FRAILTY AND ANABOLIC HORMONES IN AGEING MEN

A thesis submitted to the University of Manchester for the degree of
Doctor of Philosophy
in the Faculty of Medical and Human Sciences

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<tbody>
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<td>1 Repetition Maximum</td>
</tr>
<tr>
<td>6MWT</td>
<td>6 Minute Walk Test</td>
</tr>
<tr>
<td>95% CI</td>
<td>95% Confidence Intervals</td>
</tr>
<tr>
<td>ADL</td>
<td>Activities of Daily Living</td>
</tr>
<tr>
<td>ALF</td>
<td>Aggregate Locomotor Function test</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis Of Co-Variance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis Of Variance</td>
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<td>AUC</td>
<td>Area Under the Curve</td>
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<tr>
<td>BDI</td>
<td>Becks Depression Inventory</td>
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<tr>
<td>BIA</td>
<td>Bioelectrical Impedance Analysis</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<td>CHS</td>
<td>Cardiovascular Health Study</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross Sectional Area</td>
</tr>
<tr>
<td>CSHA</td>
<td>Canadian Study of Health and Aging</td>
</tr>
<tr>
<td>DHEA</td>
<td>Dehydroepiandrosterone</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone Sulphate</td>
</tr>
<tr>
<td>DHT</td>
<td>Dihydrotestosterone</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>E2</td>
<td>Oestradiol</td>
</tr>
<tr>
<td>ELSA</td>
<td>English Longitudinal Study of Ageing</td>
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<td>EMAS</td>
<td>European Male Ageing Study</td>
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<td>EPI DOS</td>
<td>Epidemiology of Osteoporosis Study</td>
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<tr>
<td>FI</td>
<td>Frailty Index</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
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<td>GH</td>
<td>Growth Hormone</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
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<tr>
<td>Health ABC</td>
<td>Health Aging and Body Composition study</td>
</tr>
<tr>
<td>HIMS</td>
<td>Health in Men Study</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HOMA</td>
<td>Homeostatic Model Assessment</td>
</tr>
<tr>
<td>IADL</td>
<td>Instrumental Activities of Daily Living</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like Growth Factor-1</td>
</tr>
<tr>
<td>IGF-BP</td>
<td>Insulin-like Growth Factor Binding Protein</td>
</tr>
<tr>
<td>IKE-PT</td>
<td>Isokinetic Knee Extension Peak Torque</td>
</tr>
<tr>
<td>IKF-PT</td>
<td>Isometric Knee Flexion Peak Torque</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IME-PT</td>
<td>Isometric Knee Extension Peak Torque</td>
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<td>IMF-PT</td>
<td>Isometric Knee Flexion Peak Torque</td>
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<td>InCHIANTI</td>
<td>Invecchiare in Chianti</td>
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<tr>
<td>IQR</td>
<td>Inter Quartile Range</td>
</tr>
<tr>
<td>LASA</td>
<td>Longitudinal Aging Study Amsterdam</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<tr>
<td>LH</td>
<td>Luteinising Hormone</td>
</tr>
<tr>
<td>LOH</td>
<td>Late Onset Hypogonadism</td>
</tr>
<tr>
<td>MAMC</td>
<td>Mid upper Arm Muscle Circumference</td>
</tr>
<tr>
<td>MMAS</td>
<td>Massachusetts Male Aging Study</td>
</tr>
<tr>
<td>MrOS</td>
<td>Osteoporotic Fractures in Men Study</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PASE</td>
<td>Physical Activity Scale for the Elderly</td>
</tr>
<tr>
<td>PPT</td>
<td>Physical Performance Test</td>
</tr>
<tr>
<td>pQCT</td>
<td>Peripheral Quantitative Computed Tomography</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate Specific Antigen</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RASM</td>
<td>Relative Appendicular Skeletal Muscle Mass</td>
</tr>
<tr>
<td>ROC curve</td>
<td>Receiver Operating Characteristic curve</td>
</tr>
<tr>
<td>RRR</td>
<td>Relative Risk Ratio</td>
</tr>
<tr>
<td>SARM</td>
<td>Selective Androgen Receptor Modulator</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>SF36</td>
<td>Short Form 36 Questionnaire</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex Hormone Binding Globulin</td>
</tr>
<tr>
<td>SOF</td>
<td>Study of Osteoporotic Fractures</td>
</tr>
<tr>
<td>SPPB</td>
<td>Short Physical Performance battery</td>
</tr>
<tr>
<td>T</td>
<td>Testosterone</td>
</tr>
<tr>
<td>TE</td>
<td>Testosterone Enanthate</td>
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<tr>
<td>TILDA</td>
<td>The Irish LongituDinal Study on Ageing</td>
</tr>
<tr>
<td>TOM trial</td>
<td>Testosterone in Older Men trial</td>
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<tr>
<td>WBC</td>
<td>White Blood Cells</td>
</tr>
<tr>
<td>WHAS</td>
<td>Women's Health and Aging Study</td>
</tr>
<tr>
<td>WHI-OS</td>
<td>Women's Health Initiative Observational Study</td>
</tr>
</tbody>
</table>
Abstract

The University of Manchester
Matthew David Liam O’Connell
Doctor of Philosophy
Frailty and Anabolic Hormones in Ageing Men
March 2011

Frailty can be broadly defined as the vulnerable health status that occurs in older adults. With the ageing population understanding frailty is becoming an increasingly important issue. While no consensus exists on the exact definition of frailty, two models have become prominent in geriatric research. These are the frailty phenotype, a model that measures frailty according to the syndromic aggregation of 5 physical criteria and the frailty index (FI), a broad index of age related health deficits. While recent years have seen a substantial increase in research into frailty, there remains a relative paucity of data from European studies and studies in men. Of the many mechanisms suggested to contribute to frailty there has been particular interest in the role of declining levels of anabolic hormones, partly because replacement of these hormones represents a potential strategy for managing this condition. The broad aim of this thesis was to explore the condition of frailty and its relationship to anabolic hormones, particularly testosterone (T) in ageing European Men. This project involved analysis of data from 2 studies: The European Male Ageing Study (EMAS), a longitudinal cohort study of 3369 men from 8 European centres and a trial of T treatment in 262 men with low testosterone and symptoms of frailty. A set of phenotypic frailty criteria were developed for use in the EMAS, using this model the prevalence of frailty was 2.6%. This increased with age from 0.1% in men aged 40-49 up to 6.7% in men aged 70-79. This model was compared against an FI, the correlation between the two models was moderate, r=0.41, and both models were related to incident falling at 2 year follow up; Ordinal OR (95% CI), 3.15 (1.75 to 5.66) for the frailty phenotype and 5.28 (3.35 to 8.32) for the FI in adjusted analyses. In the hormone analyses frailty was related to lower free T according to both models, Ordinal OR (95% CI); 1.19 (1.02 to 1.39) for the phenotype and β-coefficient (95% CI); 0.006 (0.003 to 0.009) for the FI (FI values range from 0-0.7). Free T was particularly related to the sarcopenia criteria OR (95% CI); 1.40 (1.09 to 1.80). Frailty was also related to LH, FSH and SHBG. Deficiency in multiple anabolic hormones was related to frailty, in adjusted analyses each additional deficiency was associated with an RRR (95% CI); 1.71 (1.38 to 2.13) increased risk of phenotypic frailty and a β-coefficient (95% CI); 0.016 (0.012 to 0.02) increase in FI score. The trial analyses focussed on a 6 month post treatment follow up phase. It was found that gains in lean mass and muscle strength were not maintained at 6 months post treatment. The adjusted difference between groups at 6 months post treatment for knee extensor strength was 4.0 (-3.9 to 11.9) Nm compared to 8.1 (-0.2 to 16.5) Nm at the end of treatment, similarly the difference in lean mass declined from 1.2 (0.8 to 1.7) kg at end of treatment to 0.3 (-0.1 to 0.8) kg at 6 months post treatment. In summary, the frailty phenotype was adapted and validated for use in the EMAS study. Analyses using this model and the trial follow up analyses are supportive of an influence of T on lean body mass in ageing men. The other hormone relationships seen suggest frailty may be broadly related to changes in the endocrine system in ageing men. The lack of sustained benefit from T treatment combined with the relationships with multiple endocrine markers suggests more complex management strategies may be required for this condition.
Declaration

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- Kieron O’Connell, my twin brother and fellow PhD student whose experiences have been similar enough to allow empathy and yet sufficiently removed from mine to have kept our conversations interesting.
- My parents for supporting my decision to undertake further study and attempting to instil a sense of perspective about the whole thing.
- The many staff involved in the studies used for this thesis, whose efforts make this kind of large scale collaborative research possible.
- Lastly, I must thank the men who volunteered to take part in these studies.
Preface

‘Where youth grows pale and spectre thin and dies’

from Ode to a Nightingale, John Keats, 1820

The image of the frail elder is not new. However, this issue has never been more relevant than it is today. At the dawn of the 21st century developed countries are experiencing unprecedented population ageing. With these trends set to continue, the recent appreciation of frailty as a definable condition may prove a timely innovation. It is hoped the understanding of this condition may mature alongside this greying of the population.

This population ageing is driven by falling birth rates and particularly by increases in life expectancy. These increases in life expectancy are thanks largely to improvements in medical science. We have reached the privileged situation where the study of ageing itself has become a major imperative. It is well established that some older adults are more vulnerable to earlier functional decline than others. Frailty as a concept represents a way to capture this vulnerability. Therefore the study of frailty may lead to new insights into the detrimental effects of ageing and ultimately to ways to intervene and manage these effects and thus promote a healthy and functional old age.

Previous studies of ageing have been dominated primarily by women, simply because historically, women were more likely to reach ages considered worthy of study. Hormonal ageing is well characterised in women but much less so in men. Understanding hormonal ageing and its consequences in men represents a small step towards understanding frailty.
This thesis begins by introducing frailty then moving on to definition and description. The hormonal correlates of this condition in men are then addressed. The first 4 results chapters use data from the epidemiological European Male Ageing Study and concern specifically: The development of models to measure frailty in this study, assessment of the prevalence of this condition across the 8 centres involved in the study, detailed exploration of the relationships between frailty and the sex hormones of hypothalamic pituitary testicular axis and exploration of the relationship between frailty and multiple hormonal deficiency. Lastly the issue of hormonal therapies is approached, specifically the issue of whether short term testosterone treatment can lead to sustained benefits in frail men once treatment is withdrawn.
Chapter 1 Introduction

1.1 Introduction

The term ‘frailty’ in the broadest sense describes the vulnerable health status that occurs at advanced ages and is considered to be highly prevalent in older adults [1]. This condition is thought to arise due to declines across multiple interrelated physiological systems, leading to a loss of homeostatic reserve and resiliency and so this vulnerability [2, 3]. Frailty represents a relatively new concept and field of study. In recent years much effort has been made into defining this condition and exploring its aetiology.

1.2 Measuring frailty

While there is general agreement on the key underlying features of frailty, no such consensus exists on its measurement. A variety of models have been proposed to capture this condition, these include: The frailty phenotype, a model based on 5 physical criteria [4], a simplified 3 criteria version of this model has also been proposed [5]. The frailty index (FI), an index of age related health deficits [2]. The FRAIL scale including; Fatigue, Resistance, Ambulation, Illness and Loss of weight, this scale combines self reported physical symptoms with a count of co-morbidities [6]. The functional domains model, a model that measures frailty based on declines in 4 key health domains; physical, cognitive, nutritive and sensory [7]. The Edmonton frail scale, a 5 category scale grading patients from robust to severely frail based on indicators across 10 health domains [8]. The easy prognostic score, a scale based on 9 easy to collect predictors from multiple health domains [9]. Studies from the Longitudinal Aging Study Amsterdam (LASA) cohort, have assessed frailty using a similar 9 criteria multi domain model [10]. Frailty has also been assessed by the ability to perform functional tasks
As frailty represents a vulnerable health state, these models are usually validated in terms of their ability to predict adverse outcomes. In this context, the propensity towards adverse outcomes provides some objective evidence of this vulnerability.

1.2.1 The frailty phenotype

Currently, the most widely accepted frailty model is the frailty phenotype developed by Fried and colleagues [4]. This model measures frailty according to the presence of 5 physical criteria, identified by expert consensus as the core manifestations of frailty in older patients [4]. The criteria are: Slow walking speed, shrinking/sarcopenia, low energy level or exhaustion, weakness and low physical activity [4]. It is hypothesised that these criteria represent key components in a mutually exacerbating cycle of declining energetics and reserve, termed the ‘frailty cycle’ [4]. A 3 tier classification is used whereby people with 3 or more of these criteria are classed as frail, 1-2 criteria as prefrail or intermediate frail and 0 as robust or non frail [4]. Frailty as assessed by this model is suggested to represent a particular physiological syndrome that while related to disability and co-morbidity is distinct from either [4, 12].

This model was first operationalised and validated in the Cardiovascular Health Study (CHS), frailty was related to increased risk of falls, progression of disability, hospitalisation and death [4]. The model’s ability to identify older adults at increased risk of adverse outcomes has since been confirmed in other large cohort studies of ageing men and women [13-15]. As the original measures are not usually available in existing data sets, these studies used adapted versions of the model [13-15]. A study in older women suggested the model, and concept of frailty as a medical syndrome, shows some internal construct validity, with the 5 criteria tending to aggregate in a manner akin to a medical syndrome [16].
A criticism of this frailty model has been that it only includes physical symptoms and therefore does not address the full breadth of the condition [17]. This is at least partially true, however the 5 criteria may be seen as the core manifestations and minimal classification criteria of a broader syndrome [3]. In support of this, frailty assessed using this model has been found to relate to other ageing phenotypes including declining cognitive function [18] and increased bodily pain [19], as well as to poorer health related quality of life [20].

### 1.2.2 The frailty index

The other prominent model in frailty research is the frailty index (FI) [2]. This model defines frailty according to the number of health deficits a person displays, expressed as a proportion of the total number measured, meaning that values range between 0-1 [2]. The model is based on the premise that at the population level at least, the number, rather than the nature of deficits may be more informative about overall health [2]. Any deficit that satisfies the following conditions can be included:

1. Variables must be related to health status
2. Deficits must be related to age
3. Deficits must not saturate too early

Within this framework any age related changes in functioning across physical, psychological and sociological domains, as well as age related morbidities may be included [21]. Benign symptoms of ageing such as greying hair would not be included, neither would presbyopia which is almost universal by age 50 [21]. Providing these conditions are met, enough variables are included and enough systems are sampled, the precise deficits used do not matter. The flexibility of this model is evident from the characteristic right skewed distribution and limit of around 0.67 (two thirds of deficits measured) that have been reliably reproduced across many diverse data sets [22-26]. As
the composite deficits must be age related, the model also shows a reliable relationship with age [2]. Furthermore in each sample bootstrapping procedures have shown that any random subset of deficits show comparable characteristics to each other and the overall index [22-26]. An example of the variables and cut-points used to create an FI is shown in table 1.1. Like the frailty phenotype, the FI has been extensively validated in terms of its ability to predict adverse outcomes, including mortality, institutionalisation and further deficit accumulation [23, 27, 28]. Notably, the FI has been found to correlate more strongly to mortality than chronological age in community dwelling and institutionalised samples [23]. A version of this model has been devised for use in the European Male Ageing Study (EMAS) [29], and is featured in the later chapters of this thesis.

In contrast to the frailty phenotype, the FI does not define frailty as a syndrome or use discrete categories for classification. Instead the FI represents a state variable, allowing gradation of health status from robust through to very frail [2]. However, the FI can be stratified into distinct categories to address particular research questions [27, 28, 30]. These include assessing the progression and outcomes at different levels of the FI, and comparison with other models [28, 30, 31].
Table 1.1: An example of the variables and cut-points used in a frailty index

<table>
<thead>
<tr>
<th>List of variables included in the frailty index</th>
<th>Cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Help Bathing</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help Dressing</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help getting in/out of Chair</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help Walking around house</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help Eating</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help Grooming</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help Using Toilet</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help up/down Stairs</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help lifting 10 lbs</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help Shopping</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help with Housework</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help with meal Preparations</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help taking Medication</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Help with Finances</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Lost more than 10 lbs in last year</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Self Rating of Health</td>
<td>Poor = 1, Fair = 0.75, Good = 0.5, V. Good = 0.25, Excellent = 0</td>
</tr>
<tr>
<td>How Health has changed in last year</td>
<td>Worse = 1, Better/Same = 0</td>
</tr>
<tr>
<td>Stayed in Bed at least half the day due to health (in last month)</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Cut down on Usual Activity (in last month)</td>
<td>Yes = 1, No = 0</td>
</tr>
<tr>
<td>Walk outside</td>
<td>&lt;3 days = 1, ≤ 3 days = 0</td>
</tr>
<tr>
<td>Feel Everything is an Effort</td>
<td>Most of time = 1, Some time = 0.5, Rarely = 0</td>
</tr>
<tr>
<td>Feel Depressed</td>
<td>Most of time = 1, Some time = 0.5, Rarely = 0</td>
</tr>
<tr>
<td>Feel Happy</td>
<td>Most of time = 0, Some time = 0.5, Rarely = 1</td>
</tr>
<tr>
<td>Feel Lonely</td>
<td>Most of time = 1, Some time = 0.5, Rarely = 0</td>
</tr>
<tr>
<td>Have Trouble getting going</td>
<td>Most of time = 1, Some time = 0.5, Rarely = 0</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>Heart attack</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>CHF</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>Stroke</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>Cancer</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>Chronic Lung Disease</td>
<td>Yes = 1, Suspect = 0.5, No = 0</td>
</tr>
<tr>
<td>MMSE</td>
<td>&lt;10 = 1, 11–17 = 0.75, 18–20 = 0.5, 20–24 = 0.25, &gt;24 = 0</td>
</tr>
<tr>
<td>Peak Flow</td>
<td>≤ 340</td>
</tr>
<tr>
<td>Shoulder Strength</td>
<td>≤ 12</td>
</tr>
<tr>
<td>BMI</td>
<td>≤ 18.5, ≥ 30 as a deficit. 25–≤30 as a ‘half deficit’</td>
</tr>
<tr>
<td>Grip Strength</td>
<td>For BMI ≤ 24, GS ≤ 29  For BMI 24.1–28, GS ≤ 30  For BMI &gt;28, GS ≤ 32</td>
</tr>
<tr>
<td>Walk time - Usual Pace</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Walk time - Rapid Pace</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

Adapted from Searle et al 2008 [21]

For continuous variables different cut-points were used for men and women. Only male cut-points are shown here.
1.2.3 Comparison of frailty models

In an effort to further validate and refine definitions of frailty, recent studies have focussed on comparing the different models proposed [5, 30-37]. The models have been compared in terms of their agreement with one another and their relative abilities to predict adverse outcomes. Correlations between the frailty phenotype and FI have ranged from 0.45 in men and women aged 65 and older from the English Longitudinal study of Ageing (ELSA) [38] to 0.92 in a small sample of older adults, including independent elderly, day hospital and continuing care patients [34]. Moderately strong correlations between the models of 0.65 and 0.61 were seen in the community dwelling [31] and institutionalised cohorts [37] from the Canadian Study of Health and Aging (CSHA) study respectively. The FI has also been found to correlate quite strongly, 0.72 to a modified version of the ‘easy prognostic score’ in a population based sample of older Italian adults [36]. In a study comparing the phenotype, FI and functional domains models in a large sample of community based and institutionalised older adults, it was found that 76.1% of people classed as frail according to the phenotype model, and 72.1% classed as frail according to the FI, were also classed as frail by at least 1 other model [32]. The functional domains model showed a weaker overlap of around 50% with the other 2 models [32].

The FI has generally been found to relate more strongly to adverse outcomes including mortality and hospitalisation than the phenotype [30, 31, 37]. In the one study to attempt a quantitative comparison using data from the CHS study, it was found that the frailty phenotype tended to underestimate the risk of death in more people than the FI based on mortality risk across 9 groups derived from applying a 3 level frailty classification to both models [30]. It may be that the broader nature of the FI allows more comprehensive assessment of overall health. Studies comparing the frailty phenotype
and the simplified 3 criteria model designed in the Study of Osteoporotic fractures (SOF) have generally shown little difference between the models in their ability to predict key geriatric outcomes including falls, fractures and death [5, 33, 35].

1.3 Prevalence of frailty

The prevalence of frailty is dependent on the definition used. Estimates range from 7-26% in comparable samples of older adults using the different models [4, 7, 15, 25]. Regardless of the definition, there is universal agreement that frailty is strongly age related and most prevalent at advanced ages [4, 7, 15, 25].

The majority of studies suggest frailty is more common in women than men. The prevalence of physical frailty, as assessed by the frailty phenotype has been found to be higher in women than men in both US and European samples [4, 39]. Similarly, a pooled analysis of 11 population and institutionalised samples from 4 developed countries, found that at a given age women tend to have higher FI values than men [23]. In contrast, frailty assessed by the functional domains model was more common in men than women from a non institutionalised US sample [7]. This may relate to the high frequency of cognitive and sensory deficits in men from this study [7]. Despite the higher prevalence most studies suggest a better prognosis for frail women compared to frail men [23, 40]. The relationship between phenotypic frailty and mortality was stronger for men than women in a sample of older Mexican Americans [40]. In agreement, FI studies suggest that at any given level of deficit accumulation men are more likely to die than women [23].

In addition to gender comparisons, race comparisons have been performed in US studies, comparing the prevalence of frailty in African American or Hispanic groups
compared to European Americans [41-43]. The prevalence of frailty was found to be higher in African Americans compared to European Americans in the CHS study [43]. Another study found the prevalence of frailty was higher in Mexican Americans compared to European Americans from the San Antonio Longitudinal study [41]. Interestingly, the Mexican Americans were less likely to become frail during the 9.9 year follow up to this study [42]. The reasons for these race differences are not clear, one factor may be differences in socioeconomic status between races. Socioeconomic status shows clear relationships with frailty, a report from the Women’s Health and Aging Study (WHAS) 1 & 2 studies found that lower income or level of education were associated with a greater odds of being frail [44]. Furthermore, in this study the association between race and frailty was attenuated by adjustment for these socioeconomic markers [44].

1.4 Lifestyle influences

An individual’s lifestyle may have an important influence on the rate at which they become frail with age. Lifestyle factors that have received attention in recent studies include smoking, physical activity and nutrition [45-51].

A recent study found that long term heavy smokers had higher levels of frailty, as measured by the FI, compared to non-smokers in a large sample of men and women aged over 65 from the CSHA cohort [47]. This relationship was unaffected by the removal of deficits known to be related to smoking, such as coughs and hypertension from the index [47].

Physical activity is generally thought to be an important factor in frailty [4]. Recent longitudinal studies have supported an influence of activity levels on the development
and progression of frailty [48, 50]. A report from the Health Aging and Body Composition (Health ABC) study found that sedentary lifestyle was associated with increased odds of incident frailty, defined by poor physical function [11], compared to more active lifestyles [50]. This effect remained significant after adjustment for confounding in the most active, planned exercise group [50]. In another study, higher levels of exercise were associated with improvements in health status over 5 years, measured using the FI in older adults from the CSHA cohort [48].

Inadequate nutrition in older people may be an important contributor to sarcopenia and frailty. The relationship between frailty and particular nutrients has been a focus of recent investigations [45, 46, 49, 51]. Low protein and total energy intakes were associated with frailty in a cross-sectional analysis of older men and women [45]. Similarly, a study in 24,417 women aged 65 to 79 found higher intakes of dietary protein were associated with lower risk of incident frailty at 3 year follow up [46]. Micronutrient deficiencies, assessed by either self reported intake or serum levels have been associated with frailty in cross sectional studies of older men and women [45, 49]. Deficiencies of multiple micronutrients were strongly related to frailty in these studies [45, 49]. Similar results were seen in a longitudinal study in disabled older women, in which baseline nutritional deficiencies were related to higher risk of frailty at follow up [51].

1.5 The biology of frailty

The development of models to measure frailty, in particular the frailty phenotype has spawned a multitude of investigations into the biological mechanisms underpinning this condition. While a vast range of markers have been explored, the majority of studies have focussed on 5 distinct yet related areas. These are oxidative stress and changes to
the metabolic, cardiovascular, inflammatory and endocrine systems. There has also been specific interest recently in the potential effects of myostatin on sarcopenia, the loss of muscle mass with ageing, which may underlie frailty.

1.5.1 Oxidative stress
A small number of recent studies have assessed the relationship between oxidative stress and frailty [52, 53]. A moderate relationship between higher levels of frailty and 8-hydroxy 2-deoxyguanosine (8-OHdG), a marker of oxidative protein damage, was seen in a small study of 90 elderly Chinese men and women [53]. In a larger study, frailty was related to low levels of vitamin E, a key antioxidant and indirect marker of oxidative stress in men and women from the InCHIANTI study [52]. In another study, high protein carbonyl levels, another indicator of oxidative protein damage, were strongly related to grip strength in disabled older women [54].

1.5.2 Metabolic changes
Frailty has been traditionally viewed as a wasting condition, however recent studies have suggested a U-shaped relationship between Body Mass Index (BMI) and frailty, where people with low or very high BMI are more likely to be frail [15, 38]. A series of large cohort studies have shown a relationship between high BMI and the frailty phenotype in older men and women [15, 38, 55]. It is possible this may reflect a mechanical effect, with increased resistance from the extra mass contributing to reduced physical function and energy levels. However, this relationship has also been shown using the more inclusive frailty index [38]. Furthermore, regardless of how frailty is measured, this relationship is stronger in people with high waist circumference, a measure that reflects the metabolically active visceral fat [38]. Therefore it seems likely that this relationship reflects the adverse metabolic or inflammatory effects of obesity.
In agreement, frailty has been related to adverse changes in metabolic markers in a number of studies [56-58]. Frailty was related to elevated fasting blood glucose, as well as higher levels of both glucose and insulin in response to an oral glucose tolerance test in participants form the CHS study [58]. Similarly, higher levels of glycated haemoglobin, an indicator of mean blood glucose levels, were related to frailty in women from the WHAS 1 and 2 cohorts [57]. Finally, in a prospective study, insulin resistance, defined by homeostasis model assessment score (HOMA), was related to incident frailty at 5 and 9 year follow up in older men and women [56]. However the related metabolic syndrome was not clearly related to frailty in this study [56].

One mechanism through which insulin resistance may contribute to sarcopenia and frailty, may be a loss of sensitivity to insulin of muscle protein metabolism [59]. Basal protein metabolism does not differ between young and older adults, however responses to particular stimuli, including insulin appear to be blunted in older adults [59]. Wilkes et al [60] studied protein balance across the leg, using leucine and phenylalanine tracer techniques, in response to infusion of insulin at a dose designed to mimic the response to a low glycaemic index meal. Following insulin infusion, it was found that leg protein breakdown, evidenced by arterio-venous difference in amino acids, was suppressed by 59% using phenylalanine and 47% using leucine in young men and only 23% and 12%, respectively in older men [60]. This suggests older adults may experience less suppression of muscle proteolysis in response to feeding.

Elevated blood glucose in frail older adults may be indicative of, or contributive to, this general insulin resistance. However, high blood glucose may also contribute to frailty directly through several mechanisms including muscle capillary damage and neuropathy. A report from the Health ABC study found that muscle strength and
quality, but not muscle mass were lower in diabetic compared to non diabetic older adults [61]. These effects were worse in those with higher blood glucose and longer duration of diabetes [61]. These data are consistent with an intrinsic effect of these metabolic derangements on muscle force output, possibly relating to a neurological effect or a change in muscle composition.

1.5.3 Cardiovascular/haematological

Phenotypic frailty has been strongly associated with chronic heart failure in ageing men and women [62]. Interestingly, relationships have also been found between frailty and subtler cardiovascular perturbations [58, 62-66]. Frailty was related to the presence of atherosclerosis and other subclinical cardiovascular disease in the CHS cohort [62]. Changes in haematological markers have also been related to frailty. In small studies lower haematocrit levels have been seen in frail patients compared to age matched controls [64, 65]. This relationship has also been shown in a large sample of older women from the WHAS 1 and 2 cohorts [63]. Haemoglobin levels also correlated negatively to the FI in a small study that included frail patients, along with age matched and young adult controls [64]. In a cross-sectional analysis from the CHS study, higher levels of blood coagulation markers factor VIII and D-dimer were seen in frail participants compared to controls [58]. More recently in a large, prospective, nested case control study including 900 women who developed incident frailty after 3 years follow up and 900 controls, higher levels of D-dimer and tissue plasminogen activator were weakly related to incident frailty [66]. However no relationship with incident frailty was seen for Factor VIII or fibrinogen in this study [66]. These studies broadly support a relationship between frailty and changes in haematological markers, however it is not clear which, if any of these markers, have particular importance.
1.5.4 Inflammation

The immunological system and in particular chronic inflammation has been the most intensively studied of the biological correlates of frailty. As discussed above, inflammatory co-morbid conditions, cardiovascular disease, anaemia and obesity have all been related to frailty [38, 55, 62, 63]. Furthermore, in a recent cross-sectional study the presence of particular pairs of inflammatory co-morbid conditions, anaemia and depression and anaemia and pulmonary disease were associated with a synergistically higher likelihood of frailty in older women [67]. Although this effect was not seen for all pairs of conditions studied [67]. Similarly, frailty has been associated with chronic infections [68-70]. Human Immuno-deficiency Virus (HIV) infection was associated with premature frailty in a cohort of men from a high risk group [68]. Cytomegalovirus (CMV) infection has been associated with both prevalent and incident frailty in older women [69, 70]. HIV pathology may lead to frailty through a variety of mechanisms, such as muscle wastage, concomitant infections and side effects from medications. However, CMV is a relatively benign infection that while frequently asymptomatic is characterised by chronic inflammation. Notably levels of Interleukin-6 (IL-6) appear to modify the relationship between this infection and frailty [69, 70].

The relationship between inflammation and frailty has been explored directly in a number of studies. In small case control studies, frail patients have been found to have higher levels of inflammatory markers, IL-6 and C-reactive protein (CRP) compared to controls [65, 71, 72]. In a similar study, frail patients showed more skewed T-cell distributions, including a larger subpopulation of CCR5+ T-cells, indicative of greater immunological impairment compared to controls [71]. While in another study, blood mononuclear cells from frail patients showed a higher IL-6 release and impaired proliferative responses to antigen insult compared to controls [73]. A study in 110 older
patients found higher levels of CRP, IL-6 and Tumour Necrosis Factor-03B1 in frailer patients, these 3 markers were also positively correlated to the FI [74].

Larger studies on inflammatory biomarkers and frailty have shown equivocal findings. Frailty was associated with both high IL-6 and white blood cell (WBC) count in a cross-sectional study of older women [75]. This association was stronger in the presence of high levels of both markers [75]. A recent study in both disabled and high functioning older women, suggested high neutrophil and monocyte counts were associated with frailty, while other WBC subpopulations were not [76]. However, these relationships did not reach significance in the high functioning cohort [76]. High CRP was associated with prevalent frailty in CHS cohort [58]. In a separate analysis from this study higher CRP levels were associated with incident frailty over 5 and 9 year follow ups [56], although IL-6 was not related to incident frailty after adjustment for confounders [56]. An analysis from the LASA cohort using their 9 indicator model of frailty found a relationship between higher CRP levels and incident, but not prevalent frailty [77], no relationship between frailty and IL-6 in either analysis [77]. A cross-sectional analysis from the WHAS 1 and 2 cohorts did not show a relationship between CRP levels and frailty [55], while a large nested case control study in older women did not show any association between either CRP or IL-6 and frailty [66]. Differences between studies may be related to the differences in the populations studied, the biomarkers assessed, or possibly the assays used. The LASA study used a broader frailty model than the other studies described here, the lack of relationship with IL-6 here may also be due to the relatively insensitive assay used [77]. The other studies have all used versions of the frailty phenotype in cohorts of similar ages. Most of these studies have focussed on older women; it is possible that the inclusion of men in the CHS study may have
contributed to the clearer relationships in that study. Other differences between studies may relate to use of different assays or differential handling of confounders.

The molecular mechanisms underlying the increased inflammatory activation in frail patients, have been explored in 2 studies based on a case control study of 16 frail patients and 16 matched controls [78, 79]. The first study assessed gene expression in CD4+ monocytes taken from both groups in response to a lipopolysacharide challenge [78]. It was found that compared to controls, monocytes from frail patients showed increased up regulation of 7 stress responsive genes in response to the challenge [78]. This effect was clearest for CXC chemokine ligand 10 (CXCL-10). In the second study frail patients showed higher resting expression of CXCL-10 which was highly correlated with serum IL-6 levels [79].

It is possible inflammatory cytokines may contribute directly to frailty through their catabolic effects, or indirectly through their interactions with the haematological and endocrine systems [65, 80]. However, it is currently unclear whether inflammation in frail elders represents a primary driver of the frailty process, a compensatory adaptation to viral antigens or other subclinical pathology, or a marker of a separate pathological mechanism such as oxidative stress [81].

1.5.5 Myostatin
Myostatin, or growth differentiation factor-8, is a myokine that inhibits skeletal muscle growth [82]. Myostatin circulates in plasma as a part of a latent complex bound to several proteins including follistatin related protein and growth and differentiation associated protein-1 [82]. Inactivating mutations on the myostatin gene have been associated with extreme muscularity in animals and humans [83, 84]. Studies in mice
have also shown that manipulating myostatin levels or function in adult animals may affect skeletal muscle mass and function [85, 86]. Studies in humans have been very limited, in part due to the lack of a reliable and sensitive assay [82]. Two recent studies have compared serum myostatin levels in young and older men, finding respectively no difference [87] and lower levels in the older compared to the younger men [82]. Myostatin levels were not related to lean mass or muscle function in either of these studies [82, 87]. These results suggest that myostatin, while a potent regulator of muscle growth, may not be primarily involved in the process of sarcopenia or the development of frailty.

1.5.6 Endocrine dysregulation

It has been suggested that changes in hormone levels may contribute to the development of frailty with ageing [3]. Levels of testosterone (T), growth hormone and dehydroepiandrosterone (DHEA) and vitamin D all decline with age, while levels of cortisol tend to increase slightly [88-90]. Levels of these hormones in older people are also influenced by general health status and health behaviours [91-94]. Furthermore deficiencies of anabolic hormones in younger adults are characterised by features similar to human ageing, reduced muscle and bone mass and increased fat mass, which can be repaired through hormonal replacement [95, 96].

