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Muscle mass, metabolic quality and physical function in frail, older people with non-weight bearing fractures: a cohort study protocol

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ABSTRACT

Introduction

For older people with frailty, the loss of muscle mass, metabolic quality and strength during periods of immobility can be detrimental, contributing to lower mobility and loss of independence following the immobile period. This study aims to understand the rate and extent of loss in muscle size, muscle strength, metabolic and physical function that older people experience when they are non-weight bearing. It will evaluate associations and predictors of these losses with the aim of identifying useful biomarkers to use in the future either as individual targets for potential interventions or as markers to predict the risk of deterioration following periods of immobility.

Methods and analysis

A longitudinal cohort study, with an embedded sub-study, will assess older people during 6 weeks of immobility following an acute non-weight bearing fracture. Participants will be followed up at 4 months to assess changes in muscle measures and function from baseline. The sample size will be 60 participants ≥70 years. Muscle mass will be assessed using bioelectrical impedance analysis, quadriceps ultrasound, whole body muscle mass by isotope tracer and MRI. Handgrip and knee extension strength will be measured. Outcomes include changes in muscle mass and strength and clinical outcomes. Descriptive statistics will be used to report the baseline characteristics. Repeated measures ANOVA will be used to analyse the mean changes in muscle size and strength over time. Logistic regression models will be used to examine the relationship between whole-body and muscle level biomarkers, changes in muscle mass or strength and adverse clinical and functional outcomes.

Discussion

This study contributes to the translational research challenge set by the NIHR for Biomedical Research Centres. It uses a multi-disciplinary approach with novel techniques to develop a comprehensive mechanistic understanding of musculoskeletal deterioration associated with immobility and frailty in older patients with non-weight bearing fractures.

INTRODUCTION

Across all ages, periods of immobility can lead to muscle atrophy, insulin resistance, reduced mitochondrial content, increased muscle lipid deposition and reduced muscle function (1-4). This may be particularly detrimental to older people with sarcopenia as they start from a position of lower muscle mass and quality, compared to younger individuals (5, 6). Sarcopenia is proposed to contribute to the vulnerability that characterises physical frailty. Both conditions form part of a self-perpetuating cycle that increase an individual's susceptibility to the risks of acute muscle loss and negative metabolic health changes when that individual becomes physically inactive (7). This, in turn, makes rehabilitation more difficult and contributes to reduced mobility, and the increased risk of dependency, hospitalisation and mortality (8-10).

Unfortunately, there are occasions where immobility is unavoidable such as the therapeutic immobilisation of, and restricted or non-weight bearing through, an affected limb for 6-8 weeks required to heal many common fragility factures (11, 12). Little is known about the clinical characteristics of older people who sustain such injuries, but many, especially those with frailty, are discharged from acute hospitals to care homes, and exposed to further risks of immobility. The impact of immobilisation upon the trajectory of the decline in their skeletal muscle mass, quality and function is also unknown. It is presumed these patients experience significant loss of muscle during the non-weight bearing period, influencing their subsequent physical functioning and clinical outcomes.

Understanding the mechanistic links between immobility and age-related muscle loss is necessary for designing targeted interventions to limit the losses and associated adverse outcomes. Some underlying changes and measurable characteristics may be detected prior to alterations in the physical phenotype using both invasive and non-invasive research techniques to examine muscle metabolic health status (4, 13-15). Identifying such measurable characteristics, known as biomarkers, that are indicative of underlying changes have two potential benefits. Firstly, biomarkers can be used to target or personalise interventions on an individualised basis because they represent the underlying physiological and metabolic states of clinical health conditions. Secondly, some biomarkers are strong indicators of longer-term health outcomes, and can

potentially be used as proximal end points in translational research studies before larger scale trials with clinical end points are undertaken.

The aims of this study are to quantify the trajectory of decline in muscle mass, muscle strength and lower limb muscle thickness in older people immobilised for the management of non-weight bearing fractures (main cohort study). The existing level of muscle loss and behaviour will be characterised using more detailed metabolic and molecular measurements at the whole-body and muscle levels i.e. a "deep phenotyping" (sub-study) that may predict and explain the magnitude of decline observed. Achieving these aims will help guide further translational research targeting the ill-understood physiology of the musculoskeletal system in severe frailty states.

