Muscle phenotyping exploratory sub-study (MPES) Sub-study 2 (Appendix 1)

Contents

1.1 Sub-study summary 2
1.2 Sub-study setting 2
2.1 Sub-study participants 2
2.2 Sub-study inclusion criteria 3
2.3 Sub-study exclusion criteria 3
2.4 Tier 2 and 3 Sub-study (Biopsy and COSIAM) only exclusion criteria 3
3.2 Surface HD EMG device Protocol 5
3.3 Muscle biopsy and COSIAM technique 8
3.2 Sub-study visit descriptions 9
3.2.1 Visit 1a and 1b – Days 1 and 2 9
3.2.2 Visit 2 – Day 29 ± 7 days 11
3.2.3 Visit 3 – Day 57 ± 7 days 12
4.1 Ethical, regulatory and governance considerations 12
5.1 Financing 12
6.1 Modification History 13

1.1 Sub-study summary

The muscle phenotyping exploratory (MPES) sub-study has two main aims within the wider aims of the CHESTY study. Firstly, it aims to test the feasibility, safety and tolerability of using surface electromyography (EMG) metrics during physical assessments among both participants with respiratory disease and healthy volunteers and ingested tracer based muscle turnover methods. The second aim is to perform muscle-level deep phenotyping and estimate baseline skeletal muscle mass as well as muscle protein synthesis and muscle protein breakdown using muscle biopsy and tracer methodology among patients hospitalised for an AECOPD with comparison to both patients with stable COPD and age-matched controls without chronic respiratory disease. Surface EMG will be performed using the CE marked 32 surface high density (HD) electrode array and transmitter (OT Bioelettronica srl, Turin, Italy) described in section 3.2. Muscle protein mass and turnover will be assessed using the COSIAM technique described in section 3.3.

The study will be nested within the CHESTY study utilising the same recruitment methods, study team, site, assessments of physical function and strength, muscle biopsies and questionnaires.

The sub-study aims to address the same primary and secondary objectives defined by the main CHESTY Study protocol utilising novel and detailed methods as described below.

1.2 Sub-study setting

The MPES sub-study will be conducted only at the Glenfield Hospital site. This is a tertiary NHS respiratory centre with significant experience in conducting research studied among subjects with stable chronic disease, unstable acute disease and among healthy volunteers.

2.1 Sub-study participants

This sub-study aims to recruit three groups of participants with up to 20 participants in each group:

- Group A Patients admitted to hospital for an AECOPD
- Group B Patients with stable COPD (≥28 days since completion of treatment for moderate AECOPD and/or ≥90 days from discharge to usual place or residence following hospitalisation for any reason)
- Group C Age-matched controls without chronic respiratory disease

Screening and recruitment for this sub-study will occur both among current participants of the CHESTY study where participants who have provided suitable consent may be contacted and offered participation in this sub-study as well as among new patients as per the CHESTY Study screening and recruitment processes described in section 8.2 of this protocol.

There will be no formal age or sex matching across sub-study participant groups with groups 1 and 2 recruited non-selectively within the inclusion and exclusion criteria. Significant experience of both inpatient and stable COPD patient recruitment at this centre has demonstrated that participants are consistently have a normally distributed mean age of 70

years and an interquartile range of 65-75 years with a close to 50 % male to female split therefore control subjects across this age range will be targeted with a 1:1 male to female ratio.

2.2 Sub-study inclusion criteria

1. Meets eligibility criteria for the CHESTY Study Core study participants or CHESTY Study Healthy Controls

2. Is one of:

    a) Patient admitted to hospital for a clinician defined AECOPD requiring treatment with systemic corticosteroids, antibiotics or both.

    b) Patient with stable clinician defined COPD
        - established on inhaled bronchodilator therapy
        - FEV1/FVC ratio < 0.7 (if available)
        - not currently exacerbating or in immediate recovery phase from exacerbation or hospitalisation defined as:

            ≥28 days since completion of treatment for moderate AECOPD

            and/or

            ≥90 days from discharge to usual place or residence following hospitalisation for any reason

    c) Healthy (age-matched) control with no self-reported underlying respiratory, cardiovascular, inflammatory or metabolic chronic disease (tier 1 only)

3. Willing and able to consent to participate in MPES sub-study

2.3 Sub-study exclusion criteria

1. Any significant musculoskeletal or inflammatory disease or disorder (except those related to COPD) which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.

