UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Human Development and Health

The CaSIO Study
Cachexia: Skeletal muscle loss and Inflammation in Older women

by

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ABSTRACT

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CACHEXIA: SKELETAL MUSCLE LOSS AND INFLAMMATION IN OLDER WOMEN

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Cachexia is an inflammatory syndrome characterised by skeletal muscle loss. It is well described in association with single disease processes where it is associated with poor outcomes and in which interventions are emerging. Older people may be particularly vulnerable to cachexia as a consequence of age related skeletal muscle loss and inflammation which are both associated with adverse outcomes; despite this the syndrome is not well understood in this group. This thesis describes the prevalence of cachexia in hospitalised older women and explores its association with hospital outcomes and all cause mortality.

148 older women were characterised in terms of demographics, cachexia, skeletal muscle and inflammation during an acute hospital admission; data collection was repeated in the community six months later. Outcome data relating to the hospital admission were ascertained at the point of discharge and all cause mortality was identified at two year follow up.

The average age of the women at baseline was 86 years (interquartile range: 83-89) and the prevalence of cachexia was 28% (36/131). Twenty nine percent of the 17 women who had cachexia at baseline and who were also seen at 6 month follow up had recovered. Cachexia was associated with increased risk of hospital acquired complication (odds ratio (95% confidence interval [CI]): 2.4 (0.97-6.13), p=0.06) and two year all cause mortality (hazard ratio [HR] (95%CI): 2.6 (1.4, 4.8), p<0.01); associations remained after adjustment for confounders including age and co-morbidity. Lower muscle strength and greater inflammatory burden at baseline were associated with increased risk of two year all cause mortality. HR and 95% CI per kilogram decrease in grip strength, and per standard deviation change in white cell count, CRP, albumin and IL-8 were: 0.95 (95%CI 0.9,1.0), p=0.03; 1.4 (95%CI 1.1,1.9), p=0.02; 1.3 (95%CI 1.0,1.7), p=0.05; 0.7 (95%CI 0.6,0.9), p<0.01; and, 1.4 (95%CI 1.0,2.0), p=0.04 respectively.

This novel study has shown that cachexia is highly prevalent among hospitalised older women and recovery may be possible. This study has also shown that cachexia is associated with especially poor hospital outcomes and longer term mortality and is a better discriminator of these outcomes than weight loss alone; skeletal muscle loss and inflammation are implicated. Direct interventions for cachexia are currently lacking for older people although there is a growing evidence base for both interventions for cachexia in younger people and also for muscle and weight loss in older people; further research is needed.

Chapter 1 Table of Contents

List of tables

Results Appendix Tables

List of figures

List of photographs

Declaration of authorship

I, Daniel Baylis

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The CaSIO Study

Cachexia: Skeletal muscle loss and Inflammation in Older women

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission

Signed: .....

Date:.....

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I would also like to acknowledge my colleagues at the MRC Lifecourse Epidemiology Unit and the geriatricians at Southampton General Hospital who helped inspire my career choice and continue to mentor me today. Specific thanks to my supervisors Avan Aihie Sayer and Helen Roberts who have made the process both enjoyable and rewarding thanks to just the right levels of approachable support and guidance. Thanks to Holly Syddall who in addition to being an exceptionally good scientist is able to communicate her knowledge in a useful, enjoyable and responsive manner and to Gemma Rood and Annemarie Colby for practical assistance with the field work. Also I am grateful to the team at the Centre for Musculoskeletal Ageing Research within the University of Birmingham for their support, assistance and teaching in the laboratories, particular thanks to Janet Lord and Hema Chahal for making the experience so enjoyable. Acknowledgements would not be complete without mentioning my friend and colleague Harnish Patel who has provided peer support throughout the whole process from day one.

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List of abbreviations

- AAP Acute phase protein
- ACTH Adrenal corticotrophic hormone
- ADL Activities of daily living
- BIA Bioelectrical impedance analysis
- CaSIO Cachexia: Skeletal muscle loss and Inflammation in Older Women
- CCF Congestive cardiac failure
- CMV Cytomegalovirus
- COPD Chronic obstructive pulmonary disease
- CRP C-reactive protein
- DAMPS Damage associated molecular patterns
- DHEA(S) Dehydroepiandrosterone (Sulphate)
- DXA Dual energy x-ray absorptiometry
- ELISA Enzyme-linked immunosorbent assay
- ESBL Extended-spectrum Beta-Lactamases
- EWGSOP European working group on sarcopenia in older people
- FoxO Forkhead box O
- GDS Geriatric depression scale
- GP General practitioner
- HAC Hospital acquired complication
- HIV Human immune-deficiency syndrome
- HPA Hypothalamic pituitary adrenal
- IGF Insulin-like growth factor
- IL Interleukin
- IWGOS International working group on sarcopenia
- Kg Kilogram
- LEU Lifecourse Epidemiology Unit
- MMSE Mini mental state examination
- MRC Medical Research Council
- MRSA Methicillin-resistant Staphylococcus aureus
- Neutrophils Neutrophil count
- NIHR National Institute for Health Research
- N/n Number
- OR Odds ratio
- PPR Pattern recognition receptors
- ROS Reactive oxygen species
- SD Standard deviation
- SNAQ Simplified nutritional appetite questionnaire
- SUHT Southampton University Hospitals NHS Trust
- TNF Tumour necrosis factor
- UK United Kingdom
- US United States
- WBC White blood cell
- WCC White cell count

Chapter 1 : Introduction

1.1 Cachexia

1.1.1 Cachexia

Cachexia is the principle focus of this thesis; it is a condition that was first described by Hippocrates, "*The shoulders, clavicles, chest and thighs melt away. This illness is fatal*". Cachexia is widely recognised as a wasting syndrome that occurs in association with severe illness and has high mortality; it is little studied in older adults who are the second principle focus of this thesis.

1.1.2 Older adults: *setting the scene*

1.1.2.1 Changing demographics

The United Kingdom (UK) population is projected to increase by more than 4 million to 65.6 million over the 10 year period, 2008 to 2018, and exceed 70 million by mid-2029 [1]. The shape of the population is also changing as the birth rate declines and people live to older ages. Life-expectancy in the UK is currently 77.4 years (males) and 81.6 years (females). By 2051, the projected life expectancy at birth will have risen to 84 years and 88 years respectively [2].

Life expectancy at age 65 is also rising. Currently, a 65 year-old male can expect to live another 17.4 years, and a woman another 20.0 years. By 2051, their equivalents will expect to live a further 21 years; almost double the life expectancy of a 65 year-old man a century earlier [2].

As the population ages, the numbers at the oldest ages will increase the fastest. There are presently 1.3 million people in the UK aged 85 and over. This number is projected to increase to 1.8 million by 2018 and to 3.3 million by 2033 [1]. Furthermore, the number of people aged 90 and above is projected to triple by 2033, the number aged above 95 quadruple, and the number of centenarians will rise from 11,000 to 80,000 in twenty years; a more than sevenfold increase [1].

1.1.2.2 Hospitalisation of older adults

Hospitalisation is an important part of the management of acutely unwell older adults. However, it can also lead to adverse consequences including the breakdown of formal and informal care, loss of social networks, premature admission to residential care and hospital acquired complications; aside from the impact on healthcare services [3].

Yet despite this, hospital admissions are rising rapidly. Within the UK, there are still more than 2 million unplanned admissions per year for people over 65, accounting for more than 51,000 acute beds at any one time [4]. Furthermore, admissions amongst this group are increasing fastest: 3% per year compared with 2% for all age groups [3]. Possible explanations for these trends include a greater emphasis on community care leading to shorter but more frequent admissions, differing admission criteria and referral pathways, breakdown in out-of-hours services, medical advances, an increasingly medicalised society and demographic changes [5]. Currently, 60% of people over 65 have at least one hospital admission in the year before their death and in the majority of cases these admissions are multiple. Aside from the health and social significance, an additional consequence is that 40% of the UK National Health Service budget is spent on healthcare for older people, (approximately £30bn) and this group accounts for more than 68% of hospital inpatient bed days [4;6]. An important strategy to help tackle these issues is to improve the recognition of subgroups of older people who are at risk of multiple hospital admissions and poor outcomes to implement preventative strategies and inform prognostication.

1.1.2.3 Hospitalised older adults and evidence based medicine

From the above evidence, a key challenge of the 21st century is to better understand the ageing process in order to improve healthy life-expectancy and extend successful, independent living. Yet despite this, older people are relatively understudied (*Table 1-1*).

Table 1-1: Results from searches on the engine Pub Med.gov, March 2013

Search term	Number of Pub Med articles	UK prevalence	Ratio
Frail	8,879	1,158,000	0.008
Older adults	172,539	11,580,000	0.015
Coronary Heart disease	243,473	3,000,000	0.081
Dementia	138,276	750,000	0.184
HIV	251,174	83,000	3.026
Cancer	2,742,669	156,000	17.58
Creutzfeldt-Jakob disease	6,727	27	249.2

The majority of clinical trials exclude people over 65 years of age and research into older people is commonly set amongst community cohorts who tend to be healthier and leading more active lifestyles. The frailest individuals are a challenging group often excluded due to the complexities of their physical conditions and social and ethical dimensions concerning recruitment and consent [7-9].

A strategy to identify these individuals is to recruit older people from the acute hospital – a cohort who are losing functional capacity and making frequent transitions between community and healthcare settings. One of the challenges in this group is discriminating between people who will have a short and uncomplicated hospital admission and return to successful independent living in the community versus those who will experience a difficult admission and poorer longer term outcomes [10]. Such differentiation would help target interventions and also assist with palliative care where a recognised barrier is the ability to identify patients at a higher risk of mortality earlier in their illness trajectory. Prognostication is important but often neglected part of patient management, especially for

discussions of goals of care, treatment preferences, advanced care planning and clinical therapeutic options [10-12].

A national move to single sex wards necessitates that this thesis is conducted in women rather than both sexes. Women live longer than men and, according to a 2009 BUPA census, account for 72% of residential and nursing home residents and a greater number of acute hospital admissions. For example, in Southampton in 2012, 72% of admissions over 80 years were female. The impact of this is considered within the discussion.

1.1.2.4 Older adults and clinical outcomes

This thesis will consider two principal outcomes that are of direct relevance to older adults. The first relates to hospital admission – specifically, the likelihood of developing a complication as a consequence of the admission, the admission length and the likelihood of being discharged back home. The second outcome considered in this thesis is all-cause mortality over a two year follow up period.

Admission to hospital is associated with a variety of health service and patient centred clinical outcomes. These are complex, closely interrelated and include admission length, readmission rates (within 28 days of discharge) and the incidence of hospital acquired complication (HAC), adverse drug reactions, mortality and the likelihood of discharge to usual residence.

On average, older patients experience worse outcomes from hospital admission than their younger counterparts, including longer admission lengths and higher readmission rates; figures that are increasing year-on-year [13-15]. Longer hospital admissions and readmissions are positively associated with increased likelihood of developing a HAC and mortality and negatively associated with the likelihood of being discharged back to pre-admission accommodation [11;16]. Furthermore, older people with complex multi-morbidity, particularly those with poor renal, hepatic and cardiac function are at an additional risk of adverse events which include infections (chest, urinary, skin, gastrointestinal), thromboembolic disease, pressure sore progression and falls [16]. Cognitive impairment compounds the risk further due to poor compliance with medications and an inability to recognise that problems exist or to ask for help [17]. Cascade iatrogenesis, with serial development of medical problems contributes to the increased exposure of older people to medical interventions in

general. Therefore HAC are dangerous and a leading cause of preventable death in this group. They are also responsible for increased length of admission and significant disability; incidence estimates in older people are 10-15% [16;18].

Following discharge from hospital, outcomes relating more specifically to individuals include functional measures of independence, disability (activities of daily living, ADLs), morbidity and mortality. Hospitalisation frequently precipitates a decline in ADLs and high rates of institutionalisation, morbidity and mortality [19]. The prognosis for older adults discharged with a new or additional disability in ADLs is poor, with only 30% returning to their preadmission level of functioning by one year [12]. Mortality rates one year post-discharge amongst patients who experience a decline in ADLs following hospital admission are 26%, rising to 45% amongst individuals with risk factors that include age, admission length, change in discharge residence and co-morbidity [11].

1.1.3 Cachexia: Definition and diagnosis

Identifying cachexia [Greek, *kakos* 'bad', *hexia* 'condition'] may offer an opportunity to differentiate older people at risk of especially poor outcomes. To facilitate diagnosis the syndrome has recently been described by consensus definition as "a complex metabolic syndrome associated with underlying illness and characterised by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults". Cachexia must be distinguished from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism. Diagnostic criteria have also been defined, *Table 1-2* [20].

Table 1-2: Cachexia diagnostic criteria

Unintentional weight loss of at least 5% in 12 months†
(or body mass index <20 kg/m^2)

Plus at least 3 of:

1. Decreased muscle strength[1]
2. Fatigue
3. Anorexia
4. Low fat-free mass[2]
5. Abnormal biochemistry
    a. Increased inflammatory markers[3]
    b. Anaemia (Hb <12g/dl)
    c. Low serum albumin (<32g/l)

†Oedema free

[1]Lowest tertile grip strength [21]
[2]Lean tissue depletion, i.e. mid-arm circumference <10th percentile for age and gender
[3]Any inflammatory marker above 2 standard deviations of the age and sex matched means, e.g. CRP >5.0 mg/l and IL-6 >4.0pg/ml

1.1.4 Prevalence and health related costs of cachexia

Cachexia is an underestimated and under-diagnosed consequence of any chronic or severe inflammatory process including infection, chronic obstructive pulmonary disease (COPD), congestive cardiac failure (CCF), chronic kidney disease, inflammatory arthritis and acquired immune deficiency syndrome (*Table 1-3*). Cachexia is believed to be common in daily practice, increase with age and be around 1-2% in the community dwelling general population; its prevalence amongst older people remains largely unknown [22]. Associated economic and healthcare costs are also poorly documented perhaps due to the association of cachexia with its primary disease rather than being a syndrome in its own right and also as a consequence of poor recognition and documentation.

Table 1-3: Prevalence of cachexia in specific conditions

Condition	Estimated prevalence of cachexia (%)
Congestive heart failure	20
Chronic renal failure (on dialysis)	40
Chronic obstructive pulmonary disease	20
Cancer	30
Rheumatoid arthritis	10
Acquired Immune Deficiency Syndrome	35 (pre-HAART†), 10 (post-HAART†)

†Highly Active Anti-Retroviral Therapy

Cachexia is associated with adverse health outcomes including reduced quality of life, increased susceptibility to infection, morbidity and mortality; twelve month mortality rates range from 15% in COPD and 30% in CCF to 80% in certain cancers [23]. Clinical studies have shown that the preservation of body fatness and skeletal muscle in cachectic patients can decrease mortality risk [24]. Studies in older adults are lacking, particularly studies linking cachexia to adverse clinical outcomes such as those during the receipt of hospital care and long term mortality.

1.1.5 Pre-cachexia

Previously cachexia was believed to be associated with the end stages of a disease process. However, it has more recently been reported that cachexia occurs far earlier in the course of a disease. Indeed, there is now good evidence that cachexia is an early phenomenon, emphasising the importance of timely recognition and treatment. For example, studies of gastrointestinal tract and lung cancer patients demonstrated that up to 80% of patients had already experienced some degree of weight loss prior to diagnosis [25]. Furthermore, several of the metabolic and biochemical changes believed to be responsible for the cachexia phenotype were present at diagnosis even in the absence of the defining clinical feature of weight loss [26].

Sub-classification of cachexia according to stage helps to increase awareness of the condition as well as facilitate early recognition and timely interventions. It is also useful for

research purposes, especially for the development of biomarkers [27]. For the recognition of individuals at high risk of developing cachexia and the facilitation of early preventative interventions *pre-cachexia* has also been defined, *Table 1-4* [27].

Table 1-4: Pre-cachexia diagnosis

Pre-cachexia requires the presence of all of the following criteria:

1. Underlying chronic disease
2. Unintentional weight loss of ≤5% of usual body weight during the last 6 months
3. Chronic or recurrent systemic inflammatory response
4. Anorexia

1.1.6 Mechanisms of cachexia

Cachexia is an inflammatory condition associated with skeletal muscle loss; it cannot be reversed with nutritional supplementation indicating that complex metabolic changes have occurred. Excessive production of pro-inflammatory cytokines is the most common cause of the muscle loss associated with cachexia and is discussed in greater detail in section *1.3 Inflammation and ageing* [23]. Chronic administration of pro-inflammatory cytokines either alone or in combination is capable of reducing food intake and reproducing the distinct features of cachexia although evidence for the role of cytokines in older people with cachexia is limited [28]. An observational study of 140 frail older people in continuing care and day hospital settings found that the frailest individuals also had features of cachexia and that this group had significantly higher levels of IL-6 [29].

1.1.6.1 Effects on skeletal muscle

The principle phenotypic feature of cachexia is weight loss which occurs principally in the form of skeletal muscle loss. The mechanisms underlying this are highly conserved due to the benefits of cytokine mediated skeletal muscle atrophy during acute inflammation for repair and the provision of energy. In this situation, specific proteins within skeletal muscle are targeted to yield amino-acids which are subsequently consumed in the liver for the

synthesis of acute-phase proteins such as C-reactive protein (CRP). Unfortunately this has severe implications during sustained inflammation which leads to skeletal muscle wasting occurring at especially accelerated rates due to the amino acid composition mismatch between skeletal muscle and acute-phase proteins. This mismatch necessitates large amounts of skeletal muscle to generate small amounts of acute-phase protein and in part explains the degree of associated weight loss.

Down regulation of the insulin-like growth factor (IGF)-1 signal transduction pathway is also implicated in the pathogenesis of skeletal muscle loss in cachexia. Catabolic states such as sepsis and cancer have been associated with reduced levels of circulating IGF-1 [30]. Furthermore, IGF-1 mRNA expression in the gastrocnemius muscle of rats bearing the AH-130 hepatoma, a well-characterized model of cachexia progressively decreased to 50% of controls [31]. This is discussed in greater detail in section *1.2.4 Systemic influences on skeletal muscle decline.*

1.1.6.2 Systemic effects

In addition to their effect on skeletal muscle, cytokines also lower serum albumin concentrations, enhance lipolysis and are responsible for the anorexia associated with cachexia. Pro-inflammatory cytokines cause decreased activity of the hypothalamic orexigenic signal neuropeptide Y, which stimulates hunger and reduces energy expenditure [32]. Cytokines also delay gastric emptying and increase feelings of fullness [33].

These effects are amplified through interactions with the endocrine system. Leptin is a hormone principally secreted by adipose tissue that plays a key role in the homeostasis of weight control, ultimately causing appetite suppression and increased energy expenditure. Pro-inflammatory cytokines stimulate the expression and release of leptin and /or mimic the hypothalamic effect of excessive negative feedback signalling from leptin. This leads to the prevention of the normal compensatory mechanisms in the face of both decreased food intake and body weight [25].

1.1.7 Cachexia and older people

1.1.7.1 Vulnerability to cachexia

Older people are particularly vulnerable to developing cachexia even during minor illnesses. This is as a consequence of illness, age-related loss of muscle, reduced physical activity, dysgeusia, dysosmia, orogingival disease, low mood, cognitive impairment, and social isolation [34;35;36]. Age-related anorexia is mediated centrally by inflammatory processes and peripherally through gastric dysmotility and impaired fundal compliance resulting in early satiation due to the rapid passage of food into the stomach antrum. Other factors include an age-related increase in the satiating effect of cholecystokinin and increased concentrations of islet amyloid polypeptide which is a peptide hormone secreted by pancreatic β-cells at the same time as insulin that causes a reduction of food intake, slows gastric emptying and inhibits digestive secretion. Hyperleptinaemia and hypogonadism further contribute to the physiologic anorexia of old age [23]. Older people lose, on average, 0.1-0.2kg of weight per year [37].

1.1.7.2 Prevalence of cachexia in older people and relations with outcomes

Despite this increased vulnerability, detailed studies on cachexia in older adults are lacking although in the United States (US) a prevalence of 20% was reported amongst nursing home residents, suggesting that it is likely to be common [23]. Although cachexia has never been studied in older people in relation to outcomes, its two principle components, skeletal muscle loss and inflammation, have been characterised in older people and associations with poor outcomes, including mortality, are well established. It is therefore likely that that cachexia in older people is not only common but represents a syndrome that unifies skeletal muscle loss and inflammation to identify individuals with especially poor outcomes.

1.1.8 Cachexia: summary

Cachexia is a complex metabolic syndrome associated with underlying illness and characterised by loss of muscle and inflammation; it is associated with adverse outcomes and has recently been defined by consensus definition. Cachexia is poorly characterised in older people despite this group being particularly vulnerable, growing in numbers and often experiencing worse outcomes from, e.g. hospital admissions.

It is hypothesised that cachexia in older people is not only common but represents a syndrome that unifies skeletal muscle loss and inflammation. This thesis aims to test the utility of identifying cachexia within a cohort of older people in both hospital and community environments and justify the importance of this approach by establishing associations with adverse outcomes. It also aims to explore the role of skeletal muscle loss and inflammation in cachexia in older people.

1.2 Skeletal muscle

Weight loss is the major diagnostic component of the cachexia syndrome. When an older person loses weight, fat-free mass (predominantly skeletal muscle) is lost to a considerably greater degree than fat mass [38]. Skeletal muscle loss has been extensively studied in older people in relation to ageing; therefore, this thesis explores the role of skeletal muscle loss in the pathogenesis and significance of cachexia in older adults. This section offers a brief description of normal skeletal muscle anatomy and physiology before describing the changes that occur with age, the syndrome of sarcopenia and associations with morbidity and mortality.

1.2.1 Normal skeletal muscle anatomy and physiology

Normal skeletal muscle consists of longitudinally arranged groups of muscle fibres (myofibres) that are innervated by a single motor neurone to form a motor unit. Generation of strength (e.g. grip strength) is proportional to the number of motor units recruited and precision movements are attained by a reduced number of myofibres per motor unit. This is especially important in specialist muscles such as those involved with eye movements and also in the maintenance of balance which can be tested using the one legged (flamingo) stand.

Myofibres are classified into two major groups according to myosin heavy chain content. Type I myofibres have a high capacity for oxidative phosphorylation with high myoglobin levels and mitochondrial density. They are characterised by a slow contraction time and relative resistance to fatigue. Type II myofibres have a greater reliance on glycolytic enzymes and are used for short, powerful bursts of movement and typically have a fast contraction time but tend to fatigue rapidly. The relative proportions of myofibre types within a specific skeletal muscle affect its strength and endurance. For example, axial muscles involved in the maintenance of posture have a higher ratio of type I myofibres for sustained controlled contractions than those involved with locomotion where shorter, more powerful bursts are needed.

Microscopically, myofibres are multinucleated single cells containing long protein bundles called myofibrils (*Figure 1-1*). These are the basic contractile units of muscle, incorporating thin and thick filaments, primarily actin and myosin respectively, organised longitudinally

into repeated subunits called sarcomeres giving skeletal muscle its striated appearance. Pools of satellite cells lie quiescent between the basal lamina and plasma membrane of myofibres. Upon injury or mechanical loading, satellite cells are activated and start to proliferate, adding new myonuculei to myofibres in order to achieve hypertrophy or replenish the satellite cell pool. This process is important in the maintenance and repair of skeletal muscle through adulthood and into old age. Age related changes to skeletal muscle anatomy and physiology are manifest through deterioration in physical function domains such as strength, speed, balance and endurance. Physical function tests including gait speed and the timed get-up-and-go are useful measures of skeletal muscle that simultaneously test these functional domains.

1.2.2 Skeletal muscle contraction

Contraction is initiated by the propagation of an action potential into myocytes via T-tubules. This causes calcium ion influx through voltage gated ion channels and from the sarcoplasmic reticulum. Calcium binds to Troponin C on thin filaments leading to the allosteric modulation of tropomyosin and subsequent unblocking of myosin binding sites, enabling binding of myosin to actin and the release of ADP plus a phosphate. The bond is broken via the binding of ATP to myosin which is subsequently ‘re-primed’ through hydrolysation back to ADP. Calcium is then actively pumped back into the sarcoplasmic reticulum and the cycle finishes when levels of intra-cellular calcium fall causing the re-blocking of myosin binding sites. The speed with which this process occurs is largely dependent on ion fluxes within myofibres which, in-turn, will affect the speed of skeletal muscle contraction and therefore physical function.

Figure 1-1: Functional structure of skeletal muscle

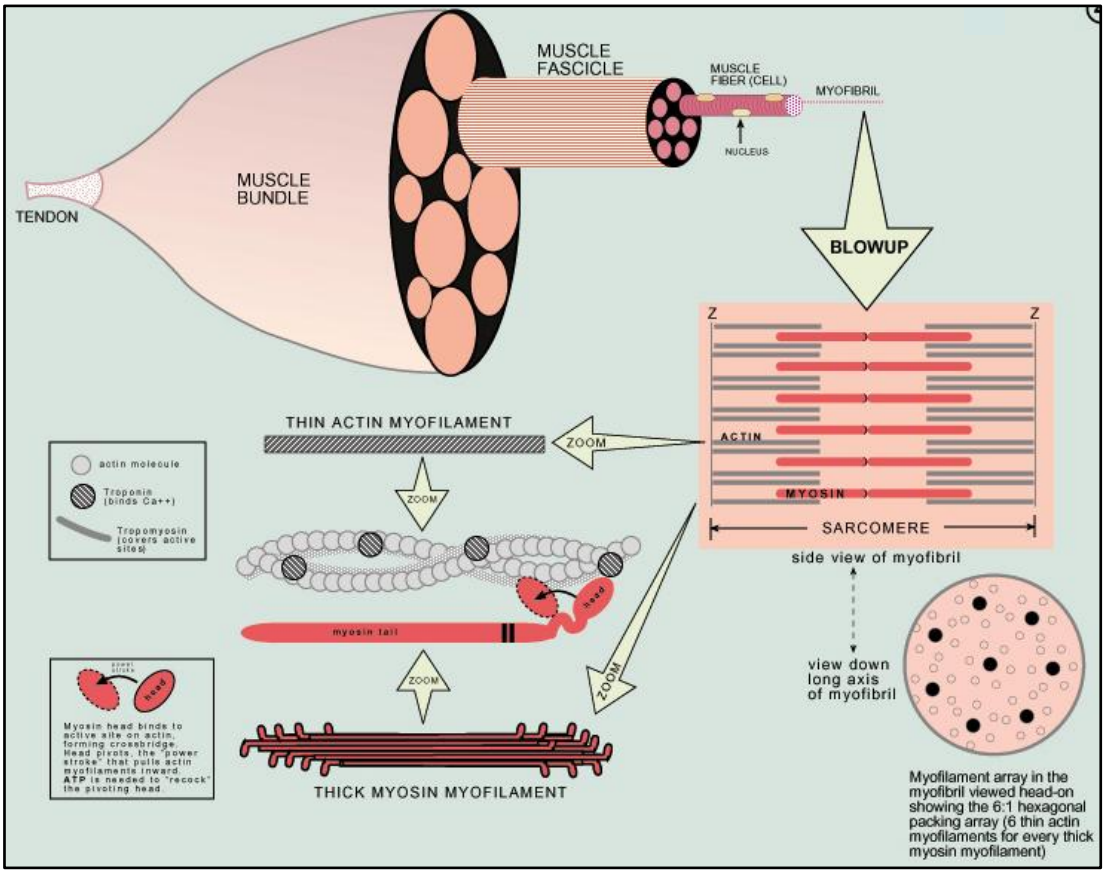


Diagram kindly provided by Mark Meyer

1.2.3 Anatomical, molecular and metabolic changes to skeletal muscle with age

1.2.3.1 Anatomical changes to skeletal muscle with age

Muscle mass and strength peaks in early adulthood and subsequently declines with age from approximately the fifth decade. In individuals over the age of 50 years, muscle mass is lost at a rate of 1-2% per year and strength at a rate of 1.5-3% per year [39]. Determinants of muscle ageing can be considered using a lifecourse approach (*Figure 1-2*)[40] and include early life influences that determine maximum muscle mass and strength in addition to mid- and later life influences that affect rate of decline, *Table 1-5* [41].

Figure 1-2: Muscle ageing, a lifecourse approach [40]

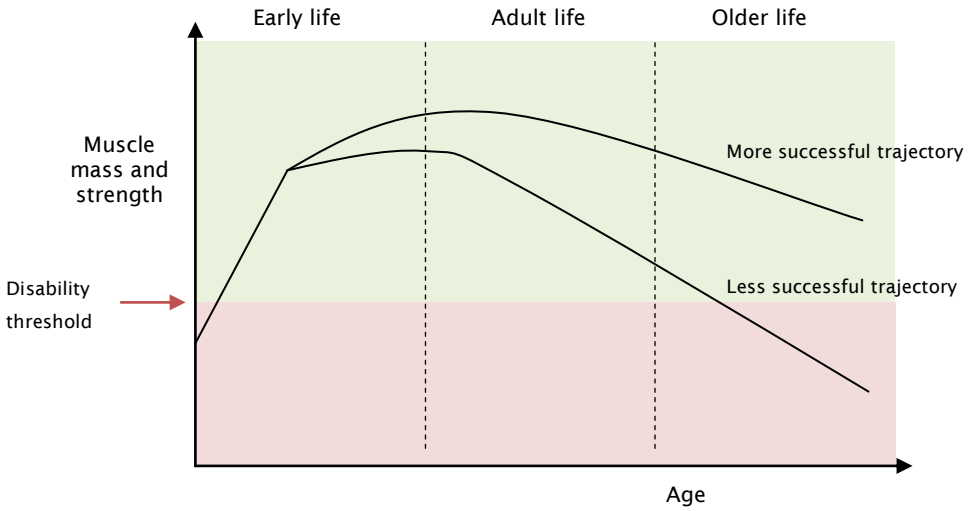


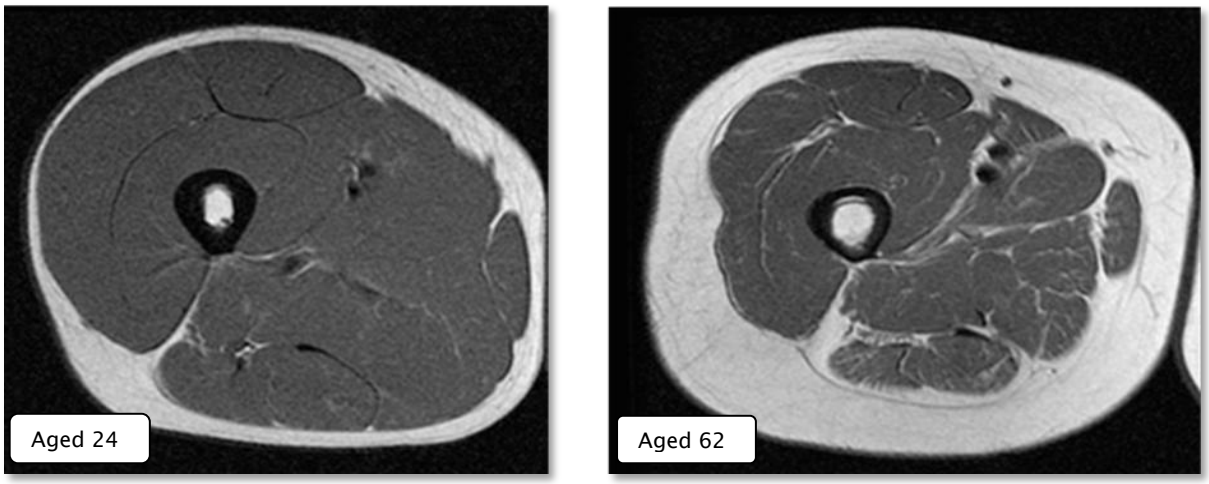
Table 1-5: Lifecourse influences on muscle ageing

Intrauterine	Maternal influences
	Early size and growth
	Genetic and epigenetic variation
Childhood	Size, growth and body composition
	Nutrition
	Physical activity
	Co-morbidity
Adulthood	Size and body composition
	Nutrition
	Physical activity
	Co-morbidity
	Ageing:
	Intrinsic (e.g. skeletal muscle)
	Extrinsic (e.g. neurodegeneration, immune-endocrine axis, anorexia of ageing)

1.2.3.2 Molecular changes to skeletal muscle with age

Macroscopically, ageing of skeletal muscle is associated with a reduction in muscle mass and infiltration of adipose and connective tissue (*Figure 1-3*). This loss of muscle may be missed by clinicians because *total* body size and leg circumference may not decline but remain constant or even increase due to a corresponding increase in fat mass [42].

Figure 1-3: Cross-sectional MRI images at the thigh



Microscopically, ageing is associated with a global decline in myofibre number as well as a reduction in size; there is some evidence for a preferential effect on type II myofibres [43]. Skeletal muscle is infiltrated with connective tissue and non-contractile proteins that reduce structural integrity; this is accompanied by a reduction in blood flow. Satellite cells become more resistant to activation and reduce in number, causing reduced regenerative capacity and impaired myofibre maintenance [40;44].

Remodelling occurs which is partly driven by changes to innervation. Myofibres undergo a continuous denervation and reinnervation due to the loss of motor neurones in the spinal cord; overall there is a 10-15% decrease in motor neurones with age [45]. This contributes to the loss and atrophy of myofibres, a reduction in motor unit number and an increase in motor unit size. Furthermore, regeneration by surviving axons is often incomplete and disorganised causing uncoordinated muscle contraction. Overall, these changes reduce skeletal muscle mass, strength, co-ordination and precision leading to deteriorations in physical functioning [40;45].

1.2.3.3 Metabolic changes to skeletal muscle with age

Muscle protein synthesis declines with age by approximately 28%, particularly affecting the myosin heavy chain and contributing to reduced contractile function. Although there are few age related changes to the enzymes of the glycolytic pathway, there is a reduction in mitochondrial volume and activity which causes a reduced muscle respiratory capacity, partly explaining the reduction in aerobic endurance that is noted with ageing [46].

Within the sarcoplasmic reticulum there is a reduction in the expression of faster Ca-ATPase proteins and increased expression of slower calcium-channel release proteins. This results in a slower flux of calcium into myofibres following depolarisation and contributes to the slowing of muscle contraction that is seen with age [47]. Again, this contributes to deterioration in functioning, particularly balance which is a specific risk factor for falls in older people [48].

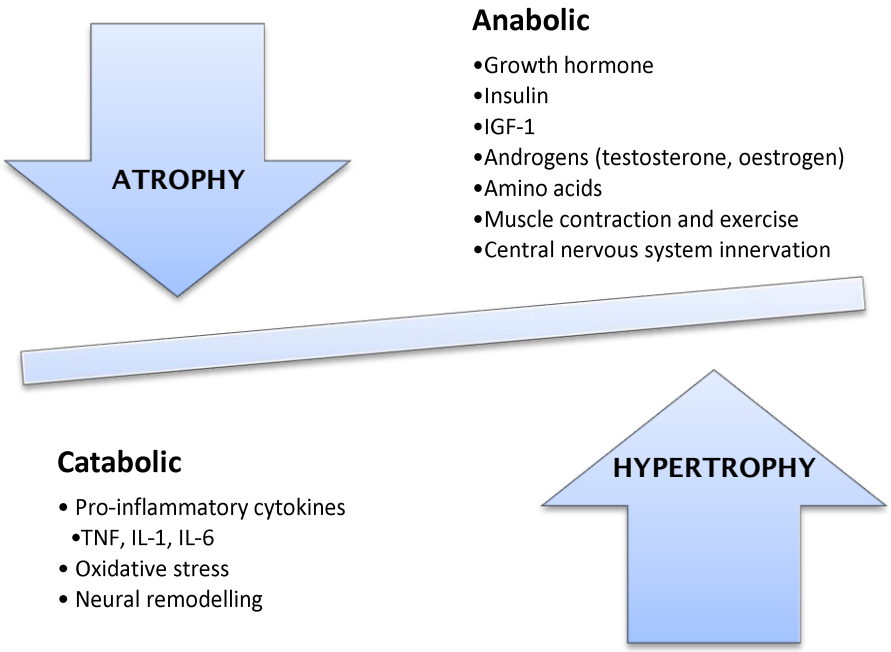
Table 1-6: Age associated changes to skeletal muscle

Decreased muscle mass and cross-sectional area
Reduced contractile function
Infiltration of adipose and connective tissue; reduced structural integrity
Decreased type II myofibre number and size
Decreased type I myofibre number and possibly size
Decreased motor units
Decreased blood flow
Reduced satellite cell number and increased resistance to activation
Decreased protein synthesis, especially myosin heavy chain
Decreased mitochondrial volume and activity; reduced anaerobic endurance
Slower calcium flux; slowing of muscle contraction

1.2.4 Systemic influences on skeletal muscle decline

Muscle is a dynamic organ that is constantly under both anabolic (hypertrophic) and catabolic (atrophic) influences; this balance determines myofibre size and as a consequence, total muscle mass, strength and physical performance. The immune-endocrine axis is strongly implicated and it is likely that the skeletal muscle changes associated with cachexia are mediated via a greater shift towards catabolism, *Figure 1-4*.

Figure 1-4: Anabolic and catabolic influences on skeletal muscle

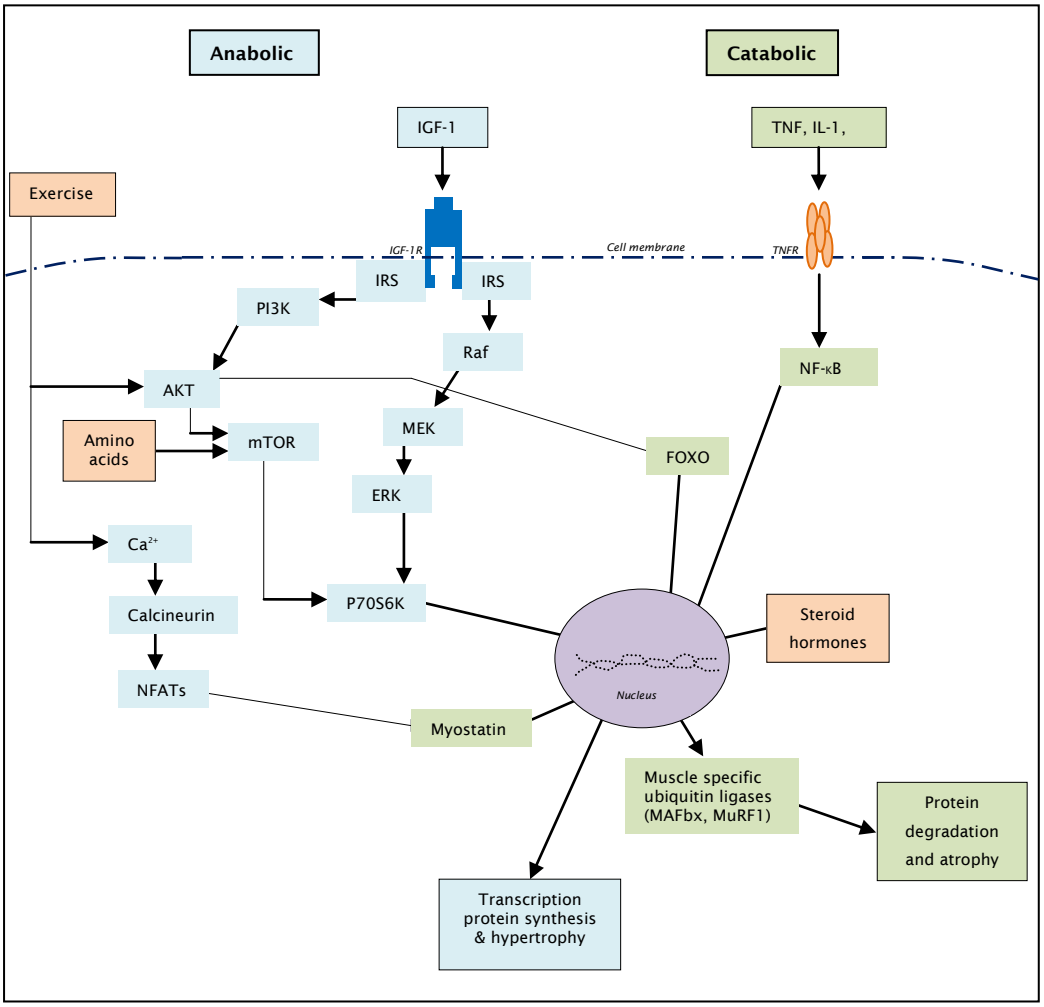


Anabolic signals that are reduced in cachexia include insulin and amino acids. However, as previously discussed, the principle implicated anabolic pathway associated with skeletal muscle loss occurs via a reduction in IGF-1, a polypeptide hormone that is predominantly secreted by the liver in response to growth hormone; a relationship key to growth and development [44]. When activated, IGF-1 receptors on skeletal muscle trigger intracellular signalling cascades that stimulate muscle protein synthesis as well as proliferation and differentiation of satellite cells [49]. IGF-1 also has anti-apoptotic effects on muscle cells and inhibits the ubiquitin-proteasome system and proteolysis (*Figure 1-5*) [50-52]. Perhaps unsurprisingly, transgenic mice over expressing IGF-1 in skeletal muscle have a hypertrophic phenotype. IGF-1 levels decline with age causing a shift in balance away from anabolism and towards catabolism. This is compounded by anabolic blunting, a term used to describe an

observed decline in response to anabolic effectors and a down-regulation of anabolic intracellular signalling molecules [44].

Forkhead box O (FoxO) transcription factors play a central role in transducing catabolic signals in skeletal muscle myocytes. In its normal state FoxO is phosphorylated and inactivated by anabolic signals (including IGF-1), via intracellular protein kinases. As the balance shifts towards catabolism and away from anabolism, FoxO translocates into the nucleus causing the transcription of skeletal muscle specific E3 ubiquitin ligases [44;53]. These label skeletal muscle proteins for degradation and are believed to be the major catabolic pathway in the skeletal muscle atrophy associated with cachexia.

Figure 1-5: Intracellular signalling pathways involved in myocyte morphology



1.2.5 Sarcopenia

Sarcopenia, from the Greek words *sarx* (flesh) and *penia* (poverty), was first described by Evans and Rosenburg in their 1991 book *Biomarkers: The 10 determinants of ageing you can control,* following an ageing and nutrition conference held in Albuquerque, New Mexico in 1989 [54]. It is used within clinical and research environments and is defined as the loss of muscle mass and function with age [55].

1.2.5.1 Prevalence and health related costs of sarcopenia

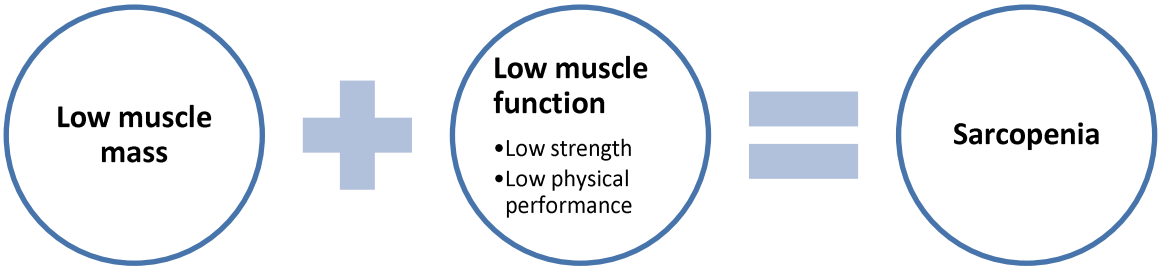
Sarcopenia is common with prevalence estimates ranging, according to definitions, from 9% to 18% over the age of 65, rising to 30% in men over 80 and even higher in hospitalised patients [41;56].

Healthcare costs for sarcopenia in the United States were estimated to be $18.5 billion which represented approximately 1.5% of total healthcare expenditure in 2000, a figure higher than the economic costs of osteoporosis and hip fracture despite the analysis not considering the contribution of sarcopenia to falls [57]. Figures are unavailable in the United Kingdom but similar proportions would be expected suggesting a healthcare cost to the UK of approximately $2.5 billion / £1.6 billion.

1.2.5.2 Sarcopenia diagnosis

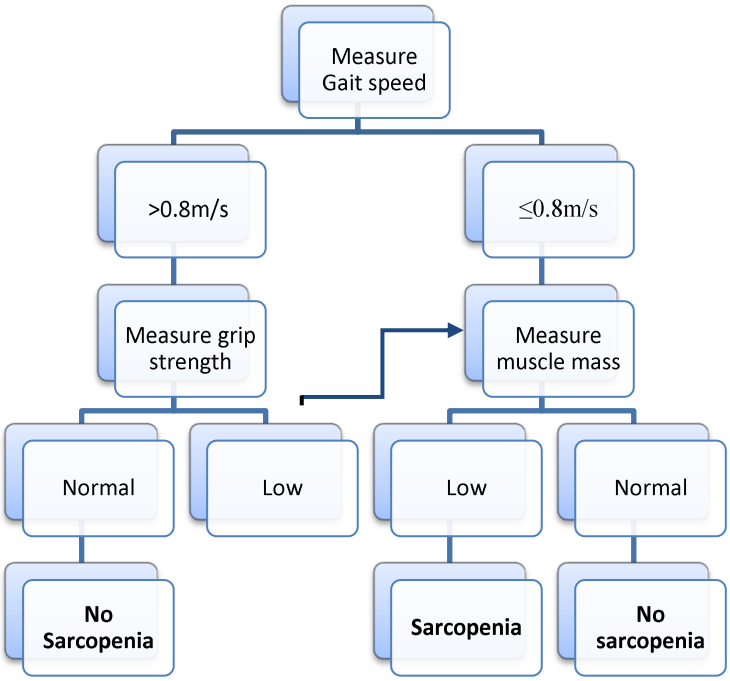
Since its original description over 20 years ago the study of sarcopenia has been inconsistent due to differing definitions used. Therefore, consensus definitions have recently been defined by both the European working group on sarcopenia in older people (EWGSOP) and the International working group on sarcopenia (IWGOS). EWGSOP define sarcopenia as 'a syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death'; the diagnosis depends on demonstrating both low muscle mass and low muscle function, *Figure 1-6*. The IWGOS definition is similar but with different cut-offs used and a greater emphasis on muscle mass [55;58].

Figure 1-6: European Working Group criteria for the diagnosis of sarcopenia



The age-related loss of muscle mass and function is a continuous variable. However, identifying individuals with or without sarcopenia requires defined cut-off points and a number of alternative approaches have been proposed. The EWGSOP recommends using an approach similar to osteoporosis where skeletal muscle mass is adjusted for height and compared against a healthy younger population with cut-off points at two standard deviations below the mean reference value [55]. With muscle mass measured by dual energy x-ray absorptiometry (DXA) scanning this equates to a cut-off point of 7.26 kg/m^2 for men and 5.5 kg/m^2 for women. Suggested cut-off points when measuring hand-grip strength are <30kg in men and <20kg in women. It is striking that when using these grip-strength definitions in older hospitalised populations the majority of patients will have sarcopenia [56]. An algorithm to facilitate the diagnosis of sarcopenia in older people has also been developed (*Figure 1-7*) and has recently been used to demonstrate a UK prevalence of 4.6% and 7.9%, among men and women (mean age 67 years) of the Hertfordshire Cohort Study [59]

Figure 1-7: Diagnosis of sarcopenia in older people [55]



1.2.5.3 Sarcopenia versus cachexia

The relationship between sarcopenia and cachexia is subject to much debate [27]. It is unclear whether they are disease processes at differing ends of the same spectrum or whether they are distinct entities. The current rationale is that cachexia is one cause of sarcopenia but the two processes are not the same. Sarcopenia occurs universally whereas cachexia only occurs in the presence of severe or accumulative inflammatory processes. Thus, most cachexic individuals are also sarcopenic but few people with sarcopenia are also cachexic. The hallmark of cachexia is a sustained, measurable systemic inflammatory response with associated weight loss. Sarcopenia is a multifactorial age-associated condition of skeletal muscle.

1.2.6 Skeletal muscle assessment techniques

The assessment of muscle mass and muscle function is critical to determining not just sarcopenia but also cachexia and frailty and it therefore an important aspect of this thesis.

1.2.6.1 Assessment of muscle mass

A wide range of techniques can be used to characterise muscle mass in both clinical and research environments (*Table 1-7*). Whole body and peripheral computed tomography as well as magnetic resonance imaging scans are very precise and can accurately differentiate muscle from other soft tissues of the body making these methods the gold standards. For clinical purposes, dual energy x-ray absorptiometry (DXA) is a suitable alternative technique and is readily available, cheaper and involves minimal radiation [60].

Bioelectrical impedance analysis (BIA) can be used to estimate the volume of fat and lean body mass. This technique utilises portable equipment so can be used in a range of settings including hospitalised patients confined to bed and in people's homes. Under standardised conditions BIA is reproducible and correlates reasonably well with gold standards [61]. However, standard conditions can be difficult to reproduce in older populations with co-existing morbidity and polypharmacy and the use of BIA is controversial and not recommended by the IWGOS [58].

Table 1-7: Tools for diagnosing skeletal muscle

Variable	Research environment	Clinical environment
Muscle mass	Computed Tomography	Bioimpendance analysis
	Magnetic resonance imaging	Dual energy x-ray absorptiometry
	Dual energy x-ray absorptiometry	Anthropometry
	Bioimpendance analysis	
Muscle strength	Handgrip strength	Handgrip strength
	Quadriceps strength	Peak expiratory flow
	Peak expiratory flow	
Physical performance	Short physical performance battery	Short physical performance battery
	Usual gait speed	Usual gait speed
	Timed up and go test	Timed up and go test

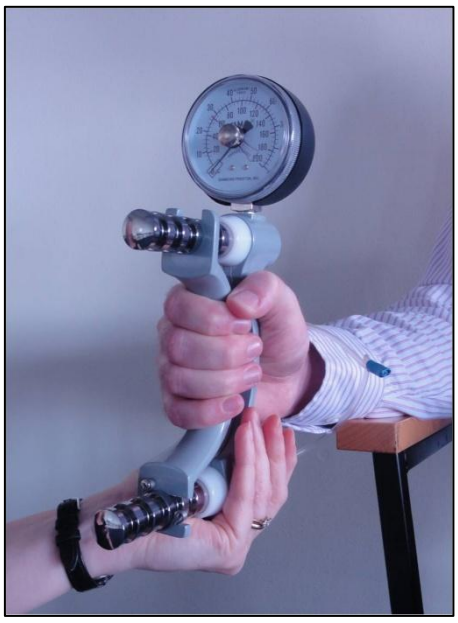
1.2.6.2 Assessment of muscle strength

Muscle strength is often characterised using isometric hand grip strength measurement. This correlates with lower limb muscle strength and is independently associated with poor clinical outcomes as well as poor mobility and demonstrates a linear relationship with activities of daily living scores [62]. Measurement is by handheld dynamometry and the Jamar® dynamometer is most widely used; a standardised protocol has been described (Southampton Protocol) [62]. An alternative method uses quadriceps strength although this technique suffers from being cumbersome and costly to perform and from the lack of standardised equipment or protocols [63]. Furthermore, it is not recommended in the EWGSOP guidelines.

