k-fold cross-validation (k = 10). This was carried out using the '*onesigma*' function within the '*pls*' package, which calculates the lowest number of components that minimises the cross-validation prediction error within one standard error of the overall best available model (Hair et al., 2021). The contribution of each predictor variable to the model was then assessed using the '*jack-knife*' function in RStudio. The predictive performance of both models was assessed using a separate test dataset (n = 30), not included in the original sample, that was used to train the models. This dataset was employed to predict skeletal muscle EI, with predictive accuracy determined by comparison with the actual EI values. To account for multiple comparisons made between PLSR models 1 and 2, and to thereby reduce the risk of type one errors, a Bonferroni adjustment was applied to the significance level reducing from the original 0.05 to 0.025.

Insert Table 1 here.

Results

The PLSR model 1 for RF EI contained two components based on the RMSEP minimisation and R^2 maximisation within the k-fold cross validation, using the '*onesigma*' analysis (Table 2). The RMSEP was 30.4 A.U. and the model explained ~52 % of the variance in RF EI. Model

2 also contained two components following the '*onesigma*' analysis (Table 2), the RMSEP was 31.8 A.U. and the model explained ~46 % of the variance in VL EI.

Insert Table 2 here.

The contribution of predictors to each model is described in Table 2. Non-diet related factors including age, self-reported physical activity and biological sex were amongst the largest contributors to EI in the RF and VL. Age was positively associated with EI in both muscles, indicating poorer muscle quality in older individuals, whereas physical activity scores were inversely associated, which indicates better muscle quality in more active individuals. Females were selected as the reference for biological sex in both models, which was positively associated with EI indicating poorer muscle quality in females compared with males. Daily absolute protein and selenium intake were inversely associated with EI in both muscles, whereas dietary fibre intake was inversely associated with the RF (but not the VL) and omega-3 fatty acids (n-3 FAs) positively with VL EI only.

The group mean ratio for energy intake:BMR was 1.354 which was not within the Goldberg cut-off values at group level of 1.545-1.657, suggesting that the sample in the current study may have underestimated dietary intake. Individually, 27 % of participants were deemed to be energy misreporters (25 under- and 2 over-reporters) with energy intake:BMR ratios outside of the individual Goldberg cut-offs of 1.129-2.268. The sensitivity analysis excluding these participants (n = 73) revealed similar results to the original analysis. Most of the significant predictors from the original analysis were maintained following removal of energy misreporters. Age (RF: β = 10.451, P = 0.03, VL: β = 9.661, P = 0.03) and biological sex (RF: β = 14.949, P = 0.01, VL: β = 13.688, P = 0.03) remained significant predictors in both muscles. Dietary protein (β = -7.599, P <0.001, VL: β = -7.321 , P = 0.01), fibre (RF: β = -4.502, P = 0.01, VL: β = -5.414, P = 0.01) and selenium (β = -7.429, P = 0.01, VL: β = -5.556, P = 0.05) were also significant for both muscles, with n-3 FAs no longer a significant predictor of EI in the VL but did reach statistical significance in the RF (RF: β = 3.013, P = 0.04; VL: P = 0.11).

The proportion of the variance in EI that was explained in the additional testing dataset was 35.4 % and 30.3 % in model 1 (RMSEP = 38.12 A.U, mean absolute error = 26.83 A.U) and model 2 (RMSEP = 36.04 A.U, mean absolute error = 27.90 A.U), respectively.

Discussion

The aim of the current study was to explore the potential effects of habitual daily nutrient intake on EI of two quadriceps muscles. PLSR models were able to explain ~52% and ~46% of the variance in RF and VL EI, respectively. In addition, the predictive capacity of both models was assessed on an additional testing dataset (*n* = 30) which demonstrated that model 1 explained ~35 % and model 2 ~30 % of the variance in EI in the RF and VL, respectively. These preliminary findings are the first to show that diet-related factors (absolute protein, selenium, fibre and n-3 FAs) could be associated with skeletal muscle EI, as a measure of overall muscle quality. It is important to note, however, that the current study is the first exploratory analysis and further work is required before any inference of causality and to fully elucidate any potential mechanisms. Nevertheless, four dietary predictors were revealed as being significantly associated across both models, irrespective of the concurrent inclusion of non-diet related factors, which are previously established contributors to skeletal muscle EI. It is noteworthy that, on average, individuals in this sample generally consumed adequate quantities of these nutrients within the context of reference nutrient intakes (RNI). For example, the reference intakes for dietary protein, 0.75 g/kg/BM/d (SACN, 2012), fibre, 30 g/d (SACN, 2014), and n-3 FAs, 1.1 – 1.6 g/d (Trumbo et al., 2002), were all achieved or surpassed on average in the current study, whereas dietary selenium (60 – 75 μg/d) intake was slightly low (Department of Health, 1991).

Total absolute daily protein intake was inversely associated with EI and was highlighted as a potential predictor across both muscles within the context of the current analysis. It is well established that dietary protein has a key role in net muscle protein balance via its role as a potent stimulus for myofibrillar protein synthesis (MPS) (Witard et al., 2014). Postprandial hyperaminoacidaemia, and subsequent delivery and uptake into skeletal muscle, increases MPS rates (Pennings et al., 2012). It is plausible, therefore, that those consuming greater quantities of dietary protein in the current study were better able to maintain a positive net protein balance (Pennings et al., 2012) via chronic, transient mammalian target of rapamycin complex one (mTORC1) pathway-mediated stimulation of MPS (Cuthbertson et al., 2005). It is well established that stimulation of MPS to exceed the levels of myofibrillar protein breakdown (MPB), typically induced via resistance exercise in conjunction with dietary protein ingestion, initiates the positive net protein balance that results in skeletal muscle hypertrophy (or mass maintenance) over time (Phillips et al., 2005). Given the relationship between skeletal muscle EI and muscle thickness, as a measure of muscle contractile area (Akima et al., 2017), it seems logical that a shift towards greater contractile material over time could partially explain the potential relationship between dietary protein intake and the echogenicity of the quadriceps skeletal muscles observed in the current study. However, due to the exploratory and observational nature of the current study it is not possible to claim causal inference from these findings and any potential mechanistic explanations are, at this moment, speculative. Whilst this is, to the authors' knowledge, the first study to directly investigate, and report upon, the potential relationship between EI and dietary protein intake, these findings are supported by previous research. Analysis of supplementary data files from a previous study assessing the relationship between muscle mass and ultrasound-derived quality also revealed an inverse linear relationship between daily protein intake, assessed via three-day weighed food diaries, and ultrasound-derived RF EI, albeit not reported directly in the manuscript (see Supplementary File S2) (Johnson et al., 2021).