2.4 Tier 2 and 3 Sub-study (Biopsy and COSIAM) only exclusion criteria

(These exclusion criteria are designed to ensure the safety of the muscle biopsy, consequently they only apply to the tiers 2 and 3 which require muscle biopsy procedures to assess endpoints. Potential participants may be offered participation in tier 1 of this sub-study without a biopsy being performed even if they meet these additional exclusion criteria.)

1. Impaired blood clotting (family or medical history of impaired blood clotting e.g., haemophilia)
2. Current use of warfarin, therapeutic doses of low molecular weight heparin or any direct oral anti-coagulant (DOAC) drugs (including but not limited to betrixaban, apixaban, edoxaban, rivaroxaban, dabigatran)

3. Current use of anti-platelet agents (other than aspirin at a dose ≤ 75 mg / day)

3.1 Sub-study assessments

		Visit 1a	Visit 1b	Visit 2	Visit 3[£]
		Day 1	Day 2	Day 29 ± 7 days[+]	Day 57 ± 7 days[+]
Tier 1	Exploratory blood sampling	X		X	X
	Questionnaires	X		X	X
	DXA Body Composition[&]	X		X	X
	BIA Body Composition	X		X	X
	MRI Thigh	X			X
	Physical Activity Monitor	X		X[£]	
	Physical assessments performed while wearing surface HD EMG device				
	SPPB including force plate	X~	X~	X	X
	Quadriceps isometric and isokinetic dynamometry including fatiguability (Cybex)	X~	X~	X	X
	Handgrip strength and fatiguability	X~	X~	X	X
Tier 2					
	Fasted blood sample	X~	X~	X	X
	Skeletal muscle biopsy	X~	X~	X	X
Tier 3	**Muscle mass (D3-Cr tracer method)**				
	D3-Cr tracer drink ingestion	X			
	24 hr urine collection & urine spot tests at 54 & 72 hr [#]		X		
	Muscle Protein Synthesis (MPS)				
	Saliva sample before and 2 hr post-D_2O ingestion		X*	X*	
	D_2O tracer drink ingestion every 7 days		X^	X^	
	Skeletal muscle biopsy [$]		X	X	X
	Muscle Protein Breakdown (MPB)				
	D3-MH tracer drink ingestion day before visit ^	X		X	X
	Repeated blood sampling (5 samples over 3 hrs)		X	X	X

~Assessments will only be completed once during visit 1. Participants in Tier 1 and tier 2 will complete these at visit 1a and will not be asked to attend Visit 1b. Participants in Tier 3 will complete these assessments at visit 1b.

*Saliva samples will be collected by participants weekly on days when taking top up drinks.

[#] 24 hr urine collection plus 54 and 72 hour urine spot tests to be collected by participants if at home or by study team for hospitalised participants who remain inpatients during this time. Sample collection from participants homes will be arranged by the study team after the 72 hour sample has been produced.

[$] Tissue from this biopsy will be used for MPS analysis and all muscle structural and molecular measures (this is not an additional biopsy).

^ Weekly top up tracer drinks will be supplied to participants at visit 1b and 2 to be consumed at home every 7 days between visits.

[+]Participants in tier 3 will not have a ±7 day window for these visits

[£]Only participants recruited during hospitalisation for an AECOPD (group a) will be invited to visit 3, participants recruited with stable COPD healthy controls will not be asked to attend this visit.

[&]A total maximum of four DXA scans will be performed on any single participant within the CHESTY study including those performed as part of this sub-study.