1.5.6.1 Cortisol

Studies in small groups of older men and women have shown equivocal relationships between cortisol and muscle mass and function [97-99]. In a large cross-sectional study relationships were seen between higher cortisol levels and poorer functional performance in older men and women [100]. In a prospective study higher salivary cortisol was related to loss of grip strength at 3 year follow up in older men and women,
however no relationship was seen between lean body mass and either serum or salivary cortisol in either cross-sectional or longitudinal analyses [101]. This study also suggested a potential modifying effect of genetic variation of the glucocorticoid receptor on these relationships [101]. In a study using salivary sampling at 7 time points over a 24 hour period, frailty was related to both higher mean levels and blunted diurnal variation in cortisol in older women [102]. This relationship may be due to the catabolic effects of cortisol [103]. This study also provides evidence of impaired endocrine regulation in frail women.

1.5.6.2 DHEA/DHEAS

A large study in older men and women found a relationship between lower DHEAS and higher levels of frailty, with stronger relationships evident in those with lower BMI [104]. Similarly, a small case control study found lower DHEAS levels in frail patients compared to controls [80]. DHEAS levels were related to calf muscle cross-sectional area (CSA) and lower extremity strength in men aged 60-79 from the Invecchiare in Chianti (InCHIANTI) study, but not in other age groups [105]. Similarly DHEA and DHEAS were related to Physical Performance Test (PPT) score below threshold levels in men aged 55-85 [106], however neither DHEA nor DHEAS were related to grip strength in this study. The relationships seen in all these studies were relatively weak and not entirely consistent.

Recent studies have moved away from the single hormone measurement approach to assess how changes in DHEAS relate to key outcomes [107, 108]. Trajectories in DHEAS levels measured at 3-6 time points over an 8 year follow up period were related to all cause mortality in older adults [107]. Persons displaying both high variability and sharp declines in DHEAS levels were at particularly high risk of death [107]. Changes
in DHEAS levels have been shown to track with changes in physical and cognitive function in very old women, but not in men [108]. It may be that fluctuations in DHEAS levels represent a marker of declining physiological regulation.

Further evidence that DHEAS may represent a passive marker rather than an active mediator in the development of frailty may be seen in the largely disappointing effects of DHEAS supplementation in older adults. While small early studies suggested DHEAS treatment may improve muscle mass and strength in the healthy elderly [109, 110], larger studies in healthy older adults and men with low muscle strength have not supported this effect [111-113]. However, a recent study in women with symptoms of frailty, found DHEAS supplementation lead to small improvements in muscle strength and physical function, although interestingly not muscle mass, when added to a low intensity exercise program [114].

1.5.6.3 Vitamin D

The relationship between Vitamin D, usually measured in its hydroxylated form and major circulating metabolite, 25-hydroxyvitamin D [25(OH)D], and frailty has been the focus of recent study. A number of studies have explored the relationship between vitamin D and physical frailty using the frailty phenotype model [115-118]. A report from the InCHIANTI study found a relationship between lower levels of vitamin D and higher odds of frailty in older Italian men, but not in women [117]. A relationship between low vitamin D intake and frailty was also seen in this cohort [45]. In another study a relationship was seen between low levels of vitamin D and frailty in 5048 non-institutionalised older US adults [118]. Similarly, a recent study in a large sample of older US women found a cross-sectional relationship between low levels of vitamin D and frailty, along with a modestly increased risk of frailty at 4.5 years follow up in
initially non frail women with low vitamin D levels at baseline [116]. Curiously this study showed a U-shaped relationship, with high vitamin D levels also related to higher odds of frailty at baseline [116]. In a parallel study, by the same group, in a cohort of older US men, low levels of vitamin D were associated with higher frailty status in cross-sectional, but not prospective analyses [119]. Another study found a relationship between low vitamin D and the frailty phenotype, along with a weaker relationship with the broader Edmonton frail scale in a group of 215 older Taiwanese adults, recruited into a trial of integrative geriatric care for frailty [115]. A further study, using a 9 marker, multiple domain frailty scale, found that low vitamin D was related to both prevalent and incident frailty in men and women from the LASA study [10].

The relationship between vitamin D levels and symptoms of physical frailty including low muscle mass, strength and physical function has also been assessed in a number of studies [120-125]. Higher levels of vitamin D were consistently related to better lower limb function, assessed by walk speed and chair rising ability in both active and inactive older US adults from the National Health And Nutrition Examination Survey (NHANES) study [122]. Similarly, low levels of vitamin D were related to poorer grip strength and performance on the Short Physical Performance Battery (SPPB) in men from the InCHIANTI study and to grip strength in women from this study [125]. However, little relationship was seen between vitamin D levels and functional performance in sample of 495 older Japanese women living in Hawaii [126]. In a prospective study, vitamin D was related to loss of muscle mass and strength in older adults from the LASA study [125]. However, vitamin D levels were not related to loss of grip or knee extensor strength in women aged 75+ from the Epidemiology of Osteoporosis (EPIPROMOS) study [120], or disabled older women from the WHAS-1 cohort [124].
These differences in findings may be due to differences in outcomes assessed, vitamin D assays or possibly the populations studied. The women in the EPIDOS study were generally older than the populations in other studies [120], while those from the WHAS-1 were among the most disabled US older women [124]. It may be that the participants included in these studies covered a smaller range of functional abilities, limiting the ability to detect associations. Similarly, the lack of relationship between physical function and vitamin D in the study on Japanese women may be due to the high vitamin D levels and functional ability in these women [126].

The nature of any relationship between vitamin D and frailty is unclear. Vitamin D levels are affected by sunlight exposure, physical activity and dietary intake [92, 127], as such vitamin D may represent a marker of general health and lifestyle. Adjustment for these factors tended to reduce the strength of relationships between vitamin D and frailty [116-118, 121-123]. Furthermore exclusion of ‘low physical activity’ from the frailty model partially attenuated the relationship between frailty and vitamin D seen in the InCHIANTI study [117]. Additionally it appears vitamin D supplementation has little effect on muscle strength in most adults [128]. It is also notable that vitamin D levels relate to many other symptoms of poor health in ageing, including cognitive function, pain, depression and bone health [129-132]. All of these points lend credence to the suggestion that vitamin D may be a non-specific marker of health status in the elderly.

It is also possible that low vitamin D may contribute to the development of frailty. In the majority of studies an independent relationship between vitamin D and frailty remained after accounting for the key confounders [116-118, 122, 123]. An active
influence of vitamin D on frailty is biologically plausible. Vitamin D receptors have been indentified on skeletal muscle [133, 134], and vitamin D deficient patients display impairments in muscle performance that can be largely repaired by replacement [135]. Low vitamin D levels may also influence frailty indirectly through secondary hyperparathyroidism. Primary hyperparathyroidism patients display decrements in muscle function that can be improved by surgical treatment [136]. High parathyroid hormone (PTH) was related to frailty or physical function in some [117, 123, 125], but not all studies [120]. Furthermore adjustment for PTH partially attenuated the relationship between vitamin D and frailty in the InCHIANTI study [117], suggesting this phenomenon may partly explain the relationship between vitamin D and frailty. PTH could directly influence frailty through its apparent effects on muscle function [136]. It is also possible this represents another marker of changing biological regulation in relation to frailty.

1.5.6.4 Growth Hormone/Insulin-like Growth Factor-1 (IGF-1)

The most commonly used marker of growth hormone levels and actions is serum IGF-1. Lower IGF-1 levels have been found in a small group of frail patients compared to controls [80]. Studies on key features of frailty have shown equivocal results. Early studies tended to show positive correlations between IGF-1 levels and lean body mass by DXA in middle aged and older people, although these were not entirely consistent between genders [137-139]. In a prospective study, higher IGF-1 levels were related to smaller losses of lean mass over 2 years in men aged 72-92, but not in women [140]. In a large recent study no relationship was seen between total body lean mass by DXA and IGF-1 in older men or women [141]. In a study of 526 persons aged 20-102 with a mean age of 65, free IGF-1 levels were weakly positively related to grip strength and lower limb power [142], although the relationship with grip strength was non significant after
adjustment for confounding. Another study showed a positive relationship between IGF-1 and grip strength in oldest old women, along with a borderline significant association in middle aged women, but no association in either group of men [143]. This study also failed to show any meaningful relationship between IGF-1 and physical function or ADL disability [143]. In another study IGF-1 was positively related to physical function in older women below a threshold level estimated at 50ug/litre, however no relationship was seen for body composition or muscle strength [144]. Similarly, low IGF-1 was related to walking limitation, but not significantly related to mobility limitation or disability in older women [145]. Other studies have shown no association between any of these outcomes and IGF-1 in large samples of healthy older people and those with mobility limitations [146-148].

There are a number of possible explanations for this lack of agreement. One possibility is differential handling of confounders. One such confounder is BMI, one study in the oldest old reported an interaction between IGF-1 and BMI in relation to functional outcomes [149]. Free IGF-1 was shown to be positively related to grip strength walk speed and physical performance only in obese participants [149]. It is also possible that serum IGF-1 may not be the best marker of growth hormone activity in ageing people, around 99% of IGF-1 in serum is bound to IGF-binding proteins (IGF-BPs) that act as modifiers of the effect of IGF-1 on its receptor [150]. IGF-1 bioactivity assays that provide a measure of these combined effects have been suggested as an alternative [150], but the relationship between these measures and functional outcomes have not yet been explored. However, a number of studies have assessed relationships with the IGF-BPs. Higher levels of IGF-BP2 were related to poorer physical performance and muscle strength in a sample of 403 older men, while no association was seen for IGF-1 [151]. Similarly, in a prospective study higher IGF-BP1 was associated with poorer grip
strength and physical performance and a higher risk of disability at follow up, while IGF-1 and IGF-BP3 were not [146]. However, IGF-BP3 has shown some relationship with physical performance measures in other studies [143].

Since the early study of Rudman et al [152] there has been considerable interest in the ‘rejuvenative’ effects of growth hormone (GH) treatment in older people. In placebo controlled trials GH injections have been found to increase lean mass in healthy older men and women with low or normal GH levels [153, 154]. Increases of 2 - 3.1 kg have been seen with varying dosages [153, 154], however these gains are accompanied by a high frequency of adverse effects, particularly at higher doses [153, 155]. More recent studies have used ghrelin mimetics, these act as GH secretagogues serving to stimulate pulsatile GH secretion, giving a more physiological profile compared to GH injection [156, 157]. These agents have been used in healthy and prefrail older people and show similar effects on lean mass compared to GH, but with milder adverse effects [156, 157]. However in contrast to GH injection, ghrelin based agents may serve to increase body fat [156].

Despite these increases in lean mass, effects on muscle strength and functional outcomes in response to GH treatment have been very modest. GH treatment for 6 months showed no effect on muscle strength or exercise performance in healthy older men and women [153, 154]. Similarly, treatment with growth hormone secretagogue, MK-677 did not improve functional outcomes in healthy older people [156]. Treatment with a similar agent, Capromorelin, lead to small improvements in physical function in prefrail older adults [157]. In agreement, studies in healthy young people suggest GH treatment has very limited effects on strength and exercise performance [158, 159]. The explanation for this lack of effect appears to be that the majority of lean mass gained
from GH treatment is not muscle tissue. GH treatments increase whole body water content; this exaggerates effects on lean mass measured by DXA [158, 159]. In addition to this, GH does have genuine anabolic effects, evidenced by increased whole body protein synthesis in response to treatment [160]. However, this effect is not specific to muscle contractile protein. Increases in lean tissue in response to GH treatment may largely reflect increases in connective tissue. Short term GH treatment in young men may increase collagen synthesis in muscle and tendon, without apparent increases in myofibril protein synthesis [161].

1.5.6.5 Testosterone

The hormone that has received the most attention in frailty research is testosterone (T). Expanded versions of this section form the basis of two review articles (see Publications).

1.5.6.5.1 Observational studies

Studies exploring the relationship between T and frailty have shown equivocal results [162-166]. No relationship was seen between free or total T and the frailty phenotype in men from the Massachusetts Male Aging Study (MMAS) cohort [165]. In another study using the same model, low bioavailable T was related to frailty in a cross-sectional analysis of men from the Osteoporotic Fractures in Men Study (MrOS) cohort, this relationship was not significant in longitudinal analyses [163]. However, no relationship was seen between frailty and total T in this study [163]. Similarly, lower free T was related to frailty assessed by the FRAIL scale in men from the Health In Men Study (HIMS) study, this relationship remained significant in longitudinal analyses [164]. A weaker relationship was also seen with total T in cross-sectional analyses from this study, this did not persist in longitudinal analyses [164]. In smaller studies, lower levels
of both total and free T were related to phenotypic frailty in a sample of 108 older Taiwanese men and women [166], while total T was not related to frailty in a small sample of heart failure patients [162].

In addition to these recent studies on frailty, there is a substantial literature on the relationship between T and key components of frailty. The majority of studies in middle aged and older men have shown relationships between T levels and measures of lean body mass [137, 139, 167-169]. These relationships were generally modest, with androgen levels estimated to explain between 2% [169] and 13% [137] of the variation in lean mass. However a number of recent studies have not supported this relationship [170-172]. No difference in lean BMI was seen across quartiles of bioavailable T in men from the MrOS study [171]. Similarly, no difference in lean mass was seen across levels of free T in a large sample of older men from the MrOS Sweden cohort [172]. While no difference in calf muscle cross sectional area (CSA) by pQCT scans across testosterone groupings was seen in men from the InCHIANTI study [170].

T levels have been inconsistently related to measures of muscle strength. Grip strength has been found to be related to T levels in ageing men in the majority of studies [137, 169-171, 173, 174]. A small study in elderly Afro-Caribbean men showed positive correlations between both free and total T and measures of upper and lower limb strength [173]. In larger studies using adjustment for key confounders, including age, BMI and co-morbidities, T levels have been found to be weakly, but significantly related to leg extensor strength in 403 community dwelling older men [169] and to leg power in men from the MrOS study [171]. However no relationship was found between T levels and grip strength in men from the MMAS study. Similarly, a recent prospective
study found no difference in 3 year declines in grip and knee extensor strength according baseline testosterone levels [175].

The relationship between T and physical function has been similarly unclear. T levels were found to correlate modestly to measures of upper and lower limb function in a small study in elderly men [173]. Larger, more rigorous studies have shown less consistent results [106, 171, 174-176]. In a report from the MrOS study men in the lowest quartile for bioavailable T (<1.75ng/dL) performed marginally worse on tests of chair rising and walking ability [171]. Weak cross-sectional relationships were seen between androgen levels and performance in combined tests of lower limb function and locomotor ability in European men [174]. A weak positive relationship was seen between total T levels and performance on the Physical Performance Test (PPT) below a threshold level estimated at 451ng/dL was seen in the MMAS cohort [106]. A similar pattern was seen for chair rising performance, but this did not reach significance p=0.81 [106]. Comparable results to total T were seen for bioavailable T in this study [106]. In a recent study higher free T levels were related to faster walking speed and improved performance on the SPPB in men from the Framingham Offspring Study. In prospective analyses from this study low free T was associated with both incident and worsening mobility limitation at 6 year follow up [176]. No clear relationships were seen between total T and function or mobility in this study [176]. In another prospective study no relationship was seen between baseline T levels and 3 year declines in lower limb functional performance in 2 large cohorts of ageing men [175].

The reasons for the discrepancies between studies are not altogether clear. One suggested explanation could be genetic variation in the sensitivity of the androgen receptor [177]. Longer CAG repeat lengths within the androgen receptor gene are
associated with decreased receptor sensitivity [177]. However, a recent study found no relationship between androgen receptor CAG repeat length and frailty in men from the MMAS study [178]. Small differences between studies may relate to differences in age, the type of frailty assessment, and in longitudinal studies, length of follow up. For example, the clearest relationship between T and frailty was seen in the HIMS study, men in this study had a minimum age of 70, which was higher than most other studies [164]. Also the frailty construct used in this study includes morbidity, while other studies have adjusted for morbidities as confounders. In general longer follow up lengths may allow greater progression of frailty, or functional disability in initially high functioning men, allowing more scope to detect significant effects.

In addition to T a number of other elements of the sex hormone system have been investigated in relation to frailty in ageing men. Higher Sex Hormone Binding Globulin (SHBG) was related to phenotypic frailty in the MMAS cohort [165]. Higher Luteinising Hormone (LH) was related to the FRAIL scale in the HIMS study [164]. While higher levels of this hormone were related to low muscle mass and strength in European men [179]. However, these relationships have not been observed in all studies [163, 164].

1.5.6.5.2 Interventional studies

The use of T treatment as a function promoting therapy has been studied in both healthy [180, 181], and more recently frail older men [181-183]. The effects of T on muscle mass and strength are dose dependent, with comparable effects seen in both young and older men [184, 185]. However, due to safety concerns the majority of studies in older men have focussed on near physiological replacement doses. These dosages may be
more informative about the potential physiological role of age related changes in T in the development of frailty.

T treatment at physiological doses has usually been shown to increase lean body mass in older men by around 1-2 kg using oral or topical preparations [112, 153, 181, 183, 186-191]. Larger gains of around 4 kg have been seen with injectable T enanthate (TE) preparations [180, 192]. This may be due to the different pharmacological profiles, injectable TE results in less stable T levels with supraphysiological peaks and troughs, a profile that may be more potently anabolic [181]. Clinician administered injectable treatments also minimise the adherence issues inherent to self administered preparations. A minority of small studies failed to show an increase in lean mass in response to T administration, this may be due to insufficient treatment duration [193, 194], insensitive assessment of lean mass by Bioelectrical Impedance Analysis (BIA) [195] or inclusion of men with normal T levels [154].

In contrast to growth hormone treatment the effects of T appear to be highly specific to muscle mass [196]. In an ultrasound imaging study, T treatment was found to preserve gastrocnemius muscle thickness [196]. Furthermore muscle biopsy studies in young and older men have shown dose dependent increases in cross sectional area of both type 1 and type 2 muscle fibres in response to T treatment [197, 198].

It has been suggested the primary anabolic action of T may be to stimulate the differentiation of pluripotent mesenchymal stem cells into myogenic lineage [199]. A cell culture study, using embryonic mouse cells found that incubation of these cells in androgen solutions (either T or dihydrotestosterone (DHT)), resulting in dose dependent increases in myogenic cells and myogenic markers [200]. These effects were
accompanied by corresponding decreases in adipogenic cells and markers of adipogenic lineage [200]. Biopsy studies have confirmed that these cells contain androgen receptors in humans [201]. Human studies have also demonstrated increases in satellite cell numbers and activation in response to T treatment [198, 202]. T also appears to influence muscle protein metabolism [194, 203-205]. T treatment over the short and medium term has been shown to stimulate muscle protein synthesis in young men [204, 205]. Four weeks T treatments lead to increases in muscle protein synthesis in older men with low T levels [194]. While 6 months treatment has been shown to decrease fasting protein degradation in older men [192]. These anabolic effects on muscle lend credence to the suggestion that changes in T levels may be actively involved in the development of frailty. Furthermore causal modelling studies in large populations of men suggest the effects of T on strength and physical function are due to these effects on body composition [206, 207].

Despite this clear effect on muscle mass, improvements in muscle strength have been less consistent. A number of studies have reported improvements in grip strength in response to androgen treatment [180, 208, 209], while others have not [181, 182, 186, 189-191, 195]. This may be due to small differences in the testing protocols used or simply to the variability of this measurement. Similarly, some studies have reported improvements in lower limb strength [182, 187, 192], while others have failed to show any effect of T treatment [180-182, 187, 189, 191, 192]. It is possible the gains in muscle mass in some studies may be too small to result in increases in strength, a recent study estimated that in order to increase leg or chest press strength through hormonal intervention gains in lean mass of at least 1.6 kg were required [210]. This may explain the lack of effect in some studies, although increases in lean mass were generally in this range. Furthermore there were notable discrepancies between improvements in strength
and the amount of lean mass gained. In these cases the differences between studies may relate to the different strength assessments used. Studies using 1 Repetition Maximum (1 RM) protocols have shown 11% increases in leg extension strength [187] and 15kg increases in leg press [192] alongside gains in lean mass of 1 and 4 kg respectively in healthy older men. A study in older men with mobility limitations showed similar improvements in leg press strength, using this technique [182]. In contrast studies using isokinetic dynamometry have either shown very small improvements or no improvement in lower limb strength compared to placebo, despite gains in lean mass of 1-4 kg and treatment for up to 3 years [181, 183, 189, 191, 211]. Improvements in knee extension strength of 6% have been shown in frail men using isometric dynamometry [181].

Greater improvements in 1 RM performance compared to dynamometry have also been seen in resistance training studies [212-214]. In these studies the difference is probably because 1 RM protocols often involve the same mode of exercise as used in the training while dynamometry assessments use different modes of contraction. In this context dynamometry may detect changes in the inherent force generating capacity of a muscle group, while improvements in 1 RM additionally reflect a specific neural adaptation to training [212, 214]. It is not clear why a similar effect may be seen in response to T treatment when gains in strength should be entirely due to muscle anabolism. Due to the high load in 1 RM contractions the contraction velocity is quite slow, it has been suggested that this may be more similar to that seen during isometric contractions [214]. It may be that the faster contraction speeds used in most isometric dynamometry studies give less accurate assessments of muscle strength in older men, in whom both the contraction velocity and rate of force development are reduced [215, 216].
The majority of studies in both healthy and frail older men have shown little effect of T treatment on physical function [181, 183, 186, 189, 195, 211, 217]. Studies in healthy men have failed to show improvements in functional tasks including, tests of balance, gait speed, chair rising, step height and functional reaching [186, 189, 195, 211, 217]. Similarly studies in frail men have shown no overall effect of T on an array of functional tasks [181, 183], although in 1 trial improvements on some scales were seen in older (≥75 years) and frailer (≥2 of Fried et al’s criteria) men [181]. One study in healthy men did show an improvement in performance of a composite functional task in response to 3 years T treatment [180]. While a study in mobility limited older men found an improvement in loaded stair climbing, where participants carried weights in both hands analogous to carrying shopping, but not in gait speed or unloaded stair climbing [182].

There are a number of reasons why increases in muscle mass and strength may not lead to improvements in physical function. Firstly it is possible that increases in strength from T treatment may have been too small lead to functional improvement in some studies. Secondly, the relationship between muscle strength and physical function is nonlinear. At a certain level dependent on the difficulty of the task used, the relationship plateaus, such that further increases in strength will not result in improvements in physical function [218-220]. It is likely that in the majority of studies the participants were above the most sensitive strength ranges for the assessments used prior to T treatment. In agreement, a recent study suggests more difficult tasks allow more discrimination in functional performance even in mobility limited older men [221]. Furthermore even supraphysiological doses of T may not improve performance on traditional function assessments in healthy older men [222]. This may explain why improvements were seen on the difficult weighted stair climb and composite tasks, but
not on other functional scales [180, 182]. The fact functional improvements were confined to only the most functionally limited men in another trial is also consistent with this framework [181]. Finally, physical function is dependent upon a number of factors in addition to strength [223], with strength making a varying contribution to the performance of different tasks [224]. It may be that T needs to be combined with exercise or other functional training in order to engender broad spectrum functional improvements [225].

The anabolic effects of T appear to peak within the first 6 months of treatment, increases in muscle mass may then be maintained for the duration of treatment (up to 3 years) in healthy older men [180, 189, 191, 225]. A small study found the effects of androgen treatment, with the orally active androgenic anabolic steroid, oxandrolone on muscle mass and strength declined within 12 weeks of treatment cessation in healthy older men [226]. However, little is known about the post treatment effects of physiological T treatment.

1.5.6.5.3 Safety
There have been long standing concerns over the safety of T therapy in older men [227, 228]. The weight of evidence now suggests that the effects of T treatment on the prostate are mild [227, 229]. A meta analysis of 19 trials in men aged >45 suggested that while prostate events were more common in T treated men, this could largely be explained by monitoring biases [227]. T treatment raises Prostate Specific Antigen (PSA) levels, leading to more frequent prostate biopsies in treated men against a high background prevalence of sub-clinical prostate cancer in normally ageing men [227]. The potentially adverse cardiovascular effects of T have recently received widespread attention following the termination of the Testosterone in Older Men (TOM) trial due to
a high frequency of adverse cardiovascular events in T treatment men [182]. However other similar trials have shown no increase in adverse events in response to T treatment in healthy and frail older men [181, 183, 186]. Meta-Analyses of placebo-controlled studies in adult and older men, suggest the most frequent adverse effect of T is a dose dependent increase in haematocrit and haemoglobin which can lead to clinically significant erythrocytosis [227, 228, 230, 231]. This effects may be due to suppression of hepcidin [232]. The high event rate in the TOM trial may be partially due to the higher dose of T used leading to clinical relevant rises in haematocrit. Alternatively it may be that the TOM trial included men at greater risks of cardiovascular events, with a high prevalence of obesity, hypertension, diabetes, and hyperlipidaemia [182].

1.5.6.6 Multiple hormonal deficiency

Most of the hormones discussed show equivocal relationships with frailty. One possible reason for this maybe due to redundancy between hormones [233, 234], where low levels of one anabolic hormone can be compensated for by maintained levels of other hormones. A recent study in older women looked at the relationship between low levels of 3 hormonal markers from free T, IGF-1 and DHEAS and frailty [235]. It was found that women with low levels of 2 or 3 hormonal markers were more likely to be frail compared to those with normal levels [235]. Multiple hormonal deficiencies have also been shown to predict mortality in older men from the InCHIANTI study [236] and in heart failure patients [237]. However, in a recent study, no relationship was found between low levels of multiple anabolic hormones and muscle strength decline in older men and women [238].
1.5.7 Endocrine inflammatory interactions

A cardinal feature of frailty is believed to be decline across multiple physiological systems. While the majority of studies have focused on single systems it is important also to understand the nature of interactions between systems in the pathogenesis of frailty [239, 240]. One such interaction is that between changes in the endocrine and inflammatory systems. A study in older women found a joint U-shaped relationship between IGF-1 and white blood cells and frailty [241]. Women with high levels of white blood cells and low or high levels of IGF-1 were found to be at higher risk of frailty, those with low levels of both markers were also found to have a somewhat elevated risk [241]. In a separate analysis of the same cohort, women with low levels of IGF-1 and high levels of IL-6 were found to be at greater risk of walking limitation, mobility limitation and IADL disability at 3 year follow up [145]. Furthermore the size of the odds ratio (OR) for both markers was greater than the sum of the individual ORs for each of the outcomes [145]. This may suggest potential synergy between the 2 markers, whereby the combination of the 2 has an additional effect to both of the markers individually [145]. A study in men and women aged 20-102 showed the opposite effect, in an analyses stratified by IL-6 tertiles, free IGF-1 was only significantly related to grip strength and leg power in participants with the lowest IL-6 levels [142].

1.5.8 Multiple systems

Frailty has been related to markers from a variety of systems, one interpretation of this may be that frailty is related to generalised biological dysregulation. This suggestion is compatible with the belief that frailty is a multisystem disorder. Recent studies have assessed this hypothesis directly with intriguing results [242-244]. Frailty was related to dysregulation across 6 physiological systems including the haematological,
inflammatory, hormonal, adiposity, neuromuscular and micronutrient systems, in older women [4]. The relationship between the number of abnormal systems and frailty was nonlinear, providing possible support for the hypothesis that frailty relates to aggregate burden of physiological dysregulation [2]. Other studies have focussed on the relationship between frailty and a different measure of cumulative dysregulation, allostatic load [243, 244]. This is a summary measure reflecting the cost of life course adaptation to stress including markers from the neuro-endocrine, immunological and cardiovascular systems [245]. Higher allostatic load score was moderately related to frailty in a cross-sectional study of women from the WHAS 1 and 2 cohorts [244]. Similarly, in a prospective study in high functioning men and women aged 70-79, higher allostatic load score was associated with increased risk of frailty at 3 year follow up [243].

1.6 Summary

A universally accepted definition of frailty is still lacking, however the development of validated models to assess this condition represents a major recent advance in this field. Much progress has been made in the study of frailty using these models. However, the majority of work has been carried out in the US and Canada, there is therefore a need to expand this effort to further populations. The most widely used model has been the frailty phenotype, however the use of this model in existing datasets is dependent on variable availability and new adaptations require additional validation within their datasets. While recently more studies have started to focus on men, there remains an imbalance with frailty being more intensively studied in women. The sex hormone system has been a focus of these studies in men, however, for a number of reasons including the use of different frailty measures, T assays and populations, results have been equivocal. Most trials of T treatment have included healthy men and there is a lack
of data on the longer term effects of treatment. While frailty is believed to relate to declines across multiple physiological systems the majority of studies have focussed on single biological correlates.

The broad aim of this thesis was to explore the condition of frailty and its potential hormonal contributors in ageing European men. This addresses both the gender and contextual imbalance seen in previous studies. An initial aim was to produce and validate a version of the frailty phenotype for use in a European cohort study of ageing men. The majority of previous studies have used single frailty models, most commonly the frailty phenotype. It was decided that while a consensus definition is lacking, the two most widely accepted models, the frailty phenotype and frailty index, would be used alongside each other to more completely explore this condition. While a number of studies have focussed on T and frailty, this thesis aimed to address gaps in this literature, including the relationships with different frailty models and the lack of knowledge of post treatment effects. A further aim was to use more detailed hormonal data to explore some of the theoretical underpinnings of frailty.
Chapter 2 Scope of this Thesis

2.1 Hypothesis

The overarching hypothesis of this thesis was that changes in anabolic hormone levels, particularly androgens, are involved in the development of frailty in ageing men.

2.2 Resources

This project focussed on analysis of data from two studies on the theme of hormonal ageing in men. These were, the European Male Ageing Study (EMAS), a large observational cohort study in men from 8 European centres and the Schering testosterone interventional trail, a clinical trial of T treatment in older men with symptoms of frailty. Details of these studies are provided in the ‘Methods’ section.

2.3 Aims and objectives

The overall aim of the project was to use the two datasets to explore the condition of frailty and its hormonal contributors in ageing men. The specific aims were:

1. To adapt the CHS frailty phenotype model for use in the EMAS and to compare and validate this model alongside the EMAS frailty index
2. To assess the prevalence of frailty across the 8 European centres in the EMAS
3. To explore the relationships between sex hormones of the Hypothalamic Pituitary Testicular (HPT) Axis and frailty in the EMAS, in terms of hormone levels and defined states of hormonal abnormality
4. To explore the relationship between deficiencies of multiple anabolic hormones and frailty in the EMAS
5. To assess the effects of testosterone treatment in prefrail and frail men at 6 months post treatment
Chapter 3 Methods

The work presented in this thesis is based on analysis of data from two studies on ageing in men: The European Male Ageing Study (EMAS), a longitudinal cohort study of the symptoms of ageing in men, and the Schering testosterone interventional trial, a large clinical trial of testosterone treatment in elderly men with low testosterone and symptoms of frailty. The detailed methods for both these studies are reported elsewhere [181, 246]. Here follows a brief outline of the methods for the two studies. The tables included in this chapter are reproduced from Lee et al 2009 [246].

3.1 The European Male Ageing Study (EMAS)

3.1.1 Design

This was a multicentre longitudinal cohort study of men from 8 European centres. The work presented in this thesis is based on cross-sectional analysis of the baseline data, with the exception of the incident falls data collected at 2 years follow up.

3.1.2 Participants

Men aged 40-79 years were recruited using population based sampling frames from 8 European centres: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmo (Sweden), Szeged (Hungary), Manchester (United Kingdom), Santiago de Compostela (Spain), and Tartu (Estonia). The study aimed to recruit approximately 400 men from each centre. This was because the primary aim of EMAS was to determine the decline in T over a 4-5 year period. Initial power calculations suggested this number of men were needed to provide 80% power ($\alpha =0.05$) to detect differences in T declines between centres, assuming at least 2 fold variation around a mean decline of 0.4-1.5% per year estimated from previous studies [246]. Stratified random sampling was used
with the aim of recruiting equal numbers of men to each of the four 10 year age bands (40-49, 50-59, 60-69 & 70-79).

The analyses presented in this thesis include men from all age bands sampled. While frailty may be primarily a condition of older ages, studies using the frailty index have shown that differences in frailty can be meaningfully graded across the adult lifespan, with higher FI at any age associated with worse prognosis [247, 248]. Similarly, symptoms of phenotypic frailty have been found to be relatively common in middle aged adults [165, 249]. As described in chapter 4, the phenotypic criteria were defined in the older men (aged ≥65). These criteria were then applied to the whole cohort, with their prevalence in relation to age forming part of their initial validation. Finally, exploratory analyses for the later chapters suggested results were similar in the older subgroup and full cohort.

### 3.1.3 Assessments

Initially, participants completed a postal questionnaire, this included questions about general health education and physical activity. Participants were asked about any morbidities they were receiving treatment for including heart disease, high blood pressure, diabetes and prostate disease. Smoking was recorded as either current, past or non smoker, and alcohol intake was quantified in terms of typical frequency of consumption during the preceding month, in days per week.

Participants then underwent a comprehensive assessment of their health status including an interviewer assisted questionnaire and a range of physical and cognitive function tests. The assisted questionnaire included the Short Form 36 (SF-36) [250] to assess health related quality of life, the Physical Activity Scale for the Elderly (PASE) [251] to
estimate physical activity levels and the Beck’s Depression Inventory (BDI) [252] to assess depressive symptoms. Physical function was assessed using the Physical Performance Test (PPT) [253] and the Tinetti test for gait and balance [254]. The 7 item PPT was used, this did not include stair climbing, but did include a 15.4 metre self selected gait speed test. The Tinetti assessments included the 5 chair stands test, in which participants were asked to rise from a chair 5 times at their own pace.

Height and weight were measured using standard calibrated instruments. Body mass index (BMI) was calculated as body mass (Kg) divided by the square of height (m²). Body composition was assessed by anthropometry, including limb circumferences and skin fold thicknesses for the entire cohort. A subset of 800 men from Manchester and Leuven also underwent Dual-energy X-ray Absorptiometry (DXA) scans to assess lean and fat mass.

3.1.4 Hormone measurements
A single fasting morning (before 10.00 h) venous blood sample was used for all measurements. T and E2 were measured by gas chromatography-mass spectrometry (GC-MS). LH, FSH, DHEAS and SHBG were measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Vitamin D levels were determined using an equilibrium radioimmunoassay. IGF-1 levels were measured by chemiluminescence immunoassay. Free T levels were derived from total T, SHBG and albumin concentrations using the Vermeulen formula [255].

3.1.5 Response rates
The overall response rate for full participation in EMAS was 40% (Table 3.1). There were substantial differences in response rates across centres, ranging from 24% in
Szeged to 60% in Florence (Table 3.1). Adjusting for those who died or moved away following contact, the response rate was slightly higher at 41% (Table 3.1). Restricting analyses to the centres who undertook follow up contact of initial non responders the response rate was 44% (Table 3.1). The total number of men recruited into the study was 3369. Of the centres, Leuven recruited the most men at 451 and Manchester the fewest at 396 (Table 3.1)

3.1.6 Comparison with non responders

Information on non responders was obtained from population registers, postal questionnaires, from those who completed them, but did not participate in the full study, and from a non responder telephone survey. Compared to those who took part, non responders tended to be slightly older, and to have left education at a younger age (Table 3.2). Non responders were more likely to report poor or fair general health, they were less likely to report experiencing bodily pain in the last month and more likely to exercise for at least half an hour per day (Table 3.2). There no differences between groups for other health behaviours or morbidities (Table 3.2).