In a similar manner to the inverse relationship reported with dietary protein intake, dietary selenium was also negatively associated with EI across both the RF and VL muscles in the current study and with similarly large estimates. This is important as the mean selenium intake across this sample (51 µg/d) was slightly lower than the UK RNI (Department of Health, 1991). This was confirmed via a post hoc Z-test ($P < 0.05$, data not shown) and could have occurred due to variable availability of selenium data in food composition tables. To the authors knowledge, no study has assessed the effects of dietary selenium intake on measures of muscle quality. However, the potential 'myoprotective' effects of selenium against conditions such as sarcopenia have been tentatively alluded to. For example, case-control studies have shown that individuals with low dietary selenium intake and serum selenium concentrations exhibit lower skeletal muscle mass and a greater risk of sarcopenia diagnosis (Verlaan et al., 2017, Chen et al., 2014). The mechanistic underpinnings of these findings are poorly understood; however, previous research has considered that selenium may have an indirect effect on skeletal muscle mass, as it can facilitate the secretion of anabolic hormones such as insulin-like growth factor 1 (IGF-1) (Karl et al., 2009, Maggio et al., 2010). Whilst high consumption of dietary selenium can cause toxicity and has been shown to have an inhibitory effect on IGF-1 concentrations in rats (Grønbaek et al., 1995), the group mean intake of selenium in the current study was notably lower than both the tolerable upper intake limit of 400 µg/d reported for humans (Risher, 2011) and current UK RNI values (Department of Health, 1991). Given the role of IGF-1 in the mTORC1 pathway for MPS (Barclay et al., 2019), it is possible that habitual dietary selenium may have a similar effect to protein on EI, by facilitating maintenance of contractile material over time. However, circulating concentrations of IGF-1 were not assessed in the current study and therefore this theory can only be speculative. Indeed, the mean age of the current sample was 38 years and any potential effects of IGF-1 on skeletal muscle properties would be more likely to occur in older populations (Van Nieuwpoort et al., 2018). Further research should therefore be considered to investigate the potential association between dietary selenium and skeletal muscle EI, as well as any potential underlying mechanisms via effects on IGF-1 concentrations. Despite this, selenium and protein intake were the strongest dietary predictors of EI in the current study, and it is possible that their effects are, at least in part, synergistic. For example, dietary sources rich in selenium such as meat and fish are also high quality protein sources, and dietary protein intake is also positively associated with circulating IGF-1 concentrations (Bihuniak and Insogna, 2015).

Other dietary factors that were associated with muscle EI in the current study, albeit to a lesser extent, were n-3 FA ($\beta = 2.223$) and there was a trend towards dietary fibre ($\beta = -5.215$, $P = 0.034$) intake, although this did not reach statistical significance following the Bonferroni adjustment. This was not consistent across both muscles, with the direction of the associations and the effect sizes differing between the potential predictors. The trend towards fibre intake as a potential contributor to the model highlighted an inverse association with RF EI (as well as the VL following the sensitivity analysis), which is congruent with previous cross-sectional research, potentially suggesting a beneficial effect on skeletal muscle mass in older adults. Higher amounts of dietary fibre intake had greater skeletal muscle mass index (appendicular lean mass relative to body mass), independent of physical activity and protein consumption (Montiel-Rojas et al., 2020). Further, relative total body lean mass and relative appendicular lean mass are positively associated with dietary fibre intake among individuals aged 40 years and above (Frampton et al., 2021). Whilst exact mechanisms are yet to be elucidated, it has been speculated that there are beneficial effects on the gut microbiome, resulting in reduced circulating myodegenerative inflammation, which may lead to greater MPS rates in those consuming fibre in greater quantities (Jiao et al., 2015). Notably, however, dietary fibre did not reach statistical significance following the Bonferroni adjustment suggesting the potential for a type one error owing to the multiple comparisons drawn in the current statistical analysis. Alternatively, this could also be explained by the inclusion of dietary fibre with numerous heavily weighted predictor variables (such as age, biological sex and dietary protein), indicating that it may have been overpowered by other variables included in the model. Further research should therefore be conducted in order to investigate the potential relationship between dietary fibre and skeletal muscle EI.

n-3 FAs were positively associated with VL EI in the current study, which perhaps diverges from conventional thought that supplementing with n-3 FAs may have beneficial effects on muscle health (Smith, 2016). Supplementation with long-chain n-3 FAs has been shown to ameliorate postprandial MPS rates, via enhanced mTORC1 pathway signalling, in response to amino acid ingestion (Smith et al., 2011). This could explain gains in thigh muscle volume observed in a follow-up study, which occurred concomitantly with a reduction in IMAT infiltration, reported in both older men and women following six months of supplementation (Smith et al., 2015). This considered, it might be expected that habitual n-3 FA intake would have had an inverse relationship with muscle EI; however, this was not the case in the current exploratory analysis. There appears to be a lack of evidence to support or explain this positive relationship between n-3 FAs and muscle EI. However, it is possible that a combination of short- and long-chain n-3 FAs co-ingested as part of the habitual diet does not have the same beneficial effect that has been observed in previous studies supplementing with long-chain FAs, only. Indeed, mean habitual intake of long chain n-3 FAs such as eicosapentaenoic (0.02 ± 0.08 g/d) and docosahexaenoic acid (0.05 ± 0.14 g/d) estimated via the three-day weighed food diaries in the current study provide an indication of co-ingestion with short-chain n-3 FAs such as alpha linoleic acid (0.32 ± 0.41 g/d), as would be expected from the habitual diet. In addition, in the sensitivity analysis, that excluded energy misreporters, n-3 FAs were no longer a significant predictor of EI in the VL, but were for the RF. This highlights the need for further research to investigate this potential relationship, perhaps using alternative research design to accurately demonstrate a cause-effect relationship.

Considering the breadth of existing evidence, it is unsurprising that age, self-reported physical activity and biological sex were revealed to be associated with skeletal muscle EI. Whilst there have been some inconsistencies in certain muscle groups (Paris et al., 2021), lower-limb muscles have been consistently reported to have greater skeletal muscle EI in older individuals across studies, including in the current exploratory analysis (Fukumoto et al., 2015, Strasser et al., 2013, Palmer and Thompson, 2017). Previous research also supports the findings that physical activity predicts EI, with inverse relationships between variables, as reported in the current study (Osawa et al., 2017). In a four-year longitudinal study, a reduction in quadriceps EI was reported among older individuals categorised into high self-reported physical activity levels (≥ twice per week) compared to a low physical activity control group (≤ once per week)

(Fukumoto et al., 2018). Likewise, biological sex was also associated with EI, with females exhibiting higher values compared with males in the current study. This is congruent with previous research, demonstrating higher EI, across a range of muscles, in both younger (Arts et al., 2010, Mangine et al., 2014, Akagi et al., 2018) and older (Arts et al., 2010, Akagi et al., 2018, Kawai et al., 2018) females compared to males.

