Investigating the biological and clinical characterisation of sarcopenia and anorexia in patients with cancer

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List of abbreviations

1RM	1 Repetition Max
5-FU	5 Fluorouracil
A	Anorectic
ABC	Advanced Breast Cancer
ACE score	Adult Comorbidity Evaluation Score
ACE	Angiotensin Converting Enzyme
ActA	Activin A
AgRP	Agouti-Related Peptide
AIIR	Activin II Receptor
AIIRB	Angiotensin II Receptor Blocker
AKT	Protein Kinase B
ALL	Acute Lymphoblastic Leukaemia
ALM	Appendicular Lean Mass
ALP	Alkaline Phosphatase
ANCHOR	ANorexia in Cancer patients: assessment of the gut HORmone and cytokine profile and body composition, and the impact of dietetic support in patients with gastrointestinal cancer study
ARC	Arcuate Nucleus
AST	Aspartate Transferase
AUC	Area Under the Curve
BCM	Body Cell Mass
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
BSA	Body Surface Area
C	Cycle
Ca	Calcium
CARG	Cancer And Aging Research Group
CART	Cocaine And Amphetamine Regulated Transcript
CCK	Cholecystokinin
CFS	Clinical Frailty Scale
CGA	Comprehensive Geriatric Assessment
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease

CM	Centimetres
CNS	Central Nervous System
CRASH	Chemotherapy Risk Assessment Scale for High-Age Patients
CRP	C-Reactive Protein
CT	Computer Tomography
CVO	Circumventricular Organs
DCR	Disease Control Rate
DFS	Disease Free Survival
DHEA	Dehydroepiandrosterone
DLT	Dose Limiting Toxicity
DMN	Dorsomedial
DMT	Dose Modifying Toxicity
DPYD	Dihydropyrimidine Dehydrogenase
DXA	Dual X-Ray Absorptiometry
EBC	Early Breast Cancer
EEC	Enteroendocrine Cells
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EORTC	European Organisation for Research and Treatment of Cancer
EPA	Eicosapentaenoic Acid
ESPEN-SIG	European Society for Clinical Nutrition and Metabolism Special Interest Groups
ESMO	European Society for Medical Oncology
FDA	Food And Drug Administration
EWGSOP	European Working Group on Sarcopenia in Older People
F	Female
FAACT/ACS	Functional Assessment of Anorexia/Cachexia Therapy Anorexia Cachexia Subscale
FFM	Fat Free Mass
G	Grams
GDF	Growth-Differentiation Factor
GGT	Gamma-Glutamyl Transferase
GI	Gastrointestinal
GIP	Gastric Inhibitory Peptide

GLP-1	Glucagon Like Peptide-1
GOJ	Gastro-Oesophageal Junction
Hb	Haemoglobin
HbAc1	Glycated Haemoglobin
HER-2	Human Epidermal Growth Factor Receptor 2
HIV	Human Immunodeficiency Virus
HGS	Hand Grip Strength
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
HU	Hounsfield Units
HV	Healthy Volunteer
ICV	Intra-cerebroventricular
IL-1	Interleukin 1
IL-6	Interleukin 6
IM	Intramuscular
IWGS	International Working Group on Sarcopenia
K	Potassium
Kg	Kilograms
L	Litre
L3	3rd Lumbar vertebra
LBM	Lean Body Mass
LDH	Lactate Dehydrogenase
LIF	Leukaemia Inhibitory Factor
LPS	Lipopolysaccharide
M	Male
Mg	Magnesium
MM	Muscle Mass
MRI	Magnetic Resonance Imaging
MTOR	Mammalian Target Of Rapamycin
MUAC	Mid-Upper Arm Circumference
Na	Sodium
NA	Not available/applicable or Non-Anorectic
NLR	Neutrophil: Lymphocyte Ratio
NPY	Neuropeptide Y

NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NSCLC	Non-Small Cell Lung Cancer
OG	Oesophagogastric
OS	Overall Survival
PD	Progressive Disease
PFS	Progression Free Survival
Ph	Phosphate
PIF	Proteolysis Inducing Factor
POMC	Proopiomelanocortin
PP	Pancreatic Polypeptide
PPI	Proton Pump Inhibitor
PR	Partial Response
PS	Performance Status
PSN	Peripheral Sensory Neuropathy
PUFAs	Polyunsaturated Fatty Acids
PVN	Paraventricular Nucleus
PYY	Peptide YY
QLQ-C30	Quality Of Life Questionnaire C30
RNA	Ribonucleic Acid
RR	Relative Risk
S	Seconds
SD	Stable Disease
SMA	Skeletal Muscle Area
SMD	Skeletal Muscle Density
SMI	Skeletal Muscle Index
SPPB	Short Physical Performance Battery
STS	Sit-To-Stand test
T 1,2,3	Type 1,2,3
TBK	Total Body Potassium
TGF-β	Transforming Growth Factor B
TNF- α	Tumour Necrosis Factor Alpha
TSH	Thyroid Stimulating Hormone
UGI	Upper GI
US	United States of America

UK	United Kingdom
VMN	Ventromedial Nucleus
VTE	Venous Thromboembolism
α-MSH	Alpha-Melanocyte Stimulating Hormone

Abstract

Advanced oesphagogastric (OG) tract cancers are commonly associated with appetite loss (anorexia), muscle loss (sarcopenia) and malnutrition. Outcomes for advanced disease are poor with average survival under 1 year and anorexia, sarcopenia and malnutrition are individually associated with poor prognosis. Sarcopenia is common in frail patients but it's correlation with frailty scores is not fully understood. Furthermore, the underlying pathophysiology of anorexia and sarcopenia are incompletely understood.

The aim of this work was to deeply characterise the prevalence and patterns of anorexia and sarcopenia in patients with advanced OG cancers and investigate a potential mechanism for anorexia through an assessment of gut hormones.

Anorexia is highly prevalent (63%) and trends towards an association with poorer survival but not treatment toxicity. Weight loss was significant at baseline and was associated with survival at more severe levels. Levels of the anorexigenic gut hormone PYY were raised in patients with cancer anorexia compared to those with no anorexia and this may suggest a potential mechanism for this symptom. Insulin, GIP and GLP-1 responses were blunted in anorexic patients, but this did not produce a raised glucose level and the cause for this is unclear.

Sarcopenia was also highly prevalent, but did not correlate with frailty scores, survival, or treatment toxicity.

A scoping review of medications trialled for sarcopenia identified positive results for androgens, growth hormone and newer agents targeting muscle catabolism. However, gains are frequently small and not associated with an increase in function. This raises the question of what would be considered a meaningful outcome for patients with advanced cancer.

This work provides a foundation for extended research into nutritional symptoms in patients with advanced OG cancers. Careful patient characterisation is important in researching these patients to allow for individualised treatment plans.

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1.1 Introduction

It is well recognised that poor nutritional and muscle mass status are associated with poor outcomes for patients with advanced cancer, both as independent symptoms and within the syndrome of cachexia. This is both in terms of cancer survival and treatment toxicity.

Currently, treatment options for managing anorexia are limited. It is known that it can progress to malnutrition and cachexia. Sarcopenia has become an area of much cancer research over the last decade. However, in order to become a biomarker with practical utility for patients with advanced cancer, it needs to able to be used to either a) guide treatment decisions, that is, whether or not to treat b) help guide the amount of treatment; modified choice of treatment or dose reductions or c) identify patients who need an intervention to improve their muscle mass. Otherwise, it is not inherently more useful than existing, more subjective measures of patient assessment, such as performance status (PS).

The aim of this work is to provide a deep characterisation of anorexia and sarcopenia in patients with advanced cancer of the oesphagogastric (OG) tract, where both symptoms are particularly prevalent. In addition, I aim to complete a detailed scoping review of the literature surrounding pharmaceutical agents trialled to treat sarcopenia. This work has a longer-term view (outside the scope of this thesis) of identifying further areas of investigation and potentially treatment for these debilitating symptoms to improve outcomes for patients.

In this introduction I will discuss the existing knowledge and areas of uncertainty around the pathophysiology of anorexia in patients with advanced cancer and the current issues around the use of sarcopenia as a prognostic and predictive marker.

The focus of my work is specifically on sarcopenia, and anorexia but because of these symptoms overlap with the syndromes of frailty and cachexia some of the data on these topics will be included within this introduction.

1.1.1 Cancer

It is estimated that there were 19.3 million new cases of cancer worldwide in 2020, and 9.9 million deaths [1].

It has long been recognised that weight loss associated with cancer is associated with poor outcomes [2], and it is often quoted that 20% of cancer deaths are due to the syndrome of cachexia [3] though this statistic is hard to verify.

1.1.2 Oesophagogastric tract cancers

Cancers of the oesphagogastric tract include cancers of the upper oesophagus, through to the stomach. Most commonly they are squamous cell carcinomas or adenocarcinomas, with lower rates of other histological subtypes including neuroendocrine tumours. Internationally oesophageal and gastric cancers are the 10th and 6th most common cancers respectively [4]. There is significant international variation, with a rate of 32.5/100,000 in eastern Asia, compared to 8.2/100,000 in western Europe for stomach cancer [4]. In the UK oesophageal and stomach cancers represent the 14th and 17th most common cancers, and the 7th and 14th most common cause of cancer mortality [5]. Rates of squamous cell cancer have fallen over the years, predominantly thought to be related to reduced rates of smoking, whereas rates of oesophageal adenocarcinoma have increased [6]. Survival is poor, particularly for advanced disease, with only 21% of patients with advanced disease living for 1 year from diagnosis [5].

Common symptoms at presentation include dysphagia (difficulty swallowing), odynophagia (pain on swallowing), anorexia, gastro-oesophageal reflux, anaemia, and weight loss. Delayed gastric motility may also contribute to symptoms, either due to direct obstruction from a pyloric tumour, or other causes [7]. It is unknown what proportion of appetite loss is related to dysphagia and delayed gastric emptying.

Stenting is sometimes used to help symptoms of dysphagia. Stents are often successful, but can also cause pain, increased symptoms of gastro-oesophageal reflux and be associated with risks including migration and rarely perforation [8].

Palliative chemotherapy is the standard of care treatment for patients with upper GI cancers not amenable to surgery or chemoradiotherapy. The standard of care internationally for adenocarcinomas and squamous cell carcinomas is a combination of platinum and fluorouracil chemotherapy, with some variation as to exact regimen. Initially trialed as triplet regimens [9], there has been a move towards doublet regimens as these are associated with lower toxicity but without reductions in survival [10]. Our institution gives capecitabine and oxaliplatin given as 6 x 21-day cycles and followed by surveillance. Patients with over-expression of human epidermal growth factor receptor-2 (HER-2) may be treated with the combination of cisplatin, capecitabine and trastuzumab as 6 x 21-day cycles, which showed an increased overall survival (OS) benefit of 13.8 months [11] compared to 11.1 months for chemotherapy alone. Trastuzumab is then given in maintenance. More recently trials showing benefit for the addition of the immunotherapy agents pembrolizumab [12] to chemotherapy for oesophageal and gastro-oesophageal junctional tumours and nivolumab [13] for gastric tumours have been published, and seen these combinations move into clinical practice.

1.1.3 Anorexia and sarcopenia

Anorexia, usually defined simply as “loss of appetite”, is a common symptom in patients with advanced cancer [14]. Furthermore, it is a highly distressing symptom. Eating is an important part of daily life, and social activities, therefore, an inability to eat may be highly isolating for patients [15].

The exact prevalence of cancer anorexia has been estimated to be as high as 61% of patients [14], with significant variation between different primary sites of disease and stage of disease at presentation.

Yet, despite many years of study, the pathophysiology of cancer anorexia remains incompletely understood. This is in part because the physiology of appetite remains incompletely understood, but also because the overlapping positions of anorexia and cachexia syndrome have complicated investigation. In this work I will focus on the biochemical mechanisms for control of appetite, but it cannot be ignored that psychological, societal, and cultural factors impact on eating behaviours as well.

Sarcopenia is defined as the “progressive and generalised loss of skeletal muscle mass and strength, with a risk of adverse outcomes such as physical disability, poor quality of life and death” [16]. Sarcopenia has become an increasing area of interest in oncology research over the last decade. A PubMed search for the terms “cancer” and “sarcopenia” in 2022 shows 706 results published in 2021, compared with 40 published a decade earlier in 2011. Because sarcopenia may present as part of the syndrome of cachexia, and it has been incorporated into definitions of malnutrition and cachexia [17, 18].

Sarcopenia can result from acute or chronic illnesses but is also part of the process of aging. Throughout adult life muscle mass remains relatively stable unless subject to other stimuli, such as exercise or illness. However, from around the 5th decade of life total muscle mass decreases by around 1-2% per year [19, 20]. This is associated with a relative increase in the proportion of body fat [21], and so the reduced muscle mass presents without loss of overall body mass. It may be considered as similar to bone density, which gradually decreases with aging (osteopenia), and upon reaching a critical threshold, becomes a pathological condition (osteoporosis) with negative health effects. In the case of sarcopenia, frailty is the pathological endpoint. Indeed these two processes have been shown to be closely correlated [22]. Sarcopenia may be considered primary, part of aging, or secondary; as a result of other factors such as disuse or disease. Primary and secondary sarcopenia may overlap. In both primary and secondary sarcopenia there is an imbalance between muscle anabolism and catabolism. In secondary sarcopenia accelerated muscle wasting is seen. Both within context of cancer cachexia and independently, sarcopenia has been demonstrated to prognostic of both survival and toxicity in a range of disease types and stages and type of treatment, including upper and lower gastrointestinal (GI) malignancies, breast, lung cancer and others [23-27].

1.1.4 Cancer Cachexia

Cachexia has previously been defined as “a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment” [28]. It has more recently been defined as “a disease-related subtype of malnutrition identified by malnutrition screening, at least one phenotypical criterion and

systemic inflammation" [29]. Systemic inflammation may be measured using markers such as CRP or neutrophil: lymphocyte ratio (NLR). NLR has been demonstrated to be associated with weight loss and cachexia in a number of cancer types [30]. A high NLR has been demonstrated to be an independent poor prognostic biomarker in advanced OG cancer [31].

In the state of cachexia there is rapid muscle wasting. It has been suggested that in some patients with cancer, sarcopenia may represent a "pre-cachexia" state [17]. Cancer cachexia is common in the last months of life but may be present much earlier in a patient's cancer journey.

Cachexia is well recognised to be a negative prognostic sign and is widely reported to account for 10-20% of cancer deaths [32]. It should be noted that this figure is quoted from a 1932 article [33] and more recent data on the prevalence of this condition is difficult to locate. A 1975 retrospective analysis found the rate of death predominantly due to cancer cachexia, as opposed to more direct cancer-induced organ failure, of 1% [34]. A systematic review found rates of >5% weight loss in 35% of patients with cancer in the UK and Ireland [35]. The uncertainty around rates of cachexia is partly because definitions of cancer cachexia have varied throughout the years. This heterogeneity of definition may also have contributed to the lack of success in identifying successful treatments for cachexia, as heterogenous populations have been studied. To date there are no treatments that can reverse cancer cachexia, with the exception of successful treatment of the cancer itself. However, the ability to deliver anti-cancer therapy may be limited by cachexia, or effective treatments may be lacking.

Furthermore, the pathophysiology underlying the individual independent components of cachexia (anorexia and sarcopenia), and the correlation between these physiological effects and clinical phenotypes and outcomes remain incompletely understood.

The relationship between sarcopenia, anorexia and cachexia is shown in figure 1.

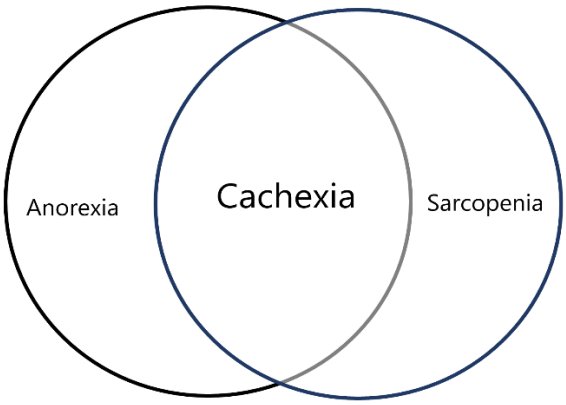

Figure 1.1: The intersecting relationship between anorexia, sarcopenia, and cachexia

1.1.5 The scale of the issue; anorexia

Anorexia may be experienced as lack of hunger, early satiety, aversion to food or lack of enjoyment of food. The exact prevalence of the symptom of anorexia remains unclear because anorexia may precede more objective measures of malnutrition such as significant weight loss, which is more widely reported. However, some degree of alteration in appetite was reported in 61% of a study of 128 patients with advanced cancer [14], with other studies reporting the prevalence of anorexia in patients with advanced cancer of 40% and 48% [36, 37]. Until the development symptom scales such as of the Functional Assessment of Anorexia/Cachexia Therapy Anorexia Cachexia subscale (FAACT A/CS) [38], shown in table 1.1 or the European Organisation for Research and Treatment of Cancer (EORTC) OG-25 shown in table 1.2, appetite was more commonly measured using simple visual analogue scales. Only the study reporting anorexia rates of 40% used the FAACT A/CS scale to assess patients and this included patients with heterogenous cancer types, with the others using self-reported symptoms.

Data about rates of anorexia in advanced OG cancer are limited. A study of patients with a mix of both localised and advanced OG cancers reported significant anorexia rates of 64% in a cohort of 152 patients [39].

Table 1.1: FAACT/ACS scale					
	Not at all	A little bit	Some-what	Quite a bit	Very much
I have a good appetite	0	1	2	3	4
The amount I eat is sufficient to meet my needs	0	1	2	3	4
I am worried about my weight	4	3	2	1	0
Most food tastes unpleasant to me	4	3	2	1	0
I am concerned about how thin I look	4	3	2	1	0
My interest in food drops as soon as I try to eat	4	3	2	1	0
I have difficulty eating rich or “heavy” foods	4	3	2	1	0
My family or friends are pressuring me to eat	4	3	2	1	0
I have been vomiting	4	3	2	1	0
When I eat, I seem to get full quickly	4	3	2	1	0
I have pain in my stomach area	4	3	2	1	0
My general health is improving	0	1	2	3	4

Malnutrition in cancer patients has been reported in a range of studies with a prevalence of anywhere between 19-71% of patients [32], a widely reported study by Dewys et al. reported a prevalence of 50% weight loss [2]. Rates of malnutrition are highest amongst patients with tumours of the upper gastro-intestinal tract. Amongst patients with gastro-oesophageal and pancreas cancer 25% of patients present with overt malnutrition at their first oncology appointment [36].

Table 1.2: EORTC OG-25 symptom scale					
	During the past week:	Not at all	A Little	Quite a bit	Very much
1	Have you had problems eating solid foods?	1	2	3	4
2	Have you had problems eating liquidised or soft foods?	1	2	3	4
3	Have you had problems drinking liquids?	1	2	3	4
4	Have you had trouble enjoying your meals?	1	2	3	4
5	Have you felt full up too quickly after beginning to eat?	1	2	3	4
6	Has it taken you a long time to complete your meals?	1	2	3	4
7	Have you had difficulty eating?	1	2	3	4
8	Have you had acid indigestion or heartburn?	1	2	3	4
9	Has acid or bile coming into your mouth been a problem?	1	2	3	4
10	Have you had discomfort when eating?	1	2	3	4
11	Have you had pain when you eat?	1	2	3	4
12	Have you had pain in your stomach area?	1	2	3	4
13	Have you had discomfort in your stomach area?	1	2	3	4
14	Have you been thinking about your illness?	1	2	3	4
15	Have you worried about your health in the future?	1	2	3	4
16	Have you had trouble with eating in front of other people?	1	2	3	4
17	Have you had a dry mouth?	1	2	3	4
18	Have you had problems with your sense of taste?	1	2	3	4
19	Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
20	Have you had difficulty swallowing your saliva?	1	2	3	4
21	Have you choked when swallowing?	1	2	3	4
22	Have you coughed?	1	2	3	4
23	Have you had difficulty talking?	1	2	3	4
24	Have you worried about your weight being too low?	1	2	3	4
25	Answer this question only if you lost any hair: If so, were you upset by the loss of your hair?	1	2	3	4

1.1.6 The scale of the issue; sarcopenia

A cross-sectional study of adults in the UK identified female sex, older age, lower educational level, higher deprivation, being underweight and chronic diseases as being associated with a higher likelihood of sarcopenia [40]. In combination with frailty, it is associated with increased rates of mortality from cardiovascular and respiratory diseases, cancer, and all-cause mortality [41]. In this large cross-sectional study sarcopenia was present in some patients without frailty, but no-one had frailty or pre-frailty without co-existent sarcopenia [40]. The prevalence of sarcopenia varies, in part because there is no single definition, or method of assessment used for it. When assessing the same population using different criteria sarcopenia may be present in between 0-15% of healthy older people [42].

Much research has been done into the implications and treatment of sarcopenia as part of geriatric frailty syndromes, however more recently it has become a particular area of interest for oncology studies. An area of particular concern is sarcopenic obesity, where patients who are overweight or obese will not be recognised as sarcopenic yet may have significantly reduced muscle mass underlying their total body mass on investigation [43]. These patients may not, therefore, be identified as at increased risk. Due to relative increased fat mass to total body mass these patients may also be at higher risk of toxicity due to altered pharmacokinetics or associated co-morbidity.

1.2.1 Biochemical control of normal appetite

Current evidence suggests that the biochemical control of appetite is a complex neurochemical balance between central and peripheral neurones, neuropeptides, and hormones. The aim of this system is to maintain a balance between energy intake and expenditure.

1.2.2 Central nervous system signalling

The main appetite centre within the brain is the arcuate nucleus of the hypothalamus (ARC) where neurones integrate hormonal and metabolic signals and transmit these deeper into the brain. The ARC lies close to an area of the brain with an incomplete blood brain barrier, the circumventricular organs (CVO) [44] making it ideally located for sensing peripheral hormonal and nutrient signals. Neurones within the ARC form and release the neuropeptides neuropeptide Y (NPY) and Agouti-related peptide (AgRP) which have an appetite stimulating roles [45]. These peptides are active in the paraventricular (PVN), dorsomedial (DMN) and ventromedial (VMN) nuclei and perifornical area. It is known that NPY has an appetitive (meal finding) action, and AgRP a consummative one [45, 46]. Once activated, NPY and AgRP have an inhibitory effect on two other populations of neurones which release the anorexigenic neuropeptides proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART). Thus, in the fasted state expression of POMC is low and expression of NPY and AgRP is high [47, 48]. Neurones expressing POMC and CART are distributed along similar pathways to NPY and AgRP [49], although the exact signalling pathways for CART are not fully understood.

The POMC, CART, NPY and AgRP neurones express receptors for, and have their actions mediated by, hormones within the enteroendocrine (EEC) system including cholecystokinin (CCK), pancreatic polypeptide (PP), gastric inhibitory peptide (GIP) glucagon-like peptide-1 (GLP-1) and oxyntomodulin, peptide YY (PYY), ghrelin, leptin, insulin and glucagon [50] which are released from enteroendocrine and pancreatic endocrine cells within the GI tract. They are also influenced by circulating levels of glucose [51, 52], free fatty acids [53], and possibly by circulating amino acids [54-56]. They are also influenced by signals received from stretch receptors in the stomach as well as other signals of current metabolic requirements as discussed in more detail below.

Downstream from POMC, NPY and AgRP neurones, the melanocortin system has a significant impact on control of appetite. The melanocortin-3 receptor (MC3R) and melanocortin-4 receptor (MC4R) are expressed in AgRP and POMC neurones [57]. It has been demonstrated that MC4R agonists decrease food intake, and antagonists increase it [58]. Once activated to stimulate hunger, NPY and AgRP in the ARC block the MC3R and MC4R, AgRP is a potent MC4R antagonist [45]. Genetic changes leading to loss or inactivation of the MC4R have been shown to be related to obesity [59], however evidence suggests that hunger signals mediated by AgRP remain intact in this situation [46], suggesting that the mechanism by which AgRP drives feeding is not only by antagonising satiety signals. Activated POMC neurones release proopiomelanocortin, which cleaves to α-melanocyte stimulating hormone (α-MSH). Amongst other activities α-MSH activates the melanocortin receptor 4 (MC4R) and acts to suppress food intake.

NPY/AgRP neurones are downregulated by leptin and other signals of adiposity and nutritional intake, whereas POMC neurones are activated by them.

Signals are transmitted to the brain via the vagus nerve. Vagal afferents in the stomach and intestine have mechanoreceptors which detect distension, and also directly receive signals from gut hormones [60]. Activation of mechanoreceptors in the stomach has been shown to cause inhibition of AgRP neurones in the hypothalamus [60] thus decreasing appetite.

1.2.4 Peripheral hormonal mediators

Hormonal signals from the small intestine and pancreas, CCK, PP, GIP, GLP-1, PYY, ghrelin, and glucagon have short-term impacts on appetite, in response to food intake. However, their role in physiological control of normal eating has been challenged: it is likely that the hedonic and reward aspects of eating over-ride these signals in health [61]. Longer term control is regulated by leptin, released from adipocytes, and insulin. Ghrelin is the only known orexigenic hormone, with the others all having an anorexigenic effect.

In the fasting state levels of CCK, PP, PYY, GLP-1 and oxyntomodulin are low and levels of ghrelin are raised. Ghrelin is released from enteroendocrine cells in the stomach in response to an empty stomach, it signals via the vagus nerve to the brain [62] and stimulates appetite via AgRP neurones [63], it also promotes gastric secretions and gut motility.

The initial feedback of satiety signals comes from gastric distension acting upon mechanoreceptors which signal via vagal afferents. This alone can cause the termination of a meal, but after a larger volume is ingested than would otherwise be taken [64]. However it is also known that some of a meal enters the small intestine before it's completion [65], triggering the release of hormones and a negative feedback mechanism is initiated to slow gastric emptying and reduce pancreatic and gut enzyme production to allow further digestion [66].

