An evaluation of the use of nutritional biomarkers in MND: a prospective, observational, longitudinal, cohort study

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Nutritional Engineering

"I call for the development of nutritional engineers – to call attention to the need for a person who carries through an enterprise and brings about a result (…) the international nutrition community directs a disproportionately small amount of its research and training to applied nutrition issues." [1,2]

"Malnutrition cannot be reduced to any single causality (…) suffering must be addressed in the short term by the available technical solutions. More work is needed on both the long-term causes of malnutrition and on methods of prophylaxis and treatment for nutritional diseases." [1]

Alan Berg, (1993) pg. 615.

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Abstract

People living with motor neuron disease (MND) frequently struggle to consume an adequate caloric intake. Often compounded by hypermetabolism, this can lead to a dysregulated energy homeostasis and malnutrition. This is negatively associated with functional change, associated with a poorer prognosis and reduced survival. It is therefore important to accurately monitor changes in nutritional state and ensure energy intake is appropriate for individual energy demand. Current clinically-accessible approaches to nutritional assessment are far from optimal, with measurements of percentage weight change from diagnosis recommended in the ESPEN guidelines. This PhD project has involved the set-up, conduct and analysis of a prospective, longitudinal, observational cohort study which aimed to propose a suitable and pragmatic approach for the assessment of nutritional state in people living with MND.

Twenty-four people living with MND were recruited to this study and invited to attend up to four study visits over a nine-month period. Nutritional state was assessed using: i) anthropometric measurements and indices; ii) 24-hour dietary recall; iii) biochemical analytes measured from blood and 24-hour urinary collections; iv) resting energy expenditure using indirect calorimetry. Disease severity was assessed using the ALSFRS-R mapped to the King's College Staging System. Bivariate correlation analyses indicated associations between assessments of nutritional state and disease severity. Repeated measures analyses identified statistically significant longitudinal changes in nutritional state.

Triceps skinfold thickness and arm muscle area were highly correlated with weight and BMI, suggesting these measurements could indicate fat and fat free mass in the clinic. Excreted 24-hour urinary sodium and potassium demonstrated statistically significant associations with body composition, suggesting these analytes may have a role as objective markers of body composition, but this requires further investigation. Resting energy expenditure is highly variable at the individual level and requires constant measurement; clinically-applicable methods of measuring resting energy expenditure need to be developed.

Abbreviations

Abbreviation	Full wording
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale Revisited
BAI	Body Adipose Index
BDA	British Dietetic Association
BMI	Body Mass Index
BPGs	Best Practice Guidelines
CCC	Confirmation of capacity and capability
CNAQ	Council on Nutrition of Appetite Questionnaire
CRP	C-Reactive Protein
DEXA	Dual-Energy X-Ray Absorptiometry
EE	Energy Expenditure
EPIC	European Prospective Investigation into Cancer and Nutrition
ESPEN	European Society for Clinical Nutrition and Metabolism
FFM	Fat Free Mass
FM	Fat Mass
HRA	Health Research Authority
IC	Indirect Calorimetry
IRAS	Integrated Research Application System
LDL	Low-Density Lipoprotein
MND	Motor Neurone Disease
MNDA	Motor Neurone Disease Association
mREE	Measured Resting Energy Expenditure
MUAC	Mid Upper Arm Circumference
MUST	Malnutrition Universal Screening Tool
mTDEE	Measured Total Daily Energy Expenditure
NDNS	National Diet and Nutrition Survey
NIV	Non-invasive ventilation
NPAAS	Nutrition and Physical Activity Assessment
NRI	Nutritional Risk Index
OPEN	Observing Protein and Energy Nutrition
PABA	Para-Aminobenzoic Acid
PBMCs	Peripheral Blood Mononuclear Cells
PBP	Progressive Bulbar Palsy
PEG	Percutaneous Endoscopic Gastrostomy
PEM	Protein-Energy Malnutrition
plwALS	People Living with Amyotrophic Lateral Sclerosis
plwMND	People Living with Motor Neurone Disease

PMA	Progressive Muscular Atrophy
pTDEE	Predicted Total Daily Energy Expenditure
QoL	Quality of Life
QNRI	Geriatric Nutritional Risk Index
REC	Research Ethics Committee
REE	Resting Energy Expenditure
SACN	Scientific Advisory Committee on Nutrition
SIRT1	Sirtuin 1
STH NHS FT	Sheffield Teaching Hospitals NHS Foundation Trust
TDEE	Total Daily Energy Expenditure
TSF	Triceps Skin Fold
UK	Urinary Potassium
UoS	University of Sheffield
UN	Urinary Nitrogen
UNa	Urinary Sodium
ΔREE	Percentage Of Accurate Prediction
24hr	24-hour

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1 Introduction to the Thesis

1.1 Thesis overview

Motor neuron disease (MND) encompasses an incurable heterogeneous group of progressive neurodegenerative motor syndromes involving the gradual degeneration and ultimate death of motor neurons. This leads to the weakness and wasting of muscles controlling movement, speech and breathing, resulting in death typically from respiratory failure approximately two-to-three years post diagnosis. Symptoms such as dysphagia and a decreased dexterity secondary to muscle weakness contribute to a sub-optimal caloric intake, which may lead to malnutrition and accelerated weight loss. The presence of hypermetabolism, i.e., the state of an increased resting energy expenditure, can result in a dysregulated energy homeostasis and thus exacerbate the nutritional challenges for people living with MND. Individuals with the greatest energy imbalance exhibit a faster rate of functional decline and shorter survival.

Given the negative association between malnutrition and prognosis in MND, nutritional management may be pivotal in the multidisciplinary care of people living with MND. However, this is complicated and multifaceted. Despite indications to suggest that the maintenance of a healthy nutritional state will prolong survival, the regular assessment of nutritional state is frequently overlooked in the palliative care of people living with MND. In clinical practice, nutritional assessment is most commonly conducted by observing changes in anthropometric measurements and related indices (e.g., weight and body mass index); however, this approach is limited and fundamentally unreliable in MND.

1.2 Aims and objectives

The aim of this PhD was to propose a suitable and pragmatic approach for the assessment of nutritional state in people living with MND. These methods need to be applicable in the clinic to enable the continuous monitoring of nutritional state in people living with MND from the point of diagnosis. To do this, the following objectives were conducted:

1. Identify the current challenges with nutritional assessment in MND;

2. Identify a suite of techniques (i.e., 'a nutritional toolkit') that can be used to deeply phenotype the nutritional status of people living with MND;
3. Investigate and demonstrate the role of these techniques to assess the nutritional status of a cohort of people living with MND;
4. Assess the relevance of these techniques to assess the severity and progression of MND;
5. Develop a framework for determining the most suitable tool, or combination of tools to assess nutritional status in MND.

A multipronged approach was adopted to deeply phenotype the nutritional state of a heterogeneous cohort of people living with MND in relation to disease severity and progression. This is shown schematically in Figure 1.1. Assessments of body composition, self-reported 24-hour dietary intake, resting energy expenditure and biochemical analytes measured from the blood and 24-hour urinary collections were collected from 24 participants living with MND. Participants were invited to attend up to four study visits over a nine-month period. Assessments of nutritional state were correlated against assessments of disease severity, using a self-administered ALSFRS-R questionnaire mapped to the King's College Staging System.

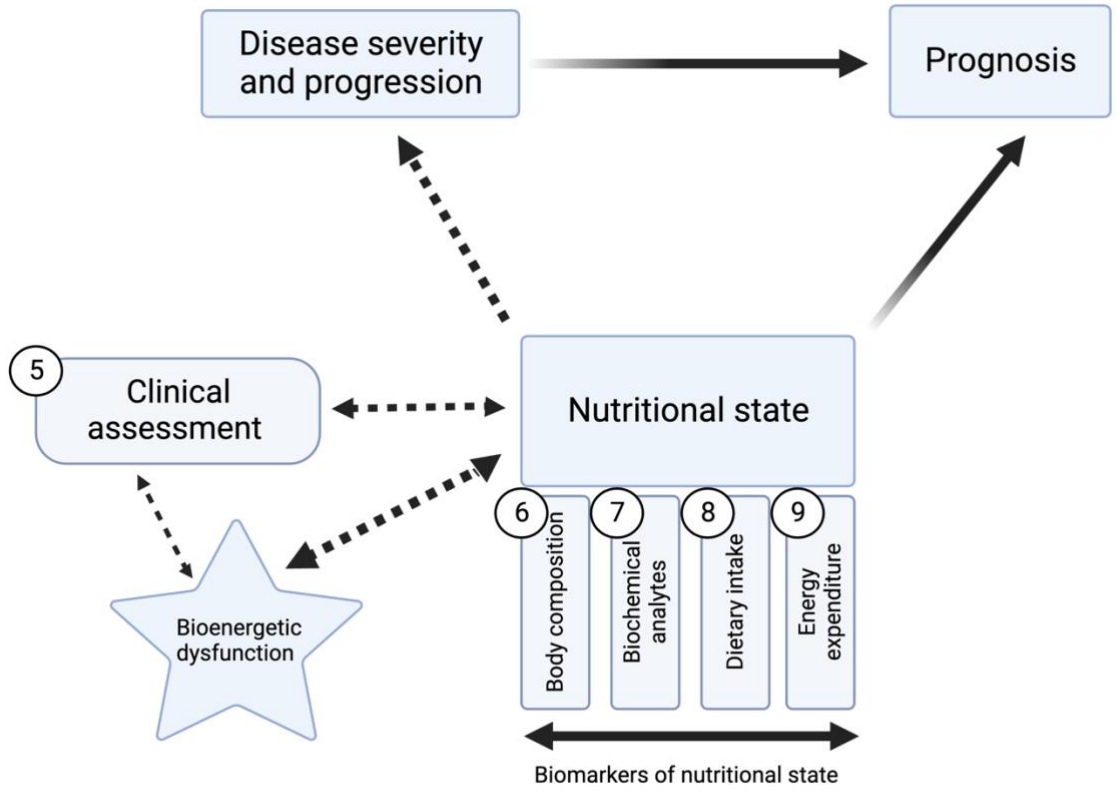


Figure 1.1 A schematic to demonstrate the multipronged approach to the nutritional assessment of a cohort of individuals living with MND. The circled numbers indicate the results chapters that investigate and demonstrate the role of each of these techniques to assess the nutritional status of a cohort of people living with MND. Created with BioRender.com.

1.3 Research hypothesis

The hypothesis for this PhD was that the biochemical analytes measured from the blood and 24-hour urinary collections of participants **without** a pro-inflammatory or hypermetabolic state would provide an accurate assessment of the nutritional state. It was expected that serum biochemical markers of nutritional status would be affected by physiological characteristics of MND, such as metabolism, muscle catabolism and inflammation, whilst biomarkers of dietary exposure would provide accurate measures of dietary intake, prior to a decline in nutritional state and subsequent weight loss.

For these results to have a meaningful clinical benefit, it was hoped that an observed decrease in the concentration of a specific or collection of biochemical analyte(s) would highlight a decline in specific or generalised nutrient intake. This would indicate the need for a personalised nutritional intervention to rebalance the nutritional state, *prior* to the onset of irreversible malnutrition and weight loss (Figure 1.2).

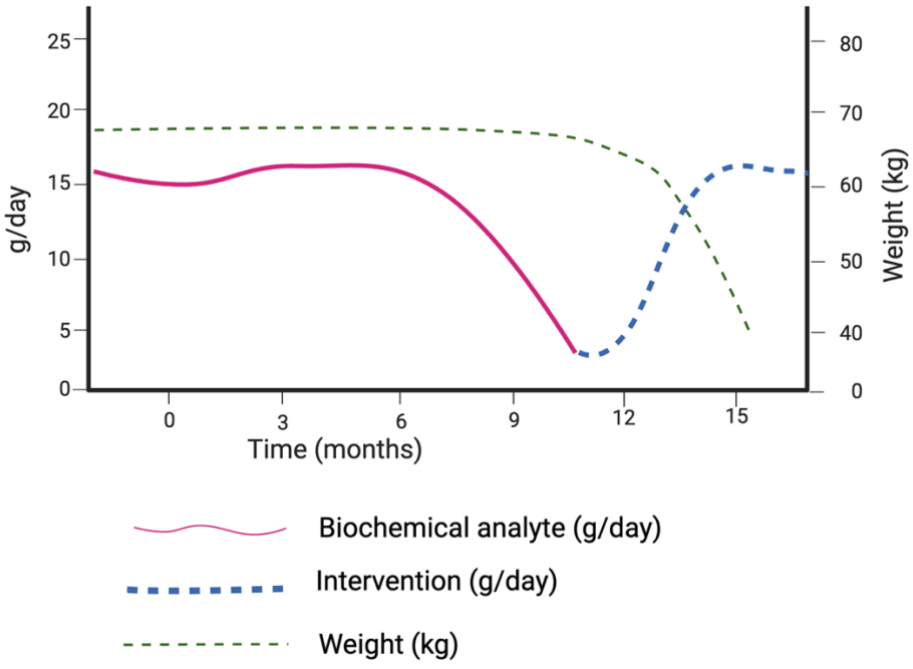


Figure 1.2 A schematic of the study hypothesis. An example of biochemical analyte concentration (grams/day) is depicted in red. A steep decline between six and nine months demonstrates an expected decline in biochemical analyte concentration. The decline in biochemical analyte concentration would indicate the need for personalised nutritional

intervention. A successful intervention is indicated by the blue dashed line to biochemical concentration levels to 'normal'. Weight (kilograms) is depicted by the dashed green line. Created with BioRender.com.

1.4 Thesis structure

This is a hybrid-style thesis predominantly presented in monograph format alongside two manuscripts written for publication **(sections 3 and 9.3).** Contributions from the thesis author to these manuscripts is acknowledged at the beginning of both of these sections.

Chapter Two describes the clinical features of MND, and focuses on objective one of this PhD project: to identify the current challenges associated with nutritional assessment in MND. This chapter presents the current literature for the assessment of nutritional state in MND, including consideration for the complexities when conducting nutritional assessment in this cohort. **Chapter Three** is an extension of Chapter Two, focussing specifically on the conduction of a scoping review to map the evidence available for the assessment of energy expenditure in MND.

Chapter Four outlines the materials and methods used in this study to achieve the second objective of this PhD study: to identify a suite of techniques, or a 'nutritional toolkit', that can be used to deeply phenotype the nutritional status of people living with MND. This chapter briefly introduces the preliminary feasibility study that was conducted ahead of the MND cohort study using samples donated from healthy participants (**section 4.1**). This study was conducted to enable protocol development and establish proof of concept. Supporting documents for this study can be found in **Appendix A-C**. Chapter Four also presents the clinical set up for the conduction of this observational PhD study, including development of the study protocol and participant-facing documents, public and patient involvement and ethical approval (**section 4.2**). Supporting documents can be found in **Appendix D-H**. Finally, Chapter Four describes the study assessments that were conducted at each study visit to enable the conduction of a multi-pronged assessment of the nutritional state of this MND cohort (**section 4.3**).

Chapter Five presents and describes participant recruitment and engagement (**section 5.1**), as well as the demographic and clinical parameters of this cohort (e.g., sex, age, MND phenotype, disease duration and disease severity) (**section 5.2**). **Section 5.3** discusses the characteristics of this cohort in the context of the wider MND population. **Chapters Six, Seven, Eight and Nine** investigate and demonstrate the role of the techniques described in Chapter Four to assess the nutritional status of this MND cohort. **Chapter Six** presents the assessment of body composition of this cohort; **Chapter Seven** presents the measurement of biochemical analytes collected blood samples and excreted 24-hour urinary collections; **Chapter Eight** presents the assessment of nutritional intake data from an online 24-hour dietary recall questionnaire; and **Chapter Nine** presents the assessment of energy expenditure in relation to the identification of hypermetabolism. Chapters 5-9 are structured in this order to: i) examine the interplay between the different assessments of nutritional state; and ii) assess the relevance of these techniques to assess the severity and progression of MND (objective four). The structure of this thesis is outlined in Figure 1.3.

Chapter Ten draws on the key outcomes of each results chapter in relation to the current literature. This chapter discusses the considerations for this research and proposes the potential clinical and research applications of these findings in order to propose a framework for determining the most suitable tool, or combination of tools to assess nutritional status in MND (objective five). Future directions for this research are outlined in **Chapter Eleven**, which briefly describes additional preliminary research conducted in the final stages of this PhD study to set up for a subsequent prospective case-control longitudinal study.

Figure 1.3 A schematic to demonstrate the thesis structure and interactions between the results chapters (Chapters 5-9), General Discussion (Chapter 10), and Taking This Work Forward (Chapter 11). Created with BioRender.com.

2 Background and Rationale

2.1 Motor neuron disease

First described in 1850 [3], motor neuron disease (MND) encompasses a heterogeneous group of progressive neurodegenerative motor syndromes. The UK prevalence of MND is 5.69 per 100,000 person years [4], with risk known to vary depending on sex, age and a family history of MND. MND risk is greater in males of all ages [5,6], with a sex ratio of 1.5:1 [4]. Symptom onset is rare below 40 years of age, with younger onset most often observed in men and hereditary MND [5], with incidence peaking at 70-80 years of age [7].

It is widely considered that MND is a syndrome as opposed to a single disease, and the exact causes of MND remain unconfirmed [8]. Whilst approximately 90% of MND diagnoses have no known hereditary link, and are thus termed sporadic, the remaining ten percent are attributed to more than 25 inherited genetic mutations [9] and therefore termed familial. The most common inherited genetic mutations are located in the *C9orf72, SOD1, TARDBP (TDP-43)* and *FUS* genes [10]. It is thought that the onset of MND is multifactorial in nature, with evidence to suggest that neuroinflammation, mitochondrial dysfunction [11], RNA metabolism [12], glutamate excitotoxicity [13], oxidative stress [14] and protein aggregation [15] all play causative roles, as reviewed by Bonafede et al., (2017) [16].

2.1.1 MND symptoms

MND involves the progressive degeneration and ultimate death of upper (corticospinal and corticobulbar) and lower (spinal and bulbar) motor neurons. This leads to weakness, fasciculation and wasting of the muscles controlling movement, speech and breathing [17]. The loss of upper and lower motor neurons can originate in any motor region and typically presents asymmetrically at symptom onset followed by involvement of additional regions as the disease progresses. Limb onset is the most common site of origin, accounting for 75% of cases [18]. Symptom onset in the upper limbs typically involves unilateral distal weakness, causing a decreased dexterity and a tendency to drop items; wasting and thinning of the thenar eminence and first dorsal interosseous muscle is common in these individuals [19]. Lower limb symptoms include foot drop, an increased likelihood of falling and excessive fatigue

when walking [19]. Twenty percent of people living with MND, and more commonly women, present with bulbar symptoms of dysarthria and dysphonia, with dysphagia more likely to follow later in the disease [5]. Patients with respiratory onset (5% of cases [19]) often experience shortness of breath when sitting or with minimal exertion, known as dyspnoea, or when supine (orthopnoea) which can cause overnight hypoventilation and headaches upon waking. Non-motor pathways controlling behavioural and personality changes can also be affected. It is estimated that up to 50% of patients develop mild cognitive changes, whilst < 15% develop frontotemporal dementia (FTD), known as MND-FTD [20,21].

2.1.2 Diagnosis

Diagnosis frequently occurs after the exclusion of alternative conditions, such as cervical myeloradiculopathy. A clinical examination of the upper and lower motor neuron signs is supported by electromyography [22]. Upper motor neuron signs may include brisk reflexes and spasticity. Lower motor signs include muscle wasting, fasciculations and reduced or absent reflexes [19]. Clinical heterogeneity and the absence of a definite diagnostic test cause diagnostic delays of an average of 10-16 months from symptom onset [23]. A longer diagnostic delay has been associated with a slower progression to respiratory involvement [24].

2.1.3 MND phenotypes

People living with MND can be phenotypically grouped according to site of onset and motor neuron involvement. Amyotrophic lateral sclerosis (ALS), the most common MND phenotype, comprises 65-85% of MND cases [25] and involves a combination of upper and lower motor neurons. Progressive muscular atrophy (PMA) involves the progressive loss of lower motor neurons. There are variations within the PMA phenotype, and there is debate surrounding the classification of PMA as a sub-type of ALS; people diagnosed with PMA tend to live longer than those with ALS, however the risk factors for a shorter survival were found to be the same as those for ALS [26]. The involvement of only lower motor neurons is referred to as the flail-arm variant, which is most common in men [27]. By contrast, involvement of only the upper motor neurons is called primary lateral sclerosis (PLS) [28].

2.1.4 Prognosis

MND is incurable. In most cases, MND results in the death of patients through respiratory failure approximately two-to-three years post diagnosis [29,30]. However, individual prognosis varies depending on age, site of onset and MND phenotype [29]. Symptom onset earlier in life or diagnosis with flail-arm or PLS variants are associated with a better prognosis, whilst bulbar onset is more commonly linked with a worse prognosis.

2.1.5 Treatment

Riluzole, the only effective drug treatment for MND, has been found to extend survival by approximately three months [31,32]. However, symptomatic treatment and palliative care has been found to have a better effect on survival. For example, non-invasive ventilation (NIV) has been found to prolong survival in people living with MND by up to seven months [33], whilst multidisciplinary care extends survival by 7.5 months when compared to patients attending general neurological clinics [34–36]. Perhaps one of the most controversial arguments in the care of people living with MND is nutritional intervention, specifically the timing and application of gastrostomy [37]. It was previously recommended that gastrostomy insertion should occur upon the development of bulbar symptoms, (i.e., when the patient is experiencing dysphagia and/or dehydration) and a trend towards weight loss, but no later than when ≥ 10% weight loss from diagnosis or ≤ 50% of predicted functional vital capacity is exceeded [38–41]. Results from the ProGas study - which aimed to compare gastrostomy insertion approaches in relation to safety and clinical outcomes - suggested that earlier gastrostomy insertion at 5% weight loss could be of greater clinical benefit to the patient [42]. Whilst patients report palliative benefits to early gastrostomy insertion and utilisation (e.g., a reduced anxiety of choking), the current evidence that gastrostomy insertion improves survival or nutritional state is unconvincing, and further dedicated research is required [43].

2.2 Nutritional challenges in MND

The following literature focuses on the first objective of this thesis: to identify the current challenges associated with the nutritional assessment of people living with MND.

2.2.1 Malnutrition

Nutritional status is defined as the condition of an individual's health, in relation to the intake and utilisation of nutrients, i.e., the extent to which the metabolic demands for energy and nutrients are met [44]. A decline in nutritional state can lead to irreversible protein-energy malnutrition, which is accompanied by a significant and irreversible loss of fat mass (FM) [45]. Malnutrition, described as the disturbance of equilibrium between protein balance, physiological requirement levels, and a change in the availability or use of nutrients [46–48] is associated with a 3.5-fold increased risk of death in MND [49]. Malnutrition is estimated to affect between 16 and 55% of people living with MND [49,50], however the exact prevalence is difficult to determine due to the array of nutritional indices and thresholds utilised [51]. For example, malnutrition has been indicated in MND cohorts using a percentage weight loss of ≥ 5-10% from usual body weight before the onset of MND symptoms [52,53], as well as BMI thresholds of < 18.5 kg/m^2 [49] or < 20 kg/m^2 [54].

The reasons for malnutrition in MND are many, but are most commonly due to the presence of dysphagia and mastication weakness, with up to 30% of people living with MND reported to present with a reduced ability to swallow at diagnosis [55]. A sub-optimal caloric intake has been reported in 70-94% of people living with MND [50,56]. In addition, symptoms secondary to muscle weakness, such as a reduced mobility and/or dexterity, may cause difficulties in shopping, chopping, cooking and bringing food to mouth [57,58]. This becomes particularly prominent in individuals without appropriate care and support. Psychologically, a reduced appetite [59], an increased risk of choking, and embarrassment anxiety about eating in public can cause negative-associations with feeding, leading to food avoidance and even anorexia in extreme situations [60].

2.2.2 Hypermetabolism

Malnutrition can be further compounded by an increased energy demand - demonstrated by a higher-than-predicted resting energy expenditure (REE) for age, weight and gender - known as 'hypermetabolism' [61,62]. Approximately 50-68% of people living with sporadic MND are reported to experience hypermetabolism [61–65]. Hypermetabolism is associated with an increased catabolism of carbohydrate, lipids and proteins [66], as well as an increase in excreted urinary nitrogen, leading to a negative nitrogen balance [67]. Reduced body energy stores in the form of malnutrition-associated loss of fat mass [68] and an increase in resting energy expenditure [69] can therefore result in a dysregulated energy homeostasis and thus exacerbate the nutritional challenges for people living with MND [44]. Individuals with the greatest energy imbalance exhibit a faster rate of functional decline and shorter survival [70–75].

2.2.3 "Nutritional risk"

Nutrition risk refers to the possibility of an individual experiencing an adverse nutritional state. For example, in the context of an individual estimated to be consuming an inadequate energy intake respective to their predicted total energy demand, this highlights the risk of the individual entering into a negative energy balance and developing malnutrition if no intervention is provided. This will be referred to as a 'risk of malnutrition' in this thesis. A single measurement to indicate nutritional risk does not, however, conclusively identify individuals who are malnourished.

2.3 Nutritional Management in MND

Given the negative association between malnutrition and prognosis in MND [39,76], nutritional management may be pivotal in the multidisciplinary care of people living with MND. However, there is an absence of a universal, mandatory approach to screening and assessing nutritional state in MND clinical care. Whilst guidelines for nutritional screening and assessment exist for general practice, these are not specific or validated in MND [40,41,77–81]. Moreover, guidelines for the clinical management of MND include brief, generalised reference to the importance of nutritional assessment [40,41]. The ESPEN guideline for clinical nutrition in neurology is the most targeted guideline, containing 22 recommendations for nutritional therapy in ALS [60]. In

the UK, the British Dietetic Association (BDA) 'Model and Process' cycle is recommended [82]. This process broadly includes: 1) nutritional screening, 2) continuous assessment and 3) development and implementation of personalised nutritional interventions (Figure 2.1).

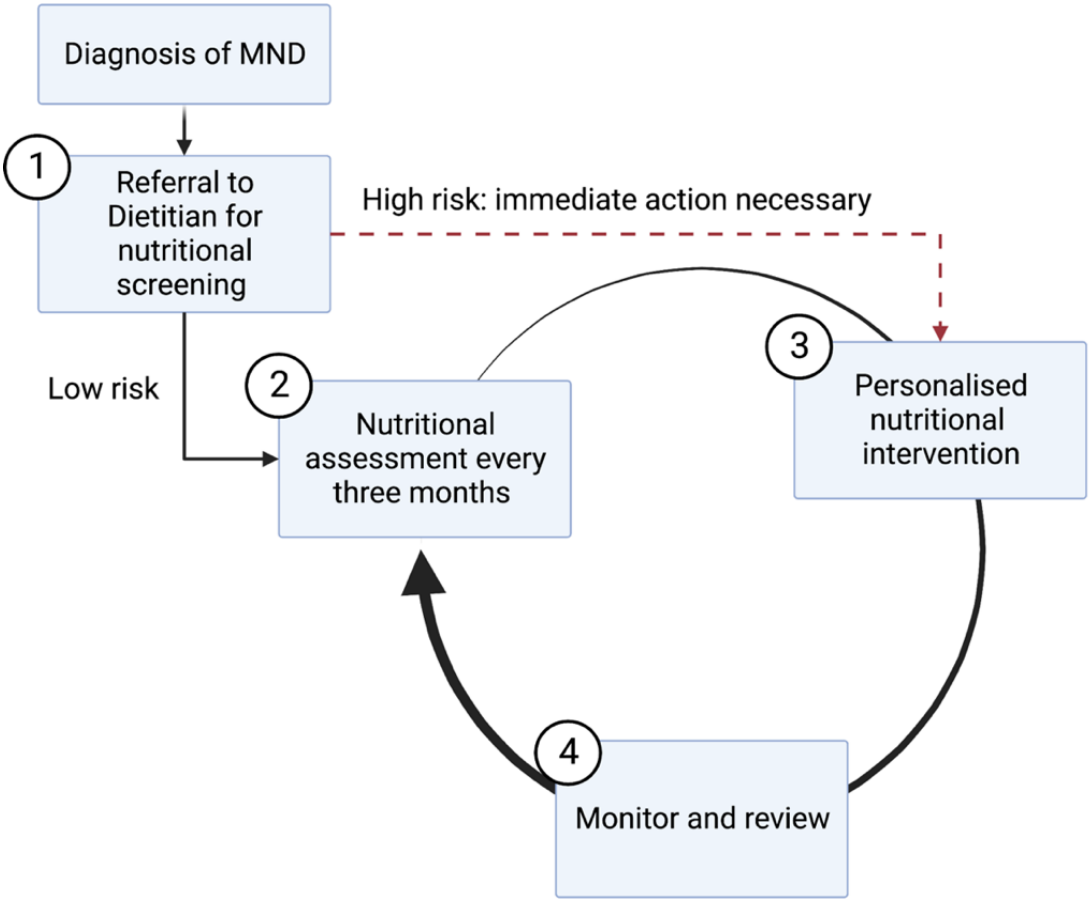


Figure 2.1 Cyclical process of nutritional screening and assessment. Created with BioRender.com.

Nutritional screening assesses individuals for their risk of an inadequate nutritional intake. The results of this screen will indicate the necessary next steps for targeted nutritional therapy [83], with the goal to prevent the onset of (or counteract) malnutrition in order to increase or maintain quality of life and extend survival. The average delay between diagnosis and nutritional screening in MND is five months [83]. Referral to a dietitian at diagnosis is paramount to ensure nutritional screening and continuous assessment occurs. Whilst this should be standard procedure, a UK survey of dietitians reported that nutritional screening occurs in only 42% of organisations in the UK; with 44% of referrals being made 'too late' to be of patient benefit [84].

Nutritional screening tools developed across a variety of health and disease-related conditions have been adopted in MND clinical care to conduct nutritional screening in people living with MND. Examples include the Malnutrition Universal Screening Tool (MUST) [85], the Nutritional Risk Index (NRI) [86] and the Council on Nutrition of Appetite Questionnaire (CNAQ) [87].

Malnutrition Universal Screening Tool (MUST)

The MUST is a five-step screening method incorporating BMI and recent weight loss to identify adults who are most at risk of malnutrition across all care settings [85] . When used to screen a cohort of 43 people living with MND for their risk of malnutrition after diagnosis, 91% of study participants were deemed 'high risk' demonstrating the importance of nutritional screening in MND care.

Nutritional Risk Index (NRI)

The NRI is a simple, objective screening tool to estimate the risk of nutrition-related morbidity. It is calculated from serum albumin levels and the percentage change from premorbid body weight [86]. The NRI has been shown to predict malnutrition more reliably when compared against the MUST [88,89]. The geriatric nutritional risk index (GNRI) has been modified from the NRI to overcome difficulties encountered when weighing elderly individuals, using ideal body weight calculated using the Lorentz formula instead of percentage body weight change [90]. The GNRI has previously been used to assess nutritional state in MND due to the similarities observed for chewing, swallowing and weighing an elderly

population [91]. The GNRI could therefore be used for nutritional screening of people living with MND.

Council on Nutrition of Appetite Questionnaire (CNAQ)

The CNAQ was designed to assess appetite and predict weight loss in general medicine [87]. Whilst not originally designed for use in MND, the CNAQ has previously been used to assess the relationship between a severe loss of appetite (indicated by a score of less than 28/40) and weight loss, disease progression, respiration and energy intake in three cohorts of people living with MND. Loss of appetite was first monitored in 51 people living with ALS over a six-month period [92]. 65% of participants presented a severe loss of appetite who recorded an average weight loss of > 5% over the same time period. A second, independent study monitored appetite and weight change at three time points over three-to-four monthly intervals in 62 people living with ALS [59]. Twenty-nine percent of these participants reported a loss of appetite which was associated with a greater loss of weight and fat mass. Appetite score was found to worsen with disease progression, assessed with the amyotrophic lateral sclerosis functional rating scale (ALSFRS-R). A third study of 61 people living with ALS demonstrated a severe loss of appetite in 18% of participants. This negatively correlated with a weight loss from diagnosis and was significantly associated with a reduced energy intake, independent of dysphagia [93].

After identification of individuals most at risk of malnutrition at diagnosis, nutritional state should be reassessed every three months [77]. As defined by the BDA, nutritional assessment is the "systematic process of collecting and interpreting information in order to make decisions about the nature and cause of nutrition related health issues that affect an individual" [48]. The regular assessment of nutritional state should therefore theoretically identify an insufficiency of: a) dietary intake or b) dysregulation of nutrient metabolism, to prevent the onset of malnutrition [94]. However, the conduction of nutritional assessment in the care of people living with MND is complicated and multifaceted.

In the absence of a standardised protocol to monitor and intervene in the nutritional care of people living with MND, a comprehensive approach must be taken based on availability of tools to the clinician, as well as suitability to the individual living with MND [95]. Defined by

Potischman in 2003, “A nutritional biomarker can be any biological specimen that is an indicator of nutritional status with respect to intake or metabolism of dietary constituents. It can be a biochemical, functional or clinical index of status of an essential nutrient or other dietary constituent” [96]. Table 2.1 presents examples of nutritional biomarkers suggested by the BDA that could be used clinically to understand the nutritional state of people living with MND [48,82]. The following literature frames previous application of these nutritional biomarkers in assessing the nutritional state of individuals living with MND, with particular reference to any known limitations that should be considered.

Table 2.1 Examples of nutritional biomarkers.

Nutritional biomarkers	Examples
Anthropometric measurements and indices for the assessment of body composition (i.e., fat and fat-free mass)	**Measurements:** Weight, height, skinfold thickness and body circumferences (e.g., mid upper arm circumference, hip and waist). **Indices:** Body mass index (BMI), body adipose index (BAI). **Technology:** Bioimpedance analysis, dual-energy X-ray absorptiometry (DEXA), whole body air-displacement plethysmography.
Biochemical analytes	Serum proteins, micronutrients, inflammatory and metabolic parameters.
Clinical assessment	Physical appearance, medication, blood pressure, functional vital capacity, amyotrophic lateral sclerosis functional rating scale (revised) (ALSFRS-R), handgrip strength and sit to stand timings.
Dietary assessment	Food frequency questionnaires, 24hr recall, weighed food diaries, dietary reference values, energy-fluid requirement calculations.
Social and physical activities of daily living	Cooking, shopping, eating, cleaning, and working.

2.4 Nutritional assessment in MND

2.4.1 Use of anthropometric measurements and indices to assess body composition

Anthropometric measurements and indices can be used as rapid, accessible and cheap methods to monitor body composition in a clinical setting. A combination of two or more anthropometric measurements or indices is considered to indicate the nutritional state of an individual [97]. Regular weight measurements are currently the most common method to assess nutritional state in people living with MND in clinical practice. A weight loss of 5-10% from before the onset of the first MND symptoms is currently used to indicate malnutrition [53] and is associated with a shorter disease duration [98]. However, from a practical perspective, weight measurements are not necessarily easy to obtain from patients who have poor mobility secondary to MND. For example, during the ProGas clinical trial – a national study involving 24 MND care centres investigating the safety and clinical outcomes of gastrostomy insertion of 330 people living with MND - valid weight measurements were able to be recorded for only 53% of patients [99]. The authors stated this was in part due to participant attrition, but also the impracticality of weighing wheelchair-bound individuals [99]. A hoist can be used if patients are unable to transfer onto weighing scales, though this is not always accessible or feasible.

Furthermore, weight loss in people living with MND is not indicative of body composition changes experienced throughout disease progression. Loss of muscle mass secondary to denervation and subsequent muscular atrophy may be initially masked by an increase in fat mass associated with an increased sedentary lifestyle due to decreased mobility [100]. Malnutrition-associated fat loss may occur later in the disease progression, as well as loss of fluid from dehydration. In addition, due to the heterogeneous nature of MND, muscle and fat mass may be lost asymmetrically within each individual [101]. Weight measurements therefore do not provide adequate detail regarding body composition and may have limited value in accurately monitoring nutritional changes in people living with MND.

2.4.1.1 Body Mass Index

Weight measurements carry little value without an appreciation of the height and body composition of the individual concerned. Weight and height measurements enable calculation of body mass index (BMI) [102]. BMI is an 'index of obesity' categorised into four groups by the Word Health Organisation (WHO) in 2016: underweight (< 18.5 kg/m^2), normal (≥ 18.5-24.9 kg/m^2), overweight (≥ 25-29.9 kg/m^2) or obese (≥ 30 kg/m^2). However, the term 'obese' implies a measure of adiposity, or fat mass. As with weight measurements, BMI does not enable differential identification of fat from muscle mass, or the anatomical distribution of fat mass [102]. The isolated use of BMI to monitor nutritional status is known to be unsuitable due to the reliance on accurate weight and height measurements, not to mention considerations for sex, age and ethnicity [103,104].

A lower BMI is thought to be associated with an increased risk for MND [105,106], whilst a greater reduction rate in BMI from premorbid is associated with a shorter survival in MND [107,108]. When considering the relationship between BMI and MND clinical outcomes, it is important to distinguish between the relationship of BMI against disease *severity* (i.e., the ALSFRS-R total score) to assess disability, and between BMI and disease *progression* (i.e., the change in ALSFRS-R score from either symptom onset or baseline measurements). Results can be misleading if this distinction is not made clear. For example, in 2015, Park et al., demonstrated a positive relationship between BMI and disease severity using the ALSFRS-R, with a significant reduction in body weight and BMI observed in participants in the lowest ALSFRS-R tertile [91]. This suggested that a lower BMI is associated with worse functionality and increased disability. However, when the relationship between BMI and disease progression was analysed by Reich-Slotky et al., (2013), a non-linear relationship was observed between BMI and ALSFSR-R [109]. The authors demonstrated that in individuals with a BMI of < 30 kg/m^2, a higher BMI was associated with a slower ALSFRS-R decline, whilst in individuals with a BMI > 30 kg/m^2, a higher BMI was associated with a faster decline.

In acknowledgement of the non-specific criterion for diagnosing malnutrition in general medicine (as described in section 2.2.1), the European Society of Clinical Nutrition and Metabolism (ESPEN) group conducted a modified Delphi process (i.e., an iterative process to integrate expert opinions) to develop a diagnostic criterion for malnutrition [110]. The ESPEN

group recommended that malnutrition should be indicated using a BMI of < 20 kg/m^2 in conjunction with unintended weight loss of 5-10% [110]. This allows for changes in body composition experienced in highly-catabolic diseases (such as MND) to be detected, when patients may lose up to 10% of their body weight but remain within the 'normal' BMI ranges.

2.4.1.2 Skinfold thicknesses and body circumferences

Skinfold thicknesses (e.g., triceps skinfold (TSF)) can be used in clinical practice as proxy estimates of subcutaneous fat mass (FM) distribution [111]. TSF is measured at the mid-point between the acromion and olecranon processes and is an index of subcutaneous fat mass [111]. Body circumference measurements (e.g., mid-upper arm circumference (MUAC) and calf circumference) encompass measurements of the bone, muscle and subcutaneous fat. MUAC percentiles have been outlined for males and females ≥ 50 years of age (Table 2.2) [112]. This data was generated in the third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) [113]. The suitability of MUAC measurements as surrogate indicators of BMI has previously been demonstrated in hospitalised populations [114,115]. A MUAC of < 23.5 cm for both men and women is typically indicative of a BMI < 20 kg/m^2, and indicates a risk of malnutrition [116–118].

Table 2.2 Mid-upper arm circumference measures of central tendency for men and women over 50 years of age. Data was examined in the third National Health and Nutrition Examination Survey [113] and presented in Kuczmarski et al., (2000) [112].

	Mean	Median
Male		
50-59	33.7	33.7
60-69	32.8	32.7
70-79	31.5	31.3
80+	29.5	29.5
Female		
50-59	32.5	32.0
60-69	31.7	31.2
70-79	30.5	30.1
80+	28.5	28.4

TSF and MUAC can be used together to calculate arm muscle area (AMA), a proxy for upper arm lean body mass, or fat free mass (FFM) [116,119]. The use of arm anthropometry has previously been used to assess nutritional state in cohorts of individuals living with MND. In 1997, Kasarskis et al., evaluated the feasibility of using arm anthropometry to assess the clinical status of 18 people living with ALS [120]. Changes in AMA were found to significantly correlate with body mass changes over a six-month period. The authors concluded that AMA could be used as a simple, inexpensive method to monitor muscular atrophy in ALS.

Salvioni et al., (2015) examined the relationships between arm anthropometry with clinical, nutritional, respiratory and functional parameters of 111 people living with ALS [121]. In this study, TSF was found to positively correlate with bulbar and limb functions, whilst AMA correlated with only limb function. Furthermore, a decline in function, assessed using the ALSFRS positively correlated with AMA, and negatively with TSF. The delay between referral and nutritional assessment was significantly correlated with AMA, indicating that the longer the delay before nutritional intervention, the greater the loss of muscle mass.

As these anthropometric measurements do not directly measure FM or FFM, these measurements are perhaps more accurately considered indirect predictors of body composition. Anthropometric indices are inherently based on underlying theoretical assumptions linked to the characteristics (i.e., body composition in relation to age and gender) of the individuals from which they were derived [122]; the accuracy of these indices is therefore population-dependent. The accuracy, and reliability, of anthropometric measurements and indices in a cohort experiencing lower-than-predicted FFM, such as in MND, is therefore reduced, as demonstrated by Ioannides et al., 2017 [123].

Reliable anthropometric measurements assume that the point of measurement is representative of whole-body composition and symmetry of limb muscles. However, the asymmetric body composition of people living with MND constantly changes throughout disease progression, which is complex and difficult to monitor. A single TSF or circumference measurement is therefore insufficient to detail these changes. From a quality control perspective, TSF measurements can be heavily influenced by gross- (inappropriate measurement location or calliper use), observational- (observer bias) or instrumental- (absence or inappropriate calibration) errors [124]. Moreover, TSF measurements may be

practically difficult to obtain in individuals living with MND, due to reduced upper limb mobility. TSF can also be erroneous due to inadequate hydration, which decreases skinfold TSF measurements [125,126]. For these reasons, the use of arm anthropometry may not be a reliable or accurate method to assess body composition in people living with MND.

Measurements of calf circumference have also been demonstrated to indicate nutritional state through positive correlations with BMI in a variety of study populations, e.g., 170 elderly (> 60 years of age) inpatients [127], as well as 2000 Iranian women aged between 15-49 [128]. The use of calf circumference measurements to estimate muscle mass has been recommended by the European Working Group on Sarcopenia in Older People [129]. A calf circumference value of < 31 cm, determined by the WHO expert committee [130] has been demonstrated to clinically indicate the presence of sarcopenia, as well as predict physical performance and survival in older individuals [131]. This may be transferable to MND, however, there is no evidence to show prior assessment of nutritional state using measurements of calf circumference in MND.

2.4.2 Use of advanced technology to assess body composition

2.4.2.1 DEXA

DEXA uses two low-doses of radiation to measures whole body composition, including bone, fat and skeletal mass whilst in a supine position [132]. DEXA has previously been demonstrated to estimate FM and FFM with an inter-study variation of < 5% (where quality control and user-technique is conducted appropriately) [133]. However, the use of DEXA as a standardised approach to monitor body composition in MND is limited due to its expense and impracticalities for use in an MND cohort due to supine-associated respiratory complications. Nevertheless, DEXA has previously been used to assess body composition in MND [68,134–137]. These studies are presented in **Chapter 3, section 3.4**.

2.4.2.2 Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) is a rapid, cheap and non-invasive method to assess body composition. BIA uses a small electrical current (50 or 100 kHz) through localised areas of the body to determine conductivity [138]. Muscle, fat and bone all have differing water contents and therefore, different impedance (i.e., the resistance of the electrical current) [139].

As FM (muscle and body water) has a high water-content, impedance is lower and electricity flows quicker; in contrast, bone and FM have a low water content, causing a higher impedance and slower electrical current.

Total body water is assumed to comprise 73% of FFM. FM is then calculated as the difference between FFM and total body weight. BIA assumes human anatomy to be cylindrical with minimal-to-no intra-individual variation in height or width. As a result, BIA is known to overestimate FFM, in relation to FFM estimated using DEXA[140,141]. As of 2020, 25 equation models have been developed to correct for the estimation of FFM from BIA measurements [142].

The suitability of BIA to assess body composition in MND was evaluated by Desport and colleagues in 2003 [143]. FFM estimated using BIA (BIA-FFM) at frequencies of 50 and 100 kHz were compared against FFM estimates using DEXA (DEXA-FFM) and skinfold-thickness measurements in a cross-sectional study involving 47 people living with MND. The authors found significant differences between the estimations of BIA-FFM using three predictive equations (namely, the Segal, Zillikens and Deurenberg equations) and DEXA-FFM in 87.5% of their study cohort. They therefore determined the use of BIA in this manner clinically unacceptable for estimating FFM in MND and developed an MND-specific predictive equation to estimate FFM following BIA impedance measurements. This equation incorporated variables of weight, height squared and TSF at a frequency of 50 kHz, and has subsequently been used to measure body composition in further cohorts of MND [144–147]. More information about these studies is presented in **Chapter 3**.

2.4.2.2.1 Air displacement plethysmography

Whole body air displacement plethysmography (ADP) indirectly measures the volume of an individual by measuring the volume of air displaced within an enclosed chamber, known as a plethysmograph. ADP is less invasive than DEXA and is conducted in a seated position, rather than supine. This makes it more suitable for individuals who cannot recline, but individuals still need manoeuvre into the plethysmograph, involving a step. An example is the Cosmed BodPod ® system, which uses whole-body densitometry [148]. The BodPod has been proven to

be a robust, reliable, reproducible (coefficient of variation: 0.15%) method for assessing body composition in a large heterogenous sample of 980 adults in duplicate [149].

2.4.3 Biochemical analytes

Biochemical analytes measured from whole blood products (plasma, serum and peripheral blood mononuclear cells (PBMCs)), or excretion samples (urine and faeces) [150,151] reflect the short-term dietary intake or metabolism of macro- and micro-nutrients [152]. Two main classifications of biochemical analytes have therefore been broadly defined in the literature: 'biomarkers of nutritional status' (**section 2.4.3.1**) and 'biomarkers of dietary exposure' (**section 2.4.3.2**) [153].

2.4.3.1 Biomarkers of nutritional status

Biomarkers of nutritional status provide a representative measure of nutritional state which encompasses the symbiotic relationship between nutrition and metabolism; reflecting not only dietary intake, but also the integrated absorption and processing of nutrients [153,154]. These biomarkers can be heavily influenced by physiology and disease [153]. Therefore, when considering the suitability, efficacy and reliability of using serum biochemical analytes as a complementary approach to assess nutritional state in MND, it would be prudent not to ignore the possible influences of physiological and homeostatic mechanisms that are likely to be at play in a cohort of people living with MND. Examples of serum biochemical markers of nutritional status are albumin, prealbumin, transferrin, ferritin and creatinine, as well as the lipid profile, consisting of cholesterol (total cholesterol, HDL- and LDL- cholesterol) and triglycerides.

The following critical analysis of primary publications frames the potential utility of serum biochemical analytes as biomarkers of nutritional status in MND. This not only relates to comparisons against existing methods of nutritional assessment in MND, but also with consideration for a selection of physiological characteristics of the disease: inflammation, malnutrition, body composition, and resting energy expenditure.

Inflammatory state

An elevated inflammatory state is known to affect the concentration of serum albumin, prealbumin, transferrin and ferritin; whereby the serum analyte concentration decreases with systemic inflammation as a result of hepatic reprioritisation of protein synthesis [155–158]. This relationship occurs independently of nutritional status [159]. A raised inflammatory response can also be associated with increased muscle catabolism and an elevated REE, both of which can contribute to a state of malnutrition if untreated [160].

Serum albumin, prealbumin, transferrin and creatinine were analysed for their utility to assess nutritional state in a cross-sectional cohort study conducted by Chelstowska et al., (2020) involving 203 people living with MND [161]. The relationship between serum analytes and the inflammatory state of study participants was analysed by examining the concentrations of routine basic inflammatory markers: C-reactive protein, erythrocyte sedimentation rate, white blood cell count, lymphocyte number and fibrinogen. Participants with increased markers of inflammation (*n* = 40) were excluded from further analyses.

Moreover, an independent cross-sectional study of 638 people living with MND [162] demonstrated a significant decrease in the concentration of serum albumin – but not creatinine – in the presence of increased inflammatory markers. This again highlights the importance of including the assessment of inflammatory state when evaluating the suitability of nutritional biomarkers in people living with MND.

Malnutrition

As described in **section 2.2.1**, malnutrition arises when the body's demand for energy exceeds intake, causing a negative energy balance. In the aforementioned study by Chelstowska et al., (2020) serum analytes measured from participants without inflammation were correlated with assessments of nutritional state using BMI, with a BMI of < 18.5 kg/m^2 used to indicate malnutrition in this cohort [161]. The concentration of serum analytes in participants identified as malnourished was compared against those without malnutrition (BMI > 18.5 kg/m^2). The authors found prealbumin and transferrin to be significantly lower in those indicated to be malnourished. The authors also reported a weak positive significant correlation between creatinine and BMI within the study population. In agreement with Chelstowska, Park et al., (2015) presented a significant positive relationship between creatinine and BMI in a South

Korean MND cohort (n = 193), whereby a greater BMI is accompanied by a greater creatinine concentration [163]. In contrast, Chiò et al., (2014) reported the absence of a relationship between creatinine and BMI in a cross-sectional validation cohort study of 683 Italian MND participants [162]. Furthermore, these studies do not agree on the relationship between albumin and BMI, with Park presenting a significant weak, positive correlation [163], and Chelstowska and Chiò both reporting no relationship [164].

These inconsistent results across international publications and MND cohorts indicates any relationship presented within a single cohort may not be representative of the wider MND population. However, it is important to note here that the study by Park et al., (2015) did not include analysis of participant inflammatory state; therefore, the relationship between BMI and creatinine or albumin levels observed in this publication should be interpreted with caution.

Body composition

MND-associated muscle catabolism is associated with a loss of FFM [165,166]. As 94% of serum creatinine derives from skeletal muscle turnover [167], the concentration of serum creatinine is inherently linked to the proportion of skeletal muscle. For example, serum creatinine was shown to correlate with a weak positive association with FFM in the Italian MND cohort previously described by Chiò and colleagues [162]. A longitudinal study investigating the use of serum analytes to monitor changes in the FFM of 42 participants living with MND was conducted by Jesus et al., (2019) [168]. They found 62% of participants demonstrated a decline in FFM by an average of 12.6% over one year. In these participants, LDL-cholesterol significantly decreased, and ferritin significantly increased. Creatinine was not included in the analysis in this study.

The subtle but quantifiable relationship between serum proteins and BMI or FFM may predict early changes in body composition, which may indicate disease severity and progression. This highlights the importance of continuous, early monitoring of nutritional state before the onset of malnutrition and associated decline in weight.

Disease severity and progression

Serum biochemical markers have previously been used to monitor nutritional state in relation to disease progression in MND. In the study by Chelstowska et al., (2020) [161] serum analytes measured from participants without inflammation were correlated with clinical parameters, specifically: MND phenotype, disease duration, dysphagia severity and ALSFRS-R score. A significant relationship between prealbumin and MND phenotype was shown, with highest concentrations observed in progressive muscular atrophy (PMA) and lowest in progressive bulbar palsy (PBP). As PBP is associated with bulbar onset, it is not surprising that prealbumin levels were also significantly decreased in patients with severe dysphagia, scoring 1 or 2 in the ALSFRS-R bulbar subscore, compared to those without bulbar weaknesses. Serum albumin was found to negatively correlate with disease duration, where significantly higher concentrations of albumin were observed in participants with a disease duration of less than 24 months.

In the aforementioned study by Park and colleagues [163], BMI, GNRI **(described in section 2.3)** and serum albumin and creatinine were used to assess nutritional state in relation to nutrient intake (estimated using 24-hour recall diaries), and disease severity (assessed using the ALSFRS-R). BMI and GNRI were shown to negatively correlate with MND severity, specifically the ALSFRS-R bulbar subscore; serum albumin was found to be significantly lower in participants with an ALSFRS-R score of < 36/48; and reported energy and macronutrient intake were significantly lower in those in the lowest tertile of ALSFRS-R.

The study conducted by Chiò et al., (2014) also showed a significant positive correlation between albumin and creatinine with ALSFRS-R total score [162]. The relationship between creatinine and ALSFRS-R was independently corroborated by Mitsumoto et al., (2020) who demonstrated creatinine positively correlated with ALSFRS-R in a longitudinal study of 276 Columbian participants living with MND [169]. Serum creatinine has also been demonstrated to inversely correlate with the annual decline of ALSFRS-R of 92 Japanese individuals with MND [170]. This study also demonstrated a negative relationship with the annual decline of ALSFRS-R for serum concentrations of cholesterol and LDL-cholesterol, however, a positive correlation was observed between ALSFRS-R annual decline and ferritin.

It is therefore widely agreed that the concentrations of serum creatinine and albumin decrease with a lower ALSFRS-R score, with indications that serum cholesterol and LDL-cholesterol follow suit.

2.4.3.2 Biomarkers of dietary exposure

Biomarkers of dietary exposure directly assess the dietary intake of food, nutrients and minerals, or dietary intake patterns [171,172]. When assessing dietary intake in any population, regardless of disease state, nutritional biomarkers associated with exposure of water-soluble nutrients can be analysed through the collection of urinary samples [171–176]. 24-hour (24hr) urinary collections overcome any diurnal variations in excretion patterns that would bias results from mid-stream 'spot' urinary samples [177–180]. Daily dietary intake variation in humans is such that an individual is unlikely to be in nutrient balance from one 24-hour collection; consecutive collections are therefore needed to gain reliable results [179,181–183]. It has been suggested that a minimum of three 24hr urinary collections are required, a minimum of one month apart [180,184].

It is important to ensure 24hr urinary collections are complete. There is controversy in the literature regarding how completeness is assessed. Many believe oral doses of para-aminobenzoic acid (PABA) should be taken to assess the complete collection of 24hr urinary samples [185–188]. For example, PABA was used in the National Diet and Nutrition Survey (NDNS) to assess completeness of 24hr urinary samples for the measurement of salt intake from urinary sodium in healthy adults [189]. However, it has been suggested that completion of a self-reported document of urinary collection, detailing collection times and any missed voids or spillages is sufficient to replace the necessity for PABA tablets [190]. This approach is highly-dependent on the compliance and honesty of participants, and samples should be weighed upon collection. When 24-hour urine was collected as part of the National Health and Nutrition Examination Survey, samples were considered 'incomplete' and discarded if any of the following criteria were not met: provision of the start and end times of the collection; a final collection volume of > 400 ml; a total collection time of > 22 hours [191].

24hr total urinary nitrogen (TUN), sodium (UNa) and potassium (UK) are estimates of dietary exposure to protein, potassium and salt intake, respectively. These analytes are described as

recovery biomarkers, whereby the output values are directly related to dietary intake over a defined time period [172,192]. However, the accurate measurement of recovery biomarkers requires patients to be weight stable, and not experiencing illness or trauma [190]. The use of TUN, UNa and UK has not previously been investigated in MND. The following section summarises the utility of these biochemical analytes in general medicine.

When first evaluated for reliability, the correlation coefficient between protein intake and TUN excretion was found to be 0.99 following a 28-day controlled metabolic study [181]. The use of TUN as a biomarker of protein intake in healthy populations has been validated through comparisons with intake questionaries [183,193–195] and a large-scale patient population in the European Prospective Investigation into Cancer and Nutrition (EPIC) study [196]. However, as TUN measured from 24-hour urinary samples reflects changes in total body protein mass, the analysis of 24hr TUN assumes that the individual is in nitrogen balance [183]. It cannot be assumed that an individual living with MND is in nitrogen balance, due to the potential influence of a hypermetabolic state, which is thought to increase the excretion of TUN, leading to a negative nitrogen balance [67].

Sodium intake has been shown to positively correlate with UNa excretion (r = 0.76) in healthy individuals consuming their normal diets [197]. When sodium intake is constant, sodium measured from a complete 24hr urinary collection accounts for 90% of all sodium intake [198,199]. The reliability of UNa can be reduced by physiological factors such as sweating, metabolism, hypertension, and hydration [48], resulting in invalid estimations of sodium intake [200–202] or unreliable 24hr urinary collections [199,203]. An extensive review of 29 studies assessing the use of UNa as a biomarker of sodium intake was published by Cogswell et al., (2015) [204], but most notably, UNa has been used to assess adult dietary salt intake in the cross-sectional NDNS study since 2005 [189,205–208]. The results from this study demonstrated that salt intake was significantly associated with body fat mass [209]. Both sodium intake and UNa have been demonstrated to significantly correlate with BMI and body composition assessments, such as body circumferences, percentage of body fat and lean body mass [210].

Potassium intake has been shown to positively correlate with UK excretion (r = 0.82) in healthy individuals consuming their normal diets [197,211]. Potassium is a widespread nutrient present in a large variety of food groups and is therefore generally indicative of a broader dietary intake;

24hr urinary potassium is therefore often used to validate dietary intake assessment tools [212–215]. However, measurement of UK from 24-hour urinary collections to indicate potassium intake has been found to demonstrate high inter and intra-individual [216] variability [199,217,218].

2.4.4 Assessment of dietary intake

2.4.4.1 Dietary assessment tools

It is important to accurately estimate an individual's dietary intake to establish appropriate nutritional goals and ensure consumption of an adequate caloric intake to balance energy expenditure. Intake diaries are a subjective dietary assessment tool used to estimate caloric and nutrient intake within a defined time period. Depending on the purpose of the study, intake diaries can be self-administered (completed by the participant or carer in the absence of a researcher) or interviewer-led (completed by a researcher in the presence of the participant); online or paper-based; and take the form of weighed food diaries, food frequency questionnaires or 24-hour recall diaries.

Weighed food diaries

Weighed food diaries involve the self-documentation of the weight of ingredients, final weight of cooked mean and any food waste. Weighed food diaries give the most accurate assessment of dietary intake [219]; however, measurements and recordings have to occur prospectively. This can be highly burdensome which is associated with poor participant engagement and inaccurate weight measurements which may inadvertently lead to under-reporting [220].

Food frequency questionnaires

Food frequency questionnaires (FFQs) are retrospective methods for reporting the frequency of consumption over 'long' periods of time (i.e., weeks, months or years). FFQs can be interviewer led, or self-administered. There is no standard FFQ, which makes them useful and adaptable for application in large population studies; however, Cade et al., (2004) conducted a semi-systematic review in order to develop a framework for the development, validation and use of FFQs [221]. An example is the development and validation of multiple FFQs specific to the study population of interest within the European Prospective Investigation into Cancer and Nutrition (EPIC) study [222].

24-hour dietary recall

24-hour dietary recall is reported to be the most accurate dietary intake assessment for estimating intakes of energy, protein, potassium and sodium [215,223] intake over short periods of time. However, a single 24-hour recall lack of ability to capture day-to-day variation [224].

No dietary assessment tool is free from error. A comprehensive review was published by Livingstone and Black (2003) detailing the widespread problems associated with the underreporting of energy intake when compared against energy expenditure, assessed using doubly-labelled water [225]. Livingstone and Black demonstrated that the poor precision of recording or measuring dietary intake can result in an invalid dietary recall through misreporting or large measurement errors [226]. These errors lead to false findings and insufficient information to accurately predict an individual's caloric intake [227–230]. In addition, the extent to which food is cooked and the combination of nutrients consumed together can influence the absorption and processing of nutritional content [153]. Limitations therefore need to be acknowledged, and, where possible, compensated for. Dietary assessment tools must be validated in relation to an objective marker of intake, i.e., biochemical analytes [231,232] .

The Dietary Assessment Tool NETwork (DIET@NET) project established Best Practice Guidelines (BPGs) to help researchers determine the most suitable dietary assessment tool for the assessment of energy and/or nutrient intake [233]; these guidelines are available on the "Nutritools" website [234].

2.4.4.2 Dietary reference values

Defined in 1991 by the Department of Health, Dietary Reference Values (DRVs) for food energy intake provide an estimate of the energy needs of the UK population [235]. Whilst DRVs are not recommendations for dietary intake at an individual level, it is still important to consider where an individual falls within a given population. The Estimated Average Requirement (EAR) is an estimate of average energy intake required to match expenditure and prevent weight loss for a given population [236]. The EAR has, by definition, similar probabilities of an individual consuming sufficient - or insufficient - intake for a particular nutrient. Reported energy and nutrient consumption can be compared against the EAR to indicate the risk of an individual not consuming an adequate nutritional intake leading to an adverse nutritional state (i.e., 'nutritional risk', described in **section 2.2.3**). The greater the

difference between estimated intake and the EAR, the greater the 'risk' of under- or over-feeding. The reference nutrient intake (RNI) for any given nutrient is defined as the amount of nutrient that is sufficient, or more than sufficient, for 97.5% of a population; however, this is excessive for the majority of the population [236]. In opposition to this is the lower reference nutrient intake, which is sufficient for 2.5% of a population, but inadequate for the majority of the population [235]. A schematic to explain the relationship between the EAR, LRNI and RNI is presented in Figure 2.2.

The most recent DRVs for energy, carbohydrate and fat intake were derived from the Scientific Advisory Committee on Nutrition (SACN) Dietary Reference Values for Energy (2011) in relation to the general population [237]. Grams of carbohydrate per day (calculated as 50% of total dietary energy) were obtained from the SACN Carbohydrate and Health (2015) [238]. Grams per day of fat (calculated as 35% of food energy) and protein reference values were obtained from DRV values for Food Energy and Nutrients for the UK (1991) [235]. The energy requirements of people living with MND may vary from the values estimated for the general population, but have not yet been outlined.

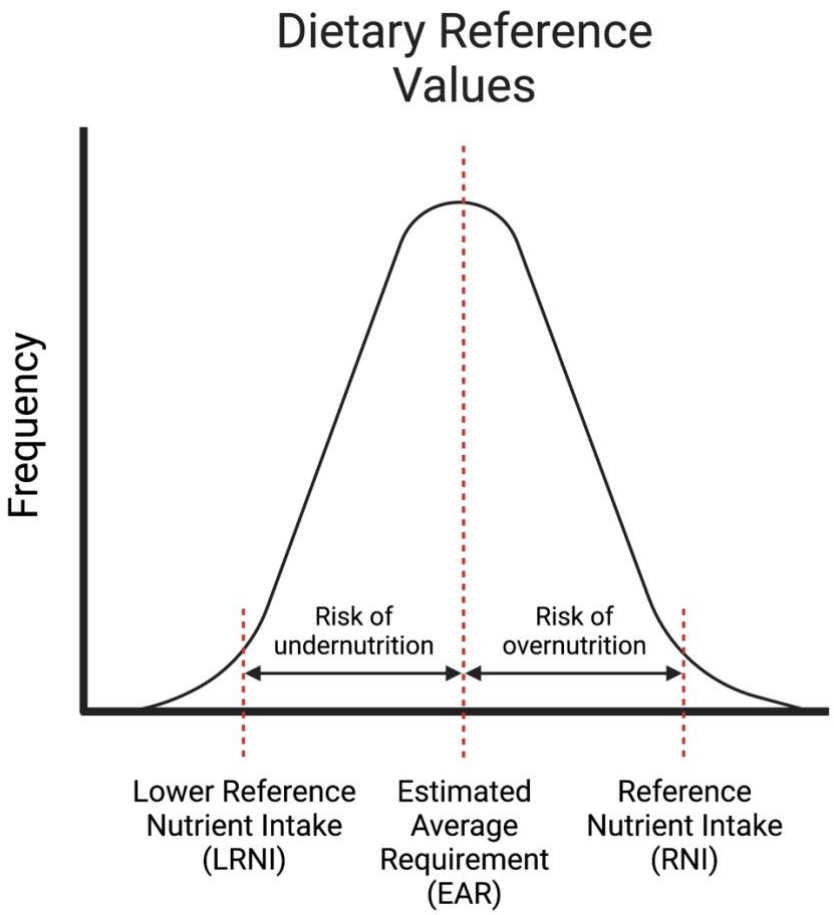


Figure 2.2 Dietary reference values to demonstrate the estimated average requirement (EAR), lower reference nutrient intake (LRNI) and reference nutrient intake (RNI). Created with BioRender.com

2.4.4.3 Assessment of dietary intake in MND

There is debate dating back to the 1980's surrounding the most optimal form of nutritional intake to prevent the onset and decrease the severity and progression of MND. Dietary intake was first evaluated in MND by Slowie et al., (1983) who used a 24-hour dietary recall to estimate that 14 out of 20 (70%) participants consumed less energy than the RDA (i.e., at risk for energy deficit) [50]. This energy deficit was supported by the conduction of a cohort study by Kasarskis et al., (1996) when the ad libitum dietary intake of 16 people living with MND was recorded over three days (one weekend day and two weekdays) [56]. When this data was averaged and expressed as a percentage of RDA, Kasarskis reported an inadequate energy consumption in 94% of participants, whilst 88% of the same individuals exceeded the recommended daily protein intake [56].

Regardless of caloric consumption, people living with MND will inevitably experience a loss of lean muscle mass attributed to motor neuron degeneration [239]. However, Nau et al., (1995) demonstrated that energy balance is not intrinsically linked to overall body weight, but rather is proportional to body composition, with small increases in fat mass able to compensate for a loss of lean mass [137]. In fact, a higher-than-average premorbid body fat has been linked to increased survival in MND [240]. This is because fat mass has a higher caloric value than lean mass of the same density [137]. For this reason, it is thought that despite a reduction in weight, people living with MND can maintain an energy balance upon consumption of a high-fat diet. However, Nau advised caution regarding the potential implications of a high fat diet on the physiology and function of people living with MND [137] .

The safety and tolerability of an enterally administered hypercaloric diet (high-carbohydrate and high-fat) was investigated in a double-blind, placebo-controlled randomised phase 2 clinical trial of 20 people living with MND [241]. The results from this study indicated that the consumption of a fat- and carbohydrate-rich hypercaloric diet was safe in people living with MND [241]. The potential benefit of a high-calorie, high-fat diet was further supported when the results of a double-blind multicentre prospective study in 201 people living with MND reported that consumption of a high-calorie, high-fat diet identified a significant survival benefit in a subgroup of fast-progressing participants [242]. However, these results did not demonstrate benefit to the wider MND population. Targeted nutritional care according to the

stratification of people living with MND may therefore aid in the maintenance of nutritional status. Advocation for a high-calorie diet contrasts with the developing notion that a reduction of caloric intake by 10-30% increases lifespan as observed in other neurodegenerative conditions, such as Alzheimer's [243], Parkinson's disease [243,244], Huntington's [245] and stroke [246]. This is referred to as the caloric restriction paradigm, first described by McCay et al., 1935 [247].