The findings of the current study are restricted to the variables included in each model. Four nutrients were associated with EI in the RF, VL or both, notwithstanding their inclusion with variables that are well-established to influence EI. If the dietary predictors contributed less to EI than revealed in the present results, this would have resulted in them being overpowered by age, physical activity and biological sex. This may explain, at least in part, the discrepancies observed between quadriceps muscles in nutritional factors associated with EI, with dietary fibre and n-3 FAs offering only a smaller contribution to each model it is possible that they were overpowered by the stronger predictors. It is, however, accepted that there are other variables that could potentially influence muscle EI, such as genetic factors, that were not incorporated within the models. The inclusion of a strength measurement in the current study, for example, could have explained a greater proportion of the variance in skeletal muscle EI than observed in the current analysis and should therefore be investigated in any potential future studies (Bali et al., 2020).

Inherent limitations associated with dietary assessment tools may have influenced the nutritional intake values observed in the current analysis. The accuracy of three-day weighed food records has specifically been questioned as they may not be totally representative of an individual's habitual diet and are subject to a potential Hawthorne effect (Thompson and Subar, 2017). However, prospective recording of dietary intake is typically regarded as a more accurate method of assessment compared to alternative techniques such as food frequency questionnaires and diet recall, owing to the reduced reliance on memory recall (Yang et al., 2010, Crawford et al., 1994). Weighed food records are therefore often employed as a reference tool when validating alternative dietary assessment techniques (Mueller-Stierlin et al., 2021). Furthermore, whilst it was traditionally thought that seven day weighed food records should be considered the 'gold standard', more recent recommendations allude to recording periods less than four days to reduce the risk of participant fatigue and subsequent reductions in recorded dietary intake that may occur as a result (Thompson and Subar, 2017). Previous research has shown that assessments across two weekdays and one weekend day can provide appropriate representation of habitual diet (Fyfe et al., 2010).

In addition, the findings of the current analysis should be interpreted with appropriate levels of caution owing to the observational and cross-sectional nature. The present data should be interpreted within the context of the sample and analysis conducted to identify potential relationships between nutritional factors and skeletal muscle EI, that require subsequent follow-up investigation to discern any potential mechanistic underpinning. Despite this, the findings of this preliminary, exploratory study highlight potential associations between specific nutrients and EI for future consideration by clinicians and researchers alike.

In conclusion, the findings from this exploratory study suggest, for the first time, that diet-related factors such as daily intake of dietary protein, selenium, fibre and n-3 FAs *may* be associated with skeletal muscle EI. Whole food products such as meat, fish and poultry as well as fresh fruits and vegetables are good dietary sources of these nutrients, and habitual consumption of a well-balanced combination of these products are typically recommended in dietary guidelines. Due to the exploratory nature of the current study, the exact mechanisms underpinning these findings are currently speculative, therefore future work should seek to elucidate the potential role of the specific nutrients and further develop understanding of the potential effects of nutritional predictors on ultrasound-derived measures of skeletal muscle quality.

References

ADDISON, O., MARCUS, R. L., LASTAYO, P. C. & RYAN, A. S. 2014a. Intermuscular fat: a review of the consequences and causes. *International Journal of Endocrinology,* 2014.

ADDISON, O., YOUNG, P., INACIO, M., BAIR, W.-N., G PRETTYMAN, M., A BEAMER, B., S RYAN, A. & W ROGERS, M. 2014b. Hip but not thigh intramuscular adipose tissue is associated with poor balance and increased temporal gait variability in older adults. *Current aging science,* 7**,** 137-143.

AHMED, S., SINGH, D., KHATTAB, S., BABINEAU, J. & KUMBHARE, D. 2018. The effects of diet on the proportion of intramuscular fat in human muscle: a systematic Review and Meta-analysis. *Frontiers in Nutrition,* 5**,** 7.

AKAGI, R., SUZUKI, M., KAWAGUCHI, E., MIYAMOTO, N., YAMADA, Y. & EMA, R. 2018. Muscle size-strength relationship including ultrasonographic echo intensity and voluntary activation level of a muscle group. *Archives of Gerontology and Geriatrics,* 75**,** 185-190.

AKAZAWA, N., KISHI, M., HINO, T., TSUJI, R., TAMURA, K., HIOKA, A. & MORIYAMA, H. 2023. Longitudinal relationship between muscle mass and intramuscular adipose tissue of the quadriceps in older inpatients at different activities of daily living levels. *Clinical Nutrition ESPEN,* 53**,** 175-181.

AKIMA, H., YOSHIKO, A., TOMITA, A., ANDO, R., SAITO, A., OGAWA, M., KONDO, S. & TANAKA, N. I. 2017. Relationship between quadriceps echo intensity and functional and morphological characteristics in older men and women. *Archives of gerontology and geriatrics,* 70**,** 105-111.

ARTS, I. M., PILLEN, S., SCHELHAAS, H. J., OVEREEM, S. & ZWARTS, M. J. 2010. Normal values for quantitative muscle ultrasonography in adults. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine,* 41**,** 32-41.

BAECKE, J. A., BUREMA, J. & FRIJTERS, J. E. 1982. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *The American journal of clinical nutrition,* 36**,** 936-942.

BALI, A. U., HARMON, K. K., BURTON, A. M., PHAN, D. C., MERCER, N. E., LAWLESS, N. W. & STOCK, M. S. 2020. Muscle strength, not age, explains unique variance in echo intensity. *Exp Gerontol,* 139**,** 111047.

BARCLAY, R. D., BURD, N. A., TYLER, C., TILLIN, N. A. & MACKENZIE, R. W. 2019. The role of the IGF-1 signaling cascade in muscle protein synthesis and anabolic resistance in aging skeletal muscle. *Frontiers in Nutrition,* 6**,** 146.

BIHUNIAK, J. D. & INSOGNA, K. L. 2015. The effects of dietary protein and amino acids on skeletal metabolism. *Molecular and cellular endocrinology,* 410**,** 78-86.

BLACK, A. E. 2000. Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metabolic rate. A practical guide to its calculation, use and limitations. *International journal of obesity,* 24**,** 1119-1130.

CARESIO, C., MOLINARI, F., EMANUEL, G. & MINETTO, M. A. 2015. Muscle echo intensity: reliability and conditioning factors. *Clinical Physiology and Functional Imaging,* 35**,** 393-403.

CHEN, Y.-L., YANG, K.-C., CHANG, H.-H., LEE, L.-T., LU, C.-W. & HUANG, K.-C. 2014. Low serum selenium level is associated with low muscle mass in the community-dwelling elderly. *Journal of the American Medical Directors Association,* 15**,** 807-811.

CORREA-DE-ARAUJO, R., HARRIS-LOVE, M. O., MILJKOVIC, I., FRAGALA, M. S., ANTHONY, B. W. & MANINI, T. M. 2017. The need for standardized assessment of muscle quality in skeletal muscle function deficit and other aging-related muscle dysfunctions: a symposium report. *Frontiers in Physiology,* 8**,** 87.

CRAWFORD, P. B., OBARZANEK, E., MORRISON, J. & SABRY, Z. 1994. Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9-and 10-year-old girls. *Journal of the American Dietetic Association,* 94**,** 626-630.