METHOD

Study design

The study will use a single-centre prospective longitudinal cohort design to determine the trajectory of muscle atrophy and muscle strength loss in older patients immobile for 6 weeks with a non-weight bearing fracture. To identify biomarkers responsible for the muscle changes, a sub-study group will be selected from the main cohort for additional muscle metabolism measurements, referred to as 'deep phenotyping'.

All participants will be assessed at 5 time points over 16 weeks following their acute fracture. Initial data and preinjury information will be collected as soon as feasible from fracture. Subsequent measurements will be performed at 1 week post fracture (to capture the early changes), at 3 weeks post fracture (to determine the rate of the muscle loss), at 6 weeks post fracture (when the majority of the patients will be ending their immobile period) and at 16 weeks post fracture (when the majority of patients will have undergone rehabilitation and have returned home).

Study setting

Eligible participants will be recruited over 2 years from the Queen's Medical Centre in Nottingham, a 1,300 bed teaching hospital serving over 2.5 million people in the surrounding community.

Participants

Main cohort

All patients admitted to hospital with an acute non-weight bearing fragility fracture will be screened for eligibility. Patients must give consent or, for those lacking the mental capacity to do so, have a personal consultee who agrees to their inclusion.

The inclusion criteria for the main study

1. Acute fragility fracture (treated with or without surgical fixation) and prescribed non-weight bearing management of that limb by an orthopaedic specialist for at least 4 weeks.
2. Aged 70 years or over
3. Unable to walk during the non-weight bearing period, limited to transfers or bedrest only.

Exclusion criteria

1. Imminent risk of death
2. Concurrent hip fracture
3. Lower leg amputation
4. Bedbound prior to admission
5. Are unable to consent for themselves and have no personal consultee.

Deep phenotype sub-study

Participants will fulfil the main study criteria and additional eligibility for the deep phenotype sub-study based on the complexity and relative contraindications for the deep phenotype measures. One gender (female) and lower limb injuries have been selected for the inclusion to the sub-study group to reduce the background variation in muscle measurements between participants.

Exclusion criteria for the deep phenotype sub-study

1. Diagnosed dementia or who lack capacity to understand the sub-study measures
2. Acutely unwell (defined by Early Warning Score ≥3)

3. Creatinine clearance <30mls/min. The D3-creatine test relies on renal excretion and has not been tested in those with low renal function (15).

4. Prescribed anticoagulants (warfarin or direct oral anticoagulant). It is felt to be an unnecessary risk to reverse therapeutic anticoagulation for a muscle biopsy.

5. Chronic use of anti-inflammatory medications and paracetamol for more than 1 month prior to start of study as these drugs alter the rate of muscle protein synthesis (16, 17).

6. Any contraindications for magnetic resonance scan

Sampling, recruitment and consent

Consecutive admissions will be screened for eligibility and recruited. Initial visits will take place on the acute hospital wards with follow-up visits in the community (at home or in a care home). The study will be fully explained, verbally and with printed information to all potential participants and their capacity to consent will be assessed using the two-stage process based on the Mental Capacity Act 2005. If potential participants do not have capacity to consent, a relative or carer will be consulted to act as the participant's personal consultee and advise on the potential participant's inclination to be involved in the research. Those participants who lack capacity initially but subsequently regain capacity during the study will be informed fully of the study, asked if they would like to continue and if they agree, they will be asked to sign the consent form.

Data collection

Table of measures to be performed at each time point

Measurement		Visits (no. of weeks after fracture) 0	1	3	6	16
	Whole cohort					
Participant characteristics - medical records	Age, gender, type of residence, comorbidities, medication prescribed, type of fracture, type of surgery, admission blood tests, height.	x				
Participant Interview	Cognition – Montreal Cognitive assessment	x				x
	Level of dependency – Barthel Index	x				x
	Frailty – Fried frailty phenotype	x				x
Participant measurements	Body composition – Weight and Bioelectrical impedance assay	x	X	X	x	x
	Thigh muscle thickness – ultrasound	x	X	X	x	x
	Muscle strength - Handgrip strength and knee extension strength	x	X	X	x	x
Outcome measures – medical records	Mortality, discharge destination, medical complications, changes in medications, length of stay and re-admissions					x
	Additional measurements for sub-study group					
Participant measurements	Whole body muscle mass:					
	- determined using D3 creatine tracer	x				
	- determined using magnetic resonance imaging					x
	Muscle Biopsy	x				
	Blood tests for inflam-ageing	x				

Table 1 Study measurements performed at each time point.