3.1.7 Interim postal questionnaire

The EMAS was designed as a longitudinal study involving a 4 year follow up period between waves 1 and 2. In between these waves additional outcome data was collected using a postal questionnaire sent out to all participants at approximately 2 years after baseline assessments. The main outcome collected was incident falls, participants were asked to report their number falls over the preceding 12 months, with the possible responses of ‘0’, ‘1’ or ‘2 or more’
### Table 3.1: Participation rates in the European Male Ageing Study by centre

<table>
<thead>
<tr>
<th>Centre</th>
<th>Country</th>
<th>Number surveyed</th>
<th>Number of participants in full study</th>
<th>Participation rate (%)</th>
<th>Died or moved house</th>
<th>Corrected participation rate (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florence</td>
<td>Italy</td>
<td>723</td>
<td>433</td>
<td>59.9</td>
<td>19</td>
<td>61.5</td>
</tr>
<tr>
<td>Leuven</td>
<td>Belgium</td>
<td>1,189</td>
<td>451</td>
<td>37.9</td>
<td>20</td>
<td>38.6</td>
</tr>
<tr>
<td>Lodz</td>
<td>Poland</td>
<td>843</td>
<td>408</td>
<td>48.4</td>
<td>64</td>
<td>52.4</td>
</tr>
<tr>
<td>Malmö</td>
<td>Sweden</td>
<td>918</td>
<td>409</td>
<td>44.6</td>
<td>46</td>
<td>46.9</td>
</tr>
<tr>
<td>Manchester</td>
<td>UK</td>
<td>1,064</td>
<td>396</td>
<td>37.2</td>
<td>44</td>
<td>38.8</td>
</tr>
<tr>
<td>Santiago</td>
<td>Spain</td>
<td>1,155</td>
<td>406</td>
<td>35.2</td>
<td>85</td>
<td>37.9</td>
</tr>
<tr>
<td>Szeged</td>
<td>Hungary</td>
<td>1,789</td>
<td>431</td>
<td>24.1</td>
<td>Unknown</td>
<td>24.1</td>
</tr>
<tr>
<td>Tartu</td>
<td>Estonia</td>
<td>735</td>
<td>435</td>
<td>59.2</td>
<td>Unknown</td>
<td>59.2</td>
</tr>
<tr>
<td>EMAS total</td>
<td></td>
<td>8,416</td>
<td>3,369</td>
<td>40</td>
<td>278</td>
<td>41.4</td>
</tr>
<tr>
<td>Centre mean†</td>
<td></td>
<td>1,052</td>
<td>421</td>
<td>43.3</td>
<td>46</td>
<td>44.9</td>
</tr>
</tbody>
</table>

*Participation rate after excluding those who died or moved house
†Mean of centre participation rates

### Table 3.2: Self-reported characteristics of participants and non-participants in the European Male Ageing Study

<table>
<thead>
<tr>
<th></th>
<th>Full study participants</th>
<th>Telephone survey of non-responders</th>
<th>Postal questionnaire only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 3,369</td>
<td>n = 361</td>
<td>n = 594</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.0 (11.0)</td>
<td>59.6 (11.7)</td>
<td>62.8 (11.7)†b</td>
</tr>
<tr>
<td>Age left education (years)</td>
<td>20.9 (7.7)</td>
<td>18.7 (7.3)b</td>
<td>19.1 (7.8)b</td>
</tr>
<tr>
<td>Number of morbidities (0-17)</td>
<td>1.2 (1.3)</td>
<td>-</td>
<td>1.3 (1.4)</td>
</tr>
<tr>
<td>Number of children (0-15)</td>
<td>1.9 (1.2)</td>
<td>-</td>
<td>1.9 (1.3)</td>
</tr>
<tr>
<td>General health fair or poor</td>
<td>33.4</td>
<td>34.8</td>
<td>37.9b</td>
</tr>
<tr>
<td>Walking or cycling 3 ½ hour per day</td>
<td>65.3</td>
<td>62.1</td>
<td>70.2b</td>
</tr>
<tr>
<td>Ever smoked cigarettes (vs. never)</td>
<td>70.5</td>
<td>72.9</td>
<td>69.6</td>
</tr>
<tr>
<td>Current smoker (vs. non-smoker)</td>
<td>21.1</td>
<td>33.0b</td>
<td>20.6</td>
</tr>
<tr>
<td>Alcohol consumption 3 1 day per week</td>
<td>56</td>
<td>-</td>
<td>57.3</td>
</tr>
<tr>
<td>Heart condition (yes vs. no)</td>
<td>16.6</td>
<td>-</td>
<td>18.3</td>
</tr>
<tr>
<td>High blood pressure (yes vs. no)</td>
<td>29</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>Diabetes (yes vs. no)</td>
<td>7.7</td>
<td>-</td>
<td>9.9</td>
</tr>
<tr>
<td>Pain in the past month (lasting 3 1 day)</td>
<td>59.4</td>
<td>-</td>
<td>43.8b</td>
</tr>
</tbody>
</table>

*p < 0.05 t-test or chi-square test between participants vs. non-response telephone survey subjects
b*p < 0.05 t-test or chi-square test between participants vs. postal questionnaire only subjects
3.2 The Schering Testosterone Interventional Trial

3.2.1 Design
This was a single-centre, randomized, double-blind, placebo-controlled parallel group study. The trial involved 6 months allocation to placebo or active T treatment, participants were then reassessed 6 months after completion of treatment.

3.2.2 Sample size and power
Preliminary data [195] indicated inter-patient standard deviations of 27% in lower limb muscle strength assessments. A conservative estimate was taken that this represented the coefficient of variation (of the change (i.e. a low intra-individual correlation) giving 115 participants per arm to provide 80% power to detect a 10% improvement in the primary endpoint (IME-PT) at 5% significance level. This number was increased to 130 to allow for an estimated 13% dropout rate.

3.2.3 Randomisation procedure
Participants were randomised into active and placebo groups in blocks of 10 by a computer generated sequence produced by the trial pharmacist. The pharmacist had no contact with research participants.

3.2.4 Interventions
The active group were treated with T gel (Testogel® 1%, Bayer Schering Pharma, Berlin, Germany) at a starting dose of 50 mg/day, the placebo group received a matched placebo gel. The dose of the gel was adjusted to 75 or 25 mg/day according to serum T at day 10 and 3 months. Dose adjustment was undertaken if circulating T levels were outside the target range of 18-30nmol/L, the placebo group therefore received the maximum dose. No interventions were given during the 6 month post treatment phase.
3.2.5 Participants

Community-dwelling men aged $\geq$65yrs were recruited by advertisements or mailed invitations from family doctor registers and screened for presence of frailty from November 2004 to August 2006, according to the criteria of Fried et al [4]. These comprised unintentional weight loss, self-reported exhaustion, low physical activity, slow walk time and low grip strength. The exact criteria used, while based on the same model [4], were different to those used to define frailty in the EMAS study in the later chapters of this thesis. Those with one or more of these five criteria for frailty and a morning total $T \leq 12$ nmol/L or calculated free $T \leq 250$ pmol/L were eligible for inclusion in the study. Exclusion criteria were: prostate cancer, benign prostatic hyperplasia (International Prostate Symptom Score, IPSS$>21$), chronic renal impairment (serum creatinine $>180$ mmol/L), active liver disease, moderate to severe peripheral vascular disease, severe chronic obstructive pulmonary disease, congestive heart failure (New York Heart Association Functional Classification $\geq 2$), angina requiring nitrate spray $>$once/wk, untreated sleep apnoea, major psychiatric illness, medications interfering with sex steroid metabolism, stroke causing persistent motor weakness, active systemic disease of muscle and joint, and cognitive impairment (Mini Mental State Examination, (MMSE) score $<18$). The study was approved by the Central Manchester Research Ethics Committee and written, informed consent obtained from each participant.

3.2.6 Blinding

The research participants, outcome assessor, study physician and other research staff remained blinded to treatment allocation throughout the study. The dose of the gel was adjusted by a clinician not involved in outcome assessment or other participant
monitoring. Precautions were taken to ensure that the outcome assessor, monitoring clinician and research nurses remained unaware of trial medication type and dose for individual participants. The presence or absence of dose adjustment for a given participant did not provide sufficient information to determine which treatment they were receiving, except possibly in 27 participants who received a dose reduction.

3.2.7 Outcomes

The primary outcome was knee extensor strength assessed by isometric peak torque (IME-PT) and isokinetic peak torque (IKE-PT), using isometric and isokinetic dynamometry (Isokom). Secondary outcomes included: lower and upper limb muscle strength assessed by isometric knee flexion peak torque (IMF-PT), isokinetic knee flexion peak torque (IKE-PT) and grip strength respectively, physical function tests, body composition and quality of life. All outcome assessments were carried out by a single assessor at baseline, at 6 months (end of treatment) and at 12 months (post-treatment follow up).

Isokinetic dynamometry to assess IKE-PT and IKE-PT was performed at an angular velocity of 90°/sec. Extension and flexion contractions were performed separately with a 2 minute rest period between each set of trials. For each set of trials the participants performed 2 sub maximal practice contractions, before performing 5 maximum voluntary contractions. A 60 second rest was allowed between each contraction. Isometric dynamometry at 90° knee joint angle for extension contractions (IME-PT) and full knee extension for flexion contractions (IMF-PT) was performed following a similar protocol with 2 sub maximal practice contractions followed by three maximum voluntary contractions. Contractions were held for 5 seconds. A 2 minute rest period
was allowed between sub maximal and maximal contractions with 25 seconds rest between each maximal contraction.

Physical function tests included the Aggregate Locomotor Function test (ALF) [256], the PPT [253], 6-minute-walk-test (6MWT) [257] and Tinetti gait and balance test [258]. The ALF includes three components; timed 8-meter walk, timed sit-to-stand transfer and timed stair ascent and descent. In the timed 8m walk, the men were asked to walk a distance of 10 metres at a comfortable walking pace, along a flat corridor. In the timed sit-to-stand test, the men were asked to walk a distance of 8 meters and sit on a chair, rise and walk back. In the timed stair climbing, the men were asked to ascend and descend a set of steps (three 20 cm high steps and four 15cm high steps). Each test was performed multiple times and a mean taken from; 3 repetitions for the walk and transfer tests and 4 for the stair test. As in the EMAS the 7 item PPT was used, the Tinetti gait and balance assessment in this study did not include the 5 chair stands test. Self-reported physical activity was estimated from the PASE questionnaire [259]. Functional assessments were performed in a pre-specified sequence at one clinic visit with rest periods in between.

Body composition (lean and fat mass) was measured by DXA; QDR 4500 Discovery scanner, Hologic Inc, Bedford, MA). Quality of Life was assessed by the Aging Males Symptom score (AMS) [260], a self administered questionnaire.

3.2.8 Hormone measurements

Levels of T, LH, FSH and SHBG were measured by chemiluminescent immunoassay with a Roche Elecys E170 platform at baseline, 10 days, 3 months, 6 months and 12 months. Free T was calculated using the Vermeulen formula [255].
3.2.9 Data cleaning, collation and creating trial database

The author was responsible for compiling the data set for the 12 month follow up phase of the trial and constructing the follow up and overall trial databases used for the analyses presented in this thesis.

Trial data was collected on paper clinical research files. From these files the data was entered into a Microsoft Access database. Following completion of data entry and accuracy checks, the final data for the 12 month study point was extracted from the entry database into spreadsheet format. These spreadsheets were then rearranged into randomisation number order in order to match the format of the 6 month database. From these spreadsheets individual files for each section of the data (dynamometry, PPT, ALF, individual questionnaires etc) were created using SPSS 15.0. Scoring and coding of study variables from this raw data was performed according to previously agreed standards applied to the baseline and 6 month data. Once the files were organised in this manner, any unexplained missing data was identified and manual checks of individual participants study files were undertaken in order to find any remaining reasons for this missingness. At this stage, potential withdrawals during this follow up phase, i.e. those missing all 12 month data were checked and verified from the participant records. The data files were also screened for any outstandingly unusual values, which, like the missing data, were checked against the participant files. Once this process was complete for all 12 month data, the individual files were combined to give an overall 12 month data base which was then added to the overall trial database. This was done using SPSS codes adapted from those written by SA Roberts for building the earlier database files.
To identify any errors in data entry, data checking was then carried out in this new file by plotting the 12 month data points against their equivalents at baseline and 6 months in scatter plots. While some change may be seen over 12 months it would be expected that the measurements at each time point should be highly correlated. Any outstanding disagreements with the earlier data were identified and the entered data was checked against the raw data from the participant’s clinical research files, and corrected where required.

3.3 Statistical analyses

The detailed analytical methods used for this thesis are described in their respective results chapters. The author performed all analyses described herein, and in consultation with SA Roberts and A Tajar, designed all analyses presented. All analyses were performed in STATA version 8.2.
Chapter 4 Development and Validation of Frailty Models for use in the European Male Ageing Study

4.1 Introduction

Defining frailty as a medical condition has proved complex [261]. The design of models to assess this condition has been a major recent innovation in this field [2-4]. The most widely used model is the frailty phenotype designed in the CHS study [4]. This model has been repeatedly modified for use in different data sets due to variable availability [15, 16, 165, 249]. These adapted models have frequently been independently validated and show comparable predictive ability for adverse outcomes to the original criteria [13, 15, 16].

Another frequently used model is the frailty index [4, 10], wherein frailty or vulnerability is quantified according to the accumulation of health deficits [2]. Like the frailty phenotype this model has been applied across multiple data sets [11, 12]. However, the precise deficits measured for this model are not considered important with the count or burden of deficits being the key factor in measuring a patient’s frailty [2]. Provided the deficits selected meet certain criteria (described in the Introduction chapter) and enough systems are sampled, this model shows reproducible characteristic across different data sets regardless of precise composition of deficits [21-23]. This model therefore represents a potentially useful tool in the validation of a modified set of frailty criteria. Comparison of these disparate models may also offer key insights into the nature of frailty and hence has been the subject of some research [30-32, 37].

The core feature of any frailty model is the ability to predict adverse outcomes, and thus show ability to quantify the vulnerability central to this condition. Falls are a key
geriatric outcome associated with progression of disability and reductions in quality of life [262]. Furthermore, both falls and frailty have been suggested to represent functionally linked manifestations of complex system failure [263]. As such, falls have been frequently used in the validation and comparison of frailty models [5, 14, 33, 35].

The broad aims of the work described in this chapter were to design and validate models to measure frailty in the EMAS data. Specifically these included: 1) Development of a modified set of frailty phenotype criteria, based on the original CHS study model, using variables available at the baseline phase of EMAS 2) Describe the characteristics of the EMAS frailty index, 3) Asses convergent validity by comparing these two models, 4) Assess criterion validity of the two models in terms of predictive ability for falls.
4.2 Methods

4.2.1 Participants

The analyses presented in this chapter are based on men enrolled in the baseline phase of the EMAS study. Part of the frailty criteria validation was based on men enrolled in the baseline phase of the Schering trial.

4.2.2 The EMAS frailty phenotype

The phenotypic frailty criteria developed by Fried and colleagues in CHS cohort [4] needed to be substantially modified for application in the EMAS study. Candidate variables were identified from review of the literature for the 5 criteria, slow walk speed, shrinking or sarcopenia, exhaustion, low physical activity and weakness. Where no equivalent variables were available, surrogate markers were correlated against objective measurements in a subset of EMAS men and men from the baseline phase of the Schering testosterone interventional study. Details of this selection process along with the final EMAS criteria are included in the results section of this chapter. The final EMAS frailty criteria are shown in table 4.2, in the ‘Results’ section of this chapter.

Once the model was developed differences in participant characteristics between frailty states were assessed using Analysis of Variance (ANOVA) for continuous variables and Chi-square for discrete variables.

4.2.3 The EMAS frailty index

The EMAS frailty index was developed in collaboration with K Rockwood’s team, the model’s original designers. The work was carried out by A Tajar. The EMAS frailty index was constructed according to standard methods [21]. In order to be included in the frailty index variables had to satisfy 3 criteria:

1) The variables must be deficits associated with health status.
2) A deficit’s prevalence must generally increase with age.

3) The deficits must not saturate too early, i.e., although an individual deficit becomes more common with age, it should not become universal at an early age.

Forty three deficits were included in the EMAS FI. These included items related to activities of daily living, International Prostate Symptom Scores, cognitive function tests, self reported morbidities and drugs taken to manage morbidities. The original EMAS FI also included the slow walking speed criteria from the frailty phenotype [29]. As a purpose of this chapter was comparing the 2 models, this variable was excluded from the FI used in this thesis, leaving forty two variables in the index. The included deficits are listed in Table 4.8, in the ‘Results’ section of this chapter.

Conceptually, the FI does not stratify patients according to levels of frailty [2]. However, for the purpose of comparison with the frailty phenotype this model has previously been divided into a 3 level variable, based on the robust, prefrail and frail classification system used for the frailty phenotype [4]. In this study a 3 level FI variable was created with cut-points for each category based on the prevalence of phenotypic frailty states. The cut-points used were 0.17 for prefrail and 0.4 for frail. This classification meant the prevalence of frailty was similar using either model, allowing for fair comparison between them.

4.2.4 Cross comparison of models

The relationship between the frailty phenotype and the FI was explored using descriptive statistics including correlations and graphical exploration of the distribution of the FI by phenotypic frailty categories.
4.2.5 Frailty and falls prediction

Data on falls was collected as part of the 2-year interim EMAS questionnaire. Participants were asked to report their number of falls in the preceding 12 months, with the possible responses; 0, 1, or 2 or more. The relationship between frailty and falls was assessed in two ways. First using ordinal logistic regression with falls as a three level ordinal outcome (0, 1 & \( \geq 2 \)). This approach effectively models the odds of moving up the levels of the dependent variables, in this case more falls. This approach was chosen to include all the falls outcome data and take account of any trend across the 3 levels. This analysis was performed for the frailty phenotype and the 3 tier FI. Two models were fitted, the first was unadjusted and the second adjusted for age and centre. Next, to further assess the predictive ability of the two frailty models for falls, Receiver Operating Characteristics (ROC) curves for recurrent falls outcome were fit for each model and compared. This technique produces a plot of the sensitivity or true positive rate vs the specificity or false positive rate for a given predictor and outcome. The area under the curve represents a summary measure of the sensitivity and specificity. Values range from 0.5 indicating no better than random discrimination, as might be achieved by using the toss of a coin to identify positive events, up to 1.0 indicating 100% sensitivity and specificity.
4.3 Results

4.3.1 Designing the frailty criteria

Following review of the literature suitable variables were selected for the low activity, exhaustion and slowness criteria. Physical Activity Scale for the Elderly score was selected for use for the low activity criteria, the Beck’s Depression Inventory energy and fatigue items were chosen for the exhaustion criteria and the 50 foot PPT walk was selected for the slowness criteria. These represent equivalent measures to those used in the original definition, and have been included in variations of the frailty phenotype [13, 72, 165, 264].

There was no measure of weight loss available at the baseline phase of EMAS, there were also no whole body lean mass measures available. However, whole body lean mass by DXA was available on a subset of EMAS men and data on limb circumferences by anthropometry were available for the whole cohort. Anthropometric measures based on limb circumferences have previously been used as simple markers of sarcopenia or low lean body mass in elderly and patient groups [265-268]. In order to further support the use of these markers in the EMAS and select the most suitable measure for this criterion, mid calf circumference, mid upper arm circumference, mid upper arm muscle circumference (arm circumference – \( \pi \) x triceps skin fold) and the mean of arm and calf circumference were correlated against a previously defined, and widely accepted, measure of sarcopenia, Relative Appendicular Skeletal Muscle Mass (RASM), the muscle mass of the limbs scaled to the height squared [269] in the subgroup of 726 EMAS men who underwent DXA scans.

Of the potential sarcopenia markers, mid upper arm circumference correlated more strongly to RASM than did mid calf circumference (Figure 4.1). Correlation coefficients
for mid upper arm circumference and mid upper arm muscle circumference were similar. The correlation with the mean of arm and calf measurements was similar to that of the arm alone. Given these similar correlations, arm muscle circumference was preferred for this criterion as it is has already been established as a simple marker of sarcopenia in elderly and patient groups [266, 267], and is designed to be less sensitive to differences in body fat than raw limb circumferences.

There were no pure measures of muscle strength available in the EMAS data. The only potential option was the 5 chair stands test. This is a commonly used measure of lower limb strength and function in the elderly [223, 270] and has been used in previous frailty models [5, 33, 35]. To offer some validity to the use of the 5 chair stands test as a measure of muscle strength, a similar measure of sit-to-stand transferring time, taken from the ALF was correlated against measures of muscle function in the frail elderly men at baseline in the Schering T interventional study. The strength measures were grip strength by dynamometry, knee extensor strength by isometric dynamometry and knee extensor power by isokinetic dynamometry. The correlations seen were modest (Table 4.1).

Fried et al’s original frailty criteria were designed in a sample of men and women aged 65-101 [4], with cut-points for most criteria set at the lowest 20% for each gender. Correspondingly, cut-points for the EMAS frailty criteria were taken from the men aged 65 and over. It was felt this was closer to the original definition and would give a more realistic estimation of the prevalence of frailty in this younger cohort. The cut-point for the slowness and low activity criteria was set at the bottom 20% from the 65+ age group. In the original model, the shrinking or sarcopenia criterion was set at 10lbs unintentional weight loss in the preceding year, this measure had a very low prevalence
at 6% compared to 18-22% for the other criteria [4]. To reflect this lower prevalence and the relatively greater severity of the original criterion, compared to the other criteria, a lower cut-point, the bottom 10%, was chosen for the sarcopenia criterion. The weakness criterion was set as the lowest 10% and those unable to complete the 5 repetitions. The combination of this lower cut-point and ‘unable’ gave a more similar prevalence to the other criteria, particularly at younger ages. The final frailty criteria next to the original CHS criteria are shown in Table 4.2.

Once the final 5 criteria were selected, frailty was defined according to the established 3 tier classification: Men with 0 criteria were classed as robust, men with 1-2 as prefrail and men with 3 or more as frail.
Figure 4.1: Correlations between relative appendicular skeletal muscle mass and potential lean mass markers in European Male Ageing Study DXA subgroup. A) Calf circumference, B) arm circumference, C) arm muscle circumference and D) mean of calf and arm circumference.

A) Calf circumference

B) Arm circumference

$r=0.49$

$r=0.64$
Table 4.1: Spearman’s rank correlations between measures of muscle performance and sit-to-stand transfer time

<table>
<thead>
<tr>
<th>Measure</th>
<th>Transfer time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grip strength</td>
<td>-0.195</td>
</tr>
<tr>
<td>Knee extensor strength</td>
<td>-0.325</td>
</tr>
<tr>
<td>Knee extensor power</td>
<td>-0.403</td>
</tr>
<tr>
<td>Criteria</td>
<td>Cardiovascular Health Study (CHS)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sarcopenia / Shrinking</td>
<td>&gt;10lb body weight lost unintentionally in past year</td>
</tr>
<tr>
<td>Weakness</td>
<td>Grip strength - lowest 20% by gender &amp; BMI</td>
</tr>
<tr>
<td>Poor endurance</td>
<td>Exhaustion – Centre for Epidemiological Studies-Depression scale:</td>
</tr>
<tr>
<td></td>
<td>Answered ‘Most of the time’ or ‘a moderate amount of the time’ to</td>
</tr>
<tr>
<td></td>
<td>I felt that everything I did was an effort or I could not get going</td>
</tr>
<tr>
<td>Slowness</td>
<td>Walking Time/15 feet - slowest 20% by gender and height.</td>
</tr>
<tr>
<td>Low activity</td>
<td>Kcals/week - lowest 20% by gender. Males &lt;383</td>
</tr>
<tr>
<td></td>
<td>Estimated from the Minnesota Leisure Time Activity questionnaire</td>
</tr>
</tbody>
</table>
4.3.2 Overview of the EMAS frailty phenotype

Complete data on all frailty measures was available for 3053 men with a mean age of 59.8 ± 11 years (Table 4.3). The most frequently missing frailty criterion was low activity at 7.5%, the other criteria ranged from 1.2-2.3% missing data (Table 4.4). Those missing frailty data tended to be slightly older and slightly frailer, according to FI scores, SF36 scales and walking speed, than those with complete data on all criteria (Table 4.3). There were also some differences in missing data across centres, with the least missing data, 4.2% seen in Santiago and the most, 13% in Malmo (Table 4.5). All subsequent analyses were restricted to men with complete frailty data.

The prevalence of frailty in EMAS was 2.6% and prefrailty 26.8% (Table 4.6). Of the individual criteria the most prevalent was low activity at 10.7%, and the least prevalent sarcopenia at 5.6% (Table 4.6). Frail men had a higher prevalence of morbidities and were more likely to smoke (Table 4.7). Frail men also tended to have a slightly higher BMI, to have left education slightly earlier, and were less likely to consume alcohol frequently, although these trends were not significant (Table 4.7).

The prevalence of frailty increased with age in a curvilinear fashion from 0.1% in the 40-49 age group to 6.7% in the 70-79% group (Figure 4.2). The prevalence of all criteria increased with age, the age relationship was strongest for the low activity and slowness criteria (Figure 4.3). The exhaustion criteria showed a weaker relationship with age and had a relatively high prevalence in the middle aged men (Figure 4.3).
### Table 4.3: Characteristics of men with complete and missing frailty measures

<table>
<thead>
<tr>
<th></th>
<th>Complete Frailty Measures</th>
<th>Missing Frailty Measures</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3053</td>
<td>316</td>
<td></td>
</tr>
<tr>
<td>age</td>
<td>59.8 ± 11.0</td>
<td>62.1 ± 10.9</td>
<td>0.0004</td>
</tr>
<tr>
<td>Frailty Index</td>
<td>0.13 ± 0.11</td>
<td>0.15 ± 0.13</td>
<td>0.0006</td>
</tr>
<tr>
<td>SF 36 physical component</td>
<td>50.1 ± 8.1</td>
<td>48.7 ± 9.2</td>
<td>0.01</td>
</tr>
<tr>
<td>SF 36 mental component</td>
<td>51.7 ± 9.2</td>
<td>49.3 ± 11.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>15m walk time</td>
<td>13.5 ± 3.1</td>
<td>14.4 ± 5.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean ± SD

### Table 4.4: Overview of missing frailty measures

<table>
<thead>
<tr>
<th>Frailty</th>
<th>n</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frailty</td>
<td>316</td>
<td>9.4</td>
</tr>
<tr>
<td>Slowness</td>
<td>55</td>
<td>1.6</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>50</td>
<td>1.5</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>41</td>
<td>1.2</td>
</tr>
<tr>
<td>Low activity</td>
<td>251</td>
<td>7.5</td>
</tr>
<tr>
<td>Weakness</td>
<td>78</td>
<td>2.3</td>
</tr>
<tr>
<td>Frailty</td>
<td>Total</td>
<td>Florence</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Frailty</td>
<td>316 (9.4)</td>
<td>39 (9.0)</td>
</tr>
<tr>
<td>Slowness</td>
<td>55 (1.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>50 (1.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>41 (1.2)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Low Activity</td>
<td>251 (7.5)</td>
<td>36 (8.3)</td>
</tr>
<tr>
<td>Weakness</td>
<td>78 (2.3)</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

Data are count (%)
### Table 4.6: Phenotypic frailty in the European Male Ageing Study

<table>
<thead>
<tr>
<th>Frailty states</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robust</td>
<td>2157 (70.7)</td>
</tr>
<tr>
<td>Prefrail</td>
<td>818 (26.8)</td>
</tr>
<tr>
<td>Frail</td>
<td>78 (2.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frailty criteria</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slowness</td>
<td>310 (10.2)</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>171 (5.6)</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>255 (8.4)</td>
</tr>
<tr>
<td>Low activity</td>
<td>325 (10.7)</td>
</tr>
<tr>
<td>Weakness</td>
<td>201 (6.6)</td>
</tr>
</tbody>
</table>

Data are count (%)
Table 4.7: Baseline characteristics of men in the European Male Ageing Study by frailty status

<table>
<thead>
<tr>
<th>n</th>
<th>Total (100.0)</th>
<th>Robust (70.7)</th>
<th>Prefrail (26.8)</th>
<th>Frail (2.6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at baseline (years)</td>
<td>59.8 ± 11.0</td>
<td>57.6 ± 10.4</td>
<td>64.6 ± 10.6</td>
<td>70.0 ± 8.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>27.7 ± 4.1</td>
<td>27.7 ± 3.8</td>
<td>27.7 ± 4.7</td>
<td>28.1 ± 5.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Age left education (years)</td>
<td>20.8 ± 7.5</td>
<td>21.0 ± 7.4</td>
<td>20.5 ± 7.9</td>
<td>19.9 ± 8.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Count (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status (current)</td>
<td>648 (21.4)</td>
<td>429 (20.0)</td>
<td>197 (24.4)</td>
<td>22 (28.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Alcohol intake (≥5 days/ week)</td>
<td>711 (23.4)</td>
<td>513 (23.9)</td>
<td>183 (22.6)</td>
<td>15 (19.7)</td>
<td>0.57</td>
</tr>
<tr>
<td>Morbidity (1 or more)</td>
<td>1561 (51.8)</td>
<td>944 (44.4)</td>
<td>547 (67.8)</td>
<td>70 (89.7)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P values are ANOVA for continuous variables and Chi-square test for categorical variables.
Figure 4.2: Relationship between phenotypic frailty and age

Figure 4.3: Predicted probability of each frailty criterion by age.
4.3.3 Overview of the EMAS frailty index

The FI shows a number of characteristics that are reproducible across different cohorts and variable compositions [2, 21]. These include a strong age relationship and right skewed distribution, indicating low levels of frailty in most people, the EMAS showed these characteristics (Figure 4.4). The FI also shows a characteristic limit of approximately 0.67, or two thirds of the possible number of deficits, that has been reproduced across many diverse population samples and index formulations [2, 21]. Accordingly, the 99% limit for the EMAS FI was 0.68.
Table 4.8: Health variables and cut-points for the European Male Ageing Study frailty index

<table>
<thead>
<tr>
<th>Origin</th>
<th>Variable</th>
<th>Cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF36</td>
<td>Rating general health</td>
<td>Excellent/ Very good =0, Good=0.5, Fair/ Poor =1</td>
</tr>
<tr>
<td>SF36- Activity Daily Living</td>
<td>Help Feeding yourself</td>
<td>Limited / Limited a little=1, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Help Walking in your home</td>
<td>Limited / Limited a little=1, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Help Bathing and dressing yourself</td>
<td>Limited / Limited a little=1, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Walking 1 km</td>
<td>Limited =1, Limited a little=0.5, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Walking more than 1 km</td>
<td>Limited =1, Limited a little=0.5, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Climbing one flight of stairs</td>
<td>Limited =1, Limited a little=0.5, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Climbing several flights of stairs</td>
<td>Limited =1, Limited a little=0.5, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Unable to do moderate activity</td>
<td>Limited =1, Limited a little=0.5, Not Limited=0</td>
</tr>
<tr>
<td></td>
<td>Unable to do vigorous activity</td>
<td>Limited =1, Limited a little=0.5, Not Limited=0</td>
</tr>
</tbody>
</table>

During the past 4 weeks have you had any of the following problems

| SF36                      | Accomplish less than you would like as a result of your physical health | All/Most of time=1, Some time=0.5, Little time/ None=0             |
| SF36                      | Cut down on the amount of time spent on work or other activities as a result of emotional problems | All/Most of time=1, Some time=0.5, Little time/ None=0             |

Questions are about how you feel and how things have been with you during the past 4 weeks

| SF36                      | Full of life                  | Little time/ None=1, Some time=0.5, All/Most of time=0                  |
| SF36                      | In the dumps                  | All/Most of time=1, Some time=0.5, Little time/ None=0                  |
| SF36                      | Down hearted                  | All/Most of time=1, Some time=0.5, Little time/ None=0                  |
| SF36                      | Tired                         | All/Most of time=1, Some time=0.5, Little time/ None=0                  |

SF36-During the last six months have you experienced Serious illness or injury to yourself

<table>
<thead>
<tr>
<th>Beck depression inventory</th>
<th>Change in sleep pattern</th>
<th>Less/ Lot more=1, Same/ More=0</th>
</tr>
</thead>
<tbody>
<tr>
<td>International Symptom Score</td>
<td>Concentration</td>
<td>Worst/Worse=1, Fair=0.5, Ok=1</td>
</tr>
<tr>
<td>Prostate Symptom Score</td>
<td>Over the past month, how often have you had to</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Postpone urination</td>
<td>Always,&gt;50%=1, about or less than 50%=0.5,Not at all, &lt; 20%=0</td>
</tr>
<tr>
<td></td>
<td>Night urinate</td>
<td>2 or more =1, 0 or 1 =0</td>
</tr>
<tr>
<td></td>
<td>Weak Stream</td>
<td>Always,&gt;50%=1, about or less than 50%=0.5,Not at all, &lt; 20%=0</td>
</tr>
</tbody>
</table>

Self reported morbidities

|                           | Heart condition           | Yes=1, No=0                                                              |
|                           | High blood pressure       | Yes=1, No=0                                                              |
|                           | Bronchitis                | Yes=1, No=0                                                              |
|                           | Asthma                    | Yes=1, No=0                                                              |

85
<table>
<thead>
<tr>
<th>Disease</th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Liver condition</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Kidney condition</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Prostate disorder</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Thyroid disorder</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Cancer ever</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Stroke ever</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Cardio vascular disorder</td>
<td>Yes=1, No=0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate drugs</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Heart Failure drugs</td>
<td>Yes=1, No=0</td>
</tr>
<tr>
<td>Diabetic drugs</td>
<td>Yes=1, No=0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Continuous variable cut points-highest or lowest 10th centile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognition</td>
</tr>
<tr>
<td>Copying - Rey-Osterrieth Complex Figure (ROCF)</td>
</tr>
<tr>
<td>Delayed reproduction - Rey-Osterrieth Complex Figure (ROCF)</td>
</tr>
<tr>
<td>Camden Topographical Recognition Memory (CTRM)</td>
</tr>
<tr>
<td>Digit-Symbol Substitution (DSST) test</td>
</tr>
<tr>
<td>Tinetti</td>
</tr>
</tbody>
</table>
Figure 4.4: Characteristics of the European Male Ageing Study frailty index. A) Distribution of the frailty index, B) Relationship between the frailty index and age.
4.3.4 Comparison of the EMAS frailty phenotype and frailty index

The two frailty models correlated moderately with one another $r=0.41$ for the whole cohort, using the three level frailty phenotype and continuous FI (Table 4.9). The correlation was similar in the older men ($\geq 65$), and lower in the younger men ($<65$) $r=0.29$. The relationship did not differ substantially when measuring phenotypic frailty as the categorical frailty variable or number of frailty criteria (Table 4.9).