CRUZ-JENTOFT, A. J., BAHAT, G., BAUER, J., BOIRIE, Y., BRUYÈRE, O., CEDERHOLM, T., COOPER, C., LANDI, F., ROLLAND, Y. & SAYER, A. A. 2019. Sarcopenia: revised European consensus on definition and diagnosis. *Age and ageing,* 48**,** 16-31.

CRUZ-JENTOFT, A. J., HUGHES, B. D., SCOTT, D., SANDERS, K. M. & RIZZOLI, R. 2020. Nutritional strategies for maintaining muscle mass and strength from middle age to later life: A narrative review. *Maturitas,* 132**,** 57-64.

CUTHBERTSON, D., SMITH, K., BABRAJ, J., LEESE, G., WADDELL, T., ATHERTON, P., WACKERHAGE, H., TAYLOR, P. M. & RENNIE, M. J. 2005. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *The FASEB Journal,* 19**,** 1-22.

DELMONICO, M. J., HARRIS, T. B., VISSER, M., PARK, S. W., CONROY, M. B., VELASQUEZ-MIEYER, P., BOUDREAU, R., MANINI, T. M., NEVITT, M. & NEWMAN, A. B. 2009. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *American Journal of Clinical Nutrition*.

DEPARTMENT OF HEALTH 1991. *Dietary Reference Values for Food Energy and Nutrients for the Uk: Report of the Panel on Dietary Reference Values of the Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*, HM Stationery Office.

EUROPEAN FOOD SAFETY AUTHORITY 2013. Example of a Protocol for Identification of Misreporting (Under- and Over-Reporting of Energy Intake) Based on the PILOT-PANEU Project. *EFSA J,* 11**,** 1-17.

FRAMPTON, J., MURPHY, K. G., FROST, G. & CHAMBERS, E. S. 2021. Higher dietary fibre intake is associated with increased skeletal muscle mass and strength in adults aged 40 years and older. *Journal of Cachexia, Sarcopenia and Muscle,* 12**,** 2134-2144.

FUKUMOTO, Y., IKEZOE, T., YAMADA, Y., TSUKAGOSHI, R., NAKAMURA, M., TAKAGI, Y., KIMURA, M. & ICHIHASHI, N. 2015. Age-related ultrasound changes in muscle quantity and quality in women. *Ultrasound in Medicine & Biology,* 41**,** 3013-3017.

FUKUMOTO, Y., YAMADA, Y., IKEZOE, T., WATANABE, Y., TANIGUCHI, M., SAWANO, S., MINAMI, S., ASAI, T., KIMURA, M. & ICHIHASHI, N. 2018. Association of physical activity with age-related changes in muscle echo intensity in older adults: a 4-year longitudinal study. *Journal of Applied Physiology,* 125**,** 1468-1474.

FYFE, C. L., STEWART, J., MURISON, S. D., JACKSON, D. M., RANCE, K., SPEAKMAN, J. R., HORGAN, G. W. & JOHNSTONE, A. M. 2010. Evaluating energy intake measurement in free-living subjects: when to record and for how long? *Public health nutrition,* 13**,** 172-180.

GARRETT, J. 2020. Vastus Lateralis Echo Intensity Correlates with Muscular Strength and Endurance in Young Men and Women.

GOODPASTER, B. H., CARLSON, C. L., VISSER, M., KELLEY, D. E., SCHERZINGER, A., HARRIS, T. B., STAMM, E. & NEWMAN, A. B. 2001. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *Journal of Applied Physiology,* 90**,** 2157-2165.

GRØNBAEK, H., FRYSTYK, J., ØRSKOV, H. & FLYVBJERG, A. 1995. Effect of sodium selenite on growth, insulin-like growth factor-binding proteins and insulin-like growth factor-I in rats. *Journal of endocrinology,* 145**,** 105-112.

HAIR, J. F., HULT, G. T. M., RINGLE, C. M. & SARSTEDT, M. 2021. *A primer on partial least squares structural equation modeling (PLS-SEM)*, Sage publications.

ISAKA, M., SUGIMOTO, K., YASUNOBE, Y., AKASAKA, H., FUJIMOTO, T., KURINAMI, H., TAKEYA, Y., YAMAMOTO, K. & RAKUGI, H. 2019. The usefulness of an alternative diagnostic method for sarcopenia using thickness and echo intensity of lower leg muscles in older males. *Journal of the American Medical Directors Association,* 20**,** 1185. e1-1185. e8.

JIAO, J., XU, J.-Y., ZHANG, W., HAN, S. & QIN, L.-Q. 2015. Effect of dietary fiber on circulating C-reactive protein in overweight and obese adults: a meta-analysis of randomized controlled trials. *International Journal of Food Sciences and Nutrition,* 66**,** 114-119.

JOHNSON, N. R., KOTARSKY, C. J., HACKNEY, K. J., TRAUTMAN, K. A., DICKS, N. D., BYUN, W., KEITH, J. F., DAVID, S. L. & STASTNY, S. N. 2021. Measures derived from panoramic ultrasonography and animal-based protein intake are related to muscular performance in middle-aged adults. *Journal of Clinical Medicine,* 10**,** 988.

KARL, J. P., ALEMANY, J. A., KOENIG, C., KRAEMER, W. J., FRYSTYK, J., FLYVBJERG, A., YOUNG, A. J. & NINDL, B. C. 2009. Diet, body composition, and physical fitness influences on IGF-I bioactivity in women. *Growth Hormone & IGF Research,* 19**,** 491-496.

KAWAI, H., KERA, T., HIRAYAMA, R., HIRANO, H., FUJIWARA, Y., IHARA, K., KOJIMA, M. & OBUCHI, S. 2018. Morphological and qualitative characteristics of the quadriceps muscle of community-dwelling older adults based on ultrasound imaging: classification using latent class analysis. *Aging clinical and experimental research,* 30**,** 283-291.

KOBAYASHI, K., YAGI, M., TATEUCHI, H., OTA, M., UMEHARA, J., SAKATA, H., OKADA, S. & ICHIHASHI, N. 2023. Effect of age on shear modulus, muscle thickness, echo intensity of the upper limb, lower limb, and trunk muscles in healthy women. *European Journal of Applied Physiology,* 123**,** 797-807.

KUSCHEL, L. B., SONNENBURG, D. & ENGEL, T. Factors of Muscle Quality and Determinants of Muscle Strength: A Systematic Literature Review. Healthcare, 2022. MDPI, 1937.

LANZA, M. B., RYAN, A. S., GRAY, V., PEREZ, W. J. & ADDISON, O. 2020. Intramuscular fat influences neuromuscular activation of the gluteus medius in older adults. *Frontiers in Physiology,* 11**,** 614415.

LEMIEUX, F., FILION, M., BARBAT-ARTIGAS, S., KARELIS, A. & AUBERTIN-LEHEUDRE, M. 2014. Relationship between different protein intake recommendations with muscle mass and muscle strength. *Climacteric,* 17**,** 294-300.