More recently, studies have reported use of FFQs to estimate dietary intake in two independent MND cohorts. These studies have focussed on the comparison of dietary intake against the risk and severity of MND. The dietary intake of 77 Korean participants living with MND was investigated in a case-control study [248]. Participants retrospectively recalled the frequency and quantity of 63 food items (grouped into nine categories) during the year prior to diagnosis with MND using interviewer-led FFQs. Consumption of fruit and ß-carotene was found to be negatively associated with the risk of developing MND.

The association between dietary intake and function in MND was investigated in a cross-sectional, multicentre study in 302 people living with ALS, with a symptom duration of 18 months or less [249]. Nutrient intake was measured using a self-administered modified FFQ to focus on antioxidant nutrients (e.g., vitamins A, C and E, ß-carotene). The data reported within the FFQ was automatically converted to estimates of average daily nutrient intake using a standardised reference database on the system 'Nutritionquest' [250]. The authors found that a greater intake of antioxidants and carotenes in fruit and vegetables was associated with higher functionality, assessed using the ALSFRS-R [249].

2.4.5 Assessment of energy expenditure

The reason for assessing energy expenditure in MND is two-fold. Primarily, in order to evaluate whether an individual is in energy balance, estimated energy intake must be compared against energy expenditure. Secondly, the presence of a hypermetabolic state (i.e., a greater-than-predicted resting energy expenditure [61,62]), is associated with an increased catabolism of carbohydrate, lipids and proteins [66]. Therefore, the potential influence of a hypermetabolic state needs to be considered alongside the measurement of biochemical analytes.

Resting energy expenditure (REE) is the 'non-active' energy (i.e., respiration, macronutrient utilisation and thermoregulation) expended by the body over a 24-hour period [251]. In healthy adults, it is assumed that REE comprises approximately 60-70% of total daily energy expenditure (TDEE), with the remaining energy expenditure made up from exertions through physical activity levels (PAL) and diet-induced thermogenesis [1] [252]. In healthy individuals, REE is mainly influenced by age, sex and FFM [253]; in MND, REE has been shown to positively correlate with FFM and neutrophil count, but negatively correlate with age [254]. REE can rapidly and dynamically alter according to the central nervous system pathways that regulate energy homeostasis [255]. For example, REE can increase as a result of overfeeding, inflammation or metabolic acidosis, or decrease due to cachexia, underfeeding, metabolic alkalosis [256]. The accurate determination of energy expenditure is imperative to avoid the harmful consequences of an energy imbalance. REE can either be predicted, using predictive energy equations, or measured, using indirect calorimetry.

A scoping review was therefore conducted to identify the approaches that have previously been used to assess energy expenditure in people living with MND and subsequently determine the most suitable, feasible method for assessing resting energy expenditure in this cohort. This scoping review is presented in a manuscript format in the next chapter.

[1] TDEE = REE + PAL + diet induced thermogenesis

2.5 Summary

This chapter has met the first objective of this thesis, *to 'identify the current challenges with nutritional assessment in MND'*. In summary, it is understood that assessments of body composition (fat and fat free mass) are more informative in understanding nutritional state than weight or BMI measurements. This is because changes in muscle mass are directly related to disease-associated muscle-wasting following muscle denervation, whereas malnutrition-associated fat loss follows later as a result of prolonged energy imbalance. A balanced nutritional state is therefore inherently related to the consumption of an appropriate nutritional intake which matches the total daily energy expenditure of that individual. Nutritional intake, energy expenditure and the metabolism of useable energy therefore all need to be assessed. Reliable estimates of nutritional intake therefore need to be collected, which need validation using a panel of biochemical analytes. The role of biochemical analytes in understanding the dietary intake and nutritional state of people living with MND are currently not well understood or evidenced; however, it is well established that the potential influence of a pro-inflammatory or hypermetabolic state needs to be measured alongside.

3 A scoping review to map the evidence for measuring energy expenditure in MND

This scoping review will be submitted for publication following thesis submission. The thesis author was responsible for the review conceptualisation (identification of the research question); methodology (identification of primary research literature and study selection); data extraction; data synthesis; and writing of the original draft, review and editing.

3.1 Review methodology

This scoping review has followed the five-step framework outlined by Arksey and O'Malley [257], i.e., 1) identification of the research question; 2) identification of primary research literature; 3) study selection; 4) data extraction; and 5) data synthesis.

3.1.1 Identification of the research question

The research question was: "what approaches (i.e., devices, technology, protocols) have been used to measure energy expenditure in people living with MND?"

3.2 Aims and objectives

The aim of this review was to map the evidence around approaches to measure energy expenditure in people living with MND. The objectives were defined according to the PICOS framework (Population, Intervention, Comparator, Outcome and Study design) [258] as shown in

Table 3.1**.**

1. To identify and map the methods (i.e., devices, protocols, equations and outcome measures) used to measure energy expenditure in adults living with MND to identify the strongest approach to assess energy expenditure in MND;
2. To identify the most suitable approach to indicate hypermetabolism in MND.

Table 3.1 PICOS Criteria.

PICOS Criteria	Description
Participants / population(s)	Adults living with motor neuron(e) disease
Intervention(s)	Measurements of energy expenditure: • Indirect Calorimetry • Plethysmography • Doubly labelled water
Comparator(s)	None/Healthy controls
Outcome(s)	Primary outcome: • Methodology, protocols and devices used to assess energy expenditure in people living with MND. Secondary outcome: • Comparisons against measure energy expenditure • Use of energy expenditure to classify hypermetabolism in MND
Study design	• Primary research articles; quantitative studies; cross-sectional or longitudinal; case-control or cohort. • International • No date restrictions • In humans

3.3 Study design

This scoping review considered the study designs conducted to measure energy expenditure in adults living with MND. This included randomized controlled trials, analytical observational studies, including prospective and retrospective cohort studies, case-control studies, cross-sectional studies and longitudinal studies.

3.3.1 Search strategy

A comprehensive search of three major biomedical and healthy sciences databases, i.e., MEDLINE via Ovid, CINAHL via EBSCO and Web of Science was undertaken on the 19th January 2023 to identify primary research articles on the topic. The search strategy, including all identified keywords and index terms, was developed in MEDLINE (search example in Figure 3.1) and subsequently adapted for CINAHL and Web of Science. Keyword terms were optimised using wildcards and truncations and combined with medical subject headings (MeSH) using Boolean Operators. Only studies conducted in humans and published in the English language were included. Search results were not limited by publication date. Reference lists of key studies were screened by hand and 'cited by' articles on PubMed to identify additional studies.

1	Motor neuron disease/ or Amyotrophic lateral sclerosis/ or motor neuron* disease.mp. or MND.mp. or ALS.mp. [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	81962
2	nutritional status/ or nutrition assessment/ or nutrition therapy/ or malnutrition/ or malnutrition.mp. or nutrition* assessment.mp. or nutrition* monitoring.mp. or *nutrition/ or malnutrition.mp. [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	112750
3	energy metabolism/ or basal metabolism/ or oxygen consumption/ or metabolism/ or *energy expenditure/ or energy demand.mp. or resting energy expenditure.mp. or REE.mp. or total daily energy expenditure.mp. or TDEE.mp. or basal energy expenditure.mp. or resting metabolic rate.mp. or RMR.mp. or basal metabolic rate.mp. or BMR.mp. or hypermetabolism.mp. or *metabolism/ [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	225548
4	calorimetry, indirect/ or plethysmography/ or indirect calorimetry.mp. or IC.mp. or whole body air displacement plethysmography.mp. or bodpod.mp. or doubly-labelled water.mp. or DLW.mp. or predictive energy equations.mp. [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	86306
5	1 and 2 and 3 and 4	8
6	limit 5 to (english language and humans)	7
7	1 and 2	320
8	1 and 2 and 3	28
9	limit 8 to (english language and humans)	24
10	1 and 3 and 4	25
11	limit 10 to (english language and humans)	21

Figure 3.1 An example of the search strategy used in MEDLINE via Ovid.

3.3.2 Study selection

Following the search, all identified citations were collated and uploaded into Mendeley Reference Manager (version 2.88.0) and duplicates removed. Titles, abstracts and full texts were systematically screened for eligibility according to the PICOS eligibility criterion Table 3.2). To minimise bias, an independent second reviewer also assessed the titles and a random selection of abstracts. Discrepancies were resolved by discussion.

Table 3.2 PICOS eligibility criterion.

PICOS Criterion	Inclusion criteria	Exclusion criteria
Participants / population(s)	• Adults living with motor neuron(e) disease (≥18 years) • Studies conducted in participants with a confirmed diagnosis of MND at any stage and any MND phenotype • Studies conducted in humans	• Studies in children (<18 years) • Studies conducted in healthy participants or any condition other than MND • Non-human studies
Intervention(s)	• Studies that measured energy expenditure by means of indirect calorimetry, plethysmography and doubly labelled water	• Studies that did not measure energy expenditure (e.g., the sole use of predictive energy equations)
Comparator(s)	• N/A	• N/A
Outcome(s)	Primary: • Studies that describe methods, protocols and devices used to measure direct or indirect output values when measuring energy expenditure in MND: ○ mREE ○ VO_2/VCO_2 ○ RQ Secondary: • Studies that compare measures of energy expenditure against predictions of resting energy expenditure to determine accuracy • Studies that present thresholds used to indicate hypermetabolism	• Studies that did not measure energy expenditure in MND

Study design	• Primary quantitative research journal articles • Cross-sectional or longitudinal • Case control or cohort • International • Studies available in full text • Published in the English language	• Qualitative studies • Reviews; systematic reviews, opinion pieces, editorials, letters, commentaries • Non-English language • Studies unavailable in full text

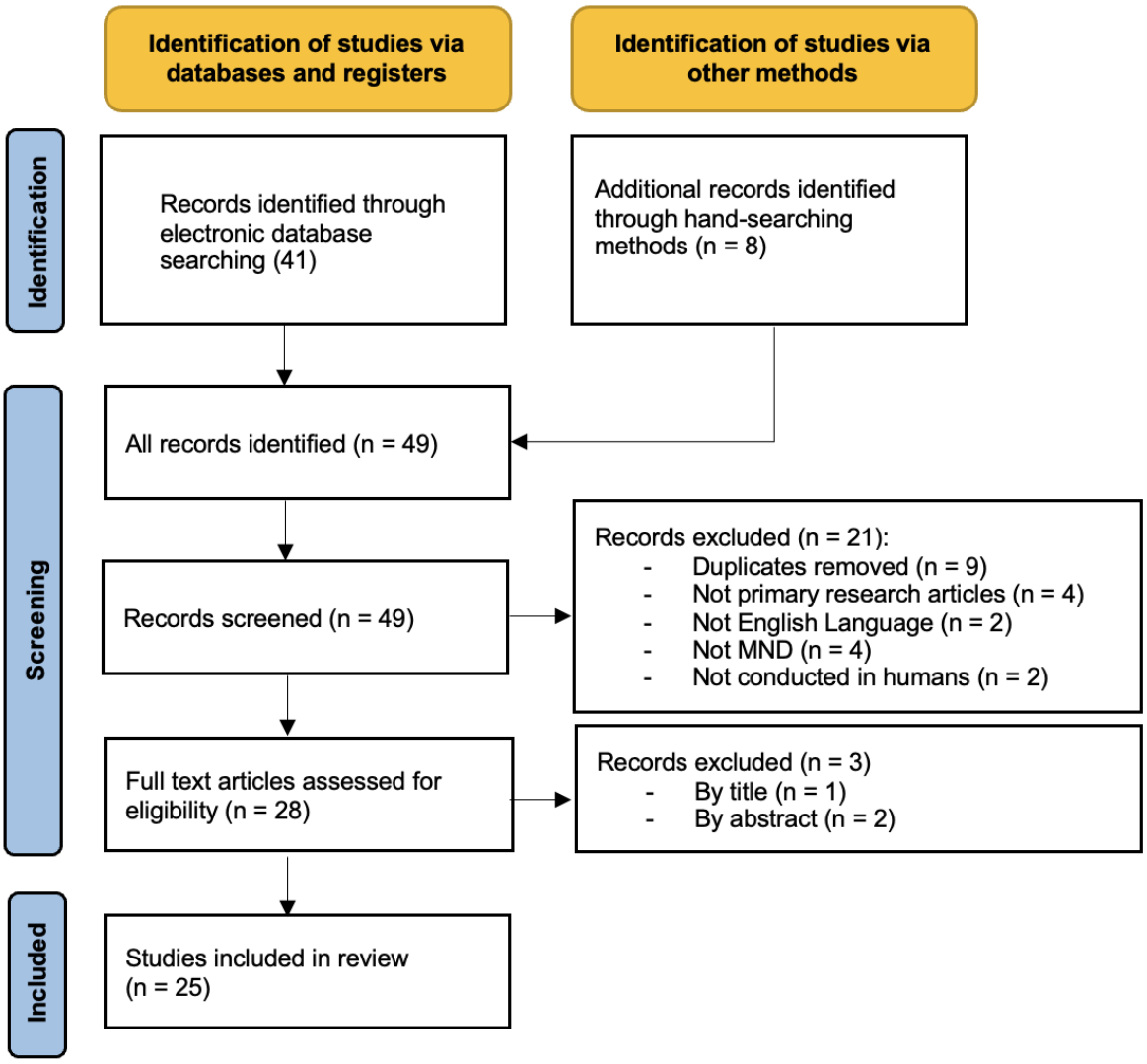
3.3.3 Eligible studies

A total of 25 primary research articles were identified that met the acceptance criteria and addressed the research question. The results of the search and the study inclusion process is presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping review (PRISMA-ScR) flow diagram Figure 3.2 [259]. A list of all included studies is presented in Table 3.3.

3.3.4 Data Extraction

Data was extracted from the identified papers using a data extraction tool developed by the reviewers. The data extracted included study population demographics, study design, aims and key findings relevant to the research question. Conclusions, strengths and weaknesses are reflected on in the discussion.

Figure 3.2 A PRISMA flow diagram to illustrate the study selection process.



3.4 Results

3.4.1 Study characteristics

The study characteristics including details of the study populations within the included studies are outlined in Table 3.3. The 25 included studies were published over a 24-year timespan between 1995 and 2023. Included studies were performed across ten countries, with the majority of research conducted in France (n = 10/25 (40%)). Of note, no studies were conducted with people living with MND in the UK.

3.4.2 Study design

The designs of each study are presented in Table 3.3. Twelve included studies 12/25 (48%) were longitudinal in design. However, energy expenditure changes between different study time points were reported in only three (25%) studies (study IDs: 5 [260], 6 [261] and 8 [262]). Nine studies (36%) were case-control; the control group was age- and sex-matched in six of these studies (66.7%), denoted by a 'Y+' in Table 3.3, control group column.

Table 3.3 Studies included in the scoping review. Data presented as mean ± SD or median (IQR), as reported in the primary literature. If median was presented without IQR, then median is indicated by * to distinguish from mean. ANTH: anthropometric measurement; Y+ indicates sex and age-matched control group; '-' indicates data not retrieved.

Study ID	**Author**	**Year**	**Study sites**	**Case-control or cohort**	**Prospective or retrospective**	**Observational or interventional**	**Cross-sectional or longitudinal**	**Country**	**Cast participants (n)**	**Age** (*median)	**Sex ratio** (F/M)	**Control group** (*n*)	**EE assessment**	**Body composition assessment**
1	Nau [68]	1995	Single	Case-control	Prospective	Observational	Longitudinal	America	12	50.9	0/12	Y (6)	IC	DEXA
2	Kasarskis [69]	1996	Single	Cohort	Prospective	Observational	Longitudinal	America	16	58	8/8	N	IC	ANTH, BIA
3	Desport [263]	2001	Single	Case-control	Prospective	Observational	Cross-sectional	France	62	63 ± 11	30/32	Y (31)	IC	BIA
4	Sherman [264]	2004	Single	Cohort	Prospective	Observational	Cross-sectional	America	34	61.7 ± 8.85	18/16	N	IC	BIA

Study ID	Author	Year	Study sites	Case-control or cohort	Prospective or retrospective	Observational or interventional	Cross-sectional or longitudinal	Country	Cast participants (n)	Age (*median)	Sex ratio (F/M)	Control group (*n*)	EE assessment	Body composition assessment
5	Desport [260]	2005	Single	Cohort	Prospective	Observational	Longitudinal	France	168	-	0.97 (5/163)	N	IC	BIA
6	Bouteloup [261]	2009	Multi	Cohort	Prospective	Observational	Longitudinal	France	61	64.3 ± 9.9	31/30	N	IC	DEXA
7	Funalot [265]	2009	Single	Case-control	Prospective	Observational	Cross-sectional	France	11	60.7 ± 8.8	5/6	Y+ (33)	IC	BIA
8	Vaisman [262]	2009	Single	Case-control	Prospective	Observational	Longitudinal	Israel	33	59	11/22	Y+ (33)	IC	DEXA
9	Siirala [266]	2010	Single	Cohort	Prospective	Observational	Longitudinal	Finland	5	55*	1/4	N	IC	-
10	Ellis [147]	2011	Single	Cohort	Prospective	Observational	Cross-sectional	America	56	54.89 ± 11.98	25/31	N	IC	ANTH, BIA

Study ID	Author	Year	Study sites	Case-control or cohort	Prospective or retrospective	Observational or interventional	Cross-sectional or longitudinal	Country	Cast participants (n)	Age (*median)	Sex ratio (F/M)	Control group (*n*)	EE assessment	Body composition assessment
11	Georges [267]	2014	Single	Cohort	Prospective	Observational	Cross-sectional	France	16	68*	4/12	N	IC	-
12	Kasarskis [268]	2014	Single	Cohort	Prospective	Observational	Longitudinal	America	80	58.7 ± 11.9	28/52	N	IC & DLW	BIA
13	Shimizu [269]	2017	Single	Cohort	Prospective	Observational	Cross-sectional	Japan	26	64.5 (62.1-70.0)	13/13	N	DLW	DLW
14	Jesus [145]	2018	Single	Cohort	Prospective	Observational	Longitudinal	France	315	65.9 (56.5-73.7)	154/161	N	IC	ANTH, BIA
15	Lunetta [270]	2018	Single	Case-control	Prospective	Observational	Cross-sectional	Italy	50	66 (9.81)	16/34	Y+ (32)	IC	BIA
16	Steyn [271]	2018	Single	Case-control	Prospective	Observational	Longitudinal	Australia	58	61	20/38	Y+ (58)	IC	Plethysmography

Study ID	Author	Year	Study sites	Case-control or cohort	Prospective or retrospective	Observational or interventional	Cross-sectional or longitudinal	Country	Cast participants (n)	Age (*median)	Sex ratio (F/M)	Control group (*n*)	EE assessment	Body composition assessment
17	Jesus [144]	2019	Single	Cohort	Prospective	Observational	Cross-sectional	France	315	65.9 (56.5-73.7)	154/161	N	IC	ANTH, BIA
18	Jesus [146]	2020	Single	Cohort	Prospective	Observational	Cross-sectional	France	315	66.6 (56.9-74.1)	154/161	N	IC	ANTH, BIA
19	Fayemendy [272]	2021	Multi	Case-control	Prospective	Observational	Cross-sectional	France	287	66.4	142/145	Y (75)	IC	ANTH, BIA
20	Kurihara [273]	2021	Single	Cohort	Retrospective	Observational	Cross-sectional	Japan	42	70 (61-74)	20/22	N	IC	BIA
21	Nakamura [274]	2021	Single	Cohort	Retrospective	Observational	Cross-sectional	Japan	48	71 (65-75)	23/25	N	IC	BIA
22	Cattaneo [275]	2022	Multi	Cohort	Retrospective	Observational	Longitudinal	Italy; France	847	63.79*	375/472	N	IC	BIA

Study ID	**Author**	**Year**	**Study sites**	**Case-control or cohort**	**Prospective or retrospective**	**Observational or interventional**	**Cross-sectional or longitudinal**	**Country**	**Cast participants (n)**	**Age** (*median)	**Sex ratio** (F/M)	**Control group** (*n*)	**EE assessment**	**Body composition assessment**
23	He [276]	2022	Single	Case-control	Prospective	Observational	Longitudinal	China	93	53.0 (43.0–60.0)	50/97	Y+ (147)	IC	BIA
24	Nakamura [277]	2022	Single	Cohort	Retrospective	Observational	Cross-sectional	Japan	78	71 (66-75)	40/38	N	IC	BIA
25	Dorst [278]	2023	Multi	Case-control	Prospective	Observational	Longitudinal	Germany, Sweden	60	48.7 ± 14.9	36/24	Y+ (73)	IC	BIA

3.4.3 Interventions

Publications were grouped according to the method of energy expenditure measurement. Out of the 25 publications, 24 (96%) used indirect calorimetry and two (8%) used doubly labelled water (DLW) (Table 3.3; method of EE assessment). Across the 24 publications that used indirect calorimetry, eight different indirect calorimeters devices were cited (Table 3.4). Two multi-centre studies (IDs: 19 [272] and 22 [275] used a different device at each site. The majority of reported calorimetry modes were set up with a canopy hood (14/19 (73.7%)).

Table 3.4 Indirect calorimetry devices. '-' indicates data not reported.

Manufacturer	Name	Mode	Number of studies	Study ID
Beckman Instruments Inc	Horizon	-	1	2
Datex Engström	Deltatrac II	Canopy hood	7	3, 5, 6, 7, 8, 9, 19
Cosmed	Quark RMR	Canopy hood	4	14, 16, 17, 18
		Oronasal mask	1	11
		-	2	19, 25
Medgraphics Corp	ULTIMACardiO2	Oronasal mask	1	23
SensorMedics corporation	Vmax Spectra V29N	Canopy hood	2	10, 22
Carefusion	Vyntus CPX	Canopy hood	1	22

Metascope	Cybermedic	-	2	1, 4
Minato Medical Science	Aeromonitor AE310S	Oronasal mask	3	20, 21, 24

3.4.4 Outcomes

3.4.4.1 Primary outcomes

Table 3.5 outlines the extracted data regarding the reported protocols and outcome measures for the conduction of IC. Data was extracted only if explicitly stated within the text. Data not reported is shown as '-'. Studies that did not report protocols for the conduction of IC (i.e., information regarding fasted period, body position or duration of recording) were checked for citations claiming reference to standardised protocols; data was extracted from these references, if appropriate. Of the studies that reported fasting ahead of IC (n = 21), quantifiable fasted periods ranged between five and twelve hours. Studies that stated the occurrence of an 'overnight fast' could not be quantified (n = 7). The reported duration of IC assessment varied between ten minutes to one hour, with washout periods - where data was discounted - reported in seven studies, varying between five and ten minutes. There was no consistency between the reported position of the head and torso ('body position') when conducting measurements; of the 18 publications that provided information on body position, only two publications (ID 16 and 22) provided the exact angle of measurement.

Output values (mREE, VO_2, VCO_2 or RQ) were reported in 20/24 (83.3%) studies using IC; however, there was no consistency in reporting the measures of central tendency for mREE. Siirala et al., 2010 [266] (ID 9) was the only included study to specify every output value measured or derived from IC, as well as outlining the principles of indirect calorimetry.

Table 3.5 Extracted indirect calorimetry protocol data. Data is presented as mean ± one standard deviation or median (IQR). '-' indicates data not reported.

ID	Fasted duration (hours)	Body position	Rested period (minutes)	Washout period (minutes)	Duration of recording (minutes)	VO_2 (ml/min)	VCO_2 (ml/min)	mREE	RQ
1	-	-	-	-	≥ 20	-	-	-	-
2	Overnight	-	-	-	-	-	-	-	0.81 ± 0.03
3	≥ 10	Supine or semi-seated	≥ 20	-	20	-	-	1561.6 ± 342.3	0.81 ± 0.04
4	Overnight	Reclined	-	5	20	-	-	Ventilated 1654.9 ± 362.9 Not ventilated 1340.8 ± 471.6	-
5	≥ 10	Supine or semi-seated	≥ 20	-	20	-	-	1521.9 ± 307.5	-
6	Overnight	Supine or semi-seated	20	-	30-45	-	-	1449.0 ± 300.7	-
7	≥ 10		20-30		20	-	-	fALS 1784 ± 340	-

ID	Fasted duration (hours)	Body position	Rested period (minutes)	Washout period (minutes)	Duration of recording (minutes)	VO_2 (ml/min)	VCO_2 (ml/min)	mREE	RQ
		Supine or semi-seated						SALS 1582 ± 300	
8	12	Supine	20	10	60	-	-	1467 ± 218	0.81 ± 0.06
9	12	Supine	-	-	30	165 (± 25)	137 (± 24)	1130 ± 170 1060 (960-1480)	0.82 ± 0.08
10	-	-	-	10	30	-	-	1488.84 ± 326.05	-
11	Overnight	Semi-seated	20	-	15	-	-	Spontaneous breathing 1197.3 (1054.7-1402.6) NIV 1149.2 (970.8-1309.5)	-
12	Overnight	-	-	-	-	-	-	1539 ± 366	-
14	12	Supine	-	-	-	-	-	1503 (1290-1698)	-
15	-	-	-	-	-	-	-	1413.7 ± 314.9	-

ID	Fasted duration (hours)	Body position	Rested period (minutes)	Washout period (minutes)	Duration of recording (minutes)	VO_2 (ml/min)	VCO_2 (ml/min)	mREE	RQ
16	12	35°	10	5	15	-	-	-	-
17	12	Supine	-	-	-	-	-	1514 ± 298.7 1503 (1290-1698)	-
18	12	Supine	-	-	30	-	-	1503 (1290-1698)	-
19	12	Supine	-	-		-	-	1500 (1290-1693)	-
20	Overnight	Supine	30	-	10	-	-	1254 (1082-1500)	0.84 (0.81-0.91)
21	Overnight	Supine	30	-	10	-	-	-	-
22	12	35°	10-20	5	20	-	-	1430.00 (1239-1650)	-
23	≥ 6	Semi-supine	-	5	16	-	-	-	-
24	-	-	-	-	-	-	-	-	-
25	≥ 5	Supine	20	5	16	-	-	1598 (1376-1885	

3.4.4.2 Secondary outcomes

Predictive energy equations as comparators against mREE

To standardise the terminology used across these publications, all assessments of estimating, calculating or predicting resting energy expenditure will be referred to as 'predictions of REE' (pREE) in this review. Within the 24 studies that conducted IC, ten (41.7%) provided predicted values of REE using a variety of predictive energy equations (Table 3.6). The Harris-Benedict (1919) equation was by far the most frequently used equation, referenced in all ten studies.

Table 3.6 Comparison of mREE against methods of pREE. Data is presented as mean ± one standard deviation or median (IQR).

ID	mREE	HB (1919)	HB (1984)	Mifflin-St Jeor	Ireton-Jones	FAO/WHO /UNU	World Schofield	De Lorenzo	Johnstone	Owen	Fleisch	Nelson	Wang	Shimizu	Rosenbaum
3	1561.6 ± 342.3	1334 ± 234.7													
4	Ventilated 1654.9 ± 362.9	1461													
	Not ventilated 1340.8 ± 471.6	1505.0													
5	1521.9 ± 307.5	1334 ± 234.7													
6	1449.0 ± 300.7	1315.5 ± 242.2													

ID	mREE	HB (1919)	HB (1984)	Mifflin-St Jeor	Ireton-Jones	FAO/WHO /UNU	World Schofield	De Lorenzo	Johnstone	Owen	Fleisch	Nelson	Wang	Shimizu	Rosenbaum
9	1130 ± 170	1580 (1190-2020)		1557 (1399-1909)		1656 (1374-2039)				1726 (1183-1879)	1630 (1210-1938)				
10	1488.84 ± 326.05	1522 ± 39		1431 ± 37	1660 ± 40										
12	1539 ± 366	1596 ± 283		1523 ± 283						1589 ± 250			1315 ± 264		1508 ± 203
17	1514 ± 298.7	1356 ± 222.2	1375 ± 212.8	1285 ± 241.6		1421 ± 213.2	1381 ± 207.1	1376 ± 224.9	1326 ± 215.5	1418 ± 206.9	1398 ± 189		1281 ± 224		1369 ± 178
19	1500 (1290-1693)	1327 (1195-1496)													
20	1254 (1082-1500)	1146 (1060-1275)												1660 (1531-1923)	

Accuracy of pREE

In this review, the calculation of the accuracy between predicted and measured REE is termed the REE variation. Table 3.7 presents the extracted equations, thresholds and results for three studies which explicitly reported values for the REE variation. In addition to these studies, Vaisman (ID 8) described the percentage of their study population whose pREE were in the accepted predicted range (17/33 (51.5%)). The most common accuracy threshold was ± 10%. These studies showed pREE to be accurate in 27.3-63% of the study populations at a threshold of ± 10%, with the mean REE variation ranging between -14.8–13.9%. Of note, Jesus et al., 2019 (ID 17) calculated the REE variation *a posteriori* for five publications that are included in this review. They presented REE variation to range between -14.6-62.8% [144].

The production of both negative and positive REE variations occurs as a result of both the over- and under-prediction of pREE when compared against mREE (Table 3.6). Moreover, there is no agreement across these studies for the most suitable predictive equation, e.g., Ellis et al., found the Mifflin-St Jeor (MSJ) equation to show the greatest accuracy (REE variation of 2.7%) in the highest proportion of study participants (63%), whilst Jesus et al., demonstrated the MSJ to have a mean REE variation of -14.8%, accurate in 27.3% of the study population.

Table 3.7 Resting energy expenditure variation. Data presented as mean ± one standard deviation. HB: Harris-Benedict; MSJ: Mifflin-St Jeor; SD: standard deviation.

ID	Author	Year	Equation	Acceptable threshold	pREE equation	REE variation (Mean ± SD)	Accurate (% of study population)
4	Sherman	2004	(pREE – mREE) / mREE x 100	< 20%	HB 1919	18.6 ± 14.9	67.6
					Fusco	25.6 ± 23.8	-
					Ireton-Jones	21.09 ± 17.5	-
					'Weight-based'	20.6 ± 14.3	-
10	Ellis	2011	-	± 10%	HB 1919	3.7	52
					MSJ	-2.7	63
					Ireton-Jones	13.9	46
17	Jesus	2019	(pREE-mREE)/mREE x 100	± 10%	HB 1919	-9.4	45.1
					HB 1984	-7.9	49.8
					World Schofield	-7.1	43.5
					De Lorenzo	-8.1	50.2

ID	Author	Year	Equation	Acceptable threshold	pREE equation	REE variation (Mean ± SD)	Accurate (% of study population)
					Johnstone	-11.1	36.9
					MSJ	-14.8	27.3
					WHO/FAO	-4.9	54.9
					Owen	-4.3	57.5
					Fleisch	-6.7	54.0
					Wang	-14.3	32.1
					Rosenbaum	-7.4	46.7

Use of energy expenditure measurements to indicate hypermetabolism

Due to variation in the terminology used to describe the difference between measured and predicted resting energy expenditure, and for ease of reporting, all extracted results and information will be presented using the same term, 'metabolic index, as outlined in Table 3.8. The comparison of pREE against mREE has been used to indicate hypermetabolism in ten of the included studies (Table 3.9).

Table 3.8 Terminology for the metabolic index (%).

Defined terminology in this review	Terminology in paper	Study IDs
Metabolic index (%)	ΔREE	19, 20
	Metabolic index	16 & 23
	REE variation	18
	Metabolic ratio	25

There is no consensus on the comparator, equation, or threshold by which to identify hypermetabolism in MND, as outlined in Table 3.9. If the equation for calculating the hypermetabolic threshold was not explicitly outlined within the publication, then it was recorded as '-' within the data extraction form. It is important to highlight discrepancies in the reporting of the metabolic index; which is inherently linked to the equation applied. E.g., the hypermetabolic threshold is presented as either 10 or 110%. The equations and threshold values are presented and described exactly as reported within each publication, with exception for the predictive equation terminology, as outlined in the introductory paragraph of **section 3.4.4.2.**

Seven studies used a hypermetabolic threshold of ≥ 10/110%, whilst three used a threshold of ≥ 20/120%. The study by Jesus et al., 2020 (ID 18) is counted on both occasions, as the

percentage of the study population who were identified as hypermetabolic was presented at thresholds of 10 and 20%.

Twelve studies used predictive energy equations as comparators against mREE to indicate hypermetabolism within their study populations (Table 3.9). In a similar manner as determining accuracy, the identification of hypermetabolism across the studies was dependent on the predictive energy equation used, as well as the adopted metabolic index (i.e., the percentage increase of mREE over pREE). The majority (11/12 (91.7%)) of these studies compared mREE against the HB equation, with hypermetabolism defined as a > 10/110% increase in measured REE against the HB equation in seven (58.3%) studies. Using this comparison, the incidence of hypermetabolism is reported to be between 40 and 100% of the study populations included in this review. When the hypermetabolic threshold was increased to 20/120% using the HB equation, the prevalence remained within this range (23.1-45.2%). Where the hypermetabolic thresholds were not stated, comparisons cannot be drawn.

Steyn et al., (ID 17) solely used the Nelson predictive energy equation [279] to consider fat and fat free mass. This identified 41% of the cohort to be hypermetabolic. This is lower than the proportion of study participants identified as hypermetabolic by Jesus et al., 2020 (53.3%) when hypermetabolism was identified using the Nelson equation at a threshold of 20%. Interestingly, rather than using predictive equations as a comparator, Nakamura et al., (2021;2022) (IDs 21 and 24) indicated hypermetabolism by comparing mREE against lean soft tissue mass (LSTM) measured by BIA (Table 3.9). This identified 23.91-47% of participants to be hypermetabolic.

Table 3.9 Calculation of hypermetabolism. Continuous data is presented as mean ± one standard deviation and/or median (IQR). fALS: familial amyotrophic lateral sclerosis; LSTM: lean soft tissue mass; mREE: measured resting energy expenditure; pREE: predicted resting energy expenditure; sALS: sporadic amyotrophic lateral sclerosis; SD: standard deviation.

ID	Predictive equation	Equation	Threshold (%)	Metabolic index (%)	Hypermetabolic participants (%)	
3	Harris-Benedict	-	-		67.7	
5	Harris-Benedict	-	110	14.1 ± 12.5	62.3	
6	Harris-Benedict	(mREE-pREE)/pREE	≥ 10	10.5 ± 10.9	47.54	
7	Harris-Benedict	mREE/pREE	110	fALS: 127 ± 9 sALS: 112 ± 12	fALS: 100 sALS: 52	
14	Harris-Benedict	[(mREE-pREE)/pREE] x 100	> 10	11.8 (3.7-19.8)	55.24	
16	Nelson	-	120	115 ± 21	41	
					10%	**20%**
18	HB 1919	(mREE-pREE)/pREE	10 / 20	-	55.2	23.1
	HB 1984				49.8	20.0
	World Schofield				46.7	19.7
	De Lorenzo				49.2	20.0
	Johnstone				64.1	28.9
	MSJ				72.7	47.9
	WHO/FAO				38.4	14.9
	Owen				35.2	14.6
	Fleisch				44.4	16.2

ID	Predictive equation	Equation	Threshold (%)	Metabolic index (%)	Hypermetabolic participants (%)	
	Wang				67.6	42.9
	Rosenbaum				49.1	22.6
	Nelson				76.3	53.3
19	Harris-Benedict	[(mREE-pREE)/pREE] x 100	> 10	11.5 (3.6-19.3)	55	
20	Harris-Benedict	mREE/pREE	-	1.07 (0.99-1.16)	-	
21	LSTM	mREE/LSTM	≥ 38 kcal/kg	36.4 (34.4-40.5)	23.91	
22	Harris-Benedict	[(mREE−pREE)/pREE] × 100	≥ 10	7.0 (-2.00-15.94)	40	
23	Harris-Benedict	mREE/pREE	≥ 120	121.7 ± 38.0	45.2	
24	LSTM	mREE/LSTM	≥ 38 kcal/kg	37.1 (34.5-41.2)	47	
25	Harris-Benedict	mREE / pREE	-	-	-	

Longitudinal analysis in resting energy expenditure and hypermetabolism

Whilst Kasarskis stated that REE was measured longitudinally (two-to-three time points over a six-month period), the average value for the repeated measures was presented, rather than individual time points. Longitudinal change cannot therefore be commented on; however, the metabolic index was shown to increase with proximity to death [280]. Desport et al., (2001) (ID 3) also presented the metabolic index in relation to proximity to death [263]. However, this appeared to remain stable.

Longitudinal measurements of mREE for subsequent time points were presented by Desport (2005) (ID 5 [260]), Bouteloup (2009) (ID 6 [261]), and Vaisman (2009) (ID 8 [262]). Desport et al., demonstrated a statistically significant reduction in the mREE values of 44 people living with ALS over an average delay of 332 days. The metabolic index was also found to decrease, but did not reach significance. In contrast, Bouteloup et al., reported no significant change in the mREE or metabolic index in 20 participants over a 12-month period, observing that the metabolic state remained consistent for 80% of the study participants. Bouteloup et al., concluded that hypermetabolism is a continuous phenomenon from diagnosis. Finally, Vaisman also demonstrated that REE measured in ten participants living with MND decreased over a six-month period, but did not reach significance.

3.4.4.3 Assessment of body composition

Measures of REE should be adjusted for assessments of FFM (mREE/FFM) to provide an assessment of the oxygen consumption per unit of FFM (kcal/kg). mREE/FFM was shown to significantly increase over time in the studies conducted by Bouteloup and Vaisman, which is accompanied by significant reductions in the FFM of the same participants. In contrast, Desport (2005) presented a significant negative relationship between mREE/FFM and the proximity to death, which is not accompanied by significant reductions in FFM within the cohort.

The publications that measured body composition alongside energy expenditure are shown in Table 3.3; body composition assessment. Twenty-three (92%) publications provided methods for the assessment of body composition. Bioelectrical impedance analysis (BIA) was the most commonly reported assessment of body composition, used in 18/23 (78.3%)

publications. It is worth noting, Kasarskis et al., (2014) (ID 12) details the use of bioelectrical spectroscopy; however, for purposes of this review, all bioelectric impedance analyses are grouped under BIA. Other methods of body composition assessment included: anthropometric (ANTH) measurements (e.g., triceps skinfold thickness (TSF), mid-upper arm circumference (MUAC) and arm muscle area (AMA)) (6/23); dual energy x-ray absorptiometry (DEXA) (3/23); whole-body air displacement plethysmography (1/21); and doubly-labelled water (1/21). In direct contrast to the results of longitudinal assessment of mREE presented above, mREE adjusted for assessments of FFM (mREE/FFM) showed significant increases over time. Bouteloup and Vaisman both reported that mREE/FFM - assessed using DEXA in both instances - significantly increased over time. However, Desport demonstrated mREE/FFM decreased with proximity to death.

3.5 Discussion

3.5.1 Protocols and techniques

Of the studies not reporting protocol information, three citations were identified. Desport (2001;2005) cited two review papers [281,282] which provided in-depth descriptions of the principles of IC, but did not outline protocols for conducting IC. These citations therefore cannot provide further evidence to detail how these assessments were conducted. Ellis, cited Compher et al., [283], a comprehensive systematic review which addressed the best practice methods to measure resting metabolic rate - alternative terminology for REE - in adults. This review defines the minimum appropriate parameters for the conduction of IC in adults: fasting (≥ 5 hours); resting ahead of measurement (10-20 minutes); duration of recording (10 minutes with the first five minutes discarded); and appropriate coefficient of variation values (< 10%) to assess steady state and ensure accuracy of recording.

3.5.2 Predictive energy equations as comparators against mREE

This review has identified that the Harris-Benedict 1919 equation is the most commonly used predictive energy equation within the included studies. When accuracy was defined as a difference of ± 10% from the mean mREE, the HB 1919 equation was shown to be accurate in 45.1-52% of the examined study populations, at group level. Ellis et al., identified the MSJ equation to be the most accurate predictive equation, however, the authors excluded individuals with a BMI < 18 or > 30 kg/m^{2,} and suggested these equations may be more accurate in individuals with a 'healthy' nutritional state [147].

Following identification of the inaccuracy of existing predictive energy equations, Jesus et al., (2019) developed an ALS-specific predictive equation incorporating FFM and FM using BIA [144]. This ALS-specific equation was demonstrated to accurately estimate REE in 65% of the study population (at a threshold of ± 10%). It would be interesting to know the proportion of this study population identified as hypermetabolic using the newly constructed formula.

3.5.3 Identification of hypermetabolism

The equations to calculate accuracy are simply the inverse of the metabolic index calculation. It therefore follows that the greater the underprediction of pREE, the greater the value of the metabolic index. Identification of hypermetabolism using estimates of FFM may therefore be more suitable for use in a cohort that does not follow inherent assumptions underlying the predictive equations, such as in MND. Moreover, since FFM is regarded as the biggest driver of REE [284], contributions of FFM should be considered in its prediction, e.g., the Nelson equation [279]. However, when used as a comparator against mREE by Jesus et al., (2020) [146] the Nelson equation was found to underpredict REE by the greatest margin, therefore also having the greatest influence on the metabolic index. For example, when the hypermetabolic threshold was set to 10% with the nelson equation, 76.3% of their study population was identified as hypermetabolic; this decreased to 53.3% when the threshold was adjusted to 20%. Similarly, use of the HB 1919 equation identified hypermetabolism in 55.2% of the same study population at a metabolic index of ≥ 10%, and 23.1% at ≥ 20%. The substantial discrepancy in identifying hypermetabolism using different thresholds and predictive equations is exemplified in Table 3 of Jesus et al., (2020) [146] which demonstrates statistically significant differences in the number of hypermetabolic participants according to predictive equations compared against use of the HB 1919 equation at a hypermetabolic threshold of 10%.

3.5.4 Respiratory hypothesis

Kasarskis (1996) [69] was the first to highlight an increasing metabolic index in relation to death. This developed the notion of the 'respiratory hypothesis', which proposed the increasing metabolic index was a result of increased energy demand from respiratory muscles. In agreement with Kasarskis, Siirala et al., [266] and Georges et al., [267] both presented a lower mREE in mechanically ventilated people living with MND. The authors concluded this was due to the decreased ventilatory burden of those on mechanical ventilation.

The respiratory hypothesis was challenged by Sherman, who demonstrated that mREE was increased in mechanically ventilated individuals, whilst those who were not ventilated had a lower-than-predicted mREE [264]. Further dispute of the respiratory hypothesis was published

by Desport et al., (2001;2005) [260,263] and Funalot [265], who both reported no correlation between metabolic level and functional vital capacity (FVC).

3.5.5 Longitudinal changes in metabolic state

As presented in the results section, there is conflicting evidence for longitudinal changes in mREE, the metabolic index and mREE adjusted for FFM (mREE/FFM). Whilst differences in longitudinal results can be commented on, a direct comparison (and therefore statements of agreement or disagreement) should be avoided due to differing study population sizes, follow-up durations and frequencies across the studies. In addition, the proximity to death at time of REE measurement was not always reported. Moreover, the clinical and anthropometric parameters of the study populations need to be considered for differences which may drive changes in the mREE, and subsequently the metabolic index and mREE/FFM ratio.

Measured REE was frequently adjusted for FFM in these publications in an attempt to minimise bias resulting from the difference in body size and composition of study participants. When analysing the longitudinal changes in mREE/FFM reported by Desport (2005) [260], Bouteloup [261] and Vaisman [262], it is important to consider that the study populations may demonstrate different compositions of FFM, which may be attributed to different stages of disease progression. For example, mREE/FFM is significantly increased over time in the studies conducted by Bouteloup and Vaisman, which is accompanied by significant reductions in the FFM of the same participants. In contrast, Desport (2005) presented a significant negative relationship between mREE/FFM and the proximity to death, which is not accompanied by significant reductions in FFM within the cohort [260]. The opposing results between Desport 2005 and Kasarskis (1996) [69] regarding the metabolic index in relation to death could be explained by the absence of adjustment to FFM by Kasarskis.

The method of body composition assessment should also be considered. Most of the included studies used BIA as an assessment of body composition. However, BIA is an indirect assessment, relying on derivation equations largely developed in healthy populations to calculate fat- and fat free- mass. Ellis [147] and Jésus [144–146] reported the use of a “validated ALS-

specific equation" for calculation of FM and FFM using weight, triceps skinfold thickness and BIA measurements [136].

He et al., (2022) categorised changes in metabolic index according to King's staging [276]. They presented a significant reduction in the metabolic index between King's stage 1 and 5. Moreover, participants in this study identified as hypermetabolic had a significant increase in FFM, and significant reduction in FM. He et al., proposed "energy metabolism in ALS followed an inverted U-shaped change with disease progression, beginning with a continuous increase in the preclinical stage with the turning point around symptom onset" (Figure 3.3).

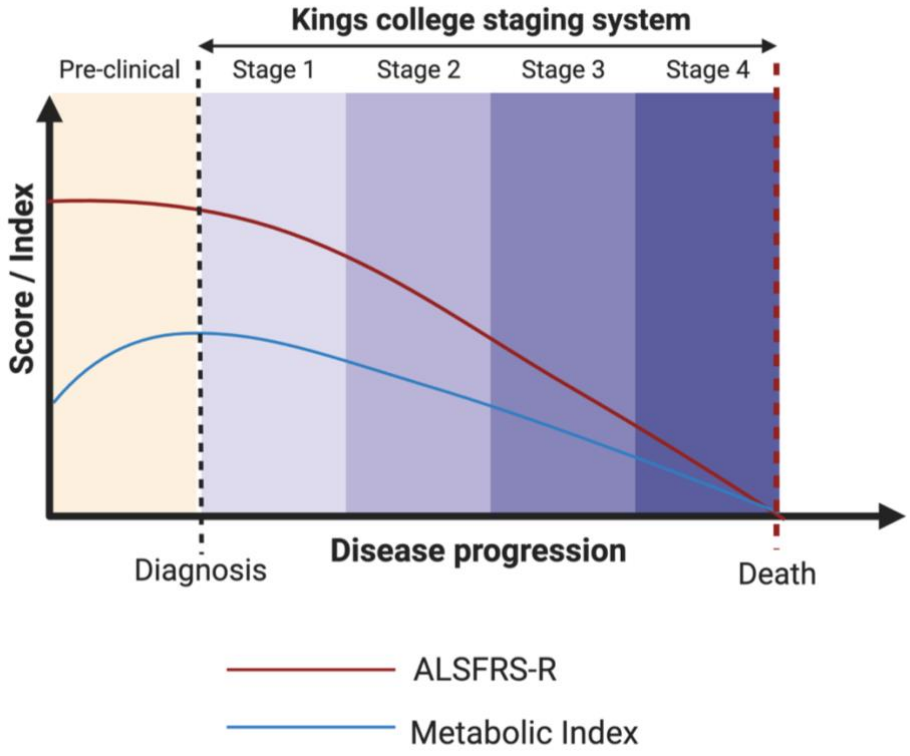


Figure 3.3 Hypothesis for dynamic changes in the metabolic index. Figure adapted with permission from He et al., 2022 [276] (Figure 5, page 1453). Created with BioRender.com

Dorst et al., 2023 [278] conducted a prospective longitudinal study to compare the metabolic rate of 60 presymptomatic ALS gene carriers with 73 individuals from the same families without pathogenic mutations. Surprisingly, the mREE and metabolic index in the presymptomatic ALS carriers was significantly *lower* than that of the controls. They found a negative correlation between metabolic index and expected time to onset, demonstrating an

increasing REE with proximity to expected disease onset. When this knowledge is combined with the results presented by Desport (2005), Vaisman and He, this supports the hypothesis of dynamic alteration proposed by He et al.

3.5.6 Hypermetabolism in relation to disease progression and severity

Jesus et al., (2018) [145] and Steyn [271] both demonstrated that the presence of hypermetabolism, identified with a metabolic threshold of ≥ 20%, was associated with an increased disease progression (indicated by a steeper ALSFRS-R slope) and increased risk of death. Interestingly, Nakamura (2021) observed that hypermetabolic individuals presenting with malnutrition had a longer survival than those with a normal weight [274]. The malnourished group was characterised by elevated LDL-cholesterol levels with a higher body fat percentage. This agrees with the hypothesis put forward by Marin et al., (2011) [53] and progressed by Jesus et al., (2018) [145] that a higher fat mass may be protective in MND.

The study by Cattaneo and colleagues was the only publication to report findings for hypometabolism within the MND cohort [275]. They observed that the hypometabolic group reached classic MND milestones (i.e., time from onset to gastrostomy or NIV) at a significantly longer time interval, showing better prognosis. In contrast, the hypermetabolic group was shown to have a steeper progression and shorter delay to reach milestones, confirming a worse prognosis and shorter survival in those with hypermetabolism.

3.6 Conclusion

Resting energy expenditure should be measured using indirect calorimetry; however, there is an absence of a standardised, validated protocol for conducting indirect calorimetry in people living with MND. Hypermetabolism is currently identified in MND by comparisons of measured resting energy expenditure against predictions of resting energy expenditure. However, the number of individuals indicated to be hypermetabolic is dependent on the predictive energy equation used, and the metabolic index threshold applied. The most commonly utilised predictive energy equation is the Harris-Benedict 1919 equation, regardless of the knowledge that this equation has been shown to be inaccurate in approximately half of the MND study populations included in this review. Measured resting

energy expenditure should be normalised against estimates of fat free mass; however, this technology isn't always available or practical in either a clinical or research setting.

4 Materials and Methods

This chapter will focus on the second objective of this PhD study: to identify and develop a suite of techniques (or 'nutritional toolkit') that can be used to deeply phenotype the nutritional status of people living with MND.

4.1 Feasibility study

A feasibility study was first conducted using biosamples provided by healthy participants. This preliminary study aimed to enable clinical and laboratory protocol optimisation, establish practicalities involved in biosample handling, storage and analysis and provide proof of concept. This development and optimisation stage was critical to ensure the production of robust and reliable data in the subsequent MND cohort clinical study. If reliability and accuracy was not determined at each stage, no further work occurred. Further information on the feasibility study can be found in **Appendix A-C**. The rest of this chapter will focus on the materials and methods involved in the MND cohort study, described below.

4.2 MND cohort study

4.2.1 Research design

This was a single-centre, prospective, longitudinal, observational cohort study entitled 'Nutritional Biomarkers in MND'. Samples and measurements were collected from a cohort of people living with MND at up to four time points within a nine-month period (baseline (month zero), month three, month six and month nine). The longitudinal collection of samples and measurements allowed for the analysis of intra-individual variation over time.

4.2.2 Research setting

Participant approach and recruitment took place within the Sheffield MND Care and Research Centre, Royal Hallamshire Hospital (Sheffield Teaching Hospitals NHS Foundation Trust). The study visits took place at the Advanced Wellbeing Research Centre, Sheffield Hallam University, Olympic Legacy Park.

4.2.3 Development of study documents

The protocol, participant information sheet (PIS), informed consent form (ICF) and all participant-facing documentation, standard operating procedures and protocols were written by the author of this thesis. Patient and public involvement (PPI) was sought prior to this study through the Sheffield Motor Neurone Disease Research Advisory Group (SMND-RAG). The SMND-RAG is a group set up to facilitate public involvement in research, consisting of patients, relatives of patients and lay public members. Initial drafts of the patient-facing documents were shared with this group and discussed at an SMND-RAG meeting. Feedback from this group contributed in shaping and formatting the study protocol and the patient-facing documents for ethics submission.

4.2.4 Ethical approval

Favourable opinion was sought and provided by the London-Fulham Research Ethics Committee (REC) (21/PR/0092) and Health Research Authority (HRA) through the Integrated Research Application System (IRAS) central allocation system (292618). Local NHS Trust governance approvals, i.e., formal confirmation of capacity and capability (CCC), was granted by the study sponsor, Sheffield Teaching Hospitals NHS Foundation Trust (STH NHS FT) (STH21332).

4.2.5 Amendments

Two substantial amendments were requested during this study. These amendments enabled the addition of: 1) collection of additional blood samples for the isolation of PBMCs (detailed in **section 4.3.4.1.3**), and 2) additional morphological assessments for body composition (detailed **in section 11.1**).

4.2.6 Participant Recruitment

To encompass the heterogeneous manner of MND, participation was not restricted by MND phenotype, site of onset or disease duration.

Inclusion criteria

- Age ≥ 18 years
- Participant was willing and able to give informed consent for participation in the study. If the participant was unable to provide written consent due to physical disability, an independent witness was present at the informed consent discussion and signed the consent form on the participant's behalf.
- Diagnosis of MND (definite, probable, possible or suspected), as defined by the El-Escorial criteria [285].

Exclusion criteria

- Age < 18 years
- Underlying significant co-morbidity (e.g., thyroid disease, cancer, or other disease) that would affect survival or metabolic state, independent of MND.
- Significant decision-making incapacity preventing informed consent by the potential participant because of a major mental disorder, (e.g., major depression), or severe cognitive decline (e.g., severe dementia).

4.2.7 Sample size and statistical opinion

Because of the exploratory nature of the study, it was determined that a sample size of 25 would be sufficient. This was guided by consultation with a professional statistician specialising in medical statistics within the University of Sheffield statistical service unit and exploratory research methodology published in similar literature [286].

4.2.8 Participant approach and informed consent

Potential participants, meeting all of the inclusion and none of the exclusion criteria, were identified and approached by a member of the clinical team within the STH NHS FT. Potential participants were provided with an invitation letter (**Appendix D**) and the PIS (**Appendix E)**. If interested in the study, the potential participant then contacted the author of this thesis to express their interest. The participant was allowed as much time as wished to consider the information, as well as the opportunity to question the author of this thesis over the phone or email.

Eligible and willing participants were asked to provide consent for participation in the study by completing the ICF (**Appendix F**) before any study-specific procedures were performed. For participants lacking ability to provide informed consent themselves, a witness (other than the person taking consent and independent from the thesis author, e.g., a family member) were present at the informed consent discussion and signed the consent form on the participant's behalf. Process consent was obtained in person at each study visit to ensure continued consent for participation in this study. A detailed visual representation of the participant recruitment process is shown in Figure 4.1.

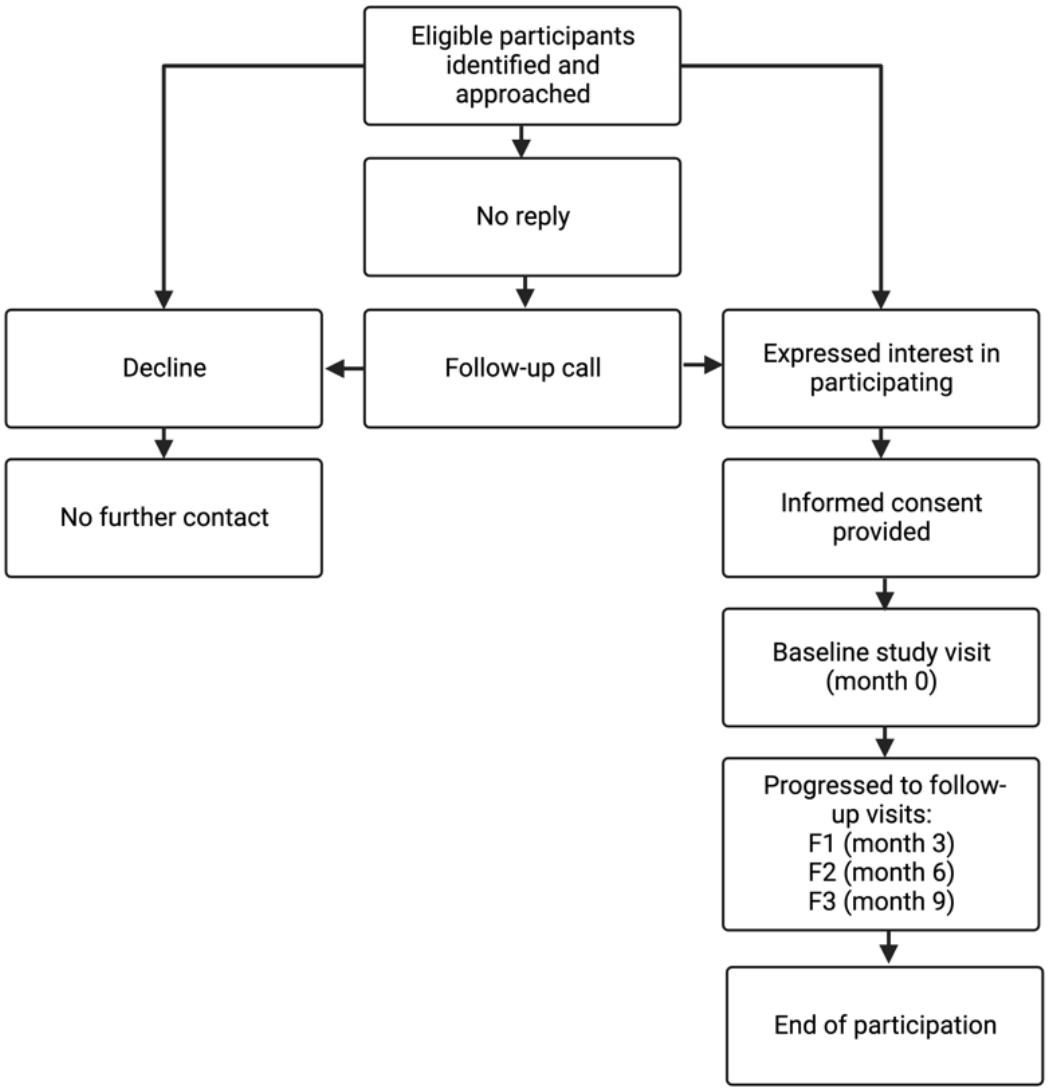


Figure 4.1 Participant recruitment process.

4.2.9 Participant discontinuation/withdrawal

Each participant had the right to withdraw from the study at any time without prejudice to future care and with no obligation to give the reason for withdrawal. In addition, the thesis author could discontinue a participant from the study at any time if it was considered necessary for any reason, including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening);
- Significant protocol deviation;
- Withdrawal of consent;
- Loss to follow up.

The reason for withdrawal and the number of study visits completed was recorded in the Clinical Research File (CRF), 'End of study pro forma'. If a participant chose to withdraw from the study, data collected until that point continued to be used. Any remaining sample(s) were destroyed if the participant specifically requested this, but data from any sample analyses already performed was retained. This was made clear in the PIS and ICF. Withdrawn participants were not replaced.

4.2.10 Data handling, recording and record keeping

At recruitment, each participant was assigned a unique alphanumeric study identifier (e.g., Pt01, whereby participant is abbreviated to Pt and the numerical value indicates participant's study number, based on the order of recruitment). The unique identifier was used to label all collected samples prior to analysis and storage.

A detailed data management and data quality issues was set out in a data management and monitoring plan. Data input and quality was the responsibility of the members of the thesis author. All data handling complied with the Data Protection Act 1998 and General Data Protection Regulation (GDPR). At the end of the study, essential documentation will be archived in accordance with STH NHS FT and University of Sheffield requirements. Any surplus

tissue is set to be destroyed at the end of the study, following guidance given in Code E of the Human Tissue Authority (HTA) [287].

4.3 Study assessments

Each participant was given the opportunity to provide clinical (assessments of disease severity and progression, the presence and/or use of percutaneous feeding and the use of ventilatory support), anthropometric, resting energy expenditure and dietary intake assessments, as well as to provide blood samples and 24-hour urinary collections. An opt-out approach was applied to enable participants to decline participation in isolated study assessments without withdrawing from the study. This option was used more as participation through the study (and therefore disease) progressed. Where possible, assessments were conducted remotely (e.g., the collection of 24-hour urine, the online self-reported dietary intake, and the ALSFRS-R).

4.3.1 Assessments of disease severity and progression

Disease severity was assessed using the revised ALS functional rating scale (ALSFRS-R) [288], mapped to the King's College staging system [289]. The ALSFRS-R is a self-administered validated functional, health outcome and quality of life questionnaire [290,291] (**Appendix G**). The ALSFRS-R is used to assess changes in physical functioning in people living with MND by subjectively comparing current functionality to a time before the onset of MND symptoms. The ALSFRS-R is comprised of 12 questions with a maximum score of 48 (four points per question) and can be divided into subscores for bulbar, fine motor, gross motor and respiratory assessment [292]. A decline in the overall or subscores of the ALSFRS-R is associated with a decreased functionality indicating an increase in the severity of the disease. MND progression can be monitored by assessing the change in ALSFRS-R functional score (ΔALSFRS-R) throughout disease duration using the following equation:

$$\Delta\text{ALSFRS-R} = (48 - \text{ALSFRS-R total score at time of assessment}) / \text{disease duration (months)}.$$

Disease duration was defined as the interval between patient-reported symptom onset and date of the baseline study visit, in months. The ALSFRS-R has been demonstrated to consistently decline by 0.92 units per month in participants engaging in clinical trials [293].

The King's College Staging System is a five-stage system based on the weakness or wasting of neurological regions, with stage five being death [289]. A reduction in any ALSFRS-R subscore maps to weakness in one neurological region. King's stages one, two and three are categorised by the number of regions involved. For example, a reduction in ALSFRS-R bulbar subscore of ≤ 11/12 indicates weakness in one neurological region, which maps to King's stage one; a reduced fine motor subscore as well as a reduced bulbar subscore suggests weakness in two regions, which maps to King's stage two, and so on. Stage four is divided in two (4A and 4B). If more than 50% of all dietary intake is consumed enterally, with administrative reliance on a care giver, this would result in a King's stage of 4A. Respiratory failure, defined by the need for non-invasive ventilation (NIV), would map to King's stage 4B [294].

4.3.2 Anthropometric measurements and indices to assess body composition

Body weight measurements were either taken at each study visit or recorded from participant recall. If weighed during the study visit, participants were asked to remove all heavy clothing and items from pockets. Body weight was recorded to the nearest 0.1 kg (SECA 875).

Participants were asked to recall their body weight (kg) at the time of symptom onset and diagnosis. This was confirmed by medical notes where possible. Percentage weight change from before the onset of MND symptoms (premorbid) was calculated using the following equation:

$$\text{Percentage weight change (\%)} = \frac{\text{Premorbid body weight (kg)} - \text{current body weight (kg)}}{\text{Premorbid body weight (kg)}} \text{x } 100$$

Body weight at diagnosis was used where premorbid measurements were not recorded or reported. A negative result was indicative of a gain in weight, and a positive result indicated a loss of weight.

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (SECA 213). BMI was calculated as body mass divided by height squared (kg/m^2) [102]:

$$\text{BMI } (\text{kg/m}^2) = \frac{\text{Body weight (kg)}}{\text{Height (m)}^2}$$

MUAC [2] was measured on both arms to the nearest 0.1 cm using a non-elasticated anthropometric measuring tape (SECA). TSF [3] was measured using a Harpenden Skinfold Caliper (Harpenden) in triplicate on both arms to the nearest 0.2 mm. AMA (cm^2), a surrogate marker of upper arm FFM was calculated by [119]:

$$\text{AMA } (\text{cm}^2) = \frac{[\text{MUAC} - (\text{TSF x } \pi)]^2}{(4 \text{ x } \pi)}$$

Calf circumferences [4] from each leg were measured in a seated position to the nearest 0.1 cm using the anthropometric measuring tape.

4.3.2.1 Using anthropometry to indicate the risk of developing malnutrition

Adopting the diagnostic criteria proposed by the ESPEN group, the nutritional risk (described in **section 2.2.3**) of developing malnutrition was indicated in this cohort using two of the following: weight loss of ≥ 5% from premorbid, a BMI of ≤ 20 kg/m^2, a MUAC of < 23.5 cm, or a calf circumference of < 31 cm. Due to the asymmetric wasting nature of MND, a result below the threshold for either the left- or right-hand side MUAC or calf circumference measurement was accepted to indicate a risk of malnutrition.

[2] MUAC is the circumference of the left upper arm and is measured at the midpoint between the tips of the shoulder and elbow.

[3] TSF is the width of a fold of skin taken over the triceps muscle. TSF is measured at the mid-point between the acromion and olecranon processes and is an indices of subcutaneous fat mass [111].

[4] Calf circumferences are measured at the largest part of the calf. This should be measured when the calf is relaxed, at an angle of 90°.

4.3.3 Assessment of dietary intake

The Best Practice Guidelines interactive tool developed by the DIET@NET project, "Nutritools" [234] (described in **section 2.4.4.1**), was used to identify the most suitable online dietary assessment tool to record a self-administered retrospective recall of energy, macro- and micro-nutrient intake in adults over a 24-hour period (Figure 4.2). The filters for an online short (24-hour) retrospective dietary recall for energy, macro- and micro-nutrients were selected in order to capture dietary intake from the same 24-hour time window as the 24-hour urinary collection. Intake24 was the only validated dietary assessment tool identified in this library.

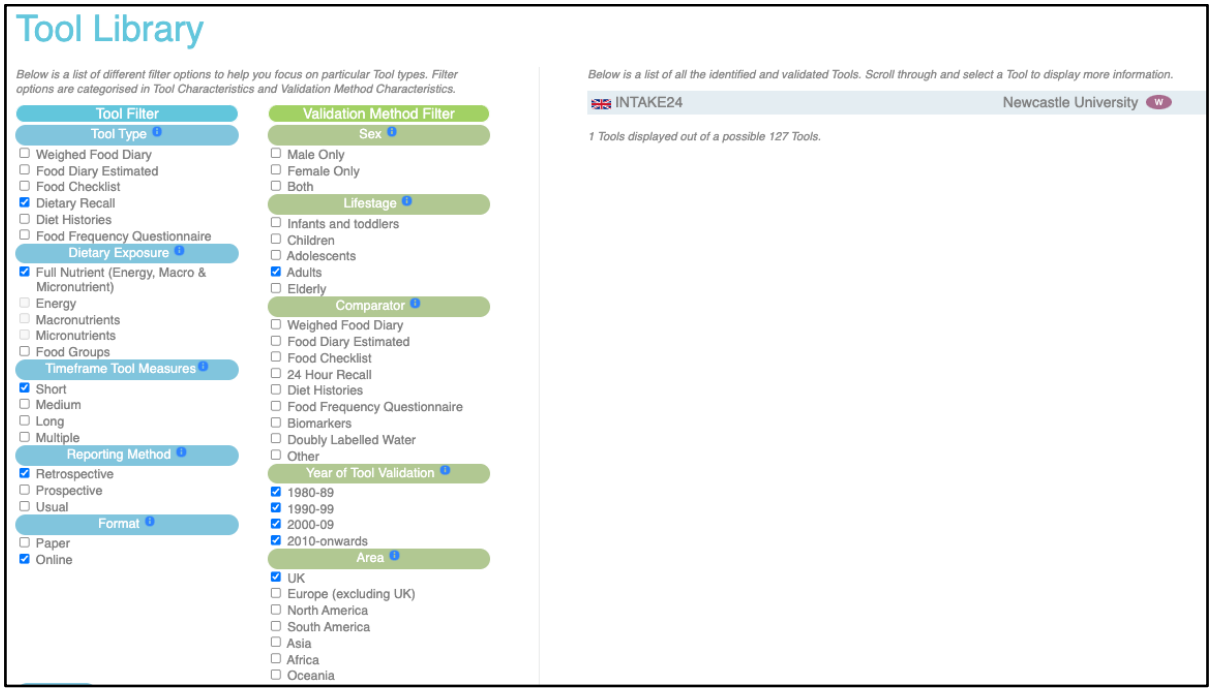


Figure 4.2 A screenshot of the DIET@NET project dietary assessment tool [234].