1.2.6.3 Assessment of physical performance

Skeletal muscle function may also be assessed using physical performance measures which include gait speed, one legged stands and chair rises. Of these assessments, gait speed is the most widely used in clinical environments and also the most practicable in older people [64].

Photograph 1: Jamar® hand held dynamometer



1.2.7 Associations with co-morbidity and body composition

Skeletal muscle loss is associated with a range of co-morbidities and all cause mortality [21]. This includes increased morbidity from osteoporosis, type 2 diabetes mellitus, ischemic heart disease and chronic lung disease [65-68]. It is also associated with a number of functional limitations and disability in community dwelling people of middle and older age [69-71].

There is a clear relationship between skeletal muscle and bone mass throughout the lifecourse. For example, the Sarcopenia and Hip fracture study reported that 75% of participants with hip fracture were also sarcopenic. Over one year follow-up, 56% fell at least once, 28% had recurrent falls and 12% sustained a new fracture; 5% hip fracture [72].

Furthermore, the Hertfordshire Cohort Study reported an inverse relationship between grip strength and falls within the last year and Joint American and British Geriatric Society guidelines for the prevention of falls in older people describe muscle weakness as the single biggest intrinsic risk factor for falling with an attributed relative risk of 4.4 [73-75].

Associations between the loss of skeletal muscle and outcomes relating to acute hospital admissions have also previously been demonstrated; it is negatively associated with the likelihood of discharge to usual residence and positively associated with longer lengths of stay in surgical, oncology and rehabilitation environments [56;76;77].

1.2.7.1 Frailty

Skeletal muscle atrophy is strongly implicated in the syndrome of frailty which describes a state of increased vulnerability resulting from decreased physiological reserves, multi-system dysregulation and a limited capacity to maintain homeostasis [78]. Frailty is of increasing global importance, impacting on all forms of adult healthcare as well as socioeconomic policy. A person who is frail is less likely to be living independently at home and people who are frail have an increased likelihood of morbidity and mortality [79;80]. It also has predictive validity for adverse health outcomes including falls, disability and receipt of hospital care [79;81]. Prevalence estimates were recently reported as 8.5% for women and 4.1% for men among community dwelling people aged 64 to 74 years [82]. The numbers of frail older people will increase as populations' age, particularly as numbers at the oldest ages are increasing the fastest.

Physical frailty is likely to be highly prevalent within hospitalised older people and also shares considerable overlap with sarcopenia and cachexia. Indeed, the Fried criteria, which are the most widely implemented objective approach to the classification of physical frailty, define the syndrome on the basis of weight loss, weakness, exhaustion, slowness and low activity [79]. This thesis offers an opportunity to explore the degree of overlap between the syndromes of sarcopenia, cachexia and frailty; a relationship which is currently unclear.

1.2.8 Skeletal muscle: summary

The change to skeletal muscle with age is an important process implicated in age related disease and also declines in physical functioning. Sarcopenia is the loss of muscle mass and function with age; it has recently been defined and is associated with a variety of clinical outcomes. The loss of skeletal muscle is an important component of cachexia and is understudied in hospitalised older adults. It may, in part, explain associations between cachexia and poor outcomes and will be explored in greater detail within this thesis.

1.3 Inflammation and ageing

The second major characteristic of cachexia is inflammation. The changes that occur to the immune system with age are a fertile area of ageing research and increasingly studied in relation to outcomes. Therefore, this thesis explores the role of inflammation in the pathogenesis and significance of cachexia in older adults. The following section initially presents an overview of the immune system followed by a discussion of how it changes with age and is associated with age related disease, skeletal muscle and clinical outcomes.

1.3.1 The immune system: an overview

The first reference to immunity dates back to the plague of Athens in 430BC when it was noticed that people who recovered from the disease could then nurse others without contracting it for a second time. It has since been recognised that the immune system is an intricate biological defence system of structures and processes that protect an organism from disease. It is highly evolved and combines physical and mechanistic barriers (skin, mucous membranes, cough reflex) with two principle components; innate and acquired immunity.

1.3.1.1 The innate immune system

The innate immune system is evolutionarily older and found in all plants and animals; it is non-specific and confers no memory. The innate immune system directly removes foreign bodies and co-ordinates, amplifies and limits immune responses by the recruitment of immune cells to the site of infection via the production of cytokines, activation of complement cascades and the acquired immune system. Cells of the innate immune system recognise pathogens via pattern recognition receptors (PRR) expressed on surface membranes and within the cytoplasm. These include toll-like receptors and purinergic receptors.

White blood cells (WBC) of the innate immune system are the products of multipotent haemopoietic cells in the bone marrow and are not tissue specific. Phagocytes are the commonest WBC and are subdivided into neutrophils (most abundant), dendritic cells (at skin and mucous membranes sites for antigen presentation) and macrophages/monocytes,

(monocytes are principally resident in the spleen and respond rapidly to infection via division and migration to tissues where they become macrophages). The principle role of phagocytes is to destroy antigens through a combination of phagocytosis (engulfing the foreign body) and the release of strong oxidising agents; phagoctyes also secrete cytokines.

Other cells of the innate system include natural killer cells which destroy compromised host cells such as those infected with virus or tumour cells and also mast cells which are found in mucous membranes and connective tissue and release histamine-heparin rich granules when activated.

The complement system consists of 25 small proteins or protein fragments secreted in an inactive form by the liver. These are then activated either indirectly by antibodies (classical pathway) or directly by antigen (alternate and lectin pathways). Upon activation the protease C3-convertase cleaves and activates protein C3 to trigger a series of downstream complement activations that ultimately amplifies the immune response and results in a membrane attack complex. The complement system enhances the phagocytosis of antigen (opsonisation), attracts phagocytes (chemotaxis), ruptures cell membranes (cell lysis) and causes the clumping together of antigen complexes to facilitate their removal.

1.3.1.2 The acquired immune system

The acquired immune system recognises foreign (non-self) antigen, mounts an effective, tailored immune response and generates memory cells to facilitate the rapid removal of antigens encountered on subsequent occasions. It is formed from leukocytes derived from multipotent haemopoietic cells in the bone marrow. A key process in the maturation of leukocytes is the process of somatic hypermutation which involves a super-fast programmed series of mutations to the variable regions of immunoglobulin genes when leukocytes divide. This generates a repertoire of immunoglobulins so that leukocytes with the greatest affinity for a specific antigen can then be selected; somatic mutations are then passed to subsequent cell lines to generate memory.

T-cells and B-cells are the principle leukocytes. T-cells mature in the thymus to become either CD4 or CD8 positive, a process involving positive and negative selection processes which only 2% of leukocytes survive. Several subsets exist, each with a different function (*Table 1-8*).

Table 1-8: T-cell sub-sets

T helper cells	CD4 positive, recognise antigen presented by MHC class II peptides	Assist other immune cells via the secretion of cytokines. Can differentiate into different subsets (1, 2, 3) according to cytokine profile excreted and type of immune response
Cytotoxic T cells	CD8 positive, recognise antigen presented by MHC class I peptides	Destroy cells infected by virus and tumour cells
Memory T cells	May be either CD4 or CD8 positive	Rapidly expand upon re-exposure to their antigen
Regulatory T cells	CD4 positive	These cells 'apply the breaks' towards the end of an immune reaction and remove auto-reactive T-cells that escaped negative pressure in the thymus
Natural Killer T cells	Recognise antigen presented by CD1d	These are different to the natural killer cells of the innate immune system. They can assume the function of T helper and cytotoxic T cells

B-cells mature in specialist lymphoid organs such as the spleen and lymph nodes; millions of different types develop, each with unique antigen recognition ability on its surface (B-cell receptor, BCR). B-cells require activation via binding antigen to its BCR and co-stimulation from a T helper cell; this causes the B-cell to divide, forming plasma B-cells and memory B-cells. Plasma B cells produce large amounts of antibody that sticks to the foreign antigen thereby facilitating its removal by the innate immune system and are also professional antigen presenting cells. Memory B-cells live for a long time and can respond quickly by division in the event of re-exposure to an antigen.

1.3.1.3 Acute phase proteins

Acute phase proteins (APPs) comprise non-specific physiological and biochemical responses to most forms of inflammation, tissue damage, infection and neoplasia [83]. These proteins are rapidly up-regulated, principally in hepatocytes, under the control of cytokines originating at the site of pathology. Positive APPs have a range of functions including the destruction or inhibition of microbes, generation of a pro-thrombotic state, increasing vascular permeability and promoting the chemotaxis of immune cells. An example is C-reactive protein which binds to dead or dying cells in order to activate the complement system. Other examples of positive AAPs include complement, amyloid, ferritin and haptoglobin. Negative AAPs reduce in concentration during inflammation in-order to preserve amino acids for the production of positive AAPs. Examples are albumin and transferrin.

1.3.2 Immunosenescence

The immune system is highly conserved across vertebrate species, and from an evolutionary perspective has undergone strong pressures to maximise survival to allow procreation. The significant improvements in human survival and lifespan to well beyond child bearing ages have been totally 'unpredicted' by evolution. As a consequence, human immune systems are exposed to considerable additional antigenic exposure outside the forces of natural selection. It is in this situation that immunity begins to exert negative effects on human ageing (antagonistic pleiotropy) leading to gradual systemic failures [84;85].

The immune system of older people declines in reliability and efficiency with age resulting in greater susceptibility to pathology as a consequence of inflammation e.g. cardiovascular disease, Alzheimer's disease, autoreactivity and vaccine failure as well as an increased vulnerability to infectious disease [86;87]. These changes are further compounded by reduced responsiveness and impaired communication between all cells of the immune system. The overall change to the immune system with age is termed immunosenescence and has a multifactorial aetiology; a consequence of the complexity of the immune system as well as of multiple genetic and environmental influences [86]. Immunosenescence of the innate immune system is characterised primarily by reduced cellular superoxide production and capability for phagocytosis. Involution of the thymus and reduced responsiveness to new antigen load due to a reduced naïve: memory cell ratio and expansion of mature cell clones, characterises immunosenescence of the acquired immune system (*Table 1-9*).

Table 1-9: Immunosenescence, *adapted from* [86]

Hematopoietic stem cells	-	Reduced capacity for renewal
	-	Possible association with telomere attrition
Innate immunity	-	Reduced barrier function, increased challenges
	-	Inflammaging
	-	Neutrophils: Reduced phagocytic ability of opsonised bacteria and impaired superoxide production
	-	Macrophages: Reduced levels of MHS class II complexes, reduced phagocytic ability and impaired superoxide production
	-	Dendritic cells: Impaired capacity to phagocytose apoptotic cells, resulting in pro-inflammatory response; impaired migration
	-	Natural Killer cells: absolute increase in numbers but reduced cytotoxicity, associated with increased morbidity and mortality
T-cells	-	Thymus atrophy
	-	Reduced naïve cells leaving thymus, severely contracted T-cell repertoire after 70 years
	-	Impaired expansion and differentiation
	-	Increased pro-inflammatory cytokine release, reduced IL-2 production
	-	Increased memory and effector cells
	-	Impaired T-cell help of B-cells
	-	Reduced regulatory T-cells – possible increased inflammation and autoreactivity
	-	Expanded clones of CMV+ve (and HSV, EBV)† CD8+ cells, dominating the T-cell repertoire and limiting response to other pathogens
B-cells	-	Reduced number of mature B-cells leaving bone marrow
	-	Increased memory B-cells, decline in naïve (CD27 –ve) B-cells
	-	Reduced responsiveness to stimulatory molecules
	-	Impaired antibody response to vaccination

†CMV, cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus

1.3.3 Inflammaging

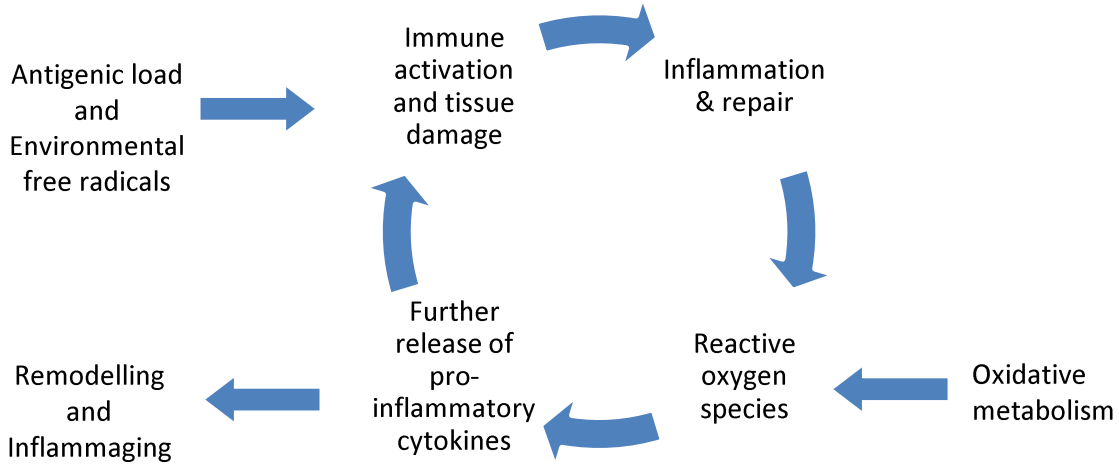
Research into age-related changes of the immune system is gathering pace as its importance within the context of multiple age-related pathologies is realised. As part of this advance, Franceschi and colleagues described the phenomenon of 'inflammaging' at the turn of the millennium as part of the spectrum of immunosenescence [88]. Inflammaging denotes an upregulation of the inflammatory response that occurs with age resulting in a low-grade chronic systemic pro-inflammatory state. It is characterised by raised levels of pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF); all of which have been shown to rise with age and be involved in the pathogenesis of most age-associated diseases [89;90]. C-reactive protein, an acute phase protein produced by the liver in response to IL-6 is also a useful marker of inflammaging and is more commonly

used in clinical practice and also a robust predictor of risk of cardiovascular and other diseases [91;92].

1.3.3.1 Aetiology

Inflammaging is believed to be a consequence of a cumulative lifetime exposure to antigenic load caused by both clinical and sub-clinical infections as well as from exposure to non-infective antigens [84]. The consequence is an inflammatory response, tissue damage and the production of reactive oxygen species (ROS) which elicit the release of additional cytokines, principally from cells of the innate immune system, but also from the acquired immune response [93]. The result is a vicious cycle driving immune system remodelling and favouring a chronic pro-inflammatory state where pathophysiological changes, tissue injury as well as healing proceeds simultaneously. Irreversible cellular and molecular damage that is not clinically evident slowly accumulates over decades (*Figure 1-8*).

Figure 1-8: Cycle of inflammaging



Immunosenescence of the acquired system contributes to inflammaging via a reduced T-cell repertoire and increased oligoclonal expansion of memory and effector-memory cells [94]. This imbalance results in reduced ability to clear novel pathogens (elongating infection duration and antigen exposure) as well as increasing functionally distinct T-cell populations which have an amplified pro-inflammatory phenotype [95].

The $CD8^+$ T-cell population is altered to a greater extent than the $CD4^+$ population and is associated with specificity to single antigens, particularly latent viral infections such as Cytomegalovirus (CVM), Epstein-Barr and Varicella Zoster. Increases in antigen specific cells with age are associated with an increase in terminally differentiated 'senescent' cells which occupy a large proportion of immune space. These cells, particularly $CD8^+$, are extremely potent producers of inflammatory cytokines and have been heavily associated with reduced anti-viral immunity and inflammatory related pathologies [96]. With age, these antigen specific cells do not proliferate as effectively, and upon mitogenic and viral stimulation, produce more IL-6 and TNF than their younger counterparts [97]. Subsequently antigenic load is a known driver of inflammaging and has been associated with increased IL-6 and mortality [98]. Therefore, T-cell immunosenescence is characterised by an increased pro-inflammatory phenotype and most likely contributes to inflammaging in a manner dependent on previous exposure to and reactivation of antigenic challenges and exhaustion of T-cell repertoire.

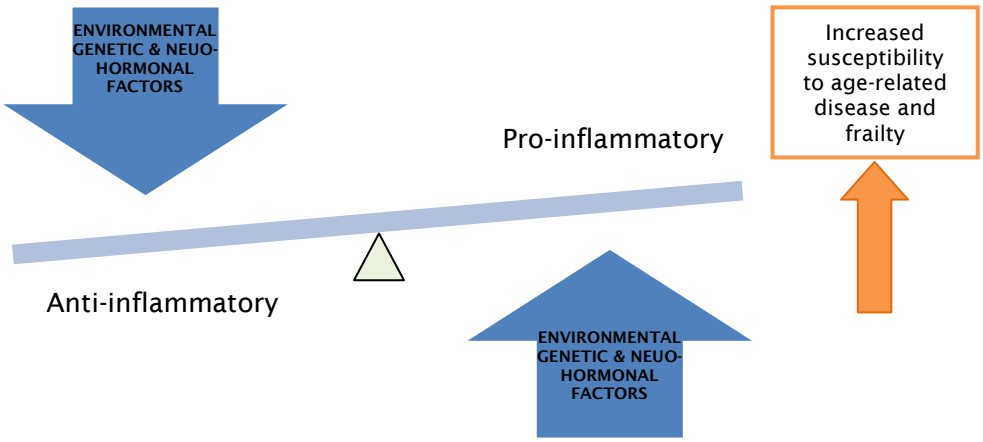
Of the innate immune system, monocyte/macrophages are suggested to contribute to inflammaging more than any other cell type. Monocyte changes with age contribute to inflammaging by a functional shift towards a pro-inflammatory phenotype and reduced function [99]. Monocytes consist of 3 distinct subtypes, differentiated by expression of CD14 and CD16; $CD14^{++}/CD16^-$ (classical), $CD14^{++}/CD16^+$ (intermediate) and $CD14^+/CD16^{++}$ (non-classical), with varying degrees of functional capabilities [100]. The $CD16^+$ positive monocytes constitutively produce more IL-6, IL-1β and TNF basally and with stimulation, with older people having a significantly larger proportion of $CD16^+$ cells than the young [101;102]. Furthermore, the $CD16^+$ population have increased adherence and migrate towards endothelial lesions via CX3CR1, contributing to increased atheroma plaque formation [103]. Subsequently, monocytes can contribute to inflammaging by chronically increased production of inflammatory cytokines and elongation of the immune response.

1.3.3.2 Resilience

A two-hit hypothesis states that the extent to which an individual is vulnerable to inflammaging and its physiological consequences is dependent firstly on the degree of increased pro-inflammatory environment and then on the resilience of that individual to withstand it. This is reflected in-part by the balance of increasing levels of pro-inflammatory cytokines with anti-inflammatory cytokines, and also concentrations of soluble cytokine receptors and cytokine receptor antagonists. These are believed to affect the degree to

which an individual is susceptible to antigenic load, the rate at which age related pathologies progress and individual vulnerability to frailty, *Figure 1-9* [85;89]. Indeed, centenarians, a population deemed to have aged successfully, have strong and effective anti-inflammatory responses with a reduced pro-inflammatory capacity [104]. This degree of susceptibility is multifactorial and a consequence of complex genetic and environmental interactions as well as the ageing process itself.

Figure 1-9: Relationship between pro- and anti-inflammation and age related disease



1.3.3.3 Susceptibility

There is now convincing evidence that susceptibility to inflammaging is, in-part, genetically determined through polymorphisms of promoter regions of genes that either affect cytokine production rates or alter their protein structure to produce a functional variant. For example, polymorphisms in the promoter C/G 174 on the IL-6 gene have been shown to affect serum IL-6 concentrations [105]. Similar associations have been found in alleles for IL-10 and polymorphisms of the toll-like receptor 4 residing on the membrane of dendritic cells and macrophages. These positively affect inflammatory responses and are associated with an increased risk of acute myocardial infarction [106;107].

Telomeres may also be implicated; these are protein complexes made from several thousand repetitive DNA sequences located at the end of chromosomes to protect them from deterioration or fusion. Telomere repetitive sequences shorten with each cell division,

eventually reaching a critical number leading to cellular senescence and death. There is evidence that this process is associated with inflammation although this is controversial and the direction of this relationship is unclear and could be bi-directional. For example, raised IL-6 and TNF was associated with shorter telomere length in 1,962 individuals of the Health, Ageing and Body Composition Study [108]. Shorter telomere length has also been found in cohorts of patients with chronic inflammatory diseases of liver, kidney and lung [109]. Negative relationships with telomere length have also been demonstrated with CRP and IL-6 [110;111].

A decrease in autophagy mechanisms occurs to varying degrees with age and further contributes to pro-inflammatory environments [112]. Autophagy is a cellular housekeeping mechanism that is responsible for the removal of dysfunctional intracellular protein (e.g. dead organelles, damaged scaffold proteins) via lysomal degradation. This prevents the activation of inflammasomes; intracellular multi-protein sensors which stimulate the inflammatory response after recognising danger signals emanating from proteins such as the intra-cellular damage-associated molecular patterns (DAMPS) that occur as a consequence of either tissue injury or necrosis. The consequence of a decline of autophagy with age is therefore an increased activation of the inflammasome and greater pro-inflammatory responses [113].

Ageing is associated with a linear accumulation of adipose tissue, both around the viscera and via the fatty infiltration of several organs including liver, bone and muscle [114]. This adipose tissue, once thought of as an inert, is now considered to be a major endocrine and paracrine organ [115]. To date approximately one hundred adipokines have been identified, falling into several functional groups; many are pro-inflammatory [116]. For example, the classic adipokine, leptin, not only has a primary role in energy homeostasis but also causes the stimulation and differentiation of monocytes into macrophages, activates NK-lymphocytes and induces the production of a number of pro-inflammatory cytokines including TNF and IL-6 [117]. Consequently, greater degrees of adiposity are directly associated with greater pro-inflammatory environments, partly via pro-inflammatory adipokines and also due to immune cells residing within adipose tissue.

1.3.4 Anti-inflammaging

As with all complex organisms, single biological systems rarely work in isolation. There is extensive cross-talk between the immune and endocrine axes which, together with the neural system, form the major communication network in the body [118]. For example, in infected tissue, local cells produce mediators which can either appear in the circulation or stimulate sensory nerves to announce the problem at distant sites, such as the brain, which may respond by activating a hormonal axis (e.g. hypothalamic-pituitary-adrenal) or neural system (e.g. autonomic). In a very similar way, an ageing cell or a group of ageing cells within an organism may be announced to distant sites in the body and generate multiple age-related interactions of the endocrine and immune axes [118].

1.3.4.1 Cortisol

Cortisol is a glucocorticoid hormone secreted by the adrenal gland in response to the pituitary secretion of adrenal corticotrophic hormone (ACTH) which itself is stimulated by corticotrophin releasing hormone from the hypothalamus; together these form the HPA axis. Cortisol plays key roles in the stress response and is also immunosuppressive [119]. Higher circulating cortisol levels have been observed in critically ill intensive care patients and also in association with septic shock and trauma [120;121]. Free cortisol undergoes diurnal variation with levels highest at approximately 8am and ageing is associated with a small rise in baseline free cortisol levels and also an increase in circadian levels (greater peaks and troughs). Therefore a stressful insult in an older individual results in a greater rise in free cortisol than in younger controls [122].

Neuronal cells within the HPA axis contain multiple cytokine receptors, particularly for IL-1, IL-6 and TNF, and it has been demonstrated in humans' *in-vivo* that injection of IL-6 or TNF induces a marked change in HPA axis; an inevitable physiological response to inflammaging is an increase in circulating cortisol levels [123-125].

This mechanism has been termed *anti-inflammaging* and although it represents an appropriate attempt to counter the inflammaging process it may also have negative implications. These include the paradox of both inflammaging and the global immunosuppression seen with increasing age in addition to associations with frailty (and

possibly cachexia) via catabolic effects on several tissue types such as liver (gluconeogenesis), muscle (protein catabolism) and bone (resorption) [125].

1.3.4.2 Dehydroepiandrosterone

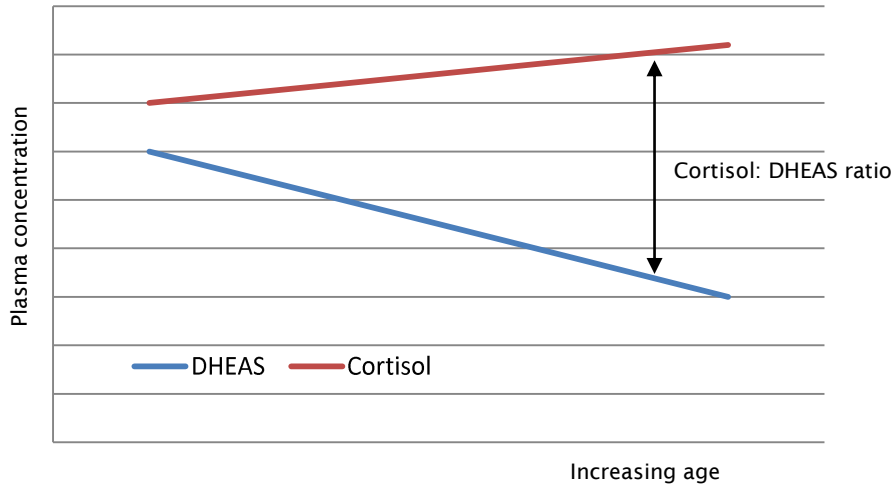
Dehydroepiandrosterone (DHEA) and its sulphated precursor, DHEA-Sulphate (DHEAS) have opposing actions to cortisol and may protect individuals from the negative effects of anti-inflammaging. They are secreted from the adrenal cortex as well as from the testis and ovary, albeit in smaller amounts, and subsequently converted to sex steroids; they account for 30-50% of testosterone in men and 100% of oestrogen in post-menopausal women. Like cortisol, DHEAS is also secreted in response to ACTH but serum levels are more stable as a consequence of stronger binding to albumin [126]. Circulating DHEAS concentrations reach a maximum in early adulthood and subsequently decline with age to approximately 10-20% of maximal levels by 70 years [127]. There is large inter-individual variability in circulating DHEAS concentrations; some post-menopausal women have barely detectable serum concentrations whilst others have normal values. The fall in DHEAS is accompanied by a parallel fall in androgens resulting in women with low DHEAS levels experiencing a deficit of sex steroids for their remaining lifetime and negative implications for the ageing process [127].

More recently an immunomodulating role for DHEAS has been realised [119]. The molecule counteracts the effects of cortisol via antagonism of glucocorticoid receptors (GR) either directly or via its downstream metabolites; low levels are associated with chronic inflammatory conditions and DHEAS may also directly inhibit GR production. Its importance as a counter-balance to cortisol has been demonstrated in a number of *in-vitro* studies where the immunosuppression of neutrophil function that occurs with cortisol has been successfully overcome by co-incubation with DHEAS [128]. Additionally, the steroid is an enhancer of IL-2 secretion from T-helper 1 (Th1) cells and negatively affects secretion from T-helper 2 (Th2) cells. Therefore, it has been suggested that observed declines in DHEAS levels with age, at least in part, explains the cytokine dysregulation seen with age and specifically, the shift towards Th2 cytokine profiles.

1.3.4.3 Cortisol:DHEAS ratio

From the above discussion it is apparent that both cortisol and DHEAS have opposing effects relating to the immune system. Cortisol increases with age and causes immune-suppression whilst DHEA(S) falls with age, antagonises the effects of cortisol and is immune modulating. Therefore, when considering the effects of the HPA axis on inflammaging the ratio of cortisol to DHEAS (*Figure 1-10*) may more accurately reflect the HPA axis rather than either cortisol or DHEAS alone. An *in-vivo* study of cortisol and DHEAS in patients with septic shock post hip-fracture showed that higher cortisol, lower DHEAS, and higher cortisol: DHEAS ratio correlated with poorer clinical outcomes [121]. This ratio has also been positively associated with metabolic syndrome and all-cause, cancer and other medical cause mortality in Vietnam veterans [129;130].

Figure 1-10: Changes in adrenocorticoid hormones with age



Cortisol:DHEAS ratios have the potential for manipulation via DHEAS supplementation. To date, DHEAS supplementation has been trialled in a number of chronic inflammatory conditions usually with non-beneficial outcomes; however the doses used have always been low. The only true beneficial effect of DHEAS supplementation has been seen in patients with Systemic Lupus Erythematosus where the drug successfully reduces disease activity and the number of exacerbations [131-133].

1.3.5 Associations with age related disease

Inflammaging and in-particular elevations in levels of TNF, IL-6, IL-1 and CRP are strong independent risk factors for morbidity and mortality in older people [10]. Epidemiological and mechanistic studies suggest that many age-related diseases are initiated or worsened by systemic inflammation including neurodegeneration and atherosclerosis (*Table 1-10*) [134;135]. For example, pro-inflammatory cytokines have been shown to interact with the processing and production of Aβ peptide, the pathological hallmark feature of Alzheimer's disease (AD) and autopsy studies have shown that the brains of patients who had AD have considerably higher levels of pro-inflammatory markers than high-pathology controls that had pathological features of AD without its symptoms [136].

Table 1-10: Inflammaging and age-associated diseases, *adapted from* [90]

Disease	Implicated cytokines
Atherosclerosis	TNF
Ischaemic heart disease	TNF, IL-1β
Cerebrovascular disease	TNF, IL-1β, IL-6
Anaemia	TNF, IL-1, IL-6
Pulmonary disease	TNF, IL-1β, IL-6
Thromboembolic disease	IL-6
Sarcopenia	TNF, IL-6
Osteoporosis and arthritis	TNF, IL-1β, IL-6
Autoimmune disease	TNF, IL-1β, Il-6, IFN-γ
Anorexia	TNF
Type II diabetes mellitus	TNF, IL- 1β, IL-6, IGF1
Dementia	TNF, IL-1β, IL-6
Frailty	IL-6

Inflammaging also affects the anabolic-catabolic balance within myocytes, causing a shift towards catabolism and progression of skeletal muscle atrophy. As with other age-related disease processes, it is believed that the degree of shift towards catabolism affects the rate of skeletal muscle atrophy; cachexia in older people may represent the more extreme end of this spectrum. A number of community based observational studies have demonstrated associations between pro-inflammatory cytokine profiles and skeletal muscle loss. For example, cross-sectional analysis from the InCHIANTI study demonstrated associations

between inflammation (IL-6, IL-1R, CRP), poor physical performance and reduced muscle strength and Schaap showed associations between TNF and 5 year change in muscle mass [137;138]. Similar findings have been reported in the Framingham Heart Study (IL-6) and the Longitudinal Aging Study Amsterdam (IL-6, CRP) and have recently undergone systematic review [139]. Inflammaging is also implicated in the progression of osteoporosis and dementia, both strongly implicated in the frailty syndrome [134;140].

Higher cortisol has been associated with increased mortality in patients with stroke [141], sepsis [142], heart failure [143] and sarcopenia [144]. Low levels of DHEA have been demonstrated in patients with chronic inflammatory diseases including inflammatory bowel disease; rheumatoid arthritis; systemic lupus erythematosus; and pemphigus [145]. Moreover, associations have also been established between low DHEAS concentrations and cardiovascular disease, sarcopenia, osteoporosis and all cause mortality [146-148].

Inflammaging is also associated with adverse functional outcomes including disability and incident frailty; IL-6 is strongly implicated and associations are likely to be multifactorial via age-related disease and direct effects on skeletal muscle [90]. There are only a limited number of observation studies of inflammaging and functional outcomes in older people especially in the context of hospital admission. Longitudinal data from the Health ABC study showed that IL-6, TNF and CRP concentrations were consistently associated with major health-related events including disability and mortality in community dwelling older people and that associations were dose-responsive [149;150].

The cumulative consequence of having a greater degree of inflammaging and anti-inflammaging is increased susceptibility to, and a faster progression of, all age-related diseases. This results in an increased vulnerability to stressors, reduced functional ability and is associated with development of the frailty syndrome [10]. Interestingly, a ten-year study of 254 healthy, community dwelling older people demonstrated that those individuals with baseline markers of greater inflammaging (white cell count) and anti-inflammaging (cortisol:DHEAS) were significantly more likely to be frail at 10 year follow-up. The authors speculate that this may offer a tool to screen for people who are 'less robust' in late middle age to allow early intervention before functional decline and the development of frailty [10].

Degrees of inflammaging in hospitalised older people have yet to be characterised in detail, especially in a longitudinal manner. Further, no studies have looked in detail at the relationship between inflammaging and cachexia in hospitalised older people despite this

group experiencing poor clinical outcomes and having some of the most pro-inflammatory systemic environments.

1.3.6 Biomarkers of inflammation considered within this thesis

There is no single defined marker of inflammation. Therefore this thesis considers a range of cellular and molecular markers that have been chosen to represent pro- and anti-inflammatory balances, anti-inflammaging and also positive and negative acute phase proteins. The rationale for using these biomarkers is presented in the following section.

1.3.6.1 White cell count

White cell count (WCC) is a numerical count of the number of white blood cells within one cubic millimetre of blood. All types of white blood cells are reflected within the WCC and it therefore allows representation of the cellular activity of both innate and acquired constituents. WCC is raised in response to infection and also in non-infective processes such as malignancy and as a consequence of certain medications. Neutrophil count is a numerical count of the number of neutrophils within one cubic millimetre of blood.

The role of these cells within inflammaging has been discussed previously. Raised WCC has been positively associated with cardiovascular disease, cancer and all-cause mortality [151]. It has also been associated with frailty in both cross-sectional and longitudinal studies in older people [10;152].

1.3.6.2 Interleukins-1, 6, 8 and 10

IL-1 is principally involved in the regulation of haematopoiesis and inflammation. When administered to humans with bone marrow failure it reduces the duration of thrombocytopenia but also causes unacceptable levels of inflammation including severe hypotension [153].

The IL-1 family originally included IL-1α and IL-1β and has since expanded to incorporate a total of 11 members. They are secreted by leukocytes and B-cells in response to many forms of tissue damage including the loss of membrane integrity and ischemia; relations with reactive oxygen species are controversial [154]. Principle target cells are within the immune

system where they cause maturation, proliferation and activation of T-helper cells, B-cells, NK-cells and macrophages. IL-1 also triggers an amplification of the immune response via the acute phase proteins and promotes cellular migration across vascular endothelial walls.

IL-6 is secreted by a variety of cells including T-cells, macrophages, myocytes, neural tissue and osteoblasts in response to inflammation, bacteria, viruses and tissue injury. It is a highly versatile cytokine with pleiotrophic actions on immune cells and a number of different cell types. For example, IL-6 is implicated in liver regeneration, haemopoiesis, bone remodelling, gliogenesis and, importantly, the acute phase response [155]. IL-6 also has anti-inflammatory properties – antagonising the actions of TNF and IL-1 and stimulating the production of IL1-Ra and IL-10.

IL-6 expression is dysregulated in a number of age related diseases including atherosclerosis and dementia. A recent systematic review of inflammation in sarcopenia reported that IL-6 and TNF were the most widely studied cytokines and consistent cross-sectional and longitudinal relationships exist [139]. Interestingly, IL-6 (and TNF) is secreted by myocytes in response to acute exercise. This phenomenon is poorly understood but may be related to the cytokine induced production of heat-shock proteins that 'chaperone' molecules during stressful situations [156].

IL-6 is strongly associated with cachexia and also a proposed criterion in its new clinical definition; levels in terminally ill cancer cachexic patients have been found to be dramatically elevated one week prior to death [157]. A study of murine models of colon cancer found that severe muscle wasting was associated with a 10-fold increase in IL-6 levels when compared to wild type. Furthermore, this was prevented by the genetic ablation of IL-6 in these mice signifying that IL-6 is necessary for the development of muscle wasting [158].

IL-8 is the third pro-inflammatory cytokine considered within this thesis. It is primarily produced by macrophages and induces chemotaxis in other granulocytes (e.g. neutrophils) causing them to migrate towards a site of infection. IL-8 secretion is triggered by oxidative stress and induces further increases in oxidative stress mediators. Therefore, it is often associated with, and drives local inflammation and may be implicated as a propagator of inflammaging, *Figure 1-8*. Furthermore, IL-8 may be produced early in the inflammatory response and persist for days or even weeks, in contrast to most other inflammatory cytokines which are produced and cleared within a few hours [159]. Therefore, IL-8 may be a more consistent marker of inflammaging despite being relatively understudied when

compared to IL-1 and IL-6. Within the Memory and Morbidity in Augsburg Elderly (MEMO) Study, cross-sectional associations were demonstrated with lower cognitive function and the cytokine is also implicated in the development of cachexia [160;161]. Associations have been demonstrated with cardiovascular diseases although these are inconsistent [162].

IL-10 is considered within this thesis for its anti-inflammatory actions and therefore as a measure of resilience to inflammaging. IL-10 is produced by various cells of the immune system and suppresses the antigen presenting capacity of APCs. IL-10 also inhibits NK cells and the production of pro-inflammatory cytokines and interferon. Knock-out mice for IL-10 develop inflammatory bowel disease because they cannot mitigate the immune response to intestinal flora [163].

In summary, IL-1, IL-6 and IL-8 are all pro-inflammatory and have catabolic affects on skeletal muscle; IL-10 is anti-inflammatory and anabolic. The inclusion of these cytokines enables a representation of this balance which may be conceptualised as a 'barometer' of inflammaging.

1.3.6.3 Tumour necrosis factor

Tumour necrosis factor was previously (and often still is) called TNF-α to distinguish it from TNF-β. Two forms of TNF-β have since been identified and the protein has been renamed LT-α and LT-β; a reference its original name, lymphotocin. Therefore, the name TNF-β is no longer used making TNF-α an orphan term not used in this thesis.

TNF is produced by a wide range of cells including cells of the immune system, adipocytes, myocytes and neuronal cells in response to pathogens and IL-1. Its principle role is to up-regulate the inflammatory system via the production of acute phase proteins, IL-1 and IL-6. TNF also stimulates phagocytosis and facilitates neutrophil migration to tissues. Furthermore, it acts centrally to cause the release or corticotrophin releasing hormone and generate fever.

TNF binds to specific receptors on skeletal muscle myocytes, inducing intracellular signalling cascades and, ultimately, the activation of nuclear transcription factor-κB, (NF-κB). This triggers muscle wasting through an upregulation of the ubiquitin-proteasome pathway

and also leads to increased expression of iNOS which converts L-arginine to citrulline, releasing Nitric Oxide (NO) in the process. Under specific conditions, NO reacts with super oxide anions (O_2^-) to form peroxynitrite, a toxic molecule and direct inhibitor of MyoD causing muscle fibre loss [164].

Evidence for the role of TNF in human skeletal muscle wasting comes from early studies of patients with cancer cachexia which found that serum levels were associated with negative nitrogen balance and loss of muscle mass. More recently the role of the TNF signalling pathway has been clearly demonstrated as a significant part of human cachexia pathology [23]. A study of skeletal muscle from cancer and AIDS patients with cachexia found that those patients had consistently higher levels of TNF mRNA and protein. Furthermore, they found that the level of active forms of the TNF receptor was also significantly increased in addition to increased inducible nitric oxide synthase (iNOS) mRNA [165].

1.3.6.4 C-reactive protein

CRP is a positive acute phase protein and a sensitive systemic marker of inflammation and tissue damage [83]. In healthy adults its median concentration is 0.8mg/l, but, following acute-phase stimulus by IL-6, serum concentrations may rise by up to 10,000-fold, peaking after 48 hours. CRP values show no seasonal or diurnal variations and are unaffected by meals or medications.

Raised CRP was initially associated with atherosclerosis and an increased likelihood of myocardial infarction, stroke and progression of peripheral artery disease [166-170]; a recent meta-analysis showed a relative risk of 2.0 for future coronary events with a baseline CRP level in the highest tertile compared to the lowest [166]. Raised levels of CRP have also been associated with other chronic diseases including respiratory, renal, neurological, hepatic, gastrointestinal, rheumatic and neoplastic and also cachexia. Cross-sectional and longitudinal relationships also exist with sarcopenia [139].

1.3.6.5 Albumin

Albumin is a negative acute phase protein which reduces in concentration during inflammation; low serum albumin is associated with subclinical inflammation [171]. Low albumin concentrations are also associated with inadequate dietary intake and a number of non-infective disease states [172]. Thus, in the absence of other causes, low albumin is a powerful non-specific marker of inflammation, catabolic states and nutrition. It is also a transporter for numerous anabolic hormones and is therefore especially relevant to the population being studied within this thesis.

Albumin forms part of the diagnostic criteria of cachexia (*Table 1-2*) and is strongly associated with a greater risk of cardiovascular disease disability and mortality [173-176]. Cross-sectional studies have reported associations with low muscle strength and muscle mass in older people and inconsistent relationships with muscle have also been seen longitudinally [177;178;171].

1.3.7 Inflammation: summary

Inflammaging describes the up-regulated chronic pro-inflammatory response with age; it occurs universally and is characterised by a rise in the levels of pro-inflammatory cytokines. The consequence of inflammaging depends on susceptibility plus a number of resilience factors including the balance with anti-inflammatory cytokines and the influence of the endocrine axis, notably cortisol and DHEAS.

Inflammaging is associated with age related disease and a number of adverse outcomes. It is possible that greater degrees of inflammaging may be implicated in the inflammatory syndrome of cachexia within older people; this is explored within this thesis.

1.4 Cachexia: skeletal muscle loss and inflammation in older people - summary

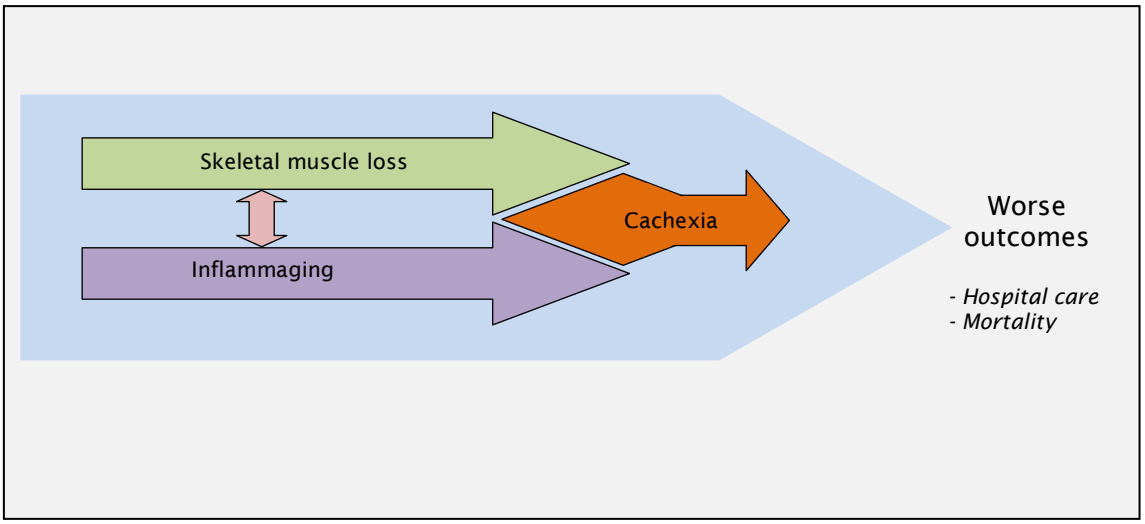
Cachexia is a complex metabolic syndrome associated with inflammation and characterised by weight loss, predominantly the loss of skeletal muscle mass. Cachexia is well studied in specific diseases where it is associated with very poor outcomes. However, little is known about cachexia in older adults, especially hospitalised older adults and in a longitudinal manner related to outcome. Considering cachexia according to its principle components, skeletal muscle loss and inflammation may offer an opportunity to better understand the syndrome within this group.

The loss of skeletal muscle mass with age is an important part of ageing and provides links between age-related disease and functional outcomes. Sarcopenia is the loss of skeletal muscle mass and function with age. It occurs universally and has recently been defined by consensus definition. Changes to skeletal muscle across the lifecourse are ultimately the consequence of changes to the delicate balance of anabolic and catabolic signals on individual myocytes. This is multifactorial in origin although the immune system is strongly implicated and is often the common final pathway.

Inflammaging describes the up-regulation of the inflammatory response that occurs with age. Like sarcopenia, it also occurs universally, resulting in a low-grade chronic systemic pro-inflammatory state characterised by raised levels of pro-inflammatory cytokines; degrees of inflammaging are thought to affect the rate at which individuals' age. Biomarkers of inflammaging may be used to represent an immune-endocrine axis which is implicated in the pathogenesis of muscle loss as well as being associated with a number of age-related diseases, functional outcomes and mortality. Detailed studies of inflammaging in hospitalised older adults are lacking despite this group having some of the most pro-inflammatory immune environments.

The hypothesis of this thesis is that cachexia is highly prevalent but under-recognised in hospitalised older women and important because it is associated with poor clinical outcomes; this is partly explained by the loss of skeletal muscle and inflammaging, *Figure 1-11.*

Figure 1-11: Relationships in CaSIO hypothesis



Chapter 2 : Objective and Aims

2.1 Objective

To characterise cachexia in a cohort of hospitalised older women to explore the role of skeletal muscle loss and inflammation, define its prevalence and justify its importance by establishing associations with clinical outcomes.

2.2 Aims

1. To determine the prevalence and correlates of cachexia in hospitalised older women.

2. To characterise muscle loss and inflammaging hospitalised older women over a six month period.

3. To explore the role of muscle loss and inflammaging in linking cachexia to short and long term clinical outcomes.

Chapter 3 : Methods

3.1 Background, design & recruitment

3.1.1 The CaSIO study: development

The CaSIO study was designed by the author for the purposes of this thesis. It was a prospective, cohort study set on the Medicine for Older People wards at Southampton General Hospital.

CaSIO was a sub-study of the Southampton Mealtime Assistance Study (SMAS), supported by the National Institute of Health Research (NIHR) and the Biomedical Research Unit at the University of Southampton. The study population for both the SMAS and CaSIO studies comprised hospitalised older adults and conducting CaSIO as a sub-study of SMAS therefore conferred clear logistical benefits.

Ethics approval was obtained from the National Research and Ethics Service (NRES) as an amendment to the Mealtime Assistance Study. This involved rewriting the main SMAS protocol to include the CaSIO data collection components and longitudinal home visits. Following submission to the Main Research Ethics Committee: Southampton A, approval was achieved, reference number – 09/H0502/93, amendment one, dated March 12th 2010 (*Appendix, page 192*).

Approval was also obtained via a similar process from the local Research and Development department of the study sponsor, Southampton Hospitals University NHS Trust (SUHT).

Training and approval from the Medical Research Council Lifecourse Epidemiology Unit (Mr. Keith Gardner) was obtained to prepare, log and store serum samples using the unit laboratory and freezer facilities. An induction programme and training was also undertaken to permit the use of laboratories and facilities within the University of Birmingham at the Queen Elizabeth Hospital (Mrs. Hema Chahal).

3.1.2 Design

CaSIO was a prospective cohort study based on two Medicine for Older People wards with follow up at six months in the community and the collection of mortality data continuing for approximately two years.

3.1.2.1 Sample size calculation

Reference data for sample size calculations was obtained from widely available hospital episode statistics and also relevant studies of older people. Biomarkers of the immune-endocrine axis were assumed to be normally distributed. Of the available studies, Alastair Kerr looked at grip strength as a predictor of length of stay in 120 hospitalised older adults and John Morley had defined the prevalence of cachexia in nursing home residents. A longitudinal study of frail older people by Thomas Gill provided helpful guidance with reference to the longer term clinical outcomes of the CaSIO study [23;56;179].

The principal calculation was based on the association between grip strength and the length of hospital admission. A sample size of 100 was estimated to have 80% power to detect a 0.28 standard deviation change in length of stay per standard deviation change in grip strength at the 5% significance level. This translated to approximately 1 day difference in length of stay per 5 kilogram change in grip strength. Additionally, from Kerr's study, this sample size enabled the power to detect a 3% increase in likelihood of discharge to usual destination for every 1kg increase in grip strength at baseline at the 5% significance level. Due to anticipated loss to follow-up (e.g. approaching 50% mortality) the sample size was increased by a factor of 1.5 to 150.

This calculated sample size also provided enough power to establish longitudinal associations. For example, with reference to the biomarkers, a sample size of 120 gave the power to detect a 1.8kg change in grip strength and an OR of 1.9 for cachexia per SD change in inflammatory marker.

3.1.3 Recruitment

All patients admitted to the study wards (G5 and G6, Department of Medicine for Older People, SUHT) were eligible to participate in the study. Recruitment was in a non-selected prospective manner until the required number was achieved.

Patients were screened within 24 hours of admission onto the study wards (or on Monday morning if admitted during a weekend). Potential participants were approached and provided with verbal and written information about the study (*Appendix, page 193*). They were given a minimum of 2 hours to consider the information before being approached for a second time to address any concerns and sign a consent form if willing to participate (*Appendix, page 199*).

3.1.3.1 Inclusion criteria

The CaSIO study was designed with wide inclusion criterion (*Table 3-1*) in order to maximise generalisability to the reference population and ultimately facilitate translation of the findings into clinical practice. Therefore, all patients over the age of 70 years admitted onto the study wards were deemed appropriate for screening and the majority of screened patients were over 80 years old due to hospital policy defining these as being suitable for the medicine for older people wards.

Patients needed to have capacity to provide informed consent judged by their ability to comprehend, retain and recall the information provided in the information sheet. This was decided on a case-by-case basis by the author who received training. Where capacity issues were unclear, advice was sought from a consultant geriatrician, Dr Helen Roberts, and the clinical team responsible for the patient. Patients who were either too unwell to be approached or in a potentially reversible state of delirium, (short-term confusion caused by illness, change in circumstances), were reviewed on a daily basis for at least three days before being excluded from the study.

Table 3-1: Inclusion Criteria

In-patients, over 70 years, admitted to G5 or G6 Medicine for Older People wards in SUHT
Informed consent from the patient

3.1.3.2 Exclusion criteria

Exclusion criteria are presented in *Table 3-2*. Patients admitted into side rooms were excluded as they represented an unjustifiable infection control risk. Patients admitted onto the study wards and screened but not recruited, due to exclusion criteria or unwillingness to participate, were recorded with a brief explanation for the exclusion or failure to recruit.