Levels of CCK, PP, GLP-1 and PYY rise as they are released from enteroendocrine cells in the intestine [67-71]. Details about the release and effect of these hormones are shown in table 1.3a and 1.3b.

Gut hormones act upon neurones within the ARC but also in reward centres to generate feelings of satiety and reduce appetite. Additional actions of CCK include gallbladder contraction and inhibition of gastric emptying [69]. GIP induces gallbladder contraction and induces insulin secretion [72] and GLP-1 also inhibits gastric emptying [70] and causes secretion of insulin as well as contributing to central glucose homeostasis [73]. Oxyntomodulin inhibits pancreatic enzyme and gastric acid secretion [74, 75].

Pancreatic polypeptide also induces pancreatic enzyme secretion [76] and delays gastric emptying [77].

In animal and human models infusions of CCK[78, 79], PP [67], PYY agonists[80], GLP-1[70, 81], oxyntomodulin[82, 83] and glucagon[84] have been shown to reduce food intake, or increase satiety. However, most of these studies used supraphysiological doses.

Table 1.3a: appetite modulating hormones

Hormone	Site of production	Time of release following a meal	Effect on appetite	Other effects
Cholecysto-kinin	I cells, duodenum and jejunum	Early release 0-15 minutes after meal plateau at 1-2h	Anorexigenic	Stimulates gallbladder contraction and inhibition of gastric emptying
Pancreatic Polypeptide	Pancreatic islet cells	Levels rise early following meal to peak at 20-50 minutes	Anorexigenic	Pancreatic hormone release
Peptide YY	Distal small intestine L cells	Levels rise to peak 1-2h after meal then plateau	Anorexigenic	Increases ileal absorption, gallbladder and pancreatic secretion. Inhibits gastric emptying
Gastric inhibitory peptide	K cells in duodenum and jejunum	Rapidly rise to peak 15-30 minutes after a meal, plateau, then fall after 90 minutes	Anorexigenic	Stimulate gallbladder contraction
Glucagon-like peptide 1	Distal small intestine L cells L cells	Rise to peak 40-80 minutes after meal then slowly fall	Anorexigenic	Increases insulin secretion, decreases glucagon, inhibits gastric emptying

Table 1.3b: appetite modulating hormones continued				
Hormone	**Site of production**	**Time of release following a meal**	**Effect on appetite**	**Other effects**
Oxynto-modulin	Distal small intestine L cells	Released 5-10 minutes after a meal. Peak levels at 30 minutes	Anorexigenic	inhibits pancreatic enzyme and gastric acid secretion
Leptin	Adipocytes and gastric chief and P cells	Diurnal release, levels may rise and peak 5-10h after over-feeding	Anorexigenic	Enhances release of other gut hormones including GLP-1 and CCK
Insulin	Pancreatic islet cells	Levels rise to peak around 1.5h after a meal	Anorexigenic	Stimulates uptake and storage of circulating glucose. Enhances release of anorexigenic gut hormones
Glucagon	Pancreatic islet cells	Levels fall within 30 minutes of hyperglycaemia	Anorexigenic	Stimulates breakdown of glycogen to increase circulating glucose and gluconeogenesis
Ghrelin	Gastric EEC cells	Falls within 30 minutes after a meal	Orexigenic	Growth hormone secretion, increased gastric secretion and motility

Insulin is released from the pancreas in response to food-intake to stimulate uptake and storage of circulating glucose. It also has an inhibitory effect on NPY neurones and data from animal models shows an anorexigenic effect [85, 86]. There is some evidence that the opposing catabolic hormone, glucagon, may increase satiety and reduce food intake in humans [87] and animal models [84], via a centrally mediated suppression of ghrelin

levels, however whether this is a direct effect of glucagon, or as a result of increased circulating glucose levels is not clear.

Leptin is released from fat cells and acts centrally to inhibit NPY and AgRP neurones [48] and stimulates POMC and CART neurones [88]. In obese individuals leptin levels are high, yet appetite is not reduced [89], possibly suggesting a degree of leptin resistance similar to the recognised phenomenon of insulin resistance. Leptin and insulin exert longer term control of appetite by potentiating the effect of other anorexigenic hormones. Receptors for leptin and insulin are found on intestinal L cells and increase the secretion of GLP-1 [90] and it has been demonstrated that leptin and CCK co-stimulate vagal neurones [91].

Leptin, insulin, GLP-1, PYY and ghrelin have all been identified in saliva [92], suggesting they may be able to modulate taste and smell receptors which could also have an impact on appetite and food intake. The interplay between hormonal and neuronal signals is shown in figure 1.2.

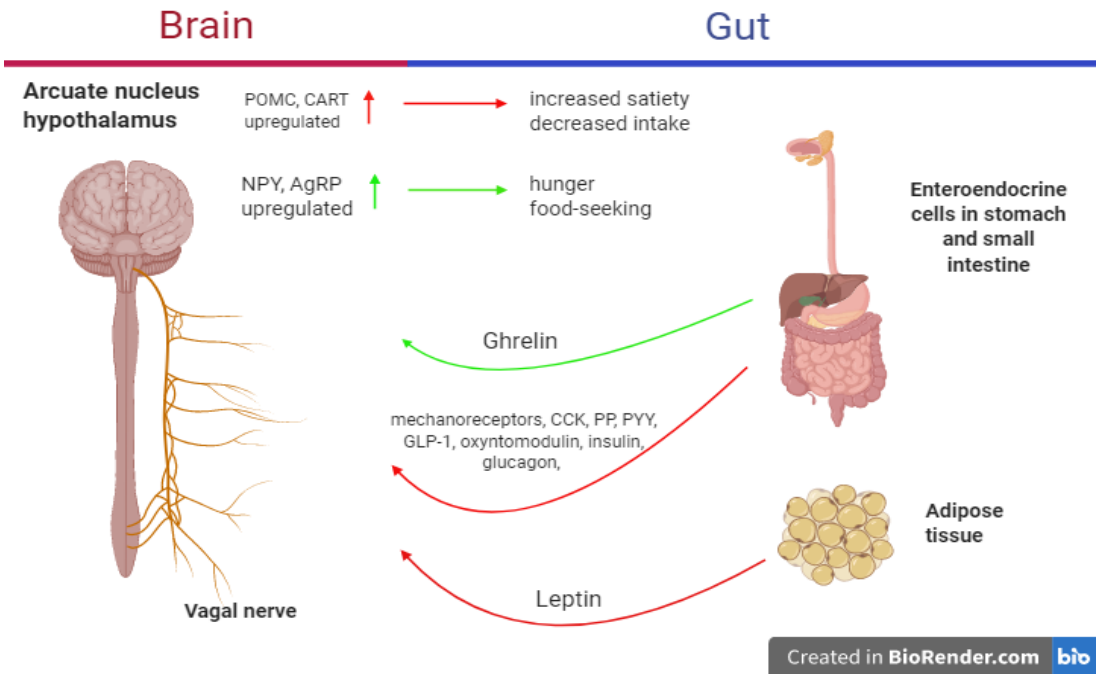

Figure 1.2: Enteroendocrine control of appetite.
Red = satiety signals, green = hunger signals. CCK = cholecystokinin, PP = pancreatic polypeptide, PYY = Peptide YY, GLP-1= glucagon-like peptide-1. NPY = neuropeptide Y, AgRP = Agouti related Protein, POMC = pro-opiomelanocortin, CART = cocaine amphetamine regulated transcript

1.2.5 Other hormonal mediators

Glucocorticoids have long been recognised to have an effect on appetite and food intake. High levels of endogenous glucocorticoids due to pituitary adenomas being associated with obesity were described in the early 20th century by Cushing (for whom the disease was named) [93]. It has been demonstrated in animals that adrenalectomy reduces expression of both POMC and AgRP neurones and food intake, with food intake gradually increasing with exogenous glucocorticoid replacement [94]. In humans it is well recognised that chronic use of exogenous steroids can lead to weight gain. Even a short course of steroids can increase food intake [95, 96] and corticosteroids are one of the only medications shown to improve appetite in cancer anorexia [97]. However, the exact mechanism of this effect remains unclear. It has been demonstrated that giving steroids increases circulating leptin levels [98-100] and one theory is that glucocorticoids may contribute to leptin resistance.

1.2.6 Nutrient mediators

The effect of nutrients on appetite has been demonstrated in both human and animal models. Intravenous infusion of 2-deoxy-D-glucose, which competitively inhibits glycolysis, caused increased food intake in humans [101], whilst infusing glucose increased satiety [102]. It appears that glucose may impact hunger by increased or decreased NPY and AgRP expression [51].

Infusion of fatty acids reduced food intake in baboons [53], and infusion of some amino acids reduced food intake in rats [56], possibly via action on vagal afferents [55].

External to these biochemical changes, appetite is influenced by multiple other psychological, physical, and environmental factors. Animal models suggest that these higher level functions are separate from the ones detailed above, in that animals who have had surgical disconnection between hind and forebrain still demonstrate the same satiety response [103].

The increasing prevalence of obesity is, however, strong evidence that these physiological mechanisms are readily overridden with over-consumption resulting to an overweight state: this is equally true in animal models. This makes more sense from an evolutionary perspective, suppressing food intake after light consumption makes little

sense except through a contemporary human prism. The role of these pathways in disease-related reductions in nutritional status is a growing area of interest.

1.3.1 Cancer anorexia

The exact pathophysiology of cancer anorexia remains unclear. As with the control of appetite itself, it represents multiple complex, overlapping mechanisms.

It has long been recognised that chronic diseases, including advanced cancer, represent inflammatory states and that this is a significant contributor to the state of cachexia. However, anorexia exists in many cancer patients without the presence of cachexia and whilst there may be some overlap, it is likely that separate mechanisms exist.

1.3.2 Inflammatory mediators of cancer anorexia

Cytokines, circulating proteins with immunomodulatory properties, have been proposed as mediators of cancer anorexia. Multiple inflammatory cytokines have been reported to be raised in patients with newly diagnosed cancer [104, 105]. The CVO (the relatively permeable area of the blood-brain barrier near the hypothalamus), senses inflammatory mediators as well as detecting peripheral signals of nutritional state. The hypothalamus then upregulates the inflammatory response by production of inflammatory cytokines from glial cells [106].

Supporting evidence for cytokines as mediators of anorexia comes from animal models where it has been demonstrated that the inflammatory cytokines tumour necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) are induced in the hypothalamus of tumour bearing rodents [107]. Furthermore, infusions of TNF-α, IL-1 and another inflammatory cytokine interleukin-6 (IL-6) reduce food intake in rodents [108-116]. Treatment with a TNF-α, IL-1 or IL-6 antagonist was shown to partially reverse this effect [117-119]. In patients with cancer and cachexia it has been shown that they have higher levels of circulating TNF-α and IL-6 than non-cachectic patients [120-123], although data about the association of these cytokines with anorexia and weight loss are conflicting [120, 124]. It is important to note that cytokine pathways are overlapping, and giving infusions of one cytokine will induce others, so individual effects have to be interpreted with caution. To date, trials investigating single cytokine antagonists in humans have not shown significant effect as treatments for cancer cachexia [125, 126].

1.3.4 Central mechanisms of anorexia

Different effects of inflammatory cytokines upon anorexia have been proposed. It has been demonstrated that IL-1 receptors are found on POMC and CART neurones within the hypothalamus and intracerebroventricular (ICV) infusions of IL-1, or other pro-inflammatory mediators have been demonstrated to increase activity within these neurones [127, 128], increase release of α-MSH, and reduce the activity of AgRP neurones. The action of IL-1 on NPY neurones is less clear, with conflicting data about messenger ribonucleic acid (mRNA) expression (a marker of gene expression) following administration of IL-1. Chronic infusions of IL-1 have been demonstrated to reduce NPY mRNA expression in rodents [129], but this effect can be reversed by ICV infusion of NPY [112] and other studies have shown no effect [128] on NPY expression.

In tumour-bearing rats NPY and AgRP neurones are downregulated and release of the peptides is reduced [130], an effect which resolves following tumour resection [131]. Tumour-bearing rats also have a reduced response to ICV infusion of NPY [132] but did demonstrate increased food intake following ICV administration of AgRP [133].

Levels of circulating NPY were low in a study of patients with advanced cancer and anorexia, compared to non-anorectic patients [134]. Interestingly, megestrol acetate, one of the only treatments with efficacy for treating cancer anorexia, was shown to increase NPY levels within the ARC of rats [135]. These data suggest that reduced or altered NPY signalling, and upregulated POMC signalling within the hypothalamus plays a role in the development of cancer anorexia.

A further action of IL-1 may be to raise both peripheral and central levels of tryptophan, the pre-cursor molecular of the neurotransmitter serotonin [136]. Serotonin is known to reduce NPY signalling and increase melanocortin signalling [137]. In a study by Dwakarsing et al. [138] two populations of mice with tumour induced cachexia were studied, one group inoculated with lung cancer cells showed reduced food intake, whereas the other, inoculated with colorectal cancer cells compensated and maintained their weight. They showed no difference in NPY/AgRP gene expression but the group of mice with reduced food intake had higher levels of hypothalamic serotonin. Levels of tryptophan have been shown to be elevated both peripherally and centrally in tumour-

bearing rodents [114, 131] and serotonin has been demonstrated to have inhibitory effects on NPY neurones. Increased serotonin release, and 5HT2c receptor activation is seen with the cytotoxic drug cisplatin. This mechanism is thought to underly the well-recognised side effects of nausea, vomiting and anorexia seen with this drug. Cisplatin-associated nausea and vomiting are effectively treated with 5HT-3 receptor antagonists [139], but the symptom of anorexia may persist despite this. Serotonin appears to exert its anorexigenic effects via the MC4R [137] and it has been demonstrated that in tumour-bearing rats MC4R signalling is not inhibited by NPY or ghrelin as might be expected. Conversely, MC4R-knockout mice are resistant to tumour-induced anorexia [140], and IL-1 induced anorexia may be reversed with infusion of AgRP which is a potent MC4R antagonist [133].

Other immuno-modulatory mediators have been posited, including MyD88, TIR-domain-containing adapter-inducing interferon-β [141] and leukaemia inhibitory factor (LIF). MyD88 is a protein involved in inflammatory signalling in the IL-1 receptor family and it had been shown that in the absence of MyD88, mice were protected against anorexia induced by the inflammatory molecule lipopolysaccharide (LPS), though not weight loss [142, 143]. This effect has also been demonstrated in tumour-bearing mice [144]. In animal models LIF has been demonstrated to induce anorexia, it is upregulated in response to LPS and led to reduced feeding in sheep [145]. This effect was counteracted by administration of AgRP suggesting a possible action via the MC4R, which is also supported by data from mice models showing increased α-MSH release in response to LIF [146]. Plasma growth-differentiation factor-15 (GDF-15) is another inflammatory mediator subject to recent attention. Work in mice and primates shows that infusion of GDF-15 leads to reduction in food intake [147, 148], although other work suggests that GDF-15 mediated weight loss is related to increased lipolysis and functions independently of anorexia [149]. It was shown to be raised in patients with advanced cancer and weight loss [150].

1.3.6 Gut hormones in cancer anorexia

Evidence for the role of gut hormones in cancer anorexia is limited. However, there is evidence for them playing a role in other forms of gastrointestinal inflammation, therefore, a role in cancer-related anorexia is plausible.

The most studied hormones in this scenario are leptin (produced by adipose tissue) and ghrelin. However insufficient evidence exists to suggest they play a significant role. As might be expected in a low-adiposity state, it has been demonstrated that ghrelin levels are raised in tumour-bearing mice and rats [151-153] patients with lung cancer and cachexia [154], and in other cachectic diseases such as congestive cardiac failure [155]. However, a small study of children with acute lymphoblastic leukaemia (ALL) demonstrated low levels of ghrelin at diagnosis, compared with healthy controls. These fluctuated throughout chemotherapy treatment and settled at higher mean level, but this remained lower than those seen in controls [156]. In tumour-bearing mice administration of ghrelin did increase food intake, though not to levels seen in non-tumour bearing animals [151, 153]. Ghrelin levels were reduced by corticotropin-releasing factor in tumour-bearing rats [157]. Ghrelin is down-regulated by IL-1 [158], and this may have an effect of delayed gastric emptying.

Leptin levels have been demonstrated to rise in response to IL-1 and TNF-α [159, 160], but levels of leptin were low in tumour-bearing mice [153], and in one study started to drop before the development of anorexia [161]. Leptin levels lower than expected have been reported in studies of patients with advanced cancer [104, 105] and were not correlated with nutritional state. Another study showed no difference in leptin levels between anorectic and non-anorectic patients with advanced cancer [134], although the sample size in this study was small.

Functional magnetic resonance imaging (MRI) allows non-invasive assessment of human neuronal signalling by assessment of cerebral blood flow. A study by Molfino et al. [162] compared a group of patients with non-small cell lung cancer (NSCLC) and anorexia, a group with NSCLC and no anorexia, and a group of controls. Patients were identified using the FAACT A/CS score and submitted to an overnight fast before the study, which was performed at the same time for each patient. They underwent functional MRI imaging before and after a test meal of an Ensure supplement drink. Anorectic patients showed reduced hypothalamic activity compared to non-anorectic patients in response to food, but there were no significant differences in leptin, ghrelin, or cytokine levels between the two patient groups [162]. This would suggest that a central signalling abnormality, most likely an enhanced anorexigenic response in the

ARC is predominant in patients with anorexia, and not a peripheral signalling effect. However, other gut hormones than leptin and ghrelin were not assessed in this study.

Data on the other gut hormones in patients with cancer is limited. One study of patients with advanced cancer investigated CCK levels, and found no difference between anorectic and non-anorectic patients in circulating levels [134]. Some data support the role of PYY in cancer anorexia. In children with ALL and cancer anorexia PYY levels were raised at baseline compared to healthy controls and increased further in response to chemotherapy, before finally returning to baseline levels. However, another study demonstrated no differences in PYY levels between patients with cancer and cachexia, patients with cancer without cachexia, and a group of age, gender, body mass index (BMI) and race matched controls [163]. Other evidence supporting a potential role for PYY in reduced appetite comes from other diseases causing intestinal inflammation. A study of patients with Crohn's disease demonstrated increased PYY levels following an overnight fast compared with healthy controls, and these remained high after a test meal [164]. In this study GLP-1 levels were also investigated but showed no significant differences between groups. Elevated PYY levels have been reported as raised in patients with tropical sprue [165] and elevated CCK levels in Giardia enteritis [166]. In the study of Giardia enteritis CCK levels were associated with anorexia and once the disease was treated both CCK levels and appetite returned to normal levels. Patients with Crohn's disease experience inflammation of the small bowel, particularly the ileum which is the site of PYY-releasing L cells. Therefore, it is not clear whether direct inflammation contributes to the raised levels seen in these patients, or whether more systematic inflammation may also cause raised PYY levels. Animal models of gut inflammation have provided evidence that CCK is directly responsible for the anorexic effect; CCK-null mice displayed no anorexia despite significant gut inflammation [167], and the process was dependent on CD-4 T-cells [168]. Enhanced EEC function may therefore be an appropriate adaptive response and a component of the innate immune response to injury.

A study in mice investigated the impact of the cytotoxic chemotherapy drug 5-fluorouracil on the levels of PYY and GLP-1 in mice. In response to infusion of the drug the mice showed raised levels of GLP-1 and PYY compared to controls, and this was associated with reduced food intake and weight loss [169].

Recent work investigating the effect of the cytokine GDF-15 demonstrated that its' effect may be via neurones containing cholecystokinin [148], however little is known about the peripheral effect on this peptide.

1.3.7 Other contributors to cancer anorexia

Patients with tumours of the upper gastro-intestinal tract, particularly the stomach, frequently report symptoms of early satiety, this may be due to the reduced luminal space as a result of the tumour, delayed gastric emptying, or increased signalling via mechanoreceptors.

Another functional MRI study assessed responses of visual stimuli limbic areas in anorectic vs non-anorectic patients [170]. Patients without anorexia demonstrated response to unpleasant food stimuli, particularly, whereas patients with anorexia demonstrated no response at all. Patients with cancer anorexia often report a desire for food, but that when faced with it, they feel unable to eat it, which might suggest an increased unpleasant stimulus response, rather than a flattened one. A small study of patients with testicular cancer demonstrated that even before commencing chemotherapy patients had an altered smell threshold compared to controls [171], however none of these patients had anorexia at baseline. On commencing platinum-based chemotherapy they reported a transient reduction in taste and loss of appetite.

Eating-related distress is a frequently reported symptom [172], and anxiety and depression are frequently reported amongst patients with cancer [173]. Depression may have an impact on appetite in all patients and may be a contributing factor for some patients with cancer.

1.3.8 Gastrointestinal symptoms and nutritional status as a prognostic biomarker in patients with oesphagogastric cancers

There is limited data specifically about the impact of appetite loss on outcomes in patients with OG cancers. Studies have investigated the impact within context of quality-of-life scoring. A UK study prospectively investigating the impact of individual factors within the EORTC QLQ-C30 quality of life tool included 83 patients with advanced disease and 69 who underwent curative treatment [39]. They reported low rates of dysphagia within the cohort, but rates of anorexia score below 50 (suggesting

significant symptoms) were 64%. Multiple individual symptoms were associated with cancer-specific survival within this study, and appetite loss was strongly associated with poorer survival across the cohort [39]. A prospective study of 110 patients with oesophageal squamous cell carcinoma demonstrated that physical function and dysphagia were prognostic of survival [174]. Data taken from the Dutch cancer registry demonstrated that patient-reported symptoms of dysphagia, appetite loss and eating restrictions as measured by the EORTC QLQ-C30 were higher in patients with advanced disease (N=129) than those with potentially curable disease. Multiple symptom scales were associated with poorer survival in both curable and advanced disease including appetite loss (HR 1.08, p 0.01) [175]. An Italian study of 143 patients hospitalised for palliative management of Oesphagogastric cancer reported anorexia in 49% of patients using a 5-point symptom scale. Anorexia was strongly correlated with nausea, vomiting, dysphagia to liquids (but not solids), dysgeusia and was associated with higher weight loss (13.3kg vs 9.8kg in patients without anorexia). A retrospective UK study of 182 patients with OG cancers reported rates of anorexia of 69% and demonstrated a marked survival difference between patients with anorexia as measured by the FAACT C/S scale and those without [176]. Patients with a score of >37 had a median survival of 19.3 months, compared to 6.7 months for those with a score of ≤37.

A retrospective study of 388 patients with cancers throughout the GI tract, including oesophageal and stomach cancers, reported weight loss in 85% of patients [177]. The highest weight loss was seen in patients with stomach cancer, and the presence of 3 or more gastrointestinal symptoms was associated with increased weight loss. The presence of 3 or more GI symptoms was associated with poorer survival, 8.3 months compared to 19.5 months for those with no symptoms. Those with weight gain had longer survival than those with stable weight or weight loss. It should be noted that this is a heterogenous group and there are different treatments and median expected survival times across this disease sites.

Studies have demonstrated that poorer nutritional status in advanced OG cancers is associated with poorer quality of life [178, 179].

A prospective study of 116 patients with gastric cancer demonstrated that those with moderate-severe malnutrition had increased rates of both haematological and non-

haematological toxicity and poorer overall survival, 74 days for those with severe malnutrition, vs 237 days for those with no malnutrition [180]. Whilst another study of older patients with GI cancers, including just over 25% with stomach cancer reported that rates of malnutrition increased after one cycle of chemotherapy [181]. The negative impact of nutritional status on survival has also been shown in a large retrospective study of 1664 patients with metastatic gastric cancer [182] and other studies have demonstrated that ongoing weight loss during chemotherapy is associated with poorer survival in advanced OG cancer [183, 184].

1.3.9 Summary anorexia

In summary, cancer anorexia appears to be the result of altered signalling at NPY, AgRP, POMC and CART neurones, and altered serotonin levels within the hypothalamus. These effects are mediated by multiple inflammatory cytokines and the exact process underlying this remains incompletely understood. Changes in gut hormones in patients with cancer anorexia are incompletely understood. There does not appear to be a role for altered leptin signalling, and the role of ghrelin and other hormones is unclear. There is, however, evidence suggesting that CCK and PYY may have a role in anorexia in other inflammatory states.

Anorexia is common in upper GI cancers and associated with other symptoms, weight loss and poorer survival. Poorer nutritional status may be associated with increased treatment toxicity and poorer survival in advanced OG cancer.

1.4 Sarcopenia

1.4.1 Measures of body composition

Research into sarcopenia in patients with cancer has markedly increased since techniques were developed allowing muscle mass to be measured accurately on routine CT scans. Prior to this, other measures such as anthropomorphic measurements, hand grip strength and bioelectrical impedance analysis were more commonly used.

Bioelectrical impedance analysis (BIA) measures lean body mass via a device which passes a small electrical current through the body [185]. Because the passage of the current is different through water-rich muscle compared with other tissues, BIA is able to provide a measurement of the fat-free mass relative to total body water, based on

principles of electrical resistance. It is portable, inexpensive and requires relatively little training to use, however its' sensitivity has been shown to be inferior to other methods [186] and can be influenced by things such as oedema, ascites, hydration status and food intake.

Dual X-ray absorptiometry (DXA) calculates whole body mass and fat-free mass (represented in kg) and shows superior sensitivity to BIA. It is well validated and can differentiate between lean mass and fat mass, however it requires separate machinery which may not be available in all centres. Therefore, focus has more recently fallen onto CT scan measures, since these form part of routine cancer care. It has been shown that measurement of muscle mass at the level of the 3rd lumbar vertebrae act as an accurate correlate for total body muscle and fat mass [187], and that CT measures predict fat-free mass as strongly as DXA [186].