Each of the phenotypic frailty categories showed distinct cumulative density distributions of deficit accumulation (Figure 4.5). Median FI increased at higher levels of phenotypic frailty, from 0.08 in robust men to 0.37 in frail men (Table 4.10). The 99% limit was also higher in prefrail and frail men compared to robust and was higher in prefrail men than frail (Table 4.10). Median FI increased with increasing number of frailty criteria, the trend was similar for the 99% limit, but this effect was not consistent at higher counts of criteria (Table 4.10). An FI score of $\geq 0.2$ suggests a moderate level of frailty [31], the majority of robust men had lower scores than this, while the majority of frail men had FI scores above this value (Figure 4.5). The distribution of the FI shifted to the right, from close to a gamma shaped distribution in robust men to a more normal distribution in frail men (Figure 4.6). This indicates a transition from mostly low FI values in robust men to a greater range of FI levels in frail men.

Table 4.11 shows the agreement and disagreement between models for classifying participants as robust, prefrail and frail, when dividing the FI according to the prevalence of phenotypic frailty. 71.1% of men were classed in the same frailty category by both models. Discrepancies in classification were most frequently between the robust or frail men and the intermediate prefrail group. Few men were classified as frail by one model and robust by the other. The kappa for the agreement between
models was 0.33, indicating a fair agreement according to commonly used standards [271].
Table 4.9: Correlations between frailty measures

<table>
<thead>
<tr>
<th>Frailty Phenotype &amp; FI</th>
<th>Whole cohort</th>
<th>Older men ≥65</th>
<th>Younger men &lt;65</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=3053</td>
<td>n=1082</td>
<td>n=1971</td>
</tr>
<tr>
<td>Frailty Phenotype &amp; FI</td>
<td>0.41</td>
<td>0.40</td>
<td>0.29</td>
</tr>
<tr>
<td>No. Frailty criteria &amp; FI</td>
<td>0.42</td>
<td>0.42</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Data are Spearman's rank correlation coefficients.

Table 4.10: Frailty index by phenotypic frailty

<table>
<thead>
<tr>
<th>Frailty state</th>
<th>Median</th>
<th>99% limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robust</td>
<td>0.08</td>
<td>0.46</td>
</tr>
<tr>
<td>Prefrail</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td>Frail</td>
<td>0.37</td>
<td>0.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. criteria</th>
<th>Median</th>
<th>99% limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.08</td>
<td>0.46</td>
</tr>
<tr>
<td>1</td>
<td>0.15</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>0.24</td>
<td>0.67</td>
</tr>
<tr>
<td>3</td>
<td>0.36</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>0.37</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>0.46</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Table 4.11: Agreement and disagreement between frailty models

<table>
<thead>
<tr>
<th>Index</th>
<th>Robust</th>
<th>Prefrail</th>
<th>Frail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robust</td>
<td>1792 (58.7)</td>
<td>423 (13.9)</td>
<td>5 (0.2)</td>
</tr>
<tr>
<td>Prefrail</td>
<td>358 (11.7)</td>
<td>346 (11.3)</td>
<td>40 (1.3)</td>
</tr>
<tr>
<td>Frail</td>
<td>7 (0.2)</td>
<td>49 (1.6)</td>
<td>33 (1.1)</td>
</tr>
</tbody>
</table>

Kappa = 0.33
Figure 4.5: Cumulative distribution of deficit accumulation by phenotypic frailty states

Figure 4.6: Density distribution of deficits for people classed as robust, prefrail and frail by the phenotypic definition
4.3.5 Frailty and risk of falling

Data on falls were available for 2482 men with complete frailty data. Men missing falls data tended to be slightly younger and slightly frailer than those with data (Table 4.12). Rates of falling were low at 2 year follow up, with 13.2% of men reporting 1 fall and 7.8% reporting 2 or more in the preceding 12 months (Table 4.13). There was a clear trend for higher rates of falls with increasing levels of frailty using either model (Table 4.12). This trend was stronger for the frailty index, using this model 27.4% of frail men reported 2 or more falls in the preceding 12 months and 22.6% reported 1 fall, compared to 19.6% and 21.4% respectively for the frailty phenotype (Table 4.13).

Both frailty models showed significant relationships with falling in the ordinal logistic regression analysis (Table 4.14). Adjustment for age and centre had little effect on the strength of the relationships between the frailty models and falling. After adjustment the odds of reporting higher numbers of falls compared to robust men was, Ordinal OR (95% CI); 1.74 (1.33 to 2.28) for prefrail men and 3.15 (1.75 to 5.66) for frail men. The relationships were stronger for the FI at 2.11 (1.80 to 2.49) for prefrail men and 5.28 (3.35 to 8.32) for frail men (Table 4.14).

The Area Under the ROC Curve (AUC) for the frailty phenotype was 0.59 and for the frailty index 0.66 (Figure 4.7). While neither model was a strong predictor of recurrent falls, the AUC for the frailty index was slightly, but significantly higher than that for the frailty phenotype.
**Table 4.12: Characteristics of men with complete and missing falls data**

<table>
<thead>
<tr>
<th></th>
<th>Complete Falls data</th>
<th>Missing Falls Data</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2482</td>
<td>571</td>
<td></td>
</tr>
<tr>
<td>age</td>
<td>60.0 ± 10.9</td>
<td>59.0 ± 11.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Frailty Index</td>
<td>0.12 ± 0.10</td>
<td>0.15 ± 0.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. frailty criteria</td>
<td>0.4 ± 0.7</td>
<td>0.5 ± 0.8</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

**Table 4.13: Falls by frailty category according to both models**

<table>
<thead>
<tr>
<th>Number of falls</th>
<th>Overall</th>
<th>Robust</th>
<th>Prefrail</th>
<th>Frail</th>
<th>Robust</th>
<th>Prefrail</th>
<th>Frail</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1961 (79.0)</td>
<td>1453 (82.1)</td>
<td>475 (23.3)</td>
<td>33 (58.9)</td>
<td>1527 (82.6)</td>
<td>403 (70.6)</td>
<td>31 (50.0)</td>
</tr>
<tr>
<td>1</td>
<td>327 (13.2)</td>
<td>208 (11.8)</td>
<td>107 (16.3)</td>
<td>12 (21.4)</td>
<td>223 (12.1)</td>
<td>90 (15.8)</td>
<td>14 (22.6)</td>
</tr>
<tr>
<td>2</td>
<td>194 (7.8)</td>
<td>108 (6.1)</td>
<td>75 (11.4)</td>
<td>11 (19.6)</td>
<td>99 (5.4)</td>
<td>78 (13.7)</td>
<td>17 (27.4)</td>
</tr>
</tbody>
</table>

Data are count (%). Chi-square P<0.0001 for both frailty models.
Table 4.14: Ordinal logistic regression models for the relationship between frailty and incident falls

<table>
<thead>
<tr>
<th>Frailty Phenotype</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ordinal OR (95%CI)</td>
<td>P</td>
<td>Ordinal OR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>Prefrail</td>
<td>1.78 (1.45 to 2.20)</td>
<td>&lt;0.0001</td>
<td>1.74 (1.33 to 2.28)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Frail</td>
<td>3.30 (1.94 to 5.62)</td>
<td>&lt;0.0001</td>
<td>3.15 (1.75 to 5.66)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Frailty Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefrail</td>
<td>2.06 (1.66 to 2.55)</td>
<td>&lt;0.0001</td>
<td>2.11 (1.80 to 2.49)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Frail</td>
<td>5.13 (3.14 to 8.38)</td>
<td>&lt;0.0001</td>
<td>5.28 (3.35 to 8.32)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are ordinal OR for risk of falls compared against the referent robust category for each model

Adjusted model includes age and centre
Figure 4.7: Receiver Operating Characteristic curves for the risk of recurrent falls by frailty

Closed circles - Frailty phenotype: AUC 0.59. Open circles - Frailty Index: AUC 0.66. Difference between models; $P=0.0004$
4.4 Discussion

In this chapter the frailty phenotype defined in the CHS study [4] was adapted for use in the EMAS data set. This modified frailty model showed an appropriate prevalence and the expected relationship with age. Convergent validity was assessed by exploring the relationship between this model and the EMAS FI and construct validity tested in terms of the ability of this model’s to predict incident falls.

4.4.1 Frailty in EMAS

Defined according to the phenotype developed here (Table 4.2), the prevalence of frailty in this cohort of European men aged 40-79 was low; 2.5%. Compared to 6.9% in the CHS cohort [4], 4% in men aged 65+ from the MrOS cohort [13], 7.7% in the MMAS cohort [165], 16.3% in the Women’s Health Initiative - Observational Study (WHI-OS); a very large study of women aged 65-79 years [15], and 25% in disabled older women from the WHAS-1 cohort [272]. These differences may reflect differences in the cohorts studied or in the operationalisation of the criteria. The low prevalence in this cohort seems appropriate given the relative youth of the participants. The exclusively male gender of participants may also have contributed to the low prevalence of frailty seen; in mixed cohorts the prevalence of phenotypic frailty is generally lower in men than women [4].

In the oldest age group (70-79 years) the prevalence of frailty was 6.7%. This is comparable to the 5.4% reported in Australian men of the same age group [273]. It is slightly higher than the 4% reported in men aged 65+ in the Osteoporotic Fractures in Men study [13] and the 4.9% in men from the Cardiovascular Health Study [4] and considerably lower than the 11% reported in men aged 70-79 from the Massachusetts Male Aging Study (MMAS) [165]. Small differences between comparable cohorts may
relate to differing operationalisation of the frailty criteria. The MMAS included middle aged men, however cut-points for the criteria were defined from the whole cohort instead of limited to the older (≥65 years) men as in the present study.

4.4.2 Relationship between frailty and age

The prevalence of frailty showed the expected increase with age (Figure 4.2). This is consistent with all previous studies and is a core feature of frailty [4, 13, 15, 249, 274]. This relationship provides the first support for the validity of the EMAS frailty phenotype. In agreement with a recent multi-centre European study [249] the symptoms of frailty were not rare in middle aged men, with exhaustion being the most prevalent symptom (Figure 4.3). This criterion also showed the weakest age relationship. It appears, exhaustion, as assessed in this and other studies [4, 5, 13], lacks specificity for the age-related constructs central to frailty and may be associated with factors such as work, financial and family pressures with contrasting adverse consequences compared to older frail men. Similarly, stress-related and mental symptoms did not correlate with age in a Finnish study on 40-70-year-old men [275]. Use of self-reported measures of exhaustion may overestimate the prevalence of frailty, especially in younger populations. The weakness and sarcopenia criteria were less strongly age related than the slowness and low activity measures. The lower prevalence of these criteria in the younger men is partly due to the lower cut-point used. However, compared to exhaustion both of these criteria showed a more pronounced increase in prevalence in the oldest ages.

4.4.3 Relationship between the two frailty models

As previously reported [15, 17, 18], convergence was seen between the frailty phenotype and index. Frailer men, defined by the frailty phenotype, showed higher FI
scores. The majority of robust men had low scores, while the majority of frail men had high FI scores (Figure 4.5 & 4.6). Prefrail men tended to have intermediate scores, but overlapped considerably with the robust and prefrail groups (Figure 4.6). The models showed a 71% agreement on the frailty classification of participants (Table 4.11), when using comparable 3 level ordinal variables for each model. This is similar to a recent report from the Health and Retirement Study, although the cut-points used there were different [32],

The correlation between models was 0.41 (Table 4.9). This is lower than the 0.65 seen in the community dwelling cohort in the Canadian Study of Health and Aging [31] and the 0.61 seen in the institutionalised cohort from this study [37], but is comparable to the 0.45 seen in the English Longitudinal Study of Ageing (ELSA) [38]. A small study in equal groups of independent elderly, day hospital and continuing care patients showed a correlation of 0.92 between the 2 models [34]. The reason for the difference in strength of relationships is not clear. It is possible that the weaker relationship seen in this study may be related to the younger age of participants, however the relationship was not appreciably different when restricted to participants aged 65 and over (Table 4.9). Differences between studies may be due to operationalisation of the models or to different rates of frailty. It may be that with the low prevalence of frailty in this cohort, this study lacks the range of values needed to show a good correlation.

The convergence between models demonstrates some validity for both these models in capturing frailty. As the FI is reproducible across different cohorts [23, 276], the relationships seen support the validity of the modified EMAS frailty criteria for assessing frailty.
4.4.4 Frailty and falling

Consistent with previous studies [4, 5, 14, 33], the risk of falling was higher in frailer men according to either model. The ability to predict adverse outcomes is the core feature of any frailty model, as this demonstrates the ability to classify older adults of vulnerable health status. Falls are a key geriatric outcome associated with further adverse sequelae [262], and are thought to be functionally linked to frailty [263]. As such this relationship provides some criterion validity for the EMAS frailty phenotype and FI models.

The relationship between falling and frailty was stronger for the FI compared to the frailty phenotype. It might have been expected that the predominantly physical nature of the frailty criteria would mean the frailty phenotype was more predictive of falls compared to the FI. However, the results here are consistent with those reported previously for mortality [30]. Overall these findings are consistent with the suggestion that the broader nature of the FI may allow a more comprehensive assessment of health status [30]. Neither model was a strong predictor of falls (Figure 4.7). This is probably because frailty models are designed to grade non-specific vulnerability, rather than risk of particular outcomes. The predictive values of the models for falls are similar to those previously reported in older men and women [5, 33]

4.4.5 Limitations and strengths

This study has a number of limitations. Due to variable availability the frailty phenotype needed to be substantially modified. This is not a limitation per se, as consistent with many studies using this model [13, 15, 272], the adapted model appears to function appropriately in measuring frailty. However, ideally the frailty phenotype would be assessed using identical measures to those from the CHS study [4]. The use of modified
criteria may to some extent limit the conclusions that can be drawn from comparing this model to the frailty index. The EMAS criteria, however, are not without potential advantages. Mid upper arm muscle circumference can predict functional decline and mortality [267, 268]. Furthermore, compared to unintentional weight loss, this marker relies on objective measurement rather than self report; and is more specific to loss of muscle mass, which is believed to be a central feature of the frailty syndrome [3]. While chair rising ability is dependant on factors in addition to strength [223], lower limb assessments have obvious functional relevance and are more predictive of subsequent disability compared to upper limb assessments [277]. Unlike grip strength, chair standing does not require specialised equipment, and can be easily applied in clinical and community settings.

A main focus of this chapter has been the validation of the EMAS frailty phenotype. The ability to validate this model is somewhat limited by the absence of prospective data on additional adverse outcomes. Mortality in particular is most commonly used to demonstrate the predictive validity of frailty measures [2, 9, 16, 21-23, 43]. A further limitation is the relative youth of the cohort this meant the prevalence of frailty and rates of falls were very low. The falls variable itself relied upon recollection of falls in the preceding 12 months, while many studies use more frequent and rigorous data collection to ensure accuracy of recall [21, 22]. However, the expected relationships between frailty and falling were still seen.

As described in the Methods chapter, the response rate for EMAS was 41% and men who entered the study tended to be slightly healthier than those who declined to take part [246]. Furthermore men missing frailty measures tended to be slightly frailer than those with complete data. It is therefore likely that the prevalence of frailty reported
here would be lower than the true prevalence in the population from which the sample was drawn. Nevertheless, the results here represent an internal comparison of responders included in the EMAS, and the frailty models appear to effectively grade frailty within this study population. The study involved predominantly Caucasians European men and results should be extrapolated beyond this setting with caution.

The design of EMAS also has notable strengths, these include the large population based sample drawn from 8 European centres and the use of standardised methodologies across these 8 centres. Strengths of this analysis include the use of the two frailty models to provide mutual validation to one and other. Defining the frailty criteria in men aged 65 and older may provide a more realistic estimate of the prevalence of frailty compared to other studies in younger cohorts in which frailty was defined in middle aged as well as older men [165].

4.4.6 Implications and future work

This works suggests that modified criteria may be used to measure frailty as defined by Fried and colleagues. This supports the ability to adapt this model for use in further data sets. The frailty models correlated with one another, the convergence between the disparate models supports the existence of frailty as a definable entity. However, the strength of the correlations was modest and there were considerable overlaps in FI score between the different levels of phenotypic frailty. These discrepancies suggest that while no consensus definition of frailty exists, both models may be used in a complementary manner. Alternatively use of a particular model should be justified in relation to the particular research question. For example, the frailty phenotype may be more useful for selecting participants to receive an anabolic intervention, while the FI may be more effective at grading mortality risk in geriatric patients.
Future research could use prospective data to extend the analyses with falls reported here to further adverse outcomes. This would allow more comprehensive validation and comparison of the models.

4.4.7 Summary

This chapter described the development and validation of the EMAS frailty phenotype. An adapted version of the frailty phenotype was defined. The EMAS frailty index was also described. The frailty phenotype model demonstrated the expected relationship with age, and showed convergent validity through its relationship to the frailty index and criterion validity through its relationship with falls outcomes. Subsequent chapters will use this model and the frailty index to describe the prevalence of frailty in men from 8 European centres and to explore potential biological correlates of this condition.
Chapter 5 Prevalence of Frailty in Middle Aged and Older Men from 8 European Centres

5.1 Introduction

Despite recent expansion of the research effort into frailty, there remains comparatively little data on the prevalence of frailty in Europe. The majority of work with the frailty phenotype has been performed in US data sets [4, 13, 16], although recent studies have started to use this model in Europe [249, 274, 278]. The bulk of the work on the FI has been in Canadian or Chinese cohorts [25-27, 279], although work with this model has also included cross national studies [23]. These 2 models have rarely been applied simultaneously to assess frailty in a given cohort. These models measure frailty in contrasting ways and as seen in the previous chapter show some, but not complete agreement. It may therefore prove informative to assess the prevalence of frailty using both of these models.

Disparities in frailty between different ethnic groups have been reported [41, 43], though these data typically derive from a single country. There is a lack of data comparing levels of frailty within larger geographic regions. Such data are important, as evidence of between country variations in frailty may provide clues towards identifying potential novel mechanistic pathways. A large scale recent study of 10 European countries reported higher levels of frailty, measured using the frailty phenotype in Southern compared to Northern European countries [249]. This study did not include data on the transitional eastern European countries. Studies have generally shown higher mortality rates from treatable medical conditions, along with poorer quality of life and fewer healthy life years in these countries [280, 281]. Previous reports from the EMAS have shown poorer cognitive function in these countries along with impaired sexual function and quality of life [282, 283]. Frailty provides a useful summary health
measure that may predict progression of disability and risk of adverse outcomes in ageing populations [4, 272]. As such assessing differences in frailty in across Europe, a population with disparities in health status and behaviours may provide useful data from both mechanistic and preventative perspectives.

The aims of this chapter were: 1) to compare the prevalence of frailty and its constituent criteria in middle aged and elderly men across 8 European centres including transitional nations using 2 alternative frailty measures. 2) To evaluate selected population characteristics as potential explanations for centre differences in frailty.
5.2 Methods

5.2.1 Participants

All analyses were performed on the subset of 3053 EMAS men with complete frailty measures.

5.2.2 Frailty measurement

Frailty was measured using the frailty phenotype and the FI. In order to give comparative estimates of frailty across Europe using both models, the FI was trichotomised into a 3 tier categorical variable. While the FI is not routinely used to classify people into frailty categories, studies have suggested an FI score $\geq 0.08$ can be considered prefrail and $\geq 0.25$ frail [28, 31]. Due to the different purposes of analyses, these are different to the cut-points used in chapter 4. In chapter 4 a comparable prevalence of frailty was needed to perform the comparative analyses in the fairest manner. Here the purpose is to estimate the prevalence of frailty in the 8 European centres using each of these models, hence these suggested cut-points for the FI classifications are used.

5.2.3 Statistical analyses

Differences between centres in frailty and other key participant characteristics were assessed using ANOVA for continuous variables and Chi-square test for discrete variables. Differences in frailty between centres were evaluated using multinomial logistic regression for the frailty phenotype. This model simultaneously compares the chance of being frail against robust and the chance of being prefrail against robust compared against a referent group. In this case this meant comparing the centres against a referent centre. Leuven (Belgium) was chosen as the referent centre due to its large sample size. Multiple linear regression was used for the FI. For direct comparison to the
frailty phenotype an additional multinomial logistic regression was used to assess centre differences in the 3 tiered frailty index variable.

Firstly unadjusted models were fitted for the effect of centre on levels of frailty. Subsequent models were designed to evaluate the influence of variables that may explain the difference in frailty between centres, these included; demographic, socioeconomic and lifestyle variables: Model 1 included age, model 2 included age and years of education, as a surrogate for socioeconomic status and model 3 included age, education and lifestyle variables; BMI and smoking. To account for the potential U-shaped relationship between BMI and frailty this variable was included as both a linear and a quadratic term. Smoking was coded as current, ex and never smoking. An additional model, model 4 was fitted for the frailty phenotype that included morbidities (coded as 0 vs 1 or more) along with the age, education and lifestyle variables. This model was not run for the FI as this frailty model does not separate morbidity from frailty.
5.3 Results

5.3.1 Prevalence of frailty and frailty criteria across centres

Prevalence and centre differences for frailty and other key participant characteristics are shown in Table 5.1. Overall the prevalence of frailty was higher using the FI; 14.7%, compared to 2.6% for the phenotypic definition (Table 5.1). Similarly, the overall prevalence of prefrailty was 45.5% using the FI groupings and 26.8% using the frailty phenotype (Table 5.1). The prevalence of frailty differed substantially between centres according to either model, as did the prevalence of all 5 frailty criteria. Trends for differences in the FI between centres were similar using either the continuous or 3 tiered variable (Table 5.1).

The prevalence of frailty was higher for all centres using the suggested frailty index classification, compared to the frailty phenotype (Figure 5.1), reflecting the higher overall prevalence seen using this model. Although the overall prevalence was higher for the FI, the relative differences in frailty across centres were similar using either model (Figure 5.1). Using the frailty phenotype, the highest prevalence of frailty was seen in Tartu 4.9%, and the lowest in Florence 1.0%. The highest levels of prefrailty were seen in Lodz, at 35.8% and Santiago, at 35.5% and the lowest, 17.7% in Manchester (Table 5.1). Similar patterns were seen for the FI with the highest median level 0.14 seen in Lodz and Tartu and the lowest 0.06 in Malmo, levels in Manchester and Florence were also low at 0.08 (Table 5.1). The prevalence of frailty was generally higher in the eastern and southern European centres compared to the northern and western (Figure 5.1). However, the prevalence of frailty was relatively low in Szeged, particularly as measured by the frailty phenotype (Figure 5.1). The FI generally showed clearer differences between countries in levels of frailty, particularly in the less frail countries (Figure 5.1).
Different clusters of frailty criteria were seen at different centres (Figure 5.2). The centres with the highest levels of frailty Tartu, Lodz and Santiago had generally high levels of all criteria. Manchester had generally low frequencies of all criteria, while Szeged had low rates of all criteria except exhaustion and slowness. Florence, a centre with a low prevalence of overall frailty had high levels of sarcopenia and low activity and a very low prevalence of slowness and weakness. Similarly, Malmo had a low prevalence of slowness, sarcopenia and exhaustion and a higher prevalence of low activity and weakness (Figure 5.2).

5.3.2 Centre differences in key health characteristics

Of the other, potentially explanatory characteristics, mean age differed only slightly, by just over 2 years between centres (Table 5.1). There were significant differences in all other characteristics across the 8 centres. Mean BMI ranged from 26.9 ± 3.8 in Leuven to 28.8 ± 5.3 in Tartu. Prevalence of morbidity (1 or more condition) was lowest in Malmo and Manchester at 42% and highest in Lodz at 67.6%. Manchester had the fewest current smokers at 40 men (11%) and Tartu had the most, 111 men (29.3%), followed by Szeged at 99 men (28.4%). Rates of smoking were somewhat high in the Southern European centres, Florence and Santiago at 24%. The mean age on leaving education ranged from 16.3 ± 5.7 years in Florence, to 25.2 ± 8.8 years in Szeged (Table 5.1).
## Table 5.1: Prevalence of frailty and other characteristics by European centre

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Florence</th>
<th>Leuven</th>
<th>Malmo</th>
<th>Manchester</th>
<th>Santiago</th>
<th>Lodz</th>
<th>Szeged</th>
<th>Tartu</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>3,053</td>
<td>394</td>
<td>417</td>
<td>356</td>
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<td>389</td>
<td>358</td>
<td>391</td>
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<td><strong>Frailty Phenotype</strong></td>
<td></td>
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</tr>
<tr>
<td>Robust</td>
<td>2157 (70.7)</td>
<td>294 (74.6)</td>
<td>293 (70.3)</td>
<td>254 (71.4)</td>
<td>288 (80.7)</td>
<td>235 (60.4)</td>
<td>217 (60.6)</td>
<td>307 (78.5)</td>
<td>269 (68.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prefrail</td>
<td>818 (26.8)</td>
<td>96 (24.4)</td>
<td>115 (27.6)</td>
<td>98 (27.5)</td>
<td>63 (17.7)</td>
<td>138 (35.5)</td>
<td>128 (35.8)</td>
<td>77 (19.7)</td>
<td>103 (26.3)</td>
<td></td>
</tr>
<tr>
<td>Frail</td>
<td>78 (2.6)</td>
<td>4 (1.0)</td>
<td>9 (2.2)</td>
<td>4 (1.1)</td>
<td>6 (1.7)</td>
<td>16 (4.1)</td>
<td>13 (3.6)</td>
<td>7 (1.8)</td>
<td>19 (4.9)</td>
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<td><strong>Frailty Index</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Robust</td>
<td>1215 (39.8)</td>
<td>179 (45.4)</td>
<td>161 (38.6)</td>
<td>212 (59.6)</td>
<td>168 (47.1)</td>
<td>149 (38.3)</td>
<td>81 (22.6)</td>
<td>155 (39.6)</td>
<td>110 (28.1)</td>
<td></td>
</tr>
<tr>
<td>Prefrail</td>
<td>1389 (45.5)</td>
<td>184 (46.7)</td>
<td>206 (49.4)</td>
<td>125 (35.1)</td>
<td>154 (43.1)</td>
<td>182 (46.8)</td>
<td>190 (53.1)</td>
<td>171 (43.7)</td>
<td>177 (45.3)</td>
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<tr>
<td>Frail</td>
<td>449 (14.7)</td>
<td>31 (7.9)</td>
<td>50 (12.0)</td>
<td>19 (5.3)</td>
<td>35 (9.8)</td>
<td>58 (14.9)</td>
<td>87 (24.3)</td>
<td>65 (16.6)</td>
<td>104 (26.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Median (IQ range)</strong></td>
<td>0.10 (0.05 - 0.19)</td>
<td>0.08 (0.04 - 0.15)</td>
<td>0.11 (0.05 - 0.17)</td>
<td>0.06 (0.02 - 0.13)</td>
<td>0.08 (0.04 - 0.14)</td>
<td>0.11 (0.05 - 0.19)</td>
<td>0.14 (0.08 - 0.24)</td>
<td>0.11 (0.05 - 0.19)</td>
<td>0.14 (0.07 - 0.25)</td>
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<tr>
<td>Slowness</td>
<td>310 (10.2)</td>
<td>4 (1.0)</td>
<td>48 (11.5)</td>
<td>23 (6.5)</td>
<td>33 (9.2)</td>
<td>52 (13.4)</td>
<td>51 (14.3)</td>
<td>40 (10.2)</td>
<td>59 (15.1)</td>
<td>&lt;0.0001</td>
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<td>Sarcopenia</td>
<td>171 (5.6)</td>
<td>31 (7.9)</td>
<td>27 (6.5)</td>
<td>17 (4.8)</td>
<td>18 (5.0)</td>
<td>30 (7.7)</td>
<td>27 (7.5)</td>
<td>2 (0.5)</td>
<td>19 (4.9)</td>
<td>&lt;0.0001</td>
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<td>Exhaustion</td>
<td>255 (8.4)</td>
<td>14 (3.6)</td>
<td>27 (6.5)</td>
<td>13 (3.7)</td>
<td>14 (3.9)</td>
<td>44 (11.3)</td>
<td>54 (15.1)</td>
<td>42 (10.7)</td>
<td>47 (12.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low Activity</td>
<td>325 (10.7)</td>
<td>62 (15.7)</td>
<td>35 (8.4)</td>
<td>46 (12.9)</td>
<td>19 (5.3)</td>
<td>65 (16.7)</td>
<td>49 (13.7)</td>
<td>13 (3.3)</td>
<td>36 (9.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weakness</td>
<td>201 (6.6)</td>
<td>6 (1.5)</td>
<td>31 (7.4)</td>
<td>32 (9.0)</td>
<td>13 (3.6)</td>
<td>48 (12.3)</td>
<td>21 (5.9)</td>
<td>18 (4.6)</td>
<td>32 (8.2)</td>
<td>&lt;0.0001</td>
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<td><strong>General Characteristics</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.8 ± 11.0</td>
<td>60.0 ± 11.1</td>
<td>60.1 ± 10.9</td>
<td>59.2 ± 10.9</td>
<td>59.7 ± 11.2</td>
<td>60.1 ± 11.3</td>
<td>61.1 ± 10.6</td>
<td>58.9 ± 11.1</td>
<td>59.1 ± 10.9</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.7 ± 4.1</td>
<td>27.1 ± 3.9</td>
<td>26.9 ± 3.8</td>
<td>27.1 ± 3.9</td>
<td>27.6 ± 3.7</td>
<td>28.2 ± 3.8</td>
<td>27.4 ± 3.8</td>
<td>28.3 ± 4.3</td>
<td>28.8 ± 5.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age left education</td>
<td>20.8 ± 7.5</td>
<td>16.3 ± 5.7</td>
<td>20.3 ± 4.9</td>
<td>22.0 ± 9.1</td>
<td>17.7 ± 3.6</td>
<td>18.1 ± 6.1</td>
<td>23.5 ± 8.1</td>
<td>25.2 ± 8.8</td>
<td>23.7 ± 7.5</td>
<td>&lt;0.0001</td>
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<tr>
<td>Current Smoker</td>
<td>648 (21.6)</td>
<td>92 (23.7)</td>
<td>70 (17.1)</td>
<td>61 (17.6)</td>
<td>40 (11.3)</td>
<td>90 (23.8)</td>
<td>99 (28.4)</td>
<td>85 (21.9)</td>
<td>111 (29.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Morbidities (1+)</td>
<td>1561 (51.8)</td>
<td>201 (51.5)</td>
<td>195 (47.1)</td>
<td>149 (42.0)</td>
<td>148 (42.1)</td>
<td>211 (54.5)</td>
<td>234 (67.6)</td>
<td>222 (58.1)</td>
<td>201 (52.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are count (%), mean ± SD or median (25 -75 IQ range). P values are ANOVA for continuous variables and Chi-square for discrete variables.
Figure 5.1: Prevalence of frailty by centre according to A) The frailty phenotype, B) The frailty index
Figure 5.2: Prevalence of frailty criteria by centre

Slowness

Sarcopenia
5.3.3 Regression analyses for centre differences in frailty

In unadjusted analyses the odds of frailty was higher for men in Tartu compared to the referent Leuven, this relationship was of marginal statistical significance; RRR (95% CI) 2.3 (1.02 to 5.17) p=0.044 (Table 5.2). Adjustment for age and education strengthened this relationship to 3.1 (1.3 to 7.2) p=0.008. Adjustment for lifestyle factors; BMI and smoking reduced this relationship, to a similar level as the unadjusted model; OR 2.4 (1.02 to 5.7) p=0.045. Adjustment for morbidity did not substantively affect this relationship (Table 5.2). Unlike frailty, odds of prefrailty were not high in Tartu (Table 5.2). Odds of both frailty and prefrailty were higher in Santiago and Lodz, although only the relationship with prefrailty reached significance; 1.5 (1.1 to 2) p=0.009 for both centres. These relationships were largely unaffected by adjustment for age, education and lifestyle, although the higher odds of prefrailty in Lodz was marginally non significant in the fully adjusted model (Table 5.2). Odds of frailty and prefrailty were lower in Manchester and Szeged compared to Leuven, this relationship reached significance for prefrailty; 0.6 (0.4 to 0.8) p=0.001 for Manchester and 0.6 (0.5 to 0.9) p=0.008 for Szeged. The strength of these relationships were largely unchanged by adjustment for confounders, however the relationship with Szeged was reduced to borderline significance in the fully adjusted model 0.7 (0.48 to 1.02) p=0.06 (Table 5.2). Odds of frailty and prefrailty were also low in Florence, but this effect only reached significance only for frailty, after adjustment for BMI and smoking (Table 5.2).

Levels of frailty using the FI were lower in Manchester, Florence and Malmo and higher in Lodz and Tartu compared to the referent Leuven (Table 5.3). These relationships were largely unaffected by adjustment for age, education or lifestyle factors. It was notable however that as with the frailty phenotype adjustment for age and education tended to strengthen the relationship between Tartu and higher levels of
frailty, while adjustment for lifestyle factors tended to weaken it. Levels of frailty were also slightly higher in Szeged, Effect size (95% CI); 0.012 (-0.003 to 0.026) p=0.107, this effect reached significance after adjustment for age; 0.018 (0.005 to 0.030) p=0.005. Relationships seen were similar using the continuous or 3 tiered FI variables (Tables 5.3 & 5.4).