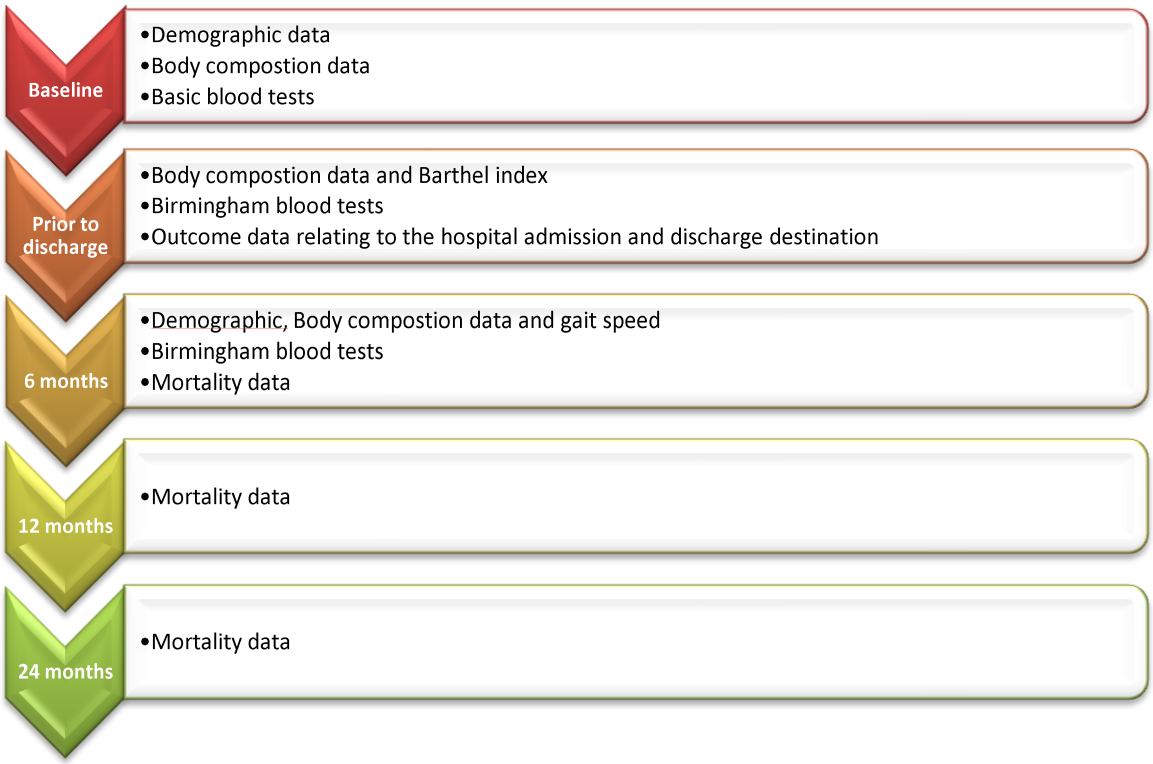
Table 3-2: Exclusion Criteria

Lacks capacity to give consent
Too unwell (activating the modified early warning system [MEWS], palliative care)
Admission into a side room

3.2 Protocol overview

Data was collected for a maximum period of twenty four months at five separate data-collection time points. All questionnaire assessments throughout the study were aided with the use of visual analogue scales, equipment was regularly checked, serviced and calibrated and inter- and intra-observer variation were performed (*Appendix 2, page 202*).

Figure 3-1: Five data collection time-points in the CaSIO study



3.2.1 Baseline data collection stage

Baseline data collection occurred following recruitment to the study, this was usually within 24 hours of admission to the study ward. Demographic data and basic blood results were extracted from the medical notes. Body composition data was collected and Barthel index, mini mental state examination (MMSE) and geriatric depression scale (GDS) scores were also obtained at this stage in addition to activity and falls questions.

3.2.2 Discharge data collection stage (prior to discharge)

Limited data was re-collected prior to discharge to assess how the population had changed during the admission. Participants were identified for the second data collection stage (prior to discharge) when their medical team signed the SUHT 'Medically fit for discharge' form. At this stage grip strength and Barthel index was reassessed and a blood test was collected for analysis in Birmingham at a later date. In the event of a delay between being declared medically fit for discharge and actually leaving hospital the period was documented and, if greater than one week, the data was recollected.

Clinical outcome data was collected from the medical notes following discharge. This included length of hospital stay, discharge destination and the number of hospital acquired complications.

3.2.3 Follow up data collection stage (in community at 6 months)

The follow up data collection stage occurred when the participants were seen in the community at their usual place of residence. At this stage body composition data, Barthel index, MMSE and GDS scores and falls and activity assessments were all repeated. Additionally, a blood test taken for repeat immune-endocrine analysis and Fried frailty and gait speed was also assessed.

3.2.4 Fourth and fifth data collection stages (mortality data)

Mortality data was collected at 12 and 24 months. This was ascertained via both hospital and primary care health care records. The date and cause of death was documented.

3.3 Data collection

The following section contains an in-depth description of the data collected in the study.

3.3.1 Demographic data collected at baseline

Following recruitment into the study, the following data was extracted from the medical notes:

- Name, date of birth
- Address, phone number
- General Practitioner (GP) and next of kin
- Marital status
- Smoking status (current, ex-, never)
- Alcohol intake
    - None
    - Mild (less than one alcoholic drink per week)
    - Moderate (less than one alcoholic drink per day)
    - Heavy (equal or more than one alcoholic drink per day)
- Category of domicile
- Care provided in the community (formal, informal)
- Reason for admission
- Active diagnoses
- Past medical history
- Current medications

3.3.2 Other data collected at baseline

In addition to demographic and body composition data, the following information was collected when the participant was recruited into the study:

3.3.2.1 Barthel Index

The Barthel index consists of 10 items that measure a person's daily functioning, specifically their activities of daily living and mobility status. The items include feeding, transfers from chair to bed, grooming, toileting, bathing, walking on a level surface, stairs assessment, dressing and continence of bowels and bladder [180;181]. This was completed with the assistance of the nursing staff responsible for the participant at the time.

3.3.2.2 Falls questions

Assessment of falls in older people is difficult due to recall bias and underreporting of falls by approximately 22%; previous cohort studies have suggested that this bias is minimised by limiting the recall period to 6 – 12 months [182].

For these reason, participants were asked the single question:

> "Over the last six months how many times have you fallen?"

Response was aided by a visual analogue scale and categorised into;

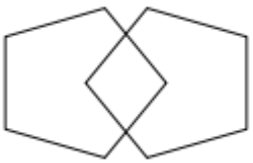
1. Never
2. Rarely
3. Monthly
4. Weekly
5. Daily

3.3.2.3 Mini-mental state examination (MMSE)

The MMSE (*Figure 3-2*) is a widely used and well validated method for grading the cognitive state of patients [183]. It contains 30 questions, assessing several cognitive domains including orientation, repetition, arithmetic, language, comprehension and basic motor skills. Normal mild cognitive impairment is defined a score of 24-27, moderate impairment as 16-23 and severe impairment as 0-15 although it is widely acknowledged that performance is affected by factors other than cognition such as age, education level and ethnicity [184].

Figure 3-2: Mini-mental state examination

Participant Name: _____ Date: _____

Maximum Score	Patient's score	Questions
5		What is the Year? Season? Month? Day? Date?
5		What is this Country? City? District? Hospital? Ward?
3		Repeat these three objects: Apple, Penny Table
5		Spell WORLD backwards (Alternatively subtract 7 from 100 and continue x5)
3		Can you recall those 3 objects?
2		Ask the participant to name 2x objects you show them (Pencil, Glass)
1		Repeat this sentence "No ifs ands or buts"
3		Follow this command: Place the index finger of your right hand onto your nose and then onto your left ear
1		Read this and do what it says: **CLOSE YOUR EYES**
1		Write a simple sentence _____
1		Copy this diagram: 
30		

3.3.2.4 Geriatric Depression Scale (GDS)

Depression is a clinical diagnosis based on a comprehensive assessment, not exclusively a scoring system taken at one time point. However, the GDS a 15-point questionnaire has been designed to identify people with symptoms suggestive of depression. It is well validated and widely used in older populations; higher scores suggest that depression is more likely (*Figure 3-3*) [185].

Figure 3-3: Geriatric Depression Scale

Are you basically satisfied with your life?	Yes/No
Have you dropped many of your activities or interests?	Yes/No
Do you feel that your life is empty?	Yes/No
Do you often get bored?	Yes/No
Are you in good spirits most of the time?	Yes/No
Are you afraid that something bad is going to happen to you?	Yes/No
Do you feel happy most of the time?	Yes/No
Do you often feel helpless?	Yes/No
Do you prefer to stay at home, rather than doing new things?	Yes/No
Do you feel you have more problems with memory than most?	Yes/No
Do you think it is wonderful to be alive?	Yes/No
Do you feel pretty worthless the way you are now?	Yes/No
Do you feel full of energy?	Yes/No
Do you think your situation is hopeless?	Yes/No
Do you think that most people are better off than you are?	Yes/No

3.3.2.5 Basic blood results

Basic blood results are frequently requested as part of patient care. They are performed by SUHT pathology department using standardised automated techniques that have the high accuracy and repeatability required for clinical care. This information was extracted from the medical records at baseline and prior to discharge:

- White Cell Count ($x10^9$/L)
- Neutrophil count ($x10^9$/L)
- C-reactive protein (mg/L)
- Albumin (g/L)

3.3.3 Body composition data

3.3.3.1 Assessment of weight loss and body mass index (BMI)

With shoes off, the participants were weighed using digital chair scales. Weight was measured to the nearest 0.1kg. Scales were calibrated and checked on a three monthly basis.

Height was calculated using conversion charts to estimate body height from ulna length [186] (*Appendix, page 201*) as recalled height is unreliable and measured height not practical.

BMI was calculated from height and weight using the formula:

$$BMI\ (kg/m^2) = weight\ (kg) / height\ (m)^2$$

3.3.3.2 Assessment of muscle strength

Grip Strength

Isometric upper limb muscle strength was measured three times in each limb alternating between right and left hands using a Jamar® handheld hydraulic dynamometer, (*Promedics, UK*); the *Southampton Protocol* was followed [62]. Participants were sat in a standard chair with their forearm resting on the chair arm and their feet flat on the floor. After ensuring that they understood what they needed to do, they were given standardised encouragement to squeeze the dynamometer as tightly as possible, for as long as they could until they were instructed to relax. Repeated measures allowed for practice and tiring effects. The highest of six measurements to the nearest 0.1kg were used in subsequent analyses. The dynamometer was calibrated before and during the study (*Lafayette Instrument Europe Ltd, Loughborough, UK*) and inter- as well as intra-observer studies were conducted during fieldwork to ensure compatibility between observers (*Appendix, page202*).

Photograph 2: Measurement of grip strength

Use of a standardised chair was not possible in the community due to lack of available space and safety and comfort concerns regarding the size of the chair. Therefore, the Southampton protocol was modified and the participant sat in a chair that was chosen to provide maximal support and comfort.

Quadriceps strength

Every 10th participant was selected prior to discharge to participate in measurement of quadriceps strength. Isometric quadriceps strength was measured on a specifically designed chair developed by the University of Southampton, (Dr. Dinesh Samuel, Faculty of Health Sciences).

Participants were asked to sit on the middle of the chair in an upright position. They were secured to the chair via a seatbelt around the waist. The dominant leg was connected to a force meter via a leash secured just above the medial malleolus. The force meter was connected to a laptop and force generator which was analysed (newtons) by *Labview®, version 8.6, Microsoft Windows®*.

To perform the test participants were asked to extend their leg at the knee against the leash using standard encouragement and for a fixed period of time. The test was repeated 3 times and maximum value recorded on the data entry sheets. Equipment was regularly calibrated using Newton weights.

3.3.3.3 Assessment of muscle mass

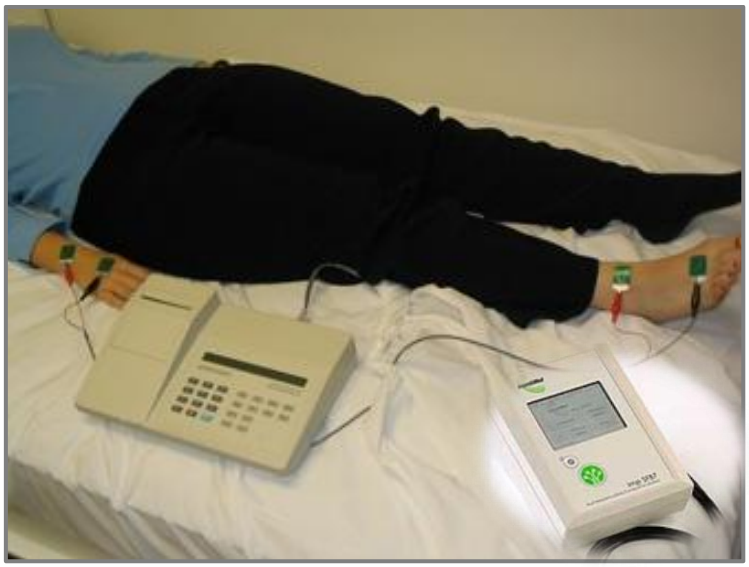
Multifrequency bioelectrical impendence

Multifrequency bioelectrical impedance (MBI) and skin-fold anthropometry were recorded on participants to assess muscle mass at baseline. MBI estimates the volume of fat and lean body mass. The test is reported as safe, easy to use, inexpensive and reproducible; it has been used as a technique for over ten years and results correlate well with magnetic resonance imaging (MRI) [61]. Reference values have been validated in a number of different populations, including older men and women, thus, it may be considered as a portable alternative to other methods [55].

With the participant lying prone, an ImpediMed SFB7, tetra polar bioelectrical impedance machine was used to perform MBI analysis according to a defined protocol. Age, height and weight data was entered and four disposable sticky electrodes placed on the participants' limbs, (2 on hand and 2 on foot). Results were captured, stored and analysed on the

provided software. Equipment was calibrated and checked prior to each use with an electronic calibrating device provided by the manufacturer.

Photograph 3: Multifrequency bio-electrical impendence



Skin-fold anthropometry

Skin-fold anthropometry is a safe, non-invasive and easy bedside technique widely used to evaluate percentage body fat in older people [187]. Training was provided to measure skinfold thickness using a standard calliper (Holtain Tanner/Whitehouse skinfold calliper, Holtain Ltd, Crymych, UK). Measurements were all of the non-dominant side unless there was a clinical reason to use the dominant side. Measurements of triceps skin-fold thickness were taken with the participants sitting in a 'holding tray' position, with their right arm held by the side and flexed at 90˚ at the elbow. The length of the arm was measured from the acromion process to the head of the ulna and the half way point established. This point was marked with a cross and the arm circumference was measured. The calliper was applied to a fold of skin with the cross at its apex and the dial read to record skin-fold thickness after a count of five seconds. A mean of three recordings was taken.

Dual-energy x-ray absorpitometry (DXA)

A DXA scan was performed by trained operators on a random selection of participants. This gave an output of body composition measures including fat mass, lean mass, bone mineral content and density at the hip and lumbar vertebrae (*Hologic Discovery, auto whole body, Version 12.5*).

Difficulty was experienced when some participants were asked to lie flat; therefore the standard protocol was modified with the addition of a single pillow under the head.

3.3.3.4 Other assessments required to define cachexia: fatigue and anorexia

Fatigue was assessed with a protocol developed by Fried using two questions from the CES-D Depression scale [188;189]. Participants were asked two yes/no questions; when a participant answered 'yes' to either of these questions it was considered positive for the presence of fatigue.

1. "Over the past week do you feel that everything was an effort?"
2. "Over the past week do you feel that you could not get going for at least three days?"

Anorexia was assessed using the Simplified Nutritional Appetite Questionnaire (SNAQ), 4 questions that are well validated in older populations *Figure 3-4* [190].

Figure 3-4: Simplified Nutritional Appetite Questionnaire

My appetite is:	
A	Very poor
B	Poor
C	Average
D	Good
E	Very good
Food tastes:	
A	Very bad
B	Bad
C	Average
D	Good
E	Very good
When I eat I feel full after eating:	
A	Only a few mouthfuls
B	One third of a meal
C	One half of a meal
D	Most of the meal
E	I hardly ever feel full
Normally I eat:	
A	Less than one meal a day
B	One meal a day
C	Two meals a day
D	Three meals a day
E	More than three meals a day

A=1, B=2, C=3, D=4, E=5

Score of ≤ 14: significant risk of ≥5% weight loss over 6 months

3.3.4 Operationalisation of consensus definitions

3.3.4.1 Cachexia and pre-cachexia

The definitions of cachexia and pre-cachexia and the techniques employed to measure them were based on recommendations from the fourth International cachexia meeting [20]. Assessment of weight loss, body mass index (BMI), muscle strength, fatigue, anorexia, fat free mass, biochemisty and chronic disease status was used to identify those individuals with cachexia or pre-cachexia.

Cachexia was defined as:

- One or both of:
  - Weight loss of at least 5% of total body weight loss in the preceding twelve months
  - Body mass index less than 20 kg/m^2
- Plus at least three of:
  - Reduced muscle strength
    - Lowest tertile grip strength from reference population
  - Fatigue
  - Anorexia
  - Low fat-free mass
    - Lowest 25th percentile for age and gender
  - Evidence of sustained or repeated episodes of inflammation
    - According to raised inflammatory markers, haemoglobin less than 12g/dl or albumin less than 32g/l

A participant had pre-cachexia if they demonstrated all of the following criteria:

1. Underlying chronic disease
2. Unintentional weight loss (oedema free) of no more than 5% of total body weight in the preceding 6 months
3. Chronic or recurrent systematic inflammatory response
4. Anorexia or anorexia related symptoms

Weight loss at baseline was calculated from the measured weight compared to the participant's usual reported weight, where possible corroboration was sought from historical medical records. Weight loss in the community was ascertained by comparing current weight to baseline weight. When weight loss was attributable to fluid loss as a result of diuretic use or other causes (e.g. dialysis), this data was ignored. Body mass index was calculated from measured weight and height and low muscle strength was defined by grip strength dynamometry and a cut-off of ≤15kg was used, representing the lowest third from a reference population [56]. Low fat free mass index was determined according to bioelectrical impedance analysis using a cut-off of less than 14.7kg/m^2 [191]. The presence or absence of chronic underlying illness and chronic or recurrent systematic inflammatory response was obtained from the hospital and primary care medical notes. The term 'chronic' was applied to any health problem that persists indefinitely or which frequently recur to an extent that requires repeated treatment, or which is the result of an acute episode that has resolved but left the patient with a permanent problem of handicap or disability [192].

3.3.4.2 Sarcopenia

Sarcopenia was defined according to the European Working Group on Sarcopenia in Older People (EWGSOP) consensus approach and identified at baseline where it was considered as an additional binary exposure [55]. This definition was discussed in greater detail within the introduction (*page 20*) and an exploration of sarcopenia within the study population is considered within the appendix (*page 210*).

3.3.5 Protocol alterations

The CaSIO study was unique in terms of the frailty of the study population and number of tests being employed to characterise them. Therefore, some of these tests needed to be modified or discontinued.

Firstly, it was not possible to accurately measure gait speed in the busy clinical environment of the study wards due to lack of space and safety concerns. Therefore, skeletal muscle was assessed directly via the measurement of mass and strength.

Testing of quadriceps strength required the participants to be taken to a non-clinical environment (*Gait Laboratory, Southampton General Hospital)* and sit on a cumbersome and large chair. They then had a cuff attached to their ankle to the measure force generated during knee extension. This was problematic as it felt unsafe taking participants away from the clinical environment, participants had great difficulty getting on and off the chair and limb pathology (oedema, ulcers, and fragile skin) often made it impossible to safely attach the ankle cuff. Furthermore, muscle strength data was also being collected using grip strength dynamometry which has a greater evidence base and is more translatable. Therefore, quadriceps strength testing was discontinued.

Similar problems were encountered with DXA scanning. The majority of participants were unable to get onto the scanning table without considerable effort or even at all. When on the table they were unable to lie flat due to spinal deformities or cardio-respiratory disease; even using a modified protocol with two pillows. Therefore, DXA scanning was also discontinued after 5 failed attempts.

3.4 Data collected at 6 month follow up

3.4.1 Overview of follow-up

Participants were seen in the community at their usual place of residence, 6 months following discharge from hospital for the collection of follow up data.

The participant's GP was contacted prior to follow up to ensure there were no significant changes in circumstances such as new accommodation, illness or death. The participant was then contacted by telephone and a mutually convenient morning appointment arranged.

In the event of loss to follow up due to participant death, withdrawal from study or other cause, information was collected from primary care and hospital records regarding events since discharge and where appropriate, the cause of death.

The following data was collected in the community, detailed methodology is provided only where not previously described:

a) Demographic and other information was updated
b) Body composition assessments
    i. Height, weight, BMI, grip strength, fatigue and anorexia questions
c) Gait speed
d) Mental health questionnaires
    i. Mini mental state examination
    ii. Geriatric depression scale
e) Barthel index
f) Fried frailty assessment
g) Falls questions
h) Birmingham blood tests

3.4.2 Demographic and other information updated

Information was collected on the following:

a) Change in accommodation
b) Change in caring arrangements
c) New medical diagnoses
d) Medication changes
e) Heath care contacts and readmissions into hospital

3.4.3 Weight and height measurements in the community

Weight was measured on a portable electronic stand-on weighing scales (Salter, UK). These scales were regularly checked, calibrated and compared to the ward version sit-on scales. Height was recorded from baseline.

3.4.4 Gait speed

Participants were instructed to wear their regular shoes and use their customary walking aid (noted as: none, stick, frame, other) and the test was performed in a private area removed from external distractions such as television or family members. A standardised electronic chronograph stopwatch was used to record the time taken to complete the test which was then recorded on the data sheet to the nearest 1/100th of a second.

Participants were instructed to stand fully upright with their arms by their sides and toes positioned 3 meters from the white plastic line on the floor. They were instructed that on the observer's word "go" they were to walk at their own comfortable pace until the observer instructed them to stop. After the participant crossed the white line the participant was instructed to stop. The participant was not informed of the distance they were supposed to be walking to ensure that they would not slow down in advance of the line. This method has been used previously [69].

Photograph 4: Assessment of gait speed

3.4.5 Fried frailty assessment

Assessment of frailty was according to the Fried criteria defined as the presence of three or more of the following: unintentional weight loss, weakness, self-reported exhaustion, slow walking speed and low physical activity. Weight loss over the six month follow-up period was calculated and weakness was defined according to grip strength. Self-reported exhaustion was assessed using the Fried protocol (*page 64*) and walking speed assessed as previously described (*page 70*). Physical activity was assessed using the question "In the past year have you spent most of your time in a chair or bed?"

3.5 Immune endocrine analysis

An overview of immune endocrine analyses across the CaSIO study is presented in *Table 3-3* and expanded within the following sections.

Table 3-3: Overview of immune endocrine analysis across study

Baseline	Prior to discharge	6 month follow up
Routine blood tests	Routine blood tests	Birmingham battery of tests
	Birmingham battery of tests	

3.5.1 Immune endocrine analysis: Southampton

3.3.6.1 Routine blood tests

The following blood results were extracted from the hospital pathology system at baseline and also prior to discharge:

- White Cell Count ($x10^9$/L)
- Neutrophil count ($x10^9$/L)
- C-reactive protein (mg/L)
- Albumin (g/L)

3.3.6.2 Venesection

Blood samples were taken between 9 and 11am both in hospital and in the community for subsequent analysis. Written consent was taken and the full sterile SUHT venesection protocol was followed. Sharps and clinical waste were disposed of using either hospital facilities or portable sharps and clinical waste bins in the community.

Participants were sitting in a comfortable position with an arm rested on a pillow. A disposable tourniquet was applied to the upper arm and an appropriate area for venesection (usually the anterior cubital fossa) was identified and cleaned using Sterets Alcowipes®. A

vacutainer®*(Vacutainer Systems, Becton Dickson and Company, UK*) and butterfly needle was used to collect 18ml venous blood into 1x plain clotted and 1x silica clot activator vacutainers. The silica clot activator vacutainers were sent to the pathology laboratories at SUHT for immediate analysis for cortisol and dehydroepiandrosterone sulphate, (DHEAS).

When in the community, blood samples were collected in an identical manner as when in hospital. Needles were disposed of in sharps bins, (Sharpsguard® orange com-plus 1 Liter Sharps Bin, *Daniels*™) and contaminated waste in a sealed yellow bag. Both were taken back to hospital after analysis for disposal by SUHT.

3.3.6.1 Preparation and storage of serum

Serum was prepared and stored at the MRC Lifecourse Epidemiology Unit. Plain tubes were left upright at room temperature for 60 – 120 minutes to allow a clot to form. They were then spun in a centrifuge (*Allegra GR, Beckman Coulter USA*) at 3000 cycles per minute for 10 minutes. The serum was drawn from the sample and aliquots were prepared in 3ml tubes, labelled, logged and frozen at -80˚C until analysis at the University of Birmingham.

Immune endocrine analysis was performed at the Centre for Musculoskeletal Ageing Research within the University of Birmingham. This has the knowledge, experience, equipment and economies of scale to enable detailed, state of the art and high quality analysis. The author spent a two week residential period working at the laboratory to perform all the tests under the supervision of Mrs Hema Chahal, an experienced technician and Professor Janet Lord.

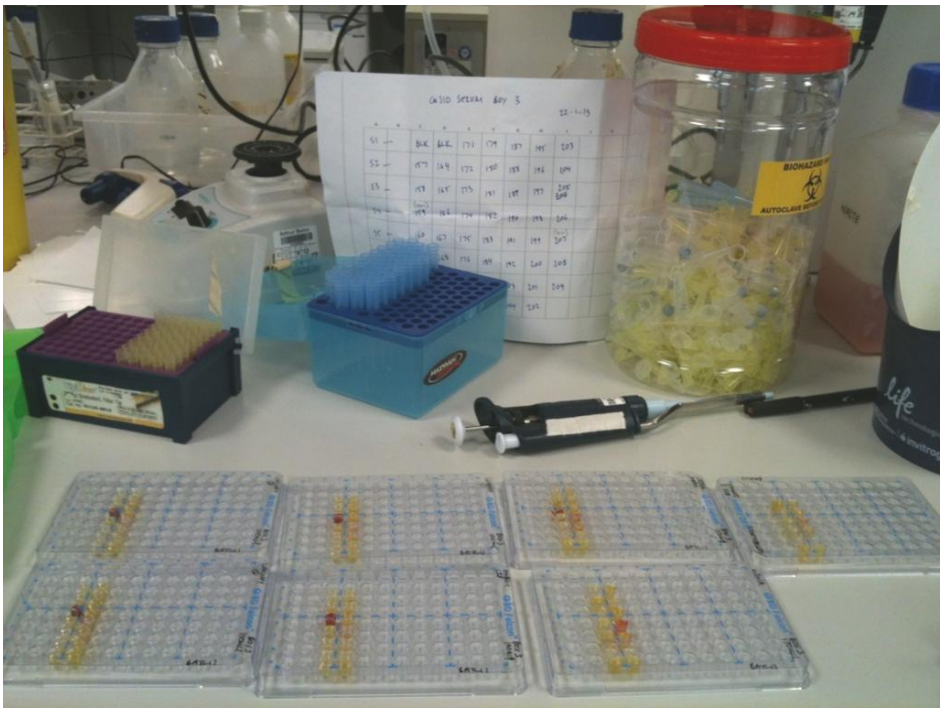
3.5.2 Immune endocrine analysis: Birmingham

The following section provides an overview of the immune endocrine analyses performed during this period.

3.5.2.1 Preparation of samples

Prior to analysis stored serum was transported on dry ice to Birmingham using a courier firm and then transferred into laboratory freezers at -80˚C. After thawing they were divided into 7 x 200µL wells within 10x8 Falcon plates for subsequent analysis in the various tests.

Photograph 5: 7x Falcon plates of divided serum samples prior to analysis



3.5.2.2 Summary of tests

The following tests were performed using enzyme-linked immunosorbent assay (ELISA) techniques:

- Cortisol (ng/ml)
- DHEAS (ng/ml)

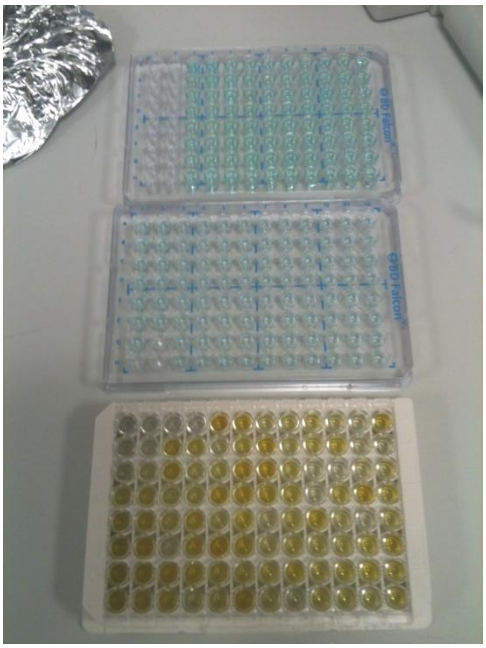
The following tests were performed using Bio-Plex Pro Assays:

- Inflammation battery: IL-1, IL-8, IL-10, TNF (all pg/ml)

3.5.2.3 Enzyme-linked immunosorbent assays

These were performed using kits from IBL International, Germany (www.IBL-International.com) and employ a sandwich-enzyme immunoassay for the qualitative determination of protein in serum. The wells of the microtitre strips are coated with purified anti-protein antibody. Target protein in the serum binds specifically to the immobilised antibody and then is recognised by a second enzyme marked antibody. After a substrate reaction the protein concentration is determined according to colour intensity. The principles and techniques are broadly similar regardless of target protein and therefore only the methods relating to the detection of Cortisol is described in this section.

Photograph 6: Sample dilution process prior to ELISA



The standard and control components were constituted using diluted buffer solution. Standard concentration solutions of 800, 400, 200, 100, 50, 20 and 0 ng/mL were then produced using serial dilutions with dilutent buffer. 20 µL of dilutent buffer was added to each well of the microtitre plate. 20 µL of each standard concentration was added plus 2 x control solutions and 78 x 20 µL of different serum samples per plate. Plates were then covered with adhesive foil, mixed thoroughly for 10 seconds and incubated at ambient room temperature to 37˚C for between 0.5 and 24 hours depending on the protein being tested. Incubation solutions were then discarded and the plates were washed three to five times with 300 µL of diluted wash buffer. Plates were then inverted and tapped on tissue paper to remove excess solution.

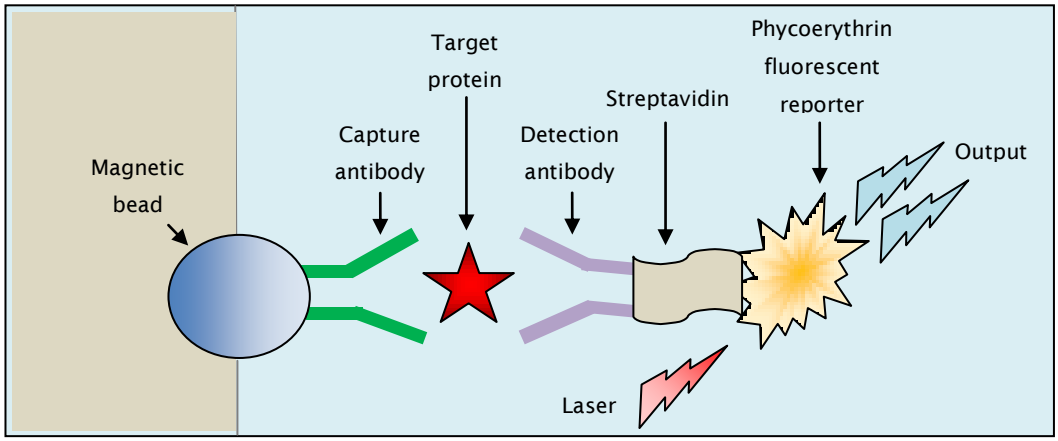
100 µL of TMB substrate solution was pipetted into each well; the plate was covered and incubated for a further 15 minutes at ambient room temperature. 100 µL of stop solution was then added into each well, followed by mixing. Finally, the back of the wells were cleaned and the plate was inserted into a photometer to measure optical density at various wavelengths depending on test. Results were then calculated using Prism® computer software (www.graphpad.com) and the calibration curve derived from the standard solutions. These were transferred to Microsoft excel, cleaned and added to the CaSIO database prior to analysis.

3.5.2.4 Bio-Plex Pro Assays

Bio-Plex Pro assays are magnetic bead-based multiplex assays designed to measure multiple biomarkers in a single serum sample. They come as factory made kits, designed to measure specific groups of proteins. Within the CaSIO study the 'inflammatory kit' was used which measures IL-1, IL-6, IL-8, IL-10 and TNF.

The principles of Bio-plex assays are similar to a sandwich ELISA (*Figure 3-5*). The individual wells of each Bio-plex plate are lined with magnetic beads coated with the specific capture antibody to the target protein of interest. The serum sample is added and then, after a series of washes to remove unbound protein, a detection antibody is added to create a sandwich complex prior to the final detection stage using a streptavidin-phycoerythrin conjugate which serves as a florescent indicator. Magnetic washing simplifies plate processing, provides increased throughput, and results in decreased variability and increased reproducibility. Data is acquired using a Luminex reader (*Photograph 7*) which uses a laser to illuminate fluorescent dyes within each bead, providing levels of luminance proportional to target protein concentration.

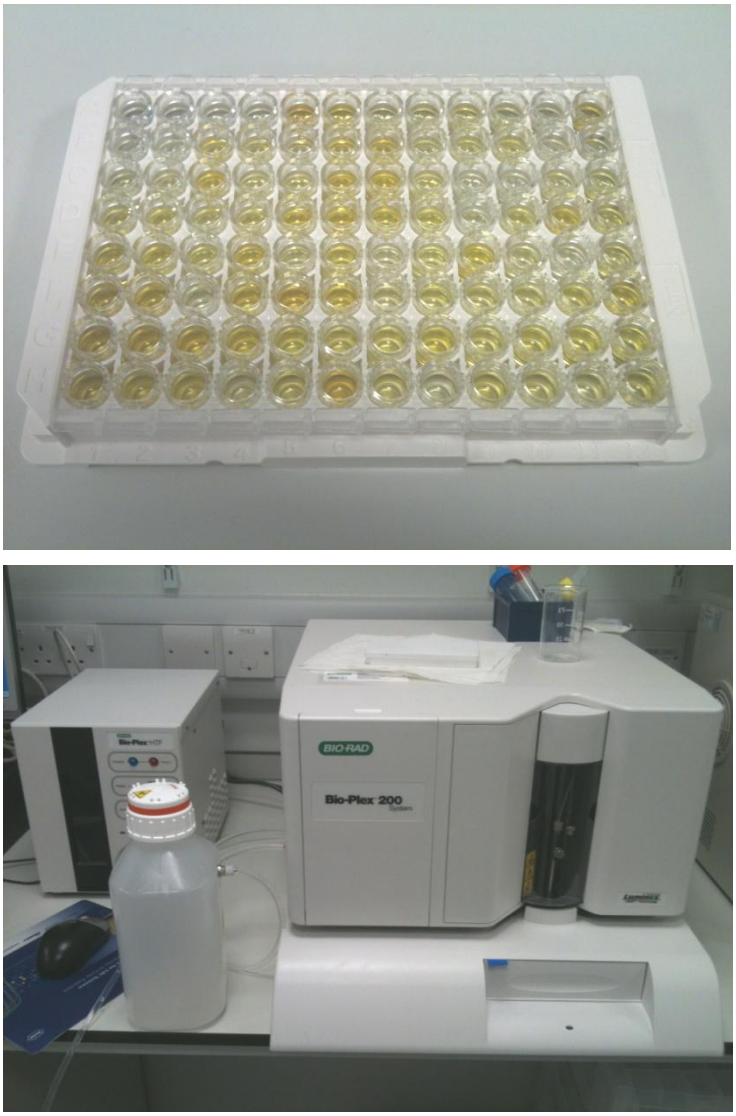
Figure 3-5: Principles of Bio-plex assays



The initial step in performing the analysis was to prepare the equipment by warming up and calibrating the Luminex machine and priming the wash station. Standard solution was then reconstituted using 500µL of Bio-plex standard dilutent and then a serial 4-fold dilution standard series was generated eight times with vortexing between transfers. Serum samples were then also diluted fourfold using standard dilutent and finally 288µl of coupled beads were diluted with 5,472µl of assay buffer to give a total volume of 5,760µl and then vortexed for 30 seconds.

To perform the assay, filter plates were first washed with Bio-plex pro II wash solution and then 50 µl of diluted coupled beads were added to each well using a multichannel pipette. Wells were then washed twice and 50µl each of standards and samples were added to each well, changing the pipette tip after each transfer. Plates were then incubated at room temperature for one hour. During this period, detection antibodies were prepared by 20x dilution with detection antibody dilutent (288µl antibody to 5,472µl dilutent). After one hour, plates were washed three times and 25µl of detection antibody added to each well. Plates were then covered and incubated on a shaker at room temperature for a further 30 minutes. During this period, 100x dilution of Streptavidin-PE was prepared by diluting 60 µl of concentrated solution with 5,940µl of buffer. After the second period of incubation, the plate was washed three times and 50µl of diluted Streptavidin-PE was added to each well using a multi-channel pipette. This was incubated on a shaker at room temperature for a further 10 minutes prior to three washes and the addition of 125µl assay buffer to each well. Plates were then vigorously shaken for 30 seconds before being placed on the Luminex reader for quantification of target protein. The output gives results as a Microsoft Excel file which was added to the main CaSIO dataset prior to cleaning and analysis.

Photograph 7: Bioplex luminex reader and prepared plate



3.6 Outcomes

3.6.1 Short term clinical outcome data collected from the medical notes

Following discharge into the community (or alternative care setting), the following data was extracted from the medical notes:

1. Details of hospital admission episode
    a. Length of stay
    b. Hospital acquired infections, (defined as new course of antibiotics):
        i. Clostridium Difficile
        ii. Methicillin-resistant Staphylococcus aureus (MRSA)
        iii. Extended-spectrum Beta-Lactamases (ESBL)
        iv. Hospital acquired pneumonia
        v. Urinary tract infection
    c. Other hospital complications (from the nursing notes):
        i. Diarrhoea, vomiting
        ii. Pressure sore progression

2. Details of discharge
    a. New medications provided on discharge
    b. Discharge destination
    c. Care package provided on discharge

3.6.2 All cause mortality

All participants were followed up with respect to mortality data at six and twelve months and on a day defined according to a median follow up time of twenty four month following discharge. A complete data set was ensured via access to both hospital and primary care records. On the two occurrences when a participant had moved out of region, the participant's GP was contacted to establish current status.

3.7 Data handling and analysis

3.7.1 Data handling

Data collection sheets were stored in a locked cabinet within the University of Southampton as per sponsor policy. The data was double entered at the MRC LEU and subsequently cleaned prior to analysis.

3.7.2 Statistical analysis plan

The following statistical analysis plan was made after data collection and prior to the initial analysis; *Table 3-4, page 82* provides a summary of measured and derived variables available for analysis within the CaSIO study.

Data was initially cleaned and basic checks were performed, extreme outliers were removed from the dataset and new variables were generated. Cytokine data was log transformed to handle skew. The population was then described according to recruitment and follow up and the group lost to follow up was compared to those followed up using Chi-squared or Fishers exact tests and ANOVA.

The study population was described at baseline and follow up; data was summarised using means, standard deviations, median, inter-quartile ranges, frequencies and percentages. Cachexia was then explored at baseline and follow up, including tracking of cases across the follow up period. Cross-sectional associations were analysed using Pearson's correlation co-efficient, t-tests, analysis of variance, cross-tabulations and chi-squared tests and regression analyses.

Associations related to hospital acquired outcomes were then analysed. Odds ratios of the likelihood of developing a hospital acquired complication according to baseline exposures were generated and then those with a p-value less than 0.10 were included within a mutually adjusted model. The associations between baseline exposures and the length of hospital admission and discharge destination were considered using Cox's proportional hazards model and time to a negative event with discharge home being considered as a censored observation. Negative events were defined as death or discharge to a more dependant

setting; discharge to rehabilitation was considered in various analyses as a negative or positive event or removed from the analysis.

Associations with mortality were also considered using Cox's proportional hazards model, defining a negative event as mortality and censored events being alive at: 6 months, 12 months and at the end of the study. Experimental analyses were run defining readmission to hospital as an additional negative event.

Therefore the following schema was followed when analysing the data:

1. Data cleaning and generation of new variables, e.g. cachexia, sarcopenia, frailty
2. Description of recruitment and follow up
3. Description of population at baseline and in the community
4. Description of cachexia
5. Cross-sectional relationships with cachexia at baseline and follow up
6. Associations with outcomes relating to the receipt of hospital care
    a. Hospital acquired complication
    b. Length of stay and discharge destination
7. Associations with all cause mortality
    a. 6 months
    b. 6 months, including readmission to hospital as an additional negative event
    c. 12 months
    d. Until end of study

Table 3-4: Variables available for analysis within CaSIO

Time point	Measured variable	Derived variable
Baseline	Marital status	BMI
	Smoking status	
	Alcohol status	Sarcopenia
	Category of domicile	
	Care provided	Pre-cachexia
	Reason for admission	
	Past medical history	Cachexia
	Medications	
	Barthel score	Anorexia
	Mini Mental State Examination	
	Geriatric Depression Scale	
	SNAQ (anorexia)	
	Fatigue and falls questions	
	Height and weight	
	Grip strength	
	Bioelectrical impedance	
	Anthropometry	
	Basic blood tests results	
Discharge	Barthel score	
	Weight	
	Grip strength	
	Basic blood test and Birmingham blood test results	
6 months	Barthel score	Sarcopenia by grip strength
	Mini Mental State Examination	
	Geriatric Depression Scale	Pre-cachexia
	SNAQ (anorexia)	
	Fatigue and falls questions	Cachexia
	Weight	
	Grip strength	Anorexia
	Birmingham blood test results	
	Healthcare contacts / readmissions	Fried frailty
	Change to medications	
	Change to package of care	
Outcomes	Length of stay	
	Discharge destination	
	Change to package of care	
	Readmissions within 28 days	
	Mortality at 6, 12 and 24 months	

Chapter 4 : Results

4.1 Recruitment and follow up

4.1.1 Recruitment

Recruitment to the CaSIO study started on June 1st 2010, was completed by November 30th 2010, and is summarised in *Figure 4-1*. Over that time 550 patients were admitted onto the study wards of whom 54% were excluded from the study due to a variety of reasons (*Table 4-1*). 58% of those eligible to participate in the study were successfully recruited.

Figure 4-1: Recruitment to CaSIO study

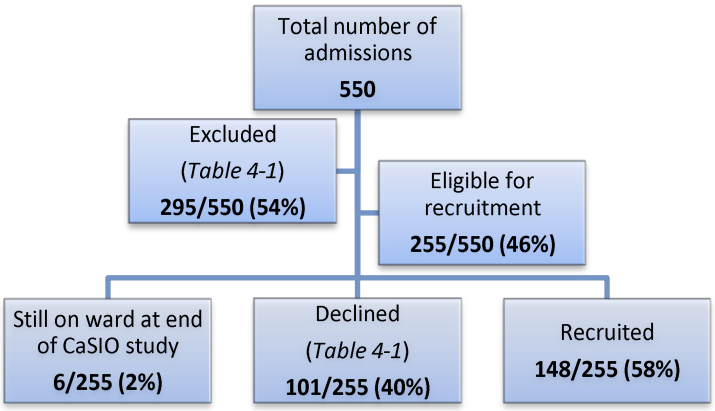


Table 4-1: Reasons for excluding patients and reasons given to not participate

Reasons for exclusion from study	Reasons given not to participate in study
Barrier nursed (52/295 [18%])	No reason given (43/101 [43%])
Lacks capacity (49/295 [17%])	Too much (19/101[19%])
Discharged prior to consenting (31/295 [11%])	Not interested (13/101 [13%])
Time constraints (27/295 [9%])	Feels too unwell (10/101 [10%])
Too unwell or dying (24/295 [8%])	Feeling too tired / too much effort (4/101 [4%])
Nil by mouth (12/295 [4%])	Is hoping to go home (3/101 [3%])
Ward closed (12/295 [4%])	Poor hospital experience (3/101 [3%])
Already on study (readmitted) (9/295 [3%])	Family advice (2/101 [2%])
Other reason given (12/295 [4%])	Sceptical (1/101 [1%])
Reason not recorded (67/295 [23%])	Other reason given (3/101 [3%])

4.1.2 Follow up

Inpatient mortality was 2% and overall mortality was 43% at the end of the study. 71% of participants were successfully followed up at their usual residence; 17% were not followed up at six month, mainly due to difficulties making contact with participants via telephone. A description and comparison of participants lost to follow up is presented in *Table 4-3* below and also in the results appendix (*Table RA 1, page 205*). All participants were accounted for within the mortality data collection regardless of status at 6 months. The end of the study was defined by a date, 13th October 2012, which was on average approximately two years from discharge (median time to end of study, 23 months).

Figure 4-2: Follow up at 6 months plus mortality

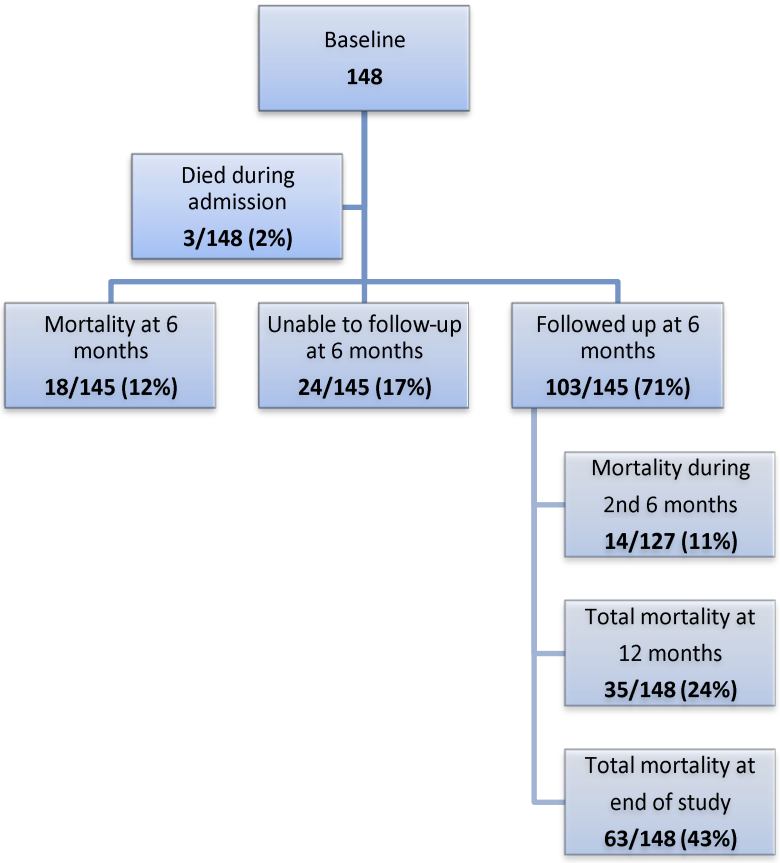


Table 4-2: Reasons for loss to follow-up at 6 months (excluding mortality)

Unable to contact participant	20/24 (83%)
Participant moved out of region	2/24 (8%)
Participant withdrawal from study	2/24 (8%)

Table 4-3: Comparison of 6 month follow up group with those lost to follow-up

	Lost to follow-up *n = 24*	Followed up *n = 103*	*p-value*
	n/d(%)	n/d(%)	
Age (years) †	87 (5.0)	86 (4.5)	*0.565*
Maximum grip strength (kg) †	13 (6.9)	13 (5.4)	*0.700*
Category of domicile			
Private home, alone	14/24 (58)	58/103 (56)	*0.144*
Private home, others	4/24 (17)	25/103 (24)	
Sheltered/warden	3/24 (13)	11/103 (11)	
Residential	3/24 (13)	7/103 (7)	
Nursing	0/0 (0)	2/103 (2)	
Prior care (yes)	15/22 (68)	59/98 (60)	*0.629*
Barthel index†	62 (27)	66 (29)	*0.566*
MMSE†	21 (5)	25 (4)	*<0.001*
GDS†	3.6 (2.9)	4.3 (2.5)	*0.201*
Fatigue (yes)	10/18 (56)	74/82 (90)	*0.001*
Anorexia (yes)	10/18 (56)	43/82 (53)	*1.000*
Inactive (yes)	5/17 (29)	39/82 (48)	*0.188*
Falls (yes)	11/18 (61)	56/81 (70)	*0.576*
Discharge bloods†			
WCC (log $x10^9$/L)	1.96 (0.3)	2.05 (0.4)	*0.285*
Neutrophils (log $x10^9$/L)	1.53 (0.4)	1.63 (0.5)	*0.335*
CRP (log mg/L)	2.41 (1.1)	2.34 (1.2)	*0.748*
Albumin (log g/L)	3.41 (0.2)	3.45 (0.2)	*0.447*
Cortisol (log ng/ml)	6.0 (0.7)	6.0 (0.5)	*0.580*
DHEAS (log ng/ml)	0.25 (0.8)	0.26 (0.64)	*0.956*
Cortisol:DHEAS	0.45 (1.1)	0.21 (0.8)	*0.317*

n/d numerator denominator; p-values from Chi-squared or Fishers exact tests; †mean (SD), p-values from ANOVA; bloods log transformed

4.2 Description of population at baseline

4.2.1 Population characteristics at baseline: Data collected from medical notes

The baseline characteristics of the 148 participants as obtained from the medical notes are displayed in *Table 4-4* and *Table 4-5*. The median time from hospital admission to transfer onto the study ward (baseline) was 2 days (IQR: 1 to 3 days).

4.2.1.1 Descriptive characteristics

All participants were female and the median age was 86 years (range 75 to 100); 5 were below 80 years of age. 97% of participants were non-smokers or ex-smokers and 3% were current smokers. 78% did not drink alcohol and only 5% reported drinking moderate to heavy amounts of alcohol. The majority (73%) of participants were widows and 6% were living in a care home at the time of admission.

4.2.1.2 Mode and reason for hospital admission, medications and past medical history

74% of participants were admitted via the Accident and Emergency department; the remainder (26%) were admitted directly from the community via primary care or community geriatricians. The commonest reasons for hospital admission were falls, shortness of breath, chest pain and collapse; all participants were taking at least one prescribed medication when admitted to hospital and the median number of medications was 10. 182 separate co-morbidities were reported in the past medical history with a median number of 4 per person; only 2 participants had no known past medical history. The commonest chronic conditions were hypertension (57%), type 2 diabetes mellitus (24%) and atrial fibrillation (20%).

