Energy metabolism in amyotrophic lateral sclerosis:
Assessment of body composition and energy expenditure

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Abstract

Background and objectives:

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the relentless loss of upper motor neurones (UMN) and lower motor neurones (LMN), leading to progressive weakness. The cause of ALS is unknown and there are currently no curative treatments. The median survival is 18 months from diagnosis, however, the rate of disease progression and survival vary significantly among individual ALS patients.

In the absence of a cure for ALS, discovery of factors that modify disease progression is critical. An understanding of processes that influence the course of ALS could shed light on disease mechanisms and identify therapeutic targets that could be manipulated in order to prolong survival.

Systemic energy metabolism is emerging as an important modifying factor in ALS. Of clinical significance, adiposity [in the form of fat mass (FM)] and nutritional status modify survival in ALS. Therefore, the assessment of FM and implementation of nutritional interventions to maintain FM are important components of routine care in ALS patients. Anthropometric measurements, including body mass index (BMI) and body adiposity index (BAI) are commonly used to predict adiposity in order to guide nutritional management. However, the accuracy of these measurements in ALS patients is currently unknown.

Hypermetabolism [an increase in measured resting energy expenditure (REE) compared to a predicted REE] has been reported in some ALS patients and is also a possible modifier of disease progression. If this is a causal relationship, then strategies to correct or compensate for increased energy expenditure could possibly modify disease course. However, further studies to clarify the incidence, clinical correlations and consequences of hypermetabolism must be established.

The objectives of this thesis were to determine whether commonly used anthropometric measurements are accurate predictors of FM in ALS patients and to study the incidence and clinical correlations of hypermetabolism in ALS patients.
Methodology and main findings:

Anthropometric measurements of BMI and BAI were compared to percentage FM derived from air displacement plethysmography (ADP) in 44 ALS patients and 35 age- and sex-matched healthy controls. Using Bland-Altman analyses it was found that both anthropometric measurements were less accurate predictors of FM in ALS patients than in controls and that BMI and BAI provided a poor estimate of FM in ALS patients. In a longitudinal assessment of 29 ALS patients, neither BMI nor BAI consistently reflected the change in FM. These results indicate that an isolated measure of BMI and BAI is not an accurate indicator of adiposity in ALS and that longitudinal measurements could be misleading.

REE was measured via indirect calorimetry (mREE) and compared to a predicted REE (pREE, derived from a model that accounts for body composition) in 50 ALS patients and 50 age- and sex-matched healthy controls. Hypermetabolism was defined as a metabolic index (mREE/pREE x100) ≥ 120. Individuals with a metabolic index <120 were considered to be normometabolic. Hypermetabolism was found in 16% of controls and 40% of ALS patients. Hypermetabolic ALS patients had a higher LMN disease burden (assessed by clinical examination) and a greater short-term functional decline (assessed by the revised ALS functional rating scale, ALSFRS-R) than normometabolic patients.

Conclusions and future directions:

This study found that in ALS patients, BMI and BAI are not accurate predictors of FM and that they provide a poor indicator of change in FM over time. It is therefore likely that changes in BMI and BAI in ALS patients occur independent to changes in FM alone and could depend on muscle atrophy and re-distribution of fat.

It was also found that when body composition is accounted for, the incidence of hypermetabolism is greater in ALS patients than in healthy matched controls. An association between hypermetabolism and LMN disease burden was observed in ALS patients. As LMN disease burden reflects dysfunction of motor units, it is hypothesized that hypermetabolism in ALS could arise from abnormal motor units which include dysfunctional LMNs, disrupted neuromuscular junctions and denervated muscle. Furthermore, hypermetabolism was associated with a greater functional decline in ALS.
patients who were studied over time. In the light of these findings it is hypothesized that hypermetabolism could drive progression of ALS and lead to a vicious cycle of denervation, hypermetabolism and further disease progression.

Overall, the results of this thesis suggest that BMI and BAI are inadequate markers of nutritional status in ALS and that hypermetabolism is an important metabolic consideration in ALS patients. More accurate ALS-specific predictors of FM are required to guide nutritional therapies and further clinical and physiological studies are needed to understand the cause and prognostic implications of changes in body composition and hypermetabolism.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Contributions by others to the thesis

Pamela A. McCombe, Robert D. Henderson, Shyuan T. Ngo and Frederik J. Steyn assisted in the study conception and design, interpretation of research data and critical review of the thesis.

Shyuan T. Ngo, Frederik J. Steyn and Jia Dai Mi assisted in participant assessments.

Susan Heggie and Kathryn Thorpe carried out the revised ALS functional rating scale (ALSFRS-R) assessments.

Statement of parts of the thesis submitted to qualify for the award of another degree

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Amyotrophic lateral sclerosis, Body composition, Nutrition, Adiposity, Anthropometric measurements, Air displacement plethysmography, Hypermetabolism, Indirect calorimetry, Resting energy expenditure, Metabolism

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List of Abbreviations

ADP, air displacement plethysmography
AgRP, agouti-related protein
ALS, amyotrophic lateral sclerosis
ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale
ATP, adenosine triphosphate
BAI, body adiposity index
BMI, body mass index
C9orf72, chromosome 9 open reading frame 72
CNS, central nervous system
DEXA, dual-energy x-ray absorptiometry
DM, diabetes mellitus
DP, Deanna protocol
ENS, enteric nervous system
ETC, electron transport chain
FALS, familial amyotrophic lateral sclerosis
FeCO₂, fraction of expired air that is carbon dioxide
FeO₂, fraction of expired air that is oxygen
FFA, free fatty acids
FFM, fat free mass
FiCO₂, fraction of inspired air that is carbon dioxide
FiO₂, fraction of inspired air that is oxygen
FM, fat mass
FTD, frontotemporal dementia
FUS, fused in sarcoma
FVC, forced vital capacity
GDNF, glial cell-derived neurotrophic factor
GLUT4, glucose transporter type 4
GSK-3, glycogen synthase kinase-3
IC, indirect calorimetry
ICU, intensive care unit
kcal, kilocalories
kcal/day, kilocalories per day
KD, ketogenic diet
LMN, lower motor neurone
MB, mean bias
MND, motor neurone disease
mREE, measured resting energy expenditure
MRI, magnetic resonance imaging
NIV, non-invasive ventilation
NPY, neuropeptide Y
P, pressure
PEG, percutaneous endoscopic gastrostomy
POMC, proopiomelanocortin
pREE, predicted resting energy expenditure
RBWH, Royal Brisbane and Women’s Hospital
REE, resting energy expenditure
RR, respiratory rate
SEM, standard error of the mean
SNIP, maximal sniff nasal inspiratory pressure
SOD1, superoxide dismutase 1
STD, standard deviation
TARDBP, transactive response DNA binding protein
TCA, tricarboxylic acid cycle
TDEE, total daily energy expenditure
TEF, thermic effect of food
THC, tetrahydrocannabinol
UMN, upper motor neurone
V, volume
VCO₂, carbon dioxide production
VO₂, oxygen consumption
WHO, World Health Organisation
Chapter 1: Introduction and review of the literature

1.1 Amyotrophic lateral sclerosis

1.1.1 Definition, diagnosis and clinical features

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the degeneration and loss of upper motor neurones (UMN) and lower motor neurones (LMN), leading to progressive paralysis (Al-Chalabi et al., 2012). The lifetime risk is approximately 1 in 350 (Alonso et al., 2009), although, for unknown reasons, age and sex modify an individual’s risk. ALS is rare before the 4th decade of life. The incidence peaks in the 7th decade of life, followed by a decline in incidence in people over 80 years of age (Deloitte, 2015). Males have 1.5 fold greater risk of developing ALS than females, however, the sex ratio varies with age and males predominate in younger patient groups (McCombe and Henderson, 2010). A recent estimate of the prevalence of ALS in Australia was 8.7 per 100,000, which equates to over 2000 Australians living with ALS at any one time (Deloitte, 2015, Talman et al., 2016).

The clinical hallmark of ALS is the concomitant presence of progressive UMN and LMN deficits involving the brainstem and multiple spinal cord regions. As there are currently no specific diagnostic tests for ALS, after exclusion of alternative causes, identification of this combination of clinical features is used to diagnose ALS according to the El Escorial diagnostic criteria (Brooks et al., 2000, Ludolph et al., 2015).

There is heterogeneity in the clinical features of ALS. Clinical signs of dysfunction of LMNs (weakness and muscle wasting) and/or UMs (spasticity and increased deep tendon reflexes) can begin in any motor region and are generally followed by involvement of other regions over time (Turner et al., 2010). Limb-onset disease occurs most commonly (in ~70% of cases), followed by bulbar-onset disease (in ~25%) and rarely, in ~5% of cases, trunk or respiratory involvement occurs first (Ferguson and Elman, 2007). Individual patients also vary with regards to the relative prominence of UMN and LMN signs, the rate and pattern of progression and the presence of extra-motor features (Pradat and Bruneteau, 2006).
Given the clinical heterogeneity, in an attempt to classify individuals with ALS into meaningful clinical groups, different ‘phenotypes’ have been proposed. These classifications include distinctions based on the site of disease onset, predominant spinal level of involvement (Talman et al., 2016), and the degree of UMN and LMN clinical signs (Ravits et al., 2007). In most individuals with ALS, a combination of UMN and LMN signs is evident at diagnosis. However, rarely, individuals present with only LMN (referred to as the ‘flail’ variant) or only UMN signs (referred to as primary lateral sclerosis or PLS). The distinction of these phenotypes has prognostic value as the ‘flail’ and PLS variants have a longer survival than other forms of ALS (Talman et al., 2016).

Although defined by motor features, recent observations suggest that clinical involvement in ALS extends beyond the motor system (McCombe et al., 2017). Cognitive impairment, including behavioural, cognitive and language deficits, affect 5% of patients. In these patients there is significant clinical and neuropathological overlap with frontotemporal dementia (FTD) (Mitsuyama and Inoue, 2009, Montuschi et al., 2015). Given the expanding phenotype of ALS, its clinical heterogeneity (del Aguila et al., 2003) and the absence of specific diagnostic markers, there is often delay in its diagnosis (Talman et al., 2016, Knibb et al., 2016). Therefore to aid in the diagnosis of ALS, clinical (Brooks et al., 2000, Ludolph et al., 2015) and electrodiagnostic criteria (de Carvalho et al., 2008) are widely employed in research and clinical settings.

There are currently no curative treatments for ALS and the median survival is only 18 months from diagnosis (Knibb et al., 2016). Neuromuscular respiratory failure is the commonest cause of death; therefore, non-invasive ventilation (NIV) is used in some patients to prolong survival and improve quality of life (Radunovic et al., 2013). Riluzole, an inhibitor of presynaptic glutaminergic transmission, exerts a modest survival benefit (Miller et al., 1996). Edaravone, an anti-oxidant approved for use in ALS patients in Japan, has shown some benefit in a subgroup of patients (Abe et al., 2014, Abe et al., 2017) and in 2017 it was approved by the United States Food and Drug Administration (Hardiman and van den Berg, 2017). However, given the limited benefits offered by the currently available drug treatments for ALS, optimal care of ALS patients focuses on symptomatic and palliative therapies delivered in specialized multidisciplinary clinics (Rooney et al., 2015).
1.1.2 Hypotheses on pathogenesis

Since the first descriptions of the characteristic clinical and pathological features of ALS by Jean-Martin Charcot in the nineteenth century (Katz et al., 2015), much has been elucidated regarding the cellular mechanisms that contribute to the pathogenesis of ALS. Proposed pathogenic mechanisms include mitochondrial dysfunction (Hervias et al., 2006, Muyderman and Chen, 2014), glutamate excitotoxicity (Heath and Shaw, 2002, Vucic and Kiernan, 2006) and abnormal protein aggregation (Mackenzie et al., 2007, Blokhuis et al., 2013). These mechanisms are summarized in Figure 1.1. Over 20 genetic mutations have been implicated in the pathogenesis of ALS (Al-Chalabi et al., 2012, Renton et al., 2014). These include mutations in the superoxide dismutase 1 (SOD1) (Rosen et al., 1993), transactive response DNA binding protein (TARDBP) (Kabashi et al., 2008), fused in sarcoma (FUS) (Kwiatkowski et al., 2009, Vance et al., 2009) and chromosome 9 open reading frame 72 (C9orf72) (DeJesus-Hernandez et al., 2011) genes. However, only 10% of all cases are familial (Renton et al., 2014). A recent large meta-analysis suggests an oligogenic basis for sporadic ALS (Zou et al., 2017).

Despite these genetic and molecular advances, the underlying cause of ALS is not fully understood. Recent theories suggest that a complex combination of genetic, environmental and molecular mechanisms plays a role in the development of ALS (Kiernan et al., 2011). Current theories propose a six-step model of disease (Al-Chalabi et al., 2014). However, it is possible that the multistep process leading to the development of ALS is variable among individuals. Although common underlying aetiologies could exist, it is likely that the specific combination of factors that contribute to disease could differ among individuals (Al-Chalabi et al., 2014).
The mechanisms underlying neurodegeneration in ALS are multifactorial and operate through inter-related molecular and genetic pathways. Specifically, neurodegeneration in ALS could result from a complex interaction of glutamate excitotoxicity, generation of free radicals, cytoplasmic protein aggregates, SOD1 enzymes, combined with mitochondrial dysfunction, and disruption of axonal transport processes through accumulation of neurofilament intracellular aggregates. Mutations in TARDBP and FUS result in formation of intracellular aggregates, which are harmful to neurons. Activation of microglia results in secretion of proinflammatory cytokines, resulting in further toxicity. Ultimately, motor neurone degeneration occurs through activation of calcium-dependent enzymatic pathways. ALS, amyotrophic lateral sclerosis. From Kiernan et al. (Kiernan et al., 2011).
1.1.3 Factors that modify survival in ALS

The rate of disease progression and the length of survival in ALS are variable among individuals (Chiò et al., 2011). Indeed, phenotypic heterogeneity can be observed between individuals with the same genetic mutation (Al-Chalabi et al., 2012). Therefore there has been a search for factors that influence the progression of disease (Chiò et al., 2009). In addition to interventions such as riluzole therapy (Miller et al., 1996) and NIV (Radunovic et al., 2013), a number of patient and disease factors have been found to affect prognosis (Knibb et al., 2016).

Older age and bulbar involvement at disease onset (Chiò et al., 2009), cognitive impairment (Elamin et al., 2011), LMN dysfunction (Devine et al., 2016) and respiratory muscle weakness (Pinto et al.) predict a shorter survival time. There are also some genes that modify the clinical course of ALS (Meyer et al., 2005, Fogh et al., 2016, Lopez-Lopez et al., 2014, Taylor et al., 2016). In the absence of a cure, discovery of factors that modify disease progression is critical. An understanding of processes that influence the course of ALS could shed light on disease mechanisms and identify therapeutic targets that can be manipulated in order to prolong survival.

1.1.4 Metabolism as a modifier of ALS

Systemic energy metabolism is emerging as an important modifying factor in ALS and is the focus of this thesis. Mounting evidence suggests that alterations in metabolism can affect the development and progression of disease. The following sections will discuss this in detail.
1.2 Metabolic dysfunction in ALS


Increasing evidence suggests that altered metabolic homeostasis occurs in some ALS patients and could modify the course of ALS. Reduced body energy stores in the form of fat mass (FM) (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a, Paganoni et al., 2011) and increased resting energy expenditure (REE) (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014) appear to be of clinical importance in some ALS patients. The evidence that metabolic needs increase relative to disease severity and duration in ALS (Kasarskis et al., 1996) suggests that the pathological changes that occur with worsening disease could contribute to altered energy needs.

It appears that the interplay between pathogenic mechanisms and physiological responses to ALS results in a state of chronic negative energy balance that worsens patient prognosis [reviewed in (Dupuis et al., 2011, Ahmed et al., 2016b)]. It is therefore possible that modification of the metabolic imbalance could improve patient outcome and provide a therapeutic target in ALS. However, to develop effective treatment strategies that improve metabolic homeostasis, prognosis and quality of life for ALS patients, it is first necessary to understand the mechanisms of dysregulated metabolism and the impact on the disease process.

This section of the thesis presents the evidence for altered energy balance in ALS, focusing on alterations in body composition and REE. Underlying mechanisms that could affect energy balance and affect disease progression are discussed and summarised in Figure 1.2. Possible therapeutic strategies that could improve metabolic imbalance and modify the course of ALS are also considered.
Figure 1.2 Possible mechanisms of altered energy balance in ALS: This shows factors that could potentially lead to altered energy balance in ALS. Factors which could reduce energy supply are displayed on the left in green and factors which could increase energy expenditure are displayed on the right in red. This combination of reduced energy supply in parallel with increased energy demand create an energy deficit, which could be linked to a more rapid disease progression and a poorer outcome in ALS. Abbreviation: RR, respiratory rate.
1.2.1 Reduced energy stores in ALS

Fat mass in ALS
A large body of evidence suggests that there is an association between endogenous energy stores in the form of FM, and the development and progression of ALS. Increased body fat before the diagnosis of ALS is associated with a prolonged survival (Gallo et al., 2013). It has been found that ALS patients are generally lean, and they are more likely to report premorbid slimness or athleticism (Scarmeas et al., 2002).

A decline in body mass and FM occurs throughout the course of ALS (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a) and a more rapid rate of reduction in adiposity predicts a worse survival (Stambler et al., 1998, Shimizu et al., 2012). Furthermore, the observation of markedly reduced amounts of white adipose tissue in the SOD1 mouse (a widely studied preclinical model of ALS) in the asymptomatic phase of disease suggests that loss of FM could occur early in the disease process (Dupuis et al., 2004).

Clinical studies have found that adiposity, measured as FM, is a predictor of mortality (Gallo et al., 2013, Lindauer et al., 2013), disease progression (Shimizu et al., 2012), and survival (Paganoni et al., 2011) in ALS patients. Body mass index (BMI), a widely used surrogate marker of adiposity, is an independent predictor of survival in ALS (Paganoni et al., 2011). Paganoni et al found a ‘U-shaped’ relationship between survival and BMI. The lowest mortality was seen in patients who were mildly obese [BMI 30–35, World Health Organisation (WHO) obese class I (WHO, 1995)] In contrast, mortality was highest in underweight patients (BMI \( \leq 18.5 \)) (Paganoni et al., 2011) (Figure 1.3). Similarly, Shimizu et al, observed a shorter survival in ALS patients who experienced a more rapid rate of reduction in BMI (Shimizu et al., 2012), and Stambler et al observed a shorter survival in ALS patients with greater weight loss (Stambler et al., 1998). Taken together these findings suggest that body energy stores, in the form of FM, could be associated with survival in ALS.
Figure 1.3 Kaplan–Meier survival curves of 427 ALS patients stratified according to their BMI. Censored patients are indicated by crosses on the corresponding survival curve. The greatest survival probability occurred in patients who were mildly obese (BMI 30–34.99) and the lowest survival probability was in underweight patients (BMI ≤18.5). Blue line: BMI≤18.5; red line: BMI 18.5–24.99; green line: BMI 25–29.99; brown line: BMI 30–34.99; purple line: BMI 35–39.99, light green line: BMI >40 (log rank, p = 0.00055). Adapted from Paganoni et al. (Paganoni et al., 2011). Abbreviation: BMI, body mass index.
**Nutritional interventions**

Studies that showed benefits following nutritional interventions that increase energy supply in ALS patients provide further evidence of an association between energy stores and progression of ALS. It has been shown that hypercaloric oral food supplementation (provision of more calories than would normally be recommended) can stabilize or moderately increase body weight (Dorst et al., 2013) and increase arm muscle area and circumference (Silva et al., 2010) in ALS patients. These results suggest that as well as maintaining FM, dietary supplementation could also prevent loss of muscle mass in ALS patients.

Given the association between loss of adiposity and poorer survival (Paganoni et al., 2011, Shimizu et al., 2012, Gallo et al., 2013, Lindauer et al., 2013), there is increasing interest in the maintenance of FM in ALS via invasive nutritional interventions (Mazzini et al., 1995, 27, Muscaritoli et al., 2012, Wills et al., 2014). Nutritional supplementation is a rapidly emerging area in the field of ALS therapeutics (Rosenfeld and Ellis, 2008) and enteral nutrition has become an important aspect of therapy in some ALS patients.

Gastrostomy feeding, the most common route of delivery of enteral nutrition, has been shown to improve survival (Wills et al., 2014) and enhance quality of life (Mazzini et al., 1995) in ALS patients. The American Academy of Neurology (Miller et al., 2009) and the European Federation of Neurological Societies (Andersen et al., 2012) recommend gastrostomy, particularly in patients who have bulbar involvement. Gastrostomy is therefore widely used in ALS patients who develop dysphagia (ProGas, 2015).

Although increasing clinical evidence suggests that oral (Silva et al., 2010, Dorst et al., 2013) and enteral (Mazzini et al., 1995, Wills et al., 2014, Dorst et al., 2015) nutritional supplementation are beneficial in ALS, a balance between maintaining weight and avoiding excess gain of fat must be achieved. In most populations, excessive weight gain is detrimental to cardiovascular health (Poirier et al., 2006). Furthermore, in ALS patients, excessive weight gain could impair respiratory muscle function or increase carer burden during passive mobilization of paralyzed patients (Héritier et al., 2015).
In order to optimize nutritional management, accurate measures of adiposity are vital. Quantification of FM provides information regarding the body’s storage of energy. In conjunction with biochemical markers of nutrition (Zazra, 1997), this information is critical in guiding nutritional management, particularly in patients who are receiving oral supplementation or enteral nutrition (Heffernan et al., 2004). Anthropometric predictors, for example BMI and body adiposity index (BAI), are commonly used in clinical practice to estimate FM due to their practicality, but it is unknown whether anthropometric predictors of FM are accurate in ALS patients. This is clinically important as anthropometric predictors are used to guide nutritional management, which could improve outcomes in ALS patients (Silva et al., 2010, Dorst et al., 2013, Wills et al., 2014). Therefore, assessment of the accuracy of anthropometric predictors of FM in ALS is warranted.

**Reduced food intake**

Inadequate energy intake is likely to contribute to the weight loss that occurs in ALS patients. Food intake is governed by appetite and the capacity to consume and digest food. These factors could be affected in ALS as a result of anorexia, fatigue, dysphagia, respiratory dysfunction and upper limb weakness. Additionally, cognitive impairment, which occurs in up to 50% of ALS patients (Murphy et al., 2016), could impair an individual's capacity to plan meals, obtain ingredients and prepare meals.