Whole cohort

Participant characteristics

Participant demographics will be accessed from medical records including age, gender, type of residence (home, institution), comorbidities (using Charlson Co-morbidity Index (18) and number of recorded comorbidities), medication prescribed, type of fracture and type of surgery (if performed). Height and weight will be assessed to calculate body mass index (weight in kilograms divided by the square of height in meters). Measuring height is often not feasible in this older, frail immobile population, therefore ulnar length will be used as a surrogate (19).

Participant Interview

Participants will be interviewed at the initial visit and at 16 weeks to assess for changes in their cognition, level of physical dependency and physical frailty.

Cognitive function

The Montreal Cognitive Assessment (MoCA) is a widely used screening test for mild cognitive impairment, administered in approximately 10 minutes (20). It assesses various cognitive domains including concentration, attention, memory, executive function, language, calculations, orientation, conceptual thinking and visual skills. The maximum score is 30, although a score above 25 represents normal cognitive function. The MoCA demonstrates excellent test-retest reliability (correlation coefficient = 0.92, $p<0.001$) and good internal consistency (Cronbach alpha= 0.83) (20).

Level of dependency

The 20 point modified Barthel Index of activities of daily living (21) will be used to determine dependency pre-injury and at 16 weeks. The Index assesses 10 functional tasks of daily living including personal hygiene, bathing, feeding, toileting, stair climbing, dressing, bowel control, bladder control, mobility and transfers. The individual is scored depending on the independence in each task and lower scores indicate higher dependency. It has been validated as a prognostic tool in stroke rehabilitation and is recommended for assessment in older adults (22). A self-reported Barthel Index will be used, this has good correlation with the performance based test ($r>0.97$) (23), and where participants are unable to self-report, a proxy report will be used.

Frailty

The frailty phenotype consists of 5 components, with the presence of 3 or more items indicating frailty and 1-2 items representing pre-frailty (7). Typically, the components of weight loss, exhaustion and low physical activity are self-reported, while gait speed and grip strength are measured values. However, due to immobility it is not possible to measure gait speed in this cohort. For this reason, participants will be screened for frailty using a validated frailty scale (FRAIL) (24). This consists of 4 simple questions self-reporting components of the frailty phenotype and 1 question (number of illnesses) based on the Rockwood Scale (25). A bespoke cumulative frailty index will also be calculated using pre-injury data from the modified Barthel Index, the number of comorbidities and the MoCA score.

Participant measurements

Measurements of body composition, muscle thickness and muscle strength will be performed in all participants over the immobility period. They will be performed on the non-injured side of the body.

Body composition

Bioelectrical impedance assay (BIA) measures the resistance of a body as a conductor to a small electrical current. Participants will lie supine on a non-conducting surface with electrode stickers on the dorsal surface of their hands and feet.

Thigh muscle thickness

Portable ultrasound will be used to measure the thickness of the *vastus lateralis* muscle of the non-injured leg to detect any longitudinal changes in muscle size. Using the technique reported by Franchi et al 2017 (26), participants will lie supine with their knee in full extension and measurements will be taken midway along their femur (a point defined as the mid distance from the greater trochanter to the lateral border of the femoral condyle). With the probe placed longitudinally to the thigh and aligned to the fascicle plane, the muscle thickness will be recorded as the distance between the superficial and deep aponeuroses. This technique has good construct validity compared to magnetic resonance imaging measures (26).

Muscle strength

Handgrip strength will be assessed using a Jamar hydraulic hand dynamometer (Sammons Preston, Model 5030J1). The dynamometer has a dual scale readout which displays isometric grip force form 0-90 kg (0-200 lb) and a handle which easily adjusts for five grip positions to accommodate hand size. Participants will be encouraged to squeeze with maximal strength and will have three trials with the best value being used for analysis.

The strength of knee extensor muscles will be measured using a hand-held Layfette manual muscle tester (Model 01165) (27). Participants will be seated on the edge of the bed and asked to push with maximal effort against the muscle tester held in the researcher's hand. Participants will have three trials with the best value used for analysis.

Sub-study measurements

The sub-study measurements will be performed at the participant's bedside with the exception of the magnetic resonance scan.