Participants in the MPES sub-study will be asked to complete the same physical assessments as described in the main CHESTY study protocol with the only change being that they will be asked to wear a surface HD EMG device while completing these assessments.

Blood sampling and questionnaires will be undertaken at MPES sub-study visits as described in the main CHESTY PIS in addition to those described below relating to the Tier 2 and 3 procedures.

Exploratory biosamples – blood (plasma and serum), urine, saliva, skeletal muscle tissue and RNA/DNA from samples collected as part of this sub-study will be stored for use in future research or analyses. These samples may be used to investigate various biomarkers related to skeletal muscle function, inflammation, immune response, metabolic function, genetic factors, and disease progression. Future research may also include molecular analyses, such as gene expression profiling and genomic studies. All sample collection and storage will be conducted in accordance with ethical guidelines, the Human Tissue Act (HTA) and require participants' consent for biobanking and future research use.

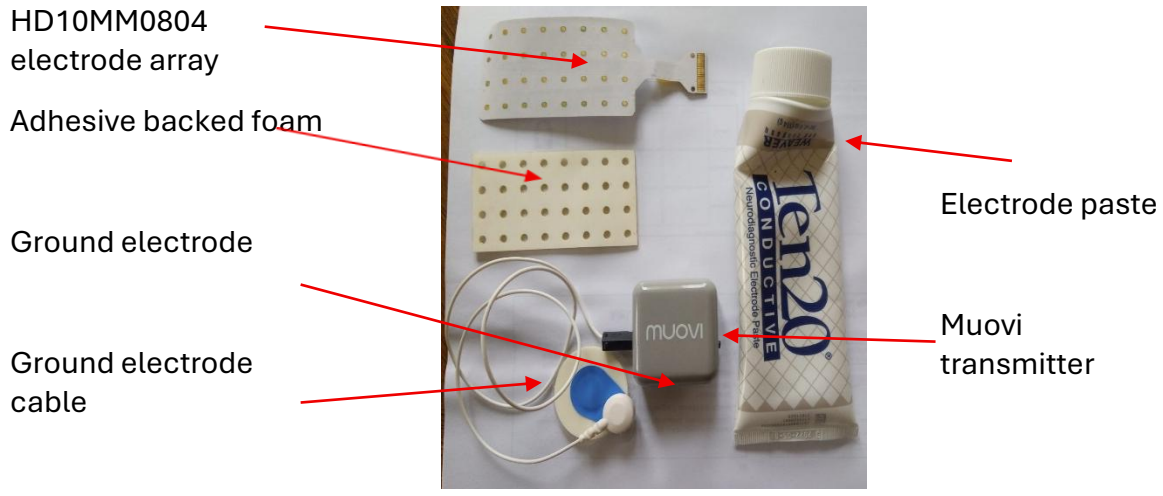
3.2 Surface HD EMG device Protocol

The participant will change into shorts / skirt/ dress/ hospital gown to expose the skin over the front of their thighs.

a) Equipment

The OT Bioelettronica HD10MM0804 electrode array and the Muovi transmitter (Bioelettronica, Italy) will be used for collecting EMG and transmitting the data to a tablet via local Wi-Fi. Bioelettronica is certified as an electronic device with intended use for life science research and is CE marked. The Muovi transmitter also contains an inertial measurement unit (IMU) which transmits signals as quaternions.

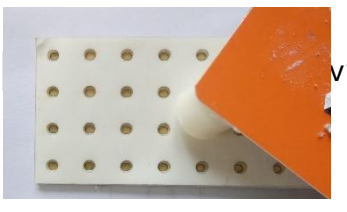
Additionally, a thin foam double adhesive backed foam, electrode paste, ground electrode and ground electrode cable will be used.