MAGGIO, M., CEDA, G. P., LAURETANI, F., BANDINELLI, S., DALL'AGLIO, E., GURALNIK, J. M., PAOLISSO, G., SEMBA, R. D., NOUVENNE, A. & BORGHI, L. 2010. Association of plasma selenium concentrations with total IGF-1 among older community-dwelling adults: the InCHIANTI study. *Clinical Nutrition,* 29**,** 674-677.

MANGINE, G. T., FUKUDA, D. H., LAMONICA, M. B., GONZALEZ, A. M., WELLS, A. J., TOWNSEND, J. R., JAJTNER, A. R., FRAGALA, M. S., STOUT, J. R. & HOFFMAN, J. R.

2014. Influence of gender and muscle architecture asymmetry on jump and sprint performance. *Journal of Sports Science & Medicine,* 13**,** 904.

MANINI, T. M., CLARK, B. C., NALLS, M. A., GOODPASTER, B. H., PLOUTZ-SNYDER, L. L. & HARRIS, T. B. 2007. Reduced physical activity increases intermuscular adipose tissue in healthy young adults. *The American Journal of Clinical Nutrition,* 85**,** 377-384.

MCCANCE, R. A. & WIDDOWSON, E. M. 2014. *McCance and Widdowson's the Composition of Foods*, Royal Society of Chemistry.

MONTIEL-ROJAS, D., NILSSON, A., SANTORO, A., FRANCESCHI, C., BAZZOCCHI, A., BATTISTA, G., DE GROOT, L. C., FESKENS, E. J., BERENDSEN, A. & PIETRUSZKA, B. 2020. Dietary fibre may mitigate sarcopenia risk: findings from the NU-AGE cohort of older European adults. *Nutrients,* 12**,** 1075.

MUELLER-STIERLIN, A. S., TEASDALE, S. B., DINC, U., MOERKL, S., PRINZ, N., BECKER, T. & KILIAN, R. 2021. Feasibility and acceptability of photographic food record, food diary and weighed food record in people with serious mental illness. *Nutrients,* 13**,** 2862.

NAGAE, M., UMEGAKI, H., YOSHIKO, A., FUJITA, K., KOMIYA, H., WATANABE, K., YAMADA, Y. & KUZUYA, M. 2021. Echo intensity is more useful in predicting hospital-associated complications than conventional sarcopenia-related parameters in acute hospitalized older patients. *Experimental Gerontology,* 150**,** 111397.

OKURA, K., IWAKURA, M., KAWAGOSHI, A., SUGAWARA, K., TAKAHASHI, H. & SHIOYA, T. 2022. Objective physical activity level is associated with rectus femoris muscle echo-intensity in patients with chronic obstructive pulmonary disease. *The Clinical Respiratory Journal,* 16**,** 572-580.

OSAWA, Y., ARAI, Y., OGUMA, Y., HIRATA, T., ABE, Y., AZUMA, K., TAKAYAMA, M. & HIROSE, N. 2017. Relationships of muscle echo intensity with walking ability and physical activity in the very old population. *Journal of Aging and Physical Activity,* 25**,** 189-195.

OTA, M., IKEZOE, T., KATO, T., TATEUCHI, H. & ICHIHASHI, N. 2020. Age-related changes in muscle thickness and echo intensity of trunk muscles in healthy women: comparison of 20–60s age groups. *European Journal of Applied Physiology,* 120**,** 1805-1814.

PALMER, T. B. & THOMPSON, B. J. 2017. Influence of age on passive stiffness and size, quality, and strength characteristics. *Muscle & Nerve,* 55**,** 305-315.

PARIS, M. T., BELL, K. E., AVRUTIN, E. & MOURTZAKIS, M. 2020. Ultrasound image resolution influences analysis of skeletal muscle composition. *Clinical Physiology and Functional Imaging,* 40**,** 277-283.

PARIS, M. T., BELL, K. E., AVRUTIN, E. & MOURTZAKIS, M. 2022. Association of strength, power, and function with muscle thickness, echo intensity, and lean tissue in older males. *Applied Physiology, Nutrition, and Metabolism,* 47**,** 521-528.

PARIS, M. T., LETOFSKY, N. & MOURTZAKIS, M. 2021. Site-specific skeletal muscle echo intensity and thickness differences in subcutaneous adipose tissue matched older and younger adults. *Clinical Physiology and Functional Imaging,* 41**,** 156-164.

PENNINGS, B., GROEN, B., DE LANGE, A., GIJSEN, A. P., ZORENC, A. H., SENDEN, J. M. & VAN LOON, L. J. 2012. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *American Journal of Physiology-Endocrinology and Metabolism,* 302**,** E992-E999.

PHILLIPS, S. M., HARTMAN, J. W. & WILKINSON, S. B. 2005. Dietary protein to support anabolism with resistance exercise in young men. *Journal of the American College of Nutrition,* 24**,** 134S-139S.

PILLEN, S., TAK, R. O., ZWARTS, M. J., LAMMENS, M. M., VERRIJP, K. N., ARTS, I. M., VAN DER LAAK, J. A., HOOGERBRUGGE, P. M., VAN ENGELEN, B. G. & VERRIPS, A. 2009. Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound in Medicine & Biology,* 35**,** 443-446.

PINEL, S., KELP, N. Y., BUGEJA, J. M., BOLSTERLEE, B., HUG, F. & DICK, T. J. 2021. Quantity versus quality: Age-related differences in muscle volume, intramuscular fat, and mechanical properties in the triceps surae. *Experimental Gerontology,* 156**,** 111594.

RADAELLI, R., BOTTON, C. E., WILHELM, E. N., BOTTARO, M., BROWN, L. E., LACERDA, F., GAYA, A., MORAES, K., PERUZZOLO, A. & PINTO, R. S. 2014. Time course of low- and high-volume strength training on neuromuscular adaptations and muscle quality in older women. *Age,* 36**,** 881-892.

RADAELLI, R., BOTTON, C. E., WILHELM, E. N., BOTTARO, M., LACERDA, F., GAYA, A., MORAES, K., PERUZZOLO, A., BROWN, L. E. & PINTO, R. S. 2013. Low-and high-volume strength training induces similar neuromuscular improvements in muscle quality in elderly women. *Experimental gerontology,* 48**,** 710-716.

RECH, A., RADAELLI, R., GOLTZ, F. R., DA ROSA, L. H. T., SCHNEIDER, C. D. & PINTO, R. S. 2014. Echo intensity is negatively associated with functional capacity in older women. *Age,* 36**,** 1-9.

REEVES, N. D., NARICI, M. V. & MAGANARIS, C. N. 2004. Effect of resistance training on skeletal muscle-specific force in elderly humans. *Journal of applied physiology,* 96**,** 885-892.

RISHER, J. 2011. *Toxicological profile for selenium (Update)*, DIANE Publishing.

SACN 2012. *Dietary reference values for energy*, The Stationery Office.

SACN. 2014. *Draft Carbohydrates and Health Report, Scientific Consultation: 26 June to 1 September 2014* [Online]. London: Public Health England. Available: https://www.gov.uk/government/publications/sacn-carbohydrates-and-health-report [Accessed 19 July 2023].