Appetite is regulated by central neuronal circuits in the hypothalamus, brainstem and limbic system, as well as peripheral organs including the gastrointestinal tract and adipose tissue (Ahima and Antwi, 2008). The ‘gut-brain’ connection refers to the release, in response to food intake, of neuropeptides and hormones that act in the brain to alter hunger and satiety (Ahima and Antwi, 2008). In health, this system maintains metabolic homeostasis by balancing energy intake and storage; however, considerable evidence suggests that these processes are impaired in ALS (Slowie et al., 1983, Kasarskis et al., 1996, Vaisman et al., 2009). Indeed, anorexia is frequent in subjects with ALS, and reports indicate a deficit of calorie consumption in ALS patients that ranges from 70% (Slowie et al., 1983) to 84% (Kasarskis et al., 1996) of recommended daily energy needs.
Poor nutritional status is negatively associated with disease severity in ALS, and the intake of nutrients tends to decrease as disease progresses (Park et al., 2015b). In ALS, anorexia and malnutrition is likely to be driven by multiple neuroendocrine and physical factors. For example, a reduction in ghrelin, a hormone that stimulates appetite (Kojima and Kangawa, 2002), is seen in ALS (Ngo et al., 2015, Czell et al., 2015) and administration of ghrelin to the SOD1 mouse model of ALS prolongs survival through increased calorie intake (Matsuo T, 2013). It could be argued that ghrelin promotes survival through stimulating appetite, however this remains untested.

Recent observations show that ALS could be associated with dysfunction of processes that would normally regulate food intake. Treatment with pioglitazone in ALS patients and the SOD1 mouse failed to promote weight gain, despite confirmation of peripheral action of pioglitazone (Vercruysse et al., 2016). Pioglitazone normally promotes food intake via the central melanocortin system, a key hypothalamic neuronal network known to regulate food intake and metabolism (Sohn, 2015). Therefore, a lack of weight gain in ALS patients and mice treated with pioglitazone suggests possible dysfunction of this system.

Indeed, interrogation of multiple mouse models of ALS (harbouring mutations for SOD, TARPD and FUS genes) confirmed altered abundance and/or expression of two key hypothalamic regulators of food intake, proopiomelanocortin (POMC) and agouti-related protein (AgRP) (Vercruysse et al., 2016). Additionally, impairments in serotonin-mediated POMC activity were observed in the SOD1 mouse (Vercruysse et al., 2016). This also suggests that in ALS there could be impairment of neuronal circuitry that regulates food intake and calorie balance. Collectively, these observations suggest that calorie imbalance in ALS could occur as a consequence of defects in central processes that regulate appetite.

Irrespective of the mechanism in ALS, the failure to consume sufficient calories to meet energy needs could result in energy deficit, which could exacerbate weakness and fatigue, thus further impairing nutrient intake to create a ‘vicious cycle’ of suboptimal energy supply.
1.2.2 Altered energy expenditure in ALS

Hypermetabolism in ALS

In addition to the reduced energy intake discussed above, another plausible contributor to the loss of energy stores in ALS is an increase in resting energy expenditure (REE) known as hypermetabolism.

REE reflects the energy required to maintain vital functions in a fasted state. In healthy adults, REE accounts for up to 75% of overall energy expenditure and is highly variable among individuals (Donahoo et al., 2004). REE is determined by body composition, predominantly fat free mass (FFM) (Johnstone et al., 2005), the metabolically active component of the body that includes muscle and other organs. A deviation of the measured REE (mREE) from predicted REE (pREE) can be in the direction of hypometabolism (i.e. when the mREE is lower than the pREE) or hypermetabolism (i.e. when the mREE is greater than the pREE).

Hypermetabolism has been reported in mouse models of ALS (Dupuis et al., 2004, Niessen et al., 2007) and ALS patients (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014) (patient studies are compared in table 1.1). Hypermetabolism in ALS can be considered paradoxical because loss of FFM secondary to neurogenic muscle wasting (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a) would be expected to reduce REE (Johnstone et al., 2005).

However, it is important to note that existing studies of hypermetabolism in ALS (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014) have limitations. These reports of hypermetabolism have been derived by comparing the mREE to the pREE calculated from the Harris-Benedict equation, which is an estimate that could be misleading in individuals with ALS as it does not consider body composition (Weijs, 2011). The limitations of pREE models are discussed in detail in Chapter 2 section 2.2.1.
Additionally, the existing studies of hypermetabolism in ALS consider an individual to be hypermetabolic when the mREE is $\geq 110\%$ of the pREE (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014). However, studies of patients with other diseases such as insulin resistance, diabetes and liver cirrhosis, in whom hypermetabolism is common (Muller et al., 1993), often use a stricter threshold to indicate hypermetabolism; a mREE $\geq 120\%$ of the pREE (Muller et al., 1999, Perseghin et al., 2002). It is currently unknown how the use of this stricter threshold could affect studies of hypermetabolism in ALS patients.

Furthermore, although hypermetabolism has been suggested to occur early in ALS (Dupuis et al., 2004, Niessen et al., 2007, Bouteloup et al., 2009), it is notable that the majority of reports of hypermetabolism are in patients with low revised ALS Functional Rating Scale (ALSFRS-R) scores which indicate more advanced disease (see table 1.1). Therefore, it is not clear when hypermetabolism becomes apparent during the course of ALS, and it is yet to be determined which individuals with ALS are at risk of becoming hypermetabolic. Additionally, few studies in ALS include age- and sex-matched healthy controls as a comparison group, potentially biasing the conclusions. These are important considerations that need to be clarified before attempting to compensate for increased energy demands.
Table 1.1 Predictions of hypermetabolism in ALS.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>ALSFRS-R</th>
<th>Method of prediction</th>
<th>Prevalence of hypermetabolism relative to predicted REE</th>
<th>Prevalence of hypermetabolism relative to a matched control population</th>
<th>Additional key study findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasarskis et al. 1996</td>
<td>16 ALS, 0 cons</td>
<td>N/A</td>
<td>mREE measured by IC compared to pREE from HB* Repeated measures for 12 subjects (serial measures for an individual were averaged)</td>
<td>4 of 16 (25%)</td>
<td>N/A</td>
<td>• Progressive reductions in BMI and FM relative to proximity to death • mREE/pREE increased as death approached</td>
</tr>
<tr>
<td>Desport et al., 2001</td>
<td>62 ALS, 31 cons</td>
<td>29.0±/-7.0</td>
<td>Mean mREE from IC compared between ALS group and control group. mREE measured by IC compared to pREE from HB in ALS group*</td>
<td>42 of 62 (67.8%)</td>
<td>Mean mREE of ALS group was 10.1% greater than the control group (p = 0.03) Prevalence of hypermetabolism in each group not presented</td>
<td>• No correlation between mREE and FVC, fasciculation or spasticity scores in ALS group • mREE remained stable as ALS subjects approached death (in contrast to (Kasarskis et al., 1996))</td>
</tr>
<tr>
<td>Desport et al., 2005</td>
<td>168 ALS, 0 cons</td>
<td>28.0±/-7.1</td>
<td>mREE measured by IC compared to pREE from HB* Repeated measures for 44 subjects</td>
<td>105 of 168 (62.3%)</td>
<td>N/A</td>
<td>• mREE correlated with age, sex, phenotype, malnutrition, weight, FFM, and ALSFRS • FFM or mREE, corrected for FFM, did not decrease relative to proximity to death (in contrast to (Kasarskis et al., 1996))</td>
</tr>
<tr>
<td>Funalot et al., 2009</td>
<td>11 FALS, 33 SALS, 0 cons</td>
<td>27.6±/-7.3 in FALS group 29.2±/-6.6 in SALS group</td>
<td>mREE measured by IC compared to pREE from HB*</td>
<td>11/11 (100%) in FALS group 17/33 (52%) in SALS group</td>
<td>N/A</td>
<td>• mREE in FALS patients was not influenced by the degree of neurological or respiratory involvement</td>
</tr>
</tbody>
</table>

* mREE from IC compared to pREE from HB
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>mREE, no cons</th>
<th>mREE measured by IC compared to pREE from HB*</th>
<th>N/A</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouteloup et al., 2009 (Bouteloup et al., 2009)</td>
<td>61 ALS, no cons</td>
<td>30.7±/−4.8</td>
<td>Repeated measures for 45 subjects</td>
<td>N/A</td>
<td>mREE did not change over time, despite deteriorating neurological, nutritional and respiratory parameters</td>
</tr>
<tr>
<td>Kasarskis et al., 2014 (Kasarskis et al., 2014)</td>
<td>80 ALS, 0 cons</td>
<td>35.7±/−5.5</td>
<td>TDEE measured directly by using the doubly labeled water method and mREE measured by IC</td>
<td>N/A</td>
<td>Highlighted the variation between equations to predict cREE; Physical function and body composition are key when estimating TDEE</td>
</tr>
</tbody>
</table>

Notes: *Subjects considered hypermetabolic when mREE ≥ 110% of pREE. Abbreviations: ALSFRS-R, Revised Amyotrophic lateral sclerosis functional rating scale; BMI, body mass index; FALS, familial ALS; cons, control subjects; FFM, fat free mass; FM, fat mass; FVC, forced vital capacity; HB, Harris-Benedict equation; IC, Indirect calorimetry; mREE, measured resting energy expenditure; N/A, not assessed; pREE, predicted REE; SALS, sporadic ALS; TDEE, total daily energy expenditure.
Activity and diet dependent energy needs

Other aspects of energy requirement could also be altered in ALS. Total daily energy expenditure (TDEE) is a composite of REE, activity-dependent energy expenditure, and the thermogenic effect of food (TEF), which reflects the energy required to digest, process and absorb nutrients (D'Alessio et al., 1988). It is well recognised that TDEE varies greatly among individuals with ALS; TDEE may exceed measured REE by 2000 kilocalories per day (kcal/day) in some individuals, whereas TDEE may equal REE in other individuals (Kasarskis et al., 2014).

A possible explanation for this variation in TDEE could be the variability in physical activity among individuals with ALS. This variability would be expected to depend on whether there is lower limb weakness, which impairs mobility. Furthermore, fatigue, which is common in ALS (Lou, 2008) and correlates with disease severity (McElhinney et al., 2009), can also affect physical activity and therefore could also alter TDEE in ALS.

Another potential explanation for the heterogeneity of TDEE in ALS is a change in TEF. TEF represents up to 15% of TDEE. While TEF varies relative to body weight (Reed and Hill, 1996, Gepner et al., 2015) and physical activity (Stob et al., 2007), it is also dependent on gastrointestinal motor function and autonomic nervous system activation (Stob et al., 2007). Although gastrointestinal abnormalities are not universally recognised in ALS, there are reports of weight loss (Jawaid et al., 2010a), decreased gastric motility (Toepfer et al., 1999a) and dysfunction of the autonomic nervous system (Baltadzhieva et al., 2005) in some ALS patients, which could affect the TEF.

Altered lipolysis

The findings of reduced FM in ALS patients (Paganoni et al., 2011) and reduced adipose tissue accumulation in animal models of ALS (Dupuis et al., 2004) have sparked the hypothesis that there is altered lipolysis in ALS. Lipolysis is the process of catabolism of cellular lipid droplets to generate free fatty acids (FFA), which are essential energy substrates (Lass et al., 2011). Lipolysis occurs predominantly in adipose tissue and is regulated by a number of enzymes and hormone signaling pathways (Lass et al., 2011). The hypothesis that lipolysis is increased in ALS is supported by the finding of elevated
plasma FFA in patients with ALS (Mueller and Quick, 1970, Pradat et al., 2010) as well as an increased rate of lipolysis (Dodge et al., 2013) and reduced FFA uptake and storage (Dupuis et al., 2004) in animal models of ALS.

In addition to accelerated lipolysis, there is evidence that use of glycogen (an important alternative fuel reserve stored in liver and skeletal muscle which yields glucose molecules) is decreased in ALS. In SOD1 mice, there appears to be a switch in muscle energy use towards lipid oxidation, rather than glucose utilization, resulting in glycogen accumulation (Palamiuc et al., 2015).

Increased glycogen storage in the central nervous system (CNS) and peripheral tissues of SOD1 mice (Dodge et al., 2013), and glycogen accumulation in neurones and glial cells of grey and ventral white matter of human ALS spinal cord (Dodge et al., 2013) are also indicative of reduced glucose metabolism and increased lipolysis. At the hormonal level, increased levels of tumor necrosis factor alpha, which induces lipolysis, (Rondinone, 2006), and increased levels of adiponectin, which regulates glucose and fatty acid oxidation (Rondinone, 2006), have been detected in ALS patients (Ngo et al., 2015).

An increase in lipolysis could deplete endogenous fat stores and result in reduced FM as seen in ALS (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a). This could result in depletion of endogenous energy supply to neurones. This is significant because it implies that maintaining fat stores in order to provide FFA as an alternative energy substrate in ALS patients could improve energy supply to neurones and therefore potentially improve neuronal survival.

Muscle atrophy and denervation
Animal studies suggest that muscular atrophy secondary to denervation can alter systemic energy needs (Nunes and de Mello, 2005). In rats, denervation of skeletal muscle is associated with a reduction in glucose uptake, glycogen synthesis and insulin sensitivity (Nunes and de Mello, 2005). Denervation atrophy in the SOD1 mouse leads to diminished entry of pyruvate into the Krebs cycle, thus hampering glucose oxidation in skeletal muscle.
Moreover, muscular atrophy is associated with a reduction in the expression of glucose transporters (Henriksen et al., 1991, Coderre et al., 1992). While yet to be confirmed, it is feasible that widespread and synchronous denervation of muscle and accompanying muscle atrophy in ALS could underlie many of the metabolic changes observed in patients.

Respiratory failure
Patients with ALS experience varying degrees of respiratory muscle weakness (Gay et al., 1991). Decline in respiratory function eventually leads to hypercapnia, which is associated with an increase in respiratory rate and reduced survival (Czudaj et al., 2009). It is plausible that an increased respiratory rate in some ALS patients could increase energy requirements at rest and during periods of exertion. However, contrary to this theory, mREE does not appear to correlate with alterations in forced vital capacity (FVC, a standard clinical measure of respiratory function) in ALS patients (Desport et al., 2005, Bouteloup et al., 2009). As mild to moderate respiratory muscle weakness may not be detected by measuring FVC alone, a more sensitive measure such as the maximal sniff nasal inspiratory pressure (SNIP) (Lyall et al., 2001), might better correlate with REE but has yet to be studied in relation to energy expenditure in ALS patients.

Neuronal hyperexcitability
Neurones are metabolically active cells as energy is required to transport proteins along axons and for the active transfer of ions across the cell membrane to support excitability and conduction of impulses (Ashrafi et al., 2017). Given their high-energy demands, neurones are vulnerable to damage when in a state of energy restriction (Shaw and Eggett, 2000, Ashrafi et al., 2017).

Abnormally increased excitability of motor neurones, also known as hyperexcitability, is proposed to play a role in the pathophysiology of ALS (Vucic and Kiernan, 2006, Vucic et al., 2008). Maintenance of the neuronal cell resting potential is an active process requiring the hydrolysis of adenosine triphosphate (ATP) via the Na+/K+-ATPase (Lodish et al., 2000). Hyperexcitable neurones have an increased Na+ influx, thus maintenance of the resting potential in a hyperexcitable neurone requires the hydrolysis of excessive cellular ATP. Although, it is not known how this increased ATP use (via the Na+/K+-ATPase) affects overall cellular energy balance in hyperexcitable neurones, it is
plausible that it results in increased cellular energy use. This increased energy requirement could exacerbate neuronal dysfunction and, if widespread, could contribute to an overall whole body increase in energy requirements.

**Neuronal repair and inflammation**

The processes of neuronal repair in ALS could significantly affect total energy requirements. In ALS, loss of lower motor neurons (LMN) leads to denervation of muscle fibres. In muscle, this results in the production of a range of factors that are trophic for motor axons (Henderson et al., 1983), including insulin-like growth factors (Day et al., 2001, Shavlakadze et al., 2005) and myogenic regulatory factors (Walters et al., 2000). Denervated muscle cells undergo a change in the expression of genes encoding cell cycle regulators and extracellular matrix components (Batt et al., 2006). Denervated muscle cells also secrete immune molecules (interleukins) and metabolic factors (Pedersen, 2011).

These responses lead to the sprouting of new axons and re-innervation of muscle fibres by axons from other motor units. Successful re-innervation during the early stages of ALS is demonstrated by the neurophysiological finding of large motor units in ALS patients (Duleep and Shefner, 2013), but as disease progresses there is a failure in adequate re-innervation, followed by muscle atrophy (Bonaldo and Sandri, 2013). In the event of mass synchronous denervation, as seen in ALS, the energy costs associated with collateral re-innervation would be significant and could contribute to increased neuronal energy demand.

Neuro-inflammatory mechanisms that serve to protect and repair dying motor neurones in ALS are poorly understood, however, they are likely to be energy expensive. A number of neuroprotective models have been proposed including those involving CD4+ effector T cells which modulate the central microglial and astrocytic response to injury (Beers et al., 2008, Jones et al., 2015).

As well as being part of a protective response, neuro-inflammation can have a direct pathogenic effect in ALS [reviewed in (McCombe and Henderson, 2011)]. For example, microglial dysregulation (Beers et al., 2006) and increased pro-inflammatory markers have been observed in mouse models of ALS (Hensley et al., 2003) and in ALS patients (Ngo et al., 2015). Therefore, inflammation could play a role in both protective and
pathogenic mechanisms in ALS. This is an important consideration in energy balance as inflammation is associated with alterations in metabolic activity characterized by increased energy demands, which can lead to fundamental shifts in metabolism (Kominsky et al., 2010). In this way, chronic inflammation in ALS could contribute to escalating energy demands in patients.

**Altered cortical metabolism**

With evidence of altered cortical function in over 50% of ALS patients (Murphy et al., 2016), it is possible that a change in brain metabolism could contribute to altered energy needs in ALS patients. Indeed, positron emission tomography studies in ALS patients have demonstrated alterations in brain glucose metabolism in regions beyond the motor cortex (Dalakas et al., 1987, Hatazawa et al., 1988, Ludolph et al., 1992, Cistaro et al., 2012, Pagani et al., 2014).

The importance of altered cortical metabolism in changing energy needs in ALS is unclear at present because both increased and decreased glucose utilization in the CNS are reported (Dalakas et al., 1987, Hatazawa et al., 1988, Ludolph et al., 1992, Cistaro et al., 2012, Pagani et al., 2014). It is plausible that increased use of glucose by neurones, astrocytes or microglia, could increase brain metabolic activity and contribute to increased resting energy requirements; however, this requires further evaluation and remains speculative.

**Alterations in the gut**

In addition to alterations in energy expenditure, abnormal gut function could also affect energy balance in ALS. Optimal absorption of nutrients requires an intact gastrointestinal tract, a functioning enteric nervous system (ENS), and a healthy gut microbiome. Observations that the ENS and the CNS share common structural and chemical features (Gershon, 1997, Gershon, 1999), and that gastrointestinal dysfunction occurs in neurodegenerative diseases including Parkinson’s disease (Jost, 2010) and Huntington’s diseases (van der Burg et al., 2011), support the hypothesis that gastrointestinal alterations could occur in ALS, thereby altering the absorption of energy.
Links between the motor system and the ENS have been found in animal models. A lack of glial cell-derived neurotrophic factor (GDNF, a potent protective factor for motor neurons) in a mouse model leads to considerable loss of ENS neurones (Sanchez et al., 1996). The TARDBP mouse model of ALS exhibits intestinal dysfunction characterised by a progressively thinned colon, swollen small intestine and increased TDP-43 protein accumulation in the myenteric nerve plexus, which contributes to death independent of muscular weakness (Guo et al., 2012, Hatzipetros et al., 2014). Furthermore, the receptor tyrosine kinase Ret, which transduces GDNF signaling, is highly expressed by a specific subtype of enteric neurones in the human ENS (Luesma et al., 2014).

Autonomic dysfunction has been described in ALS patients (Baltadzhieva et al., 2005), with reports of subclinical gastrointestinal motor dysfunction (Toepfer et al., 1999a), delayed colonic transit times (Toepfer et al., 1997), delayed gastric emptying (Toepfer et al., 1999b), and an increased prevalence of constipation (Nubling et al., 2014). Furthermore, a study of percutaneously fed ALS patients found that 7 of 10 experienced weight loss that was unexplained by dysphagia, hypermetabolism or inadequate diet (Zhang, 2011). This further implicates malabsorption as a potential mechanism for weight loss in ALS.

Another important consideration in the absorption of nutrients is the gut microbiome, which comprises up to 100 trillion microorganisms (Backhed et al., 2005) that provide beneficial functions including nutrient absorption, vitamin synthesis and digestion of fiber (Maslowski and Mackay, 2011). The composition of the gut microbiome is influenced by diet, and gut microbes play a role in modulating inflammatory and immune responses (Kranich et al., 2011, Maslowski and Mackay, 2011), susceptibility to infection, and regulation of weight and nutrition (Kau et al., 2011, Turnbaugh and Gordon, 2009, Goodrich et al., 2014). Alterations in the gut microbiome could also potentially contribute to alterations in host metabolism (Goodrich et al., 2014, Guinane and Cotter, 2013). Whether this is the case in ALS is yet to be explored.

In the SOD1 mouse model of ALS abnormal intestinal permeability, associated with increased inflammatory cytokines in the gut and an altered gut microbiome, has been observed (Wu et al., 2015b). Furthermore, correction of the gut microbiome with butyrate in this model has been shown to restore gut integrity and prolong survival (Zhang et al., 2017). While gut microbes have not been studied in ALS patients, it is plausible that the
dietary changes that occur in ALS (Slowie et al., 1983, Kasarskis et al., 1996) could contribute to adaptations in the gut microbiome. Moreover, autonomic dysfunction in ALS (Baltadzhieva et al., 2005) could contribute to altered bowel transit times, thereby affecting microbial growth and diversity (Kamada and Kao, 2013).