In general, the two models showed similar results for which centres had higher odds of frailty, with the exceptions of Santiago and Szeged. Santiago showed higher odds of prefailty using the phenotypic definition (Table 5.2), but no clear difference from the referent centre (Leuven) using the FI (Tables 5.3 & 5.4). Szeged had lower odds of prefailty according to the frailty phenotype (Table 5.2) and higher levels of frailty measured by the FI (Tables 5.3 & 5.4)
<table>
<thead>
<tr>
<th>Centre</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Prefrail Frail</td>
</tr>
<tr>
<td></td>
<td>RRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Leuven</td>
<td>Referent</td>
<td>0.25</td>
</tr>
<tr>
<td>Florence</td>
<td>0.8 (0.6 to 1.1)</td>
<td>0.25</td>
</tr>
<tr>
<td>Malmo</td>
<td>1.0 (0.7 to 1.4)</td>
<td>0.92</td>
</tr>
<tr>
<td>Manchester</td>
<td>0.6 (0.4 to 0.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Santiago</td>
<td>1.5 (1.1 to 2.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>Lodz</td>
<td>1.5 (1.1 to 2.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>Szeged</td>
<td>0.6 (0.5 to 0.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Tartu</td>
<td>1.0 (0.7 to 1.3)</td>
<td>0.88</td>
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</table>

<table>
<thead>
<tr>
<th>Centre</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Prefrail Frail</td>
<td>Prefrail Frail</td>
</tr>
<tr>
<td></td>
<td>RRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Leuven</td>
<td>Referent</td>
<td>0.17</td>
</tr>
<tr>
<td>Florence</td>
<td>0.8 (0.6 to 1.1)</td>
<td>0.17</td>
</tr>
<tr>
<td>Malmo</td>
<td>1.1 (0.8 to 1.6)</td>
<td>0.62</td>
</tr>
<tr>
<td>Manchester</td>
<td>0.6 (0.4 to 0.9)</td>
<td>0.006</td>
</tr>
<tr>
<td>Santiago</td>
<td>1.7 (1.2 to 2.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Lodz</td>
<td>1.5 (1.1 to 2.1)</td>
<td>0.018</td>
</tr>
<tr>
<td>Szeged</td>
<td>0.7 (0.5 to 1.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Tartu</td>
<td>1.0 (0.7 to 1.4)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Model 1 includes age, Model 2 includes age & education, Model 3 includes age, education, BMI & smoking, Model 4 includes age, education, BMI, smoking and morbidities.
Table 5.3: Multiple linear regression models for differences between centres in frailty index score

<table>
<thead>
<tr>
<th>Centre</th>
<th>Unadjusted Effect size (95% CI)</th>
<th>P</th>
<th>Model 1 Effect size (95% CI)</th>
<th>P</th>
<th>Model 2 Effect size (95% CI)</th>
<th>P</th>
<th>Model 3 Effect size (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florencia</td>
<td>-0.016 (-0.031 to -0.002)</td>
<td>0.026</td>
<td>-0.016 (-0.028 to -0.003)</td>
<td>0.013</td>
<td>-0.021 (-0.034 to -0.009)</td>
<td>0.001</td>
<td>-0.022 (-0.034 to -0.009)</td>
<td>0.001</td>
</tr>
<tr>
<td>Malmo</td>
<td>-0.036 (-0.051 to -0.021)</td>
<td>&lt;0.0001</td>
<td>-0.031 (-0.044 to -0.019)</td>
<td>&lt;0.0001</td>
<td>-0.028 (-0.041 to -0.015)</td>
<td>&lt;0.0001</td>
<td>-0.028 (-0.041 to -0.015)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Manchester</td>
<td>-0.021 (-0.036 to -0.007)</td>
<td>0.005</td>
<td>-0.019 (-0.032 to -0.007)</td>
<td>0.003</td>
<td>-0.023 (-0.036 to -0.010)</td>
<td>&lt;0.0001</td>
<td>-0.021 (-0.034 to -0.009)</td>
<td>0.001</td>
</tr>
<tr>
<td>Santiago</td>
<td>0.006 (-0.008 to 0.020)</td>
<td>0.42</td>
<td>0.006 (-0.006 to 0.018)</td>
<td>0.34</td>
<td>0.002 (-0.011 to 0.014)</td>
<td>0.782</td>
<td>-0.003 (-0.015 to 0.009)</td>
<td>0.65</td>
</tr>
<tr>
<td>Lodz</td>
<td>0.050 (0.035 to 0.065)</td>
<td>&lt;0.0001</td>
<td>0.045 (0.033 to 0.058)</td>
<td>&lt;0.0001</td>
<td>0.048 (0.035 to 0.061)</td>
<td>&lt;0.0001</td>
<td>0.044 (0.031 to 0.057)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Szeged</td>
<td>0.012 (-0.003 to 0.026)</td>
<td>0.107</td>
<td>0.018 (0.005 to 0.030)</td>
<td>0.005</td>
<td>0.023 (0.011 to 0.036)</td>
<td>&lt;0.0001</td>
<td>0.017 (0.005 to 0.030)</td>
<td>0.007</td>
</tr>
<tr>
<td>Tartu</td>
<td>0.052 (0.038 to 0.067)</td>
<td>&lt;0.0001</td>
<td>0.057 (0.045 to 0.069)</td>
<td>&lt;0.0001</td>
<td>0.062 (0.049 to 0.074)</td>
<td>&lt;0.0001</td>
<td>0.051 (0.039 to 0.064)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Model 1 includes age, Model 2 includes age & education, Model 3 includes age, education, BMI & smoking.
Table 5.4: Multinomial logistic regression models for the differences between centres in Frailty Index categories

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>_prefrail</td>
<td>_frail</td>
<td>_prefrail</td>
<td>_frail</td>
</tr>
<tr>
<td></td>
<td>RRR (95% CI)</td>
<td>P</td>
<td>RRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Leuven</td>
<td>Referent</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Florence</td>
<td>0.8 (0.6 to 1.1)</td>
<td>0.14</td>
<td>0.6 (0.3 to 0.9)</td>
<td>0.021</td>
</tr>
<tr>
<td>Malmo</td>
<td>0.5 (0.3 to 0.6)</td>
<td>&lt;0.0001</td>
<td>0.3 (0.2 to 0.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Manchester</td>
<td>0.7 (0.5 to 1.0)</td>
<td>0.03</td>
<td>0.7 (0.4 to 1.1)</td>
<td>0.105</td>
</tr>
<tr>
<td>Santiago</td>
<td>1.0 (0.7 to 1.3)</td>
<td>0.76</td>
<td>1.3 (0.8 to 1.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>Lodz</td>
<td>1.8 (1.3 to 2.6)</td>
<td>&lt;0.0001</td>
<td>3.5 (2.2 to 5.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Szeged</td>
<td>0.9 (0.6 to 1.2)</td>
<td>0.33</td>
<td>1.4 (0.9 to 2.1)</td>
<td>0.17</td>
</tr>
<tr>
<td>Tartu</td>
<td>1.3 (0.9 to 1.7)</td>
<td>0.15</td>
<td>3.0 (2.0 to 4.6)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Model 1 includes age, Model 2 includes age & education, Model 3 includes age, education, BMI & smoking.
5.4 Discussion

The main finding of this chapter was that different levels of frailty were seen in different European centres, with generally higher levels in Eastern European centres compared to Northern and Western. These differences were apparent however frailty was measured. Differences between centres were not apparently explained by differences in age, education or lifestyle using the measures available in EMAS.

Differences in levels of frailty across European countries have been previously reported [249]. However, this is to my knowledge the first study to assess frailty in the transitional Eastern European countries. Overall levels of frailty were generally higher in the centres from these countries, although the prevalence of phenotypic frailty were relatively low in Szeged. While no comparable data exist for these countries, the higher levels of frailty seen in these centres are consistent with most data on variations in mortality and general health in the ageing European population. Old age mortality rates are higher in the transitional countries [281]. Alongside this trend it has been estimated that the number of healthy life years, years without disability, remaining at age 50 is fewer in these countries [280]. These findings also agree with earlier reports from EMAS, which showed poorer quality of life and lower cognitive function in men from these centres, compared to the other centres [282, 283]. Higher levels of frailty have been previously reported in Spain compared to Northern European countries [249]. In this study this effect was seen for the frailty phenotype, but not for the frailty index.

The underlying causes of these centre differences in frailty are not clear. Age, while strongly related to frailty did not differ appreciably across centres (Table 5.1). Adjustment was made for key lifestyle variables; BMI and smoking, both of which varied across centres (Table 5.1) and are known to relate to frailty [38, 47]. Adjustment
was also made for years of education, a surrogate measure of socioeconomic status, another key predictor of frailty [44]. Adjustment for these factors had little effect on the apparent centre differences in frailty. In some cases, adjustment for these factors actually strengthened relationships, particularly for the FI.

It is possible that the variables used may not fully reflect variations in these factors across centres. Age at leaving education in particular may be an imprecise measure, considering the different education systems operating across Europe. It is also possible that men in the transitional centres were more likely to return to education in later life, meaning their apparent level of education may not reflect their lifetime socioeconomic status. In this context, education may not be an accurate surrogate of socioeconomic status when comparing across these centres. This may also explain why adjustment for education actually strengthened some of the country differences between centres. Alternatively it may be that there are other contextual factors involved at the difference centres that counterbalance the usual effects of the factors studied here. Indeed, there are a myriad of other factors that may contribute to the geographic variation seen in levels of frailty including, quality of health care, dietary intakes, or cultural differences in physical activity and other health behaviours. It is also possible these differences reflect biological differences between centres, such as variation in levels of vitamin D or chronic inflammation, or possibly, genetic differences. Further exploration into regional differences in frailty may potentially lead to novel aetiological insights into this condition.

There were some interesting measurement points to highlight from this data. Consistent with previous reports [28], the prevalence of frailty was higher according to the suggested frailty index classification compared to the frailty phenotype (Figure 5.1).
This effect is largely a function of the cut-points used for defining the models, the cut points chosen here may not be definitive, but are based on previous work. The continuous index was divided into categories using cut-points based on previous studies [28, 31]. Similarly, the cut-points for the frailty phenotype defined within a subset of this dataset, were designed to correspond to the original model [4]. It might be expected that the multi-domain frailty index would identify more people as frail than the primarily physical phenotype.

Levels of frailty and prefrailty were not always consistently different across centres, with a high prevalence of frailty, but not prefrailty seen in Tartu (Figure 5.1). More strikingly, Malmo showed a relatively high prevalence of prefrailty alongside a low prevalence of frailty (Figure 5.1). This may be due to the differing patterns of frailty criteria reported at different centres (Figure 5.2). In Malmo, the high level of prefrailty was primarily due to the low activity and weakness criteria. Similarly, the relatively high prevalence of prefrailty in Florence was primarily due to high frequencies of low activity and sarcopenia. These differences between centres may reflect cultural differences in reporting activity or energy levels and possibly even in the performance of physical function tasks such as self selected walk speed. It is also possible that these findings represent translational or measurement differences in criteria between centres, although every effort was made to control for these effects in the design of EMAS [246]. Ethnic variation in height and BMI, variables used to standardise the frailty criteria, has been suggested to contribute to the different prevalence of frailty seen in different ethnic groups [41]. It is possible this effect may have contributed to the centre differences seen, however in this study only slowness was normalised to height. These findings demonstrate the difficulty of comparing levels of frailty between countries, or other sub-populations using this model.
Compared to the frailty phenotype, the frailty index generally showed stronger effects and more discrimination between countries (Figure 5.1). Differences in frailty between centres were evident using either the continuous or 3 tiered version of this variable (Tables 5.3 & 5.4). The broader and more inclusive nature of this model may allow for the greater sensitivity of this model to small differences in frailty. The inclusion of 42 variables may smooth any effects of differential reporting between centres that appear to influence the 5 item phenotype model. It should be noted that part of the difference could be due to the different prevalence of frailty for each model, however results were similar when matching the prevalence of FI frailty to that of the phenotype (not shown). It is also possible that differences in effects using the two models relate to differences at the referent centre, although both models ranked Leuven similarly for frailty in relation to other centres.

As with the frailty phenotype the prevalence of frailty was high in Tartu using the 3 tiered FI model, while the prevalence of prefrailty was low. A similar trend across categories was seen in Szeged. A possible explanation could be that the progression of frailty differs across centres, with prefrail men in some centres more likely to progress to full frailty more quickly than in other centres. This suggestion could be tested in future analyses of the EMAS follow up data.

The two models generally agreed on the trends in frailty across centres, this agreement may partly allay any concerns over the measurement issues discussed. There were however two notable disagreements between the models. In Santiago the frequencies of frailty and prefrailty was high according to the frailty phenotype, but not the FI. While in Szeged the prevalence of frailty was low using the phenotype and relatively high
using the FI. This may reflect a difference between the primarily physical frailty measured by the phenotype and the broader frailty assessed by the FI. These discrepancies may also be related to measurement biases in the frailty criteria, as discussed above. The low level of phenotypic frailty in Szeged was mainly due to very low levels of sarcopenia and low activity. Mid upper arm muscle circumference, the measure used to define the sarcopenia criterion is strongly related to BMI, the high BMI in this centre may therefore have contributed to the low prevalence of this criterion. Similarly, the frequency of low activity may be influenced by cultural trends in activity levels and/or their reporting.

Frailer men were more likely to be missing frailty measures (Chapter 4, Table 4.3). There were also differences in missing frailty measures between centres (Chapter 4, Table 4.5). It is possible that some of the centre differences seen may be related to this differential missing data between centres. Santiago had the least missing data at 4.2%. The high prevalence of phenotypic frailty in this centre may be partly due to the low frequency of missing data compared to the other centres, meaning this centre included more men who could be assessed as frail. This provides a further possible explanation for the difference between models seen at this centre.

5.4.1 Limitations and strengths

This study had a number of limitations. Most obviously the study was performed entirely in men and while health trends in European men correlate strongly to those in women [280], our results cannot be immediately generalised to European women. The expected trends in frailty were seen across the European countries, however men were recruited into the study from a limited area around each centre. Frailty measured at these centres may not be entirely representative of the centre’s parent country.
Additionally, as discussed in chapter 3, the response rate for the study was 41% further limiting the generalisability of the findings. Finally, as discussed, exploration of missing data suggests differences in missing according to both frailty and centre. The possibility that this missing data influenced the centre effects seen can therefore not be excluded.

The cohort is slightly younger than the ideal age for assessing levels of frailty across Europe. The prevalence of frailty is highest at the oldest ages [4], unfortunately data were not available on men aged 80 and older. Frailty in this older age group in Europe is an important issue for future studies to address. This younger age group also meant the overall prevalence of frailty in the study was very low. This will have limited the ability to detect differences between the sub-populations at each centre.

The use of the modified criteria should also be acknowledged, it is possible that using the original measures may have given different prevalence estimates or allowed for more discrimination between centres. The criteria included two notable departures from the original model. Firstly, ‘Weakness’ was assessed using chair rising ability rather than grip strength, this is more a test of functional ability than a specific measure of muscle strength. The task involved, rising 5 times from a chair, is comparatively easy, and participants were allowed to do the test in their own time, this may have reduced the test sensitivity in healthier men. However, the criterion was operationalised as a dichotomous variable with only the slowest 10% and those unable to complete the task classified as positive, this approach should allow sufficient discrimination for inclusion in the frailty model. Secondly, sarcopenia or shrinking was assessed using mid upper arm muscle circumference, rather than unintentional weight loss as in the original model. The use of a more specific lean mass marker may have given a different pattern across centres compared to weight loss. Anthropometric measures are relatively
inaccurate for assessing lean mass compared to scanning techniques such as DXA [284], however they are sufficiently sensitive for use in large cohort studies [267]. The criteria used here have advantages over those used in another recent European survey, which relied heavily on self report data [249]. Alongside these limitations with the frailty criteria, as discussed above, the measurement of some of the confounding variables may have been sub-optimal. This limits the ability to discern the influence of these factors on the centre differences in frailty.

Relevant strengths of the EMAS study include the large sample size, the inclusion of centres representing all areas of Europe including the Eastern transitional centres and the use of rigorously standardised methodologies across these centres. The use of both frailty models allows confidence in the results seen and provides novel comparative data for assessing frailty across different populations.

5.4.2 Implications and future work

The pattern in frailty across centres was in agreement with previously reported differences in rates of mortality and levels of health measures across Europe. Frailty measurement may provide a useful summary of health status in ageing European men. Future work on EMAS could use prospective data to explore the progression and outcomes of frailty across the different centres. As discussed, it is possible that men in certain centres are more likely to become frailer or to die or suffer an adverse event compared to others due to differences in health care provision or other sociological factors. Similarly, it is possible that frail men in some countries may be more able to recover in health. This information would provide important data for the management of frailty. More research is needed into understanding the different prevalence of frailty across centres, exploring geographical variation may lead to new mechanistic insights.
into frailty. A further useful target in frailty research may be to characterise frailty in further populations outside of the US, Canada, Europe and China. From this study it appears both the frailty phenotype and frailty index may be effective for this purpose.

5.4.3 Summary

In this multicentre study of European men aged 40-79, differences in frailty were seen between European centres, with generally higher levels of frailty in the transitional Eastern European countries. The differences in frailty across centres were similar using both models, suggesting the adapted criteria used for the frailty phenotype were sufficiently sensitive to 'true' variations in frailty across centres. These differences were not apparently explained by differences in age, education, or lifestyle factors, at least as measured in EMAS. It is possible they may reflect cultural or policy differences across Europe or possibly biological or genetic variation between countries. More research is needed into understanding the disparities in levels of frailty across Europe.
Chapter 6 Relationships between the Hypothalamic Pituitary Testicular Axis and Frailty in Ageing Men

6.1 Introduction

Frailty is believed to arise due to declines across multiple physiological systems [3]. One system suggested to be involved in this process is the sex hormone or Hypothalamic Pituitary Testicular (HPT) axis in ageing men [285]. Declining levels of T in ageing men parallel age related losses of muscle mass [286, 287], while exogenous T has potent anabolic effects in older men [185].

Two large studies in middle aged and older men failed to show a relationship between total T and frailty [163, 165]. Recent studies have suggested possible relationships between the unbound fractions of T and frailty [163, 164]. Studies on key aspects of frailty including sarcopenia, muscle strength and physical function have generally shown weak relationships between T and muscle mass [137, 139, 168], and less consistent effects on strength and function [106, 174, 175].

The majority of previous studies have focussed on T, traditionally seen as the active component and key output of the HPT axis in men. However, frailty may be related to changes in homeostatic regulation [2, 3, 240]. Changes in the HPT axis in ageing men are evident through perturbations of other components of the axis, most notably in elevated levels of gonadotrophins and SHBG [94]. Both SHBG and LH have shown some relationship with frailty [165, 179].

Most studies on the biological correlates of frailty have focussed on single biomarkers, without regard to changes within biological systems or genuine states of deficiency.
Ageing men with low T levels can be classified into 2 groups: Primary hypogonadism, in which low T levels are accompanied by high LH levels, reflecting age related testicular deficit and secondary hypogonadism, in which low T is accompanied by low LH, reflecting pituitary changes, related primarily to obesity [288]. Work on EMAS has recently identified a potential third group, compensated hypogonadism in which T levels are maintained by increased LH output, this condition may precede primary hypogonadism [288]. The existence of ‘genuine’ T deficiency in older men is controversial. A recent report from EMAS attempted a definition of true age related T deficiency, termed Late Onset Hypogonadism (LOH). To be classified as LOH men must have low T combined with multiple symptoms of T deficiency [289].

The aim of this chapter was to explore the relationships between frailty and the HPT axis in ageing men using both major frailty models. This included consideration of: 1) All components of the axis 2) defined states of axis function, 3) symptomatic hormonal deficiency (late onset hypogonadism).
6.2 Methods

6.2.1 Participants
Analyses were performed on men from the baseline phase of the EMAS. Men lacking complete data for all frailty criteria (n=316) and men with known pituitary, adrenal or testicular disease or those taking androgen affecting drugs (n=125, after excluding men missing frailty measures) were excluded from all analyses. The final analysis sample included 2928 men.

6.2.2 Frailty measurement
Frailty was measured using both the frailty phenotype and the frailty index.

6.2.3 Definitions of hormonal conditions
Hormonal conditions were defined using hormone levels and symptoms of androgen deficiency. Firstly a four-category variable of gonadal status was constructed using just hormone levels with 2 thresholds: a T level of 10.5 nmol/L and an LH level of 9.4 U/L. The T cut-off point was similar to that used in previous studies [288]. The LH cut point equated to the 97.5\textsuperscript{th} centile (the upper limit of normal) in the youngest age group in the EMAS, men aged 40-44 years [288]. The four categories were eugonadal (T $\geq$10.5 nmol/L and LH $\leq$9.4 U/L), secondary hypogonadism (T $< 10.5$ nmol/L and LH $\leq$9.4 U/L), primary hypogonadism (T $< 10.5$ nmol/L and LH $> 9.4$ U/L), and compensated hypogonadism (T $\geq$10.5 nmol/L and LH $> 9.4$ U/L) [288]. Next the clinical syndrome of LOH was defined as low T combined with symptoms of androgen deficiency. Men were considered to have LOH if they had total T $<11$ nmol/L and free T $<220$ pmol/L and 3 sexual symptoms: poor morning erections, low frequency of sexual thoughts, and erectile dysfunction [289]. These criteria represent a parsimonious definition of hypogonadism. The hormone thresholds used reflect higher odds of sexual symptoms.
below these T levels. The inclusion of multiple sexual symptoms is due to the multiple causes of these symptoms in ageing men. Inclusion of the three symptoms that tended to cluster most clearly with low T compensates for the lack of specificity of each of the items used [289].

6.2.4 Statistical analyses

Differences in hormone levels between frailty categories were assessed using ANOVA and between hormones and the FI were assessed using Spearman’s rank correlations. Differences in the frequency of frailty and frailty criteria between the different hormonal groups were assessed using Chi-square tests and differences in levels of the FI were assessed using ANOVA.

The relationships between single hormones and the frailty phenotype were evaluated using ordinal logistic regression. This approach effectively looks at the odds of having a higher level of frailty. Binary logistic regression was used for the individual criteria. Relationships between hormones and the FI were evaluated using linear regression. The hormone variables were standardised for these analyses as z-scores ((raw score – mean)/standard deviation). This approach models the change in frailty per Standard Deviation (SD) change in hormone levels. As lower levels of total T, free T, bioavailable T, DHT and DHEAS were associated with greater frailty status, inverse variables for these hormones were used in the modelling. This gives effect sizes relating to decreases in these hormones. This approach allows easy comparison of the strength of relationships for the different hormones.

Three models were fitted for both frailty measures, the first model was unadjusted. The second included: European centre, smoking status (never smoker, ex smoker or current
smoker) and BMI (included as both a linear and quadratic term to reflect the potential U-shaped nature of the relationship between BMI and frailty). These are potential confounders of the relationships between sex hormones and frailty that are largely independent of age. In addition to these factors the third model included age (as a linear covariate). A fourth model was fitted for the frailty phenotype that adjusted additionally for morbidities (coded as 0 or ≥1). Morbidities were not included in the FI analyses as they are not considered separate to frailty according to this model and are therefore included in the FI.

The relationships between gonadal status groups and LOH and frailty were assessed using the same modelling and adjustment strategy used for the single hormones. In each case the men classed as eugonadal, according to each definition were used as the referent group.
6.3 Results

6.3.1 Single hormones

After excluding men on hormone affecting medications 2094 men were robust, 763 were prefrail and 71 were frail (Table 6.1). Levels of total T differed only marginally between frailty groups, ranging from, mean ± SD; 16.6 ± 5.8 nmol/L in robust men to 15.9 ± 6.3 nmol/L in frail men (Table 6.1). Free T levels were lower in frail men; 241.5 ± 77.7 pmol/L compared to robust men; 301.9 ± 86.0 pmol/L, with intermediate levels seen in prefrail men; 271.2 ± 85.2 pmol/L (Table 6.1). Similar trends were seen for bioavailable T, and DHEAS (Table 6.1). Higher levels of LH were seen in the higher frailty categories, rising from 5.7 ± 3.6 U/L in robust men, up to 8.1 ± 6.1 U/L in frail men (Table 6.1). Similarly, elevated levels of FSH, SHBG and Total E2 were seen in prefrail and frail men, compared to robust men (Table 6.1). Levels of free E2, bioavailable E2, and DHT did not differ substantially across frailty categories (Table 6.1).

The FI correlated positively with total T, free T, bioavailable T and DHEAS and negatively with LH, FSH, SHBG, Total E2 and DHT (Table 6.2). Correlations with total T and total E2 were very weak r= -0.09 and 0.08 respectively (Table 6.2). Correlations with the other hormones were stronger, ranging from 0.19 for SHBG up to -0.34 for DHEAS (Table 6.2). Free E2 and bioavailable E2 did not correlate with the FI (Table 6.2).

In unadjusted analyses lower free T, bioavailable T and DHEAS and higher levels of LH, FSH, SHBG and total E2 were all related to frailty. Most of these relationships were similar in strength, ranging from, Ordinal OR (95% CI); 1.34 (1.24 to 1.45) for a 1 SD increase in FSH, up to 1.59 (1.45 to 1.74) for a 1 SD decrease in bioavailable T
A weaker relationship of 1.15 (1.06 to 1.25) was seen for a 1 SD increase in Total E2 (Table 6.3). The ordinal model used effectively looks at the odds of moving up the levels of the outcome per standard deviation change in hormone levels. For example, the Ordinal OR (95% CI); 1.59 (1.45 to 1.74) seen for bioavailable T equates to a 59% increased odds of having a higher level of frailty per 1 SD decrease in bioavailable T. Adjustment for BMI and smoking tended to slightly increase the strength of these relationships, while adjustment for age reduced them substantially (Table 6.3). The relationship with total E2 was attenuated following adjustment for age; 1.04 (0.95 to 1.14) p=0.41, but the relationships with the other hormones persisted (Table 6.3). Further adjustment for morbidities marginally reduced the strength of most relationships, such that association ranged from 1.08 (1.01 to 1.15) for DHEAS up to 1.21 (1.03 to 1.41) for bioavailable T (Table 6.3).

Consistent with the frailty phenotype, the FI was related to free T, bioavailable T, DHEAS, LH, FSH, SHBG and Total E, also to Total T and DHT in unadjusted analyses (Table 6.4). In this analysis the effect sizes equate to changes in FI per standard deviation change in hormone levels. Adjustment for BMI and smoking attenuated the relationships with total T and DHT (Table 6.4). Adjustment for age substantially reduced the strength of all relationships, such that the relationships with bioavailable T, total E2, and DHEAS were no longer significant. However, all other relationships remained significant, ranging in strength from, Effect size (95% CI); 0.006 (0.001 to 0.010) for SHBG to 0.013 (0.009 to 0.017) for LH (Table 6.4).

Relationships between sex hormones and the individual frailty criteria are shown in Table 6.5. With the exception of bioavailable E2 and DHT, all hormones were related to the slowness criterion. Adjustment for confounding, particularly age reduced the
strengths of these relationships, however SHBG, Total E2 and DHEAS remained significantly related to this criterion. The relationship with LH remained borderline significant, OR (95% CI); 1.11 (1.00 to 1.23) p=0.054. Free T was related to the sarcopenia criterion, this relationship was revealed following adjustment for BMI increasing from 1.15 (0.97 to 1.35) p=0.109 in unadjusted analyses to 1.87 (1.47 to 2.40) p<0.0001, in the centre, BMI and smoking model. This relationship remained after adjustment for age and morbidities; 1.40 (1.09 to 1.80) p=0.008. A similar effect was seen for bioavailable T, while FSH also remained related to sarcopenia in adjusted analyses. In adjusted analyses, all hormones were related to the exhaustion criteria except free E2, bioavailable E2 and DHT (Table 6.5). In fully adjusted analyses, free T, LH and SHBG remained related to the exhaustion criterion and bioavailable T and total E2 remained borderline related (Table 6.5). In unadjusted analyses, free T, bioavailable T, LH, FSH, SHBG, Total E2 and DHEAS were related to low activity and weakness (Table 6.5). Adjustment for age largely attenuated these relationships (Table 6.5).
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Robust</th>
<th>Prefrail</th>
<th>Frail</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2928</td>
<td>2,094</td>
<td>763</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>16.5 ± 5.9</td>
<td>16.6 ± 5.8</td>
<td>16.4 ± 6.3</td>
<td>15.9 ± 6.3</td>
<td>0.42</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>292.4 ± 87.0</td>
<td>301.9 ± 86.0</td>
<td>271.2 ± 85.2</td>
<td>241.5 ± 77.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>7.1 ± 2.2</td>
<td>7.4 ± 2.2</td>
<td>6.6 ± 2.2</td>
<td>5.7 ± 1.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Luteinising Hormone (U/L)</td>
<td>6.2 ± 4.4</td>
<td>5.7 ± 3.6</td>
<td>7.2 ± 5.7</td>
<td>8.1 ± 6.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>8.5 ± 8.8</td>
<td>7.7 ± 7.1</td>
<td>10.3 ± 11.3</td>
<td>12.9 ± 16.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>42.6 ± 19.7</td>
<td>40.5 ± 17.9</td>
<td>47.2 ± 21.3</td>
<td>55.6 ± 33.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>74.0 ± 24.6</td>
<td>73.1 ± 23.4</td>
<td>75.9 ± 27.1</td>
<td>82.0 ± 28.0</td>
<td>0.0007</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>0.61</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>51.3 ± 17.0</td>
<td>51.6 ± 16.6</td>
<td>50.6 ± 18.0</td>
<td>51.4 ± 17.7</td>
<td>0.39</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>1.3 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>1.4 ± 0.9</td>
<td>0.71</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate (nmol/L)</td>
<td>4.7 ± 2.8</td>
<td>5.0 ± 2.8</td>
<td>4.0 ± 2.5</td>
<td>3.0 ± 2.0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean ± SD. P value from unadjusted ANOVA

nmol/L = nanomoles per litre, U/L = units per litre, pmol/L = picomoles per litre
### Table 6.2: Correlations between sex hormones and the frailty index

<table>
<thead>
<tr>
<th>Hormone</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>-0.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>-0.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>-0.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Luteinising Hormone (U/L)</td>
<td>0.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>0.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>0.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>0</td>
<td>0.92</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>-0.01</td>
<td>0.49</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>-0.07</td>
<td>0.0001</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate (nmol/L)</td>
<td>-0.34</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are Spearman’s rank correlation coefficients.
Table 6.3: Ordinal logistic regression models for the relationships between sex hormones and the frailty phenotype

<table>
<thead>
<tr>
<th>Sex Hormone</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ordinal OR (95% CI)</td>
<td>P</td>
<td>Ordinal OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>1.05 (0.97 to 1.14)</td>
<td>0.22</td>
<td>1.12 (0.99 to 1.26)</td>
<td>0.061</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>1.53 (1.40 to 1.68)</td>
<td>&lt;0.0001</td>
<td>1.60 (1.40 to 1.84)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>1.59 (1.45 to 1.74)</td>
<td>&lt;0.0001</td>
<td>1.66 (1.44 to 1.91)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Luteinising Hormone (U/L)</td>
<td>1.37 (1.27 to 1.48)</td>
<td>&lt;0.0001</td>
<td>1.38 (1.26 to 1.51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>1.34 (1.24 to 1.45)</td>
<td>&lt;0.0001</td>
<td>1.36 (1.32 to 1.40)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>1.45 (1.34 to 1.57)</td>
<td>&lt;0.0001</td>
<td>1.44 (1.33 to 1.56)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>1.15 (1.06 to 1.25)</td>
<td>0.001</td>
<td>1.14 (1.04 to 1.25)</td>
<td>0.006</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.00 (0.92 to 1.09)</td>
<td>0.98</td>
<td>1.00 (0.90 to 1.12)</td>
<td>0.99</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>0.95 (0.87 to 1.03)</td>
<td>0.206</td>
<td>0.95 (0.85 to 1.07)</td>
<td>0.41</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>0.97 (0.89 to 1.06)</td>
<td>0.49</td>
<td>1.01 (0.92 to 1.10)</td>
<td>0.85</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate(nmol/L)</td>
<td>1.58 (1.43 to 1.73)</td>
<td>&lt;0.0001</td>
<td>1.64 (1.47 to 1.85)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

OR are ordinal OR for 1SD decrease in total, free and bioavailable Testosterone, DHEAS and Dihydrotestosterone and for 1SD increase in the other hormones
Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age, Model 3 includes European centre, BMI, smoking, age and morbidities
<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect size (95% CI)</td>
<td>P</td>
<td>Effect size (95% CI)</td>
<td>P</td>
<td>Effect size (95% CI)</td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>0.009 (0.005 to 0.013)</td>
<td>&lt;0.0001</td>
<td>0.002 (-0.003 to 0.007)</td>
<td>0.37</td>
<td>0.001 (-0.003 to 0.005)</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>0.030 (0.026 to 0.034)</td>
<td>&lt;0.0001</td>
<td>0.026 (0.023 to 0.029)</td>
<td>&lt;0.0001</td>
<td>0.006 (0.003 to 0.009)</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>0.030 (0.027 to 0.034)</td>
<td>&lt;0.0001</td>
<td>0.026 (0.021 to 0.031)</td>
<td>&lt;0.0001</td>
<td>0.004 (-0.001 to 0.009)</td>
</tr>
<tr>
<td>Luteinising Hormone (U/L)</td>
<td>0.027 (0.023 to 0.030)</td>
<td>&lt;0.0001</td>
<td>0.028 (0.021 to 0.035)</td>
<td>&lt;0.0001</td>
<td>0.013 (0.009 to 0.017)</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>0.026 (0.022 to 0.030)</td>
<td>&lt;0.0001</td>
<td>0.026 (0.022 to 0.031)</td>
<td>&lt;0.0001</td>
<td>0.010 (0.007 to 0.013)</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>0.020 (0.016 to 0.023)</td>
<td>&lt;0.0001</td>
<td>0.027 (0.021 to 0.033)</td>
<td>&lt;0.0001</td>
<td>0.006 (0.001 to 0.010)</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>0.011 (0.007 to 0.015)</td>
<td>&lt;0.0001</td>
<td>0.009 (0.003 to 0.015)</td>
<td>0.01</td>
<td>0.002 (-0.001 to 0.006)</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>0.002 (-0.002 to 0.006)</td>
<td>0.29</td>
<td>-0.002 (-0.009 to 0.006)</td>
<td>0.62</td>
<td>-0.0004 (-0.0054 to 0.0047)</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>0.001 (-0.003 to 0.005)</td>
<td>0.68</td>
<td>-0.003 (-0.008 to 0.003)</td>
<td>0.32</td>
<td>0.002 (-0.002 to 0.005)</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>0.006 (0.002 to 0.011)</td>
<td>0.002</td>
<td>-0.002 (-0.008 to 0.004)</td>
<td>0.46</td>
<td>0.003 (-0.002 to 0.008)</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (nmol/L)</td>
<td>0.029 (0.025 to 0.033)</td>
<td>&lt;0.0001</td>
<td>0.029 (0.020 to 0.038)</td>
<td>&lt;0.0001</td>
<td>0.003 (-0.003 to 0.008)</td>
</tr>
</tbody>
</table>

Effects sizes are change in FI score for 1SD decrease in total, free and bioavailable testosterone, DHEAS and Dihydrotestosterone and for 1SD increase in the other hormones.

Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age.
<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total testosterone (nmol/L)</strong></td>
<td>1.19 (1.04 to 1.36)</td>
<td>1.10 (0.97 to 1.26)</td>
<td>1.07 (0.95 to 1.20)</td>
<td>1.04 (0.90 to 1.21)</td>
</tr>
<tr>
<td><strong>Free testosterone (nmol/L)</strong></td>
<td>1.78 (1.54 to 2.06)</td>
<td>1.72 (1.46 to 2.03)</td>
<td>1.20 (1.00 to 1.43)</td>
<td>1.16 (0.95 to 1.41)</td>
</tr>
<tr>
<td><strong>Bioavailable testosterone (nmol/L)</strong></td>
<td>1.82 (1.58 to 2.11)</td>
<td>1.77 (1.50 to 2.09)</td>
<td>1.18 (0.99 to 1.40)</td>
<td>1.14 (0.94 to 1.39)</td>
</tr>
<tr>
<td><strong>Luteinising Hormone (U/L)</strong></td>
<td>1.36 (1.24 to 1.50)</td>
<td>1.39 (1.24 to 1.55)</td>
<td>1.12 (1.01 to 1.23)</td>
<td>1.11 (1.00 to 1.23)</td>
</tr>
<tr>
<td><strong>Follicle Stimulating Hormone (U/L)</strong></td>
<td>1.30 (1.19 to 1.43)</td>
<td>1.32 (1.20 to 1.46)</td>
<td>1.06 (0.94 to 1.20)</td>
<td>1.05 (0.92 to 1.20)</td>
</tr>
<tr>
<td><strong>Sex Hormone Binding Globulin (nmol/L)</strong></td>
<td>1.36 (1.23 to 1.51)</td>
<td>1.49 (1.34 to 1.66)</td>
<td>1.13 (1.04 to 1.22)</td>
<td>1.13 (1.04 to 1.23)</td>
</tr>
<tr>
<td><strong>Total oestradiol (pmol/L)</strong></td>
<td>1.30 (1.16 to 1.46)</td>
<td>1.27 (1.14 to 1.40)</td>
<td>1.15 (1.05 to 1.26)</td>
<td>1.16 (1.05 to 1.29)</td>
</tr>
<tr>
<td><strong>Free oestradiol (pmol/L)</strong></td>
<td>1.13 (1.00 to 1.28)</td>
<td>1.08 (0.93 to 1.26)</td>
<td>1.10 (0.97 to 1.25)</td>
<td>1.12 (0.98 to 1.27)</td>
</tr>
<tr>
<td><strong>Bioavailable oestradiol (pmol/L)</strong></td>
<td>1.08 (0.96 to 1.23)</td>
<td>1.04 (0.91 to 1.18)</td>
<td>1.11 (1.00 to 1.23)</td>
<td>1.12 (1.01 to 1.25)</td>
</tr>
<tr>
<td><strong>Dihydrotestosterone (nmol/L)</strong></td>
<td>1.09 (0.95 to 1.25)</td>
<td>0.99 (0.87 to 1.12)</td>
<td>1.07 (0.98 to 1.17)</td>
<td>1.06 (0.96 to 1.17)</td>
</tr>
<tr>
<td><strong>Dehydroepiandrosterone sulphate (nmol/L)</strong></td>
<td>2.06 (1.73 to 2.45)</td>
<td>2.05 (1.77 to 2.37)</td>
<td>1.17 (1.04 to 1.32)</td>
<td>1.14 (1.01 to 1.28)</td>
</tr>
<tr>
<td><strong>Total testosterone (nmol/L)</strong></td>
<td>0.68 (0.59 to 0.78)</td>
<td>1.25 (0.98 to 1.58)</td>
<td>1.24 (0.97 to 1.59)</td>
<td>1.21 (0.95 to 1.54)</td>
</tr>
<tr>
<td><strong>Free testosterone (nmol/L)</strong></td>
<td>1.15 (0.97 to 1.35)</td>
<td>1.87 (1.47 to 2.40)</td>
<td>1.44 (1.10 to 1.89)</td>
<td>1.40 (1.09 to 1.80)</td>
</tr>
<tr>
<td><strong>Bioavailable testosterone (nmol/L)</strong></td>
<td>1.18 (1.00 to 1.40)</td>
<td>1.98 (1.61 to 2.43)</td>
<td>1.46 (1.16 to 1.84)</td>
<td>1.42 (1.14 to 1.78)</td>
</tr>
<tr>
<td><strong>Luteinising Hormone (U/L)</strong></td>
<td>1.30 (1.17 to 1.44)</td>
<td>1.35 (1.14 to 1.59)</td>
<td>1.13 (0.96 to 1.32)</td>
<td>1.11 (0.95 to 1.29)</td>
</tr>
<tr>
<td><strong>Follicle Stimulating Hormone (U/L)</strong></td>
<td>1.32 (1.19 to 1.46)</td>
<td>1.38 (1.26 to 1.50)</td>
<td>1.16 (1.05 to 1.29)</td>
<td>1.15 (1.04 to 1.27)</td>
</tr>
<tr>
<td><strong>Sex Hormone Binding Globulin (nmol/L)</strong></td>
<td>1.76 (1.56 to 1.99)</td>
<td>1.42 (1.15 to 1.76)</td>
<td>1.09 (0.83 to 1.43)</td>
<td>1.09 (0.83 to 1.43)</td>
</tr>
<tr>
<td><strong>Total oestradiol (pmol/L)</strong></td>
<td>0.96 (0.82 to 1.14)</td>
<td>1.01 (0.85 to 1.21)</td>
<td>0.88 (0.71 to 1.09)</td>
<td>0.89 (0.72 to 1.10)</td>
</tr>
<tr>
<td><strong>Free oestradiol (pmol/L)</strong></td>
<td>0.67 (0.55 to 0.81)</td>
<td>0.84 (0.67 to 1.06)</td>
<td>0.83 (0.66 to 1.05)</td>
<td>0.84 (0.67 to 1.05)</td>
</tr>
<tr>
<td><strong>Bioavailable oestradiol (pmol/L)</strong></td>
<td>0.64 (0.53 to 0.77)</td>
<td>0.79 (0.64 to 0.97)</td>
<td>0.82 (0.66 to 1.02)</td>
<td>0.83 (0.67 to 1.02)</td>
</tr>
<tr>
<td><strong>Dihydrotestosterone (nmol/L)</strong></td>
<td>0.59 (0.52 to 0.68)</td>
<td>0.89 (0.74 to 1.08)</td>
<td>0.98 (0.81 to 1.19)</td>
<td>0.95 (0.79 to 1.15)</td>
</tr>
<tr>
<td><strong>Dehydroepiandrosterone sulphate (nmol/L)</strong></td>
<td>1.35 (1.12 to 1.63)</td>
<td>1.81 (1.36 to 2.40)</td>
<td>1.14 (0.84 to 1.54)</td>
<td>1.10 (0.82 to 1.47)</td>
</tr>
</tbody>
</table>

**Table 6.5: Logistic regression models for the relationships between sex hormones and frailty criteria**
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>1.14 (1.02 to 1.27)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>1.23 (1.10 to 1.38)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>1.19 (1.05 to 1.35)</td>
<td>0.008</td>
<td>1.16 (1.05 to 1.29)</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.08 (0.94 to 1.23)</td>
<td>0.28</td>
<td>1.02 (0.92 to 1.14)</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>1.07 (0.93 to 1.22)</td>
<td>0.35</td>
<td>1.02 (0.90 to 1.16)</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>1.12 (0.96 to 1.30)</td>
<td>0.14</td>
<td>0.98 (0.81 to 1.18)</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate (nmol/L)</td>
<td>1.24 (1.07 to 1.45)</td>
<td>0.005</td>
<td>1.27 (1.02 to 1.58)</td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>1.13 (0.99 to 1.28)</td>
<td>0.066</td>
<td>1.09 (0.92 to 1.29)</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>1.52 (1.33 to 1.74)</td>
<td>&lt;0.0001</td>
<td>1.48 (1.21 to 1.81)</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>1.67 (1.45 to 1.92)</td>
<td>&lt;0.0001</td>
<td>1.64 (1.32 to 2.02)</td>
</tr>
<tr>
<td>Luteinising Hormone (U/L)</td>
<td>1.25 (1.14 to 1.37)</td>
<td>&lt;0.0001</td>
<td>1.26 (1.12 to 1.42)</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>1.25 (1.14 to 1.37)</td>
<td>&lt;0.0001</td>
<td>1.27 (1.18 to 1.36)</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>1.31 (1.18 to 1.45)</td>
<td>&lt;0.0001</td>
<td>1.37 (1.25 to 1.51)</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>1.18 (1.05 to 1.33)</td>
<td>0.005</td>
<td>1.15 (1.00 to 1.31)</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.10 (0.97 to 1.24)</td>
<td>0.13</td>
<td>1.07 (0.92 to 1.24)</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>1.00 (0.88 to 1.13)</td>
<td>0.95</td>
<td>0.97 (0.85 to 1.11)</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>1.03 (0.90 to 1.17)</td>
<td>0.69</td>
<td>0.98 (0.86 to 1.12)</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate (nmol/L)</td>
<td>1.74 (1.48 to 2.03)</td>
<td>&lt;0.0001</td>
<td>1.79 (1.36 to 2.36)</td>
</tr>
<tr>
<td>Weakness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>1.08 (0.92 to 1.26)</td>
<td>0.34</td>
<td>1.06 (0.87 to 1.29)</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>1.37 (1.17 to 1.61)</td>
<td>&lt;0.0001</td>
<td>1.33 (1.11 to 1.60)</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>1.42 (1.21 to 1.68)</td>
<td>&lt;0.0001</td>
<td>1.37 (1.12 to 1.67)</td>
</tr>
<tr>
<td>Luteinising Hormone (U/L)</td>
<td>1.19 (1.07 to 1.32)</td>
<td>0.001</td>
<td>1.19 (1.04 to 1.37)</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (U/L)</td>
<td>1.22 (1.10 to 1.36)</td>
<td>&lt;0.0001</td>
<td>1.23 (1.11 to 1.36)</td>
</tr>
<tr>
<td>Sex Hormone Binding Globulin (nmol/L)</td>
<td>1.27 (1.12 to 1.44)</td>
<td>&lt;0.0001</td>
<td>1.30 (1.14 to 1.48)</td>
</tr>
<tr>
<td>Total oestradiol (pmol/L)</td>
<td>1.19 (1.03 to 1.37)</td>
<td>0.016</td>
<td>1.15 (0.99 to 1.33)</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.08 (0.93 to 1.25)</td>
<td>0.31</td>
<td>1.05 (0.93 to 1.18)</td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>1.03 (0.89 to 1.20)</td>
<td>0.71</td>
<td>1.01 (0.90 to 1.13)</td>
</tr>
<tr>
<td>Dihydrotestosterone (nmol/L)</td>
<td>1.00 (0.86 to 1.17)</td>
<td>0.96</td>
<td>0.96 (0.78 to 1.17)</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate (nmol/L)</td>
<td>1.65 (1.36 to 1.99)</td>
<td>&lt;0.0001</td>
<td>1.59 (1.20 to 2.09)</td>
</tr>
</tbody>
</table>

OR are for 1SD decrease in total, free and bioavailable testosterone, DHEAS and Dihydrotestosterone and for 1SD increase in the other hormones
Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age, Model 3 includes European centre, BMI, smoking, age and morbidities
6.3.2 Gonadal status groups

274 men were classed as having compensated hypogonadism, 57 had primary hypogonadism and 342 had secondary hypogonadism. The remaining 2218 men were considered eugonadal. 5.5% of men in the compensated hypogonadal group were classed as frail compared to 1.9% of eugonadal men (Table 6.6). The prevalence of prefrailty was also higher in men with compensated hypogonadism at 38% and higher still in men with primary hypogonadism at 57.9%, compared to 24.1% in eugonadal men. Only 42.1% of men with primary hypogonadism were classed as robust compared to 74.0% of eugonadal men and 56.6% of compensated hypogonadal men (Table 6.6).

The prevalence of prefrailty and frailty did not differ substantially in the secondary hypogonadal group compared to eugonadal. Levels of the FI were higher in the compensated and primary groups at, median (25th - 75th inter quartile range), 0.18 (0.08 - 0.26) and 0.17 (0.13 - 0.24) respectively, compared to 0.08 (0.05 - 0.15) in eugonadal men (Table 6.6)

The prevalence of slowness, sarcopenia, exhaustion and low activity varied across the gonadal groupings (Table 6.6). The prevalence of slowness was high in the compensated group at 18.6%, compared to 8.1% in eugonadal men. The compensated group also showed relatively high frequencies of sarcopenia, exhaustion and low activity (Table 6.6). The primary group had a high prevalence of low activity at 31.6% compared to 9% in the eugonadal group. This group also had a high prevalence of slowness at 19.3%. The prevalence of weakness did not differ significantly across the groups (Table 6.6)

In unadjusted analyses the compensated, Ordinal OR (95% CI); 2.23 (1.73 to 2.88) and primary hypogonadism 3.37 (2.05 to 5.54) were both related to higher levels of frailty
compared against the referent eugonadal group (Table 6.7). Adjustment for centre, BMI and smoking strengthened the relationship with primary hypogonadism and slightly reduced the relationship with compensated (Table 6.7). Adjustment for age and morbidities attenuated these relationships, although the relationship with primary hypogonadism remained close to significance, 1.79 (0.97 to 3.31) p=0.062 (Table 6.7). Secondary hypogonadism was not clearly related to phenotypic frailty.

In unadjusted analysis all 3 groups were associated with higher levels of the FI (Table 6.8). The strongest relationship was seen for the primary group, Effect size (95% CI); 0.087 (0.059 to 0.114) and the weakest for the secondary, 0.023 (0.012 to 0.035). Adjustment for BMI attenuated the relationship with secondary hypogonadism (Table 6.8). Adjustment for age greatly reduced the strength of relationship for the other 2 groups (Table 6.8). In this model only compensated hypogonadism remained significantly related to the FI, 0.022 (0.012 to 0.032) p=0.01. The relationship with primary hypogonadism, while similar in strength did not approach statistical significance 0.029 (-0.014 to 0.072) p=0.16.

Of the individual frailty criteria, sarcopenia was related to both primary and secondary hypogonadism in adjusted analyses, OR (95% CI); 2.28 (1.25 to 4.14) for the primary group and 3.09 (1.35 to 7.10). These relationships were revealed only after adjustment for BMI changing from 1.41 (0.50 to 3.95) p=0.52 in the unadjusted model to 4.44 (2.26 to 8.70) P<0.0001 in the BMI, smoking and centre adjusted model for the primary group (Table 6.9). A similar effect was seen for the secondary group (Table 6.9). Compensated hypogonadism was associated with a higher likelihood of all 5 frailty criteria, but these relationships did not persist after adjustment for age (Table 6.9).
Similarly, adjustment for age attenuated the relationships seen between primary hypogonadism and the slowness and low activity criteria (Table 6.9).
Table 6.6: Prevalence of frailty by gonadal status groups

<table>
<thead>
<tr>
<th>Hormone levels</th>
<th>Normal (T≥10.5 LH ≤9.4)</th>
<th>Compensated hypogonadism (T≥10.5 LH &gt;9.4)</th>
<th>Primary hypogonadism (T &lt;10.5 LH&gt; 9.4)</th>
<th>Secondary hypogonadism (T &lt;10.5 LH ≤9.4)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2,218</td>
<td>274</td>
<td>57</td>
<td>342</td>
<td></td>
</tr>
<tr>
<td>Robust</td>
<td>1641 (74.0)</td>
<td>155 (56.6)</td>
<td>24 (42.1)</td>
<td>246 (71.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prefrail</td>
<td>535 (24.1)</td>
<td>104 (38.0)</td>
<td>33 (57.9)</td>
<td>86 (25.2)</td>
<td></td>
</tr>
<tr>
<td>Frail</td>
<td>42 (1.9)</td>
<td>15 (5.5)</td>
<td>0 (0.0)</td>
<td>10 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Slowness</td>
<td>180 (8.1)</td>
<td>51 (18.6)</td>
<td>11 (19.3)</td>
<td>36 (10.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>113 (5.1)</td>
<td>33 (12.0)</td>
<td>4 (7.0)</td>
<td>12 (3.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>161 (7.3)</td>
<td>36 (13.1)</td>
<td>4 (7.0)</td>
<td>28 (8.2)</td>
<td>0.009</td>
</tr>
<tr>
<td>Low activity</td>
<td>200 (9.0)</td>
<td>40 (14.6)</td>
<td>18 (31.6)</td>
<td>34 (9.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weakness</td>
<td>133 (6.0)</td>
<td>27 (9.9)</td>
<td>4 (7.0)</td>
<td>24 (7.0)</td>
<td>0.104</td>
</tr>
<tr>
<td>Frailty Index</td>
<td>0.08 (0.05 - 0.15)</td>
<td>0.18 (0.08 - 0.26)</td>
<td>0.17 (0.13 - 0.24)</td>
<td>0.12 (0.06 - 0.19)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are count (%) or median (25 - 75 IQ range)
### Table 6.7: Ordinal logistic regression models for the relationships between gonadal status groups and the frailty phenotype

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Compensated</td>
<td>2.23 (1.73 to 2.88)</td>
<td>&lt;0.0001</td>
<td>2.16 (1.66 to 2.81)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary</td>
<td>3.37 (2.05 to 5.54)</td>
<td>&lt;0.0001</td>
<td>4.03 (2.17 to 7.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.12 (0.87 to 1.45)</td>
<td>0.37</td>
<td>1.03 (0.71 to 1.49)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

OR are ordinal OR for higher frailty status compared against the referent Eugonadal group
Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age, Model 3 includes European centre, BMI, smoking, age and morbidities

### Table 6.8: Multiple linear regression models for the relationships between gonadal status groups and the frailty index

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect size (95% CI)</td>
<td>P</td>
<td>Effect size (95% CI)</td>
</tr>
<tr>
<td>Compensated</td>
<td>0.062 (0.049 to 0.075)</td>
<td>&lt;0.0001</td>
<td>0.064 (0.053 to 0.076)</td>
</tr>
<tr>
<td>Primary</td>
<td>0.087 (0.059 to 0.114)</td>
<td>&lt;0.0001</td>
<td>0.085 (0.042 to 0.128)</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.023 (0.012 to 0.035)</td>
<td>&lt;0.0001</td>
<td>0.003 (-0.007 to 0.014)</td>
</tr>
</tbody>
</table>

Effect sizes are for difference in FI compared to the referent Eugonadal group
Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age
Table 6.9: Logistic regression models for the relationships between gonadal status groups and frailty criteria

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Slowness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensated</td>
<td>2.59 (1.84 to 3.64)</td>
<td>&lt;0.0001</td>
<td>2.59 (1.71 to 3.92)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary</td>
<td>2.71 (1.38 to 5.32)</td>
<td>0.004</td>
<td>2.64 (1.66 to 4.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.33 (0.91 to 1.94)</td>
<td>0.14</td>
<td>0.99 (0.82 to 1.19)</td>
<td>0.92</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensated</td>
<td>2.55 (1.69 to 3.84)</td>
<td>&lt;0.0001</td>
<td>3.11 (1.86 to 5.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary</td>
<td>1.41 (0.50 to 3.95)</td>
<td>0.52</td>
<td>4.44 (2.26 to 8.70)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.68 (0.37 to 1.24)</td>
<td>0.208</td>
<td>3.19 (1.50 to 6.76)</td>
<td>0.003</td>
</tr>
<tr>
<td>Exhaustion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensated</td>
<td>1.93 (1.31 to 2.84)</td>
<td>0.001</td>
<td>1.94 (1.35 to 2.81)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primary</td>
<td>0.96 (0.34 to 2.70)</td>
<td>0.95</td>
<td>1.00 (0.46 to 2.18)</td>
<td>0.11</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.14 (0.75 to 1.73)</td>
<td>0.54</td>
<td>0.78 (0.50 to 1.24)</td>
<td>0.29</td>
</tr>
<tr>
<td>Low activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensated</td>
<td>1.72 (1.20 to 2.49)</td>
<td>0.003</td>
<td>1.73 (1.12 to 2.68)</td>
<td>0.013</td>
</tr>
<tr>
<td>Primary</td>
<td>4.66 (2.62 to 8.29)</td>
<td>&lt;0.0001</td>
<td>4.91 (2.18 to 11.05)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.11 (0.76 to 1.63)</td>
<td>0.58</td>
<td>0.94 (0.57 to 1.57)</td>
<td>0.82</td>
</tr>
<tr>
<td>Weakness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensated</td>
<td>1.71 (1.11 to 2.65)</td>
<td>0.015</td>
<td>1.65 (0.81 to 3.37)</td>
<td>0.17</td>
</tr>
<tr>
<td>Primary</td>
<td>1.18 (0.42 to 3.32)</td>
<td>0.75</td>
<td>1.22 (0.61 to 2.46)</td>
<td>0.58</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.18 (0.75 to 1.86)</td>
<td>0.46</td>
<td>1.00 (0.72 to 1.38)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

OR are for odds of frailty criteria in the hypogonadal groups compared against the referent Eugonadal group

Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age, Model 3 includes European centre, BMI, smoking, age and morbidities.
6.3.3 Late onset hypogonadism

Using the criteria proposed by Wu et al [289], 59 men were classed as having LOH and 2676 as eugonadal (Table 6.10). The prevalence of both frailty and prefrailty was high in hypogonadal men: 6.8% of LOH men were frail compared to 2% of eugonadal and 45.8% were prefrail compared to 24.8% (Table 6.10). Median FI was also notably higher in LOH men, 0.20 (0.13 - 0.33) compared to 0.10 (0.05 - 0.17) in eugonadal men (Table 6.10). The prevalence of slowness, exhaustion and low activity was high in hypogonadal men (Table 6.10). The prevalence of weakness was higher in the LOH men compared to eugonadal, but this difference did not reach statistical significance (Table 6.10). The prevalence of sarcopenia was low in LOH men, 1.7% compared to 5.4% in eugonadal men (Table 6.10).

LOH was related to higher phenotypic frailty status in unadjusted analyses, Ordinal OR; 3.08 (1.86 to 5.12) p<0.0001 (Table 6.11). This relationship was reduced following adjustment for age and was no longer significant, 1.34 (0.79 to 2.29) p=0.28 (Table 10)

LOH was related to higher levels of the FI, Effect size (95% CI); 0.131 (0.104 to 0.157) p<0.0001. This relationship was reduced substantially but remained significant in fully adjusted analyses, 0.059 (0.018 to 0.100) p=0.01 (Table 6.11)

LOH was associated with greater likelihood of the slowness, exhaustion and low activity criteria. None of these relationships remained after adjustment for age (Table 6.12). There was no clear relationship between LOH and sarcopenia or weakness (Table 6.12)
<table>
<thead>
<tr>
<th></th>
<th>Eugonadal</th>
<th>Hypogonadal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2676</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Robust</td>
<td>1959 (73.2)</td>
<td>28 (47.5)</td>
<td></td>
</tr>
<tr>
<td>Prefrail</td>
<td>664 (24.8)</td>
<td>27 (45.8)</td>
<td></td>
</tr>
<tr>
<td>Frail</td>
<td>53 (2.0)</td>
<td>4 (6.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Slowness</td>
<td>224 (8.4)</td>
<td>17 (28.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
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<td>145 (5.4)</td>
<td>1 (1.7)</td>
<td>0.208</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>195 (7.3)</td>
<td>9 (15.3)</td>
<td>0.021</td>
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<tr>
<td>Low activity</td>
<td>254 (9.5)</td>
<td>13 (22.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Weakness</td>
<td>163 (6.1)</td>
<td>7 (11.9)</td>
<td>0.069</td>
</tr>
<tr>
<td>Frailty Index</td>
<td>0.10 (0.05 - 0.17)</td>
<td>0.20 (0.13 - 0.33)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are count (%) or median (25 – 74 IQ range)
### Table 6.11: Relationship between late onset hypogonadism and frailty by both frailty models

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Frailty Phenotype</td>
<td>3.08 (1.86 to 5.12)</td>
<td>&lt;0.0001</td>
<td>2.77 (1.64 to 4.66)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Frailty Index</td>
<td>0.131 (0.104 to 0.157)</td>
<td>&lt;0.0001</td>
<td>0.111 (0.074 to 0.148)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are Ordinal OR (95% CI) P for the frailty phenotype and Effect size (95% CI) P for the frailty index compared against the referent eugonadal group.

Model 1 includes centre, smoking and BMI, Model 2 includes centre, smoking, BMI and age, model 3 includes centre, smoking, BMI, age and morbidities.

### Table 6.12: Relationships between late onset hypogonadism and frailty criteria

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Slowness</td>
<td>4.43 (2.48 to 7.91)</td>
<td>&lt;0.0001</td>
<td>3.63 (1.65 to 7.99)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>0.30 (0.04 to 2.19)</td>
<td>0.24</td>
<td>1.22 (0.15 to 9.69)</td>
<td>0.85</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>2.29 (1.11 to 4.73)</td>
<td>0.025</td>
<td>1.54 (0.66 to 3.56)</td>
<td>0.32</td>
</tr>
<tr>
<td>Low activity</td>
<td>2.69 (1.44 to 5.06)</td>
<td>0.002</td>
<td>2.42 (1.45 to 4.03)</td>
<td>0.001</td>
</tr>
<tr>
<td>Weakness</td>
<td>2.08 (0.93 to 4.64)</td>
<td>0.075</td>
<td>1.56 (0.82 to 2.98)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

OR are for frailty criteria compared against the referent Eugonadal group.

Model 1 includes European centre, BMI and smoking, Model 2 includes European centre, BMI, smoking and age, Model 3 includes European centre, BMI, smoking, age and morbidities.
6.4 Discussion

The main findings of this study were that along with free testosterone many other components of the HPT axis showed some relationships with frailty in middle aged and older European men. Additionally relationships with frailty were seen for states of impaired gonadal function and symptomatic T deficiency. The relationships seen were broadly consistent using either frailty measure.

6.4.1 Sex hormones and frailty

In this study no relationship was found between total T and frailty, however it was measured. This agrees with the majority of previous studies in middle aged and older men [163, 165]. One recent study did show a relationship between total T and frailty in men aged 70-88 using the FRAIL scale [164]. This difference in findings may relate to the different frailty measures used, or the greater age of the men in that study.

Lower free T related to higher levels of frailty according to both models, while lower bioavailable T was associated with higher levels of phenotypic frailty, but was not significantly related to the FI after adjustment for age. The free fractions of T have frequently shown some relationship with frailty in other studies: Lower free T related to the FRAIL scale in elderly men in both cross-sectional and longitudinal analysis [164]. Low bioavailable T related to the frailty phenotype in elderly men, although this relationship could not be confirmed longitudinally [163]. A recent study also reported a relationship between low free T and the development of mobility limitations in men from the Framingham Offspring Study [176]. However, in another study no relationship was seen between the frailty phenotype and free T [165]. Similarly, no prospective relationship could be found between bioavailable T and declines in muscle strength and physical function, in 2 large cohorts of ageing men [175]. The reasons for these
different findings are not entirely clear but may relate to differing frailty measures, age of samples or hormone measurement.

LH was positively related to both frailty measures. This agrees with previous studies that have shown relationships between higher LH and the FRAIL scale [164] and an inverse relationship with muscle mass and strength [179]. The results in this chapter confirm this relationship using the 2 most widely accepted frailty models. The relationship between FSH and frailty has not been previously described, but might be expected as levels of FSH form part of the same feedback loop with LH.

Consistent with an earlier report from the MMAS study [165], higher SHBG was associated with the frailty phenotype. The results presented here extend this earlier finding by also revealing an association between higher SHBG and higher FI level (Table 6.4). In a recent study SHBG was not related to the FRAIL scale [164]. As previously reported DHEAS was inversely related to the frailty phenotype [104], but was not related to the FI after adjustment for age.

The relationship between lower free and bioavailable T and the sarcopenia criteria agrees with previous evidence of a relationship between T and muscle mass in ageing men [137, 139, 167-169]. The relationships seen between SHBG, the gonadotrophins and E2 with slowness and exhaustion have not been previously reported.

6.4.2 Gonadal status groups and frailty

The 3 defined gonadal status groups; primary, secondary and compensated hypogonadism represent distinct states of HPT axis abnormality in ageing men. Relating defined states of axis function to frailty follows logically from the belief that
the development of frailty relates to dysregulation of biological systems [3, 240], however this approach has not yet been applied to any system in the study of frailty. In the condition of compensated hypogonadism the HPT axis appears to be compensating for age related deficits in testicular function by increasing the LH signal to the testes in order to maintain T output [288]. Primary hypogonadism then represents the culmination of this process wherein T levels can no longer be maintained by increased LH drive [288]. Secondary hypogonadism in which both LH and T levels are low represents a separate condition relating to pituitary rather testicular dysfunction [288]. While compensated and primary hypogonadism are primarily age related, secondary is primarily related to obesity [288].

In this chapter both compensated and primary hypogonadism showed some relationship with frailty This is consistent with the hypothesis that age related change in the HPT axis may be related to frailty. After adjustment for age, only compensated hypogonadism remained significantly related to the FI, although the relationship with primary hypogonadism was similar in strength. Neither compensated nor primary hypogonadism were significantly related to the frailty phenotype after adjustment for age, although the relationship with primary hypogonadism remained close to significance. The directions and magnitudes of relationships were the same using either model and the differences in statistical significance may reflect limitations in power due to the small numbers. Secondary hypogonadism was not related to phenotypic frailty, and while it was associated with a slightly higher FI score, this effect was explained by adjustment for BMI. Interestingly both primary and secondary hypogonadism were related to the sarcopenia criteria while compensated hypogonadism was not, after adjusting for age. This may suggest that low T regardless of aetiology relates to this frailty criterion in ageing men. Along with the single hormone relationships with free
and bioavailable T, this may be suggestive of a causal effect of T on this outcome. Men with compensated hypogonadism have been shown previously to have mainly physical symptoms [288]. In this chapter these men were found to be more likely to have any of the 5 frailty criteria, however this effect was explained by adjustment for age and health status.

6.4.3 Late onset hypogonadism and frailty
LOH represents a clinical state of T deficiency in ageing men. The criteria used to assess this condition in this study have only recently been defined [289]. Men with LOH have frankly low levels of both total and free T, combined with multiple symptoms of androgen deficiency. This approach moves beyond the biochemical classification discussed in the previous section to consider the relationship between symptomatic T deficiency and frailty. With frailty suggested to arise from dysregulation, or failure [2] of biological systems it might be expected that this condition may relate to clear states of hormonal deficiency such as LOH.

This condition was related to higher levels of frailty using both models. Adjustment for age, BMI and smoking greatly reduced the strengths of these relationships. For the FI, LOH status was associated 0.13 higher FI score, following adjustment this was reduced to a 0.06 higher score. Adjustment for these factors seemed to largely explain the relationship between LOH and phenotypic frailty, although the lack of significance seen may also reflect the small numbers of frail and LOH men.

6.4.4 Nature of relationships between the HPT axis and frailty
The relationships seen between sex hormones and frailty were relatively weak. These findings are in line with the majority of work on the biological correlates of frailty [102,
These results are also consistent with the understanding of frailty as a multisystem disorder [239].

Adjustment for key confounders particularly age, morbidities and BMI substantially reduced the relationships between hormones and frailty in all analyses. Changes in sex hormone levels in ageing men are affected by age and health status [93, 94, 290]. It is notable that the pattern of hormonal change in relation to frailty, higher gonadotrophins and SHBG and lower free T, is similar to that seen with advancing age [94]. It is possible that hormonal changes in ageing men represent markers of overall decline, rather than causal contributors to frailty. The gonadotrophins and SHBG have not been believed to have biological effects beyond the HPT axis itself. Elevated levels of these hormones suggest some change in the regulation of the HPT axis in frailer men. The higher gonadotrophin levels suggest increasing compensation for declining testicular function is required to maintained T output in older men. Subclinical changes in the regulation of biological systems and progressive adapted responses like this have been suggested to underlie the development of frailty [239, 240, 291]. It is possible that changes in the regulation of well defined systems like the HPT axis reflect the broader age related changes in homeostatic regulation underlying frailty. Interestingly these regulatory hormones related more strongly to the broader FI than the physical phenotype. They also tended to relate to the slowness and exhaustion criteria both of which are broad frailty markers, potentially influenced by multiple physiological systems.

Low T levels may also represent a marker of overall decline, however it is also possible that declining T levels may be causally related to the development of frailty. Lower free and bioavailable T related more clearly to the physical frailty phenotype than the
broader FI. Furthermore both the continuous hormone relationships and gonadal group analyses were suggestive of a relationship between T levels and the sarcopenia criterion. T has potent effects on skeletal muscle in both young and older men [184, 185]. T stimulates myogenic differentiation of mesenchymal stem cells [199, 200], activates muscle satellite cells [198, 202] and increases muscle protein synthesis [192, 194], resulting in increased muscle fibre size and contractile protein content [197, 198]. It is possible T may influence the development of frailty through its influence on lean mass. In support of this, studies using path analysis and structural equation modelling suggest the effects of T on important aspects of frailty including strength and physical function are largely secondary to their effects on body composition [206, 207]. Therefore it is possible that T levels may influence the development of frailty through this effect on lean mass.

The relationships with sarcopenia for free and bioavailable T, primary hypogonadism and secondary hypogonadism were apparent only after including BMI in adjusted analyses. It appears men with low T are more prone to low muscle mass relative to their body size. This effect may also suggest that subtracting arm fat from the triceps skin fold does not completely account for fat mass using this measure.

6.4.5 Limitations and strengths

A limitation of this work was that the operationalisation of the criteria was different to the original model [4]. While this version of the frailty phenotype has been validated in this data set (chapter 4), it is possible that the modified criteria used may have contributed to the relationships observed. The use of mid upper arm muscle circumference to assess sarcopenia is now well supported [267]. However, compared to the use of weight loss in the original model, this measure is a more specific marker of
lean mass. It is possible this relationship would not be seen if ‘weight loss’ was used for this criterion. However, loss of lean mass is hypothesised as the central event of this frailty model and may underlie most of its core features [4]. As such the use of a lean mass marker in place of non specific weight loss seems a reasonable interpretation of this criterion.

Recent guidelines [284], have questioned the accuracy of anthropometric markers for assessing sarcopenia, with BIA suggested as a viable low cost alternative. This technique was not available in EMAS phase 1, but has been included in phase 2. Use of BIA would have the additional advantage of minimising measurement error between the different investigators involved in a multicentre study. Nevertheless, limb circumferences have been shown to predict consequences of sarcopenia, including declines in physical function and mortality [267]. Furthermore, the relationship with T, a primary regulator of muscle mass in men, seen in this chapter underlines the correlation seen between mid upper arm muscle circumference and RASM (chapter 4) in supporting the use of this marker for assessing sarcopenia in the EMAS.

This work also had some more general limitations. As described in chapters 3 and 4, the findings are based on predominantly Caucasian middle aged and older European men and may not apply to other groups of men. Also, the participants were home-dwelling volunteers recruited in selected centres and may not be representative of the general population. Furthermore, as discussed in chapters 3 and 4, issues of participant response and missing data may mean the sample used was slightly healthier than the general population from which it was drawn. The low prevalence of frailty and hormonal disorders in this relative young, healthy population will have limited the ability to detect relationships. Finally, in a cross-sectional study, it is not possible to determine the
directionality of observed relationships. Further prospective data are required to
determine the temporal relationship between changes in sex hormone levels and frailty
in ageing men.