SAHNI, S., MANGANO, K. M., HANNAN, M. T., KIEL, D. P. & MCLEAN, R. R. 2015. Higher protein intake is associated with higher lean mass and quadriceps muscle strength in adult men and women. *The Journal of nutrition,* 145**,** 1569-1575.

SMITH, G. I. 2016. The effects of dietary omega-3s on muscle composition and quality in older adults. *Current nutrition reports,* 5**,** 99-105.

SMITH, G. I., ATHERTON, P., REEDS, D. N., MOHAMMED, B. S., RANKIN, D., RENNIE, M. J. & MITTENDORFER, B. 2011. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *The American Journal of Clinical Nutrition,* 93**,** 402-412.

SMITH, G. I., JULLIAND, S., REEDS, D. N., SINACORE, D. R., KLEIN, S. & MITTENDORFER, B. 2015. Fish oil–derived n− 3 PUFA therapy increases muscle mass and function in healthy older adults. *The American Journal of Clinical Nutrition,* 102**,** 115-122.

STOCK, M. S. & THOMPSON, B. J. 2021. Echo intensity as an indicator of skeletal muscle quality: applications, methodology, and future directions. *European Journal of Applied Physiology,* 121**,** 369-380.

STRASSER, E. M., DRASKOVITS, T., PRASCHAK, M., QUITTAN, M. & GRAF, A. 2013. Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age,* 35**,** 2377-2388.

THERKELSEN, K. E., PEDLEY, A., HOFFMANN, U., FOX, C. S. & MURABITO, J. M. 2016. Intramuscular fat and physical performance at the Framingham Heart Study. *Age,* 38**,** 31.

THOMPSON, F. E. & SUBAR, A. F. 2017. Dietary assessment methodology. *Nutrition in the Prevention and Treatment of Disease***,** 5-48.

TRUMBO, P., SCHLICKER, S., YATES, A. A. & POOS, M. 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids.(Commentary). *Journal of the american dietetic association,* 102**,** 1621-1631.

VAN NIEUWPOORT, I., VLOT, M., SCHAAP, L., LIPS, P. & DRENT, M. 2018. The relationship between serum IGF-1, handgrip strength, physical performance and falls in elderly men and women. *European journal of endocrinology,* 179**,** 73-84.

VERLAAN, S., ASPRAY, T. J., BAUER, J. M., CEDERHOLM, T., HEMSWORTH, J., HILL, T. R., MCPHEE, J. S., PIASECKI, M., SEAL, C. & SIEBER, C. C. 2017. Nutritional status, body composition, and quality of life in community-dwelling sarcopenic and non-sarcopenic older adults: A case-control study. *Clinical Nutrition,* 36**,** 267-274.

VITALE, J. A., MESSINA, C., ALBANO, D., FASCIO, E., GALBUSERA, F., CORBETTA, S., SCONFIENZA, L. M. & BANFI, G. 2021. Appendicular muscle mass, thigh intermuscular fat infiltration, and risk of fall in postmenopausal osteoporotic elder women. *Gerontology,* 67**,** 415-424.

WILHELM, E. N., RECH, A., MINOZZO, F., BOTTON, C. E., RADAELLI, R., TEIXEIRA, B. C., REISCHAK-OLIVEIRA, A. & PINTO, R. S. 2014. Concurrent strength and endurance training exercise sequence does not affect neuromuscular adaptations in older men. *Experimental gerontology,* 60**,** 207-214.

WITARD, O. C., JACKMAN, S. R., BREEN, L., SMITH, K., SELBY, A. & TIPTON, K. D. 2014. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *The American Journal of Clinical Nutrition,* 99**,** 86-95.

WOLD, S., SJÖSTRÖM, M. & ERIKSSON, L. 2001. PLS-regression: a basic tool of chemometrics. *Chemometrics and intelligent laboratory systems,* 58**,** 109-130.

WU, J., LUO, H., REN, S., SHEN, L., CHENG, D. & WANG, N. 2022. Enhanced echo intensity of skeletal muscle is associated with poor physical function in hemodialysis patients: a cross-sectional study. *BMC Nephrology,* 23**,** 1-9.

YANG, Y. J., KIM, M. K., HWANG, S. H., AHN, Y., SHIM, J. E. & KIM, D. H. 2010. Relative validities of 3-day food records and the food frequency questionnaire. *Nutrition research and practice,* 4**,** 142-148.

YITZCHAKI, N., ZHU, W. G., KUEHNE, T. E., VASENINA, E., DANKEL, S. J. & BUCKNER, S. L. 2020. An examination of changes in skeletal muscle thickness, echo intensity, strength and soreness following resistance exercise. *Clinical Physiology and Functional Imaging,* 40**,** 238-244.

YOSHIDA, Y., MARCUS, R. L. & LASTAYO, P. C. 2012. Intramuscular adipose tissue and central activation in older adults. *Muscle & nerve,* 46**,** 813-816.

YOSHIKO, A., KAJI, T., SUGIYAMA, H., KOIKE, T., OSHIDA, Y. & AKIMA, H. 2017. Effect of 12-month resistance and endurance training on quality, quantity, and function of skeletal muscle in older adults requiring long-term care. *Experimental gerontology,* 98**,** 230-237.

YOUNG, H. J., JENKINS, N. T., ZHAO, Q. & MCCULLY, K. K. 2015. Measurement of intramuscular fat by muscle echo intensity. *Muscle & nerve,* 52**,** 963-971.

Figure 1. Example from a participants' B-mode ultrasound image of the rectus femoris in the transverse plane (top left) and panoramic image of the vastus lateralis (top right) for assessment of skeletal muscle echo intensity together with the corresponding gray-scale pixel intensity histograms (below each image). Yellow dashed lines represent the region of interest used to determine echo intensity within the muscle belly. Red dashed lines indicate the borders of the dermal layer (top line) and the muscle aponeurosis (bottom line), and the red arrows represent the subcutaneous fat layer. Rectus Femoris, RF; Vastus Lateralis, VL; Vastus Intermedius VI.

Figure 2. Hierarchical structure for food diary analysis conducted in the online dietary analysis software Nutritics (nutritics.com). Food items listed in the three-day weighed food diaries are primarily selected from the UK McCance and Widdowson database. In cases where a food item is not present in this database foods were carefully selected from either the 'Nutritics-sourced Foods, Supplements and Additives' or the 'GS1 Brandbank Live Feed' databases. If a food item was not present in any of the selected databases, with full nutritional information available, details were either requested from the manufacturer or information was combined between two sources to produce a new food item. Once a new food item had been created, records were kept for re-use across participants.

Table 1. Participant characteristics and predictor variables in the training (*n* = 100) and testing (*n* = 30) datasets for predicting rectus femoris and vastus lateralis echo intensity using partial least squares regression. All data are presented as mean ± SD and the range.