Via these mechanisms, the microbiome could serve as a link between dietary intake, energy metabolism and disease state in ALS. Indeed, it has been proposed that gut microbes are important in the development and progression of other neurodegenerative diseases (Wang and Kasper, 2014, Scheperjans et al., 2015). Overall, the hypothesis that the gut microbiome modulates brain activity through the ‘gut-microbiota-brain axis’ (Fang, 2015) provides a link between the gut and pathologies affecting the brain. In doing so, it opens novel avenues of investigation in the field of the gut-brain axis in ALS.

**Impaired glucose handling**

Impaired glucose handling and function of insulin is of particular interest in ALS because reduced glucose availability could affect overall energy balance and exacerbate neuronal dysfunction, particularly in the CNS, which relies predominantly on glucose as fuel. Since the 1960s, there have been reports of impaired glucose tolerance and insulin resistance in ALS (Cumings, 1962, Ionasescu and Luca, 1964, Steinke and Tyler, 1964, Saffer et al., 1977, Gotoh et al., 1972, Reyes et al., 1984, Pradat et al., 2010).

A systematic review of seven, predominantly retrospective, observational studies found no increase in the prevalence of diabetes mellitus (DM) in ALS (Lekoubou et al., 2014), although the incidence of abnormal glucose tolerance tests in ALS patients ranges from 19% to 72% (Ionasescu and Luca, 1964, Mueller and Quick, 1970, Koerner, 1976, Harno et al., 1984, Armon et al., 1991, Pradat et al., 2010). Therefore subtle alterations in glucose handling could occur in ALS.

Abnormal glucose metabolism does not appear to affect survival or disease severity (Lekoubou et al., 2014, Paganoni et al., 2015); however, one Taiwanese study reported an increased risk of ALS in those with DM (Sun et al., 2015). By contrast, a Danish study found that type 2 DM is protective for ALS (Kioumourtzoglou et al., 2015) and individuals with DM have been found to have a an average of 4 years later onset of ALS than those without DM (Jawaid et al., 2010b).
While the data examining the relationship between ALS and impaired glucose handling is limited and inconclusive, the finding of hyperglycaemia in ALS patients with severe muscle wasting (Collis and Engel, 1968, Gotoh et al., 1972, Shimizu et al., 2011) raises the possibility that reduced muscle mass may lead to a global disruption in glucose homeostasis.

Approximately 90% of insulin-mediated glucose uptake occurs in skeletal muscle (Sinacore and Gulve, 1993). Thus, muscle wasting in ALS could affect glucose handling by altering insulin-mediated translocation to the muscle membrane of the glucose transporter type 4 (GLUT4), which is critical in regulating cellular glucose uptake and whole body glucose homeostasis (Henriksen et al., 1991). Congruent with this, muscle wasting is correlated with abnormal glucose tolerance in some ALS patients (Collis and Engel, 1968, Gotoh et al., 1972), however this correlation is not universal (Mueller and Quick, 1970) and controlling for muscle wasting does not affect the occurrence of impaired glucose handling (Saffer et al., 1977). Thus, muscle wasting, while a possible contributor, is unlikely to be the sole explanation for abnormal glucose handling in all ALS cases.

Animal models of ALS have revealed insights into possible mechanisms of altered glucose handling. In the SOD1 mouse, there is impaired glucose use in motor tracts in conjunction with significant depletions in cortical ATP (Browne et al., 2006), and expression of key enzymes involved in gluconeogenesis (the process of endogenous glucose production) are up-regulated in the liver with a concomitant increase in fasting glycaemia (Dupuis et al., 2004).

In ALS patients, studies examining insulin and glucose responses using a compound that stimulates secretion of insulin, indicate impaired synthesis or release of insulin from the pancreas (Saffer et al., 1977, Steinke and Tyler, 1964). Recent findings in ALS patients provide further evidence to support an alteration of insulin release and function in ALS (Ngo et al., 2015). Reduced circulating levels of gastric inhibitory polypeptide in ALS patients (Ngo et al., 2015), could result in insufficient insulin release (McIntosh et al., 2009), and reduced expression of circulating pancreatic polypeptide in ALS patients (Ngo et al., 2015) could contribute to reduced peripheral insulin action.
Mitochondria, the main site of oxidative phosphorylation and production of ATP, play a pivotal role in bioenergetics and survival of the cell. Mitochondria are also a major source of oxygen free radicals and act as regulators of cell death via the intrinsic pathway of apoptosis. The nervous system consumes substantial amounts of energy and oxygen, so mitochondria are critical to its function [reviewed in (Xavier et al., 2015)]. Mitochondrial dysfunction significantly affects neuronal function and survival by increasing neuronal sensitivity to a variety of insults such as oxidative stress and bioenergetic defects (Xavier et al., 2015).

There is ample evidence that pathological changes in ALS are closely associated with changes in mitochondrial morphology and bioenergetics (Hervias et al., 2006, Muyderman and Chen, 2014). Such observations have led to the hypothesis that mitochondrial dysfunction plays an important role in the pathogenesis of ALS, and a large number of studies in ALS patients and animal models have found mitochondrial abnormalities in neuronal and non-neuronal tissues [reviewed in (Martin, 2011)].

Animal studies have revealed mitochondrial dysfunction in the pre-symptomatic and early phases of disease (Mattiazzi et al., 2002, Damiano et al., 2006, D'Alessandro et al., 2011, Carri and Cozzolino, 2011, Tan et al., 2014), implying that mitochondrial dysfunction is an early event that triggers disease, rather than the end product of neuronal cell degeneration.

Mitochondrial dysfunction leads to the accumulation of oxygen free radicals and reduced ATP production from oxidative phosphorylation, reducing cell function and energy availability, and leading to cell death (Biala et al., 2015, Lionaki et al., 2015). Mitochondrial dysfunction in ALS is therefore a potential mechanism of disrupted cellular energy production, which could have significant upstream affects on neuronal function and whole body energy balance.
1.2.3 Attempts to correct metabolic imbalance in ALS

Factors that contribute to energy imbalance in ALS and how these changes could exacerbate disease progression have been reviewed above. Strategies and/or drugs to improve this energy imbalance could possibly slow disease progression and improve survival in ALS and are therefore attractive areas for exploration. A number of therapies considering this approach have been hypothesized and tested in ALS (selected strategies are illustrated in Figure 1.4).

As discussed above in section 1.2.1, an emerging therapeutic approach in ALS is dietary modification and supplementation (Rosenfeld and Ellis, 2008). Improving nutrition can improve the outcome in ALS [reviewed in (Ngo et al., 2014)] and provide benefit in animal models (Dupuis et al., 2004) and ALS patients (Mazzini et al., 1995, Silva et al., 2010, Dorst et al., 2013, Wills et al., 2014). For this reason some authors advocate early enteral nutrition (Greenwood, 2013).

However, despite the notion that increased calorie intake compensates for increased energy demands and improves outcome in ALS, the exact composition of dietary intervention, and when to initiate dietary changes, are yet to be determined. This is particularly important as significant weight gain in some ALS patients may impair respiratory muscle function and mobilization (Héritier et al., 2015). The best method for monitoring the response to nutritional interventions has yet to be determined, and it is currently unknown whether anthropometric predictors of FM are accurate in ALS and can be used to guide nutritional advice.

Other dietary modifications that aim to alter energy substrate supply and/or promote anaplerosis (replenishing depleted metabolic intermediates), including the ketogenic diet (Zhao et al., 2006), the "Deanna protocol" (Fournier et al., 2013, Ari et al., 2014) and triheptanoin (Tefera et al., 2016), could potentially be useful in treating the energy deficit in ALS, but need further formal testing.
Drug therapies that improve metabolic imbalance could improve outcome in ALS, but this is a largely unexplored area. It is postulated that agents that stimulate glucose transport (such as glycogen synthase kinase-3 inhibitors) and block hyperexcitability (including retigabine, flecainide and diazoxide), as well as mitoprotective drugs (agents that preserve mitochondrial function), could be beneficial (Table 1.2).

Given the link between ALS and glucose intolerance, therapies that improve insulin resistance in type 2 diabetes have been explored in ALS. The anti-diabetic drug pioglitazone improved motor performance, delayed weight loss, attenuated motor neuron loss, and extended survival in a SOD1 mouse model (Kiaei et al., 2005), however, pioglitazone failed to show efficacy in a phase II clinical trial of ALS patients (Dupuis et al., 2012). Furthermore, it has been suggested that anti-diabetic drugs are detrimental in ALS in light of emerging evidence that some features of the metabolic syndrome may be protective in ALS (Jawaid et al., 2014). Since the trial of pioglitazone, no further diabetic agents have been studied in ALS and therefore the uncertainty of benefit from anti-diabetic drugs remains.
Figure 1.4 Proposed treatment strategies aimed at restoring metabolic homeostasis to slow disease progression in ALS. This shows strategies that could potentially correct or compensate for the energy deficit in ALS patients. Enteral nutrition is generally accepted to be a safe strategy to maintain body weight in ALS (Wills et al., 2014). However, further studies to determine the optimal diet composition and the ideal time to initiate enteral feeding are required. Non-invasive ventilation has been shown to improve quality of life and survival in ALS (Bourke et al., 2003). The other strategies in the figure are proposed based on their theoretical plausibility; however they require further validation to determine safety and efficacy in ALS. More information on these experimental strategies is included in Table 1.2. Abbreviations: CNS, central nervous system; GSK-3, glycogen synthase kinase-3.
Table 1.2 Tested strategies that could improve metabolic imbalance in ALS

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Potential mechanism to improve metabolic imbalance in ALS</th>
<th>Summary of evidence in ALS or models of ALS</th>
<th>Possible future directions</th>
</tr>
</thead>
</table>
| Increase calorie intake through dietary supplementation and enteral nutrition | • Maintains energy stores and compensates for increased energy demands  
• Provides energy substrate for lipolysis, which is increased in ALS | **Patient data**  
• High caloric food supplements stabilized body weight in ALS patients (Dorst et al., 2013)  
• Oral supplementation increased BMI in ALS patients (Silva et al., 2010)  
• PEG feeding maintained BMI and reduced mortality in bulbar onset ALS (Mazzini et al., 1995)  
• Hypercaloric nutrition with ‘Jevity’ (contains 29% of calories from fat) via PEG was safe and tolerable in ALS patients (Wills et al., 2014)  
• High caloric intake (1,500 kcal/day) via PEG prolonged survival (Dorst et al., 2015)  
• Trials currently recruiting: ‘Oral Nutritional Supplementation in ALS Patients’ (ClinicalTrials.gov) and ‘Efficacy, Safety and Tolerability of High Lipid and Calorie Supplementation in ALS’ (ClinicalTrials.gov) | • Determine the optimal composition of diet and calorie content to maintain weight in ALS  
• Longitudinal studies that examine disease progression relative to dietary intake and metabolic parameters  
• Develop consensus guidelines on initiating enteral feeding |
| Boost the supply of alternate energy substrates including ketone bodies, intermediates in the TCA cycle and medium chain fatty acids | • Ketones are an alternate energy substrate for neurons  
• Energy deficit might be improved by providing alternative substrate (acetyl-CoA) for the TCA cycle and the ETC for enhanced ATP production  
• Beneficial in other disorders of altered neuronal metabolism (Baranano and Hartman, 2008) | **Patient data**  
• ‘Safety and Tolerability of the KD in ALS Trial’ (ClinicalTrials.gov) terminated May 2015, results not published | **Animal studies**  
• KD maintained motor function and reduced spinal cord motor neuron death in SOD1 mice (Zhao et al., 2006)  
• DP improved motor scores and improved survival in SOD1 mice (Ari et al., 2014)  
• Caprylic triglyceride attenuated progression and protected spinal cord motor neuron loss in SOD1 mice (Zhao et al., 2012) | **Studies in ALS models to elucidate mechanisms**  
• Trials in ALS patients to determine safety and efficacy |
<table>
<thead>
<tr>
<th>Inhibit GSK-3</th>
<th>Animal studies</th>
<th>Animal studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• GSK-3 has been proposed to play a role in the pathogenesis of ALS (Grimes and Jope, 2001)</td>
<td>• Administration of a GSK-3 inhibitor delayed the onset of symptoms and prolonged survival in SOD1 mice (Koh et al., 2007)</td>
<td>• Studies in ALS models to elucidate mechanisms.</td>
</tr>
<tr>
<td>• Blocking the action of GSK-3 enhances glycogen synthesis and stimulates glucose transport in skeletal muscle (Jope and Johnson, 2004), which might enhance energy utilization in skeletal muscle</td>
<td>• Increased GSK-3 activity found in motor neuronal model of ALS; GSK-3 inhibition prevented motor neurone cell death (Koh et al., 2005)</td>
<td>• Trials in ALS patients to determine safety and efficacy.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Improve mitochondrial function</th>
<th>Patient data</th>
<th>In vitro studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Agents that improve mitochondrial function might reduce oxidative stress and optimize mitochondrial electron transport and ATP synthesis.</td>
<td>• Phase III trial of dexpramipexole (Mora et al., 2013), phase II trial of Coenzyme Q10 (Kaufmann et al., 2009) and a pilot study of tauroursodeoxycholic acid (Elia et al., 2016) showed no benefit</td>
<td>• Methyl pyruvate treatment improved mitochondrial ATP production in a SIGMAR1 model of ALS (Tagashira et al., 2014).</td>
</tr>
<tr>
<td></td>
<td>• A phase I study of BIIB067 is currently underway (ClinicalTrials.gov)</td>
<td>• SS-31 improved mitochondrial electron transport and ATP synthesis (Birk et al., 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Studies in ALS models to elucidate mechanisms.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Larger studies in ALS patients of agents that show benefit in animal models</td>
</tr>
</tbody>
</table>

**Animal studies**
- Administration of a GSK-3 inhibitor delayed the onset of symptoms and prolonged survival in SOD1 mice (Koh et al., 2007)
- Increased GSK-3 activity found in motor neuronal model of ALS; GSK-3 inhibition prevented motor neurone cell death (Koh et al., 2005)

**In vitro studies**
- Methyl pyruvate treatment improved mitochondrial ATP production in a SIGMAR1 model of ALS (Tagashira et al., 2014).
- SS-31 improved mitochondrial electron transport and ATP synthesis (Birk et al., 2014)
<table>
<thead>
<tr>
<th>Block neuronal hyperexcitability</th>
<th>Patient data</th>
<th>Animal studies</th>
<th>In Vitro studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Blocking neuronal hyperexcitability could correct neuronal energy imbalance by reducing excessive energy use</td>
<td>• Phase II trial of talampanel (Pascuzzi et al., 2010) and a Phase II/III trial of memantine (de Carvalho et al., 2010) showed no significant benefit</td>
<td>• Diazoxide improved survival in SOD1 mice (Pugliese et al., 2013)</td>
<td>• Retigabine blocked neuronal hyperexcitability and improved motor neurone survival (Wainger et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>• Phase II trial of retigabine on neuronal excitability in ALS currently underway (ClinicalTrials.gov)</td>
<td>• Studies in ALS models to elucidate mechanisms</td>
<td>• Studies in ALS patients of agents that show benefit in animal models</td>
</tr>
<tr>
<td></td>
<td>• A pilot study found that flecainide stabilized axonal membrane function. Not powered to detect benefit (Park et al., 2015a)</td>
<td>• Larger studies in ALS models to elucidate mechanisms</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALS, Amyotrophic lateral sclerosis; BMI, body mass index; DP, Deanna protocol; ETC, electron transport chain; GSK-3, glycogen synthase kinase-3; kcal/day, kilocalories per day; KD, ketogenic diet; PEG, percutaneous endoscopic gastrostomy; TCA, tricarboxylic acid cycle; THC, tetrahydrocannabinol.
1.3 Summary of metabolism in ALS and rationale for the thesis

The death of motor neurones in ALS is likely to occur secondary to a combination of genetic susceptibility and environmental factors. However, the considerable heterogeneity in the clinical features of ALS and the different clinical courses seen in patients with the same pathogenic mutation indicate that other factors modify the disease. There is evidence that altered energy metabolism occurs in ALS and that the resulting energy imbalance could modify disease progression.

**Fat mass in ALS**

Of clinical significance, FM and nutritional status modify survival in ALS (Mazzini et al., 1995, Stambler et al., 1998, Dupuis et al., 2004, Silva et al., 2010, Paganoni et al., 2011, Shimizu et al., 2012, Dorst et al., 2013, Wills et al., 2014). The assessment of FM can be used to guide nutritional management, which is emerging as an important aspect of routine care in ALS patients. Although anthropometric measurements are widely used in clinical practice to estimate FM, it is currently unknown whether they are accurate predictors of FM in ALS patients.

Anthropometric measurements, including BMI (Eknoyan, 2008) and BAI (Bergman et al., 2011), are commonly used to predict adiposity in clinical and research settings. Anthropometric measurements could be convenient predictors of FM in ALS patients, particularly in those who have significant physical disability or respiratory impairment, which could preclude the use of other measures of FM, such as dual-energy x-ray absorptiometry (DEXA) or magnetic imaging resonance (MRI) (discussed in Chapter 2, section 2.1). Furthermore, as the monitoring of responses to nutritional intervention requires longitudinal assessment of FM, the rapid and practical nature of anthropometric measures makes them convenient for repeated use in a clinical setting.

Despite the high practicality of using anthropometric measures in ALS, their accuracy in this patient group is currently unknown. BMI (Norgan, 1994, Garrido-Chamorro et al., 2009) and BAI (Johnson et al., 2012) have been shown to be inaccurate predictors of FM in populations with altered muscle mass. Therefore, it is plausible that the alteration
in body composition that occurs in ALS (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a) could reduce the predictive accuracy of anthropometric measurements.

Given the importance of accurately assessing FM for optimal nutritional care, inaccurate or misleading estimates of adiposity could have adverse clinical consequences. It is therefore of clinical interest to assess whether BMI and BAI are accurate estimates of FM in ALS patients.

**Hypermetabolism in ALS**

There have been studies demonstrating hypermetabolism in some patients with ALS (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014), however, the cause of hypermetabolism is unknown. The few studies that have assessed clinical correlates of hypermetabolism do not identify its underlying mechanisms (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009).

Given than hypermetabolism has been observed in relation to proximity to death in ALS patients (Kasarskis et al., 1996), metabolic abnormalities are hypothesized to be associated with a poor disease outcome in ALS. This is of interest because if this is a casual relationship, then strategies to correct or compensate for increased energy requirements could be used to modify disease course. For example, improved nutrition, which could potentially compensate for the higher energy needs associated with hypermetabolism, has been shown to reduce mortality in ALS patients (Mazzini et al., 1995, Wills et al., 2014, Dorst et al., 2015).

Importantly, previous studies of hypermetabolism in ALS have limitations and modified approaches could be explored. Existing studies have not accounted for the alteration in body composition that occurs in ALS (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014). This could be important, as body composition is the greatest determinant of REE (Johnstone et al., 2005). It is currently unknown how accounting for body composition affects predictions of REE in ALS. Also previous studies have assessed ALS patients with advanced disease...
without matched controls. Therefore, studies of ALS patients earlier in their disease course and assessed in comparison to matched controls are currently lacking.

Metabolic abnormalities that are found in ALS patients suggest possible mechanisms through which disease could be modified. Strategies to correct or compensate for energy imbalance in ALS could benefit neuronal survival, and are therefore promising avenues to improve quality of life and survival in patients living with ALS. However, before progress can be made, further knowledge on the incidence, clinical correlations and consequences of metabolic dysfunction must be established.

1.4 Objectives of the Thesis

1) To determine whether the commonly used anthropometric measurements BMI and BAI are accurate predictors of FM in ALS patients compared to age- and sex-matched controls.

2) To study hypermetabolism in ALS patients and age- and sex-matched controls by:
   
   a) Investigating the incidence of hypermetabolism using an REE prediction model that accounts for body composition.

   b) Investigating clinical factors that are associated with hypermetabolism in ALS patients.

   c) Determining whether hypermetabolism affects disease progression in ALS patients by measuring changes in the ALSFRS-R score over a 4-month period.
Chapter 2: Body composition and resting energy expenditure (REE): Background and assessment

Reduced body energy stores in the form of fat mass (FM) (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a) and increased resting energy expenditure (REE) (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014) are key clinical features of the metabolic disturbance that has been reported in some ALS patients. These aspects of metabolism will be investigated in the thesis. In order to develop the methodology and to provide scientific context, this chapter discusses the general background of body composition and REE including a review of common methods for their assessment.

2.1 Body composition

An interest in the relationship between nutrition, altered body composition and disease dates back to the time of Hippocrates (Cardenas, 2013). Today, knowledge of body composition is fundamental to the study of human physiology, metabolism and nutrition (Kondrup et al., 2003, Kyle et al., 2006).

Body composition refers to the relative components of the total body mass. Body composition can be conceptualized in five compartments; atomic, molecular, cellular, tissue or whole body compartments (Wang et al., 1992). For the clinical and nutritional purposes relevant to this thesis, body composition will be considered in a two-compartment model. This model partitions the body into FM and fat free mass (FFM) (Forbes, 1999). FFM comprises the metabolically active body components including muscle, internal organs, bone and connective tissue. Many techniques exist to investigate body composition. Commonly used techniques are discussed in the following sections and the advantages and disadvantages are summarized in Table 2.1.
Table 2.1 Commonly used techniques to assess body composition. Advantages and disadvantages are discussed in general and in relation to ALS patients, particularly those with respiratory dysfunction who are unable to lie flat.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometric measurements (for example BMI (Eknoyan, 2008) and BAI (Bergman et al., 2011))</td>
<td>Rapid, simple, portable comfortable, inexpensive, safe. Do not require specialized equipment or training. Practical and efficient.</td>
<td>Population specific, poor accuracy in individuals with altered fat free mass as is commonly the case in ALS.</td>
</tr>
<tr>
<td>Air displacement plethysmography</td>
<td>Rapid, comfortable, and safe. Automated and non-invasive. Practical and efficient.</td>
<td>Expensive equipment. Not portable. Not suitable for individuals with claustrophobia. May be inaccurate in individuals with fluid shifts (such as oedema, ascites or dehydration) or extremely altered body composition.</td>
</tr>
<tr>
<td>Magnetic resonance imaging</td>
<td>Accurate. Provides an estimate of regional body composition.</td>
<td>Expensive equipment and technical staff. Requires highly skilled interpretation. Only quantifies FM that is present in adipose tissue (does not quantify total FM). Not practical in ALS patients with respiratory dysfunction as requires lying supine for long periods.</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; BAI, body adiposity index; ALS, amyotrophic lateral sclerosis; FM fat mass.
2.1.1 Anthropometric measures

Anthropometric measurements, including body mass index (BMI) and body adiposity index (BAI) are commonly employed methods of assessing body composition in clinical and research practice. They are accessible, quick and efficient ways to estimate FM. However, they are in fact ‘predictors’ of body composition, as they do not directly measure body composition. Anthropometric measurements are derived from mathematical models that take into account demographic indices and raw measurements of physical properties of the body, primarily dimensions of body size and shape, and apply various theoretical assumptions (Wells and Fewtrell, 2006).