Table 4-4: Baseline characteristics: age, medication and co-morbidity burden

	n	Median	Interquartile range
Age (years)	148	86	83, 89
Number of co-morbidities	148	5.0	4,8
Number of medications	148	10	8,14

Table 4-5: Baseline characteristics: smoking, alcohol, marital status, domicile, reason for admission and active co-morbidities

	Frequency (numerator / denominator)	Percentage
Sex		
Female	148/148	100
Smoking		
Non-smoker	101/148	68
Ex-smoker	43/148	29
Current smoker	4/148	3
Alcohol		
None	115/148	78
Minimal	26/148	18
Moderate	6/148	4
Heavy	1/148	1
Marital status		
Single	13/133	10
Married	23/133	17
Widowed	97/133	73
Category of domicile		
Private home, alone	84/148	57
Private home, with others	32/148	22
Sheltered/warden	23/148	16
Residential home	9/148	6
Package of care (excluding those in residential home)	73/139	53
Reason for admission		
Falls	30/148	20
Shortness of breath	29/148	20
Chest pain	13/148	9
Collapse	11/148	7
Acute confusion	10/148	7
Mobility impairment	7/148	5
Generally unwell	5/148	3
Dizziness	4/148	3
Diarrhoea	3/148	2
Abdominal pain	3/148	2
Leg pain	3/148	2
Other	29/148	20
10 commonest co-morbidities		
Hypertension	84/148	57
Type 2 diabetes mellitus	35/148	24
Atrial fibrillation	29/148	20
Ischemic heart disease	28/148	19
Hypothyroidism	20/148	14
Osteoporosis	19/148	13
Renal failure	18/148	12
ST elevation myocardial infarction	18/148	12
Cerebrovascular accident (stroke)	18/148	12
High cholesterol	18/148	12

4.2.2 Population characteristics at baseline: Data collected directly

A description of the 148 CaSIO participants with respect to additional baseline characteristics measured as part of the CaSIO study is presented in *Table 4-6* to *Table 4-8.*

4.2.2.1 Body composition measures

The median height was 158cm and the median weight was 60kg, median BMI was 24kg/m^2. Weight was measured in 141 participants and weight loss was ascertained in 131; 10 participants were excluded as a consequence of weight loss due to treatment of congestive cardiac failure and other causes. The medians for multifrequency bioelectrical impendence (fat mass and fat free mass) and skin fold anthropometry on admission were 18kg and 40kg for fat mass and fat free mass respectively and 16cm for mean triceps skin fold thickness.

4.2.2.2 Functional assessments

The median Barthel index was 67. Median grip strength was 12.5kg. Nearly one half (48%) of participants reported spending most of their time in a chair or in bed (inactive) and two thirds (68%) had experienced at least one fall in the preceding 6 months.

4.2.2.1 Cognitive assessments

The median MMSE score was 25 and the median GDS was 4.

4.2.2.3 Inflammatory biomarkers

There was a raised inflammatory burden at baseline as evidenced by a median C-reactive protein of 20 mg/L (normal range <5 mg/L) and mildly raised range of white cell and neutrophil counts (normal WCC 4.0–11.0 x10^9/L and neutrophil count 2.0-7.5 x10^9/L). Prior to discharge CRP was mildly elevated at 12 mg/L; WCC and neutrophil count had fallen to within normal levels, *Table 4-8.*

4.2.2.4 Data collected prior to discharge

Limited data were re-collected prior to discharge to assess how the population had changed during the admission. Barthel index was rescored prior to discharge from hospital on all participants and if the period between baseline data collection and discharge was greater

than 5 days then weight, BMI and grip strength measurements were also repeated. In comparison with the measurements taken at baseline, Barthel index had improved, weight and BMI were somewhat lower and maximum grip strength was unchanged. These results are presented in *Table RA 2*, *page 206*.

Table 4-6: Population characteristics at baseline

	n	Median	Interquartile range
Height (cm)	148	158	155, 161
Weight (kg)	141	60	52, 70
Body Mass Index (kg/m^2)	141	24	21, 28
Maximum grip strength (kg)	139	13	9, 16
Bioelectrical impendence:			
Fat mass (kg)	93	18	14, 24
Fat-free mass (kg)	93	40	35, 47
Fat-free mass index (kg/m^2)†	93	16	14, 18
Muscle mass index (kg/m^2)˚	92	8	6, 10
Mean triceps skinfold (cm)	124	16	11, 20
Barthel index	147	67	41, 93
Mini mental state exam	144	25	22, 27
Geriatric depression scale	108	4	2, 6

†Fat-free mass index [191]; ˚Skeletal muscle mass index [61]

Table 4-7: Mobility, falls, cognition and mood symptoms at baseline

	Frequency (numerator / denominator)	Percentage
Mobility		
Inactive†	55/115	48
Active	60/115	52
Falls		
Never	37/115	32
Rarely	58/115	50
Monthly	15/115	13
Weekly	3/115	3
Daily	2/115	2
Mini mental state examination score		
≥ 23	87/144	60
< 23	57/144	40
Geriatric depression scale score		
0-5	81/108	75
6-10	23/108	21
>10	4/108	4

†Spends most of the day in a chair or bed

Table 4-8: Inflammatory biomarkers collected during hospital admission

Biomarker	n	Median	Interquartile range
At baseline			
C-reactive protein (mg/L)	146	20	4.0, 67
White cell count (x10^9/L)	148	10	7.2, 13
Neutrophil count (x10^9/L)	148	7.3	5.1, 11
Albumin (g/L)	138	34	31, 37
Prior to discharge			
C-reactive protein (mg/L)	145	12	5.0, 28
White cell count (x10^9/L)	147	7.8	6.2, 9.4
Neutrophil count (x10^9/L)	147	5.1	3.9, 6.5
Albumin (g/L)	142	31	28, 36
IL-1 (pg/ml)	110	0.3	0.0, 1.2
IL-6 (pg/ml)	110	8.5	3.7, 23
IL-8 (pg/ml)	111	13	8.8, 20
IL-10 (pg/ml)	109	2.6	2.1, 3.2
TNF (pg/ml)	109	6.0	0.0, 27
Cortisol (ng/ml)	102	450	360, 520
DHEAS (ng/ml)	111	390	130, 660
Cortisol:DHEAS	98	1.2	0.6, 2.4
IL-6:IL-10	108	2.6	1.2, 5.5
IL-8:IL-10	111	4.8	3.1, 6.7

4.3 Description of population at follow up

A description of the study participants at 6 month follow up is presented within the following section.

4.3.1 Loss to follow up

103 participants were followed up in the community; the median time to follow up was 6.0 months (IQR 5.7-6.4 months). 16% of participants were lost to follow up and 14% had died; a comparison with those lost to follow up was provided earlier in this chapter and can also be found in *Table RA 1*, (*page 205*). Participants in this category were more likely to have worse cognitive function and less likely to report fatigue at baseline.

4.3.2 Population characteristics: changes in circumstances and healthcare utilisation

18% of participants had a new address at 6 month follow up and 40% had experienced an escalation in their usual package of care, (*Table 4-9*). 40% of all participants were readmitted to hospital at least once over the 6 month period and of these, 19% were admitted multiple times; one participant was admitted on 4 separate occasions over 6 months.

Table 4-9: Category of domicile, care needs and readmissions at 6 month follow-up

	Frequency (numerator / denominator)	Percentage
New permanent address at 6 months	18/103	18
Category of domicile		
Private home, alone	58/103	56
Private home, with others	25/103	24
Sheltered/warden	11/103	11
Residential home	7/103	7
Nursing home	2/103	2
Package of care escalated since discharge†	38/94	40
Readmission to hospital		
Yes	41/103	40
No	62/103	60

†Of the 94 participants who were not in a residential or nursing home

4.3.3 Population characteristics at follow up: data collected directly

4.3.3.1 Body composition measurements at follow up

The median weight was 57kg with a median BMI of 23 which represented a median weight loss of 3kg over the 6 month follow up period.

4.3.3.2 Functional assessments and frailty at follow up

The median Barthel index was 84, (interquartile range 62-96) and the mean grip strength was 14kg. 63% of participants said that they spent most of their time in a chair or in bed and 49% had experienced at least one fall since discharge from hospital. The majority of participants were frail or pre-frail at follow up, *Table 4-10* and *Table 4-11*; frailty was not characterised at baseline.

4.3.3.4 Cognitive assessments

Median MMSE was 26 and GDS was 5, *Table 4-10.*

Table 4-10: Population characteristics at follow up

	n	Median	Interquartile range
Height (cms)	91	158	155, 161
Weight (kg)†	91	57	49, 68
Body mass index (kg/m^2)	91	23	20, 27
Weight change (kg)	91	-3	-9, 2
Grip strength (kg)†	103	14	3, 28
Barthel index	103	84	62, 96
Mini-mental state examination	103	26	23, 28
Geriatric depression scale	81	5	2, 6

†Mean and standard deviation

Table 4-11: Functional and cognitive markers at follow up

	Frequency (numerator / denominator)	Percentage
Mobility		
Inactive†	64/103	62
Active	39/103	38
Falls over last 6 months		
Never	53/103	52
Rarely	31/103	30
Monthly	17/103	17
Weekly	2/103	2
Daily	0/103	0
Frailty status		
No frailty	12/89	14
Pre-frail	15/89	17
Frail	62/89	70
Mini mental state examination score		
≥ 23	71/102	70
< 23	31/102	30
Geriatric depression scale score		
0-5	53/81	65
6-10	26/81	32
≥ 10	2/81	3

†Spends most of the day in a chair or bed

4.3.3.5 Inflammatory biomarkers

The inflammatory biomarkers characterised at 6 month follow up are presented in *Table 4-12.*

Table 4-12: Inflammatory biomarkers at follow up

	n	Median	Interquartile range
CRP (mg/L)	84	7.6	2, 35
IL-1 (pg/ml)	96	0.4	0,0.9
IL-6 (pg/ml)	92	15	0, 30
IL-8 (pg/ml)	96	9.2	0, 18
IL-10 (pg/ml)	95	10	0, 38
Cortisol (ng/ml)	97	123	9, 146
DHEAS (ng/ml)	95	180	73, 518
Cortisol:DHEAS	87	0.6	0, 1.1
IL-6:IL-10	94	4.2	0, 7.4
IL-8:IL-10	96	3.9	0, 8.4

4.3.4. Comparison of data collected at baseline and follow up

Table 4-13 describes the 103 participants with data collected at baseline and follow up. Mean grip strength at six month follow up was 1kg higher than at baseline (*p=0.009*) and the average Barthel index was 17 points higher (*p<0.001*) when compared with baseline but was no different at follow up than at discharge. Participants were less active (52% vs. 48%, *p<0.001*) and had an improved MMSE score (26 vs. 25, *p=0.017*) possibly due to follow up bias. Participants at follow up had elevated IL-8 and cortisol (*p=0.088* and *<0.001*) in comparison with baseline and pro- to anti-inflammatory ratios were also increased as evidenced by greater cortisol:dheas and IL-8:IL-10 ratios (*p<0.001* and *0.018*). Gait speed and, therefore, frailty was characterised at follow up but not at baseline.

Table 4-13: Comparison of variables at baseline and follow up

	n	Baseline *Mean (SD)*	6 month follow up *Mean (SD)*	p-value for difference
Age at baseline (years)	103	86 (5)	86 (4)	*0.765*
Barthel index (at baseline)†	103	67 (41-93)	84 (62-96)	*<0.001*
Barthel index (at discharge)†	103	87 (57-97)	84 (62-96)	*0.120*
Max. grip strength (kg)	97	13 (5.4)	14 (6.2)	*0.009*
Inactivity (% yes) ˚	82	48	52	*<0.001*
Falls (% yes)˚	81	68	63	*0.015*
MMSE (score)†	100	25 (22-27)	26 (23-28)	*0.017*
GDS (score)	80	4 (2-5.5)	5 (2-6)	*0.095*
IL-1 (log pg/ml)	17	0.4 (0.6)	0.6 (1.1)	*0.324*
IL-6 (log pg/ml)	47	2.3 (1.4)	2.2 (1.6)	*0.588*
IL-8 (log pg/ml)	28	2.7 (0.6)	3.1 (0.8)	*0.088*
IL-10 (log pg/ml)	48	1.3 (1.2)	1.5 (1.2)	*0.247*
CRP (log mg/ml)	79	2.2 (1.2)	2.1 (1.7)	*0.511*
Cortisol (log ng/ml)	64	4.1 (1.3)	4.8 (0.4)	*<0.001*
DHEAS (log ng/ml)	67	5.7 (1.0)	5.3 (1.6)	*0.016*
Cortisol:DHEAS	60	0.2 (0.9)	0.4 (1.2)	*<0.001*
IL-8:IL-10	28	1.4 (0.9)	2.0 (0.9)	*0.018*
IL-6:IL-10	47	0.9 (0.9)	1.0 (1.3)	*0.710*

p-values from paired t-tests unless otherwise stated; blood tests from discharge and log transformed
†Median (IQR), p-values from paired Wilcoxon signed-rank tests
˚Percentages, p-values from exact McNemar's chi squared tests

4.4 Description of cachexia at baseline

4.4.1 Anorexia and fatigue assessment at baseline

The median SNAQ score was 14; 53% of participants scored less than or equal to 14 indicating significant symptoms of anorexia. 79% of participants felt that everything they did was an effort and 78% reported that they had significant difficulties getting going in the morning.

Table 4-14: Anorexia and fatigue at baseline

	Frequency (numerator / denominator)	Percentage
Anorexia (SNAQ)		
No evidence	54/116	47
Likely	62/116	53
Fatigue questions		
Everything an effort	92/116	79
Cannot get going	91/116	78

4.4.2 Pre-cachexia and cachexia: prevalence at baseline

Pre-cachexia was defined in 2010 by Muscaritoli and colleagues as having all of the following: chronic disease, weight loss≤5%, systemic inflammatory response and anorexia (*page 5*) [27]. All participants had chronic disease as defined by Rose and evidence of systemic, recurrent inflammatory response over the preceding 6 months [192]. Therefore, the prevalence of pre-cachexia within CaSIO participants on admission was 5% as defined according to the degree of weight loss (≤5%) and the presence of anorexia. Cachexia was identified on the on the basis of demonstrating weight loss of greater than 5% or a BMI of less than 20 plus at least three of: reduced muscle strength, low fat free mass, fatigue, anorexia, evidence of inflammation [20]. Using this definition 28% of the population had cachexia, *Table 4-15*.

The number of study participants with pre-cachexia was too small to permit further useful statistical analyses; therefore, participants with pre-cachexia were combined with the 'no cachexia' group to generate a binary variable *cachexia.* Using this variable, 28% of participants had cachexia and 72% did not have cachexia.

Table 4-15: Prevalence of pre-cachexia and cachexia on admission

	Frequency (numerator / denominator)	Percentage
No cachexia	88/131	67
Pre-cachexia	7/131	5
Cachexia	36/131	28

4.4.3 Cross-sectional associations with cachexia at baseline

Cachexia was associated with older age, a lower MMSE score, higher GDS score, fatigue, anorexia, inactivity and lower grip strength. These participants were also more functionally dependent according to category of domicile, package of care and Barthel index although these associations lacked significance at the 5% level. Cachexia was also significantly associated with WCC and neutrophil count (*Table 4-16*).

Table 4-16: Cross-sectional associations with cachexia at baseline

	Cachexia: No	Yes	*p-value*	*Age adjusted p-value*
	n/d(%)	n/d(%)		
Age (years)†	86 (4.5)	88 (4.9)	*0.002*	---
Category of domicile				
Private home, alone	58/95 (61)	20/36 (56)	*0.609*	*0.266*
Private home, others	17/95 (18)	10/36 (28)		
Sheltered / warden	15/95 (16)	4/36 (11)		
Residential	5/95 (5)	2/36 (6)		
Package of care (yes)	54/95 (57)	23/33 (70)	*0.303*	*0.442*
Barthel index†	68 (30)	62 (26)	*0.223*	*0.488*
MMSE score†	25 (4)	22 (4)	*<0.001*	*0.024*
GDS score†	3.6 (2)	5.6 (3)	*0.001*	*0.003*
Fatigue (yes)	60/73 (82)	31/32 (97)	*0.059*	*0.081*
Anorexia (yes)	30/72 (42)	25/32 (78)	*0.001*	*0.001*
Inactivity (yes)	27/73 (37)	20/30 (67)	*0.006*	*0.010*
Falls (yes)	46/72 (64)	23/31 (74)	*0.366*	*0.322*
CRP (log mg/L) †	2.7 (1.6)	3.1 (1.8)	*0.384*	*0.365*
WCC (log $x10^9$ /L)†	2.2 (0.4)	2.4 (0.5)	*0.021*	*0.029*
Neutrophils (log $x10^9$ /L)†	1.9 (0.5)	2.2 (0.6)	*0.014*	*0.018*
Albumin (log g/L)†	3.5 (0.2)	3.5 (0.2)	*0.248*	*0.286*
Grip strength (kgs)†				
≤10	23 (26)	16 (44)	*<0.001*	*<0.001*
11-15	26 (30)	17 (47)		
16+	38 (44)	3 (8)		

†Mean(SD), n/d numerator denominator, p-values from logistic regression for cachexia; blood results log transformed

4.4.4 Cross-sectional associations with muscle strength at baseline

Cross-sectional associations with grip strength at baseline are presented in *Table 4-17*. Values are presented according to tertiles of grip strength to illustrate trend. Lower grip strength was associated with older age, more dependent category of domicile, presence of a package of care, worse functional status (Barthel index), worse cognition, more anorexia and greater inactivity and falls; these remained significant after adjustment for age.

Table 4-17: Cross-sectional associations with grip strength at baseline

	Grip (kgs): ≤10	11-15	16+	*p-value*	*Age adjusted p-value*
	n/d(%)	n/d(%)	n/d(%)		
Age (years)†	87 (4.8)	87 (4.5)	85 (4.0)	*0.010*	---
Category of domicile					
Private home, alone	23/49 (47)	28/45 (62)	30/45 (67)		
Private home, others	9/49 (18)	9/45 (20)	9/45 (20)	*0.026*	*0.004*
Sheltered / warden	12/49 (25)	6/45 (13)	5/45 (11)		
Residential	5/49 (10)	2/45 (4)	1/45 (2.2)		
Package of care (yes)	33/45 (73)	30/42 (71)	18/45 (40)	*0.002*	*<0.001*
Barthel index†	55 (27)	70 (28)	71 (27)	*0.004*	*0.014*
MMSE score†	23 (4.5)	24 (3.8)	25 (3.7)	*0.005*	*0.017*
GDS score†	4.5 (2.7)	4.1 (2.0)	3.8 (3.2)	*0.088*	*0.113*
Fatigue (yes)	34/37 (92)	32/36 (89)	31/40 (78)	*0.150*	*0.201*
Anorexia (yes)	26/38 (68)	19/36 (53)	15/39 (39)	*0.017*	*0.013*
Inactivity (yes)	23/37 (62)	20/35 (57)	11/39 (28)	*<0.001*	*0.001*
Falls (yes)	27/37 (73)	26/35 (74)	23/40 (58)	*0.057*	*0.049*
CRP (log mg/L)	3.3 (1.6)	2.6 (1.6)	2.8 (1.7)	*0.400*	*0.448*
WCC (log $x10^9$ /L)	2.3 (0.4)	2.3 (0.5)	2.3 (0.4)	*0.675*	*0.727*
Neutrophils (log $x10^9$ /L)	2.0 (0.5)	2.0 (0.6)	2.0 (0.5)	*0.803*	*0.849*
Albumin (log g/L)	3.5 (0.2)	3.5 (0.2)	3.6 (0.2)	*0.066*	*0.179*

†Mean(SD), n/d numerator denominator; p-values from logistic regression for grip strength; blood results log transformed

4.5 Description of cachexia at follow up

4.5.1 Anorexia and fatigue assessment at follow up

The median SNAQ score was 14 with 51% of participants scoring less than or equal to 14 indicating significant symptoms of anorexia. 48% felt that everything they did was an effort and 43% reported that they had difficulty getting going in the morning.

Table 4-18: Anorexia and fatigue assessment at follow up

	Frequency (numerator / denominator)	Percentage
Anorexia (SNAQ)		
No evidence	50/103	49
Likely	53/103	51
Fatigue questions		
Everything an effort	50/103	48
Cannot get going	45/103	43

4.5.2 Pre-cachexia and cachexia: prevalence at follow up

At follow up the prevalence of pre-cachexia had increased from 5% to 8% and cachexia from 27% to 34%, *Table 4-19.*

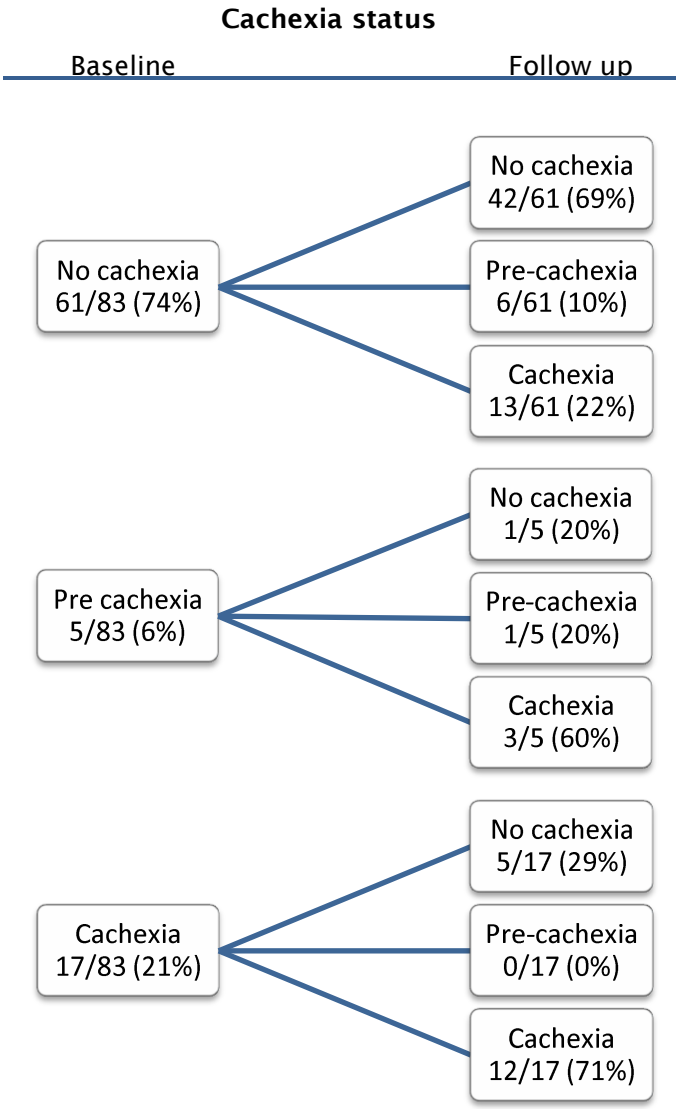
Table 4-19: Prevalence of cachexia in the community

	Frequency (numerator / denominator)	Percentage
No cachexia	48/83	58
Pre-cachexia	7/83	8
Cachexia	28/83	34

4.5.3 Cachexia tracking over follow up period

83 participants had cachexia characterised at baseline and at follow up. *Figure 4-3* illustrates that 69% of participants without cachexia at baseline remained without cachexia at follow up; 22% developed cachexia. Equally, the majority (71%) of participants with cachexia at baseline also had cachexia at follow up; however, 29% did not have cachexia. Participants with pre-cachexia at baseline were more likely to have cachexia at follow up than those without cachexia at baseline.

Figure 4-3: Cachexia at baseline and follow up



4.5.4 Cross-sectional associations with cachexia at follow up

Table 4-20 presents the cross-sectional associations seen with cachexia at follow up. These were similar to the findings at baseline (*Table 4-16*) demonstrating significant associations with lower MMSE score, higher GDS score, fatigue, anorexia and immune-endocrine biomarkers (cortisol, DHEAS).

Table 4-20: Cross-sectional associations with cachexia at follow up

	Cachexia: Yes	No	*p-value*	*Age adjusted p-value*
	n(%)	n(%)		
Age (years) †	86 (4.9)	85 (4.4)	*0.554*	---
Category of domicile				
Private home, alone	12/28 (43)	29/55 (53)	*0.853*	*0.916*
Private home, others	6/28 (21)	11/55 (20)		
Sheltered/warden	4/28 (14)	7/55 (13)		
Residential	2/28 (7)	4/55 (7)		
Nursing	4/28 (14)	4/55 (7)		
Barthel score†	71 (31)	82 (20)	*0.328*	*0.068*
MMSE†	24 (5)	27 (3)	*0.006*	*0.001*
GDS†	5.4 (2.6)	3.9 (2.9)	*0.029*	*0.072*
Fatigue (yes)	24/28 (86)	26/55 (47)	*0.001*	*0.001*
Anorexia (yes)	19/28 (68)	23/55 (42)	*0.025*	*0.028*
Inactive (yes)	17/28 (61)	34/55 (62)	*1.000*	*0.984*
Falls (yes)	15/28 (54)	26/55 (47)	*0.645*	*0.525*
3m walk (secs)†	2.2 (0.6)	2.4 (0.8)	*0.374*	*0.270*
Frailty status				
Non-frail	0/27 (0)	12/49 (25)	*<0.001*	*0.004*
Pre-frail	2/27 (7)	12/49 (25)		
Frail	25/27 (93)	25/49 (51)		
CRP (log mg/L)†	2.2 (2.9)	2.5 (4.0)	*0.817*	*0.868*
IL-1 (log pg/ml)†	0.7 (1.2)	1.0 (0.9)	*0.216*	*0.318*
IL-6 (log pg/ml)†	2.2 (1.8)	1.8 (1.4)	*0.235*	*0.349*
IL-8 (log pg/ml)†	3.0 (0.8)	3.1 (0.7)	*0.620*	*0.368*
IL-10 (log pg/ml)†	1.5 (1.4)	1.1 (0.8)	*0.028*	*0.303*
Cortisol (log ng/ml)†	4.7 (0.4)	4.9 (0.5)	*0.048*	*0.056*
DHEAS (log ng/ml)†	5.1 (1.4)	6.0 (2.0)	*0.042*	*0.042*
Cortisol:DHEAS†	0.4 (1.1)	0.6 (1.2)	*0.725*	*0.615*
IL-6:IL-10†	1.0	1.7	*0.277*	*0.564*

†Mean(SD), n/d numerator denominator; p-values from logistic regression for cachexia; blood results log transformed

Cross-sectional associations with muscle strength at follow up were similar to those at baseline and are presented within the appendix, *Table RA 3, page 207*.

4.5.5 Relationships of cachexia with muscle strength and frailty at follow up

70% of participants were frail at six month follow up; a further 17% were pre-frail (*Table 4-11*). Cross-sectional associations with frailty were similar to associations with muscle strength and are presented within the results appendix, *Table RA 4* on *page 208.*

4.6 Cachexia and outcomes relating to the hospital admission

4.6.1 Description of short-term outcomes

The following section describes the short-term outcome variables: length of hospital stay, number of hospital acquired complications and mortality; changes to discharge destination, changes to package of care and readmission within 28 days.

4.6.1.1 Length of hospital stay

Median length of hospital admission was 15 days (IQR 9-24, range 2-99 days).

4.6.1.2 Hospital acquired complication

21% of participants experienced at least one hospital acquired complication during admission, the commonest complications were: hospital acquired pneumonia (10%); diarrhoea and/or vomiting (7%) and urinary tract infection (4%). In total, 11% of participants were moved from an open ward into a side room for barrier nursing (*Table 4-21*). No participant was diagnosed with a new thromboembolic event.

4.6.1.3 Mortality

2% of participants died during the initial hospital admission.

Table 4-21: Hospital acquired complications and mortality

	Frequency (numerator / denominator)	Percentage
Hospital acquired complication (overall)	31/145	21
Hospital acquired pneumonia	15/145	10
Diarrhoea and/or vomiting	11/145	7
Urinary tract infection	6/145	4
Progression of pressure sore	2/145	1
ESBL†	1/145	1
MRSA˚	1/145	1
Thromboembolic event	0/145	0
Received barrier nursing	16/145	11
Death during admission	3/148	2

†Extended spectrum beta-lactamase; ˚Methicillin resistant staphylococcus aureus

4.6.1.4 Discharge destination

78% of participants were discharged to a permanent place of residence, 22% were transferred to a community health care site for further rehabilitation. Of those discharged to a permanent place of residence, 91% went back to their usual residence and 9% were discharged to a more dependent setting in a residential or nursing home. 74% of participants who were discharged to a private home or sheltered/warden accommodation were in receipt of a formal care package, *Table 4-22*.

Table 4-22: Discharge destinations

	Frequency (numerator / denominator)	Percentage
Discharge destination		
Previous residence	103/145	71
More dependent setting	10/145	7
Rehabilitation	32/145	22
Destination among if not discharged to rehabilitation		
Private home, alone	53/113	47
Private home, with others	22/113	22
Sheltered/warden	17/113	15
Residential home	12/113	11
Nursing home	6/113	5
Package of care provided†	70/95	74

†Formal meal provision or personal care amongst those not discharged to a residential or nursing home

4.6.1.5 Readmission to hospital within 28 days

19 (13%) participants were readmitted to hospital within 28 days of discharge.

4.6.2 Associations between cachexia and other baseline characteristics with hospital acquired complication

Table 4-23 presents the associations between characteristics at baseline and the likelihood of developing a hospital acquired complication. These relationships were then mutually adjusted to identify baseline characteristics associated with HAC, *Table 4-24*. Barthel index and cachexia at baseline were strongly associated with developing a hospital acquired complication (no interaction was found between these two variables). *Figure 4-4* illustrates that the presence of cachexia, regardless of functional status, significantly increases the likelihood of developing a hospital acquired complication during the admission for those with a Barthel index less than 85.

Table 4-23: Associations between baseline characteristics and hospital acquired complication

Baseline characteristic	n	OR†	Confidence interval	*p-value*
Cachexia (yes vs. no)	121	2.44	0.97 – 6.13	*0.059*
Age (per year older)	148	1.08	0.99 – 1.18	*0.069*
Max. grip (per kg increase)	139	0.98	0.89 – 1.03	*0.887*
CRP (SDS)	146	1.31	0.87 – 1.96	*0.196*
WCC (SDS)	148	1.32	0.88 – 1.97	*0.177*
Neutrophils (SDS)	148	1.40	0.93 – 2.10	*0.105*
Albumin (SDS)	138	0.84	0.57 – 1.24	*0.393*
Anorexia (yes vs. no)	116	2.34	0.88 – 6.20	*0.089*
Fatigue (yes vs. no)	116	1.94	0.53 – 7.19	*0.319*
Barthel index (per point increase)	139	0.98	0.97 – 0.99	*0.008*
Inactive (yes vs. no)	115	1.94	0.76 – 4.92	*0.165*
History of falls (yes vs. no)	115	1.25	0.75 – 2.08	*0.402*
GDS score (per point increase)	108	1.06	0.89 – 1.26	*0.518*
MMSE score (per point decrease)	144	1.07	0.96 – 1.19	*0.232*

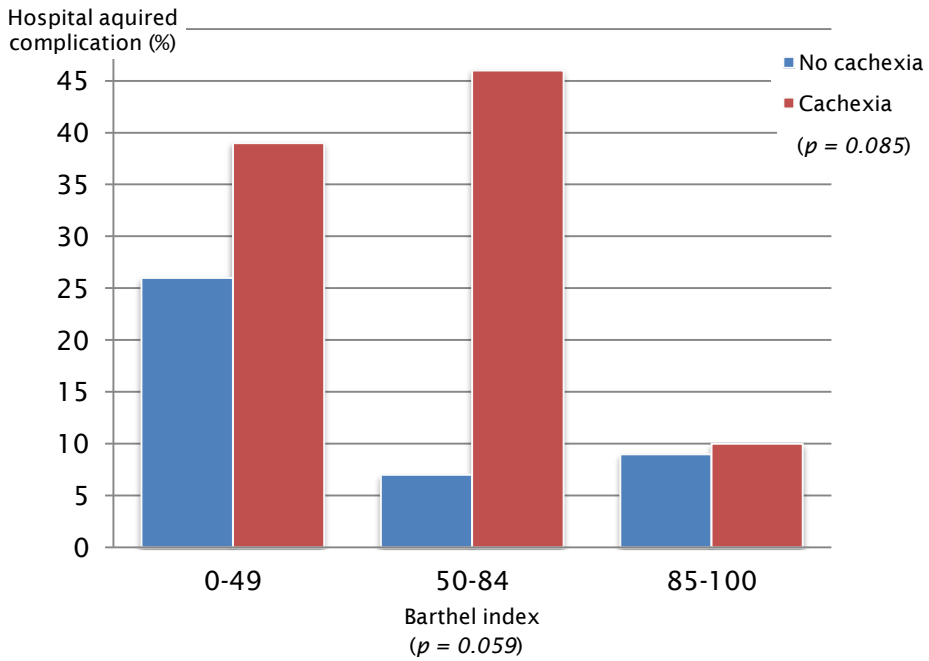
†OR = Odds ratio and 95% confidence intervals; p-values from logistic regression; SDS, Standard Deviation Score

Table 4-24: Associations between baseline characteristics and hospital acquired complication, mutually adjusted, (n = 118)

Baseline characteristic	OR†	Confidence interval	*p-value*
Cachexia (yes vs. no)	2.73	0.87 – 8.59	*0.085*
Age (per year older)	1.05	0.94 - 1.17	*0.370*
Barthel index (per point increase)	0.98	0.96 – 1.00	*0.059*
Anorexia (yes vs. no)	1.38	0.43 – 4.51	*0.588*

†OR = Odds ratio and 95% confidence intervals; p-values from logistic regression. Baseline characteristics included on the basis of p<0.10 in *Table 4-23*

Figure 4-4: Prevalence of hospital acquired complication according to Barthel index and cachexia, p-values after mutual adjustment



4.6.3 Associations between cachexia and other baseline characteristics with admission length and discharge destination

Table 4-25 presents the associations between baseline characteristics and time to: death or discharge to a more dependent setting (new residential home or new nursing home), n = 45. For the purposes of this analysis, discharge to a rehabilitation setting was also regarded as a 'failure event'; removing these participants (*n=32, 22%*) entirely from the analyses did not significantly alter the results.

Table 4-25: Associations between baseline characteristics and to discharge to a more dependent setting or mortality

Baseline characteristic	n	HR	Confidence interval	*p-value*
Cachexia (yes vs. no)	121	1.00	0.48 - 2.11	*1.000*
Age (per year older)	148	1.06	1.00 – 1.12	*0.049*
Max grip (per kg increase)	139	1.02	0.95 - 1.10	*0.575*
Sarcopenia (yes vs. no)	98	0.94	0.42 - 2.15	*0.889*
WCC (SDS)	148	1.16	0.79 - 1.54	*0.553*
Neutrophils (SDS)	148	0.99	0.72 - 1.37	*0.960*
CRP (SDS)	146	1.03	0.78 - 1.39	*0.825*
Albumin (SDS)	138	1.35	0.94 - 1.94	*0.107*
Anorexia (yes vs. no)	116	0.93	0.45 - 1.91	*0.838*
Fatigue (yes vs. no)	116	1.89	0.57 - 6.30	*0.302*
Barthel index (per point increase)	147	1.00	0.99 - 1.01	*0.804*
Inactive (yes vs. no)	115	1.23	0.57 - 2.68	*0.600*
History of falls (yes vs. no)	115	1.12	0.75 - 1.66	*0.590*
GDS score (per point increase)	108	1.08	0.95 - 1.22	*0.268*
MMSE score (per point increase)	144	0.98	0.91 - 1.05	*0.464*

HR = Hazard ratio and 95% confidence intervals; p-values from Cox's proportional hazards model; SDS, Standard Deviation Score

It was considered that the lack of associations in these analyses may be explained by extrinsic factors impacting on length of stay, especially pre-existing support arrangements that would facilitate an easier discharge despite higher functional dependency. Therefore, in order to control for pre-existing community support, analyses were repeated but restricted to the sub-set of participants who were living independently at home prior to admission. These additional analyses, presented in *Table 4-26*, did not show any new significant associations, perhaps due to loss of power, *n=59 to 84*. Results were unaltered if Cox models were stratified by category of domicile.

Table 4-26: Associations between baseline characteristics and the time to discharge to usual residence or mortality in participants who were living independently prior to admission

Baseline characteristic	n	HR	Confidence interval	*p-value*
Cachexia (yes vs. no)	74	1.37	0.54 – 3.52	*0.510*
Age (per year older)	84	1.00	0.93 – 1.08	*0.929*
Max grip (per kg increase)	81	0.97	0.87 – 1.07	*0.503*
Sarcopenia (yes vs. no)	59	1.29	0.39 – 4.20	*0.676*
WCC (SDS)	84	1.16	0.79 – 1.72	*0.453*
Neutrophils (SDS)	84	1.02	0.70 – 1.50	*0.902*
CRP (SDS)	83	1.04	0.73 – 1.48	*0.811*
Albumin (SDS)	79	1.27	0.85 – 1.89	*0.241*
Anorexia (yes vs. no)	69	0.94	0.38 – 2.32	*0.892*
Fatigue (yes vs. no)	69	1.04	0.30 – 3.63	*0.951*
Barthel index (per point increase)	84	0.99	0.97 – 1.01	*0.234*
Inactive (yes vs. no)	70	1.03	0.39 – 2.72	*0.958*
History of falls (yes vs. no)	69	1.03	0.59 – 1.78	*0.927*
GDS score (per point increase)	65	1.08	0.90 – 1.29	*0.408*
MMSE score (per point increase)	82	0.97	0.89 – 1.05	*0.468*

HR = Hazard ratio and 95% confidence intervals; p-values from Cox's proportional hazards model; SDS, Standard Deviation Score

4.7 Cachexia and all-cause mortality

The primary longitudinal outcome assessed in this thesis was all cause mortality. This is explored within the following sections with regard to the associations between baseline characteristics and mortality at six and twelve months and at the end of the study.

4.7.1 Mortality in CaSIO study

2% of participants died during hospital admission and an additional 12% died within the first six months and 9% within the second six months. Overall, 41% of participants had died by the end of the study.

Table 4-27: Mortality in CaSIO study

	numerator / denominator (%)	Cumulative at time-point n (%)
Died during hospital admission	3/148 (2)	3 (2)
Died during 6 month follow up	18/148 (12)	21 (14)
Died during 6-12 month follow up	14/148 (9)	35 (24)
Died from 12 months to end of study	25/148 (17)	60 (41)

4.7.2 All cause mortality

4.7.2.1 Associations between baseline characteristics and six month all cause mortality

Associations between the baseline characteristics with six month mortality are presented in the following tables, using both unadjusted and age adjusted models. Older age, cachexia, higher WCC on admission, lower Barthel index and higher IL-8 and cortisol on discharge were all significantly associated with increased mortality at six months. In the mutually adjusted model WCC on admission remained significant and a survival curve for this is presented in *Figure 4-5*. All significant associations were robust after adjustment for smoking status, alcohol status and the number of co-morbidities (data not shown).

It was considered that hospital readmission is also a negative outcome and therefore these analyses were subsequently repeated defining a failure event as time to death *or readmission to hospital*. All significant associations disappeared as a consequence of this

alteration although more of an association with BMI and a history of falls was apparent; these analyses are presented in *Table RA 5* (*page 209*).

Table 4-28: Associations with six month mortality, univariate analyses, unadjusted and age adjusted

Exposure (at baseline unless stated)	n	HR	CI	*p-value*	*p-value†*
Cachexia (yes vs. no)	118	3.60	1.14 – 11.3	*0.029*	*0.094*
Age (per year older)	145	1.15	1.05 – 1.26	*0.002*	---
Max. grip (per kg increase)	136	0.94	0.86 – 1.03	*0.171*	*0.405*
Sarcopenia (yes vs. no)	96	2.57	0.78 – 8.45	*0.119*	*0.477*
Weight (higher, kg)	138	1.00	0.97 – 1.03	*0.894*	*0.619*
BMI (per point increase)	138	1.00	0.92 – 1.09	*0.990*	*0.498*
Anorexia (yes vs. no)	115	1.09	0.41 – 2.93	*0.863*	*0.972*
Fatigue (yes vs. no)	115	3.95	0.52 – 30.0	*0.185*	*0.202*
Barthel index (per point increase)	144	0.99	0.97 – 1.00	*0.156*	*0.345*
Barthel index on discharge (per point increase)	142	0.98	0.97 – 1.00	*0.021*	*0.191*
Barthel score change over admission (per %)	141	0.99	0.96 – 1.01	*0.330*	*0.696*
Inactive (yes vs. no)	114	2.55	0.88 – 7.35	*0.082*	*0.154*
History of falls (yes vs. no)	114	0.95	0.52 – 1.71	*0.821*	*0.866*
Geriatric depression scale (per point increase)	108	1.01	0.83 – 1.23	*0.920*	*0.797*
Mini mental state exam (per point increase)	141	1.01	0.89 – 1.13	*0.938*	*0.434*
Category of domicile	145				
Private home, alone		1.00	Baseline		
Private home, shared		1.37	0.47 – 4.00	*0.568*	*0.164*
Sheltered/warden		0.37	0.05 – 2.86	*0.338*	*0.339*
Residential home		2.06	0.45 – 9.41	*0.351*	*0.648*
Immune-endocrine biomarkers (per SDS)					
C-reactive protein	143	0.88	0.55 – 1.42	*0.600*	*0.563*
White cell count	145	1.68	1.05 – 2.70	*0.032*	*0.027*
Neutrophil count	145	1.44	0.90 – 2.31	*0.130*	*0.106*
Albumin	135	1.00	0.63 – 1.58	*0.987*	*0.949*
C-reactive protein on discharge	141	1.28	0.80 – 2.05	*0.303*	*0.609*
White cell count on discharge	144	1.34	0.86 – 2.09	*0.202*	*0.183*
Neutrophil count on discharge	144	1.23	0.78 – 1.97	*0.389*	*0.330*
Albumin on discharge	139	0.75	0.52 – 1.08	*0.119*	*0.236*
IL-1 on discharge	32	0.86	0.31 – 2.36	*0.780*	*0.984*
IL-6 on discharge	97	1.34	0.78 – 2.29	*0.293*	*0.484*
IL-8 on discharge	106	1.79	0.99 – 3.22	*0.054*	*0.109*
IL-10 on discharge	106	0.90	0.46 – 1.76	*0.765*	*0.768*
Cortisol on discharge	102	2.03	1.20 – 3.41	*0.008*	*0.043*
DHEAS on discharge	107	1.00	0.57 – 1.75	*0.991*	*0.625*
Cortisol:DHEAS on discharge	100	1.14	0.58 – 2.25	*0.700*	*0.254*
IL-8:IL-10 on discharge	106	1.00	0.56 – 1.81	*0.994*	*0.989*
IL-6:IL-10 on discharge	96	1.10	0.62 – 1.95	*0.750*	*0.977*

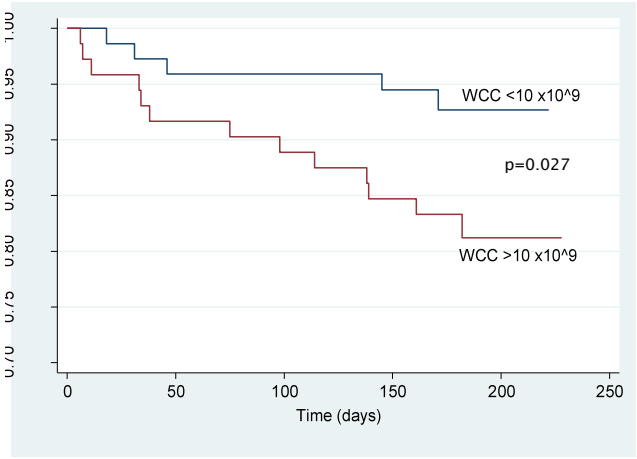
HR = Hazard ratio and 95% confidence interval for unadjusted model only; p-values from Cox's proportional hazards model; SDS, Standard Deviation Score; †age adjusted

Table 4-29: Associations with six month mortality, mutually adjusted model (n=84)

Exposure (at baseline unless stated)	HR	CI	*p-value*
Cachexia (yes vs. no)	2.02	0.32 – 12.8	*0.454*
Age (per year older)	1.16	0.90 – 1.49	*0.245*
Barthel index on discharge (per point increase)	1.00	0.96 – 1.04	*0.983*
White cell count (per SDS)	4.21	1.46 – 12.1	*0.008*
IL-8 on discharge (per SDS)	1.38	0.61 – 3.13	*0.441*
Cortisol on discharge (per SDS)	1.30	0.49 – 3.41	*0.597*

HR = Hazard ratio and 95% confidence interval in a mutually adjusted model; p-values from Cox's proportional hazards model; SDS, Standard Deviation Score. Baseline exposures included on the basis of p<0.07 in *Table 4-28*.

Figure 4-5: Kaplan-Meier six month survival curves according to WCC on admission; p-value from unadjusted model; y-axis, proportional survival



4.7.2.2 Associations between baseline characteristics and twelve month all cause mortality

Associations between baseline characteristics and immune-endocrine biomarkers with twelve month mortality are presented in the following tables, using both unadjusted and age adjusted models, *Table 4-30*. Greater age, lower grip strength, cachexia, lower Barthel index on discharge, higher CRP, IL-8 and cortisol and lower albumin on discharge were all associated with increased risk of 12 month mortality. Associations with cachexia were robust after adjusting for age and, after mutual adjustment, cachexia emerged as the strongest predictor of mortality at 12 months (see *Table 4-31*). All significant associations were robust after adjustment for smoking status, alcohol status and the number of co-morbidities (data not shown). Associations with WCC lost significance.

Table 4-30: Associations with twelve month mortality, univariate analyses, unadjusted and age adjusted

Exposure (at baseline unless stated)	n	HR	CI	*p-value*	*p-value†*
Cachexia (yes vs. no)	118	3.58	1.57 - 8.18	*0.002*	*0.010*
Age (per year older)	145	1.10	1.02 - 1.18	*0.008*	---
Max. grip (per kg increase)	136	0.94	0.88 - 1.00	*0.061*	*0.162*
Sarcopenia (yes vs. no)	96	1.52	0.57 - 4.06	*0.399*	*0.814*
Weight (higher, kg)	138	1.00	0.97 - 1.02	*0.800*	*0.762*
BMI (per point increase)	138	1.01	0.95 - 1.07	*0.874*	*0.446*
Anorexia (yes vs. no)	115	1.22	0.58 - 2.56	*0.595*	*0.593*
Fatigue (yes vs. no)	115	1.03	0.42 - 2.54	*0.951*	*0.968*
Barthel index (per point increase)	144	1.00	0.98 - 1.01	*0.569*	*0.932*
Barthel index on discharge (per point increase)	142	0.99	0.98 - 1.00	*0.066*	*0.397*
Barthel score change over admission (per %)	141	0.98	0.96 - 1.00	*0.068*	*0.190*
Inactive (yes vs. no)	114	1.57	0.74 - 3.32	*0.237*	*0.325*
History of falls (yes vs. no)	114	1.02	0.67 - 1.56	*0.926*	*0.894*
Geriatric depression scale (per point increase)	108	0.95	0.82 - 1.10	*0.497*	*0.315*
Mini mental state exam (per point increase)	141	0.97	0.89 - 1.06	*0.487*	*0.963*
Category of domicile	145				
Private home, alone		1	Baseline		
Private home, shared		1.47	0.66 – 3.30	*0.350*	*0.114*
Sheltered/warden		0.42	0.10 – 1.80	*0.243*	*0.233*
Residential home		2.56	0.86 – 7.60	*0.091*	*0.231*
Immune-endocrine biomarkers (per SDS)					
C-reactive protein	143	1.01	0.71 - 1.44	*0.957*	*0.968*
White cell count	145	1.16	0.82 - 1.66	*0.399*	*0.333*
Neutrophil count	145	1.13	0.79 - 1.60	*0.508*	*0.441*
Albumin	135	0.81	0.60 - 1.09	*0.155*	*0.169*
C-reactive protein on discharge	141	1.37	0.96 - 1.96	*0.084*	*0.195*
White cell count on discharge	144	1.06	0 .75 -1.51	*0.730*	*0.597*
Neutrophil count on discharge	144	1.09	0.77 - 1.54	*0.643*	*0.533*

Albumin on discharge	139	0.73	0.56 - 0.96	*0.022*	*0.047*
IL-1 on discharge	32	0.76	0.30 - 1.94	*0.571*	*0.604*
IL-6 on discharge	97	1.21	0.80 - 1.84	*0.360*	*0.566*
IL-8 on discharge	106	1.52	0.98 - 2.36	*0.063*	*0.098*
IL-10 on discharge	106	0.85	0.51 - 1.40	*0.520*	*0.514*
Cortisol on discharge	102	1.66	1.11 – 2.48	*0.014*	*0.081*
DHEAS on discharge	107	1.05	0.69 - 1.61	*0.815*	*0.964*
Cortisol:DHEAS on discharge	100	1.21	0.74 – 1.96	*0.447*	*0.431*
IL-8:IL-10 on discharge	106	1.19	0.73 - 1.94	*0.496*	*0.508*
IL-6:IL-10 on discharge	96	1.24	0.80 - 1.94	*0.336*	*0.626*

HR = Hazard ratio and 95% confidence interval for unadjusted model only; p-values from Cox's proportional hazards model. SDS, Standard Deviation Score; †age adjusted.

Table 4-31: Associations with twelve month mortality, mutually adjusted model, (n=83)

Exposure (at baseline)	HR	CI	*p-value*
Cachexia (yes vs. no)	4.51	1.21 – 16.8	*0.025*
Age (per year older)	1.12	0.99 – 1.28	*0.078*
Max. grip (per kg increase)	0.99	0.87 – 1.14	*0.934*
Barthel index on discharge (per point increase)	1.01	0.98 – 1.03	*0.639*
IL-8 (per SDS)	1.06	0.60 – 1.88	*0.837*
Cortisol (per SDS)	1.15	0.63 – 2.12	*0.652*

HR = Hazard ratio and 95% confidence interval in a mutually adjusted model; p-values from Cox's proportional hazards model. SDS, Standard Deviation Score. Baseline exposures included on the basis of p<0.07 in *Table 4-30*.

4.7.2.3 Associations between baseline characteristics and all cause mortality to the end of the study

Participants were followed up with respect to mortality status on 13th October 2012; this gave a median total follow up time from the date of discharge of 23 months (IQR 15 – 25 months). Associations between baseline characteristics and immune-endocrine biomarkers with mortality up to this point are presented in *Table 4-32*, using both unadjusted and age adjusted models. Older age, lower grip strength, anorexia, cachexia, lower Barthel index on discharge and higher WCC, neutrophils, CRP, IL-8 and lower albumin were all associated with increased mortality by the end of the study. Associations remained significant after adjusting for age with the exception of grip strength, Barthel index and CRP whose associations with end of study mortality were attenuated. All significant associations were robust after adjustment for smoking and alcohol status and number of co-morbidities (data not shown). Associations with age and cachexia remained after mutual adjustment although those with Barthel index on discharge, grip strength, anorexia and WCC were attenuated. The survival curves for selected associations are presented in *Figure 4-6*.