Although there is some evidence that cross-sectional area and muscle strength are not directly correlated [188], potentially due to fat infiltration of muscle, CT measures of muscle mass have been demonstrated to be predictive of outcomes in a range of cancers through combining assessments of cross-sectional area and density, as measured by Hounsfield units (HU). Skeletal muscle density (SMD) is thought to represent a more accurate marker of muscle strength. Prior to losing mass, muscles undergo fatty infiltration and so lose density prior to losing mass. CT measurements of skeletal muscle area are calculated most commonly at the level of the 3rd lumber vertebra (L3), however other levels have been validated for measurement, including the 4th thoracic vertebra (T4) and the 2nd cervical vertebra (C2). Skeletal muscle area (SMA) is calculated using neural learning software. This can then be adjusted for height to give the skeletal muscle index (SMI).

1.4.2 Defining sarcopenia

Sarcopenia has been defined by consensus group definitions, from the European Working Group on Sarcopenia in Older People (EWGSOP) [16], the European Society for Clinical Nutrition and Metabolism Special Interest Group (ESPEN-SIG) [18], and the International Working Group on Sarcopenia (IWGS) [189]. The EWGSOP guidelines were recently updated [190]. These definitions are shown in table 1.2 and use combined measures of lean body mass and muscle strength in their criteria.

There is no international standard defining a cut-off level for sarcopenia using CT measures. Two previous significant studies of patients with cancer used different cut-offs for SMI; ≤38.5 cm^2/m^2 for women and ≤52.4 cm^2/m^2 for men was used by Prado et al. [191], and Martin et al. [43] used ≤41 cm^2/m^2 for women and ≤43 cm^2/m^2 for men. These criteria have been widely used in other oncology studies. However, other criteria developed using a healthy volunteer cohort (kidney transplant donors) defined sarcopenia 34.4 cm^2/m^2 for women and 45.4 cm^2/m^2 [192]. The European Working Group on Sarcopenia in Older People (EWGSOP) guidelines recommend using a cut-off 2 standard deviations below the mean of a healthy young adult population, rather than a specific disease population [16], this would suggest that the healthy-volunteer definitions created by Derstine et al. [192] should be used. However, in the EWGSOP2 updated guideline, criteria were given which extrapolated from DXA measurements to identify CT measured cut-offs of and 55 cm^2/m^2 and 39 cm^2/m^2 for males and females respectively, therefore falling closest to the Prado cut-offs.

Table 1.4: sarcopenia definitions

Group	Definition	Measure
EWGSOP2 2018	(1) Low muscle strength Plus one of: (2) Low muscle quantity or quality or (3) Low physical performance	Grip strength <27kg Males (M): <16kg Females (F) Chair stand >15s for 5 rises Gait speed: ≤0.8 m/s DXA/ BIA: <7.0 kg/m2 Males and <6.0 kg/m^2 Females CT or MRI measured muscle mass
ESPEN-SIG 2010	I. A low muscle mass, II. Low gait speed,	•DXA: i.e. a percentage of muscle mass ≥2 standard deviations below the mean measured in young adults of the same sex and ethnic background. •Gait speed e.g. walking speed below 0.8 m/s in the 4-m walking test
IWGS 2011	Reduced muscle mass and function	•gait speed of than 1 m/s •lean mass less than 20^{th} percentile of values for healthy young adults. • appendicular fat lean mass/ $height^2$ (aLM/Ht^2) of ≤ 7.23 kg/ m^2 men and in women at ≤ 5.67 kg/ m^2.

DXA: Dual X-ray absorptiometry, BIA: bio-electrical impedance analysis, CT: computer tomography, MRI: magnetic resonance imaging, m/s metres per second

The commonly used CT cut-offs are shown in table 1.5:

Table 1.5: sarcopenia cut-off criteria (skeletal muscle index, SMI) from CT measures		
Criteria	Female cut-off for sarcopenia (cm^2/m^2)	Male cut-off for sarcopenia (cm^2/m^2)
Martin et al .	41.0	43.0
Prado et al.	38.5	52.4
Derstine et al.	34.4	45.4
EWGSOP2	39	55

The populations used in the Martin and Prado criteria were patients with cancers of the lung and GI tract across a variety of stages. These cancers predominantly present in older adults [193] and the mean age within the Martin paper was 64 years. Additionally, these patient groups may be expected to have high levels of sarcopenia due to the effect of malnutrition and co-existent respiratory diseases, although in the original Prado cohort only 15% of patients were sarcopenic. Given that the Prado and Martin cut points are significantly higher than those of Derstine et al., they identify many more patients as sarcopenic. In my own previous work 40% of patients were sarcopenic by Prado criteria, compared with 17% by Derstine criteria.

1.4.3 Sarcopenia as a prognostic biomarker

There is a wealth of evidence that sarcopenia is a biomarker for poor prognosis in patients with cancer. Many hundreds of papers exist supporting this and it has been confirmed in a large meta-analysis [194]. It should be noted that there was significant heterogeneity in this meta-analysis, patients with solid tumours from multiple different primary sites were used, and multiple different cut points for diagnosing sarcopenia were included. Unsurprisingly the prevalence of sarcopenia varied greatly, between 19 and 74%. Nevertheless, sarcopenia was associated with poorer OS with a hazard ratio (HR) of 1.44, $p < 0.001$. Sarcopenia was also associated with disease free survival (DFS), but not progression free survival (PFS) in this analysis, it was a negative prognostic marker for patients with both early stage and metastatic disease. Because it is known that muscle is infiltrated with fat as part of the process of muscle wasting,

another area of investigation has been myosteatosis, measured by muscle density. A recent meta-analysis of patients with colorectal cancer showed that reduced muscle density was associated with poorer OS, both when co-existent with sarcopenia and independently of it, HR 1.51, p0.002 [195].

What is not known from these papers is what the underlying cause of sarcopenia was in these patients. There is no radiological way to differentiate disease-related sarcopenia from sarcopenia of old age. One way to differentiate this would be to assess the rate of change, however many of these studies assess sarcopenia at baseline only. Furthermore, in studies that do longitudinally assess sarcopenia, most patients are subject to either surgery or systematic anti-cancer therapy, both of which may hasten muscle loss themselves. Studies have assessed differences in markers of sarcopenia before, and after cancer diagnosis. In one longitudinal study of older adults, there was no difference in baseline physical performance measures such as grip strength between patients who went on to develop cancer and those who did not [196]. The time to cancer diagnosis was between 2 and 4 years in this study and it may be that the time to cancer development explains the lack of difference seen. In another longitudinal study enrolling older adults, patients underwent annual assessments including DXA assessment of appendicular lean muscle mass, hand grip strength and gait speed [197]. In individuals who developed cancer, they noted a reduction in gait speed prior to diagnosis. Following diagnosis, a deterioration in appendicular lean mass (ALM) was seen, most significantly in patients with metastatic cancer. Again, the time from baseline to diagnosis of cancer was up to 8 years. Patients without cancer showed a steady deterioration in indices of sarcopenia throughout the period of follow-up [197]. A small retrospective study of patients treated for colorectal cancer showed that progressive sarcopenia, between baseline CT and one performed 6-18 months after diagnosis was associated with poorer survival [198].

1.4.5 Sarcopenia as a predictive biomarker

Traditional drug dosing in cancer treatment is done by body surface area (BSA), yet it is known that this has a poor association with fat-free mass. There may be wide ranges in drug distribution and clearance between patients with the same BSA [199, 200]. Therefore, there is interest in sarcopenia as a predictive biomarker. Prado et al. found that patients receiving treatment for colon cancer with the cytotoxic drugs leucovorin

and 5-flourouracil (5-FU) who had a low proportion of skeletal muscle in relation to their BSA, had a higher incidence of a dose-limiting toxicity (DLT) [201]. Another study identified a threshold for significantly increased risk of peripheral sensory neuropathy (PSN) from oxaliplatin treatment based on a dose per kilogram (kg) per lean body mass. Below this threshold no patients experienced significant PSN, whereas above it 44% of patients did [202]. A study of patients with oesophago-gastric cancer receiving oxaliplatin and capecitabine found that muscle density but not mass was associated with grade 3-4 toxicity, and sarcopenic obesity associated with grade 2 or worse PSN [203]. Two studies investigated patients with early (EBC) and advanced breast cancer (ABC) and reported rates of sarcopenia of 38% and 54% respectively [204, 205]. Low skeletal muscle gauge (a measure of SMI adjusted for muscle density) was associated with an increased risk of grade 3-4 chemotherapy toxicity, RR 2.00, p 0.003 for patients with EBC. There was also an increased risk of hospitalisation for both cohorts. Furthermore, in a prospective study of patients with ABC receiving capecitabine it was found that 50% of sarcopenic patients had toxicity after their first cycle, compared with 20% of non-sarcopenic patients [27]. Conversely, A small retrospective study of patients receiving gemcitabine nab-paclitaxel for advanced pancreatic cancer did not identify any significant differences in SMA between patients who experienced first cycle toxicity vs those who did not [206]. Both these studies assessed toxicity after the first cycle of treatment, whereas the two studies of patients with EBC and ABC assessed toxicity throughout the treatment course. It may be that sarcopenic patients experience more toxicity through cumulative effects. It has been hypothesised that dosing according to body composition may reduce the risk of chemotherapy toxicity, but prospective data is currently lacking.

The impact of different anti-cancer treatments on sarcopenia should also be considered. Some of the molecular targets of commonly used oral targeted agents are involved in protein synthesis, via the PI3K/AKT/MTOR pathway. As such the treatment itself may negatively impact on muscle mass, independently of tumour and patient factors. In a study of patients receiving the tyrosine kinase inhibitor sorafenib for renal cell carcinoma, 37% of sarcopenic patients had a DLT [207]. There was no difference in rates of sarcopenia over time between patients demonstrating disease control compared to those with disease progression [208], suggesting that this may be drug effect rather than disease-related muscle wasting. There is limited evidence about the impact of other

targeted treatments on body composition, however. A recent meta-analysis investigating the effect of immunotherapy treatments in patients with lung cancer and sarcopenia reported no association between sarcopenia and drug toxicity [209].

Sarcopenia and particularly sarcopenic obesity may impact on the pharmacokinetics (the study of the movements of a drug into, through and out of the body) of medications given the altered ratio of lean body mass to fat mass and thus an altered volume of distribution [210], and changes in proportion of metabolic enzymes such as dihydropyrimidine dehydrogenase (DPYD) which has high activity levels in skeletal muscle [211]. Therefore, in early phase trials sarcopenia could potentially alter the toxicity profile and maximum tolerated dose (used in dose finding) of a drug if a significant number of patients with sarcopenia were enrolled. To date however, there is conflicting data as to whether patients with sarcopenia have more toxicity in early phase trials [212, 213].

1.4.6 Sarcopenia, fitness, and frailty assessments

Currently, fitness for cancer treatment is most commonly assessed by clinicians using the Eastern Co-operative Group Performance Status (PS) [214]. The PS runs on a scale of 0-5 with 0 representing good fitness with no restrictions and 5 representing death. Most clinical trials require patients to be PS 0-1 which indicates that they are unrestricted in activities of daily living, or only minimally restricted. This means that trial data is taken from the fittest patients and its' applicability to patients in the "real world" of cancer treatment may be limited. Another frequently used and more detailed scale is the Karnofsky performance status [215].

The benefits of PS are that it is a quick, simple, and effective marker of patient fitness; it can be delineated by simple questions in clinical practice and has been shown to be predictive of toxicity and prognosis in patients at various stages of disease [216, 217]. However, there is recognition that PS has limitations. One significant limitation is its' subjectivity [217] and another concern is that it does not well represent the fitness of older patients and may therefore lead to under-treatment in this group. It is also well recognised that older patients are under-represented in clinical trials [218], despite patients over 65 years of age representing the majority of cancer patients [219]. Much research has therefore been done into different frailty screening tools, such as the G8

[220] or Rockwood scale [221], and the use of the comprehensive geriatric assessment (CGA) as a more detailed and effective assessment of fitness in older patients with cancer.

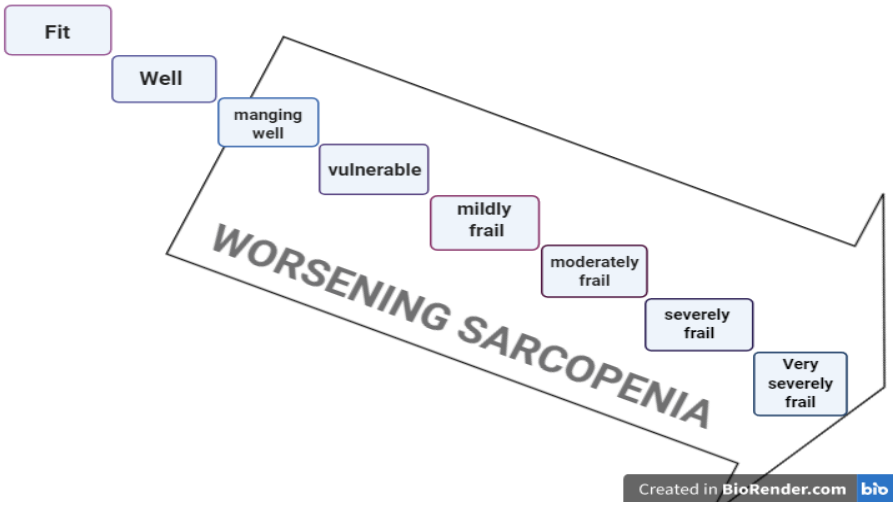

Figure 1.3: a putative relationship between sarcopenia and frailty as based on the Rockwood score

Frailty, defined by Fried et al [222], is a clinical syndrome including weakness, fatigue and weight loss. It is associated with loss of independence and increased vulnerability to stressors such as illness and indeed, treatments. Although associated with co-morbidity, frailty does not require the presence of it as cachexia does. However, like cachexia, frailty may show significant overlap between the individual phenomena of anorexia and sarcopenia. An indicative relationship between sarcopenia and frailty is shown in figure 1.3.

Frailty screening tools are designed to identify patients who may benefit from a comprehensive geriatric assessment [223], rather than to diagnose frailty themselves. A common finding is that with the use of frailty screening and CGA more older patients get aggressive treatment, and more patients get no anti-cancer treatment at all [224-226]. Whilst frailty is associated with poorer outcomes, fit older patients may tolerate treatment as well as younger patients [227, 228].

Frailty screening tools may include objective measures of fitness such as hand-grip strength or the “timed-up and go” test which measures the time taken for a patient to get up from a chair and walk a set distance. Other assessments of physical performance

exist such as the short physical performance battery (SPPB) which assesses gait speed, chair stand and balance, it has been demonstrated to accurately predict disability in older community-based populations [229]. These may have better sensitivity for identifying patients at risk of treatment toxicity than PS [216].

The CGA is considered the gold standard assessment for older frail patients. There is evidence that it increases the number of older patients who are independently living, but evidence to support its impact on mortality and cost-effectiveness is limited [230]. A significant advantage of the CGA is that gives specific information about the needs of a patient, such as walking aids or modifications to their home. However, it requires specific training, and is usually undertaken by a multi-disciplinary team including geriatricians rather than oncologists, and this has prevented the CGA from being widely incorporated into oncology practice. Oncologists' subjective assessments of patients fitness do not correlate well with the CGA [231]. There is some evidence that the CGA may predict toxicity from cancer treatment [224] and other tools have been developed to predict treatment toxicity in older patients according to their fitness levels, such as the Cancer and Aging Research Group (CARG) score [232] and Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) score [233].

Whilst frailty screening, SPPB and CGA give useful predictive information about patient fitness, the time taken to perform them and necessity for trained staff and specialist equipment may limit uptake. They were not developed for use specifically in patients with cancer. They are only validated for patients aged over 65, however younger patients with cancer may have reduced fitness as a result of the disease and the phenomenon of cancer cachexia. Furthermore, to date, we have limited data about the correlation between the phenotype of frailty and the underling body composition. In a large prospective biobank study assessing sarcopenia and frailty using the Fried criteria no patients had pre-frailty or frailty without sarcopenia [41]. Whilst multiple studies have noted that sarcopenia appears to be prognostic of survival within each individual ECOG PS group, there is only limited data correlating it with frailty scores in patients with cancer. Zwart et al. [234] performed a prospective analysis of CT muscle mass in patients with head and neck cancer. The study found very high rates of sarcopenia, and it correlated well with frailty as assessed by the widely used G8 screening tool ($r=0.38$, $p<0.001$). In another study by Williams et al. SMI did not correlate well with frailty,

assessed by their own scoring system [235]. There was a correlation noted between frailty and skeletal muscle gauge in this study, again suggesting the importance of considering muscle density in relation to sarcopenia.

1.4.7 Pathophysiology of sarcopenia of aging

Sarcopenia of aging is characterised by a progressive decrease in the number and size of muscle fibres [236] leading to decreased muscle mass and function [237]. In addition to this, tendons lose water and become stiffer. In both cancer cachexia, and aging, there is a preponderance to lose type 2, fast twitch, muscle fibres [238, 239]. The mechanisms underlying this process are not fully understood, but ultimately lead to an imbalance between anabolism and catabolism. Proposed mechanisms include:

- upregulation of catabolism (which will be discussed in more detail in the next section)
- increased apoptosis of satellite cells (muscle fibre precursor cells) [240]
- deterioration of neuro-muscular junction function [241], leading to progressive denervation of muscle fibres and potentially uncoupling of excitation-contraction
- decreased mitochondrial protein synthesis [242]
- increased reactive oxygen species triggering catabolism [243]
- decreased myosin heavy chain synthesis [244]

The causes of these processes are also not fully understood, but likely to be multifactorial, including reduced use, nutritional changes, inflammation, and other environmental factors.

Although there appears to be an imbalance between anabolism and catabolism, it has been widely demonstrated that older people with sarcopenia [245], including patients with chronic diseases [246, 247], retain the ability to build muscle in response to resistance exercises. Evidence suggests that exercise is able to improve signalling at the neuro-muscular junction [248, 249].

The role of nutrition is unclear. In a large cross-sectional study sarcopenia was not correlated with reported energy or protein intake [250] but was associated with lower

income. This was reflected in a recent UK cross sectional study [40] and in this study self-reported higher intake of protein and carbohydrates was associated with a lower likelihood of sarcopenia. In studies investigating nutritional supplements compared with, or in addition to, resistance exercise in older people, nutritional supplements alone were not able to increase muscle mass or strength [251]. Nor did they add anything to resistance exercise alone. It has been demonstrated that older patients do show an anabolic response to amino acid supplements [252, 253], though not to the same degree as younger patients, so it may be that the type of nutritional supplement is significant here. In another study older patients required larger amounts of protein to stimulate anabolism than younger patients [254].

Hormonal changes have been considered as potential drivers of sarcopenia. Levels of testosterone and other adrenal androgens decrease with age [255, 256], and there is some evidence that muscle mass may be increased with testosterone supplementation [257, 258]. The use of testosterone replacement is not recommended for treating muscle mass alone, in the absence of other symptoms of hypogonadism [259]. Similarly, oestrogen based hormone replacement therapy (HRT) is known to improve bone density in post-menopausal women, and has been demonstrated to show an associated increase in muscle mass [260]. However, because of known side effects, the use of HRT has to be cautious, and is not recommended for sarcopenia alone. It is thought that reduced growth hormone secretion may contribute to reduced anabolism with aging[261], and although growth hormone replacement has been trialled [262, 263] in older patients it is not routinely used. No significant changes in body composition were reported in a trial of the androgen dehydroepiandrosterone [264].

The role of inflammation in the sarcopenia of aging is unclear. Inflammation is thought to play a role in many processes of aging, however, in a study investigating inflammatory cytokines, no differences were found between young and older participants [265] with the exception of IL-6. Higher baseline levels of CRP have been negatively correlated with muscle mass in older patients [266]. There is evidence that exercise reduces CRP and possibly also IL-6 levels in older adults [267, 268]. Hofmann et al. assessed circulating levels of multiple biomarkers of muscle status in older women, including inflammatory cytokines [269]. There was a positive correlation between insulin-like growth factor 1 (IGF-1) and muscle mass. This would be expected

as IGF-1 is known to promote anabolism via the PI3K/AKT/MTOR pathway [270]. There was a negative correlation between GDF-15, an inflammatory cytokine and muscle mass. However, no biomarker was shown to reliably predict sarcopenia. Included in this panel was myostatin, an extracellular cytokine known to inhibit muscle growth. Other studies have reported higher myostatin mRNA levels in sarcopenic patients [271]. A phase two trial of a myostatin antibody showed increased muscle mass and function in older patients [272], though this does not appear to have been developed further to date. Ultimately the role of inflammation in the sarcopenia of aging remains unclear currently.

1.4.8 Pathophysiology of muscle wasting in advanced cancer

The inflammatory state found in cancer cachexia is thought to underly the accelerated muscle wasting seen in this condition. Theoretically, markers of inflammation may be able to differentiate muscle wasting secondary to advanced cancer from the reduced muscle mass associated with aging. Inflammatory cytokines may increase protein degradation via upregulation of the following pathways:

- Ubiquitin-proteosome pathway: a pathway involved in multiple cellular processes including normal protein degradation. Molecules are tagged with ubiquitins and degraded by proteosomes.
- The autophagy/lysosomal pathway
- The calcium dependent enzymes (calpains) pathway

Different pathways of sarcopenia are shown in figure 1.4. Putative factors in the mediation of these pathways are proteolysis inducing factor (PIF), myostatin, activin A (ActA) and inflammatory cytokines. Activins and Inhibins are protein complexes which are part of the Transforming Growth Factor β (TGF-β) cytokine superfamily [273]. They have roles in multiple biological processes. Another cytokine of this family is myostatin, also known as growth differentiation factor 8 (GDF-8). Released from muscle cells it acts to prevent muscle cell growth and differentiation.

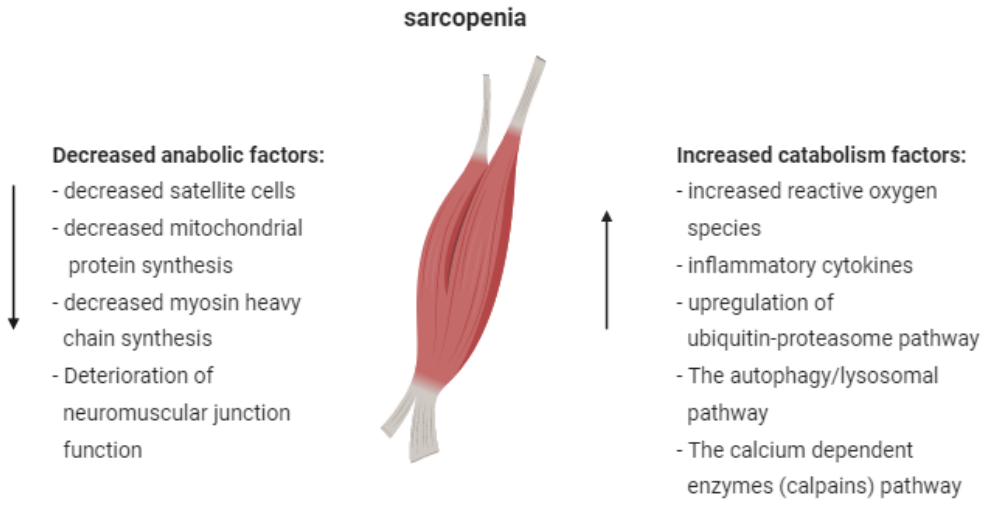

Figure 1.4:mechanisms of sarcopenia (created with biorender.com)

Injection of PIF into mice caused rapid weight loss, with decreased muscle synthesis and increased degradation [274]. This effect appears to be due to activation of the ubiquitin-proteosome pathway [275, 276]. Following upregulation of the ubiquitin-proteosome pathway reduced protein synthesis is seen, due to activation of RNA-dependent protein kinases. However, though PIF was detected in humans with cancer, it was not shown to be associated with muscle loss [277]. Upregulation of genes encoding for ubiquitin ligases is seen in response to inflammatory cytokines [278], leading to protein degradation through action on the NF-κB pathway and the p38 MAP kinase pathway [279, 280]. High expression of genes in the ubiquitin-proteosome pathway have been detected in patients with cancer [281, 282]. There has been interest in the drug bortezomib, a proteasome and NF-κB inhibitor licensed for use in haematological malignancies, as a potential therapeutic drug for cancer cachexia. But it has yet to demonstrate any significant effect on muscle mass in patients with cancer [283].

The inhibitory role of myostatin on anabolism was demonstrated by McPherron et al. in mice. Myostatin knockout mice developed increased muscle bulk [284]. They also identified deletion mutations for the myostatin gene in cattle with a larger muscle phenotype [285]. The overexpression of activins has been demonstrated to lead to

muscle wasting [286]. Both myostatin and ActA take effect via inhibition of the PI3K/AKT/mTOR pathway. Blockade of the activin receptor ActRIIB led to reversal of muscle wasting and prolonged survival in cachectic mice [287]. ActA release is stimulated in response to TNF-α [288], which can also directly inactivate the AKT/PI3K pathway [289]. High ActA levels were seen in cancer patients and appear to correlate with cachexia, and poor prognosis [290, 291]. Interestingly, in a study by Loumaye et al. of patients with advanced lung and colorectal cancer, high ActA levels were seen in cachectic patients, but low levels of myostatin [290].

Because of the role of inflammation in the muscle wasting seen in patients with cancer, anti-inflammatory medications have been trialled as potential treatments of cachexia, though with limited success to date as will be discussed in chapter 5.

1.4.9 Treating sarcopenia

Theoretically, in advanced cancer, if sarcopenia has a negative effect on both quality of life and prognosis, then increasing muscle mass should have a positive effect. It is important to note that strong evidence supporting this theory is lacking.