In addition to the large sample size and well standardised assessments, the use of mass
spectrometry T measurement is a major strength of the EMAS study. The analysis
strategy moved beyond the previous single hormone association studies to consider the
HPT axis as a whole, including gonadal status and symptomatic hormonal deficiency.
This approach conceptually approaches the widespread theory that frailty relates to
dysregulation in biological systems [2, 3, 239]. As there remains no consensus on how
to measure frailty the use of the two most widely accepted models in a complementary
manner may also be considered a strong point.

6.4.6 Implications and future work

This work has a number of potential implications for the study of frailty. The
relationship between free T and frailty in the absence of a relationship with total T
suggests, at least where frailty is concerned that free T may be the more biologically
relevant measure of the circulating hormone in ageing men. It is possible that
monitoring older men for low free T levels may better identify those in need of T
therapy. However, it should be noted that the accuracy of the formula used to calculate
free T in these study has been questioned [292]. The relationships seen between other
components of the HPT axis and frailty may suggest that similar in depth study of other
biological systems may yield further insights into frailty.

Interestingly there was considerable, but not complete agreement on the relationships
between the HPT axis and frailty using either model. This suggests both models
approach the same condition and may be distinct, yet related manifestation of frailty
with broadly similar aetiologies. The small differences between models, however, highlight that while frailty remains incompletely defined there is a need to use all available tools in order to further our understanding of this condition.

6.4.7 Summary

In addition to T, many other components of the sex hormone axis showed some relationship with frailty, as did states of impaired gonadal function and testosterone deficiency. These findings are compatible with the hypothesis that frailty is related to age-related changes in biological systems and suggest in depth study of well defined systems may allow insights into this condition. Approaches such as those described in this chapter could be applied to other biological systems in relation to frailty.

A version of part of the FI analyses presented in this chapter was developed into a paper lead by A Tajar (see Publications). Part of the frailty phenotype analyses presented here formed the basis of an oral presentation given at the Endocrine Society Annual meeting 2010 (see Publications).
Chapter 7 Relationship between Multiple Anabolic Hormonal Deficiencies and Frailty in Ageing Men

7.1 Introduction

The results presented in chapter 5 show that frailty was related to free T in men from the EMAS study. This is consistent with the majority of previous reports [163, 164, 166], however this relationship has not been seen in all studies [165]. Furthermore in all cases this relationship has tended to be fairly weak [163, 164, 166]. Similar findings have been seen for a range of other candidate biomarkers [293]. Frailty is believed to be a multisystem disorder and recent studies have supported this hypothesis [242-244]. Frailty has been related to allostatic load, a summary measure of the physiological cost of life course adaptation to stress involving particularly the neuro-endocrine, immunological and cardiovascular systems [245], in older men and women [243, 244]. Similarly frailty was related to dysregulation across 6 key physiological systems, haematological, inflammatory, hormonal, adiposity, neuromuscular and micronutrient, in older women [242]. In this context it may be that particular abnormalities, such as low T may contribute to frailty in only a proportion of cases, possibly explaining the somewhat inconsistent relationships seen.

While T is an important anabolic stimulus in men, there are a number of other hormonal factors that are believed to be involved in regulating muscle mass in older people. These include growth hormone and IGF-1, DHEAS and vitamin D. Each of these hormones has shown some relationship to frailty in large cohort studies [77, 104, 117, 118, 241].

Frailty is suggested to result from a loss of homeostatic redundancy [234, 294]. Furthermore some authors contend that the number of deficits may be more informative
than the specific deficits themselves [2]. It is possible that maintained levels of other anabolic hormones may provide sufficient anabolic stimuli to compensate for the loss of individual hormones. If this is the case it might be expected that multiple deficiencies in anabolic hormones would relate more clearly to frailty than any particular hormone. A recent study tested this hypothesis in elderly women, it was found that deficiency in 2 or 3 hormones from free T, DHEAS and IGF-1 was strongly related to frailty [235]. This relationship has not been explored in men, in whom the pattern of hormonal ageing is very different to that seen in women. Physical frailty is more common in women than men [4]. This may be due to the higher muscle mass seen in men, which in turn is partly due to their higher levels of anabolic hormones [295]. It is therefore important to determine the functional consequences of multiple anabolic hormonal deficiencies in ageing men. Deficiencies in anabolic hormones have been related to mortality in ageing men and congestive heart failure (CHF) patients, with multiple deficiencies indicative of particularly poor prognosis [236, 237].

The aim of this study was to explore the relationship between multiple anabolic hormonal deficiencies and frailty in ageing men from the EMAS cohort. It was hypothesised that multiple deficiencies in anabolic hormones would be related to frailty.
7.2 Methods

7.2.1 Participants
Analyses were performed on the baseline phase of the EMAS. 316 men lacking complete data for all frailty measures and 125 men with known pituitary, adrenal or testicular disease or those taking androgen affecting drugs were excluded from analyses. The final analysis sample included 2928 men.

7.2.2 Frailty measurement
Frailty was measured using the frailty phenotype and the frailty index.

7.2.3 Hormonal deficiencies score
The hormonal deficiency score included levels of 4 key hormones that decline with age and/or are believed to be important to the health of ageing men, these were; free T, DHEAS, IGF-1 and vitamin D. In the absence of accepted cut offs for the hormones studied, men in the lowest quartile for each hormone was classed as having low levels or being deficient in this hormone. Defining relative hormonal deficiency is this manner is a common method in epidemiological research into frailty biomarkers [235]. The cut-points for each hormone are shown in Table 7.1. The 4 deficiencies variables were then summed to give a hormonal deficiency score from 0-4 for each participant, the prevalence of each level of deficiency score is shown in table 7.3.

7.2.4 Statistical analyses
The prevalence of hormonal deficiencies was compared across frailty states using Chi-square tests. Differences in frailty and other key characteristics were compared across hormonal deficiency scores using ANOVA for continuous variables and Chi-square for discrete variables.
The relationship between hormonal deficiency score and frailty was assessed using multinomial logistic regression. The relationship between hormonal deficiency scores and the continuous FI was modelled using linear regression. The relationship between individual frailty criteria and hormonal deficiency score was assessed using logistic regression. The relationship between single hormonal deficiencies and frailty was modelled in the same way as for the overall score, using multinomial logistic regression for the frailty phenotype and linear regression for the frailty index.

For the frailty analyses, the hormonal deficiency score was treated as both a linear and a categorical variable. The linear variable was used to assess the overall trends in the relationship between hormonal deficiencies and frailty. As a previous study in women suggested the relationship between hormonal deficiencies and frailty may be nonlinear [235], the categorical variable was included to check whether there were any evidence of nonlinearity in this relationship. Due to the small number of men with 4 deficiencies, the top 2 levels, 3 and 4 deficiencies were pooled for this analysis.

As described for the sex hormone analysis in chapter 6, the key confounders were considered to be age, BMI, morbidities, centre and smoking. Therefore in addition to the unadjusted model, 2 models were fit for both frailty measures; model 1 included BMI, smoking and centre and model 2 included BMI, smoking centre and age. A third model was fit for the frailty phenotype that additionally adjusted for the presence of morbidity. Morbidity was not included in the FI analysis as this model does not separate morbidities from frailty. Only the unadjusted and fully adjusted models are presented for the single hormone analyses.
7.3 Results

7.3.1 Hormonal deficiency score

The cut-points shown in table 7.1 represent the 25th centile for each hormone. Therefore the overall prevalence of each deficiency was ~25% (Table 7.2). The prevalence of each deficiency increased markedly in a stepwise fashion across levels of frailty (Table 7.2). This effect was similar across all hormones, but strongest for low free T, with the prevalence increasing from 21% in robust men to 53.7% in frail men. The prevalence of overall hormonal deficiency scores are shown in table 7.3. 971 men (35.2%) had 1 deficiency and 548 men (19.9%) had 2. Only 26 men (0.9%) had all 4 deficiencies, and 1017 (36.9%) had no deficiencies (Table 7.3).
### Table 7.1: Hormonal deficiency thresholds

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free testosterone</td>
<td>≤234 pmol/L</td>
</tr>
<tr>
<td>Insulin-like Growth Factor 1</td>
<td>≤104 mg/L</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>≤15.5 ng/mL</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulphate</td>
<td>≤2.5 nmol/L</td>
</tr>
</tbody>
</table>

### Table 7.2: Prevalence of hormonal deficiencies by frailty status

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Robust</th>
<th>Prefrail</th>
<th>Frail</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free T</td>
<td>723 (25.0)</td>
<td>433 (21.0)</td>
<td>254 (33.5)</td>
<td>36 (53.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IGF-1</td>
<td>728 (25.1)</td>
<td>456 (21.9)</td>
<td>239 (31.6)</td>
<td>33 (48.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>703 (25.1)</td>
<td>447 (22.3)</td>
<td>231 (31.3)</td>
<td>25 (39.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DHEAS</td>
<td>728 (25.1)</td>
<td>438 (21.0)</td>
<td>256 (33.8)</td>
<td>34 (51.5)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P values are Chi-square

### Table 7.3: Prevalence of multiple anabolic deficiencies

<table>
<thead>
<tr>
<th>Number of deficiencies</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1017 (36.9)</td>
</tr>
<tr>
<td>1</td>
<td>971 (35.2)</td>
</tr>
<tr>
<td>2</td>
<td>548 (19.9)</td>
</tr>
<tr>
<td>3</td>
<td>197 (7.1)</td>
</tr>
<tr>
<td>4</td>
<td>26 (0.9)</td>
</tr>
</tbody>
</table>

Data are count (%)
7.3.2 Characteristics of men with hormonal deficiencies

The prevalence of frailty increased with increasing hormonal deficiency score from 0.5% in men with no deficiencies to 11.5% in men with 4 deficiencies, while prefrailty increased from 17.8% to 57.7%. Correspondingly, the prevalence of robustness decreased from 81.7 to 30.8 across the hormonal deficiency categories (Figure 7.1). The prevalence of all frailty criteria increased with increasing deficiency score, this relationship was most pronounced for the slowness criteria (Figure 7.2). Median FI increased from 0.06 in men with no deficiencies to 0.27 in men with 4 deficiencies (Figure 7.3). Men with more deficiencies tended to be older and have more co-morbid conditions (Table 7.4). BMI increased across deficiency score peaking at 29.2 ± 5.0 in the 3 deficiency group (Table 7.4). Men with more deficiencies were more likely to be current smokers (Table 7.4).
Figure 7.1: Prevalence of phenotypic frailty by number of hormonal deficiencies

![Graph showing prevalence of frailty by number of hormonal deficiencies.]

Figure 7.2: Prevalence of frailty criteria by number of hormonal deficiencies

![Graph showing prevalence of frailty criteria by number of hormonal deficiencies.]

166
Figure 7.3: Distribution of the frailty index by number of hormonal deficiencies

Frailty Index

Anabolic Deficiencies

0 1 2 3 4
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of deficiencies</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>54.6 ± 9.4</td>
<td>59.5 ± 10.6</td>
</tr>
<tr>
<td>Morbidities</td>
<td>0.5 ± 0.9</td>
<td>0.8 ± 1.1</td>
</tr>
<tr>
<td>BMI</td>
<td>27.0 ± 3.4</td>
<td>27.5 ± 4.1</td>
</tr>
<tr>
<td>Count (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol ≥5 days/week</td>
<td>238 (23.5)</td>
<td>221 (22.9)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>212 (21.2)</td>
<td>225 (23.6)</td>
</tr>
</tbody>
</table>

P values are ANOVA for continuous, and Chi-square for discrete variables.
7.3.3 Relationship between multiple hormonal deficiencies and frailty

The hormonal deficiency score was clearly related to frailty (Table 7.5). Each additional deficiency was associated with an RRR (95% CI); 2.70 (2.11 to 3.45) higher odds of frailty and a 1.57 (1.44 to 1.71) higher odds of prefrailty (Table 7.5). These relationships were not greatly affected by adjustment for BMI, smoking and centre, but were substantially reduced by adjustment for age (Table 7.5). Further adjustment for morbidities tended to slightly weaken the relationships (Table 7.5). After full adjustment, the relationships were 1.71 (1.38 to 2.13) for frailty and 1.18 (1.03 to 1.35) for prefrailty.

Similar relationships were seen for the FI, with each additional deficiency associated with an, Effect size (95% CI); 0.040 (0.036 to 0.04) increase in FI score (Table 7.5). Like the phenotype, adjustment for BMI, smoking and centre did not greatly affect the relationship, while adjustment for age reduced the strength considerably down to 0.016 (0.012 to 0.02).

As vitamin D levels are affected by sun exposure and this hormone was included in the hormone score, an additional model including season was fit for both frailty measures. This did not meaningfully affect the relationship between hormonal deficiency score and frailty and so is not presented here. Similarly, exclusion of men taking vitamin D or calcium supplements did not meaningfully affect the relationships seen (data not shown).

Hormonal deficiency score was related to all frailty criteria, with relationships ranging from, OR (95% CI); 1.77 (1.56 to 2.00) for slowness to 1.50 (1.30 to 1.73) for weakness, per 1 deficiency increase in hormone score (Table 7.6). Adjustment for
confounders, particularly age and morbidities reduced the strengths of these relationships, such that they ranged from 1.20 (1.10 to 1.32) for weakness to 1.30 (1.18 to 1.44).

When treating the hormone score as a categorical variable, there were trends towards a non-linear relationship between hormonal deficiencies with frailty, with disproportionately stronger effects at greater numbers of deficiencies (Table 7.7). The RRR (95% CI) for frailty was 3.59 (1.30 to 9.93) for 1 deficiency, 10.79 (4.05 to 28.72) for 2 deficiencies and 25.45 (9.21 to 70.35) for 3 or 4 deficiencies compared against 0 deficiencies (Table 7.7). A similar pattern was seen for prefrailty, although the overall effects were weaker (Table 7.7). Adjustment for age greatly reduced the size of effects and the general pattern was smoothed towards a more linear relationship (Table 7.7). Odds of frailty were 1.93 (0.77 to 4.81) for 1 deficiency, 3.15 (1.43 to 6.98) for 2 deficiencies and 6.57 (2.64 to 16.35) for 3 or 4 deficiencies. Adjustment for morbidity furthered reduced the strength of relationships (Table 7.7).

The presence of a single hormonal deficiency was associated with an, Effect size (95% CI); 0.035 (0.026 to 0.044) p<0.0001 higher FI score, 2 deficiencies with 0.081 (0.071 to 0.092) p<0.001 higher score and 3 or 4 with an, 0.120 (0.105 to 0.134) p<0.0001 higher score, compared to men with no deficiencies (Table 7.8). As with the frailty phenotype, adjustment for centre, BMI and smoking had little effect on the relationship between hormonal deficiencies and the FI, while adjustment for age reduced the strength of the relationships substantially (Table 7.8).

Deficiency in any of the hormones was related to frailty, prefrailty and higher FI scores (Table 7.9). These effects were reduced by adjustment for age and other confounders for
free T, IGF-1 and DHEAS (Table 7.9). However, these adjustments did not greatly affect the relationship between low vitamin D and frailty. In adjusted analyses low vitamin D remained clearly related to prefrailty, frailty and to higher FI scores. Low free T remained related to frailty, RRR (95% CI); 2.00 (1.26 to 3.16), and to slightly higher FI scores, the relationship with prefrailty was close to significance 1.29 (0.97 to 1.72), p=0.085. Low DHEAS remained related to slightly higher FI scores, but not clearly with frailty or prefrailty (Table 7.9). Low IGF-1 tended to relate to frailty, but this did not reach significance, 1.62 (0.84 to 3.10).
Table 7.5: Relationships between hormone deficiency score and frailty

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRR (95% CI)</td>
<td>P</td>
<td>RRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Prefrailty</td>
<td>1.57 (1.44 to 1.71)</td>
<td>&lt;0.0001</td>
<td>1.55 (1.40 to 1.73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Frailty</td>
<td>2.70 (2.11 to 3.45)</td>
<td>&lt;0.0001</td>
<td>2.72 (2.11 to 3.50)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Effect size (95% CI)</td>
<td>P</td>
<td>Effect size (95% CI)</td>
<td>P</td>
<td>Effect size (95% CI)</td>
</tr>
<tr>
<td>FI score</td>
<td>0.040 (0.036 to 0.04)</td>
<td>&lt;0.0001</td>
<td>0.037 (0.033 to 0.04)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Model 1 adjusts for BMI, smoking and centre, model 2 adjusts for BMI, smoking, centre and age, model 3 adjusts for BMI, smoking, centre, age and morbidities.

RRR are for odds of prefrailty or frailty per 1 deficiency increase in hormone score, Effect sizes are change in FI score per 1 deficiency increase in hormone score.
<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Slowness</td>
<td>1.77 (1.56 to 2.00)</td>
<td>&lt;0.0001</td>
<td>1.73 (1.55 to 1.93)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>1.39 (1.19 to 1.63)</td>
<td>&lt;0.0001</td>
<td>1.77 (1.52 to 2.07)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>1.56 (1.37 to 1.79)</td>
<td>&lt;0.0001</td>
<td>1.48 (1.33 to 1.64)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low Activity</td>
<td>1.70 (1.51 to 1.92)</td>
<td>&lt;0.0001</td>
<td>1.67 (1.42 to 1.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weakness</td>
<td>1.50 (1.30 to 1.73)</td>
<td>&lt;0.0001</td>
<td>1.45 (1.32 to 1.60)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Model 1 adjusts for BMI, smoking and centre, model 2 adjusts for BMI, smoking, centre and age, model 3 adjusts for BMI, smoking, centre, age and morbidities.

OR are for odds of frailty criteria per 1 deficiency increase in hormone score.
Table 7.7: Relationship between number of hormonal deficiencies and the frailty phenotype

<table>
<thead>
<tr>
<th>Number of deficiencies</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRR (95% CI)</td>
<td>P</td>
<td>RRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td><strong>Prefrailty</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.73 (1.40 to 2.15)</td>
<td>&lt;0.0001</td>
<td>1.63 (1.36 to 1.95)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>2.53 (1.99 to 3.22)</td>
<td>&lt;0.0001</td>
<td>2.43 (1.96 to 3.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3/4</td>
<td>3.93 (2.86 to 5.40)</td>
<td>&lt;0.0001</td>
<td>3.85 (2.39 to 6.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Frailty</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.59 (1.30 to 9.93)</td>
<td>0.014</td>
<td>3.48 (1.48 to 8.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>10.79 (4.05 to 28.72)</td>
<td>&lt;0.0001</td>
<td>10.77 (4.78 to 24.25)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3/4</td>
<td>25.45 (9.21 to 70.35)</td>
<td>&lt;0.0001</td>
<td>26.07 (9.08 to 74.81)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Model 1 adjusts for BMI, smoking and centre, model 2 adjusts for BMI, smoking, centre and age, model 3 adjusts for BMI, smoking, centre, age and morbidities. RRR compares odds of frailty or prefrailty for each level of the hormone score against the referent category of 0 deficiencies.
### Table 7.8: Relationship between number of hormonal deficiencies and the frailty index

<table>
<thead>
<tr>
<th>Number of deficiencies</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect size (95% CI)</td>
<td>P</td>
<td>Effect size (95% CI)</td>
</tr>
<tr>
<td>1</td>
<td>0.035 (0.026 to 0.044)</td>
<td>&lt;0.0001</td>
<td>0.031 (0.015 to 0.047)</td>
</tr>
<tr>
<td>2</td>
<td>0.081 (0.071 to 0.092)</td>
<td>&lt;0.0001</td>
<td>0.073 (0.057 to 0.090)</td>
</tr>
<tr>
<td>3/4</td>
<td>0.120 (0.105 to 0.134)</td>
<td>&lt;0.0001</td>
<td>0.111 (0.099 to 0.123)</td>
</tr>
</tbody>
</table>

Model 1 adjusts for BMI, smoking and centre, model 2 adjusts for BMI, smoking, centre and age

Effect sizes are difference in FI score compared against the referent no deficiencies category
Table 7.9: Relationship between single hormonal deficiencies and frailty

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRR (95% CI)</td>
<td>P</td>
<td>RRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td><strong>Prefrailty</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low free T</td>
<td>1.90 (1.58 to 2.28)</td>
<td>&lt;0.0001</td>
<td>1.29 (0.97 to 1.72)</td>
<td>0.085</td>
</tr>
<tr>
<td>Low IGF-1</td>
<td>1.64 (1.36 to 1.97)</td>
<td>&lt;0.0001</td>
<td>1.06 (0.88 to 1.28)</td>
<td>0.56</td>
</tr>
<tr>
<td>Low vitamin D</td>
<td>1.59 (1.32 to 1.92)</td>
<td>&lt;0.0001</td>
<td>1.58 (1.24 to 2.01)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low DHEAS</td>
<td>1.92 (1.60 to 2.31)</td>
<td>&lt;0.0001</td>
<td>0.97 (0.73 to 1.29)</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Frailty</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low free T</td>
<td>4.38 (2.68 to 7.17)</td>
<td>&lt;0.0001</td>
<td>2.00 (1.26 to 3.16)</td>
<td>0.003</td>
</tr>
<tr>
<td>Low IGF-1</td>
<td>3.35 (2.06 to 5.46)</td>
<td>&lt;0.0001</td>
<td>1.62 (0.84 to 3.10)</td>
<td>0.15</td>
</tr>
<tr>
<td>Low vitamin D</td>
<td>2.29 (1.37 to 3.84)</td>
<td>0.002</td>
<td>2.22 (1.23 to 3.99)</td>
<td>0.008</td>
</tr>
<tr>
<td>Low DHEAS</td>
<td>3.99 (2.43 to 6.54)</td>
<td>&lt;0.0001</td>
<td>1.27 (0.82 to 1.98)</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Frailty Index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low free T</td>
<td>0.057 (0.049 to 0.066)</td>
<td>&lt;0.0001</td>
<td>0.015 (0.002 to 0.028)</td>
<td>0.031</td>
</tr>
<tr>
<td>Low IGF-1</td>
<td>0.044 (0.036 to 0.053)</td>
<td>&lt;0.0001</td>
<td>0.008 (-0.002 to 0.017)</td>
<td>0.091</td>
</tr>
<tr>
<td>Low vitamin D</td>
<td>0.030 (0.021 to 0.039)</td>
<td>&lt;0.0001</td>
<td>0.029 (0.016 to 0.041)</td>
<td>0.001</td>
</tr>
<tr>
<td>Low DHEAS</td>
<td>0.070 (0.062 to 0.079)</td>
<td>&lt;0.0001</td>
<td>0.018 (0.003 to 0.033)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Adjusted model includes BMI, smoking, centre, age and *morbidities not included in Frailty Index model
Each model compares odds of frailty or difference in FI score between low and normal levels of each hormone
7.4 Discussion

The main finding of this chapter was that the burden of anabolic hormonal deficiencies was related to frailty in ageing European men, independently of age and other key confounders. This relationship was evident however frailty was measured.

These results are in agreement with an earlier study in women [235], and further extend their findings through demonstrating that this relationship is not limited to physical frailty, but is also evident for the more inclusive frailty index. These findings are also consistent with the relationship seen between anabolic deficiencies and mortality in older men and male CHF patients [236, 237]. However, one study failed to find a relationship between the burden of anabolic deficiencies and 6 year declines in grip strength in older men and women, although catabolic burden was associated with this decline [238]. This discrepant finding may be due to the inclusion of both men and women, the use of grip strength alone instead of broader frailty measures or possibly the somewhat arbitrary way anabolic deficiencies are defined in these studies.

Hormonal deficiency was quite strongly related to frailty with each additional deficiency associated with an approximately 70% greater odds of frailty in fully adjusted analyses. The relationship with prefrailty was weaker with each additional deficiency associated with an approximately 20% increased odds of prefrailty. Similar results have been seen in women [235]. This likely reflects the heterogeneous health status in this intermediately frail group of men.

Previous studies in women have suggested a nonlinear pattern between increasing physiological impairment and frailty [235, 242]. This effect has been reported in relation to multiple hormonal deficiencies [235] and to dysregulation across
physiological systems [242]. It has been suggested that while low numbers deficits or impairments can be compensated for, there exists a threshold level of homeostatic impairment that undermines an organism's overall adaptive capacity and leads ultimately to frailty and its adverse sequelae [242]. The results here are somewhat consistent with this suggestion, with 3 or 4 deficiencies strongly associated with frailty and weaker effects seen for 1 or 2 deficiencies, although this pattern was less clear after adjustment for age.

7.4.1 Nature of the relationship between multiple hormonal deficiencies and frailty

It is plausible that the relationship seen reflects a loss of redundancy in the anabolic regulation of muscle mass and strength. It seems reasonable to suggest that loss of a single anabolic influence can be compensated for by the other systems, but that impairment in multiple anabolic regulatory systems removes this safety net and leads to loss of muscle mass and function and so frailty. In support of this suggestion, IGF-1, T and vitamin D have known receptors on skeletal muscle [134, 200, 296-298] although no receptor for DHEA has yet been cloned [235]. Furthermore there is some evidence for an anabolic effect of each of these hormones in the elderly [114, 128, 153, 185]. T and growth hormone have potent anabolic effects when given exogenously to older men, particularly when given together in combined treatment [153, 154, 181, 185, 188]. There is less evidence for an active effect of DHEA, most studies have shown no effect of this hormone on muscle mass or strength [109, 112, 113]. A recent study found DHEA in combination with gentle exercise increased muscle strength in older women, although no effect was seen on muscle mass [114]. Similarly, there is little evidence for an active effect of vitamin D, although a recent meta-analysis suggested a modest effect on muscle strength in deficient patients [128].
The relative lack of support for an active effect of the latter two hormones raises the possibility that the anabolic deficiencies seen here are passive markers of the overall physiological dysregulation that underlies frailty [107]. In this case apparently greater burdens of anabolic deficiency may reflect escalating homeostatic dysregulation. It was notable that adjustment for chronological age greatly reduced the strength of the relationships between hormonal deficiencies and frailty measures. Similarly, adjustment for morbidities reduced the strength of the relationship between the frailty phenotype and the anabolic deficiencies score. Furthermore, levels of these hormones are affected by lifestyle factors, and health status [91-94, 299]. As such they can be considered markers of general health or successful ageing in men. Additionally levels of anabolic hormones may be reduced following acute stressors such as surgery [300]. In this context hormone levels may be passive markers of an additional causal stressor. It is possible that in frail men, in whom physiological regulation is impaired it may take longer for hormone levels to normalise post stressors.

While it is intuitive to suggest that multiple anabolic deficiencies may be causally related to frailty a reasonable case can be made for each of the possibilities described. It is beyond the scope of this analysis to determine the casual nature of these relationships.

7.4.2 Limitations and strengths

The study population was a predominantly Caucasian European sample of middle aged and older men and caution is required in generalising to other populations. Most importantly these findings cannot be generalised to women, however a recent study suggest similar relationships exist in older women [235]. As described in chapters 3 and 4, participant response and missing data issues mean the sample used may be slightly
healthier than the general population. Finally, this was a cross-sectional analysis which means, as discussed above, the direction of the relationships seen cannot be determined.

The main analytical limitation was the use of essentially arbitrary thresholds for defining hormonal deficiency. This approach is widely used in epidemiological research in the absence of clearly defined deficiency levels for many biomarkers. However, given the age of the population it may be that these cut-points do not represent deficient levels of the hormones. The cut-points were chosen to balance the need to identify physiologically relevant low hormone levels with the statistical need for sufficient numbers to perform meaningful analyses. As such the analyses presented here can be understood as a relative comparison within this cohort of relatively healthy middle aged and older men. It might be expected that the relationships seen would be stronger in an older frailer population with lower hormone levels.

There are a couple of conceptual points that should be acknowledged. Firstly, while ageing is associated with adverse changes in anabolic and catabolic balance, no catabolic markers were considered in this study. Frailty has been related to higher mean cortisol levels in older women [102]. While potentially catabolic changes in inflammatory cytokines are among the most studied frailty biomarkers [81] and have shown synergistic interactions with changes in anabolic hormones in relation to frailty, functional decline and mortality [80, 145]. Other potentially important contributors to muscle loss with ageing were also not included: Myostatin inhibits muscle growth and may promote muscle catabolism in older adults [59]. While a loss of sensitivity to the anti-catabolic effects of insulin may also be involved in sarcopenia [60]. Future studies may aim to integrate all of these effects for a comprehensive overview of anabolic-catabolic balance in frailty.
Secondly, while T, DHEA, and IGF-1 are all similarly regulated by the hypothalamic pituitary axis and may have interactive actions on muscle [192, 193, 301], vitamin D is perhaps a different kind of marker that is dependent primarily on diet and lifestyle [92]. Vitamin D was included in this study due to its potential additional anabolic influence, in order to give the most complete summary of anabolic hormonal status in ageing men.

This chapter shares the key strength of EMAS discussed in the previous results chapters, including large sample size, standardised assessments and mass spectrometry testosterone measurement.

A strength of this analysis was the comprehensive overview of anabolic hormonal status in ageing men. Frailty was assessed using the 2 most well established models. While both of these models can be considered imperfect, the general agreement on the results using either model, suggest a genuine relationship between frailty, as an entity and multiple anabolic hormonal deficiency.

7.4.3 Implications and future work
The results of this study are in line with the growing body of evidence supporting the theory that frailty is a multi-system disorder [235, 242-244, 302]. Future studies may usefully focus on multisystem changes in relation to frailty, instead of the more common single biomarker approach. The further implication of this is that, as other authors have observed [233], the single deficiency, single replacement model of treatment that has proved effective in younger people with particular endocrine conditions, may be inappropriate in frail men. The multisystem nature of frailty may require more complex management strategies.
A potential target for future research may be optimisation of overall anabolic hormone status in older men. This could be achieved through multiple hormonal replacements. Studies combining T and GH treatments have shown encouraging results in older men [153, 154, 188], however there remain some concerns over the adverse effects of this combination [153]. Other approaches, such as combining T and DHEA have shown less encouraging results [112]. Although in this study doses of both hormones were low. It is possible that the benefits of low dose multiple hormone replacement may not be apparent in short term studies. Alternatively anabolic hormone levels could be maintained through alternative strategies, including maintenance of physical activity and healthy body weight and management of co-morbid conditions. Such an approach would have numerous additional benefits beyond the effect of hormone optimisation. In this scenario hormone supplementation would remain an option for patients with persistently low levels of any hormones.

7.4.4 Summary

In summary multiple anabolic hormonal deficiency was related to frailty in this study of middle aged and older European men. This finding is consistent with the hypothesis that frailty relates to the loss of homeostatic redundancy.
Chapter 8 Durability of Testosterone Effects in Prefrail and Frail Elderly Men

8.1 Introduction

The preceding chapters have focussed on the definition and biological correlates of frailty. The effort into understanding this condition is largely aimed at generating potential strategies for its management. Resistance training increases muscle mass and strength in the elderly and may also lead to functional improvements [303, 304]. Another potential avenue for treatment is the use of pharmacological anabolic interventions [225]. Such treatments do not require the equipment or supervision necessary for a resistance training program and may be less burdensome on the frail elderly. Among the first wave of potential therapies is the use of anabolic hormone replacement [188], with T replacement in elderly men receiving particular interest.

Our group recently reported an increase in lean body mass, muscle strength and quality of life in frail elderly men in response to 6 months T treatment in a randomised prospective placebo-controlled study [181]

The effects of T treatment on muscle mass in elderly men appear to reach their peak by about 6 months of treatment; this increase in muscle mass can then be maintained (without further increment) with continued treatment for up to 3 years [180, 189, 191]. It is unclear if these positive effects can be maintained once treatment is withdrawn. Since the use of T in elderly men often raises concern regarding possible adverse effects [227], it is important to determine if short-term treatment can lead to prolonged benefits beyond the duration of T exposure.
The effects of 12 weeks treatment with the anabolic-androgenic steroid, oxandrolone on muscle mass and strength in healthy elderly men were shown to decline, within 12 weeks of treatment cessation [226]. However, the offset trajectory following physiological T treatment has not been studied. The condition of frailty is believed to arise from a mutually exacerbating cycle of declining energetics and reserve: Wherein a loss of muscle mass and strength leads to declines in physical function, energy levels, and physical activity, which further potentiate the deficits in muscle mass and function [4]. Anabolic interventions like T may halt this cycle of decline, with improved strength allowing maintenance of function and activity, thus preventing or delaying further decline and progression to frailty (Figure 8.1). It is therefore possible that some beneficial effect of T treatment in frail men may be maintained once treatment is withdrawn, due to the interruption of this cycle of frailty. The aim of this chapter was to evaluate the post-treatment durability of effects at 6 months after the cessation of T treatment in prefrail and frail elderly men.
Figure 8.1: Hypothetical mechanistic pathway through which testosterone treatment may interrupt the progression of frailty
8.2 Methods

8.2.1 Participants

This chapter focuses on the post-treatment follow-up phase of the Schering testosterone interventional trial, a 6 month trial of testosterone treatment in prefrail and frail elderly men. The analyses focus on the subset of 208 men who completed assessments at 12 months.