Due to these assumptions, the accuracy of anthropometric measures is population dependent. For example, BAI is not an accurate predictor of FM in individuals with extreme levels of adiposity (Bergman et al., 2011) and it lacks accuracy in obese individuals (Geliebter et al., 2013) and healthy sedentary females (Suchanek et al., 2012). Anthropometric measures can also overestimate FM in individuals with significantly increased FFM such as body builders (Madden and Smith, 2016). The validity of anthropometric measures in individuals with reduced FFM, such as ALS patients, is currently unknown.

2.1.2 Air displacement plethysmography

Whole body air-displacement plethysmography (ADP) has been used to measure body composition for over a century, but only became available for routine use after the development of an automated system called the BodPod (Cosmed USA, Inc), in the mid-1990s (Dempster and Aitkens, 1995).

Plethysmography refers to the measurement of size, and in the case of ADP refers to the measurement of body volume. In ADP, the volume of a subject is measured indirectly by measuring the volume of air displaced inside an enclosed chamber, known as a plethysmograph. The BodPod system is comprised of a plethysmograph, electronic weighing scale, computer, software and, calibration weights and cylinder.
The BodPod has been found to be a reliable, reproducible and valid method for determining body composition (Fields et al., 2002, Noreen and Lemon, 2006, Anderson, 2007, Lee and Gallagher, 2008, Lowry and Tomiyama, 2015). It offers a rapid and automated evaluation of body composition. It avoids exposure to ionizing radiation and is practical for subjects who are otherwise challenging to assess, including the elderly, children, and individuals with physical disabilities. However, in some populations, including individuals with fluid shifts and/or extremely altered body composition, there could be inaccuracies in the measurement of body composition due to the assumptions inherent to the two-compartment model of densitometry used by the BodPod (Fields et al., 2002).

2.1.3 Dual-energy x-ray absorptiometry

Dual-energy x-ray absorptiometry (DEXA) was originally developed to measure bone mineral density, which is calculated from the differential absorption of x-rays of two different energies (Pietrobelli et al., 1996). This calculation requires quantification of overlying soft tissue, therefore values of FM and FFM are also obtained during a DEXA scan.

DEXA is widely used for the purposes of evaluating bone health, however, the equipment and technical staff required are expensive, the scan exposes the subject to ionizing radiation and the images require highly specialized interpretation. Regarding its use for assessment of body composition, although able to accurately quantify whole body FM (Toombs et al., 2012), DEXA could be unreliable for longitudinal studies of individuals who undergo significant changes in nutritional status between measurements with bias present in some disease states (Williams et al., 2006).

2.1.4 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is based on intrinsic tissue properties. MRI analyzes the absorption and emission of radio frequency energy, and generates images based on variations in the phase and frequency of the absorbed and emitted energy (de Figueiredo et al., 2011). MRI uses a multi-compartmental model to assess soft tissue, bone, organ size, muscle, fat distribution and total body water. It allows for accurate quantification of adipose tissue, skeletal muscle and organs.
The main advantage of MRI is its ability to estimate regional body composition (Müller et al., 2011). However, given the molecular basis of MRI, comparing measures from other techniques can be difficult. MRI makes assumptions regarding the fat content of adipose tissue and the density of fat, and MRI can only quantify fat that is present in adipose tissue (Wells and Fewtrell, 2006), rather than total FM which is measured by densitometry models. MRI is expensive and, requires specialized technical staff and highly skilled interpretation. The imaging acquisition is relatively slow, therefore subjects are required to lie supine inside a ‘donut’ shaped magnet for long periods of time. This can be unsuitable for individuals with claustrophobia and mental or physical disability. These disadvantages limit the use of MRI in routine metabolic assessments.

2.1.5 Summary

Assessment of body composition provides important information in the study of metabolism and nutrition. Although many methods exist for predicting and assessing body composition, as pathologist Beneke remarked in 1878, ‘Nothing is measured with greater error than the human body’. Overall, no method for assessment of body composition has been shown to be ideal in all populations. The technique of choice depends upon the characteristics of the test subject and available resources. The limitations of each method should be acknowledged in the context of the population being studied.

2.2 Resting energy expenditure

Total daily energy expenditure (TDEE) is a composite of resting energy expenditure (REE), activity-dependent energy expenditure, and the thermogenic effect of food [TEF, the energy required to digest, process and absorb nutrients (D'Alessio et al., 1988)]. REE reflects the energy required for vital functions of respiration, cardiac output and maintenance of intra-cellular environments, in a fasted state. In healthy adults REE accounts for up to 75% of overall energy expenditure (Donahoo et al., 2004) (figure 2.1).
REE is predominantly dependent on body composition (Johnstone et al., 2005) and correlates with FFM and FM in both women and men (Nielsen et al., 2000). A study in elderly individuals found that approximately 88% of the variance in REE is explained by changes in mass and body composition (Geisler and Müller, 2017). The greatest determinant of REE is FFM (Wang et al., 2000, Johnstone et al., 2005), the metabolically active component of the body comprised of internal muscle, organs, bone, water and connective tissue. REE is also linked to appetite control (Blundell et al., 2012) and adjusts in response to central nervous system pathways that regulate energy homeostasis (Keesey and Powley, 2008).

REE varies significantly relative to age, sex, body composition and general health (Johnstone et al., 2005, Geisler et al., 2016). REE displays a diurnal variation (Fredrix et al., 1990) and can be increased by the consumption of stimulants including caffeine (Astrup et al., 1990) and nicotine (Audrain-McGovern and Benowitz, 2011).
2.2.1 Predictions of REE

When REE cannot be directly measured, predicted REE (pREE) is used across many clinical disciplines to inform nutritional requirements. In particular, pREE is commonly used to guide total parenteral nutrition during critical illness (Plank and Hill, 2003). Another important function of the pREE is its use as a comparison value to the measured REE (mREE), in order to determine whether an individual’s mREE is abnormal.

There are numerous models for determining pREE that take into account various demographic, anthropometric and/or body composition measures (Frankenfield et al., 2005, Wang et al., 2001). 3 commonly used models are compared in Table 2.2A and B.

Table 2.2A Resting energy expenditure prediction models.

<table>
<thead>
<tr>
<th>Prediction model for REE (kcal/day)</th>
<th>Equation</th>
</tr>
</thead>
</table>
| Harris-Benedict (Harris and Benedict, 1918) | Males: 66.4730 + 13.7516 wt (kg) + 5.0033 ht (cm) - 6.7550 age (yrs)  
Females: 655.0955 + 9.5634 wt (kg) + 1.8496 ht (cm) - 4.6756 x age (yrs) |
| Mifflin-St Jeor (Mifflin et al., 1990) | Males: 10 x wt (kg) + 6.25 x ht (cm) – 5.0 x age (yrs) +5  
Females: 10 x wt (kg) + 6.25 x ht (cm) – 5.0 x age (yrs) – 161 |
| Modified Nelson*(Nelson et al., 1992). | 25.80 x FFM (kg) + 4.04 x FM (kg) |

Notes: *The originally described Nelson equation (Nelson et al., 1992) calculated the pREE in kilojoules/day (kJ/day), the version shown in the table is the modified version used by the BodPod body composition measuring system (Cosmed USA, Inc) to calculate the pREE in kilocalories/day (kcal/day). This adaptation can be found at: http://www.bodpod.com/hires/marketing_literature/product_news/Product_News_Estimation_of_RMR_based_on_Lean_Mass_with_Bod_Pod_EN_print.pdf
Table 2.2B Comparison of parameters considered in three commonly used pREE models. Abbreviations: Kcal/day, kilocalories per day; REE, resting energy expenditure.

<table>
<thead>
<tr>
<th>Prediction model for REE (kcal/day)</th>
<th>Considers weight</th>
<th>Considers height</th>
<th>Considers age</th>
<th>Considers sex</th>
<th>Considers body composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris-Benedict</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>Mifflin-St Jeor</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>Nelson</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>✓</td>
</tr>
</tbody>
</table>

Abbreviations: REE, resting energy expenditure; wt, weight; ht, height; FFM, fat free mass; FM, fat mass; yrs, years.

The American Dietetic Society suggests use of the Mifflin-St Jeor equation (Mifflin et al., 1990). The Harris-Benedict equation (Harris and Benedict, 1918) is also widely used, particularly in studies of ALS patients as discussed in Chapter 1 section 1.2.2 (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014).

While the Mifflin-St Jeor and Harris-Benedict equations are commonly used to predict REE (Ellis and Rosenfeld, 2011), these predictions do not account for body composition. Given that body composition, and in particular, FFM (Johnstone et al., 2005), is the major determinant of REE, these equations are inherently inaccurate in individuals with altered body composition (Weijs et al., 2008), for example ALS patients (Sherman et al., 2004, Siirala et al., 2010).
Studies have shown that REE prediction equations differ significantly (Miller et al., 2013, Flack et al., 2016) and that the Harris-Benedict equation could overestimate REE when compared to indirect calorimetry (Kisiolek et al., 2015). A study of women in different age groups suggests that the Nelson prediction, which accounts for body composition, is accurate in older individuals (Woolf, 2015). This is most likely because it accounts for the amounts of FM and FFM, which are altered in the elderly (St-Onge and Gallagher, 2010, Geisler et al., 2016). Given the important influence that body composition has on REE, it has been suggested that accounting for body composition is critical in predicting REE (Wang et al., 2000).

Overall, REE prediction models rely on various assumptions and are therefore associated with inherent errors. The evidence suggests that caution should be exercised when choosing a pREE model, and that specific population characteristics, such as age, sex and most importantly body composition, should be taken into account where possible.

2.2.2 Abnormalities of REE

A significant difference between mREE and the pREE reflects an underlying physiological disturbance and a change in energy requirements. This is most often associated with systemic illness. A decrease in the mREE relative to the pREE is known as a hypometabolic state. Hypometabolism is known to occur in association with hypothermia (Gordon, 2001) and endocrine disorders including hypothyroidism (Persani, 2012).

Conversely, an increase in REE relative to the pREE is known as a hypermetabolic state. Clinical conditions characteristically associated with hypermetabolism include hyperthyroidism (Roth and McAuliffe, 1989), pheochromocytoma (McCullagh and Engel, 1942), malignancy (Purcell et al., 2016), burns (Auger et al., 2017) and significant trauma (Rogobete et al., 2017). Given reports of hypermetabolism in ALS, the clinical consequences and possible mechanisms of hypermetabolism in other disorders will be considered.
2.2.3 Clinical consequences of hypermetabolism

Hypermetabolism is associated with a negative nitrogen balance, cachexia, insulin resistance and increased catabolism of protein, carbohydrates and triglycerides (Challoner, 1966, Nair et al., 1984, Nair et al., 1986, Bogardus et al., 1986, Chiolero et al., 1997, Charlton and Nair, 1998, Sreekumar and Nair, 2007). In severe burns, hypermetabolism is associated with significant catabolism of skeletal muscle (Newsome et al., 1973, Hart et al., 2000) and, diminished mitochondrial coupling in skeletal muscle (Porter et al., 2016) and brown adipose tissue (Yo et al., 2013). In extreme cases, hypermetabolism can be life threatening and require critical care (Wu et al., 2015a).

Much of the research on the consequences of hypermetabolism has been carried out in cancer and burns patients or in an intensive care unit (ICU) setting. It has recently been shown that hypermetabolism correlates with clinical and biological markers of cancer cachexia and is associated with a shorter survival in metastatic cancer patients (Vazeille et al., 2017). Hypermetabolism in patients with severe sepsis in ICU predicts a worse mortality (Wu et al., 2015a). Similarly hypermetabolism following severe burns is associated with increased cardiac workload, muscle wasting and increased morbidity (Herndon et al., 2001).

From a therapeutic aspect, it has been shown that effective modulation of hypermetabolism in burns patients (via beta-adrenergic blockade and reduction of endogenous catecholamine actions) reduces inflammation, and improves biochemical and clinical outcomes (Herndon et al., 2001, Lunawat et al., 2015). Given that hypermetabolism has also been speculated to lead to a worse outcome in ALS patients, these observations that modulation of hypermetabolism improves outcomes in burns patients, raise the possibility that metabolic therapies could also be beneficial in ALS.
2.2.4 Possible mechanisms of hypermetabolism

Hypermetabolism occurs across a spectrum of clinical conditions and a number of different mechanisms are thought to contribute to its development.

In thyrotoxicosis, hypermetabolism arises from the direct effects of thyroid hormone, which has widespread actions in the hypothalamus, adipose tissue and skeletal muscle, and plays a central role in regulating metabolic rate, thermogenesis and glucose metabolism (Mullur et al., 2014). Endogenous catecholamines, which increase adrenergic stress, are thought to be the primary mediators of hypermetabolism in pheochromocytoma (McCullagh and Engel, 1942), trauma and burns (Reiss et al., 1956, Wilmore et al., 1974, Porter et al., 2014).

Systemic inflammation and increased metabolism in cancerous and adipose tissue have been proposed to lead to hypermetabolism in cancer patients (Petruzzelli and Wagner, 2016). In critically ill patients, a combination of a systemic inflammatory response, oxidative stress and mitochondrial dysfunction have been suggested to contribute to the development of hypermetabolism (Rogobete et al., 2017). It is possible that a combination of overlapping mechanisms lead to hypermetabolism in different clinical contexts.

2.2.5 Clinical assessment of REE

All energy production in the body occurs from metabolism of ingested nutrients. The final common pathway of extracting the chemical energy of cellular fuels (also known as energy substrates, including carbohydrate, fat, and protein) is oxidization to carbon dioxide and water. Heat is also generated in the processes of substrate combustion.

Direct calorimetry measures total heat loss by evaporation, radiation, and conduction/convection from the body (Webb, 1980). To undergo direct calorimetry, the test subject must be confined in a thermally isolated chamber to measure heat production whilst maintaining a resting state (Webb, 1980). Direct calorimetry is time consuming, requires expensive equipment and the conditions are not practical for clinical use. The availability of direct calorimetry is limited to a few specialized research centers and since
the early 1900’s it has been largely superseded by indirect calorimetry (Ferrannini, 1988, Simonson and DeFronzo, 1990, Battley, 1995, da Rocha et al., 2006, Haugen et al., 2007, Oshima et al., 2016).

Indirect calorimetry measures whole body oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$) to estimate the total energy production (Ferrannini, 1988). “Energy production” refers to the conversion of substrates into the chemical energy of ATP plus loss of some energy during the oxidation process. Given that energy production and expenditure are equal in the steady state, i.e., at rest with no change in body temperature, the rate of energy production is an accurate estimate of energy expenditure (Ferrannini, 1988). Indirect calorimetry uses stoichiometry models of substrate oxidative reactions and applies the assumptions that all the O$_2$ consumed has been used to oxidize degradable fuels, and that all the CO$_2$ that is produced is measured by the system.

Measures of VO$_2$ and VCO$_2$ can be transformed into energy expenditure in kilocalories per day (kcal/day), with a correction for the metabolism of protein, by applying the widely used Weir equation (Weir, 1949). This equation is derived from first principles for the calorie value of a litre of oxygen metabolizing a mixture of carbohydrate, protein and fat (Weir, 1949):

Weir equation: $\text{EE (kcal/day)} = \left[ (3.941 \times \text{VO}_2) + (1.11 \times \text{VCO}_2) - (2.17 \times \text{uN}_2) \right] \times 1440$

Where EE is energy expenditure (kcal/day), VO$_2$ is O$_2$ consumption, VCO$_2$ is CO$_2$ production (L/min) and uN$_2$ is urinary nitrogen (g/day). The factor of 1440 allows for the calculation of EE in kcal/day.

Given that in the original publication, Weir et al. stated that the error in neglecting the effects of protein metabolism is low (~1% for each 12.3% of total calories arising from protein) (Weir, 1949), the Weir equation is often abbreviated to omit accounting for urinary nitrogen, which reflects protein metabolism. This abbreviated equation is used by most modern indirect calorimetry systems (Blond et al.).

Abbreviated Weir equation: $\text{EE (kcal/day)} = \left[ (3.94 \times \text{VO}_2) + (1.11 \times \text{VCO}_2) \right] \times 1440$
2.2.6 Summary

Alterations in REE reflect a change in whole body energy metabolism, which is often associated with significant systemic illness. The interpretation of alterations in REE is complex because it requires consideration of an expected or ‘predicted’ REE (pREE), which has inherent limitations. Given that body composition is the greatest determinant of REE, pREE models that account for FM and FFM appear to be the most accurate, particularly in populations with altered body composition. Hypermetabolism has been shown to be detrimental to outcomes in some populations and modification of the underlying mechanisms of hypermetabolism could be a potential therapeutic tool. Indirect calorimetry is widely considered to be the method of choice for measuring REE in most clinical and research settings.
3.1 Participants

3.1.1 Recruitment

ALS patients

Patients who attended the Royal Brisbane and Women's Hospital (RBWH) motor neurone disease (MND) clinic were given information by their treating neurologist regarding the research and asked if they were provisionally interested in participating. Patients who expressed an interest were then invited to attend the RBWH MND research clinic on a separate occasion. At the research clinic, the investigator provided further detailed information regarding all aspects of the research assessment. ALS patients were enrolled if they fulfilled the following inclusion criteria:

1) Fulfillment of the revised El Escorial criteria for probable or definite ALS (Brooks et al., 2000, Ludolph et al., 2015)
2) Fulfillment of the 2008 consensus electrodiagnostic criteria for ALS (de Carvalho et al., 2008).
3) Aged over 18 years
4) Willing and able to provide informed consent

Patients who had any of these exclusion criteria were not recruited:

1) Respiratory failure or cognitive impairment to a degree that would detrimentally affect participation in research assessments
2) Concomitant conditions or medications known to affect the parameters of interest (mainly diabetes, cancer, thyroid dysfunction, active inflammatory or infective conditions. However, those with other well-controlled chronic diseases such as hypertension and stable heart disease were not excluded).
Healthy control participants

Healthy control participants were recruited from individuals who presented to the research clinic with patients or other contacts of ALS patients (for example spouses, friends, colleagues or family members). Where possible the control participants were age- and sex-matched to the ALS patients. All controls met the following inclusion criteria:

1) Aged over 18 years
2) Willing and able to provide informed consent

Control individuals who had any of these exclusion criteria were not recruited:

1) Diagnosed with, or under investigation for an active neurological condition (however, individuals with previous neurological conditions that were no longer active, for example a prior transient ischemic attack or episodic migraine, were not excluded).
2) Concomitant conditions or medications known to affect the parameters of interest (mainly diabetes, cancer, thyroid dysfunction, active inflammatory or infective conditions. However, those with other well-controlled chronic diseases such as hypertension and stable heart disease were not excluded).

3.1.2 Ethical considerations

The University of Queensland and The RBWH human research ethics committees approved all aspects of this study. Written informed consent was obtained from all participants prior to assessments.
3.2 Clinical Assessment

3.2.1 Anthropometric assessment

Demographic data including age and sex were recorded. Participants were asked to remove jewellery and eyeglasses and to wear underwear to minimize extracorporeal weight. Body mass was determined to the nearest 0.001kg (Cosmed USA Inc.). Hip circumference was measured to the nearest 0.5cm following established guidelines (WHO, 2008). Height was measured to the nearest 0.5cm with a wall-mounted stadiometer (Novel Products Inc. Illinois, USA). Body mass index (BMI) was calculated as body mass divided by height squared (kg/m^2). Body adiposity index (BAI) was calculated from height and hip circumference as described by Bergman et al (Bergman et al., 2011).

\[
\text{BMI} = \frac{\text{Body Mass (kg)}}{\text{Height (m)}^2}
\]

\[
\text{BAI} = \left\{\frac{\text{Hip circumference (cm)}}{\text{Height (m)}^{1.5}}\right\} - 18
\]

3.2.2 Clinical assessment of ALS patients

In ALS patients, the site of disease onset (either upper limb, lower limb, bulbar or respiratory) was recorded. Disease duration was defined as the interval between the onset of the first symptom reported by the patient and the day of metabolic assessment.

ALS patients were asked whether they experienced significant weight loss (estimated to be more than ~10% of their original body weight) around the time of onset of the first ALS symptom. The answer given by the patient was confirmed with the medical records when possible.

Respiratory function tests comprising measurement of seated and supine forced vital capacity (FVC) and the maximal sniff nasal pressure (SNIP) were performed within 4 weeks of the metabolic assessment. Results were recorded as raw values and also expressed as a percentage of the predicted value.
The revised ALS functional rating scale (ALSFRS-R) (Cedarbaum et al., 1999) was recorded on the day of assessment. The ALSFRS-R bulbar, upper limb, lower limb and respiratory sub-scores were calculated as previously described by Pinto et al (Pinto and de Carvalho, 2015).

The physical examination performed by the neurologist at the RBWH MND clinic at the time of ALS diagnosis was used to determine the upper motor neurone (UMN) and lower motor neurone (LMN) score as previously described (Ravits et al., 2007, Devine et al., 2016) (Table 3.1). Each limb was given an UMN and LMN score from 0–3 based on the degree of hyper-reflexia, spasticity and clonus, or degree of wasting and weakness respectively. Scores from each limb were summed to give a total UMN and LMN score, each out of 12.
Table 3.1 Scoring system used to quantify clinical UMN and LMN involvement in each limb.