Whole body muscle mass

The D3-creatine dilution method provides a direct assessment of functional muscle mass. Oral D3-creatine will be taken up into the skeletal muscle creatine pool and converted to D3-creatinine, which is excreted in the urine (15). Urine will be collected from sub-study participants over 72 hours from administration of a 30mg oral D3-creatine dose and the D3-creatinine level will be measured using high-performance liquid chromatography/mass spectrometry (HPLC/MS) (28). The total body creatine pool size and whole body muscle mass will be calculated using the equation from Clark et al.(15).

A magnetic resonance imaging (MRI) scan (serial cross sections) will be used to quantify whole body muscle mass in this population of patients.

Muscle biopsy

Muscle biopsies will be obtained from the *vastus lateralis* muscle using a minimally invasive, micro-biopsy technique developed by Hayot et al. (29). Under sterile conditions and after local anaesthetic injection, a percutaneous biopsy needle (Bard RTM) will be passed 5-6 times to obtain tissue for analysis. The samples will be immediately frozen in liquid nitrogen to minimise *ex vivo* changes to intracellular metabolism.

The muscle biopsy samples will be used to determine temporal changes in the expression of targeted DNA, mRNA (microfluidic, low-density array gene cards) and proteins known to be associated with muscle mass regulation, deconditioning, fuel selection and mitochondrial mass and proliferation.

Outcome data

Outcome data will be collected from medical records at 16 weeks, to determine mortality, discharge destination, medical complications, total length of stay and hospital re-admissions.

Sample size

Changes in mobility and muscle function have been shown to associate with as little as 1kg change in grip strength (30). For the main study, a sample size of 48 patients would provide 80% power to detect a 2.5kg change in grip strength over 6 weeks of immobility at 5% significance level. With an anticipated dropout rate of 20%, a recruitment target is set for 60 participants.

The sub-study is not powered for outcomes, rather it will inform the feasibility of performing the deep-phenotype measurements in older hospital patients with non-weight bearing fractures. The strict inclusion criteria for the sub-study minimises the variance and allows for a small sample size.

Data analysis plan

Demographic information and baseline variables will be summarised using descriptive statistics expressed as mean (SD) for continuous variables and median (IQR) for categorical variables. Descriptive statistics summarise the characteristics of non-weight bearing patients and the key outcome variables including mortality, length of stay and hospital readmissions.

The pre-injury data from the modified Barthel Index, the number of comorbidities and the MoCA will be summed to calculate a baseline cumulative Frailty Index (the number of deficits present divided by number of deficits considered) (25) which will be compared using correlation statistics to the baseline frailty cumulative deficits score. The role of frailty on the rate of muscle mass, strength and functional loss will be analysed using regression analysis.

Repeated measures analysis of variance will be used to compare changes over time in body composition, muscle thickness and muscle strength measures. Data from the sub-study measurements will be correlated with these changes and non-invasive measures to enhance our understanding of the musculoskeletal system in the extreme frail state.

Ethics and dissemination

Ethical approval for the study was granted by Wales Research Ethics Committee 6 on 11/04/18. Amendments to the study protocol were approved on 14/11/18 and 26/3/19. The study proposal and public facing information were also reviewed by members of the Dementia and Older People Patient and Public Involvement Group (PPI) group at Nottingham University.

The appropriate permissions have been granted for access and usage of all specified databases, clinical information systems and patient records. Results from the study will be disseminated at national and international conferences, in peer-reviewed journals and in a doctoral thesis.

Protocol amendments

Significant protocol amendments have been implemented after further approval by the Sponsor, the ethics review board and the UK Health Research Authority. The methods detailed above represent the amended protocol to which participants have been recruited. The details of the original proposals are listed in Appendices 1 and 2. The amendments are listed below and explained in the text.

1. Reduction of the number and frequency of tests in the deep phenotype sub-study group
2. Alterations to the inclusion and exclusion criteria of the sub-study group to reflect the changes in tests and remove the specification for a single fracture type.
3. Removal of the requirement for participants to be discharged to a care home or community hospital from the inclusion criteria of the main study group.

In addition to D3 creatine and muscle biopsy for gene expression, the initial sub-study protocol included measures of muscle protein synthesis, muscle protein breakdown and insulin resistance, shown in Appendix 1. All the deep phenotype measures were to be performed at the first four time points (baseline, week 1, week 3 and week 6). However, following reflection on the lack of recruitment to the deep phenotype sub-study, the number and repeated nature of the measures (recurring at four time points) were

identified as barriers to recruitment as they were too onerous for participants. After consideration of what was necessary to obtain scientific knowledge to meet the study objectives and what was acceptable to participants, the protocol was amended.