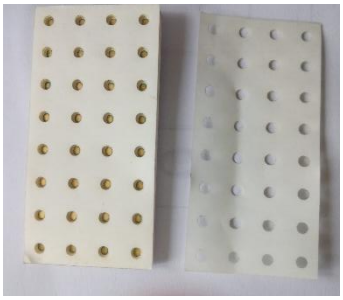


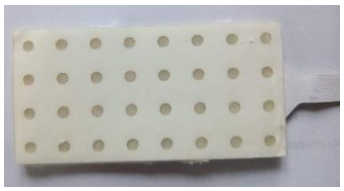
b) Preparing the electrode array

i) Remove film from foam. Apply adhesive side to electrode array ensuring all electrodes are visible.

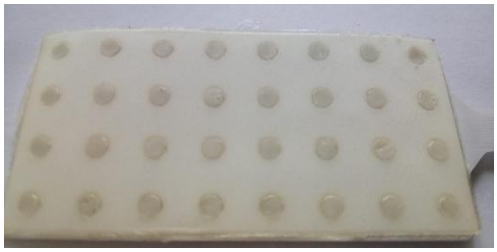
ii) Apply paste to foam ensuring all electrodes are evenly covered







iii) Remove top film making sure no excessive paste is between electrodes



c) Attaching the HD EMG electrode to the skin overlying vastus lateralis

The optimal position is 2/3rds along a line from the anterior superior iliac spine to the lateral border of the patella (Hermens et al., 2000). However, as a strap will be placed across the thigh when the participant is undergoing chair dynamometer testing, the electrode array will be placed more proximally along the line. Confirm the position of the VL by asking the participant to extend their knee against resistance and palpate the muscle contracting. Wipe the skin with an alcohol wipe and allow to dry. Apply the adhesive side of the electrode to the identified location, with the long line of the array in parallel with the line from the ASIS to the lateral border of the patella.

Attach the Muovi to the electrode array, the ground electrode to the ground electrode cable and insert the cable into the Muovi. Apply micropore tape to securely attach the electrode array and Muovi to the skin.

d) Setting up the MUOVI transmitter and Axomics software on the android tablet

i) Connecting the tablet to the Muovi transmitter
The Muovi must be fully charged before starting.
Hold the black button down for 4 seconds until the white and red dots flash simultaneously.
A white light will flash every 2 seconds.
Open the Wi-Fi settings on the android tablet and select the Muovi network to connect to.
The light will now flash twice

ii) Axomics App
Open the 'Axomics' app on the tablet
Go to 'Setup' tab and enter the session ID and Participant ID

iii) Signal check

Go to the 'Capture' tab and click on 'Not a test'

A 'Please confirm' box will popup. Click on 'Connect' followed by 'record'.

Ask the participant to stand up and walk 4m then return to the chair.

Click on 'stop'.

Click on the 'View' tab and select the most recent 'Not a Test' signal.

Check that there are good recordings from all 32 channels.

If not:

- Check the ground electrode cable is attached and the ground electrode firmly adherent to the skin
- Check the electrode array is fixed firmly to the skin with micropore tape.
- Make sure the electrode array is firmly inserted into the Muovi, with the 'Muovi' writing facing outward.
- Check the tablet is still connected to the Muovi transmitter, with intermittent two flashes of white light. Red flashes indicate the battery has low charge.
- Repeat the 'Not a Test' recording.
- If there are still issues with signals remove the EMG array, reapply foam and paste and reattach the array in a slightly different location.

iv) Recording experimental signals

Click on the 'Capture' tab and select the experimental task.

Click on 'connect' when the popup 'Please confirm box appears.

Click on the 'Recording' tab. When recording, the recording number will increase.