<table>
<thead>
<tr>
<th>UMN Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No involvement</td>
</tr>
<tr>
<td>1</td>
<td>Definite, but trace involvement</td>
</tr>
<tr>
<td></td>
<td>• Reflexes preserved in a limb with weakness, or</td>
</tr>
<tr>
<td></td>
<td>• Slight increase in tone</td>
</tr>
<tr>
<td>2</td>
<td>Moderate involvement</td>
</tr>
<tr>
<td></td>
<td>• At least one reflex brisk ($\geq 3$ on a 4-point scale), or</td>
</tr>
<tr>
<td></td>
<td>• Moderate increase in tone, or</td>
</tr>
<tr>
<td></td>
<td>• Presence of an extensor plantar response (in lower limb)</td>
</tr>
<tr>
<td>3</td>
<td>Significant and severe involvement</td>
</tr>
<tr>
<td></td>
<td>• All reflexes pathologically brisk with significant transmission or clonus, or</td>
</tr>
<tr>
<td></td>
<td>• Significant spasticity, limiting ability to move limb or walk</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LMN Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No involvement</td>
</tr>
<tr>
<td>1</td>
<td>Definite, but trace involvement</td>
</tr>
<tr>
<td></td>
<td>• Weakness $\geq 4/5$, involving one or more segments (with no segments $&lt; 4/5$), and</td>
</tr>
<tr>
<td></td>
<td>• Mild wasting</td>
</tr>
<tr>
<td>2</td>
<td>Moderate involvement</td>
</tr>
<tr>
<td></td>
<td>• Weakness $\geq 3/5$, involving one or more segments (with no segment $&lt; 3/5$), and</td>
</tr>
<tr>
<td></td>
<td>• Moderate wasting</td>
</tr>
<tr>
<td>3</td>
<td>Significant and severe involvement</td>
</tr>
<tr>
<td></td>
<td>• Little or no movement (LMN weakness $\leq 2/5$) involving one or more segments, and</td>
</tr>
<tr>
<td></td>
<td>• Severe wasting</td>
</tr>
</tbody>
</table>

Notes: The grading of weakness refers to the Medical Research Council (MRC) scale for muscle power. Abbreviations: UMN, upper motor neurone; LMN, lower motor neurone. Table from Devine et al and Ravits et al (Devine et al., 2016) and (Ravits et al., 2007).
3.3 Assessment of body composition

3.3.1 Air displacement plethysmography (ADP)

Whole body air displacement plethysmography (ADP) was selected for assessment of body composition as it is rapid and performed in a seated position. ADP is therefore suitable for use in the majority of ALS patients, even those with physical disability or respiratory dysfunction. ADP also avoids exposure to ionizing radiation (in contrast to DEXA), which was of particular ethical concern in the healthy control participants.

On the same morning as the anthropometric assessment, body composition was determined by the BodPod system (Cosmed USA Inc.) according to standardized procedures (Fields et al., 2002, von Hurst et al., 2016). Full quality assurance and calibration procedures were carried out according to the manufacturers instructions each morning prior to testing. Assessment was performed at 8 am after an overnight (>12 hours) fast. The following procedure was guided by prompts generated by the BodPod computer and the BodPod software automatically performed the calculations.

Participants were asked to attend a second assessment of body composition 3 to 6 months following their first assessment.

3.3.2 ADP procedure

Participants were asked to remove jewellery and eyeglasses before the test and to wear tight fitting underwear and a swimming cap during assessment to minimize extracorporeal volume and weight. Body weight to the nearest 0.001 kg was determined by the scale linked to the BodPod. The participant was then seated inside the BodPod chamber.

The BodPod chamber is divided into two compartments separated by a common wall onto which a movable diaphragm is fixed (Figure 3.1). The front is the test chamber in which the subject sits and the back is the reference chamber. During a measurement, the BodPod is sealed by electromagnets on the door, while the diaphragm oscillates between
the two chambers. Small volume changes are produced inside the chambers by oscillation of the diaphragm and the resulting pressure changes are detected.

The volume of air inside the chamber was measured by applying Boyle’s Law, which states that at a constant temperature, volume (V) and pressure (P) are inversely related: \( \frac{P_1}{P_2} = \frac{V_2}{V_1} \). The volume of air in the test chamber was measured when the chamber was empty and then again when the participant was seated inside the chamber. Whole body volume was determined as the difference between the volume of air inside the chamber when the subject was inside, and the volume of air in the empty chamber.

**Figure 3.1 Schematic of the BodPod System (Cosmed USA, Inc).** On the left, the front measurement chamber in which the participant sits, from Bayer College of Medicine website: https://www.bcm.edu/bodycomplab/bpodschemapage.htm. On the right, the components of the BodPod and diaphragm mechanism, from the Cosmed USA website: http://bodyknow.ca/index.php/the-bod-pod/how-it-works
The predicted thoracic gas volume was calculated according to the participant’s height and age as described by Crapo et al (Crapo et al., 1982). The thoracic gas volume was accounted for to determine the non-gas body volume. Body density was calculated according to the equation: Density = Mass divided by Volume.

Following calculation of body density, fat mass (FM) and fat free mass (FFM) were calculated by the Bodpod software using the two-compartment body composition Siri model (Siri, 1961). Test results including whole body FM and FFM (percentage and mass in kg) were generated and displayed on the BodPod computer screen (Figure 3.2).

Figure 3.2 Results screen generated by the BodPod system (Cosmed USA, Inc). The subject identifiers are blocked out in the right panel.
3.4 Assessment of resting energy expenditure

3.4.1 Participant preparation

On the same morning as the body composition assessment (described above in section 3.3), participants underwent indirect calorimetry using the Quark RMR system (Cosmed USA Inc. Omnia software version 1.5) to measure resting energy expenditure (REE). Assessment of REE was performed in the morning to control for diurnal variation (Fredrix et al., 1990) and after an overnight (>12 hours) fast, to remove the component of energy expenditure that arises from the thermogenic effect of food (TEF) (D'Alessio et al., 1988).

In the days prior to testing, to ensure that true resting conditions (Compher et al., 2006, Haugen et al., 2007) were met, participants and/or patient carers were contacted by the investigator and asked to adhere to the following restrictions:

1) For 12 hours prior to testing:
   a. Fast i.e. consume nothing other than plain water
   b. Abstain from caffeine

2) For at least 2 hours prior to testing:
   a. Abstain from nicotine
   b. Abstain from physical exertion

If participants were experiencing an inter-current illness in the days prior to assessment, for example a viral infection, the assessment was postponed until they were fully recovered. On arrival at the RBWH clinic, participants were transported in a wheelchair to the research building (to maintain a resting state) through indoor corridors (to avoid large variations in their surrounding temperature and negate the metabolic effects of changes in thermoregulation). Participants were maintained in a seated position for at least 15 minutes prior to testing.
3.4.2 Prediction of REE

The predicted REE (pREE) was computed by the BodPod software according to the modified Nelson equation taking into account FM and FFM (as shown in section 2.2.1, Table 2.2A).

3.4.3 Indirect calorimetry procedure

The classic open-circuit ventilated-hood technique (Figure 3.3) was used according to standard methods (Ferrannini, 1988, Blond et al., 2010, von Hurst et al., 2016). Thorough quality control procedures, including flow and gas calibration, were carried out according to manufacturer’s instructions prior to assessment each morning.

The participant lay supine with a 30° head up angle with the canopy hood covering the face (Figure 3.4). The testing environment was controlled at 22°C and with minimal surrounding stimulation (to avoid an adrenergic stress response which could increase metabolic rate). The participant was instructed to breathe in a relaxed comfortable manner, to refrain from talking or moving and to stay awake during the recording.

The fraction of carbon dioxide in the expired air (FeCO₂) under the canopy was maintained between 0.9-1% by manual adjustment of the flow rate through the hood by the investigator. O₂ consumption (VO₂) and CO₂ production (VCO₂) were calculated as the difference between the expired air in the hood and room air. VO₂ and VCO₂ were measured every 10 seconds and energy expenditure was determined by COSMED Omnia V1.5 software according to the abbreviated Weir equation (Weir, 1949) (see section 2.2.5).

Recordings were collected for at least 20 minutes. The recording from the first 5 minutes of assessment, during which the participant became accustomed to the environment, was excluded from analysis. A stable epoch of no less than 15 minutes was selected to calculate the average REE in kcal/day.
Fig 3.3 Schematic of the indirect calorimetry system. This shows the canopy method in a spontaneously breathing subject (other systems exist for use in ventilated subjects). The subject is placed under a clear canopy with a plastic drape to avoid air leakage. The flow generator creates a constant outward flow through the canopy. The exhaled breath is diluted by the constant flow from the flow generator (L/min), and collected by the calorimeter for gas analysis. The fraction of expired air that is oxygen (FeO₂) and carbon dioxide (FeCO₂) is measured by the gas analyzer. The fraction of inspired air that is oxygen (FiO₂) and carbon dioxide and (FiCO₂) are measured in the ambient air. From these values, the volumes of consumed O₂ (VO₂) and produced CO₂ (VCO₂) are calculated. These values are used to calculate EE using the Weir equation (see section 2.2.5). (Arrows, respiratory gas flow; solid line, gas sampling; dotted line, signal for flow analysis). Adapted from Oshima et al (Oshima et al., 2016).
3.5 Longitudinal assessment

ALS patients were invited to attend a second visit 3–6 months following their first assessment. The second visit comprised repeat anthropometric and ADP assessment of body composition and assessment of disease progression as measured by the ALSFRS-R.
3.6 Statistical methods

Statistical analysis was performed by the investigator using Prism software version 7 (Graphpad Inc, CA, USA). For all outcomes, a $p$ value <0.05 was considered statistically significant.

3.6.1 Statistical methods for objective one

**Correlations between anthropometric measures and FM in ALS patients and controls**

Linear regression analysis was used to examine the relationships between each anthropometric measurement (BMI and BAI) and percent FM derived from ADP. Pearson’s correlation coefficient from univariate analysis was also determined for each relationship.

**Agreement between anthropometric measures and FM in ALS patients and controls**

The agreement between each anthropometric measure and percent FM derived from ADP was assessed by the Bland-Altman approach (Bland and Altman, 1986). The difference versus the mean of the two methods was plotted for each participant. The Bland-Altman analysis evaluates the bias between the mean differences of the two methods, and estimates an agreement interval, within which 95% of the differences of one method fall, compared to the other (Giavarina, 2015). In each plot the results were compared to the line of equality, which reflects complete agreement between methods.

**Longitudinal changes in anthropometric measures and FM in ALS patients**

The net change in BMI, BAI and percent FM derived from ADP between longitudinal assessments was calculated for each ALS patient. Linear regression analysis was used to examine the strength of the relationship between a change in BMI and BAI versus percent FM derived from ADP.
3.6.2 Statistical methods for objective two

Metabolic Index to define hypermetabolism:
pREE was derived from the modified Nelson equation (Chapter 2 section 2.2.1 table 2.2A). Metabolic index was calculated as:

\[
\frac{\text{measured REE (mREE)}}{\text{predicted REE (pREE)}} \times 100
\]

Hypermetabolism was defined as a metabolic index ≥ 120 (Perseghin et al., 2002). In this study, participants with a metabolic index <120 were defined as normometabolic. The proportion of each cohort that reached the definition for hypermetabolism was calculated. The metabolic indices of the ALS and control cohorts were displayed in a frequency histogram.

Clinical factors associated with hypermetabolism:
Demographic, energy expenditure, body composition and clinical parameters were compared between normometabolic and hypermetabolic ALS patients. Quantitative means were compared using an unpaired Student's t-test and ratios or percentages were compared using Fishers exact test.

Linear regression analysis was used to examine the relationships between the metabolic index and demographic, body composition and clinical parameters. Pearson’s correlation coefficient from univariate analysis was also determined for each relationship.

Longitudinal analysis:
For ALS patients who attended a second assessment, the individual net changes in ALSFRS-R scores and body composition data between the first and second assessment were calculated. Quantitative mean changes were compared between normometabolic and hypermetabolic patients using an unpaired Student’s t test.
Chapter 4: Results and summary of findings

4.1 Results for objective 1

A version of this section of the thesis was published in IOANNIDES, Z. A., STEYN, F. J., HENDERSON, R. D., MCCOMBE, P. A. & NGO, S. T. 2017. Anthropometric measures are not accurate predictors of fat mass in ALS. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, published online 27 Apr 2017. This paper can be found in Appendix 2.

4.1.1 Participant demographics and body composition data

44 ALS patients and 35 age- and sex-matched healthy control participants underwent anthropometric and body composition analysis as described in Chapter 3. Baseline demographic and clinical data is shown in table 4.1. No ALS patients were receiving enteral nutrition at the time of their first assessment.

Anthropometric and fat mass (FM, derived from air displacement plethysmography, ADP) data is shown in table 4.2. There were no significant differences in body mass index (BMI) or body adiposity index (BAI) between the ALS and control cohorts. Although a lower total body mass and higher percentage FM was observed in the ALS cohort, this did not reach statistical significance.
Table 4.1 Demographic and clinical data of 35 control and 44 ALS participants.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=35)</th>
<th>ALS (n=44)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:Female (n)</td>
<td>25:10 (71.43% male)</td>
<td>32:12 (72.73% male)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.48±1.18 (46.64-73.74)</td>
<td>61.09±1.32 (46.02-79.55)</td>
<td>0.37</td>
</tr>
<tr>
<td>Age of males (years)</td>
<td>59.35±1.53 (46.64-73.74)</td>
<td>59.49±1.61 (46.02-79.55)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Age of females (years)</td>
<td>59.86±1.37 (52.78-66.54)</td>
<td>65.37±1.81 (55.79-74.83)</td>
<td>0.23</td>
</tr>
<tr>
<td>Time Since Diagnosis (months)</td>
<td>N/A</td>
<td>9.90±1.58 (0.77-47.00)</td>
<td>N/A</td>
</tr>
<tr>
<td>ALSFRS-R (of 48)</td>
<td>N/A</td>
<td>38.50±0.64 (28.00-46.00)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Notes: Data presented as mean ± SEM (range). Exact age at assessment was calculated in years from date of birth to date of assessment. p value for comparison of controls and ALS patients; quantitative means compared using an unpaired Student’s t-test, age ratios compared using the Fishers exact test. Abbreviations: ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; N/A, not assessed; SEM, standard error of mean.
Table 4.2 Anthropometric and fat mass data of 35 control and 44 ALS participants.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=35)</th>
<th>ALS (n=44)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Mass (kg)</strong></td>
<td>82.23±3.11 (50.61-119.90)</td>
<td>76.37±2.16 (52.19-112.80)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.65±0.79 (17.93-37.25)</td>
<td>25.64±0.52 (19.17-34.13)</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>98.91±1.84 (81.00-123.00)</td>
<td>98.75±1.25 (81.00-116.00)</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>BAI</strong></td>
<td>24.84±0.81 (16.42-43.25)</td>
<td>25.93±0.79 (15.96-37.39)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Fat mass (%)</strong></td>
<td>30.55±1.36 (10.00-53.60)</td>
<td>34.01±1.73 (7.20-55.60)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Fat mass (kg)</strong></td>
<td>26.89±2.05 (5.07-57.97)</td>
<td>26.49±1.52 (5.63-51.46)</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Notes: Data presented as mean ± SEM (range). p value for comparison of controls and ALS patients using an unpaired Student’s t-test. Abbreviations: BAI, body adiposity index; BMI, body mass index; Fat mass determined by air displacement plethysmography.

4.1.2 Correlations between anthropometric measures and fat mass

Positive correlations were observed between BMI and percentage FM in controls ($r²=0.34; r=0.59; p<0.01$) and ALS patients ($r²=0.29; r=0.54; p<0.01$) as shown in figure 4.1A. Similarly, positive correlations were observed between BAI and percentage FM in controls ($r²=0.61; r=0.78; p<0.01$) and ALS patients ($r²=0.56; r=0.75; p<0.01$) as shown in figure 4.1B. The slopes of the regression lines of the ALS and control cohorts were significantly different (for comparison of BMI vs. FM slopes, $p=0.03$ figure 4.1A, and for comparison of BAI vs. FM slopes, $p=0.04$ figure 4.1B).
Figure 4.1 Correlations between percentage fat mass and body mass index (A) and body adiposity index (B). The slopes of the regression lines of the ALS and control cohorts were significantly different [for comparison of BMI vs. FM slopes, \( p=0.03 \) (A), for comparison of BAI vs. FM slopes, \( p=0.04 \) (B)]. Abbreviations: BAI, body adiposity index; BMI, body mass index. Fat mass as measured by air displacement plethysmography.
4.1.3 The agreement between anthropometric measures and fat mass

Bland-Altman analyses to examine the agreement between each anthropometric measurement and percentage FM, revealed a positive mean bias (MB) for all comparisons (figure 4.2A to 4.2D). The MBs of the ALS cohort were greater than those of the control cohort. For analyses comparing BMI to percentage FM, the MB of Controls = 4.22 and the MB of ALS patients = 8.38. For analyses comparing BAI to percentage FM, the MB of Controls = 5.91 and MB of ALS patients = 8.08.

The 95% confidence intervals of limits were greater in the ALS analyses (-12.14 to 28.89 for analyses comparing BMI to percentage FM and -9.06 to 25.23 for analyses comparing BAI to percentage FM, figure 4.2C and D) than the control analyses (-7.41 to 15.84 for analyses comparing BMI to percentage FM and -3.22 to 15.04 for analyses comparing BAI to percentage FM figure 4.2A and B).
Figure 4.2 Bland–Altman analyses of anthropometric measures against fat mass.

Controls are indicated by white circles (A and B) and ALS patients are indicated by blue circles (C and D). The mean bias (MB; broken red line) illustrates the average discrepancy between methods. Broken grey lines illustrate the 95% confidence interval of limits of agreement (mean bias ± 2 STD). Abbreviations: ALS, amyotrophic lateral sclerosis; BAI, body adiposity index; BMI, body mass index; MB, mean bias; STD, standard deviation. Fat mass refers to percentage fat mass determined by air displacement plethysmography.
4.1.4 Longitudinal assessment of ALS patients

29 ALS patients (22 male and 7 female) were re-assessed ~6 months (mean 5.65 ± 0.40, range of 2.80 to 9.73 months) following their initial assessment. Demographic, anthropometric and fat mass data is shown in Table 4.3. The mean ALSFRS-R score significantly decreased between assessments, reflecting a decline in function between the first and second assessment. One patient (patient 4 in table 4.4) commenced regular enteral nutrition via a percutaneous endoscopic gastrostomy (PEG) between assessments.

While the mean BMI did not change, the mean BAI increased between assessments (p=0.04). However, the increase in mean BAI was by less than 1 unit. The mean percentage and total FM (kg) did not change between assessments, however, individual increases or decreases in FM were observed. The individual changes in BMI, BAI and fat mass are shown in figure 4.3 and table 4.4. There was a wide range of change in both FM (kg) and percentage FM over the ~6 month interval. The net change of FM (kg) ranged from a loss of 9.36kg fat to a gain of 6.96kg fat (Table 4.4).

The change in FM was not consistently reflected by either anthropometric measurement. In some individuals the direction of change of BMI or BAI was opposite to the direction of change in FM (Figure 4.4 and table 4.4).
Table 4.3 Longitudinal demographic, anthropometric and fat mass data of 29 ALS patients.

<table>
<thead>
<tr>
<th></th>
<th>1st assessment</th>
<th>2nd assessment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at Assessment (years)</strong></td>
<td>60.60±1.65 (46.04-79.55)</td>
<td>61.06±1.65 (46.49-79.82)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Time from Diagnosis (months)</strong></td>
<td>10.84±2.12 (0.90-47.10)</td>
<td>16.48±2.14 (4.60-51.07)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>ALSFRS-R (out of 48)</strong></td>
<td>38.48±0.75 (28.00-45.00)</td>
<td>36.79±0.77 (26.00-45.00)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Body Mass (kg)</strong></td>
<td>77.01±2.78 (55.50-112.80)</td>
<td>76.36±3.02 (55.64-114.90)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.45±0.68 (20.18-34.13)</td>
<td>25.25±0.80 (18.10-34.78)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>97.12±1.66 (81.00-116.00)</td>
<td>97.91±1.83 (82.50-114.00)</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>BAI</strong></td>
<td>24.70±0.96 (15.96-35.63)</td>
<td>25.40±1.05 (15.93-36.80)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Fat mass (%)</strong></td>
<td>33.07±2.00 (11.70-50.40)</td>
<td>32.90±2.13 (7.70-49.80)</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Fat mass (kg)</strong></td>
<td>25.81±1.93 (6.94-51.46)</td>
<td>25.66±2.07 (4.42-49.84)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Notes: Data presented as mean ± SEM (range). p value for comparison of measures between 1st and 2nd assessment using a paired Student's t-test. Abbreviations: ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; BAI, body adiposity index; BMI, body mass index; N/A, not assessed. Fat mass determined by air displacement plethysmography.
Figure 4.3 Longitudinal changes in fat mass and anthropometric measures. Scatter plots showing net changes in percentage fat mass (A) and BMI and BAI (B) in 29 ALS patients over a ~6 months interval (mean 5.65 ± 0.40, range of 2.80 to 9.73 months). Abbreviations: BAI, body adiposity index, BMI, body mass index; FM, fat mass determined by air displacement plethysmography.
### Table 4.4 Individual longitudinal anthropometric and fat mass data of 29 ALS patients

Net change in values ($\Delta$) from first assessment to the second assessment after a ~6 month interval (mean 5.65 ± 0.40, range of 2.80 to 9.73 months). 1st refers to the measure at the first assessment.