Interventions may be nutritional, exercise based or pharmaceutical. In terms of nutritional interventions studies in patients with advanced cancer have demonstrated a benefit to survival from nutritional counselling and early supportive care [292-294]. Where body composition has been assessed most patients gained fat rather than muscle [295] and a positive impact of nutritional support on muscle mass alone has not conclusively been demonstrated in patients with cancer [296]. Nutritional support is nevertheless likely to be an important part of treating sarcopenia in patients with malnutrition. Some data shows that patients with a higher body mass index (BMI) live longer with cancer, in contradiction to the data around sarcopenic obesity, though this data is conflicting [297, 298]. This may be in part due to studies combining patients with different cancer types and stages, but also likely reflects the poor association between BMI and muscle mass. However, optimal nutritional management of obese patients with cancer has not been clearly elucidated. There is evidence, albeit of varying quality, to support a positive impact of nutritional interventions in older, frail patients [299] and so nutritional management is likely to form a key part of any intervention for sarcopenia.

Exercise interventions in the form of prehabilitation have been trialled in patients with localised cancer undergoing surgery and have demonstrated evidence of improvements in physical function and reduced post-operative complications [300, 301]. In patients with advanced cancer data suggests exercise interventions may have beneficial effects on quality of life and fatigue [302, 303] but there is limited longer term data available in either of these groups.

Potential physical limitations for patients with advanced cancer, and the need for rapid results mean that pharmaceutical agents to increase muscle mass represent an attractive option. These will be discussed in more detail in chapter 5; however, I will briefly summarise some key existing data related to cancer patients here. The most common pharmaceutical agents for treating cancer associated weight loss and cachexia; megestrol acetate and dexamethasone have never been shown to have significant effects on muscle mass, increasing weight through fluid and fat increase in the case of megestrol [304, 305], and not demonstrated to positively impact weight at all in the case of dexamethasone [306].

Newer agents, for example anamorelin, a ghrelin receptor agonist, have demonstrated an increase in muscle mass. However, anamorelin did not gain regulatory approval in the United States or Europe as it did not demonstrate improvements in quality-of-life or grip strength [307].

Anti-inflammatories have frequently been trialled in patients with cancer, because evidence suggests that inflammation underlies the muscle wasting seen in cachexia [308, 309], but in at least one trial of healthy adults they have shown a negative effect on anabolism [310], which re-iterates the importance of careful patient selection for trials in patients with cancer.

Most pharmaceutical agents trialled in patients with cancer were trialled on their own, without nutritional or exercise interventions. Improvements in mass but not function are a frequent finding of trials of pharmaceutical agents in a range of patient groups where drugs are trialled alone [311-314]. This may be because improvements in function require triggering of the neuromuscular junction via exercise [248, 315] rather than just an increase in muscle fibre size. However, where drugs have been trialled in older adults alongside exercise, they rarely show a benefit over exercise alone [316, 317].

Theoretically, increased muscle mass without increased function may potentially have benefits for cancer patients. Given that increased toxicity is thought to be related to altered pharmacokinetics where there are significant ratios of fat mass to lean mass, an increase in muscle mass may counteract this. This has not yet been tested in a trial setting however, and the lack of improvements of quality-of-life in trials of anamorelin argue against this. Furthermore, muscle mass increases metabolic rate, and therefore may increase calorie demand which could be a challenge for patients with significant cancer related anorexia to meet.

1.5 Summary

In summary, anorexia and sarcopenia are highly prevalent in patients with cancer. The mechanisms underlying anorexia in patients with cancer are incompletely understood, particularly the role of the enteroendocrine system. Effective treatments for cancer related anorexia are limited. The only treatments with known efficacy are glucocorticoids and megestrol acetate, however, the use of both is limited due to associated toxicity. Effective treatment for anorexia could help increase patients' oral intake and reduce the clinical deterioration seen as a result of malnutrition.

Research in sarcopenia in patients with cancer has been limited by varying definitions and methods of assessment. Sarcopenia as measured on CT imaging could represent a simple, objective method of fitness assessments for patients, but large-scale, prospective research is lacking on how sarcopenia correlates with physical function and frailty. There is only limited data supporting the use of pharmaceutical agents to support muscle mass in patients with cancer, and no treatments specifically trialled for this use are licenced within the UK. Better understanding of patient fitness could allow for more personalised treatment plans, with less associated cancer treatment toxicity.

1.6. Hypothesis and Aims

1.6.1 Hypothesis

- Understanding the prevalence of anorexia, and characterising patterns of anorexia and nutritional symptoms in patients with upper GI cancers will allow better understanding of this symptom, and potentially help identify strategies for managing it.
- Investigation of the patterns of release of gut hormones in patients with cancer will increase the understanding of the role of the enteroendocrine system in cancer anorexia.
- Investigation of the correlation between sarcopenia as measured on CT scan with frailty scores and treatment outcomes will increase the understanding of the potential utility of this tool in routine cancer care
- Understanding the existing data around the medications used for treating sarcopenia across different treatment settings will allow for the investigation of agents for use in patients with cancer which may lead to more effective treatments for this condition.

1.6.2 Aims

The aims of this project are as follows:

1. I aim to investigate the prevalence of anorexia in patients with upper GI cancers and try to characterise patterns of anorexia in this group.

2. I aim to investigate the role of gut hormones in cancer anorexia. To do this I will investigate gut hormone levels in patients with cancer anorexia compared with those without significant anorexia and correlate this with inflammatory cytokines and other biochemical markers.

3. I aim to investigate the correlation between sarcopenia as measured on CT scans, frailty as measured by screening tools and cancer treatment outcomes.

4. I aim to perform a systematic scoping review of treatments that have been investigated to date for sarcopenia.

2. Characterising patterns of anorexia and malnutrition in patients with upper GI cancers

2.1. Introduction

There is limited data specifically about the prevalence of gastrointestinal symptoms and the impact of appetite loss on outcomes in patients with OG cancers. There is some existing data suggesting that anorexia and dysphagia are associated with poorer survival [39, 174].

There is some limited prospective data [180], and more retrospective data [182] that malnutrition is associated with poorer survival in advanced OG cancer. Studies have demonstrated that poorer nutritional status in advanced OG cancers is associated with poorer quality of life [178, 179].

A retrospective study at The Christie hospital of 182 patients with oesphagogastric cancers reported rates of anorexia of 69% and demonstrated a marked survival difference between patients with anorexia as measured by the FAACT C/S scale and those without [176]. Patients with a score of >37 had a median survival of 19.3 months, compared to 6.7 months for those with a score of ≤37.

There is limited prospective data to identify how much of anorexia and malnutrition is related to dysphagia and how much to other issues, such as systemic inflammation. There is a significant unmet need to understand and manage these symptoms to allow for optimisation of patients with advanced OG cancers to receive systemic therapy.

2.2 Study design, aims, hypothesis and power calculations

2.2.1 Study design and aims

I aimed to deeply, prospectively, characterise nutritional symptoms in patients with OG cancer receiving treatment at a tertiary cancer centre, The Christie Hospital NHS Foundation Trust. To facilitate this, I led on the development of the Anorexia in Cancer patients: assessment of the gut HORmone and cytokine profile and body composition, and the impact of dietetic support in patients with gastrointestinal cancer (ANCHOR) study. ANCHOR is a single-site prospective observational study with a pilot sub-study,

investigating the rates of anorexia in patients with OG cancer, and the association between sarcopenia, nutritional status and fitness measured by various tests. I developed the protocol in collaboration with supervisors and co-investigators and led on development of all study materials, patient information leaflets and the ethics application. I also submitted a grant application to fund the study costs which was successfully approved (more information below).

Retrospective data from an unselected cohort at our centre demonstrated a significant difference in median survival between patients who lost ≥3% weight between baseline visit and first cycle of chemotherapy and those whose weight remained more stable, 6.4 vs. 10.5 months [176]. Weight loss of ≥3% to cycle 1 was seen in 36% of patients. Since this initial data was collected it has become standard practice for all new patients identified as having nutritional needs to have dietician input.

The aim of this study is to prospectively validate that work. ANCHOR aims to recruit up to 500 patients from a tertiary oncology centre serving a large population with overall poor health outcomes. The study will represent one of the largest prospectively collected cohorts of patients with advanced OG cancers. In addition to this it will include deep, biomarker led characterisation of patients, using validated tools which has only been performed in limited cohorts to date.

Patient participation was undertaken on existing patients in the OG cancer clinic about the study design. Patients reported positive feedback that they felt the study design was acceptable and that they appreciated that research was being performed in this area that has significant impact on their quality of life.

2.3 Methodology

2.3.1 Power calculations

Retrospective work at our institution showed rates of 3% weight loss to cycle 1 of 36%. Given that all patients presenting for treatment have dietician assessment, whereas this was mixed in the retrospective sample, I conservatively estimated potential prevalence of 20%.

Power calculations suggest that for an estimated population of 120 eligible patients per year, we would need a sample size of 81 to detect this, with 95% confidence.

We hypothesise that patients with early weight loss of ≥3% weight between baseline and cycle 1 of chemotherapy have poorer survival, and therefore the proportion of patients alive at 1 year would be lower. Based on trial data, suggesting around 50% of patients remain alive at 1 year, and assuming a rate of early weight loss of 20%, a sample size of 399 would be able to detect a 15% difference in rate of patients alive at 1 year with 80% power (80 patients with 3% weight loss and 319 without). If the prevalence of 3% weight loss were to turn out to be closer to around 30%, then a sample size of 343 would be able to detect a 15% difference in rate of patients alive at 1 year with 80% power (86/257).

Previous data from our institution demonstrated anorexia rates of 69% retrospectively with a marked survival difference. Power calculations suggest that with an expected rate of 69% anorexia to detect a survival difference with 80% power would require 409 patients, and 224 events.

The ANCHOR trial is expected to recruit up to 500 patients during its recruitment period. I am presenting data from the first year of data collection and 60 patients recruited under a separate prospective project completed as part of the ukCAT database (see ethical approval details below).

It is expected therefore that data collected within the first year of the study will be able to confirm prevalence of anorexia and early weight loss and guide overall recruitment of the trial.

Furthermore, the aim is to provide a deep characterisation of the nutritional status of the patients, identify patterns of malnutrition within this cohort and investigate the relationship between nutritional characteristics and outcomes including chemotherapy toxicity and survival. Since there is only very limited data on the impact of nutritional status on chemotherapy toxicity, I've not performed power calculations for this. Instead, this initial data presentation is designed to guide the recruitment of the remainder of the study.

2.3.2 Patient selection

Patients were prospectively recruited, initially under an ethics approval for the use of CT scans to investigate sarcopenia (to be discussed further in chapter 4), and then within context of the ANCHOR trial.

Patients presenting with locally advanced or metastatic oesophageal, gastro-oesophageal junction (GOJ) or gastric cancer were included. Patients with localised disease who were otherwise deemed unsuitable for radical treatment were also included. Squamous cell carcinoma, adenocarcinoma and undifferentiated carcinomas were included, but patients with neuroendocrine carcinoma were excluded. Patients enrolled in other treatment trials were included. Patients were excluded if they had systemic therapy or significant radiotherapy within the previous 5 years for any cancer, were undergoing curative intent treatment or were unable to understand the study sufficiently to consent.

All patients planned to commence 1st line, palliative chemotherapy were invited into the study. Some patients were invited to undertake some additional tests, including a cardiopulmonary exercise test and for some patients an assessment of gut hormones (discussed in chapter 3). The study schema is shown in figure 2.1.

Full inclusion and exclusion criteria are as follows:

<u>Inclusion criteria: Cohort A</u>

1. Patients with de novo stage IV gastric, GOJ or oesophageal cancer, or more localised disease that is otherwise not amenable to curative intent treatment
2. Histologically proven adenocarcinoma, squamous cell carcinoma or poorly differentiated carcinoma
3. Patients should be chemotherapy or immune therapy naïve. Patients who have received previous chemotherapy or radiotherapy for another indication may be included if treatment was given with curative intent and was >5 years ago. Patients with relapsed disease who had previously had surgical intervention only were included.
4. Patient must be 18 years of age or above
5. Patient must be able to understand the study information given to them and be willing to give consent for trial participation

6. Patients should be commencing a course of palliative chemotherapy treatment with the upper GI team at the Christie Hospital

Exclusion criteria Cohort A:

1. Patients unable to give informed consent
2. Patients not undergoing systemic anti-cancer treatment at The Christie hospital, for example patients not deemed fit enough for treatment, patients having alternative treatments such as radiotherapy or surgery, or patients referred for 2nd opinions.

Patients initially planned to commence chemotherapy but who did not due to a deterioration of physical condition or other change in circumstances were included on an intention-to-treat basis.

2.3.3 Baseline assessments

Demographic data were recorded prospectively for all patients including:

- baseline weight and weight change within the preceding 6 months
- height
- body mass index (BMI)
- dysphagia score (O'Rourke)
- reported weight loss
- routine blood tests taken at clinic visit including full blood count and biochemistry including CRP where available
- body composition as calculated from baseline CT imaging

Systemic inflammation was defined using NLR equal to or greater than 3. The decision to use 3 as a cut-off for significant systemic inflammation was based on existing data [318].

Patients enrolled within the ANCHOR trial underwent nutritional assessment at first study visit including:

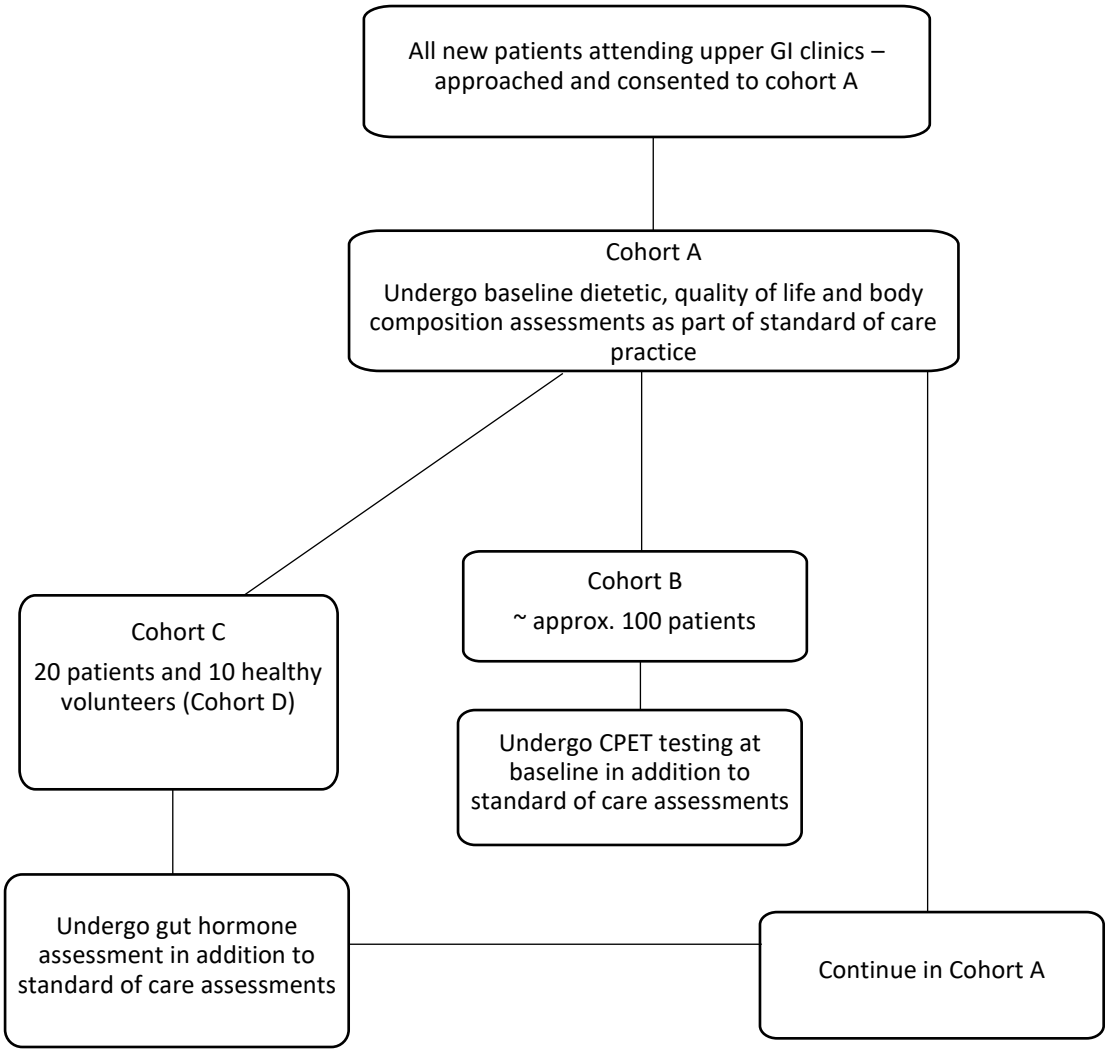

Figure 2.1: study schema

- hand grip strength (HGS) measured using dynameter
- sit to stand test (STS)
- mid-upper arm circumference (MUAC)
- Functional Assessment of Anorexia/Cachexia Therapy Anorexia/Cachexia subscale (FAACT A/CS) questionnaire
- QLQ-OG25 quality of life questionnaire (OG-25)

2.3.3.2 ANCHOR test details:

The following tests were undertaken on patients recruited to ANCHOR by study sub-investigators following appropriate training.

Hand Grip test: Grip strength was performed using a Jamar digital dynameter. With the patient sat on a chair with the shoulder adducted and elbow flexed at 90º (non-dominant arm) they were asked to "squeeze" the handle of the dynamometer device. This is repeated 3 times with 30 second (s) interval. The best Hand Grip Strength (HGS) result in kilograms (kg) is recorded.

Sit-to-stand test: the patient is seated on a chair, from start of the test they are asked to stand without the use of arms to assist, then sit back down 5 times. The total time to complete the test was recorded in seconds.

Mid-Upper Arm Circumference: The mid-point of the upper arm is identified, using a flexible non-stretch tape. This was laid at the midpoint between the acromion and olecranon processes on the shoulder blade and the ulna. The arm circumference was then measured in centimetres cm with the arm in full extension.

Patients underwent dietetic support from a registered dietician at baseline and throughout their first course of chemotherapy (usually 6 cycles of treatment, lasting 18 weeks), if required following initial assessments. Dietician support including nutritional supplements was available to patients as required throughout their treatment.

Prospective data was collected on weight change, body composition change, anorexia score and quality of life scores. Other than quality-of-life score patients did not undergo any interventions that are not part of usual standard of care.

Assessments including the FAACT/CS and OG-25 scores, MUAC, HGS and STS were repeated at mid-point and end of chemotherapy.

2.3.4 Study duration

Specific reasons for discontinuing a participant from study procedures were:

- investigator decision

- safety reasons
- incorrect enrolment e.g., the participant does not meet the required inclusion/exclusion criteria for the study
- participant death
- participants decision to withdraw
- completed visits up to the end of trial time point, following which patients were followed for survival data only.

2.3.5 Ethics approval

Ethics approval for analysis of CT scans is provided under the ukCAT database. This large project allows for sarcopenia analysis of CT imaging performed at The Christie NHS Foundation Trust or imported to our imaging systems for clinical purposes.

An application was made to the ukCAT database to allow for the sarcopenia analysis of all patients recruited to the ANCHOR trial. However, since the ukCAT ethics approval allows for some data collection, the initial 60 patients in this analysis were recruited under the application to the ukCAT data project whilst the full ANCHOR study approval was awaited. This was approved under ukCAT database application number 2020-017. The ukCAT database was approved by the North-west research ethics committee, Haydock, 28th February 2017.

The initial 60 patients recruited therefore had data collected from standard-of-care investigations such as height, weight and demographic information but did not undergo the full investigations listed above.

The ANCHOR trial was approved by Fulham Research Ethics Committee (IRAS ID: 286840, REC reference 21/PR/0298), April 8th, 2021, and further patients were recruited under this.

2.3.6 Study funding

The study was funded through a grant awarded by the Manchester Academic Health Science Centre cancer domain. This covered all the costs of gut hormone assay kits and analysis. Some additional funding to cover in-house study costs and additional materials was provided from the upper GI medical oncology team research funds.

2.3.7 Statistical analysis

Statistical analysis was undertaken using SPSS (IBM, version 25, 2017). Proportions were analysed with descriptive statistics, differences between groups with χ^2, Fisher's exact test or non-parametric tests as appropriate.

Survival was analysed using Kaplan-Meier analysis and cox regression. Correlations were assessed using logistic regression. Cut-off for survival was taken on September 17th, 2022.

Advice was provided from the University of Manchester's statistical support service, particularly regarding power calculations. However, all statistics were then performed by me.

2.4 Results

2.4.1 demographics

The first 60 patients were prospectively recruited between January 2021 and July 2021. Following confirmation of additional ethics approval for the wider ANCHOR study a further 98 patients were recruited with additional quality of life and anthropomorphic data, giving a total of 158 patients for analysis. At final analysis all but 2 patients had completed first line chemotherapy, with a median follow-up of 12 months.

Patient demographics are shown in table 2.1

Weight loss was reported by 111 (70%) of patients. Mean weight loss for the cohort overall at baseline was 7.8kg, but if selected to patients reporting weight loss only, was 11.1kg.

Dysphagia was present in 92 (58%) patients, with 61 (38%) reporting no swallowing issues and data missing in 5 (3.2%).

Table 2.1 Patient demographics			
		N	%
Sex		118	75
		40	25
Median age (range)	67 (33-91)		
Age ≥70		74	46.5
Mean height (range)	Male	173cm (158-187cm)	
	Female	158cm (147-170cm	
Mean weight (range)	Male	79.3kg (45-141kg)	
	Female	66.1kg (40.7-112kg)	
Mean BMI (range)	BMI 26.3 (12.7-50.9)		
Weight loss in the preceding 6 months to assessment *of patients who had lost weight	Any	111	70
	*≥3% of baseline	104	94
	*≥5% of baseline	93	84
	*≥10% of baseline	78	70
N co-morbidities	0	43	27
	1-2	89	56
	≥3	26	17
Performance status	0-1	120	76
	2-3	38	24

Primary site of disease and histological details are shown in table 2.2.

Table 2.2: Tumour characteristics		
Primary disease site	N	%
Oesophagus upper third	4	2.5
Oesophagus mid third	12	7.6
Oesophagus lower third	53	33.5
GOJ T1	12	7.6
GOJ T2	12	7.6
GOJ T3	13	8.2
Stomach	51	32.3
Duodenal	1	0.6
Histology		
Adenocarcinoma	131	82.9
Squamous	23	14.6
Undifferentiated	4	2.5
HER-2 status (undifferentiated and adenocarcinoma only)		
HER-2 positive	29	21.5
negative	99	73.3
unknown	7	5.2
Disease extent		
Localised disease	23	14.6
Metastatic	135	85.4
Sites metastases		
Lymph Node only	58	36.7
Liver	48	30.4
Peritoneal (including local lymph node) only	23	14.6
Other	29	18.4
GOJ = gastro-oesophageal junction, HER=2 Human Epidermal Growth Factor Receptor 2		

2.4.2.2 Biochemical and inflammatory markers

Mean biochemical and inflammatory marker values are shown in table 2.3.

Table 2.3 Biochemical and inflammatory markers		
Mean value marker (normal range) N = 158		
Albumin (35-50)	41	
Sodium (133-146)	137	
Creatinine (44-97)	74	
Hb (120-165)	124	
Neutrophils (2-7.5)	7.04	
Lymphocytes (1.5-4.0)	1.56	
NLR	5.33 (range 1.0-23.1)	
Mean CRP (<5) N=47	53.4	
Patients with Hb <120 N =63	N	%
Iron deficiency present	36	56
Iron deficiency absent	9	14
Iron deficiency unavailable	18	28

A CRP was not routinely tested, and was available in 47 patients, of these 47 patients, 30 had Hb <120, mean CRP 56.2, and 18 had Hb ≥120, mean CRP 39.0, p 0.34. A normal CRP was found in 10% of patients in both anaemic and non-anaemic groups.

Mean neutrophil: lymphocyte ratio (NLR) was 7.0 (range 1.0-23.1), with 114/158 (72%) patients having an NLR >3. CRP correlated with NLR, r 0.58, p <0.001.

Distribution of NLR is shown in figure 2.2

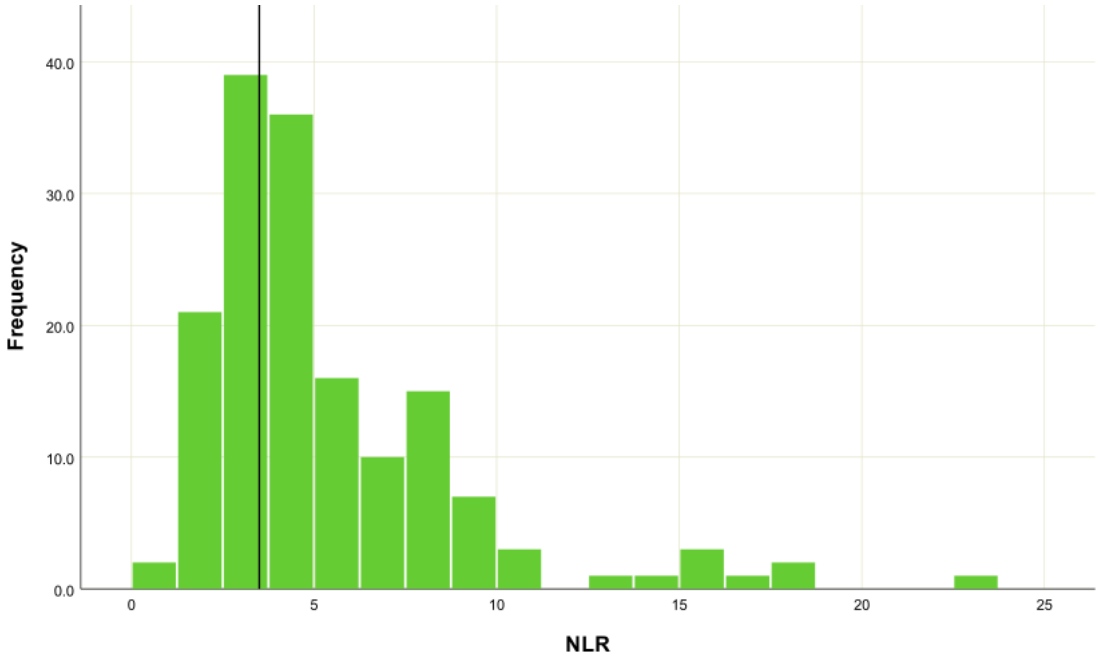

Figure 2.2: Histogram of neutrophil:lymphocyte ratio (NLR) with marker at 3.5.