Table 4-32: Associations with end of study mortality, univariate analyses, unadjusted and age adjusted

Exposure (at baseline unless stated)	n	HR	CI	*p-value*	*p-value†*
Cachexia (yes vs. no)	118	2.63	1.44 – 4.79	*0.002*	*0.026*
Age (per year older)	145	1.11	1.05 – 1.17	*<0.001*	---
Max. grip (per kg increase)	136	0.95	0.90 – 1.00	*0.032*	*0.144*
Sarcopenia (yes vs. no)	96	1.16	0.54 – 2.49	*0.710*	*0.701*
Weight (higher, kg)	138	0.98	0.97 – 1.00	*0.123*	*0.438*
BMI (per point increase)	138	0.97	0.93 – 1.02	*0.271*	*0.790*
Anorexia (yes vs. no)	115	1.88	1.01 – 3.50	*0.046*	*0.034*
Fatigue (yes vs. no)	115	1.49	0.67 – 3.35	*0.332*	*0.421*
Barthel index (per point increase)	144	0.99	0.98 – 1.00	*0.134*	*0.356*
Barthel index on discharge (per point increase)	142	0.99	0.98 – 1.00	*0.022*	*0.343*
Barthel score change over admission (per %)	141	0.99	0.98 – 1.01	*0.370*	*0.979*
Inactive (yes vs. no)	114	1.72	0.94 – 3.14	*0.077*	*0.126*
History of falls (yes vs. no)	114	1.32	0.82 – 1.57	*0.459*	*0.355*
Geriatric depression scale (per point increase)	108	0.98	0.87 – 1.11	*0.778*	*0.459*
Mini mental state exam (per point increase)	141	0.96	0.90 – 1.02	*0.228*	*0.817*
Category of domicile	145				
Private home, alone		1.00	Baseline		
Private home, shared		1.24	0.66 – 2.32	*0.507*	*0.156*
Sheltered/warden		0.83	0.37 – 1.89	*0.658*	*0.485*
Residential home		1.54	0.55 – 4.40	*0.414*	*0.805*
Immune-endocrine biomarkers (per SDS)					
C-reactive protein	143	1.18	0.91 – 1.55	*0.226*	*0.279*
White cell count	145	1.40	1.06 – 1.85	*0.018*	*0.014*
Neutrophil count	145	1.34	1.02 – 1.76	*0.038*	*0.030*
Albumin	135	0.73	0.58 – 0.91	*0.007*	*0.006*
C-reactive protein on discharge	141	1.32	1.00 – 1.73	*0.048*	*0.162*
White cell count on discharge	144	1.26	0.97 – 1.64	*0.090*	*0.057*
Neutrophil count on discharge	144	1.28	0.98 – 1.68	*0.068*	*0.038*
Albumin on discharge	139	0.76	0.61 – 0.94	*0.010*	*0.025*
IL-1 on discharge	32	0.74	0.33 – 1.67	*0.468*	*0.473*
IL-6 on discharge	97	1.23	0.90 – 1.68	*0.186*	*0.350*
IL-8 on discharge	106	1.41	1.02 – 1.95	*0.040*	*0.061*
IL-10 on discharge	106	0.86	0.60 – 1.23	*0.404*	*0.411*
Cortisol on discharge	28	0.84	0.46 – 1.55	*0.586*	*0.383*
DHEAS on discharge	102	1.33	0.95 – 1.87	*0.101*	*0.516*
Cortisol:DHEAS on discharge	107	1.02	0.75 – 1.39	*0.907*	*0.773*
IL-8:IL-10 on discharge	100	1.10	0.77 – 1.57	*0.616*	*0.184*
IL-6:IL-10 on discharge	106	1.29	0.87 – 1.89	*0.202*	*0.212*

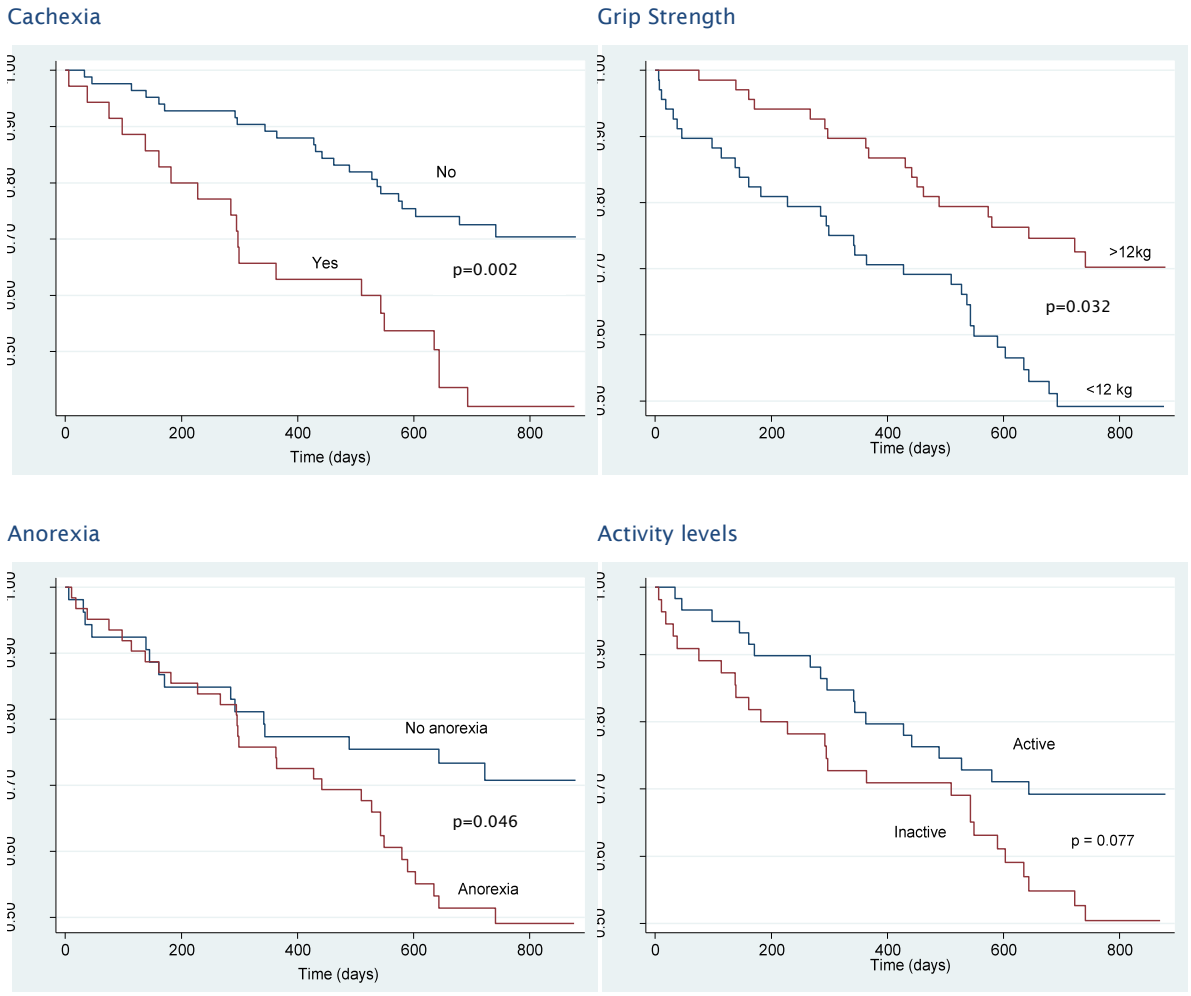
HR = Hazard ratio and 95% confidence interval for unadjusted model only; p-values from Cox's proportional hazards model; SDS, Standard Deviation Score; †After adjusting for age.

Table 4-33: Mutually adjusted associations with end of study mortality, n = 93

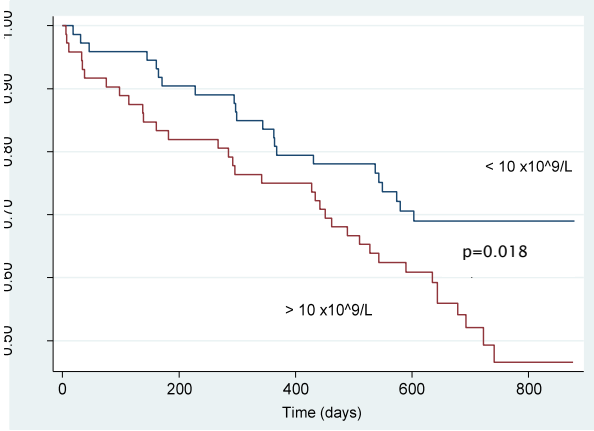
Exposure (at baseline unless stated)	HR	CI	*p-value*
Cachexia (yes vs. no)	2.18	0.98 – 4.84	*0.055*
Age (per year older)	1.11	1.03 – 1.20	*0.004*
Max. grip (per kg increase)	1.01	0.93 – 1.09	*0.877*
Anorexia (yes vs. no)	2.20	0.93 – 5.21	*0.075*
Barthel index on discharge (per point increase)	1.00	0.99 -1.02	*0.807*
White cell count (per SDS)	1.03	0.97 – 1.10	*0.348*

HR = Hazard ratio and 95% confidence interval in a mutually adjusted model; p-values from Cox's proportional hazards model. SDS, Standard Deviation Score. Baseline exposures included on the basis of p<0.07 in *Table 4-32* (WCC was used as only immune-endocrine biomarker to avoid over adjustment).

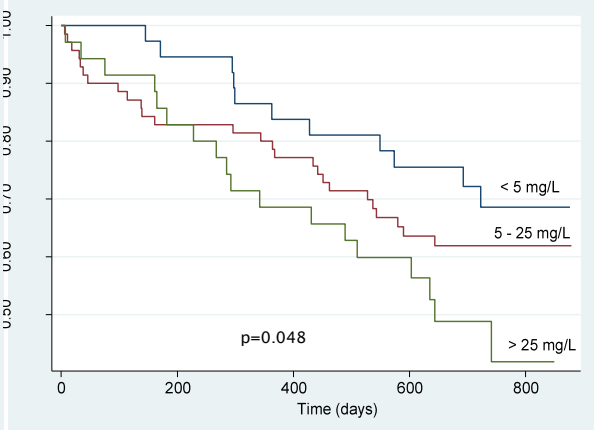
Figure 4-6: Kaplan-Meier end of study survival curves according to selected exposures, y-axis representing proportional survival and p-values from unadjusted analyses as in *Table 4-32*



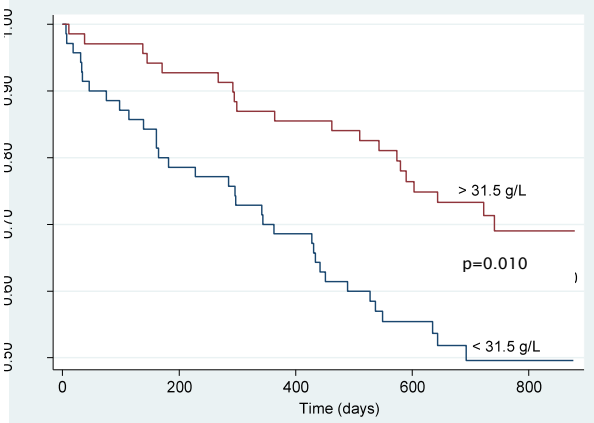
White Cell Count



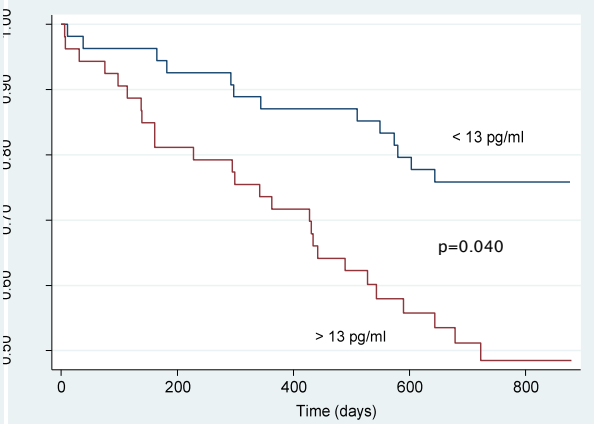
C-reactive protein



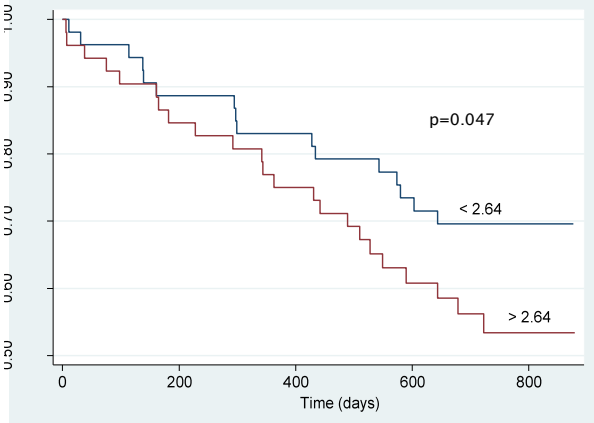
Albumin



IL-8



IL6: IL10 ratio



Weight loss

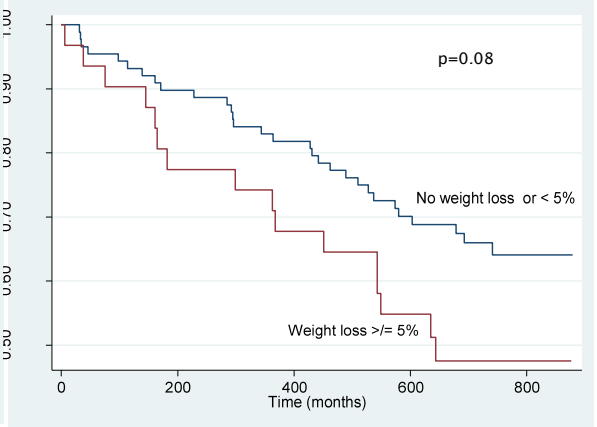


Table 4-34: Likelihood of mortality over the duration of the study according to cachexia and weight loss

	Exposure			
Likelihood of mortality at:	Cachexia		% weight loss	
	Hazard ratio	*p-value*	Hazard ratio	*p-value*
6 months	3.6	*0.03*	2.2	*0.15*
12 months	3.6	*<0.01*	1.7	*0.20*
End of study	2.6	*<0.01*	1.7	*0.08*

p-values from Cox's proportional hazards model, unadjusted model. Hazard ratios for cachexia according to yes vs. no; for weight loss according to none or <5% weight loss vs. ≥5% weight loss

Table 4-35: Likelihood of mortality over the follow up period according to components of the cachexia definition, mutually adjusted model

	6 months		12 months		End of study	
	HR	*p-value*	HR	*p-value*	HR	*p-value*
Weight loss	Baseline		Baseline		Baseline	
0-5%	1.2	*0.87*	1.5	*0.54*	4.4	*<0.01*
≥5%	4.1	*0.05*	2.3	*0.11*	3.2	*<0.01*
BMI <20	1.0	*0.93*	1.0	*0.96*	1.0	*1.00*
Max. grip strength (per SDS)	0.8	*0.47*	0.7	*0.16*	0.7	*0.08*
Anorexia (yes vs. no)	0.4	*0.24*	1.0	*0.96*	1.4	*0.45*
Fatigue (yes vs. no)	4.2	*0.21*	0.6	*0.40*	1.1	*0.85*
WCC (per SDS)	2.6	*0.02*	1.2	*0.52*	1.4	*0.15*

HR = Hazard ratio, p-values from Cox's proportional hazards model

Table 4-36: Influence of grip strength, inflammaging (WCC) and cachexia on mortality over duration of study in mutually adjusted model (model 1) and also adjusted for age and Barthel index (model 2)

	6 months		12 months		End of study	
	HR	*p-value*	HR	*p-value*	HR	*p-value*
Model 1						
Max. grip (per kg)	1.0	*0.61*	1.0	*0.99*	1.0	*0.61*
WCC (per SDS)	2.4	*0.84*	1.0	*0.87*	1.2	*0.32*
Cachexia (yes)	3.4	*0.09*	4.3	*<0.01*	2.5	*<0.01*
Model 2						
Max. grip (per kg)	1.0	*0.48*	1.0	*0.66*	1.0	*0.99*
WCC (per SDS)	2.1	*0.04*	1.1	*0.69*	1.2	*0.26*
Cachexia (yes)	2.6	*0.18*	4.7	*<0.01*	2.1	*0.04*
Age (years)	1.1	*0.33*	1.1	*0.15*	1.1	*<0.01*
Barthel index	1.0	*0.40*	1.0	*0.75*	1.0	*0.96*

HR, hazard ratio, p-values from Cox's proportional hazards model

4.7.3 Associations between cachexia at 6 month follow up with end of study mortality

Associations between characteristics at 6 month follow up with mortality by the end of the study (median time, 17 months) were also explored, *Table 4-37*. Older age and a history of falls were associated with increased mortality over the follow up period. In a mutually adjusted model older age, falling and cachexia were significant predictors (p<0.08), *Table 4-38*. Selected survival graphs are presented in *Figure 4-7*.

Table 4-37: Associations between 6 month characteristics with end of study mortality, univariate unadjusted and age adjusted analyses

Exposure (at 6 month follow up)	N	HR	CI	*p-value*	*p-value†*
Cachexia (yes vs. no)	83	1.56	0.73 - 3.37	*0.254*	*0.279*
Age (per year older)	103	1.11	1.03 - 1.19	*0.004*	---
Max. grip (per kg increase)	103	0.99	0.94 - 1.05	*0.742*	*0.965*
Weight (per kg increase)	89	0.97	0.94 – 1.01	*0.087*	*0.274*
Body mass index (per point increase)	89	0.95	0.88 – 1.02	*0.207*	*0.583*
Anorexia (yes vs. no)	101	0.91	0.80 - 1.03	*0.130*	*0.103*
Fatigue (yes vs. no)	102	0.78	0.39 - 1.56	*0.480*	*0.119*
Barthel index (per point increase)	102	0.99	0.98 - 1.01	*0.404*	*0.727*
Inactivity (yes vs. no)	102	1.50	0.71 - 3.16	*0.289*	*0.404*
History of falls (yes vs. no)	101	5.15	1.05 – 4.41	*0.036*	*0.114*
Geriatric depression scale (per point increase)	79	1.03	0.89 - 1.18	*0.708*	*0.511*
Mini mental state exam (per point increase)	101	0.98	0.90 - 1.06	*0.557*	*0.842*
Gait speed (longer, seconds)	73	1.02	0.99 - 1.05	*0.317*	*0.375*
Category of domicile	103				
Private home, alone		1.00	Baseline		
Private home, shared		1.08	0.44 - 2.66	*0.859*	*0.780*
Sheltered / warden		0.91	0.30 - 2.75	*0.869*	*0.554*
Residential home		1.41	0.41 - 4.88	*0.586*	*0.644*
Nursing home		1.73	0.57 - 5.22	*0.330*	*0.603*
Frailty status (Fried)	88				
Non-frail		1.00	Baseline		
Pre-frail		1.72	0.31 - 9.38	*0.532*	*0.440*
Frail		2.43	0.57 -10.37	*0.230*	*0.242*
Inflammation biomarkers (per SDS)					
CRP	80	0.78	0.53 - 1.15	*0.210*	*0.305*
IL-1	53	0.93	0.56 - 1.53	*0.769*	*0.535*
IL-6	59	0.68	0.45 - 1.02	*0.060*	*0.464*
IL-8	30	0.81	0.45 - 1.44	*0.468*	*0.349*
IL-10	53	0.66	0.32 - 1.35	*0.255*	*0.611*
Cortisol	92	0.98	0.65 - 1.47	*0.920*	*0.707*
DHEAS	83	1.32	0.90 – 1.97	*0.158*	*0.981*
Cortisol:DHEAS	82	0.84	0.54 - 1.30	*0.430*	*0.826*

HR = Hazard ratio and 95% confidence interval for unadjusted model only; p-values from Cox's proportional hazards model; SDS, Standard Deviation Score; †Age adjusted

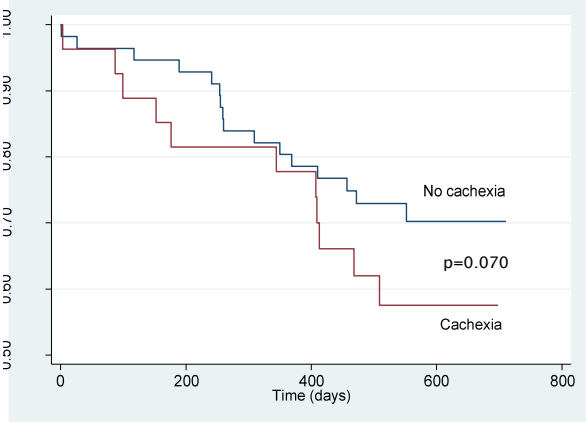
Table 4-38: Associations of 6 month characteristics with end of study mortality, mutually adjusted model (*n=80*)

Exposure (at 6 month follow up)	HR	CI	*p-value*
Cachexia (yes vs. no)	2.00	0.86 – 4.69	*0.070*
Age (per year older)	1.10	1.00 – 1.19	*0.045*
Max. grip strength (per kg increase)	0.99	0.89 – 1.07	*0.633*
CRP (per SDS)	1.00	1.00 – 1.00	*0.116*
History of falls (yes vs. no)	1.56	0.96 – 2.56	*0.074*

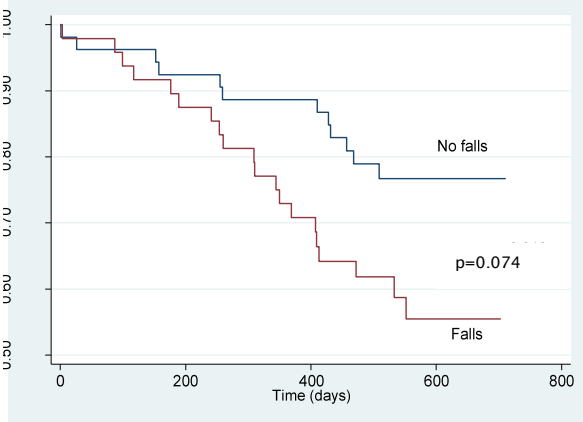
HR = Hazard ratio and 95% confidence interval for mutually adjusted model; p-values from Cox's proportional hazards model. Exposures included on the basis of p<0.07 in *Table 4-37*

Figure 4-7: Kaplan-Meier end of study survival curves according to selected 6 month follow up characteristics. Y-axis represents proportional survival and p-values from *Table 4-38.*

Cachexia



Falls



Chapter 5 Discussion

The CaSIO study has characterised 148 hospitalised older women focusing on cachexia during hospital admission and health outcomes at follow up in the community. This chapter will firstly discuss the results that have been presented in this thesis, placing them in context relative to the wider scientific literature. Second, consideration will be given to the clinical translation of the findings from the CaSIO study. Third, the strengths and limitations of the thesis will be addressed. Finally, recommendations for future research suggested by this thesis will be outlined.

5.1 The CaSIO study population

5.1.1 Description of population characteristics

This section considers the general characteristics of the CaSIO study population in comparison with the target population of hospitalised older women. A more detailed discussion of the representativeness of the study sample and possible sources of selection bias may be found within the limitations section.

5.1.1.1 Demographics

The average age of the women who participated in the CaSIO study was 86 years which is similar to hospital data on the average age of patients admitted to the Medicine for Older People wards. The prevalence of being a widow was 73% among CaSIO women and increased with age (*p=0.01*); this is similar to national figures amongst women of this age group [193]. The death of a spouse and being single is recognised as a risk factor for depression [194] and, indeed, widowhood was associated with high geriatric depression score (*p=0.06*) among CaSIO participants. Only three percent of participants were current smokers, a prevalence which is comparable with UK data which show that the prevalence of smoking declines with age and is approximately 7% among women aged over 65 years [195]. The reasons for this decline are likely to be multifactorial and include: reduced enjoyment of smoking with age; effects of co-morbidity; increased survival among non-smokers and socioeconomic factors such as isolation, cost and change of accommodation to care homes. National data suggest that alcohol consumption also declines with age for similar reasons to smoking [195] and this was reflected among CaSIO participants, the majority of whom did not consume alcohol and

only 5% of whom reported moderate to heavy alcohol consumption. Information characterising socioeconomic status was not collected within the CaSIO study.

The majority of study participants were admitted to hospital via the Accident and Emergency department and there was a high degree of co-morbidity as evidenced by an average of 5 active conditions within the past medical history and participants taking an average of 10 regular mediations. These figures are slightly lower than the average of 15 medications per person as identified by a recent observational study of frail older people within a day hospital setting [196].

5.1.1.2 Baseline functional status

The CaSIO study population had poor functional status at baseline with a median Barthel index of 67, interquartile range of 41-93 and only 9% of participants scoring the maximum of 100. One half of participants described spending most of their time in either a chair or a bed and 68% had experienced at least one fall in the preceding 6 months. These functional characteristics are reflected in living arrangements with 22% of participants having been admitted from either sheltered accommodation or residential care, and one half of the participants who lived at home being in receipt of a formal package of care; figures comparable with other studies of hospitalised older people in the UK [197]. None of the CaSIO participants had been admitted to hospital from a nursing home; this may be a consequence of nursing home residents being towards the end of their lives and therefore either managed in a palliative capacity in the community or, if admitted to hospital, not being eligible to participate in a study such as CaSIO owing to severity of illness or lacking the capacity to provide informed consent.

5.1.1.3 Baseline cognitive status

Within the UK, prevalence estimates for dementia in acute hospital settings increase with age and range from 13-63% depending on the population studied. A recent systematic review of cognitive impairment amongst older hospitalised people reported that 27% have previously diagnosed dementia, 50% have cognitive impairment and 27% have delirium [198]. Within the CaSIO study, MMSE was assessed at baseline and represents a 'snap-shot' in time rather than a formal cognitive assessment; low scores may represent longstanding cognitive deficit (dementia), a short-term cognitive decline due to illness, hospital admission (delirium) and may also be affected by non-cognitive factors including background educational level, ethnicity and sensory impairment. The median MMSE score was 25 with a range of 8 to 30 which, within the limitations of MMSE discussed, indicates a significant

degree of cognitive impairment amongst CaSIO participants and is likely to be an underestimate due to the exclusion criteria defined for the CaSIO study.

A number of patients with lower MMSE scores had capacity to give consent to participate in CaSIO. The issues surrounding cognitive impairment and the capacity to give informed consent are complex; in the case of the CaSIO study, individuals who consistently demonstrated an understanding of the study and a willingness to participate on more than one occasion were deemed to have capacity to provide consent for the specific task of participating in the study. This illustrates the importance of having researchers trained with specific skills to conduct research in older people in order to be as inclusive with regard to participation as possible.

Similar to the MMSE, the GDS score only provides a snap-shot of depressive symptoms and GDS scores are subject to the effects of day-to-day fluctuations in mood and environmental and clinical factors. A full diagnosis of depression is more complex to obtain and was beyond the scope of this study. However, one quarter of the study population gave predominantly negative responses in the GDS which is consistent with estimates of depression from other studies of older hospitalised patients which range from 5-58%, with a mean prevalence of 29% [199]. It is likely that data from CaSIO participants provides an underrepresentation of the prevalence of depressive symptoms within the target population due to the tendency for people who are more depressed to be unwilling to participate in research studies and the selection bias that would have occurring when screening out patients with dementia, a condition that is known to be associated with depression.

5.1.1.4 Population at follow up

70% of participants were followed up at six months. This group were less likely to have been cognitively impaired at baseline which is probably a reflection of the logistical difficulties of contacting and arranging appointments with people who have cognitive impairment. Therefore, as previously discussed, follow up bias would have been introduced at this stage although, with the exception of fatigue, no differences were identified between any other baseline characteristics according to follow up status, including baseline GDS score.

The study population at follow up were more functionally dependent than prior to being discharged from hospital. Participants were living in more dependent settings at follow-up with 10% having moved into a nursing home and 37% having experienced an escalation of their usual package of care. They also reported being more inactive and, perhaps as a consequence, were experiencing fewer falls and reported a higher median GDS score, indicating a greater degree of depressive symptoms. These results are consistent with

published literature demonstrating an overall decline in functional status following hospital admission [11;12;19].

44% of participants had been readmitted to hospital over the six month period, often on multiple occasions. Readmissions were highest in months 1 and 5 following discharge; this could reflect an initial early period of increased vulnerability to becoming unwell followed by an increased likelihood of readmission with time.

5.1.1.5 Frailty at follow up

The majority (70%) of participants were frail at follow up and a further 17% were pre-frail; just 14% were non-frail. These frailty prevalences are considerably higher than those identified in a recent systematic review which reported that 11% of community-dwelling older persons were frail, rising to 16% in those aged 80-85 and 26% in those aged over 85 years; overall 42% were pre-frail [200]. These discrepancies are likely to reflect the substantial differences between populations of older people who are transitioning in and out of healthcare settings as opposed to older people living independently within the community. This reinforces the importance of the CaSIO study's strategy of recruiting older people from a hospital setting in order to study a group of rarely characterised vulnerable older people.

5.1.1.6 Summary: study population versus target population

In spite of limitations in the data collection for the CaSIO study, (see *section 5.7.2*), the study participants were broadly comparable with the wider population of hospitalised older women in terms of demographic and functional characteristics. However, the study participants are likely to have been less unwell, less confused and to have reported fewer depressive symptoms at baseline.

5.1.2 Description of cachexia at baseline and follow up

5.1.2.1 Description of anorexia at baseline and follow up

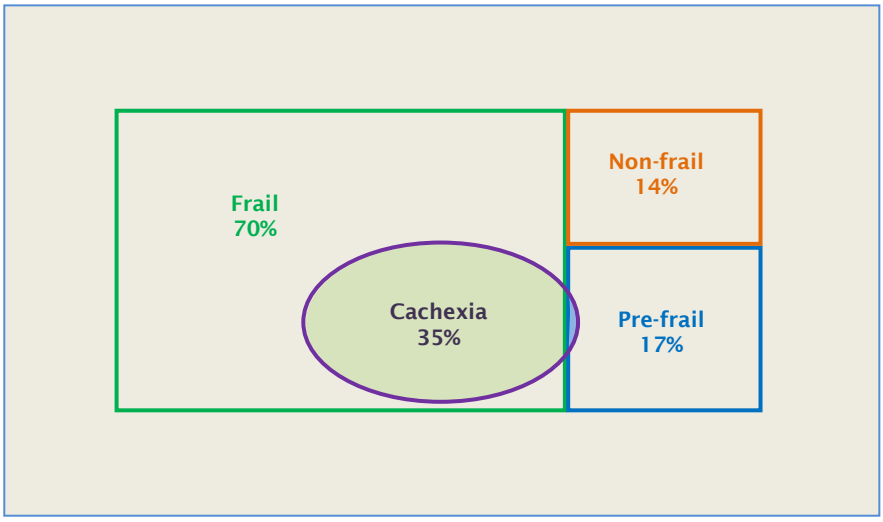
The prevalence of symptoms suggesting anorexia within the CaSIO study at baseline was 47%. This is considerably higher than an estimated anorexia prevalence of 33% as recently reported amongst older women (average age, 82 years) within acute hospital and rehabilitation settings [201]; this difference in prevalence perhaps reflects the older age and degree of multimorbidity within the CaSIO population. As described in the introduction to this thesis, a high prevalence of anorexia symptoms is unsurprising among the CaSIO participants who, not only represent the frailest older people, but have also been admitted to a hospital setting and have a greater inflammatory burden. Prevalence at follow up was similar (51%) which is likely to reflect ongoing inflammation and anorexia of ageing [36].

Anorexia, as measured by the simplified nutritional assessment questionnaire (SNAQ), was quick to perform and acceptable to CaSIO participants; it has been shown to be effective at predicting anorexia-related weight loss in older adults which, in turn, is associated with falls, hip fractures, compromised immunity, pressure ulcers, frailty and mortality [190]. This previous research suggests that it is important to identify anorexia in older adults in order to address it through comprehensive assessment to treat reversible causes by, for example, reviewing medications, treating co-morbidity and dietary assessment.

5.1.2.2 Description of cachexia at baseline and follow up

28% of the study population had cachexia at baseline and 5% had pre-cachexia. This is the first description of the prevalence of cachexia in older people in an acute hospital setting and is higher than the reported prevalence of 20% in a care home setting [23]. By 6 month follow up the prevalence of cachexia had increased further to 34% and pre-cachexia to 8% which is a substantial increase, especially in the context of reduced survival rates among the participants with cachexia at baseline. Participants with cachexia represented a distinct group within an already frail population, *Figure 5-1*.

Figure 5-1: Venn diagram of frailty and cachexia at follow up



The CaSIO study therefore suggests that cachexia has a similar prevalence among hospitalised and recently hospitalised older women as it does amongst cancer patients; furthermore, its prevalence is higher than in other chronic diseases including chronic obstructive pulmonary disease and congestive heart failure [23]. Due to the large and growing numbers of frail older people, this group is likely to contribute to the greatest cachexia burden within the general population. Despite this it is likely that cachexia is under-recognised and under-treated in older people which may be due to conflation with other syndromes (e.g. frailty) or under documentation and also a lack of evidence regarding its clinical significance in terms of outcomes plus a lack of established interventions.

5.1.2.2 Description of skeletal muscle loss at baseline and follow up

According to the European working group for sarcopenia in older people (EWGSOP) definition the overall prevalence of sarcopenia within the CaSIO study at baseline was 26%. Most studies to date have characterised sarcopenia in community dwelling older people and previously published prevalence estimates are difficult to compare owing to methodological differences between studies. For example, research in New Mexico amongst community dwelling women over 80 years used DXA scanning to define the prevalence of sarcopenia as 24% and, in a separate study, its prevalence in community dwelling women over 85 years was just 13% when defined using BIA [202;203]. However, a recent study within hospitalised older adults (mean age 83) that also used the EWGSOP definition and BIA to characterise fat free mass reported a prevalence of sarcopenia of 25%, very similar to findings from the CaSIO study [194].

Skeletal muscle loss may also be defined using grip strength alone and a cut-off in women of less than 20kg has been proposed by Laurentani [204]. If this definition is applied, the proportion of CaSIO participants classified as having sarcopenia at baseline rises from 26% to 87% (mean maximum grip strength, 13kg). This prevalence is perhaps more plausible among a population of frail older women and is similar to prevalence estimates from other studies of grip strength such as InCHIANTI (mean grip strength in women aged >85 years, 14.5kg) and the study by Kerr in a hospitalised population equivalent to CaSIO (median grip strength in women aged 75-101 years, 16kg) [56].

The CaSIO study also provided the opportunity to compare sarcopenia with frailty at follow up. *Figure 5-2* illustrates the substantial overlap between frailty (defined according to weight loss, low activity, exhaustion, gait speed, muscle strength) and sarcopenia (defined according to grip strength of less than 20kg) among the CaSIO participants. These results suggest that, on the basis of current definitions, sarcopenia and this definition of frailty overlap considerably and may even describe the same age-related processes. In CaSIO, the additional assessments that were needed to characterise frailty were of little added diagnostic value over and above the assessment of low grip strength alone.

Figure 5-2: Venn diagram of frailty and sarcopenia at follow up

5.1.2.3 Description of immune-endocrine biomarkers at baseline and follow up

Inflammation, in addition to the loss of skeletal muscle, is an important component of cachexia. The CaSIO study identified inflammation on the basis of routine blood tests at baseline and routine blood tests plus cytokines, cortisol and DHEAS analysis at discharge; additional analyses were conducted at follow up. The ratio of pro- to anti- inflammaging markers (IL-6/IL-8 vs. IL-10 and cortisol vs. DHEAS) was also assessed.

At baseline most clinical markers of inflammation were off baseline as a consequence of the admitting diagnosis. By discharge these had returned to normal levels with the exception of CRP which, although substantially reduced in comparison to levels at admission, remained slightly elevated at 12mg/L. This observation suggests that CRP is the 'last in the chain' of inflammatory markers to settle following acute inflammation due to its production from the liver being stimulated by IL-6. It is reassuring to note that the more advanced immune-endocrine analysis at discharge was taken at a point beyond the acute inflammatory insult and therefore should represent more stable baseline levels of inflammation. However, cortisol and DHEAS are likely to have remained off baseline at discharge due to greater activation of the HPA axis in association with the stress of a hospital admission; this should be considered when interpreting results involving these biomarkers.

By 6 month follow up CRP, cortisol and DHEAS had fallen to presumed baseline levels. IL-1, IL-6 and IL-10 were all significantly higher than on discharge and the ratio IL-6:IL-10 had also risen indicating a generally more pro-inflammatory environment when compared to baseline; this may represent a greater degree of inflammaging with time. A limitation of this study is the lack of longitudinal data for immune-endocrine biomarkers; these were only taken at one follow up time point (6 months) and numbers were low and lacked characterisation of the cellular immune system. Larger numbers, more comprehensive characterisation and more time points are needed in order to explore longitudinal associations in more detail and to draw conclusions about changes in the inflammatory environment with time.

5.2 Cachexia: cross-sectional associations

5.2.1 Cross sectional associations with cachexia

Cachexia in younger patient populations is usually associated with a single diagnosis. This was not the case in the CaSIO study where participants were more likely to have a number of morbidities that collectively contributed to cachexia via the generation of a pro-inflammatory, catabolic environment rather than a single morbidity such as cancer or congestive cardiac failure. It is likely that the aetiology of cachexia within older people represents the extremes of inflammaging set against the multifactorial background of genetic variation, accumulative antigenic load, co-morbidity and acute illness. Additionally, the CaSIO population had an increased vulnerability to cachexia due to age-related skeletal muscle loss and reduced physical activity, physiologic anorexia, low mood, cognitive impairment, and social isolation [34-36].

Cachexia was associated with lower grip strength, more anorexia symptoms and greater fatigue among CaSIO participants; this was unsurprising as these clinical characteristics are all components of its definition. Individuals with cachexia were also more likely to be older, score lower on MMSE, higher on GDS and have a greater pro-inflammatory environment than individuals without cachexia.

5.2.2 Cross-sectional associations with skeletal muscle loss

Low muscle strength was associated with older age, living in more dependent environments, greater functional impairment, inactivity, falling and anorexia. Lower scores on MMSE and higher GDS scores were also associated with low muscle strength and all associations, with the exception of GDS score, remained significant after adjustment for potential confounders including age. Similar cross-sectional associations were identified at follow up where lower grip strength was also associated with slower gait speed and greater prevalence of frailty.

These findings are consistent with reports elsewhere in the literature [74;75;205]. Loss of skeletal muscle is an intrinsic part of the ageing process and is also associated with age related disease; it was therefore unsurprising to find a high prevalence of low muscle strength in the CaSIO study. Associations between low muscle strength and functional outcomes occur directly through loss of power, speed and precision movement and also through associations with multi-morbidity such as cardiovascular disease [21;65-68;69-71]. Low muscle strength is the biggest single intrinsic risk factor for falls and shares a number of common aetiologies with anorexia including age, multi-morbidity and inflammation [74;75;205].

Furthermore, poor dietary intake as a consequence of anorexia will hasten skeletal muscle decline and, in turn, will contribute to the inflammatory component of anorexia via the direct secretion of myokines from muscle [206].

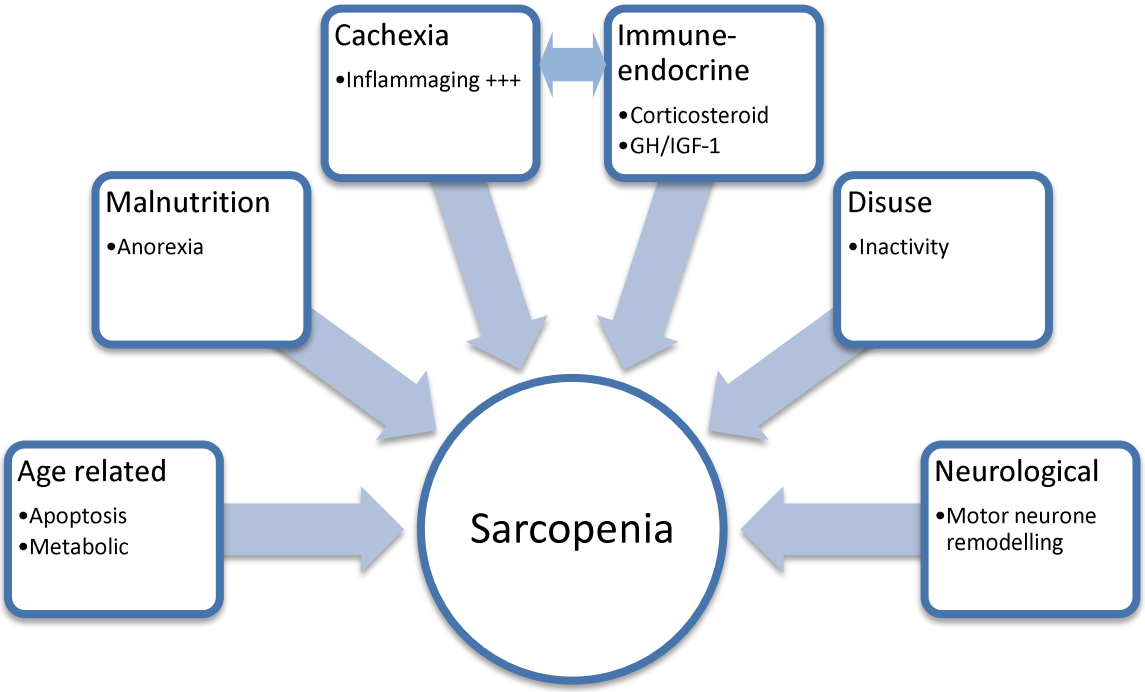
Associations have also previously been demonstrated between loss of skeletal muscle mass and cognitive decline which is also an indicator of biological ageing [207]. There is much debate in the literature regarding the nature and degree of this interdependency and whether a common unifying mechanism is responsible. Possible explanations include: 'brain-aging' – a general age related decline in white matter and cerebella integrity; neurovascular effects occurring simultaneously with muscle wasting; telomere shortening and the environmental impacts of socioeconomic status and risky health behaviours including smoking, obesity and low physical activity [207]. Depression is associated with cognitive decline in older people and also with worse physical functioning although this has rarely been demonstrated in association with grip strength and never in hospitalised older people. The role of depression within the lifecourse is complex, as it is both a cause and consequence of worse cognitive and physical health [208;209].

5.2.3 Sarcopenia versus cachexia

It was anticipated that the majority of CaSIO participants with sarcopenia would not have cachexia but that all participants with cachexia would also have significant degrees of sarcopenia. This hypothesis was confirmed with 84% of all participants having a maximum grip strength of less than 20kg, but only a minority of these having cachexia.

Both sarcopenia and cachexia are catabolic conditions with a common pathway characterised by the loss of muscle mass. However, it is likely that sarcopenia relates specifically to loss of skeletal muscle mass and function with a multifactorial aetiology of which inflammation is one of the drivers, as opposed to cachexia which is a purely inflammatory process and in older people may represent one end of the spectrum of inflammaging; cachexia causes skeletal muscle loss and is therefore one of the many causes of sarcopenia. Sarcopenia causes weight loss and is one of the reasons older people are at a greater risk of developing cachexia. This is in agreement with the current dogma that cachexia is one cause of sarcopenia but the two disease processes are not the same, *Figure 5-3* [210].

Figure 5-3: Conditions potentially leading to sarcopenia, *adapted from* [210]



The importance of the distinction between 'normal' age-related loss of skeletal muscle mass and function (sarcopenia) and cachexia becomes apparent when considering their implications within the study population. There were cross-sectional associations between grip strength and functional measures such as Barthel index, category of domicile and the provision of a package of care whereas cachexia was not associated with these functional characteristics but was more strongly associated with characteristics such as cognition, fatigue, anorexia and inflammation. This is important when considering longer term outcomes; cachexia, rather than muscle strength, was far more closely associated with perhaps the most significant intrinsic outcome, mortality.

Therefore, it seems that the diagnosis of cachexia captures a different set of pathological processes than the age related loss of skeletal muscle mass and function and offers a different perspective within the CaSIO study population. Presence of cachexia appears to translate in to individuals having worse hospital related and longer term outcomes following admission; this is discussed in greater detail in the following sections.

5.3 Cachexia and hospital related outcomes

5.3.1 Hospital related outcomes: comparisons with national data

Consistent with both local and national data, the median length of stay among CaSIO participants was 15 days[13]; the distribution of length of stay was positively skewed with one participant remaining in the acute hospital environment for 99 days. This specific participant experienced a prolonged hospitalisation due to complex negotiations regarding accommodation arrangements following discharge rather than physical or medical factors. The incidence of hospital acquired complication (HAC) was 21% among CaSIO participants which is in keeping with reports in the literature[16]; CaSIO 28-day readmission rates were 13% compared with 14% nationally [16;211].

Although rates of hospital acquired complication and 28-day readmission were broadly comparable with published data, inpatient mortality among CaSIO participants was only 2% in contrast with a departmental rate of 15%. This difference is likely to be due to selection bias as a consequence of exclusion of the most unwell patients from the recruitment process [14].

5.3.2 Hospital acquired complication

Lower Barthel index and the presence of cachexia at baseline were associated with greater likelihood of hospital acquired complication. The odds of a HAC were 1.02 fold greater per unit decrease in Barthel score (p=0.008) and were 2.44 fold greater among patients with cachexia at baseline in contrast with those without cachexia (p=0.059). The association between cachexia and HAC was not statistically significant at the traditional 5% level; nonetheless, the magnitude of association was noteworthy. In combination, a CaSIO participant with baseline Barthel index greater than 68 and without cachexia was almost four times less likely to develop a HAC in contrast with a participant with a Barthel index of less than 68 and with cachexia.

Patients with cachexia are more likely to develop HAC because of complex, systemic processes which are a consequence of multi-morbidity and accumulative inflammation. They have little energy and protein reserve to defend against additional stresses which, especially whilst in hospital, come in multiple forms including pathogens, bed rest, under-nutrition and invasive procedures. In addition to this, participants with a greater functional deficit are less able to self care; and therefore are more vulnerable to HAC as a consequence of, for example, pressure sore progression due to immobility and pneumonia due to feeding and positioning difficulties.

The implications of HAC are well documented; 15% lead to impairment or disability lasting more than 6 months and another 10% contribute to patient death - all lead to a mean increase in hospital stay of 8 days [16]. Furthermore, a recent study of HAC amongst hospitalised older people in the UK reported that 50% of adverse events are preventable and 30% are associated with disability or death, not to mention the significant psychological and social impact on patients and their families and high associated healthcare costs [18].

The CaSIO study results suggest that identification of cachexia in hospitalised older people may be useful to help differentiate sub-groups of patients at a greater risk of HAC in order to facilitate targeted intervention within the context of finite healthcare resource [212]. This is important because there is a significant evidence base that interventions to prevent HAC in older people can be effective. Such interventions were recently reviewed [213] and include the avoidance of hospital acquired infection by: aspiration precautions [214;215]; adherence to antibiotic and catheter care guidelines [216;217]; hand washing [218]; avoidance of pressure sores by regular turning [217]; nutritional interventions [219] and risk assessment [220;221]; early mobilisation and the use of non-hospital settings to reduce functional decline [222]; and use of medication review [223;224], exercise programmes [225] and risk assessment to reduce falls [226].

There were some limitations when considering HAC within the CaSIO study. First, the true burden of adverse events may have been underestimated due to the possibility of underreporting in the medical notes and possible failure to record all adverse events on the study data collection sheets. However, medical record reviews have previously been found to be good at detecting adverse events [16;227] and care was taken to record data that were as comprehensive and complete as possible for the CaSIO participants.

Second, it is possible those CaSIO participants with cachexia had longer hospital admissions and that this exposed them to more hospital pathogens and, as a consequence, a greater likelihood of developing HAC. However, an association between cachexia and increased risk of HAC is biologically plausible as described above and, moreover, no association was evident between cachexia and length of stay which suggests that the association between cachexia and HAC in the CaSIO study was not simply due to the mediating effect of length of stay. Unfortunately the date of development of a HAC was not recorded in the CaSIO study which means that it is not possible to further disentangle the possible circular nature of the associations between cachexia, HAC and length of stay; it is recommended that future studies include documentation of the date of HAC in order to perform time-to-event analyses. Unsurprisingly, development of a HAC is recognised as being associated with longer hospital admission [16;213].

5.3.3 Length of hospital admission and discharge destination

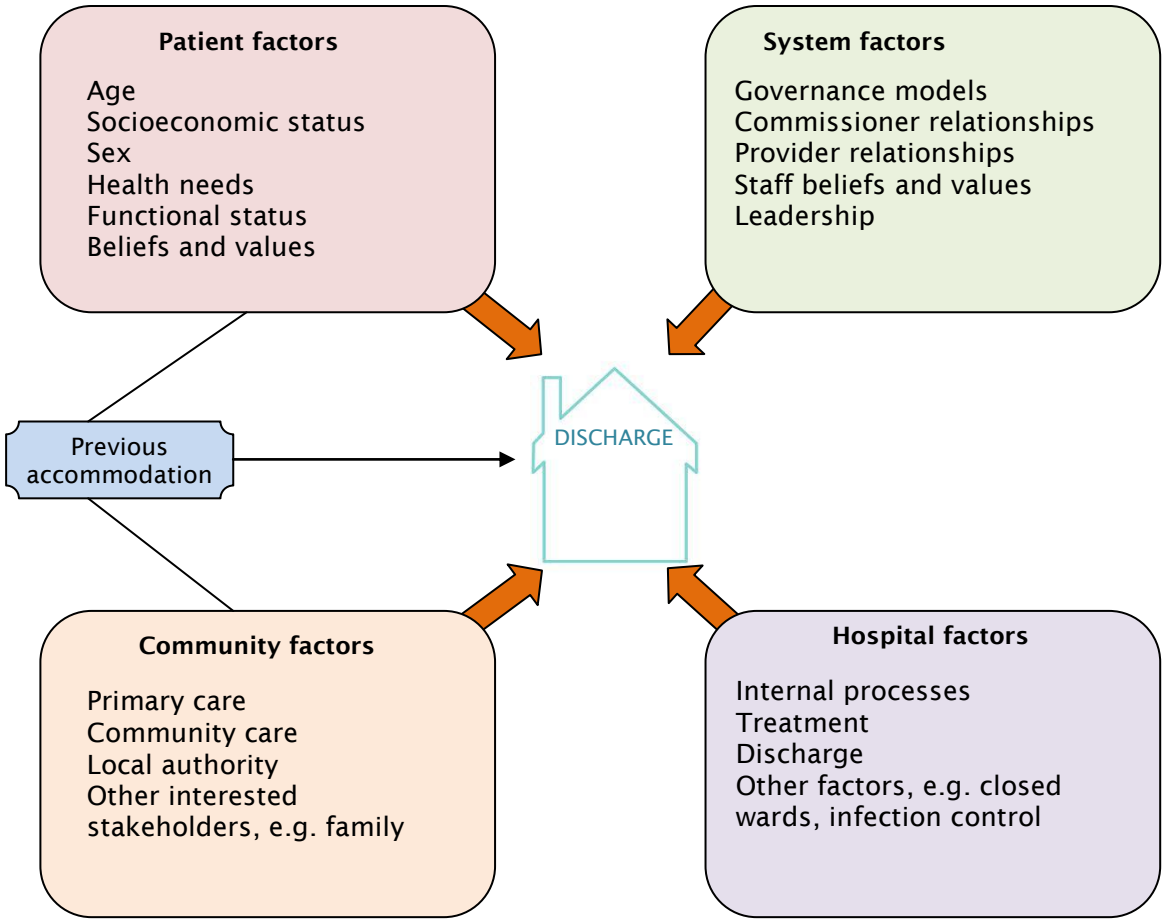
70% of CaSIO participants were successfully discharged back to their usual place of residence although often with an increased formal care package; the remainder were discharged to more dependent settings or died. No participant was discharged to a less dependent setting. These patterns of discharge destination reflect the loss of functional status that can be precipitated by a hospital admission in older people; unsurprisingly, participants with a lower Barthel index were more likely to be discharged to a more dependent setting [12].