<table>
<thead>
<tr>
<th>Patient number and sex</th>
<th>Assessment interval (months)</th>
<th>1st BMI</th>
<th>Δ BMI</th>
<th>1st BAI</th>
<th>Δ BAI</th>
<th>1st FM (%)</th>
<th>Δ FM (%)</th>
<th>1st FM (kg)</th>
<th>Δ FM (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>6.30</td>
<td>26.78</td>
<td>1.07</td>
<td>25.37</td>
<td>0.42</td>
<td>29.50</td>
<td>2.40</td>
<td>25.00</td>
<td>3.14</td>
</tr>
<tr>
<td>2 M</td>
<td>5.30</td>
<td>20.94</td>
<td>1.18</td>
<td>25.89</td>
<td>-1.33</td>
<td>15.70</td>
<td>8.70</td>
<td>9.70</td>
<td>6.28</td>
</tr>
<tr>
<td>3 M</td>
<td>9.57</td>
<td>23.07</td>
<td>-1.05</td>
<td>18.64</td>
<td>0.07</td>
<td>27.10</td>
<td>-1.50</td>
<td>21.86</td>
<td>-1.86</td>
</tr>
<tr>
<td>4 M*</td>
<td>7.50</td>
<td>22.44</td>
<td>-3.02</td>
<td>19.46</td>
<td>-0.66</td>
<td>18.10</td>
<td>-10.40</td>
<td>12.01</td>
<td>-7.59</td>
</tr>
<tr>
<td>5 M</td>
<td>2.80</td>
<td>24.16</td>
<td>0.46</td>
<td>23.09</td>
<td>2.52</td>
<td>28.80</td>
<td>1.60</td>
<td>22.20</td>
<td>1.63</td>
</tr>
<tr>
<td>6 M</td>
<td>6.77</td>
<td>27.33</td>
<td>0.86</td>
<td>23.02</td>
<td>3.81</td>
<td>33.00</td>
<td>0.90</td>
<td>28.42</td>
<td>1.68</td>
</tr>
<tr>
<td>7 F</td>
<td>7.70</td>
<td>31.45</td>
<td>-0.08</td>
<td>25.97</td>
<td>-1.18</td>
<td>31.60</td>
<td>-0.60</td>
<td>34.58</td>
<td>-0.73</td>
</tr>
<tr>
<td>8 M</td>
<td>9.73</td>
<td>25.72</td>
<td>-0.75</td>
<td>24.76</td>
<td>-2.16</td>
<td>39.50</td>
<td>-0.60</td>
<td>31.10</td>
<td>-1.38</td>
</tr>
<tr>
<td>9 F</td>
<td>8.40</td>
<td>32.18</td>
<td>0.67</td>
<td>32.11</td>
<td>-0.86</td>
<td>44.30</td>
<td>0.10</td>
<td>43.64</td>
<td>1.00</td>
</tr>
<tr>
<td>10 M</td>
<td>7.27</td>
<td>25.72</td>
<td>-5.07</td>
<td>21.16</td>
<td>0.42</td>
<td>30.30</td>
<td>-6.90</td>
<td>24.68</td>
<td>-9.36</td>
</tr>
<tr>
<td>11 F</td>
<td>8.33</td>
<td>20.18</td>
<td>-0.25</td>
<td>18.07</td>
<td>0.67</td>
<td>11.70</td>
<td>-0.30</td>
<td>6.94</td>
<td>-0.26</td>
</tr>
<tr>
<td>12 M</td>
<td>7.80</td>
<td>23.50</td>
<td>0.38</td>
<td>21.99</td>
<td>0.00</td>
<td>42.20</td>
<td>2.10</td>
<td>29.71</td>
<td>1.99</td>
</tr>
<tr>
<td>13 F</td>
<td>6.77</td>
<td>24.23</td>
<td>-0.49</td>
<td>21.31</td>
<td>0.00</td>
<td>30.70</td>
<td>0.50</td>
<td>22.76</td>
<td>-0.10</td>
</tr>
<tr>
<td>14 F</td>
<td>6.07</td>
<td>27.45</td>
<td>0.93</td>
<td>25.76</td>
<td>1.53</td>
<td>41.20</td>
<td>2.00</td>
<td>34.00</td>
<td>2.92</td>
</tr>
<tr>
<td>15 M</td>
<td>6.60</td>
<td>22.49</td>
<td>-0.14</td>
<td>22.41</td>
<td>0.00</td>
<td>27.70</td>
<td>-0.90</td>
<td>17.60</td>
<td>-0.66</td>
</tr>
<tr>
<td>16 F</td>
<td>5.87</td>
<td>23.08</td>
<td>0.23</td>
<td>18.95</td>
<td>0.85</td>
<td>21.20</td>
<td>1.60</td>
<td>15.34</td>
<td>1.31</td>
</tr>
<tr>
<td>17 M</td>
<td>5.43</td>
<td>21.67</td>
<td>-0.66</td>
<td>15.96</td>
<td>0.98</td>
<td>12.30</td>
<td>-1.30</td>
<td>9.28</td>
<td>-1.24</td>
</tr>
<tr>
<td>18 M</td>
<td>2.80</td>
<td>34.13</td>
<td>0.65</td>
<td>27.69</td>
<td>0.41</td>
<td>45.60</td>
<td>-2.20</td>
<td>51.46</td>
<td>-1.62</td>
</tr>
<tr>
<td>19 M</td>
<td>3.53</td>
<td>20.54</td>
<td>-2.44</td>
<td>16.94</td>
<td>-1.01</td>
<td>22.90</td>
<td>-5.30</td>
<td>15.76</td>
<td>-5.11</td>
</tr>
<tr>
<td>20 F</td>
<td>3.23</td>
<td>28.27</td>
<td>-0.47</td>
<td>26.35</td>
<td>-0.29</td>
<td>34.10</td>
<td>-2.80</td>
<td>27.40</td>
<td>-2.96</td>
</tr>
<tr>
<td>21 M</td>
<td>3.27</td>
<td>27.76</td>
<td>0.88</td>
<td>21.42</td>
<td>2.76</td>
<td>33.20</td>
<td>0.80</td>
<td>31.87</td>
<td>1.83</td>
</tr>
<tr>
<td>22 M</td>
<td>2.87</td>
<td>31.19</td>
<td>0.30</td>
<td>25.69</td>
<td>0.62</td>
<td>37.70</td>
<td>1.20</td>
<td>38.12</td>
<td>1.51</td>
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<tr>
<td>23 M</td>
<td>3.07</td>
<td>24.64</td>
<td>1.16</td>
<td>30.31</td>
<td>5.26</td>
<td>34.60</td>
<td>8.50</td>
<td>22.77</td>
<td>6.96</td>
</tr>
<tr>
<td>24 M</td>
<td>5.23</td>
<td>27.34</td>
<td>-0.78</td>
<td>35.63</td>
<td>-1.03</td>
<td>43.10</td>
<td>-1.50</td>
<td>28.68</td>
<td>-1.77</td>
</tr>
<tr>
<td>25 M</td>
<td>6.60</td>
<td>24.85</td>
<td>-0.53</td>
<td>29.26</td>
<td>0.23</td>
<td>47.00</td>
<td>-0.40</td>
<td>32.57</td>
<td>-0.97</td>
</tr>
<tr>
<td>26 M</td>
<td>3.53</td>
<td>22.10</td>
<td>-1.78</td>
<td>28.49</td>
<td>-1.11</td>
<td>49.40</td>
<td>-5.00</td>
<td>28.82</td>
<td>-4.99</td>
</tr>
<tr>
<td>27 M</td>
<td>4.37</td>
<td>25.49</td>
<td>1.39</td>
<td>33.34</td>
<td>2.54</td>
<td>43.50</td>
<td>1.00</td>
<td>27.35</td>
<td>2.14</td>
</tr>
<tr>
<td>28 M</td>
<td>3.97</td>
<td>27.87</td>
<td>1.25</td>
<td>33.89</td>
<td>2.91</td>
<td>50.40</td>
<td>-0.60</td>
<td>36.83</td>
<td>1.23</td>
</tr>
<tr>
<td>29 M</td>
<td>3.03</td>
<td>21.54</td>
<td>0.33</td>
<td>23.31</td>
<td>3.93</td>
<td>32.60</td>
<td>4.00</td>
<td>18.07</td>
<td>2.54</td>
</tr>
</tbody>
</table>

Notes: *Patient number 4 began receiving regular enteral nutrition via a percutaneous endoscopic gastrostomy (PEG) 5 months after the first assessment. Abbreviations: BAI, body adiposity index; BMI, body mass index; F, female; FM, fat mass determined by air displacement plethysmography; M, male.
Positive correlations were observed between the change in both anthropometric measurements and the change in percentage FM. A change in BMI versus a change in percentage FM is shown in figure 4.4A ($r^2=0.62$ $r=0.79$ $p<0.01$). A change in BAI versus a change in percentage FM is shown in figure 4.4B ($r^2=0.20$ $r=0.44$ $p=0.02$).

**Figure 4.4** Correlations between the net changes in anthropometric measures and percentage fat mass. Linear regression showing 95% confidence band of line of best fit for change in BMI (A) or change in BAI (B) relative to change in the percentage fat mass determined by air displacement plethysmography. Male and female ALS patients are indicated in blue and red respectively. Abbreviations: BAI, body adiposity index; BMI, body mass index; FM, fat mass.
4.1.5 Summary of findings

Using ADP as the standard, the accuracy of BMI and BAI in predicting adiposity was assessed in 44 ALS patients. The accuracy in ALS patients was compared to that of 35 healthy age- and sex-matched controls. Longitudinal assessments were performed in 29 ALS patients to determine the accuracy of BMI and BAI in predicting changes in FM over time.

While BMI and BAI correlated with percentage FM, Bland-Altman analyses showed that there was a lack of agreement between either anthropometric measure and percentage FM. BMI and BAI were both associated with a positive bias when compared to percentage FM. Furthermore, comparisons of the results between the ALS patients and healthy matched controls suggest that both anthropometric measurements are worse predictors of FM in ALS than in controls.

In a longitudinal assessment of ALS patients there was no change in the mean FM, although individual changes in FM ranging from a loss of 9.36kg to a gain of 6.96kg were observed. A change in BMI and BAI correlated with a change in FM. However, neither BMI nor BAI consistently reflected the change in FM. Indeed, the direction of change of BMI and BAI was opposite to the direction of change in FM in some patients.

These results indicate that an isolated measure of BMI and BAI is not an accurate indicator of adiposity in ALS and that assessing longitudinal changes in these anthropometric measures could be misleading.
4.2 Results for objective 2

Fifty ALS patients and 50 age- and sex-matched controls underwent assessment of body composition and resting energy expenditure (REE) as described in Chapter 3.

4.2.1 Clinical characteristics of the ALS patients

The clinical characteristics of the ALS patients are shown in Table 4.5. In the majority of patients, the lower limbs were the site of onset of disease. One female ALS patient, aged 72, was known to be a carrier of the \( C9orf72 \) mutation and had a diagnosis of frontotemporal dementia (FTD). The mean duration of disease from symptom onset was 25.84 ± 2.71 (2.53–89.73) months at the time of assessment. The average ALSFRS-R score was 38.04 ± 0.55 (28–46). Eighteen patients (36%) reported significant weight loss at the time of onset of first ALS symptom. One ALS patient had been receiving enteral nutrition via a PEG for 2 months prior to assessment.
Table 4.5 Clinical data for 50 ALS patients.

<table>
<thead>
<tr>
<th>Disease parameters</th>
<th>ALS Total cohort (n=50)</th>
<th>ALS Male cohort (n=34)</th>
<th>ALS Female cohort (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UL onset n (%)</td>
<td>14 (28.00)</td>
<td>12 (35.29)</td>
<td>2 (12.50)</td>
</tr>
<tr>
<td>LL onset n (%)</td>
<td>21 (42.00)</td>
<td>13 (38.24)</td>
<td>8 (50.00)</td>
</tr>
<tr>
<td>Bulbar onset n (%)</td>
<td>14 (28.0)</td>
<td>8 (22.53)</td>
<td>6 (37.50)</td>
</tr>
<tr>
<td>Resp onset n (%)</td>
<td>1 (2.00)</td>
<td>1 (2.94)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Disease duration</td>
<td>25.84±2.71 (2.53-89.73)</td>
<td>26.65±3.37 (2.53-89.73)</td>
<td>24.14±4.66 (6.83-82.47)</td>
</tr>
<tr>
<td>ALSFRS-R scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score (of 48)</td>
<td>38.04±0.55 (28-46)</td>
<td>38.47±0.59 (32-46)</td>
<td>37.13±1.17 (28-43)</td>
</tr>
<tr>
<td>Bulb score (of 12)</td>
<td>10.28±0.29 (3-12)</td>
<td>10.38±0.32 (3-12)</td>
<td>10.06±0.61 (3-12)</td>
</tr>
<tr>
<td>UL score (of 8)</td>
<td>6.30±0.26 (2-8)</td>
<td>6.21±0.32 (2-8)</td>
<td>6.50±0.43 (3-8)</td>
</tr>
<tr>
<td>LL score (of 8)</td>
<td>4.50±0.29 (1-8)</td>
<td>4.94±0.33 (3-8)</td>
<td>3.56±0.52 (1-8)</td>
</tr>
<tr>
<td>Resp score (of 12)</td>
<td>11.18±0.22 (6-12)</td>
<td>11.00±0.32 (6-12)</td>
<td>11.56±0.18 (10-12)</td>
</tr>
<tr>
<td>Clinical examination scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UMN score (of 12)</td>
<td>6.28±0.42 (0-12)</td>
<td>6.50±0.53 (0-12)</td>
<td>5.80±0.74 (0-10)</td>
</tr>
<tr>
<td>LMN score (of 12)</td>
<td>3.40±0.35 (0-11)</td>
<td>3.32±0.39 (0-8)</td>
<td>3.56±0.74 (0-11)</td>
</tr>
<tr>
<td>Respiratory function tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seated FVC (% of predicted)</td>
<td>91.64±2.84 (59-129)</td>
<td>93.76±3.26 (64-129)</td>
<td>87.13±5.55 (59-127)</td>
</tr>
<tr>
<td>Postural change in FVC (%)</td>
<td>-11.34±1.62 (-55.38-8.23)</td>
<td>-11.96±1.95 (-55.38-2.57)</td>
<td>-9.95±2.95 (-34.44-8.23)</td>
</tr>
<tr>
<td>SNIP (% of predicted)</td>
<td>60.15±3.42 (16-108)</td>
<td>60.29±4.65 (16-108)</td>
<td>59.88±4.67 (30-99)</td>
</tr>
</tbody>
</table>

Notes: Data presented as mean ± SEM (range). Disease duration refers to the interval in months between symptom onset and assessment. ALSFRS-R sub-scores determined as described by Pinto et al (Pinto and de Carvalho, 2015). Clinical examination scores (UMN and LMN scores) determined as described by Ravits et al (Ravits et al., 2007). Abbreviations: UL, upper limb; LL, lower limb; ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; Bulb, bulbar; Resp, respiratory; UMN, upper motor neurone; LMN, lower motor neurone; FVC, forced vital capacity; SNIP, maximal sniff nasal inspiratory pressure.
4.2.2 Body composition and REE data

Demographic and body composition data of all participants are presented in Tables 4.6 A to C. There was no difference between the control and ALS cohorts in sex ratio or age. The mean weight and BMI of the ALS cohort were lower than the controls, however, these did not reach statistical significance (Table 4.6 A).

The mean fat free mass (FFM) in kg of ALS patients was significantly lower than that of controls (Table 4.6 A). The mean weight, BMI and FFM (kg) of the male ALS cohort were significantly lower than the male control cohort (Table 4.6 B). When compared to the female control cohort, the mean percentage FFM of the female ALS cohort was lower and the mean percentage FM was higher.

REE data for the total, male and female cohorts of the ALS and control participants is shown in Table 4.6 A to C. The predicted REE (pREE, derived from the modified Nelson equation as described in Chapter 2 section 2.2.1 and table 2.2) was significantly lower in the total, male and female ALS cohort compared to the respective control cohort. There was no difference in the mean mREE between each ALS cohort and the respective control cohort. The mean mREE corrected for FFM (REE/FFM) of the each ALS cohort was higher than the respective control cohort, however, only the difference between the REE/FFM of the male ALS and male control cohorts was statistically significant.

Metabolic index data [measured REE (mREE) / pREE x 100] is shown in Table 4.6 A to C). The mean metabolic index of the total ALS cohort was higher than that of the total control cohort however this did not reach statistical significance. The metabolic index of the male ALS cohort was significantly higher than that of the male control cohort (p<0.01).
Tables 4.6 A to C: Age, body composition and resting energy expenditure data for the control and ALS cohorts. Table A shows data for the total ALS and control cohorts, B shows data for the male cohorts and C shows data for the female cohorts.

Table 4.6 A. Age, body composition and resting energy expenditure data for the total control and total ALS cohorts

<table>
<thead>
<tr>
<th></th>
<th>Control Total cohort (n= 50)</th>
<th>ALS Total cohort (n=50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
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<td>61.74±1.21 (46.02-79.55)</td>
<td>0.16</td>
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<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.42±2.23 (52.15-119.90)</td>
<td>76.66±2.06 (48.77-112.80)</td>
<td>0.06</td>
</tr>
<tr>
<td>BMI (kg/m)</td>
<td>27.03±0.59 (19.27-37.25)</td>
<td>25.96±0.60 (19.00-42.49)</td>
<td>0.21</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>55.91±1.65 (35.26-74.49)</td>
<td>50.27±1.56 (29.53-74.82)</td>
<td>0.01</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>26.57±1.38 (11.79-55.01)</td>
<td>27.63±1.58 (3.10-66.25)</td>
<td>0.62</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>68.11±1.23 (46.30-85.00)</td>
<td>64.68±1.66 (35.10-92.80)</td>
<td>0.10</td>
</tr>
<tr>
<td>FM (%)</td>
<td>31.87±1.23 (15.00-53.70)</td>
<td>35.31±1.67 (7.20-64.90)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Resting energy expenditure data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pREE</td>
<td>1549±43.27 (1016-2105)</td>
<td>1392±40.03 (878-2070)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>mREE (kcal per day)</td>
<td>1669±53.47 (817-2413)</td>
<td>1602±64.00 (641-2532)</td>
<td>0.43</td>
</tr>
<tr>
<td>mREE/FFM (kcal/kg per day)</td>
<td>30.02±0.63 (17.93-40.14)</td>
<td>32.12±1.00 (11.57-45.59)</td>
<td>0.08</td>
</tr>
<tr>
<td>Metabolic Index</td>
<td>107.81±2.04 (65.20-139.27)</td>
<td>114.47±3.05 (62.48-147.73)</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table 4.6 B. Age, body composition and resting energy expenditure data for the male control and male ALS cohorts

<table>
<thead>
<tr>
<th></th>
<th>Control Male cohort (n= 34)</th>
<th>ALS Male cohort (n=34)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>58.65±1.37 (42.41-73.74)</td>
<td>60.39±1.60 (46.02-79.55)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>89.50±1.95 (67.42-119.90)</td>
<td>80.92±2.40 (52.19-112.80)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m)</td>
<td>27.90±0.54 (20.36-35.04)</td>
<td>25.90±0.65 (19.00-34.13)</td>
<td>0.02</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>62.89±1.08 (46.13-74.49)</td>
<td>55.93±1.22 (40.64-74.82)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>26.69±1.49 (11.79-52.80)</td>
<td>25.87±1.84 (3.10-51.46)</td>
<td>0.73</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>70.77±1.15 (52.20-85.00)</td>
<td>69.36±1.73 (54.40-92.80)</td>
<td>0.50</td>
</tr>
<tr>
<td>FM (%)</td>
<td>29.23±1.15 (15.00-47.80)</td>
<td>30.63±1.74 (7.20-45.60)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Resting energy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expenditure data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pREE</td>
<td>1729±29.23 (1318-2105)</td>
<td>1547±32.53 (1169-2070)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>mREE (kcal per day)</td>
<td>1870±39.52 (1246-2413)</td>
<td>1827±54.60 (1235-2532)</td>
<td>0.52</td>
</tr>
<tr>
<td>mREE/FFM (kcal/kg per day)</td>
<td>29.82±0.53 (23.61-37.68)</td>
<td>32.78±0.95 (23.56-42.19)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Metabolic Index</td>
<td>108.25±1.67 (87.68-131.87)</td>
<td>118.23±2.83 (89.49-147.46)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table 4.6 C. Age, body composition and resting energy expenditure data for the female control and female ALS cohorts

<table>
<thead>
<tr>
<th></th>
<th>Control Female cohort (n= 16)</th>
<th>ALS Female cohort (n=16)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>61.21±1.57 (52.81-74.05)</td>
<td>64.60±1.47 (55.79-74.83)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.39±3.27 (52.15-102.60)</td>
<td>67.60±2.40 (48.77-102.10)</td>
<td>0.96</td>
</tr>
<tr>
<td>BMI (kg/m)</td>
<td>25.20±1.36 (19.27-37.25)</td>
<td>26.09±1.31 (19.29-42.49)</td>
<td>0.64</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>41.07±0.82 (35.26-47.64)</td>
<td>38.23±1.93 (29.53-62.93)</td>
<td>0.19</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>26.32±3.00 (12.58-55.01)</td>
<td>31.37±2.85 (17.47-66.25)</td>
<td>0.23</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>62.46±2.46 (46.30-76.00)</td>
<td>54.74±2.13 (35.40-67.40)</td>
<td>0.02</td>
</tr>
<tr>
<td>FM (%)</td>
<td>37.49±2.48 (15.00-47.80)</td>
<td>45.26±2.14 (32.60-64.90)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Resting energy expenditure data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pREE</td>
<td>1166±26.48 (1016-1451)</td>
<td>1063±28.30 (878-1255)</td>
<td>0.01</td>
</tr>
<tr>
<td>mREE (kcal per day)</td>
<td>1240±62.64 (817-1619)</td>
<td>1125±74.79 (641-1562)</td>
<td>0.25</td>
</tr>
<tr>
<td>mREE/FFM (kcal/kg per day)</td>
<td>30.42±1.66 (17.93-40.14)</td>
<td>32.78±0.95 (23.56-42.19)</td>
<td>0.93</td>
</tr>
<tr>
<td>Metabolic Index</td>
<td>106.87±5.43 (65.20-139.27)</td>
<td>106.46±7.17 (62.48-147.73)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Notes for tables 4.6 A to C: All data is presented as mean ± SEM (range). Metabolic index, mREE/pREE x 100. p values from an unpaired students t-test. Abbreviations: ALSFRS-R, the revised ALS functional rating scale; BMI, body mass index; FFM, Fat-free mass; FM, fat mass; kcal, kilocalories; mREE, measured REE; pREE, predicted REE according to the modified Nelson equation (as described in Chapter 2 section 2.2.1 and table 2.2).
4.2.3 The incidence of hypermetabolism

The incidence of hypermetabolism (defined as metabolic index $\geq 120$) is shown in Figure 4.5 A. Hypermetabolism was observed in 8 (16%) of the controls and 20 (40%) of the ALS patients. Four (50%) of the hypermetabolic controls were males and 15 (75%) of the hypermetabolic ALS patients were males. The pattern and range of spread of metabolic index in both cohorts are shown in Figure 4.5 B, where it can be seen that the frequency histogram of the ALS cohort is shifted to the right compared to that of the control cohort. The female ALS patient with FTD had a metabolic index of 66.36.