2.4.3 Anorexia and anthropometrics

Baseline data was available for 98 patients recruited under the ANCHOR trial, which included the FAACT C/S score, OG-25 and anthropometrics. Anorexia was defined as a FAACT C/S score of ≤ 37.

Weight loss was present in 70/98 (71.4%) of patients.

Mean FAACT C/S score was 31.8 (range 6-48), and 62 (63%) of patients were anorexic with a score of ≤37 at baseline.

Mean mid-upper arm circumference was 29.5cm for men, 27.2cm for women (range 19.3-38.6cm), mean hand grip strength was 29.1kg for men and 20.0kg for women (range 9.9-50.1kg), mean sit-to-stand test time was 12.0 seconds (range 5.5-24.4s).

Highest OG-25 scores at baseline were for eating restrictions, anxiety, and weight loss.

Distribution of anorexia scores in shown in figure 2.3.

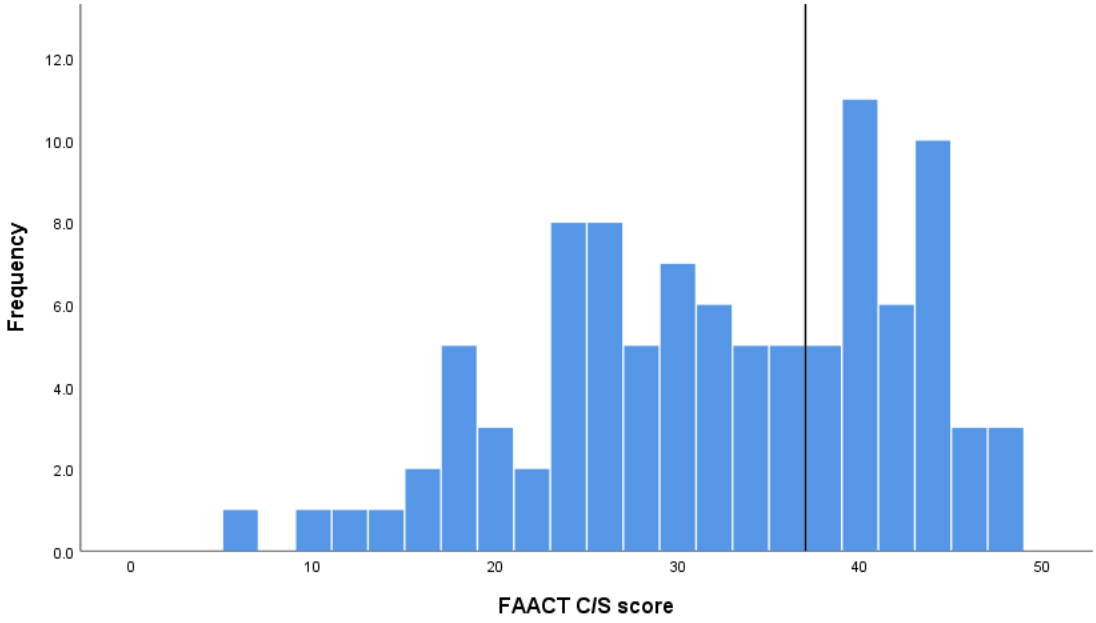

Figure 2.3: distribution of FAACT C/S scores, marker bar at 37 (diagnostic of anorexia)

2.4.4 Patterns of nutritional status

Presence of absence of anorexia, dysphagia and a stent were assessed by disease site. This data is presented in table 2.3a and 2.3b.

Anorexia was significantly more common in tumours of the upper-middle oesophagus (66.6% vs 47.5% in GOJ T3 and stomach tumours), as was dysphagia (83.3 vs 35%). However, anorexia was present in almost half of patients with disease in the stomach and GOJ Type 3. There was no significant difference in mean FAACT A/CS score across disease sites. Mean score for upper-middle oesophagus 30.2, lower oesophagus and GOJ T1/2 31.7 and for GOJ T3 and stomach 32.5, p 0.78. Dysphagia was less common in the stomach and GOJ and there was not a significant difference in proportions of stented patients between disease sites.

Anorexia was more common in the presence of dysphagia with 45/58 (78%) of patients with dysphagia being anorectic, compared to 22% having dysphagia and normal appetite, p 0.01. The relative patient numbers experiencing anorexia and dysphagia is shown in figure 2.4.

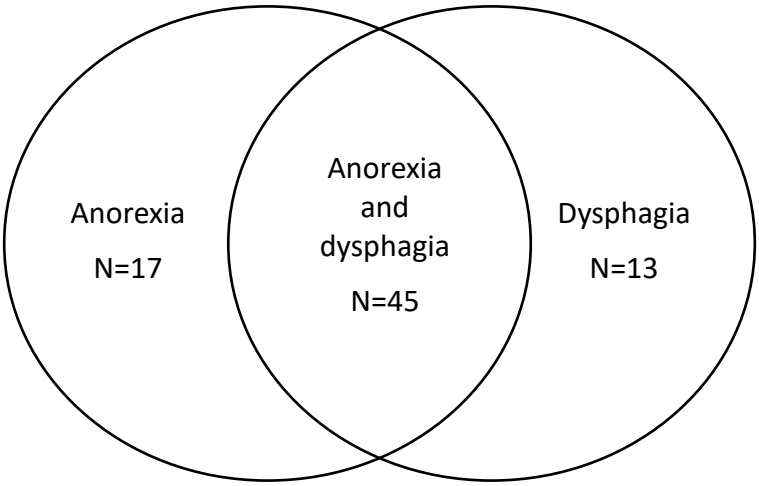

Figure 2.4: Venn diagram showing overlap of anorexia and dysphagia

Anorexia was common in patients reporting weight loss at baseline, present in 53/70 patients with anorexia scores reporting weight loss, 75.7% compared with 9/28 (32.1%) of patients with no weight loss p <0.01. To assess whether other symptoms could be contributing to anorexia at different disease sites the OG-25 score for food restriction, pain and reflux was also assessed by disease site (see table 2.3b). There were no significant differences between groups for mean scores.

Table 2.4a: Nutritional symptoms and stent prevalence by disease site

Disease site	**Anorectic**			**Dysphagia**			**Stented**		
	N	%	P	N	**%**	**p**	**N**	**%**	**p**
Oesophageal upper and middle third	8	66.6		10	83.3		4	33.3	
Oesophageal lower third, GOJ T1/2	35	76.1	0.02	34	73.9	<0.01	11	23.9	0.63
GOJ T3/Stomach	19	47.5		14	35.0		8	20.0	

GOJ = gastro-oesophageal junction

When assessed according to the presence of anorexia on Mann-Whitney testing, there was a significant difference in % weight lost prior to diagnosis, p 0.001, but no significant difference in OG-25 food restriction score p 0.71, or reflux score p 0.21

between anorectic and non-anorectic patients. There was a significant difference for pain scores, p 0.008. There was no difference in median values for HGS p 0.08, MUAC p 0.53 or NLR p 0.10 between anorectic and non-anorectic patients.

Table 2.4b: nutritional symptoms and stent prevalence by disease site

	Mean OG-25 food restriction score	p	**mean OG-25 reflux score**	p	**mean OG-25 pain score**	p	**Mean FAACT score**	p
Oesophageal upper and middle third	28.2	0.75	4.6	0.26	26.8	0.14	29.1	0.85
Oesophageal lower third, GOJ T1/2	35.7		16.6		17.7		31.8	
GOJ T3/Stomach	45.0		28.4		32.8		32.2	

GOJ = gastro-oesophageal junction, T = Type

2.4.5. Presence of cachexia

I identified patients with cachexia using the ESMO 2021 definition of cachexia of the presence of weight loss defined by 5% weight loss and systemic inflammation[29]. Based on the available information for this cohort, I defined this as an NLR of ≥3.

Cachexia was present in 74 patients at baseline. Anorexia was present in 67 of these patients (90.5%). Patients with cachexia had a higher mean PS and CFS (0.7 vs 1.3 and 2 vs 3 respectively). There were no marked differences in other physical fitness markers or OG-25 scores, in fact cachectic patients had better mean OG-25 scores for the key indicators assessed. Mean FAACT A/CS score was 27 for cachexic patients vs 36 for non- cachectic patients. The proportion of cachectic patients was similar between disease sites and adenocarcinoma and squamous cell carcinoma histologies. All patients with undifferentiated carcinoma had cachexia. Cachexia was present in 55% of patients with liver metastases, 43% of patients with peritoneal disease and 33% of patients with lymph node only disease.

2.4.6 Treatments received

Data on treatment received and treatment related outcomes was available in 158 patients, minimum follow-up 121 days. Median time from first assessment to cycle 1 of chemotherapy was 16 days (range 6-74).

The majority of patients were planned to receive oxaliplatin and capecitabine chemotherapy alone and 16.5% of patients were planned to commence cisplatin, capecitabine and trastuzumab.

Data on treatment received is detailed in table 2.4 (additional information on chemotherapy received is available in appendix 1, table 2.4b). Only 49.4% of patients completed all planned chemotherapy (74 patients receiving 6 cycles of chemotherapy and 3 receiving 4 planned cycles of FLOT), dose delays and reductions were common, occurring in 58.9% and 41.1% of patients respectively, 10.1% patients stopped treatment due to toxicity. Disease control rate (DCR) was 62.6% (if including only patients who received at least 1 cycle of chemotherapy, DCR was 69.2%), no assessment of response was available for 24.7% of patients.

Table 2.5 treatment received		
Cycles received N = 158	**N**	**%**
0	15	9.5
1	18	11.4
2	12	7.6
3	13	8.2
4	16	10.1
5	8	5.1
6	74	46.8
Not available	2	1.3
Reason treatment stopped N=156		
Complete	77	49.4
Toxicity	10	6.4
Disease progression	19	12.5
Clinical deterioration or other co-morbidity	19	12.2
Death	20	12.8
Declined pre-start	4	2.6
Died before start	7	4.5
Treatment tolerance N=143 (excluding patients never started)		
Dose delays/omissions	93	65.5
Dose reductions	65	45.8
Admission toxicity related	51	35.9
Admission disease related	43	30.9
Stopped due to toxicity	16	11.3
Best response N= 158		
PR	58	36.7
SD	41	25.9
PD	20	12.7
NA	39	24.7
PR = partial response, SD = stable disease, PD = progressive disease, NA = not available/applicable		

2.4.6 Change in nutritional factors on treatment.

Of 143 patients who received at least 1 cycle (C) of chemotherapy, mean weight change between baseline and cycle 1 was -0.2%. Weight loss was present in 42% of patients, 12.5% of patients lost ≥3% body weight between baseline assessment and cycle 1.

Of 111 patients who received at least 3 cycles of chemotherapy, 61.2% lost weight, with 34.2% losing ≥3% weight between C1 and C3. Mean weight loss between baseline and C3 was -1.7% (range -24.3% to 13.7%).

Of 74 patients who received 6 cycles of chemotherapy, 54.1% lost weight, with 36.5% losing ≥3% body weight. Mean weight change between baseline and C6 was -2.1% but mean weight change between C3 and C6 was +0.4% reflecting a pattern in some patients to lose weight initially and then gain.

FAACT A/CS score was available at mid-point for 65 patients and mean score was 35. FAACT A/CS score was available after 6 cycles of treatment for 39 patients and was 35.

Mean value for HGS post C3 was 26.0kg, mean change in HGS between baseline and C3 for 65 evaluable patients was -0.5kg (-1.5%), range -17.3kg - +10.6kg and mean change in MUAC was -0.8cm (-2.9%) range -5.3cm to +3.0cm. A gain in HGS was seen in 26 (40%) of patients.

In 39 evaluable patients mean value for HGS post C6 was 26.1kg, mean change in HGS between C3 and C6 was -1.8kg (-5.5%) range -6.4kg to +8.8kg, mean change in MUAC between C3 and C6 was -1.5cm (-2.4%) range -4.8cm to +3.9cm.

Mid-treatment OG-25 scores were available for 66 patients, end-of-treatment OG-25 scores were available for 41 patients. The majority of symptoms showed improvement in mean scores across treatment, particularly for dysphagia, eating restrictions, odynophagia, and anxiety. Worsening symptom scores were reported for dry mouth, taste, body image and cough. This data is shown in table 2.5 and figure 2.5.

Table 2.6 mean OG-25 scores and on-treatment change					
	Baseline mean	mid treatment mean N=66	mean change	End of treat mean N=41	mean change
Dysphagia	23.8	10.9	-12.9	14.1	-9.7
Eating restrictions	37.7	28.7	-9.1	29.1	-8.6
Reflux	20.7	15.6	-5.1	22.9	2.2
Odynophagia	29.6	14.3	-15.3	16.7	-13.0
Pain	24.7	20.5	-4.2	18.7	-6.0
Anxiety	70.8	51.8	-19.1	45.9	-24.9
Eating with others	18.8	12.8	-6.0	10.8	-8.0
Dry mouth	25.6	37.4	11.8	27.5	1.9
Taste	12.1	24.1	12.0	29.9	17.8
Body image	14.8	19.0	4.2	19.6	4.8
Swallowing saliva	11.1	5.1	-6.0	8.5	-2.6
Choking on saliva	10.8	6.7	-4.1	11.1	0.3
Coughing	21.5	22.5	1.0	25.2	3.7
Issues talking	5.4	6.7	1.2	6.0	0.5
Weight loss	29.9	28.7	-1.2	22.2	-7.7

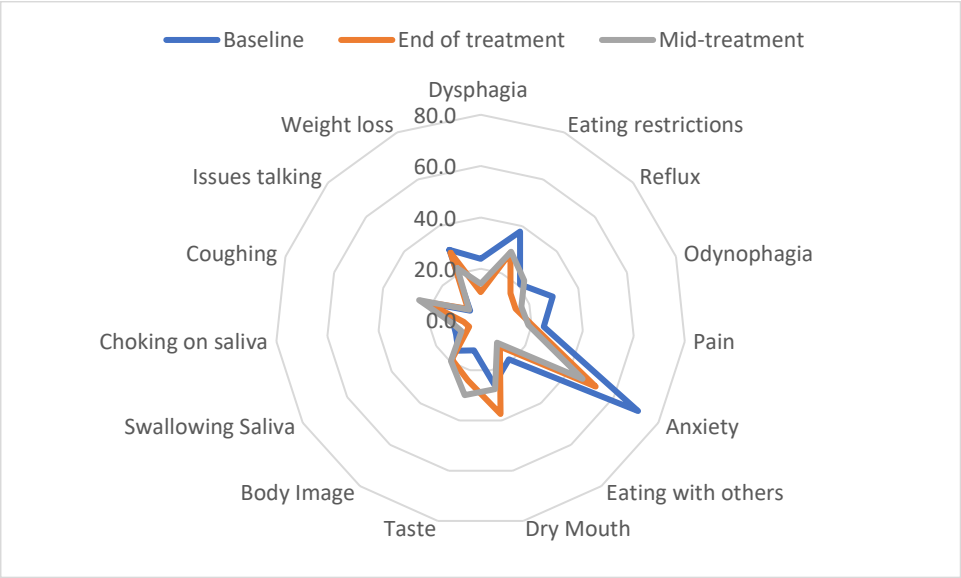

Figure 2.5: change in OG-25 scores on treatment, whole cohort

The improvement in scores could be related to a drop-off in collection in patients with worse scores, and potentially worse clinical condition overall, who were not well enough to attend for repeat tests. I therefore repeated the analysis using only the 41 patients who had results available for all time-points and this demonstrated similar results as shown in table 2.6 and figure 2.6.

Table 2.7 Change in OG scores – patients with all results available only N =41

	Baseline mean	mid treatment mean	mean change	End of treat mean	mean change
Dysphagia	21.0	7.6	-13.4	14.1	-6.9
Eating restrictions	33.7	20.7	-13.0	28.2	-5.5
Reflux	26.0	13.5	-12.5	22.9	-3.1
Odynophagia	27.6	12.2	-15.5	16.7	-11.0
Pain	26.0	19.8	-6.2	18.7	-7.3
Anxiety	69.5	47.3	-22.2	47.1	-22.4
Eating with others	14.6	11.7	-2.9	10.8	-3.8
dry mouth	23.6	43.2	19.6	27.5	3.9
Taste	10.6	23.4	12.8	29.9	19.3
Body Image	12.2	16.2	4.0	19.7	7.5
Swallowing saliva	11.4	3.6	-7.8	8.5	-2.8
choking on saliva	10.6	2.7	-7.9	11.1	0.5
coughing	21.1	26.1	5.0	25.2	4.1
issues talking	7.3	7.2	-0.1	6.0	-1.3
weight loss	21.9	22.5	0.6	22.2	0.3

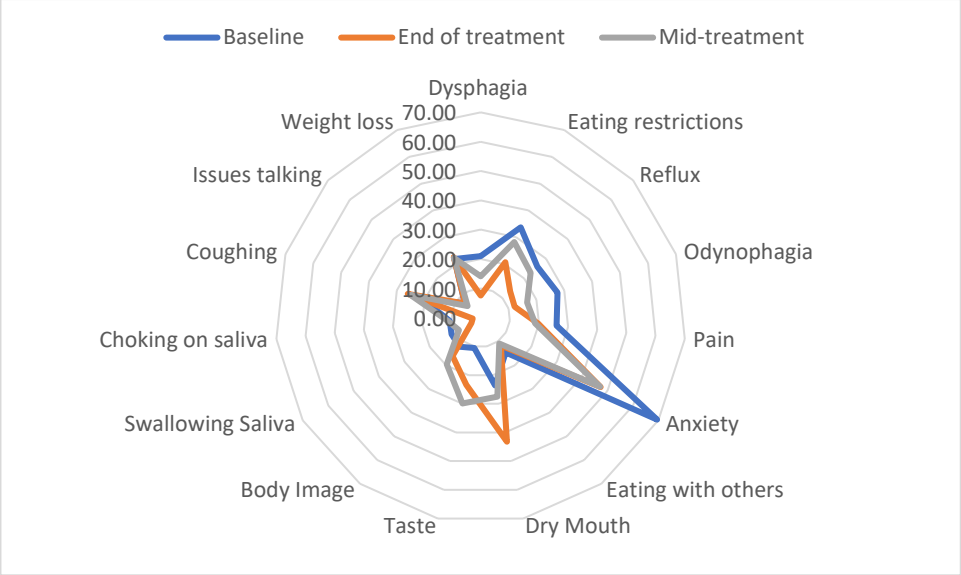

Figure 2.6: on-treatment OG-25 scores, cohort with all results available

2.4.7 Survival and toxicity outcomes

With a median follow-up time of 12 months, median progression free survival (PFS) for the whole cohort was 5.0 months (95% CI 4.2-5.9 months) from study recruitment (1st oncology visit). Median overall survival (OS) was 10.0 months (95% CI 7.2-12.8 months).

2.4.7.2 Association of nutritional factors with survival and treatment outcomes

Baseline weight loss and symptoms

Presence of weight loss at baseline was not associated PFS HR 0.86 (95% CI 0.56-1.34, p 0.86). This remained the case for baseline weight when assessed by ≥3%, ≥5% or ≥10% weight loss; HRs 1.11 (0.77-1.60, p 0.58), 1.30 (0.91-1.86, p 0.15) and 1.25 (0.88-1.79, p 0.22) respectively.

Patients with weight loss at baseline had reduced mOS compared to patients without, though this did not reach statistical significance except for the ≥5% threshold. For patients with ≥3% weight loss in the preceding 6 months mOS was 9 months vs 12 (HR 1.27, 95% CI 0.80-2.40, p 0.30), at 5% baseline weight loss this was 8 months vs 12 (HR 1.53, 95% CI 0.97-2.41, p 0.05) and at ≥10% weight loss 8 months vs 12 (HR 1.57, 95% CI 1.01-2.43, p 0.15).

Of patients who received at least 1 cycle of chemotherapy (n=143) 76.9% had at least one toxicity outcome. Baseline weight loss was also not associated with toxicity outcomes on logistic regression, for example weight loss ≥10% was not associated with admissions due to toxicity HR 1.44 (95% CI 0.71-2.92, p 0.32), dose reductions HR 0.66 (95% CI 0.34-1.30, p 0.23) or dose delays, HR 1.13 (95% CI 0.56-2.29, p 0.72). There was a trend to an association with cessation of chemotherapy due to toxicity, HR 3.36, 95% CI 0.91-12.35, p 0.07. Patients with ≥10% weight loss were more likely to receive a baseline dose reduction, 41.8% vs 28.6% p 0.07, which may have impacted on this association.

There was no significant association between baseline dysphagia and PFS HR 0.78 (0.54-1.13, p 0.17), nor with OS HR 1.29 (0.81-2.12, p 0.27).

Early on-treatment weight loss

Weight loss of ≥3% between baseline and C1 was not associated with PFS HR 1.30 (0.67-2.54, p 0.44), and was not significantly associated with OS, HR 1.75 (95% CI 0.91-3.36, p 0.09). Median survival for those with ≥3% weight loss between baseline and C1 was 8.0 months compared with 14 months without ≥3% weight loss (log rank p 0.09) shown in figure 2.7. Of 73 patients who received at least 1 cycle of chemotherapy and had at least 1 year of follow-up 2/9 (22.2%) who had ≥3% weight loss to cycle 1 were alive at 1 year, compared with 48.4% of patients with <3% weight loss, a difference of 26.2%.

Weight loss between baseline and C1 was not significantly associated with increased dose delays HR 2.62 (95% CI 0.54-12.66, p 0.23), dose reductions HR 0.69 (95% CI 0.20-2.31, p 0.54), admissions due to toxicity HR 1.9 (95% CI 0.57-6.39, p 0.29) or cessation of treatment due to toxicity HR 0.63, (95% CI 0.07-5.32, p 0.67).

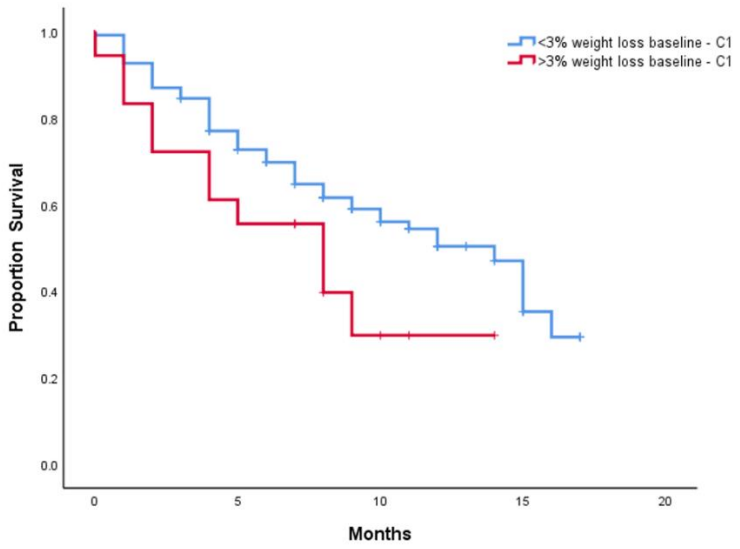

Figure 2.7: overall survival according to presence of 3% weight loss between baseline and cycle 1

On-treatment weight loss

The presence of ongoing weight loss between C1 and C3 was not associated with PFS, HR 1.25 (95% CI 0.79-1.98, p 0.34) nor with OS, HR 1.45 (95% CI 0.78-2.69, p 0.24). However, mean weight change between C1 and C3 was a larger decrease for patients whose best response to treatment was disease progression (PD) -2.8% vs -1.6% for patients with disease control (p 0.39) and more patients with ≥3% weight loss between C1-3 had PD as best response (21.6% vs 5.40% for those with no weight loss), p 0.06.

Anorexia

There was no significant association between the presence of anorexia as defined by a FAACT C/S score of ≤37 and PFS; HR 0.99 (95% CI 0.62-1.60, p 0.98), nor with OS, HR 1.89 (95% CI 0.92-3.92, p 0.09) shown in figure 2.8. However, severe anorexia, defined as FAACT C/S score ≤30, was associated with OS, HR 2.42, p 0.009. If the FAACT score was analysed as a continuous variable HR for OS was 0.98 (95% CI 0.96-1.01, p 0.30).

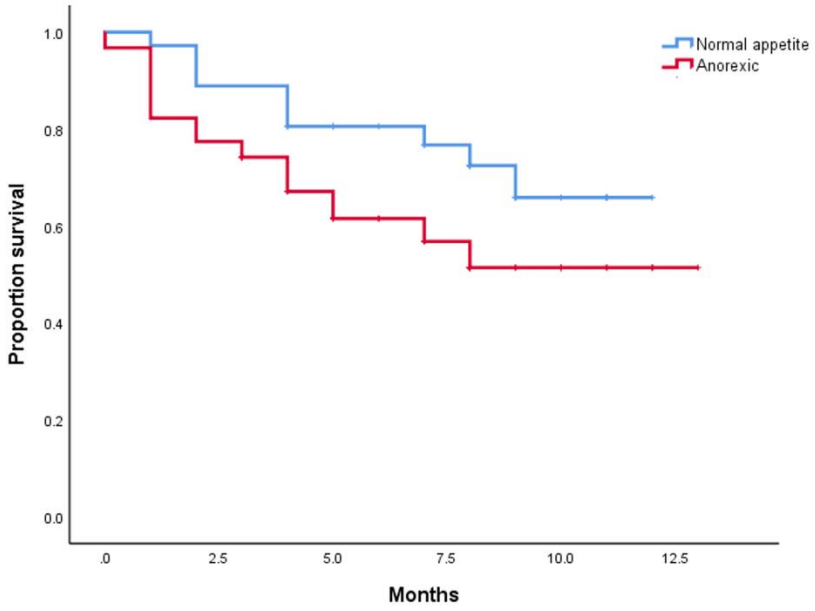

Figure 2.8: overall survival according to presence or absence of anorexia as assessed by FAACT C/S score

Fewer patients with anorexia had PD as best response 15.4% vs 18.2% for those with normal appetite, this did not reach statistical significance, p 0.42.