Click on 'stop'

The first 2 tasks are 'Rest chair at begin' and 'Rest standing at begin'

The last 2 tasks are 'Rest chair at end' and 'Rest standing at end'

The purpose of recording these resting signals at the beginning and end is to ensure there are no changes in signal properties during the duration of the experiment.

v) Checking recorded signals

Ideally after each recording open the 'View Tab' and check quality of signals.

vi) Uploading signals

At the end of the experiment upload all recorded pseudonymised data to the Newcastle University secure file drop system.

vii) Shutting down

Ensure the device is switched off after use by holding the black button until just a red dot appears. The lights should now be switched off and the device is off

3.3 Muscle biopsy and COSIAM technique

Skeletal muscle samples will be collected from participants who have been offered and consented to Tier 2 of this sub-study at each sub-study visit. Participants will be asked to fast overnight prior to the visit and the quadriceps micro-biopsy technique will be used with ultrasound guidance where appropriate. Tissue will be subjected to cellular and molecular analysis to determine the skeletal muscle phenotype. Analysis may include assessment of the transcriptional profile; proteomics; and cellular and sub-cellular morphology (immunohistochemistry and electron microscopy).

The Combined Oral Stable Isotope Assessment of Muscle (COSIAM) (Cegielski et al, 2022) will be used to assess muscle mass, rates of muscle protein synthesis and muscle protein breakdown. This technique requires stable isotopes (D3-creatine; deuterated water, D_2O; and D3-3-methylhistidine) to be consumed as a drink with bio-sampling of skeletal muscle, blood, urine and saliva (Cegielski et al, 2022). Patients will consume stable isotopic tracers in the form of a drink. These contain naturally occurring, non-radioactive deuterium atoms incorporated into methyl-[D3]-creatine (D3-Cr), deuterated water (D_2O), and D3-methyl histidine (D3-MH).

The objective of the muscle biopsy and COSIAM tracer sub-study is to elucidate the cellular mechanisms underpinning changes in skeletal muscle function and mass during and after hospital admission for an acute exacerbation of COPD with comparison to individuals with stable COPD and age matched controls.

What are we measuring?

Cellular & molecular analysis of skeletal muscle phenotype – Muscle biopsy samples may be collected and processed in four ways in order to facilitate subsequent analysis: (a) snap frozen directly from tissue extraction; (b) cryopreserved in OCT embedding medium; (c) immediately fixed in EM fixative (2.5% glutaraldehyde in 0.1M sodium cacodylate buffer or PBS pH 7.3); (d) cells/fibres harvested from fresh samples for mitochondrial function, cellular modelling and/or sequencing related readouts.

Muscle Mass - The D3-Cr tracer method allows an accurate alternative to imaging methods (e.g., DXA, MRI or CT) to quantify whole-body skeletal muscle mass. Assessment of the ratio of labelled to unlabelled creatine excreted in urine permits accurate estimation of the amount of total creatine in the muscle and subsequently muscle mass. This will allow calibration and comparison to other methods used in this sub-study among participants with COPD.

Muscle Protein Synthesis - Measurement of the accumulation of D_2O into muscle tissue protein (via the amino acid alanine) allows the rate of MPS to be estimated from consumption of the D_2O drink, collection of blood and saliva samples and muscle biopsies.

Muscle Protein Breakdown - The ratio in blood of labelled D3-MH to unlabelled methylhistidine (MH) (which is released from muscle during the process of muscle protein breakdown in the body) allows the calculation of the rate of muscle protein breakdown (MPB) between 20-24 hours post D3-MH ingestion, assessed by tracer dilution via repeat blood sampling over a 3-hour time period.

What will be asked of participants?

The COSIAM tracer methodology requires that visit 1 is split over two consecutive days. At visit 1a participants will be asked to provide a blood/saliva sample and urine void before consuming drinks containing D3-Cr, D_2O, and D3-MH. Collection of all urine produced for 24-hr after drinking the D3-Cr and a saliva sample is collected after consuming the D2O drink. At visit 1a the last of the 24-hr urine sampling will conclude and repeated blood sampling and skeletal muscle biopsy will be performed. Patients will then take top-up D_2O drinks weekly throughout the sub-study. Skeletal muscle biopsy samples at visits 2 and 3 will be used to determine the cumulative MPS rate over this period. Patients will consume D3-MH 24 hours before the follow-up visits 2 and 3 and further blood sampling and skeletal muscle biopsy will be performed at these visits to measure the rates MPB and MPS respectively at visits 2 and 3.