Intake24.co.uk [295] is an open-source, self-completed computerised dietary recall system, which has been established for its convergent- and criterion- validity, through comparison studies with interviewer-led 24hr recall methods [296–298], iterative testing [299], field testing [300,301]. Intake24 has been validated by comparison against measures of total energy expenditure using doubly-labelled water [302]. Intake24 has been used since October 2019 as the dietary assessment method for the UK National Diet and Nutrition Survey (NDNS) Rolling Programme [303].

The Intake24 system guides the user with prompts and photo aids to complete a chronological 24-hour recall diary retrospectively. However, the user had the option to complete the diary prospectively if they wished. Intake24 has a high-tolerance for spelling mistakes, to enable ease of use. It has also been populated with a food database covering over 2300 foods, which is coded to the NDNS nutrient databank [300]. Once a food-type has been identified, the system provides the option to estimate portion size by its proportion of the plate or glass, rather than entering caloric values for each ingredient (an example is shown in Figure 4.3). Intake24 provides an automated output for: energy; macro- (e.g., fat, carbohydrate, protein, sugars) and micro- (e.g., vitamins, sodium, potassium) nutrient food groups.

Participants received guidance on how to complete the online recall diary when they consented to participate in the study. Participants were also offered the option of completing a paper-based recall, to be transferred onto the Intake24 system with the assistance of the thesis author at their study visit. The thesis author did not influence the participant recall, and paper recalls were not transferred to the Intake24 questionnaire without the presence of the participant or carer.

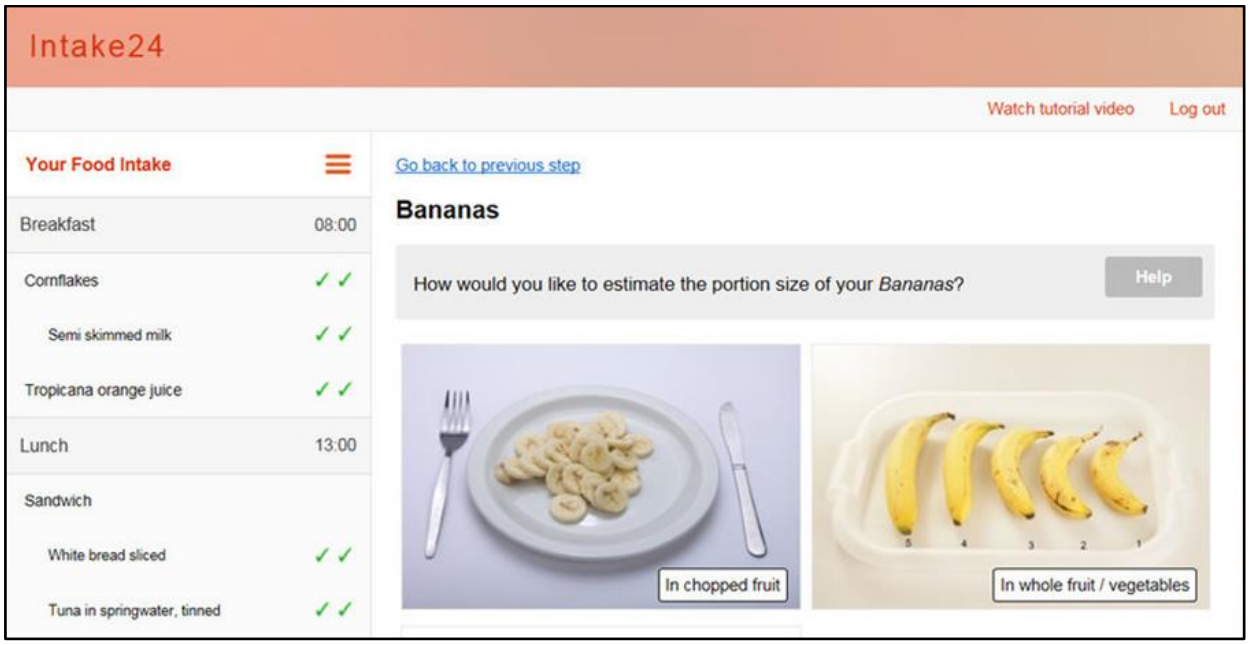


Figure 4.3 A screenshot example of the Intake24 questionnaire [304].

4.3.3.1 Comparison against UK Dietary Reference Values

Estimates of macronutrient (carbohydrate, fat and protein) intake using Intake24 were compared against the UK Government estimated average requirement (EARs) for energy and macronutrients intake, with recommended values given for sex and age group, as shown in Table 4.1 [305]. Reported intake that was below the EAR was used as an indicator of nutritional risk. Reported dietary intake was evaluated for reliability by bivariate correlation analysis against the concentration of biochemical analytes.

Table 4.1 Public Health England Government Dietary Recommendations for energy and macronutrient intake [305].

	≤ 64		65 - 74		≥ 75	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Energy (kcal/day)	2500	2000	2342	1912	2294	1840
Protein (g/day)	55.5	45	53.5	46.5	53.3	46.5
Fat (g/day)	97	78	91	74	89	72
Carbohydrate (g/day)	333	267	312	255	306	245

4.3.4 Biosample collection, processing and analysis

4.3.4.1 Blood samples

A blood sample of up to 75 ml was undertaken with aseptic technique. A tourniquet was applied and the skin overlying the vein was cleaned with an alcohol-based skin wipe. A 21- or 23-gauge butterfly needle was used with a vacutainer system to obtain the blood samples. Blood was sent to the Medical Laboratory at the Northern General Hospital, Sheffield, and was tested for the following nutritional analytes: serum albumin, prealbumin, creatinine, retinol-binding protein, transferrin, ferritin, lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol triglycerides) and eGFR (Table 4.2). The concentration of routine inflammatory parameters: C-Reactive Protein, full blood count, erythrocyte sedimentation rate and fibrinogen were also measured to exclude or confirm a pro-inflammatory state.

Table 4.2 Biochemical analytes for the assessment of nutritional state and dietary intake

Analyte	Marker of
Serum	
Creatinine	Muscle mass
Albumin	Protein intake
Prealbumin	Protein intake
Retinol-binding protein	Vitamin A intake
Transferrin	Iron intake and protein-energy malnutrition
Ferritin	Iron intake / deficiency
Lipid profile (cholesterol, HDL-C, LDL-C, triglycerides)	Cholesterol and triglyceride concentration
eGFR	Marker of kidney function and malnutrition
24-hour urine	
24-hour urinary sodium	Sodium/Salt intake
24-hour urinary potassium	Potassium intake
24-hour urinary urea	Protein intake
24-hour total urinary nitrogen	Protein intake

Additional blood was drawn and processed by the thesis author – sometimes with the support of a laboratory technician at the University of Sheffield (UoS) – to obtain plasma [5], serum [6] and peripheral blood mononuclear cells (PBMCs) [7]. Collected biosamples were processed immediately upon collection (pictured schematically in Figure 4.4), as per the Human Tissue Act 2004, before being aliquoted and stored in a -80 ^{o}C freezer or liquid nitrogen Dewar within the UoS.

[5] The liquid component of the blood with no cells.

[6] Plasma without the fibrinogen and clotting factors.

[7] PBMCs are isolated from peripheral blood and identified as any blood cell with a round nucleus. Examples include monocytes, lymphocytes and dendritic cells).

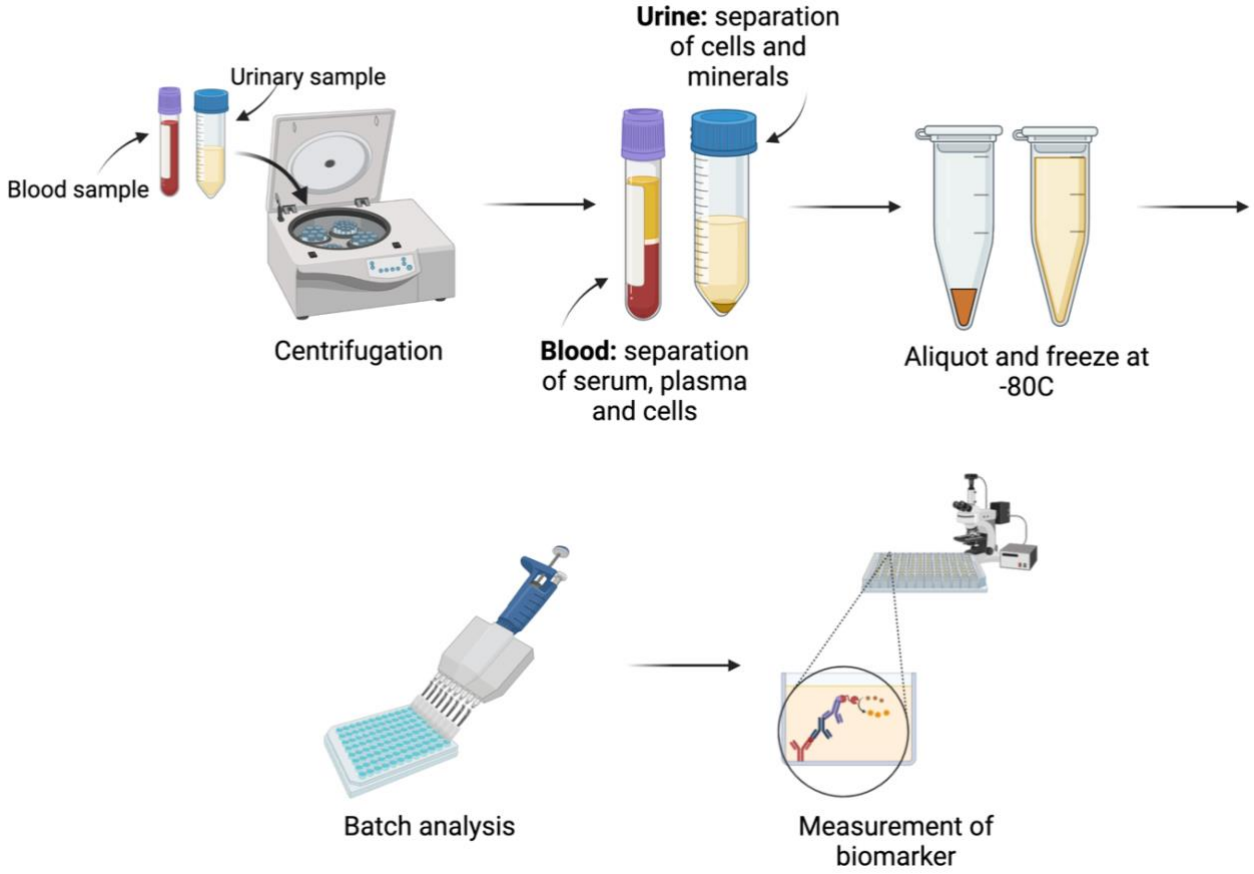


Figure 4.4 A schematic to demonstrate blood and urinary sample processing. Blood was drawn and processed to obtain plasma, serum and peripheral blood mononuclear cells (PBMCs). 24-hour urinary collections were weighed, mixed and a representative sample was taken from the collection. Collected biosamples were centrifuged immediately upon collection before being aliquoted and stored in a -80 ^{o}C freezer or liquid nitrogen Dewar within the UoS. Batch analysis was then conducted on these samples to measure biochemical analytes of interest.-Created with BioRender.com.

4.3.4.1.1 Serum isolation

Whole blood collected in SST-vacutainers were kept upright and allowed to clot on ice for 30-60 minutes. Samples were centrifuged at 2350 g for 10 minutes at 4 °C, resulting in a serum supernatant.

4.3.4.1.2 Plasma isolation

Whole blood collected in EDTA-treated vacutainers was centrifuged at 800 g for ten minutes at room temperature. Any plasma demonstrating haemodialysis was discarded. The plasma supernatant was removed and centrifuged at 2350 g for 10 minutes at 4 °C.

4.3.4.1.3 Peripheral blood mononuclear cell isolation

After removal of plasma supernatant, the remaining whole blood was diluted with sterile PBS at a ratio of 1:1. Ficoll-density centrifugation was conducted with lymphoprep, a density gradient media, at a ratio of 2:1 before centrifugation at 710 g for 40 minutes, brake 1, acceleration 1, at room temperature. The resulting PBMC buffy layer was removed and washed twice with sterile PBS by centrifugation at 430 g for 10 minutes at 4 °C. PBMCs were resuspended at a concentration of 7 million cells per ml of freezing media (10% DMSO in FBS) (Figure 4.5).

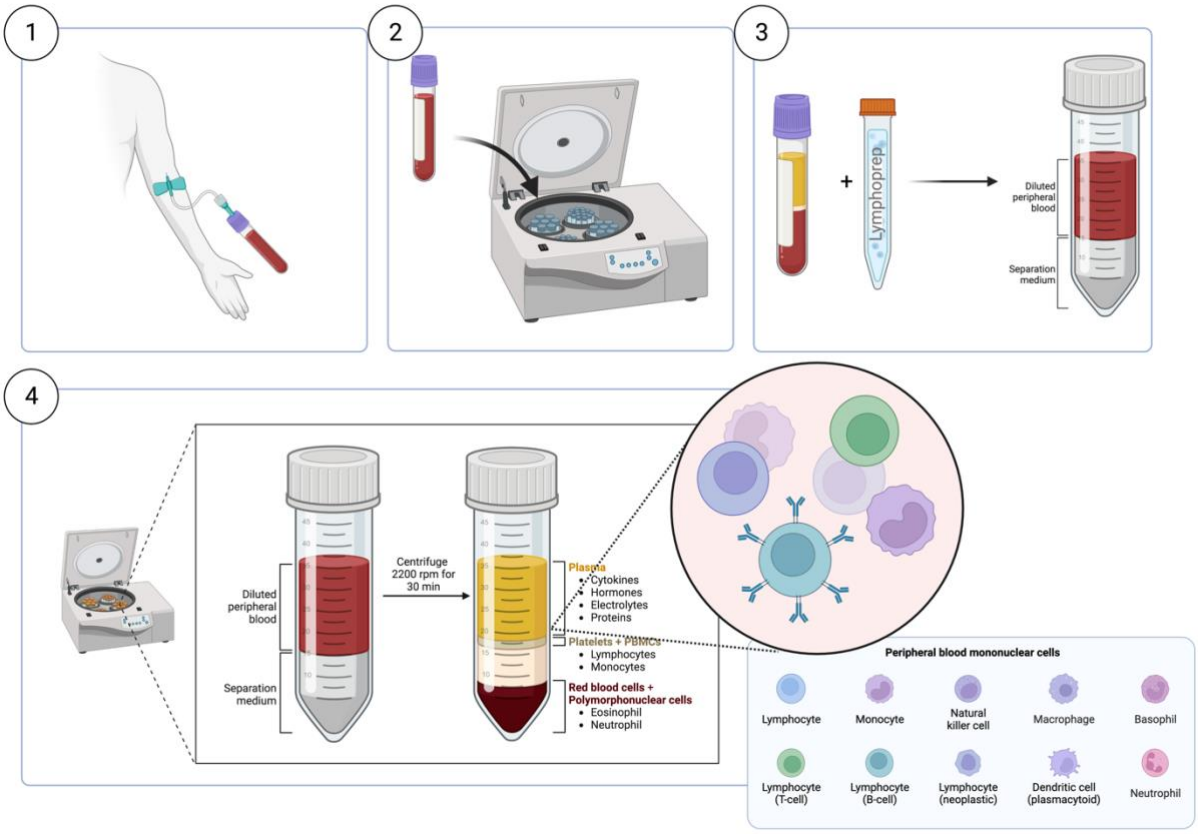


Figure 4.5 Peripheral blood mononuclear cell isolation process from whole blood. Blood was drawn (1) and centrifuged (2) to obtain plasma. After removal of the plasma supernatant, the remaining whole blood was diluted with sterile PBS at a ratio of 1:1 (3). Ficoll-density centrifugation was conducted with lymphoprep at a ratio of 2:1 before centrifugation at 710 g for 40 minutes at room temperature (4). The resulting PBMC buffy layer was removed and washed twice with sterile PBS by centrifugation at 430 g for 10 minutes at 4 °C. PBMCs were resuspended at a concentration of 7 million cells per ml of freezing media (10% DMSO in FBS). Created with BioRender.com.

4.3.4.2 24-hour urinary collection

The NHANES study procedures manual was used as a guide for the development of the 24-hour urinary collection and processing standard operating procedure in this study [191]. Participants were asked to collect all urine from one 24-hour period the day prior to their study visit (participant instructions in **Appendix H**). Participants discarded their first urinary

excretion of the day, and collected all following excretions for the next 24-hours. Participants completed the 24-hour collection on the same day as their study visit, and returned their containers at their study visit on this day. To ensure adherence to the provision of a complete 24-hour urinary collection, participants were requested to record the start and end timings of their collection, as well as timings of all samples collected and details of any spillages or missed collections (collection sheet in **Appendix H**). 24-hour urinary collections were deemed complete if the following criteria were met:

- The start and end times were provided;
- The final volume was > 400 ml;
- The total collection time was > 22 hours.

24-hour urinary collections were weighed, pH-checked and aliquots taken for analysis of 24-hour urinary potassium, sodium and urea by the Medical Laboratory team at the Northern General Hospital, Sheffield. The remaining 24-hour urinary collection was returned to the UoS where the author of this thesis and supporting laboratory technician centrifuged a representative sample of the collection at 2000 g for five minutes at 4°C to remove cellular and solid material (Figure 4.4). The supernatant was aliquoted and stored at -80 °C or liquid nitrogen within UoS premises until analysis for total urinary nitrogen was conducted on each sample.

4.3.4.3 Using biochemical analytes to indicate a risk of developing malnutrition

Reductions in serum or 24-hour urinary biochemical analytes below pre-defined reference ranges provided by the Northern General Laboratory Medicine protocols [306] were used to indicate a risk for malnutrition.

4.3.4.4 Measurement of total urinary nitrogen (TUN): Micro-Kjeldahl analysis

To gain an estimate of an individual's protein intake, total urinary nitrogen (TUN) from 24-hour urinary collections was analysed using Micro-Kjeldahl analysis (Gerhardt Analytical Systems [307]). Commonly used in the food industry, MK is a three-step process comprised of digestion, distillation and titration of nitrogen-rich samples. This method was adapted by the thesis author for the analysis of TUN in urinary samples using 24-hour urinary collections from

healthy participants. These results are presented in **Appendix A**. In brief, 0.1 g of ammonium sulphate was used as a reference standard. Three millilitres of each 24-hour urinary collection were weighed and added to 98% sulphuric acid and a catalyst tablet before heating to 240 °C to digest the proteins within the sample (Figure 4.6, picture 1). Once digested, steam distillation occurred in the presence of sodium hydroxide and deionised water to release ammonia gas. Ammonia was condensed rapidly and captured in a 40% boric acid reservoir with pH indicator (Merck, Mixed indicator 5) (Figure 4.6, picture 2). Acid-base titration was then performed using a standard solution of 0.1 M hydrochloric acid (HCl). The volume of HCl required to neutralise the boric acid reservoir was incorporated into the equation below to calculate the percentage of nitrogen in each urinary sample (Figure 4.6, picture 3) using the following equation:

$$\% \text{ Nitrogen} = \frac{(\text{sample titre (ml)} - \text{blank titre (ml)} \text{ x } 0.1 \text{ x } 1.4007}{\text{weight of sample (g)}}$$

The percentage of nitrogen was converted into grams/day (g/day) by considering the volume of each 24-hour urinary collection.

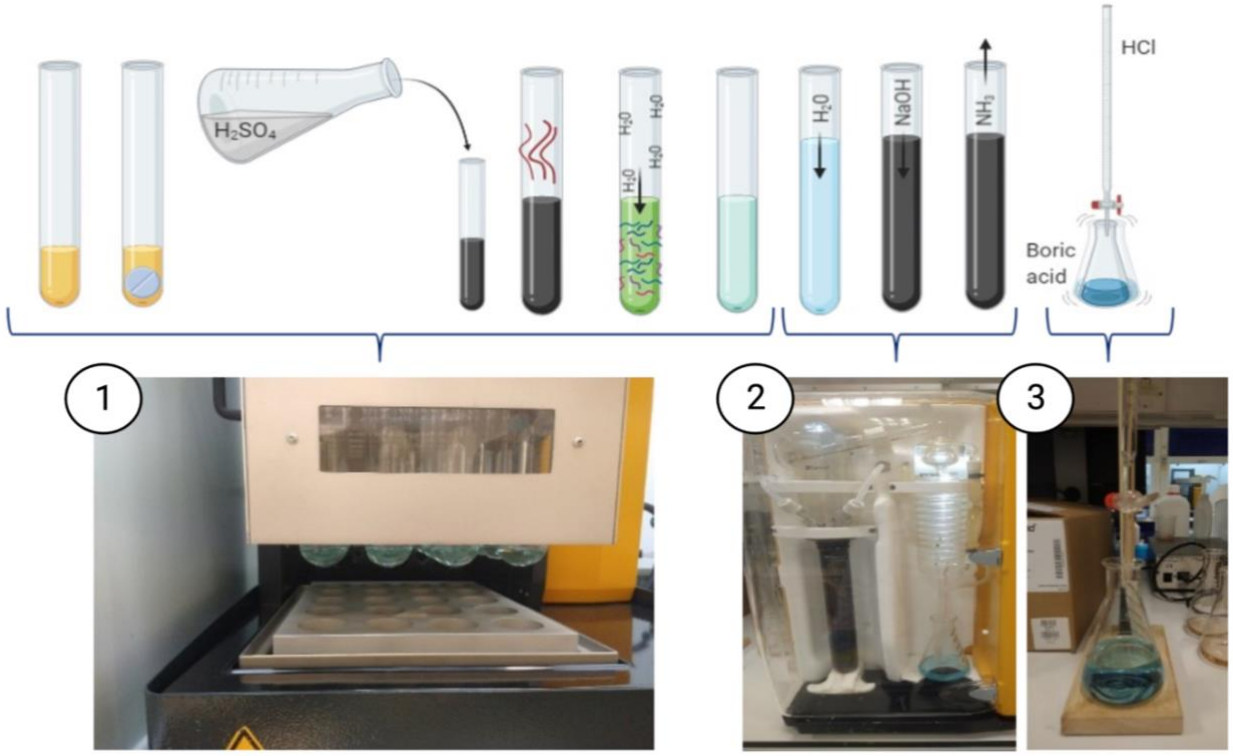


Figure 4.6 Photographs and schematics to demonstrate the Micro-Kjeldahl process. (1) Digestion: Three millilitres of each 24-hour urinary collection were weighed and added to 98% sulphuric acid and a catalyst tablet. The samples were heated to 240 °C. 0.1 g of ammonium sulphate was used as a reference standard. (2) Distillation: steam distillation occurred using sodium hydroxide and deionised water to release ammonia gas. Ammonia was condensed rapidly and captured in a 40% boric acid reservoir with pH indicator. (3) Titration: acid-base titration was then performed using a standard solution of 0.1 M hydrochloric acid (HCl). Created with BioRender.com

4.3.5 Assessment of resting energy expenditure

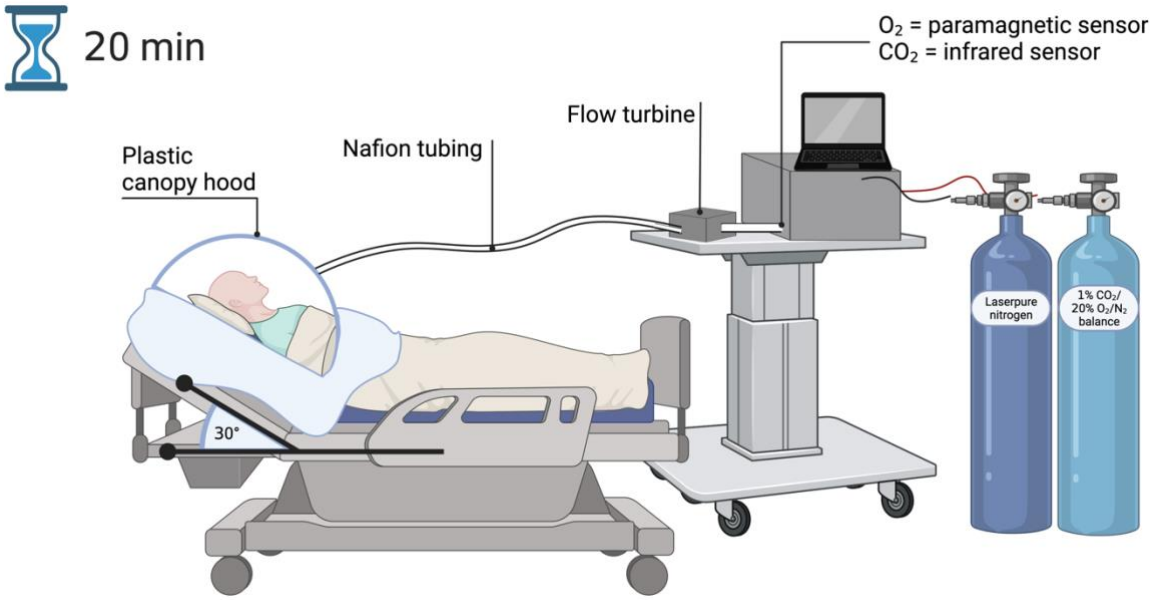


Figure 4.7 A schematic to show the conduction of indirect calorimetry. Participants were rested in a seated position for one hour prior to measurement. The GEMNutrition metabolic cart (GEM) was first calibrated using two reference gases. Indirect calorimetry was conducted using a plastic canopy hood connected to the GEM with Nafion tubing. The participant lay semi-supine at an angle of 30° from horizontal, or seated where this was not possible, throughout the measurement. Measurement lasted for 20 minutes. Created with BioRender.com.

As a result of the findings from the scoping review presented in Chapter 3, it was concluded that the most suitable approach to measure resting energy expenditure in this MND cohort was using indirect calorimetry. A protocol was developed which was considered suitable and appropriate for the study participants. This is shown schematically in Figure 4.7. The GEMNutrition (GEM) metabolic cart [308] was calibrated ahead of each measurement using Laserpure (99.998%) nitrogen and 1% CO_2/20% O_2/N_2 balance calibration gases (span calibration) and samples of room air (inspired O_2 and CO_2). Span gases were compared against predefined reference ranges [309] for quality management.

Participants were rested in a seated position for one hour prior to measurement. The measurement lasted 20 minutes in either a semi-supine (30° from horizontal) or seated

position, allowing for participant mobility and respiratory complications. The first five minutes of each measurement were discounted from analysis (the wash-out period) to flush out the room air and increase the possibility of reaching a steady state (coefficient of variation (CV) ≤ 5%). Participants did not sleep or talk during the measurement. Flow rate was adjusted to ensure the fraction of expired (F_e) CO_2 value was between 0.4 and 0.7.

As outlined in the scoping review, to ensure best practice when conducting indirect calorimetry, participants should be fasted for a minimum of five hours ahead of measurement [283]. However, a realistic, pragmatic approach was applied to measure resting energy expenditure (mREE) in this study cohort. The time of day for the study visit (and therefore calorimetry measurement) was not standardised, but instead influenced by participant and carer availability to reduce burden. Whilst fasting for five hours would be considered achievable for healthy individuals, it was not deemed appropriate to ask individuals to disrupt their daily routines, meal times and subsequent medication timings, especially when the overarching purpose of this study is to maintain a healthy nutritional state. Participants were therefore not required to be in a fasted state for this study assessment, but rather asked to recall the time of their last meal and what it was. This enabled the possibility of applying a thermogenic factor to the mREE, if deemed necessary.

4.3.6 Calculation of measured resting energy expenditure

Macronutrients (carbohydrates, fats and proteins) are chemical energy which are metabolised to provide cellular energy. This is termed 'metabolisable energy'. Metabolisable energy is quantified by the difference between energy intake and energy loss. In the presence of oxygen, carbon-based nutrients are converted into carbon dioxide, water and heat. Indirect calorimetry measures whole-body energy expenditure and substrate utilisation by calculating substrate and oxygen usage, and the subsequent quantification of by-products (i.e., the quantity of oxygen consumed, and carbon dioxide produced, respectively [310]:

$$\text{Substrate} + O_2 \rightarrow CO_2 + H_2O + \text{heat}$$

Metabolisable energy can be lost as heat, known as the thermic effect of food. This contributes to 10% of total daily energy expenditure [252]. The term 'indirect' calorimetry stems

from the indirect determination of heat production by measuring O_2 consumption and CO_2 production, rather than directly measuring heat production, as is the case in 'direct' calorimetry. Therefore, whilst the term 'energy expenditure' is commonly used, this process is actually measuring **energy production.**

4.3.6.1 Direct and indirect measurements

It is important to note the difference between direct measurements of indirect calorimetry, and subsequent derived estimates, which can introduce error. Oxygen is directly measured by a paramagnetic sensor, whilst CO_2 is detected by an infra-red analyser. These signals are detected as voltages, which are converted into digital outputs by an analog-to-digital converter. This produces direct measurements of the fraction of inspired (F_i) and expired (F_e) O_2 and F_i and F_e CO_2. Airflow rate and values of F_i and F_e O_2 and CO_2 are utilised in Haldane's transformation to calculate volumes of oxygen (VO_2) and carbon dioxide (VCO_2) [311]. VO_2 and VCO_2 can in turn be used to derive mREE using the **Weir equation** [312]:

$$\text{mREE (kcal/day)} = [(3.941 \times VO_2) + (1.106 \times VCO_2)] \times 1.44 - (2.17 \times \text{TUN})$$

Where mREE is measured resting energy expenditure (kcal/day), VO_2 is the volume of oxygen consumed (ml/min), VCO_2 is the volume of carbon dioxide produced (ml/min) and TUN is total urinary nitrogen (g/day). The protocol for TUN using Micro-Kjeldahl analysis is outlined in **section 4.3.4.4.** TUN results are presented in **section 7.1.4**. The values of 3.941, 1.106 and 2.17 reflect the caloric contributions from 1L of oxygen metabolism [312]; 1.44 reflects the minutes per day.

The inclusion of total urinary nitrogen in the Weir equation reduces measurement error to provide the most accurate derivation of mREE possible. However, in the original publication, Weir et al., reported the error in omitting protein metabolism is approximately 1% [312]. The **abbreviated Weir equation** is therefore more commonly used in automated indirect calorimetry systems [313]:

$$\text{mREE (kcal/day)} = [(3.94 \text{ X } VO_2) + (1.11 \text{ X } VCO_2)] \times 1.44$$

Carbohydrates, proteins and fat are oxidised at different rates. I.e., it takes more oxygen to metabolise one mole of fat than one mole of carbohydrate. The respiratory quotient (RQ) is

a quantifiable measure of macronutrient oxidation into energy. The RQ is calculated by the ratio of volume of carbon dioxide expired over the volume of oxygen consumed:

$$\mathrm{RQ} = \frac{\mathrm{VCO_2}}{\mathrm{VO_2}}$$

An RQ value of 0.70 is indicative of a predominant oxidation of fat, whilst a value of 1.00 identifies carbohydrate oxidation [314]. It is therefore thought that RQ can be used to estimate dietary intake [251].

At each stage of calculating mREE, there is a possibility of the introduction of random or systematic errors, which can be compounded if not identified and addressed. Thus, reporting of mREE is not always the most suitable, or pragmatic, approach and more direct values, such as VO_2 should also be provided.

4.3.7 Assessment of total daily energy expenditure

Predictive energy equations for the estimation of total daily energy expenditure (TDEE) were developed by Kasarskis et al., (2014) [134]. These predictive equations were developed in a heterogeneous cohort of 80 people living with ALS. TDEE was first measured using the doubly-labelled water method. Statistical models were then used to accurately estimate TDEE using clinically-accessible parameters [134]. The Kasarskis Model 6 predictive energy equation was applied to this cohort to estimate TDEE.

For men, the equation is:

$$\text{TDEE (kcal/day)} = [66 + (13.7 \times \text{weight (kg)}) + (5 \times \text{height (cm)}) - (6.76 \times \text{age (years)}] + (55.96 \times \text{ALSFRS-6 score}) - 168$$

And for women:

$$\text{TDEE (kcal/day)} = [655 + (9.6 \times \text{weight (kg)}) + (1.8 \times \text{height (cm)}) - (4.7 \times \text{age (years)}] + (55.96 \times \text{ALSFRS-6 score}) - 168$$

The ALSFRS-6 score is calculated from the sum of questions 1 (speech), 4 (handwriting), 6 (dress and perform self-care activities), 7 (turn in bed and adjust bed clothes), 8 (ability to walk) and 10 (shortness of breath) of the ALSFRS-R [315].

5 Recruitment and Cohort Description

The following results chapters (Chapters 5-9) focus on objective three: to investigate and demonstrate the role of the techniques described in Chapter 4 to understand the nutritional status of a cohort of people living with MND.

5.1 Recruitment

The study ran for 21 months. This included a ten-month recruitment period, an eight-month follow-up period after recruitment of the last participant, and a two-month period for final data analysis and dissemination. Data collection and analysis ran concurrently with the recruitment and follow-up periods.

Fifty-nine potential participants were approached between October 2021 and August 2022 by a member of the MND clinical team. Fifteen potential participants declined participation, 20 did not respond to the invitation and 24 (19 men, 5 women who met defined eligibility criteria) provided informed consent to participate in this study. Two participants did not complete baseline assessments: one was lost to follow-up, and the other withdrew consent. A further eight participants withdrew during the follow-up process: three provided written confirmation of withdrawal, three were lost-to-follow-up, one was withdrawn by the thesis author, and one participant died after the second follow-up study visit. Figure 5.1 shows a flowchart for the participant approach, recruitment and participation process.

Twenty-two participants completed clinical and nutritional assessments at the baseline study visit. Twenty participants completed the first follow-up assessment (F1), 17 completed the second follow-up study visit (F2) and 14 completed the third follow-up study visit (F3). For participants completing all four study visits, the average study duration was 9.79 months (± 0.58).

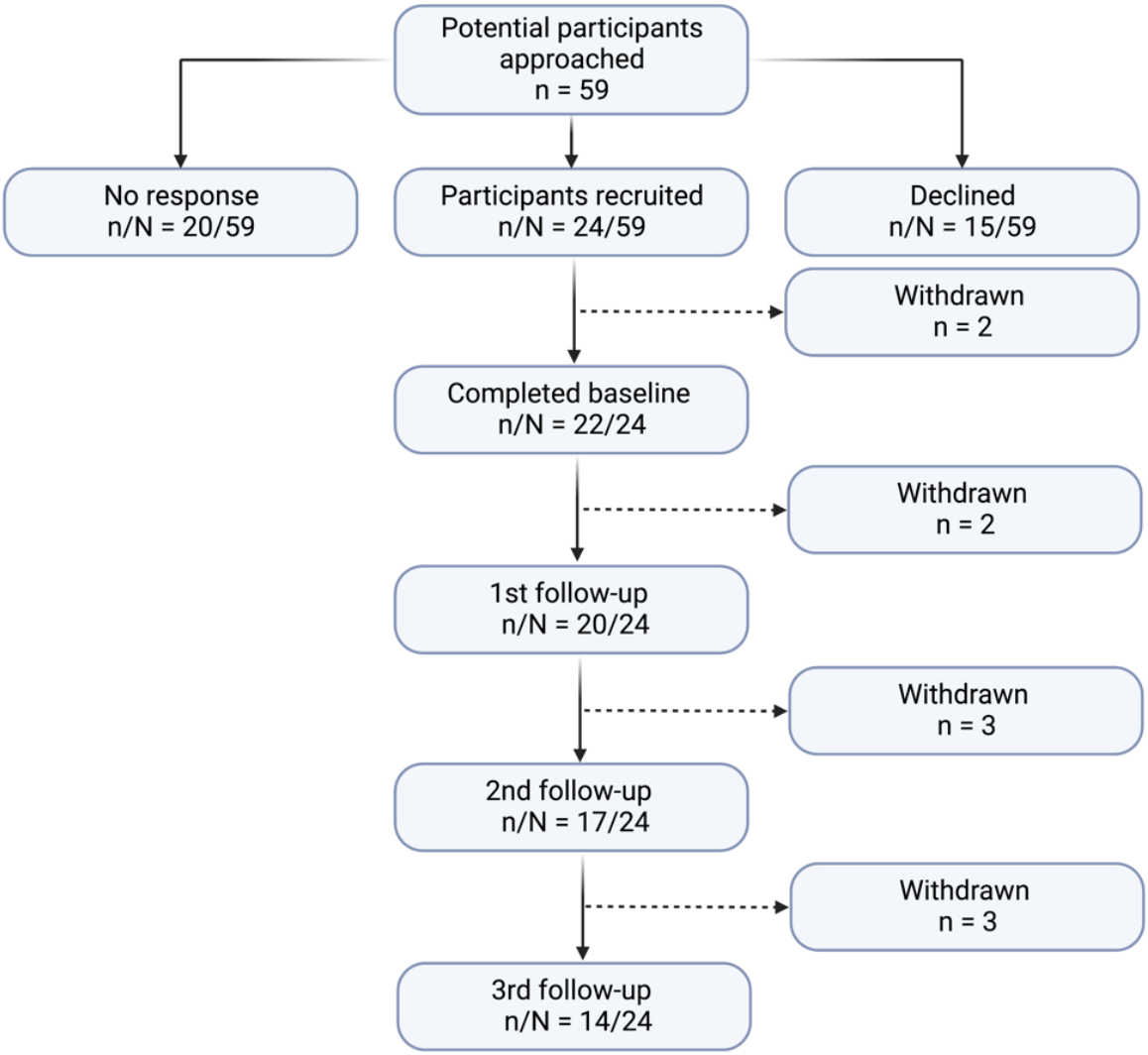


Figure 5.1 Participant approach, recruitment and participation process. Data presented as n/N. Fifty-nine potential participants were approached. Fifteen potential participants declined participation, 20 did not respond to the invitation and 24 provided informed consent to participate in this study. Ten participants withdrew throughout the duration of the study. Twenty-two participants completed the baseline study visit, 20 completed the first follow-up assessment (F1), 17 completed the second follow-up study visit (F2) and 14 completed the third follow-up study visit (F3).

5.2 Demographics and clinical information

The previous chapter presented the set up for the MND cohort study. This chapter presents the demographic (age and sex) and clinical (MND phenotype, site of disease onset (upper- or lower-limb, bulbar, respiratory or mixed), disease duration, severity and progression indices) data from all MND participants recruited to this study. This will set the scene to present the relationship between clinical severity and progression in relation to nutritional status later in the thesis.

5.2.1 Baseline results

Baseline demographic and clinical data for the study population is shown in Table 5.1. The male-to-female ratio was 19:5, with an average age of 63.64 ± 11.22 years. The age of each participant at baseline was used for all equations and longitudinal analysis. The majority of participants (7/22 (31.82%)), had a lower limb onset and ALS was the most prevalent phenotype (16/22 (72.73%)). Intra-cohort disease duration was highly variable. Whilst the average disease duration was 46.64 months (± 47.22), this was highly skewed (2.27) by two atypical slowly progressing participants with disease durations of 170 and 188 months. Fifty percent of the cohort had experienced symptoms for less than 27 months at their first study visit. The average ALSFRS-R score was 33.5/48 (± 6.25). When mapped to the King's staging system, 8/22 (36.36%) participants demonstrated a King's score of 4B, indicating respiratory failure, with 50% of study participants reporting weakness or wasting in two-to-three neurological regions. Despite the presence of prophylactic gastrostomy in five participants (22.73%), no participant utilised enteral nutrition at baseline. Fifteen (68.18%) participants did not use non-invasive ventilation (NIV); six (27.26%) used overnight NIV and one participant (4.55%) used NIV intermittently throughout the day, as well as overnight. A non-statistically significant positive relationship was observed between the length of disease duration and disease severity, assessed with the total ALSFRS-R score (Spearman's $r = 0.40$, $p = 0.06$).

5.2.2 Longitudinal changes

Table 5.1 shows the longitudinal changes in the MND clinical parameters for all study participants at all study visits. The mean ALSFRS-R total score and gross motor subscore significantly decreased between baseline and the second and third follow-up study visits; however, the change in functional score (ΔALSFRS-R) was not observed to statistically significantly decrease between baseline and any follow-up study visits.

Two participants underwent gastrostomy insertion between baseline and F3, but neither switched entirely to enteral feeding during participation in the study. Three participants increased their use of NIV: one participant reported an increase from no respiratory support to intermittent NIV use between baseline and the first follow-up study visit, and two others increased from overnight use at baseline, to 24-hour use by the third follow-up visit.

Table 5.1 Demographic and MND-clinical assessments. 22 participants completed baseline assessments, 20 participants completed follow-up 1 (F1), 17 completed follow-up 2 (F2) and 14 completed follow-up 3 (F3). Categorical data is presented as n/N (percentage of population (%)). Continuous data is presented as mean ± one standard deviation and median (IQR). Changes in longitudinal data were analysed for significance using Dunnett's mixed method for multiple comparisons test for normally distributed data or Wilcoxon matched-pairs signed rank test for non-normally distributed data. Significance observed at $p < 0.05$, highlighted in bold. ALSFRS-R: Amyotrophic Lateral Sclerosis functional rating scale – revised; SD: standard deviation; ΔALSFRS-R: change in functional score.

					P value		
	Baseline	**F1**	**F2**	**F3**	**B – F1**	**B – F2**	**B – F3**
Sex							
Male	18/22 (81.82)	16/20 (80)	15/17 (88.24)	12/14 (85.71)			
Female	4/22 (18.18)	4/20 (20)	2/17 (11.76)	2/14 (14.29)			
Age, years	63.64 ± 11.22						
	61.50 (56-72)						
MND phenotype							
ALS	16/22 (72.73)						
PMA	3/22 (13.64)						
PLS	1/22 (4.55)						
PBP	1/22 (4.55)						
unspecified	1/22 (4.55)						
Site of onset							
Bulbar	4/22 (18.18)						
Upper limb	6/22 (27.27)						
Lower limb	7/22 (31.82)						
respiratory	2/22 (9.10)						
mixed	3/22 (13.61)						

Duration since symptom onset (months)	46.64 ± 47.22 27.00 (22.75-52.50)	43.85 ± 36.79 30.00 (23.00-51.00)	50.12 ± 39.19 36.00 (25.00-63.00)	58.71 ± 41.24 43.00 (34.25-75.75)			
Disease Severity							
King's Staging							
1	3/22 (13.64)	3/20 (15.00)	1/17 (5.88)	2 (14.29)			
2	5/22 (22.73)	3/20 (15.00)	4/17 (23.53)	1 (7.14)			
3	6/22 (27.27)	6/20 (30.00)	6/17 (35.29)	7 (50.00)			
4A	0/22 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)			
4B	8/22 (36.36)	8/20 (40.00)	6/17 (35.29)	4 (28.57)			
ALSFRS-R (/48)	33.50 ± 6.25 32.00 (29.00-38.00)	32.15 ± 6.71 31.00 (26.50-39.00)	29.12 ± 8.73 29.00 (21.50-37.00)	29.07 ± 9.71 28.50 (22.00-37.50)	n = 20 0.30	n = 17 **0.003**	n = 14 **0.01**
Bulbar (/12)	9.68 ± 2.53 10 (8.75-12)	9.45 ± 2.84 10.00 (9.00-12.00)	9.29 ± 3.12 10.00 (7.50-12.00)	8.79 ± 3.64 9.00 (8.00-12.00)	n = 20 0.81	n = 17 0.25	n = 14 0.06
Fine motor (/12)	7.64 ± 2.46 7.5 (5.75-9.25)	7.10 ± 3.04 7.00 (5.25-9.00)	5.71 ± 3.24 5.00 (3.00-8.50)	5.75 ± 3.25 5.00 (3.00-8.25)	n = 20 0.99	n = 17 0.18	n = 14 0.09
Gross motor (/12)	7.27 ± 2.43 7.5 (5.75-9.00)	6.90 ± 2.77 7.00 (5.00-8.75)	5.65 ± 2.15 5.00 (4.00-7.00)	5.50 ± 2.53 5.00 (3.75-8.25)	n = 20 0.13	n = 17 **0.0001**	n = 14 **0.008**
Respiratory (/12)	8.91 ± 3.70 11 (6.5-12)	8.70 ± 3.73 11.00 (6.25-11.75)	8.47 ± 4.16 10.0 (5.00-12.00)	9.21 ± 3.79 11.00 (7.50-12.00)	n = 20 0.74	n = 17 0.20	n = 14 0.38
ΔALSFRS-R	0.59 ± 0.51 0.48 (0.18-0.76)	0.54 ± 0.39 0.40 (0.22-0.73)	0.58 ± 0.46 0.39 (0.22-0.92)	0.48 ± 0.43 0.33 (0.16-0.75)	n = 20 0.57	n = 17 0.96	n = 14 0.67
Gastrostomy							
Present	5/22 (22.73)	6/20 (30.00)	6/17 (35.29)	5/14 (35.71)			
Not present	17/22 (77.27)	17/20 (70.00)	14/17 (64.71)	9/14 (64.29)			
Non-invasive ventilation							
No respiratory support	15/22 (68.18)	12/20 (60.00)	11/17 (64.71)	10/14 (71.43)			
Intermittent use	0/22 (0.00)	2/20 (10.00)	1/17 (5.88)	1/14 (7.14)			
Overnight	6/22 (27.27)	5/20 (25.00)	3/17 (17.65)	1/14 (7.14)			

Overnight and intermittent throughout the day	1/22 (4.55)	1/20 (5.00)	0/17 (0.00)	0/14 (0.00)
24-hour use	0/22 (0.00)	0/20 (0.00)	2/17 (11.76)	2/14 (14.29)

5.3 Discussion

This thesis presents data from a heterogenous study population including participants with MND phenotypes of ALS, PMA, PLS and PBP, who presented with symptoms in the upper and lower limbs, bulbar and respiratory regions. The small cohort size makes it impractical to group these participants by phenotype or location of onset.

To gauge whether this cohort was reflective of the characteristics of the English MND population, measures of central tendency from this study cohort were compared against an analysis of the incidence of MND for England between 1998 and 2019 [4]. The median age of this cohort was 61.5 years (IQR: 56-72). This was lower than the median UK age at diagnosis of 72 years (IQR: 64-80). The male-to-female ratio of 3.8:1 is greater than the English ratio of 1.5:1, but does reflect the greater incidence reported in men [4]. By chance, this small observational study only included White British individuals; this study therefore does not reflect the wider MND population ethnic groups. The site of onset frequency in this study population differed from that expected in the wider MND population, with a greater-than-expected number of participants presenting with respiratory onset (9% compared to 5% [19]). Subsequently, the frequency of onset for limb onset (59% compared to 75% [18]) and bulbar onset (18% compared to 25% [5]) was lower-than-expected. The presentation of mixed onset in 14% of this cohort could account for this difference, but more likely, the small sample size influences this margin of error [316].

The mean baseline ALSFRS-R score for this cohort was 33.50 ± 6.25. On average this cohort had a higher ALSFRS-R score compared to other studies which assessed the nutritional and metabolic state of MND cohorts [254,317–320]. This suggests that this study included participants in the earlier stages of disease progression. The average disease duration at baseline was skewed by two slow-progressing individuals (46.64 ± 47.22), however, the median disease duration of 27 months (IQR: 22.75-52.50) perhaps indicates a bias towards inclusion of slower-progressing individuals. In addition, the average change in ALSFRS-R score at each time point was calculated to be between 0.48 and 0.59 units, demonstrating a slower-than-average disease progression in this cohort [293].

The total ALSFRS-R score was observed to statistically significantly decline over a six-to-nine-month period, demonstrating increasing severity and progression within this cohort. The attrition rate for this study from consent to the fourth study visit was 10/24 (41.67%); however, withdrawal due to death occurred in just one participant. This may have caused further bias to those with a slower-progression in the reporting of longitudinal data included in this thesis, although as this study did not capture individuals at a pre-defined stage of their disease, this bias may be weakened.

5.4 Conclusions

The clinical descriptives presented in this chapter suggest that this White British, predominantly male, slow-progressing MND cohort may not be completely indicative of the wider MND population. Therefore, it should be acknowledged that the results presented in this thesis may not necessarily transfer to another independent MND cohort with different demographical and disease characteristics. The next chapter in this thesis will present the use of anthropometric measurements and indices to estimate body composition as an assessment of the nutritional status of this MND cohort.

6 Assessment of body composition

An understanding of body composition is pivotal to the study of human physiology, metabolism and nutritional state. Forbes et al., (1999) first described body composition as a two-compartment model of fat- and fat-free mass [321]. Whilst fat mass (FM) describes the relatively-inert compartment of the body comprised mainly of adipose tissue, fat free mass (FFM) encompasses metabolically-active components including muscle (approximately 20%), as well as viscera, bone and connective tissues (approximately 80%) [322]. The purpose of this chapter is to present the assessment of nutritional state of the MND cohort using anthropometric measurements and estimates of body composition.

6.1 Baseline results

Twenty-two participants living with MND underwent anthropometric and body composition analysis, where possible. Anthropometric measurements and indices recorded at baseline are shown in Table 6.1. The average weight of the study population at baseline was 79.12 kg (male: 80.90 kg (n = 17); female: 69.03 kg (n = 3)). It was not possible to collect weight measurements from two participants (one male, one female) due to mobility restrictions. Combined with the absence of either premorbid or diagnostic weight values for 3/22 (13.64%) participants, the average percentage weight change compared to the healthy weight before symptom onset in this cohort could only be calculated for 18 participants (an average decline of 4.58% body weight ± 7.26). 50% of participants demonstrated a weight loss of > 5.88%. When the cohort was divided by median split of the percentage weight change (those with < 5.88% or > 5.88% weight loss), no statistically significant differences were observed for disease duration (p = 0.81), total ALSFRS-R score (p = 0.76) or change in functional score (p = 0.79).

The mean and median BMI values for this cohort showed that these study participants were classified as overweight (26.26 kg/m^2) at the group level. At an individual level, two participants were underweight, seven participants had a healthy BMI, eight participants were overweight and four participants were obese (Table 6.1). Restrictions in upper limb movement for Pt05 removed the ability to measure TSF and MUAC. AMA was therefore not calculated for this participant. To consider the high probability of error introduced in the TSF

readings, the coefficient of variation (CV) was calculated for the triplicate TSF values on each arm at baseline; the average CV was 3.32%. User error when measuring TSF in Pt07 meant this data was removed from analysis and AMA was not calculated for this individual.

Right- and left-arm measurements for MUAC, TSF, AMA and calf circumference were recorded and presented in Figure 6.1 to account for potential asymmetric changes in body composition. Although no significant differences were found when analysed using a paired t test, an asymmetric reduction in any marker of malnutrition contributed towards an indication of malnutrition risk at an individual level within the study cohort. Using the anthropometric threshold values outlined in **section 4.3.2.1**, a risk of malnutrition was indicated in two participants at baseline (Pt01, Pt08), as presented in Table 6.2.

Table 6.1 Anthropometric measurements at baseline (n = 22). Data is presented as mean ± one standard deviation and median (IQR). AMA: arm muscle area; BMI: body mass index; IQR: interquartile range; MUAC: mid-upper arm circumference; SD: standard deviation; TSF: triceps skinfold thickness.

	n	**Mean ± SD**	**Median (IQR)**
Weight (kg)	20	79.12 ± 17.22	83.45 (64.93-88.58)
Weight change from premorbid (%)	18	4.58 ± 7.26	5.88 (1.12-8.06)
BMI (kg/m^2)	20	26.26 ± 4.53	26.20 (22.90-29.08)
Left arm MUAC (cm)	21	29.54 ± 3.33	30.05 (27.00-31.85)
Right arm MUAC (cm)	21	29.58 ± 3.44	29.75 (27.38-32.13)
Average MUAC (cm)	21	29.56 ± 3.31	29.80 (27.20-31.90)
Left arm TSF (mm)	20	13.94 ± 6.82	13.70 (7.70-17.98)
Right arm TSF (mm)	20	13.47 ± 5.70	13.07 (9.03-17.72)
Average TSF (mm)	20	13.70 ± 5.88	14.09 (8.52-17.54)
Left arm AMA (cm^2)	20	50.39 ± 12.99	46.64 (39.71-61.94)
Right arm AMA (cm^2)	20	51.24 ± 13.24	48.47 (41.87-62.33)
Average AMA (cm^2)	20	50.81 ± 12.32	46.77 (43.97-60.73)
Left calf circumference (cm)	22	37.01 ± 3.74	36.85 (34.65-39.85)
Right calf circumference (cm)	22	37.31 ± 3.62	36.80 (34.58-39.95)
Average calf circumference (cm)	22	37.16 ± 3.47	36.65 (34.69-39.48)

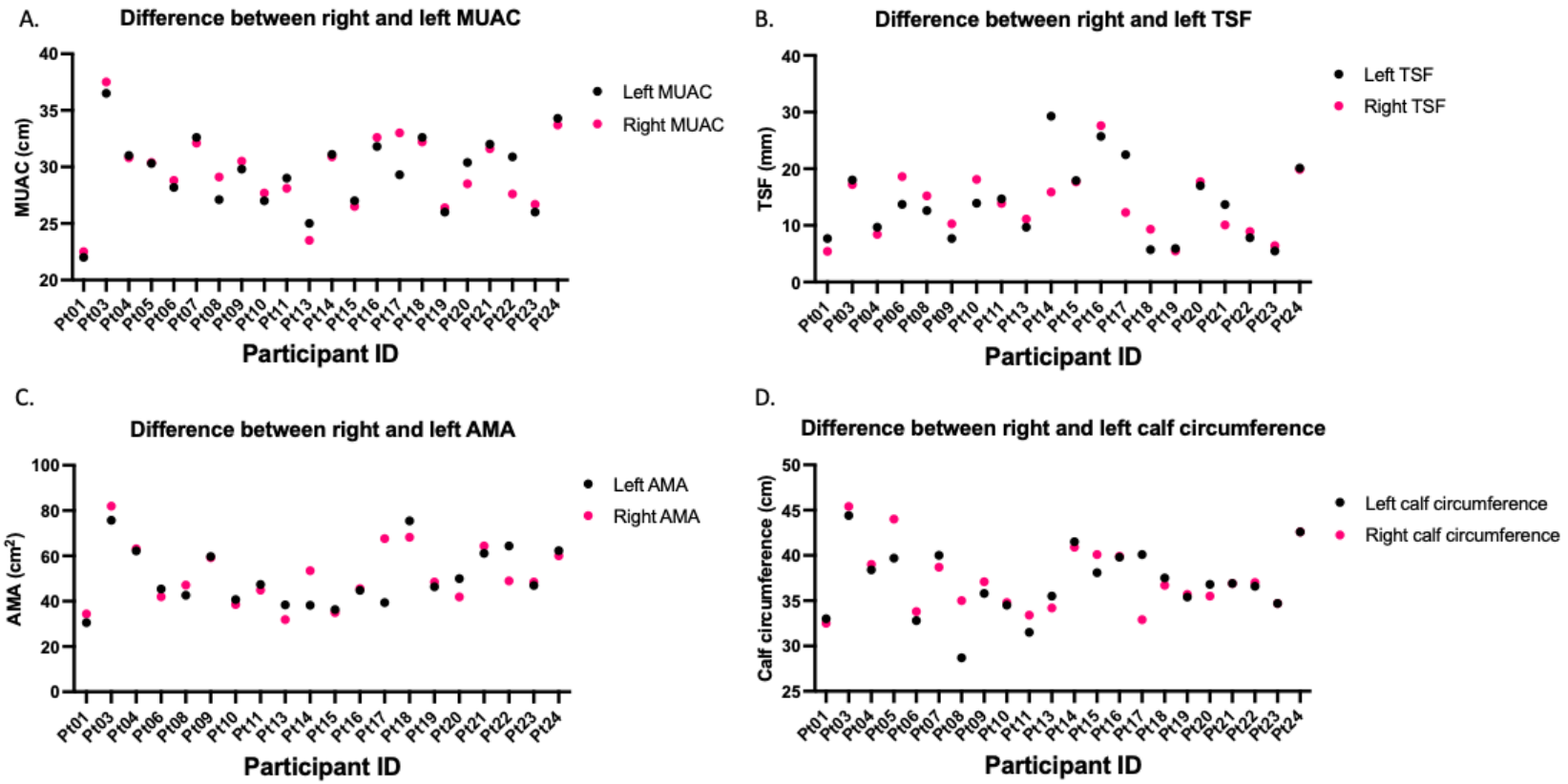


Figure 6.1 Difference between left and right measurements for (A) MUAC, (B) TSF, (C) AMA and (D) calf circumference at baseline (n = 22). Measurements for the left-hand side are shown in black, with the right-hand side measurements in pink. Statistical analysis was performed using a paired t test. Significance was determined at the $p < 0.05$ level. AMA: arm muscle area; MUAC: mid upper arm circumference; TSF: Triceps skinfold thickness.

Table 6.2 Indicators for the risk of malnutrition by percentage weight change from premorbid, MUAC and TSF at baseline (n = 22). Participants indicated to be at risk of malnutrition are highlighted in bold. Indicators of malnutrition risk are highlighted in red where the measurements fell below defined threshold values. Note: a negative weight change is indicative of a gain of weight from premorbid. Data not collected is denoted by '-'. BMI: body mass index; MUAC: mid upper arm circumference.

			MUAC (cm)			Calf circumference (cm)		
	Weight change from premorbid (%)	**BMI (kg/m^2)**	**Left**	**Right**	**Average**	**Left**	**Right**	**Average**
Pt01	4.81	17.70	22.00	22.50	22.25	33.00	32.50	32.75
Pt03	9.76	32.40	36.50	37.50	37.00	44.40	45.40	44.90
Pt04	6.07	26.40	31.00	30.80	30.90	38.40	39.00	38.70
Pt05	-1.25	34.60	30.30	30.40	30.35	39.70	44.00	41.85
Pt06	16.54	22.20	28.20	28.80	28.50	32.80	33.80	33.30
Pt07	-0.81	26.00	32.60	32.10	32.35	40.00	38.70	39.35
Pt08	15.20	25.70	27.10	29.10	28.10	28.70	35.00	31.85
Pt09		27.40	29.80	30.50	30.15	35.80	37.10	36.45
Pt10	7.09	29.00	27.00	27.70	27.35	34.50	34.80	34.65
Pt11	8.57	22.80	29.00	28.10	28.55	31.50	33.40	32.45
Pt13	7.89	23.20	25.00	23.50	24.25	35.50	34.20	34.85
Pt14	-15.95	29.10	31.10	30.90	31.00	41.50	40.90	41.20
Pt15	-	26.00	27.00	26.50	26.75	38.10	40.10	39.10
Pt16	6.39	33.00	31.80	32.60	32.20	39.80	39.90	39.85
Pt17	-	-	29.30	33.00	31.15	40.10	32.90	36.50
Pt18	-	-	32.60	32.20	32.40	37.50	36.70	37.10
Pt19	7.14	21.20	26.00	26.40	26.20	35.40	35.70	35.55
Pt20	-3.85	27.00	30.40	28.50	29.45	36.80	35.50	36.15
Pt21	1.76	26.40	32.00	31.60	31.80	36.90	36.90	36.90
Pt22	2.63	24.30	30.90	27.60	29.25	36.60	37.00	36.80
Pt23	4.84	19.20	26.00	26.70	26.35	34.70	34.70	34.70
Pt24	5.69	31.60	34.30	33.70	34.00	42.60	42.60	42.60

6.1.1 Relationships between anthropometric measurements and indices against disease severity and progression

At baseline, weight and BMI showed significant moderate-to-strong positive correlations with MUAC, TSF, AMA and calf circumference (Table 6.3). Weight, BMI, TSF, MUAC and calf circumference were found to significantly moderately negatively correlate with ALSFRS-R total score (Figure 6.2A-F). However, no significant correlations were observed for TSF, MUAC or AMA against fine motor subscore (Figure 6.2G-I), or for calf circumference with gross motor subscore (Figure 6.2J).

Participants were grouped by ALSFRS-R score by the median split technique, with participants with an ALSFRS-R score lower than 32 categorised as 'low' and those above as 'high'. A Mann-Whitney U test was conducted to compare the BMI between the two ALSFRS-R groups. In agreement with the significant negative correlations observed between the BMI and ALSFRS-R, a significant reduction in BMI was observed for those with a 'high' ALSFRS-R score (U = 20, p = 0.03). Weight was also demonstrated to decrease in those with a 'high' ALSFRS-R score, but this did not reach significance (U = 23, p = 0.06). Participants with a lower BMI demonstrated a slower disease progression, calculated by dividing the change in total ALSFRS-R score by disease duration (months), although this relationship was not significant (Spearman's r = 0.19, p = 0.43). When assessed for the relationship with disease duration, neither TSF, MUAC, AMA nor calf circumference correlated with disease duration (TSF: p = 0.77; MUAC: p = 0.66; AMA: p = 0.72; calf circumference: p = 0.81).

Table 6.3 Correlation coefficients and significance between weight and BMI against anthropometric measures and proxies for body composition at baseline. Bivariate correlation analysis was performed using either the Pearson's or Spearman's correlation coefficient according to the distribution of data analysed by the Shapiro-Wilk test. Significance was determined at $p < 0.05$. Significant results are highlighted in bold. AMA: arm muscle area; MUAC: mid-upper arm circumference; TSF: triceps skinfold thickness.

	n		**Weight (kg)**	**BMI (kg/m^2)**
Left arm MUAC (cm)	*21*	*r*	0.83	0.73
		p	**<0.001**	**0.0002**
Right arm MUAC (cm)	21	*r*	0.81	0.76
		p	**<0.0001**	**0.0001**
Average MUAC (cm)	21	*r*	0.83	0.76
		p	**<0.0001**	**0.0001**
Left arm TSF (mm)	20	*r*	0.67	0.73
		p	**0.002**	**0.0007**
Right arm TSF (mm)	20	*r*	0.49	0.74
		p	**0.04**	**0.0004**
Average TSF (mm)	20	*r*	0.62	0.77
		p	**0.007**	**0.0002**
Left arm AMA (cm^2)	20	*r*	0.53	0.44
		p	**0.02**	0.07
Right arm AMA (cm^2)	20	*r*	0.66	0.50
		p	**0.003**	**0.03**
Average AMA (cm^2)	20	*r*	0.62	0.49
		p	**0.007**	**0.04**
Left calf circumference (cm)	22	*r*	0.85	0.68
		p	**<0.0001**	**0.001**
Right calf circumference (cm)	22	*r*	0.88	0.82
		p	**<0.0001**	**<0.0001**
Average calf circumference (cm)	22	*r*	0.89	0.77
		p	**<0.0001**	**<0.0001**

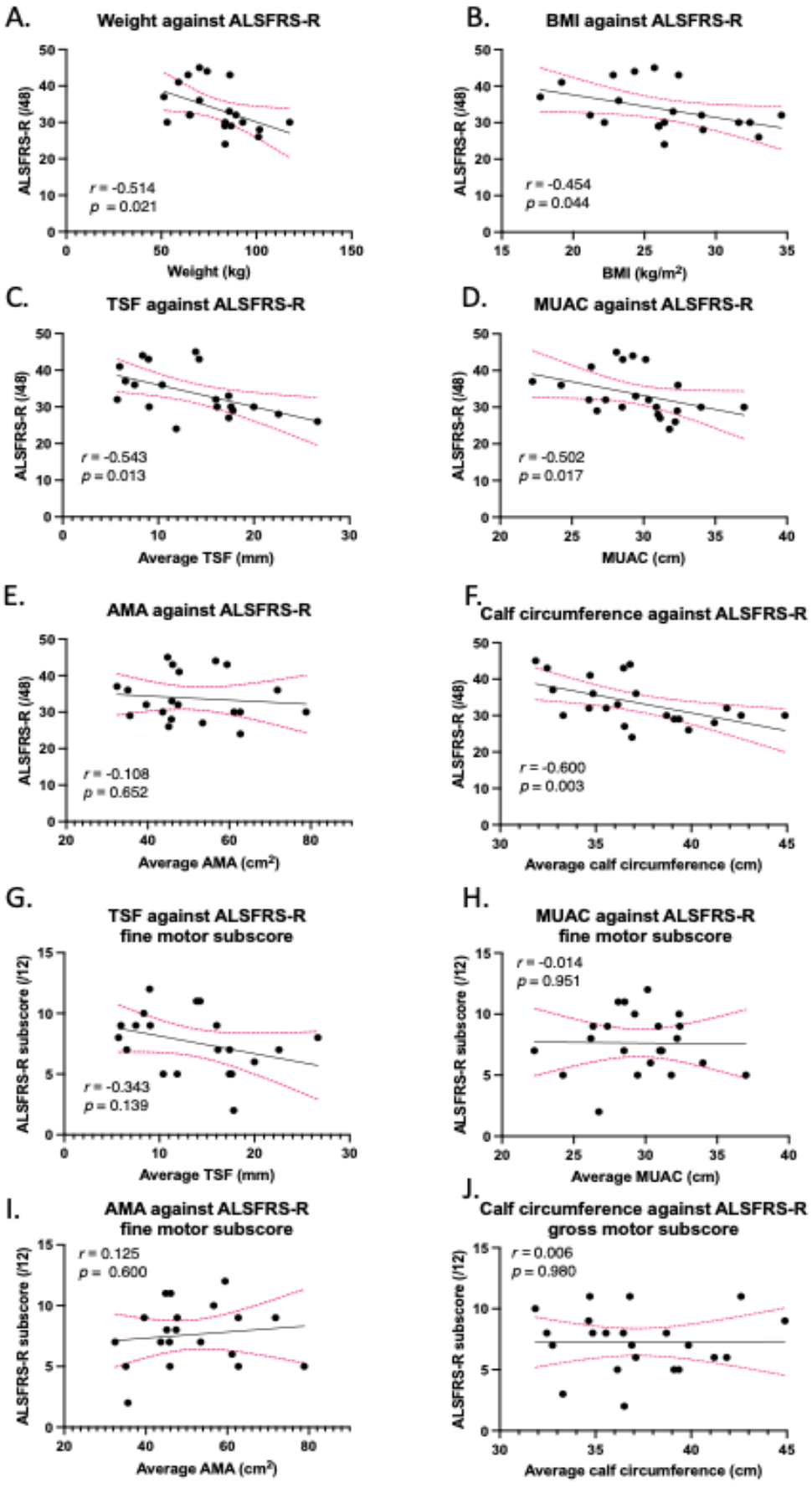

A.
Weight against ALSFRS-R
ALSFRS-R (/48)
r = -0.514
p = 0.021
Weight (kg)
B.
BMI against ALSFRS-R
ALSFRS-R (/48)
r = -0.454
p = 0.044
BMI (kg/m²)
C.
TSF against ALSFRS-R
ALSFRS-R (/48)
r = -0.543
p = 0.013
Average TSF (mm)
D.
MUAC against ALSFRS-R
ALSFRS-R (/48)
r = -0.502
p = 0.017
MUAC (cm)
E.
AMA against ALSFRS-R
ALSFRS-R (/48)
r = -0.108
p = 0.652
Average AMA (cm²)
F.
Calf circumference against ALSFRS-R
ALSFRS-R (/48)
r = -0.600
p = 0.003
Average calf circumference (cm)
G.
TSF against ALSFRS-R fine motor subscore
ALSFRS-R subscore (/12)
r = -0.343
p = 0.139
Average TSF (mm)
H.
MUAC against ALSFRS-R fine motor subscore
ALSFRS-R subscore (/12)
r = -0.014
p = 0.951
Average MUAC (cm)
I.
AMA against ALSFRS-R fine motor subscore
ALSFRS-R subscore (/12)
r = 0.125
p = 0.600
Average AMA (cm²)
J.
Calf circumference against ALSFRS-R gross motor subscore
ALSFRS-R subscore (/12)
r = 0.006
p = 0.980
Average calf circumference (cm)

Figure 6.2 Comparison of anthropometric indices for body composition against disease progression. ALSFRS-R total score against (A) Weight (kg), (B) BMI (kg/m^2), (C) TSF (mm), (D) MUAC (cm), (E) AMA (cm^2), (F) Calf circumference (cm). ALSFRS-R fine motor subscore against (G) TSF (mm), (H) MUAC (cm) and (I) AMA (cm^2). (J) ALSFRS-R gross-motor subscore against calf circumference (cm). Bivariate correlation analysis was performed using either the Pearson's or Spearman's correlation coefficient according to the distribution of data analysed using the Shapiro-Wilk test. Significance was determined at the $p < 0.05$ level. AMA: arm muscle area; BMI: body mass index; MUAC: mid-upper arm circumference; TSF: triceps skinfold thickness.