Of 92 patients with available FAACT C/S scores who received at least 1 cycle of chemotherapy, 57 (62.0%) had anorexia, and of these 80.7% experienced a toxicity outcome. Of 35 patients without anorexia, 62.8% received a toxicity outcome (p 0.09) and patients with anorexia were not significantly more likely to have a baseline dose reduction (33.3% vs 27.3%, p 0.64). On regression analysis anorexia was not associated with the presence of dose delays, HR 1.93 (95% CI 0.81-4.64, p 0.14), dose reductions HR 0.96, (0.41-2.24, p 0.92), admissions related to toxicity, HR 1.37 (95% CI 0.53-3.51, p 0.51), or cessation due to toxicity HR 1.37 (95% CI 0.53-3.51, p 0.51).

Anthropometric and strength measures

There was no significant correlation between MUAC and PFS HR 0.97, (95% CI 0.91-1.03, p 0.33) nor OS, HR 0.95, (95% CI 0.88-1.04, p 0.26).

No significant correlation was present between HGS and PFS, HR 0.99, (95% CI 0.97-1.02, p 0.64), nor in loss of grip strength between C1 and C3 HR 1.45 (95% CI 0.47-4.43, p 0.52). HGS at baseline was not significantly associated with OS; HR 0.97 (95%

CI 0.94-1.01, p 0.12) and change in HGS on treatment was not associated with OS; HR 1.17 (95% CI 0.33-4.17, p 0.80).

2.4.7.3 Correlation between biochemical factors and survival

Correlation between biochemical factors and OS is shown in table 2.7.

Neutrophil lymphocyte ratio was associated with PFS HR 1.07 (95% CI 1.03-1.12 p 0.002) and OS HR 1.07 (95% CI 1.02-1.12, p 0.01). CRP was available for 47 patients and mean value was 50.0 mg/L, it was not significantly associated with PFS HR 1.01 (95% CI 1.00-1.01, p 0.06) but was with OS HR 1.01 (95% CI 1.01-1.02, p 0.01).

On multivariate analysis no biochemical factor maintained a statistically significant association with overall survival.

Table 2.8 univariate analysis association biochemical factors and overall survival

	Sig.	HR	95.0% CI	
			Lower	Upper
Creatinine (µmol/L)	0.66	0.99	0.98	1.01
Sodium (mmol/L)	0.18	0.98	0.97	1.01
Albumin (g/L)	**<0.001**	0.90	0.86	0.94
NLR	**0.01**	1.06	1.01	1.12
Hb (g/L)	**0.02**	0.99	0.98	0.99

Hb = haemoglobin, NLR = neutrophil:lymphocyte ratio

2.4.8 Comparison of differences between anorectic and non-anorectic patients

Of 98 patients with FAACT C/S scores available, anorexia was present in 62 patients (63%). Comparison of clinical features between anorectic and non-anorectic patients is shown in table 2.8a and 2.8b.

Table 2.9a: comparison of anorectic vs non-anorectic patients					
	Non-anorectic N=36	**%**	**Anorectic N=62**	**%**	**Total**
Male	25	69.4	54	87.1	79
Female	11	30.6	8	12.9	19
mean age	67.9		63.4		
primary disease site					
Oesophagus upper third	0	0.0	3	4.8	3
Oesophagus middle third	4	11.1	5	8.1	9
Oesophagus lower third	7	19.4	21	33.8	28
GOJ T1	3	8.3	5	8.1	8
GOJ T2	1	2.9	9	14.5	10
GOJ T3	3	8.3	5	8.1	8
Stomach	18	50.0	14	22.6	32
Histology					
Adenocarcinoma	32	88.9	48	77.4	80
Squamous cell carcinoma	3	8.3	11	17.8	14
undifferentiated	1	2.8	3	4.8	4
Sites of metastatic disease					
liver	13	36.1	23	37.1	36
lymph node only	13	36.1	17	27.4	30
Peritoneal (including local lymph node)	4	11.1	6	9.7	10
Weight change					**P value**
No reported weight loss	19	52.8	9	14.5	0.001
Weight loss < 10%	8	22.2	18	29.0	0.39
Weight loss ≥ 10%	9	25.0	35	56.5	0.02
GOJ = gastro-oesophageal junction, T = Type					

Table 2.9b: Symptoms and clinical features					
	Non-anorectic N=36		**Anorectic N=62**		
	N	%	N	%	p
Dysphagia	13	36.1	45	72.6	0.058
PS 0-1	33	91.7	46	74.2	**0.04**
PS 2-3	3	8.3	16	25.8	
	Mean value		Mean value		P
Mean OG dysphagia score	22.5		22.3		0.86
Mean food restriction score	37.7		36.5		0.71
Mean reflux score	21.2		18.2		0.21
Mean pain score	31.0		19.7		**0.008**
mean HGS (males only) *kg*	28.4		29.4		0.57
Mean MUAC (males only) *cm*	30.0		29.2		0.36
Mean STS *seconds*	12.5		11.7		0.31
Mean CFS	2		3		0.009
ACE-Comorbidity score	0.9		0.9		0.44
	N	**%**	**N**	**%**	**p**
Smoker	3	8.3	15	24.2	**0.05**
Alcohol	15	41.7	28	45.2	0.48
PPI	23	63.9	30	48.4	0.58
	Mean value		**Mean value**		**p**
Albumin	42.5		41.3		0.18
Sodium	136.1		137.6		0.97
Creatinine	78.1		75.2		0.6
CRP	28.1		55.9		0.58
Haemoglobin	129.2		123.4		**0.03**
Neutrophil lymphocyte ratio	4.3		5.7		0.16

HGS = hand grip strength, kg = kilograms, MUAC = mid-upper arm circumference, cm = centimetres, STS = sit to stand test, PS = performance status, CFS = clinical frailty scale, PPI = proton pump inhibitor, CRP = c-reactive protein

Of 40 patients with stomach and GOJ T3 cancer, 14 had dysphagia and 26 had no dysphagia. Of dysphagic patients 12/14 were anorectic.

I compared baseline factors between anorectic and non-anorectic patients with gastric and GOJ T3 cancer and no dysphagia (N=26) to try to identify any other relevant factors to anorexia in this group. There were no significant differences noted other than a higher proportion of smokers in the anorectic group, 6/9 (66%) and these differences are summarised in table 2.9. There were no gender differences, p 0.42.

Table 2.10: comparison of characteristics for patients with T3 GOJ or Stomach cancer and no dysphagia, comparing between anorectic and non-anorectic patients

Values	No anorexia N=17	%	Anorectic N=9	%	p
Liver metastases	6	60.0	3	33.3	0.64
Lymph node only metastases	5	50.0	2	22.2	0.54
Peritoneal/ local lymph node only	2	20.0	2	22.2	0.43
HER-2 positive	2	20.0	1	11.1	0.70
Mean age at diagnosis	68.9		58.1		0.07
Current Smoking	1	10.0	5	55.6	**0.01**
Current Alcohol use	7	60.0	3	33.3	0.47
PPI use	9	80.0	6	66.7	0.40
Mean CRP	31.9		83.4		0.27
NLR >3	12	90.0	7	77.8	0.54
Mean NLR	4.6		4.3		0.75
Mean Hb	124.2		117.2		0.49

HER-2 = human epidermal growth factor receptor-2, PPI = proton pump inhibitor, CRP = c-reactive protein, NLR = neutrophil:lymphocyte ratio, Hb = haemoglobin

2.4.9 Grouping patients

To try to further characterise patients by nutritional status I grouped patients from the ANCHOR cohort (N=98) into 3 groups as shown in figure 2.9. Patients with “no malnutrition, symptom low” were defined as those with no weight loss and no dysphagia or anorexia. Patients with “nutritional symptoms, or malnutrition” were defined as those with either weight loss (either <5% or without inflammation),

dysphagia or anorexia, and patients with “cachexia” those with ≥5% weight loss and a NLR ≥3.

Patients with no malnutrition, symptom low N= 15	Patients with nutritional symptoms or malnutrition but not cachexia N=34	Patients with cachexia N=49

Figure 2.9: patient grouping

Median follow-up for this cohort is 12 months at the time of analysis. Survival for the 3 cohorts is shown in figure 2.10. The difference in survival by group was significant by log rank analysis, p 0.006, median survival was not reached for the first two groups and 8 months for the group with cachexia. There is no significant survival difference between the symptom low group and nutritional symptom group.

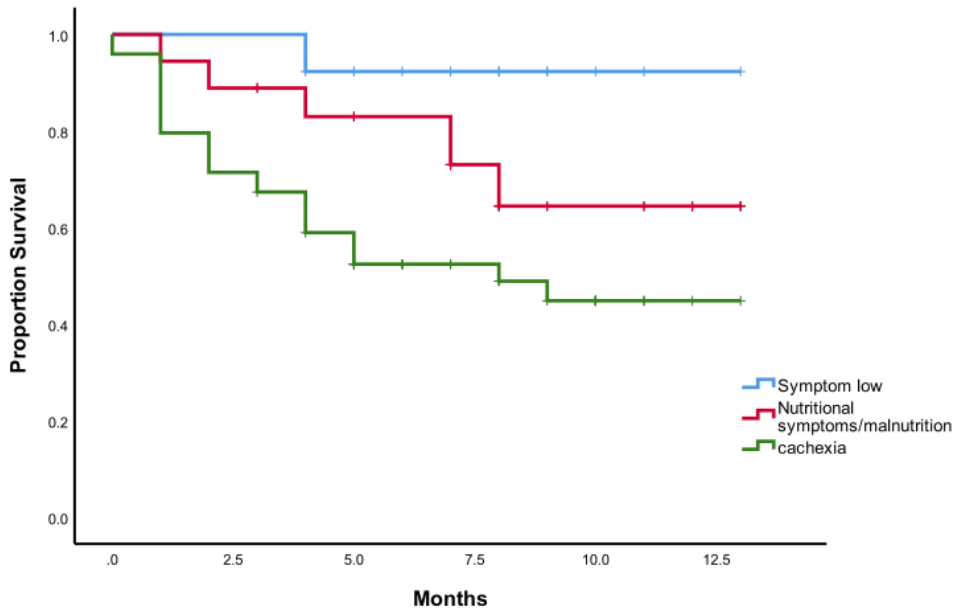

Figure 2.10: overall survival according to patient group

In order to query the impact of inflammation in this cohort, I split the group of “nutritional symptoms or early malnutrition” but without meeting cachexia definitions

into those with malnutrition/risk and an NLR ≥3 compared to those without. Patients with features of malnutrition but no inflammation appear to have poorer OS than those with inflammation, log rank across groups p 0.01 as shown in figure 2.11.

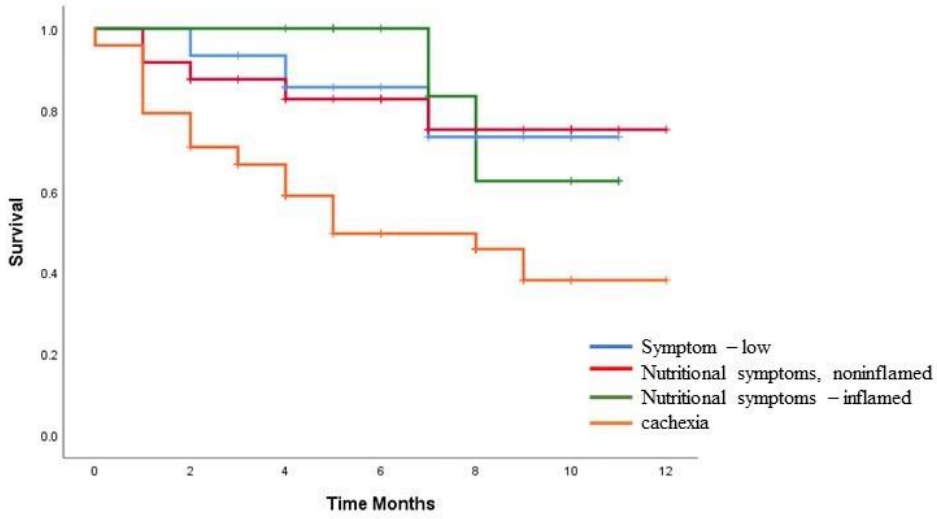

Figure 2.11: overall survival according inflammation status

2.5 Discussion

2.5.1 Prevalence of weight loss and anorexia

This prospectively collected cohort of patients with upper GI cancers demonstrates that anorexia, malnutrition, and cachexia are common in patients with upper GI cancers.

The cohort represents typical demographics of patients with upper GI cancers, predominantly male and older. It confirms that weight loss is common at baseline, and is frequently severe, with 70% of patients having lost ≥10% weight at baseline and some patients having lost nearly one third of their body mass. Anaemia was common, present in 41% of patients and systemic inflammation was present in 71% of patients.

Anorexia was present in 63% of patients at baseline. In this cohort anorexia was more common in the upper GI tract, but it remained present in nearly half of patients with cancers of the stomach or GOJ T3.

Dysphagia was also more common in patients with tumours of the oesophagus compared to stomach and there appears to be significant overlap of dysphagia and anorexia. However, there were patients with anorexia in the absence of dysphagia, suggesting that mechanical obstruction alone may not account for all anorexia. Only 13 patients were stented at baseline, and all of these patients reported anorexia. Anorexia in the absence of dysphagia was more common in patients with stomach tumours. It may be that mechanical effects do impact in this group as well, due to local mass effect triggering vagal nerve signalling, or delayed gastric outlet obstruction. Alternatively, it could be that a different mechanism contributes to anorexia in this group. As discussed in the introduction, there is evidence to suggest that inflammation may contribute to anorexia, however, as assessed by an NLR of ≥3, inflammation was present in many patients with no anorexia. Other possible mechanisms of anorexia will be considered further in the next chapter, but it may be that NLR is not affected by inflammatory cytokines that impact on appetite.

Multiple factors can impact upon appetite. Smoking, a recognised appetite suppressant was more common in a small subset of patients with no dysphagia but anorexia, though this would not account for all of the anorexia noted in the cohort. It is recognised that anxiety and low mood can impact on appetite. Anxiety scores on the OG-25 scale were

high at baseline and improved for those available at mid and end points, as did FAACT A/CS scores. However, this may be impacted by selection bias, as there was significant drop-out.

Cachexia, defined by the presence of 5% weight loss and systemic inflammation defined by a NLR of ≥3 was present in half of patients and was more common in patients with liver metastases as is well recognised. Survival was poorer for patients with cachexia. Analysed as a continuous variable NLR was strongly associated with survival in this cohort, CRP was only available in a limited number of patients and was not significantly associated with survival, hence the choice of NLR to define systemic inflammation. The decision to use 3 as a cut-off for significant systemic inflammation was based on existing data [318], however various cut-points have been used [319] and selecting a higher value may have had more sensitivity.

Existing criteria for malnutrition [320] use the phenotypic criteria of ≥5% weight loss. I decided to investigate weight loss at multiple cut-points, as existing data demonstrates that ≥3% weight loss between baseline and 1st cycle of chemotherapy is associated with poorer survival [184].

Weight loss during treatment was common, more so at mid-point (65%) than at end of treatment (55%). This was despite patients having dietician support through treatment. Compliance with nutritional advice and support was not monitored, which could account for some of this ongoing weight loss. It may that anti-cancer therapy can accelerate muscle loss contributing to weight loss on therapy. Mean weight loss was greater for patients who had progressive disease as their best response to treatment, p 0.05, suggesting that ongoing weight loss may represent a biomarker for disease activity, but with limited sensitivity.

2.5.2 Correlations of nutritional symptoms with toxicity and survival

Weight loss did not significantly correlate in this cohort with survival, both at baseline and on treatment, with the exception of ≥5% weight loss at baseline. In other cohorts weight loss of ≥ 3% between baseline and C1 has been significantly associated with poorer OS but was not in this cohort. This was previously significantly associated until the addition of more patients with shorter follow-up, and so this is a finding that may

change with data maturation. Equally weight changes were not associated with treatment toxicity. This is in contrast to other data [180], though this study defined malnutrition via weight change calculated against albumin. Albumin levels did correlate with poorer survival in multivariate analysis in my cohort.

Whilst there was clear trend towards improved survival for patients with no anorexia, this did not reach statistical significance. The survival data for the cohort is immature, with median follow-up time for the ANCHOR cohort of patients being 9 months, therefore this significance may change with time. In previous retrospective work from our institution, a very marked difference in survival was noted between non-anorectic and anorectic patients, with all non-anorectic patients living for at least 1 year from baseline. In this prospective cohort, whilst median survival was not reached for patients without anorexia, there have been survival events in this cohort suggesting that this very marked survival difference is not present here.

Patients with poorer nutritional status could be expected to find chemotherapy more challenging. Patients with anorexia had a higher rate of toxicity compared to those without, 80.7% vs 62.8% of non-anorectic patients. However, on regression analysis in this cohort neither anorexia nor weight loss was associated with treatment toxicity, which was prevalent within the cohort. Again, as only just over half of patients completed all chemotherapy, and nearly 20% received either 0 or 1 cycles of treatment, further data collection may elucidate these relationships more clearly.

Anorexia scores, where available for all 3 timepoints, improved on average, as did all OG-25 markers, with the exception of dry mouth and taste, both of which are impacted by chemotherapy.

2.5.3 Patient grouping

The aim of this work was to better characterise the nutritional status of patients and identify those who do better, and those may who benefit from support. A small cohort of patients had no evidence of malnutrition at baseline, and neither of the key symptoms of anorexia of dysphagia, yet they had similar survival to patients with nutritional symptoms.

The cohort of patients with no evidence of malnutrition had predominantly stomach tumours (11/15) but there were no other clear indicators to select them out. There was a mix of localised and metastatic disease including liver metastases, there were a range of ages, including some very young patients and some patients did have other symptomology, such as reflux or pain, evidenced by OG-25 scores.

Small numbers of patients present with minimal gastro-intestinal symptoms. In my experience these are often older patients, whose investigations were often commenced in response to iron deficiency anaemia. This may suggest a different disease biology. There is limited data in the literature to classify this group. Data exists comparing patients with alarm symptoms to those who presented with "simple dyspepsia" without alarm symptoms. These studies have demonstrated that alarm symptoms which include signs of advanced disease such as weight loss, GI bleeding and abdominal masses, were associated with poorer prognosis [321, 322]. Anaemia was considered an alarm symptom in these studies and so this doesn't fit with the group above. Other data suggests anaemia is associated with poorer prognosis [323].

There are some patients with the presentation of anaemia but no other GI symptoms within the small cohort above (6/15 had anaemia), but as already detailed, there are also patients who have other symptoms. This suggests that good nutritional status may be the significant protective contributing factor, supported by the clear reduction in survival seen in patients with or at risk of malnutrition but without cachexia.

The differing magnitude of impact of nutrition vs inflammation upon outcomes is not clear from this data. Patients with cachexia, based on ≥5% weight loss and inflammation had clearly poorer OS than those with good nutritional state or with nutritional symptoms not meeting cachexia definition. Surprisingly, time to first event was longest in the cohort with nutritional symptoms and a high NLR. It is not clear from this data how the impact of malnutrition and inflammation intersect to influence outcomes. However, sub-groups are very small, and definite conclusions could not be based on these numbers.

2.5.4 Limitations

The main limitations of this work are that numbers are relatively small. This means that sub-group analyses are of limited power. Furthermore, there was no pre-specified plan for sub-group analyses, which could have introduced bias. However, as part of the work was aiming to identify signals to guide recruitment in the rest of the study, this is something that will be addressed as recruitment continues.

There is a moderate amount of heterogeneity within the cohort, particularly in terms of treatments received. There was significant drop-off in data availability at mid-point and end-of treatment for the FAACT C/S and OG-25 scores and anthropometrics. This is due in part unavoidable loss to follow-up but also missing data. However, as this is only an initial analysis of ongoing work it provides a foundation and direction for ongoing research.

2.5.6 Future directions

The ultimate aim of this work was to better characterise the patterns of anorexia, weight loss and cachexia in patients with upper GI cancers, with a longer-term view to aid optimal management.

This initial analysis of data from the ANCHOR trial gives the beginnings of a picture, but further data collection will be vital to more strongly elucidate the subgroup patterns present. What is clearly demonstrated is that, unfortunately, outcomes for patients with upper GI cancers remain poor, particularly for those with cachexia.

There was a 25% difference in proportion of patients alive at 1 year with ≥3% weight loss between baseline and cycle 1, but with nearly 7x as many patients not experiencing this weight loss. Power calculations show that we will need to recruit 240 patients to confirm this effect.

Given poorer survival for patients with evidence of risk or presence of early malnutrition compared to those without, it would suggest that aggressive nutritional management may be of benefit. There is some existing evidence to show that nutritional support can improve outcomes in patients with advanced upper GI cancers [324].

A key focus of ongoing data collection in ANCHOR will be investigating the impact of stenting in these patients. Responses to stents in terms of ability to eat are variable, and so patients may have had a degree of ongoing dysphagia. Existing data suggests 60% of patients achieve eating some solids but not full diet after stenting [325].

There is limited data about the impact of stenting on appetite. Data exists using the EORTC OES-18 questionnaire, which includes 3 questions that investigate appetite specifically, and with the EORTC QLQ-C30 which includes an appetite loss symptom scale. These show that post stenting patients report an improvement in appetite but are still reporting moderate appetite loss symptoms [326, 327]. Anecdotally, many patients report a desire to eat but an inability to do so due to dysphagia. Others report having no appetite until their stent and a marked improvement since. Therefore, elucidating this relationship formally may help identify those whose anorexia is predominantly due to mechanical obstruction, compared to others where different mechanisms may be at play. I aimed to investigate mechanisms underlying anorexia in patients with GOJ and gastric cancer further, and this is discussed in the next chapter.

The relationship and intersection between inflammation and nutritional factors remains to be further elucidated. As discussed in my introduction, definitions of cachexia have varied over time, but with weight loss and inflammation generally accepted to be key, as discussed extensively. In an ideal world, we would be able to identify patients who just need nutritional support versus those who need an additional treatment for the cachexia, or alternatively patients with pre-cachexia who may benefit from a more aggressive treatment. However, from the limited data I have so far this remains unclear. The cut-off of ≥5% weight loss is arbitrary and with further data collection I hope to be able to elucidate patterns of malnutrition and cachexia in more detail.

On another note, some definitions of cachexia have included criteria such as refractory to anti-cancer treatment or refractory to nutritional support. Patients within this cohort who met the current ESMO definition of cachexia did experience responses to therapy, and to nutritional support, including quite marked weight gain in some patients. The patients who were refractory to both cancer treatment and nutritional support represent a much smaller sub-cohort.

Finally, none of the nutritional factors investigated in this cohort so far correlated with toxicity. This is in contrast to some other studies. In chapter 4 I will present data looking at two aspects of sarcopenia, one, is how well it correlates to physician fitness assessments, but the other is how it correlates to toxicity and inflammation.

3. Investigating the gut hormone profile of patients with cancer anorexia

3.1 Introduction

As discussed in the introduction, the pathogenesis of cancer anorexia is multifactorial and still not comprehensively understood. It likely reflects a complex combination of paraneoplastic metabolic processes, mechanical obstruction, psychological factors, and dysregulation of various molecular pathways of immunity and inflammation.

The central nervous system plays a key role to appetite regulation [328, 329]. The hypothalamus controls food intake by responding to various neuronal, mechanical, and hormonal afferent stimuli that receives from the periphery. The other important components of this circuit are the enteroendocrine cells (EEC) which are found in the intestinal mucosa. They detect various nutrients in the gut lumen and respond to them with the secretion of peptides and hormones. Established agents that participate in this gut-brain axis are CCK, insulin, leptin, ghrelin, GLP-1, amylin, PP and PYY [330, 331].

It is recognised that the EEC activity is enhanced in some inflammatory bowel disease (e.g. Crohn's) and this influences appetite via the gut-brain axis signalling resulting in early satiety [164].

Many cancer patients exhibit evidence of systemic inflammation at diagnosis, as pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α can be produced both by tumour cells, as well as from the host response to the tumor [332, 333]. This increase in cytokines is strongly implicated in producing anorexia [334].

The interplay between gut hormones and cytokines in the development of cancer anorexia is incompletely understood.

The most studied hormones in patients with advanced cancer are leptin (produced by adipose tissue) and ghrelin. However insufficient evidence exists to suggest they play a significant role. As might be expected in a low-adiposity state, it has been demonstrated that ghrelin levels are raised in patients with lung cancer and cachexia [154].

Data on the other gut hormones in patients with cancer is limited. One study of patients with advanced cancer investigated CCK levels and found no difference between anorectic and non-anorectic patients in circulating levels [134]. Some data support the role of PYY in cancer anorexia, in children with ALL and cancer anorexia PYY levels were raised at baseline compared to healthy controls and increased further in response to chemotherapy, before finally returning to baseline levels. However, another study demonstrated no differences in PYY levels between patients with cancer and cachexia, patients with cancer but no cachexia and a group of age, gender, body mass index (BMI) and race matched controls [163].

3.2 Study aims and hypothesis

Patients were recruited within the ANCHOR trial, as detailed in chapter 2. The aim of this part of the study is to characterise the gut hormone and cytokine profile in cancer patients (pre-prandial and postprandial), identify possible differences between patients with anorexia and those who with normal appetite, and healthy controls, and establish any possible contribution of EEC activity in cancer anorexia.