![Bar chart showing the incidence of hypermetabolism and normometabolism](image1)

**A: The incidence of hypermetabolism**

![Frequency histogram showing the spread of metabolic index](image2)

**B: The distribution of metabolic index**

**Figure 4.5 Metabolic index of controls and ALS patients.** (A) Bar chart showing the incidence of hypermetabolism (defined as metabolic index $\geq 120$) and normometabolism (defined as metabolic index < 120). (B) Frequency histogram showing the spread of metabolic index in control (white bars) and ALS (blue bars) cohorts. The distribution of the ALS cohort is shifted to the right compared to that of the control cohort. Abbreviation: ALS, amyotrophic lateral sclerosis.
4.2.4 Clinical correlates of hypermetabolism

Comparison of the characteristics of hypermetabolic and normometabolic ALS patients

Table 4.7 compares the parameters of the hypermetabolic ALS patients with those of the normometabolic ALS patients. There was no difference in age, sex ratio, body composition data or clinical data between hypermetabolic patients and normometabolic patients. mREE was significantly higher in hypermetabolic patients.

Table 4.7 Comparison of demographic, anthropometric and energy expenditure data in normometabolic and hypermetabolic ALS patients.

<table>
<thead>
<tr>
<th></th>
<th>Normometabolic ALS patients (n=30)</th>
<th>Hypermetabolic ALS patients (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.02±1.61 (46-80)</td>
<td>59.82±1.77 (46-76)</td>
<td>0.20</td>
</tr>
<tr>
<td>Smoker: non smoker ratio</td>
<td>1:29</td>
<td>2:18</td>
<td>0.56</td>
</tr>
<tr>
<td>Male: Female ratio</td>
<td>19:11</td>
<td>15:5</td>
<td>0.54</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.99±2.77 (48.77-109.41)</td>
<td>80.66±2.92 (58.35-112.81)</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI</td>
<td>25.34±0.68 (18.10-32.15)</td>
<td>26.89±1.10 (20.54-42.49)</td>
<td>0.21</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>25.45±1.83 (3.10-41.29)</td>
<td>30.91±2.73 (9.27-66.25)</td>
<td>0.09</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>50.62±2.08 (31.30-74.82)</td>
<td>49.74±2.51 (29.52-66.10)</td>
<td>0.79</td>
</tr>
<tr>
<td>mREE (kcal/day)</td>
<td>1414±75.34 (641-2099)</td>
<td>1883±80.32 (1280-2532)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>pREE (kcal/day)</td>
<td>1381±54.58 (878-2070)</td>
<td>1409±59.08 (878-1791)</td>
<td>0.75</td>
</tr>
<tr>
<td>Metabolic Index</td>
<td>101.35±3.07 (62.48-199.84)</td>
<td>134.14±2.12 (120.40-147.73)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Notes: Data presented as mean ± SEM (range). Metabolic Index; mREE/pREE x 100; p value from an unpaired Student’s t-test for comparison of quantitative means and from Fishers exact test for comparison of ratios. Abbreviations: BMI, body mass index; kcal, kilocalories; mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure according to the modified Nelson equation as described in Chapter 2 section 2.2.1 and table 2.2.
Clinical data is shown in Table 4.8. There was no difference in disease duration, the ratio of individuals taking riluzole, the incidence of self-reported weight loss at symptom onset or site of disease onset between hypermetabolic and normometabolic patients.

There was no difference in the ALSFRS-R scores between hypermetabolic and normometabolic patients. Regarding the clinical examination scores as described by Ravits et al (Ravits et al., 2007), the total lower motor neurone (LMN) score was higher in the hypermetabolic patients than the normometabolic patients ($p=0.04$). The mean upper motor neurone (UMN) score was lower in the hypermetabolic group but not statistically significantly so.
Table 4.8 Comparison of clinical data in normometabolic and hypermetabolic ALS patients.

<table>
<thead>
<tr>
<th></th>
<th>Normometabolic ALS patients (n=30)</th>
<th>Hypermetabolic ALS patients (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (months)</td>
<td>27.98±3.57 (6.83-89.73)</td>
<td>22.84±4.31 (2.53-86.43)</td>
<td>0.37</td>
</tr>
<tr>
<td>Taking riluzole: not taking riluzole ratio</td>
<td>14:16</td>
<td>9:11</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Self-reported weight loss:no weight loss ratio</td>
<td>12:18</td>
<td>6:14</td>
<td>0.56</td>
</tr>
<tr>
<td>Limb onset: other site of onset</td>
<td>19:11</td>
<td>16:4</td>
<td>0.35</td>
</tr>
<tr>
<td>Bulbar onset: other site of onset</td>
<td>10:20</td>
<td>4:16</td>
<td>0.35</td>
</tr>
<tr>
<td>Respiratory onset: other site of onset</td>
<td>1:29</td>
<td>0:20</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

**ALSFRS-R Scores**

<table>
<thead>
<tr>
<th></th>
<th>Normometabolic ALS patients (n=30)</th>
<th>Hypermetabolic ALS patients (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total score (of 48)</td>
<td>38.53±0.73 (28-46)</td>
<td>37.33±0.89 (28-45)</td>
<td>0.29</td>
</tr>
<tr>
<td>Bulbar subscore (of 12)</td>
<td>10.37±2.17 (3-12)</td>
<td>10.15±1.87 (7-12)</td>
<td>0.72</td>
</tr>
<tr>
<td>Upper limb subscore (of 8)</td>
<td>6.47±1.89 (2-8)</td>
<td>6.05±1.70 (3-8)</td>
<td>0.43</td>
</tr>
<tr>
<td>Lower limb subscore (of 8)</td>
<td>4.70±0.41 (1-8)</td>
<td>4.20±0.39 (1-8)</td>
<td>0.54</td>
</tr>
<tr>
<td>Respiratory subscore (of 12)</td>
<td>11.07±1.32 (6-12)</td>
<td>11.35±0.28 (8-12)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**Clinical examination scores**

<table>
<thead>
<tr>
<th></th>
<th>Normometabolic ALS patients (n=30)</th>
<th>Hypermetabolic ALS patients (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMN score (of 12)</td>
<td>6.38±0.56 (0-12)</td>
<td>2.88±0.66 (0-10)</td>
<td>0.85</td>
</tr>
<tr>
<td>LMN score (of 12)</td>
<td>2.97±0.42 (0-8)</td>
<td>4.42±0.57 (1-11)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Respiratory function tests**

<table>
<thead>
<tr>
<th></th>
<th>Normometabolic ALS patients (n=30)</th>
<th>Hypermetabolic ALS patients (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seated FVC (% of predicted)</td>
<td>91.64±2.84 (59-129)</td>
<td>93.76±3.26 (64-129)</td>
<td>0.30</td>
</tr>
<tr>
<td>Postural change in FVC (%)</td>
<td>-11.34±1.62 (-55.38-8.23)</td>
<td>-11.96±1.95 (-55.38-2.57)</td>
<td>0.45</td>
</tr>
<tr>
<td>SNIP (% of predicted)</td>
<td>60.15±3.42 (16-108)</td>
<td>60.29±4.65 (16-108)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Notes: Data presented as mean ± SEM (range). ‘Taking riluzole’ was defined as taking riluzole regularly as prescribed for at least 1 month prior to assessment. ALSFRS-R sub-scores as described by Pinto et al (Pinto and de Carvalho, 2015). p value from an unpaired Student’s t-test for comparison of quantitative means and from Fishers exact test for comparison of ratios. Abbreviations: ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; UMN, upper motor neurone; LMN, lower motor neurone; UMN and LMN scores determined as described by Ravits et al (Ravits et al., 2007); FVC, forced vital capacity; SNIP, maximal sniff nasal inspiratory pressure.
Clinical correlations of metabolic parameters in ALS patients

Correlation coefficients from univariate analyses of the REE variables of ALS patients versus age at assessment, age at diagnosis of ALS, body-composition variables, ALSFRS-R score, UMN and LMN scores, and respiratory function tests are shown in Table 4.9.

The mREE significantly correlated with weight and FFM. However, when the REE was corrected for FFM (mREE/FFM), the only variable that significantly correlated with mREE/FFM was the total LMN score. Similarly, the only variable that significantly correlated with the metabolic index was the total LMN score. In contrast, the total UMN score poorly correlated with mREE, mREE/FFM and the metabolic index. Linear regression plots of the metabolic index versus the ALSFRS-R, FVC and, UMN and LMN scores are shown in Figure 4.6.
Table 4.9 Correlation coefficients for REE variables against age, composition and clinical parameters. Correlation coefficients from univariate analyses. *p<0.05

<table>
<thead>
<tr>
<th></th>
<th>Measured REE</th>
<th>Measured REE/FFM</th>
<th>Metabolic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at assessment</td>
<td>-0.42</td>
<td>-0.18</td>
<td>-0.19</td>
</tr>
<tr>
<td>Age at diagnosis of ALS</td>
<td>-0.40</td>
<td>-0.19</td>
<td>-0.19</td>
</tr>
<tr>
<td>Weight</td>
<td>*0.67</td>
<td>0.30</td>
<td>0.27</td>
</tr>
<tr>
<td>BMI</td>
<td>0.31</td>
<td>0.30</td>
<td>0.19</td>
</tr>
<tr>
<td>FFM</td>
<td>*0.66</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>ALSFRS-R</td>
<td>0.06</td>
<td>-0.25</td>
<td>-0.14</td>
</tr>
<tr>
<td>UMN score</td>
<td>0.15</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>LMN score</td>
<td>0.22</td>
<td>*0.43</td>
<td>*0.43</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>-0.04</td>
<td>-0.13</td>
<td>-0.14</td>
</tr>
<tr>
<td>SNIP (% predicted)</td>
<td>-0.02</td>
<td>-0.06</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

Notes: UMN and LMN scores determined as described by Ravits et al (Ravits et al., 2007). Abbreviations: BMI, body mass index; FFM, fat free mass; ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; UMN, upper motor neurone; LMN, lower motor neurone. FVC, forced vital capacity; SNIP, maximal sniff nasal inspiratory pressure; REE, resting energy expenditure; Metabolic Index; measured resting energy expenditure (REE)/predicted REE x 100.
Figure 4.6 Correlations between the metabolic index and clinical parameters in ALS patients. A significant correlation was observed between the LMN score and metabolic index (measured REE/predicted REE x 100). Abbreviations: ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; FVC, forced vital capacity (% of predicted); SNIP, maximal sniff nasal inspiratory pressure (% of predicted); UMN, upper motor neurone; LMN, lower motor neurone.
Longitudinal assessment of ALS patients

33 ALS patients underwent a second assessment of ALSFRS-R and body composition analysis and after an interval of 3.8 months [115.7 ± 6.12 (82–218) days]. Of these patients, 20 were normometabolic and 13 were hypermetabolic at the first assessment. Net changes in ALSFRS-R, weight and body composition data between assessments are shown in Table 4.10.

Table 4.10 Changes in clinical and composition data in 33 ALS patients. Net changes in variables between assessments 3.8 months [115.7 ± 6.12 (82–218) days] apart in 33 ALS patients.

<table>
<thead>
<tr>
<th>Change in variables</th>
<th>Normometabolic ALS cohort (n=20)</th>
<th>Hypermetabolic ALS cohort (n=13)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALSFRS-R score</td>
<td>-0.10±0.64 (-6-8)</td>
<td>-2.46±0.80 (-8-2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.48±0.59 (-6.75-3.04)</td>
<td>-0.80±0.10 (-8.17-84.32)</td>
<td>0.77</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>-0.21±0.54 (-3.61-3.92)</td>
<td>-0.20±0.80 (-5.61-5.92)</td>
<td>0.99</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>-0.28±0.33 (-3.13-2.26)</td>
<td>-0.72±0.66 (-4.93-4.74)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Notes: Normometabolic patients had a metabolic index <120 at the first assessment and hypermetabolic patients had a metabolic index ≥ 120 at the first assessment. Metabolic Index; measured resting energy expenditure (REE)/predicted REE x 100. p value from an unpaired Student’s t-test. Abbreviation: ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale.

The individual changes in normometabolic and hypermetabolic patients are shown in Figure 4.7. The patient who was receiving enteral nutrition via a PEG was normometabolic and lost 2.35kg of FM and 0.37kg of FFM between assessments.

There was no difference in weight or body composition changes between the hypermetabolic patients and the normometabolic patients (Figure 4.7 C-E). However, the decline in ALSFRS-R was significantly greater in hypermetabolic patients than in normometabolic patients (p=0.02) (Figure 4.7 A and E).
Figure 4.7 Comparison of individual changes in clinical and body composition measures in 13 hypermetabolic and 20 normometabolic ALS patients. The decline in ALSFRS-R score was greater in hypermetabolic patients than normometabolic patients ($p=0.02$). Abbreviations: ALSFRS-R, the revised Amyotrophic Lateral Sclerosis functional rating scale; FFM, fat free mass.
4.2.5 Summary of findings

A detailed assessment of body composition and energy expenditure was performed in 50 ALS patients and 50 healthy controls in order to investigate the incidence and clinical associations of hypermetabolism. The healthy controls were well matched to the ALS patients in age and sex distribution. A subset of ALS patients (n=33) attended a second assessment ~4 months later to determine whether hypermetabolism is associated with a change in disease progression.

The mean ALSFRS-R score of the ALS patients was 38.04. FFM was significantly lower in the ALS patients than in the controls. After accounting for body composition, a larger proportion of ALS patients were hypermetabolic compared to the controls (40% in ALS patients and 16% in controls).

There was no association between hypermetabolism and the duration, severity, or site of onset of disease or, respiratory function. The proportion of patients who were taking riluzole and who reported weight loss at symptom onset did not differ between hypermetabolic and normometabolic patients. The LMN score determined from clinical examination was significantly higher in hypermetabolic patients than in normometabolic patients and was correlated with the metabolic index in all ALS patients.

In longitudinal studies there was no difference in change of body weight or composition between hypermetabolic and normometabolic patients. A greater decline in mean ALSFRS-R score was observed in hypermetabolic patients than in normometabolic patients.

This study confirms that a large proportion of ALS patients are hypermetabolic. It is the first to show that hypermetabolism is associated with a higher LMN disease burden and a greater short-term functional decline than normometabolism.
Chapter 5: Discussion, conclusions and future directions

In this thesis, studies of the validity of anthropometric predictors of fat mass (FM) and of hypermetabolism, using techniques that account for body composition, were carried out in ALS patients and matched controls.

5.1 Anthropometric predictors of fat mass in ALS

A version of this section of the thesis was published in Ioannides, Z. A., Steyn, F. J., Henderson, R. D., McCombe, P. A. & NGO, S. T. 2017. Anthropometric measures are not accurate predictors of fat mass in ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, published online 27 Apr 2017. This paper can be found in Appendix 2.

The first objective of this thesis was to assess the accuracy of anthropometric predictors of FM. This is relevant to routine clinical care of ALS patients as predictors of FM could be useful in guiding nutritional management that has been shown to improve outcomes in ALS patients (Mazzini et al., 1995, Silva et al., 2010, Dorst et al., 2013, Wills et al., 2014).

It was found that body mass index (BMI) and body adiposity index (BAI) were not accurate predictors of FM as measured by air displacement plethysmography (ADP) in ALS patients. Furthermore, it was shown that longitudinal changes in BMI and BAI did not accurately reflect changes in FM.
5.1.1 The complex relationship between anthropometric measures and FM

The relationship between BMI, BAI and FM is dependent on sex, age, body shape, ethnicity, and the extent and distribution of adiposity (Norgan, 1994). In non-ALS populations, BAI has been shown to over- or underestimate FM in individuals with low or high levels of adiposity, respectively (Bergman et al., 2011). Similarly, studies have found limitations associated with anthropometric measures as predictors of FM in populations with low BMI (Norgan, 1994), obese individuals (Geliebter et al., 2013) and healthy sedentary females (Suchanek et al., 2012).

A strength of this study was the inclusion of an age- and sex-matched healthy control population. Comparisons between the ALS patients and controls showed that BMI and BAI were less accurate predictors of FM in ALS patients than in healthy individuals. These findings suggest that factors specific to ALS could reduce the accuracy of anthropometric predictors. Altered body composition in ALS patients (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a) is a plausible explanation for these results.

It is known that BMI is inaccurate in individuals with altered fat free mass (FFM), for example, in athletes who have increased muscle mass relative to FM (Garrido-Chamorro et al., 2009). ALS is characterized by a reduction in muscle mass secondary to neurogenic muscle wasting (Kinsley L, 2015 Feb 12). Muscle wasting would result in a lower overall body weight, which could reduce BMI. A change in BMI in ALS could therefore be a reflection of muscle wasting and not a change in FM.

Hip circumference [the key determinant of BAI and generally accepted as a proxy for abdominal adiposity (Bergman et al., 2011)] could also be affected by factors intrinsic to the disease process in ALS. For example, redistribution of fat to the abdominal region in ALS (Lindauer et al., 2013, Ahmed et al., 2016b) could affect hip circumference, and therefore influence BAI. In the ALS patients who underwent longitudinal assessments, while the mean BMI and FM measured by ADP did not change, the mean BAI increased between assessments ($p=0.04$). This suggests that hip circumference increased between assessments despite there being no change in the mean FM measured by ADP. This supports the hypothesis that a change in BAI in ALS patients could occur secondary to a change in the distribution but not the overall amount of fat.
Ethnicity can also affect body composition estimates (Wagner and Heyward, 2000). The original description of BAI was validated in a black African American population (Bergman et al., 2011). However, in the present study, the vast majority of the ALS patients in this study were of white Caucasian background. This is in keeping with the higher incidence of ALS in white Caucasian individuals (compared to black, Asian and Hispanic individuals) in large population studies (Cronin et al., 2007, Rechtman et al., 2015). Therefore, applying BAI to an ALS population that predominantly includes white Caucasian individuals, could account for some of the inaccuracy observed between BAI and FM in this study.

Altered CNS processes that regulate metabolism could also modify measures of FM in ALS. The CNS controls food intake via multiple neuronal populations. Key networks exist in the arcuate nucleus of the hypothalamus which involve the anorexigenic (appetite-suppressing) pro-opiomelanocortin (POMC) neurones and the orexigenic (appetite-increasing) neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons (Sohn, 2015). The balance between these hypothalamic networks is crucial in maintaining energy homeostasis. Alterations of these complex hypothalamic networks in ALS, either via a pathological process or an adaptive response to disease, could affect energy intake and therefore modulate energy storage in the form of FM.

Emerging evidence supports the hypothesis that the hypothalamus could play a role in mediating fat storage in ALS patients. Hypothalamic atrophy has been observed in ALS patients and hypothalamic volume has been found to correlate with BMI, especially in individuals with familial ALS (Gorges et al., 2017). Furthermore, observations in ALS patients and the SOD1 mouse of a lack of weight gain following treatment with pioglitazone, which normally promotes food intake via modulation of hypothalamic networks, indicate that the key hypothalamic neuronal networks that control food intake could be dysfunctional in ALS (Vercruysse et al., 2016). Additional studies of neuronal fibre density in the arcuate nucleus of the hypothalamus in SOD1, TARDP and FUS mice, which show a reduction in hypothalamic neurones producing POMC and an increase in those producing AgRP (Vercruysse et al., 2016), also suggest an alteration in these neuronal types.
Therefore, pre-clinical and clinical studies suggest that in ALS there could be impairment of hypothalamic neuronal circuitry that regulates food intake and calorie balance, which could affect measures of FM. Although this is plausible based on knowledge of the normal role of these circuits, further studies elucidating the relationship between measures of body composition and the function of these central neuronal networks in ALS patients are needed.

5.1.2 Limitations of the study

Limitations of this study include potential error associated with the use of ADP as the standard measure of FM. ADP is a reliable and reproducible method for determining body composition (Noreen and Lemon, 2006, Anderson, 2007, Lee and Gallagher, 2008) and clinical studies suggest that ADP and DEXA measurements of body fat agree within 1% (Fields et al., 2002). However, it is important to note that this relationship has not been studied in ALS. ADP was selected for measurement of FM because many ALS patients are unable to lie flat due to respiratory impairment, which could preclude the use of DEXA or MRI. Error was minimized by adherence to a strict protocol to minimize extracorporeal weight and volume.

Due to the progressive nature of ALS, many of the patients were unable to attend a second assessment. The longitudinal assessments were therefore limited to 29 patients. Replication of these finding in a larger cohort of patients is needed.
5.2 Hypermetabolism in ALS

The second objective was to study hypermetabolism in ALS patients and age- and sex-matched controls. This study found that a larger proportion of ALS patients were hypermetabolic compared to controls (40% in ALS patients and 16% in controls). This confirms previous reports of hypermetabolism in ALS (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014). Additionally, this study made the novel observation that hypermetabolism was associated with a greater lower motor neurone (LMN) disease burden and a greater short-term functional decline in ALS patients.

5.2.1 A novel approach to assessing hypermetabolism in ALS

Given the limitations of previous studies of hypermetabolism in ALS (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014), which were discussed in Chapter 1 section 1.2.2, efforts to improve upon existing methodologies were made.

This study was novel in accounting for body composition when determining pREE. This is a critical consideration because body composition, which has a significant influence on REE (Nielsen et al., 2000, Wang et al., 2000, Johnstone et al., 2005, Geisler and Müller, 2017), is altered in ALS. A decline in FM and FFM (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a) have been reported in ALS. Indeed, in this study, the FFM of the ALS patients was significantly lower than that of controls. Therefore, when studying hypermetabolism in ALS, allowing for body composition was considered to be important. While a number of REE prediction models account for FFM (Wang et al., 2000), the Nelson model (Nelson et al., 1992) was used in this study as it accounts for FM and FFM, which are both affected in ALS (Nau et al., 1995, Kasarskis et al., 1996, Desport et al., 1999, Desport et al., 2000, Jawaid et al., 2010a).
Another strength of this study of hypermetabolism is the characteristics of the cohorts. Inclusion of a healthy control cohort that was well matched to the ALS cohort for sex and age, highlights that the higher incidence of hypermetabolism observed in ALS patients compared to healthy controls is likely to be a consequence of changes specific to ALS.