6.2 Longitudinal anthropometric measurements

Table 6.4 presents the longitudinal anthropometric measurements and indices for all participants at all study visits. Statistically significant differences were observed between baseline and the first follow-up for left-arm MUAC, and between baseline and the third follow-up for right-arm MUAC.

The percentage change for all anthropometric measurements was calculated for 14 participants between the baseline assessment and the third study visit (Figure 6.3). The baseline measurement was set to 100%, with the measurement recorded at the third study visit expressed as a percentage of the baseline value. Seven participants (50%) of the longitudinal cohort lost weight from premorbid over the nine-month study duration (with an average decline of 5%), but four participants demonstrated increases in weight from premorbid by the final study visit (Figure 6.3B). Similarly, nine participants demonstrated decreases in BMI over the nine months, but one participant demonstrated no change and three participants were observed to have increases in their BMIs (Figure 6.3C).

A Pearson's moment correlation analysis was conducted to investigate the relationship between the percentage of change in body weight between baseline and F3 against the percentage change in the other anthropometric measurements for body composition. No significant correlations were observed for MUAC (n = 13, r = 0.26, p = 0.39), TSF (n = 11, r = 0.34, p = 0.31) or AMA (n = 11, r = -0.28, p = 0.40). However, a significant moderate positive correlation was observed between the percentage change in body weight against the change in calf circumference (n = 13, r = 0.68, p = 0.01).

Table 6.4 Longitudinal anthropometric measurements and indices. Data presented as mean ± one standard deviation and median (IQR). The number of participants with each anthropometric assessment at each time point is indicated as n/N (%). Longitudinal changes analysed using Dunnett's mixed-effects analysis for multiple comparisons. Significance at $p < 0.05$. Significant results highlighted in bold. AMA: arm muscle area; BMI: body mass index; F1-3: follow-up study visits 1-3; IQR: interquartile range; MUAC: mid-upper arm circumference; TSF: triceps skinfold thickness.

	Study time point				P value		
Anthropometric measurement or indices	**Baseline**	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
Weight (kg)	n = 20/22 79.12 ± 17.22 83.45 (64.93-88.58)	n = 19/20 79.53 ± 17.61 82.00 (63.00-88.20)	n = 17/17 81.02 ± 16.59 84.00 (66.10-91.20)	N = 14/14 80.83 ± 14.42 82.60 (69.35-89.90)	n = 19 0.84	n = 16 0.28	n = 13 0.53
Weight loss from baseline (%)	n = 18/22 4.58 ± 7.26 5.88 (1.12-8.06)	n = 17/20 4.90 ± 7.50 3.94 (2.14-9.34)	n = 14/17 4.51 ± 9.87 3.37 (-0.56-12.81)	n = 11/14 5.01 ± 9.69 6.83 (-5.93-14.81)	n = 17 0.97	n = 14 >0.99	n = 11 0.99
BMI (kg/m^2)	n = 20/22 26.26 ± 4.53 26.20 (22.90-29.08)	n = 19/20 26.26 ± 4.44 26.10 (23.90-28.70)	n = 17/17 26.15 ± 4.59 26.00 (21.85-30.40)	n = 14/14 26.15 ± 3.79 26.55 (23.17-28.73)	n = 19 >0.99	n = 16 0.97	n = 13 0.98
Left arm MUAC (cm)	n = 22/22 29.54 ± 3.33 30.05 (27.00-31.85)	n = 20/20 29.00 ± 3.42 29.55 (26.30-31.48)	n = 16/17 29.36 ± 3.65 29.80 (25.93-32.50)	n = 14/14 29.30 ± 3.39 30.15 (27.00-31.38)	n = 20 **0.049**	n = 16 0.88	n = 14 0.84

	Study time point				P value		
Anthropometric measurement or indices	**Baseline**	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
Right arm MUAC (cm)	n = 22/22 29.58 ± 3.44 29.75 (27.38-32.13)	n = 20/20 29.26 ± 3.39 29.40 (26.63-31.38)	n = 16/17 29.30 ± 3.90 30.15 (26.93-32.38)	n = 14/14 28.90 ± 3.82 29.35 (26.58-32.05)	n = 20 0.44	n = 16 0.61	n = 14 **0.04**
Average MUAC (cm)	n = 21/22 29.56 ± 3.31 29.80 (27.20-31.90)	n = 20/20 29.13 ± 3.36 29.60 (26.46-31.28)	n = 16/17 29.33 ± 3.69 29.90 (26.70-32.59)	n = 14/14 29.10 ± 3.56 29.75 (27.09-31.70)	n = 20 0.12	n = 16 0.69	n = 14 0.23
Left arm TSF (mm)	n = 20/22 13.94 ± 6.82 13.70 (7.70-17.98)	n = 19/20 12.83 ± 6.00 12.33 (8.73-16.83)	n = 16/17 13.31 ± 5.37 13.07 (8.65-18.68)	n = 14/14 13.46 ± 5.06 13.90 (9.93-17.59)	n = 17 0.85	n = 14 0.96	n = 12 0.99
Right arm TSF (mm)	n = 20/22 13.47 ± 5.70 13.07 (9.03-17.72)	n = 19/20 13.01 ± 5.84 12.08 (7.87-17.13)	n = 16/17 14.03 ± 5.60 15.01 (8.87-18.27)	n = 14/14 13.02 ± 4.00 13.30 (10.12-16.43)	n = 17 0.95	n = 14 0.91	n = 12 0.96
Average TSF (mm)	n = 20/22 13.70 ± 5.88 14.09 (8.52-17.54)	n = 19/20 12.92 ± 5.80 12.13 (8.60-18.23)	n = 16/17 13.67 ± 5.40 13.87 (8.81-18.80)	n = 14/14 13.24 ± 4.41 12.98 (10.48-16.91)	n = 17 0.88	n = 14 >0.99	n = 12 0.97
Left arm AMA (cm^2)	n = 20/22 50.39 ± 12.99 46.64 (39.71-61.94)	n = 19/20 49.92 ± 11.33 49.44 (41.46-59.57)	n = 16/17 50.94 ± 9.88 52.44 (42.72-59.91)	n = 14/14 50.52 ± 10.01 50.86 (44.33-56.85)	n = 17 0.996	n = 14 0.995	n = 12 >0.99
Right arm AMA (cm^2)	n = 20/22 51.24 ± 13.24 48.47 (41.87-62.33)	n = 19/20 50.65 ± 11.05 51.80 (44.19-56.99)	n = 16/17 49.92 ± 10.94 49.69 (44.37-58.02)	n = 14/14 49.69 ± 11.83 48.26 (44.12-58.11)	n = 17 0.97	n = 14 0.76	n = 12 0.71

	Study time point				P value		
Anthropometric measurement or indices	**Baseline**	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
Average AMA (cm^2)	n = 20/22 50.81 ± 12.32 46.77 (43.97-60.73)	n = 19/20 50.28 ± 10.87 49.32 (44.31-57.66)	n = 16/17 50.43 ± 9.79 51.60 (44.46-58.73)	n = 14/14 50.11 ± 10.43 50.53 (44.76-60.29)	n = 17 0.99	n = 14 0.996	n = 12 0.98
Left calf circumference (cm)	n = 22/22 37.01 ± 3.74 36.85 (34.65-39.85)	n = 20/20 37.02 ± 3.60 37.25 (34.45-39.33)	n = 16/17 37.25 ± 3.75 37.00 (34.53-39.40)	n = 14/14 37.15 ± 3.50 37.95 (35.25-39.00)	n = 20 >0.99	n = 16 0.51	n = 14 0.92
Right calf circumference (cm)	n = 22/22 37.31 ± 3.62 36.80 (34.58-39.95)	n = 20/20 37.60 ± 3.41 37.65 (34.70-40.20)	n = 16/17 38.13 ± 3.23 37.90 (35.23-40.03)	n = 14/14 38.13 ± 2.99 38.20 (35.18-40.00)	n = 20 0.57	n = 16 0.07	n = 14 0.11
Average calf circumference (cm)	n = 22/22 37.16 ± 3.47 36.65 (34.69-39.48)	n = 20/20 37.31 ± 3.42 37.53 (34.42-39.66)	n = 16/17 37.69 ± 3.36 37.58 (34.61-40.39)	n = 14/14 37.64 ± 3.09 38.23 (34.99-39.17)	n = 20 0.72	n = 16 0.13	n = 14 0.28

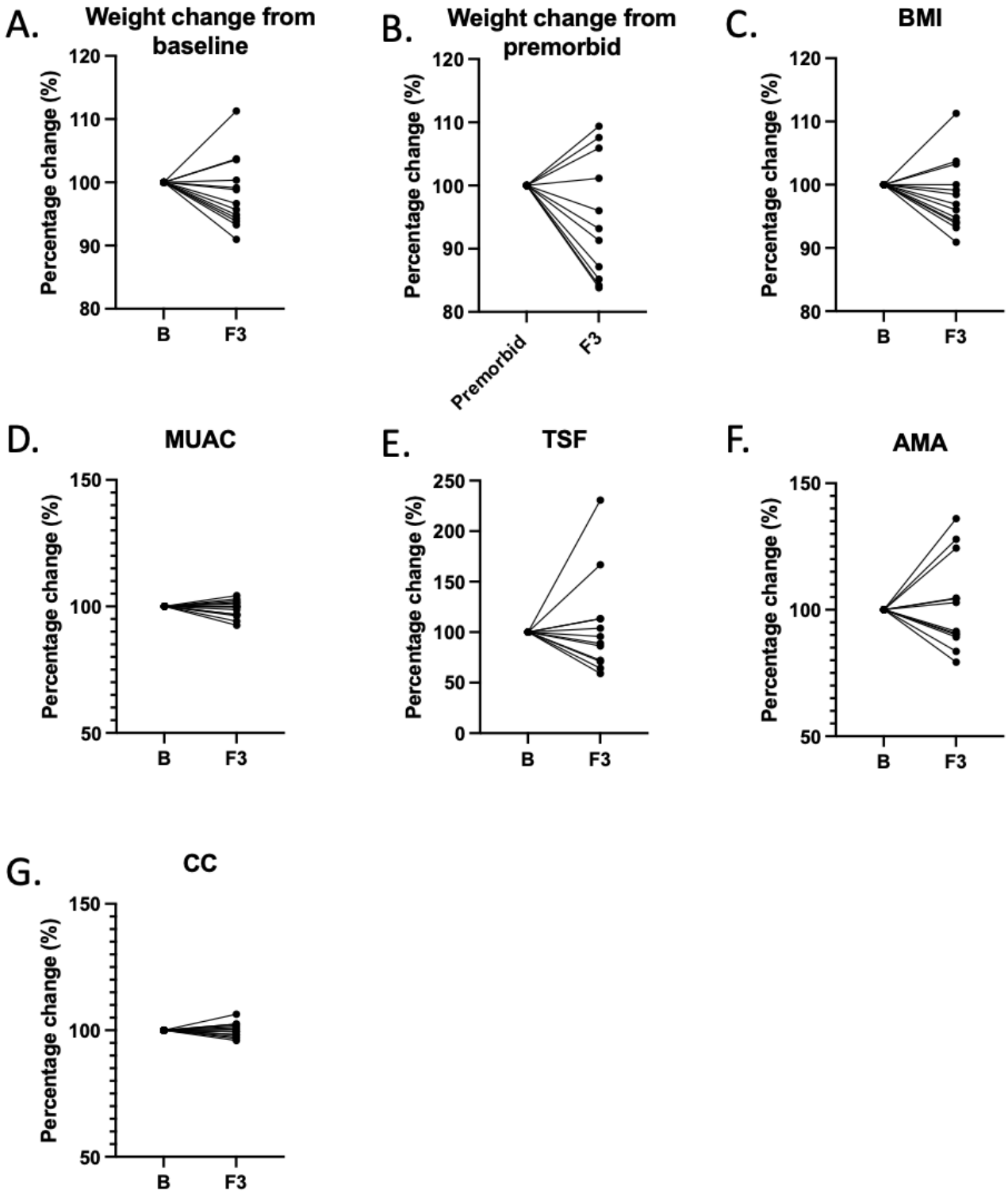


Figure 6.3 Intra-cohort analysis of the percentage change in anthropometric assessments between baseline and F3. A) Weight, kg (n = 13). B) Percentage change in weight (n = 11). C) BMI, kg/m^2 (n = 13). D) Mid-upper arm circumference (MUAC), cm (n = 14). E) Triceps skinfold

thickness (TSF), mm (n = 12). F) Arm muscle area (AMA), cm^2 (n = 12). G) Calf circumference (CC), cm (n = 14). B: baseline study visit; BMI: body mass index; F3: third follow-up study visit.

6.3 Discussion

The purpose of this chapter was to assess the suitability of using anthropometric measurements and indices as estimates of body composition in order to assess the nutritional state of this MND cohort. In 2021, the average weight of the UK population was 85.1 kg for men and 71.8 kg for women [323]. This cohort therefore demonstrated lower than average weight measurements for both sexes. However, as presented in Table 6.1, this cohort are categorised as overweight at group level at baseline (average BMI: 26.26 kg/m^2 ± 4.53). As described in **section 2.4.1,** measurements of weight and BMI do not describe the presence or distribution of fat and muscle mass. As this PhD study was not initially designed to assess body composition, techniques such as BIA and DEXA were not included in the study design. Instead, proxy estimates of arm muscle area (AMA) (calculated using the mid-upper arm circumference and triceps skinfold thickness (TSF), as described in **section 4.3.2**) were used to estimate fat free mass, whilst TSF was used as an estimate of fat mass.

As MND is characterised by a reduction in muscle mass secondary to muscle denervation, the positive relationship between AMA and weight at baseline is unsurprising – the smaller the AMA value, the lower the overall body weight measurement, in turn reducing BMI. An increase in TSF was also found to be significantly associated with increases in weight and BMI; it stands to reason that a loss of muscle mass will lead to a decreased mobility, causing a more sedentary lifestyle. Often, this reduction in physical activity level will cause a reduction in the total energy expenditure of the individual, which may not be accompanied by a proportional reduction in dietary intake. This could explain the increase in fat mass observed within this cohort (Figure 6.3E).

6.3.1 Relationship of nutritional state assessed by body composition with disability and disease progression

Percentage weight loss from premorbid has previously been used to indicate survival in MND, with a weight loss of 5-10% from premorbid associated with a shorter disease duration [53]. However, the absence of a statistically significant decrease in disease duration, or increase in the severity and progression of those with a weight loss of greater than 5.88% from before

disease onset did not support the previously suggested use of percentage weight loss from premorbid to predict survival or disease severity in this cohort.

TSF and MUAC were also observed to significantly negatively correlate with disease severity in this cohort. This supports the results in the previously described study by Salvioni et al., (2015) [121], and agrees with the observation that an increased disease severity is associated with a sedentary lifestyle and increased fat mass. It was surprising however, that no significant correlations were observed between TSF or MUAC against the fine motor subscore (Figure 6.2G). Similarly, whilst the calf circumference was also found to significantly negatively correlate with ALSFRS-R total score, no correlation was observed between calf circumference and the gross motor ALSFRS-R subscore. Therefore, whilst these measurements of skinfold thickness and body circumferences are significantly related to ALSFRS-R total score, they do not necessarily indicate a regionalised loss of function. As these measurements are indirect estimates of localised fat mass rather than skeletal muscle, and therefore not directly linked to function and strength, this may explain this dissociation. Moreover, it was expected that AMA would positively correlate with ALSFRS-R. However, this relationship was not observed in this cohort (Figure 6.2E). This may be because the AMA is a crude estimate of upper arm muscle mass, derived from two estimates of fat mass. It would be interesting to see whether survival analysis data from this cohort agreed with the hypothesis that a higher fat mass is protective in MND [53]. As discussed in **section 5.3**, this cohort are considered to be in the early stages of MND. If this cohort were followed for a longer duration, it would be expected that the relationship between the TSF and the ALSFRS-R would invert once TSF started to decline following the onset of malnutrition-associated fat loss.

Whilst a greater weight and BMI were significantly associated with an increased disease severity in this cohort, as indicated by a reduced ALSFRS-R total score (Figure 6.2), those with a lower BMI demonstrated a slower disease progression. This result was not expected. Previous data have shown that a higher BMI is considered to be protective in MND, as summarised in systematic review of eight studies including 6,098 people living with MND conducted by Dardiotis et al., (2018) [324].

Whilst no significant changes were observed over the nine-months for any anthropometric measurement or assessment of body composition in the longitudinal cohort (Table 6.4), the

intra-cohort longitudinal changes presented in Figure 6.3 demonstrate the high variability observed for the 14 participants who completed all four study visits (Figure 6.3). Interestingly, the participant who presented the largest percentage change in TSF (+130%) (Figure 6.3E), also demonstrated the second largest decline in AMA (-16.5%) (Figure 6.3F). Whilst the percentage increase in TSF for this individual seems unfeasible, the average coefficient of variation was 4.6% for the triplicate baseline measurements and 3.6% for the third follow up study visit, which is appropriate. This variation highlights that an assumption of continuous decline in skeletal muscle, or increase in fat mass, cannot be made, and body composition should be assessed regularly. This dataset could be further interrogated to assess the ratio between TSF:AMA to monitor changes in nutritional state.

No significant correlations were observed between the percentage of body mass change (from either premorbid or baseline) and percentage change in MUAC, TSF or AMA over the nine-month period. This does not agree with the conclusion by Kasarskis et al., (1997) that arm anthropometry can be used to monitor muscular atrophy in MND [120]. However, a significant positive correlation was observed between the percentage of body weight change and percentage change in calf circumference between the baseline and third follow-up study visits (p = 0.01) for the 14 participants who completed all four study visits. This suggests that changes in calf circumference could be used as a surrogate marker to monitor weight loss in people living with MND.

6.3.2 Assessing the risk of malnutrition using anthropometry

Measurements and indices of body composition have been used to identify malnutrition in this cohort. As described in **section 4.3.2.1,** the risk of malnutrition was indicated in this MND cohort by lower-than-accepted measurements for two of the following assessments: a percentage weight loss of ≥ 5% from premorbid, a BMI of ≤ 20 kg/m^2, a MUAC of < 23.5 cm or a calf circumference of < 31 cm. These thresholds were used to indicate the risk of an adverse nutritional state in this study. An immediate observation from this dataset is the asymmetric wasting of the upper and lower limbs (Figure 6.1). This highlights the necessity of recording and presenting the left- and right-hand side measurements separately, as well as the average measurement for both sides. For this reason, malnutrition risk was indicated if a measurement fell below the threshold value for one limb only.

The data presented in this chapter suggests that a MUAC of < 23.5 cm is indicative of a BMI of ≤ 20 kg/m^2 in this cohort. Whilst this threshold is recommended for use in generic dietetic care [116,117], the role of MUAC in understanding nutritional state in MND has not previously been reported. As this cohort only included one participant with MUAC < 23.5 cm, this needs further evaluation in a larger cohort with participants recording lower BMI and MUAC values. A novel finding for this study was the significant positive relationship for calf circumference against body weight and BMI in this cohort at baseline; a reduction in calf circumference < 31 cm was associated with a ≥ 5% weight loss from premorbid.

Using these anthropometric thresholds, a risk of malnutrition was identified in 2/22 (9.1%) of this study cohort at the baseline study visit (Table 6.2: Pt01 and Pt08). This estimate of malnutrition is lower than the previously estimated incidence of malnutrition of 16-55% of people living with MND [49,50]. This may be explained by the implementation of a reduction in two markers of malnutrition; if only one marker of malnutrition was applied, then 12/22 participants (54.5%) would be indicated to be malnourished in this cohort at baseline, which would then fall within the previously estimated incidence of malnutrition in MND.

It is interesting to observe that not all markers of malnutrition simultaneously decreased. For example, Pt01 recorded 'lower-than-acceptable' values for BMI and MUAC, but measurements of calf circumference remained above the threshold for malnutrition. This individual was diagnosed with lower limb onset ALS, with a disease duration of 19 months at baseline (below the 25th percentile for disease duration (Table 6.1). This participant also reported functional declines in fine and gross motor subscores, mapping to a King's stage of 2 and a change in functional score of 1.95 (Table 6.1), placing them above the 75th percentile for disease severity. Pt08 was indicated to be malnourished by a weight loss of 15.20% from premorbid, and a left calf circumference of 28.7 cm, but yet was still categorised as 'overweight' according to BMI thresholds (BMI: 25.70 kg/m^2). This participant had a disease duration of 26 months, with symptom onset presenting as left foot drop. This individual was diagnosed with lower limb onset, but their MND phenotype was unspecified. They also reported weakness in their fine and gross motor subscore, mapping to a King's stage of 2 and a change in functional score of 1.73, also placing them in the fourth quartile for disease severity in this cohort. This demonstrates that an individual can be indicated to be

malnourished, but remain within the range of a 'healthy' BMI. These data also suggest that a risk of malnutrition indicated using anthropometric measurements and indices is associated with a greater disease severity in this cohort.

6.3.3 Conclusion

The use of upper arm and lower leg anthropometry has been demonstrated to reflect weight and BMI in this study cohort and are therefore suggested to indicate nutritional state in this cohort; however, the reliability and robustness of these measurements need further validation in a larger study population. Whilst weight, BMI, MUAC, TSF and calf circumference were demonstrated to reflect disease severity, these measurements provide localised, crude estimates of body composition. It would perhaps be more informative to estimate whole body fat and fat free mass using advanced techniques such as whole-body plethysmography, BIA or DEXA.

7 Measurement of biochemical analytes

The purpose of this chapter is to investigate the role of biochemical analytes measured from the serum and 24-hour urinary collections from the MND cohort as a tool to assess the nutritional state of people living with MND. This chapter first considerations the concentration of serum analytes (**section 7.1.1**), with and without consideration of a pro-inflammatory state, before investigating the 24-hour urinary analytes (**section 7.1.4**), with and without consideration for the completeness of the collection. The measurements of the biochemical analytes presented in this chapter are correlated against assessments of function and disease severity (presented in **Chapter 5**) and body composition (**Chapter 6**), to investigate the interplay within the multipronged approach to understanding the nutritional state of this MND cohort. This chapter sets up for the evaluation of the role of biochemical analytes as objective markers of dietary intake, reported in **Chapter 8.**

7.1 Baseline results

Blood (n = 19) and 24-hour urinary collections (n = 22) were collected from participants at the baseline study visit (Table 7.1). It was not possible to obtain blood samples from three participants. PBMC isolation was added as a substantial amendment on the 2nd February 2022, therefore additional whole-blood collected for plasma and PBMC isolation at UoS was only collected in 11/22 (50%) participants at baseline. 24-hour urinary collections were obtained from all study participants at baseline; however, only 17 of these collections (77.3%) were self-reported as complete, with no spillages or missed samples.

Table 7.1 Biosamples collected from the study cohort at baseline (n = 22).

	24-hour urinary collection		Venepuncture		
	Collected	**Completeness of collection**	**Serum**	**Plasma**	**PBMCs**
Pt01	✓	Complete	✓		
Pt03	✓	Complete	✓		
Pt04	✓	Complete	✓		
Pt05	✓	Unusable	✓		
Pt06	✓	Incomplete	✓		
Pt07	✓	Complete	✓		
Pt08	✓	Complete	✓		
Pt09	✓	Complete	✓	✓	✓
Pt10	✓	Incomplete	✓	✓	✓
Pt11	✓	Complete	✓		
Pt13	✓	Complete			
Pt14	✓	Complete	✓	✓	✓
Pt15	✓	Complete	✓	✓	✓
Pt16	✓	Complete	✓	✓	✓
Pt17	✓	Complete	✓	✓	✓
Pt18	✓	Complete	✓	✓	✓
Pt19	✓	Incomplete	✓	✓	✓
Pt20	✓	Incomplete			
Pt21	✓	Complete			
Pt22	✓	Complete	✓	✓	✓
Pt23	✓	Complete	✓	✓	✓
Pt24	✓	Complete	✓	✓	✓
n/22	**22**	**Complete: 17/22 (77.3%)**	**19**	**11**	**11**
		Incomplete: 5/22 (22.7%)			

7.1.1 Serum biochemical analytes at baseline

The measures of central tendency for the serum biochemical analytes collected at baseline are presented alongside the Northern General Hospital (NGH) Laboratory Medicine reference ranges [306] (where provided) in Table 7.2. Bivariate correlation analysis was conducted to explore the relationship between the baseline serum biochemical analytes and clinical and anthropometric parameters (Table 7.3). No statistically significant relationships were observed between the serum biochemical analytes and disease duration or change in functional score (ΔALSFRS-R). However, when the relationship between the serum analytes and the severity of disability using the ALSFRS-R was investigated, serum retinol-binding protein was observed to significantly, moderately negatively correlate with the ALSFRS-R total score (r = -0.46, p = 0.05), whilst cholesterol (r = -0.52, p = 0.02), LDL-cholesterol (r = -0.59, p = 0.01) and non-HDL-cholesterol (r = -0.59, p = 0.01) significantly negatively correlated with bulbar function, assessed using the ALSFRS-R bulbar subscore. This suggests worsening dysphagia is associated with an increased concentration of cholesterol, LDL-cholesterol and non-HDL-cholesterol. When compared to King's staging, moderate positive, significant relationships were observed with total HDL-cholesterol (r = 0.55, p = 0.02) and retinol binding protein (r = 0.54, p = 0.02).

In relation to body composition, serum albumin was observed to strongly, negatively correlate with the percentage weight change from premorbid (r = -0.66, p = 0.01). Cholesterol significantly negatively correlated with left-arm (r = -0.47, p = 0.05) and average MUAC (r = -0.46, p = 0.05), as well as right calf circumference (r = -0.47, p = 0.04), whilst transferrin significantly positively correlated with left-arm TSF (r = 0.51, p = 0.04). Creatinine demonstrated a significant positive correlation with left-arm (r = 0.51, p = 0.04) and average (r = 0.51, p = 0.04) AMA.

Table 7.2 Serum biochemical analytes at baseline (n = 19). Data is presented as mean ± one standard deviation and median (IQR). The number of participants with measurements for each biochemical analyte is indicated as n/N (%). IQR: interquartile range; SD: standard deviation.

	Reference ranges	n/N Mean ± SD Median (IQR)
Creatinine (mol/L)	Male: 62 - 106 Female: 44 - 80	19/19 66.63 ± 17.54 65.00 (53.00-74.00)
Albumin (g/L)	35 - 50	19/19 46.47 ± 2.27 46.00 (45.00-48.00)
Prealbumin (g/L)	Male: 0.2 - 0.5 Female: 0.1 - 0.4	19/19 0.27 ± 0.04 0.28 (0.23-0.30)
Cholesterol (mmol/L)		19/19 4.97 ± 0.93 5.00 (4.40-5.50)
Triglyceride (mmol/L)		19/19 1.49 ± 0.61 1.50 (1.00-1.90)
HDL cholesterol (mmol/L)		19/19 1.67 ± 0.80 1.49 (1.24-1.90)
LDL cholesterol (mmol/L)		17/19 2.87 ± 0.78 2.50 (2.10-3.25)
Non-HDL cholesterol (mmol/L)		19/19 3.46 ± 0.90 3.40 (2.80-3.90)
Total HDL cholesterol ratio (mmol/L)		19/19 3.51 ± 1.06 3.10 (2.70-4.50)
Ferritin (µg/L)	Males: 30 – 400 Females < 60 yr: 15-150 Females > 60 yr: 30-400	19/19 251.68 ± 114.09 226.00 (155.00-337.00)
Transferrin (g/L)	2.0-3.2	19/19 2.39 ± 0.32 2.35 (2.09-2.62)
Retinol binding protein (mg/L)	20 - 40	19/19 53.53 ± 12.22 55.00 (41.00-65.00)

	18/19
eGFR EPI	86.06 ± 9.79
	90.00 (88.25-90.00)

Table 7.3 Correlation of all baseline serum biochemical analytes against demographic, clinical and anthropometric data (n = 22). Correlation analysis was conducted using Pearson's moment correlation analysis for normally distributed data, or Spearman's (†) rank-order correlation analysis for non-normally distributed data. Significance observed at $p < 0.05$. Significant results highlighted in bold. ALSFRS-R: amyotrophic lateral sclerosis functional rating scale - revised; AMA: arm muscle area; BMI: body mass index; MUAC: Mid-upper arm circumference; TSF: triceps skinfold thickness; ΔALSFRS-R: change in the amyotrophic lateral sclerosis functional rating scale - revised.

		Creatinine (mol/L)	Albumin (g/L)	Prealbumin (g/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L) †	LDL cholesterol (mmol/L)	Non-HDL cholesterol (mmol/L)	Total HDL-cholesterol (mmol/L)	Ferritin (µg/L)	Transferrin (g/L)	Retinol-binding protein (mg/L)	eGFR †
Demographic and Clinical														
Age (years)	*r*	-0.02	-0.38	-0.25	-0.04	-0.06	0.33	-0.04	-0.10	-0.26	0.15	-0.32	0.01	-0.52
	p	0.95	0.11	0.29	0.86	0.80	0.17	0.89	0.69	0.29	0.55	0.18	0.96	**0.03**
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
Disease duration (months) †	*r*	0.02	0.07	0.22	-0.09	0.21	-0.25	0.05	0.18	0.22	-0.22	0.15	0.10	-0.30
	p	0.93	0.77	0.37	0.73	0.38	0.30	0.86	0.46	0.36	0.38	0.55	0.69	0.23
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
King's staging †	*r*	-0.14	0.16	0.33	0.29	0.20	-0.39	0.37	0.42	0.55	0.25	0.27	0.54	0.20
	p	0.56	0.50	0.17	0.23	0.42	0.10	0.14	0.07	**0.02**	0.31	0.27	**0.02**	0.44
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
ALSFRS-R total subscore (/48) †	*r*	0.16	-0.17	-0.41	-0.06	-0.23	0.33	-0.24	-0.23	-0.41	-0.20	-0.26	-0.46	-0.31
	p	0.50	0.50	0.08	0.81	0.35	0.17	0.35	0.34	0.08	0.41	0.29	**0.049**	0.21

		Creatinine (mol/L)	Albumin (g/L)	Prealbumin (g/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L) †	LDL cholesterol (mmol/L)	Non-HDL cholesterol (mmol/L)	Total HDL-cholesterol (mmol/L)	Ferritin (µg/L)	Transferrin (g/L)	Retinol-binding protein (mg/L)	eGFR †
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
ALSFRS-R bulbar subscore (/12)	*r*	0.07	0.01	-0.09	-0.52	-0.13	-0.11	-0.59	-0.59	-0.33	-0.41	0.32	-0.09	-0.04
	p	0.76	0.98	0.72	**0.02**	0.59	0.65	**0.01**	**0.01**	0.18	0.08	0.18	0.71	0.87
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
ALSFRS-R Fine Motor subscore (/12)	*r*	-0.02	-0.27	-0.17	0.19	-0.10	0.38	0.05	-0.03	-0.41	0.10	-0.15	-0.21	-0.03
	p	0.93	0.26	0.49	0.45	0.69	0.11	0.84	0.92	0.08	0.68	0.55	0.38	0.91
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
ALSFRS-R Gross Motor subscore (/12)	*r*	0.30	-0.01	-0.43	-0.16	-0.20	0.15	-0.18	-0.20	-0.20	-0.25	-0.20	-0.37	-0.23
	p	0.22	0.96	0.06	0.51	0.40	0.55	0.48	0.42	0.40	0.31	0.42	0.13	0.36
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
ALSFRS-R Respiratory subscore (/12) †	*r*	0.05	-0.13	-0.16	0.03	-0.13	0.25	0.06	0.01	-0.23	-0.12	-0.33	-0.35	-0.20
	p	0.85	0.59	0.52	0.91	0.60	0.31	0.81	0.97	0.35	0.62	0.17	0.15	0.43
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
ΔALSFRS-R †	*r*	-0.12	-0.02	0.09	0.16	0.03	0.04	0.11	0.04	0.06	0.25	0.03	0.23	0.29
	p	0.62	0.93	0.72	0.51	0.92	0.87	0.68	0.86	0.79	0.30	0.89	0.34	0.24
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
Anthropometric														
Weight (kg)	*r*	0.45	0.08	0.19	-0.44	-0.16	-0.35	-0.21	-0.29	0.09	-0.26	0.17	0.35	-0.12
	p	0.07	0.75	0.47	0.08	0.54	0.17	0.46	0.26	0.74	0.32	0.51	0.16	0.66
	n	17	17	17	17	17	17	15	17	17	17	17	17	16

		Creatinine (mol/L)	Albumin (g/L)	Prealbumin (g/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L) †	LDL cholesterol (mmol/L)	Non-HDL cholesterol (mmol/L)	Total HDL-cholesterol (mmol/L)	Ferritin (µg/L)	Transferrin (g/L)	Retinol-binding protein (mg/L)	eGFR †
Percentage weight change from premorbid (%)	*r*	-0.07	-0.66	-0.42	0.08	-0.10	0.09	-0.07	-0.07	-0.41	0.32	-0.25	-0.21	-0.01
	p	0.82	**0.01**	0.12	0.78	0.72	0.75	0.81	0.81	0.13	0.25	0.36	0.45	0.98
	n	15	15	15	15	15	15	14	15	15	15	15	15	14
BMI (kg/m^2)	*r*	0.11	-0.09	-0.02	-0.27	-0.12	-0.01	-0.02	-0.16	0.05	-0.24	0.18	0.22	-0.32
	p	0.69	0.73	0.93	0.30	0.64	0.97	0.95	0.53	0.86	0.35	0.48	0.40	0.23
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Left MUAC (cm)	*r*	0.39	-0.18	0.18	-0.47	-0.10	-0.32	-0.22	-0.30	-0.02	-0.07	0.11	0.35	-0.08
	p	0.10	0.47	0.47	**0.04**	0.68	0.18	0.40	0.22	0.95	0.78	0.65	0.14	0.74
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
Right MUAC (cm)	*r*	0.33	-0.25	0.12	-0.44	-0.13	-0.29	-0.20	-0.29	-0.05	0.00	0.06	0.37	-0.12
	p	0.16	0.31	0.62	0.06	0.59	0.23	0.43	0.22	0.84	0.99	0.82	0.13	0.63
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
Average MUAC (cm)	*r*	0.37	-0.22	0.15	-0.46	-0.12	-0.35	-0.22	-0.30	-0.03	-0.03	0.09	0.36	-0.11
	p	0.12	0.37	0.54	**0.046**	0.63	0.15	0.41	0.21	0.90	0.89	0.73	0.13	0.65
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
Left TSF (mm)	*r*	-0.11	0.46	0.38	-0.26	0.20	-0.35	-0.02	-0.05	0.36	-0.09	0.51	0.43	0.33
	p	0.67	0.07	0.14	0.31	0.44	0.17	0.94	0.84	0.16	0.72	**0.04**	0.08	0.21
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Right TSF (mm)	*r*	-0.06	0.09	0.03	-0.26	-0.01	-0.16	-0.03	-0.15	0.09	-0.05	0.34	0.18	0.09
	p	0.83	0.73	0.92	0.32	0.98	0.55	0.92	0.57	0.73	0.86	0.18	0.48	0.74

		Creatinine (mol/L)	Albumin (g/L)	Prealbumin (g/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L) †	LDL cholesterol (mmol/L)	Non-HDL cholesterol (mmol/L)	Total HDL-cholesterol (mmol/L)	Ferritin (µg/L)	Transferrin (g/L)	Retinol-binding protein (mg/L)	eGFR †
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Average TSF (mm)	*r*	-0.09	0.31	0.23	-0.28	0.11	-0.32	-0.03	-0.10	0.25	-0.08	0.46	0.34	0.25
	p	0.73	0.23	0.37	0.28	0.66	0.21	0.93	0.70	0.33	0.77	0.06	0.18	0.35
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Left AMA (cm^2)	*r*	0.51	-0.48	-0.10	-0.30	-0.27	-0.13	-0.17	-0.25	-0.22	-0.02	-0.19	0.06	-0.39
	p	**0.04**	0.054	0.70	0.25	0.29	0.63	0.54	0.33	0.39	0.93	0.46	0.82	0.13
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Right AMA (cm^2)	*r*	0.45	-0.32	0.10	-0.33	-0.17	-0.42	-0.19	-0.22	-0.08	0.03	-0.12	0.30	-0.29
	p	0.07	0.21	0.71	0.20	0.52	0.09	0.51	0.39	0.77	0.91	0.66	0.24	0.27
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Average AMA (cm^2)	*r*	0.51	-0.43	0.00	-0.33	-0.24	-0.31	-0.19	-0.25	-0.16	0.00	-0.17	0.19	-0.37
	p	**0.04**	0.09	0.99	0.19	0.36	0.23	0.49	0.33	0.54	0.99	0.52	0.46	0.16
	n	17	17	17	17	17	17	15	17	17	17	17	17	16
Left calf circumference (cm)	*r*	0.38	0.12	0.33	-0.33	-0.04	-0.50	-0.09	-0.11	0.25	-0.21	0.17	0.42	0.002
	p	0.11	0.62	0.17	0.18	0.87	**0.03**	0.75	0.65	0.30	0.40	0.49	0.07	0.99
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
Right calf circumference (cm)	*r*	0.37	0.12	0.002	-0.47	-0.14	-0.29	-0.30	-0.32	0.04	-0.30	0.11	0.18	-0.15
	p	0.12	0.63	0.99	**0.04**	0.58	0.22	0.24	0.18	0.86	0.22	0.66	0.46	0.55
	n	19	19	19	19	19	19	17	19	19	19	19	19	18
	r	0.40	0.13	0.18	-0.42	-0.09	-0.41	-0.20	-0.23	0.16	-0.27	0.15	0.32	-0.09

		Creatinine (mol/L)	Albumin (g/L)	Prealbumin (g/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L) †	LDL cholesterol (mmol/L)	Non-HDL cholesterol (mmol/L)	Total HDL-cholesterol (mmol/L)	Ferritin (µg/L)	Transferrin (g/L)	Retinol-binding protein (mg/L)	eGFR †
Average calf circumference (cm)	*p*	0.10	0.61	0.46	0.08	0.71	0.08	0.44	0.35	0.52	0.27	0.54	0.18	0.72
	n	19	19	19	19	19	19	17	19	19	19	19	19	18

7.1.2 Markers of inflammation

As highlighted in **section 2.4.3.1**, it is important to assess for a pro-inflammatory state alongside the measurement of serum hepatic biochemical analytes, such as albumin, pre-albumin and transferrin. The blood concentrations of routine, basic inflammatory markers are presented in Table 7.4, which shows the measures of central tendency, NGH Laboratory Medicine reference ranges [306] and the proportion of study participants with elevated markers of inflammation, expressed as n/N (%).

An increase in at least one inflammatory parameter was found in 9/19 (47.4%) of participants (seven males, two females). The most commonly increased inflammatory marker was fibrinogen, which was elevated in 8/19 (42.1%) of study participants at baseline. Platelets and lymphocyte cell count were not elevated in any participants. The study cohort were therefore divided into two groups; those with at least one elevated marker of inflammation ("Pro-inflammatory group") (n = 9), and those without any elevated markers of inflammation ("no inflammation group") (n = 10).

Table 7.4 Markers of inflammation for the study cohort at baseline (n = 19). Data is presented as mean ± one standard deviation and median (IQR). The number of participants with elevated inflammatory marker concentrations is indicated as n/N (%). IQR: interquartile range; SD: standard deviation.

Biosample	Inflammatory Marker	Mean ± SD Median (IQR)	Reference ranges	Elevated n/N (%)
Whole blood	Erythrocyte sedimentation rate (mm/hr)	10.79 ± 10.03 6.00 (5.00-20.00)	Male: 1 - 10 Female: 1 - 15	5/19 (26.32)
	White cell count ($x10^9$/L)	6.49 ± 1.99 6.10 (5.40-7.10)	3.5 - 9.5	2/19 (10.53)
	Platelets ($x10^9$/L)	248.05 ± 69.34 241.00 (184.00-310.00)	150 - 400	0/19 (0.00)
	Lymphocytes ($x10^9$/L)	1.46 ± 0.52 1.26 (1.06-1.92)	1.0 - 3.0	0/19 (0.00)
Plasma	Fibrinogen (g/L)	3.81 ± 0.89 3.90 (3.20-4.30)	2.0 – 4.0	8/19 (42.11)
Serum	C-Reactive Protein (mg/ml)	3.19 ± 6.87 1.60 (0.80-2.00)	0.00 - 5.00	2/19 (10.53)

7.1.3 Serum biochemical analytes by inflammatory state

Table 7.5 compares the measures of central tendency for the serum biochemical analytes measured at baseline for the two inflammatory groups. To examine whether there was a statistical difference in the serum hepatic proteins or lipid profile analytes between the groups with or without inflammation, normally distributed data was analysed using Welch's t-test and non-normally distributed data was analysed using the Mann-Whitney U test. No statistically significant differences were observed between the inflammatory groups for any serum biochemical analyte. To confirm that a pro-inflammatory state did not interfere with serum hepatic proteins or the lipid profile, Pearson's and Spearman's correlation analysis was conducted to determine the relationship between serum biochemical analytes and markers of inflammation (Table 7.6). Statistically significant moderate, negative relationships were found between serum creatine and Erythrocyte sedimentation rate (ESR) (r = -0.68, p = 0.001), white cell count (r = -0.48, p = 0.04), fibrinogen (r = -0.63, p = 0.004) and C-reactive protein (CRP) (r = -0.50, p = 0.03). eGFR was observed to significantly moderately positively correlate against white cell count (r = 0.51, p = 0.03) and C-reactive protein (r = 0.55, p = 0.02).

For each of the significant relationships for serum creatinine and eGFR identified in Table 7.6, the study participants were again grouped according to their inflammatory state, this time specific to the inflammatory marker of interest. For example, those with an elevated fibrinogen concentration were categorised as "pro-inflammatory", and those with fibrinogen below the maximum reference level as "no inflammation"). Mann-Whitney U tests were performed to evaluate whether the concentration of serum analytes was significantly increased or decreased in those with inflammation, compared to those without inflammation. The concentration of serum creatinine demonstrated a significant reduction in those with elevated levels of ESR, compared to those with normal levels of ESR (Figure 7.1; Mann-Whitney U = 12, p = 0.03).

It was therefore concluded that a pro-inflammatory state did not interfere with the concentration of serum hepatic proteins or the lipid profile in this cohort. However, the pro-inflammatory state was associated with a decrease in serum creatinine.

Table 7.5 Comparisons of serum biochemical analyte concentrations across inflammatory groups/non-inflammatory groups. Data presented as mean ± one standard deviation and median (IQR). Normally distributed data was analysed using Welch's t-test; non-normally distributed data was analysed using Mann-Whitney test. Significance at $p < 0.05$ level. IQR: interquartile range; SD: standard deviation.

	No inflammation group	Inflammation group	P value
Creatinine (mol/L)	n = 10 69.50 ± 11.34 68.00 (63.50-77.00)	n = 9 63.44 ± 22.93 56.00 (50.50-66.00)	0.07
Albumin (g/L)	n = 10 45.90 ± 1.79 45.50 (44.75-48.00)	n = 9 47.11 ± 2.67 46.00 (45.50-48.50)	0.27
Prealbumin (g/L)	n = 10 0.27 ± 0.05 0.27 (0.23-0.30)	n = 9 0.27 ± 0.05 0.28 (0.24-0.31)	0.92
Cholesterol (mmol/L)	n = 10 5.33 ± 0.86 5.20 (4.45-5.78)	n = 9 4.69 ± 0.97 4.90 (4.05-5.35)	0.23
Triglyceride (mmol/L)	n = 10 1.49 ± 0.68 1.50 (0.95-1.95)	n = 9 1.50 ± 0.56 1.50 (1.00-1.85)	0.97
HDL cholesterol (mmol/L)	n = 10 1.82 ± 1.02 1.47 (1.23-2.03)	n = 9 1.52 ± 0.48 1.61 (1.05-1.93)	0.95
LDL cholesterol (mmol/L)	n = 8 2.90 ± 0.86 2.85 (2.20-3.30)	n = 9 2.47 ± 0.68 2.40 (2.05-3.00)	0.27
Non-HDL cholesterol (mmol/L)	n = 10 3.71 ± 1.03 3.70 (2.80-4.53)	n = 9 3.18 ± 0.66 3.40 (2.50-3.65)	0.20
Total HDL cholesterol ratio (mmol/L)	n = 10 3.70 ± 1.20 3.70 (2.58-4.78)	n = 9 3.29 ± 0.89 3.10 (2.70-3.60)	0.62
Ferritin (µg/L)	n = 10 243.20 ± 76.62 240.00 (179.75-305.50)	n = 9 261.11 ± 149.97 226.00 (151.50-412.50)	0.75
Transferrin (g/L)	n = 10 2.27 ± 0.20 2.31 (2.07-2.49)	n = 9 2.52 ± 0.39 2.62 (2.13-2.85)	0.11
Retinol binding protein (mg/L)	n = 10 50.80 ± 12.25 45.00 (40.75-61.75)	n = 9 56.56 ± 12.16 58.00 (46.00-65.00)	0.32

	n = 9	n = 9	
eGFR EPI	86.44 ± 6.64	85.67 ± 12.63	0.46
	90.00 (84.50-90.00)	90.00 (89.50-90.00)	

Table 7.6 Correlation analysis between markers of inflammation and serum biochemical analytes (n = 19). Correlation analysis was conducted using Pearson's moment correlation analysis for normally distributed data, or Spearman's (†) correlation analysis for non-normally distributed data. Significance observed at the $p < 0.05$ level, highlighted in bold. CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

		Marker of inflammation					
Serum biochemical analyte		**ESR (mm/hr)**	**White cell count ($x10^9$/L)**	**Platelets ($x10^9$/L)**	**Lymphocytes ($x10^9$/L)**	**Fibrinogen (g/L)**	**CRP (mg/ml)**
Creatinine (mol/L) (n = 19)	*r*	-0.68	-0.48	-0.39	-0.22	-0.63	-0.50
	p	**0.001**	**0.04**	0.10	0.36	**0.004**	**0.03**
Albumin (g/L) (n = 19)	*r*	0.01	0.05	-0.11	-0.05	0.04	0.08
	p	0.96	0.84	0.65	0.86	0.86	0.76
Prealbumin (g/L) (n = 19)	*r*	-0.06	-0.05	-0.08	0.11	-0.41	-0.17
	p	0.82	0.84	0.75	0.64	0.09	0.49
Transferrin (g/L) (n = 19)	*r*	0.33	0.19	0.13	0.27	0.06	0.25
	p	0.17	0.43	0.59	0.26	0.80	0.30
Cholesterol (mmol/L) (n = 19)	*r*	0.12	-0.18	-0.10	-0.17	-0.28	-0.14
	p	0.62	0.45	0.69	0.50	0.24	0.57
Triglyceride (mmol/L) (n = 19)	*r*	0.11	0.29	0.33	0.12	-0.14	0.25
	p	0.65	0.23	0.17	0.61	0.56	0.30
HDL-Cholesterol (mmol/L) (n = 19)	*r*	0.13	-0.14	0.00	-0.23	0.06	-0.36
	p	0.59	0.58	0.99	0.34	0.81	0.14
LDL-Cholesterol (mmol/L) (n = 17)	*r*	-0.09	-0.10	-0.25	-0.10	-0.26	-0.08
	p	0.72	0.71	0.34	0.71	0.31	0.78
Non-HDL Cholesterol (mmol/L) (n = 19)	*r*	-0.10	-0.14	-0.06	-0.08	-0.33	-0.05
	p	0.68	0.56	0.81	0.74	0.17	0.84
Total HDL Cholesterol ratio (mmol/L) (n = 19)	*r*	-0.18	0.14	-0.08	-0.02	-0.21	0.24
	p	0.47	0.57	0.74	0.92	0.40	0.32
Ferritin (µg/L) (n = 19)	*r*	0.15	0.15	0.36	-0.04	0.22	0.23
	p	0.54	0.55	0.14	0.88	0.36	0.34
Retinol binding protein (mg/L) (n = 19)	*r*	0.12	0.04	0.01	0.06	-0.18	0.24
	p	0.63	0.87	0.98	0.82	0.47	0.32
eGFR (n = 18)	*r*	0.35	0.51	0.48	0.22	0.24	0.55
	p	0.16	**0.03**	0.04	0.39	0.34	**0.02**

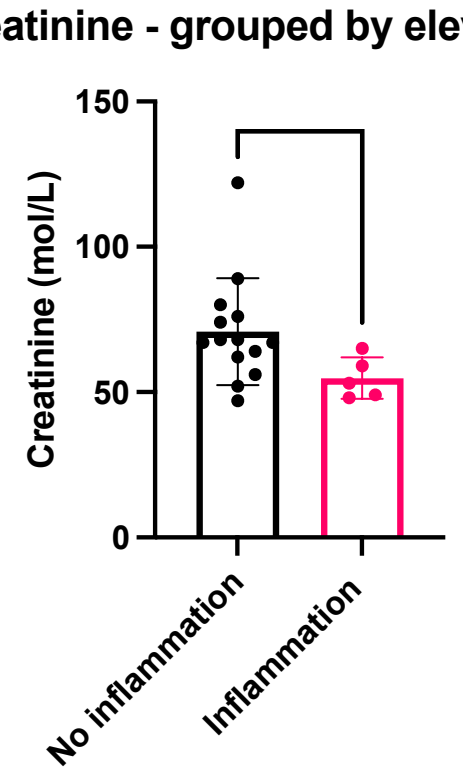


Figure 7.1 Creatinine grouped by erythrocyte sedimentation rate (ESR) concentration. Those with a normal ESR level were categorised as 'no inflammation' (n = 14) and those with an elevated ESR level were categorised as 'inflammation' (n = 5). Significance was analysed using the Mann-Whitney test to compare the ranks of non-parametric data (U = 12, p = 0.03*). Significance at $p < 0.05$.

7.1.4 24-hour urinary biochemical analytes

Table 7.7 shows the measures of central tendency and NGH Medical Laboratory reference ranges (where provided) [306] for the 24-hour urinary biochemical analytes for all 22 participants at baseline. As for the serum analytes, Pearson's and Spearman's correlation analyses was conducted to compare the baseline 24-hour urinary biochemical analytes against baseline clinical and anthropometric parameters (Table 7.8). 24-hour urinary sodium and potassium both statistically significantly moderately positively correlated with weight (sodium: $r = 0.68$, $p = 0.001$; potassium: $r = 0.55$, $p = 0.01$), left MUAC (sodium: $r = 0.42$, $p = 0.05$; potassium: $r = 0.49$, $p = 0.02$) and calf circumference (sodium: $r = 0.49$, $p = 0.02$; potassium: $r = 0.56$, $p = 0.01$). 24-hour urinary sodium also demonstrated a significant moderate positive correlation with left ($r = 0.62$, $p = 0.003$) and average TSF ($r = 0.52$, $p = 0.02$), whilst 24-hour urinary potassium moderately positively correlated with MUAC (average MUAC: $r = 0.49$, $p = 0.02$) and AMA (average AMA: $r = 0.53$, $p = 0.02$).

Table 7.7 24-hour urinary biochemical analytes at baseline (n = 22). Data is presented as mean ± one standard deviation and median (IQR). IQR: interquartile range; SD: standard deviation; TUN: total urinary nitrogen.

	Reference Ranges	Mean ± SD Median (IQR)
24-hour urinary sodium (mmol/L)		121.50 ± 53.36 116.00 (76.25-143.75)
24-hour urinary potassium (mmol/L)		65.14 ± 21.93 63.00 (53.75-81.25)
24-hour urinary urea (mmol/L)	200-600	333.77 ± 115.08 344.50 (228.00-406.25)
24-hour TUN (g/day)		9.93 ± 3.42 10.66 (6.80-12.04)

Table 7.8 Correlation of complete 24-hour urinary biochemical analytes against demographic, clinical and anthropometric data at baseline (n = 22). Correlation analysis was conducted using Pearson's correlation coefficient for normally distributed data or Spearman's correlation coefficient (†) for non-normally distributed data. Significance observed at $p < 0.05$. Significant results highlighted in bold. ALSFRS-R: amyotrophic lateral sclerosis functional rating scale - revised; AMA: arm muscle area; BMI: body mass index; MUAC: Mid-upper arm circumference; TSF: triceps skinfold thickness; TUN: total urinary nitrogen; ΔALSFRS-R: change in the amyotrophic lateral sclerosis functional rating scale - revised.

		24-hour urinary sodium (mmol/L)	24-hour urinary potassium (mmol/L)	24-hour urinary urea (mmol/L)	24-hour TUN (g/day)
Age (years)	*r*	-0.10	0.06	0.06	0.02
	p	0.67	0.81	0.80	0.94
	n	22	22	22	22
Disease duration † (months)	*r*	0.16	-0.05	-0.01	-0.07
	p	0.48	0.84	0.95	0.77
	n	22	22	22	22
King's staging †	*r*	0.30	0.40	0.08	0.02
	p	0.17	0.06	0.73	0.92
	n	22	22	22	22
ALSFRS-R total subscore † (/48)	*r*	-0.20	-0.18	0.10	0.12
	p	0.38	0.42	0.67	0.61
	n	22	22	22	22
ALSFRS-R bulbar subscore † (/12)	*r*	0.11	-0.07	0.09	0.04
	p	0.64	0.77	0.68	0.86
	n	22	22	22	22
ALSFRS-R Fine Motor subscore (/12)	*r*	-0.08	-0.01	0.18	0.18
	p	0.71	0.97	0.43	0.41
	n	22	22	22	22
ALSFRS-R Gross Motor subscore (/12)	*r*	-0.01	0.11	0.36	0.32
	p	0.96	0.63	0.10	0.14
	n	22	22	22	22
ALSFRS Respiratory subscore † (/12)	*r*	-0.33	-0.33	-0.10	-0.07
	p	0.13	0.13	0.65	0.77
	n	22	22	22	22
ΔALSFRS-R	*r*	-0.11	0.13	0.15	0.12
	p	0.64	0.57	0.50	0.61

		24-hour urinary sodium (mmol/L)	24-hour urinary potassium (mmol/L)	24-hour urinary urea (mmol/L)	24-hour TUN (g/day)
	n	22	22	22	22
Anthropometric parameters					
Weight (kg)	*r*	0.68	0.55	0.37	0.26
	p	**0.001**	**0.01**	0.11	0.27
	n	20	20	20	20
Percentage weight change † (%)	*r*	-0.31	-0.22	-0.26	-0.34
	p	0.21	0.39	0.30	0.17
	n	18	18	18	18
BMI (kg/m^2)	*r*	0.43	0.28	0.004	-0.07
	p	0.06	0.24	0.99	0.78
	n	20	20	20	20
Left MUAC (cm)	*r*	0.42	0.49	0.25	0.14
	p	**0.05**	**0.02**	0.27	0.54
	n	22	22	22	22
Right MUAC (cm)	*r*	0.35	0.47	0.19	0.06
	p	0.11	**0.03**	0.40	0.80
	n	22	22	22	22
Average MUAC (cm)	*r*	0.39	0.49	0.22	0.10
	p	0.07	**0.02**	0.32	0.66
	n	22	22	22	22
Left TSF (mm)	*r*	0.62	0.09	0.11	0.09
	p	**0.003**	0.71	0.64	0.69
	n	20	20	20	20
Right TSF (mm)	*r*	0.32	-0.08	-0.21	-0.26
	p	0.17	0.75	0.36	0.27
	n	20	20	20	20
Average TSF (mm)	*r*	0.52	0.02	-0.04	-0.07
	p	**0.02**	0.95	0.87	0.77
	n	20	20	20	20
Left AMA (cm^2)	*r*	0.02	0.45	0.19	0.07
	p	0.94	**0.05**	0.43	0.76
	n	20	20	20	20
Right AMA (cm^2)	*r*	0.20	0.54	0.32	0.18
	p	0.39	**0.01**	0.17	0.45
	n	20	20	20	20
Average AMA (cm^2)	*r*	0.12	0.53	0.27	0.13
	p	0.62	**0.02**	0.25	0.57

		24-hour urinary sodium (mmol/L)	24-hour urinary potassium (mmol/L)	24-hour urinary urea (mmol/L)	24-hour TUN (g/day)
	n	20	20	20	20
Left calf circumference (cm)	*r*	0.52	0.60	0.30	0.23
	p	**0.01**	**0.003**	0.17	0.31
	n	22	22	22	22
Right calf circumference (cm)	*r*	0.39	0.44	0.22	0.13
	p	0.07	**0.04**	0.32	0.56
	n	22	22	22	22
Average calf circumference (cm)	*r*	0.49	0.56	0.28	0.19
	p	**0.02**	**0.01**	0.21	0.39
	n	22	22	22	22

7.1.4.1 Considering the completeness of 24-hour urinary collections

It was important to consider the potential influence of an incomplete (i.e., missing samples or spillages) or inappropriately collected/unusable (i.e., collected for an inappropriate duration or first sample not discarded) 24-hour urinary collection on the concentration of the urinary biochemical analytes. Unpaired t-tests were used to compare the concentrations of the 24-hour urinary biochemical analytes in samples that were complete against samples that were incomplete, or unusable (Table 7.9). Statistically significant reductions were observed in the concentrations of 24-hour urinary urea (p = 0.02) in the incomplete/unusable group. Participants with incomplete or unusable collections (n = 5) were therefore removed from a repeated Pearson's moment correlation analyses for 24-hour urinary urea to investigate whether the presence of incomplete urinary samples alters the relationship with clinical and anthropometric parameters (n = 17). No statistically significant relationships were observed.

Table 7.9 24-hour urinary biochemical analytes grouped by completeness of collection at baseline (n = 22). Data presented as mean ± one standard deviation and median (IQR). Analysis was conducted using an unpaired t-test. Significance at $p < 0.05$. Significant results highlighted in bold. IQR: interquartile range; TUN: total urinary nitrogen.

	Complete n/N = 17/22	**Incomplete/unusable n/N = 5/22**	**P value**
24-hour urinary sodium (mmol/L)	124.82 ± 53.09 121.00 (86.00-144.00)	110.20 ± 58.90 111.00 (53.50-166.50)	0.60
24-hour urinary potassium (mmol/L)	68.12 ± 20.42 67.00 (56.00-82.500)	55.00 ± 26.29 56.00 (31.50-78.00)	0.25
24-hour urinary urea (mmol/L)	363.41 ± 104.42 382.00 (272.50-410.50)	233.00 ± 97.37 216.00 (142.00-332.50)	**0.02**
24-hour TUN (g/day)	10.69 ± 3.17 10.95 (8.00-12.60)	7.34 ± 3.21 6.59 (4.38-10.69)	0.052

7.1.5 Longitudinal changes in biochemical analytes

Longitudinal analysis of the serum and 24-hour urinary biochemical analytes was conducted for all participants (where samples were collected) over the nine-month study period - regardless of inflammatory state or completeness of the urinary collection. Normally distributed data was analysed using a mixed effects analysis for multiple comparisons. Non-normally distributed data was analysed using the Wilcoxon t-test (Table 7.10). Statistically significant reductions were observed between baseline and the first follow-up for serum creatinine and TUN, as well as between baseline and the second follow-up for 24-hour urinary sodium and between baseline and the third follow-up for serum creatinine and HDL-cholesterol.

The decline in serum creatinine (Δcreatinine) was calculated by the formulae: (creatinine at baseline – creatinine at third follow up) / months between baseline and the third follow-up study visit. The Δcreatinine ranged between -0.41 and 2.25. A Pearson's moment correlation analysis was conducted to compare the relationship between the Δcreatinine and ΔALSFRS-R. This was not found to be statistically significant (r = 0.57, p = 0.08).

Table 7.10 Longitudinal changes in biochemical analytes. Data presented as n/N; mean ± one standard deviation; and median (IQR). Longitudinal data was analysed for significance using the mixed effects analysis for multiple comparisons for normally distributed data, or the Wilcoxon t-test for non-normally distributed data. Significance at p = <0.05 level, highlighted in bold. B = baseline study visit; F1-3: first, second or third follow-up study visit; IQR: interquartile range; SD: standard deviation.