	Training Sample (*n* = 100)		**Testing Sample (*n* = 30)**	
Participant Characteristics	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Stature (m)	1.72 ± 0.08	1.52 – 1.91	1.69 ± 0.08	1.56 – 1.85
Body mass (kg)	73.2 ± 16.1	48.4 – 130.6	72.1 ± 14.5	53.8 – 122.7
BMI (kg/m^2)	24.4 ± 4.1	17.3 – 40.0	25.0 ± 4.4	19.0 – 40.0
Subcutaneous fat thickness (cm)	1.07 ± 0.58	0.19 – 2.53	0.94 ± 0.64	0.17 – 3.36
RF Echo Intensity (A.U)	125.15 ± 37.55	55.87 – 222.97	127.80 ± 46.83	67.96 – 258.02
VL Vpan Echo Intensity (A.U)	126.28 ± 38.60	35.53 – 225.67	120.21 ± 43.54	56.71 – 247.37
Predictors (*n* = 19)				
Age (years)	38 ± 15	18 – 79	38 ± 16	18 – 69
Biological Sex (M/F)	45/55	–	13/17	–
Physical activity score	8.65 ± 1.41	5.13 – 12.38	8.41 ± 1.48	5.75 – 10.75
Total Energy Intake (kcal/d)	2124 ± 674	587 – 4937	2336 ± 648	1485 – 3658
Total Protein (g/d)	87.1 ± 37.6	22.6 – 203.8	106.7 ± 56.9	34.7 – 273.2
Total Fats (g/d)	84.6 ± 36.0	15.2 – 183.1	90.1 ± 35.1	30.9 -191.8
Total CHO (g/d)	240.2 ± 84.5	84.8 – 624.4	265.7 ± 81.9	135.5 – 449.1
Saturated Fats (g/d)	25.3 ± 13.8	3.8 – 71.7	30.9 ± 14.7	8.7 – 73.2
n-3 FAs (g/d)	1.9 ± 2.6	0.2 – 21.3	1.5 ± 1.0	0.4 – 4.2
n-6 FAs (g/d)	10.3 ± 6.6	0.1 – 30.3	9.8 ± 6.6	0.9 – 34.6
Fibre (g/d)	32.5 ± 15.1	6.2 – 83.3	33.4 ± 16.7	9.1 – 80.4
Calcium (mg/d)	1011 ± 704	151 – 6021	1129 ± 707	357 – 4190
Iron (mg/d)	17.2 ± 7.4	5.5 – 37.0	20.4 ± 15.1	7.5 – 76.4
Magnesium (mg/d)	553 ± 1274	123 – 13011	465 ± 195	181 – 907
Potassium (mg/d)	5293 ± 13987	788 – 141186	4059 ± 2163	1499 – 11300
Selenium (µg/d)	51.5 ± 29.3	7.7 – 148.6	77.2 ± 49.1	16.0 – 226.4
Iodine (µg/d)	107 ± 89	6 – 376	155 ± 155	9 – 800
Vitamin D (µg/d)	17.9 ± 92.7	0.0 – 910.8	7.8 ± 9.4	0.4 – 68.9
Vitamin A (µg/d)	977.7 ± 806.1	39.9 – 4487.3	1073 ± 1012	344 - 5086

RF, rectus femoris; VL, vastus lateralis, Vpan, panoramic image; A.U., arbitrary units; n-3 FAs, omega-3 fatty acids; n-6 FAs, omega-6 fatty acids. There were no differences in participant characteristics between training and testing samples, assessed via an independent samples t-test (P>0.05).

Table 2. Two-component partial least squares regression predicting rectus femoris and vastus lateralis echo intensity from habitual nutrient intake (and non-diet related factors) and the contribution of each predictor variable.

Model 1						**RF EI**
Components						2
Adjusted RMSEP						30.37
Adjusted R^2						0.52
Model 2						**VL EI**
Components						2
Adjusted RMSEP						31.75
Adjusted R^2						0.46
		Model 1			**Model 2**	
Predictor variables	**β-Estimate**	***t***	***P***	**β-Estimate**	***t***	***P***
Age (years)	8.927	3.522	0.006*	9.742	4.937	0.001*
Biological Sex (ref = f)	13.501	6.582	<0.001*	13.182	5.900	<0.001*
Physical activity score	-4.534	-2.874	0.018*	-6.245	-2.310	0.046
Total Energy Intake (kcal/d)	0.107	0.072	0.944	-0.368	-0.426	0.680
Total Protein (g/d)	-7.617	-4.119	0.003*	-7.480	-6.214	<0.001*
Total Fats (g/d)	1.727	1.271	0.236	0.633	0.481	0.642
Total CHO (g/d)	0.348	0.257	0.803	0.361	0.225	0.827
n-3 FAs (g/d)	2.223	1.744	0.115	3.145	3.775	0.004*

n-6 FAs (g/d)	1.635	1.071	0.312	0.810	0.335	0.745
Fibre (g/d)	-5.215	-2.498	0.034	-3.887	-2.059	0.070
Calcium (mg/d)	0.858	0.300	0.771	1.635	0.618	0.552
Iron (mg/d)	-2.938	-1.074	0.311	-2.491	-2.136	0.061
Magnesium (mg/d)	-2.107	-1.198	0.262	-2.392	-0.737	0.480
Potassium (mg/d)	-2.130	-1.050	0.321	-2.878	-2.077	0.068
Selenium (µg/d)	-7.144	-3.234	0.010*	-4.775	-2.698	0.024*
Iodine (µg/d)	1.500	0.730	0.484	2.565	0.717	0.492
Vitamin D (µg/d)	1.201	1.888	0.092	0.726	0.100	0.344
Vitamin A (µg/d)	1.881	1.212	0.256	1.300	0.755	0.469

RMSEP, root mean square error of prediction; CV, cross-validation; RF, rectus femoris, VL, vastus lateralis; CHO, carbohydrates; n-3 FAs, omega-3 fatty acids; n-6 FAs, omega-6 fatty acids. * denotes statistical significance following Bonferroni adjustment ($P < 0.025$).