Appendix 2 details the original inclusion and exclusion criteria for the deep phenotype sub-study. Originally inclusion in the sub-study was limited to one type of fracture (ankle fracture) to limit the presumed variability between participants. However, after four months of screening only 1 patient met the eligibility criteria for the sub-study (compared to 25 patients for the main study). Discussion among the research team proposed that the inclusion of other lower limb fractures would not alter the ability to compare participants because all measurements involved the non-fractured limb. The reduction in sub-study measures altered the eligibility criteria, in particular, the removal of an insulin glucose tolerance test meant people with diabetes did not need to be excluded from the sub-study.

Appendix 2 also details the original inclusion criteria for the main cohort study, which selected participants based on their discharge destination to a care home or community hospital for their non-weight bearing period. The researchers were unaware that the number of patients returning directly home with family or care agency support accounted for around 15-20% of the population group. Predicting a participant's discharge destination within the first few days of admission was also a challenge and despite remaining non-weight bearing, initially a few recruited participants were withdrawn when their discharge plans changed. The destination of non-weight bearing for patients (home or a care home) is determined by clinical practitioners reflecting the clinical or social circumstances for the patients and it was agreed that there was no scientific justification to exclude older patients going directly home from the study provided their mobility remained limited to transfers or bedrest.

DISCUSSION

This longitudinal cohort study focuses on examining the rate and extent of losses in muscle size, metabolic quality and strength that older people experience when they are non-weight bearing, the associations and predictors of these losses and how these losses match to adverse clinical events.

The proposed study has several strengths including the longitudinal design of the study and the combination of clinical and deep-phenotyping techniques. Discerning measurable

traits in the muscles of older people with frailty through deep phenotyping will provide a detailed understanding of muscle behaviour and physiology at the cellular level in this patient group. Similarly, measurements at multiple time points will increase knowledge and understanding of key changes that could be future intervention targets to prevent functional declines. Bedrest studies in volunteers have shown changes in muscle architecture and metabolism begin rapidly; alterations in muscle protein synthesis occur within a few hours of immobility, while detectable loss of muscle mass occurs within a few days (31, 32). In comparison, alterations to the clinical phenotype often become apparent after immobilisation, hence the need to identify biomarkers to target and potentially prevent such outcomes. The deep-phenotype sub-study will provide better understanding of how aged muscle behaves in relation to the muscle measurement changes in older people with frailty.

This will be one of the first times the non-invasive technique of D3-creatine to measure whole-body muscle mass will be applied to frail populations in a clinical setting. Although the technique has been validated in healthy populations who may or may not have frailty, it is expected the clinical population will have higher degrees of frailty.

The study will use several techniques to quantify muscle size, metabolic quality and strength. This will allow comparison of methods for measuring muscle size, metabolic quality and strength, and will explore the use of newer approaches that quantify whole body muscle mass and compare with those assessing a single muscle group to examine the distribution of muscle loss during immobility.

The frailty, acute fracture, associated complications and high risk of deterioration are reasons for conducting research in this population, but they are also barriers to participation. The protocol amendments have tried to address some of the barriers and reduce the non-recruitment risk, especially for the deep phenotype sub-study, although it is acknowledged challenges remain. Members of the research team have recruited to methodologically similar studies in less frail participants and have recruited very frail older people into other studies, but these methods have not been attempted in these frail patients. For the recognition of sarcopenia in older frail patients in clinical settings, there is a need to increase understanding of the most accurate and clinically feasible techniques.

There are others limitations to the study. There is a risk of participant selection bias if the non-weight bearing patients who consent to take part in the study differ from those who decline. Attrition is an added risk for any longitudinal study and especially for older

patients with a high degree of frailty. This has been accounted for in the sample size calculation. Comparison of characteristics between participants with non-participants and those lost to follow-up will be possible in relation to routinely collected hospital data to assess how closely representative the sample population will be of older patients hospitalised with non-weight bearing fractures. More information on musculoskeletal declines could be gathered through using more frequent time points, but at this stage it is not felt it is acceptable to ask older people with frailty to participant in overly burdensome studies and that increased burden would either adversely reduce recruitment or retention.

Although the study will assess patients with non-weight bearing fractures, it is hoped the detailed insight of how muscle mass and function change during immobility in this frail group could be extrapolated to provide better understanding of deconditioning in older people with frailty who may be immobile from other causes including acute illness or hospital stay.