					P value		
	Baseline	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
Serum analytes							
Creatinine (mol/L)	n = 19/22 66.63 ± 17.54 65.00 (53.00-74.00)	n = 17/20 66.59 ± 17.92 61.00 (56.00-75.00)	n = 13/17 68.09 ± 18.50 64.00 (55.00-78.00)	n = 10/14 59.40 ± 15.81 60.00 (45.25-69.75)	n = 15 **0.02**	n = 12 0.42	n = 10 **0.03**
Albumin (g/L)	n = 19/22 46.47 ± 2.27 46.00 (45.00-48.00)	n = 17/20 46.29 ± 2.52 46.00 (45.00-47.50)	n = 13/17 46.31 ± 2.43 47.00 (44.50-47.50)	n = 10/14 46.20 ± 3.29 46.00 (44.00-47.75)	n = 15 0.98	n = 12 0.99	n = 10 0.96
Prealbumin (g/L)	n = 19/22 0.27 ± 0.04 0.28 (0.23-0.30)	n = 16/20 0.27 ± 0.05 0.27 (0.23-0.39)	n = 12/17 0.29 ± 0.05 0.29 (0.25-0.33)	n = 9/14 0.28 ± 0.05 0.28 (0.24-0.33)	n = 14 0.98	n = 11 0.18	n = 9 0.61
Cholesterol (mmol/L)	n = 19/22 4.97 ± 0.93 5.00 (4.40-5.50)	n = 17/20 4.91 ± 1.03 4.90 (4.30-5.60)	n = 13/17 4.89 ± 0.84 5.00 (4.40-5.35)	n = 10/14 4.98 ± 0.66 4.95 (4.40-5.43)	n = 15 0.96	n = 12 0.96	n = 10 >0.99
Triglyceride (mmol/L)	n = 19/22 1.49 ± 0.61 1.50 (1.00-1.90)	n = 17/20 1.57 ± 0.68 1.40 (1.05-2.05)	n = 13/17 1.58 ± 0.70 1.40 (1.00-1.95)	n = 10/14 1.71 ± 0.83 1.50 (1.18-2.00)	n = 15 0.20	n = 12 0.24	n = 10 0.12
HDL cholesterol (mmol/L)	n = 19/22 1.67 ± 0.80 1.49 (1.24-1.90)	n = 17/20 1.46 ± 0.44 1.37 (1.20-1.83)	n = 13/17 1.52 ± 0.46 1.55 (1.23-1.86)	n = 10/14 1.44 ± 0.35 1.38 (1.19-1.83)	n = 15 0.15	n = 12 0.27	n = 10 **0.002**

					P value		
	Baseline	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
LDL cholesterol (mmol/L)	n = 17/22 2.67 ± 0.78 2.50 (2.10-3.25)	n = 12/20 2.63 ± 0.83 2.50 (2.03-3.30)	n = 12/17 2.63 ± 0.74 2.60 (2.10-3.13)	n = 10/14 2.77 ± 0.59 2.70 (2.43-3.40)	n = 9 0.99	n = 11 0.99	n = 9 0.91
Non-HDL cholesterol (mmol/L)	n = 19/22 3.46 ± 0.90 3.40 (2.80-3.90)	n = 17/20 3.45 ± 0.88 3.70 (2.65-4.10)	n = 13/17 3.78 ± 0.69 3.30 (3.20-3.70)	n = 10/14 3.55 ± 0.80 3.55 (3.10-4.08)	n = 15 >0.99	n = 12 0.97	n = 10 0.97
Total HDL cholesterol ratio (mmol/L)	n = 19/22 3.51 ± 1.06 3.10 (2.70-4.50)	n = 17/20 3.58 ± 1.01 3.70 (2.70-4.10)	n = 13/17 3.47 ± 0.95 3.40 (2.75-4.10)	n = 10/14 3.70 ± 1.12 3.70 (2.70-4.85)	n = 15 0.93	n = 12 0.99	n = 10 0.81
Ferritin (µg/L)	n = 19/22 251.68 ± 114.09 226.00 (155.00-337.00)	n = 17/20 240.94 ± 93.79 248.00 (161.50-315.00)	n = 13/17 226.62 ± 100.75 250.00 (123.00-308.50)	n = 10/14 261.70 ± 102.21 277.50 (184.50-340.75)	n = 15 0.58	n = 12 0.29	n = 10 0.82
Transferrin (g/L)	n = 19/22 2.39 ± 0.32 2.35 (2.09-2.62)	n = 15/20 2.46 ± 0.35 2.41 (2.18-2.70)	n = 12/17 2.38 ± 0.37 2.34 (2.24-2.67)	n = 10/14 2.37 ± 0.26 2.39 (2.12-2.56)	n = 13 0.48	n = 11 >0.99	n = 10 0.98
Retinol binding protein (mg/L)	n = 19/22 53.53 ± 12.22 55.00 (41.00-65.00)	n = 15/20 51.01 ± 19.48 57.00 (38.00-65.00)	n = 13/17 55.62 ± 13.16 54.00 (45.50-67.00)	n = 8/14 55.75 ± 9.60 57.00 (46.75-63.75)	n = 13 0.82	n = 12 0.35	n = 8 0.44
eGFR EPI	n = 18/22 86.06 ± 9.79 90.00 (88.25-90.00)	n = 15/20 84.27 ± 10.71 90.00 (82.00-90.00)	n = 12/17 84.33 ± 10.89 90.00 (84.25-90.00)	n = 8/14 87.38 ± 7.03 90.00 (89.25-90.00)	n = 10 >0.99	n = 8 0.63	n = 6 0.75
24-hour urinary analytes							
24-hour urinary sodium (mmol/L)	n = 22/22 121.50 ± 53.36 116.00 (76.25-143.75)	n = 20/20 101.05 ± 47.22 105.50 (60.50-137.50)	n = 16/17 107.63 ± 46.40 89.00 (73.25-132.25)	n = 13/14 96.39 ± 41.62 87.00 (65.60-126.00)	n = 20 0.08	n = 16 **0.04**	n = 13 0.17

	Baseline	F1	F2	F3	P value B-F1	P value B-F2	P value B-F3
24-hour urinary potassium (mmol/L)	n = 22/22 65.14 ± 21.93 63.00 (53.75-81.25)	n = 20/20 59.90 ± 25.78 60.00 (39.75-77.25)	n = 16/17 63.38 ± 21.38 57.50 (46.50-79.50)	n = 13/14 57.92 ± 18.66 51.00 (47.50-68.00)	n = 20 0.43	n = 16 0.98	n = 13 0.31
24-hour urinary urea (mmol/L)	n = 22/22 333.77 ± 115.08 344.50 (228.00-406.25)	n = 20/20 303.00 ± 127.26 315.00 (198.00-377.00)	n = 16/17 338.25 ± 122.42 302.00 (256.00-370.50)	n = 12/14 341.33 ± 112.22 322.00 (256.00-411.50)	n = 20 0.13	n = 16 0.34	n = 12 0.91
24-hour TUN (g/day)	n = 22/22 9.93 ± 3.42 10.66 (6.80-12.04)	n = 20/20 8.74 ± 3.72 8.82 (5.73-11.17)	n = 16/17 9.57 ± 3.40 8.83 (6.99-10.75)	n = 13/14 9.73 ± 3.35 10.03 (6.76-11.06)	n = 20 **0.05**	n = 16 0.16	n = 13 0.68

7.2 Discussion

7.2.1 Influence of a pro-inflammatory state

As described in **section 2.4.3.1** the interplay between a pro-inflammatory state and the concentration of serum hepatic proteins has been well documented. For that reason, routine basic markers of inflammation were measured alongside the measurement of serum biochemical analytes. A pro-inflammatory state was identified in almost half (9/19 (47.4%)) of this study cohort (where blood samples were obtained). In direct contrast to results presented by Chiò et al., (2014) [164], no significant relationships were observed between the inflammatory markers and either the hepatic proteins (albumin, prealbumin and transferrin), or the serum lipid profile. A pro-inflammatory response was therefore not demonstrated to affect the concentration of these serum biochemical analytes in this cohort and individuals with elevated expression of inflammatory markers were not discounted from the bivariate correlation analysis to assess the relationship between biochemical analytes and the assessment of nutritional state.

The data presented in this chapter have however demonstrated that serum creatinine was significantly negatively correlated with markers of inflammation (ESR, white cell count, fibrinogen and CRP); specifically, an elevated ESR concentration was associated with a significant reduction in serum creatinine. A raised inflammatory response is known to be associated with increased muscle catabolism [160]. As serum creatinine is an established by-product of muscle catabolism [167], it was hypothesised that serum creatinine would be significantly positively associated with AMA, the proxy marker of muscle mass in this cohort [162]. As the results presented in **Chapter 6** demonstrated that AMA significantly reflects body weight, it was expected that serum creatinine would also demonstrate a positive relationship with body weight. Whilst creatinine did significantly positively correlate with AMA at baseline, serum creatinine also demonstrated a positive relationship with weight; however, this was not found to be significant (p = 0.07). Therefore, it can be concluded that an elevated inflammatory state observed in this cohort is associated with increased muscle catabolism, identifiable using serum creatinine as a proxy marker for muscle mass.

7.2.2 Relationship with BMI

No serum or 24-hour urinary biochemical analytes were found to correlate with BMI in this cohort. This was surprising, as serum creatinine has previously been demonstrated to significantly positively correlate with BMI in two independent cross-sectional MND cohort studies [162,325]. Whilst the absence of a correlation with serum albumin agrees with the findings by Chiò et al., (2014) [162] this contrasts with Park et al., (2015), who demonstrated a significant negative relationship between albumin and BMI [91]. This disparity may be associated by the influence of a pro-inflammatory state, as Park et al., did not reportedly consider the influence of an inflammatory state.

7.2.3 Relationship with body composition

As described in **section 2.4.1,** the calculation of percentage weight loss from before MND onset (premorbid) has commonly been used as an indicator of declining nutritional state. The baseline data presented in this chapter demonstrates that the lowest albumin concentrations were observed in individuals reporting the greatest percentage weight loss from premorbid.

A decrease in serum transferrin is known to indicate severe protein-energy malnutrition [326,327]. Serum transferrin was found to positively correlate with left-arm TSF in this cohort. As protein-energy malnutrition is associated with a decline in fat mass, the relationship between transferrin and TSF suggests that serum transferrin could be used as a marker of fat mass in MND.

24-hour urinary sodium and potassium were both observed to positively correlate with weight, MUAC and calf circumference. 24-hour urinary sodium also significantly positively correlated with TSF, whilst 24-hour urinary potassium positively correlated with MUAC and AMA. As body circumference measurements encompass bone, muscle and fat mass, they cannot reliably distinguish between the contributions from fat or fat free mass. These data therefore suggest that sodium measured from 24-hour urinary collections could be indicative of fat mass, whilst potassium may be a better reflection of fat free mass. Neither 24-hour urinary urea or total urinary nitrogen were observed to demonstrate relationships with any proxy measures of body composition.

7.2.4 Assessment of malnutrition risk

Serum analyte concentrations below the clinically acceptable reference ranges have previously been used to indicate malnutrition [325]. The concentration of serum biochemical analytes was therefore investigated for participants already indicated to be at risk of malnutrition (Pt01 and Pt08: Table 6.2). No reductions in the concentration of serum analytes were identified for Pt01. However, Pt08 demonstrated lower-than-normal concentrations for serum creatinine, prealbumin and transferrin. This individual was also found to have a lower-than-average AMA (44.85 cm^2) and TSF (13.90 mm) compared to the rest of this cohort at baseline, with elevated levels of ESR, fibrinogen and CRP. In addition to Pt08, a further four participants presented with serum analyte concentrations below the reference ranges. The study participants indicated to be at risk of an adverse nutritional state, which may lead to malnutrition, are shown in Table 7.11. Four of these participants presented decreased levels of creatinine, two presented decreases in transferrin and one in prealbumin. Three of the participants indicated to be at risk of malnutrition using reductions in the concentration of serum biochemical analytes demonstrated a ≥ 5% weight loss from premorbid, ranging between a loss of 6.07% and 15.20%. One individual was unable to provide a weight measurement to calculate percentage weight loss from premorbid and BMI. Whilst no malnutrition-risk thresholds have been previously suggested for AMA and TSF, two participants with reductions in serum creatinine demonstrated AMA assessments lower than the group median (< 46.77 cm^2, Table 6.1) and one participant with reduced levels of transferrin measured TSF lower than the group median (< 14.09 mm, Table 6.1).

Table 7.11 Markers of a risk of malnutrition by weight change from premorbid (%), BMI, MUAC, calf circumference and serum biochemical analytes at baseline (n = 22). Markers of a risk of malnutrition are highlighted in red. Participants indicated to be at risk of an adverse nutritional state are highlighted in bold. Note: a negative weight change is indicative of a gain of weight from premorbid. Data not collected is denoted by '-'. BMI: body mass index; MUAC: mid-upper arm circumference.

			MUAC (cm)			Calf circumference (cm)			Serum biochemical analytes		
	Weight change from premorbid (%)	**BMI (kg/m²)**	**Left**	**Right**	**Average**	**Left**	**Right**	**Average**	**Creatinine (mol/L)**	**Transferrin (g/L)**	**Prealbumin (g/L)**
Pt01	4.81	17.70	22.00	22.50	22.25	33.00	32.50	32.75	68	2.51	0.23
Pt03	9.76	32.40	36.50	37.50	37.00	44.40	45.40	44.90	122	2.22	0.28
Pt04	6.07	26.40	31.00	30.80	30.90	38.40	39.00	38.70	52	2.03	0.26
Pt05	-1.25	34.60	30.30	30.40	30.35	39.70	44.00	41.85	48	2.62	0.27
Pt06	16.54	22.20	28.20	28.80	28.50	32.80	33.80	33.30	53	2.45	0.28
Pt07	-0.81	26.00	32.60	32.10	32.35	40.00	38.70	39.35	80	2.09	0.29
Pt08	15.20	25.70	27.10	29.10	28.10	28.70	35.00	31.85	49	1.96	0.18
Pt09		27.40	29.80	30.50	30.15	35.80	37.10	36.45	89	2.34	0.22
Pt10	7.09	29.00	27.00	27.70	27.35	34.50	34.80	34.65	64	1.95	0.22
Pt11	8.57	22.80	29.00	28.10	28.55	31.50	33.40	32.45	56	3.04	0.32
Pt13	7.89	23.20	25.00	23.50	24.25	35.50	34.20	34.85	-	-	-
Pt14	-15.95	29.10	31.10	30.90	31.00	41.50	40.90	41.20	67	2.76	0.32
Pt15	-	26.00	27.00	26.50	26.75	38.10	40.10	39.10	62	2.27	0.28
Pt16	6.39	33.00	31.80	32.60	32.20	39.80	39.90	39.85	65	2.94	0.3
Pt17	-	-	29.30	33.00	31.15	40.10	32.90	36.50	47	2.48	0.35
Pt18	-	-	32.60	32.20	32.40	37.50	36.70	37.10	67	2.21	0.24
Pt19	7.14	21.20	26.00	26.40	26.20	35.40	35.70	35.55	68	2.5	0.29
Pt20	-3.85	27.00	30.40	28.50	29.45	36.80	35.50	36.15	-	-	-

Pt21	1.76	26.40	32.00	31.60	31.80	36.90	36.90	36.90	-	-	-
Pt22	2.63	24.30	30.90	27.60	29.25	36.60	37.00	36.80	74	2.35	0.33
Pt23	4.84	19.20	26.00	26.70	26.35	34.70	34.70	34.70	76	2.02	0.26
Pt24	5.69	31.60	34.30	33.70	34.00	42.60	42.60	42.60	59	2.68	0.21

7.2.5 Relationship with disease severity and progression

Data presented in this chapter have demonstrated that disease severity – assessed by the ALSFRS-R total score and the King's College staging system - was not associated with either the concentration of either serum creatinine or albumin. These findings contrast with data published by Chelstowska and Kuzma-Kozakiewicz (2020) [320], Chiò et al., (2014) [162] and Mitsumoto et al., (2020) [169].

In order to consider longitudinal changes in the biochemical analytes, data from all participants was included at all study time points, regardless of the completeness of the 24-hour urinary collections. The results for 24-hour urinary urea should therefore be inferred with caution. As presented in **Chapter 6**, muscle mass was not found to significantly decrease in this cohort over the nine-month study period (Table 6.4). Due to the significant positive relationship observed between serum creatinine and AMA at baseline, it was expected that serum creatinine content would also remain constant over the nine-month study period. Serum creatinine was nevertheless found to significantly decrease between baseline and the first and third study visits. However, this significant decline did not reflect disease progression, assessed by the change in ALSFRS-R score over the same period.

7.3 Conclusions

The comparison of the baseline results for the serum biochemical analytes with and without consideration of an elevated inflammatory profile demonstrated the influence of a pro-inflammatory state on the concentration of serum creatinine. The relationships observed between serum creatinine and transferrin with body composition suggest that these analytes may be useful indicators of malnutrition risk. The decline in these analytes irrespective of changes in body composition suggests that these biochemical analytes may decrease ahead of a decline in BMI and body composition, which would support this thesis hypothesis.

The incorporation of 24-hour urinary biochemical analytes as assessments of nutritional state in MND is novel. The statistically significant decline in 24-hour urinary urea in incomplete or unusable 24-hour urinary collections at baseline demonstrates the importance of protocol

adherence when providing 24-hour urinary samples. Furthermore, no statistically significant relationships were observed between 24-hour urinary urea or total urinary nitrogen against body composition, disease severity or disease progression. This suggests that 24-hour urinary urea and total urinary nitrogen cannot be used to assess the nutritional state. As the concentrations of 24-hour sodium and potassium were not demonstrated to significantly reduce in incomplete or unusable 24-hour urinary collections, this demonstrates a decreased reliance on protocol adherence. Moreover, the significant relationships demonstrated with body composition in this chapter suggest that both 24-hour urinary sodium and potassium could be utilised as robust, pragmatic biomarkers to estimate body composition in MND.

The next results chapter will use the concentrations of biochemical analytes presented in this chapter to investigate the role of using biochemical analytes as objective markers of dietary intake.

8 Assessment of nutritional intake

The purpose of this chapter was to assess the role of using an online self-reported 24-hour dietary recall questionnaire, "Intake24" to estimate the intake of energy and macronutrients. This chapter will first examine the accuracy and reliability of using Intake24 to estimate nutritional intake by comparing a select subset of reported nutritional intake data against the concentrations collected serum and excreted urinary biochemical analytes presented in Chapter 7 (**section 8.1**). This chapter will also compare the reported nutritional intake data against: i) the anthropometric assessments presented in Chapter 6 to investigate the relationship between nutritional intake and body composition (**section 8.2**); ii) disease severity (presented in Chapter 5), to investigate the relationship between nutritional intake and function (**section 8.3**); and iii) the estimated average energy and nutrient requirements of a healthy population in the UK (introduced in **section 2.4.4.2**) to investigate whether the participants in this study cohort were consuming an adequate nutritional intake (**section 8.4**).

8.1 Comparison of reported nutritional intake against biochemical analytes

In order to evaluate the reliability of using Intake24 to assess the nutritional intake in this MND cohort, Pearson's and Spearman's correlation analyses were conducted to investigate the relationship between the participant reported dietary intake data, obtained from the automated output from the Intake24 nutrient database, and the concentration of measured serum and urinary biochemical analytes. As significant reductions in the concentration of 24-hour urinary urea were observed in incomplete or unusable samples (Table 7.9), bivariate correlation analysis was conducted using only complete 24-hour urinary collections for the relationship with 24-hour urinary urea (n = 17). Significant correlations are highlighted in bold in Table 8.1. Of particular note, statistically significant, moderate positive relationships were observed between potassium intake and urinary potassium excretion (r = 0.59, p = 0.004, n = 22), as well as between protein intake and excreted total urinary nitrogen (r = 0.48, p = 0.02, n = 22).

Table 8.1 Comparisons between self-reported nutrient intake and the concentration of serum and urinary biochemical analytes at baseline. Correlation analysis was conducted using Pearson's correlation coefficient for normally distributed data, or Spearman's correlation coefficient (†) for non-normally distributed data. Significance was observed at $p < 0.05$, highlighted in bold.

		Self-reported nutrient intake										
		Sodium (mg)	Potassium (mg)	Protein† (g)	Carbohydrate † (g)	Fat (g)	Cholesterol (g)	Starch (g)	AOAC fibre † (g)	Vitamin A † (µg)	Retinol (µg)	Iron † (mg)
24-hour urinary analytes												
Sodium (mmol/L)	*r*	0.27	0.26	0.45	0.06	0.19	0.36	0.04	0.10	0.15	0.34	0.26
	p	0.23	0.24	**0.04**	0.78	0.41	0.10	0.85	0.66	0.50	0.13	0.25
	n	22	22	22	22	22	22	22	22	22	22	22
Potassium (mmol/L)	*r*	0.37	0.46	0.59	0.21	0.27	0.32	0.19	0.03	0.23	0.26	0.07
	p	0.09	**0.03**	**0.004**	0.35	0.22	0.15	0.39	0.89	0.31	0.24	0.75
	n	22	22	22	22	22	22	22	22	22	22	22
Urea (mmol/L)	*r*	0.29	-0.11	0.15	-0.004	0.30	0.37	0.03	-0.41	0.23	0.28	-0.35
	p	0.26	0.67	0.56	0.99	0.25	0.15	0.92	0.11	0.38	0.28	0.17
	n	17	17	17	17	17	17	17	17	17	17	17
Total urinary nitrogen (g/day)	*r*	0.49	0.15	0.48	0.29	0.47	0.34	0.31	0.11	0.35	0.36	0.03
	p	**0.02**	0.50	**0.02**	0.18	**0.03**	0.12	0.17	0.64	0.11	0.10	0.89
	n	22	22	22	22	22	22	22	22	22	22	22

		Self-reported nutrient intake										
		Sodium (mg)	Potassium (mg)	Protein† (g)	Carbohydrate † (g)	Fat (g)	Cholesterol (g)	Starch (g)	AOAC fibre † (g)	Vitamin A † (µg)	Retinol (µg)	Iron † (mg)
Serum analytes												
Creatinine (mol/L) †	*r*	0.23	0.22	0.35	0.11	0.38	0.22	0.03	0.14	0.04	0.04	0.30
	p	0.33	0.38	0.15	0.65	0.11	0.37	0.92	0.56	0.87	0.89	0.21
	n	19	19	19	19	19	19	19	19	19	19	19
Albumin (g/L)	*r*	0.04	0.03	0.23	-0.04	0.31	0.03	0.00	0.30	-0.06	0.21	0.54
	p	0.86	0.92	0.34	0.88	0.19	0.90	0.99	0.21	0.81	0.39	**0.02**
	n	19	19	19	19	19	19	19	19	19	19	19
Prealbumin (g/L)	*r*	0.23	0.29	0.42	0.13	0.16	0.02	0.18	0.20	0.02	-0.04	0.41
	p	0.33	0.24	0.08	0.60	0.53	0.92	0.47	0.42	0.94	0.89	0.08
	n	19	19	19	19	19	19	19	19	19	19	19
Cholesterol (mmol/L)	*r*	-0.35	0.01	-0.28	-0.31	-0.26	-0.16	-0.31	0.01	-0.42	-0.46	-0.11
	p	0.14	0.97	0.25	0.20	0.28	0.52	0.20	0.96	0.08	**0.047**	0.64
	n	19	19	19	19	19	19	19	19	19	19	19
Triglyceride (mmol/L)	*r*	0.03	0.37	0.53	0.45	0.08	0.18	0.50	0.06	-0.14	-0.11	0.13
	p	0.90	0.12	**0.02**	0.053	0.75	0.47	**0.03**	0.79	0.56	0.65	0.61
	n	19	19	19	19	19	19	19	19	19	19	19
	r	-0.46	-0.15	-0.50	-0.39	-0.19	-0.30	-0.45	0.06	-0.10	-0.14	-0.18
	p	**0.048**	0.54	**0.03**	0.10	0.44	0.21	0.054	0.79	0.67	0.57	0.46

		Self-reported nutrient intake										
		Sodium (mg)	Potassium (mg)	Protein† (g)	Carbohydrate † (g)	Fat (g)	Cholesterol (g)	Starch (g)	AOAC fibre † (g)	Vitamin A † (µg)	Retinol (µg)	Iron † (mg)
HDL-cholesterol† (mmol/L)	*n*	19	19	19	19	19	19	19	19	19	19	19
LDL-cholesterol (mmol/L)	*r*	-0.23	-0.44	-0.51	-0.49	-0.35	-0.39	-0.41	-0.22	-0.28	-0.40	-0.27
	p	0.37	0.08	**0.04**	**0.046**	0.17	0.12	0.10	0.40	0.28	0.11	0.30
	n	17	17	17	17	17	17	17	17	17	17	17
Non-HDL-cholesterol (mmol/L)	*r*	-0.19	-0.03	-0.10	-0.24	-0.22	-0.12	-0.14	-0.21	-0.40	-0.41	-0.18
	p	0.45	0.89	0.69	0.32	0.37	0.62	0.56	0.40	0.09	0.08	0.46
	n	19	19	19	19	19	19	19	19	19	19	19
Total HDL-cholesterol (mmol/L)	*r*	0.19	-0.11	0.18	0.07	-0.02	0.01	0.12	-0.22	-0.20	-0.18	-0.01
	p	0.44	0.65	0.45	0.79	0.94	0.98	0.62	0.36	0.41	0.45	0.98
	n	19	19	19	19	19	19	19	19	19	19	19
Ferritin (µg/L)	*r*	0.13	-0.19	-0.31	-0.11	-0.11	-0.27	-0.13	-0.38	0.13	-0.26	-0.32
	p	0.60	0.43	0.19	0.66	0.66	0.27	0.61	0.11	0.58	0.29	0.18
	n	19	19	19	19	19	19	19	19	19	19	19
Transferrin (g/L)	*r*	-0.11	0.42	0.34	0.22	0.13	-0.02	0.27	0.48	-0.05	0.26	0.54
	p	0.67	0.07	0.15	0.38	0.59	0.93	0.27	**0.04**	0.85	0.28	**0.02**
	n	19	19	19	19	19	19	19	19	19	19	19

		Self-reported nutrient intake										
		Sodium (mg)	Potassium (mg)	Protein† (g)	Carbohydrate † (g)	Fat (g)	Cholesterol (g)	Starch (g)	AOAC fibre † (g)	Vitamin A † (µg)	Retinol (µg)	Iron † (mg)
Retinol binding protein (mg/L)	*r*	0.05	0.22	0.19	-0.10	0.01	0.09	-0.08	-0.06	0.21	0.08	0.18
	p	0.84	0.38	0.44	0.69	0.97	0.72	0.75	0.81	0.39	0.75	0.47
	n	19	19	19	19	19	19	19	19	19	19	19

8.2 Relationship between nutritional intake and body composition

Table 8.2 demonstrates the bivariate correlation analysis conducted between reported nutritional intake and anthropometric assessments of nutritional state at baseline. Water intake was found to significantly moderately positively correlate with body weight (r = 0.48, p = 0.03, n = 20), BMI (r = 0.47, p = 0.04, n = 20) and calf circumference (r = 0.44, p = 0.04, n = 22); energy intake significantly moderately negatively correlated with BMI (r = -0.48, p = 0.03, n = 20); retinol and vitamin A intake significantly moderately positively correlated with MUAC (retinol: r = 0.43, p = 0.045, n = 22; Vitamin A: r = 0.50, p = 0.02, n = 22); Vitamin A also significantly moderately positively correlated with AMA (r = 0.46, p = 0.04, n = 20); and 24-hour urinary sodium significantly moderately negatively correlated with the percentage weight change from premorbid (r = -0.54, p = 0.02, n = 18).

8.3 Relationship between nutritional intake and disease severity

When the relationship between nutritional intake and disease severity was assessed (Table 8.3), fat intake significantly moderately positively correlated with the rate of disease progression (ΔALSFRS-R) (r = 0.46, p = 0.03, n = 22) and Vitamin A intake significantly moderately negatively correlated with the ALSFRS-R respiratory subscore (r = -0.51, p = 0.02, n = 22).

Table 8.2 Correlation of nutritional intake against anthropometric data at baseline. Correlation analysis was conducted using Pearson's correlation coefficient for normally distributed data, or Spearman's (†) correlation coefficient for non-normally distributed data. Significance observed at $p < 0.05$. Significant results highlighted in bold. AMA: arm muscle area; Av = average; L = left; MUAC: mid-upper arm circumference; R = right; TSF: triceps skinfold thickness.

					MUAC (cm)			TSF (mm)			AMA (cm^2)			Calf circumference (cm)		
Participant self-reported intake		**Weight (kg)**	**Weight change from premorbid (%) †**	**BMI (kg/m^2)**	**L**	**R**	**Av**	**L**	**R**	**Av**	**L**	**R**	**Av**	**L**	**R**	**Av**
Water (g)	*r*	0.48	-0.19	0.47	0.35	0.33	0.34	0.33	0.28	0.33	0.08	0.15	0.12	0.38	0.45	0.44
	p	**0.03**	0.44	**0.04**	0.12	0.14	0.12	0.16	0.23	0.16	0.74	0.53	0.60	0.08	**0.04**	**0.04**
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Protein (g)	*r*	0.08	-0.26	-0.15	0.13	0.07	0.10	-0.18	-0.31	-0.25	0.16	0.16	0.17	0.14	0.04	0.09
	p	0.75	0.29	0.52	0.56	0.75	0.64	0.45	0.19	0.29	0.50	0.51	0.48	0.55	0.87	0.68
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Fat (g)	*r*	-0.14	-0.28	-0.42	-0.08	-0.14	-0.11	0.03	-0.11	-0.03	-0.18	-0.17	-0.19	0.10	-0.16	-0.03
	p	0.56	0.25	0.07	0.73	0.55	0.63	0.89	0.65	0.89	0.44	0.49	0.43	0.66	0.47	0.90
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Carbohydrate (g) †	*r*	-0.19	0.15	-0.42	-0.06	-0.10	-0.08	-0.13	-0.14	-0.17	0.13	-0.03	0.06	-0.10	-0.18	-0.16
	p	0.44	0.55	0.06	0.79	0.64	0.73	0.57	0.55	0.47	0.59	0.90	0.80	0.65	0.43	0.47
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Energy (kcal) †	*r*	-0.21	-0.09	-0.48	-0.09	-0.15	-0.11	-0.18	-0.25	-0.25	0.09	-0.05	0.08	-0.07	-0.21	-0.14
	p	0.37	0.74	**0.03**	0.70	0.51	0.64	0.45	0.28	0.29	0.72	0.85	0.75	0.77	0.36	0.55

Participant self-reported intake		Weight (kg)	Weight change from premorbid (%) †	BMI (kg/m²)	MUAC (cm)			TSF (mm)			AMA (cm²)			Calf circumference (cm)		
					L	R	Av	L	R	Av	L	R	Av	L	R	Av
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Starch (g) †	*r*	-0.18	-0.03	-0.39	-0.01	-0.06	-0.03	-0.07	-0.12	-0.12	0.14	0.06	0.14	-0.09	-0.19	-0.17
	p	0.45	0.91	0.09	0.96	0.80	0.91	0.77	0.62	0.61	0.55	0.81	0.57	0.71	0.40	0.46
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Cholesterol (g)	*r*	0.19	-0.56	0.11	0.02	-0.06	-0.02	-0.08	-0.27	-0.18	0.02	0.04	0.03	0.21	0.11	0.17
	p	0.41	**0.02**	0.65	0.93	0.79	0.92	0.73	0.25	0.45	0.93	0.88	0.90	0.34	0.64	0.45
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Retinol (µg)	*r*	0.38	0.09	0.28	0.41	0.43	0.43	0.29	0.25	0.29	0.33	0.41	0.40	0.20	0.25	0.24
	p	0.10	0.74	0.23	0.06	**0.045**	**0.045**	0.22	0.29	0.22	0.16	0.07	0.09	0.37	0.27	0.29
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Vitamin A (µg) †	*r*	0.38	0.15	0.24	0.52	0.54	0.50	0.22	0.21	0.28	0.43	0.44	0.46	0.33	0.32	0.33
	p	0.10	0.55	0.32	**0.01**	**0.01**	**0.02**	0.35	0.38	0.24	0.06	0.05	**0.04**	0.14	0.15	0.14
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Sodium (mg)	*r*	0.31	-0.54	-0.04	0.38	0.27	0.33	0.28	-0.01	0.16	0.24	0.30	0.29	0.41	0.18	0.31
	p	0.19	**0.02**	0.87	0.08	0.22	0.13	0.24	0.96	0.51	0.32	0.20	0.22	0.06	0.42	0.15
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Potassium (mg)	*r*	0.16	0.01	-0.01	0.22	0.21	0.22	-0.11	-0.16	-0.14	0.26	0.27	0.28	0.10	0.04	0.08
	p	0.51	0.96	0.98	0.33	0.35	0.33	0.64	0.50	0.55	0.28	0.24	0.23	0.64	0.86	0.73
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
Iron (mg) †	*r*	-0.03	-0.21	-0.24	0.19	-0.09	0.07	0.07	0.06	0.04	0.15	-0.24	-0.05	-0.02	-0.05	0.03

Participant self-reported intake		Weight (kg)	Weight change from premorbid (%) †	BMI (kg/m^2)	MUAC (cm)			TSF (mm)			AMA (cm^2)			Calf circumference (cm)		
					L	R	Av	L	R	Av	L	R	Av	L	R	Av
	p	0.91	0.41	0.32	0.41	0.70	0.76	0.76	0.81	0.87	0.54	0.31	0.83	0.92	0.83	0.90
	n	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22

Table 8.3 Correlation of nutritional intake against disease severity (n = 22). Correlation analysis was conducted using Pearson's correlation for normally distributed data, or Spearman's (†) correlation for non-normally distributed data. Significance observed at $p < 0.05$. Significant results highlighted in bold. ALSFRS-R: amyotrophic lateral sclerosis functional rating scale – revised; ΔALSFRS-R: change in the amyotrophic lateral sclerosis functional rating scale – revised.

				ALSFRS-R subscore (/12)				
Participant self-reported intake		**King's Staging**	**ALSFRS-R total (/48)**	**Bulbar**	**Fine**	**Gross**	**Respiratory**	**ΔALSFRS-R**
Water (g)	*r*	0.24	-0.25	-0.21	0.10	-0.17	-0.40	0.13
	p	0.28	0.26	0.35	0.66	0.45	0.07	0.56
	n	22	22	22	22	22	22	22
Protein (g)	*r*	-0.03	-0.01	0.12	0.10	0.07	-0.11	-0.001
	p	0.90	0.95	0.61	0.66	0.76	0.63	0.996
	n	22	22	22	22	22	22	22
Fat (g)	*r*	0.09	-0.11	0.00	-0.06	0.13	-0.29	0.46
	p	0.69	0.62	0.99	0.78	0.56	0.19	**0.03**
	n	22	22	22	22	22	22	22
Carbohydrate (g) †	*r*	-0.24	0.22	0.35	0.27	0.03	0.04	-0.01
	p	0.28	0.33	0.11	0.23	0.89	0.86	0.96
	n	22	22	22	22	22	22	22
Energy (kcal) †	*r*	-0.17	0.12	0.22	0.20	0.08	-0.04	0.10
	p	0.44	0.59	0.33	0.36	0.73	0.87	0.66
	n	22	22	22	22	22	22	22
Starch (g) †	*r*	-0.20	0.19	0.24	0.36	0.05	0.09	-0.09
	p	0.37	0.39	0.28	0.10	0.84	0.68	0.71
	n	22	22	22	22	22	22	22
Cholesterol (g)	*r*	0.03	-0.13	0.04	-0.31	-0.12	-0.13	0.06
	p	0.91	0.57	0.88	0.16	0.60	0.56	0.78
	n	22	22	22	22	22	22	22
Retinol (µg)	*r*	0.15	-0.09	0.31	-0.02	0.34	-0.28	-0.02
	p	0.51	0.70	0.17	0.94	0.13	0.21	0.94
	n	22	22	22	22	22	22	22
Vitamin A (µg) †	*r*	0.28	-0.36	0.04	-0.07	0.14	-0.51	0.37
	p	0.21	0.10	0.87	0.76	0.55	**0.02**	0.09
	n	22	22	22	22	22	22	22
Sodium (mg)	*r*	0.23	-0.33	-0.02	-0.08	-0.19	-0.41	0.27
	p	0.31	0.14	0.94	0.72	0.40	0.06	0.22
	n	22	22	22	22	22	22	22

Potassium (mg)	*r*	0.21	0.04	0.02	0.35	-0.06	-0.41	0.20
	p	0.36	0.86	0.95	0.12	0.79	0.06	0.37
	n	22	22	22	22	22	22	22
Iron (mg) †	*r*	-0.08	-0.09	0.14	-0.06	-0.08	-0.08	0.17
	p	0.74	0.70	0.53	0.80	0.73	0.73	0.44
	n	22	22	22	22	22	22	22

8.4 Comparison of reported dietary intake against dietary reference values

The participant self-reported data for the intake of energy, carbohydrates, fats and proteins were compared against the estimated average requirement for the UK population according to the age and sex of each participant [305] (Table 8.4).

Table 8.4 Public Health England Government Dietary Recommendations for energy and macronutrients [305].

	≤ 64		65 - 74		≥ 75	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Energy (kcal/day)	2500	2000	2342	1912	2294	1840
Protein (g/day)	55.5	45	53.5	46.5	53.3	46.5
Fat (g/day)	97	78	91	74	89	72
Carbohydrate (g/day)	333	267	312	255	306	245

During the 24-hours prior to the baseline study visit, 15 participants (15/22 (68.2%); twelve males, three females) consumed less than the estimated average recommended energy and carbohydrate intake for their age and sex. Similarly, 16 participants (16/22 (72/7%; thirteen males, three females) consumed less than the estimate average requirement for fat intake whilst only two participants (9.1%; one male, one female) consumed less than the estimated average requirement for protein intake. Figure 8.1 is included as an example to demonstrate the comparison of reported energy intake against the estimated average UK requirement.

Longitudinal changes in reported energy and macronutrient intake over the nine-month study period are presented in Table 8.5. No statistically significant differences were observed for energy or macronutrient intake between baseline and any follow-up visits.

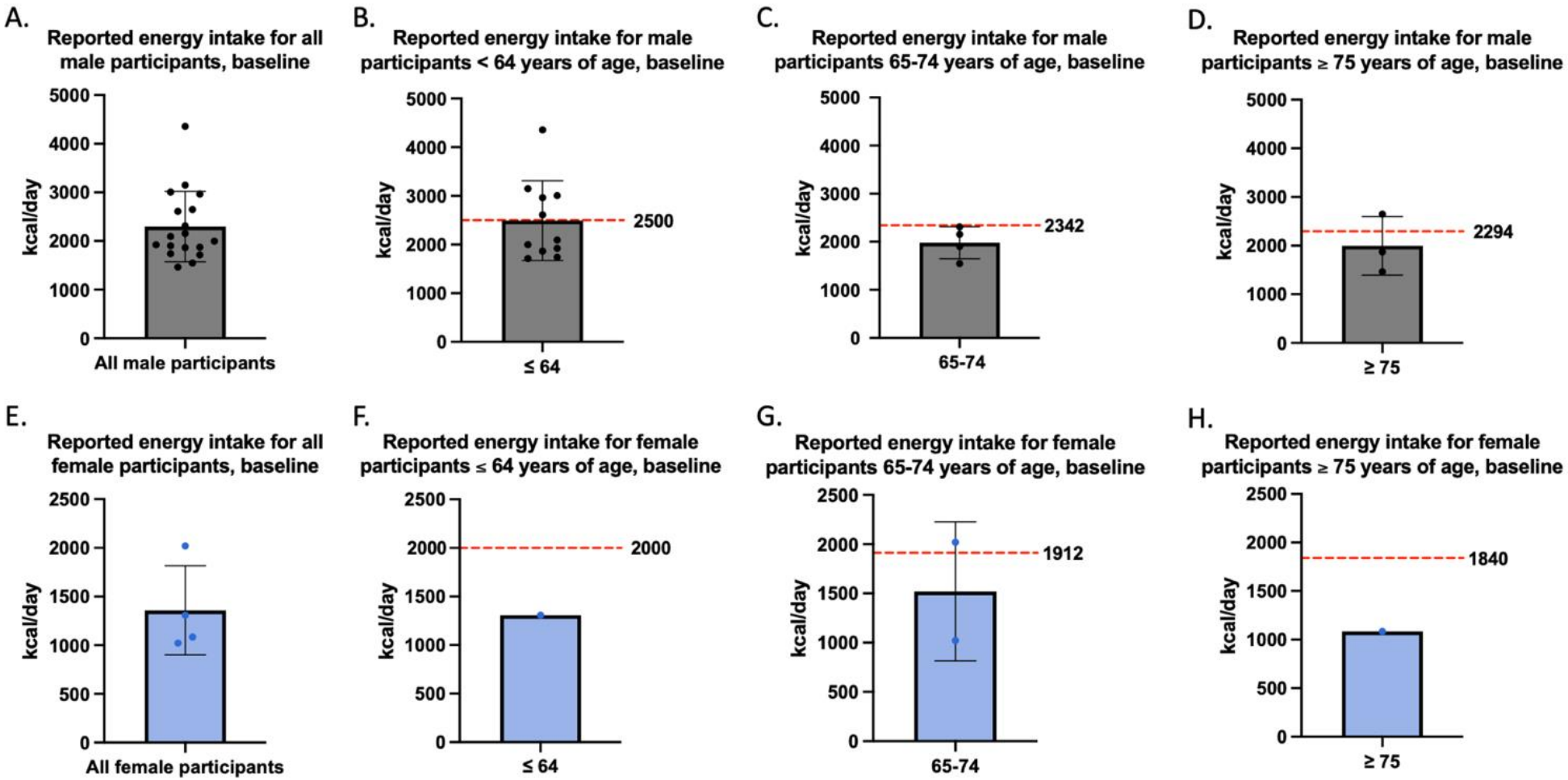


Figure 8.1 Reported energy intake (kcal/day) for all participants at baseline (n = 22). Self-reported energy intake is compared against the UK estimated average requirement (shown by the red dotted line) for age (A/E: all participants; B/F: ≤ 64 years; C/G: 65-74 years; D/H: ≥ 75 years) and sex (A-D: males; E-H: females).

Table 8.5 Longitudinal changes in energy (kcal/day) and macronutrient (g/day) intake over nine-months. Data presented as mean ± one standard deviation and median (IQR). Data checked for normality using Shapiro-Wilk analysis. Parametric analyses were conducted using Dunnett's test for mixed-effects analysis. Non-parametric analyses were conducted using Wilcoxon matched-pairs signed rank test. Significance at $p < 0.05$. B: baseline; F1-3: follow-up study visits 1-3; IQR: interquartile range

	Study time point				P value		
Self-reported nutritional intake	**Baseline n/N = 22/24**	**F1 n/N = 20/24**	**F2 n/N = 17/24**	**F3 n/N = 14/24**	**B-F1 n = 20**	**B-F2 n = 17**	**B-F3 n = 14**
Energy (kcal/day)	2125.74 ± 767.31 1961.25 (1671.90-2621.44)	2044.61 ± 718.15 1823.15 (1554.96-2726.40)	2441.30 ± 1388.44 2393.81 (1163.89-3395.12)	2258.82 ± 814.20 1953.50 (1790.89-2839.46)	0.92	0.67	0.85
Carbohydrate (g/day)	269.28 ± 137.88 239.57 (183.24-342.51)	249.03 ± 100.06 237.80 (155.19-327.44)	299.22 ± 184.77 239.61 (160.43-411.44)	274.88 ± 93.57 269.07 (221.66-322.40)	0.99	0.89	0.22
Fat (g/day)	81.22 ± 24.67 85.48 (63.46-94.23)	80.14 ± 25.63 83.02 (58.85-98.11)	100.05 ± 65.19 93.38 (46.51-125.45)	94.24 ± 50.87 80.19 (57.48-119.35)	>0.99	0.50	0.67
Protein (g/day)	82.44 ± 28.80 73.82 (67.68-95.01)	80.13 ± 32.03 76.19 (49.39-106.53)	87.86 ± 38.53 104.11 (49.30-116.16)	81.17 ± 26.88 77.36 (64.70-95.97)	0.60	0.93	0.71

8.5 Discussion

Whilst significant relationships were observed with nutrient intake for 24-hour urinary sodium, potassium and total urinary nitrogen, the accurate measurement of recovery biomarkers of dietary exposure measured in 24-hour urinary collections requires patients to be weight stable, and not experiencing illness or trauma [190]. The data presented in Chapter 6 demonstrated this cohort had an average weight loss of 4.58% compared to before the onset of MND symptoms (or premorbid). Therefore, the significant negative relationship observed between the percentage of weight loss from premorbid and sodium intake may explain the dissociation between sodium intake and excretion. Interestingly, despite a significant positive relationship observed between protein intake and total urinary nitrogen at baseline (Table 8.1), the concentration of excreted total urinary nitrogen was observed to decrease between baseline and the first follow-up visit (Table 7.10), whilst protein intake did not significantly change throughout the duration of the study (Table 8.5). This suggests that the excretion of total urinary nitrogen is not directly related to protein intake.

The significant correlations observed between serum albumin, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and transferrin did not appear to be specific to the appropriate corresponding nutrient intake. These results need further analysis to understand the relationships observed; however, as these are serum biomarkers of nutritional state (defined in **section 2.4.3.1**), they reflect not only dietary intake, but also the integrated absorption and processing of nutrients [153,154].

The significant positive relationship observed between fluid intake and calf circumference measurements suggests that this lower limb anthropometric assessment is highly influenced by fluid intake; caution should therefore be applied when using this measurement as an assessment of nutritional state.

The data presented in this chapter have demonstrated that 15/22 (68.2%) of this cohort are at risk of not consuming sufficient energy, in relation to the wider UK general population. This is in agreement with the conclusions drawn by Slowie et al., (1983) [50] and Kasarskis (1996) [56], who both demonstrated an energy consumption lower than the estimated average requirements. The over-consumption of protein in 20/22 (90.9%) of the cohort is also

reflective of the over consumption of protein reported in 87.5% of participants in the study by Kasarskis and colleagues (1996) [56]. However, comparisons of the estimated energy intake from the participant self-reported 24-hour dietary recall against the UK estimated average requirements do not indicate whether an individual's consumption is adequate, but rather frames an individual's consumption in relation to that recommended for the general public, which may not be suitable [236]. For example, an individual living with MND may have a reduced physical activity level, compared to a healthy sex- and age-matched control. They may therefore have a reduced energy demand, and consequently, a reduced energy intake below that of the estimated average requirement may be sufficient. To better indicate whether an individual is consuming an adequate energy intake, estimated energy intake should be compared against assessments of total daily energy expenditure, specific to that individual.

8.6 Conclusion

These preliminary, novel results suggest that 24-hour urinary potassium could be used as an objective biomarker of potassium intake, however further research is needed to validate these findings. Comparisons of estimated energy intake against the UK estimated average requirements do not indicate whether an individual is meeting their own energy demand. This information therefore shouldn't be used in the clinic to assess whether the nutritional intake of an individual is appropriate. To indicate whether these participants were in energy balance, the self-reported energy intake data presented in this chapter will be compared against estimates of total daily energy expenditure in Chapter 9.

9 Assessment of resting energy expenditure

As stated in **section 2.4.5,** the energy expenditure of people living with MND should be assessed for two reasons:

1. In order to evaluate whether an individual is in energy balance, estimated energy intake must be compared against energy expenditure;
2. Approximately 50-68% of people living with sporadic MND are reported to experience hypermetabolism [61–65]. The presence of a hypermetabolic state (i.e., a greater-than-predicted resting energy expenditure [61,62]), is associated with an increased catabolism of carbohydrates, lipids and proteins [66], as well as an increase in excreted urinary nitrogen, leading to a negative nitrogen balance [67]. Therefore, the potential influence of a hypermetabolic state needs to be considered when assessing the nutritional state of people living with MND.

A key outcome from the conduction of the scoping review presented in Chapter 3 was the absence of a standardised, pragmatic approach when using indirect calorimetry in individuals living with MND. **Section 9.1** will therefore present the baseline results for the indirect calorimetry parameters in this MND cohort. As discussed in Chapter 8, in order to evaluate whether an individual is in energy balance, estimated energy intake must be compared against total daily energy expenditure. **Section 9.2** continues to investigate the assessment of energy balance in understanding the nutritional status of this MND cohort. As also demonstrated in Chapter 3, predictive energy equations are frequently used for the identification of hypermetabolism in MND. **Section 9.3** is a manuscript submitted for publication which aims to critically reflect on the use of predictive energy equations as comparators against measured resting energy expenditure to indicate hypermetabolism in MND. Finally, as this thesis argues against the currently accepted approach to define hypermetabolism, **section 9.7** proposes an alternative approach for defining hypermetabolism in this cohort.

9.1 Measured resting energy expenditure

9.1.1 Quality control: GEMNutrition metabolic cart calibration

The GEMNutrition (GEM) metabolic cart was calibrated before each measurement. Figure 9.1 shows the span and inspired calibration values in reference to predefined reference ranges [309]. Span calibration was appropriately within the predefined reference ranges (with the exception of Pt21, span O_2). However, values for inspired O_2 consistently fell below the recommended reference range (20.80 - 21.27%). As the inspired calibration is a sample of room air, this does not cause concern, as the measured $F_i O_2$ will be proportional to this value. Moreover, the coefficient of variation for inspired O_2 was 0.12%, therefore demonstrating consistency across all baseline measurements.

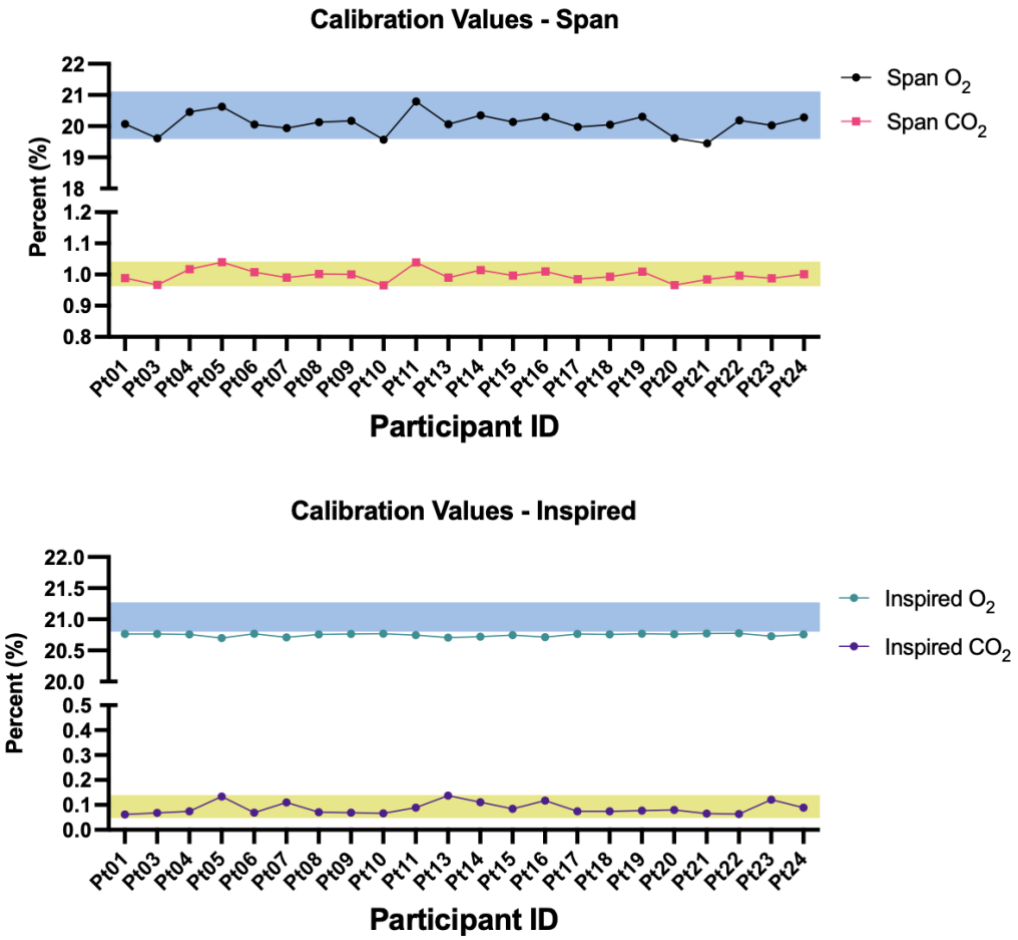


Figure 9.1 Calibration values for each participant at baseline. (Top) Calibration using span gases. Reference ranges shown in blue (span O_2) and yellow (span CO_2). (Bottom) Calibration

using inspired room air. Reference ranges shown in blue (inspired O_2) and yellow (inspired CO_2).

9.1.2 Indirect calorimetry parameters

Table 9.1 outlines the indirect calorimetry measurements recorded at baseline for all participants. The most common time of day for IC measurements was 11:55am, with an average post-prandial time of 3 hours 28 minutes for those who were not fasted (20/22 (90.91%)). The majority (19/22 (86%)) of participants were in a semi-supine position, with a head and torso angle most commonly at 30° from horizontal.

Table 9.1 Indirect calorimetry protocol and measurements for all participants at baseline (n = 22). Measures of central tendency are presented as mean ± one standard deviation and median (IQR). AMA: arm muscle area; IQR: interquartile range; mREE: measured resting energy expenditure; mREE/AMA: measured resting energy expenditure divided by arm muscle area; RQ: respiratory quotient; SD: standard deviation; VCO_2: volume of carbon dioxide expired; VO_2: volume of oxygen inspired;

Pt	Time (24hr)	Duration (min)	Fasted	Time post-prandial (hours:mins)	Body position	Angle (°)	Flow rate (L/min)	VO_2 (ml/min)	VCO_2 (ml/min)	RQ	mREE (kcal/day)	mREE/AMA (kcal/cm^2)
Pt01	11:48	20	No	3:18	Semi-supine	50	51.13	182.80	200.73	.91	1417	43.63
Pt03	11:51	20	No	3:21	Semi-supine	45	50.76	187.80	221.67	.85	1543	19.56
Pt04	12:30	23	No	2:30	Semi-supine	55	51.23	233.47	277.93	.84	1933	30.84
Pt05	12:18	20	No	4:03	Semi-supine	35	57.74	179.47	200.60	.89	1411	-
Pt06	12:15	20	No	3:45	Semi-supine	30	52.73	196.07	232.87	.84	1618	37.06
Pt07	14:55	21	No	4:55	Semi-supine	30	54.22	244.80	290.73	.84	2019	25.37
Pt08	13:05	20	No	4:05	Semi-supine	30	54.18	241.67	259.47	.93	1841	41.04
Pt09	11:59	20	No	2:59	Semi-supine	30	53.15	241.80	268.13	.90	1889	31.78
Pt10	11:15	20	No	2:30	Semi-supine	30	51.73	158.53	118.27	1.35	915	23.09
Pt11	10:59	20	No	2:44	Semi-supine	30	54.08	233.80	263.40	.89	1850	40.11
Pt13	11:37	20	Yes	-	Semi-supine	30	51.83	131.47	163.07	.81	1124	32.02

Pt14	11:39	21	No	4:09	Semi-supine	30	57.81	221.07	253.47	.87	1761	38.44
Pt15	12:20	20	No	3:05	Semi-supine	-	52.23	218.200	250.133	.88	1750	49.13
Pt16	15:25	21	No	2:25	Wheelchair	-	56.11	183.270	219.530	.84	1522	33.71
Pt17	11:15	20	No	3:15	Wheelchair	-	53.41	218.867	239.067	.92	1689	31.58
Pt18	11:12	20	Yes	-	Semi-supine	30	52.20	191.200	226.933	.84	1578	21.96
Pt19	12:08	20	No	3:08	Semi-supine	30	52.95	224.067	260.600	.86	1818	38.35
Pt20	14:51	20	No	4:21	Semi-supine	30	51.52	243.267	252.333	.96	1802	39.26
Pt21	14:25	19	No	4:25	Semi-supine	30	50.91	198.786	211.000	.94	1500	23.91
Pt22	11:29	22	No	3:29	Semi-supine	30	57.28	229.440	246.190	.93	1740	30.72
Pt23	11:22	20	No	3:37	Semi-supine	30	53.96	186.688	177.313	1.05	1291	27.07
Pt24	11:35	20	No	3:35	Sitting	40	53.91	211.667	189.667	1.12	1401	22.90
Mean ± SD (% of study population)	12:22 ± 1.19	20.32 ± 0.84		03:28 ± 0.41	Semi-supine (86.36)	33.95 ± 7.74	53.41 ± 2.16	228.32 ± 41.26	207.19 ± 30.11	0.92 ± 0.12	1610 ± 271	32.45 ± 8.03
Median (IQR)	11:55 (11:27-12:38)	20.00 (20.00-20.25)		03:25 (03:00-04:04)	Sitting (13.64)	30.00 (30.00-35.00)	53.05 (51.68-54.19)	235.97 (200.70-259.75)	214.93 (185.83-233.55)	0.89 (0.84-0.93)	1654 (1415-1824)	31.78 (24.64-38.85)
n/N (%) *Fasted* *Not-fasted*			2/22 (9.09) 20/22 (90.91)									

9.2 Prediction of total daily energy expenditure

Total daily energy expenditure was predicted (pTDEE) for this cohort using the Kasarskis Model 6 predictive energy equation [134]. The percentage difference between the measured resting energy expenditure (mREE) and predicted total daily energy expenditure for the study cohort at baseline is presented in Table 9.2. On average, mREE was found to be 69.73% of pTDEE. This demonstrates that pTDEE using the Kasarskis model 6 predictive equation is suitable for estimating TDEE in this cohort.

Table 9.2 Comparisons of measured resting energy expenditure against predicted total daily energy expenditure at baseline (n = 20). Measures of central tendency are presented as mean ± one standard deviation and median (IQR). IQR: interquartile range; mREE: measured resting energy expenditure; pTDEE: predicted total daily energy expenditure; SD: standard deviation.

Participant ID	mREE	Kasarskis Model 6 pTDEE	mREE as a percentage of TDEE (%)
Pt01	1417	2154	65.77
Pt03	1543	2728	56.57
Pt04	1930	2471	78.11
Pt05	1411	2100	67.18
Pt06	1618	1719	94.13
Pt07	2020	2349	85.99
Pt08	1841	2297	80.16
Pt09	1889	2597	72.74
Pt10	915	1880	48.67
Pt11	1850	2396	77.21
Pt13	1124	2189	51.36
Pt14	1774	2615	67.84
Pt15	1750	2226	78.63
Pt16	1523	2506	60.76
Pt19	1818	2160	84.17
Pt20	1802	2430	74.15
Pt21	1500	2025	74.07
Pt22	1746	2651	65.87
Pt23	1291	2297	56.21
Pt24	1401	2548	54.98
Mean ± SD	**1608 ± 287.47**	**2317 ± 265.37**	**69.73 ± 12.37**
Median (IQR)	**1682 (1413-1835)**	**2323 (2156-2538)**	**70.29 (57.62-78.50)**

9.2.1 Assessment of energy balance

Participant self-reported energy intake data obtained from Intake24 was expressed as a percentage of pTDEE to assess the risk of an inadequate energy consumption in relation to predicted total daily energy expenditure (Figure 9.2). If the reported energy intake was less than the pTDEE, then the individual was identified as being at risk of an inadequate energy consumption, and therefore at risk for an adverse nutritional state. Eleven participants (50%) did not consume sufficient energy to equal their pTDEE, four participants (18.18%) consumed sufficient energy to equal their pTDEE and five participants (22.73%) exceeded their pTDEE. TDEE could not be predicted in two participants where weight measurements could not be recorded.

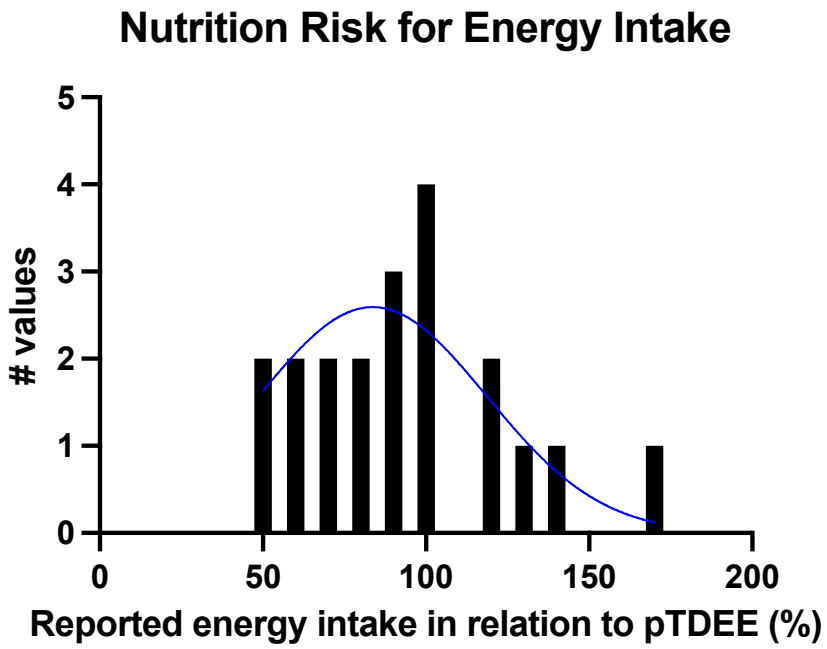


Figure 9.2 Reported energy intake expressed as a percentage of predicted total daily energy expenditure (n = 20). Gaussian distribution shown by blue line. Data was checked for – and passed - normality using the Shapiro-wilk test (p = 0.31).

9.3 A critical view of the use of predictive energy equations for the identification of hypermetabolism in motor neuron disease: a pilot study

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The next section of this thesis is presented in the form of a manuscript entitled: "A critical view of the use of predictive energy equations for the identification of hypermetabolism in motor neuron disease: a pilot study". This manuscript has been revised following peer-review, and submitted to the journal Clinical Nutrition ESPEN for publication. This manuscript considers a **subset of participants** who provided **complete 24-hour urinary collections** for the calculation of mREE using the Weir equation.

Thesis author contributions:

The thesis author was responsible for conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; validation; visualisation; writing - original draft, review & editing.

9.3.1 Abstract

Background and Aims

People living with motor neuron disease (MND) frequently struggle to consume an optimal caloric intake. Often compounded by hypermetabolism, this can lead to dysregulated energy homeostasis, prompting the onset of malnutrition and associated weight loss. This is associated with a poorer prognosis and reduced survival. It is therefore important to establish appropriate nutritional goals to ensure adequate energy intake. This is best done by measuring resting energy expenditure (mREE) using indirect calorimetry. However, indirect calorimetry is not widely available in clinical practice, thus dietitians caring for people living with MND frequently use energy equations to predict resting energy expenditure (pREE) and estimate caloric requirements. Energy prediction equations have previously been shown to underestimate resting energy expenditure in over two-thirds of people living with MND.

Hypermetabolism has previously been identified using the metabolic index. The metabolic index is a ratio of mREE to pREE, whereby an increase of mREE by ≥ 110% indicates hypermetabolism. We aim to critically reflect on the use of the Harris-Benedict (1919) and Henry (2005) energy prediction equations to inform a metabolic index to indicate hypermetabolism in people living with MND.

Methods

mREE was derived using VO_2 and VCO_2 measurements from a GEMNutrition indirect calorimeter. pREE was estimated by Harris-Benedict (HB) (1919), Henry (2005) and kcal/kg/day predictive energy equations. The REE variation, described as the percentage difference between mREE and pREE, determined the accuracy of pREE ([pREE-mREE]/mREE) x 100), with accuracy defined as ≤ ± 10%. A metabolic index threshold of ≥ 110% was used to classify hypermetabolism. All resting energy expenditure data are presented as kcal/24hr.

Results

Sixteen people living with MND were included in the analysis. The mean mREE was 1642 kcal/24hr ranging between 1110 and 2015 kcal/24hr. When REE variation was analysed for the entire cohort, the HB, Henry and kcal/kg/day equations all overestimated REE, but

remained within the accuracy threshold (mean values were 2.81% for HB, 4.51% for Henry and 8.00% for kcal/kg/day). Conversely, inter-individual REE variation within the cohort revealed HB and Henry equations both inaccurately reflected mREE for 68.7% of participants, with kcal/kg/day inaccurately reflecting 41.7% of participants. Whilst the overall cohort was not classified as hypermetabolic (mean values were 101.04% for HB, 98.62% for Henry and 95.64% for kcal/kg/day), the metabolic index ranges within the cohort were 70.75% - 141.58% for HB, 72.82% - 127.69% for Henry and 66.09% – 131.58% for kcal/kg/day, indicating both over- and under-estimation of REE by these equations. We have shown that pREE correlates with body weight (kg), whereby the lighter the individual, the greater the underprediction of REE. When applied to the metabolic index, this underprediction biases towards the classification of hypermetabolism in lighter individuals.

Conclusion

Whilst predicting resting energy expenditure using the HB, Henry or kcal/kg/day equations accurately reflects derived mREE at group level, these equations are not suitable for informing resting energy expenditure and classification of hypermetabolism when applied to individuals in clinical practice.

9.3.2 Introduction

Motor neuron disease (MND) encompasses an incurable heterogeneous group of progressive neurodegenerative motor syndromes involving the gradual degeneration and ultimate death of motor neurons. This leads to the weakness and wasting of muscles controlling movement, speech and breathing (1), resulting in death typically from respiratory failure approximately two-to-three years post diagnosis (2,3). The prevalence of MND is 3.37 per 100,000 people worldwide (4) with Amyotrophic Lateral Sclerosis (ALS), the most common form of MND, comprising an estimated 65-85% of cases (5).

Weight loss in people living with MND (plwMND) is primarily driven by the relentless progression of denervation-induced muscle wasting. Symptoms such as dysphagia and a decreased dexterity secondary to muscle weakness (6–8), contribute to a sub-optimal caloric intake, which may lead to malnutrition and further weight loss (9–12). The presence of hypermetabolism, i.e., the state of an increased resting energy expenditure (REE), can result in dysregulated energy homeostasis and thus exacerbate the nutritional challenges for plwMND (13). Individuals with the greatest energy imbalance exhibit a faster rate of functional decline and shorter survival (9,14–18).

It is therefore important to accurately estimate an individual's total daily energy expenditure (TDEE) to establish appropriate nutritional energy intake goals. REE, i.e., the amount of energy required to maintain normal physiology at rest (19), comprises 60% of TDEE, the remainder of which is exerted through physical activity and the thermic effect from food metabolism (20). REE is best calculated using indirect calorimetry, which directly measures inspired O_2 and expired CO_2 to derive measures of REE (mREE). However, indirect calorimetry may be costly, time consuming and not readily available in all clinical contexts (21). When it is not possible to perform indirect calorimetry, REE is predicted (pREE) using predictive energy equations (22). The Henry equation (23) is reported to be the most commonly utilised predictive energy equation by dietitians caring for plwMND in the UK (22).

The assessment for the presence of hypermetabolism involves the calculation of the metabolic index, i.e., the ratio of mREE to pREE, expressed as a percentage. It is accepted that a metabolic index of ≥ 110% typically indicates hypermetabolism (9,24–30). The Harris-

Benedict (HB) (1919) (31) predictive equation is frequently used as the denominator in the metabolic index calculation (8,32–36). This is despite the discouragement of the use of the HB equation in MND clinical care in the UK, as it may poorly reflect REE in approximately half of cases (37,38). Nonetheless, application of the metabolic index using the HB equation has previously indicated that 50-68% of plwMND are considered hypermetabolic (24–26,28,29).

We aim to critically reflect on the use of predictive energy equations as comparators against mREE to calculate the metabolic index in plwMND. To achieve this, we evaluated the agreement between the HB (31) and Henry (23,39) predictive energy equations, as well as calculations of kcal/kg/day (40), against mREE using indirect calorimetry in a cohort of plwMND.

9.3.3 Materials and methods

9.3.3.1 Participant recruitment

Twenty-four plwMND were recruited from the Sheffield MND Care and Research Centre, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, from October 2021 to August 2022. Favourable opinion for this research was obtained from the London-Fulham Research Ethics Committee 21/PR/0092.

9.3.3.2 Inclusion criteria

Participants included with a confirmed diagnosis of MND were invited to participate. Time since diagnosis, MND phenotype, site of onset and medication were not considered for eligibility. Exclusion criteria were limited to an underlying, unmanaged significant co-morbidity that would affect survival or metabolic state, independent of MND (e.g., thyroid disease, cancer), or significant decision-making incapacity preventing informed consent.

9.3.3.3 Data collection

This study presents cross-sectional data from baseline visits collected during a longitudinal, observational, prospective study. Study visits were conducted at the Advanced Wellbeing Research Centre, Sheffield Hallam University. The following information was collected from each participant, where possible: demographic; clinical; anthropometric; indirect calorimetry; 24hr urinary collections.

9.3.3.4 Anthropometric measurements

Weight and height measurements were recorded in light clothing and shoes in an unaided standing position. Participant-reported weight and height measurements were collected from participants unable to stand unaided for those that could recall a recent measurement. BMI (kg/m^2) was calculated using: BMI (kg/m^2) = weight (kg)/height^2 (m). Arm muscle area (AMA) was calculated using the triceps skinfold (TSF) and mid-upper arm circumference (MUAC) values for the left and right arms: AMA (cm^2) = $[MUAC - (TSF \times \Pi)]^2 / (4 \times \Pi)$ as a proxy for lean body mass (LBM).