3.2 Hypothesis

Our hypothesis is that pro-inflammatory cytokines produced by the tumour can not only affect appetite directly through the vagal and the central melanocortin system but also indirectly though enhanced EEC activity.

3.3 Study objectives and outcomes

Study objectives are as follow:

To characterise the gut hormone and cytokine profile in patients with upper GI cancer (pre-prandial and postprandial), identify possible differences between patients suffering with anorexia and those who with normal appetite, and establish any signal that enhanced EEC activity could be acting as a contributing factor in cancer anorexia. In the long term, identification of the pathophysiology of the hormonal alterations in cancer patients may help identify possible pharmaceutical targets for the cancer anorexia.

3.4 Study outcomes

Primary outcome: To characterise the gut hormone and cytokine profile in cancer patients

Secondary outcomes:

- Identify any correlation between the patients' hormonal and cytokine profile with their nutritional state and future weight loss rate.

3.5 Statistical power

Due to the exploratory nature of this pilot study, formal power calculations were not done. The study was powered on pragmatic lines, since it is designed to provide baseline data, with which to power a larger study should initial results demonstrate a signal. However, using reported population means and standard deviations [335], the sample size of 10 patients per group would allow detection of a difference of 40%, with 80% power in PYY – the gut hormone of most interest.

In case the study was unable to recruit sufficient patients with anorexia for the two-group comparison, the power to detect correlations between the FAACT A/CS scale and gut hormone levels was calculated. A sample of 10 patients with anorexia would be able to detect a correlation coefficient of 0.60 (α 0.05 and β 0.20).

Total sample size = $N = ((Z_\alpha+Z_\beta)/C)^2 + 3 = 10$.

3.6 Study inclusion

Inclusion criteria for the ANCHOR study are detailed in chapter 2. Additional inclusion and exclusion criteria for the participants in this part of the study, cohorts C and D are listed below.

Inclusion Criteria: Cohort C

1. Patients must have GOJ or gastric adenocarcinoma
2. Patients must be able and willing to fast for 8-10 hours

3. Patients in the anorexic group must have completed the FAACT A/CS questionnaire and scored ≤37 in total score **and** ≤ 2 in the appetite specific question
4. For inclusion in the non-anorexic group patients must have completed the FAACT A/CS questionnaire; the total score should be > 37

Patients not receiving chemotherapy, i.e., receiving surveillance or best supportive care were also invited to cohort C following a study amendment. As this is a single time-point assessment it was felt this would not impact on the results.

Inclusion criteria: Cohort D - healthy controls

1. Participants must be 18 years of age or above
2. Participants must be able and willing to fast for 8-10 hours
3. Participants must be able to understand the study information given to them and be willing to give consent for trial participation

Participants must have completed the FAACT A/CS questionnaire and the total score should be > 37 **and** ≥3 for the appetite specific question

Healthy controls were selected to age (within 5 years) and sex match anorexic patients within cohort C.

Exclusion Criteria Cohort C & D: all patients and healthy volunteers

1. Symptoms of dysphagia of any cause, oesophageal or gastric obstruction (assessed via medical history/O'Rourke score). Patients with O'Rourke score >2 will be excluded to try and limit heterogeneity
2. Presence of oesophageal stent or any other kind of feeding aid (nasogastric tube, nasoduodenal tube, gastrostomy, jejunostomy)
3. Presence of brain metastases or any kind of brain tumour including benign pituitary adenomas that could have an independent impact on anorexia
4. Histological diagnosis of neuroendocrine tumour, or mixed tumour.
5. Previous gastro-duodenal surgery due to altered gut hormone secretion
6. History of Inflammatory Bowel Disease (Ulcerative colitis, Crohn's disease) due to presence of local inflammation
7. History of Coeliac disease

8. History of endocrine disease (insulin dependent Diabetes mellitus, Thyroid disease, Cushing's) due to altered gut hormone secretion
9. Significant past or present eating disorder e.g., anorexia nervosa, bulimia nervosa due to potential bias
10. Current active infection (general or intestinal) as this could impact on inflammatory cytokines
11. Chronic use of immunomodulatory drugs that could impact on inflammatory cytokines (steroids, immunosuppressant drugs, recent short-term use of corticosteroids would require a two-week washout period prior to study assessments)
12. Chronic use of NSAIDS or aspirin (periodic use can be accepted) as this could impact on inflammatory cytokines
13. Patients with pacemakers (contraindication for BIA)
14. Allergy to any of the ingredients of the meal test or unwillingness to consume the particular meal (Heinz Chicken soup or Heinz Mushroom soup)

3.7 Study assessments

I undertook all study assessments. On a scheduled appointment, each participant arrived after an overnight fast from 10 pm, for a test meal study. A cannula was placed in the antecubital fossa (cephalic vein/basilic vein/median cubital vein) to facilitate blood withdrawal.

A first sample of 20mls was taken for baseline **pre-prandial** measurement (time 0). A sample of 15ml of blood was taken to analyse glucose, HbAc1, CRP, gut hormones (ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP and glucose) and a major cytokine TNF-α. A further 5ml was taken to complete the whole biochemistry profile (see below). Participants were then be given a test meal of 290g Heinz Cream of Chicken soup or Cream of Mushroom, according to patient choice. This was selected as it is a weak stimulus to gut hormones in healthy controls [336]. The meal was consumed using a mug with a spoon, within a 10-minute period. After the meal was completed serial blood samples were taken at 15, 30, 45, 60, 90, 120 minutes, these samples were of up to 5ml volume, giving a maximum total blood donation of 50ml. These samples were tested for **postprandial** levels of the abovementioned gut hormones, excluding the biochemistry samples. Participants were also asked to indicate their level of appetite on

a visual analogue score (VAS), at 0 (baseline-fasting), 15, 30, 45, 60, 90, 120 minutes. Participants were afterwards be invited to consume an ad libitum meal and the amount taken recorded and asked to complete a 24-hour food intake diary. Study schema is shown in figure 3.1

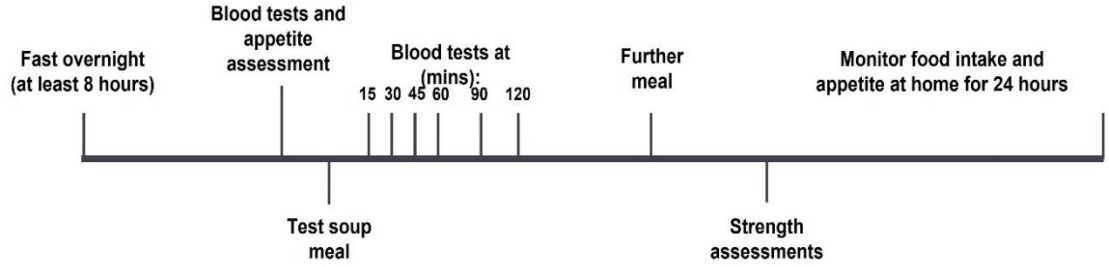

Figure 3.1: study schema

Baseline Biochemistry and Metabolic Assessment

On the initial blood sample (time 0, fasting) albumin, LDH, electrolytes (sodium (Na), potassium (K), phosphate (Ph), calcium (Ca), magnesium (Mg)), vitamin D, urea, creatinine, haemoglobin, folic acid, ferritin, vitamin B12, thyroid stimulating hormone (TSH), and C-reactive protein (CRP) were assessed for completion of the metabolic profile of each patient.

Strength and body composition assessments including hand grip strength, sit-to-stand test and mid-upper arm circumference were undertaken as detailed in chapter 2 if not already complete.

Study assessments were selected to coincide with times when patients would be having routine clinical bloods collected, usually just prior to the commencement of chemotherapy. Time points of bloods collected, and samples taken are shown in table 3.1.

Table 3.1: sample timings	
Time point	Samples
T0	**Hb and biochemical profile:** urea, creatinine albumin, LDH, electrolytes sodium, potassium, phosphate, calcium, vitamin D, Magnesium, thyroid stimulating hormone, free T4 and CRP, glucose, HbA1c, folic acid, iron studies ferritin, vitamin B12. **Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
T+15	**Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
T+ 30	**Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
T+ 45	**Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
T+ 60	**Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
T+ 90	**Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
T+ 120	**Gut hormones**: ghrelin, insulin, GLP-1, PYY, pancreatic polypeptide, GIP, glucose **Cytokines**: TNF-α
LDH = lactate dehydrogenase, GLP-1 = glucagon like protein-1, PYY = peptide YY, GIP = gastric inhibitory peptide, CRP = c-reactive protein, Hb= haemoglobin, HbA1c = glycated haemoglobin, TNF-α = tumour necrosis factor alpha.	

3.6 Sample processing

All samples were collected by me. All blood samples were collected through a peripheral cannula. Cannulas were kept patent by flushing with non-heparinized saline (0.9% sodium chloride; Becton Dickinson, New Jersey, USA) following each sample collection.

Blood samples were collected by syringe into serum separator vacutainers (Becton Dickinson, Plymouth, UK) following withdrawal of 4-5 mL of blood to clear the catheter extension.

For gut hormone samples 50µl of DPP-IV inhibitor (Merck Millipore Limited, UK) to prevent rapid inactivation of GLP-1 and 50 µl of Pefabloc (Roche Diagnostics Limited, UK) were added to prevent the degradation of active GLP-1 by DPP-IV and acylated ghrelin by protease (10 µl/mL of whole blood for both inhibitors as recommended by the manufacturers).

Samples were then kept on ice until centrifugation. Blood samples were centrifuged (Z400K, Hermle, Germany) at 1500**g** for 15 min at 4ºC. Following this, plasma was pipetted and samples were stored at -20°C within 2 hours, to allow for batching of sample processing.

Standard baseline tests including full blood count (FBC) and common biochemistry tests for electrolytes, kidney function (urea, creatinine) liver function tests (alkaline phosphatase (ALP), gamma-glutamyl transferase (gGT), aspartate tranferase (AST), bilirubin) and CRP were performed as routine in The Christie's laboratory, as part of the standard practice for these patients.

Samples analysed at Manchester Metropolitan University were transported on ice to the laboratory by car using triple layer packaging in line with packing instruction 650. Samples to be analysed externally were labelled with a study identifier, and date of birth and timepoint. Samples to be processed internally were labelled according to trust policy. Sample location was tracked on a study database stored on secure NHS servers within a password protected database.

Hormone and cytokine analysis

Unfortunately, due to supply issues secondary to the covid-19 pandemic, 4 patients underwent testing without DPP-IV added to the collection tubes and so total GLP-1 was analysed instead of active GLP-1.

I conducted the gut hormone analysis, with the support from a post-doctoral researcher with significant experience in ELISA. I contributed to all stages of the process which allowed me full understanding of the process.

Concentrations of insulin, total GLP-1, total GIP, PYY, pancreatic polypeptide, GIP, acylated ghrelin and the inflammatory cytokine TNF-α were determined using ELISA using a validated, commercially available human gut hormone multiplex assay (Milliplex MAP, Merck Millipore Ltd, UK). Serum glucose concentration was determined by manual assay using the glucose oxidase phenol 4-aminoantipyrine peroxidase (GOD-PAP) method on a semi-automated clinical chemistry analyser (Misano; Randox Laboratories Ltd, UK).

Quality controls, assay buffer, wash buffer, matrix solution, and immobilised beads solutions were prepared as per protocol. Analyte details were inputted in the software to

permit analysis. Samples were arranged vertically as indicated a well map and all samples were pipetted in duplicate. Seven serially diluted standards were used to automatically generate a seven-point standard curve. Filter plates were blocked by pipetting 200µl of Assay Buffer into each well of the microtitre plate. Plates were then sealed and mixed on a shaker for ten minutes at room temperature. The assay buffer was removed by vacuuming (<100mmHg). Any excess assay buffer was removed from the bottom of the plate by blotting onto an absorbent pad. Then 25µl of assay buffer was added to the zero standard (background) and sample wells, and 25µl of each standard and control were added into the appropriate wells. Twenty-five µl of sample plasma was then pipetted into the sample wells and 25µl of appropriate matrix solution added to the background, standards, and control wells. Following this 25µl of mixed assay beads was added to each well. Plates were then then sealed, covered with aluminium foil, and incubated with agitation on a plate shaker (600 rpm) for 16 hours at 4°C. After overnight incubation, the plates and reagents were allowed to warm to room temperature. Fluid was removed by vacuuming. The plates were then washed three times with 200µL/well of 1X wash buffer, removing the wash buffer by vacuum filtration between each wash. Next 50µl of detection antibody cocktail was pipetted into each well. The plate was sealed, covered with aluminium foil, and incubated with agitation on a plate shaker for 30 minutes at room temperature then 50µl of Streptavidin-phycoerythrin (SAPE) was added to each well containing the 50µl of detection antibody cocktail. The plates were sealed again, covered with aluminium foil, and incubated with agitation on a plate shaker for 30 minutes at room temperature. The contents were then gently removed by vacuum. Subsequently, the plate was then washed three times with 200µl/well 1X wash buffer, removing the wash buffer by vacuum filtration between each wash.

Finally, 100µl of sheath fluid was added to the wells. Plates were re-sealed, covered with aluminium foil and the beads were then re-suspended on a plate shaker for five minutes. The plate was then read on the Luminex instrument and results analysed.

3.8 Statistical analysis

Statistical analysis was undertaken using SPSS (IBM, version 25, 2017). Shapiro-Wilk normality tests demonstrated non-gaussian distribution for all hormones except for

TNF-α. Data followed a skewed, log- normal distribution and therefore was log-transformed for analysis with parametric tests.

Differences between mean values between groups were performed using ANOVA. Correlations were assessed using Pearson's correlation tests and between group comparisons by Tamhane's post-hoc test.

3.9 Results

3.9.1 Recruitment

Participants were recruited from October 2021 to May 2022. Ten patients with normal appetite were recruited, 7 with anorexia and 5 healthy controls, of whom 3 were analysed as part of this initial analysis. Only 3 healthy volunteers were analysed due to analysis kit availability.

Recruitment was slightly slower than initially expected. This was felt to be due to a reduction in number of referrals overall to the department because of the covid-19 pandemic.

3.9.2 Demographics

Demographic features of participants are shown in table 3.2. There was a similar distribution of disease sites between GOJ and gastric between the non-anorectic and anorectic groups and similar range of sites of metastatic disease. Patients with GOJ tumours, Siewert type 2 or 3, were only recruited if there was clear evidence of disease extension into the stomach on radiological imaging.

There were slightly more women in the non-anorectic group (3 women, 7 men, 30% female, vs 1 woman, 6 men in the anorectic group 14% female) and mean age was higher for NA patients, 69 years (range 43-88) than A patients, 60 years (34-80), and HVs, mean age 52 years (29-57). Otherwise, distribution of disease sites and stages was similar between groups. The proportion of patients experiencing dysphagia symptoms was slightly higher in the anorectic group.

Table 3.2: Demographic Features

study number	**Cohort**	**Age**	**PS**	**CFS**	**Current Smoking**	**Current Alcohol use**	**PPI use**	**Number comor-bidities**	**O'Rourke score**	**FAACT A/CS score**	**BMI**	**% Weight change**	**MUAC (cm)**	**HGS (kg)**	**STS (s)**
1	NA	71	0	2	No	No	0	2	1	38	28.6	-7.2	31.5	26.5	18.4
2	A	78	0	1	No	No	0	3	2	29	22.7	-16.1	26.5	27.5	12.3
3	NA	55	0	1	No	Yes	1	0	1	48	24.3	0.0	29.3	38.6	7.1
4	NA	43	0	1	No	No	1	0	1	44	22.8	0.0	26.8	20.2	9.6
5	A	34	0	1	No	No	0	0	1	25	24.0	-11.5	30.0	43.2	10.0
6	NA	66	0	1	No	Yes	0	2	1	42	29.1	-2.4	31.0	32.8	9.3
7	A	63	0	1	No	Yes	1	2	2	16	27.0	-9.7	33.3	33.8	6.1
8	NA	69	0	1	No	Yes	0	1	1	44	20.7	0.0	26.5	31.7	10.3
9	A	80	1	2	No	No	1	2	1	36	27.0	-6.0	26.2	21.0	17.6
10	A	60	0	1	Yes	Yes	1	0	1	27	27.3	-3.1	33.3	50.1	5.5
11	A	50	0	1	Yes	Yes	1	1	2	21	26.5	-10.9	31.8	36.6	.
12	NA	76	1	2	No	No	0	2	2	45	30.9	-7.3	32.5	20.6	13.6
13	NA	84	0	1	No	Yes	0	1	1	45	26.5	0.0	29.5	12.6	11.1
14	A	56	1	2	No	No	0	2	1	28	40.6	-19.4	38.3	27.8	11.8
15	NA	77	0	1	No	Yes	1	1	1	42	27.8	0.0	31.0	33.2	13.2
16	NA	62	1	2	No	No	0	3	1	46	23.7	0.0	29.0	18.4	15.5
17	NA	88	0	1	No	No	0	2	2	41	30.7	0.0	30.2	24.6	14.0
18	HV	57	0	1	No	Yes	0	0	1	46	23.2	0.0	29.0	31.4	10.4
19	HV	70	0	1	No	Yes	0	0	1	43	23.5	0.0	29.3	36.1	13.1
20	HV	29	0	1	No	Yes	0	0	1	46	26.0	0.0	31.4	44.7	10.2

NA = Non Anorectic, A= Anorectic, HV = Healthy Volunteer, PS = performance status, CFS = clinical frailty scale, PPI = proton pump inhibitor BMI = body mass index, MUAC = mid-upper arm circumference, HGS = hand grip strength, kg = kilograms, STS = sit to stand test (seconds)

Mean baseline FAACT A/CS score was 43.5 for non-anorectic patients, 26 for anorectic and 45 for healthy volunteers.

Mean BMI was similar between groups 26.5 for non-anorectic patients compared with 27.9 for anorectic patients and 24.2 for HVs. Mean weight loss was higher as would be expected for anorectic patients 10.7kg vs 1.4 kg.

3.9.3 Baseline biochemistry

Baseline biochemistry results are shown in table 3.3a and 3.3b, p value given is ANOVA with between group comparison by Tukey's honestly significant difference (HSD) test shown in brackets. Statistically significant differences were seen for iron levels, transferrin saturations, magnesium, and vitamin B12 between groups but no other comparators.

Table 3.3a baseline biochemistry comparison between groups

Marker (normal range)	Mean	Standard deviation	P value ANOVA (Tukey's HSD)
Hb g/L (120-165)			
NA	125	11.6	0.09 (0.08 NA vs HV)
A	130	12.5	
HV	144	9.1	
NLR			
NA	4.8	2.5	0.17
A	4.3	1.4	
HV	2.1	0.3	
CRP mg/L (<5)			
NA	9.5	9.8	0.45
A	19.0	30.8	
HV	0.0	0.0	
Sodium mmol/L (133-146)			
NA	140.6	1.3	0.06 (**0.05** NA vs A)
A	138.1	2.1	
HV	139.3	1.2	
Urea mmol/L (2.5-7.8)			
NA	5.6	1.6	0.96
A	6.1	2.3	
HV	5.9	2.6	
Creatinine umol/L (44-97)			
NA	93.6	32.9	0.19
A	70.6	11.2	
HV	88.7	17.2	

NA = non-anorectic A= anorectic, HV = healthy volunteer, HB = haemoglobin, NLR = neutrophil:lymphocyte ratio, CRP = c-reactive protein, AST = aspartate transferase

Table 3.3b baseline biochemistry comparison between groups			
	Mean	Standard deviation	P value ANOVA (Tukey's HSD)
Bilirubin µmol/L (0-20)			
NA	7.9	1.6	0.47
A	17.3	28.1	
HV	18.0	8.8	
AST IU/L (0-33)			
NA	31.0	10.3	0.52
A	80.9	149.6	
HV	35.0	7.0	
Albumin g/L (35-50)			
NA	42.7	2.3	0.89
A	42.7	4.3	
HV	43.7	1.5	
Mg mmol/L (0.7-1.0)			
NA	0.9	0.1	**0.05** (0.06 A vs HV)
A	0.8	0.1	
HV	0.9	0.1	
T4 pmol/L (10-22)			
NA	15.4	1.7	0.56
A	16.2	2.9	
HV	20.0	1.6	
Ferritin µg/L (22-322)			
NA	97.4	122.6	0.47
A	206.8	314.7	
HV	54.3	3.2	
Iron µmol/L (12-31)			
NA	9.3	4.4	(**0.01 NA vs HV, 0.03 A vs HV**)
A	10.0	2.8	
HV	17.6	2.5	
Transferrin saturation % (<55_			
NA	18.0	8.8	**0.05** (0.04 HV vs NA)
A	20.8	6.6	
HV	31.7	4.5	
HBA1c mmol/L (25-36)			
NA	36.2	7.0	0.81
A	38.1	3.8	
HV	36.7	6.5	
Vitamin B12 ng/L (211-911)			
NA	410.0	127.1	**0.03** (0.03 NA vs A)
A	1171.0	877.6	
HV	376.3	93.0	
Folate µg/L (>5.4)			
NA	12.2	5.2	0.57
A	9.7	7.2	
HV	8.7	2.0	
Vitamin D nmol/L (51-249)			
NA	52.9	23.1	0.88
A	53.6	30.9	
HV	61.0	6.9	

NA = non-anorectic A= anorectic, HV = healthy volunteer, Mg= magnesium, HbA1c = glycated haemoglobin

3.9.4 Gut hormone and cytokine values

GIP

Median values for anorectic patients showed a lower baseline value to NA patients and HVs, 28.5pg/ml vs 41.3pg/ml and 50.1pg/ml respectively, values across time points shown in figure 3.2. They rose to a lower peak at 30 minutes than the other groups 271.7pg/ml vs 350.4pg/ml and 449.0pg/ml respectively, though proportionately the rise for A patients was similar to others, with a 9.5 fold increase above baseline compared to an 8.5 fold increase for NA patients. The time to peak was the same as in HVs, though NA patients should a slightly early peak at 15 minutes.

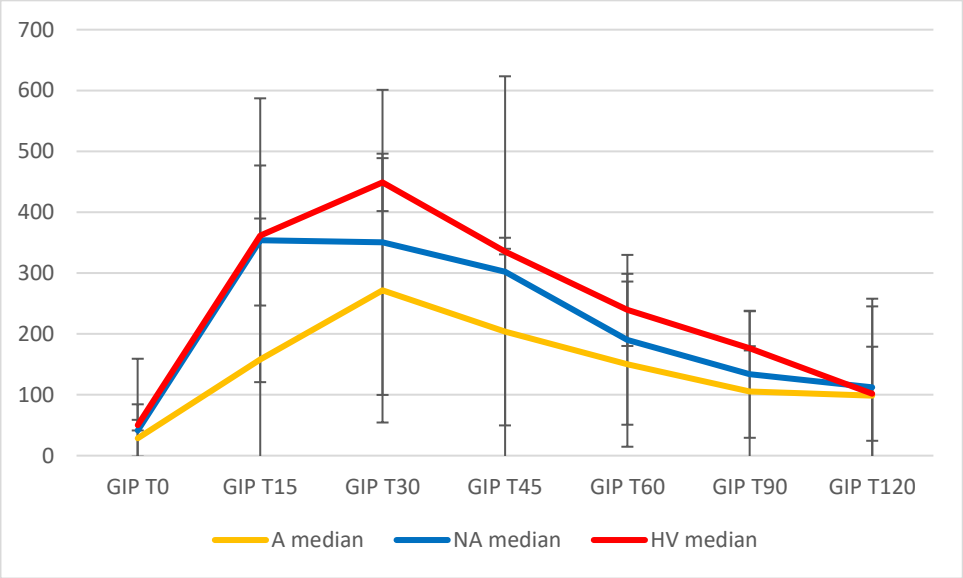

Figure 3.2: Median values (pg/ml) by group at each time-point GIP. A = anorectic, NA = non-anorectic, HV = healthy volunteer

These differences did not reach statistical significance as shown in table 3.4, full tables are available in appendix 2.

Table 3.4: GIP group comparisons – baseline and peak							
	(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
GIP T0	A	NA	-0.1	0.2	0.96	-0.75	0.54
		HV	-0.1	0.2	0.90	-0.78	0.51
GIP T30	A	NA	0.1	0.3	0.98	-0.61	0.80
		HV	-0.3	0.1	0.23	-0.63	0.14

GLP-1

Values for total GLP-1 at baseline were similar between groups, 139.1pg/ml compared to 156.1pg/ml for NA patients and 121.7pg/ml for HVs. These results are shown in figure 3.3.

Values for both NA and A patients peaked at 30 minutes, with values for A patients being numerically lower than for NA patients; 214.1pg/ml vs 276.3pg/ml. The increase above baseline was 54% for A patients and 76% for NA patients. HV patients showed a bi-modal peak at 15 minutes and then a 2nd peak at 90 minutes.