3.2 Sub-study visit descriptions

3.2.1 Visit 1a and 1b – Days 1 and 2

Visit 1 will be completed over two days with the first day described as visit 1a and the second day visit 1b. This is necessary to allow pre-tracer baseline sampling and then time for the labelled tracers to equilibrate across tissues in the body before further sampling. For participants recruited during hospitalisation for and AECOPD the study team will aim to complete both visit 1a and 1b during their hospitalisation however participants with stable COPD and the age-matched control participants will be asked to attend the research centre on consecutive days to undergo the assessments.

Visit 1a – Day 1

Participants will be asked to attend the NIHR Biomedical Research Centre – Respiratory at the Glenfield Hospital, Leicester. Tier 1 and tier 2 participants will be asked to attend in an overnight fasted state. Usual medicines can be taken with sips of water but no food (or drinks containing milk, sugar or caffeine) should be consumed for 10 hours prior to the visit. Tier 3 participants will not be asked to attend visit 1a in a fasted state.

Baseline questionnaires will be completed with study team assistance where required (30 mins).

Exploratory and baseline blood samples will be collected. (5 mins)

To perform the DXA and BIA assessment participants will be asked to lie or sit reclined if unable to lie flat on the DXA machine. The DXA scan will be performed and immediately followed by the BIA test. (20 mins)

The thigh MRI scan will be arranged to take place between other assessments. To undergo the MRI scan participants will be required to change into a hospital gown and remove all metal from their body. Suitable MRI safety questionnaires will be completed with participants prior to the scan. If a suitable time cannot be arranged on the same day due to capacity within the MRI department, the participant will be asked if they are

willing to return to the Glenfield Hospital within 7 days to undergo the MRI scan. (30 mins)

For participants completing tier 2 (but not tier 3) a thigh muscle biopsy will be performed including procedural consent and provision of post-biopsy care instructions and advice. (60 mins)

For participants completing tier 1 or tier 2, the surface HD EMG device will be secured on their thigh as described in section 3.2 above. Participants will then be asked to perform the SPPB, handgrip and quadriceps physical assessments with the surface HD EMG device in place. (45 mins)

Participants completing tier 3 will be supplied with tracer drinks (D3-Cr, D2O and D3-MH) to consume during the visit immediately after completely voiding their bladder and providing a blood/saliva sample. A saliva sample will be obtained 2 hours after consuming the tracer drink (this sample can be taken at home), volunteers will be asked to refrain from eating or drinking for at least one hour prior to every saliva collection – to avoid contamination of the saliva D2O labelling. (5 mins each but must be 2 hours apart) (5 mins)

Participants completing tier 3 will be supplied with a 24-hour urine collection bottle(s) (to take home or to be kept with them on the hospital ward) and instructions to collect **all** urine produced until they are seen at visit 1b the following day when they will be asked to return the collection bottle(s). (5 mins)

Participants will be supplied with a wrist worn physical activity monitor and asked to wear this for 7 days and return the monitor at visit 2.

Visit 1a will take up to a total of approximately 2h30m for Tier 1 participants and 3h for Tier 2 participants. Visit 1a will take up to a total of approximately 2h for Tier 3 participants who will also be asked to return the following day for visit 1b.

Visit 1b – Day 2

Only participants completing tier 3 will be asked to attend visit 1b. Participants will be asked to attend in an overnight fasted state. Usual medicines can be taken with sips of water but no food (or drinks containing sugar, milk or caffeine) should be consumed for 10 hours prior to the visit.

A peripheral venous cannula will be sited in one of the participants arms to allow serial blood sampling over 3 hours during the visit. (5mins insertion time but sampling every 45 minutes over 3 hours).