9.3.3.5 Total urinary nitrogen

Total urinary nitrogen (TUN) (g/24hr) was measured from 24-hour urinary collections following Micro-Kjeldahl analysis (41,42). To ensure adherence to the provision of a complete 24-hour urinary collection, participants were requested to record the start and end timings of their collection, as well as timings of all samples collected and details of any spillages or missed collections. Samples were deemed complete if collected over the appropriate 24-hour period and no missed collections or spillages. Incomplete collections were not included in analysis.

9.3.3.6 Measured resting energy expenditure

mREE in kcal/24hr was derived following indirect calorimetry using the GEMNutrition Gas Exchange Measurement (GEM) open-circuit metabolic cart with canopy hood. The GEM was calibrated using Laserpure nitrogen and 1% CO_2/20% O_2/N_2 calibration gases. A realistic, pragmatic approach was adopted to conduct indirect calorimetry and derive mREE in this cohort. Participants were rested in a seated position for one hour prior to measurement. Calorimetry measurement lasted 20 minutes in either a semi-supine or seated position, allowing for participant mobility and respiratory complications. The time of day for the calorimetry measurement was not standardised, but instead influenced by participant and carer availability to reduce burden; participants were therefore not required to be in a fasted state. Participants did not sleep or talk during the measurement. The first five minutes of measurements were discounted from analysis to increase the possibility of reaching a steady state (coefficient of variation (CV) ≤ 5%).

Fractional measures of inspired (Fi) and expired (Fe) O_2 and CO_2 measured directly by the GEM were derived into VO_2 and VCO_2 measurements using Haldane's transformation (43). Measures of VO_2, VCO_2 and TUN were then applied to the Weir equation to derive the mREE: mREE = ((3.941 x VO_2) + (1.106 x VCO_2)) x 1.44 - (2.17 x TUN) (44). The inclusion of total urinary nitrogen in the Weir equation reduces measurement error to provide the most accurate derivation of mREE possible.

9.3.3.7 Predicted energy expenditure

pREE was estimated in kcal/24hrs by the HB (1919) (31) and Henry (2005) (23) energy prediction equations. Both the HB and Henry equations use independent variables of weight, height, age and gender to calculate a predicted value for REE (Table 9.3). Kcal per kg body weight per day (kcal/kg/day) was also calculated based on body weight; i.e., 22 kcal/kg/day was applied to those ≤ 65 years of age, and 24 kcal/kg/day to those > 65 years (40). Participants with BMI values ≤ 18.5 or ≥ 30.0 kg/m^2 were excluded from analysis (n = 4 (25% of the original cohort)).

Table 9.3 Harris-Benedict (1919) and Henry (2005) predictive energy equations according to sex and age group.

	Sex	Age	Predictive energy equation
Harris-Benedict	**Male**	**N/A**	66.47 + (13.75 × weight (kg)) + (5.0 × height (cm)) – (6.75 × age (years))
	Female		655.09 + (9.56 × weight (kg)) + (1.84 × height (cm)) – (4.67 × age (years))
Henry	**Male**	**18-30**	(14.4 x weight (kg) + (313 x height (m)) + 113
		30-60	(11.4 x weight (kg) + (541 x height (m)) - 13
		60+	(11.4 x weight (kg) + (541 x height (m)) - 256
	Female	**18-30**	(10.4 x weight (kg) + (615 x height (m)) - 282
		30-60	(8.18 x weight (kg) + (502 x height (m)) – 11.6
		60+	(8.52 x weight (kg) + (421 x height (m)) + 10.7

9.3.3.8 Statistical analysis

Statistical analysis was conducted using IBM® SPSS® Statistics v27 and GraphPad Prism v9.3.1 (GraphPad Software Inc, La Jolla, CA, USA). Continuous variables were presented as mean ± one standard deviation (SD). Reported kcal/24hr were rounded to the nearest whole number. Mean values were compared using dependent t-tests. Normality was assessed using the Shapiro-Wilk test. Spearman or Pearson bivariate correlation analysis was performed according to the results from the Shapiro-Wilk test. Correlations were plotted with a linear regression line and 95% confidence intervals from the mean. The threshold for significance was $p \leq 0.05$ for all analyses. Bland-Altman limits of agreement analysis (mean bias ± 95% confidence intervals) was used to assess the extent of error of each predictive equation by comparison against mREE (45). Mean bias demonstrates the average difference between measured and predicted REE at group level.

9.3.3.8.1 REE Variation

The REE variation, i.e., the percentage difference between pREE and mREE (%ΔREE), to determine the accuracy of pREE when compared against mREE using indirect calorimetry was calculated using the formula: %ΔREE = ((pREE-mREE)/mREE) x 100 (37,38). Accuracy of pREE was defined as ± 10% from mREE. As indirect calorimetry measurement error is accepted at 5% (46), an error limit of ± 10% is accepted as twice the measurement error to indicate a 'true difference' (47,48). Underprediction of REE by the predictive equation produces a negative%ΔREE, whilst overprediction results in a positive%ΔREE.

9.3.3.8.2 Metabolic Index

The metabolic index (MI) percentage was calculated using the following formula: MI = (mREE/pREE) x 100 (34,35). A metabolic index threshold of ≥ 110% was used to classify hypermetabolism (47).

9.3.4 Results

9.3.4.1 Study population

Two participants withdrew consent before indirect calorimetry was conducted. Indirect calorimetry measurements were conducted on 22 people living with MND between October 2021 and August 2022. Weight measurements were neither collected nor reported from two participants. REE could therefore not be estimated for these participants, and they were excluded from analyses. Participants who did not provide complete 24-hour urinary collections for the measurement of total urinary nitrogen were also excluded from analysis (*n* = 4). The flowchart of participant inclusion is shown in Figure 9.3**.**

Of the sixteen included participants, 100% were male. Participant demographics, anthropometric measurements and disease duration from symptom onset (in months) are shown in Table 9.4. One participant opted to be fasted. The average time post-prandial was just over three and a half hours (n = 15).

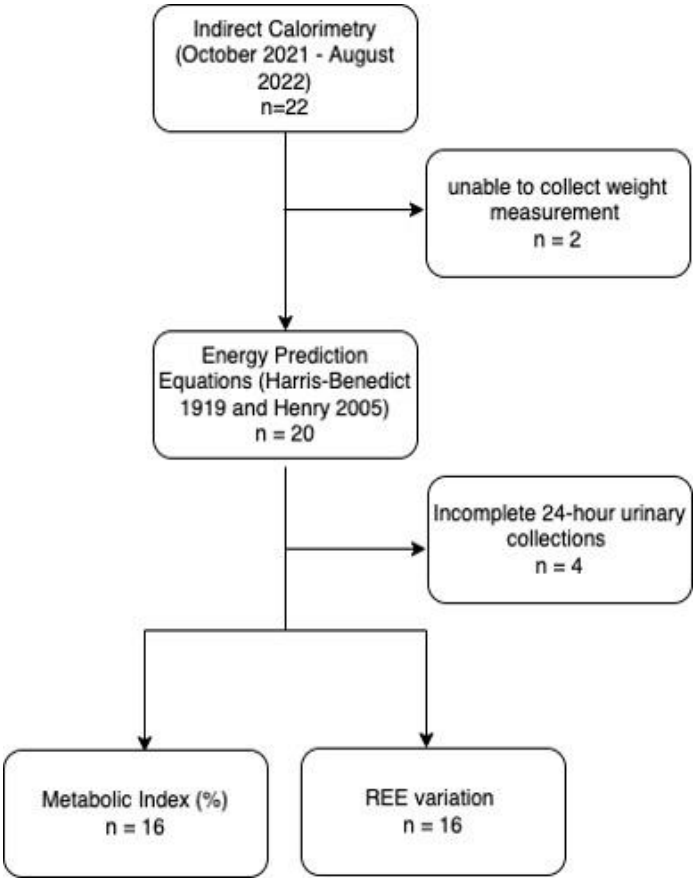

Figure 9.3 A flowchart of participants living with MND included in the study.

Table 9.4 Descriptive statistics of demographic, anthropometric and clinical characteristics of participants included in the analysis (n = 16). [a]p value of comparison between mREE and pREE. ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale (Revised); AMA: arm muscle area; BMI: body mass index; MUAC: mid upper arm circumference; mREE: measured resting energy expenditure; pREE: predicted resting energy expenditure; TSF: triceps skin fold; TUN: total urinary nitrogen; VCO_2: volume of carbon dioxide expired; VO_2: volume of oxygen inspired.

	Mean (SD)	**Median (IQR)**	**Minimum**	**Maximum**	***p***
Age	62 (12.1)	60 (55-71)	37	83	
Weight (kg)	81.89 (16.98)	83.50 (70.00-91.28)	51.40	117.48	
Height (cm)	176.33 (6.58)	176.15 (171.68-178.60)	165.10	190.50	
BMI (kg/m^2)	26.14 (4.24)	26.20 (23.48-28.26)	17.70	33.00	
Left arm AMA (cm^2)	52.62 (14.62)	48.66 (39.45-62.30)	30.54	81.45	
Right arm AMA (cm^2)	52.36 (14.60)	48.70 (42.60-62.33)	31.84	81.98	
VO_2 (ml/min)	234.05 (37.56)	248.16 (203.30-262.42)	163.07	290.73	
VCO_2 (ml/min)	211.87 (31.36)	219.64 (186.97-239.70)	131.47	244.80	
TUN (g/24hr)	11.08 (3.05)	11.24 (8.40-12.72)	6.69	17.39	

Symptom duration (months)	44 (45)	27 (18-59)	12	188	
ALSFRS-R	34.25 (7.04)	31.50 (29.00-42.50)	24.00	45.00	
mREE (kcal/24hr)	1642 (258)	1740 (1435-1848)	1110	2015	
Harris-Benedict (kcal/24hr)	1655 (265)	1644 (1398-1819)	1294	2176	0.87[a]
Henry (kcal/24hr)	1683 (231)	1671 (1485-1831)	1363	2114	0.58[a]
Post-prandial (hours : minutes)	03:31 (0.03)	03:29 (02:59-04:09)	02:25	04:55	

9.3.4.2 Measured Resting Energy Expenditure

mREE was derived for each participant from the Weir equation using the volume of oxygen inspired (VO_2), volume of carbon dioxide expired (VCO_2) and total urinary nitrogen values (Table 9.4). The mean mREE for the cohort was 1642 kcal/24hr (± 258), with individual data ranging from 1110 to 2015 kcal/24hr (Figure 9.4A/Table 9.4). This was not significantly different to the mean pREE using either the HB (1655 kcal/24hr ± 265, p = 0.87) or Henry (1683 kcal/24hr ± 231, p = 0.58) equations (Figure 9.4A/Table 9.4). Bivariate correlation analysis demonstrated a weak, positive relationship between mREE and both the HB (Pearson's r = 0.18, p = 0.50) and Henry (Pearson's r = 0.26, p = 0.33) predictive energy equations (Figure 9.4B/C).

9.3.4.3 Does body composition reflect assessments of resting energy expenditure?

Weight, height, BMI and right arm AMA were found to strongly correlate with pREE in a positive relationship for the HB and Henry equations. Age was shown to have a weak, negative relationship with both the HB and Henry equations, but this was not significant (Table 9.5). The determination of VO_2 from indirect calorimetry using the Haldane equation is advantageous as a more accurate measure of metabolic activity than derived mREE. As REE is known to be influenced by age, sex and LBM, it should therefore follow that the mREE should decrease with age and a lower LBM. However, neither VO_2 nor mREE significantly correlated with weight, height, BMI, AMA or age (Table 9.5).

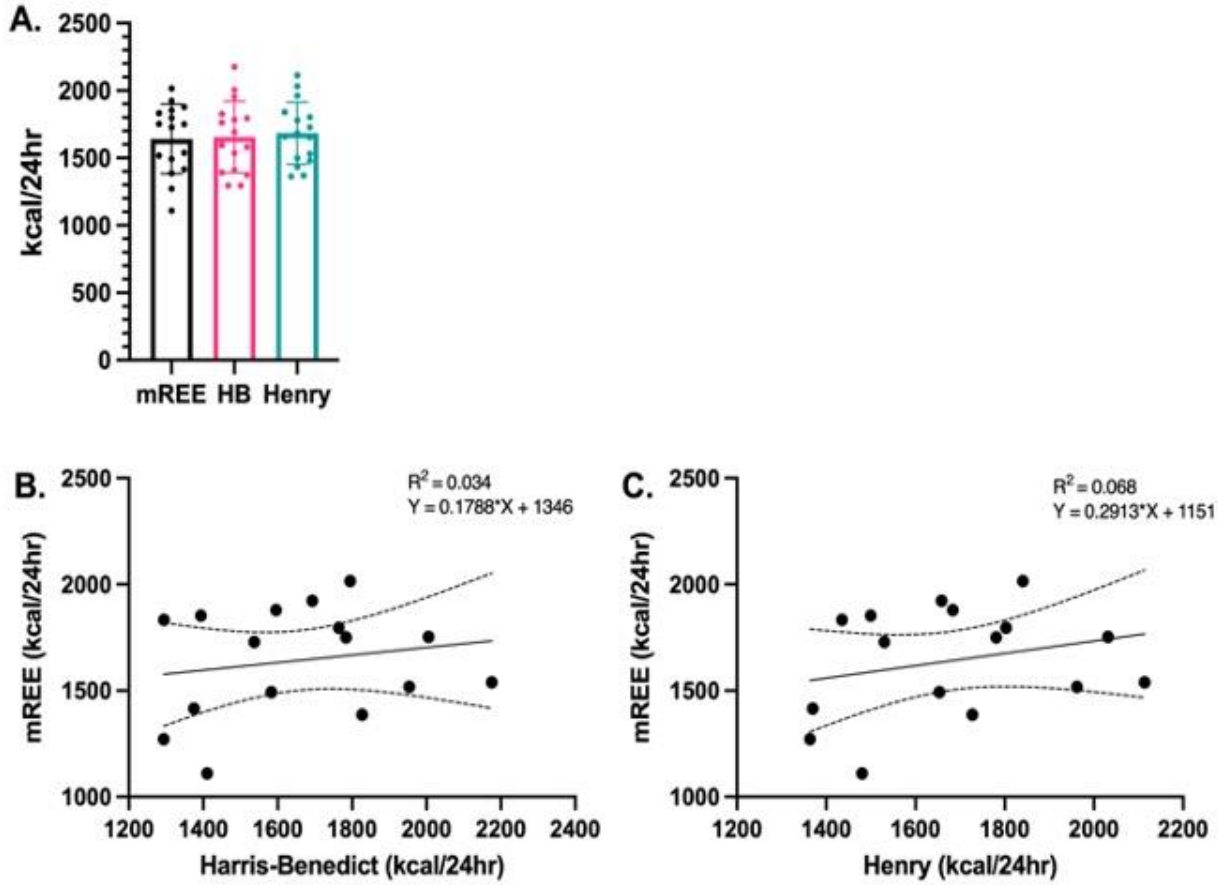

Figure 9.4 Measured and predicted resting energy expenditure (n = 16). (A) comparison of the mean ±1 SD of mREE and pREE using the HB and Henry equations. (B and C) comparison of mREE against pREE using HB and Henry equations. B and C show regression line with 95% confidence intervals. HB: Harris-Benedict; mREE: measured resting energy expenditure; pREE: predicted resting energy expenditure; SD: standard deviation.

Table 9.5 Correlations of resting energy expenditure against age and body composition (n = 16). Bivariate correlation analysis conducted using Pearson's correlation coefficient (*r*). Significance was observed at $p < 0.05$. AMA: arm muscle area; BMI: body mass index.

		HB	Henry	VO_2	mREE
Weight (kg)	*r*	0.94	0.95	0.20	0.18
	p	<0.0001	<0.0001	0.45	0.49
Height (cm)	*r*	0.78	0.80	0.19	0.16
	p	0.0003	0.0002	0.47	0.56
BMI (kg/m^2)	*r*	0.84	0.85	0.19	0.18
	p	<0.0001	<0.0001	0.49	0.51
Left arm AMA (cm^2)	*r*	0.39	0.37	0.33	0.34
	p	0.14	0.16	0.21	0.20
Right arm AMA (cm^2)	*r*	0.52	0.52	0.33	0.32
	p	0.04	0.04	0.22	0.22
Age (years)	*r*	-0.23	-0.12	0.00	-0.01
	p	0.39	0.66	0.99	0.98

9.3.4.4 Metabolic Index

mREE was compared against the HB and Henry predictive equations to calculate the metabolic index for each participant. Whilst the average value for the entire cohort did not surpass the 110% threshold (mean MI = HB: 101.04% ±20.33; Henry: 98.62% ±17.40) (Table 9.6) intra-cohort analysis revealed 6/16 (37.5%) (HB) and 5/16 (31.25%) (Henry) of participants would be categorised as hypermetabolic using the 110% threshold.

Table 9.6 Metabolic index (%) = measured resting energy expenditure compared with predicted resting energy expenditure (n = 16). Derived mREE was compared against pREE using either the Harris-Benedict or Henry equation to calculate the metabolic index (%). Hypermetabolism is indicated using a metabolic index threshold of 110%. MI: metabolic index.

	Mean (SD)	**Median (IQR)**	**Min.**	**Max.**	**Hypermetabolic participants n/N (%)**
pREE Harris-Benedict MI (%)	101.04 (20.33)	100.06 (80.90-113.32)	70.75	141.58	6/16 (37.5)
pREE Henry MI (%)	98.62 (17.40)	98.93 (81.77-112.65)	72.82	127.69	5/16 (31.3)

9.3.4.5 REE Variation

Whilst a weak, positive relationship between the HB and Henry equations against mREE exists in our cohort (Figure 9.4), correlation analysis only presents the linear relationship between two variables, but not agreement (49). Bland-Altman limits of agreement analysis (45) presented the proportional bias and accuracy between the measured and predicted REE (Figure 9.5).

The acceptable limits of agreement (LoA) were set *a priori*. There are no predefined clinically acceptable agreement limit for the error of pREE (kcal/day). The clinically acceptable limits agreed a *priori* were therefore determined as the maximum possible difference between mREE and pREE (kcal/day) to ensure accuracy, as previously defined as ≤± 10%. As the greatest mREE recorded by this cohort was 2015 kcal/day, the clinical *a priori* limits of agreement were set at ± 201.5 kcal/day. Therefore, individuals with a mREE-pREE difference of ≤201.5 kcal/day presented a REE variation of ≤± 10%.

The data presented in Figure 9.5 are single paired measurements of the 16 study participants for measured and predicted resting energy expenditure. Data was checked for normal distribution using the Shapiro-Wilk test (mREE: p = 0.45; HB: p = 0.59; Henry: p = 0.63). 95% confidence intervals were calculated for the bias and upper and lower limits of agreement. Results were calculated manually (45) and verified using GraphPad Prism software for Bland-Altman method comparison and paired t-tests.

For the Harris-Benedict Bland-Altman plot **(**Figure 9.5A), the mean proportional bias was -13.38 ± 334 kcal/day (95% confidence intervals: -164.5 - 191.2 kcal/day). The calculated LoA were −667 and 641 kcal/day. The mean proportional bias for the Henry pREE was found to be larger than that of the Harris-Benedict (Figure 9.5B), at -41.72 ± 297.5 kcal/day (95% confidence intervals: -116.8 - 200.2 kcal/day). Upper and lower calculated LoA were -624.8 and 541.3 kcal/day. For both predictive equations, the negative proportional bias demonstrates that, on average, pREE using either the HB or Henry equation is greater than the mREE, although this was not significant. The proportional bias and 95% confidence intervals fell within the *a priori* LoA, suggesting that both pREE equations are accurate at the group level. However, at least 50% of study participants (HB: 10/16 (62.5%); Henry: 8/16

(50%)) do not fall within the clinically acceptable LoA, rendering these predictive equations inaccurate for these individuals.

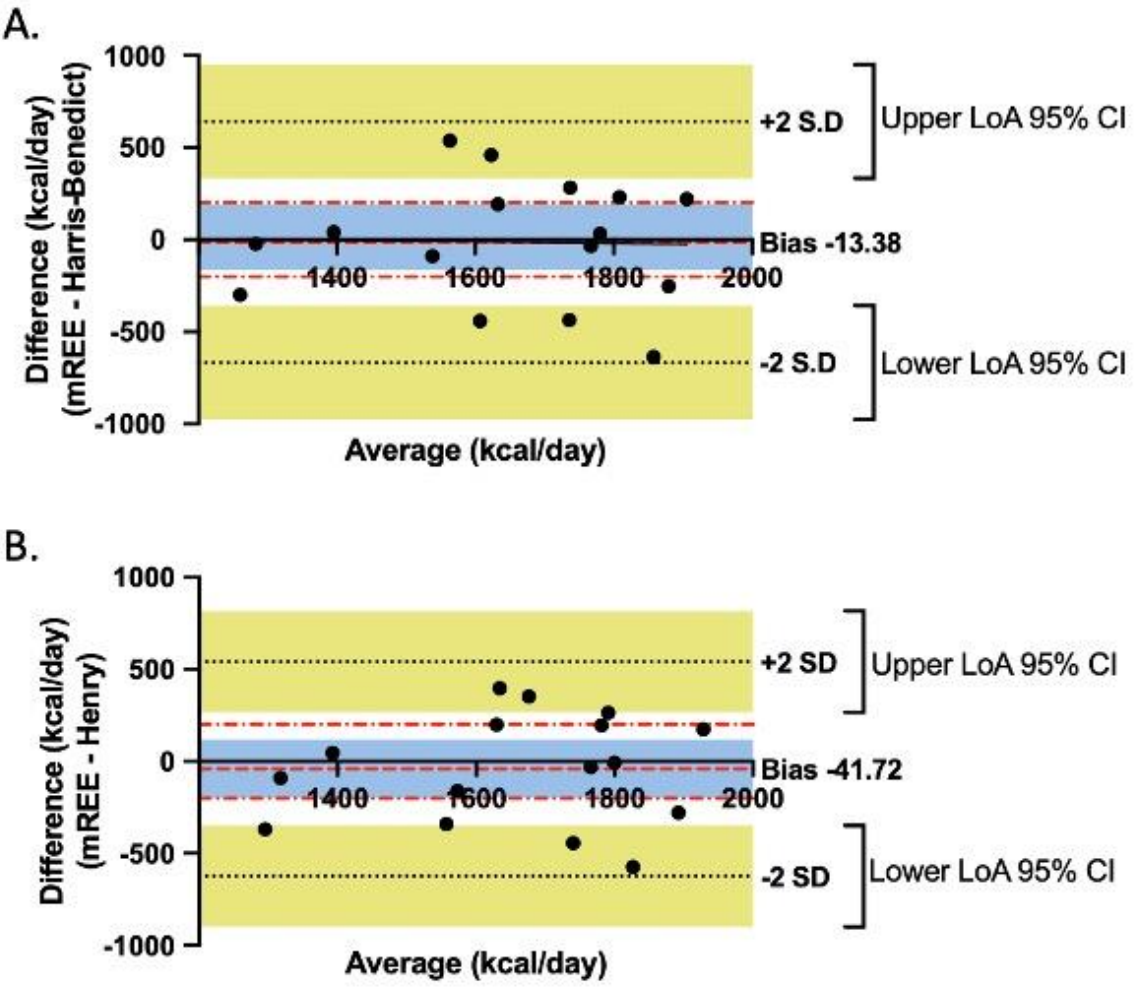

Figure 9.5 Bland-Altman method comparison between measured and predicted resting energy expenditure (kcal/day) (n = 16). (A) Harris-Benedict (1919). (B): Henry (2005). *A priori* clinically acceptable limits of agreement are indicated by the red dot-and-dash line at ± 201.5 kcal/day. The mean proportional bias between mREE and pREE is indicated by the red dashed line, with the 95% CI indicated by blue shading. Calculated upper and lower limits of agreement are shown at ± 2 standard deviations, with the 95% CI shaded in yellow. CI: confidence interval; LoA: limits of agreement; mREE: measured resting energy expenditure, pREE: predicted resting energy expenditure.

When assessed for REE variation (%ΔREE), pREE by both the HB and Henry equations accurately reflected mREE at group level (± 10%) (mean%ΔREE = HB: 2.81 ± 20.81; Henry: 4.51% ± 18.98) (Table 9.7). However, inter-individual%ΔREE analysis within this cohort revealed both the HB and Henry equations inaccurately reflected mREE for 68.7% of participants (Table 9.7).

Table 9.7 Resting energy expenditure variation: percentage difference between measured and predicted resting energy expenditure (n = 16).**%**ΔREE: percentage difference between measured and predicted resting energy expenditure.

	Mean (SD)	**Median (IQR)**	**Minimum**	**Maximum**	**Accurate n/N (%)**
pREE Harris-Benedict (%ΔREE)	2.81 (20.81)	-0.03 (-11.75-23.86)	-29.37	41.34	5/16 (31.3)
pREE Henry (%ΔREE)	4.51 (18.98)	1.08 (-11.23-22.41)	-21.68	37.33	5/16 (31.3)

To determine factors influencing over- or under-estimation of REE, independent variables forming both predictive equations, such as age, sex, weight and height were compared against the %ΔREE for participants in this cohort (Figure 9.6). %ΔREE was significantly strongly, positively correlated with weight for both the HB and Henry equation (HB: r = 0.59, p = 0.02; Henry: r = 0.54, p = 0.03) (Figure 9.6A/B). As weight is a constituent element of BMI, a similar relationship was expected between BMI and %ΔREE for both equations; but this was significant only for the HB equation (HB: r = 0.53, p = 0.04; Henry: r = 0.48, p = 0.06) (Figure 9.6C/D). These correlations demonstrated that both the HB and Henry equations overpredicted REE (a positive%ΔREE) in heavier individuals, but underpredicted REE (a negative %ΔREE) in lighter individuals, when compared with mREE. There was no correlation between arm muscle area, a proxy for lean body mass, and %ΔREE (HB: r = 0.11, p = 0.68; Henry: r = 0.05, p = 0.86).

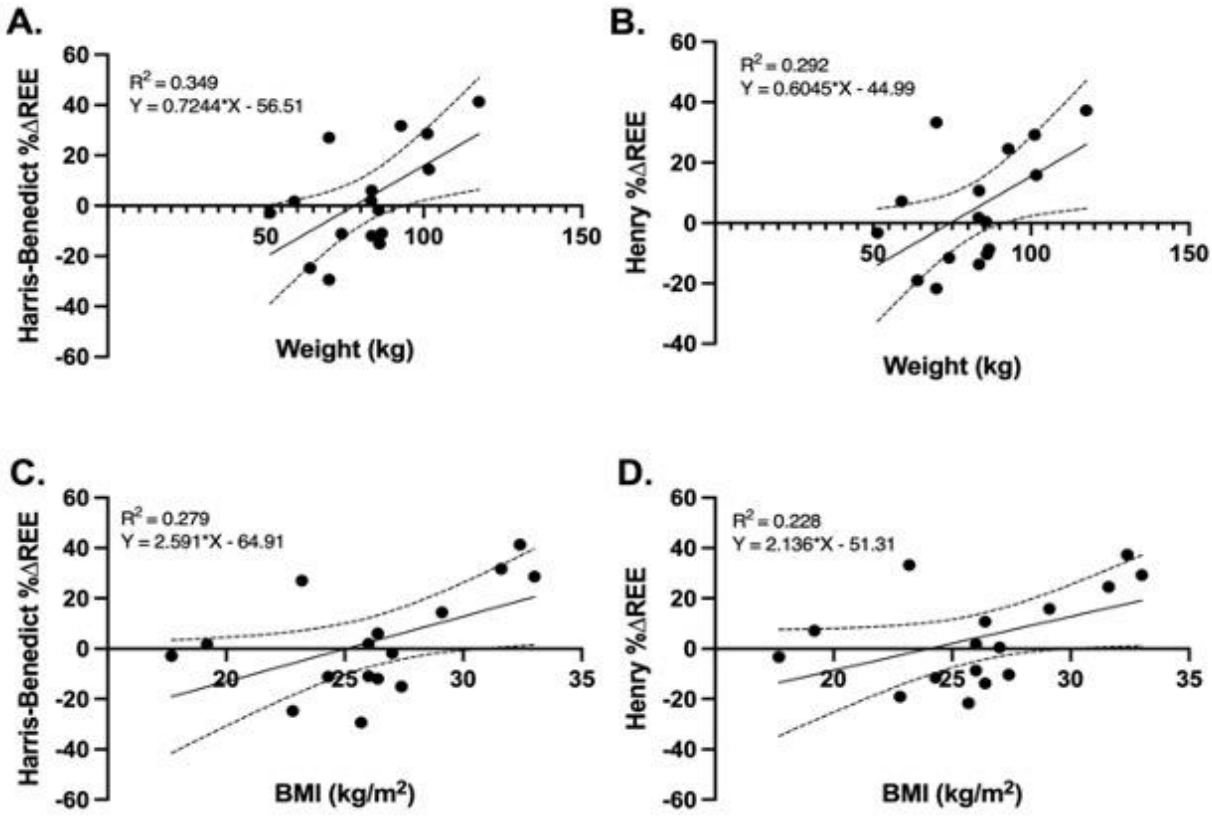

Figure 9.6 Comparing weight and BMI to percentage difference between measured and predicted resting energy expenditure (n = 16). (A/B) Weight (kg) against the %ΔREE using pREE by HB and Henry, respectively. (C and D) BMI (kg/m^2) against the %ΔREE using pREE by HB

and Henry equations. HB: Harris-Benedict; pREE: predicted resting energy expenditure; %ΔREE: percentage difference between measured and predicted resting energy expenditure.

9.3.4.6 Does REE variation influence the metabolic index?

We therefore raised the question as to whether an underprediction of REE using predictive energy equations, when compared against mREE, also biases the identification of hypermetabolism, using the 110% metabolic index threshold. It should be noted that, since both the metabolic index (mREE/pREE x 100) and REE variation ([pREE-mREE]/mREE x 100), are dependent on an accurate estimate of pREE, an underprediction of pREE (> -10%) naturally leads to the calculation of a larger negative %ΔREE and consequently an increase in the calculated metabolic index. An overprediction in pREE would result in the converse situation.

9.3.4.7 kcal/kg/day

Four participants (25% of the study population) were excluded from analysis. pREE was found to be 1798 kcal/24hrs (± 249). This was not significant when compared against mREE in the same individuals (mean mREE: 1701 ± 272 kcal/24hr; $p = 0.29$). When assessed for accuracy, the mean REE variation using kcal/kg/day was 8.00%, and was found to be accurate in 7/12 (58.3%) of participants. The average metabolic index was 95.64%, with 1/12 (8.33%) participants surpassing the metabolic threshold of 110%.

9.3.5 Discussion

In our study, mREE derived by means of indirect calorimetry and total urinary nitrogen analysis was similar to previous research findings in plwMND (32,33,36,37,50). mREE was compared to pREE using HB, Henry and kcal/kg/day equations to critically evaluate the suitability of using the metabolic index to indicate hypermetabolism in plwMND.

More than 100 predictive energy equations exist, which presume a linear relationship between REE and independent variables such as age, weight, height, and other body composition indices (51). A potential disadvantage of predictive energy equations is that they are predominantly derived from young, healthy, White British individuals; hence, may not accurately reflect mREE in critical, chronic illness (52) or MND patients (36–38,53).

9.3.5.1 What is the importance of identifying hypermetabolism?

Hypermetabolism has been shown to be a prognostic indicator of survival, functional change and weight loss (8,17,18,53–56). Therefore, it is important to identify hypermetabolism in individuals to optimise nutritional management. The metabolic index is not a readily-accessible tool that can be calculated by dietitians, and there is a need for MND-specific predictive equations that could be applied to inform appropriate dietetic nutritional management and incorporate a metabolic component.

9.3.5.2 Predictive energy equations overestimate resting energy expenditure at group level

The inclusion of the Henry equation in this study was informed by the results of a recent large UK-wide survey of dietetic practice in MND (22). The accuracy of the Henry equation when compared to mREE has not been previously assessed in MND. We found the Henry equation to accurately reflect mREE in 5/16 (31.3%) of our participants. This is the same as for the HB equation (Table 9.7). In line with previous MND literature utilising the HB equation to calculate pREE (37,38,57), our results show an overall overestimation of pREE with a lack of precision, as demonstrated by the wide limits of agreement (Figure 9.5). This intra-cohort variability resulted in an underestimation of energy requirements by up to 636 kcal/24hr, as well as overestimation by 538 kcal/24hr (Figure 9.5). To explain this variability, we conducted

bivariate correlations of continuous variables incorporated within both the HB and Henry predictive equations (i.e., weight, height and age) against mREE.

9.3.5.3 Weight informs predictions of resting energy expenditure

We have shown that weight correlates in a linear relationship with REE variation (51) (Figure 9.6A/B). This suggests that the lighter the individual, the greater the underestimation of pREE, producing a lower, negative %ΔREE, and vice versa (Figure 9.6). The clinical implications of this inaccuracy could result in under- or over-feeding patients with potentially detrimental clinical outcomes. Underfeeding would be more likely to occur in lighter individuals, contributing towards accelerated muscle wastage, malnutrition and irreversible weight loss. Conversely, a potential consequence of overfeeding (caused by an overestimation of pREE in heavier individuals) is hypercapnia (58), which can cause respiratory acidosis, inducing further respiratory implications (59).

Weight measurements represent lean and fat mass, both of which have different contributions to REE. Whilst lean body mass (including visceral organs and skeletal muscle) is highly metabolically active, fat mass (such as adipose tissue) is largely metabolically inactive (19,34,60). We have demonstrated that whilst weight and estimates of LBM significantly positively correlated with pREE in this cohort, neither weight nor LBM correlated with mREE. The reduction of skeletal muscle in plwMND deviates from the underlying assumed metabolic contributions that are observed in healthy individuals, altering REE (29,61–64). This may explain the overestimation of pREE at group level. It has been suggested that predictive equations may have increased accuracy if pre-morbid body weight was used, in place of current body weight (51).

9.3.5.4 The metabolic index does not appropriately identify hypermetabolism

The inaccuracy of these predictive energy equations led us to question the suitability of applying these equations to identify hypermetabolism in plwMND. Whilst individuals within this cohort did demonstrate a greater-than-predicted REE, we have shown that these equations are not appropriate comparators to enable the calculation of a clinically significant elevation in mREE (Figure 9.5). Moreover, we have demonstrated that the number of

individuals identified as hypermetabolic ranged between 8.33% and 37.5% depending on the predictive equation used.

9.3.5.5 Current recommendations in MND dietetic practice

The conceptualisation of predictive energy equation inaccuracy in MND is not a novel one (37,38). Whilst additional MND-specific predictive equations have been developed in this knowledge (37,40,65), these require body composition measurements such as bioelectric impedance analysis (BIA). A potential limitation of this is the inclusion of additional predictive equations within BIA analysis, which may further compound measurement error (66,67).

UK MND dietetic practice is informed by guidelines released by the British Dietetic Association (BDA) Parental and Enteral Nutrition Group (PENG). These guidelines currently recommend estimation of REE using 22-24 kcal/kg/day for plwMND (40). This calculation was devised from mREE using indirect calorimetry conducted in two cohorts of plwMND (34,65). One weakness of this approach however, is that this equation has only been validated in MND for individuals with a BMI indicating healthy or normal weight and overweight (18.5 - 30.0 kg/m^2), and it may not be appropriate for individuals with BMI extremes (68). When analysed in an appropriate sub-group from our cohort (12/16 (75%) of the original study population), one individual surpassed the metabolic index threshold of 110%. pREE using kcal/kg/day was also underestimated in the same individual, and most notably, this individual fell below the 25th percentile for weight (kg) in this cohort. This reinforces the data presented in this article, suggesting the lighter the individual, the greater the underprediction of REE, which biases towards a metabolic index ≥ 110%.

9.3.5.6 What does this mean for future research?

It would be easy to conclude that the HB, Henry and kcal/kg/day equations are unsuitable for estimating energy requirements in all individuals living with MND. However, these equations were accurate in 31.3-58.3% of participants within this cohort. It might therefore be more appropriate to develop weight or BMI guidance ranges for when these equations may appropriately reflect mREE. Application of predictive equations to individuals outwith these weight or BMI ranges would need to be utilised with caution.

9.3.5.7 Considerations

Undertaking research with this frail and often mobility restricted cohort of patients does not come without practical challenges, and researchers often have to take a pragmatic approach. Although we were able to detect statistically significant relationships, the sample size and lack of gender diversity limits our ability to draw firm conclusions for the wider MND population. Out of the 22 initially recruited participants, 16 were included in the analysis because of challenges around obtaining valid weight measurements and complete 24hr urinary collections (Figure 9.3). The all-male sample may be a result of the requirement of a 24hr urinary sample collection, which may have deterred the participation of female patients. To reduce participation burden, participants were not required to fast before indirect calorimetry measurement, and we acknowledge the chance of a component of dietary-induced thermogenesis within the obtained indirect calorimetry measurements. However, the thermogenic effect over the average post-prandial time of 3.5 hours observed in our cohort may be acceptable, considering that the thermogenic influence of a whole food meal is modest and is waning by 120 minutes (69). Intra-cohort variation in our sample was such that it was not meaningful to stratify individuals according to clinical characteristics, e.g., functional status, duration of disease from onset of symptoms, or disease severity (Table 9.4) in order to examine relationships between clinical characteristics and mREE. This research was designed as an exploratory pilot study, and as such we did not measure all possible confounders that may contribute towards REE (e.g., body temperature (70)).

9.3.6 Conclusion

Although our cohort was not hypermetabolic as a group, intra-cohort analysis revealed high variations and inaccuracies when using either the HB, Henry or kcal/kg/day predictive energy equations to estimate REE. Weight and BMI appear to be an important contributing factor to the under- or over-prediction of REE, e.g., the lighter the individual, the greater the underprediction of REE using either the HB or Henry equation. The %ΔREE appears to negatively correlate with the metabolic index, whereby the greater the underprediction of REE, the greater the metabolic index. This subsequently biases the classification of hypermetabolism towards individuals who are lighter. We suggest this %ΔREE is more likely to be attributed to the assumed metabolic contributions from a given weight included in the predictive energy equations, rather than resembling a true clinically significant raised REE.

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9.3.9 Conflict of interest

None declared

9.3.10 Rights Retention Statement

9.3.11 References

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9.4 Baseline results for all study participants

The results presented in **section 9.3** included a subset of the study cohort who provided complete 24-hour urinary collections for the calculation of mREE using the Weir equation. To assess the difference in mREE when calculated using the abbreviated Weir, the automated mREE produced by the GEM using the abbreviated Weir equation ($mREE_{GEM}$) was compared against the mREE manually calculated by the author of this thesis using the Weir equation ($mREE_{Weir}$). This comparison can only be presented for participants who provided **complete** 24-hour urinary collections (Table 9.8). Results are shown to two decimal places in this instance to enable the percentage difference between the two values to be presented clearly. The average difference between the automated mREE by the GEM and calculated mREE using the Weir equation was 0.46% ± 0.41. The following mREE data in this thesis therefore presents the $mREE_{GEM}$ for all study participants, regardless of 24-hour urinary collection completeness, unless otherwise stated.

Table 9.8 Calculating the difference between the measured resting energy expenditure using the Weir and abbreviated Weir equations at baseline for participants who provided complete 24-hour urinary collections (n = 17). $mREE_{GEM}$: automated calculation of mREE by the GEM using the abbreviated Weir equation; $mREE_{Weir}$: manually calculated mREE using the Weir equation; SD: standard deviation.

	VO_2 (ml/min)	**VCO_2 (ml/min)**	**TUN (g/day)**	**$mREE_{GEM}$ (kcal/day)**	**$mREE_{Weir}$ (kcal/day)**	**Difference ($mREE_{GEM}$ / $mREE_{Weir}$) (%)**
Pt01	200.73	182.80	6.84	1416.60	1415.46	0.08
Pt03	221.67	187.80	8.21	1542.59	1539.25	0.22
Pt04	277.93	233.47	11.94	1932.85	1923.19	0.50
Pt07	290.73	244.80	11.21	2018.63	2015.46	0.16
Pt08	259.47	241.67	11.44	1840.58	1832.55	0.44
Pt09	268.13	241.80	12.85	1889.23	1878.88	0.55
Pt11	263.40	233.80	6.69	1849.55	1852.65	-0.17
Pt13	163.07	131.47	10.95	1124.15	1111.03	1.17
Pt14	253.47	221.07	17.39	1761.44	1752.80	0.49
Pt15	250.13	218.20	7.79	1750.44	1750.12	0.02
Pt16	219.53	183.27	8.31	1522.26	1519.69	0.17
Pt17	239.07	218.87	10.77	1688.90	1681.92	0.41
Pt20	252.33	243.27	10.55	1802.17	1796.54	0.31
Pt21	211.00	198.79	9.38	1499.68	1493.67	0.40
Pt22	246.19	229.44	15.23	1740.45	1729.50	0.63
Pt23	177.31	186.69	14.27	1290.81	1272.62	1.41
Pt24	189.67	211.67	12.34	1400.76	1386.70	1.00
Mean ± SD	**234.34 ± 36.38**	**212.29 ± 30.41**	**10.95± 2.96**	**1651.24 ± 247.13**	**1644.24 ± 249.34**	**0.46 ± 0.41**

9.5 Longitudinal assessment of resting energy expenditure

The indirect calorimetry measurements were analysed longitudinally for all participants across the four study visits (Table 9.9). To account for variations within the proportions of fat free mass within the study population [328], mREE was normalised against estimates of FFM using AMA (mREE/AMA, kcal/cm^2). No statistically significant differences were observed at group level for mREE, mREE/AMA or VO_2 when the repeated measures were analysed using a mixed effects analysis for multiple comparisons for normally distributed data (mREE and VO_2), or the Wilcoxon test for non-normally distributed data (mREE/AMA). However, when the 14 individuals who completed all four study visits were assessed at an individual level, variations of up to 33.26% were observed for mREE and up to 46.97% for mREE/AMA. These longitudinal variations are shown graphically in Figure 9.7. It is important to note that the predicted REE using the Harris-Benedict (1919) equation (HB pREE) calculated at each time point did not reflect the observed fluctuations in measured resting energy expenditure, varying by a maximum of 6.41% between baseline and the third follow-up (Figure 9.8). This small variation could be explained by considering the independent variables incorporated in the Harris-Benedict predictive equation (weight, height and age), with weight being the only variable that could deviate and influence changes to the pREE. Weight has been shown to increase by an average of 2.2% in this cohort (Table 6.4) over the nine-month study period, thus explaining this minimal variation. This again highlights the necessity for regular measurements of energy expenditure, rather than predictions.

When analysed for all participants at all time points, VCO_2 was found to significantly increase between baseline and the third follow-up visit, and RQ significantly increased from baseline to all follow-up study visits (Table 9.9). When the RQ was analysed for the 14 participants who completed all four study visits, a significant increase in RQ was also observed between baseline and each subsequent follow-up study visit (Figure 9.9). It was hypothesised that the increase in RQ was related to changes in nutritional intake; however, no statistically significant relationships were observed when the RQ was compared against the self-reported carbohydrate or fat intake data from Intake24 using a Spearman's rank-order correlation analysis at baseline (carbohydrate: r = -0.02, p = 0.92, n = 22; fat: r = 0.16, p = 0.46, n = 22) or the third follow-up study visit (carbohydrate: r = 0.00, p = 0.99, n = 14; fat: r = -0.32, p = 0.27, n = 14), nor did the intake of any macronutrient alter significantly over time (Table 8.5).

Table 9.9 Longitudinal changes in measured resting energy expenditure parameters. Data is presented for each study visit. The number of participants who recorded each measurement at each time point is indicated by n/N. Continuous data is shown as mean ± one standard deviation and median (IQR). Significance was calculated between each time point using a mixed effects analysis for multiple comparisons for normally distributed data, or the Wilcoxon test for non-normally distributed data. Significance is at the $p < 0.05$ level, significant results are highlighted in bold. AMA: arm muscle area; F1-F3: follow-up study visits 1-3; HB: Harris-Benedict; IQR: interquartile range; mREE: measured resting energy expenditure; mREE/AMA: measured resting energy expenditure adjusted for arm muscle area; NDNS: national diet and nutrition survey; RQ: respiratory quotient; SD: standard deviation.

					P value		
	Baseline	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
mREE (kcal/day)	22/22 1610 ± 274.07 1654 (1416-1824)	20/20 1550 ± 226.14 1543 (1351-1711)	16/17 1577 ± 268.09 1558 (1358-1803)	14/14 1609 ± 222.52 1580 (1462-1830)	n = 20 0.63	n = 16 0.91	n = 14 >0.99
mREE/AMA (kcal/cm^2)	20/22 32.83 ± 8.08 31.90 (24.70-39.13)	19/20 31.91 ± 5.71 30.39 (29.01-33.91)	16/17 32.25 ± 7.88 30.34 (27.74-36.00)	14/14 33.66 ± 9.86 34.72 (25.39-38.19)	n = 17 0.82	n = 14 0.63	n = 12 0.79
VO_2 (ml/min)	22/22 228.32 ± 41.26 235.97 (200.70-259.75)	20/20 216.81 ± 32.33 216.30 (186.87-243.01)	16/17 219.47 ± 39.82 220.00 (186.09-254.73)	14/14 224.57 ± 32.26 218.20 (201.89-255.77)	n = 20 0.46	n = 16 0.71	n = 14 0.92
VCO_2 (ml/min)	22/22 207.19 ± 20.11 214.93 (185.83-233.55)	20/20 209.97 ± 30.33 207.77 (189.63-232.72)	16/17 217.20 ± 22.42 221.96 (182.25-239.48)	14/14 219.72 ± 28.94 217.00 (187.48-248.17)	n = 20 0.94	n = 16 0.30	n = 14 **0.05**

					P value		
	Baseline	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
RQ	22/22 0.92 ± 0.12 0.89 (0.84-0.93)	20/20 0.97 ± 0.06 0.96 (0.93-1.02)	16/17 1.00 ± 0.10 1.01 (0.92-1.11)	14/14 0.98 ± 0.06 0.99 (0.93-1.03)	n = 20 **0.01**	n = 16 **0.02**	n = 14 **0.0001**

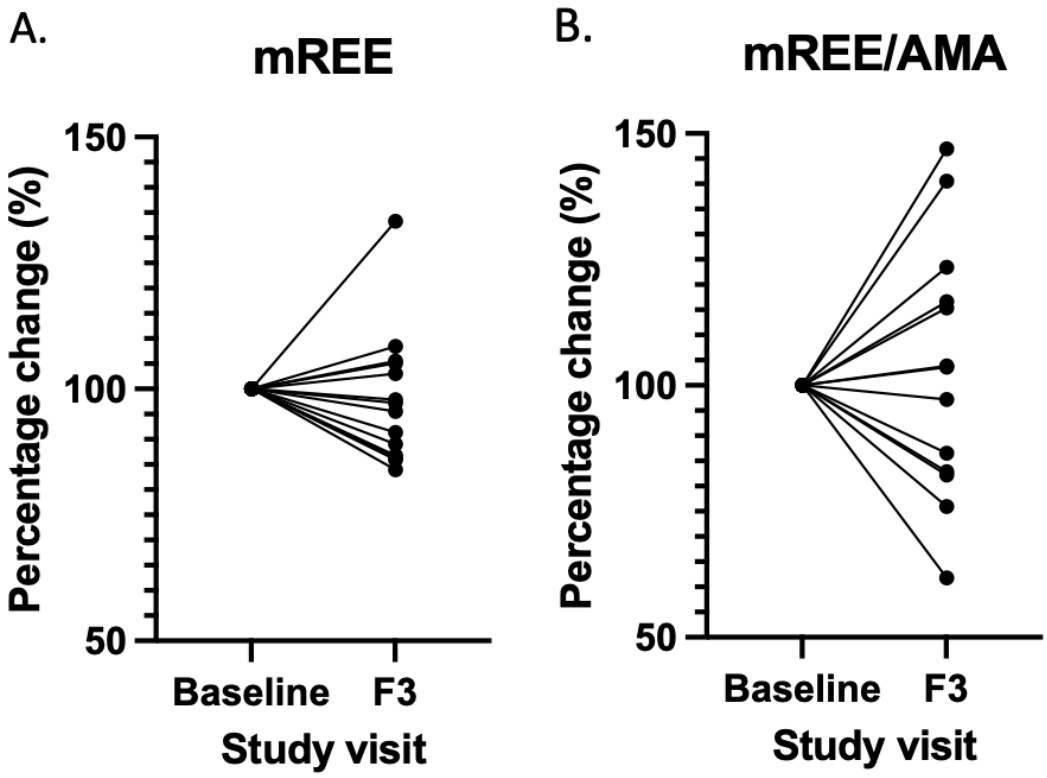

Figure 9.7 Percentage change in (A) measured resting energy expenditure (kcal/day) and (B) mREE adjusted for AMA (mREE/AMA, kcal/cm^2) between baseline and the third study visit (n = 14). AMA: arm muscle area; F3: third follow-up study visit; HB: Harris-Benedict; mREE: measured resting energy expenditure; NDNS: national diet and nutrition survey.

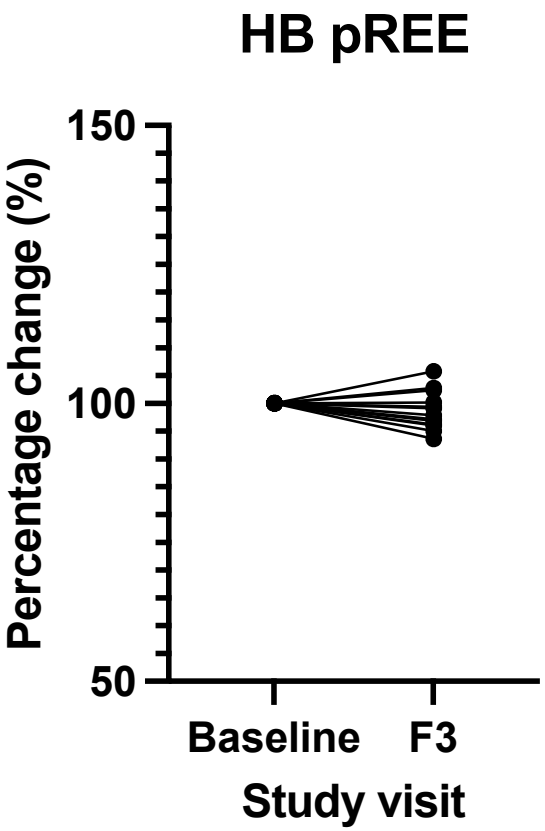

Figure 9.8 Percentage change in the Harris-Benedict predicted resting energy expenditure between baseline and the third study visit (n = 13). F3: third follow-up study visit; HB: Harris-Benedict; pREE: predicted resting energy expenditure;

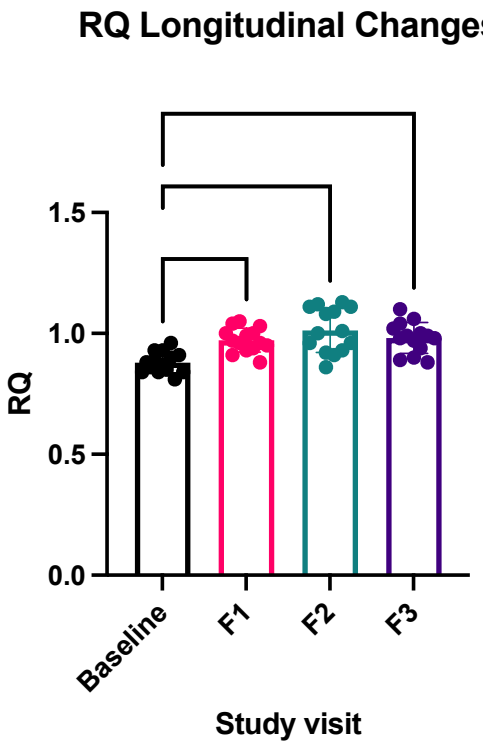

Figure 9.9 Changes in respiratory quotient over time for the longitudinal cohort (n = 14). Data is presented as mean ± one standard deviation. A repeated measures one-way ANOVA was conducted to compare RQ between baseline and follow-up study visits (Baseline-F1 p = <0.0001***; Baseline-F2 p = 0.0018**; Baseline-F3 p = 0.002***). Significance is at the p < 0.05 level. F1-F3: follow-up study visits 1-3; RQ: respiratory quotient.

9.6 Prevalence of hypermetabolism

As demonstrated in **Chapter 3**, the incidence of hypermetabolism is inherently dependent on the predictive equation utilised and the metabolic index threshold applied. A mixed-effects analysis for multiple comparisons was conducted to determine whether the metabolic indexes calculated using the Harris-Benedict, Henry and kcal/kg/day predictive energy equations were statistically significantly different: no significant differences were identified (Figure 9.10).

When the prevalence of hypermetabolism was considered at a metabolic index of 110%, hypermetabolism was indicated in 25 - 40% of the entire study cohort (Table 9.10/Figure 9.10). However, when the hypermetabolic threshold was increased to 120%, hypermetabolism was identified in 15 - 25% of participants. Moreover, hypometabolism has previously been indicated in MND by a metabolic index threshold of 90% [275]. When applying the hypometabolic threshold at 90% in this cohort, 25 - 30% of the study participant were identified as hypometabolic, depending on the predictive equation used (Table 9.10/Figure 9.10).

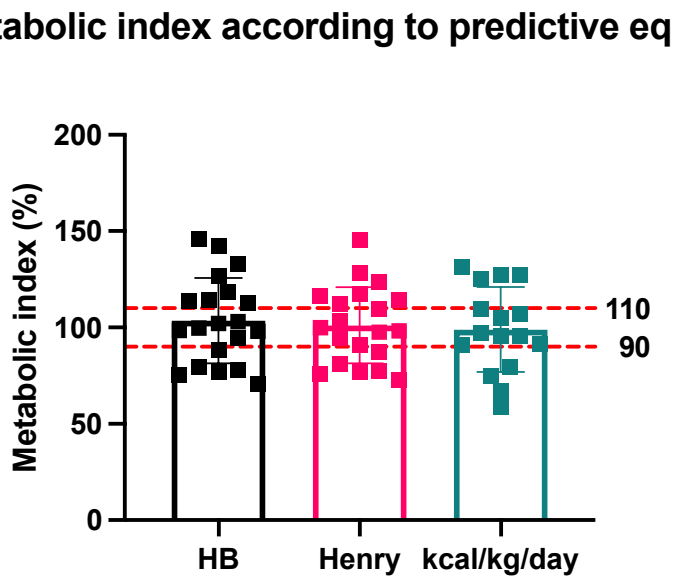

Figure 9.10 Using the metabolic index (%) to indicate hyper- and hypo-metabolism at baseline (n = 22). The upper red dashed line indicates the hypermetabolic threshold at 110%; the lower red dashed line indicates the hypometabolic threshold at 90%. Analysis was conducted using a mixed-effects analysis for multiple comparisons: HB vs Henry: p = 0.07; HB vs kcal/kg: p = 0.37; Henry vs kcal/kg: p = 0.76. HB: Harris-Benedict.

Table 9.10 Individual values of the metabolic index (%) for the study population at baseline (n = 22). MI = metabolic index; SD: standard deviation; IQR: interquartile range; *: hypermetabolic threshold at 110%; †: hypermetabolic threshold at 120%; #: hypometabolic.

	Harris Benedict	**Henry**	**kcal/kg/day**
Pt01	103.04	103.42	125.27*†
Pt03	70.90 #	72.97 #	-
Pt04	114.01*	116.36*	105.08
Pt05	98.41	97.58	-
Pt06	145.83*†	145.45*†	127.24*†
Pt07	112.53*	109.75	97.09
Pt08	142.20*†	128.25*†	109.56
Pt09	118.40*	112.21*	91.53
Pt10	75.35 #	76.69 #	58.74 #
Pt11	132.74*†	132.76*†	131.36*†
Pt13	79.72 #	75.95 #	66.91 #
Pt14	88.44 #	87.33 #	79.36 #
Pt15	98.14	98.28	95.40
Pt16	77.95 #	77.62 #	-
Pt17	-	-	-
Pt18	-	-	-
Pt19	126.53*†	117.26*	127.17*†
Pt20	102.15	99.94	95.55
Pt21	94.74	90.70	74.83 #
Pt22	113.64*	114.14*	107.27
Pt23	99.76	94.68	91.16
Pt24	76.67 #	81.08 #	-
Mean ± SD	**103.56 ± 22.20**	**101.15 ± 19.73**	**98.97 ± 22.08**
Median (IQR)	**100.95 (86.26-115.10)**	**99.11 (85.77-114.69)**	**96.32 (88.21-113.49)**
MI threshold			
110, n/N (%)	8/20 (40)	7/20 (35)	4/16 (25)
120, n/N (%)	4/20 (20)	3/20 (15)	4/16 (25)
Hypometabolic	6/20 (30)	6/20 (30)	4/16 (25)

9.7 An alternative approach to defining hypermetabolism in MND

An alternative approach to defining hypermetabolism in MND is therefore required. The National Diet and Nutrition Survey (NDNS) predicted REE for the UK general population using the average of three predictive equations which considered age, sex and body composition [329]. In this survey, the REE was predicted in MJ/day for sex and age group. MJ/day were converted into kcal/day by multiplication by 239 (Table 9.11). Individual mREE results from this study cohort were compared to the mean ± one standard deviation for the NDNS predicted REE (kcal/day), according to the sex and age of the participant. If the mREE was below one standard deviation of the NDNS average pREE – for that particular sex and age category - then the participant was classified as hypometabolic. Conversely, if the mREE was greater than, or equal to, one standard deviation higher than the mean NDNS average pREE – again, according to age and sex - then the participant was classified as hypermetabolic. Participants with mREE within the agreed limits (within ± one standard deviation of the mean) were classified as normometabolic. For example, Pt01 had a mREE of 1417 kcal/day. This was below the hypometabolic threshold of 1577.44 kcal/day for this participants age and sex (calculated by: 1792.54 - 215.11 = 1577.43), and was therefore categorised as hypometabolic.

Hypermetabolism was indicated in 7/22 (31.8%) participants, highlighted in red in Table 9.11; normometabolism in 10/22 (45.5%), highlighted in green; and hypometabolism in 5/22 (22.7%), highlighted in blue. The metabolic index (shown in brackets in Table 9.11) was calculated for each participant using the same formulae as presented in **section 9.3.3.8.2**, but this time the mREE for each participant was divided by the average NDNS pREE according to the sex and age category of that participant, expressed as a percentage. The mean cohort metabolic index was 101.6% ± 15.96 (Figure 9.11).

Interestingly, those identified as hypermetabolic using the NDNS pREE presented a metabolic index ≥ 110%. Therefore, this suggests that the metabolic index threshold of 110% previously utilised to indicate hypermetabolism in MND is appropriate, but this does not negate the caution needed when applying unsuitable predictive equations to this equation. The same cannot be said for the hypometabolic threshold previously indicated at 90%, as participants identified as normometabolic when using the NDNS pREE demonstrated a metabolic index < 90% (e.g., Pt16).

When analysed for the resting energy expenditure variation (%ΔREE), the average %ΔREE was 1.01%, demonstrating a lower accuracy than pREE using the Harris-Benedict predictive equation (0.88%), but a higher accuracy than either the Henry (2.47%) or kcal/kg/day (6.47%) pREE (**Appendix I, Table** 13.1). The NDNS pREE was accurate (at the ± 10% threshold) for 40% of the study cohort.

Table 9.11 Identification of hypermetabolism at baseline using the predicted resting energy expenditure according to sex and age from the National Diet and Nutrition Survey (n = 22). Data shown as mREE (metabolic index). MJ/day were converted into kcal/day by multiplication by 239. The hypermetabolic threshold was calculated as the NDNS pREE + one standard deviation. The hypometabolic threshold was calculated as the NDNS pREE – one standard deviation. Red highlight indicates hypermetabolic, green indicates normometabolic, blue indicates hypometabolic. mREE values are given in kcal/24hr, metabolic index is presented as %. MJ: megajoule; mREE: measured resting energy expenditure; NDNS: national diet and nutrition survey; pREE: predicted resting energy expenditure; Pt: participant; SD: standard deviation

	Female			Male		
	16-49	**50-64**	**65-91**	**16-49**	**50-64**	**65-91**
NDNS pREE						
MJ/day ± SD	6.0 ± 0.7	5.8 ± 0.6	5.3 ± 0.6	7.5 ± 0.9	7.1 ± 0.8	6.5 ± 0.7
Kcal/day ± SD	1434.03 ± 167.30	1386.23 ± 143.40	1266.73 ± 143.40	1792.54 ± 215.11	1696.94 ± 191.20	1553.54 ± 167.30
Hypometabolic threshold	1266.73	1242.83	1123.33	1577.43	1505.74	1386.23
Hypermetabolic threshold	1601.33	1529.64	1410.13	2007.65	1888.15	1720.84
mREE						
Pt01				1417 (79.03)		
Pt03						1543 (99.30)
Pt04					1933 (113.90)	
Pt05			1411 (111.42)			

	Female			Male		
	16-49	50-64	65-91	16-49	50-64	65-91
Pt06			1618 (127.77)			
Pt07					2019 (118.96)	
Pt08						1841 (118.48)
Pt09						1889 (121.61)
Pt10			915 (72.23)			
Pt11					1850 (108.99)	
Pt13						1124 (72.36)
Pt14					1761 (103.80)	
Pt15				1750 (97.65)		
Pt16					1522 (89.71)	
Pt17						1689 (108.71)
Pt18		1578 (113.81)				
Pt19					1818 (107.16)	
Pt20					1802 (106.20)	
Pt21						1500 (96.53)
Pt22					1740 (102.56)	
Pt23						1291 (83.09)
Pt24					1401 (82.55)	

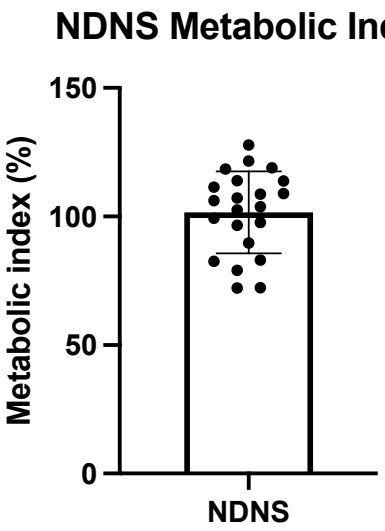

Figure 9.11 The metabolic index (%) calculated using pREE from the National Diet and Nutrition Survey at baseline (n = 22). Data is shown as mean ± one standard deviation. NDNS: National Diet and Nutrition Survey.

9.7.1 Relationship between metabolic rate, nutritional state and disease severity

As presented in **section 3.5.6**, the presence of hypermetabolism is associated with a worse prognosis and increased disease severity. **Chapter 3** demonstrated the Harris-Benedict equation to be the most utilised, reported predictive equation to calculate the metabolic index in MND. The following data compares the metabolic index calculated by the NDNS pREE ('NDNS MI') against the Harris-Benedict (1919) pREE ('HB MI') to assess the suitability of the proposed NDNS method to indicate hypermetabolism in this cohort and enable conclusions with existing MND literature to be drawn.

Participants were categorised according to King's College staging system at baseline. A one-way ANOVA was used to compare the metabolic index in participants at each stage. The metabolic index was observed to decrease with increasing disease severity, indicated by a higher King's College staging score; however, this was not significant for either the Harris-Benedict or NDNS metabolic index (HB: $p = 0.052$; NDNS: $p = 0.42$; Figure 9.12).

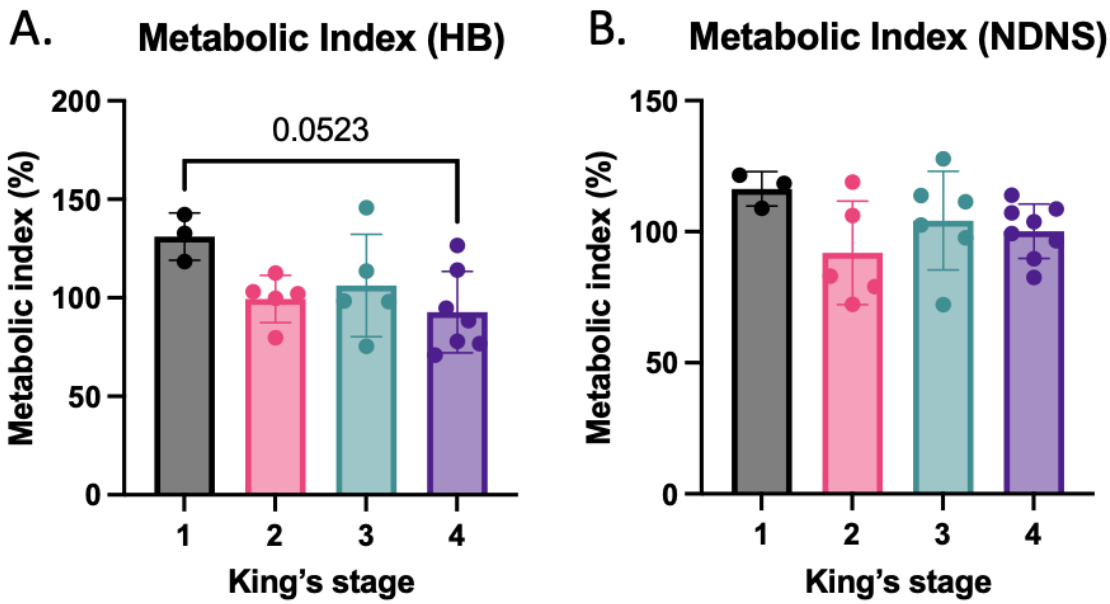

Figure 9.12 Changes in the metabolic index (%) by classification of participants to the King's College staging system using predicted resting energy expenditure estimated by A) the Harris-Benedict 1919 equation (n = 20), and B) the National Diet and Nutrition Survey (n = 22) for participants at baseline. Data was analysed between stages 1 and 4 using a one-way ANOVA (HB: $p = 0.052$; NDNS: $p = 0.421$). Significance observed at $p < 0.05$.