DECLARATIONS

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Competing interests

None

Authors' contributions

EL drafted the article. All authors read, amended and approved the final manuscript

REFERENCES

1. Bell KE, von Allmen MT, Devries MC, Phillips SM. Muscle Disuse as a Pivotal Problem in Sarcopenia-related Muscle Loss and Dysfunction. J Frailty Aging. 2016;5(1):33-41.
2. Marshall SC, Berg K. Cessation of exercise in the institutionalized elderly: effects on physical function. Physiotherapy Canada Physiotherapie Canada. 2010;62(3):254-60.
3. Deschenes MR, Holdren AN, McCoy RW. Adaptations to short-term muscle unloading in young and aged men. Med Sci Sports Exerc. 2008;40(5):856-63.

4. Crossland H, Skirrow S, Puthucheary ZA, Constantin-Teodosiu D, Greenhaff PL. The impact of immobilisation and inflammation on the regulation of muscle mass and insulin resistance: different routes to similar end-points. J Physiol. 2019;597(5):1259-70.
5. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci. 2006;61(10):1059-64.
6. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39(4):412-23.
7. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56(3):M146-56.
8. Beaudart C, Zaaria M, Pasleau F, Reginster JY, Bruyere O. Health Outcomes of Sarcopenia: A Systematic Review and Meta-Analysis. PLoS One. 2017;12(1):e0169548.
9. Landi F, Liperoti R, Russo A, Giovannini S, Tosato M, Capoluongo E, et al. Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. Clin Nutr. 2012;31(5):652-8.
10. Cederholm T, Cruz-Jentoft AJ, Maggi S. Sarcopenia and fragility fractures. Eur J Phys Rehabil Med. 2013;49(1):111-7.
11. Lin CW, Moseley AM, Refshauge KM. Effects of rehabilitation after ankle fracture: a Cochrane systematic review. Eur J Phys Rehabil Med. 2009;45(3):431-41.
12. Swart E, Bezhani H, Greisberg J, Vosseller JT. How long should patients be kept non-weight bearing after ankle fracture fixation? A survey of OTA and AOFAS members. Injury. 2015;46(6):1127-30.
13. Calvani R, Marini F, Cesari M, Tosato M, Anker SD, von Haehling S, et al. Biomarkers for physical frailty and sarcopenia: state of the science and future developments. J Cachexia Sarcopenia Muscle. 2015;6(4):278-86.
14. Wilkinson DJ, Franchi MV, Brook MS, Narici MV, Williams JP, Mitchell WK, et al. A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. Am J Physiol Endocrinol Metab. 2014;306(5):E571-9.
15. Clark RV, Walker AC, O'Connor-Semmes RL, Leonard MS, Miller RR, Stimpson SA, et al. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans. J Appl Physiol (1985). 2014;116(12):1605-13.
16. Trappe TA, Carroll CC, Dickinson JM, LeMoine JK, Haus JM, Sullivan BE, et al. Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults. American journal of physiology Regulatory, integrative and comparative physiology. 2011;300(3):R655-62.
17. Trappe TA, White F, Lambert CP, Cesar D, Hellerstein M, Evans WJ. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. Am J Physiol Endocrinol Metab. 2002;282(3):E551-6.
18. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol. 1994;47(11):1245-51.
19. Elia M RC, Stratton R, Todorovic V, Evans L, Farrer K. Malnutrition Universal Screening Tool (MUST) for adults www.bapen.org.uk2004 [
20. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695-9.
21. Collin C, Wade DT, Davies S, Horne V. The Barthel ADL Index: a reliability study. International disability studies. 1988;10(2):61-3.