No association was identified between CaSIO baseline characteristics and time to discharge to a more dependent setting or death. The factors affecting successful discharge are complex and heterogeneous and may be considered according to: patient factors; system factors; community factors and hospital factors (

Figure **5-4**). The CaSIO study considered many patient factors but only a few community factors and made no record of system or hospital factors despite these having an influence on lengths of hospital stays and discharge outcomes [4]. The inability to account for system and hospital factors may have contributed to differences between the results obtained by CaSIO and previously published studies.

The procedural challenges involved in setting up an appropriate package of care on discharge may also have compromised the ability to identify associations between CaSIO baseline characteristics and discharge destination. Once a decision has been reached that a patient requires a new and higher level of care then the process of making this transition (e.g. from a private house to a care home) is complicated and dictated by systems and organisations rather than intrinsic patient factors. This more than doubles length of stay and creates a paradox where the less functionally able patients who were already in highly supported accommodation may actually be discharged faster than those who are less functionally able but who already have appropriate care in place [4]. For example, a participant with cachexia and a low Barthel index may be more likely to be discharged to their usual accommodation due to the relative simplicity of restarting a pre-existing care package or returning to a care home. Conversely, a participant who is not cachexic but is on the cusp of developing functional impairment may take longer to discharge by virtue of the need for more intensive rehabilitation or starting a novel package of care.

Figure 5-4: Complex factors affecting success of discharge, adapted from the King's Fund [4]



In order to try to account for this paradox, analyses were repeated by restricting the CaSIO population to only those participants who were living independently at home prior to hospital admission; these study participants would be expected to present similar levels of procedural challenge in order to set up appropriate care on discharge in contrast with, for example, participants who were admitted to hospital from a nursing home. Associations between baseline characteristics and time to discharge were stronger when the sample was restricted in this manner, but remained non-significant at the 5% level, probably in part owing to reduced statistical power as a consequence of smaller sample size (only 84 participants) and also the remaining absence of availability of data on hospital and system factors which influence discharge.

An additional complicating factor in the investigation of associations between baseline characteristics and discharge destination in the CaSIO study was that a sub-set (*n=32*) of

participants were discharged to rehabilitation settings. The principal statistical analyses regarded discharge to this destination as a failure event (in addition to death or discharge to a more dependent setting) as these participants had a degree of functional impairment that required a longer period of time within a community hospital setting. However, this group represented two extremes of the population – those who were functionally able and required rehabilitation to achieve full independence and those who were functionally less able but received rehabilitation in an attempt to achieve, at best, a modest improvement. Sensitivity analyses were therefore conducted which in turn regarded a discharge to a rehabilitation setting as a successful discharge outcome, or which also excluded patients who were discharged to a rehabilitation setting from the analyses altogether; the pattern of no association between CaSIO baseline characteristics and discharge destination was unaltered in all of these sensitivity analyses.

Ideally, the period of time spent in a rehabilitation setting would be recorded so that it could be considered as an extension to the hospital admission in order to obtain more accurate information pertaining to length of stay prior to final discharge. Unfortunately, this information was unavailable within the CaSIO study and even if it had been the paradox of relatively fitter people having longer lengths of hospital stay as a consequence of the need to put in place an appropriate package of care would remain.

5.3.4 Cachexia and hospital related outcomes: conclusions

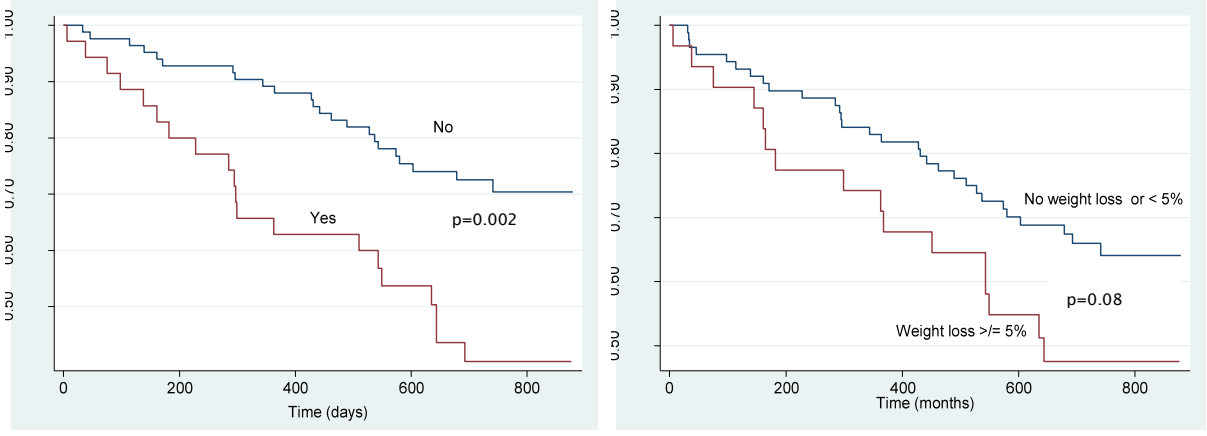
In the CaSIO study, hospitalised older women with cachexia were more likely to develop a hospital acquired complication than their counterparts without cachexia. However, cachexia was not associated with length of stay or discharge destination; this may reflect limitations in the availability of data on system and hospital factors or the fact that relatively fitter hospitalised older patients paradoxically experience a longer length of stay while waiting for an appropriate discharge package of care to be arranged. CaSIO is the first study to identify an association between cachexia among hospitalised older women and any worse short term hospital related outcome. The following section of this Discussion considers associations between cachexia and long term mortality outcome.

5.4 Associations with mortality

5.4.1 Cachexia and increased mortality

Cachexia at baseline was strongly associated with increased mortality at all stages of follow up in the CaSIO study. The magnitude of the association between cachexia at baseline and mortality was substantial with hazard ratios of 3.6 at 6 and 12 months and 2.6 at the end of the study; moreover, these associations were robust to adjustment for age, smoking habit, alcohol intake and co-morbidities, *Figure 5-5.* Cachexia has long been associated with increased mortality, indeed it was initially described as a pre-death syndrome by Hippocrates. However, its importance within older populations, especially within the context of hospitalisation has not been previously described and therefore these findings are novel. This section of the Discussion considers the reasons why hospitalised older people with cachexia are at greater risk of mortality.

Figure 5-5: Kaplan-Meier survival curves according to cachexia and weight loss; p-values from unadjusted model, y-axis, proportional survival



Cachexia Weight loss

5.4.1.1 Weight loss

Weight loss over the preceding six months is the major diagnostic criteria of cachexia; in older people this is likely to predominantly represent the loss of skeletal muscle with or without the loss of fat mass [38]. Significant weight loss in older people is common with an annual incidence of approximately 13%; sarcopenia and cachexia are two causes of weight loss in older people, other causes include anorexia, malnutrition, hypermetabolism and

dehydration [37;228]. A consequence of all of these is protein energy malnutrition which, in turn, has been associated with pressure ulcers, hip fracture, falls, sarcopenia, fatigue, anaemia, oedema, cognitive changes and derangement of the immune system. Furthermore, weight loss may be a marker of occult disease and is associated with increased drug toxicity via the loss of fat which increases concentrations of fat soluble drugs and also low albumin which increases the bioavailability of protein bound drugs [229]. It is therefore unsurprising that weight loss was associated with greater mortality within this study. This is consistent with the wider literature demonstrating that when an older person loses weight they are at a greater risk of death, even true when they were overweight at baseline; this has also previously been shown in older people following discharge from hospital settings [229-231].

Therefore, it is possible that identifying individuals with cachexia adds little extra discriminatory value to simply identifying weight loss in older people. Additional analyses were conducted to investigate this possibility using percentage weight loss in the preceding 6 months as the risk factor for mortality, *Figure 5-5* and *Table 4-34*, *page 118*. This revealed that 26% of participants experienced more than 5% weight loss over the preceding 6 months and, consistent with the literature, this group was at greater risk of mortality - especially over the longer follow up times. However, the additional criteria within the cachexia definition added meaningful discriminatory value both in terms of the degree of significance of association with mortality (p-values 0.002 and 0.08 for mortality versus cachexia and weight loss respectively, see *Figure 5-5*) and also the magnitude of association (hazard ratios 2.6 vs. 1.7 for mortality versus cachexia and weight loss respectively).

5.4.1.2 Cachexia sub-criteria

In order to better understand how each individual component of the cachexia definition augments the associations of weight loss with mortality these were considered within a mutually adjusted survival model from which a number of patterns emerged (*Table 4-35*, *page 118*). Firstly, weight loss was the biggest single predictor of mortality, especially towards the end of the study; the importance of weight loss is reflected in its role as the major diagnostic criteria for cachexia. BMI, which is used within the definition of cachexia as a surrogate marker of weight loss, appeared not to influence the likelihood of mortality which perhaps emphasises the importance of change in weight versus a low yet stable weight; this is especially relevant within frail populations such as in this study where the mean weight at baseline was 63kg. Biomarkers of inflammaging (in this case WCC) appeared to add discriminatory value for mortality throughout the study follow-up period although their effect was more pronounced in the first 6 months. The impact of low muscle strength on increased mortality was also consistently apparent throughout the study follow-up period, becoming more significant with time. Finally, the presence or absence of anorexia

and fatigue seemed to offer little additional discriminatory value in terms of prediction of mortality, perhaps because they are so closely related to inflammatory biomarkers. It may be that these sub categories of cachexia are more useful when blood biomarkers of inflammation are unavailable.

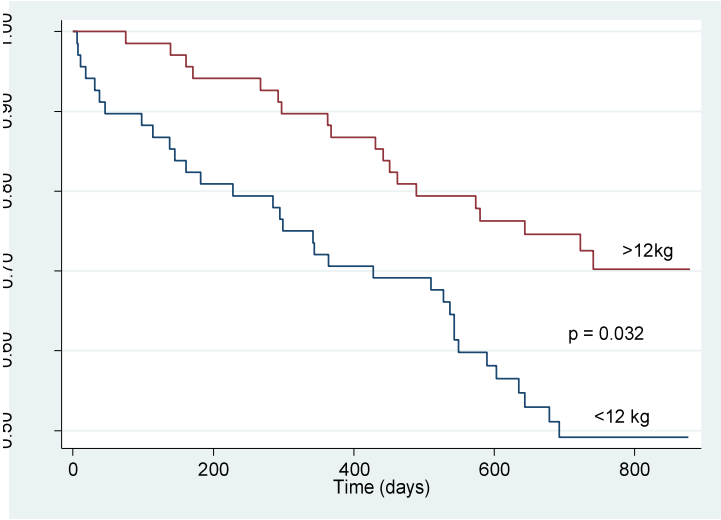
In summary, these observations suggest that the consensus definition for cachexia identifies older women at increased risk of mortality. It is likely that this arises via a combination of skeletal muscle loss and inflammation which is discussed in greater detail in the subsequent sections.

5.4.2 Loss of skeletal muscle contributing to greater mortality in cachexia

The CaSIO study has shown that the loss of skeletal muscle in hospitalised older women was associated with mortality at 6 and 12 months and at the end of the study. It is plausible, as one of the principal components of cachexia in older people, that loss of skeletal muscle therefore partially explains the relationship between cachexia and increased mortality.

However, associations between sarcopenia and mortality were not significant in the CaSIO study which may be a consequence of loss of power owing to use of a binary sarcopenia variable rather than a continuously distributed variable. Associations with grip strength were more convincing; a 1kg reduction in grip strength was associated with a 5% increase in risk of mortality. This is a significant magnitude of effect within a population whose grip strength ranges from 1-28kg and is illustrated in the survival curve in *Figure 5-6* which clearly shows a survival benefit from having a maximum grip strength at baseline which is greater than 12kg.

Figure 5-6: Kaplan-Meier survival curve according to grip strength at baseline; p-value from unadjusted model, y-axis, proportional survival



CaSIO is not the first study to demonstrate associations between loss of skeletal muscle and mortality. Gale et al used the 1973-4 UK Department of Health and Social Security survey of community dwelling people over 65 years to demonstrate that lower grip strength was associated with increased mortality from all causes over a twenty-four year follow up period [21]. Similar associations and effect sizes have been reported in other studies and also summarised in a recent meta-analysis; very recently, associations between sarcopenia and mortality have been demonstrated using the EWGSOP definition [232;233] and also following acute hospital admission over six months although this was in a younger population with a mean age of 77 years [234]. CaSIO is the first study to look at associations between sarcopenia and longer term mortality in hospitalised older people.

Several explanations may be proposed for the association between loss of skeletal muscle and mortality, including confounding. Indeed, adjustment for age did attenuate the statistical significance of the association between skeletal muscle and mortality in CaSIO which is unsurprising given that loss of muscle strength is an age related process. However, the magnitude of the hazard ratios was little altered by adjustment for age. Moreover, gender could not have confounded the association between loss of skeletal muscle and mortality as only women were included in CaSIO and associations were robust to adjustment for height. Besides these potential confounders, no standard set of additional adjustment factors is recommended in the literature [233].

It is likely that associations between loss of skeletal muscle and mortality are multi-factorial, acting via a number of different mechanisms. Firstly, degrees of muscle loss represent general health status and disease burden; associations with age-related diseases are well established [235]. Secondly, associations with mortality may act via more direct, muscle related, routes including worse physical functioning, increased dependency, frailty and increased risk of falls [70;71;74;75;236]. Finally, accelerated loss of skeletal muscle is likely to reflect intrinsic ageing processes and progressive homeostatic dysregulation across multiple systems including the immune, endocrine and neural axes [44].

5.4.3 Inflammation contributing to greater mortality in cachexia

The associations between cachexia and mortality may also be mediated, in part, via inflammation; this is explored in greater detail within the following section.

5.4.3.1 Overall patterns

The CaSIO study has shown that a greater degree of inflammation predicts long-term mortality in hospitalised older patients. Raised WCC, CRP, IL-8 and cortisol plus low albumin predicted mortality in a consistent manner and with p-values that remained significant after adjustment for confounders including age and co-morbidity. Associations were also found with IL-6 and IL-10; these were in the expected direction but were not significant at the 5% level. Furthermore, this study has demonstrated associations between subtle pro- and anti-inflammatory balances and mortality as evidenced by ratios of IL-10 with IL-6 and IL-8. No associations were found with DHEAS. For ease of reference, these findings are summarised in *Table 5-1*.

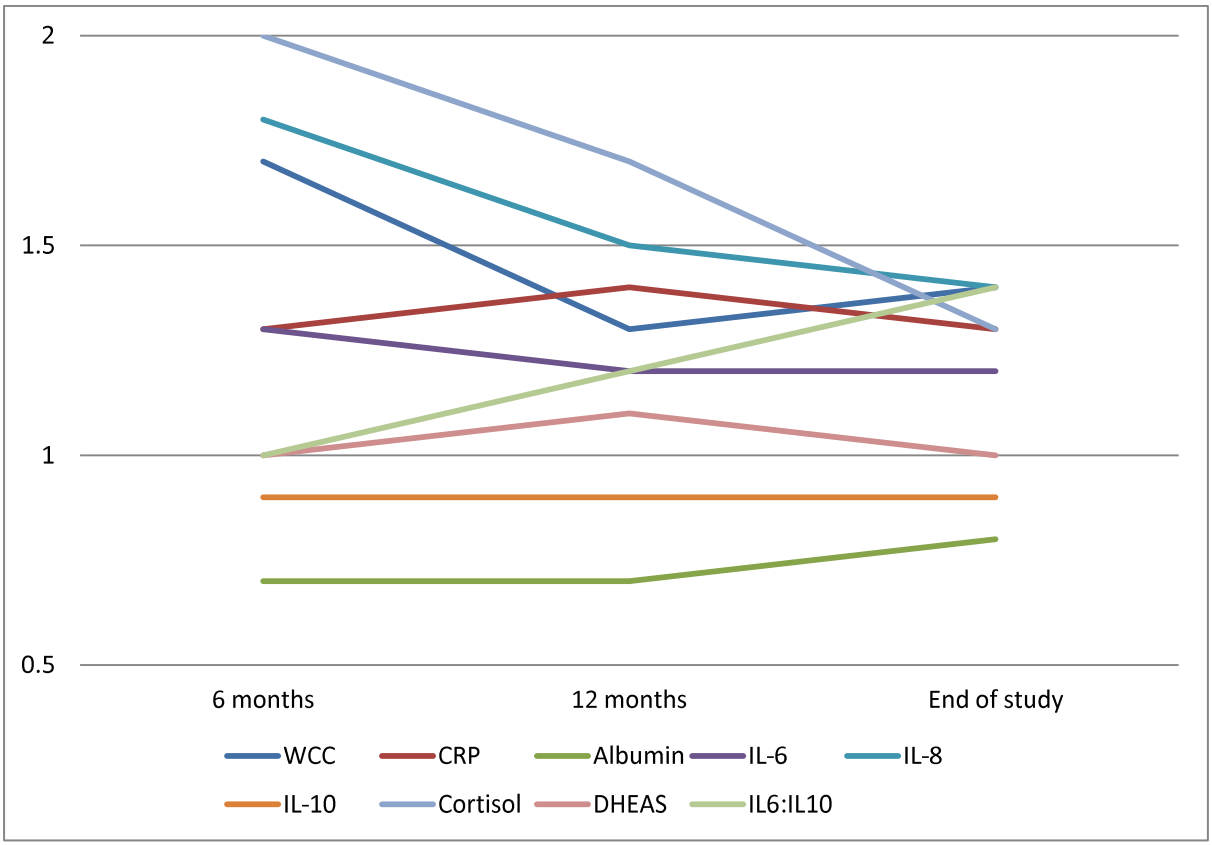
Table 5-1: Hazard ratios (HR) and p-values for associations with mortality at different time points according to immune-endocrine markers (per SDS change)

Mortality risk	6 months		12 months		End of study	
	HR	p-value	HR	p-value	HR	p-value
Admission						
WCC	1.7	*0.03*	1.2	*0.40*	1.4	*0.02*
Neutrophils	1.4	*0.13*	1.1	*0.51*	1.3	*0.03*
CRP	0.9	*0.60*	1.0	*0.96*	1.2	*0.23*
Albumin	1.0	*0.99*	0.8	*0.16*	0.7	*0.01*
Discharge						
WCC	1.3	*0.20*	1.1	*0.73*	1.3	*0.09*
Neutrophils	1.2	*0.39*	1.1	*0.64*	1.3	*0.07*
CRP	1.3	*0.30*	1.4	*0.08*	1.3	*0.05*
Albumin	0.7	*0.12*	0.7	*0.02*	0.8	*0.01*
IL-6	1.3	*0.29*	1.2	*0.36*	1.2	*0.19*
IL-8	1.8	*0.05*	1.5	*0.06*	1.4	*0.04*
IL-10	0.9	*0.77*	0.9	*0.52*	0.9	*0.40*
Cortisol	2.0	*0.01*	1.7	*0.01*	1.3	*0.10*
DHEAS	1.0	*0.99*	1.1	*0.82*	1.0	*0.90*
Cortisol:DHEAS	1.1	*0.70*	1.2	*0.45*	1.1	*0.62*
IL6:IL10	1.0	*0.99*	1.2	*0.50*	1.4	*0.05*
IL8:IL10	1.1	*0.75*	1.2	*0.34*	1.3	*0.20*

HR = Hazard ratio; biomarkers per SDS; p-values from Cox's proportional hazards model, unadjusted models presented. IL-1 and TNF removed from the results due to low numbers

Figure 5-7 is presented to demonstrate trends only; it indicates the magnitude and direction of association between selected immune-endocrine biomarkers and mortality at different follow-up times in the CaSIO study. The figure shows that all the pro-inflammatory biomarkers had hazard ratios for mortality which were consistently greater than 1.0 and IL-10 (an anti-inflammatory biomarker) and albumin (a negative acute phase protein) had hazard ratios for mortality which were consistently less than 1.0. The balance between pro- and anti- inflammatory cytokines is demonstrated more specifically by the hazard ratios for mortality versus IL6:IL10 ratio; hazard ratios for mortality per SDS increase in this ratio increased with greater follow-up time. DHEAS is the only biomarker that was not associated with mortality as evidenced by hazard ratios that were consistently 1.0. Finally, it appears that the association between mortality and inflammaging, especially cortisol, is greatest in the 6 months following admission and then wanes with time to the end of the study when hazard ratios were lowest. Taken together, these observations add weight to the argument that inflammation is associated with mortality in hospitalised older people and that inflammation may partially explain associations between cachexia and mortality. A discussion of the association between inflammation and mortality in CaSIO is presented in the following sections of this Discussion.

Figure 5-7: Associations of immune endocrine axis with mortality demonstrated as hazard ratios per SDS change of biomarker (y-axis); plotted against follow up point (x-axis)

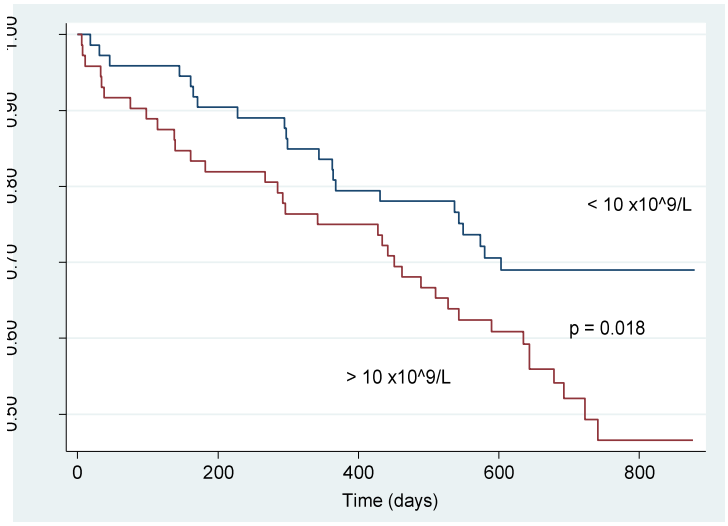


5.4.3.2 Cellular markers of inflammaging and mortality

Neutrophils are the largest constituent of white blood cells and therefore it is unsurprising that associations between neutrophil count and mortality are similar, but somewhat less marked, than those between white cell count (WCC) and mortality; this has been reported previously [237]. Therefore, this discussion will focus on consideration of the associations between WCC and mortality which are illustrated in *Figure 5-8.*

Raised WCC occurs as a consequence of infection or inflammation and also due to trauma, exercise, steroids and malignancy. In CaSIO, the median WCC was 9.8 $x10^9$/L at baseline and fell to 7.7 $x10^9$/L prior to discharge; both these are within the normal reference range of less than 11 $x10^9$/L. Associations between mortality and WCC were identified for WCC at both baseline and discharge and were robust to adjustment for age and co-morbidity. However, the associations between mortality and WCC were strongest for WCC on admission rather than discharge; this pattern was evident for mortality at all follow-up points to the end of the study and is therefore unlikely to be due to severe acute illness at baseline confounding the association between WCC and mortality. It is possible that this pattern of association is explained by residual inflammation from the acute infection causing a greater amplification of WCC range and thereby revealing associations. Alternatively, individuals with a greater long-term mortality risk may be more susceptible to higher and more deregulated inflammatory responses which take a longer time to settle; both explanations are likely to be implicated.

Figure 5-8: Kaplan-Meier survival curve according to white-cell count at baseline; p-value from unadjusted model, y-axis, proportional survival



WCC has previously been demonstrated to predict the incidence of a number of morbidities including coronary heart disease, cerebrovascular disease, hypertension, diabetes and malignancy; these were the subject of a recent review article [238]. There is also considerable evidence that raised WCC is associated with greater all cause mortality in a variety of different populations including patients with acute coronary syndrome, following acute stroke and amongst healthy community dwelling older people where it also predicts hospitalisation [237;239-242]. For example, in the Normative Aging Study, the 2000 healthy men (average age 47 years) who were followed up for 13 years were 1.8 times more likely to be dead by the end of the study if their baseline WCC was greater than 9 $x10^9$/L compared with individuals with a WCC lower than this threshold [243]. WCC has never been reported in relation to longer term mortality in older people discharged from acute hospital settings; the results from the CaSIO study are therefore novel.

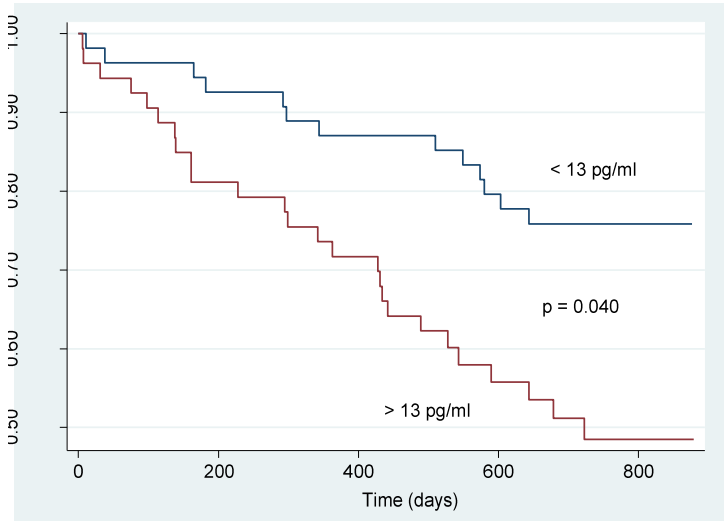
Biological mechanisms underpinning the association between WCC and mortality are likely to be bi-directional and complex, involving white blood cells mediating inflammatory processes via the production of pro-inflammatory cytokines and oxygen metabolites and also co-morbidity driving raised WCC. This contributes to a general pro-inflammatory milieu and the acceleration of ageing processes towards mortality as discussed later in *section 5.4.3.5.*

5.4.3.3 Molecular markers of inflammaging and mortality: cytokines

The CaSIO study has demonstrated that the pro-inflammatory cytokine IL-8 was significantly associated with mortality across the duration of the study and was robust after adjustment for age and co-morbidity (*Figure 5-9*). Similar associations were seen with IL-6 and, in the opposite direction, weakly with the anti-inflammatory cytokine IL-10.

Unfortunately, concentrations of IL-1 and TNF were below the detectable range of the assay used and meaningful results were only available in 32 and 28 participants respectively; these sample sizes were too small for analysis of these biomarkers in relation to mortality. This problem could possibly have been overcome by repeating the analyses using lower volumes of diluting or plasma samples. Unfortunately this was not possible in CaSIO due to the lack of sample availability.

Figure 5-9: Kaplan-Meier survival curve according to IL-8 at discharge; p-value from unadjusted model, y-axis, proportional survival



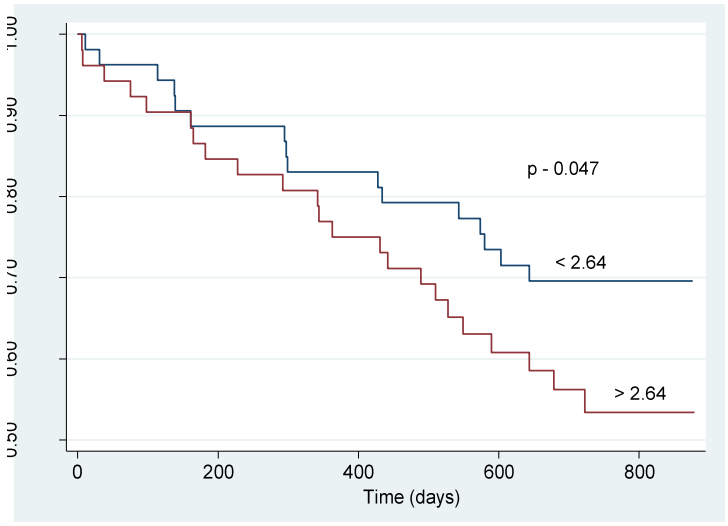
IL-8 is secreted from a variety of cells in response to inflammatory stimuli including TNF, IL-1 and oxidative stress. Its principal role is as a chemo-attractant and also in the propagation of inflammation via its long half-life triggering additional oxidative stress; it is therefore implicated in the aetiology of inflammaging. Raised levels have been seen in association with human immunodeficiency syndrome, a disease which shares a number of immune characteristics with immunoscenescence [244]. For these reasons IL-8 may be a more consistent marker of inflammaging despite being relatively understudied in comparison with IL-1 and IL-6.

Within the Memory and Morbidity in Augsburg Elderly (MEMO) Study, cross-sectional associations were demonstrated between higher IL-8 and lower cognitive function and the cytokine has also been implicated in the development of cachexia in patients with stomach cancer [160;161]. Few other studies have considered IL-8; the associations identified between IL-8 and mortality in CaSIO are novel and warrant further exploration and replication.

Associations between IL-6 and mortality in CaSIO are consistent with previously reported studies of community dwelling older people in which IL-6 was associated with all-cause, cardiovascular disease, cancer and liver-related mortality; no previous studies have investigated the association between IL-6 and mortality in hospitalised older people [245-249].

IL-10 is an anti-inflammatory cytokine that is responsible for 'putting the brakes' on inflammatory processes. It was included in CaSIO as a measure of resilience to inflammaging; no significant associations were identified between IL-10 and mortality at the 5% significance level but the possibility of a biological association was suggested via the consistent association between low IL-10 and higher mortality throughout the CaSIO follow-up period (*Figure 5-7*). To explore anti- and pro-inflammatory balance in more depth, this thesis also considered IL-10 as a ratio against its pro-inflammatory counterparts, IL-6 and IL-8. This approach indicated that a greater shift towards a pro-inflammatory milieu was associated with a greater likelihood of mortality, although this was only significant with regard to mortality at the end of the study (*Figure 5-10*). These findings are broadly consistent with previous studies which have shown that polymorphisms leading to an over expression of the IL-10 gene are found in greater numbers among centenarians (a population who are deemed to have aged successfully) and are less prevalent among people with cardiovascular disease [106]. This reinforces the importance of resilience to inflammaging and is discussed later within this section.

Figure 5-10: Kaplan-Meier survival curve according to IL6:IL10 ratio at discharge; p-value from unadjusted model, y-axis, proportional survival



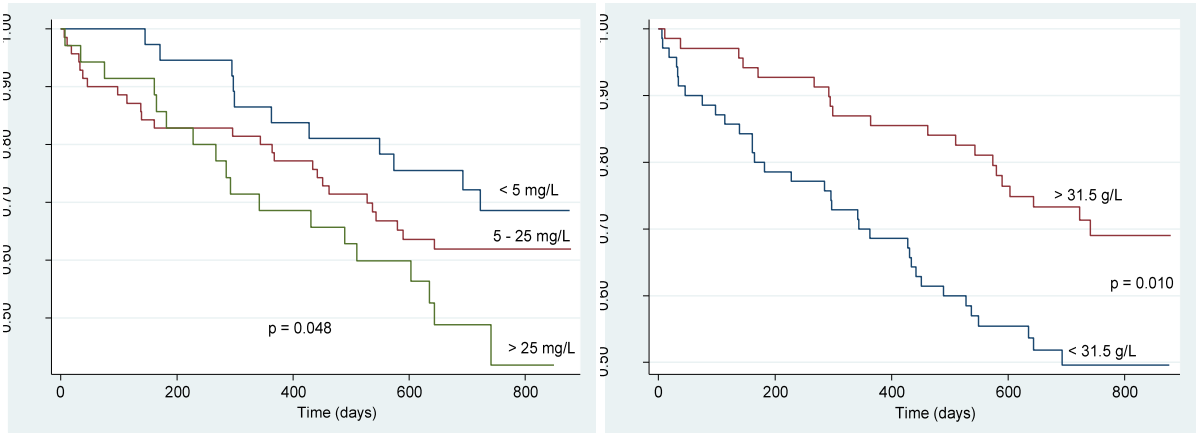
The observed differences in the patterns of association between mortality and IL-8 and IL-6 may be a consequence of their different kinetics and therefore likelihood of detection. IL-8 is relatively long lived and is resistant to temperature and enzyme degradation resulting in a considerably prolonged half life; also mRNA concentrations remain high following a single stimulation and are especially stable. These properties are in contrast to IL-6 which is relatively short lived (half life 2-4 hours) [250;251]. Evidence of this prolonged half life can be

seen in a number of disease states where high concentrations of IL-8 have been reported in association with malaria, pneumonia and chronic diseases of the respiratory, gastrointestinal and musculoskeletal systems [252-255].

5.4.3.4 Acute phase protein markers of inflammaging and mortality: C-reactive protein and albumin

Raised CRP was associated with an increased likelihood of mortality during CaSIO follow-up although associations were attenuated by adjustment for age. Low albumin was also associated with increased mortality and these associations were robust to adjustment for age and co-morbidity (*Figure 5-11*).

Figure 5-11: Kaplan-Meier survival curve according to CRP and Albumin at discharge; p-value from unadjusted model, y-axis, proportional survival



CRP Albumin

These findings are consistent with previous studies which have linked CRP with atherosclerosis and an increased likelihood of myocardial infarction, stroke and progression of peripheral artery disease; a recent meta-analysis estimated a relative risk of 2.0 for future coronary events for a baseline CRP level in the highest tertile compared with the lowest [166]. Raised levels of CRP have also been associated with progression of other chronic diseases including respiratory, renal, neurological, hepatic, gastrointestinal, rheumatic and cancer. Furthermore, CRP has been shown to predict mortality in community dwelling older people who have been followed up, in separate studies, over 4.6, 7 and 10 years respectively [245;248;256].

Low albumin may reflect a reduced rate of production as a consequence of malnutrition, liver failure or inflammation and increased loss via, for example, the renal system or burns. Albumin has a number of functions including the maintenance of oncotic pressures within

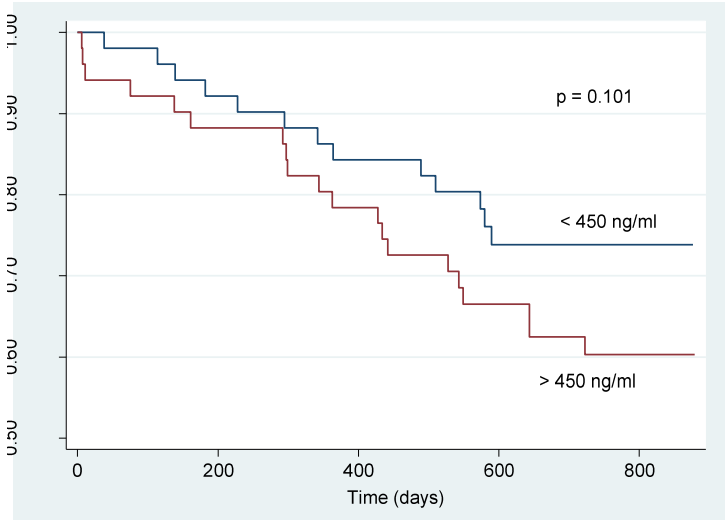
the vascular system and low levels allow fluid to leak from the interstitial spaces producing peripheral oedema and ascites. Albumin is also very important in the transportation of molecules including bilirubin, free fatty acids, drugs, and hormones. Associations between albumin and mortality have previously been demonstrated in chronic diseases such as cardiovascular disease, renal disease and cancer. Serum albumin on admission to hospital has also been shown to predict in-patient mortality and also predicted mortality among community dwelling older people and among residents of care homes [257-259].

Serum albumin has previously been shown to be associated with 6 month mortality among hospitalised older people [260] but neither CRP nor albumin has previously been used as a predictor of longer term mortality in older patients following an acute hospital admission; these findings from the CaSIO study are therefore novel.

5.4.3.4 HPA axis markers of inflammaging and mortality: Cortisol and DHEAS

Higher cortisol levels at discharge were significantly associated with increased risk of mortality during the CaSIO follow up period. DHEAS at discharge was not associated with mortality.

Figure 5-12: Kaplan-Meier survival curve according to cortisol at discharge; p-value from unadjusted model, y-axis, proportional survival



These results are consistent with previous studies demonstrating associations between higher cortisol and increased mortality in patients with stroke, sepsis and heart failure, although this has not been a consistent finding in all previous studies [141-143;261]. Cortisol is also associated with degree of severity, ongoing persistent clinical instability and mortality

in patients with community acquired pneumonia [262]. Higher baseline serum cortisol concentrations in community dwelling men who participated in the Vietnam Experience Study were associated with increased all-cause mortality over 15 years of follow-up [119]. Cortisol has never previously been shown to predict all-cause mortality among older people or within the context of discharge from an acute hospital setting; these are novel findings from the CaSIO study.

The relationship between cortisol and mortality is multi-faceted and complex and the influence of the acute hospital admission appears to be more pronounced than with the other biomarkers. Therefore, raised serum cortisol within this study is likely to be a consequence of the stresses associated with acute illness and hospital admission, which wane with time, plus anti-inflammaging responses which are constant or increase. The consequence of raised cortisol is immunosuppression and therefore increased vulnerability to disease. It also causes a greater catabolic state which impacts on all systems, including skeletal muscle, resulting in faster multi-system deterioration [125].

DHEAS may act as a protective mechanism against the negative effects of cortisol and it is therefore interesting that no associations with mortality were found with either DHEAS or its ratio with cortisol. Both of these have previously been shown to change and to be associated with poor clinical outcomes within hospital settings [121]. It is possible that an absence of association between mortality and DHEAS in CaSIO may be explained by error (due to bias inherent in the study design, measurement or assay variation), or that this pattern of association is due to chance; alternatively, it may truly be the case that no association exists between DHEAS and mortality among the CaSIO population. The median DHEAS concentration in CaSIO was 0.39μg/mL and the distribution of values was strongly positively skewed; this median level of DHEAS is low in comparison with a healthy population. In contrast the median cortisol concentration among CaSIO participants was 449ng/mL which is high in comparison to the general population. It is therefore possible that the concentrations of DHEAS within hospitalised older adults are too low to offer significant protection from the deleterious effects of cortisol. This is likely to be a consequence of reduced baseline levels of DHEAS that occur with age plus a stress induced intra-adrenal shift from adrenal androgen toward glucocorticoid biosynthesis associated with hospital admission [263]. An alternative possibility is that stress produces a shift from DHEAS to its dissociated biologically active form DHEA which is where the protective effects may have been seen, this has previously been reported but was not measured within this study; exploration of the role of DHEA and its ratio with cortisol among hospitalised older adults is therefore a natural area for future research [121].

5.4.3.5 Inflammaging and mortality: conclusions

Taken together, the results from the CaSIO study suggest that inflammaging is associated with mortality amongst older women following hospital admission; anti-inflammatory cytokines/resilience to inflammaging and anti-inflammaging may also be important. This reinforces established and emerging theories that identify inflammation as a central aetiological driver behind pathological age related processes and may also partially account for increased mortality among hospitalised older people with cachexia [84;90;264].

Participants in CaSIO had a greater degree of inflammation than a baseline reference population; this was evident within the cytokine analyses plus cellular markers of the immune system and also the acute-phase proteins which were all elevated. These levels are likely to reflect the extremes of inflammaging among a very old population with significant degrees of accumulative antigen exposure. It is also likely that the acute illness which precipitated hospital admission (plus any hospital acquired infections) had contributed to an inflammatory milieu; it is not possible to ascertain to what degree. However, at the point of immune-endocrine analysis, participants had a median CRP of 12 mg/L and WCC of 7.8 $x10^9$/L – levels most clinicians would consider as returning to baseline values within this population. Furthermore, the hospital admission could be considered as part of the continuum of antigenic exposures that has contributed to extremes of inflammaging within the study participants.

Inflammaging is manifest through raised pro-inflammatory cytokines in association with an increase in cellular markers of inflammation such as WCC and the acute phase proteins. These more generic markers of inflammation may be more clinically translatable due to their common usage and as a reflection of general inflammatory milieu rather than specific inflammatory proteins; this perhaps explains the strong associations identified between mortality and WCC and albumin in CaSIO [10]. According to the inflammaging hypothesis [88], resilience to inflammaging via, for example, a greater production of anti-inflammatory cytokines is an important mechanism to counter its negative effects. Early supporting evidence for this hypothesis is provided by the protective effects of raised IL-10 and its ratio with IL-6 and IL-8 on mortality in the CaSIO study.

There are a number of mechanisms that may explain the associations between inflammaging and mortality. These include confounding via, for example: age, gender, medication use and co-morbidity. This study was set within an all female population and further work is needed to replicate these findings within a male population. Age is directly related to accumulative antigen exposure and therefore is an essential component of inflammaging; adjustments for age would be expected to attenuate associations. However,

in the CaSIO study, many of the associations were robust to adjustment for age which suggests aetiological importance of other factors including genetically determined resilience and environmentally determined degrees of antigenic exposure across the lifecourse.

Only 14% of participants were taking medications during the hospital admission (steroids, non-steroidal anti-inflammatory drugs or disease modifying drugs) which are known to significantly affect the immune-endocrine axis. Associations were not altered substantially if these participants were excluded from analyses which suggest that they were not unduly influencing the results. Furthermore, associations were robust to adjustment for the number of medications being taken.

The role of co-morbidity in this study is complex and bi-directional. Inflammaging contributes directly to the progression of age-related disease and co-morbidity directly contributes to inflammaging via direct (cytokine secretion) and indirect (reactive species production) mechanisms - both processes contribute simultaneously and synergistically to mortality. Within this study, associations between inflammation and mortality remained significant following adjustment for the number of co-morbidities in the past medical history and were attenuated but remained significant after adjustment for Barthel index and grip strength, characteristics strongly associated with co-morbidity, functional dependence and frailty; no adjustment was made for specific co-morbidities. These findings suggest that co-morbidity may contribute to, but do not fully explain, the associations between inflammaging and mortality in the CaSIO study.

It is likely that the mechanisms underlying the association between inflammaging and mortality in the CaSIO study population are multi-faceted in nature. Firstly, inflammaging is a consequence of co-morbidity and acute illness which leads directly to mortality. Secondly, raised inflammatory activity contributes to the oxidative stress hypothesis of ageing, an evolution of the free radical theory of ageing [264]. According to this hypothesis, oxidative damage occurs to all tissues as a consequence of reactive oxygen species and other anti-oxidants including reactive nitrogen and lipid species; these cause direct tissue damage and, as discussed in the introduction, are also a major causative factor in the re-activation of immune systems and a driver of inflammaging [265]. Thirdly, pro-inflammatory cytokines cause an acceleration of age related disease. Examples include a faster progression and greater instability of atherosclerotic plaques via increased expression of endothelial adhesion molecules, faster progression of sarcopenia via the activation of catabolic signalling pathways and the direct stimulation of osteoclasts leading to more bone resorption and osteoporosis [90;140]. Finally, as discussed earlier, inflammatory activity leads to activation of the HPA axis and raised cortisol (anti-inflammaging) causing immunosuppression and a further shift towards catabolism.

It is likely that within this heterogeneous population, individuals have a range of pro-inflammatory profiles which result from immunosenescence, individual resilience and the accumulative burdens of acute and chronic disease and antigen exposure across the lifecourse. At the point of discharge from an acute hospital admission, these inflammatory profiles and the degree to which they return to a new baseline directly impact on subsequent longevity over two years via a variety of mechanisms. A proportion of hospitalised older people may also have an inflammatory profile at the more pro-inflammatory end of the spectrum, at a level typically seen among younger people in association with a single illness. This may be contributing to the cachexia syndrome in hospitalised older women and accounting for part of its association with mortality.

5.4.4 Cachexia, skeletal muscle loss and inflammation – exploring relative contributions to mortality

This thesis hypothesised that cachexia would be associated with poor outcomes in hospitalised older women as a consequence of the combined burdens of skeletal muscle loss and inflammation. The results from the CaSIO study broadly support this hypothesis.

A final set of mutually adjusted survival analysis models were used to try to identify the relative contributions of skeletal muscle loss (grip strength), inflammation (white cell count) and cachexia on mortality at 6 and 12 month follow-up and at the end of the study, without and with adjustment for age and Barthel index (as a marker of general functional status) (*Table 4-36*, *page 119*). These analyses confirmed the importance of cachexia as a risk factor for mortality at 12 month follow-up and at the end of the CaSIO study follow-up period. Inflammation at baseline was associated with 6 month mortality but not at 12 months or at the end of the study (as shown in Figure 5-8). Grip strength was not associated with mortality over and above its contribution to cachexia.

In summary, results from the CaSIO study suggest that inflammation and loss of skeletal muscle contribute to the syndrome of cachexia which is the single biggest risk factor for mortality among older women following discharge from hospital.

5.5 Clinical translation

5.5.1 Cachexia in older people, does it really matter?

This thesis aimed to characterise the syndrome of cachexia in hospitalised older women in terms of its prevalence, aetiology and clinical relevance. The results suggest that cachexia is common in hospitalised older women, a consequence of inflammation and skeletal muscle loss; it is straightforward to diagnose using the consensus definition and identifies a cohort of older people, within an already frail population, who are likely to experience especially poor outcomes. These findings become useful within the clinical setting if older people can recover from cachexia and if interventions exist to treat cachexia. These questions are explored within the following sections.

5.5.2 Can we treat cachexia in older people?

5.5.2.1 Does the CaSIO study suggest that cachexia can be treated?

21% of the participants seen at both baseline and follow up had cachexia at baseline and, of these; the majority (71%) also had cachexia at follow up. However, a minority (29%) had transitioned back into the 'no cachexia' category at follow up. This suggests that it may be possible to treat cachexia in older people with the aim of increasing the likelihood of this transition happening; there are a number of possible explanations for the apparent recoveries from cachexia which were observed within this study:

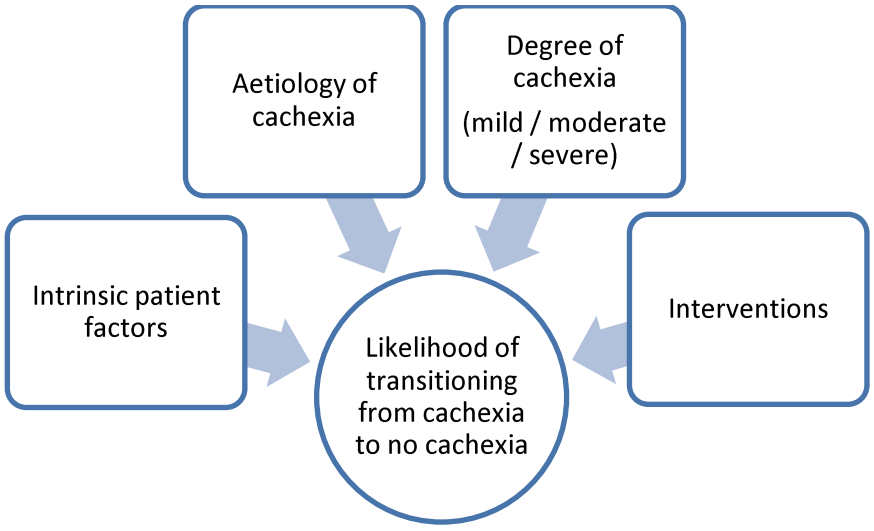
1. Participants were unwell leading up to admission and treating the underlying illness attenuated the inflammation which precipitated a return to a more anabolic state post discharge and a stabilisation of weight.
2. Participants received multiple interventions during the hospital admission including medical, pharmacological, psychosocial, nutritional and therapy which acted to successfully treat the pathophysiology of cachexia.
3. A change in discharge environment, for example an increase in the provision of support, acted as a psychosocial/nutritional intervention.
4. Transitions from cachexia to no cachexia occur independently to hospital admission or planned interventions.
5. These findings could be a result of study error due to the incorrect identification of cachexia at baseline or follow up. This explanation seems less likely for the reasons identified in the strengths and limitations section and also given the associations that

have been described in this thesis between cachexia and other participant characteristics and outcomes.

6. A combination of all of the above.

It is plausible that recovery from cachexia within the CaSIO population arose for some or all of the above reasons plus additional factors as described in *Figure 5-13*. Those intrinsic to the participant include age, sex and chronic disease burden and have less potential to be influenced regardless of intervention. The aetiology of cachexia is also likely to influence the chance of recovery; this is often multifactorial in older people, however, when cachexia is due to a few single conditions (e.g. pneumonia) it is likely to be more amenable to treatment and the individual has a higher chance of recovering from the associated cachexia. Finally, cachexia may be sub classified into mild/moderate/severe according to the degree of weight loss; younger individuals with mild cachexia are more likely to recover when compared to severe cachexia and this is probably also true in older people [27]. It is therefore possible that within this study the sub-group of participants who had not recovered by follow up had moderate or severe cachexia at baseline and that interventions in these people are less likely to be successful.

Figure 5-13: Factors contributing to the likelihood of recovering from cachexia



Greater participant numbers and characterisation of cachexia at 12 months would have enabled a more detailed exploration of this hypothesis including a greater understanding of the natural trajectory of cachexia in older people and would also have enabled a comparison of the recovery group versus the non-recovery group.

However, despite these limitations, the findings within this section remain interesting. Certain factors (e.g. age, sex) cannot be changed and others, (e.g. chronic disease) may only

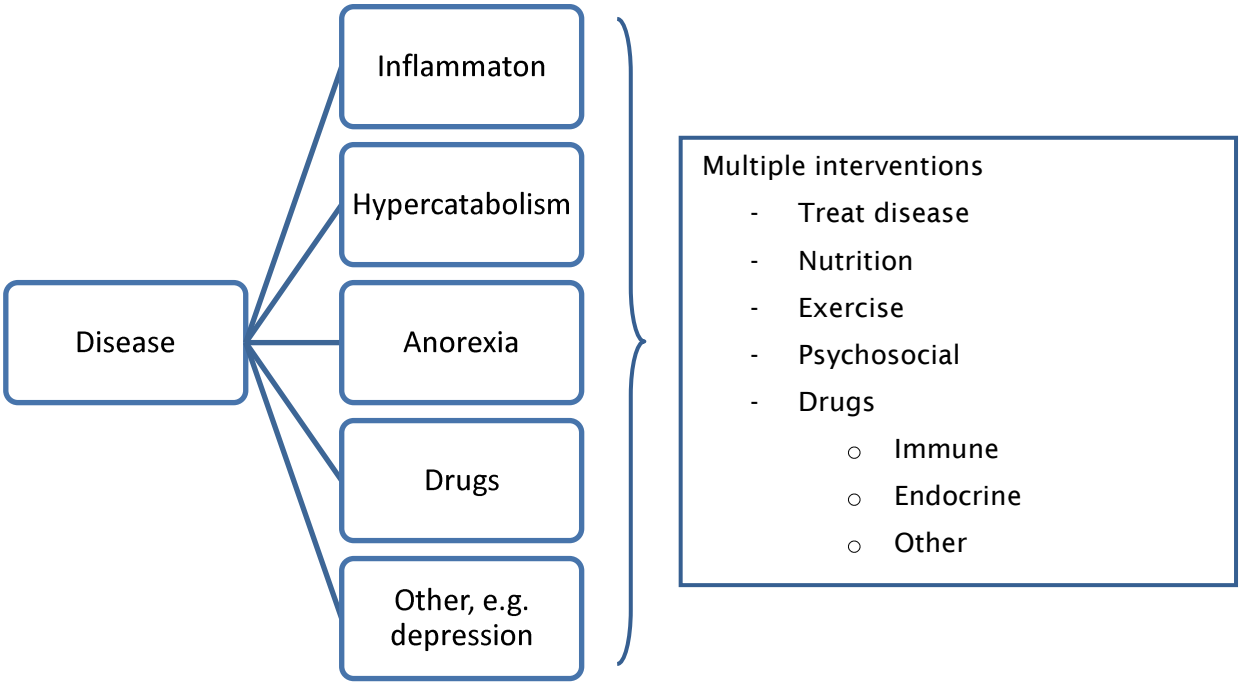
be minimally amenable to interventions. Where possible, treatment targeted directly at the aetiology of cachexia (e.g. pneumonia) may, at least in part, be identified and treated as per established clinical practice. It is likely that it is important to identify cachexia early in its trajectory when it is at the developing or mild stage and also that there is value in identifying pre-cachexia; this is consistent with cachexia that occurs in younger people and suggests there might be a role for increased awareness, screening and diagnosis amongst older people [25-27]. Interventions targeted directly at cachexia are the final step; it may be possible to exert influence over the likelihood of an older person with cachexia transitioning back to no cachexia. Further consideration of possible interventions for cachexia in older people is discussed in greater detail within the following sections.