Pearson's product-moment correlation and Spearman's rank-order correlation analyses were conducted to assess the relationship between the HB and NDNS metabolic indexes and assessments of nutritional state (body composition, nutrient intake, biochemical analytes and measurements of resting energy expenditure), as well as assessments of disease severity, duration and progression (Table 9.12). These relationships were analysed further by grouping the cohort according to the metabolic state of each participant (hyper-, normo- or hypo-metabolic). One-way ANOVA analysis was conducted to identify any statistically significant differences between the metabolic groups. Significant results to support the correlation analyses are presented in Figure 9.13.

Both the NDNS and HB metabolic indexes were significantly correlated with VO_2 and mREE in a positive relationship (HB: VO_2: r = 0.61, p = 0.004; mREE: r = 0.64, p = 0.004; NDNS VO_2 and mREE: r = 0.80, p = <0.001). These relationships are shown visually in Figure 9.13A-D, where a significant increase in both the mREE and VO_2 was observed between hypometabolic and hypermetabolic groups. The NDNS metabolic index was observed to significantly moderately negatively correlate with serum albumin (r = -0.46, p = 0.048). However, no further significant correlations were observed between the NDNS metabolic index and assessments of nutritional state or disease severity.

The HB metabolic index was also found to significantly moderately positively correlate with mREE adjusted for AMA (mREE/AMA) (r = 0.48, p = 0.04). When analysed for the relationship with disease severity, the HB metabolic index demonstrated a significant decline with an increased disease severity (i.e., a moderate negative correlation with King's staging (r = -0.48, p = 0.03) and moderate positive correlations with the ALSFRS-R total (r = 0.47, p = 0.04) and fine motor (r = 0.51, p = 0.02) scores). This relationship with disease severity was consistent with the results presented in Figure 9.12. Significant decreases in the ALSFRS-R fine motor subscore were also observed in the hypo- and normo-metabolic groups when compared against the hypermetabolic group (Figure 9.13E).

When compared against assessments of nutritional state previously investigated in this thesis, the HB metabolic index was found to significantly negatively correlate with: weight (r = -0.55, p = 0.01); BMI (r = -0.52, p = 0.02); and calf circumference (r = -0.59, p = 0.01), suggesting that

an increase in the metabolic index is associated with a decrease in the nutritional state. A statistically significant, moderate positive correlation was observed between the HB metabolic index and carbohydrate intake (r = 0.53, p = 0.02), however no significant relationships were observed for the HB metabolic index against energy intake or serum and 24-hour urinary biochemical analytes.

Table 9.12 Correlation analysis for the metabolic index calculated with the Harris-Benedict and National Diet and Nutrition Survey pREE. Correlation analysis was conducted using Pearson's correlation coefficient for normally distributed data, or Spearman's (†) correlation coefficient for non-normally distributed data. Significance is at the $p < 0.05$. Significant results highlighted in bold. ALSFRS-R: amyotrophic lateral sclerosis functional rating scale – revised; BMI: body mass index; HB: Harris-Benedict; NDNS: National Diet and Nutrition Survey; ΔALSFRS-R: change in the amyotrophic lateral sclerosis functional rating scale – revised.

	NDNS metabolic index			**HB metabolic index**		
	n	***r***	***p* value**	***n***	***r***	***p* value**
Age (years)	22	0.26	0.25	20	0.16	0.50
Disease duration (months) †	22	0.26	0.25	20	0.17	0.47
King's staging (%) †	22	-0.21	0.35	20	-0.48	**0.03**
ALSFRS-R (/48)	22	0.09	0.70	20	0.47	**0.04**
Bulbar (/12)	22	0.05	0.83	20	0.13	0.60
Fine Motor (/12)	22	0.35	0.11	20	0.51	**0.02**
Gross Motor (/12)	22	-0.42	0.05	20	-0.20	0.39
Respiratory (/12) †	22	0.08	0.72	20	0.39	0.09
ΔALSFRS-R	22	-0.19	0.39	20	-0.34	0.14
Anthropometric						
Weight (kg)	20	0.09	0.70	20	-0.55	**0.01**
Weight change from premorbid (%) †	17	-0.11	0.67	16	-0.19	0.49
BMI (kg/m^2)	20	0.06	0.82	20	-0.52	**0.02**
Left TSF (mm)	21	-0.14	0.56	19	-0.43	0.06

	NDNS metabolic index			HB metabolic index		
	n	***r***	***p* value**	***n***	***r***	***p* value**
Right TSF (mm)	21	-0.15	0.52	19	-0.34	0.16
Average TSF (mm)	21	-0.15	0.52	19	-0.40	0.09
Left AMA (cm^2)	21	0.37	0.10	19	-0.05	0.85
Right AMA (cm^2)	21	0.41	0.06	19	-0.11	0.64
Average AMA (cm^2)	21	0.41	0.07	19	-0.08	0.74
Left calf circumference (cm) †	22	-0.05	0.82	20	-0.61	**0.004**
Right calf circumference (cm) †	22	0.05	0.83	20	-0.47	**0.04**
Average calf circumference (cm) †	22	-0.04	0.87	20	-0.59	**0.01**
Reported intake						
Energy intake (kcal/24hr)	22	0.01	0.96	20	0.16	0.49
Carbohydrate (g/day)	22	0.33	0.14	20	0.53	**0.02**
Protein (g/day)	22	0.10	0.64	20	0.17	0.48
Fat (g/day)	22	-0.28	0.21	20	-0.08	0.73
Assessment of resting energy expenditure						
VO_2 (ml/min)	22	0.80	**<0.001**	20	0.61	**0.004**
RQ †	22	-0.33	0.14	20	-0.12	0.62
mREE (kcal/day)	22	0.80	**<0.001**	20	0.62	**0.004**
mREE/AMA ($kcal/cm^2$)	21	0.16	0.49	19	0.48	**0.04**
Biochemical analytes						
Serum analytes						
Creatinine (mol/L) †	19	-0.18	0.46	17	-0.25	0.33
Albumin (g/L) †	19	-0.46	**0.048**	17	-0.16	0.54
Prealbumin (g/L)	19	0.17	0.50	17	0.01	0.99
Cholesterol (mmol/L)	19	-0.09	0.72	17	0.16	0.54

	NDNS metabolic index			HB metabolic index		
	n	***r***	***p* value**	***n***	***r***	***p* value**
Triglycerides (mmol/L)	19	0.17	0.49	17	0.30	0.24
HDL cholesterol (mmol/L) †	19	-0.004	0.99	17	0.08	0.75
LDL cholesterol (mmol/L)	17	-0.22	0.39	15	0.17	0.53
Non-HDL cholesterol (mmol/L)	19	0.11	0.64	17	0.04	0.88
Total HDL cholesterol ratio (mmol/L)	19	-0.16	0.53	17	-0.19	0.45
Ferritin (µg/L)	19	0.35	0.15	17	0.39	0.12
Transferrin (g/L)	19	-0.04	0.87	17	-0.07	0.78
Retinol-binding protein (mg/L)	19	0.21	0.39	17	-0.08	0.77
eGFR †	18	0.10	0.69	16	0.39	0.14
Urinary analytes						
24hr urinary sodium (mmol/L)	22	0.02	0.91	20	-0.32	0.17
24hr urinary potassium (mmol/L)	22	0.19	0.40	20	-0.16	0.50
24hr urinary urea (mmol/L)	22	0.06	0.79	20	-0.06	0.79
24hr total urinary nitrogen (g/day)	22	0.06	0.81	20	-0.01	0.98

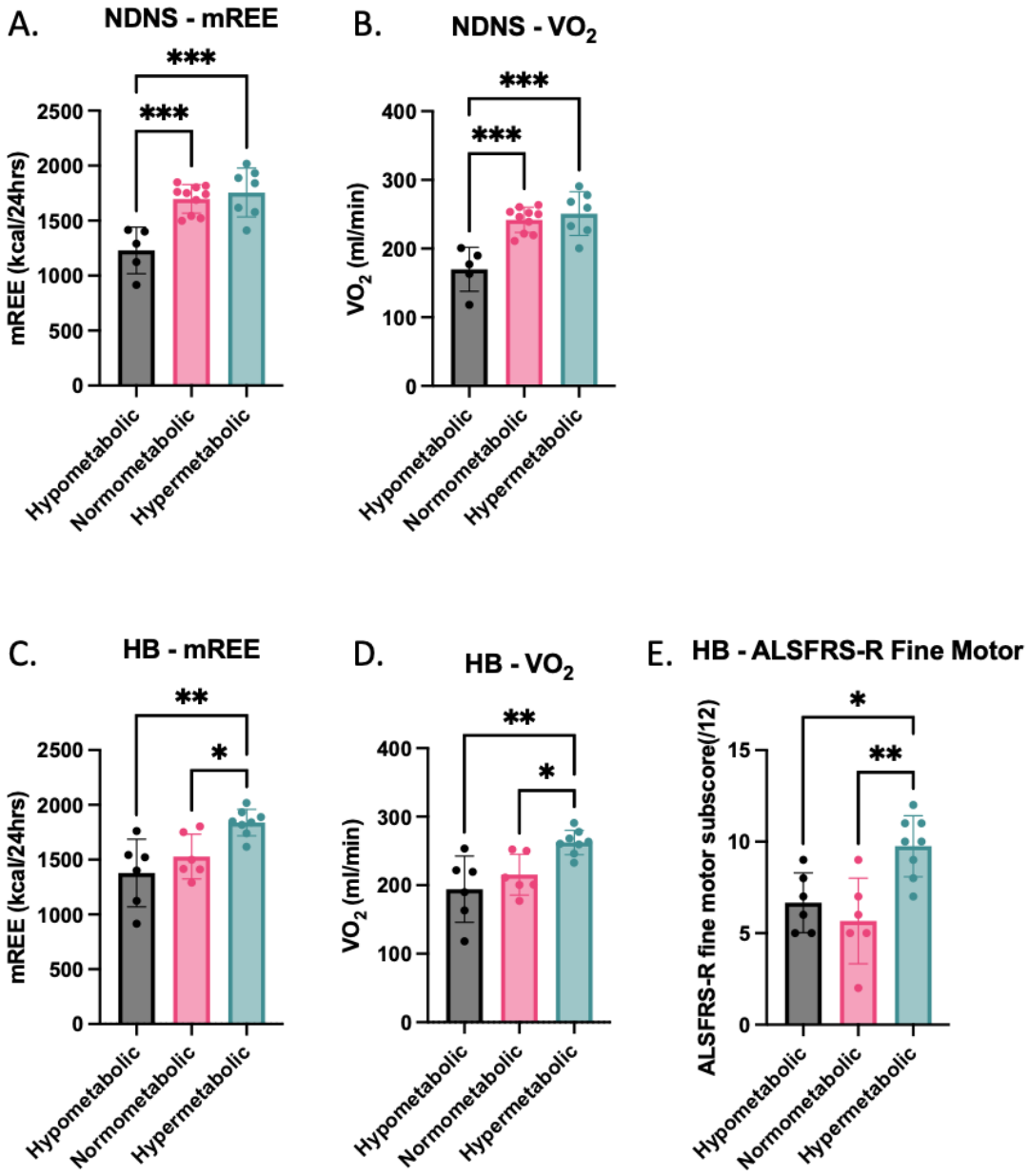

Figure 9.13 Resting energy expenditure parameters and disease severity according to metabolic category. (A/C) Measured resting energy expenditure (mREE, kcal/24hrs). (B/D) VO_2 (ml/min). (E) ALSFRS-R fine motor score. Normality was checked using Shapiro-Wilk analysis. Statistical differences between metabolic groups were analysed using a one-way ANOVA for multiple comparisons. Significance was observed at p = < 0.05*, p = <0.01* and p = <0.001*** levels.

9.7.2 Longitudinal assessment of hypermetabolism

Significance was calculated between each time point using a mixed effects analysis for multiple comparisons with Dunnett's correction for normally distributed data, or the Wilcoxon test for non-normally distributed data. No significant differences were observed at group level between baseline and any follow-up time points for either the HB or NDNS metabolic index (Table 9.13). When the 14 individuals who completed all four study visits were assessed at an individual level, variations of up to 34% were observed for the HB metabolic index and 33.25% for the NDNS metabolic index. These longitudinal variations are shown graphically in Figure 9.14. To add complexity, the observed intra-cohort variations in the metabolic index did not continuously increase or decrease in a linear manner, but rather dynamically fluctuated per time point (Figure 9.15). This can be best exemplified by considering the longitudinal changes in the NDNS metabolic index, as the NDNS pREE does not change with each time point and subsequently any changes to the metabolic index are directly related to changes in the mREE (Figure 9.15A).

Table 9.14 shows the metabolic state of each study participant at each time point using the NDNS and HB metabolic index at each time point. For the NDNS metabolic index, no participants maintained a constant metabolic state throughout participation in this study. However, for the HB metabolic index, two participants (Pts 08 and 22) remained hypermetabolic throughout participation in this study, and one participant (Pt03) remained hypometabolic. Pt03 is a perfect example of the influence different predictive energy equations have on the indication of hypermetabolism: when using the Harris-Benedict predictive equation, this participant is indicated to be hypometabolic at all time points (also previously demonstrated at baseline in Table 9.10), whereas the NDNS pREE indicates this participant to be hypermetabolic at the second and third study visits (F1 and F2, Table 9.14).

Table 9.13 Longitudinal changes in the metabolic index. Data is presented for each study visit. The number of participants who recorded each measurement is indicated by n/N. Continuous data is shown as mean ± one standard deviation and median (IQR). Significance was calculated between each time point using a mixed effects analysis for multiple comparisons with Dunnett's correction for normally distributed data, or the Wilcoxon test for non-normally distributed data. Significance is at the $p < 0.05$ level. F1-F3: follow-up study visits 1-3; HB: Harris-Benedict; IQR: interquartile range; NDNS: National Diet and Nutrition Survey.

					P value		
	Baseline	**F1**	**F2**	**F3**	**B-F1**	**B-F2**	**B-F3**
HB metabolic Index (%)	20/22 103.56 ± 22.19 100.96 (81.90-117.30)	19/20 99.94 ± 14.80 99.55 (85.71-108.83)	15/17 99.76 ± 16.06 95.35 (86.42-109.22)	13/14 101.25 ± 16.20 102.78 (93.82-111.15)	n = 19 0.52	n = 15 0.98	n = 13 0.64
NDNS metabolic index (%)	22/22 101.69 ± 15.97 105.37 (88.13-113.83)	20/20 98.65 ± 15.94 99.09 (87.27-104.84)	16/17 97.27 ± 12.99 97.17 (86.29-110.44)	14/14 99.68 ± 11.10 100.12 (94.41-108.22)	n = 20 0.78	n = 16 0.56	n = 13 0.83

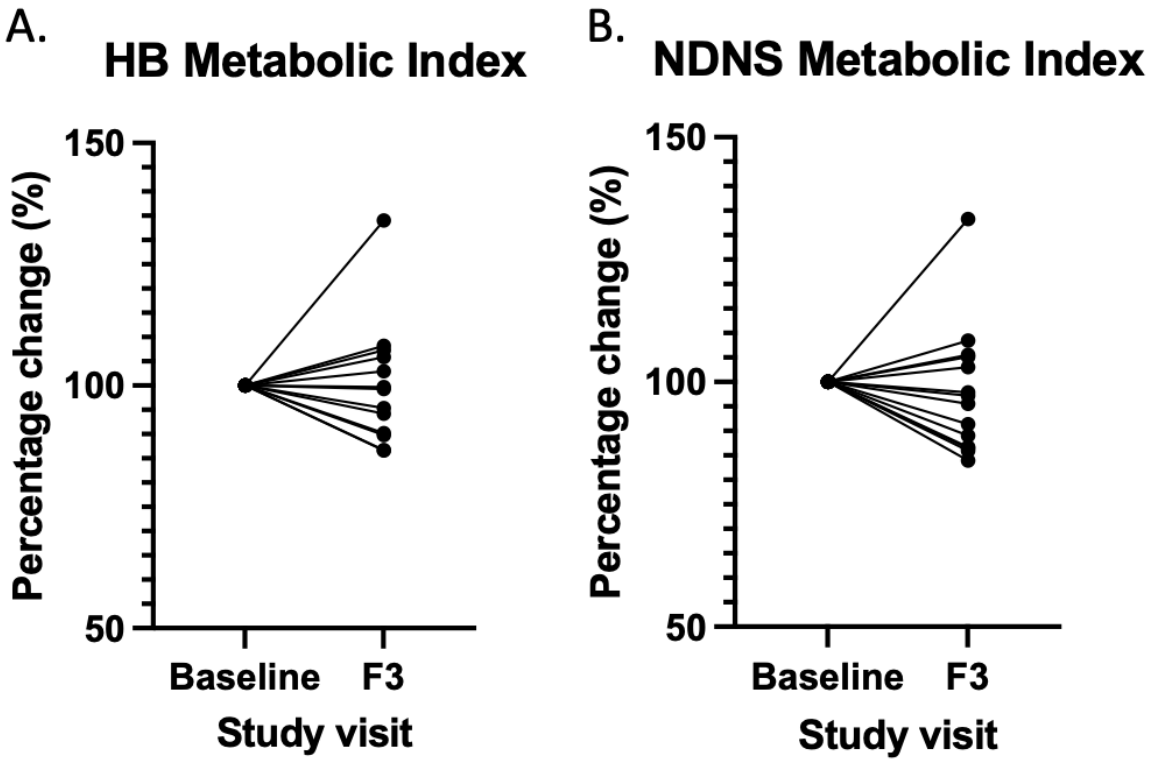

Figure 9.14 Percentage change in (A) Harris-Benedict metabolic index and (B) NDNS metabolic index between baseline and the third study visit (%) (n = 14). F3: third follow-up study visit; HB: Harris-Benedict; NDNS: National Diet and Nutrition Survey.

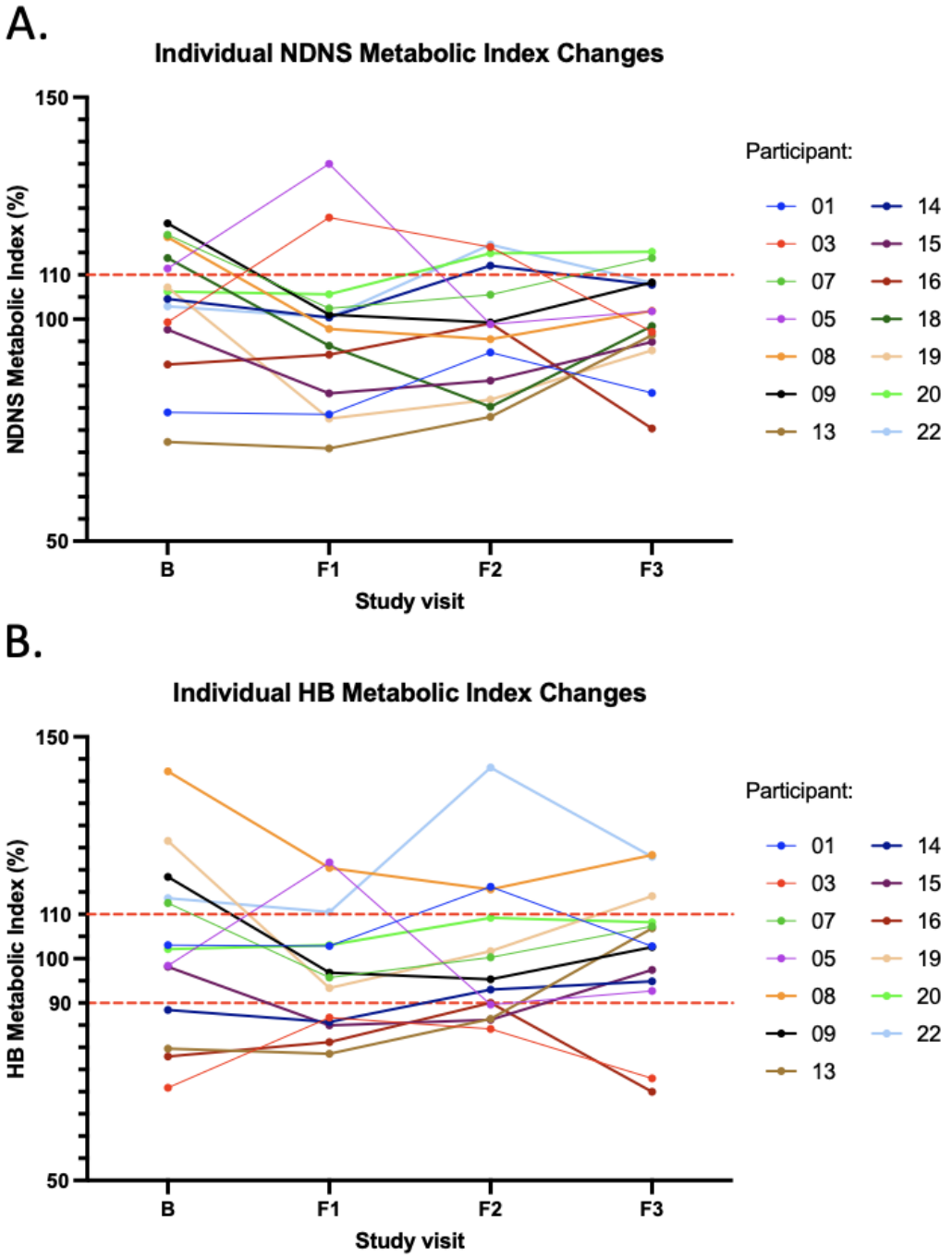

Figure 9.15 Individual longitudinal changes in the (A) NDNS metabolic index (n = 14) and (B) Harris-Benedict metabolic index (n = 13) (%). B: baseline; HB: Harris-Benedict; F1-3: follow-up study visits 1-3; NDNS: National Diet and Nutrition Survey. Red dashed lines indicate the metabolic index threshold for hypermetabolism (110%) and hypometabolism (90% - HB metabolic index only).

Table 9.14 Longitudinal metabolic index data for individual participants when using the NDNS (left) and Harris-Benedict (right) predictive equations. Hypermetabolic participants highlighted in red; hypometabolic participants highlighted in blue. B: baseline study visit; HB: Harris-Benedict; F1-3: follow-up study visits 1-3; NDNS: National Diet and Nutrition Survey: Pt: participant.

	NDNS Metabolic Index (%)				HB Metabolic Index (%)			
Participant	**B**	**F1**	**F2**	**F3**	**B**	**F1**	**F2**	**F3**
Pt01	79.03	78.57	92.5	83.4	103.04	102.85	116.19	102.78
Pt03	99.3	122.89	116.27	97.17	70.9	86.7	84.13	73.02
Pt05	111.42	134.99	98.85	101.79	98.41	121.68	89.64	92.74
Pt07	119.04	102.45	105.53	113.77	112.53	95.74	100.29	107.29
Pt08	118.48	97.81	95.48	101.88	142.2	120.46	115.59	123.34
Pt09	121.61	101.01	99.28	108.29	118.4	96.84	95.35	102.6
Pt13	72.36	70.88	77.99	96.42	79.72	78.55	86.42	106.84
Pt14	104.54	100.36	112.08	107.72	88.44	85.71	93.04	94.89
Pt15	97.65	83.3	86.15	94.89	98.14	84.97	86.19	97.47
Pt16	89.81	92.00	99.08	75.38	77.95	81.18	90.07	69.97
Pt18	113.81	94.05	80.25	98.44	-	-	-	-
Pt19	107.16	77.58	81.86	92.95	126.53	93.39	101.71	114.11
Pt20	106.20	105.63	114.86	115.18	102.15	103.05	109.22	108.19
Pt22	102.89	100.42	116.82	108.19	113.64	110.5	143.08	122.98
Hypermetabolic participants, n/14 (%)	5 (35.7)	2 (14.3)	4 (28.6)	2 (14.3)	5 (38.46)	3 (23.08)	3 (23.08)	3 (23.08)
Hypometabolic participants, n/14 (%)	2 (14.3)	4 (28.6)	4 (28.6)	2 (14.3)	4 (30.77)	4 (30.77)	4 (30.77)	2 (23.08)
Normometabolic participants, n/14 (%)	7 (50.00)	8 (57.14)	6 (42.86)	10 (71.43)	4 (30.77)	6 (46.15)	6 (46.15)	8 (61.54)

9.8 What is driving the mREE?

Pearson's and Spearman's bivariate correlation analysis were conducted to investigate the relationship between VO_2 and mREE against anthropometric assessments of body composition (Table 9.15) and participant reported nutritional intake (Table 9.16) at baseline. No significant correlations were observed between the resting energy expenditure parameters and anthropometric assessments. However, water, carbohydrate, sodium and potassium intake were all found to significantly, moderately positively correlate with mREE and VO_2 in this cohort (Table 9.16). The longitudinal analysis of carbohydrate intake is previously presented in Table 8.5. Longitudinal analysis was therefore conducted for the reported intake of sodium and potassium using data collected from Intake24. Sodium intake was found to significantly increase between baseline and the second follow-up study visit (p = 0.04, n = 17) following a mixed-effects analysis for multiple comparisons (Figure 9.16).

Table 9.15 Correlation analysis to investigate the relationship between measured resting energy expenditure and body composition at baseline. Correlation analysis was conducted using Pearson's correlation coefficient for normally distributed data, or Spearman's (†) correlation coefficient for non-normally distributed data. Significance is at $p < 0.05$. AMA: arm muscle area; BMI: body mass index; mREE: measured resting energy expenditure; MUAC: mid-upper arm circumference; TSF: triceps skinfold thickness.

				MUAC (cm)			**TSF (mm)**			**AMA (cm^2)**			**Calf circumference (cm)**		
	Weight (kg)	**Weight change from premorbid (%) †**	**BMI (kg/m^2)**	**Left**	**Right**	**Av.**	**Left**	**Right**	**Av.**	**Left**	**Right**	**Av.**	**Left**	**Right**	**Av.**
mREE (kcal/day)	0.20	0.01	-0.07	0.26	0.23	0.25	0.07	-0.11	-0.02	0.18	0.25	0.23	0.01	0.06	0.04
	0.40	0.98	0.76	0.24	0.30	0.26	0.78	0.65	0.95	0.45	0.28	0.33	0.95	0.78	0.86
	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
VO_2 (ml/min)	0.21	-0.01	-0.08	0.25	0.22	0.24	0.06	-0.11	-0.02	0.17	0.25	0.23	0.03	0.07	0.05
	0.38	0.98	0.75	0.26	0.32	0.27	0.80	0.63	0.94	0.46	0.29	0.34	0.91	0.75	0.82
	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22
RQ †	-0.18	-0.31	0.02	-0.11	-0.11	-0.12	-0.01	0.03	-0.05	0.07	-0.01	0.07	-0.18	-0.16	-0.22
	0.44	0.21	0.94	0.64	0.64	0.60	0.96	0.92	0.83	0.77	0.99	0.77	0.43	0.48	0.33
	20	18	20	22	22	22	20	20	20	20	20	20	22	22	22

Table 9.16 Correlation analysis to investigate the relationship between measured resting energy expenditure and reported nutrient intake at baseline (n = 22). Correlation analysis was conducted using Pearson's correlation coefficient for normally distributed data, or Spearman's (†) correlation coefficient for non-normally distributed data. Significance is at $p < 0.05$, significant results are highlighted in bold. mREE: measured resting energy expenditure.

	mREE (kcal/day)	VO_2 (ml/min)
Energy (kcal)	0.31	0.28
	0.16	0.21
Water (g)	0.43	0.45
	0.05	**0.04**
Protein (g) †	0.34	0.36
	0.12	0.10
Fat (g)	0.03	0.01
	0.91	0.97
Carbohydrate (g) †	0.51	0.51
	0.02	**0.02**
Starch (g)	0.43	0.38
	0.05	0.08
AOAC fibre (g) †	0.21	0.21
	0.34	0.36
Cholesterol (g)	0.01	0.01
	0.98	0.98
Retinol (µg)	-0.11	-0.12
	0.63	0.60
Vitamin A (µg) †	0.09	0.08
	0.70	0.71
Sodium (mg)	0.56	0.54
	0.01	**0.01**
Potassium (mg)	0.53	0.54
	0.01	**0.01**
Iron (mg) †	0.08	0.08
	0.74	0.73

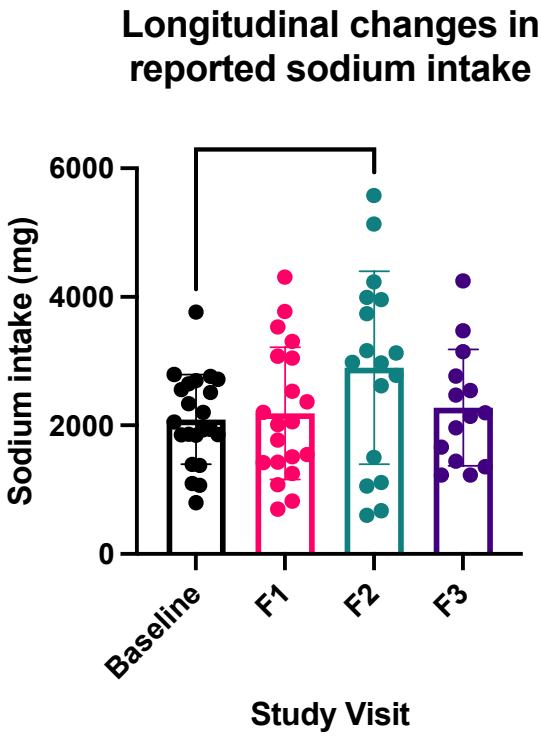

Figure 9.16 Longitudinal changes in reported sodium intake from a 24-hour dietary recall (mg). Data is presented as mean ± one standard deviation. Normality was checked using Shapiro-Wilk analysis. Statistical differences between sodium intake reported at each study visit was analysed using a mixed-effects analysis for multiple comparisons. Significance is at the $p < 0.05$ * level. Baseline-F1: $p = 0.97$, $n = 20$; Baseline-F2: $p = 0.04$*, $n = 17$; Baseline-F3: $p = 0.87$, $n = 14$. F1-F3: follow-up study visits 1-3.

9.9 Discussion

In healthy adults, it is assumed that REE comprises approximately 60-70% of total daily energy expenditure (TDEE) [252]. Data presented in this chapter have demonstrated that measured resting energy expenditure (mREE) comprised approximately 70% of predicted total daily energy expenditure (pTDEE) calculated by the Kasarskis Model 6 equation [268] (Table 9.2).

9.9.1 Assessment of energy balance

To investigate whether the energy needs of this study cohort were met, energy intake was compared against predictions of total daily energy expenditure using the Kasarskis Model 6 predictive energy equation [134]. It was suggested by Vaisman et al., (2009) that an inadequate energy intake decreases resting energy expenditure [262]. However, relationship between reported energy intake and measured resting energy expenditure was not observed in this cohort (Table 9.16).

9.9.2 Incidence of hypermetabolism

As presented in **section 2.2.2**, hypermetabolism is associated with an increased catabolism of carbohydrate, lipids and proteins [66], as well as an increase in excreted urinary nitrogen, leading to a negative nitrogen balance [67]. The primary purpose of identifying hypermetabolism in this cohort was to address whether the presence of hypermetabolism influences the concentration of biochemical analytes. The results presented in **section 9.3** have demonstrated that the inclusion of body weight is responsible for the inaccuracy of the HB and Henry predictive energy equations when compared against mREE. This data is supported by a conclusion by Ellis et al., (2011) who suggested predictive equations may be more accurate in individuals with a 'healthy' nutritional state (i.e., BMI range 18-30 kg/m^2) [147]. This chapter has demonstrated that the underprediction of resting energy expenditure in lighter individuals is proportional to the indication of hypermetabolism, thus biasing the classification of hypermetabolism to those individuals with a lower body weight.

As highlighted in the scoping review in **Chapter 3**, as FFM is regarded as the biggest driver of REE, contributions of FFM should therefore be considered in its prediction [284]. This PhD study

was not initially designed to assess body composition, therefore use of predictive equations incorporating assessments of FFM and FM such as the ALS-specific equation developed by Jesus et al., (2019) [144] was not possible for this study. Instead, proxy estimates of arm muscle area (AMA) were used to normalise measurements of resting energy expenditure (mREE/AMA) in this cohort.

Prior MND literature has presented a relationship between the proportion of FFM, FM and hypermetabolism in individuals living with MND [276]. Whilst this study observed no significant correlations between the metabolic index and AMA (Table 9.12), the metabolic index, calculated using the Harris-Benedict pREE was observed to increase with decreases in weight, BMI, calf circumference and a higher ALSFRS-R score. Combined, these data demonstrate that lighter individuals with a reduction in fat and fat free mass have a higher-than-predicted demand for energy, but experience less severe MND-related symptoms and subsequently have a higher functionality.

As presented in **Chapter 3**, the substantial discrepancy in identifying hypermetabolism using different thresholds and predictive equations can lead to statistically significant differences in the number of participants indicated to be hypermetabolic [146]. This chapter has argued against the identification of hypermetabolism using predictive energy equations developed in young White British males as the denominator, but rather has proposed the use of pREE values developed by the National Diet and Nutrition Survey (NDNS). The NDNS pREE was devised from three equations which included assessments of body composition, per sex and age category. It was hypothesised that predicting resting energy expenditure in this way may therefore be more representative of the MND population, with accuracy in a greater proportion of the MND population. When compared to the measured resting energy expenditure of participants in this cohort, the NDNS pREE was found to be accurate in 40% of this study population; a greater proportion than either the HB or Henry pREE, but less than when using kcal/kg/day.

9.9.3 Clinical and nutritional associations with hypermetabolism

Whilst this evidence supports the 'dynamic hypothesis' proposed by He et al., 2022 [276] (presented in **section 3.5.5)** it contrasts with results from Steyn [330], Desport [263] and Vaisman

[262], who all reported no agreement between hypermetabolism (indicated using the Harris-Benedict metabolic index) and the ALSFRS-R. Of note, as no participants were using percutaneous feeding, a King's score of four was indicative of respiratory failure. This agrees with Siirala [266] and Georges [267] who both presented a lower mREE in individuals using mechanical ventilation. In contrast to results presented by Jesus [145] and Steyn [271], no relationship was observed between the Harris-Benedict metabolic index and disease progression, calculated by the change in ALSFRS-R functional score (ΔALSFRS-R).

9.9.4 Does the presence of a hypermetabolic state influence biochemical analytes?

The research question of whether hypermetabolism dysregulates the concentration of biochemical analytes needed to be addressed. The only significant relationship observed was the negative correlation between the NDNS metabolic index and serum albumin. However, when the study cohort was separated into groups depending on their metabolic state (i.e., hypometabolic, normometabolic or hypermetabolic), no significant differences in the concentrations of the serum or 24-hour urinary biochemical analytes were observed. This suggests that an elevated metabolic state does not influence the concentration or reliability of biochemical analytes.

9.9.5 Longitudinal changes in resting energy expenditure

Resting energy expenditure was re-assessed at three-monthly intervals. At group level, no significant differences were observed for changes in mREE, VO_2, metabolic index, mREE or mREE/AMA over the nine-month study period. However, the significant, sequential increases in RQ suggests a preferential shift to carbohydrate oxidation over time. As participant-reported energy and macronutrient intake did not significantly increase over time (Table 8.5), this suggests that the switch to carbohydrate oxidation during the disease course was not driven by an increased carbohydrate intake in this cohort.

Individual fluctuations observed in the 14 participants who completed all four study visits demonstrated that the intra-cohort metabolic state is: i) dependent on the pREE applied as

the comparator and ii) highly variable across the study period. An individual's metabolic state is therefore highly variable, and in contrast to a statement by Bouteloup [261], is not a "continuous phenomenon". The significant relationship demonstrated between mREE and sodium intake, as well as the significant increase in sodium intake between baseline and the second follow-up study visit may contribute to these fluctuations, but further research is needed to understand this relationship. This research will be continued in a post-doctoral position by the thesis author, as detailed in **section 11.2**.

9.10 Conclusion

The NDNS pREE is representative of the general UK population for any given age and sex. Use of the NDNS metabolic index therefore removes consideration for the weight of the individual and consequently does not bias the identification of hypermetabolism. When considered in isolation, variations in mREE are not driven by body composition or participant reported energy intake, but rather were indicated to increase following increased consumption of water, carbohydrate, sodium and potassium intake. This must be considered with caution, as the data collected from the participant-reported 24-hour recall cannot be concluded to be reliable. The significant increase in RQ observed between baseline and all three follow-up visits suggests a preferential shift to carbohydrate oxidation, however sodium intake, but not carbohydrate intake was found to significantly increase over time. Further research is needed to determine whether this relationship is perhaps fortuitous, or whether the metabolic state may be driven by the metabolism of endogenous fuel sources.

10 General discussion

This PhD study aimed to propose a suitable and pragmatic approach for the assessment of nutritional state in people living with MND. This has been achieved by completing the following objectives in this thesis:

1. Identification of the current challenges with nutritional assessment in MND **(Chapters 2 and 3)**;
2. Identification of a suite of techniques (i.e., 'a nutritional toolkit') that can be used to deeply phenotype the nutritional status of people living with MND **(Chapter 4)**;
3. Investigation and demonstration of the role of these techniques to assess the nutritional status of a cohort of people living with MND **(Chapters 5-9)**;
4. Assessment of the relevance of these techniques to assess the severity and progression of MND **(Chapters 5-9)**.

10.1 Identification of the current challenges with nutritional assessment in MND

A comprehensive literature review, presented in **Chapter 2**, outlined the current challenges and multiple complexities associated with assessing the nutritional state of people living with MND. Whilst a loss of body weight compared to before the onset of MND symptoms is most commonly used to monitor declines in nutritional state, simple weight measurements are unable to differentiate changes in body composition experienced as a result of disease-associated muscle-wasting and malnutrition-associated fat loss. Assessments of body composition (fat and fat free mass) are therefore necessary to achieve a detailed understanding of nutritional state and indicate the onset of a negative energy balance. A prolonged energy imbalance is related to an inadequate nutritional intake which fails to match energy expenditure, as well as the dysregulation of useable energy metabolism. It was consequently important to assess both the nutritional intake and metabolisation of chemical energy in the form of calories and macronutrients (carbohydrates, fats, proteins).

Estimating nutritional intake using a 24-hour dietary recall is useful to gauge energy and macronutrient intake. However, to ensure the reliability of reported nutritional intake, it was

suggested that dietary assessment must be validated in relation to an objective marker of intake, such as biochemical analytes. Biochemical analytes are categorised into two categories: 1) biochemical analytes of dietary exposure, which robustly and reliably reflect dietary intake in weight stable individuals [171,172]; and 2) biochemical analytes of nutritional status, which encompasses the symbiotic relationship between nutrition and metabolism, reflecting dietary intake and the absorption and processing of nutrients [153,154]. As inflammation, muscle catabolism and hypermetabolism are known to be at play in individuals living with MND [160], the influence of a pro-inflammatory, catabolic and hypermetabolic state needed to be considered alongside measurement of biochemical analytes.

A scoping review was therefore conducted to identify the most suitable and pragmatic methods and techniques to assess resting energy expenditure, and subsequently hypermetabolism, in an MND cohort (**Chapter 3**). This scoping review highlighted a lack of MND-specific protocol for indirect calorimetry measurements, the inaccuracy of predictive energy equations when estimating resting energy expenditure, and inconsistencies when defining hypermetabolism.

10.2 Identification of a suite of techniques that can be used to deeply phenotype the nutritional status of people living with MND

Chapter 4 describes the selected techniques to deeply phenotype the nutritional state of this MND cohort. To briefly summarise the methods utilised in this study:

1. **Body composition** was assessed using upper arm anthropometric measurements of the triceps skinfold (TSF) and mid-upper arm circumference (MUAC). The TSF was used as an estimate of fat mass, whilst a proxy for muscle mass (arm muscle area, AMA) was derived using TSF and MUAC measurements. Mid-upper arm and calf circumference measurements were also measured, but it was unclear whether they would be reflective of fat or muscle mass in this cohort as this had not been previously been investigated in MND.
2. **Nutritional intake** was assessed using a self-reported 24-hour online recall diary, 'Intake24'.

3. **Biochemical analytes:** A panel of 24-hour urinary biochemical biomarkers of dietary exposure (24-hour urinary sodium, potassium, urea and total urinary nitrogen) and serum biochemical analytes of nutritional state (creatinine, albumin, prealbumin, transferrin, ferritin, retinol-binding protein and the lipid profile) were measured to investigate the role of these analytes in understanding the nutritional state;
    a) As a pro-inflammatory state is known to cause a hepatic reprioritisation of acute phase proteins (albumin, prealbumin, transferrin and ferritin), basic inflammatory markers (ESR, white cell count, fibrinogen and C-reactive protein) were measured alongside these analytes in order to rule out dysregulation of analyte metabolism due to the inflammatory response.
4. **Resting energy expenditure** was measured using the GEMNutrition indirect calorimeter;
    a) Predicted resting energy expenditure was estimated using the Harris-Benedict (1919), Henry and kcal/kg body weight energy equations, as well as using the National Diet and Nutrition Survey predictions for age and sex for the general UK population;
    b) **Hypermetabolism** was indicated by comparing the measured resting energy expenditure against predictions of resting energy expenditure to calculate the metabolic index.

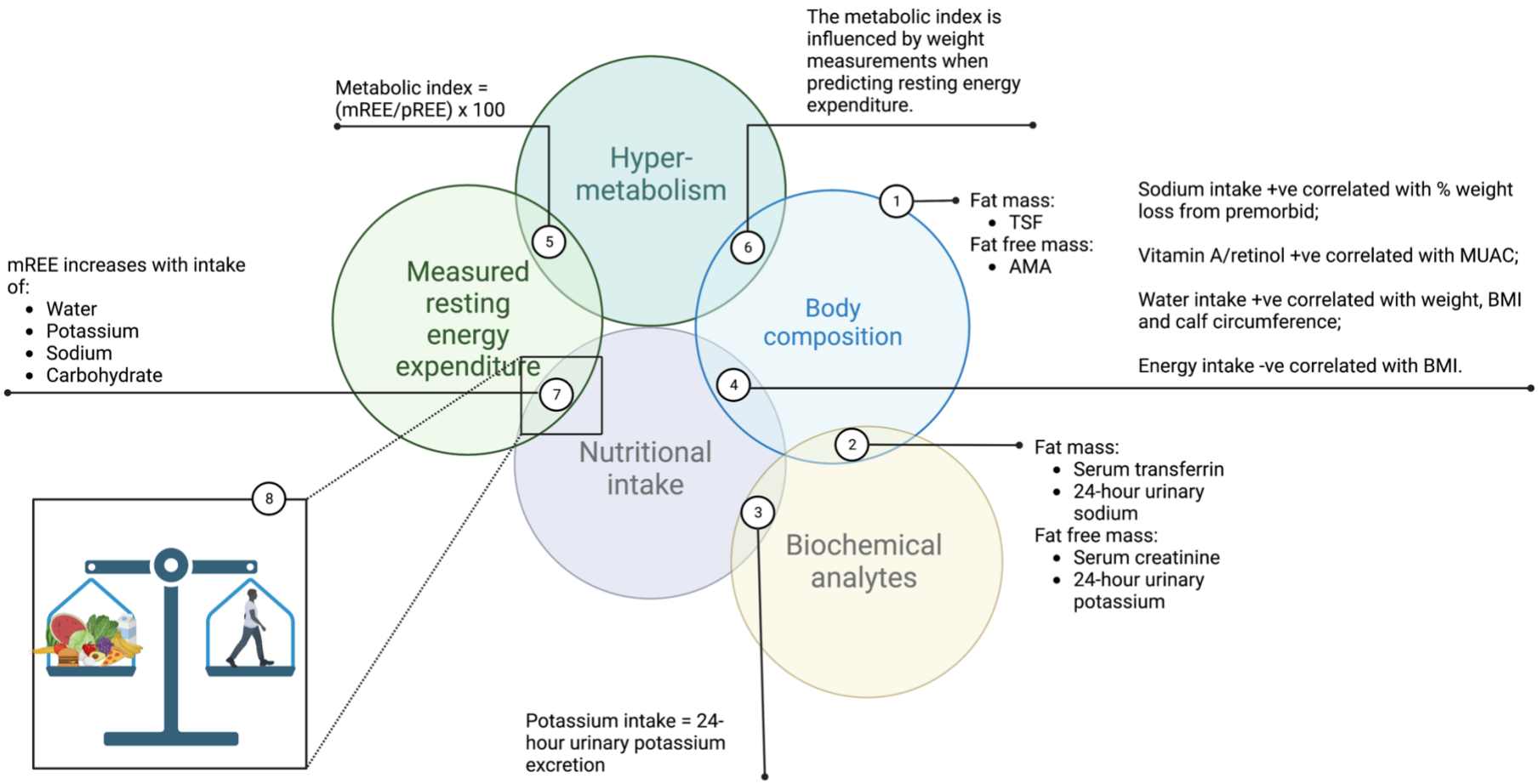

Figure 10.1 A schematic to highlight the key findings from this PhD study. The numbered circles indicate key findings described below. Created with BioRender.com

10.3 Investigation and demonstration of the suitability of these techniques to assess the nutritional status of a cohort of people living with MND

This discussion chapter will bring together to present the key, ***novel*** findings presented in **Chapters 5–9**. This can be visualised schematically in Figure 10.1. The numbered circles within the Venn diagram relate to the following paragraphs.

(1) Estimates of fat and fat free mass reflect weight and BMI

In this study, measurements of triceps skinfold thickness (TSF, mm) were used as a proxy estimate of fat mass, whilst derived measurements of arm muscle area (AMA, cm^2), using TSF and the mid-upper arm circumference (MUAC), estimated fat free mass. **Chapter 6** demonstrated that these anthropometric estimates of fat and fat free mass are indicative of nutritional state in this cohort, whereby a decrease in both fat and fat free mass accompanies a decline in body weight and BMI (

Table 6.3).

As body circumference measurements incorporate muscle, fat and bone, it was unknown whether MUAC and calf circumference would reflect fat or fat free mass in this cohort; this relationship has not been previously investigated in MND. Regardless of whether mid-upper arm and calf circumference measurements indicate fat or fat free mass, the data presented in **Chapter 6** (Table 6.2) have shown that a decline in calf circumference of < 31 cm is associated with a percentage weight loss of ≥ 5% compared to before symptom onset in this cohort. This relationship suggests that a decline in calf circumference may be indicative of a negative energy balance, which is accompanied with the loss of both fat and fat free mass. The accuracy and suitability of these measurements needs further evaluation, and the thesis author has taken steps to develop this framework, as described in **section 11.1.**

(2) Serum and 24-hour urinary biochemical analytes reflect body composition

Chapter 7 demonstrated that serum transferrin and 24-hour urinary sodium significantly positively correlated with measures of TSF. As a decline in serum transferrin concentration is known to be a marker of extreme protein-energy malnutrition [326,327], this supports the suggestion that serum transferrin may be associated with fat mass, which further strengthens the suggestion that TSF could be used as a proxy measure of fat mass. It is important to note that causal relationships are not clear from this research; i.e., it is not known whether the decline in transferrin is a result of a loss of fat mass, or whether the decline in transferrin prompts the decline in fat mass.

Sodium intake was expected to correlate with BMI, body fat and 24-hour urinary sodium [331–333]. However, in this cohort, sodium intake was not found to significantly correlate with the excretion of 24-hour urinary sodium or BMI, but did significantly positively correlate with TSF. Whilst it would be easy suggest that 24-hour urinary sodium could be associated with fat mass, consideration of the homeostatic mechanisms of sodium balance may offer an alternative, plausible explanation that increased sodium excretion may be linked to excess water intake [334]. When a Pearson's product-moment correlation analysis was conducted to investigate the relationship between reported water intake (from Intake24) and 24-hour urinary sodium excretion at baseline, a moderate, significant positive correlation coefficient

was observed (r = 0.48, p = 0.03, n = 22). As calf circumference has also been demonstrated to be influenced by water intake (Table 8.2) this may explain the significant relationships observed between 24-hour urinary sodium and calf circumference.

Serum creatinine and 24-hour urinary potassium significantly positively correlated with AMA. This suggests that these analytes could be associated with fat free mass. As discussed in **section 7.2.1**, a pro-inflammatory state indicated by an elevated erythrocyte sedimentation rate is known to increase muscle catabolism, which is associated with a decreased serum creatinine concentration [160]. The relationship between 24-hour urinary potassium and AMA is less clear. The first potential explanation is that this association is fortuitous, with no biological relevance or explanation. The second suggestion is that these results may be independently linked to the size of the individual: the larger the person, the greater the quantity of skeletal muscle, as well as the greater excretion of urine. The third explanation may be associated with dietary intake; however, whilst 24-hour urinary potassium was reflective of potassium intake and AMA, a significant relationship was not observed between potassium intake and AMA. Therefore, changes in muscle mass cannot be directly linked to potassium intake. Potassium is an intracellular cation predominantly located in skeletal muscle. A fourth suggestion may be that changes in the metabolism of potassium could be a by-product of muscle wasting. The concentration of potassium in the extracellular fluid (ECF) is highly regulated by skeletal muscle and renal function. The regulation of extracellular potassium by skeletal muscle was reviewed by McDonough et al., (2002) [335]. In brief, following a decreased intake of dietary potassium, intracellular potassium is 'buffered' into the extracellular space. Once the ECF potassium reaches maximal capacity, any surplus ECF potassium is excreted in the urine to remove the excess potassium to prevent build up and toxicity [336]. A decreased dietary intake coupled with an increase in muscle-wasting may decrease the availability of skeletal ICF potassium, decreasing the movement of potassium into the ECF, decreasing the concentration of potassium excreted in the urine. Total body potassium would need to be measured to further investigate the relationship between potassium intake, sequestration and total body composition [337]. A statistically significant relationship between GFR and either potassium intake or 24-hour urinary potassium excretion was not observed in this study, but that does not mean that these basic principles of renal physiology are not occurring.

The relationship between biochemical analytes and body composition therefore requires further evaluation. This dataset could be further interrogated to investigate the sodium:potassium ratio and its relationship with body composition. The 24-hour urinary samples collected and stored from this cohort could also be re-analysed for 24-hour urinary creatinine, which may offer a better marker of 24-hour urinary completeness [338], as well as an alternative marker of fat free mass [339]. Future work with a larger dataset could include interpolation analysis to develop thresholds for clinically meaningful changes in muscle and fat mass.

(3) The reliability of participant-reported nutritional intake

Biochemical analytes for dietary exposure have been demonstrated to reflect macronutrient intake in healthy, weight stable individuals [190]. This cohort have been demonstrated to have an average weight loss of 4.58% since the onset of MND-related symptoms, indicating that at group level, these individuals were not weight stable. Sodium intake was not significantly correlated with 24-hour urinary sodium excretion, which may agree with the literature that urinary sodium does not reliably reflect sodium intake in individuals experiencing weight loss. Interestingly, 24-hour urinary sodium excretion was found to statistically significantly decrease between baseline and the second follow-up study visit (Table 7.10), whilst estimates of sodium intake were found to significantly increase in the same individuals over the same time period (Figure 9.16). Therefore, although individuals were increasing the amount of sodium consumed, less sodium was excreted, suggesting a sequestration of sodium which may explain the lack of association in this cohort. However, errors in reported dietary intake to under- or over-estimate sodium intake, or an undeclared deviation from the 24-hour urinary collection protocol may also explain the absence of a significant relationship.

In contrast, potassium intake was found to significantly positively correlate with 24-hour urinary potassium. Furthermore, the concentration of 24-hour urinary potassium was not influenced by incomplete 24-hour urinary collections. This suggests that: i) the reported 24-hour recall of potassium intake was reliable in this study; and ii) excreted 24-hour urinary potassium may be suitable as a robust, pragmatic biomarker of potassium intake and muscle mass, regardless of weight loss and protocol adherence to 24-hour urinary collection.

(4) Are changes in body composition a direct result of changes in nutrient intake?

It is not possible in this study to differentiate between exogenous and endogenous contributions to body composition. For example, protein intake was significantly correlated with excreted total urinary nitrogen (TUN). However, as disease severity progressed, TUN was found to significantly decrease between baseline and the second study visit (a duration of three-months) for 20 participants, whilst protein intake did not significantly change. This may be indicative of muscle catabolism and/or malnutrition; however, as no relationship was observed between TUN and AMA, TUN cannot be used as a biomarker of muscle mass. Similarly, although urinary potassium was reflective of potassium intake and AMA, a significant relationship was not observed between potassium intake and AMA, therefore, changes in muscle mass cannot be directly linked to potassium intake.

(5) Identification of hypermetabolism in MND

Hypermetabolism is currently indicated by comparing measurements of resting energy expenditure against predictions of resting energy expenditure. **Chapter 3** demonstrated that the Harris-Benedict (1919) predictive energy equation was the most commonly used approach to predicting resting energy expenditure internationally [340]. However, the inaccuracy of the Harris-Benedict equation in people living with MND has been repeatedly demonstrated (as presented in **Chapter 3, section 3.4.4.2**). Whilst predictive energy equations that incorporate body composition, such as the Nelson equation [279], have been proposed to have a better accuracy in MND cohorts, the Harris-Benedict predictive equation is still reported as the most commonly compared equation against mREE using indirect calorimetry to indicate hypermetabolism in MND (Table 3.9).

As body composition was estimated by TSF (mm) and AMA (cm^2), which could not be extrapolated to values in kilograms or percentage of body weight for application in the Nelson equation, resting energy expenditure was predicted using the Harris-Benedict, Henry and kcal/kg body weight energy equations in this cohort. These predictive energy equations were compared against measured resting energy expenditure using indirect calorimetry.

(6) Does the presence of hypermetabolism influence the nutritional state?

The metabolic index, calculated using the Harris-Benedict pREE, was observed to increase with decreases in weight, BMI and calf circumference. Exciting, ***novel research*** conducted in this PhD study has demonstrated that in extreme weights, the predictive equations are further from the measured REE, e.g., the lighter the individual, the greater the underprediction of REE. This is likely because this population cohort are vastly different to the population that was used to derive these equations in the first place [340]. In turn, the greater the underprediction of the resting energy expenditure, the greater the metabolic index. This biases classification of hypermetabolism to those who are lighter, regardless of true energy demand. These data were presented, and their implications discussed in more detail, in **Chapter 9**, **section 9.3.** Data in this thesis have shown that measured resting energy expenditure using the GEMNutrition metabolic cart is approximately 70% of predicted total daily energy expenditure (Table 9.2). This is reflective of the mREE contributions observed in a healthy population, which raises the question, are people living with MND truly hypermetabolic?

(7) What drives mREE?

When the metabolic index was calculated using predicted resting energy expenditure for the UK general population in the national diet and nutrition survey (NDNS), the NDNS metabolic index was not found to correlate with nutritional state. As the point of reference was the average predicted resting energy expenditure for the UK population, given by age and sex, the NDNS metabolic index was inherently related to changes in the individual's measured resting energy expenditure. Contrary to published literature, the measured resting energy expenditure was not found to be influenced by weight or FFM, but rather mREE was found to increase with an increase in the consumption of water, carbohydrates, sodium and potassium. It could therefore be suggested that resting energy expenditure is driven by metabolisable fuel-energy contributions.

In support of this, a significant increase in RQ was observed at group level between baseline and all three follow-up visits (Figure 9.9). This suggests a preferential shift to carbohydrate oxidation over time. A significant relationship was not observed when RQ was compared against reported carbohydrate intake at baseline (r = 0.13, p = 0.57, n = 22), and reported

carbohydrate intake did not increase significantly over time (Table 8.5). This suggests that the switch to carbohydrate oxidation during the disease course was not driven by increased carbohydrate intake in our cohort. Carbohydrate oxidation can promote serum triglyceride synthesis. Although a significant increase in serum triglyceride was not observed within this cohort over time (Table 7.10), a non-significant moderate positive correlation was observed between serum triglyceride and participant self-reported carbohydrate intake at baseline (Spearman's $r = 0.45$, $p = 0.053$, $n = 19$).

(8) Calculating energy balance

The data generated in this PhD study has raised the question as to why identification of hypermetabolism is important in MND; surely reliable measurements of energy expenditure are sufficient to ensure energy intake equals energy demand? Energy intake was estimated using participant self-reported 24-hour recall diaries and compared against the UK estimated average requirements to indicate individuals at risk of not consuming adequate energy [305]. However, again, this was comparing individuals against broad, generic, predicted requirements. Instead, the Kasarskis model 6 predictive equation was used to estimate total daily energy expenditure [134]. Whilst this equation also incorporates weight, height, gender and age, it was devised in a cohort of people living with MND and considers the severity of MND-related symptoms (speech, dexterity, mobility and respiration). Comparison of reported energy intake against energy demand calculated using the Kasarskis model 6 TDEE equation gives a reliable indication of whether an individual is consuming an appropriate nutritional intake, specific to the physiological demands of that individual.

10.4 Assessment of the relevance of these techniques to assess the severity and progression of MND

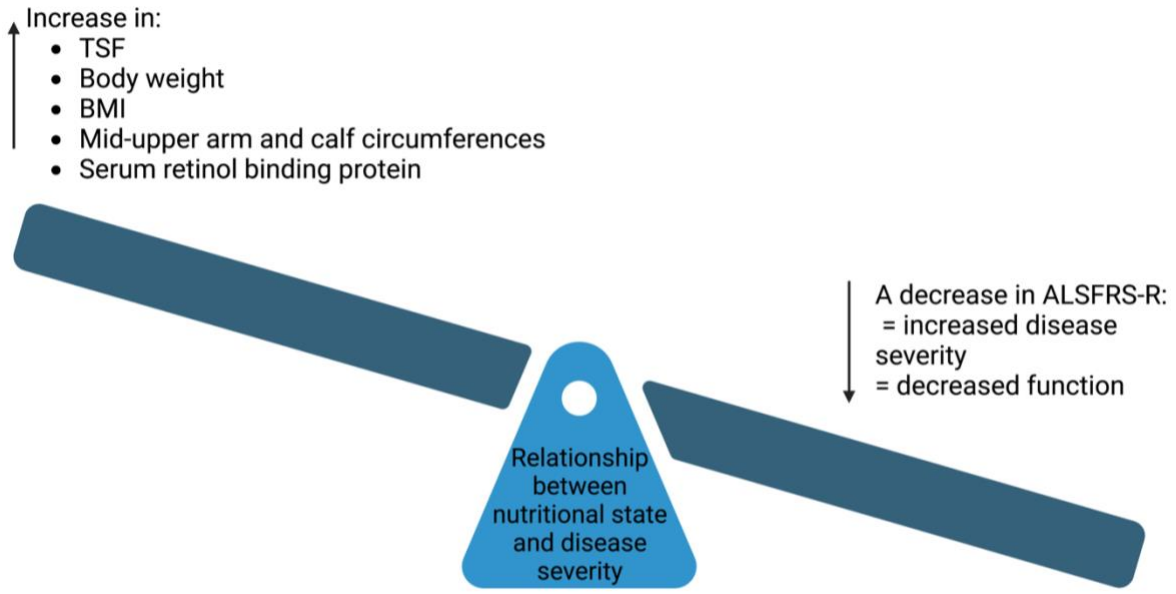

Figure 10.2 A schematic to demonstrate the relationship of assessments of nutritional state with disease severity.

In this study cohort, an increase in disease severity (indicated by a lower ALSFRS-R total score), has been demonstrated to be associated with an increase in body weight, BMI, TSF and body circumference measurements (Figure 10.2). This is counter-intuitive, however, it is important to remember that this cohort may not be representative of the wider MND cohort, as discussed in **Chapter 5, section 5.3**. At baseline assessment, this cohort was overweight with an average BMI of 26.26 kg/m^2 (Table 6.1), indicated to be in the early stages of MND progression. Therefore, whilst a single anthropometric measurement could be used to indicate disease severity, it is important that individual changes in body composition are monitored in relation to the rate of change in disease severity. It would be interesting to follow these participants longer to observe whether individuals indicated to be at risk of an adverse nutritional state (e.g., a BMI ≤ 20 kg/m^2) had a longer survival than those with a normal weight [274].

10.5 Considerations, limitations and clinical observations

This thesis presents data from a small, heterogenous cohort study of people living with MND. As discussed in **section 5.3**, this White British, predominantly male, slow-progressing, early-stage MND cohort is not indicative of the wider MND population. The results presented in this thesis may not necessarily transfer to another independent MND cohort with different demographics and disease characteristics. As this study was a pilot observational study, the study was designed to observe the potential roles of the biomarkers of nutritional state in this cohort. Whilst reference ranges have been used (where possible) to indicate deviation from expected results, the absence of an age- and sex-matched healthy control group reduces the possibility to indicate whether these results are significantly different from the normal, healthy 'expected' results.

It is fundamentally challenging to conduct a longitudinal study involving individuals with restricted mobility, communication barriers and a potentially reduced emotional capacity. The recruitment rate for participation in this study was approximately 40% (Figure 5.1). It was observed that the willingness and ability of the individual living with MND to participate was largely restricted by the capacity and inclination of the individual's carer(s). A review describing the predictors of engagement, attrition and protocol adherence in ALS trials was published by Atassi et al., 2013 [341]. A number of key findings reported in this review were also experienced in this study: 1) non death attrition rates; 2) participants with a better functional status and longer disease duration were associated with retention; and 3) protocol adherence.

Atassi et al reported non death attrition rates to be between 18 and 22%, with the most common causes were consent withdrawal and adverse events [341]. The nondeath attrition rate in this MND cohort study was 9/24 (37.5%), with disease progression and travel difficulties given as the reason for withdrawal in 3/9 (33.3%) withdrawn participants. Loss of participants to follow-up can bias results for slowly progressing participants. As described in Chapter 5, **section 5.3,** this cohort had a slower than average disease progression; however, as this study did not capture individuals at a pre-defined stage of their disease, this bias may be weakened. Furthermore, all participant data was accounted for and presented in both the cross-sectional and longitudinal analysis for this study.

The most commonly identified protocol deviation was the provision of incomplete or inappropriately collected 24-hour urinary collections 13/22 (59.1%). Feedback from participants recruited to this study indicated that the 24-hour urinary collection was the most burdensome, and was explicitly stated as the reason for withdrawal of one female participant, despite informing participants that they could opt out of that particular study assessment. Interestingly, the protocol deviation for 24-hour urinary collection was most frequently observed in individuals with a higher functionality, whilst participants with a decreased function actually demonstrated an increased adherence to this protocol; it was stated to be easier than normal toileting procedures.

All assessments of nutritional state conducted in this study were compared against disease severity assessed using a participant self-completed ALSFRS-R functionality questionnaire. However, the ALSFRS-R is not without limitation. Due to reduced dexterity, the ALSFRS-R was often completed by a carer (most frequently a spouse or family member) on behalf of the participant. It was observed that the carers were often in denial about the severity of their partners/family member's symptoms, and as such, marked the individual higher than they truly were; sometimes with ALSFRS-R total scores recorded to increase between study visits. As these questionnaires were designed to be self-completed for this study, the thesis author could not question or amend the scores provided. Furthermore, participant's symptoms often do not neatly fit into one tick box, and individuals often met the description of multiple scores, again meaning that their recorded score did not precisely reflect their symptoms. The implication of this error is that the cohort were likely to be experiencing a greater functional decline than is reported. Moreover, each question within the ALSFRS-R carries the same weighting. For example, a more life-threatening symptom, such as breathing difficulties, versus excess salivation contribute equally to the final ALSFRS-R score; this means that the total ALSFRS-R score does not fully reflect the severity of disease [342]. Use of the ALSFRS-R subscores for bulbar, fine motor, gross motor and respiratory function have been demonstrated to better reflect disease progression [343]. These limitations are widely reported, and efforts are being made to better characterise disease progression [344].

Whilst the conduction of venepuncture sounds simple in principle, it became more difficult to successfully palpate and insert a needle into participants cubital fossa with increased frailty

[345]. For example, venepuncture was successfully conducted in 19/22 (86.4%) participants at baseline, but only 10/14 (71.4%) at the fourth and final study visit. This can be attributed to a decline in muscle mass, making veins more likely to roll; a decreased elasticity of vein walls, increasing the chance of collapse which stops the blood draw; or dehydration, common in people living with MND due to increased dysphagia or increased anxiety regarding toilet access [346,347]. Whilst the effect of this could be mitigated by using a 22-gauge needle with butterfly luer adapter, or draw from the dorsal aspect of the hand, the 'three attempts and stop' approach was adopted to reduce participant burden.

A clear limitation of this study is missing anthropometric data as a result of practical limitations associated with mobility and movement. For example, weight measurements were not collected from two participants who were not able to stand freely, and it was unfeasible to measure accurate TSF measurements from participants with a reduced mobility due to restricted access to the triceps in wheelchair bound participants. Furthermore, as described in **section 2.4.1.2**, TSF measurements are highly influenced by hydration levels. To consider the influence of measurement error on inaccurate TSF measurements, coefficient of variation (CV) was calculated for triplicate TSF measurements; TSF values with CV > 5% were discounted from analysis. Combined with the absence of a correlation between TSF and estimated water intake, this increases confidence in the reliability and reproducibility of these measurements in estimating body composition.

It was interesting to observe that correlations with anthropometric measurements are not necessarily significant for both left and right anthropometric measurements, highlighting the importance of assessing asymmetric whole-body anthropometry. A further limitation of using TSF and AMA as proxy estimates of body composition is that they do not provide a measure of the proportion of fat or fat free mass per body weight; i.e., kg muscle mass per kg body weight. Therefore, it was not possible to compare the body composition of this cohort against other MND cohorts when measured using BIA, DEXA or plethysmography. Examples of publications that have measured body composition when assessing energy expenditure are presented in the scoping review, **Chapter 3, Table** 3.3**.**

The collection of dietary intake data using a retrospective 24-hour recall diary is inherently reliant on the participant (and/or) carer memory. As the quantity of dietary intake was recorded using portion size photographs on Intake24, accurate dietary reporting is reliant on the appropriate interpretation of size and depth of the images. Not all food, specifically in reference to enteral feeds that some participants were using as supplements, exists in the nutrient database used by Intake24; these supplements were added to the extracted intake data manually by the thesis author where possible, however back-of-the-packet nutritional information was not always provided by the participant. These reporting errors may lead to biases and inappropriate representation of the 24-hour intake. Furthermore, the use of a single 24-hour dietary recall every three months may be considered inadequate to capture day-to-day variability. However, this study was not originally designed to assess the longitudinal changes in dietary intake, but rather to evaluate the role of using biochemical analytes to objectively assess the nutritional state of an MND cohort. Further work can be conducted using this data set to comprehensively investigate the longitudinal changes in dietary intake.