22. Dickinson EJ. Standard assessment scales for elderly people. Recommendations of the Royal College of Physicians of London and the British Geriatrics Society. Journal of Epidemiology and Community Health. 1992;46(6):628-9.
23. Shinar D, Gross CR, Bronstein KS, Licata-Gehr EE, Eden DT, Cabrera AR, et al. Reliability of the activities of daily living scale and its use in telephone interview. Arch Phys Med Rehabil. 1987;68(10):723-8.
24. Morley JE, Malmstrom TK, Miller DK. A simple frailty questionnaire (FRAIL) predicts outcomes in middle aged African Americans. J Nutr Health Aging. 2012;16(7):601-8.
25. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. J Gerontol A Biol Sci Med Sci. 2007;62(7):722-7.
26. Franchi MV, Longo S, Mallinson J, Quinlan JI, Taylor T, Greenhaff PL, et al. Muscle thickness correlates to muscle cross-sectional area in the assessment of strength training-induced hypertrophy. Scand J Med Sci Sports. 2017.
27. Martin HJ, Yule V, Syddall HE, Dennison EM, Cooper C, Aihie Sayer A. Is hand-held dynamometry useful for the measurement of quadriceps strength in older people? A comparison with the gold standard Bodex dynamometry. Gerontology. 2006;52(3):154-9.
28. Stimpson SA, Leonard MS, Clifton LG, Poole JC, Turner SM, Shearer TW, et al. Longitudinal changes in total body creatine pool size and skeletal muscle mass using the D3-creatine dilution method. J Cachexia Sarcopenia Muscle. 2013.
29. Hayot M, Michaud A, Koechlin C, Caron MA, Leblanc P, Prefaut C, et al. Skeletal muscle microbiopsy: a validation study of a minimally invasive technique. Eur Respir J. 2005;25(3):431-40.
30. Stevens PJ, Syddall HE, Patel HP, Martin HJ, Cooper C, Aihie Sayer A. Is grip strength a good marker of physical performance among community-dwelling older people? J Nutr Health Aging. 2012;16(9):769-74.
31. Suetta C, Frandsen U, Jensen L, Jensen MM, Jespersen JG, Hvid LG, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. PLoS One. 2012;7(12):e51238.
32. Drummond MJ, Timmerman KL, Markofski MM, Walker DK, Dickinson JM, Jamaluddin M, et al. Short-term bed rest increases TLR4 and IL-6 expression in skeletal muscle of older adults. American journal of physiology Regulatory, integrative and comparative physiology. 2013;305(3):R216-23.

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APPENDIX 1 – Original schedule of procedures for the sub-study

Procedures	**Visits**				
	Baseline	**Week 1**	**Week 3**	**Week 6**	**Week 16**
Demographics	x				
Height and weight measures	x	x	x	x	x
Medical history – co-morbidities, medications	x	x	x	x	x
Participant interview – MoCA, ADLs, physical frailty questions	x				x
Review hospital records for medical complications	x	x	x	x	x
Ultrasound of muscle	x	x	x	x	x
Hand grip dynamometer	x	x	x	x	x
Quadriceps muscle strength	x	x	x	x	x
Muscle biopsy for D2O and gene expression	x	x	x	x	
Heavy water (D20) drink for muscle protein synthesis	x	x	x	x	
D3-methylhistidine (3MH) drink for muscle protein breakdown	x	x	x	X	
D3-creatine drink for whole body muscle mass	x	x	x	x	
Urine collection for 3MH and D3-creatinine	x	x	x	x	

Venepuncture for 3MH and inflammaging	x	x	x	x	
Saliva samples for D2O	x	x	x	x	
IV glucose tolerance test	x	x	x	x	
Whole body MRI scan					x

APPENDIX 2 – original eligibility criteria for the main cohort study and deep phenotype sub-study

Inclusion criteria for the main cohort study:

- ≥ 70 years,
- Acute fracture (treated with or without surgical fixation) and prescribed non-weight bearing management by the orthopaedic specialist
- Requiring a non-weight bearing bed in rehabilitation hospital or care home because they are unable to return home for the duration of the non-weight bearing restriction.
- Can provide informed consent or has a personal consultee who can provide consent on their behalf.

For deep phenotype characterisation, in addition to the above:

- Discharged from acute hospital to a community bed for the immobilisation period
- Ankle fracture without surgical fixation
- Female gender

Exclusion criteria for the overall main cohort study:

- Terminal illness or moribund
- Concurrent hip fracture
- Bed bound prior to admission
- Lower limb amputation

Additionally, for the deep phenotype characterisation:

- Poor renal function (Creatinine Clearance <30mls/min)
- Anticoagulants (warfarin, DOAC)
- Swallowing difficulties

- Severe cognitive impairment (MoCA < 10) or acute delirium
- Surgical fixation of the fracture
- Acutely unwell (defined by Early Warning Score ≥3)
- Contraindications for MRI scan (e.g. brain aneurysm clips, permanent pacemaker)
- Diabetes mellitus
- Chronic use of anti-inflammatory medications and paracetamol (>1 month) prior to start of study