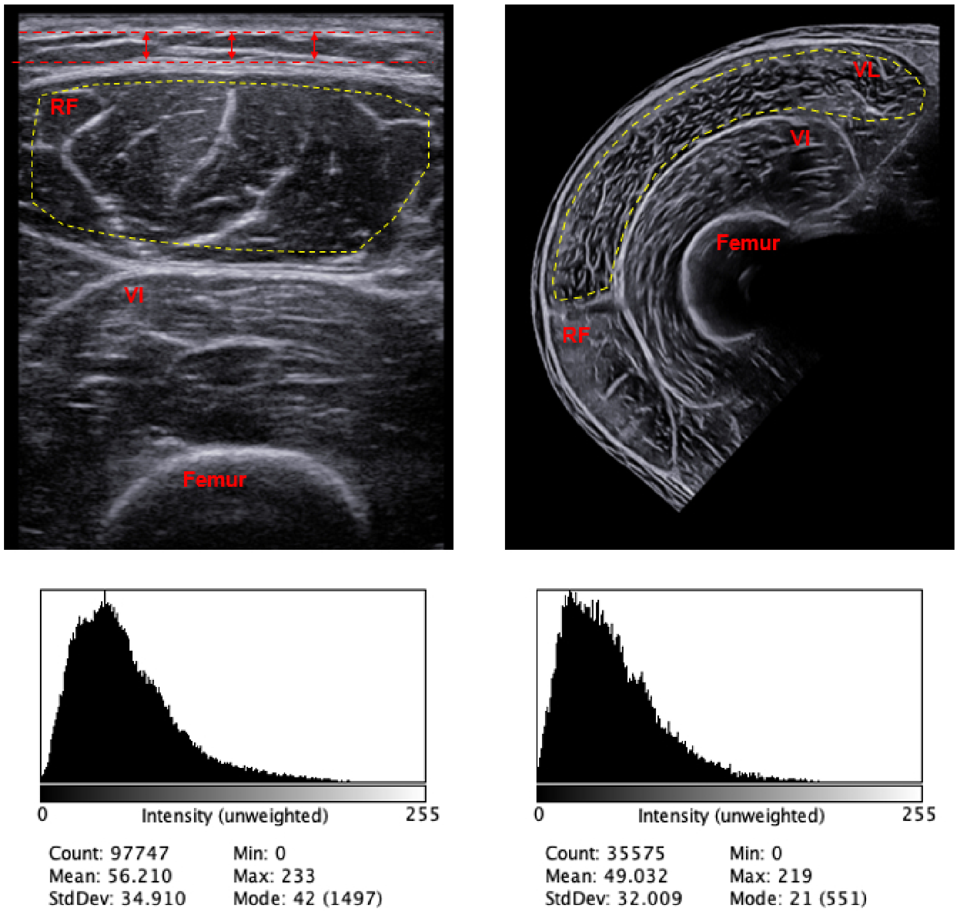
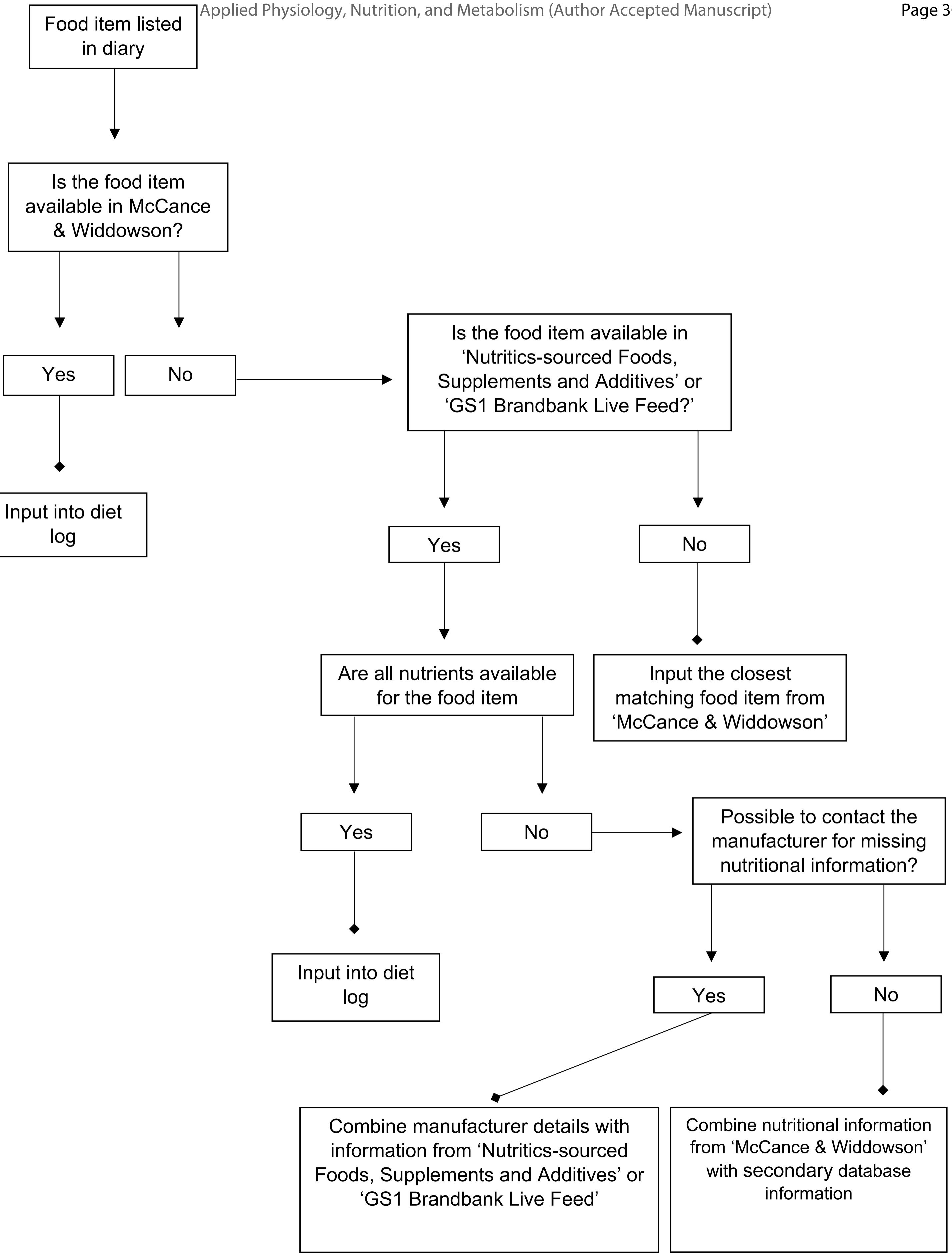


Figure 1. Example B-mode ultrasound image of the rectus femoris in the transverse plane (top left) and panoramic image of the vastus lateralis (top right) for assessment of skeletal muscle echo intensity together with the corresponding gray-scale pixel intensity histograms (below each image). Yellow dashed lines represent the region of interest used to determine echo intensity within the muscle belly. Red dashed lines indicate the borders of the dermal layer (top line) and the muscle aponeurosis (bottom line), and the red arrows represent the subcutaneous fat layer. Rectus Femoris, RF; Vastus Lateralis, VL; Vastus Intermedius VI.

75x71mm (236 x 236 DPI)


Food item listed in diary
Is the food item available in McCance & Widdowson?
Yes
No
Input into diet log
Is the food item available in 'Nutritics-sourced Foods, Supplements and Additives' or 'GS1 Brandbank Live Feed?'
Yes
No
Are all nutrients available for the food item
Input the closest matching food item from 'McCance & Widdowson'
Yes
No
Possible to contact the manufacturer for missing nutritional information?
Input into diet log
Yes
No
Combine manufacturer details with information from 'Nutritics-sourced Foods, Supplements and Additives' or 'GS1 Brandbank Live Feed'
Combine nutritional information from 'McCance & Widdowson' with secondary database information