10.6 Final conclusions

1. Assessment of body composition

Body composition sits at the heart of nutritional assessment. People living with MND inevitably experience muscular atrophy as a result of muscular denervation. This causes a reduction in strength and mobility, which leads to an increasingly sedentary lifestyle. An increase in disease severity has been associated with an increase in weight, BMI and estimates of fat mass. As the increase in fat often occurs simultaneously to the loss of muscle mass, the change in body composition remains 'invisible' to the clinician or researcher upon the measurement of body weight. This makes weight measurements not ideal for the assessment of nutritional state. The first indication of a decline in nutritional state would become detectable following the onset of malnutrition, associated with a severe and rapid decline in fat mass, and subsequent weight loss. It is therefore important to monitor changes in body composition from diagnosis to enable earlier intervention and delay the decline of nutritional state for as long as possible.

2. Ensure energy intake is appropriate for the individual's energy expenditure

Nutritional state declines due to a prolonged negative energy imbalance caused by an inadequate energy intake that does not meet energy demand. Nutritional intake and energy demand therefore need to be accurately and reliably assessed in these individuals. Biochemical analytes were used in this study to evaluate the reliability of 24-hour dietary recall using Intake24. It was hypothesised that serum biochemical markers of nutritional status would be affected by physiological characteristics of MND, such as metabolism, muscle catabolism and inflammation, whilst biomarkers of dietary exposure would provide accurate measures of dietary intake, prior to a decline in nutritional state and subsequent weight loss.

Neither the metabolic nor inflammatory state were observed to directly influence the concentration of the biochemical analytes. A pro-inflammatory state was associated with a significant reduction in serum creatinine levels; however, this is thought to be linked to the influence of inflammation on muscle catabolism. It can therefore be concluded that concentrations of serum biochemical analytes measured in this cohort are reflective of

nutritional state. Regardless of the average weight loss of approximately 5% from premorbid, 24-hour urinary potassium was observed to significantly positively correlate with potassium intake, demonstrating the potential suitability of this analyte to be used as a marker of potassium intake.

3. Hypermetabolism indicated using predictive energy equations does not reflect energy demand.

Calculation of the metabolic index by comparing measured resting energy expenditure against predictions of resting energy expenditure does not truly reflect an increased energy demand. Moreover, defining whether an individual is hypermetabolic not add clinical value in the dietetic care for people living with MND, as it does not provide a defined, accurate target for adequate energy consumption. Instead, energy expenditure should be regularly measured using indirect calorimetry.

10.6.1 Potential clinical application and impact of this research

The fifth and final objective for this PhD thesis was to propose the most suitable tool, or combination of tools, to assess nutritional status in MND. For these results to have a meaningful clinical benefit, it was hoped that an observed decrease in the concentration of a specific or collection of biochemical analyte(s) would highlight a decline in specific or generalised nutrient intake. This would indicate the need for a personalised nutritional intervention to rebalance the nutritional state, *prior* to the onset of irreversible malnutrition and weight loss (Figure 1.2). Unfortunately, the relationship between nutritional intake and biochemical analytes is inconclusive, and further research is needed to fully understand these relationships.

This study has demonstrated that anthropometric measurements of the upper arm and lower leg reliably reflect fat and fat free mass, which can be applied clinically to monitor changes in disease-related muscle atrophy and malnutrition-associated fat loss. These measurements are quick, cheap and non-invasive, and can be easily conducted in the clinic to provide an immediate indication of nutritional decline due to a negative energy balance. Whilst changes in body composition will indicate changes in nutritional state, a prolonged period of energy

deficit needs to occur before this is detectable. It is therefore important to ensure patients have an energy consumption appropriate to their individual total daily energy expenditure to prevent an energy deficit.

This study has shown that total daily energy expenditure can be reliably predicted using the Kasarskis Model 6 predictive energy equation, which is easily estimated in the clinic using the patient's age, weight, height and a brief assessment of function using a subset of the ALSFRS-R questionnaire (speech, handwriting, self-care, walking and breathing) [315]. However, this is still reliant on being able to record accurate weight measurements and collect reliable indications of disease severity. As resting energy expenditure comprises a high majority of total daily energy expenditure, resting energy expenditure should be regularly measured alongside predictions of TDEE.

This study has identified large and inconsistent variations within the resting energy expenditure of people living with MND over a nine-month period, the underlying causes of which requires further investigation. This highlights the importance of continuously measuring the energy demands of people living with MND to ensure adequate and appropriate nutritional care. However, it is not feasible to use indirect calorimetry in a clinical setting. This indicates the need for an alternative method to measure energy expenditure that could be applied in the clinic. Going forward and building on these exciting, novel findings, the aim is to investigate whether changes in 'whole-body' resting energy expenditure is reflected at the cellular level, and subsequently to identify the drivers behind these metabolic changes. The ability to take a blood sample to assess caloric intake, resting energy expenditure and fuel utilisation preferences would enable rapid, personalised and targeted nutritional care that could be routinely assessed and modified as appropriate. An overview of this research proposal for taking this work forward is detailed in **section 11.2.**

11 Taking this work forward

11.1 Improving the assessment of body composition

Measurements of TSF and AMA are crude proxy measurements for upper arm body composition. Whilst these measurements have been found to reflect weight and BMI in this study cohort, they require validation by comparison against advanced technology before they can be recommended for use in a clinical setting. In addition, these measurements do not indicate the respective contributions to the proportion of body weight (e.g., TSF is measured in mm, which does not indicate the proportion of fat mass per kg of total body weight).

Nau et al., (1995) demonstrated that a small increase in fat mass is more than adequate compensation for the energy lost in muscle mass [137]. Body composition data expressed as fat or fat free mass (kg) could be used to investigate the caloric contributions of lean mass, fat mass and total body mass. Longitudinal changes in body composition could therefore provide an estimate for the change in energy stored with disease progression. Assessment of body composition using whole body air displacement plethysmography, for example, would enable this investigation.

11.1.1 Whole body air displacement plethysmography

As stated in **section 10.5**, the suitability of body composition assessment using TSF, AMA and mid upper arm and calf circumference measurements requires validation using advanced technology**.** Inclusion of body composition assessment by whole body air displacement plethysmography (ADP) (described in **section 2.4.2)** was therefore included as a substantial amendment towards the end of this study. ADP was conducted in this study population using the Cosmed BodPod (Cosmed USA, Inc) system [348] (Figure 11.1). The BodPod ADP assessment is conducted in a seated position, making it a feasible, practical and safe assessment of body composition for participants who can transfer from chair to chair.

Figure 11.1 The BodPod system (Cosmed).

11.1.2 Size Stream full-body 3D scanner

A Size Stream SS20 Booth Scanner, shown in Figure 11.2, uses 20 infrared depth lasers and sensors to rapidly and non-invasively calculate 240 body measurements in six seconds. Measurements are recorded in triplicate. These measurements combine to form a digital 3D image of the individual in two minutes, providing body circumferences measurements with an accuracy of ± 5 mm. Measurements are conducted in a standing position, as shown in Figure 11.3, therefore limiting use of this scanner to participants who can stand unaided.

Figure 11.2 The Size Stream SS20 Booth Scanner.

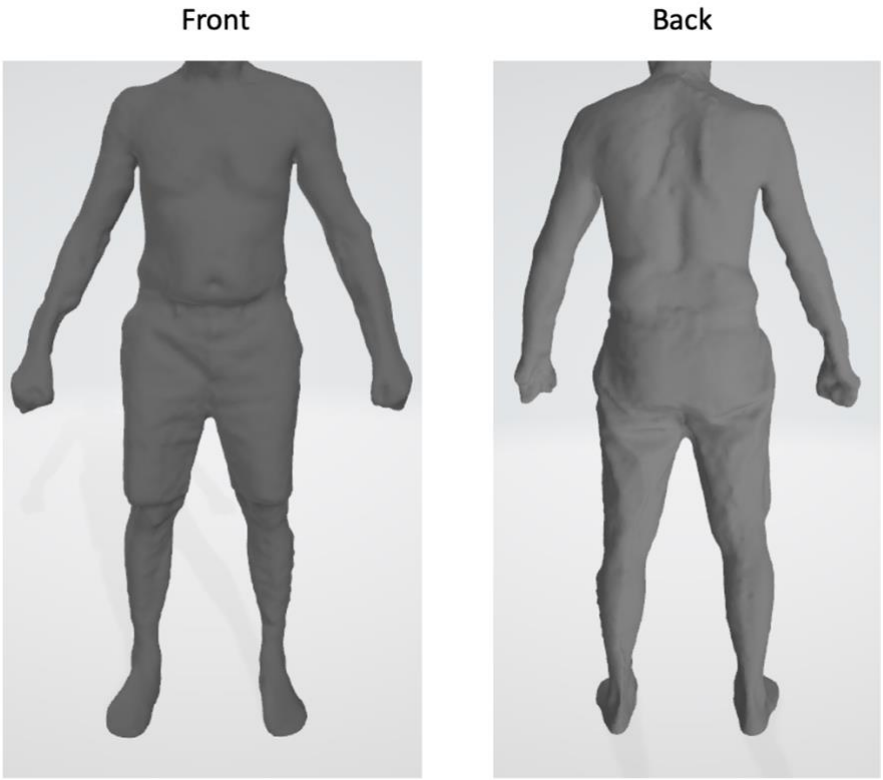

Figure 11.3 Screenshots from the 3D size scanner. Left: front; right: back. Images cropped below the head to protect participant identity.

11.1.3 Using peripheral blood mononuclear cells to understand the energy demands of people living with MND

Peripheral blood mononuclear cells (PBMCs) are a group of immune cells including monocytes and lymphocytes which reflect dietary intake [349] and metabolism [350], as well as mimic the gene expression of skeletal muscle [351]. They have therefore been suggested as a minimally invasive and rapid model to examine nutrigenomics [352]. To provide proof of concept for the measurement of metabolic activity in PBMCs, venepuncture was conducted on healthy participants as part of the preliminary feasibility study (described in **section 4.1**). PBMCs were isolated from whole blood using Ficoll-density gradient centrifugation (methodology described in **section 4.3.4.1.3**).

11.1.3.1 Sirtuin 1

Sirtuin1 (SIRT1) is described as a master metabolic regulator which can adapt transcriptional processes involved in mitochondrial function and metabolic homeostasis [353–355]. Sirtuin activity is directly influenced by the availability of nutrients, whereby caloric restriction increases SIRT1 activity [356]. SIRT1 was therefore proposed as an objective measure of caloric intake or resting energy expenditure in people living with MND. PBMCs isolated from five healthy participants were used to develop a fractionation protocol to measure SIRT1 in the cytoplasm and nucleus of PBMCs using western blotting (**Appendix J**).

11.1.3.2 Metabolic profiling of PBMCs

This work was conducted in collaboration with Dr Scott Allen. Isolated PBMCs from four healthy participants were analysed using phenotypic metabolic screening using a Biolog OmniLog™ bioanalyser [357,358] to measure the uptake and metabolism of a variety of energy substrates. The metabolic profile of the PBMCs was comparable in all four participants, whereby a preferential metabolism of carbohydrate energy sources was observed (**Appendix K, Figure** 13.2).

11.2 Post-Doctoral project outline: understanding longitudinal changes in energy expenditure in MND

11.2.1 Aim and objectives:

To understand whether whole-body energy expenditure is reflected at the cellular level, and to identify the physiological or cellular mechanisms which may influence energy expenditure in MND.

Objectives:

1. To measure resting energy expenditure to assess whole-body metabolism and macro-nutrient oxidation:

Measurements of resting energy expenditure will be collected using indirect calorimetry (GEMNutrition metabolic cart) [308]. Resting energy expenditure - together with reported energy intake using 24-hour dietary recalls (Intake24 [359–361]) and physical activity levels (accelerometers) - will be used to derive total daily energy expenditure (TDEE). (Figure 11.4, pathway 1).

2. To measure cellular metabolic activity and fuel utilisation preferences in PBMCs:

Venepuncture will be collected at the time of indirect calorimetry. Cellular metabolic activity and fuel utilisation preferences will be measured in PBMCs by: i) measuring the expression of SIRT1 using western blotting; ii) phenotypically characterising the energy metabolism pathways using Biolog Phenotype MicroArrays; and iii) measurement of mitochondrial fuel usage in live PBMCs using the Agilent Seahorse XF Mito Fuel Flex Test [362] (Figure 11.4, pathway 2).

3. To assess the influence of body composition and reported energy intake on whole-body energy expenditure and macronutrient oxidation as well as cellular substrate oxidation:

Measured resting energy expenditure will be normalised against indirect measures of body composition using whole-body air displacement plethysmography (Cosmed BODPOD) [363] and bioimpendence spectroscopy. Reported dietary intake information will be collected for the

day prior to REE measurement and venepuncture conduction using Intake24 [359–361]. Estimates of total energy and macronutrient intake will be correlated against measured resting energy expenditure, respiratory quotient, cellular metabolic profiles and mitochondrial fuel usage (Figure 11.4, pathway 3).

11.2.2 Study design

This study will be a case-control, prospective longitudinal study, involving 10 people living with MND and five age- and sex- matched healthy controls (with no known metabolically altering condition).

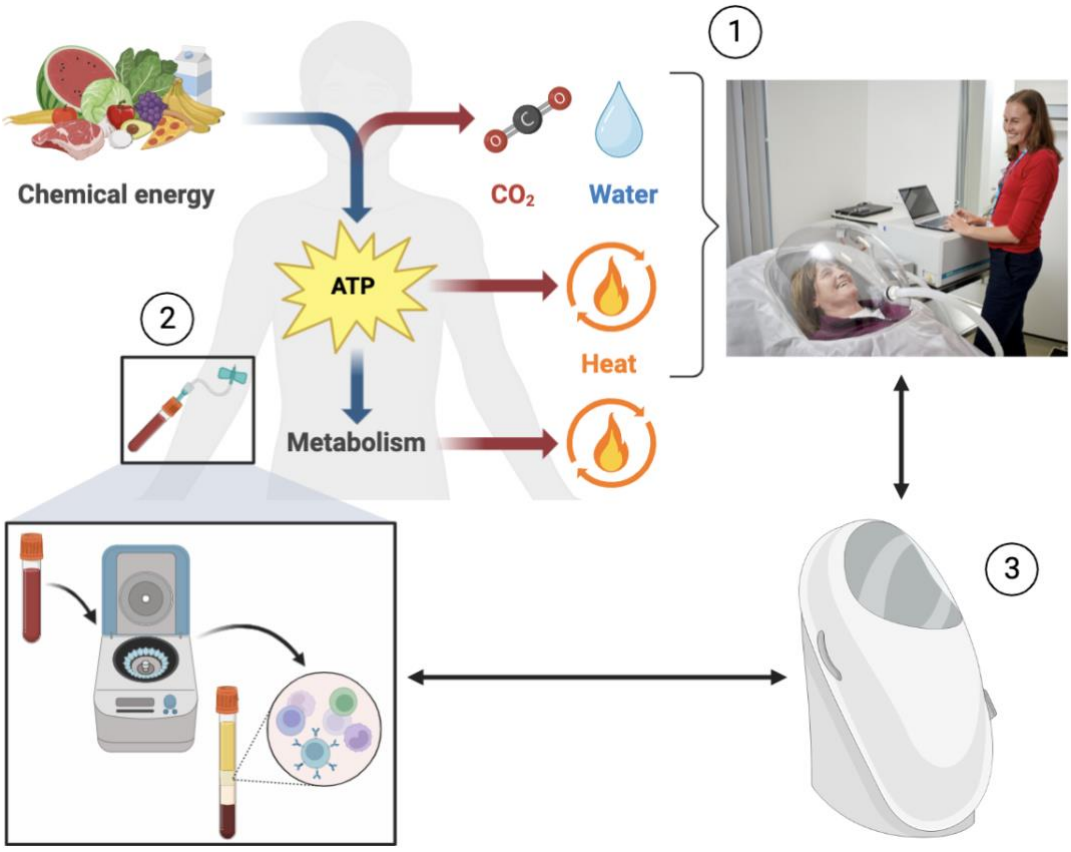

Figure 11.4 A schematic to show the postdoc study objectives. 1) Measurement of resting energy expenditure using indirect calorimetry. 2) Venepuncture and measurement of PBMC metabolic activity and fuel utilisation preferences. 3) Measurement of body composition using the Cosmed BODPOD. Created using BioRender.com

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13 Appendices

Appendix A.

Feasibility Study

This appendix describes the initial feasibility study that was first conducted using biosamples provided by healthy participants to enable the development and optimisation of clinical and laboratory protocols.

13.1 Feasibility study design

This was a single centre, observational cross-sectional study, which took place within the University of Sheffield between June 2020 and September 2021.

13.2 Ethical and Regulatory Considerations

Favourable opinion was sought and granted by the University of Sheffield Research Ethics Committee (UREC) (reference number: 032854). Three amendments were requested and approved by UREC. The final approved study protocol can be found in **Appendix B.**

13.3 Recruitment

The feasibility study was advertised by posters and emails within the Department of Neuroscience, University of Sheffield. Individuals interested in participating in the study approached the author of this thesis directly to request further information about the study. The participant information sheet (PIS) **(Appendix C)** - detailing no less than: the exact nature of the study; what it involved for the participant; the implications and constraints of the protocol and any risks involved in taking part - was provided via email to all eligible individuals who expressed an interest in participating in this study. Eligibility criteria was broadly specified as an individual >18 years of age, who was willing and capable to provide informed consent.

Each participant personally signed and dated the latest approved version of the informed consent form (ICF) **(Appendix D)** before any study-specific procedures were performed. The ICF was also dated and counter-signed by the thesis author.

13.4 Biosample collection and handling

Participants had the option to provide blood samples and/or a 24-hour urinary collection. All participants were free to choose to provide whichever sample type(s) they felt comfortable providing. The protocols for biosample processing are described in **section 4.3.4.**

All sample processing and analysis was conducted within UoS premises. Biosamples were processed and handled according to laboratory standard operating procedures already in place in the laboratory, as well as control of substances hazardous to health (CoSHH) and material safety data sheets (MSDS) specific to the biosamples and experimental substrates being used, as per the Departmental policies and standards. Collected biosamples were rendered acellular by centrifugation immediately upon collection, as per the Human Tissue Act 2004, before being aliquoted and stored in a -80^{o}C freezer within the UoS. All data derived from sample analysis was used by the research team in the development of experimental protocols for the subsequent longitudinal nutritional study in people living with motor neuron disease.

13.5 Results

Seventeen healthy participants were recruited to this feasibility study. All participants donated blood samples and five donated 24-hour urinary collections.

13.5.1 Micro-Kjeldahl: total urinary nitrogen

The developed Micro-Kjeldahl protocol is described in **section 4.3.4.4.**

Three technical repeats were conducted on three consecutive days using different aliquots of the same 24hr urinary sample from each participant (Figure 13.1). The high statistical significance demonstrates MK is a sensitive and accurate method to determine small variations in total urinary nitrogen (TUN) from 24-hour urinary samples of different participants. The inter-assay coefficient of variation values of < 3.2% demonstrate the

consistency and reproducibility of these results. This suggested that it would be possible to reliably and sensitively detect small changes in total urinary nitrogen in 24-hour urinary samples, in the subsequent MND cohort study. The TUN results are presented in Chapter 7, **section 7.1.4.**

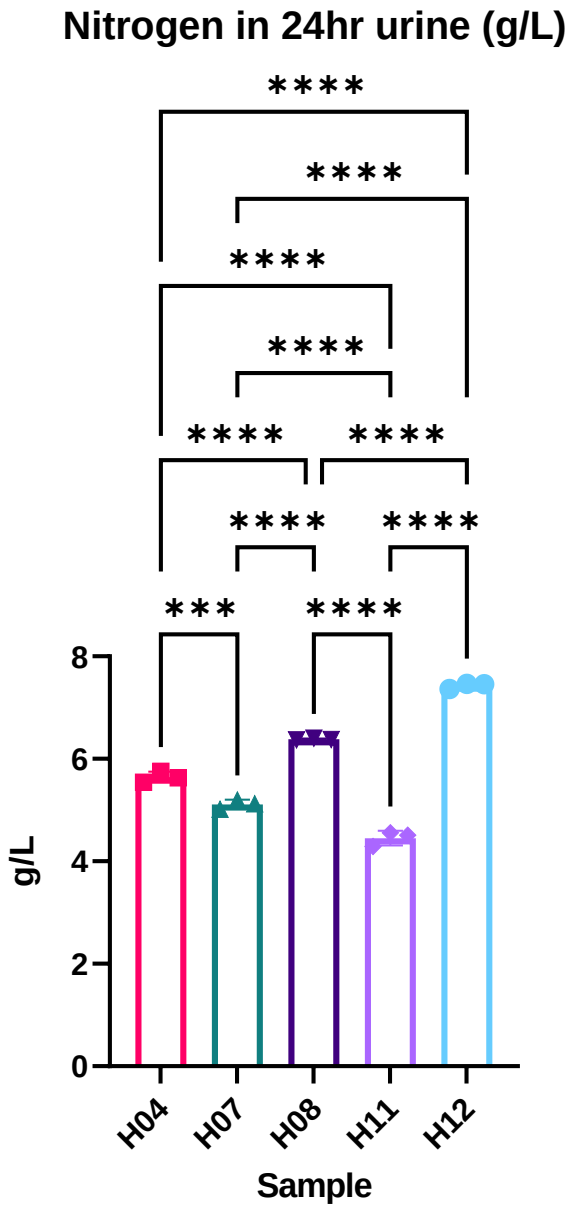


Figure 13.1 Total urinary nitrogen measured in 24-hour urinary samples from healthy participants (g/L) (n=3). Data is presented as mean ± SD. Data analysed using one-way ANOVA with Tukey's multiple comparison test. significance at $p < 0.05$. H = healthy participant.

Appendix B.

Feasibility Study: Participant Information Sheet

Optimisation of biosample handling and protocol development for the analysis of nutritional biomarkers

We would like to invite you to take part in our research study led and co-ordinated by a team at the Sheffield Institute for Translational Neuroscience (SITraN), at the University of Sheffield (UoS). Before you decide if you would like to take part, it is important that you understand why this research is being done and what it would involve for you. Please take time to read the following information carefully and discuss it with others if you wish. One of our research team will go through the information sheet with you and answer any questions you have. If, after reading this information sheet, you have any further questions, please do not hesitate to contact a member of the research team using the contact details at the end of this sheet. Thank you for reading this.

What is the purpose of this study?

Motor neuron disease (MND) is a devastating terminal illness, causing a continuing weakness of muscles responsible for movement, swallowing, and breathing. People living with this condition often have problems with their nutrition, especially those experiencing eating and drinking difficulties.

The best way to look after people is to understand the effect of these problems on their bodies and make sure they take sufficient nutrients and calories in. We can do this mainly by measuring people's weight frequently or asking them to tell us what and how much they have had to eat or drink over a specific period of time. These methods may be practical but they are not as reliable as we would want them to be.

There is a need to explore other ways to help us observe the nutrition of people living with MND. A potential way is by doing lab tests using samples of blood and/or urine to examine certain markers which can inform us about people's nutrition. However, before we actually involve people living with the condition, we are asking healthy volunteers to donate a one-off sample of blood and/or urine. We will use this process to learn more about the practicalities around collecting and keeping the samples as well as to fine-tune the lab tests to identify the markers we are interested in.

Why have I been invited?

We are aiming to collect blood and/or urinary samples from up to 20 healthy volunteers. We are contacting members of staff and students on the UoS 'Volunteers' list, who have specifically opted-in to receive emails requesting volunteers to support with UoS research

projects. You may have also seen this study advertised on generally accessible UoS web pages, social media, as well as on posters placed around the UoS.

Do I have to take part?

No. It is entirely up to you to decide whether or not to take part. We would like to encourage you to read this information sheet in your own time, discuss it with others, and consider whether you would like to participate in the study. When you have decided, we would be grateful if you could let us know by responding to this email. If you do decide to take part, you will be given this information sheet to keep, and asked to sign an informed consent form.

If you contact us indicating that you do not wish to participate, we will not contact you again about this study.

What does my participation involve?

If you contact us to express an interest in participating, a member of the study team will speak to you (in person or over the telephone) about what the study involves. If you would like to take part, you will be invited to attend The Royal Hallamshire Hospital to discuss the study in full with a member of the study team.

If you provide written consent to take part, we will then ask you to donate a sample. You can choose which sample type(s) you are happy to donate. You are free to donate whichever samples you feel comfortable providing.

- Blood sample

A trained phlebotomist (e.g., a research nurse) will collect a small blood sample (approximately 75 ml, the equivalent of around 12 teaspoons), usually from a vein in your arm.

- Urine sample

You will be asked to provide one mid-stream urine sample.

- 24-hour urinary sample

All urine from one 24-hour period will be collected; the first 'void' of the day will be discarded, and all subsequent voids for the next 24 hours will be collected.

What are the potential disadvantages and risks of taking part?

Due to the minimally invasive procedures involved, we do not anticipate that taking part poses any physical risk. Members of our research team will be available to offer any support you may need.

Blood samples:

There are small risks associated with needle injections. For most people, needle injections do not cause serious problems, however some people experience a small amount of swelling, bleeding or pain at the needle site or some people may feel faint. There may be minor temporary bruising at the site of removal. There is always a theoretical risk of introducing

infection into the skin, blood or spinal fluid with any invasive procedure, but in practice the use of sterile single-use equipment by trained staff makes this risk extremely small.

What are the potential benefits of taking part?

Whilst there are no benefits for those people participating in the project, we aim to use samples from healthy volunteers to explore and assess the practicalities of biosample collection, as well as to adapt, develop and optimise clinical and experimental protocols and procedures. This includes biosample handling, storage and experimental analysis. The results of this work will inform the design and undertaking of a large scale clinical study with people living with MND. This will enhance the chances of successfully identifying and evaluating nutritional biomarkers, and subsequently the improvement of nutritional care, in people living with MND.

Expenses and payments

Unfortunately, we will not be able to pay you for participating in the study.

Will my taking part in the study be kept confidential?

All samples will be labelled alphanumerically (e.g. SHF B H01) with no reference to your identification.

What will happen to the samples and data I give?

The sample(s) that you give will be used solely for the purpose of this research project, and will be considered as a gift. Samples will be labelled with the study code, date and sample code only and it will not be possible to identify who you are from your samples.

Where possible, all sample analysis will be conducted within UoS premises. However, we may send anonymised samples to external collaborators to help us develop our experimental protocols strictly for this study. No details that could identify participants would ever be sent with the samples. All data derived from sample analysis will be used by the UoS research team in the development of experimental protocols for a future nutritional study in people living with motor neuron disease.

What if I want to withdraw from the study?

It is important to stress that if you do agree to participate, you will be free to withdraw at any time and without giving any reason, up until the point of sample collection. Please note, that once your sample has been collected, anonymised and included within a data set, your data cannot be removed from the study beyond this point.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have a concern about any aspect of this study, you

should ask to speak with the researchers who will do their best to answer your questions. If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Sarah Roscoe in the first instance (contact details are given at the end of this information sheet). Alternatively, complaints can be made to the Principal Investigator of the study, Dr Haris Stavroulakis. If something does go wrong and you are harmed during the research, and this is due to someone's negligence, then you may have grounds for legal action for compensation against the funder of the study, but you may have to pay your own legal costs.

Who is organising and funding the study?

This research is being funded by SITraN, University of Sheffield.

Who has reviewed the study?

This study has been reviewed by the University of Sheffield Research Ethics Committee.

Further information

Sarah Roscoe: saroscoe1@sheffield.ac.uk

Haris Stavroulakis: t.stavroulakis@sheffield.ac.uk

Thank you for reading this information sheet and considering taking part in this study.
If you decide to take part, you will be given a copy of this information sheet and the signed consent form if you wish to take home with you.

Appendix C.

Feasibility Study: Informed Consent Form

Optimisation of biosample handling and protocol development for the analysis of nutritional biomarkers

Participants should complete the entirety of this consent form themselves:

Please initial the appropriate boxes	Initials
Taking Part in the Project	
I have read the participant information sheet [Version 2.0, 27/5/2020] for the above study, and I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my taking part is voluntary and that I can withdraw from the study at any time until the point of sample collection. I do not have to give any reasons for why I no longer want to take part and there will be no adverse consequences if I choose to withdraw.	
I understand that taking part in the project will involve the collection of urinary and/or blood samples, and once my sample has been collected, anonymised and included within a data set, it cannot be removed from the study beyond this point.	
I agree to take part in the study.	
How my information will be used during and after the project	
I understand that my participation in this study and all the information/samples I give will be anonymised and remain confidential.	
I consent to the storage and processing of data and samples obtained during this study as described in the participant information sheet. I consider these samples a gift to the University of Sheffield, and I understand I will not gain any direct personal or financial benefit from this.	
I understand and consent for my anonymised samples or data collected during the study to potentially be used in future research, analysis or publications.	

PARTICIPANT	
Signature:	
Print Name:	
Date:	

NB The participant must date his/her own signature

INVESTIGATOR:	I have explained the above study to the participant and obtained consent	
Signature:		
Print Name:		Date:

Project contact details for further information:

Sarah Roscoe: saroscoe1@sheffield.ac.uk

Dr Haris Stavroulakis: t.stavroulakis@sheffield.ac.uk

Save 2 copies of the consent form: 1 copy for the participant, 1 copy for the researcher

Appendix D.

Nutritional biomarkers in MND: Participant invitation letter

<Address>

<Telephone>

<Email>

<Patient name>

<Patient address>

Dear <insert patient name>

An evaluation of the role of nutritional biomarkers as indicators of nutritional status in people living with motor neuron disease: a pilot study

(Nutritional biomarkers in MND)

We would like to invite you to take part in a new research study, called "**Nutritional biomarkers in MND**", which is led by a team at the Sheffield Institute for Translational Neuroscience (SITraN), at the University of Sheffield and Sponsored by the Sheffield Teaching Hospitals NHS Foundation Trust.

The aim of the research study is to optimise the way we monitor the nutritional status of an individual living with MND and for this we are inviting people attending the Sheffield MND Research and Care Centre to take part. We have enclosed a copy of the participant information sheet and would be grateful if you would read it and consider whether you would wish to take part.

If after reading the information sheet you think you might be interested in taking part you can contact Sarah Roscoe using the contact details at the top of this letter, who would be happy to fully discuss the study with you and answer any questions. Alternatively, you can return the enclosed reply slip in the enclosed <stamped or freepost> addressed envelope. A member of our research study team will then contact you and arrange an appointment to discuss the study further.

If you are experiencing communication difficulties, please advise us on the response slip when you are most likely to have a carer/family member/friend with you, who will be able to assist your communication with us.

You do not have to decide whether to take part in the study until after you have discussed the study with a member of the research team, and have had all your questions answered. If we do not hear from you for two weeks after receipt of this letter, we may contact you once to ask you whether you would be interested in taking part in the study. If you do not wish to take part, we will not contact you again for this study.

Thank you very much for your valuable help with this research. Please contact Sarah Roscoe via email on <insert email> if you have any questions about the study.

Yours sincerely,

Dr Haris Stavroulakis

Appendix E.

Nutritional biomarkers in MND: Participant Information Sheet

An evaluation of the role of nutritional biomarkers as indicators of nutritional status in people living with motor neuron disease: a pilot study

(Nutritional biomarkers in MND)

Study reference: 21/PR/0092 / STH21332
Principal Investigator: Dr Haris Stavroulakis

Participant Information Sheet

We would like to invite you to take part in our research study, which is led by a team at the Sheffield Institute for Translational Neuroscience (SITraN), at the University of Sheffield. Before you decide if you would like to take part, it is important that you understand why this research is being done and what it would involve for you. Please take time to read the following information carefully. One of our team will go through the information sheet with you and answer any questions you have. If, after reading this information sheet, you have any further questions, please do not hesitate to contact a member of the research team using the contact details at the end of this sheet.

What is the purpose of the study?

Motor neuron disease (MND) is an illness which causes progressive weakness of muscles controlling movement, swallowing and breathing. People living with MND are at risk of malnutrition, a serious condition that happens when people do not get the right amount of nutrients, often followed by weight loss. This is a problem, because malnutrition and subsequent weight loss is linked to increased disability and shortened life expectancy.

There are many reasons why people living with MND are at risk of malnutrition. Most are associated with MND symptoms, making activities such as cooking, bringing food to mouth, chewing and swallowing increasingly difficult. As a result, people living with MND eat and drink less than they actually need to. Another important reason is that some people living with MND may also experience changes in their metabolism (a complicated chemical process inside the body that changes food into energy), whereby they use more energy than normal when at rest.

Monitoring of nutritional status is important to understand if people are malnourished, or at risk of malnutrition. In clinical practice, this is most commonly done by measuring people's body composition (for example, their weight). However, a simple weight measurement, although useful, does not provide a comprehensive view of nutritional status and may be difficult to obtain, particularly in people with advanced MND.

There is a need to come up with ways to better understand the nutritional status of an individual living with MND. We plan to do this by looking at markers in the blood and urine, and see whether levels of these markers match up with that people are telling us they are eating and drinking, and how well these markers compare to the usual measurements taken at check-up appointments, such as weight.

Why have I been invited to participate?

We have tried to keep this study as inclusive as possible; therefore, by being over the age of 18, with a confirmed diagnosis of MND and the ability to provide consent, you have been invited to take part in this study.

Do I have to take part?

No, it is entirely up to you to decide whether you would like to take part. If you decide you would like to help us with this study, you will be given an information sheet to keep and be asked to sign a consent form. You remain free to withdraw at any time, without giving reason and this will not affect your usual clinical care.

What will happen to me if I take part?

If, after reading this information sheet, you are interested in taking part in this study, a member of the study team will discuss your involvement in the study with you, and answer any questions you have. If you decide to take part, we will invite you to provide written consent, either in person, or remotely.

If you provide consent to take part, participation in the study will last for up to 9 months. A member of the research team will collect some information on up to four occasions, at approximately three-month intervals.

The study visits will be conducted at the Advanced Wellbeing Research Centre, a state-of-the-art facility, specially designed for the conduct of high quality health research. The Advanced Wellbeing Research Centre is based at the Olympic Legacy Park in Sheffield City Centre and is easily accessible by public transport and by car (including access to a free visitor car park). Where practical, assessments will be collected remotely.

At each visit, we will ask you to provide the following samples and measurements:

1) Blood sample: A trained phlebotomist (e.g., a research nurse) will collect a small blood sample (up to 75 ml, the equivalent of around 12 teaspoons), usually from a vein in your arm.
2) 24-hour urinary sample: You will collect all urine from one 24-hour period; you will discard your first urinary sample of the day, and collect all following samples for the next 24 hours. You will be provided with all necessary equipment when you provide consent to take part in the study. We acknowledge this is a time-consuming process for both yourself, and your carer(s). Please discuss this with your carer(s) before agreeing to take part in the study. If your carer(s) has any questions relating to the 24-hour urinary collection, we are more than happy to discuss this with them (contact details are at the end of this document).

We will use blood and 24-hour urinary samples to measure a range of nutritional markers, which will indicate what you have eaten and drunk in the past 24 hours. We will compare the results of these markers, with what you have told us you have consumed in your 24-hour food recall diary (see below), and how well these markers compare to the usual measurements taken at check-up appointments, such as weight. We will also measure markers of inflammation and muscle breakdown from the serum and plasma.

3) Indirect calorimetry: In order to investigate the influence of metabolism on the levels of nutritional markers in the blood and urine, we need to identify individuals who are hypermetabolic. We will do this by measuring how much energy you use at rest, using a device called an indirect calorimeter. This will involve lying down (figure 1A) (or remaining seated when this is not possible (figure 1B) under a see-through hood for around 20 minutes, whilst breathing normally and keeping very still. This will measure the amount of oxygen and carbon dioxide you exhale whilst you are resting, as well as the amount of macronutrients (e.g., carbohydrates, proteins, fats) you are burning, whilst at rest. In future applications, this will allow us to tailor an individual's nutritional intake to include more of whichever food group they need to consume more of, to remain in an energy balance.

A)  B) 

Figure 1 - Images of individuals lying down (A) and sitting upright (B) in the indirect calorimeter.

4) 24-hour food diary

In order to understand the amount of food and fluid you consume, we will ask you to complete an online food diary to record all food and fluid consumed within a 24-hour period. We will use an online tool, called Intake24, which you will complete a day prior to your routine clinical appointment, from your own home. For example, if your routine clinical appointment is on a Friday, we will ask you to complete the 24-hour food diary on the Thursday evening. This software will take you through each meal in chronological order, with prompts and diagrams to help you provide as much information and detail as possible (as shown in figure 2). This will take approximately 20 minutes to complete in one go, however you may add information throughout the day. You will receive guidance on how to complete the online recall diary when you agree to participate in the study.

No personal information will ever be stored on this system, and only the research team will be able to link this information to you or your donated samples. More information regarding privacy can be found here: https://intake24.co.uk/info/privacy#content.

If you are unable to access a computer or mobile device, or you would prefer to complete a paper copy, you will be able to complete a recall diary with us in the clinic, when you come to provide samples and measurements.

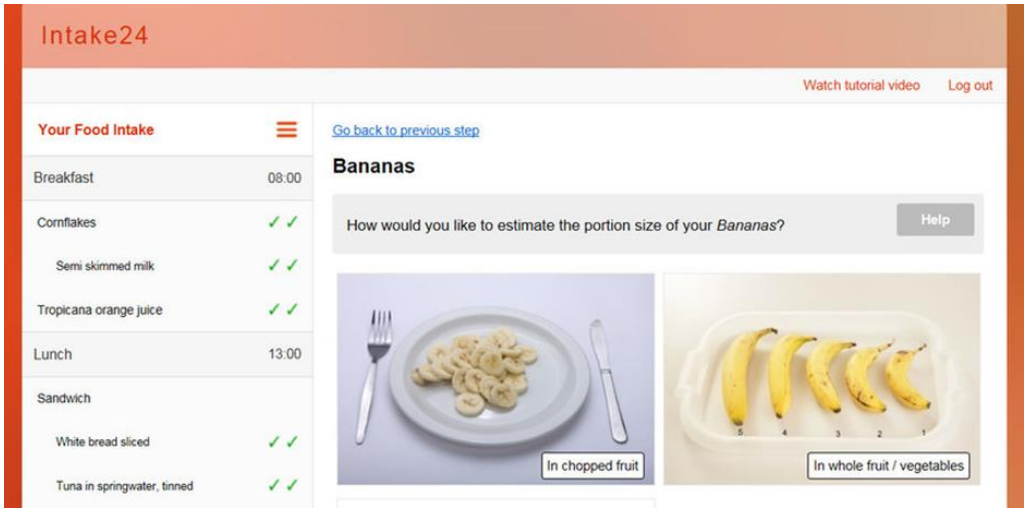
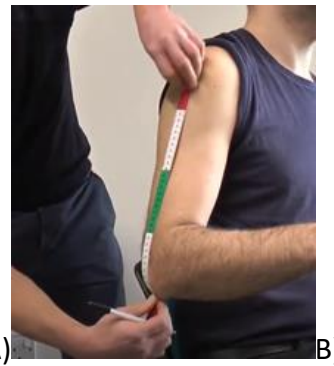


Figure 2 - An example of the Intake24 questionnaire.

5) Revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R): this is a questionnaire used to assess changes in physical function and monitor the progress of the condition in people living with MND. This is usually done as part of your routine clinical appointments. However, in the absence of this for any reason, we will ask you to complete this questionnaire at home. The research team will be able to help you with this over the phone if you have any problems. The ALSFRS-R will be available in both electronic and paper versions.

6) Anthropometry measurements: We will ask your permission to access relevant sections of your medical records whilst the study is ongoing, to collect information about your current health. In addition, we will ask to measure the length, circumference and skin thickness of your upper arm, using a tape measure and a skinfold calliper, as shown in figure 3 below.

A)  B)  C) 

D) 

Figure 3 - Pictures showing how we will measure A) the length, B) the circumference, C) a skin calliper and D) the thickness of your upper arm.

Body composition measurements

The following section details two additional, optional assessments that can be conducted at the end of your study visit if you decide you would like to complete them. These additional assessments are called 3D body scanning and whole body air displacement plethysmography (BODPOD). Details of both assessments are given below (numbers 7 and 8).

These procedures involve measurements of external body shape and composition whilst maintaining pre-defined postures (e.g., standing straight, bending arms and/or legs slightly). All test procedures will be conducted in a private, enclosed laboratory space. You will be asked to change into specific clothing, including close-fitting shorts and a swimming cap. You will also be required to remove your shirt (excluding bras or crop tops) and any jewellery, watches or other accessories throughout the session. Private changing facilities are provided. You will need to be able to change clothing by yourself, or with the support of a family member or friend; the research team will be unable to help you with this. It is highly recommended that you bring your own jacket, robe or bath towel to keep you warm between each test.

7) 3D body scanning: A Size Stream full-body 3D scanning device will be used to collect 3D images of your external body shape. The 3D scanner contains 20 separate 'depth cameras', information from these cameras is combined to form the final 3D scan. Each camera uses only visible and infra-red light and is completely harmless. From your 3D scan, we will extract a range of body size and shape measures. During the 3D body scanning procedure, you will be asked to adopt different positions (e.g., standing straight, bending arms and/or legs slightly). You will also be asked to hold your breath during the scanning procedure for approximately 10 seconds. You might be asked to complete 2-3 separate body scans, meaning the entire 3D body scanning assessment will take a maximum of 5 minutes to complete. Figure 4 is a picture of the 3D body scanner.

Figure 4 – The 3D body scanner.

8) Whole body air displacement plethysmography (BODPOD): Whole body air displacement plethysmography will then be performed using a machine called a BODPOD. The BODPOD is shown in figure 5 below. The BODPOD is the best body composition measurement system that is available for us to use. We are doing this to determine body composition (body fat and fat-free mass). We will need to take two repeat measures, lasting for 50 seconds each. On occasion, we might need to take a third measurement. A full test takes approximately 5 minutes. You will be asked to sit in a confined space and will experience pressure changes (felt within the ears) during measurement, the procedure is completely painless. If you are uncomfortable sitting within confined spaces you may wish to skip this measurement.

Figure 5 – The BODPOD.

What are the possible benefits of taking part?

This study is described as 'observational' and is not designed to provide a treatment or intervention to participants directly. In this instance, we are observing the level of certain markers in your blood and urine, trying to understand what this means for your nutrition by comparing it to more traditional measurements, such as weight. We will be unable to share with you some information e.g., what specific blood and urine marker results mean for your nutritional status, as part of the research is to understand the meaning of these results. However, we will be able to inform you, if you wish, about other information that we collect from you e.g., whether you have gained, maintained or lost weight between the study visits.

How will this research change the future care of people living with MND?

By assessing the role of nutritional biomarkers, we aim to increase our understanding of the nutritional status of people living with MND. The regular assessment of nutritional biomarkers, by collecting blood and urine at routine appointments, may allow the early identification of changes in dietary intake or patterns, predicting a decline in nutritional status before irreversible malnutrition or weight loss. We hope this will ensure that people living with MND receive timely and appropriate nutritional care, to slow the progression of the disease and maintain a better quality of life for longer. This intervention is not one size fits all, and will be specific to each individual.

Expenses and payments:

We will reimburse any travel expenses you have incurred, to a maximum of £20. Please discuss with the research team for further details.

What are the possible disadvantages and risks of taking part?

Due to the observational nature of the study, we do not anticipate that taking part poses any physical risk. Very occasionally answering questions about the impact of MND can cause upset. Dr Stavroulakis and the other members of our research team will be available to offer any support you may need.

<u>Blood samples:</u>

There are small risks associated with having a blood test. For most people, inserting a needle to take a blood sample does not cause a problem, however some people may experience a small amount of swelling, bruising, bleeding or pain at the insertion site or some people may feel faint. On very rare occasions infection may occur.

In the unlikely event of an incident requiring medical attention whilst you are at the Advanced Wellbeing Research Centre (e.g., if you feel unwell during the study visit) the research team and the security/reception of the building will coordinate calling an ambulance.

What will happen if I don't want to carry on with the study?

You will be free to withdraw at any time and without giving any reason. If you withdraw from the study, we will keep the information about you and any samples that we have already obtained. Should you decide to withdraw, this will not affect your NHS care either now, or at any point in the future.

What if there is a problem?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, you can contact Sarah Roscoe on saroscoe1@sheffield.ac.uk in the first instance. If you remain unhappy and wish to complain formally, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. Alternatively, you can contact the Research Director, Sheffield Teaching Hospitals NHS Foundation Trust, on telephone 0114 226 5938.

If you are harmed by your participation in this study, there are no special compensation arrangements. Sheffield Teaching Hospitals NHS Foundation Trust and the University of Sheffield will provide indemnity for this study. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Sheffield Teaching Hospitals NHS Foundation Trust or the University of Sheffield, but you may have to pay your legal costs.

How will we use information about you?

We will need to use information from your medical records for this research project. This information will include your name, hospital number and contact details. People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead. You will only be identified on study documentation using a unique study code that will be assigned to you. It will not be possible for anyone to be able to identify you from the study database as all the data will be coded. Only the research study team will be able to link you to the data in the study database.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What will happen to any samples I give?

The sample(s) that you give will be used solely for the purpose of this research project. Samples will be labelled with the study code, date and sample code only and it will not be possible to identify who you are from your samples.

The data and samples collected may then be analysed by the research team and their scientific collaborators as part of this study.

Where possible, all experimental analysis will be conducted by the research team at SITraN. However, your samples may be sent to hospitals, university departments, non-profit institutions, or commercial companies in the UK or worldwide for analysis as part of this study. No details that could identify you would be sent with the samples.

Samples will be destroyed at the end of the study in accordance with the Human Tissue Authority Code of Practice.

What will happen to the results of the research study?

The results of this research may be presented at scientific meetings in the UK and overseas. It will not be possible to identify you from any of the data that will be presented. The data from the study may also be published in a medical journal. You will not be identified in any report or publication.

Who is organising the research?

This research study is a collaboration between researchers at the University Sheffield and Sheffield Teaching Hospitals NHS Foundation Trust.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by The London-Fulham Ethics Committee.

COVID-19 statement

We will adhere to the strict COVID-19 safety protocols implemented by the Sheffield Teaching Hospitals NHS Foundation Trust and the Advanced Wellbeing Research Centre for research participants visiting hospital and research premises. Any samples and measurements that can be collected remotely (i.e., away from the research setting), removing the need for face-to-face contact, will be done so. Where face-to-face research is necessary, approved clinical procedures will be followed. Participants will be given an allotted and agreed time, which they will be asked to adhere to. Appropriate personal protective equipment (PPE) will be worn at all times (i.e., a properly fitting facemask) by both the researcher and the participant (unless exempt). All equipment will be sanitised between

participants. Participants who have consented to take part in the study should notify the research team if they have any symptoms prior to attending a study visit as well as if they have been asked to isolate (members of the research team will do the same).

How will my confidentiality be protected?

During your participation in the study, we will be collecting information about you. All of this information/data will be maintained in a confidential file, and only members of the research team will have access to this. We will enter all the data we collect into a secure and encrypted electronic data collection system (database), but all data will be anonymised prior to this. During the study, members of the lead research team in Sheffield will be accessing the anonymised database for the purposes of data analysis.

All samples will be labelled alphanumerically (e.g. Pt01 P) with no reference to your identification.

After the project is complete, your data will be stored in suitable data storage by the University of Sheffield or the study Sponsor for 5 years.

Sheffield Teaching Hospitals NHS FT (STH NHS FT) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information at

- https://www.sth.nhs.uk/about-us/general-data-protection-regulations.
- https://www.sheffieldclinicalresearch.org/for-patients-public/how-is-your-information-handled-in-research/
- https://www.hra.nhs.uk/information-about-patients
- or by contacting the study team.

If you wish to raise a complaint on how we have handled your personal data, you can contact our Data Protection Officer who will investigate the matter. If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO). Our Data Protection Officer is Peter Wilson and you can contact them by phone 0114 2265153 or email sth.infogov@nhs.net.

STH NHS FT will use your details, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from STH NHS FT

and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The only people in STH NHS FT who will have access to information that identifies you will be people who need to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

After the project is complete, the data will be stored in suitable data storage. None of the data from you will be made available to any persons other than those involved in this research, unless approved by the Ethics Committee. Confidentiality will be safeguarded throughout.

Contact Details:

Further information about this study can be obtained from Sarah Roscoe on saroscoe1@sheffield.ac.uk

If you would like to talk to somebody who is independent from this study, to discuss volunteering in research, please contact the NHS Patient Services Team (Sheffield Teaching Hospitals) on 0114 271 2400 or by emailing sth.pals@nhs.net.

Thank you for reading this information sheet and considering taking part in this study.

If you decide to take part you will be given a copy of this information sheet and the signed consent form to take home with you and keep.

Appendix F.

Nutritional biomarkers in MND: Informed Consent Form

An evaluation of the role of nutritional biomarkers as indicators of nutritional status in people living with motor neuron disease: a pilot study

(Nutritional biomarkers in MND)

Study reference: 21/PR/0092 / STH21332
Principal Investigator: Dr Haris Stavroulakis

Consent Form

Participant study ID:	

Volunteers should complete the whole of this consent form themselves or nominate another person to do so:

Please initial each box:

1.	I confirm that I have read and understand the information sheet dated 22/7/2022, version 4.0 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. If I withdraw, any information collected about me already will continue to be used as part of the study as explained in the information sheet.	
3.	I agree to the research team using and storing biological materials (blood and urine) collected from me during the study as described in this information sheet.	
4.	I understand that my anonymised information will be stored in a database held by the research team at the University of Sheffield.	
5.	I agree that my anonymised information can be used in future projects.	
6.	I understand my anonymised samples may be sent to hospitals, university departments, non-profit institutions or commercial companies in the UK or worldwide for analysis as part of this study. No details that could identify me will be sent with the samples. Samples will be destroyed at the end of the study in accordance with the Human Tissue Authority Code of Practice.	
7.	I understand that information collected during the study may be looked at by responsible individuals from the Sponsor (Sheffield Teaching Hospitals NHS Foundation Trust) or the regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I	

	understand and consent to my sample(s) and information collected as part of the study being used in the ways set out in the information sheet.	
9.	I agree that the research team can access my medical records to verify information about my health.	
10.	I understand that I may be asked to complete an ALSFRS-R questionnaire if this has not been done as part of my routine clinical care for any reason.	
11.	I understand I may be asked to complete optional body composition assessments, including 3D body scanning and whole body air displacement plethysmography (BODPOD).	
12.	I agree to take part in the study.	

_____	_____	_____
Full name of volunteer (BLOCK CAPITALS)	Date	Signature
_____	_____	_____
Name of witness (if volunteer unable to sign themselves due to physical disability) (BLOCK CAPITALS)	Date	Signature
_____	_____	_____
Name of study team member taking consent (BLOCK CAPITALS)	Date	Signature

Original for researcher (filed in Investigator Site File); copy to volunteer; copy in medical records where applicable.

Appendix G.

Nutritional biomarkers in MND: Self-administered ALSFRS-Revised

Participant ID:

Baseline ☐ / 3M ☐ / 6M ☐ / 9M ☐

Date of completion (dd/mm/yy): ☐☐/☐☐/☐☐

Self-administered ALSFRS-Revised

The following questions refer to how you are currently functioning at home. Please read each item carefully and base your answers on your functioning today compared to the time before you had any symptoms of MND. Please choose the answer that best fits your functional status today. Place an "x" in the box next to your answer.

Compared to the time before you had symptoms of ALS:

1. Have you noticed any changes in your speech?

☐	No change
☐	Noticeable speech differences
☐	Speech has changed, asked often to repeat words or phrases
☐	Speech has changed; sometimes need the use of alternative communication methods (i.e., computer, writing pad, letter board or eye chart)
☐	Unable to communicate verbally

2. Have you noticed any changes (increases) in the amount of <u>saliva</u> in your mouth (regardless of any medication use)?

☐	No change
☐	Slight but definite excess of saliva with or without night time drooling
☐	Moderate amounts of excessive saliva with or without minimal daytime drooling
☐	Marked amounts of excessive saliva with some daytime drooling
☐	Marked excessive saliva with marked drooling requiring a constant tissue or handkerchief

3. Have there been any changes in your ability to <u>swallow</u>?

☐	No changes (all foods and liquids)
☐	Some changes in swallowing or occasional choking episode (including coughing during swallowing)
☐	Unable to eat all consistencies of food and have modified the consistency of foods eaten
☐	Use a gastrostomy/feeding tube to supplement what is eaten by mouth
☐	Do not eat anything by mouth and receive all nutrition through a gastrostomy/feeding tube

4. Has your handwriting changed? Please choose the best answer that describes your handwriting with your dominant (usual) hand without a cuff or brace.

☐	No change
☐	Slower and/or sloppier but all the words are legible
☐	Not all words are legible
☐	Able to hold a pen but unable to write
☐	Unable to hold a pen

The following question refers to your ability to cut foods and handle utensils (feed yourself) compared to before you had symptoms of ALS. If most of your nutrition is through a gastrostomy/feeding tube, skip to **part b** of this question. If you eat most of your meals by mouth (more than 50%) answer **part a**.

5a. Cutting food and handling utensils

☐	No change
☐	Somewhat slow and clumsy (or different than before) but no assistance or adaptive equipment
☐	Sometimes need help with cutting more difficult foods
☐	Foods must be cut by someone else but can feed slowly without assistance
☐	Need to be fed

5b. Using a gastrostomy/feeding tube

☐	Use feeding tube without assistance or difficulty
☐	Use feeding tube without assistance however may be slow and/or clumsy
☐	Require assistance with closures and fasteners
☐	Provide minimal assistance to caregiver
☐	Unable to perform any of the manipulations

6. Has your ability to <u>dress and perform self-care activities</u> (i.e., bathing, teeth brushing, shaving, combing your hair, other hygienic activities) changed?

☐	No change
☐	Perform self-care activities without assistance but with increased effort or decreased efficiency
☐	Require intermittent assistance or use different methods (i.e., sit down to get dressed, fasten buttons with a fastener or your non-dominant hand)
☐	Require daily assistance
☐	Do not perform self-care activities and completely dependent on caregiver

7. Has your ability to <u>turn in bed and adjust the bed clothes</u> (i.e., cover yourself with the sheet or blanket) changed?

☐	No change
☐	Can turn in bed and adjust the bed clothes without assistance but it is slower or more clumsy
☐	Can turn in bed or adjust the bedclothes without assistance but with great difficulty
☐	Can initiate turning in bed or adjusting the bed clothes but require assistance to complete the task
☐	Helpless in bed

8. Has your ability to <u>walk</u> changed?

☐	No change
☐	Walking has changed but do not require any assistance or devices (i.e., foot brace, cane, walker)
☐	Require assistance to walk (i.e., cane, walker, food brace or hand held assistance)
☐	Can move legs or stand up but unable to walk from room to room
☐	Cannot walk or move my legs

9. Has your ability to <u>climb stairs</u> changed?

☐	No change
☐	Slower
☐	Unsteady and/or more fatigued
☐	Require assistance (i.e., using the handrail, cane or person)
☐	Cannot climb stairs

10. Do you experience <u>shortness of breath</u> or have <u>difficulty breathing</u>?

☐	No change
☐	Shortness of breath only with walking
☐	Shortness of breath with minimal exertion (i.e., talking, eating, bathing or dressing)
☐	Shortness of breath at rest while either sitting or lying down
☐	Significant shortness of breath (all of the time) and considering using mechanical ventilation

11. Do you experience shortness of breath or have difficulty breathing while lying down on your back?

☐	No change
☐	Occasional shortness of breath while lying on back but don't routinely use more than two pillows to sleep
☐	Shortness of breath while lying on back and require more than two pillows (or an equivalent) to sleep
☐	Can only sleep sitting up due to shortness of breath
☐	Require the use of respiratory (breathing) support (BiPAP or invasive ventilation via tracheostomy) to sleep and do not sleep without it

12. Do you require respiratory (breathing) support?

☐	No respiratory support
☐	Intermittent use of BiPAP
☐	Continuous use of BiPAP at night
☐	Continuous use of BiPAP at night and during the day (nearly 24 hours per day)
☐	Mechanical ventilation by intubation or tracheostomy

Appendix H.

Nutritional biomarkers in MND: 24-hour urinary collection instructions and collection sheet

Participant ID:

Baseline ☐ / 3M ☐ / 6M ☐ / 9M ☐

Participant information leaflet: How to collect your 24hr urine sample

Thank you for your time and participation in our research on the nutritional management of people living with MND.

You have been asked to complete a 24-hour urinary collection, because we want to see how well the nutritional markers in your urine indicate what you have eaten and drunk in the past 24-hours, and how well this compares to what you are telling us you are eating and drinking in your intake diary. For this reason, it is really important that you collect **all** of your urine from a 24-hour period, the day before your routine clinical appointment.

For example, if your appointment is on Friday, please start your 24-hour urine collection on Thursday morning.

What you need to do:

1. When you get up in the morning, go to the toilet, but do not collect your urine. This is the start of the 24hr collection period. Please record this time on the collection sheet (at the end of this document).
2. From now on, please collect all urine that you pass until the same time the next morning. Please record the times of each collection on the collection sheet at the end of this document - do not feel you need to complete all rows, as long as you record all urinary collections.
3. Each time you collect urine, please collect all urine in the jug, and then carefully pour it into the 2.5 L plastic container.
4. Rinse the jug with water then hand dry it (with toilet paper)
5. If for any reason a sample is missed, or spilt, please write this on the collection sheet.
6. Please try and keep the collection as cool as possible - do not sit it next to a radiator or heater.
7. Your collection will end 24hrs after it began - i.e., if you started at 7am on Thursday morning, you should try to make your last collection just before 7am on Friday morning. This is the end of your 24hr collection period. Please make a note of this time on the collection sheet.
8. When you have completed the collection, please:
    a. Make sure the lid is screwed on tightly to the 2.5 L container
    b. Return it, along with the collection sheet, to the clinic at your study visit.

Please contact us if you have any issues:

Sarah Roscoe: saroscoe1@sheffield.ac.uk

Sample collection sheet

Urinary collections	Date	Time (24hr clock)	Comments (e.g. spillage, overflow, full sample)
Start time: please flush this urine away and record the time			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
End time: final urinary collection			
Missed urinary collections	**Date**	**Time (24hr clock)**	**Comments (e.g. spillage, overflow, forgot to collect)**
1			
2			
3			
4			
5			

Appendix I.

Measured and predicted resting energy expenditure

Table 13.1 Measured and predicted resting energy expenditure for the study population at baseline (n = 22). Data shown as mean ± one standard deviation and median (IQR). mREE: measured resting energy expenditure; pREE: predicted resting energy expenditure; SD: standard deviation;%ΔREE: REE variation.

		pREE		
	mREE	**Harris Benedict**	**Henry**	**kcal/kg/day**
Pt01	1417	1375	1370	1131
Pt03	1543	2176	2114	n/a
Pt04	1930	1693	1659	1837
Pt05	1411	1434	1446	n/a
Pt06	1618	1110	1113	1272
Pt07	2020	1795	1841	2081
Pt08	1841	1294	1435	1680
Pt09	1889	1596	1684	2064
Pt10	915	1214	1193	1558
Pt11	1850	1393	1500	1408
Pt13	1124	1410	1480	1680
Pt14	1774	2006	2031	2235
Pt15	1750	1784	1781	1835
Pt16	1523	1953	1962	n/a
Pt17	1689	n/a	n/a	n/a
Pt18	1578	n/a	n/a	n/a
Pt19	1818	1437	1551	1430
Pt20	1802	1764	1803	1886
Pt21	1500	1583	1653	2004
Pt22	1746	1537	1530	1628
Pt23	1291	1294	1363	1416
Pt24	1401	1827	1728	n/a
Mean ± SD	1610 ± 274.15	1584 ± 284.56	1612 ± 265.02	1697 ± 316.25
Median (IQR)	1654 (1437-1814)	1560 (1389-1786)	1602 (1444-1787)	1680 (1427-1916)
%ΔREE		0.88 ± 21.66	2.47 ± 19.87	6.47 ± 26.81
Accurate, n/N (%)		6/20 (30)	6/20 (30)	7/16 (43.75)

Appendix J.

Cytoplasmic and Nuclear Isolation from PBMCs

Wash buffer (- PIC)	Wash buffer (+ PIC)	Cytoplasmic isolation buffer	Nuclear isolation buffer
10 mM HEPES (pH 7.6)	10 mM HEPES (pH 7.6)	10 mM HEPES (pH 7.6)	20 mM HEPES (pH 7.6)
10 mM KCl	10 mM KCl	10 mM KCl	420 mM NaCl
2 mM $MgCl_2$	2 mM $MgCl_2$	2 mM $MgCl_2$	1.5 mM $MgCl_2$
1 mM EDTA Na_2	1 mM EDTA Na_2	1 mM EDTA Na_2	0.2 mM EDTA Na_2
		0.02% Nonidet P-40	25% glycerol
	Protease inhibitors (1x)	Protease inhibitors (1x)	Protease inhibitors (1x)

Cytoplasmic extraction

1. Locate and defrost >600ul of PBMCs
2. Combine aliquots from the same participant into one eppendorf
3. centrifuge at 430g, 10 mins, 4C
4. Wash PBMCs with **wash buffer (-PIC);** centrifuge 430g, 10 min, 4C
5. Resuspend cell pellets with **500 ul cytoplasmic isolation buffer (+PIC)**
    a. **Volume tbc based on EKK results**
6. Vortex for 10 seconds
7. Incubate on ice for 15 mins
8. Centrifuge 1: 1500g, 3 mins, 4C
    a. Keep nuclear pellet on ice. Add **100ul 'wash buffer + PIC' ***
    b. Transfer supernatant to new eppendorf
9. Centrifuge 2: centrifuge supernatant at 3500g, 8 mins, 4C

    a. Transfer supernatant to new eppendorf
10. Centrifuge 3: centrifuge supernatant at 17000g, 1 min, 4C
    a. Remove supernatant. **This is the cytoplasmic extract. Keep on ice.**

Nuclear extraction

1. *The nuclear pellet should be on ice in **100 ul wash buffer + PIC** from step 8a.
2. Centrifuge at 1500 g for 3 minutes at 4C **(x2)**
3. Resuspend pellet in **100 ul nuclear isolation buffer (+PIC)**
4. Transfer into 0.2 ml eppendorf
5. Sonicate 30 seconds, 30% amplitude
6. Incubate for 15 minutes on ice
7. Transfer back into larger eppendorf to fit centrifuge holes
8. Centrifuge at 20,000 g for 10 mins
    a. **Collect supernatants containing nuclear extracts – keep on ice**
9. Determine protein content of cytoplasmic and nuclear extracts using a bradford assay.

Western blot prep:

- need to load a minimum of 10ug per well
- ADD SDS (2ul SDS for every 8ul sample; 5x → 1x)
- mix
- Heat 95C for 5 mins
- Mix
- Pulse quickly
- Freeze at -20C, upright

Appendix K.

Metabolic profiling of PBMCs

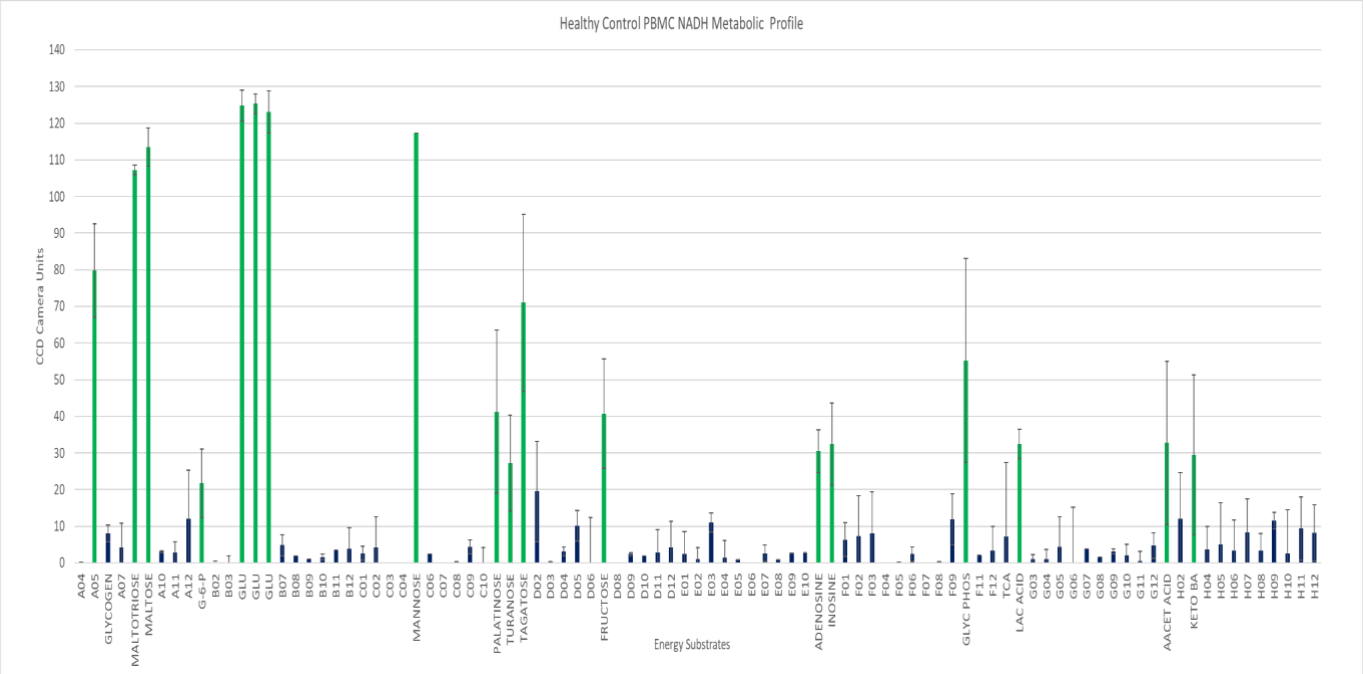


Figure 13.2 Histogram showing the metabolic profile of healthy PBMCs 24 hours post isolation. PBMCs were isolated from four healthy controls and incubated at 20K per well in PM-M1 carbohydrate screening plates for 25 hours 52. Subsequently NADH production was measured in each well of the plate (which contained a different energy substrate) using a Redox Dye (Biolog) and an OmniLog phenotypic bioanalyser (Biolog). Data presented as mean with standard deviation. Green bars denote significant NADH production with the defined metabolite, above background levels. Data generated by Dr Scott Allen.