5.5.2.2 How does cachexia in older people compare to cachexia in other diseases?

Unfortunately there is currently a very limited evidence base regarding interventions for cachexia in older people. Therefore when considering possible strategies a logical approach is to look at interventions for cachexia in younger people and also the evidence for treating the individual components of cachexia in older people i.e. weight loss, muscle loss and inflammation.

Cachexia in younger people is usually caused by a single disease which is more easily identifiable and may be treatable. Conversely, cachexia in older people seems to more generally occur as a consequence of a multifactorial aetiology which has accumulated across the lifecourse [22;23;266]; within this study cachexia was not associated with a single acute illness, co-morbidity, or co-morbidity burden. However, regardless of this difference, cachexia in younger and older people is manifest via a common pathogenesis (*Figure 5-14*). For example, the pathogenesis of COPD related cachexia in younger people is similar to cachexia in older people – an increased pro-inflammatory environment and hypercatabolism combined with anorexia, drugs (e.g. antimuscarinic bronchodilators – gastrointestinal side effects) and the psychosocial aspects of the disease which include depression and isolation [23]. Therefore, due to a common pathogenesis, it is plausible that approaches identified as successful in single disease cachexia in younger people may also be successful in cachexia in older people; this is discussed within the next section.

Figure 5-14: Pathogenesis and treatment of cachexia



5.5.2.3 Treatment of cachexia in younger people; what works best?

Cachexia in association with single diseases has been studied and interventions have been trialled with varying degrees of success. For example, the pathogenesis of cachexia in patients on dialysis for chronic kidney disease is thought to be a primary consequence of hypercatabolism and anorexia; treatment benefits have been demonstrated using a combination of dialysis, anti-inflammatory therapy (cyclooxygenase [COX]-inhibitor), erythropoietin and nutritional support – this approach increases body weight and improves survival [23;267;268]. However, evidence is conflicting and interventions are not always so successful. Studies looking at nutritional supplementation in patients with cachexia that occurs in association with COPD demonstrated no benefit and neither did testosterone replacement. Pharmacological approaches seem more successful within this group - treatment with megestrol acetate confers some advantage in terms of appetite, exercise tolerance and weight gain [269-271]. In patients with AIDS related cachexia resistance exercise, anabolic steroids and growth hormone have all been shown to increase muscle mass and strength [272;273] and megertrol acetate improves appetite, weight and quality of life; these interventions are currently approved and being used within the clinical environment [274;275].

In congestive cardiac failure, ACE inhibitors are well studied, widely available, and improve symptoms, reduce morbidity and increase survival. In the SOLVD trial ACE-inhibitors prevented weight loss in association with congestive cardiac failure and this is unlikely to be

explained solely by their haemodynamic mode of action [276]. Explanations include immune modulation via the reduction of circulating levels of atrial and brain natriuretic peptides, TNF and IL—6 and also the enhancement of endothelial function which may have a role in modifying skeletal muscle function [276]. Other cardiac medications such as beta blockers may also prevent the development of cardiac cachexia, in this case via the reduction of basal metabolic rate [277]. In a study of the treatment of cardiac cachexia with growth hormone, improvements in muscle mass were seen however numbers were small and controlled studies are lacking. More targeted immune manipulation with TNF inhibitors (Infliximab) or intravenous immunoglobulin in cardiac cachexia have also been trialled but have either shown no clinical benefit or resulted in hospitalisation; the use of anabolic steroids has prohibitive side effects [278].

From the above evidence, approaches for cachexia in younger people with single diseases appear to show variable results. They seem most likely to demonstrate benefit when interventions are multifaceted and target the underlying cause in addition to nutrition, exercise and pharmacological treatments. This is likely to be directly translatable to the treatment of cachexia in older people which has a common pathogenesis, multiple aetiologies and the potential for multiple interventions. An approach to considering these interventions is described within the next section.

5.5.3 How could we treat cachexia in older people?

The best way to treat cachexia in older people is to remove the underlying cause of the inflammatory processes that are driving the syndrome. As discussed above, this is more easily achievable when the cachexia is associated with a few defined diseases but is more difficult when the inflammation represents extremes of inflammaging and has much broader aetiologies originating from across the lifecourse; prevention is better than cure [40]. Evidence from the treatment of cachexia in younger people suggests that a multiple intervention approach is warranted which may be considered according to: nutritional, physical and pharmacological interventions.

5.5.3.1 Nutritional interventions

Multiple studies of cachexia in younger people have demonstrated that nutritional interventions, including by the provision of total parenteral nutrition, does not prevent the continuing loss of muscle or correct the underlying metabolic abnormality associated with the syndrome [20]. However, the scientific literature suggests that there may be a role for

nutritional support in conjunction with other interventions for the treatment of cachexia in older people.

Older people have a vulnerability to becoming cachexic due to dysgeusia, dysosmia, orogingival disease, low mood, cognitive impairment, and social isolation [34;35]. A consequence is the reduction of food intake by around one quarter between 40 and 70 years. This contributes to a normal age related weight loss of 0.1-0.2kg a year and puts older people at risk of developing more severe weight loss and cachexia even during minor illnesses [36]. According to the UK National Diet and Nutritional Survey, 14% of community dwelling older men are at medium or high risk of malnutrition and a recent systematic review of weight loss in older people found that reduced food intake is the biggest single risk factor [228]. Therefore, there is widely acknowledged need for nutritional interventions in older people and a growing research evidence base. Such interventions are likely to form part of a multiple approach for the treatment of cachexia in older people and include direct dietary modifications and also interventions that address the physical and psychosocial factors associated with reduced food intake.

Non-pharmacological interventions that optimise food intake may be successful and have been trialled with varying degrees of success although much of the evidence is anecdotal [37]. These include eating smaller meals more often, the use of snacks and favourite foods, minimising dietary restrictions, optimising and varying food texture (especially in patients with dementia), the provision of high energy meals at the start of the day, eating in company and the use of mealtime assistants [279]. There is also a role for rationalising medications and medical examination; a review of people who had lost weight in a residential home found that 75% were on one or more medication known to contribute to weight loss, furthermore, chronic disease, constipation, falls, pain, orogingival disease are all significant organic contributors to weight loss and are also modifiable [228]. Randomised controlled trials for such interventions have generally been small in number and non-significant. For example, a study by Splett et al. in nursing home residents' trialled a specific dietician led intervention versus usual practice and found no benefit in terms of intake or functional outcomes [280]. Similarly, a breakfast cross-over trial which compared front loading nutrition at the start of the day when appetite is generally highest to a usual meal pattern demonstrated no difference [228;281]. Conversely, the use of protected mealtimes and mealtime assistants on hospital wards does increase food intake and 'food friendly' sociable environments have also shown some benefit [282]. Further work and larger trials are needed.

Nutritional intake may also be enhanced via the use of nasogastric (NG) tube feeding or parenteral feeding. It is generally accepted that these types of nutritional interventions are only suitable for a defined period whilst a person is being treated for a reversible condition;

they are unlikely to offer a long term solution for older people with cachexia. Tokuda *et al.* investigated the influence of NG tube feeding on survival in hospitalised older people and, after multivariate adjustment, found it to be associated with significantly higher mortality (hazard ratio, 2.29; 95% confidence interval, 1.22– 4.33) [283]. NG feeding was associated with side effects including aspiration, diarrhoea, and vomiting. An alternative approach is to bypass the stomach completely with parenteral nutrition; this has previously been unsuccessful in the treatment of cachexia in younger people and is associated with a high incidence of catheter-related infections [284]. Nevertheless, it has been used in older people immediately after acute disease who are not capable of taking adequate calories or fluid and a recent single centre trial in care home residents suggested that peripheral parenteral nutrition may be used to successfully improve nutritional and functional status whilst also being safely administered with a low complication rate; again, further trials are needed [285].

The use of nutritional supplementation to improve weight, physical function and quality of life may be an achievable intervention; it is commonly provided in the form of high energy sip feeds and recently underwent Cochrane review. Of the 62 trials, the authors concluded that most studies were small and of poor quality. However, benefits were seen in terms of overall weight gain of approximately 2.2% but there was no associated reduction in mortality. When the analyses were restricted to those who were malnourished at baseline a significant reduction in mortality was seen; these findings may be translatable to older people with cachexia [286;287]. Similar evidence is less robust within community dwelling older people and those recently discharged from hospital or in care homes; additional data from large-scale multi-centre trials which use different approaches are still required [286;288].

Polyunsaturated fatty acids (PUFAs) are anti-inflammatory and anti-catabolic; their role in the treatment of cachexia is unclear but promising and may offer an additional approach. For example, eicosapentaenoic acid is an alpha-3 omega fatty acid commonly found in oily fish which suppresses IL-6 in patients with pancreatic cancer cachexia and has been associated with significant gains in body weight, lean mass and quality of life [289]. A 2007 systematic review of 17 trials (8 of good quality) looking at the benefits of PUFAs in patients with cancer cachexia concluded that oral supplementation benefited patients with advanced cancer and weight loss, and are indicated in tumours of the upper digestive tract and pancreas where they increased weight and appetite, improved quality of life and reduced post-surgical morbidity [290]. A more recent systematic review found fair evidence showing that supplementation with PUFAs was safe and may improve the quality of life and physical activity in patients with cancer. However, it did not improve energy or protein intake, appetite or survival. The authors concluded that the evidence for an effect on body weight, fat-free mass and performance status remained inconclusive [290].

Nutritional interventions which target skeletal muscle may help recovery from cachexia and there is a considerable evidence base in older people. For example, increasing daily protein intake to 1.2-1.5 g/kg has been reported in some studies to be beneficial. However, this needs to be consumed as a small amount of high quality protein with each meal and with a reduction in carbohydrate intake. Such a diet is very difficult to achieve especially considering that 40% of people over 70 years do not consume the recommended minimum dietary allowance of 0.8g/kg/day [291]. In addition to protein, Vitamin D may have a role in treating skeletal muscle and currently the evidence for supplementation in sarcopenia is strong but questions still remain about dosing, efficacy and long-term safety; further trials are needed [40]. There may also be a role for dietary supplementation with essential amino acids such as leucine which is found in leguminous products, fish and beef, and is believed to increase protein anabolism whilst reducing muscle breakdown [291].

5.5.3.2 Physical interventions

As discussed previously, physical interventions have shown some benefit in people with cachexia associated with single diseases in younger people and have also been well studied in association with age related skeletal muscle loss; they may form part of a multiple intervention approach for the treatment of cachexia in older people. It is now well established that exercise can slow the loss of skeletal muscle mass and function and inactivity accelerates loss; this will directly impact on fat free mass and weight. Resistance exercise (strength training) is the most effective mode of activity to combat age-related changes in muscle [235]. This includes the very old and those admitted to nursing homes and benefits can still be demonstrated more than 30 weeks after stopping an exercise programme. A recent Cochrane review of 121 trials including 6,700 participants, found that high intensity resistance training performed 2-3 times per week had a positive effect on muscle strength and that this was associated with a modest improvement in gait speed and getting out of a chair. The authors concluded that progressive resistance training is an effective intervention for improving strength and physical functioning in older people including functional performance of some simple and complex tasks [292]. There is also weak evidence that some other types of exercise (gait, balance, co-ordination and functional tasks; strengthening exercise; 3D exercise and multiple exercise types) are moderately effective, immediately post intervention, in improving clinical balance outcomes in older people and that such interventions are safe [48].

A number of current trials are combining nutritional supplementation with resistance exercise which remains the most effective treatment to combat age-related loss of skeletal muscle [291].

5.5.3.3 Pharmacological interventions for cachexia in older people

There are currently no pharmacological interventions that have a good evidence base for the treatment for cachexia in older people. However, drugs have been used with varying degrees of success in the treatment of cachexia in younger people and a few studies have considered drug treatments of weight loss in older people; some or all of these may be translatable to the treatment of cachexia in older people. There is also a growing evidence base for the drug treatment of skeletal muscle mass loss in older people - this is central to the cachexia process and has the potential to be used within a multifactorial approach for its treatment. These are discussed in greater detail in the following section.

Pharmacological treatment of cachexia in younger people

Drug interventions for cachexia in younger people can be broadly speaking considered within three main groups: appetite stimulants; endocrine agents and inflammatory mediators.

Of the appetite stimulants, corticosteroids are the most widely used in clinical practice; these are acknowledged to cause increased appetite and a general sense of well being but they do not cause weight gain when compared to placebo and the duration of appetite stimulation is often short-lived [293-295]. Prolonged steroid therapy produces myopathy and a wide variety of other side effects. Progesterone analogues such as megesterol acetate show greater potential as a therapeutic agent; they are thought to act as an anti-inflammatory agent, glucocorticoid and androgen and have been approved by the US Food and Drug Administration for the use as an appetite stimulant in HIV patients [279]. In a recent RCT of 133 patients with cachexia, those in the treatment arm (megestrol acetate) had a significant increase in both appetite and weight compared to placebo and a recent meta-analysis has suggested it is effective for the treatment of cachexia in younger people in terms of appetite and weight gain [296-299]. When compared to corticosteroids and anabolic agents, progesterone analogues have similar efficacy in terms of appetite stimulation and weight gain but a considerably better side effect profile; there is limited evidence for the use of megesterol acetate in older people which is discussed in more detail later [297]. Finally, of the appetite stimulators, anecdotal reports and small studies have suggested that marijuana stimulates appetite; unfortunately synthetic cannabinoids have not been demonstrated to have activity against cachexia in patients with advanced cancer, although there may be some benefit in patients with advanced HIV disease and the drug is currently licensed for this purpose in the US [300].

Anabolic steroids have been shown to be less effective than steroids or progesterone analogues as an enhancer of appetite but, as discussed later, may have a role in increasing lean body mass; trials within patients with cachexia have so far been inconclusive [298]. For example, oxandrolone, a synthetic derivative of dihydrotestosterone only caused non-significant weight gain in 155 patients on chemotherapy and these benefits were rapidly lost following the cessation of treatment whereas enobosarm, a selective androgen receptor modulator, was trialled as an intervention for weight loss due to cancer and demonstrated significant increases in lean body mass for up to four months [301].

Growth hormone has been trialled as a treatment for critical illness myopathy where it was associated with high rates of mortality which was considered to be a consequence of the diversion of amino acids and energy reserves from the acute phase response to skeletal muscle [302]. More optimistically, Ghrelin may offer an alternative therapeutic approach. Ghrelin is an appetite stimulating hormone which also induces the release of growth hormone and has anti-inflammatory properties. Early studies have demonstrated that it can be administered safely and repeated administration improves body composition, muscle wasting and functional status in cachectic patients with heart failure or COPD; studies regarding its efficacy within patients with cachexia are currently ongoing [279].

The direct manipulation of inflammatory mediators is a biologically plausible strategy for the treatment of cachexia. There have been a few small trials looking at the role of TNF inhibitors in patients with cachexia which have demonstrated no benefits in terms of appetite or body weight and also significant adverse reactions [303]. Thalidomide inhibits TNF production and has been associated with weight gain in patients with chronic infections such as HIV, however, a recent Cochrane review concluded that there was insufficient evidence to recommend its use and that more studies are needed [304].

A number of other therapeutic agents have been trialled as a treatment for cachexia in younger people which include insulin (anabolic), melatonin (TNF inhibition), metaclopramide (gastric motility), beta blockers (reduced basal metabolic rate) and serotonin antagonists (appetite stimulant). These studies have all been small and demonstrated either no, or weak, benefits in terms of weight gain and cannot currently be recommended as a treatment although further work is being undertaken. Finally, there may be a role for combination therapy, i.e. simultaneously targeting appetite and inflammation which seem to more consistently offer benefit. For example, megesterol acetate plus ibuprofen was found to be better at maintaining weight in patients with cancer than megesterol acetate plus placebo; more studies and a better understanding of drug interactions and side effects is needed [305].

Pharmacological treatment of weight loss in older people

There are few studies that have looked at the drug treatment of weight loss in older people beyond the use of nutrition based interventions; these have been small and have failed to demonstrate any significant benefit.

Mirtazipine is a serotonergic norepineprine uptake inhibitor that is commonly used in the treatment of depression and is perhaps the commonest used medication for weight loss in older people although this is an unlicensed indication and a recognised side effect. In one study set in older people it was associated with an increase in weight of 0.6 kg over 12 weeks, however, this was not statistically significant and the effect has not been seen in other studies involving the drug; no studies have been designed to specifically look at mirtazipine and weight change.

Four trials have examined pharmacologic treatment options for unexplained weight loss in older people, these were all small and only one was a blinded randomized control trial; the commonest studied medication was the progesterone derivative, megestrol acetate and one study also looked at the use of the cannibinoid, dronabinol. The largest trial was an RCT of 69 patients who were assigned to either placebo or megestrol acetate for 12 weeks. No significant differences in weight gain were reported although patients in the treatment arm showed significantly greater improvements in appetite, enjoyment of life and well-being [306]; similar benefits in older people have been reported elsewhere [279]. In a separate study based within a care home setting, megestrol acetate in combination with mealtime assistance significantly increased oral intake in frail long-term care residents but was ineffective without the mealtime assistance and a smaller study looking at the role of megesterol acetate in the treatment of anorexia in older people (n = 14 versus 10 controls) found that the drug did not significantly increase anthropometric measures when compared with controls [307]. The treatment may be more effective when combined with olanzapine [229]. Common reported side effects of megestrol acetate in these studies included hypertension, gastrointestinal upset, insomnia and impotence; reported serious adverse events included adrenal insufficiency and thromboembolic events. It is also likely that megestrol acetate blunts the beneficial effects of progressive resistance exercise training as a consequence of its catabolic actions; conversely it may enhance the benefits of resistance exercise after a course has been completed [279]. Finally, a placebo controlled cross over study looking at the effects of dronabinol in patients with Alzheimers dementia and refusing food showed a trend toward weight gain during the treatment period although this was not a statistically significant effect and dronabinol was associated with significant side effects including hallucinations, sedation and dizziness [308].

Pharmacological treatment of low skeletal muscle in older people

Despite being a fertile area of research there are currently no pharmacological treatments that are licensed to treat the loss of muscle mass and strength with age. Possible therapeutic options include the use of established drugs, targeting muscle via the endocrine axis and the use of novel agents.

Of the established drugs, it is perhaps ACE-inhibitors which have the strongest evidence for a possible role in improving physical function in older people. Onder *et al.* found that participants in the Woman's Health and Aging Study who were taking ACE-inhibitors had a lower mean 3-year decline in muscle strength compared with those on alternative antihypertensives or no antihypertensive medications [309]. Furthermore, in controlled trials, the use of an ACE-inhibitor in older people with heart failure caused by left ventricular dysfunction improved gait speed and ACE-inhibitors improved exercise capacity and maintained health related quality of life in functionally impaired older people without heart failure or left ventricular dysfunction [310;311]. Conversely, a more recent RCT set amongst community dwelling older people did not show a benefit in the use of perindopril over and above progressive exercise training [312]. Other potential drug candidates which modify vascular function have also been considered including within the Hertfordshire Cohort Study where positive associations between grip strength and nitrates and fibrates have been demonstrated although the use of ACE inhibitors, statins or thiazides within this cohort was not associated with differences in grip strength decline [313;314]. In a separate study spironolactone did not benefit physical function in older people although quality of life measures were significantly improved [315].

Endocrine supplementation may act as a mechanism to enhance muscle mass and function in older people; DHEA, growth hormone, oestrogen and testosterone have all been widely studied within this context. DHEA levels increase with exercise and it is biologically plausible that dietary supplementation may increase muscle mass and performance although the benefits of DHEA on muscle strength and physical function in older adults are inconclusive. For example, a recent systematic review was only able to identify eight studies within their inclusion criteria of which equal numbers showed positive and negative results and only one study showed improvement in a composite score measuring physical performance; the rest reported no differences between DHEA and control for any end point [316]. The authors concluded that DHEA supplementation does not appear to routinely benefit measures of physical function or performance and that further large clinical trials are necessary to better identify the clinical role of DHEA supplementation within this population.

There have been a larger number of trials looking at the use of growth hormone to improve muscle mass and function and the strongest evidence is within states of GH deficiency where supplementation has been demonstrated to increase exercise capacity [317]. In older people who are not GH deficient, supplementation often increases muscle mass but benefits on function and performance are less clear and the supplement is especially associated with side effects in older people which include fluid retention, falls, orthostatic hypotension and gynecomastia [318]. The evidence for supplementation with oestrogen is contradictory and side effects include breast cancer; there may be a role for combination treatment with exercise although more evidence is needed [319].

A significant proportion of older men are deficient in testosterone which has been associated with the loss of muscle and bone mass although the evidence regarding supplementation is variable. Of the randomised trials investigating the effects of supplementation on body composition in men, some demonstrated benefits in muscle mass but not strength or performance, some showed improvements in both mass and strength and others showed no improvement. A meta-analysis found that testosterone replacement increased fat free mass without changing body weight and only a trend towards improvements in strength was seen [320]. Currently, testosterone supplementation is prohibited by side effects which include increased prostate size and risk of prostate cancer.

More novel drug treatments that target skeletal muscle include myostatin inhibitors and creatine. Myostatin inhibits growth factor and mutations to its gene are associated with muscle hypertrophy; polymorphisms in humans correlate with measures of muscle mass, strength and physical performance [321]. Targeting the myostatin pathway may therefore have a role in the treatment of skeletal muscle loss however, human trials focus on the treatment of muscular dystrophy and are only currently in the early stages. Furthermore, there is a suggestion from animal studies that myostatin deficiency is associated with more brittle tendons and an increased risk of rupture [321]. Creatine supplementation decreases muscle relaxation time and enhances muscle protein synthesis resulting in an increased ability to perform higher intensity exercise. A recent meta-analysis showed that supplementation significantly increased total body mass in older people when used alongside resistance exercise, however, numbers were small and the authors concluded more trials were needed [322].

5.5.4 Interventions targeted at inflammation

In addition to targeting cachexia, it may be possible to target inflammaging which is implicated in the cachexia syndrome of older people. Such an approach must be considered across the lifecourse, well before the syndrome has developed. Unfortunately, no intervention currently exists that targets inflammaging and immunosenescence; two of the principal difficulties are establishing reliable biomarkers of inflammaging to enable monitoring and also an understanding of which changes are beneficial or harmful. However, as the importance of this age related process becomes established, plausible interventions are emerging based on immunomodulation via nutrition, exercise, the endocrine axis, reducing antigen exposure and using direct pharmacological approaches.

It is widely accepted that nutrition plays a role in many diseases including infection, diabetes and cardiovascular disease; all of these are linked to immune responses and extrapolations may be made to inflammaging. For example, micronutrients such as vitamins B12 and E, folate, zinc, iron, copper and selenium have been shown, in varying degrees, to enhance thymic and neutrophil function and increase T-lymphocyte proliferation [323]. Lipids including Omega-3 polyunsaturated fatty acids (PUFAs) and conjugated linoleic acid have potent anti-inflammatory properties which may be explained via their enhancement of the membrane micro-environment of immune cells [323]. PUFAs are well known to reduce the risk of cardiovascular disease, an inflammatory pathology originating from atherosclerosis [324]. Therefore, nutritional approaches may represent an effective and safe strategy to enhance immune function although further work is needed and no single approach can currently be recommended. The best use of nutrition as a modulator of inflammaging, is probably through eating a healthy diet across the lifecourse and combining this with other interventions.

Long term moderate exercise is likely to improve T-cell function, antibody production, innate immunity and thymic output. However, these interactions are complex and short burst, high intensity exercise may generate a pro-inflammatory environment whilst also being immune suppressing; further trials are needed especially with regard to the type, frequency and duration of the exercise intervention [325].

A separate strategy may be the manipulation of the immune axis via the endocrine axis although evidence for this is currently limited due to the lack of trial evidence and unacceptable side effect profiles reflecting the pleiotrophic nature of hormones. For example, oestrogen and insulin both have immune modulating actions and restoring the insulin resistance that occurs with age using anti-diabetes drugs may have a beneficial immunorestorative effect [326]. An alternative approach might be via the supplementation of

insulin-like growth factor (or its trigger, growth hormone) which promotes the survival of immune cells and also declines significantly with age. DHEAS has already been discussed within this thesis as a counter to the detrimental effects of cortisol; it also enhances natural killer cell activity and decreases circulating IL-6 [327;328]. Unfortunately, as with other endocrine interventions, there is currently no consistent trial data to recommend its use. Finally, it has been suggested that vitamin D may modulate T-cell function. Therefore, vitamin D deficiency, which is common in older people, could play a role in the development of chronic inflammatory diseases and autoimmunity; as vitamin D is commonly prescribed in older people it would be worthwhile evaluating the long term effects on immunity of this treatment [329].

Strategies to reduce antigenic load include reducing exposure to acute infections and sub-clinical bacterial infections alongside rapid identification and treatment. Vaccinations may play an important role, both in terms of booster revaccinations and vaccinations in earlier life against non-lethal viruses which reduce T-cell repertoire in later life (e.g. CMV); these types of vaccines are currently being developed within the context of reducing the incidence of Herpes Zoster and post-herpatic neuralgia [323].

Several autoimmune diseases are successfully treated with direct blockage of cytokines or their receptors. Therefore similar approaches may be considered in older people with inflammaging although this currently has the major drawback of inhibiting appropriate immune responses and increasing susceptibility to infection and disease reactivation. The use of less potent drugs (e.g. statins) is more realistic; they are also inexpensive, widely available and have well documented properties.

5.5.5 Clinical translation: conclusion

We are still a long way off a clear evidence base from which to recommend strategies to combat cachexia in older people; more research in all age groups is needed. However, the CaSIO study has illustrated the clinical importance of cachexia in older people and suggests that it is possible for older people to transition from states of cachexia to no cachexia; there may, in time, become a role for specific interventions to increase the likelihood of this happening.

The single most effective intervention for cachexia is removing the cause which is difficult in older people due to its multifactorial aetiology. More specific interventions for cachexia in younger people are based on nutrition, exercise and pharmacological approaches; they have an established and growing evidence base although larger studies and trials are needed and the overall message is unclear - some are successful, others are not.

The evidence for the treatment of cachexia specifically in older people is minimal. However, there have been studies which have addressed interventions for weight loss in older people and also the treatment of skeletal muscle loss – both key components of the cachexia syndrome. Further studies are needed and this is a fertile area of research especially as pharmacological approaches targeting skeletal muscle are gaining interest and investment [40]. Of the drug treatments currently available, progesterone analogues seem to have the most encouraging evidence base although novel drugs are currently being developed. It may also become possible in the future to treat or manipulate inflammaging itself and thereby attenuate the rate at which age related processes progress and reduce the incidence of cachexia in older people although this is some way off.

The CaSIO study has shown that cachexia is common among hospitalised older people and associated with adverse outcomes. A strong evidence base for interventions does not currently exist however strategies may emerge with time. It is likely that some of the lessons learned from previous and future studies of cachexia in younger people plus the treatment of weight loss and skeletal muscle loss in older people will be translatable to the treatment of cachexia in older people. A multiple intervention approach which targets nutrition, physical activity and drug interventions in combination with medical and psychosocial assessment is likely to be required. Further work is needed.

5.6 Strengths and limitations

5.6.1 Strengths

The CaSIO study had many strengths. First, the CaSIO data set provides a detailed characterisation of a group of frail older women both during, and after discharge from, an acute hospital admission. Frail older people remain relatively understudied in epidemiological research studies; the CaSIO study has demonstrated that it is possible to successfully recruit and collect data from hospitalised older people.

Second, data collection, laboratory work, data handling and statistical analyses were conducted to the highest standard by an experienced research team comprising of researchers from the University of Southampton and University of Birmingham with expertise in epidemiology, ageing and inflammation.

Third, efforts were made to minimise the potential impact of measurement error on the CaSIO study by using strict study protocols for data collection and measurement and also by using accurate measuring instruments that were regularly checked, serviced and calibrated during the study. In addition, intra- and inter-observer variation studies were conducted throughout the study to ensure reliability and repeatability of measures both between and within researchers involved with the data collection.

Fourth, although a healthy participant effect was unsurprisingly evident in the CaSIO study in terms of degree of ill health and confusion, the study participants were nonetheless broadly comparable with the wider population of hospitalised older women in terms of demographic and functional characteristics. Moreover, statistical analyses were internal to the CaSIO dataset. Unless associations between admission characteristics and outcomes of interest were systematically different between older women who participated in the study and those who did not, no substantial bias should have been introduced with regard to these associations. Overall, we suggest that results from the CaSIO study are likely to be generalisable to the wider population of frail, hospitalised older women.

Finally, the results from the CaSIO study will not only inform future research but are also directly translatable to the clinical environment.

5.6.2 Limitations

The CaSIO study also had a number of limitations which must be acknowledged and considered when interpreting and drawing conclusions from the study. These limitations are discussed below.

5.6.2.1 Sources of bias

Study participants were recruited in a prospective manner until the target sample size was reached. A number of exclusion criteria were pre-defined for practical and ethical reasons which unavoidably introduced selection bias. Study participants were likely to be less unwell; less confused, and did not have conditions that required isolation (e.g. diarrhoea) in comparison with the background population of hospitalised older women. However, no other exclusion criteria were applied to study in an effort to keep the impact of selection bias to a minimum.

An additional source of bias at the stage of participant recruitment was that women had to volunteer to take part in the study; those who were willing to participate may differ from those who were not. Overall, these sources of bias at the stage of participant recruitment are likely to have led to an underestimate of the prevalence of cachexia among the study participants in comparison with the wider population of hospitalised older women.

The CaSIO study was designed to minimise loss to follow up. Steps taken included provision of written and verbal information to participants and, when appropriate and possible, to their families upon discharge. In addition, study participants' GPs were contacted prior to follow up. Despite these measures 24/148 (16%) of participants were lost to follow up at six months, mainly due to difficulties in contacting the participant via the telephone to arrange an appointment. It is likely that follow up bias was introduced at this stage as evidenced by the fact that participants lost to follow up were more likely to have scored worse (lower) on the MMSE questionnaire and to have reported less fatigue at baseline *Table 4-3, page 86*. It is unsurprising that participants who were lost to follow up had lower MMSE scores at baseline; the difference in reported level of fatigue at baseline is unexpected and may be a chance finding. It is reassuring that no other differences were seen in the baseline characteristics of participants according to follow up status, including with regard to functional status.

5.6.2.2 Sources of measurement error: data collection

The study methods were designed to minimise sources of error by using well defined measurement protocols and by performing the measurements at the same time of day and during the same point in relation to the admission for all study participants. Furthermore, the technical methods used in the study were robust and reliable, methodology was consistent with best practice (e.g. conducting grip strength measurement according to the Southampton protocol) and the equipment was regularly checked, calibrated and serviced. However, despite these efforts, it is likely that a degree of measurement error was still introduced as a consequence of recall bias, methodological limitations, observer, and participant variation.

Recall bias may have been introduced as a result of asking participants to remember falls; this has been shown to be subject to underreporting by approximately 22% [182]. Therefore the CaSIO study limited recall to a period of six months which is the timeframe recommended by previous cohort studies to minimise the effects of this type of bias [182].

Recall bias may also have been introduced by asking participants to remember their usual weight in order to ascertain degrees of weight loss at baseline. Data from the US suggests that women tend to under-report their weight by an average of 1.5 kg across most age groups but by just 0.2 kg in women aged 80-89 years [330]. If apparent in CaSIO, this pattern of under-reporting would lead to slightly underestimated degrees of weight loss and consequently to an unduly low estimate of the prevalence of cachexia. Where possible in the CaSIO study, reported usual weight was verified using previously measured weight recordings elsewhere within the participants' medical records; this is the most realistically translatable system for ascertainment of degree of weight loss in the clinical environment and is similar to the techniques used by Fried et al. when using unintentional weight loss to characterise a frailty phenotype within the cardiovascular health study [188].

Missing data was introduced as a consequence of methodological limitations when characterising muscle mass due to the techniques (i.e. DXA and BIA) being either contraindicated or not tolerated. For example, it was not possible to assess muscle mass in 55/148 (37%) or participants due to the strict conditions that need to be satisfied in order to perform bioelectrical impedance analysis; bias may have been introduced as a consequence if there were differences between participants in whom it was or was not possible to perform BIA. However, these are only minor criteria within the cachexia diagnosis meaning that it was still possible to characterise cachexia for 131/148 (89%) participants at baseline. In the absence of DXA or BIA measures of muscle mass being available, grip strength was used to characterise skeletal muscle in the definitions of sarcopenia and cachexia utilised

for CaSIO. Participants with low muscle strength were assumed to have a similarly low muscle mass which has previously been validated in a study that demonstrated a high correlation between muscle strength and mass ($r = 0.79$, $p<0.0001$) [331].

Finally, sources of observer variation will have impacted on the CaSIO study but efforts were made to minimise this. Examples of observer variation include the failure of a researcher to record the same reading on repeated examination (inconsistency, intra-observer variation) and also differences in measurements recorded by different researchers (inter-observer variation). These sources of error were examined prior to the study and average differences in measurements between and within observers were small (e.g. inter- and intra-observer mean variation for grip strength, 0.2kg). Observer variation was also minimised by blinding researchers and participants to knowledge of previous results in order to avoid expectation/test execution bias. In addition, comprehensive measurement protocols were used which enable tests to be performed consistently, clearly and to high standards (protocols stipulated all aspects of measurement including set up, calibration, verbal encouragement, reading scale and recording of results). Finally, the number of researchers working on the study was kept to a minimum with the exception of coding of the Barthel index which was either calculated by the lead researcher or was ascertained from the nursing staff who are trained to use established methodology to code the Barthel index. There is also an element of subjective interpretation in coding the Barthel index particularly at its middle range where an individual is neither very dependant or very independent. It is therefore acknowledged that there is a greater potential for inter-observer variation to have affected the Barthel score than other variables in CaSIO and this should be considered when interpreting these data. However, despite these issues, it is likely that the Barthel index remains a valid assessment of activities of daily living and it has been previously shown to have good reliability when administered by face-to-face interview and by different observers as per the CaSIO study [181]. Test-retest reliability has not been studied in older people but it has been shown to be good in populations of stroke patients and it also shows good responsiveness [332].

Participant variation may occur as a consequence of an awareness of being studied (Hawthorne effect), variable motivation to respond and mood at the time of interview as well as rapport with the interviewer. It must also be acknowledged that measurements in CaSIO were taken in two different settings, hospital and home. It is possible that measured variables will have differed according to time of day, environment and as a consequence of physiological factors, e.g. acute illness, fluctuations in biomarker blood tests (circadian, hour-to-hour) and psychological factors (symptoms of depression). It was noticeable that 40/148 (27%) CaSIO participants did not have documentation of GDS score; participants often found it difficult to answer the probing questions within the GDS which were included

towards the end of the CaSIO questionnaire and it is possible that non-responders to these questions are more likely to have symptoms of depression and fatigue. With hindsight, the study methods could have been further improved by greater involvement of service users (e.g. patients, public) when designing the CaSIO study methods in order to make the data collection tools more participant friendly.

All of the potential sources of measurement error that have been discussed within this section must be considered when interpreting the results from the CaSIO study.

5.6.2.3 Sources of measurement error: blood biomarkers

A number of issues with regard to blood biomarker analysis must also be considered when interpreting these data. These include cytokine induction by anticoagulant during phlebotomy, coagulation interfering with analysis and degradation of samples during handling [333]. These were minimised by adhering to a strict protocol including the collection of samples at the same time of day, immediate processing and freezing at -80˚C, avoiding freeze-thawing and performing the analyses in a laboratory with extensive experience and expertise; data were checked prior to analysis and outliers beyond a pre-defined range were removed. Furthermore, it is important to consider that the context for blood biomarker analysis is to gauge general systemic inflammatory milieu rather than to investigate the local and biological functions of specific biomarkers.

Cytokines do not undergo diurnal variation or exhibit circadian patterns and normal reference ranges vary according to the population being studied and techniques used; they demonstrate wide confidence intervals and reference data is lacking, especially within older populations. Therefore, within-lab analyses of data are recommended and international standards are not yet developed. It is reassuring that the immune-endocrine analyses within this study demonstrated reliable intra- and inter-biomarker correlations (*Appendix, page 212 to 214*). However, in order to validate these analyses further, a reference study was used which characterised cytokine profiles within 55 subjects over 65 years using similar techniques to those used within the CaSIO study [334]. This revealed that all CaSIO data were within 1 standard deviation of the reference study and also indicated a generally greater pro-inflammatory environment which is to be anticipated within an older hospitalised population. The only discrepancy was IL-8 which was lower within the CaSIO study. This is likely to reflect the use of serum rather than plasma samples which, despite being the preferred technique, artificially lowers IL-8 concentrations due to the molecule being trapped within fibrin matrixes that are removed during preparation [333;335].

5.6.2.4 Confounding

There is no standard set of variables which must be considered for their potential confounding effect on a study such as CaSIO but gender, age, health behaviours such as smoking habit and alcohol intake, co-morbidities and socioeconomic position are often explored as potential confounders in epidemiological studies of older people.

Gender cannot have confounded the associations studied in CaSIO because all the participants were female. In addition, very few of the participants reported currently smoking or drinking alcohol; these lifestyle habits are therefore very unlikely to have had a confounding influence on the CaSIO study.

Associations between CaSIO baseline characteristics and hospital and discharge outcomes were typically somewhat attenuated by adjustment for age which is unsurprising as both skeletal muscle loss and inflammation are age related processes. However, age did not completely explain associations them and p-values often remained statistically significant at the 5% level even after adjustment for age.

Co-morbidity, the acute admitting diagnosis and medications all had the potential to act as confounders in CaSIO. Although the study population had a significant co-morbidity burden this was generally a mixture of chronic diseases such as hypertension and diabetes; there was not a high prevalence of diseases classically associated with cachexia such as cancer or congestive cardiac failure. Furthermore, all results were adjusted for the number of co-morbidities and also number of medications and this did not significantly change the results. Unfortunately, it is a limitation of CaSIO that relatively low participant numbers prohibited a more exhaustive exploration of the potential confounding impact of specific morbidities, specific acute illnesses leading to hospital admission or specific medications although there is the potential, at least in-part, to address this with additional analyses as discussed in the next section.

A further limitation of CaSIO is that it was not possible to adjust for socioeconomic status as this information was not collected; future studies would be advised to collect these data.

5.6.2.5 Scope of the data collection

The CaSIO data collection had some limitations.

First, CaSIO was a female only study due to restrictions on recruitment resulting from the national move to single sex wards. Women were chosen as the focus of CaSIO instead of men because women represent approximately 70% of frail older people [188]. However, the findings require replication in men.

Second, the assessment of gait speed (and therefore frailty) occurred at follow up without reference to an assessment at baseline. As previously discussed this was due to the practicalities of measuring gait speed on the ward; however, it could have been achievable with advanced consideration of the prohibitive issues, e.g. availability of space, patient safety.

Third, muscle mass data were not recorded at follow up which impacted on the assessment of cachexia at this time point. However, fat-free mass is only a minor diagnostic criterion for cachexia and therefore this only resulted in diagnostic uncertainty in one participant who was subsequently removed from the analyses involving this variable. This issue reinforces the need for a novel technique such as ultrasound to assess skeletal muscle mass in frail older people.

Fourth, loss of independence is a poor long term outcome for older people and this was assessed at baseline and follow up using both the Barthel index and accommodation type/support needed in the community. However, these data were not collected at 12 months or at the end of the study; at these time points it is likely that a number of participants would have moved to more dependant community settings and the study would have been further enhanced by collection of these data and their use as a long term outcome alongside mortality.

Fifth, the CaSIO study would have been improved by inclusion of a more detailed characterisation of physical functioning. A physical performance battery, including gait speed (3 metre walk), chair raises and one legged (flamingo) stand, would ideally have been completed at baseline and follow up. The timed up and go (TUG) (which records the time to rise from a chair, walk 3 metres, turn around, return to the chair and sit down) could also have been usefully included in CaSIO. These functional tests are widely used in research and clinical environments, have established methodology and are well validated and associated with worse outcomes in older people [69;70;336-339].

Finally, the study would have been enhanced by a more detailed immune-endocrine analysis including biomarkers such as IGF-1 and growth hormone; it was disappointing that the characterisation of specific immune-endocrine biomarkers, TNF and IL-1, was limited in CaSIO due to technical issues at the laboratory stage.

5.6.2.6 Sample size

Increased numbers of participants would have enabled: a more detailed analysis of the role of pre-cachexia as a precursor to cachexia; detailed exploration of the role of co-morbidity and whether specific co-morbidities, patterns of co-morbidities, admission diagnoses or medications are particularly associated with cachexia or explain associations between cachexia and worse outcomes; and a more in depth investigation of the contribution of the specific components of the cachexia syndrome to the results presented in this thesis.

5.6.2.7 Role of chance

Given the large numbers of exposures assessed at baseline and the multiple associations analysed, it is important to consider whether the results presented in this thesis may have arisen by chance. A number of procedures have been developed to deal with multiple testing, such as applying a Bonferroni correction. Such techniques were not used in this thesis due to continuing controversy regarding if and when these procedures should be used; it has been suggested they may even result in an *over*correction leading to too many type II errors (false negative results) [340].

Instead of applying a correction for multiple testing, the Bradford Hill criteria may be used to consider the evidence that is provided for association in the CaSIO study. First, it seems unlikely that the results of the CaSIO study have simply arisen by chance; rather, they are consistent with the scientific evidence described in the introduction and are also plausible and aligned with both published research and clinical experience. Second, the strength and size of the associations identified in CaSIO was strong with p-values often significant at the 1% significance level and odds/hazard ratios suggestive of substantial effect sizes. Third, the results are internally consistent (e.g. all pro inflammatory biomarkers were associated with increased mortality and anti inflammatory biomarkers with reduced mortality) and also externally consistent with other studies (cachexia is associated with mortality in other conditions). Fourth, the longitudinal design of the study enabled identification of temporal relationships between participant characteristics and subsequent outcomes; longitudinal associations provide stronger evidence for association than cross-sectional associations. Finally, a clear biological gradient was apparent in many of the associations described in this

thesis e.g. progressively higher levels of CRP were associated with greater risks of mortality, *Figure 4-6, page 116*. In summary, after consideration of the study findings relative to the Bradford Hill criteria, it is suggested that the results presented in this thesis have not arisen purely by chance.

5.6.2.8 Relevance

The consensus definition for cachexia is a valuable method for identification of individuals with cachexia in a consistent manner for research purposes. However, the clinical presentation of cachexia is familiar to the majority of practicing clinicians who, like Hippocrates, identify it as severe wasting condition associated with impending death. It may be that there is little added value in using the consensus definition over and above clinical acumen. To address this question the CaSIO study could have been enhanced by asking each participant's named clinician whether they considered their patient to have cachexia (yes/no) or to be at risk of developing cachexia (yes/no); these clinician assessments could be compared with those from the consensus definition to ascertain their agreement and to identify the 'added value' of one approach relative to the other.

Previous studies have suggested that use of a consensus definition to diagnose cachexia does identify a different set of individuals to those identified as having cachexia according to clinical acumen alone. For example, a number of retrospective studies of cachexia in cancer patients have shown that the syndrome was present at the time of initial clinical presentation, considerably earlier than recognised by the clinician [25;26]. Also, within the CaSIO study, a number of participants were identified as having cachexia despite having a normal body mass index; it is unlikely that these individuals would have been diagnosed with cachexia according to clinical acumen alone, resulting in false negatives, and therefore use of the consensus definition would facilitate earlier intervention. Conversely, change in weight rather than a low but stable weight was associated with worse outcomes in CaSIO; it is likely that a number of the participants with low but stable weight would have been diagnosed with cachexia according to clinical acumen, resulting in false positives and the identification of people who were not at an increased risk of poor outcomes.

Finally, the value of diagnosing cachexia lies with informing outcome and expectation; there is currently no established intervention and it is therefore unlikely that the results of this study are currently translatable other than to increase awareness. However, as discussed previously, there are potential interventions for skeletal muscle and weight loss in older people, and a growing evidence base for the treatment of cachexia in younger people; this thesis has begun to define cachexia in older people and will help inform future work in this area with the aim of bring benefits to older people.

5.7 Research in hospitalised older people, lessons learned

5.7.1 Recruitment and follow-up

This study has demonstrated that it is possible to conduct high quality research in acutely hospitalised older populations. All ward admissions were screened for inclusion in the study and 58% of eligible patients were successfully recruited; only 2 participants withdrew, in both circumstances due to deteriorations in health. These rates compare favourably with similar studies of hospitalised older adults. For example, in 2009, the Medical Crises in Older People study group in Nottingham, UK recruited 6% of all patients admitted to the hospital wards. This low proportion was due to a combination of factors during the recruitment process: low screening rates (43% were screened); high exclusion rates (36% were excluded) and low recruitment successes (34% of those approached) [197]. It is likely that the good rate of recruitment to the CaSIO study was achieved as a consequence of the sample population being located on only two wards which were more manageable and geographically accessible for the lead researcher. In addition, recruitment of study participants was the responsibility of the lead researcher, a geriatrician, for the entire study; this ensured that the process was conducted in a skilled and consistent manner.

Despite the relatively successful recruitment of study participants, CaSIO was designed in a generic manner rather than being tailored to the sample population. Changes to the protocol would have improved recruitment further and also enhanced the experience of patients, participants and researchers. The major lessons learned are summarised in the following sections.

5.7.1.1 Patient information sheets

The majority of patients enjoyed the opportunity to discuss the study with the researcher following the initial screening stage. Without exception patients were then immediately either enthusiastic or disinclined to participate following this initial approach. However, the CaSIO ethics requirements dictated that patients should be given an information sheet and then a further 24 hours to assimilate the information prior to signing consent forms.

It became clear that participants were largely unable to read the patient information sheet or found them too cumbersome and descriptive, providing too much detail. This often caused undue concern; patients gained the impression that involvement in the study would require

considerable effort and entail risk. A more suitable alternative would have been to use a patient information sheet which was more straightforward and easy to understand.

Furthermore, patients often expressed disappointment that the data collection could not start for at least 24 hours. On the basis of this feedback an ethics amendment was attained to allow the minimum period between initial contact and recruitment to be reduced from 24 hours to 2 hours; this was more acceptable to both participants and researchers.

5.7.1.2 Consent forms

The process of obtaining consent is important and understandably monitored by strict research governance with the overall aim of protecting patients and participants. However, in the CaSIO study, the consent form contained 14 separate items over two pages and was cumbersome. Furthermore, every consent item required initialling and then also a full signature, name and date at the bottom of the document. This style of consent is perhaps appropriate for healthy adults but in this study caused the majority of participants' considerable difficulty due to problems such as acute illness, arthritic hands, reduced co-ordination and poor vision. The protocol was therefore changed to allow only one signature at the bottom of the page, and when that was not possible, the signature of a healthcare professional who had witnessed verbal consent. Again, this was more acceptable to both participants and researchers.

5.7.1.3 Follow-up logistics

Locating and contacting participants in order to arrange 6 month follow up visits was both challenging and time consuming. Participants had often moved to an alternative residence or did not own / were unable to use a telephone. In order to obtain up to date contact information and identify any changes in circumstances, letters were sent to each participant's general practitioner (GP) one month prior to the scheduled follow up visit – unfortunately this system was reliable in less than 50% of cases. Furthermore, when telephone contact was made with the participant they had often forgotten or failed to understand their role in the study and, in some cases, an unexplained visit from a doctor caused undue concern. These problems would have been reduced by:

a) A routine telephone call to the GP surgery reception to establish up-to-date contact details and changes in circumstance;

b) The use of a formal letter to the participant in order to consolidate the telephone conversation and explain the purpose, time and date of the visit and to include a contact telephone number for further information.

5.7.2 Assessment of body composition in hospitalised older people

The study of body composition within hospitalised older adults revealed a number of logistical difficulties that were difficult or unworkable within CaSIO; it is essential that such barriers do not exclude older people from research. Rather, such difficulties emphasise the importance of tailoring study protocols to older people and confirm the need to develop reliable and sensible techniques which overcome the problems encountered in CaSIO.

Within the CaSIO study:

- The assessment of gait speed was unnecessary to define sarcopenia;
- Grip strength dynamometry was a useful tool for the assessment of muscle strength;
- Bioelectrical impendence analysis (BIA) was the only practical tool available for the assessment of muscle mass, albeit with strict caveats;
- It may be that an alternative technique, for example ultrasound assessment of muscle mass, warrants further exploration.

These points are expanded in the following sections.

5.7.2.1 Assessment of physical performance on the acute medical wards

It was not possible to find space to create the standard conditions required for testing physical performance on the acute medical wards which reflected the busy conditions of a hospital running at capacity together with infection control and space pressures. It may have been possible to arrange this at the planning stage of the study with a greater insight and prior knowledge of these issues.

The European Working Group for Sarcopenia in Older People (EWGSOP) algorithm for the identification of sarcopenia was modified in CaSIO by moving past the initial screening test of gait speed; it was considered that all CaSIO participants warranted full testing for sarcopenia at baseline by virtue of being on an acute hospital ward. At 6 month follow up all participants had a slow gait speed (less than 0.8 metres per second) which was likely to have also been the case at baseline; this suggests that screening for sarcopenia on the basis of gait speed within the CaSIO participants was unnecessary and could be bypassed by

proceeding immediately to the assessment of muscle strength and mass. This may also be the case in other studies of hospitalised frail older people.

5.7.2.2 Assessment of muscle strength

The assessment of grip strength using a Jamar dynamometer was the only technique that was acceptable and feasible across the entire study sample. This important observation is consistent with a Japanese study that investigated the optimal physical or cognitive test to screen for falls risk in frail older people. The authors conducted a feasibility study which included 3,340 older people and concluded that grip strength and gait speed were the two most practical tests and could be administered to 90% of participants regardless of functional status [64]. Furthermore, grip strength testing is widely used and well validated. This observation adds to the body of evidence describing the utility of grip strength measurements in a variety of different healthcare settings; information that is not currently well established [62;235;341].