A thigh muscle biopsy will be performed including procedural consent and provision of post-biopsy care instructions and advice. (60 mins)

The surface HD EMG device will be secured on their thigh as described in section 3.2 above. Participants will then be asked to perform the SPPB, handgrip and quadriceps physical assessments with the surface HD EMG device in place. (45 mins)

Participants will be supplied with top-up tracer drink (D_2O) to consume weekly following the biopsy and may optionally be given a 1-day top-up of D2O at visit 1b. A saliva sample will be obtained before consuming the tracer drink and a second saliva sample will be obtained 2 hours after consuming every D2O tracer drink, volunteers will be asked to refrain from eating or drinking for at least one hour prior to every saliva collection). Top-up D2O drinks and saliva sampling can be performed at home. Samples will be returned to the study team at visits 2 and 3. (5 mins)

Participants will be issued with sample bottles and storage containers to take a spot urine sample between at 54-hours and again at 72-hours after drinking D3-Cr tracer (they will be asked to void the first urine of the day and not collect this – as this represents urine collecting over the previous 8 hours). Arrangements will be made to collect these samples from participant's homes within a few days, if possible, but they should aim to store the urine in the fridge or freezer. (5 mins)

Participants will be given full instructions for activities relating to the COSIAM tracer measurements including a list of timings. Participants will go home with top-up D2O drinks to be taken weekly and a D3-MH drink to be taken the day before visit 2.

Visit 1b will take a total of approximately 4 hours.

3.2.2 Visit 2 – Day 29 ± 7 days

Participants will be asked to attend the NIHR Biomedical Research Centre – Respiratory at the Glenfield Hospital, Leicester in an overnight fasted state. Usual medicines can be taken with sips of water but no food (or drinks containing sugar, milk or caffeine) should be consumed for 10 hours prior to the visit.

Questionnaires will be completed with study team assistance where required (30 mins).

Exploratory blood samples will be collected. (5 mins)

To perform the DXA and BIA assessment participants will be asked to lie or sit reclined if unable to lie flat on the DXA machine. The DXA scan will be performed and immediately followed by the BIA test. (20 mins)

For participants completing tier 2 or tier 3 a thigh muscle biopsy will be performed including procedural consent and provision of post-biopsy care instructions and advice.

For participants completing tier 3 only, a D3-MH tracer drink will be consumed at home the day before the visit. Repeated blood sampling to establish MPB will be repeated as for visit 1. A saliva sample will also be collected immediately prior to the biopsy – to accurately estimate the D2O precursor labelling in the saliva. (60 mins)

The surface HD EMG device will be secured on participant's thigh as described in section 3.2 above. Participants will then be asked to perform the SPPB, handgrip and quadriceps physical assessments with the surface HD EMG device in place. (45 mins)

Participants completing tier 3 will be supplied with top up tracer drinks to consume weekly during the second half of the study. (5 mins)

Participants attending visit 2 will be supplied with a wrist worn physical activity monitor and asked to wear this for 7 days and return the monitor at visit 3.

Participants will go home with top-up D2O drinks to be taken weekly and a D3-MH drink to be taken the day before visit 2.

Visit 2 will take up to a total of approximately 2 hours for tier 1 participants and 4 hours for tier 2 and tier 3 participants.

3.2.3 Visit 3 – Day 57 ± 7 days

Visit 3 will be identical to visit 2 with the addition of the MRI scan which will be scheduled to occur on the same day as the participant is due to attend. Only participants recruited during a hospitalisation for AECOPD will be invited to attend this visit.

Visit 3 will take up to a total of approximately 2h30m for tier 1 participants and 4h30m for tier 2 and tier 3 participants.

4.1 Ethical, regulatory and governance considerations

As a nested sub-study within the CHESTY study, all governance and oversight will follow the processes described in the main CHESTY study protocol and/or as described by relevant University of Leicester non-CTIMP study sponsorship SOPs.

5.1 Financing

Funding for this sub-study will be supported by a grant from the Wellcome LEAP Dynamic Resilience Program.

6.1 Modification History

Amendment/Modification No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
NSM05	V1.1	09-Jul-2026	Dr Hamish McAuley	Clarifications to the protocols: a) That a total maximum of four DXA scans will be performed across both the CHESTY study and the MPES Sub-study 2 (section 3.1)