8.2.2 Statistical analyses

The primary analysis included all randomized participants completing baseline assessments on an intention to treat (ITT) basis. Where data were missing, participants were necessarily excluded from the analysis of that endpoint. The analyses presented here are based throughout on the subset of participants completing the 12 month follow up phase of the trial and so the 6 month data differ from those presented in the original trial report [181]. The outcome analysis was based on an Analysis of Covariance (ANCOVA) model for the effect of allocation on treatment outcome at 12 months, adjusting for the baseline values. Baseline frailty status, physical function (6 minute walk time) and randomisation number were included as covariates, to account for recruitment trends in retention and level of frailty. Results are expressed as adjusted mean differences with 95% confidence intervals. Differences in safety parameters between groups were evaluated using Mann Whitney tests.
8.3 Results

8.3.1 Participants

From a total of 1677 men screened, 274 met the recruitment criteria to be randomized into T (138) and placebo groups (136) (Figure 8.2). 12 men withdrew before, and 31 during the treatment phase (baseline - 6 months) [181]. A further 22 withdrew during the follow up phase (6 - 12 months). One participant in the placebo group was excluded from analyses at 12 months after independently starting T treatment prescribed from his general practitioner. Baseline characteristics were well matched between groups and did not differ in the men completing the trial compared to the randomised cohort (Table 8.1).
<table>
<thead>
<tr>
<th></th>
<th>Randomised Placebo group (n = 132)</th>
<th>Testosterone group (n = 130)</th>
<th>Completed Placebo group (n = 104)</th>
<th>Testosterone group (n = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>73.9 ± 6.4</td>
<td>73.7 ± 5.7</td>
<td>74.1 ± 6.3</td>
<td>73.8 ± 5.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.7 ± 13.4</td>
<td>81.0 ± 14.0</td>
<td>80.7 ± 14.0</td>
<td>81.0 ± 13.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 4.0</td>
<td>27.9 ± 4.1</td>
<td>27.7 ± 4.1</td>
<td>27.9 ± 4.1</td>
</tr>
<tr>
<td>Frail</td>
<td>20 (15%)</td>
<td>18 (14%)</td>
<td>16 (15%)</td>
<td>14 (13%)</td>
</tr>
<tr>
<td>Prefrail</td>
<td>112 (85%)</td>
<td>112 (86%)</td>
<td>88 (85%)</td>
<td>90 (87%)</td>
</tr>
<tr>
<td>Frailty criteria present, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exhaustion</td>
<td>65 (49%)</td>
<td>68 (52%)</td>
<td>49 (47%)</td>
<td>53 (51%)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>32 (24%)</td>
<td>26 (20%)</td>
<td>26 (25%)</td>
<td>21 (20%)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>21 (16%)</td>
<td>13 (10%)</td>
<td>17 (16%)</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Walk time</td>
<td>11 (8%)</td>
<td>9 (7%)</td>
<td>10 (10%)</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Grip strength</td>
<td>81 (62%)</td>
<td>81 (62%)</td>
<td>63 (61%)</td>
<td>67 (64%)</td>
</tr>
<tr>
<td>Number of frailty criteria present, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One criterion</td>
<td>79 (59.8%)</td>
<td>83 (63.8)</td>
<td>63 (60.6%)</td>
<td>67 (64.4%)</td>
</tr>
<tr>
<td>Two criteria</td>
<td>33 (25%)</td>
<td>29 (22.3%)</td>
<td>25 (24.0%)</td>
<td>23 (22.1%)</td>
</tr>
<tr>
<td>Three criteria</td>
<td>15 (11.4%)</td>
<td>16 (12.3%)</td>
<td>12 (11.5%)</td>
<td>13 (12.5%)</td>
</tr>
<tr>
<td>Four criteria</td>
<td>5 (3.8%)</td>
<td>2 (1.5%)</td>
<td>4 (3.9%)</td>
<td>1 (1.0%)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. or count (%).
Figure 8.2: Participant flow

8260 men, ≥ 65 yrs invited
1677 men were assessed for eligibility

Excluded (n = 1403)
- Not frail
- Raised PSA
- Prostate pathology
- T >12 nmol/l
- Lower urinary tract symptoms
- Not interested
- Not contactable
- Did not attend appointment

Randomized (n=274)

Allocated to placebo gel (n = 136)
- Received placebo gel (n = 132)
- Did not receive placebo gel (n = 4)
  - Renal failure (1)
  - Not interested (2)
  - Chest infection (1)

Withdrawals (n = 15)
- Skin rash (3)
- Sour smell (1)
- Raised PSA (1)
- Diarrhoea (1)
- Neurological problem (1)
- Acute myocardial infarction (1)
- Cancer lung (1)
- Cancer oesophagus (1)
- Pulmonary embolism (1)
- Not interested (2)
- Not contactable (2)
- Discontinued intervention (n = 2)
  - Skin rash (2)

Completed 6 months (n = 117)

Withdrawals (n = 12)
- Decline in cognitive function (1)
- Knee replacement surgery (1)
- Leg fracture (1)
- Not contactable (3)
- Not interested (5)
- Caring for ill wife (1)
- Exclusions (n=1)
  - Started T treatment independently (1)

Completed 12 months (n = 104)

Allocated to testosterone gel (n = 138)
- Received testosterone gel (n = 130)
- Did not receive testosterone gel (n = 8)
  - Not interested (2)
  - Prostate pathology (1)
  - Not contactable (2)
  - Discontinued intervention (n = 4)
    - Raised PSA (2)
    - Viral infection (1)
    - Abdominal aneurysmal surgery (1)

Withdrawals (n = 16)
- Skin rash (1)
- Raised PSA (1)
- Aggressive (1)
- Ankle swelling (1)
- Shoulder pain (1)
- Cancer lung (1)
- Angina, chest infection (1)
- Cancer oesophagus (1)
- Pulmonary embolism (1)
- Started on antiandrogen (1)
- Not interested (2)
- Not contactable (3)
- Discontinued intervention (n = 4)
  - Raised PSA (2)
  - Viral infection (1)
  - Abdominal aneurysmal surgery (1)

Completed 6 months (n = 114)

Withdrawals (n = 10)
- Chronic Inflammatory Demyelinating Polyneuropathy (1)
- Coronary Artery Bypass Surgery (1)
- Not contactable (3)
- Not interested (1)
- Raised PSA (2)
- Rash (1)
- Tenderness in breast (1)

Completed 12 months (n = 104)
8.3.2 Hormone levels

Total T levels that were raised into the target range (18 - 30 nmol/L) during treatment in the active group had declined to baseline by 6 months post treatment (Figure 8.3). The same pattern was seen for free T (Figure 8.3). LH levels that were suppressed during treatment were slightly, but not significantly, elevated at 12 months compared to baseline (Figure 8.3).
Figure 8.3: Change (mean ± SEM) in total and free testosterone and luteinising hormone over 12 months
A) Total testosterone B) Free testosterone C) Luteinising Hormone levels

A) Total testosterone (nmol/L)

B) Free testosterone (pmol/L)

C) Luteinising hormone (IU/L)
8.3.3 Muscle strength

IME-PT increased in the T group and decreased in the placebo group during treatment to give a difference between groups of 8.1 (-0.2 to 16.5) Nm at 6m. This decreased to 4.0 (-3.9 to 11.9) Nm (p= 0.32) at 12 months. Similarly the difference for IKE-PT was 0.8 (-4.6 to 6.3) Nm (p=0.76) at 12 months compared to 4.0 (-1.5 to 9.6) Nm at 6 months. No other muscle strength outcomes were significantly different between groups at 12 months (Table 8.2).

8.3.4 Body composition

Lean Body Mass (LBM) increased during treatment in the T group to give a difference between groups of 1.2 (0.8 to 1.7) kg at 6 months this declined to 0.3 (-0.1 to 0.8) kg (p=0.12) at 12 months (Table 8.2). Fat mass had decreased in the treatment group at 6 months, but this difference was not maintained at 12 months 0.0 (-0.6 to 0.6) kg (p=0.68) (Table 8.2).

Percentage changes in IME-PT and lean body mass over 12 months are summarised in Figure 8.4.
Table 8.2: Muscle strength and body composition over 12 months in testosterone and placebo groups

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time point</th>
<th>Placebo group n</th>
<th>Testosterone group n</th>
<th>Adjusted difference testosterone-placebo (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isometric knee extension peak torque (Nm)</td>
<td>Baseline</td>
<td>144.9 ± 41.5</td>
<td>142.7 ± 42.7</td>
<td>8.1 (-0.2 to 16.5)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>140.0 ± 36.9</td>
<td>145.5 ± 42.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>136.3 ± 36.1</td>
<td>137.4 ± 38.4</td>
<td>4.0 (-3.9 to 11.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>Isokinetic knee extension peak torque (Nm)</td>
<td>Baseline</td>
<td>100.5 ± 31.9</td>
<td>100.2 ± 29.6</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>102.5 ± 29.1</td>
<td>105.1 ± 30.1</td>
<td>4.0 (-1.5 to 9.6)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>102.3 ± 29.2</td>
<td>102.0 ± 28.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isometric knee flexion peak torque (Nm)</td>
<td>Baseline</td>
<td>112.9 ± 32.1</td>
<td>106.6 ± 27.5</td>
<td>6.3 (-0.2 to 16.5)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>116.1 ± 27.6</td>
<td>115.9 ± 24.8</td>
<td>4.9 (-1.4 to 11.1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>115.3 ± 30.3</td>
<td>115.2 ± 27.5</td>
<td>0.8 (-4.6 to 6.3)</td>
<td>0.76</td>
</tr>
<tr>
<td>Isokinetic knee flexion peak torque (Nm)</td>
<td>Baseline</td>
<td>60.9 ± 20.3</td>
<td>59.6 ± 19.5</td>
<td>1.3 (-1.2 to 7.1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>67.3 ± 20.5</td>
<td>68.3 ± 18.9</td>
<td>1.0 (-3.0 to 5.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>Baseline</td>
<td>31.5 ± 8.0</td>
<td>30.4 ± 6.5</td>
<td>0.8 (-0.7 to 2.4)</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>33.3 ± 6.3</td>
<td>33.4 ± 6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>29.9 ± 7.0</td>
<td>30.6 ± 7.1</td>
<td>1.4 (-0.3 to 3.1)</td>
<td></td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>Baseline</td>
<td>50.7 ± 7.6</td>
<td>51.6 ± 7.4</td>
<td>0.9 (0.8 to 1.7)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>50.7 ± 7.4</td>
<td>52.8 ± 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>50.4 ± 7.9</td>
<td>51.7 ± 7.6</td>
<td>0.3 (-0.1 to 0.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>Baseline</td>
<td>21.0 ± 7.7</td>
<td>21.5 ± 7.6</td>
<td>0.5 (-1.1 to -0.2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>20.9 ± 7.5</td>
<td>20.7 ± 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>20.7 ± 7.7</td>
<td>21.1 ± 7.2</td>
<td>0.4 (-0.6 to 0.6)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. ANCOVA P comparing adjusted mean difference between placebo and testosterone groups; adjusted for corresponding baseline value, frailty criteria, walk time and randomization number.
8.3.5 Physical function and activity

None of the physical function scales were significantly different between groups at 12 months (Table 8.3). ALF scores and six minute walk distance tended to improve over time in both groups, while performance on the PPT and Tinetti gait and balance stayed fairly constant over the 12 months (Table 8.3). There were no differences between groups in physical activity levels, measured by PASE score at 6 or 12 months.

8.3.6 Quality of life

Symptom scores for the somatic and sexual scales of the AMS decreased during treatment (improved quality of life) to a greater extent in the T group compared to placebo. No difference between groups for any scale remained at 12: -1.1 (-2.3 to 0.1) (p=0.081) for the somatic scale, 0.7 (-0.6 to 2.1) (p=0.28) for the sexual scale (Table 8.3). The percentage changes in these scales over 12 months are summarised in Figure 8.4
### Table 8.3: Physical function and quality of life measures over 12 months in testosterone and placebo groups

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time point</th>
<th>Placebo group</th>
<th>Testosterone group</th>
<th>Adjusted difference (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical function tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ALF Score (sec)</td>
<td>Baseline</td>
<td>21.8 ± 6.1</td>
<td>22.5 ± 7.2</td>
<td>-1.4 (-3.4 to 0.7)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>22.0 ± 9.2</td>
<td>21.0 ± 7.5</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>21.3 ± 6.3</td>
<td>22.0 ± 8.4</td>
<td>0.1 (-1.0 to 1.2)</td>
<td>0.75</td>
</tr>
<tr>
<td>6 min walk (m)</td>
<td>Baseline</td>
<td>394 ± 91</td>
<td>383 ± 86</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>402 ± 90</td>
<td>404 ± 79</td>
<td>9.6 (-5.0 to 24.2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>396 ± 103</td>
<td>394 ± 87</td>
<td>5.8 (-12.9 to 24.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Total PPT Score</td>
<td>Baseline</td>
<td>21.0 ± 3.9</td>
<td>21.1 ± 3.8</td>
<td>-0.5 (-0.7 to 0.6)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>21.2 ± 3.9</td>
<td>21.9 ± 3.2</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>19.5 ± 4.0</td>
<td>19.5 ± 3.2</td>
<td>0.1 (-0.7 to 0.8)</td>
<td>0.87</td>
</tr>
<tr>
<td>Tinetti Balance Score</td>
<td>Baseline</td>
<td>14.2 ± 2.1</td>
<td>14.1 ± 1.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>13.7 ± 2.0</td>
<td>13.7 ± 1.9</td>
<td>0.2 (-0.3 to 0.6)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>13.4 ± 2.3</td>
<td>13.5 ± 2.1</td>
<td>0.1 (-0.4 to 0.6)</td>
<td>0.54</td>
</tr>
<tr>
<td>Tinetti Gait Score</td>
<td>Baseline</td>
<td>11.2 ± 1.3</td>
<td>11.4 ± 1.0</td>
<td>-1 (-0.7 to 0.4)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>11.1 ± 1.3</td>
<td>11.2 ± 1.3</td>
<td>-0.1 (-0.7 to 0.4)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>10.8 ± 1.8</td>
<td>10.7 ± 1.5</td>
<td>-0.1 (-0.5 to 0.2)</td>
<td>0.29</td>
</tr>
<tr>
<td>PASE score</td>
<td>Baseline</td>
<td>129.4 ± 65.1</td>
<td>135.4 ± 75.3</td>
<td>6.0 (-27.2 to 18.2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>142.3 ± 86.1</td>
<td>155.5 ± 80.0</td>
<td>-4.5 (-27.2 to 18.2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>149.4 ± 81.7</td>
<td>159.6 ± 83.0</td>
<td>2.8 (-18.5 to 24.1)</td>
<td>0.8</td>
</tr>
<tr>
<td>Aging Males’ Symptom scales</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatic subscale</td>
<td>Baseline</td>
<td>16.2 ± 5.2</td>
<td>16.3 ± 5.1</td>
<td>-1.0 (-2.2 to 0.2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>13.6 ± 4.8</td>
<td>12.6 ± 4.1</td>
<td>-1.1 (-2.3 to 0.1)</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>15.1 ± 5.4</td>
<td>14.1 ± 4.4</td>
<td>-1.1 (-2.3 to 0.1)</td>
<td></td>
</tr>
<tr>
<td>Psychological subscale</td>
<td>Baseline</td>
<td>8.7 ± 4.3</td>
<td>9.1 ± 3.8</td>
<td>-0.2 (-0.7 to 1.1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>7.3 ± 3.1</td>
<td>7.9 ± 3.4</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>8.2 ± 3.9</td>
<td>7.6 ± 3.6</td>
<td>0.6 (-1.8 to 1.0)</td>
<td>0.091</td>
</tr>
<tr>
<td>Sexual subscale</td>
<td>Baseline</td>
<td>13.6 ± 4.4</td>
<td>14.0 ± 4.3</td>
<td>-1.3 (-2.5 to -0.1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>12.3 ± 4.5</td>
<td>11.0 ± 4.4</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>11.9 ± 5.0</td>
<td>12.6 ± 4.6</td>
<td>0.7 (-0.6 to 2.1)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. ANCOVA P comparing adjusted mean difference between placebo and testosterone groups; adjusted for corresponding baseline value, frailty criteria, walk time and randomization number.

ALF: Aggregate Locomotor Function score; PPT, Physical Performance Test; PASE, Physical Activity Scale for the Elderly.

6 MWT, Tinetti gait, and balance, ALF test scores presented represent untransformed data; they were log transformed for the purpose of analysis.
Figure 8.4: Percentage (mean ± SEM) changes over 12 months in muscle strength, lean mass and quality of life
(A) Knee extensor strength, (B) Lean Body Mass, (C) AMS Somatic symptoms, (D) AMS Sexual symptoms
C
On treatment Post treatment
Baseline 6 months 12 months
% change
Placebo Testosterone

D
On treatment Post treatment
Baseline 6 months 12 months
% change
Placebo Testosterone
8.3.7 Safety

Haematocrit and haemoglobin were significantly higher in T-treated men at 12 months; however this difference was present at baseline (Table 8.4). No other safety measures were significantly different between groups at 12 months (Table 8.4). PSA levels were slightly raised during treatment in the T-treated group and HDL levels were slightly reduced. Neither of these changes persisted at 12 months (Table 8.4). Total Cholesterol and LDL levels were not significantly affected by treatment (Table 8.4).
### Table 8.4: Safety monitoring over 12 months in testosterone and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
</tr>
<tr>
<td>Prostate specific antigen (ng/decilitre)</td>
<td>1.5 ± 0.9</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>42.1 ± 3.6</td>
<td>41.3 ± 3.7</td>
</tr>
<tr>
<td>Haemoglobin (g/decilitre)</td>
<td>14.1 ± 1.3</td>
<td>13.9 ± 1.4</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/litre)</td>
<td>1.5 (1.2 - 1.9)</td>
<td>1.6 (1.2 - 1.9)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/litre)</td>
<td>4.5 (3.9 - 5.2)</td>
<td>4.3 (3.7 - 4.9)</td>
</tr>
<tr>
<td>Triglycerides (mmol/litre)</td>
<td>1.4 (1.1 - 2.0)</td>
<td>1.3 (0.9 - 1.8)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/litre)</td>
<td>2.2 (1.6 - 2.6)</td>
<td>2.0 (1.6 - 2.7)</td>
</tr>
</tbody>
</table>

Data are mean ± SD or median (25 -75 IQ range) P values are from Mann Whitney test for difference between groups at 12 months.
8.4 Discussion

The main finding of this chapter was that the increased lean body mass, strength and quality of life after 6 months T treatment in frail elderly men, were not maintained at 6 months after the end of treatment. The results also indicate that any effects of T treatment on PSA, haemoglobin and haematocrit, and HDL are transient and not maintained post-treatment.

In the T group, muscle strength assessed by IME-PT increased by around 6% during treatment, and then declined to just above baseline by 12 months (Figure 8.4). In contrast, IME-PT declined linearly across the 12 months in the placebo group. The different trajectories between the T and placebo groups highlight the clear albeit transient effects of the short-term intervention. The other muscle strength outcomes tended to increase only slightly or remain stable over time in both groups (Table 8.2). A recent longitudinal study suggests isokinetic knee extension performance declines at a rate of 3-4% per year in older men [286]. This effect along with any effect of treatment was not apparent in this study. Isokinetic contractions may be harder to perform for older men, due to the need to exert force at a set speed over a full range of motion, compared to a static contraction. The regularity of testing over a relatively short 12 month period on this more difficult assessment may have meant that improvement in performance due to learning effects were sufficient to mask small changes in muscle force over time.

The gains in lean mass also declined to baseline once treatment was withdrawn (Figure 8.4). The decline in lean mass of 0.6% over 12 months in the placebo group was smaller than the decline in muscle strength. This is consistent with studies that have reported greater declines with age in strength compared to lean mass [286, 305]. The loss of
muscle strength with ageing is due not only to the loss of muscle mass, but to factors as diverse as muscle quality, fibre type, muscle architecture, tendon elasticity and neural coordination of muscle force output [306, 307].

The rate of decline in lean mass and muscle strength post treatment was faster in the T group compared to placebo. This probably reflects the loss of treatment effect superimposed onto the normal age related decline seen in the placebo group.

Changes in AMS score in the T group followed the same pattern as those in muscle strength and lean mass; improving with treatment and declining once treatment was withdrawn. Unlike the other outcomes, the placebo group also tended to improve in symptom scores during treatment, consistent with a placebo effect. This effect was of a smaller magnitude than evident in the T group, and declined post treatment. At 12 months the difference between groups was small and neither group had yet returned to baseline (Figure 8.4).

The improvement with T treatment and subsequent decline post treatment suggest the effects seen in this study were due to the direct influence of the changing T levels and not to any secondary treatment-related factor. The decline in muscle mass and strength post treatment suggests that this relatively short term intervention may not be sufficient to interrupt the overall progression of frailty in this heterogeneous cohort of prefrail and frail men. The potential for any intervention to produce prolonged benefits in this population may be related to the size of the treatment effect, and particularly how this affects functional ability. It is possible that greater gains in strength with more potent anabolic stimuli administered over a longer period may produce more sustained functional benefits. The effect of increasing T within the physiological range for 6
months may have been too small or too brief to affect the functional ability and lifestyle of these men and so to influence the cycle of frailty. The lack of overall effect of treatment on physical function may support this idea, while the PASE data suggest no substantial change in physical activity occurred in either group during treatment (Table 8.3). The majority of men in the study were prefrail, rather than frail. The baseline functional impairment in these men may not be severe enough for a small effect of T to engender substantial improvements.

There have been concerns about the health risks associated with the use of T in older men [182, 231]. In this study, the effects on traditional safety parameters were small during treatment and returned to baseline within 6 months after treatment. These results suggest short term treatment with physiological androgen dosages should be well tolerated, and any possible long term risks on the prostate and cardiovascular system can be avoided by short term or minimised by repeated short cycles of treatment.

The results presented here were based on the subset of men that completed assessments at 12 months to allow a clear comparison of the 6m and 12m data. In this subset some results reported previously [181], did not reach statistical significance, but the magnitude of the effects were virtually identical to that reported for the whole cohort [181] and the loss of formal significance simply reflects the smaller numbers analysed.

8.4.1 Limitations and strengths

Limitations with the trial design and outcome assessments were discussed in detail previously [181]. Briefly, inclusion was based on a single T measurement, it is normally recommended that repeated measurements are used to account for intra-individual variation [308]. However, this effect is less marked in older men [309], and the results
from the placebo group showed the stability of T levels over time. The use of any 1 of the Fried frailty criteria for screening meant the population included exhibited considerable heterogeneity. It is possible a more specific subgroup of men with low muscle strength may have seen more improvement in physical function, however the inclusion of the heterogeneous group should be more representative of frailty in the general population. The use of isokinetic dynamometry to assess muscle strength is well accepted, however, it has been suggested that the slower contraction speeds used in 1-RM protocols may be more suitable for older men [214]. Although there was no clear evidence of bias from the missing data, the possibility of frailty related missing data and consequent bias cannot be completely excluded.

A limitation with this follow-up phase was the lack of interim measurements between 6 and 12 months. It was therefore not possible to evaluate the decay time course of the treatment effects during this post treatment phase. It is possible some benefits may have remained for a short time post treatment. It is also possible that transient T deficiency from any delay in suppression of endogenous T treatment may have accelerated the decay of treatment effects in the initial post treatment phase. The overall drop out rate at 12 months was 20%, 12% occurred during treatment and a further 8% during this follow-up phase. Drop out was equal between arms and was therefore unlikely to have resulted in any bias in the results.

8.4.2 Implications and future work

These data have important implications for the management of frailty in men. There is no evidence of any ‘knock-on’ effects of T. The optimal duration of treatment is currently unknown. Further studies are needed to determine how long the beneficial effects of T persist. This highlights the importance of alternate approaches in the
management of frailty. For example, a more active multi-faceted strategy, combining an anabolic agent like T with lifestyle intervention such as increasing physical activity and/or strength training to interrupt the downward spiral into frailty. Resistance training has been found to elicit substantial gains in strength and function in even the very elderly [303, 304]. The use of T may augment the effects of an exercise program [310] and provide the initial boost required to implement a lifestyle intervention in elderly men.

The aetiology of frailty is complex and multifactorial [2, 3] and it is likely that any approach to its management will need to be similarly complex and implemented over the longer term. Although a single short term intervention may temporarily improve symptoms of physical frailty, it may not suffice to effectively reverse or ameliorate the condition permanently.

8.4.3 Summary

In summary, the beneficial effects of short term T treatment on body composition, muscle strength and quality of life declined by 6 months after treatment withdrawal. This suggests that any benefits of short-term T exposure on these parameters are entirely hormone-dependent and do not propagate secondary derivative pathways that might help break a cycle of frailty in ageing men.

The work presented in this chapter forms the basis of a manuscript published in the Journal of Clinical Endocrinology and Metabolism and was presented as a poster at The Endocrine Society Annual meeting 2010 (see Publications).
Chapter 9 Concluding Comments

The study of frailty, as understood today, began in earnest a decade ago with the publication of Fried and colleagues seminal paper proposing an operational definition for the condition [4]. While much progress has been made, our understanding of this condition remains in its infancy. In this context the studies described herein represent early exploratory investigations into this condition and its aetiology and management in ageing men. From these studies a number of themes become apparent, that are consistent with those emerging in the field at large.

From the results described in chapter 4, both the frailty phenotype, as adapted for the EMAS, and the frailty index can be considered valid tools to measure frailty. The models correlated moderately with each other suggesting they were at least partially measuring the same condition, and both were associated with adverse outcomes, in this case falling, the core feature of any frailty model. In chapter 6, free T levels were inversely associated with both models, while gonadotrophin and SHBG levels were positively associated with higher scores on either model. Similarly, in chapter 7, deficiency of multiple anabolic hormones was associated with a high risk of phenotypic frailty and high levels of deficit accumulation. The similar hormonal relationships seen with either model further suggest they are approaching a single condition, with a particular underlying biology.

The models however do not agree entirely, the correlation between them was only 0.41. In other studies the correlation has been somewhat higher, usually around 0.6 [31, 37]. Either way this suggests considerable disagreement between models. As there is currently no consensus on the key features of frailty and how they can be measured, it
can be considered that in all studies to date frailty is an unmeasured or latent construct with any of the current models representing imperfect indicators of the condition. In agreement, the different models appear to capture different, but overlapping groups of older adults [32]. In this context, the choice of model used may depend upon the particular question or purpose addressed. In chapter 4 the FI was more strongly related to incident falls than the frailty phenotype, while the outcome data available here was limited, this result agrees with other studies using longer follow ups and key outcomes including mortality [30, 31]. It may be then that the FI represents a more useful prognostic or decision making tool for geriatric practice. Additionally, the graded measurement of frailty, combined with the more inclusive nature of this model may make it more suitable for comparing levels of frailty between population groups, as seen in chapter 5. The more focussed nature of the frailty phenotype, while possible reducing its predictive relationship with adverse outcomes, may make it more appropriate for screening older people at need of function promoting therapies, as in chapter 8.

Future work on defining frailty may focus on either devising new models or refining existing ones. The FI is a flexible measure that can be applied to any existing dataset with sufficient general health data in older people. It has been suggested the model may require additional translation to make it useable in clinical and other contexts [34]. To this end studies have validated frailty indexes derived entirely from self report data and from a standardised comprehensive geriatric assessment [311, 312].

Refining the frailty phenotype may involve either the inclusion of additional components to improve predictive ability [313], or reducing it down to the minimum criteria. In chapter 4, ‘exhaustion’ was the least strongly age related criterion. Compared to the other criteria this may be less specific frailty marker, at least in a relatively young
population like the EMAS, with varying work and family pressures that may contribute to energy levels. The use of the relatively non-specific exhaustion and low activity in screening for the Schering trial, may have contributed to the lack of sustained effect seen in chapter 8. T treatment did not improve physical function in most men at 6 months [181], and so would not be expected to lead to increased activity levels that may promote the maintenance of muscle gained. Furthermore depending on the underlying cause of these self reported criteria, strength promoting treatments may not improve these symptoms of frailty. It may be more appropriate for future function promoting trials to use narrower definitions focusing on the symptoms of frailty most likely to respond to treatment as screening tools. The recent European consensus definition of sarcopenia, including low muscle mass, strength and physical function, essentially includes the 3 frailty criteria most likely to improve with anabolic therapy [284].

Free and bioavailable T were related to phenotypic frailty, while total T was not. It may be that the free fractions represent more biologically relevant measures of T in ageing men, due to rising SHBG levels. However, these relationships have not been seen in all studies [165]. This may relate to the fact T is not the only anabolic hormone in ageing men. The results of chapter 7 suggest men with deficiencies in multiple hormones were more likely to be frail. However, even in men with low levels of all 4 hormones the prevalence of frailty was only 11.5%. These hormonal results are consistent with the understanding of frailty as a multisystem disorder with many contributory factors in addition to endocrine changes.

The most interesting finding of the work contained in this thesis may be that frailty is related not only to traditionally studied mediatory hormones like T, but also to secondary, regulatory components of the sex hormone axis. The pattern of hormonal
variation in relation to frailty is similar to the pattern seen in relation to advancing age [94]. Similarly, levels of the other hormones studied in chapter 7 are age related and deficiencies in multiple hormones occurred primarily in the oldest 70-79 year old group. These relationships are suggestive of increased levels of age related biological change in frail men. These endocrine findings are similar to those seen in studies of other systems, particularly the inflammatory, haematological and metabolic systems [56, 57, 63, 75]. It appears therefore that frail older people may experience exaggerated or accelerated age related changes in physiological regulation.

The nature of the hormonal relationships seen cannot be discerned from cross-sectional data. Free T levels were related to frailty, and particularly to the sarcopenia criteria. Given the known effects of T on muscle [185], it is reasonable to suggest changing levels of T may contribute to frailty through this pathway. The loss of the lean mass gained during T treatment seen at the post treatment follow up to the Schering trial in chapter 8 further suggests T may actively contribute to muscle mass maintenance in ageing men. Ultimately it will require further longitudinal and interventional studies to establish the causal relationships between anabolic hormones and frailty.

The understanding of the biological changes related to frailty has progressed dramatically in recent years [293]. The variation in sex hormone levels in relation to frailty suggests additional insights may be gained from studying further homeostatic regulatory systems in relation to frailty. Similarly, the relationship between multiple hormonal deficiencies and frailty suggests future studies may usefully move away from the single biomarker approach to consider multiple markers in relation to this complex condition. An approach that is already beginning to emerge in recent studies [235, 242]
A range of biological markers across a number of systems have been studied in relation to frailty [57, 65, 80, 163, 241, 242]. As the field has progressed it has become increasingly clear that these disparate investigations form a coherent body of work. Frailty is associated with usually subclinical changes across several interacting systems [65, 80, 241, 293]. Future work may focus further on how changes within and across these systems relate to frailty. One possibility could be a frailty index like approach using biological data, including age related changes across key physiological systems, to give a summary measure of age related physiological change. Alternatively more sophisticated study designs may be required to capture the physiological changes associated with frailty. It has been suggested that the homeostatic changes underlying frailty may become apparent only in response to stressors [240]. Subtle endocrine changes may also become clearer with more frequent sampling, both in the short term, across 24 hours [102] and longer term, across several years [107].

Another key target will be to identify the driver behind these physiological changes, perhaps involving study of age related cellular damage. A number of studies have suggested a relationship between oxidative stress and frailty [52-54]. While the one study to explore the relationship between telomeres and frailty found no relationship between telomere length and the frailty index in a Chinese population [26].

The EMAS, with its wealth of biological and phenotypic data, will no doubt provide a valuable resource for addressing some of the suggestions described. In addition, there are a number of other European cohort studies that could be used, including the ELSA [38], SHARE [249], UK Biobank [314], and The Irish Longitudinal Study of Ageing (TILDA) [315]. Each of these studies has different strengths that should be exploited in the future study of frailty.
The use of T therapy did not lead to sustained increases in muscle mass and strength in frail and prefrail men. This finding suggests that the improvements seen were entirely hormone dependent, supporting the active effects of T on muscle mass in frail men. It is possible that more sustained improvements may be seen in a more selected group of older men. In very weak men, the small improvements in strength may be sufficient to improve function and so stimulate increases in activity, which could in turn lead to maintenance of the muscle gained. Although this effect would also clearly depend on other factors, particularly the motivation of the men to remain active. The results of chapter 6 suggest of the 5 frailty criteria, T was primarily related to sarcopenia. Future trials may therefore usefully include men with low functional ability secondary to low muscle mass and T levels.

The termination of a recent high profile clinical trial, due to high rates of adverse cardiovascular events in the T treated group has again highlighted the long standing concerns over the safety of T therapy in older men [182]. These concerns combined with the limited efficacy of T treatment [181], raise fundamental questions over the use of T. However, as seen in chapter 8, the potentially adverse effects of T can be easily resolved by treatment withdrawal. Furthermore, due to their potent and highly specific anabolic effects on muscle [184, 185, 197, 198, 202], androgens remain the most promising class of anabolic endocrine drugs that are currently available. Selective Androgen Receptor Modulators (SARMs), designed to stimulate anabolic effects on muscle and bone while avoiding adverse effects on the prostate and other tissue, probably represent the future of androgen based therapies for chronic conditions [316, 317]. These compounds address both the safety and efficacy issues with conventional T treatment, as they may be safely used in more potent pharmacological doses [316].
high specificity of these drugs extends their potential utility to additional populations. Most relevantly these non virilising androgens may be used as an anabolic therapy in frail older women.

Future studies may focus on additional therapies alongside androgens for treating frailty. In chapter 7, multiple hormonal deficiency was related to frailty, similar results have been seen in older women [235]. This suggests the use of combined hormone replacement may be effective in treating frailty. This would have the added advantage of allowing lower doses of each hormone, reducing any safety concerns. Combining androgens with other anabolic drugs such as growth hormone secretagogues [156, 157] and myostatin antagonists [318] may produce greater anabolic effects than could be achieved with safe doses of any drug alone. It is also important to consider non pharmacological therapies including dietary intervention and particularly resistance training. Progressive resistance training programs are highly effective in improving strength and function in physically frail elders [303, 319]. In addition to muscle hypertrophy, strength increases from resistance training occur due to a number of neural mechanisms [320], while gains in strength from androgen treatments can be attributed entirely to increases in fibre size [198]. These different mechanisms suggest possible synergy in combining resistance training with an anabolic drug, like T for increasing strength and function in frail patients. The use of anabolic drugs may also compensate for the blunted hypertrophic response to training seen particularly in older women [321]. One aim for a future trial may be to test whether gains in strength from androgen treatment may be maintained post treatment through the maintenance of a concurrent resistance training program.
The major general limitation of the work contained in this thesis concerns how generalisable the findings are. The relationships seen between changes in male sex hormones and frailty cannot be generalised to women. However, in the broader sense the finding that frailty relates to changes in biological systems may inform future studies in women. Furthermore the development of tissue selective androgens [316], means that data on T treatment may become relevant to managing frailty in both genders. Compared to women, frailty has been relatively understudied in men, these studies therefore begin to address a notable gap in the literature.

The EMAS includes men aged 40-79, as frailty primarily effects the oldest age groups [4], this is probably slightly younger than the ideal age range for studying frailty. The low prevalence of frailty in this younger population will have limited the power to detect associations in these studies. However, as it is believed the determinants of frailty reach back to at least late middle age [247], there may be some value in studying frailty in younger populations. The study includes a volunteer sample, the comparison with non responders suggests the men who participated were relatively healthier than the general population [246]. It is likely the results presented underestimate the prevalence of frailty in European men of this age range and possibly the strength of the hormonal relationships. Finally, the EMAS like all observational cohort studies used so far in frailty research was not designed to study frailty. While much progress has been made using these existing datasets, studies focussed specifically on frailty may be a necessary next step. The first of these studies have already been designed and begun recruiting [322, 323].

An overall limitation of both the studies used in this thesis, was the requirement for participants to attend study sessions at the research centres. This means some of the
frailest patients, those unable to attend the centres will not have been included. It may be that future frailty research takes place increasingly in the patients homes [323].

In summary, the studies presented here lend some support to the overall hypothesis that changes in hormone levels are important to the development of frailty in men. However, it is clear that in addition to hormones frailty is related to many other biological factors. Furthermore frailty is importantly related to many non biological factors, including psychological, behavioural and sociological influences. It is likely all of these factors need to be addressed in order to fully understand and optimally manage this complex condition.
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