Conversely, the assessment of quadriceps strength was impractical due to the cumbersome nature of the equipment necessary to perform the test and also lower limb pathology such as fragile skin, ulcers and oedema. These problems have previously been reported and are further compounded by a wide variety in measurement equipment used and different protocols, for example, quadriceps muscle strength varies according to position (supine vs. seated position); some issues may be overcome by using a portable alternative measurement device[342]. Quadriceps strength had been included as a marker of physical functioning in CaSIO given that it has been suggested that changes in grip strength with age do not adequately represent total loss of muscle strength and that the loss of quadriceps strength may occur at a faster rate and have a greater impact on functional status [343]. However, the assessment of grip strength has reproducible properties as a test, allows intra-individual assessments over time and inter-individual assessments within a population, and grip strength dynamometry is also simple, safe, and inexpensive and used widely in the literature and within the European consensus definition. Therefore, within the context of the problems encountered, grip strength was the most appropriate assessment of muscle strength in the CaSIO study.

5.7.2.3 Assessment of fat and muscle mass

The majority of CaSIO participants were unable to tolerate a DXA scan due to logistical difficulties mounting the couch and problems with lying supine. This difficulty is unlikely to be encountered clinically as the investigation is rarely indicated in older, frail people. The

problem has not been reported in the academic literature although difficulties have been reported with the use of other imaging modalities among frail older people. The difficulty of conducting DXA scans in CaSIO is an important observation because current opinion suggests that DXA is the best alternative to gold standards for the assessment of muscle mass in frail older people. Alternative techniques within this population are peripheral CT/MRI scans which are likely to be tolerated but are expensive and not widely available, anthropometry, or bioelectrical impendence analysis (BIA) [55;58].

Anthropometry has previously been used in other studies to characterise fat-free mass and, in younger populations, correlates well with gold standard measures [344]. However, there are relatively few studies validating anthropometric measures in hospitalised older people and within this study, triceps skinfold assessment demonstrated poor associations with BIA assessment of muscle mass. This is likely to be a consequence of the loss of skin elasticity and sub-dermal adipose tissue that occurs with age, causing skin to be pinched by the anthropometric callipers without bringing adipose tissue with it and therefore recording only dermal thickness. These findings suggest anthropometric measures in older people are vulnerable to error and add to a growing sentiment reflected in the literature [55].

BIA is reproducible and correlates well with the gold standard, MRI [61]. Within the CaSIO study BIA was performed on 62% of participants and its output was consistent with reference data available from NHANES III [345]. However, 38% of participants were excluded from BIA due to the strict conditions that are needed in order to perform accurate analyses (e.g. laying supine, no oedema, ensuring an empty bladder) and also contraindications such as fragile skin and skin ulcers that prevent the attachment of electrodes. Therefore, the use of BIA for the assessment of muscle mass in frail older people can, at best, only be cautiously recommended due to the lack of suitable alternative techniques and the prohibitively strict protocols that need to be followed to achieve accurate results. This ambiguous advice is reflected within the literature where some groups, for example, EWGSOP consider it a suitable alternative to DXA yet others, for example the International working group on sarcopenia (IWGOS), deem the technique too unreliable [55;58].

An alternative technique for the assessment of muscle mass in older people may be ultrasonography (USS) which is safe, inexpensive and widely available in healthcare settings. Furthermore, higher quality and more portable USS machines are becoming increasingly available as the technique is used in other areas of healthcare (e.g. chest drain insertion). USS has previously been evaluated against MRI as a technique for cross-sectional assessment of the vastus lateralis muscle of the quadriceps in a small group of healthy young volunteers where the technique was repeatable and demonstrated high validity ($r=0.999$) [346]. Similar results have since been seen when assessing other muscles and also

muscle mass following spinal cord injury and stroke [347;348]. Most recently the technique has been used in older people (mean age 68 years) to measure skeletal muscle mass recovery following coronary heart disease; again the technique was found to be valid and reliable when compared with CT imaging [349]. These early findings indicate that USS may have an important role in the assessment of sarcopenia among older people and suggest that further studies are needed to develop a protocol and to evaluate its properties as a measure of skeletal muscle mass in different populations.

5.7.2.4 Characterisation of body composition in hospitalised older adults: implications

In summary, a number of techniques for the characterisation of muscle mass and function lack utility in hospitalised older adults despite being recommended in major consensus papers (*Table 5-2*). Experience from the CaSIO study suggests that muscle strength might be best assessed using grip strength dynamometry and that the screening assessment of gait speed is unnecessary for the identification of sarcopenia among hospitalised older adults. The gold-standard techniques for the characterisation of muscle mass are peripheral CT/MRI scans which are unsuitable for widespread clinical use. In addition, whole body scanners are unlikely to be tolerated due to well documented problems with claustrophobia and also similar problems to those encountered with DXA scans.

These observations have major implications for the applicability of the IWGOS and EWGSOP definitions of sarcopenia among hospitalised frail older people: the IWGOS definition suggests that all people with poor physical function (97% of the CaSIO population) should undergo DXA scanning, and the EWGSOP definition prioritises the assessment of muscle mass over muscle strength. These observations are also relevant to the grading of sarcopenia on the basis of low muscle mass, with or without low muscle strength or performance, as has been proposed by a number of research groups [55;350].

Table 5-2: Assessment of sarcopenia in hospitalised older people: experience and potential alternatives based on findings from CaSIO

Assessment	Recommended	Rationale and comments
Muscle mass		
Anthropometry	No	Inaccurate, little correlation with BIA
BIA†	Maybe	Accurate under the correct conditions and lack of suitable other technique but multiple contradictions and exclusions
DXA˚	No	Poorly tolerated
pCT/MRI¥	Yes	Expensive and not widely available; radiation associated with CT. Whole body CT/MRI would be more likely to be poorly tolerated than DXA
Other		
USS*	Maybe	Safe, portable and well tolerated; results comparable to MRI/CT in early studies; needs reproducibility studies in older adults [346]
Muscle function		
Gait speed	No	Well tolerated and reproducible but of little discriminatory value as a screening test
SPPB˚˚	No	Difficult to perform and minimal additional benefit to gait speed
Grip strength	Yes	Well tolerated, accurate and reproducible; standard protocol defined [62]
Quadriceps strength	No	Equipment cumbersome and non-standard with no single protocol; often poorly tolerated or contraindication

†Bioelectrical impedance analysis; ˚Dual-energy x-ray absorptiometry; ¥ Peripheral computed tomography or magnetic resonance imaging; *Ultrasonography; ˚˚Short physical performance battery

5.8 Future work

5.8.1 Questions outstanding

This thesis has begun to describe cachexia in hospitalised older people and its association with outcomes. There are a number of outstanding questions which have arisen directly from this work and these will be considered in the following sections.

5.8.2 Additional work within the CaSIO study

5.8.2.1 Additional statistical analyses

There are a number of additional analyses that will be undertaken as part of the CaSIO study; these were not considered as part of the initial analysis plan but have since emerged as having the potential to add value to the study. For example, the ten commonest co-morbidities (*Table 4-4 page 87*) affected a number of CaSIO participants; further analysis is worthwhile to try to explore their associations with cachexia, inflammation, outcomes and to explore their role as confounders. Six of these co-morbidities are diseases of the cardiovascular system, the rest are chronic diseases of other systems; all have previously been associated with increased mortality. If associations are found between these co-morbidities and mortality in CaSIO then these co-morbidities will be included, with cachexia, in multivariate analyses to identify the relative impact of cachexia and co-morbidity on mortality. Similar analyses will be carried out on the basis of the most common reasons for admission, and the most commonly used groups of medications within CaSIO; these were aspirin, statins, ace inhibitors, loop diuretics, laxatives, calcium and vitamin D, and paracetamol. Associations with statins, ace-inhibitors and vitamin D will be of particular interest due to other research which has suggested possible benefits of these medications in-terms of muscle, physical functioning and mortality (as discussed in *section 5.5.3* of this thesis) [40;309-314;351].

5.8.2.2 Additional biomarker characterisation

Serum was stored during the laboratory phase of the CaSIO study which provides the opportunity for further analyses of biomarkers. Therefore, the next phase of additional work involves determining biomarker concentrations within the endocrine axis, specifically DHEA, IGF-1 and vitamin D. Characterisation of DHEA would help establish whether the lack of association with DHEAS in this study was due to its dissociated form, DHEA being the biologically active molecule; this has been previously been described within the academic

literature [119]. IGF-1 is the principle anabolic hormone involved in muscle hypertrophy and previous studies have shown associations with skeletal muscle; levels decline with age and low levels may be implicated with increased vulnerability to cachexia [30;31;352]. Vitamin D is widely prescribed in older people; it is also anti-inflammatory and is implicated in skeletal muscle strength and functioning although, as previously discussed, data are inconsistent. Exploration of the relationship between vitamin D and cachexia and mortality in CaSIO would augment understanding of its role within the context of accelerated skeletal muscle loss. DHEA, IGF-1 and vitamin D are all the focus of ongoing trials which aim to identify therapeutic skeletal muscle interventions and may therefore also emerge as valuable in the treatment of cachexia in older people.

Additional biomarkers to consider for future analysis in CaSIO are adipokines, biologically active molecules secreted by adipose tissue. The classic adipokine is leptin which is pro-inflammatory and also has important role in metabolism and energy homeostasis; adiponectin and ghrelin have opposite actions and are anti-inflammatory. Therefore, a greater understanding of these adipokines in hospitalised older people covers the domains of inflammation, body composition and cachexia – they may offer useful insights into the aetiology of cachexia in older people and also offer therapeutic targets [115].

Finally, the laboratory work for this study was performed at the Centre for Musculoskeletal Ageing Research at the University of Birmingham. This research group has specific expertise in characterising inflammation which has provided the opportunity to perform novel analyses to help characterise inflammation in older people. Damage associated molecular pattern molecules (DAMPs) are intracellular molecules that are released as a consequence of cellular damage and initiate and perpetuate inflammatory responses; they are emerging as a driver of inflammation in younger trauma victims (e.g. soldiers) and also those with chronic inflammatory disease (e.g. rheumatoid arthritis). Hospitalised older people experience high levels of disease, infection and immobility and it is therefore likely that they also have high levels of DAMPs; these may also be partially responsible for driving inflammation and contributing to cachexia in this group [353-355]. Examples include mitochondrial DNA and high mobility group box-1, these have yet to be characterised in older people but CaSIO provides this opportunity and also the opportunity to test for associations with cachexia, muscle, inflammation and outcomes.

Metabolomics is a technique that uses separation and then spectrometry to characterise a biological system of molecules (the metabolome) which is considered to be the final fingerprint of genetic regulation, enzymatic activity, systemic and environmental influence. All individuals therefore have a unique metabolome which represents acute and chronic processes, including inflammation. The metabolome is excreted via the kidneys and may

ultimately be detectable via a urine test - offering a straightforward way to characterise complex systemic processes within clinical environments. Characterising the metabolome in older people is novel and may represent differing degrees of accumulative and acute inflammation. Linking this with outcomes will help to explore its potential as a clinically accessible biomarker.

5.8.2.3 Additional data collection

A natural extension of CaSIO would be to study hospitalised older men as well as more women. This would constitute a significant amount of work and resources and any extension study would need to have learned from the limitations of the current study and could explore the value of novel techniques such as the ultrasound in the assessment of muscle mass.

Although not directly applicable to acutely hospitalised older people, it may be more feasible to use pre-existing cohort studies such as the Hertfordshire Cohort Study (HCS) or the 1946 National Survey of Health and Development (NSHD) to explore the findings within this thesis in more detail [356;357]. For example, the HCS comprises a cohort of community dwelling older men and women who are well characterised and currently being seen as part of active and ongoing follow up; an ethics amendment would enable collection of the data needed to characterise cachexia without significant extra time, cost or resources. It is then also likely that this cohort will be followed up into older age, enabling the exploration of longitudinal outcomes.

5.8.2.4 Intervention studies

The findings from CaSIO suggest that it may be possible for older people to change cachexia category and there is also a growing evidence base regarding the treatment of skeletal muscle loss, weight loss and inflammation in older people. However, intervention studies directly for cachexia in older people are lacking - this is an additional natural progression of the current study which could possibly be incorporated within a pre existing observational study or may warrant a separate intervention trial.

An initial example of a realistic and potentially effective intervention within the context of cachexia in older people is comprehensive geriatric assessment (CGA). This is a multidimensional interdisciplinary diagnostic process focused on determining a frail older person's medical, psychological and functional capability in order to develop a coordinated and integrated plan for treatment and long term follow up [358]. CGA addresses issues that

leave older people vulnerable to developing cachexia and also aspects of the syndrome itself; this includes the identification and treatment of medical and psychological illness, medication review and management of physical, nutritional and social needs. A recent meta-analysis of CGA amongst older people admitted to an acute hospital setting concluded that patients who received the intervention were more likely to be alive and in their own home compared with those who received care in a more conventional hospital setting [358]. The authors commented that further work is need to identify which sub-population of older people would benefit most from CGA; the identification of pre-cachexia and cachexia may offer this opportunity.

More targeted interventions for cachexia that might be trialled include nutritional, exercise and pharmacological interventions; the rationale for these approaches was discussed in *section 5.5.3* and strategies for intervention include protein and energy supplementation, leucine, vitamin D, omega 3 polyunsaturated fatty acids and resistance exercise. Pharmacological interventions offer the opportunity for randomised control trials involving established drugs such as ace-inhibitors and also novel medications, for example DHEAS, progesterone agents and thalidomide.

5.8.2.5 Other additional work

The CaSIO study revealed a number of practical difficulties in the characterisation of body composition among older people admitted to hospital. This has significant implications for the characterisation of skeletal muscle in this population and is at odds with current best practice recommendations regarding the definition of both sarcopenia and cachexia [20;39;55]. The practical difficulties encountered in the assessment of body composition in the CaSIO study suggest that there might be benefit in exploring the usefulness of ultrasound assessment of skeletal muscle mass among older people in both the community and in hospital settings.

Chapter 6 : Conclusions

Cachexia is an inflammatory syndrome characterised by skeletal muscle loss. It is well described in association with single disease processes where it is associated with poor outcomes and in which interventions are emerging. Hospitalised older women are particularly vulnerable to cachexia as a consequence of multiple risk factors which include age related skeletal muscle loss and inflammaging; genetic and environmental influences across the lifecourse are implicated.

Cachexia was common among hospitalised older women in this study and appeared to be a dynamic state from which recovery was possible. Women with cachexia were more likely to experience complications from the hospital admission and were also more likely to die during the subsequent two years; cachexia was a better discriminator of these outcomes than weight loss alone, possibly via the combined effects of muscle loss and inflammation which were both also associated with poor outcomes.

Cachexia in older people still needs to be better defined in both hospital and community settings and over longer periods of time. There may be a role for targeted interventions, especially those older people who are developing the syndrome, in order to increase the likelihood of transitioning to a non-cachexia state; further research is needed.

Chapter 7 Appendix

Appendix 1: Study documentation

A1.1 Ethics approval

(Original documentation is filed with the sponsor and available on request)

National Research Ethics Service
Southampton & South West Hampshire REC (A)
Building L27, University of Reading,
London Road, Reading, RG1 5AQ.

30th November 2010

Principle Investigator: Dr Helen Roberts, Consultant, Southampton University Hospital Trust

Study title:

Introduction of mealtime assistance onto an acute medical ward for older people

REC reference:

09/H0502/93

Amendment number:

3

Amendment date:

05 October 2010

Ethical opinion:

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

A1.2 Patient information sheet

PATIENT INFORMATION SHEET

A research study to evaluate the use of mealtime assistance on an acute medical ward for older people

LREC number: 09/H0502/93

We would like to invite you to take part in a research study. Before you decide we would like you to read the following information in order for you to understand why the research is being done and what it will involve.
Part 1 tells you the purpose of this study and what will happen to you if you take part.
Part 2 gives you more detailed information about the conduct of the study.
Take time to decide whether or not you wish to take part.

PART ONE

What is the purpose of the study?
The aim of this study is to see if the use of volunteers helping as mealtime assistants can increase the amount and quality of food patients eat. We want to know if this approach is practical and if patients and staff find it helpful.

Why have I been chosen?
We are asking all patients admitted to two acute medical wards for older people, over a two-year period, to be part of this study.

Do I have to take part?
No, it is up to you to decide whether or not to take part. If you do, you will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
For some patients the nursing staff will help with meals, whilst for others it will be trained volunteers who provide the meal-time assistance. This may include making sure you can reach your food or helping to feed you. They will only assist if you are happy and it is safe to do so. The nursing staff will have overall responsibility for mealtime assistance.
Using different ways of assisting with meal times on the two wards will allow the research team to compare the two ways of working to see which offers the most benefit to patients

and their health. To measure this we would like to collect a variety of data from you both during your hospital stay and at six months after your discharge from hospital.

1. Use of your routinely collected hospital information

We would like to collect existing data from your medical records (both paper and computer records), which would have been obtained during your time in hospital. This would include blood results, weight, nutrition score, medication being taken, information about how you manage at home, any previous and current illness and discharge details. In addition to this we will talk to your nurse and obtain information about your level of activity at the moment.

2. Body measurements

If you agree we would also like to conduct some simple tests for which you can remain clothed and be in bed, and which take about 10-15 minutes to measure. These include measuring

the circumference and length of your arm, and the thickness of your skin on your arm.
your strength by getting you to grip a measuring handle with your hands.
the composition of muscle and water in your body, using a quick and simple test called bio-impedance, which involves placing a sticker on your hand and foot, to pick up small electrical messages from your body (rather like an ecg heart tracing).

If you agree we would like to do all of these tests in the next few days, when you are ready for discharge and when you actually leave hospital, so we can compare the results.

3. Blood tests

We would like to check the levels of three vitamins in your blood, by taking a small amount of extra blood when you are having a routine blood test. After analysis no blood samples will be retained.

A few participants will have additional blood taken on two occasions whist in hospital for measurement of immune functioning. Again, these would only require a small amount of extra blood taken when you are having routine blood tests and after analysis no blood samples will be retained.

4. Questionnaires

We would like to ask you some more questions about your memory, mood, appetite and concentration at the moment. We will also be recording food and drink intake on a few days for everyone in the ward, and may monitor staff activity. When you are ready to leave the hospital, we would like to ask you and/or your next of kin to complete a short questionnaire telling us about your experience of the food, your satisfaction with mealtimes and your well-being.

Additionally we hope to interview a few patients or relatives from each ward for around 20 minutes (either on the ward or in a private room, according to their choice) about their views on mealtimes and nutrition in hospital in more depth.
Finally we would also like to contact you briefly about six months after your hospital discharge by phone to ask you how your health has been. We would contact your GP prior to this to check your current location, and the appropriateness of contacting you or your next of kin.

5. Additional optional tests

a) A single DXA body composition scan will be carried out on a few participants. This is a type of x-ray which is a fast and painless procedure which involves lying down fully clothed on a couch for 15 minutes while a scanning arm passes over your body.
b) A few participants in each ward will have their mobility over an 8 hour period assessed using a small activity monitor attached to their leg, on two days whilst they are in hospital. Specific consent will be asked for this, part of which is a medical student project supervised by the research team.
c) A few participants will have a more detailed assessment of physical performance, taking no more than 30 minutes. The strength of your leg will be measured by sitting on a chair and straightening it at the knee against resistance. You will also be asked to walk 3 metres, stand up from a chair and stand one leg whilst being timed. If you are unwilling or unable to perform any of these tasks, they will not be attempted.
d) A few participants will be visited at home after discharge from hospital; up to three times over one year. This is to monitor changes in circumstances, health and well-being. Your GP will be contacted to establish any changes that may have occurred and then an appointment will be made with you over the telephone. A trained researcher will visit you at a convenient time for no more than one hour. During this time some of the questionnaires and physical tests will be repeated. We will also take an additional blood test for immune measurements that will be disposed of after analysis.

Expenses and Payment

There is no payment for participants in this study.

Are there any risks or disadvantages associated with taking part?
There are no risks for those patients (or relatives) agreeing to just the use of routine data, bioimpedance or assessment of well-being and grip strength. Bio-impedance cannot be done on individuals who have a pacemaker or similar electronic device. Some individuals may find the body measurements slightly uncomfortable but this is a quick measurement to take and the researcher would stop at your request. The vitamin status will be assessed as part of a routine blood test although will require approx a small additional blood sample.

Specific consent will be obtained for the DXA scan which is discussed in the next section. Specific consent would be obtained for the activity monitor, which is very small and light, so little burden and no associated risks.
The individual interviews will be anonymised but we recognise that this will be an additional task lasting around 20 minutes, and patients and carers will be selected from those that are willing to consent to this specifically.

Ionising Radiation (Medical Exposure) Regulations-IRMER
A single DXA body composition scan will be carried out on participants who are fit enough to travel to the scanner on another floor at SGH and give specific consent. The radiation exposure is very low, equivalent of spending one day in Cornwall.

What are the possible benefits of taking part?
There are benefits from participating in a research study e.g. you and your clinical team will have information about your health and body composition that would not be part of your usual care. The information that is obtained during this study will allow us to determine if there is any benefit to specific meal time assistance and then make recommendations to improve future patient care.

What happens when the research study stops?
Usual mealtime assistance on both wards will resume.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might have suffered will be addressed. More detailed information on this is given in part two of the sheet.

Will my taking part in the study be kept confidential?
Yes, we will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART TWO

What if new information becomes available?
A member of the research team will tell you and discuss whether you would like to continue in the study. If you decide not to continue in the study your care will continue as usual on the ward. If you decide to continue in the study we may ask you to sign an updated consent form. If at any time the research team consider it to be in your best interest to withdraw from the study, this would be discussed with you and your care would continue as usual on the ward. If for any reason the research study stopped we would inform you.

What will happen if I don't want to carry on with the study?
You can let us know at any time if you do not wish to participate in the study. No further assessments would be made but we would like to retain the use of anonymised routine data and any data already collected. Similarly it would be important for this study to be able to record patient outcomes such as discharge details.

What if there is a problem?
If you have any cause for concern regarding your participation in the trial, please contact one of the researchers in the first instance (see contact details at the end of this sheet). If this is unsatisfactory, they will be able to direct you to an alternative person who will be able to help.
If you have a complaint, which cannot be resolved by these measures, you may wish to complain formally. You can do this through the NHS Complaints Procedure. Details can be obtained from Southampton University Hospital Trust. Southampton University Hospital Trust sponsors this study and provides indemnity against clinical negligence during the study.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. We will inform your GP and Hospital Consultant that you are participating in the study. Additionally we will contact your GP prior to the six month follow-up phone-call.
We will keep a record of your contact details so that we can contact you for follow-up but these will be stored securely and only accessed by direct members of the research team. Any other information about you will have your name and address removed so that you cannot be recognised from it. In the analysis of results, your data will be used anonymously. Our procedures for handling, processing, storing and destroying data relating to your

participation in the study are compliant with the Data Protection Act 1998. In accordance with this Hospital's regulations we are required to keep your data secure for 15 years. For the purposes of monitoring research there is a possibility that the hospital's Research and Development department will audit the data that we have collected.

What will happen to the results of the research study?
The results of the research will be published in medical scientific journals. Research staff may also present the results at conferences and local meetings. You will not be identified in any report produced.

Who is organising and funding the research?
This research is being funded by the National Institute of Health Research, part of the Department of Health.

Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the local research ethics committee and has been reviewed by the research and development team at Southampton University Hospitals NHS Trust.

This information sheet is for you to keep. If you are interested in participating in this study, please speak to your nurse who will contact the research team. Thank you very much for reading this information and considering taking part in the study.

For any further information please contact either

Dr Helen Roberts, Senior Lecturer in Geriatric Medicine

or

Norma Diaper, Senior Research Nurse

Southampton General Hospital

Telephone 023 8079 4354

A1.3 Participant consent form

PATIENTS INFORMED CONSENT FORM

A research study to evaluate the use of mealtime assistance on an acute medical ward for older people

LREC number: 09/H0502/93
Participant ID:
Name of Principal Investigator: Dr Helen Roberts

Thank you for reading the information about our research project. If you would like to take part, please read and sign this form.

PLEASE INITIAL THE BOXES IF YOU AGREE WITH EACH SECTION:

1.	I have read the information sheet version.....dated .....for the above study and have been given a copy to keep. I have been able to ask questions about the study and I understand why the research is being done. I have been informed about any risks or inconveniences involved and the conditions under which the study is to be conducted.	☐
2.	I understand that I can withdraw from the study at any time without my medical treatment or legal rights being affected.	☐
3.	I agree to my Physician being informed of my participation in this study and my GP being contacted to check the appropriateness of a follow-up contact and any changes in my contact details.	☐
4.	I understand that relevant sections of my medical notes and data collected during the study, maybe looked at by responsible individuals from the research team, from regulatory authorities or from the NHS trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
5.	I agree for someone from the research team to look at my records to obtain the information as described in the use of routinely collected hospital data part of the information sheet.	☐
6.	I agree that if I withdraw from this study, all data that has been collected up to this point can still be used, in an anonymised form in the final analysis.	☐
7.	I agree to participate in the body measurements assessment, as outlined in the information sheet.	☐

8.	I agree to participate in the questionnaires assessment as outlined in the information sheet.	☐
9.	I agree that additional blood can be taken during up to 2 routine blood tests. I understand what this blood will be used for and that all samples will be disposed of after analysis.	☐
10.	I agree to follow up data being collected after discharge from hospital and understand that this may include home visits for further assessments and blood tests as detailed in the information sheet.	☐
11.	I agree to participate in further assessment of my physical performance via the leg strength and mobility tests outlined in the information sheet.	☐
12.	I agree to my interview being audio taped and I understand that transcripts of my interview will be anonymised.	☐
13.	I agree to have a DXA Scan and the results being used by the research team.	☐
14.	I agree to have my mobility monitored by an Activpal (an activity monitor, attached to my leg for an eight hour period, twice during my hospital admission) and the results being used by the research team.	☐

.....
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Name of patient Date Signature

.....

Person taking consent (If not researcher) Date Signature

.....
Researcher Date Signature

Original for investigator site file/researcher
1 copy for participant
1 copy for medical record/hospital notes

A1.4 Data collection sheets

The data collection sheets used in CaSIO were prohibitively long to include within this thesis. For that reason all the individual questionnaires have been detailed in the methods section. Below is an example of the conversion chart used to estimate body height from ulna length.

Figure 18: Ulna length to body height conversion chart (Malnutrition Advisory Group) [186]

Measured Ulna-length (cm)	Estimated body height (m)			
	Men ≤ 65 years	Men >65 years	Women ≤ 65 years	Women >65 years
18.5	1.46	1.45	1.47	1.40
19.0	1.48	1.46	1.48	1.42
19.5	1.49	1.48	1.50	1.44
20.0	1.51	1.49	1.51	1.45
20.5	1.53	1.51	1.52	1.47
21.0	1.55	1.52	1.54	1.48
21.5	1.57	1.54	1.55	1.50
22.0	1.58	1.56	1.56	1.52
22.5	1.60	1.57	1.58	1.53
23.0	1.62	1.59	1.59	1.55
23.5	1.64	1.60	1.61	1.56
24.0	1.66	1.62	1.62	1.58
24.5	1.67	1.63	1.63	1.60
25.0	1.69	1.65	1.65	1.61
25.5	1.71	1.67	1.66	1.63
26.0	1.73	1.68	1.68	1.65
26.5	1.75	1.70	1.69	1.66
27.0	1.76	1.71	1.70	1.68
27.5	1.78	1.73	1.72	1.70
28.0	1.80	1.75	1.73	1.71
28.5	1.82	1.76	1.75	1.73
29.0	1.84	1.78	1.76	1.75
29.5	1.85	1.79	1.77	1.76
30.0	1.87	1.81	1.79	1.78
30.5	1.89	1.82	1.80	1.79
31.0	1.91	1.84	1.81	1.81
31.5	1.93	1.86	1.83	1.83
32.0	1.94	1.87	1.84	1.84

Appendix 2: Intra and inter-observer variation tables

A2.1 Grip strength

Daniel Baylis measurement of grip strength (kilograms) against the gold standard (Dr. Helen Roberts)

	Observer 1	Right 1	Right 2	Left 1	Left 2	Observer 2	Right 1	Right 2	Left 1	Left 2	1 max	2max
Subject 1	DB	32	32	34	34	Helen R	27	30	30	31	31	34
Subject 2	Helen R	36	34	33	33	DB	32	32	32	32	36	32
Subject 3	DB	36	34	36	34	Helen R	35	33	32	31	35	36
Subject 4	Helen R	44	46	36	38	DB	36	46	40	40	46	46
Subject 5	DB	14	12	14	14	Helen R	13	13	14	12	14	14
Subject 6	Helen	6	6	2	3	DB	2	2	5	6	6	6
Subject 7	DB	10	8	8	6	Helen	5	8	8	6	8	10
Subject 8	Helen R	10	10	10	9	DB	11	12	8	10	10	12
Subject 9	DB	12	10	10	10	Helen R	16	15	9	8	16	12
Subject 10	Helen R	27	28	24	22	DB	24	26	22	24	28	26
									Inter observer mean variation			**0.2**
Subject 1	DB	32	32	34	34	DB	32	30	32	30	34	32
Subject 2	DB	32	32	32	32	DB	36	36	34	32	32	36
Subject 3	DB	36	34	36	34	DB	34	30	30	28	36	34
Subject 4	DB	36	46	40	40	DB	missing	missing	missing	missing	46	missing
Subject 5	DB	14	12	14	14	DB	12	12	14	14	14	14
Subject 6	DB	2	2	5	6	DB	2	2	8	8	6	8
Subject 7	DB	10	8	8	6	DB	8	8	10	8	10	10
Subject 8	DB	11	12	8	10	DB	10	8	8	8	12	10
Subject 9	DB	12	10	10	10	DB	16	16	10	12	12	16
Subject 10	DB	24	26	22	24	DB	24	24	24	24	26	24
									Intra observer mean variation			**0.2**

Appendix 3: Scientific output

A3.1 Inflammaging review article

Reference: Baylis D, Bartlett DB, Patel HP, Roberts CR. Understanding how we age: insights into inflammaging. *Longevity & Healthspan.* 2013, 2:8

Inflammaging is characterised by the upregulation of the inflammatory response that occurs with advancing age; its roots are strongly embedded in evolutionary theory.
Inflammaging is believed to be a consequence of a remodelling of the innate and acquired immune system resulting in chronic inflammatory cytokine production.
Complex inter-related genetic, environmental and age-related factors determine an individual's vulnerability or resilience to inflammaging; including polymorphisms to the promoter regions of cytokines, cytokine receptors and antagonists; age-related decreases in autophagy and increased adiposity. Anti-inflammaging describes the upregulation of the hypothalamic-pituitary axis in response to inflammaging, leading to higher levels of cortisol which in turn may be detrimental, contributing to less successful ageing and frailty. This may be countered by the adrenal steroid dehydroepiandosterone which itself declines with age leaving certain individuals more vulnerable. Inflammaging and anti-inflammaging have both been linked with a number of age related outcomes including chronic morbidity, functional decline and mortality. This important area of research offers unique insights into the ageing process and the potential for screening and targeted interventions.

Chapter 8 Results appendix

RA 1 Complementary results tables

RA 1.1: Loss to follow up

16% of participants were lost to follow-up and still alive at 6 months. The following table compares this group with the 103 participants who were followed up at 6 months.

Table RA 1: Comparison of 6 month follow up group with those lost to follow-up

	Lost to follow-up *n = 24*	Followed up *n = 103*	*p-value*
	n/d(%)	n/d(%)	
Age (years) †	87 (5.0)	86 (4.5)	*0.565*
Maximum grip strength (kg) †	13 (6.9)	13 (5.4)	*0.700*
Category of domicile			
Private home, alone	14/24 (58)	58/103 (56)	*0.144*
Private home, others	4/24 (17)	25/103 (24)	
Sheltered/warden	3/24 (13)	11/103 (11)	
Residential	3/24 (13)	7/103 (7)	
Nursing	0/0 (0)	2/103 (2)	
Prior care (yes)	15/22 (68)	59/98 (60)	*0.629*
Barthel index†	62 (27)	66 (29)	*0.566*
MMSE†	21 (5)	25 (4)	*<0.001*
GDS†	3.6 (2.9)	4.3 (2.5)	*0.201*
Fatigue (yes)	10/18 (56)	74/82 (90)	*0.001*
Anorexia (yes)	10/18 (56)	43/82 (53)	*1.000*
Inactive (yes)	5/17 (29)	39/82 (48)	*0.188*
Falls (yes)	11/18 (61)	56/81 (70)	*0.576*
Discharge bloods†			
WCC (log $x10^9$/L)	1.96 (0.3)	2.05 (0.4)	*0.285*
Neutrophils (log $x10^9$/L)	1.53 (0.4)	1.63 (0.5)	*0.335*
CRP (log mg/L)	2.41 (1.1)	2.34 (1.2)	*0.748*
Albumin (log g/L)	3.41 (0.2)	3.45 (0.2)	*0.447*
Cortisol (log ng/ml)	6.0 (0.7)	6.0 (0.5)	*0.580*
DHEAS (log ng/ml)	0.25 (0.8)	0.26 (0.64)	*0.956*
Cortisol:DHEAS	0.45 (1.1)	0.21 (0.8)	*0.317*

n/d numerator denominator; p-values from Chi-squared or Fishers exact tests; †mean (SD), p-values from ANOVA; bloods log transformed

RA 1.2 Additional data collected prior to discharge

Limited data was re-collected prior to discharge to assess how the population had changed during the admission. Barthel index was rescored prior to discharge from hospital and if the period between baseline data collection and discharge was greater than 5 days then weight, BMI and grip strength measurements were also repeated. These results are presented in *Table RA 2*.

Table RA 2: Comparison between variables collected at baseline and prior to discharge

		On admission		On discharge		*p-value*
	n	Median	IQR†	Median	IQR†	
Barthel	141	68	41, 94	88	57, 88	*<0.001*
Weight (kg)	92	59	51, 70	58	50, 70	*0.079*
BMI	92	24	20, 28	23	20, 29	*0.093*
Maximum grip (kg)	53	13	8, 16	12	9, 16	*0.598*

†IQR Interquartile range, p-values from paired Wilcoxon signed rank tests

RA 1.3 Cross-sectional associations with muscle strength at 6 month follow-up

Table RA 3: Cross-sectional associations with grip strength at 6 month follow up

	Grip (Kg)			*p-value*
	≤12	13-16	16+	
	n/d(%)	n/d(%)	n/d(%)	
Age†	87 (4.8)	87 (4.5)	85 (4.1)	*0.531*
Category of domicile				
Private home, alone	13/37 (35)	18/32 (56)	19/34 (56)	*0.003*
Private home, others	8/37 (22)	8/32 (25)	6/34 (18)	
Sheltered/warden	6/37 (16)	4/32 (13)	5/34 (15)	
Residential	3/37 (8)	0/32 (0)	4/34 (12)	
Nursing	7/37 (19)	2/32 (6)	0/34 (0)	
Package of care (yes)	17/47 (36)	13/30 (43)	11/29 (38)	*0.156*
Barthel score†	64 (28)	82 (21)	87 (13)	*<0.001*
MMSE†	23 (4.5)	26 (3.1)	27 (3.1)	*<0.001*
GDS†	5 (2.7)	5 (3.3)	3 (2.3)	*0.028*
Fatigue (yes)	28/37 (76)	17/32 (53)	17/33 (52)	*0.069*
Anorexia (yes)	17/33 (52)	19/35 (54)	15/29 (52)	*0.982*
Inactive (yes)	27/37 (73)	20/32 (63)	16/33 (48)	*0.011*
Falls (yes)	19/36 (53)	12/32 (38)	17/33 (52)	*0.875*
3m walk (secs)†	2.6 (0.6)	2.2 (0.8)	2.1 (0.6)	*0.030*
Frailty status				
Non-frail	0/31 (0)	3/30 (10)	9/27 (33)	*<0.001*
Pre-frail	2/31 (6)	7/30 (23)	6/27 (22)	
Frail	29/31 (94)	20/30 (67)	12/27 (44)	
CRP (log mg/L)	1.7 (1.7)	2.2 (1.6)	2.1 (1.7)	*0.971*
IL-1 (log pg/ml)†	0.4 (1.2)	0.5 (0.9)	0.4 (0.6)	*0.869*
IL-6 (log pg/ml)†	2.3 (0.8)	3.1 (0.8)	2.9 (0.8)	*0.123*
IL-8 (log pg/ml)†	1.6 (1.4)	1.7 (1.4)	1.3 (1.1)	*0.430*
IL-10 (log pg/ml)†	1.6 (1.4)	1.7 (1.4)	1.3 (1.1)	*0.988*
Cortisol (log ng/ml)†	4.9 (0.4)	4.9 (0.3)	4.7 (0.5)	*0.013*
DHEAS (log ng/ml)†	5.9 (1.9)	5.2 (1.6)	5.1 (1.0)	*0.264*
Cortisol:DHEAS†	1.1 (2.0)	1.2 (1.8)	1.2 (1.6)	*0.617*
IL-6:IL-10†	7 (10)	4.4 (6.8)	1.5 (4.0)	*0.002*

†Mean(SD), n/d numerator denominator; p-values from logistic regression for grip strength; blood results log transformed

RA 1.4 Relationships with frailty

Fried frailty was assessed at 6 month follow up and this offered an opportunity to explore cross-sectional associations (presented below) and also its relationship with sarcopenia and cachexia.

Table RA 4: Cross-sectional associations with frailty at six month follow up

	Not frail (*n=27*)	Frail (*n= 62*)	*p-value*
	n/d(%)	n/d(%)	
Age (years) †	84 (3.7)	86 (4.6)	*0.203*
Category of domicile			
Private home, alone	15/27 (56)	29/62 (47)	*0.110*
Private home, others	8/27 (30)	13/62 (21)	
Sheltered/warden	1/27 (3)	12/62 (19)	
Residential	3/27 (11)	3/62 (5)	
Nursing	0/27 (0)	5/62 (8)	
Package of care (yes)	1/25 (4)	54/54 (100)	*<0.001*
Barthel index†	95 (8.4)	73 (24)	*<0.001*
MMSE score†	27 (3)	25 (4)	*0.031*
GDS score†	2.2 (2.8)	5.8 (2.7)	*<0.001*
Fatigue (yes)	6/27 (22)	54/62 (87)	*<0.001*
Anorexia (yes)	9/27 (33)	38/61 (62)	*0.020*
Inactive (yes)	6/27 (22)	49/62 (79)	*<0.001*
Falls (yes)	8/27 (30)	31/61 (51)	*0.065*
3m walk (secs)†	1.9 (0.6)	2.6 (0.7)	*<0.001*
CRP (log mcg/ml)†	2.2 (1.5)	1.9 (1.8)	*0.388*
IL-1 (log pg/ml)†	1.1 (1.2)	0.8 (1.3)	*0.773*
IL-6 (log pg/ml)†	2.2 (1.9)	2.2 (1.9)	*0.223*
IL-8 (log pg/ml)†	3.1 (1.2)	3.1 (0.7)	*0.978*
IL-10 (log pg/ml)†	1.2 (1.2)	1.7 (1.4)	*0.393*
Cortisol (log ng/ml)†	3.9 (0.8)	2.7 (2.6)	*0.323*
DHEAS (log mcg/ml)†	4.6 (0.5)	4.9 (0.4)	*0.004*
Cortisol:DHEAS†	5.1 (1.0)	5.5 (1.9)	*0.002*
IL-6:IL-10†	0.4 (0.9)	0.5 (1.2)	*0.109*

†Mean(SD), n/d numerator denominator; p-values from logistic regression for grip strength; blood results log transformed

RA 1.5 Associations with 6 month mortality or hospital readmission

58 participants were readmitted to hospital a median of 67 days after discharge (IQR 21-112 days). *Table RA 5* presents associations between baseline characteristics and mortality or readmission.

Table RA 5: Associations between baseline exposures and 6 month mortality or hospital readmission; univariate analyses, unadjusted

Exposure (at baseline unless stated)	n	HR	CI	*p-value*
Cachexia (yes vs. no)	118	1.52	0.87 - 2.66	*0.146*
Age (per year older)	145	0.98	0.93 - 1.03	*0.444*
Max. grip (per kg increase)	136	0.99	0.94 - 1.03	*0.555*
Sarcopenia (yes vs. no)	96	1.33	0.71 - 2.49	*0.377*
Weight (per kg increase)	138	1.01	1.00 - 1.03	*0.094*
BMI (per point increase)	138	1.04	1.00 - 1.08	*0.050*
Anorexia (yes vs. no)	115	0.91	0.54 - 1.56	*0.742*
Fatigue (yes vs. no)	115	1.09	0.55 - 2.17	*0.807*
Barthel index (per point increase)	144	1.00	0.99 - 1.01	*0.509*
Barthel index on discharge (per point increase)	142	1.00	0.99 - 1.00	*0.324*
Inactivity (yes vs. no)	114	1.54	0.89 - 2.65	*0.122*
History of falls (yes vs. no)	114	1.31	0.97 - 1.77	*0.081*
Geriatric depression scale (per point increase)	108	1.07	0.98 - 1.17	*0.137*
Mini mental state examination (per point increase)	141	0.99	0.93 - 1.05	*0.704*
Category of domicile	145			
Private home, alone		1.00		
Private home, shared		1.25	0.69 – 2.26	*0.462*
Sheltered/warden		1.73	0.91 – 3.26	*0.093*
Residential home		1.27	0.45 – 3.57	*0.653*
Inflammatory biomarkers (per SDS)				
C-reactive protein	143	0.82	0.63 - 1.05	*0.115*
White cell count	145	1.09	0.86 - 1.39	*0.482*
Neutrophil count	145	1.08	0.84 - 1.37	*0.547*
Albumin	135	1.06	0.81 - 1.38	*0.657*
C-reactive protein on discharge	141	0.89	0.70 - 1.13	*0.330*
White cell count on discharge	144	1.03	0.81 - 1.30	*0.814*
Albumin on discharge	139	1.02	0.80 - 1.30	*0.897*
IL-6 on discharge	97	0.87	0.65 - 1.17	*0.349*
IL-8 on discharge	106	1.21	0.92 - 1.59	*0.180*
IL-10 on discharge	106	0.79	0.57 - 1.11	*0.178*
Cortisol on discharge	102	1.14	0.84 - 1.54	*0.399*
DHEAS on discharge	107	1.01	0.77 - 1.32	*0.945*
Cortisol:DHEAS on discharge	100	1.09	0.81 - 1.47	*0.572*
IL-8:IL-10 on discharge	106	1.27	0.92 - 1.76	*0.147*
IL-6:IL-10 on discharge	96	0.97	0.73 - 1.28	*0.812*

HR = Hazard ratio and 95% confidence interval for unadjusted model only; p-values from Cox's proportional hazards model; SDS standard deviation score

RA 1.6: Defining sarcopenia within the study population

Cut-off points for muscle strength

Maximum grip strength was used to define muscle function. Consensus cut-off values and reference data in equivalent populations is lacking; the European working group on sarcopenia (EWGSOP) suggest using 20kg as a cut off of in women. Within the CaSIO population this resulted in only 13% of participants falling above this defined cut off, a proportion similar to other studies of sarcopenia in populations over 80 years in both acute hospital and rehabilitation settings [56;77]. For this reason the use of a more stringent cut-off (15kg) was also explored, which was derived from data previously published by Kerr within a population similar to the CaSIO study [56].

Using these cut offs 87% of participants fell into the sarcopenia category when using the EWGSOP definition and 66% of participants fall into the sarcopenia category when using the Kerr definition, *Table RA 6.*

Cut-off points for muscle mass

Consensus cut-off values and references in equivalent populations to CaSIO are also deficient when defining muscle mass using bioelectrical impedance assessment (BIA). EWGSOP uses reference data from the Third National Health and Nutrition Examination Survey during 1988-1994 (NHANES III) and suggests cut off points in women which utilise a skeletal muscle mass index calculation as follows: severe sarcopenia ≤5.75 kg/m^2, moderate sarcopenia 5.76-6.75 kg/m^2, normal muscle ≥6.76 kg/m^2 [61]. This data is taken from non-Hispanic white females and is limited to a maximum age group of 70-80 years [345]. Despite this being a younger population to CaSIO, the mean skeletal muscle mass figures are comparable: 42.3 kg/m^2 (SD 6.5) in NHANES III and 41.7 kg/m^2 (SD 9.6) in CaSIO. Therefore, when sarcopenia is defined using a muscle mass index cut off defined as <6.76 kg/m^2 29% of the CaSIO population fall within this category.

Schutz *et al.* studied fat-free mass index and fat mass index percentiles in Caucasians aged 18-98 years and included the category 75-98 years with a median fat-free mass of 15.9 kg/m^2. This reference data is therefore more closely translatable to the CaSIO population and this was also used to explore different cut-off points within the study using the 25th percentile, <14.7 kg/m^2, to define sarcopenia. In the CaSIO study, 33% of participants were within this category, *Table RA 6* [191].

Table RA 6: Sarcopenia cut-offs at baseline according to different grip strength and BIA cut-offs

	Frequency numerator/denominator	Percentage
Grip strength (<20kg)†		
No sarcopenia	18/139	13
Sarcopenia	121/139	87
Grip strength (<15kg)˚		
No sarcopenia	48/139	35
Sarcopenia	91/139	65
Skeletal muscle mass index (<6.76 kg/m²)*		
No sarcopenia	65/92	71
Sarcopenia	27/92	29
Fat free mass index (<14.7 kg/m²) ʸ		
No sarcopenia	61/92	67
Sarcopenia	31/92	34

†European working group definition [55]; ˚Kerr et al. [56]; *Skeletal muscle mass index [61]; ʸ Fat free mass index [191]

4.2.4.4 Exploring different definitions of sarcopenia

On the basis of these exploratory analyses sarcopenia within the CaSIO study was defined according to EWGSOP definition (†) as this was considered to be most consistent with current research practice. Sarcopenia was also described according to grip strength as a continuous variable in order to substantially increase the power to detect significant associations.

Table RA 7: Prevalence of sarcopenia at baseline according to different cut-offs selected

n/d numerator denominator (%)	Grip strength <20kg	Grip strength <15kg
Skeletal muscle mass index <6.76 kg/m²	24/92 (26%)†	17/92 (19%)
Fat free mass index <14.7 kg/m²	26/92 (28%)	20/92 (22%)

†European working group on sarcopenia in older people definition

RA 2: Inflammatory marker associations

White cell count (WCC), neutrophil count, C-reactive protein (CRP) and albumin were all performed immediately within the hospital pathology laboratories and underwent an internal control process necessary for the quality standards that are required for clinical care. These were available on 93-100% of participants at baseline and 96-99% of participants at discharge.

The more complex immune-endocrine analysis occurred on frozen samples within a laboratory setting. These were more vulnerable to error due to cytokine induction by anticoagulant during phlebotomy, coagulation interfering with analysis and degradation of samples during handling; this was especially true of samples collected in the community in non-clinical settings with a time lapse to transport samples back to the laboratory for freezing [333]. Error was minimised by adhering to strict protocol including the collection of samples at the same time of day, immediate processing and freezing at -80°C, avoiding freeze-thawing and performing the analyses in a lab with extensive experience and expertise. After cleaning and the removal of extreme outliers, results were available on 69 – 75% of participants at discharge and 29-78% at 6 months follow up. Analyses for TNF and IL-1 were commonly below the detectable range of the assays used which reduced numbers, particularly at follow up.

Table RA 8 to *Table RA 13* describe the associations between the inflammatory markers measured as part of inflammaging within this study and demonstrated that the immune-endocrine analyses behaved as expected with clear patterns between baseline and follow up and significant correlation between pro-inflammatory cytokines, acute phase proteins and cellular markers.

Table RA 8: Associations between inflammatory markers taken at baseline

	WCC	Neutrophil	Albumin
CRP	0.4	0.5	-0.4
	(<0.001)	(<0.001)	(<0.001)
WCC	---	0.9	-0.3
		(<0.001)	(0.001)
Neutrophil	---	---	-0.3
			(0.003)

Pearson's correlation coefficient; p-values given underneath in brackets

Table RA 9: Associations between immune-endocrine markers performed in both Southampton General Hospital and University of Birmingham Laboratories

	Bir. CRP	Bir. Cortisol	Bir. DHEAS
UHS CRP	0.8	---	---
	(<0.001)		
UHS Cortisol	---	0.1	---
		(0.187)	
UHS DHEAS	---	---	0.9
			(<0.001)

Pearson's correlation coefficient or Spearman's rank correlation coefficient (DHEAS only, non-parametric); p-values given underneath in brackets

Table RA 10: Associations between inflammatory markers taken on admission and discharge

		Admission			
		WCC	CRP	Neutrophil	Albumin
Discharge	WCC	0.5			
		(<0.001)			
	CRP		0.5		
			(<0.001)		
	Neutrophil			0.5	
				(<0.001)	
	Albumin				0.7
					(<0.001)

Pearson's correlation coefficient; p-values given underneath in brackets

Table RA 11: Associations between immune-endocrine markers taken on discharge

	IL-8	IL-10	Cortisol	DHEAS	CRP	WCC	Albumin
IL-6	0.5	0.7	-0.1	0.0	0.2	0.1	-0.4
	(<0.001)	(<0.001)	(0.520)	(0.717)	(0.045)	(0.219)	(<0.001)
IL-8	---	0.3	0.0	0.0	0.3	0.1	-0.4
		(0.009)	(0.809)	(0.873)	(0.001)	(0.314)	(<0.001)
IL-10	---	---	0.02	-0.02	0.0	0.0	-0.3
			(0.879)	(0.832)	(0.827)	(0.904)	(0.002)
Cortisol	---	---	---	-0.04	0.0	0.1	0.0
				(0.685)	(0.813)	(0.471)	(0.976)
DHEAS	---	---	---	---	0.0	0.0	-0.1
					(0.795)	(0.736)	(0.207)
CRP	---	---	---	---	---	0.2	-0.3
						(0.079)	(0.0002)
WCC	---	---	---	---	---	---	-0.1
							(0.272)

Pearson's correlation coefficient or Spearman's rank correlation coefficient (DHEAS only, non-parametric); p-values given underneath in brackets

Table RA 12: Associations between pro- and anti-inflammatory ratios

	IL-8:IL-10	IL-6:IL-10
Cortisol:DHEAS	0.0	0.0
	(0.718)	(0.707)
IL-8:IL-10	---	0.33
		(0.001)

Pearson's correlation coefficient; p-values given underneath in brackets

Table RA 13: Associations between inflammatory markers at discharge and 6 month follow-up

	IL-6	IL-8	IL-10	Cortisol	DHEAS	CRP
IL-6	0.3					
	(0.031)					
IL-8		0.0				
		(0.991)				
IL-10			0.5			
			(<0.001)			
Cortisol				0.183		
				(0.122)		
DHEAS					0.6	
					(<0.001)	
CRP						-0.4
						(0.032)

Pearson's correlation coefficient or Spearman's rank correlation coefficient (DHEAS only, non-parametric); p-values given underneath in brackets

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