Regarding the ALS cohort, the mean ALSFRS-R score was 38.04. Prior studies of hypermetabolism in ALS predominantly assessed patients with advanced disease (the mean ALSFRS-R scores in prior studies were between 27.6 and 35.7 see table 1.1, section 1.2.2) (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014). Given that a lower ALSFRS-R score indicates worse disability, the patients assessed in this study had less disability and were at an earlier stage in the disease process than prior studies. The current results can therefore give insights into the incidence and correlates of hypermetabolism earlier in disease than previously studied.

A stricter definition of hypermetabolism (mREE ≥ 120% of pREE) was applied in this study compared to previous studies in ALS, which used ≥ 110% as the threshold for hypermetabolism. This stricter definition was applied to be in accord with studies of patients with insulin resistance, diabetes and liver cirrhosis (Muller et al., 1999, Perseghin et al., 2002), in whom hypermetabolism is common (Muller et al., 1993). The use of a more stringent threshold for hypermetabolism is likely to explain the lower incidence of hypermetabolism in this study compared to some previous studies (Desport et al., 2001, Desport et al., 2005, Funalot et al., 2009, Bouteloup et al., 2009) as outlined in table 1.1.
5.2.2 Clinical correlates of hypermetabolism in ALS

In order to gain insights into the pathogenesis of hypermetabolism, we aimed to identify clinical factors that correlate with increasing energy expenditure. This is necessary because ALS is recognized to be a highly heterogeneous disorder (Turner et al., 2010). In particular, patients differ in the site of disease onset and in their motor features, leading to a number of different classification systems or ‘disease phenotypes’ (Talman et al., 2009). Patients also vary in the presence or absence of cognitive impairment (Xu et al., 2017) and in the presence or absence of other non-motor issues (McCombe et al., 2017).

In this study, the effect of site of disease onset and degree of upper motor neurone (UMN) and lower motor neurone (LMN) involvement on the presence of hypermetabolism was investigated. The severity of UMN and LMN involvement were recorded using a clinical scoring system that grades UMN and LMN signs in each body region from a physical examination (Ravits et al., 2007). The original authors of this grading system used it to demonstrate the focality of upper and lower motor neurone degeneration in ALS (Ravits et al., 2007). It has subsequently been used to show that LMN burden is associated with survival in ALS (Devine et al., 2016).

The site of disease onset did not differ between hypermetabolic and normometabolic ALS patients. However, it was found that hypermetabolism was associated with a significantly higher LMN score. Furthermore, the REE corrected for FFM and the metabolic index, significantly correlated with the total LMN score. Whilst these correlations do not confirm causation, they raise the possibilities that hypermetabolism could increase the severity of LMN clinical signs, or conversely, that a greater LMN burden could be a risk factor for hypermetabolism.
5.2.3 Possible sites for the origin of hypermetabolism in ALS

At present the cause of hypermetabolism in ALS remains unknown. An important unanswered question is which tissues have an increased metabolic rate? The clinical correlations revealed in this study, although not confirming causation, allow plausible sites for the origin on hypermetabolism in ALS to be hypothesized.

A greater LMN score arises when there are a greater number of dysfunctional motor units. The association between hypermetabolism and LMN disease burden suggests that motor unit abnormalities could be linked to hypermetabolism in ALS. Abnormal motor unit physiology is well described in pre-clinical models of ALS (Gurney et al., 1994, Frey et al., 2000, Schaefer et al., 2005, Kryściak et al., 2014) and ALS patients (Henderson et al., 2009, de Carvalho et al., 2012, Piotrkiewicz and Hausmanowa-Petrusewicz, 2013, de Carvalho et al., 2014).

A motor unit is made up of a motor neurone, its neuromuscular junctions and skeletal muscle fibers that are innervated by its axonal terminals (Purves D, 2001) (figure 5.1). It is possible that abnormalities at each of these sites could contribute to hypermetabolism in ALS.
Figure 5.1 The motor unit. A single motor unit comprising the lower motor neurone from the spinal cord to the neuromuscular junction and the innervated skeletal muscle fibres. (from Dictionary of Sport and Exercise Science and Medicine by Churchill Livingstone, 2008 Elsevier Limited; http://medical-dictionary.thefreedictionary.com/motor+unit)

As skeletal muscle accounts for up to 30% of total energy expenditure at rest (Rolfe and Brown, 1997) and is a major determinant of resting energy expenditure (Zurlo et al., 1990), it is a plausible site for the origin of hypermetabolism. Skeletal muscle is heavily dependent on glucose as its primary energy source under normal physiological conditions, only switching to lipid as an alternate energy source when the available glucose is unable to sustain energy requirements (Randle, 1998). Although lipid oxidation provides more ATP than glucose, it requires more oxygen per mole of ATP synthesized, thus lipid oxidation is accompanied by a higher rate of oxygen consumption (Randle, 1998, Leverve et al., 2007).

In chronic denervation, studies of rats (Pak et al., 2016) and patients (Karpati et al., 1979) show increased uptake of glucose and lipids from arterial blood, suggesting that denervation results in increased energy use. Rat studies suggest that denervation is associated with a reduction in the expression of glucose transporters (Henriksen et al., 1991, Coderre et al., 1992) and an increased turnover of proteolipid receptors (Lunt et al., 1971) which could affect the transport of energy substrate into skeletal muscle. In line with this, altered energy substrate preference in skeletal muscle, characterized by an increase in lipid oxidation and reduction in glucose utilization, has been observed in hypermetabolic
SOD1 mice (Palamiuc et al., 2015). As lipid oxidation consumes more oxygen than glucose oxidation, if this preference towards lipid metabolism in muscle occurs in a widespread fashion, it could translate into increased overall energy consumption and contribute to hypermetabolism.

Mitochondria, the main site of oxidative phosphorylation and production of ATP, play a pivotal role in bioenergetics and survival of the cell. As skeletal muscle and neuronal cells have a high-energy requirement, mitochondria are critical to sustaining their function and maintaining energy homeostasis [reviewed in (Xavier et al., 2015)]. The mitochondrial respiratory chain provides the energy necessary for mitochondrial ATP synthesis and calcium handling. Morphological alterations and functional impairments, including defects in the activities of mitochondrial respiratory chain complexes, have been detected in skeletal muscle and neuronal tissue of ALS patients (Afifi et al., 1966, Hervias et al., 2006, Martin, 2011, Pansarasa et al., 2014). It is possible that inefficient energy production via defects in the mitochondrial respiratory chain could increase muscle and neuronal energy requirements and therefore contribute to hypermetabolism.

The metabolic and physiological consequences of denervation have led to the long-standing theory that denervated muscle acts like fatigued muscle (Langley, 1916). This is possibly due to fasciculations and trophic processes such as re-innervation (Bass, 1962). With this in mind, the processes of neuronal repair that follow denervation in ALS could significantly affect energy requirements.

Neuromuscular junction disruption also occurs in ALS (Dupuis and Loeffler, 2009, Campanari et al., 2016). It has been proposed that hypermetabolism of skeletal muscle origin in SOD1 mice could lead to destruction of the neuromuscular junction and further axonal degeneration (Dupuis and Loeffler, 2009). Although the mechanism of this proposed chain of events in ALS is not known, this theory suggests that a complex relationship between hypermetabolism and neuronal loss could exist.

Denervation results in the production of a range of factors that are trophic for motor axons (Henderson et al., 1983), including insulin-like growth factors (Day et al., 2001, Shavlakadze et al., 2005) and myogenic regulatory factors (Walters et al., 2000). Denervated muscle cells undergo a change in the expression of genes encoding cell cycle regulators and extracellular matrix components (Batt et al., 2006). Denervated muscle
cells also secrete immune molecules (interleukins) and metabolic factors (Pedersen, 2011). These responses lead to the sprouting of new axons and re-innervation of muscle fibres by axons from other motor units.

Successful collateral re-innervation during the early stages of ALS is demonstrated by the neurophysiological finding of large motor units in ALS patients (Duleep and Shefner, 2013). However, as disease progresses there is a failure in adequate re-innervation, which is followed by muscle atrophy (Bonaldo and Sandri, 2013). Physiological abnormalities including abnormal LMN firing (de Carvalho et al., 2014, Howells et al., 2016) and fasciculations (Piotrkiewicz and Hausmanowa-Petrusewicz, 2013) accompany these changes. In the event of widespread denervation, as seen in ALS, the energy costs associated with these neuronal changes could be significant and potentially contribute to increased systemic energy requirements.

Although it is hypothesized that hypermetabolism in ALS originates from abnormal motor units, it is possible that a systemic derangement in energy metabolism also occurs as a related phenomenon. Indeed, altered glucose metabolism associated with denervation, is not confined to the affected muscles. Abnormal glucose metabolism has been observed in tissues distant from the site of denervation in animal studies (Nunes and de Mello, 2005). Studies of denervated rats show a generalized reduction in glucose uptake, glycogen synthesis, insulin sensitivity (Nunes and de Mello, 2005) and an enhanced effect of epinephrine on glycogen metabolism (Moruzzi and Bergamini, 1983). Given that these hormones have widespread effects throughout the body, these observations support the notion that denervation could lead to extensive changes in energy metabolism that extend beyond muscle physiology.

Much of the evidence supporting metabolic change in motor units following denervation arises from studies of denervation of isolated nerves in pre-clinical models. Extrapolating from these results, it is feasible that the mass synchronous denervation that occurs in ALS could have systemic metabolic consequences and could underlie the metabolic changes observed in patients.
Respiratory dysfunction has also been hypothesized to contribute to hypermetabolism, as discussed in section 1.2.2. This study found no correlation between the metabolic index and respiratory parameters. This is in line with previous studies showing a lack of association between forced vital capacity (FVC) and hypermetabolism in ALS patients (Desport et al., 2005, Bouteloup et al., 2009). In addition to the FVC, in this study a more sensitive measure of mild to moderate respiratory muscle weakness, the maximal sniff nasal inspiratory pressure (SNIP) (Lyall et al., 2001) was also recorded, however the lack of correlation between respiratory function and metabolic parameters remained. These results imply that factors other than respiratory dysfunction play a role in hypermetabolism.

Cortical hyperexcitability (Vucic and Kiernan, 2006, Vucic et al., 2008) is another potential contributor to hypermetabolism in ALS (discussed in section 1.2.2). However, our study showed that use of riluzole, an inhibitor of glutaminergic transmission that has been shown to modulate cortical hyperexcitability in ALS (Geevasinga et al., 2016), was not associated with a reduction in the incidence of hypermetabolism. Although riluzole has only a modest effect on hyperexcitability, this observation suggests that although hyperexcitability could contribute to hypermetabolism, other factors appear to be important.

5.2.4 Hypermetabolism as a modifier of functional decline

Longitudinal assessment of a subset of ALS patients evaluated how hypermetabolism influences changes in functional disability, weight and body composition over time. Hypermetabolic patients experienced a greater decline in function, as assessed by the ALSFRS-R, when compared to normometabolic patients. This is in line with the findings of Desport et al who observed that REE correlated with a worsening of ALSFRS-R scores in ALS patients (Desport et al., 2005). However, an earlier study by the same author did not observe a correlation between spasticity scores or level of fasciculations in ALS patients and REE (Desport et al., 2001). This reflects the limitations of clinical markers, which do not necessarily follow the underlying pathophysiological changes of ALS.
The finding of a greater functional decline in hypermetabolic patients suggests that hypermetabolism could be linked to a poorer survival. Notably, it has been shown that a transgenic mouse model engineered to exhibit muscle-specific hypermetabolism, displayed the hallmarks of ALS including neuro-muscular junction damage and motor neurone degeneration (Dupuis et al., 2009). This indicates that hypermetabolism could exacerbate neuronal loss.

Although a causal relationship between hypermetabolism and survival has not been confirmed in ALS, hypermetabolism is known to predict a shorter survival in other disorders including cancer (Vazeille et al., 2017), sepsis (Wu et al., 2015a) and burns (Herndon et al., 2001). Evidence from dietary studies in ALS suggests that hypermetabolism and survival could be linked. Increased caloric intake is associated with an improved survival in ALS and a possible mechanism of benefit is that increased energy intake compensates for the increased energy demands associated with hypermetabolism. In 2004 it was shown that a high calorie diet extended mean survival by 20% in SOD1 mice (Dupuis et al., 2004). More recently, studies in ALS patients have shown that high caloric food supplements stabilize body weight (Dorst et al., 2013), increase BMI (Silva et al., 2010) and reduce mortality (Mazzini et al., 1995, Wills et al., 2014, Dorst et al., 2015).

The occurrence of hypermetabolism was not documented in these studies that showed a benefit of increased caloric intake. However, as between 25-70% of sporadic ALS patients are hypermetabolic (Kasarskis et al., 1996, Desport et al., 2001, Desport et al., 2005, Bouteloup et al., 2009, Funalot et al., 2009, Kasarskis et al., 2014), these findings could suggest that compensating for the increased energy demands of hypermetabolism, could improve survival.

Whether hypermetabolism is associated with a worse outcome remains to be confirmed as there are conflicting findings in the previous reports. Although Karsarkis et al found that mREE/pREE increased as death approached in 16 ALS patients, Bouteloup et al, found that the survival of hypermetabolic ALS patients was not significantly different to that of normometabolic patients (Bouteloup et al., 2009). Desport et al found that mREE remained stable as 62 ALS subjects approached death (Desport et al., 2001) and that mREE, corrected for FFM, decreased relative to proximity to death in 168 ALS patients (Desport et al., 2005).
Although it would be expected that hypermetabolism would lead to negative energy balance and weight loss, there was no difference between hypermetabolic patients and normometabolic patients in the occurrence of weight loss at symptom onset or weight loss over the ~4 month follow-up period. The absence of excessive weight loss in hypermetabolic individuals suggests that compensation could occur, either as a result of increased energy intake or a reduction in other sources of energy expenditure, namely activity dependent energy needs and the thermic effect of food.

5.2.5 The hypothesized vicious cycle of hypermetabolism

As discussed above, the finding that LMN dysfunction is associated with hypermetabolism, raises the possibility that increased energy demands of diseased motor units drive hypermetabolism. In addition to this, the observation that hypermetabolism is associated with a greater functional decline in ALS patients, leads to the hypothesis that hypermetabolism could increase motor neurone vulnerability and accelerate disease progression in ALS. These two hypotheses and a prior report that greater LMN dysfunction is associated with a poorer survival in ALS patients (Devine et al., 2016), can be linked to hypothesize that hypermetabolism of motor unit origin leads to metabolic changes that could exacerbate disease progression. This is outlined in figure 5.2.
Figure 5.2 The hypothesized link between hypermetabolism, lower motor neurone dysfunction, motor unit abnormality and survival in ALS. The results of this study suggest that hypermetabolism is associated with LMN dysfunction (1) and disease progression (3). From these results it is hypothesized that hypermetabolism arises from diseased motor units (2) and that hypermetabolism leads to a vicious cycle of accelerated disease progression (4), further LMN degeneration and exacerbation of hypermetabolism. In addition to this, Devine et al (Devine et al., 2016) showed that LMN burden is associated with a worse survival (5). Therefore, it is also hypothesized that hypermetabolism is linked to poor survival (6). Blue arrows indicate known associations. Red arrows and question marks indicate hypothesized associations or relationships.
5.2.6 Limitations of the study

Methodological limitations of the use of the ADP as the measure for body composition used to predict REE are discussed above in section 5.1.2.

It should be noted that although LMN and UMN scores were assessed in an unbiased way, this clinical approach is not as rigorous as other techniques, for example neurophysiological assessments. The longitudinal portion of this study is limited by the use of ALSFRS-R as the sole marker of disease progression, the relatively short follow-up period and the lack of long-term survival data. Furthermore, due to the progressive nature of ALS, the longitudinal assessments were limited to 33 individuals.

5.3 Potential future directions

Regarding the studies of anthropometric measurements, given that neurogenic muscle wasting could contribute to the inaccuracy of predictions of FM in ALS patients, incorporation of anthropometric measurements of muscle wasting could allow the loss of muscle mass to be taken into account. For example, arm muscle area calculated from triceps skinfold thickness and mid-arm circumference, allows total body muscle mass to be predicted (Heymsfield et al., 1982). Studies assessing the combined use of BMI, BAI and anthropometric predictors of muscle mass could be useful in developing a practical approach to predicting FM specifically in ALS patients.

Redistribution of FM could also contribute to the inaccuracy of anthropometric predictors of FM in ALS patients. MRI studies of regional adiposity could therefore be useful in characterizing changes in the distribution of FM. Indeed, studies assessing whether MRI changes in body composition, including muscle wasting or redistribution of FM, correlate with anthropometric predictors in ALS patients, could be useful in the development ALS-specific predictors of adiposity. MRI could also provide quantification of FFM, which could allow for more accurate predictions of REE in future studies of hypermetabolism.
Future studies of hypermetabolism in ALS could benefit from a combination of clinical, functional and electrophysiological parameters for monitoring disease progression and, UMN and LMN disease burden. For example, motor unit number estimations (MUNE) (Gooch et al., 2014, Henderson and McCombe, 2017) could be a useful quantitative method of following motor unit physiology. Likewise, UMN disease burden could be better assessed by transcranial magnetic stimulation (TMS) techniques, which reflect corticomotoneuronal function (Vucic and Kiernan, 2013). Furthermore, longitudinal assessment of changes in LMN and UMN disease burden in relation to the onset of hypermetabolism, could provide insights into whether the progression of LMN and/or UMN involvement affect the development of hypermetabolism.

Prospective longitudinal assessments of changes in metabolic status and survival in ALS patients are necessary to determine whether hypermetabolism is associated with a worse survival. The observed associations between hypermetabolism and LMN dysfunction and disease progression, also require confirmation in a larger cohort.

Given that hypermetabolism was not associated with weight loss, investigation of other factors that could be altered in compensation for the increased energy expenditure of hypermetabolism is warranted. This would include assessments of dietary intake, appetite, activity dependent energy needs and the thermic effect of food.

It is important to note that the observations presented in the thesis are associations and although they enable the development of hypothetical mechanisms, they do not confirm causation. Physiological studies of energy substrate use and energy requirement in muscle and neuronal tissue from ALS patients relative to whole body energy expenditure are required in order to clarify whether altered motor unit energy expenditure is associated with hypermetabolism in ALS. If it is found that motor unit metabolism is altered in hypermetabolic patients, the specifically affected pathways could be modified and the effect on hypermetabolism and disease progression assessed. This could provide insights into the cause and clinical consequence of hypermetabolism.

An important unanswered question is when does hypermetabolism occur in the course of ALS? Although this study was unique in assessing patients relatively early in their disease course, it is possible that pathological changes occur prior to the onset of symptoms in individuals at risk of developing ALS (Vucic et al., 2008). Therefore,
longitudinal assessment of pre-symptomatic individuals who harbour genetic mutations for the development of ALS could reveal insights into the development of hypermetabolism in relation to the emergence of clinically apparent disease. Given that familial ALS appears to be associated with a high incidence of hypermetabolism (Funalot et al., 2009), the assessment of genetically at risk individuals could be a useful approach.

It is also unknown how the extra-motor features of ALS, such as cognitive impairment, affect metabolism. Altered metabolism (Ahmed et al., 2014a) and changes in energy expenditure (Ahmed et al., 2016c) have been reported in patients with frontotemporal dementia (FTD). Cognitive impairment similar to that seen in FTD occurs in some ALS patients (Xu et al., 2017), and ALS and FTD appear to exist at opposite ends of a common disease spectrum (Ahmed et al., 2016a, De Silva et al., 2016). However, compared to patients with ALS, patients with FTD display a distinct metabolic profile, characterized by a higher BMI (Ahmed et al., 2014b) and altered eating patterns (Ahmed et al., 2016b). These findings suggest that cognitive impairment could play a role in centrally modifying behaviours that could affect metabolism. The one ALS patient with FTD in this study was found to have a low metabolic index, however, larger studies are required of the effects on metabolism of cognitive impairment in ALS.

5.4 Overall Conclusions

ALS is a fatal disease with no cure. Identification of factors that could modify disease progression is therefore vital. Metabolic dysfunction is increasingly recognized to occur in some ALS patients and could modify the course of disease. Of clinical significance, alterations in fat mass (FM) (Desport et al., 1999, Paganoni et al., 2011) and hypermetabolism (Kasarskis et al., 1996) could be associated with a worse disease outcome. Therefore, improved methods to assess these factors and a greater understanding of their clinical associations are important.
In this thesis, the accuracy of anthropometric predictors of FM and the incidence and clinical correlations of hypermetabolism were examined in ALS patients.

Using ADP as the standard, it was found that BMI and BAI are not accurate predictors of FM and that they provide a poor indicator of change in FM over time in ALS patients. It is likely that changes in BMI and BAI in ALS patients occur independent to changes in FM alone. Changes in BMI and BAI could depend on muscle atrophy and re-distribution of fat in individual ALS patients.

Given that measures of adiposity guide nutritional therapies in ALS, inaccurate estimation of FM or misleading estimates of changes in FM could have adverse clinical consequences. When possible, more accurate measures of body composition should be sought in ALS patients.

It was also found that when body composition is accounted for, the incidence of hypermetabolism is greater in ALS patients than in healthy matched controls. This study is the first to demonstrate an association between hypermetabolism and LMN disease burden and a greater functional decline in ALS patients. In light of these results it is hypothesized that hypermetabolism arises from abnormal motor units. Furthermore, it is hypothesized that hypermetabolism could drive progression of ALS and lead to a vicious cycle of denervation, hypermetabolism and further disease progression (figure 5.2).

Overall, the results of this thesis suggest that BMI and BAI are inadequate markers of nutritional status in ALS and that hypermetabolism is an important metabolic consideration in ALS patients. ALS-specific predictors of FM are required to guide nutritional therapies and further clinical and physiological studies are needed to understand the cause and prognostic implications of changes in body composition and hypermetabolism.
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Appendices

Appendix 1. Published peer-reviewed journal article 1


This article can be found at:

https://www.karger.com/Article/Abstract/446502

Appendix 2. Published peer-reviewed journal article 2


This article can be found at:

http://www.tandfonline.com/eprint/sSHIJdg2j6Vh3nGs9Xmj/full