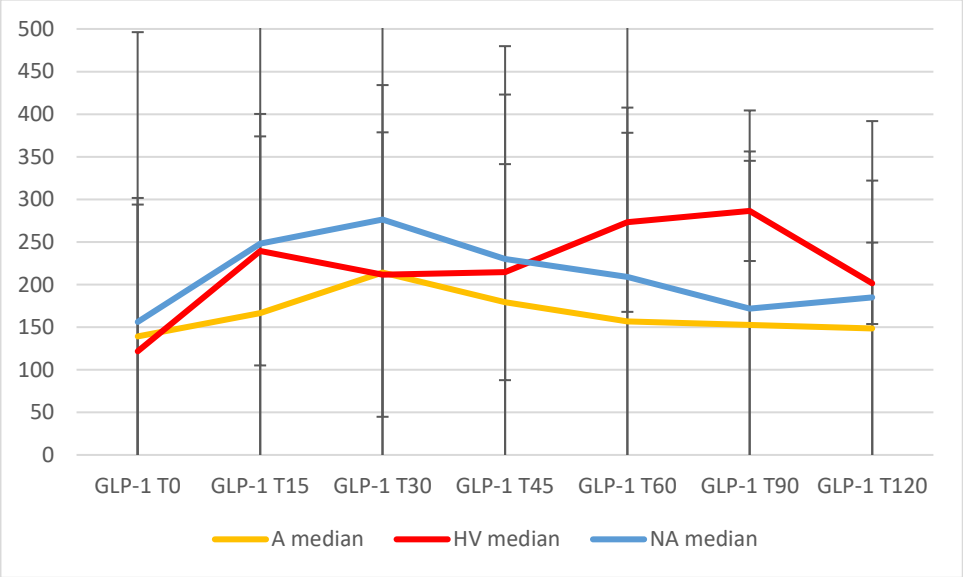

Figure 3.3: Median values (pg/ml) by group at each time-point GLP-1. A = anorectic, NA = non-anorectic, HV = healthy volunteer

Differences between groups did not reach statistical significance as shown in table 3.5.

Table 3.5: GLP-1 group comparisons – baseline and peak							
	(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
GLP T0	A	NA	0.0	0.2	1.00	-0.42	0.42
		HV	0.1	0.2	0.99	-1.00	1.12
GLP T30	A	NA	-0.1	0.2	0.90	-0.54	0.34
		HV	-0.1	0.2	0.95	-0.68	0.50

Insulin and glucose

Baseline median values for insulin were lower in anorectic patients than NA patients and HVs, 341.7pg/ml vs 707.0pg/ml and 551.2pg/ml respectively. This did reach statistical significance as shown in table 3.6.

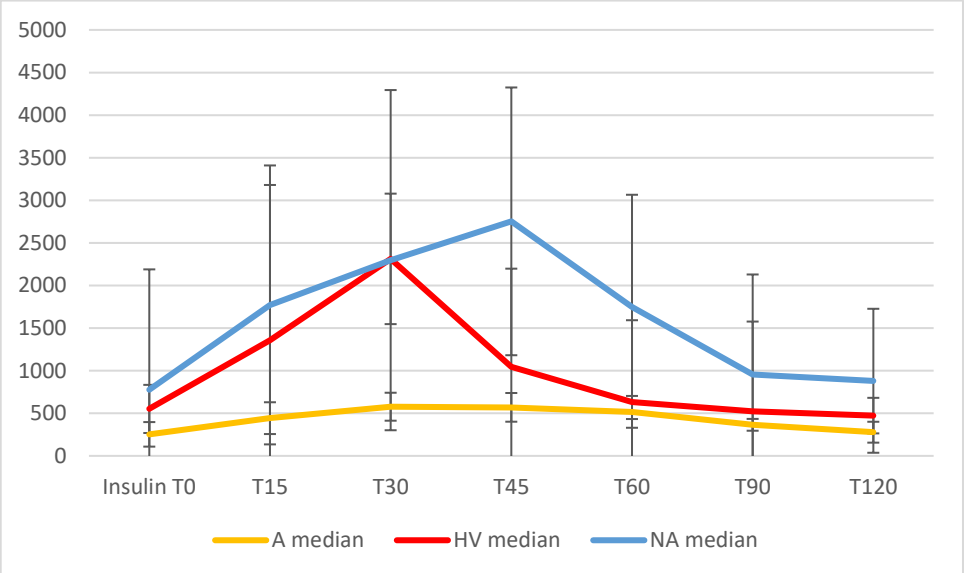

Figure 3.4: Median values by group at each time-point insulin. A = anorectic, NA = non-anorectic, HV = healthy volunteer

A patients showed a blunted response to insulin, with a small peak noted at 30 minutes of 599.2pg/ml, a 75% increase above baseline, compared to NA patients whose insulin levels peak at 45 minutes with a value of 1758.1pg/ml, an increase of 249% above

baseline, and HV patients whose levels peaked also at 30 minutes, with a value of 1046.3pg/ml, an increase of 90% above baseline as shown in figure 3.4.

Table 3.6: Group comparisons Insulin – baseline and peak

	(I) Group	(J) Group	Mean Difference	Std. Error	p	95% CI Lower Bound	95% CI Upper Bound
Insulin T0	A	NA	-0.5	0.2	**0.04**	-0.95	-0.02
		HV	-0.4	0.2	0.19	-1.05	0.25
Insulin T45	A	NA	-0.4	0.1	**0.03**	-0.78	-0.03
		HV	-0.3	0.2	0.41	-1.35	0.66

The blunted insulin effect seen in A patients did not translate to an increase in glucose levels as shown in figure 3.5. Glucose levels for A patients were very similar to NA patients at baseline, 5.2 mM/L and 5.3mM/L respectively, rising to a small peak at 45 minutes in both groups of 7.2mM/L and 6.9mM/L respectively.

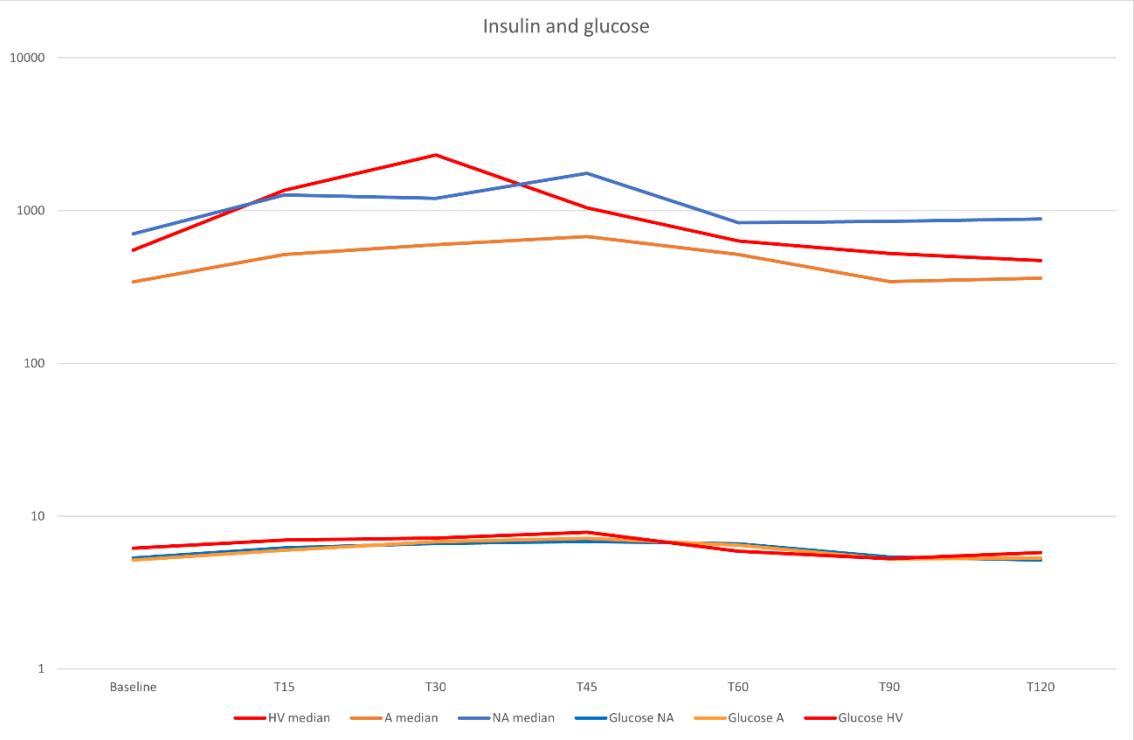

Figure 3.5: Median values by group at each time-point insulin. A = anorectic, NA = non-anorectic, HV = healthy volunteer

Pancreatic polypeptide

Median levels of pancreatic polypeptide were lower at baseline for A patients compared to NA patients and HVs, with values of 35.4pg/ml compared to 150.2pg/ml and 105.7pg/ml respectively.

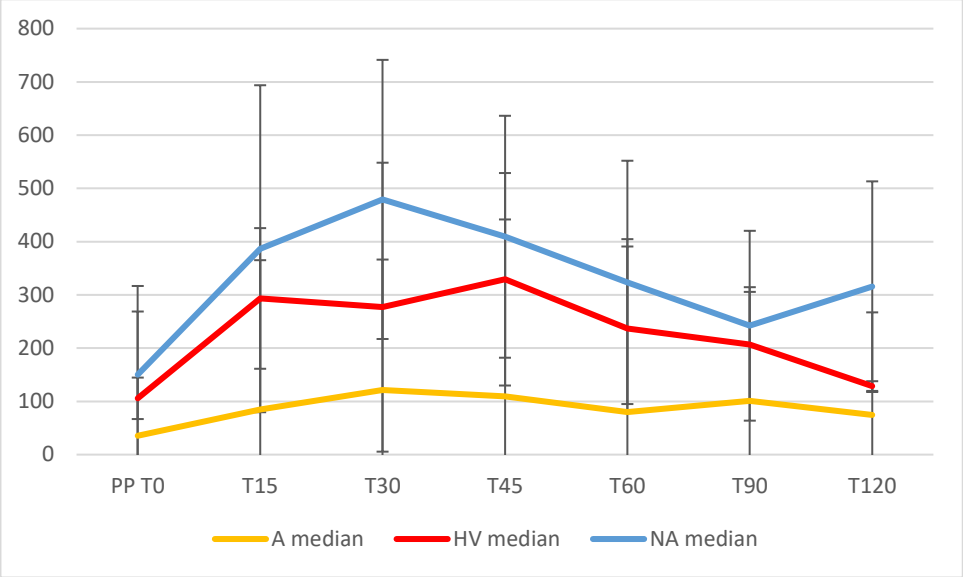

Figure 3.6: Median values by group at each time-point pancreatic polypeptide (PP). A = anorectic, NA = non-anorectic, HV = healthy volunteer

Median pancreatic polypeptide levels were lower in anorectic patients than in non-anorectic and healthy controls, this did not reach statistical significance. Levels demonstrated a blunted peak at 30 minutes (shown in figure 3.6), the same time as NA patients and slightly early than HVs which, as shown in table 3.7, did not reach statistical significance.

Table 3.7: PP Group comparisons

	(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
PP T0	A	NA	-0.3	0.3	0.65	-0.99	0.43
		HV	-0.3	0.2	0.64	-0.93	0.42
PP T30	A	NA	-0.3	0.2	0.38	-0.80	0.23
		HV	-0.3	0.2	0.42	-1.01	0.35

The proportions of rise in values were similar between groups. Median values for A patients at 30 minutes were 121.4pg/ml, a rise of 342% compared to 479.2pg/ml for NA patients, a rise of 319% and 329.3pg/ml at 45 minutes for HVs, a rise of 312%.

Peptide YY

Levels of PYY were higher at baseline for A patients and showed a more significant rise than in HVs and NA patients as shown in figure 3.7.

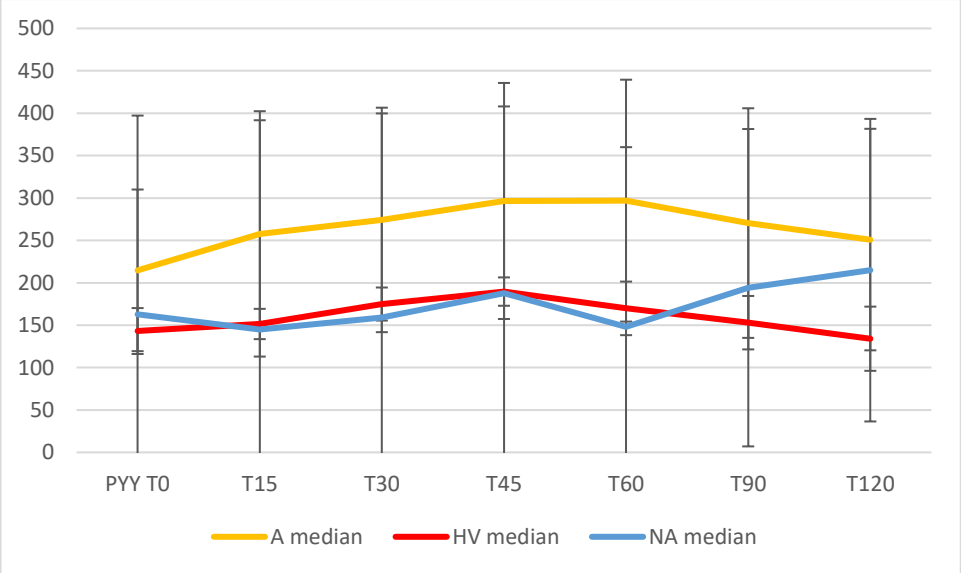

Figure 3.7: Median values by group at each time-point PYY. A = anorectic, NA = non-anorectic, HV = healthy volunteer

Median baseline value for A patients was higher NA patients; 214.69pg/ml compared to 162.9pg/ml for NA patients and 143.1pg/ml for HVs. Time to peak was 45 minutes for all groups, with more blunted peaks for NA patients and HVs, and at this time point PYY for A patients was 296.5pg/ml a rise from baseline of 38%, compared to 187.9pg/ml, a rise of 15% for NA patients and 189.6pg/ml, a rise of 33% for HVs. These differences did not reach statistical significance as shown in table 3.8.

At peak time of 45 minutes for A patients PYY was 58% higher for A patients than for NA. It should be noted that multiple samples failed analysis for PYY, with only 13/20 baseline values available. Therefore, to confirm whether this was a true finding the area under the curve (AUC) was calculated for PYY samples.

Table 3.8: PYY group comparisons							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PYY T0	A	NA	0.1	0.2	0.93	-0.44	0.65
		HV	0.1	0.1	0.87	-0.38	0.58
PYY T45	A	NA	0.0	0.2	1.00	-0.58	0.56
		HV	0.0	0.2	1.00	-0.59	0.58

Mean AUC for A patients was 25161, compared to 19190 for NA patients and 16684 for HVs. The AUC was 31% higher for A patients than for NA patients. AUC values for all hormones and cytokines are shown in table 3.9.

Table 3.9: Area under the curve values for each group				
	NA	A	HV	p
GIP	1607.5	1248.1	1612.7	0.57
GLP-1	1860.2	1615.1	1588.7	0.67
Insulin	9344.0	2785.8	8507.7	**0.04**
Glucose	36.0	36.2	40.7	0.18
PP	2085.9	1399.8	1684.3	0.46
PYY	19190.9	25161.0	16684.7	0.74
TNFa	63.3	73.6	56.4	0.38

TNF-α

TNF-α levels were 33% higher at baseline for A patients than for NA and remained raised across time-points as shown in figure 3.8. Baseline values for A patients were 12.3pg/ml compared to 9.2pg/ml for NA patients and 10.3pg/ml for HVs. This did not reach statistical significance. There was no peak in values for either group, this would not be expected for an inflammatory cytokine, no significant differences were noted at any time point, as shown in table 3.10.

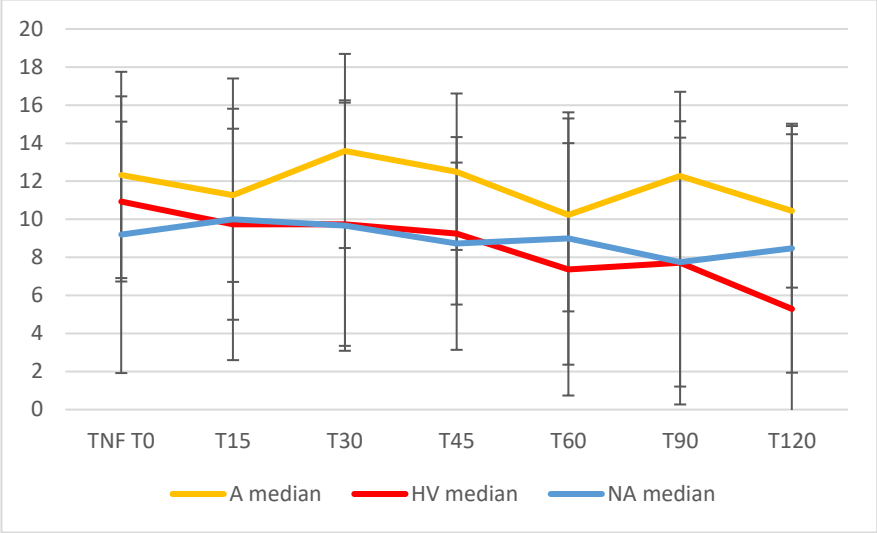

Figure 3.8: Median values by group at each time-point TNFa. A = anorectic, NA = non-anorectic, HV = healthy volunteer

Table 3.10: TNF-α group comparisons							
	(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
TNF T0	A	NA	0.0	3.1	1.00	-8.28	8.28
		HV	2.2	3.2	0.89	-8.93	13.36
TNF T30	A	NA	1.4	2.8	0.95	-6.26	9.02
		HV	1.6	4.2	0.98	-17.69	20.83

Visual analogue scores

Visual analogue appetite scales followed a similar pattern for all 3 groups, with a drop at 15 minutes, remaining low to 30 minutes and gradually increasing to T120 as shown in figure 3.9. As would be expected appetite scores were lowest for anorectic patients, and slightly higher for NA patients than for HVs.

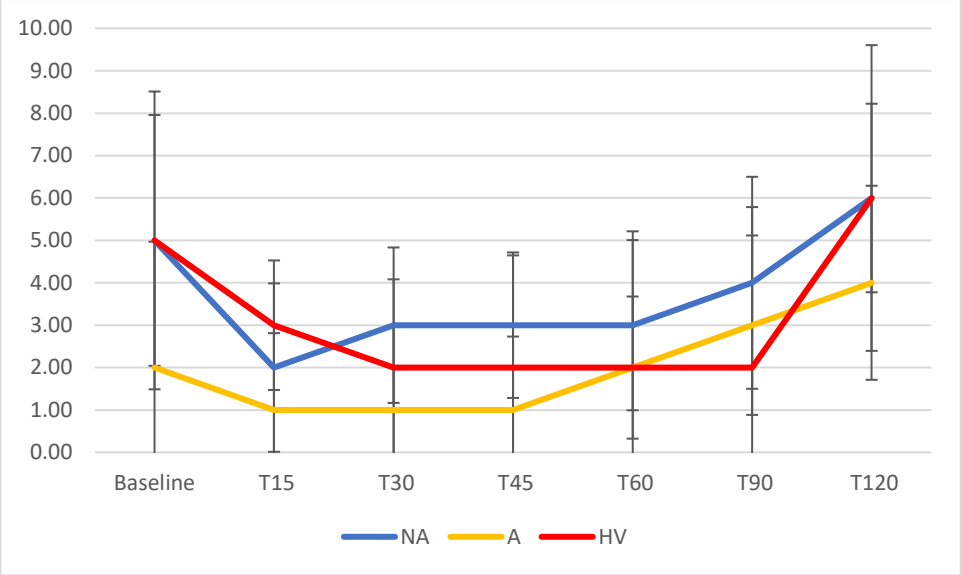

Figure 3.9: Median values by group at each time-point visual analogue scale appetite score. A = anorectic, NA = non-anorectic, HV = healthy volunteer

3.9.5 Correlations of gut hormone and cytokine values

Correlations of baseline gut hormone values with TNF-α levels are shown in table 3.11. These were significantly correlated for PYY, GLP-1, PP and insulin, with the strongest correlation being between TNF-α and PYY and TNF-α and GLP-1.

Table 3.11: Correlations between cytokines and gut hormones			
		PYY	TNF-α
PYY	Pearson Correlation	1	0.72
	Sig. (2-tailed)		**0.006**
	N	13	13
		TNF-α	PP
PP	Pearson Correlation	1	0.45
	Sig. (2-tailed)		**0.045**
	N	20	20
		TNF-α	Insulin
Insulin	Pearson Correlation	0.44	1
	Sig. (2-tailed)	**0.071**	
	N	18	18
		TNF-α	GIP
GIP	Pearson Correlation	0.18	1
	Sig. (2-tailed)	0.449	
	N	20	20
		TNF-α	GLP1
GLP1	Pearson Correlation	0.70	1
	Sig. (2-tailed)	**0.001**	
	N	20	20
GLP-1 = glucagon like protein-1, PYY = peptide YY, GIP = gastric inhibitory peptide, TNF-α = tumour necrosis factor alpha.			

3.9.6 Correlations of cytokines and gut hormones with appetite scores

Correlations of baseline cytokines and gut hormones with the FAACT A/CS score are shown in table 3.12. None of these reached statistical significance except for insulin which showed a positive correlation. TNF-α and PYY showed negative correlations with the FAACT A/CS score.

Table 3.12: Correlations between baseline gut hormone and cytokine results with FAACT A/CS score			
		FAACT A/CS score	PYY
FAACT A/CS score	Correlation Coefficient	1.0	-0.17
	Sig. (2-tailed)	.	0.57
	N	20	13
		FAACT A/CS score	PP
FAACT A/CS score	Correlation Coefficient	1.0	0.31
	Sig. (2-tailed)	.	.180
	N	20	20
		FAACT A/CS score	Insulin
FAACT A/CS score	Correlation Coefficient	1.0	0.55
	Sig. (2-tailed)	.	**0.02**
	N	20	18
		FAACT A/CS score	GLP1
FAACT A/CS score	Correlation Coefficient	1.0	0.02
	Sig. (2-tailed)	.	0.93
	N	20	20
		FAACT A/CS score	GIP
FAACT A/CS score	Correlation Coefficient	1.0	0.34
	Sig. (2-tailed)	.	0.15
	N	20	20
			TNF-α
FAACT A/CS score	Correlation Coefficient	1.0	-0.08
	Sig. (2-tailed)	.	0.73
	N	20	20
GLP-1 = glucagon like protein-1, PYY = peptide YY, GIP = gastric inhibitory peptide, TNF-α = tumour necrosis factor alpha.			

TNF-α did not correlate with CRP (correlation co-efficient 0.14, p 0.6) in our cohort, but did correlate with Hb (-0.56, p 0.001) and NLR (0.72, p <0.001).

3.10 Discussion

In this work we demonstrated differences in hormone responses between patients with oesphagogastric cancer and anorexia and patients with normal appetite.

3.10.1 Baseline characteristics and biochemical markers

As would be expected anorectic patients had a lower FAACT A/CS score, and higher OG-25 food restriction score than non-anorectic patients and healthy controls.

There were more women in the non-anorectic group, and mean age was younger in the anorectic group. There were also 2 current smokers in the anorectic patient group, with none in the NA group. Nicotine is known to suppress appetite through upregulation of POMC neurones and the MC4R receptor [337] but not through any interaction with the EEC system, however, given the small numbers within the group this could have had an impact. The split of disease location and metastatic spread was similar between groups.

Anorectic patients had a slightly higher mean CRP than non-anorectic patients, but this was predominantly driven by one patient with a markedly raised CRP and 3/7 patients had normal CRP. Mean NLR was similar between groups. This may suggest inflammation is not a key contributor to anorexia in this cohort. As will be discussed below differences in TNF-α were noted between groups which did not reach significance. The noted difference in vitamin B12 values is likely due to 1 patient in the anorectic cohort having had recent treatment for B12 deficiency and having markedly higher values than others. The significant difference in magnesium values is likely related to limited variance in this group.

Three patients with anorexia had dysphagia symptoms and 2/10 non-anorectic patients. Anorectic patients had markedly higher mean baseline weight loss, 11.0% vs 1.7% for non-anorectic patients.

3.10.2 Ghrelin

Due to storage issues with the reagent the majority of active ghrelin results were below expected range or undetectable by ELISA. These results have therefore not been analysed further.

It is unfortunate that these analyses failed as existing in-human data for ghrelin levels in advanced cancer patients is limited and conflicting, with some data showing higher levels[154], some showing lower [156] and one study showing no differences between anorectic and non-anorectic patients [162]. Ghrelin is released from the stomach in response to an empty stomach and therefore its' release in gastric cancer patients may be more impacted by the local impact of tumour or delayed gastric emptying.

3.10.3 GIP, GLP-1, insulin and glucose

Levels of GIP, GLP-1 and insulin were lower for anorectic patients compared to non-anorectic patients and healthy controls. This was an unexpected finding. The blunted response of insulin is in line with the blunted levels of the two incretin hormones GIP and GLP-1. However, this finding wasn't reflected in median glucose values, which showed a very similar rise and fall across all three groups.

The amount of carbohydrate in the study meal (13g) is relatively small and wouldn't be expected to trigger a large insulin response. Though clearly the response in anorectic patients is lower than that of non-anorectic patients and healthy volunteers.

It is recognised that insulin is involved in growth signalling and has therefore been suggested that it may be associated with poorer cancer outcomes. There is epidemiological evidence suggesting an increased cancer risk for patients with pre-diagnostic insulin resistance [338], though it should be noted that insulin resistance often co-exists with other recognised risk factors such as obesity.

There is some evidence to suggest insulin resistance in patients with advanced cancer [339-341], though this would usually lead to higher, rather than lower insulin levels and also likely be reflected in higher glucose levels. Though pancreatic beta cell failure and reduced insulin secretion can be a later feature of insulin resistance [342]. Insulin resistance is thought to be mediated by inflammatory cytokines including TNF-α [343, 344], which was raised in the anorectic cohort. However, if the reduced insulin levels seen here were secondary to failure of beta cells it would be expected that there would be evidence of established diabetes and higher glucose levels, which were not demonstrated in this cohort. Of note the BMI between groups was very similar, however

anorectic patients had lost significantly more weight and so would have had a higher pre-morbid BMI on average.

In acute phase illness GLP-1 is often raised in response to IL-6 [345, 346], however in chronic inflammation levels can drop [346]. All patients in our cohort were newly diagnosed, however time from initiation of carcinogenesis cannot be known so chronic inflammatory changes may have taken effect. However, normal fasting glucose levels in our cohort would point against insulin resistance as the explanation for these results.

If not related to insulin resistance, then another hypothesis to explain the blunted GLP-1 and insulin response could be delayed gastric emptying, delaying stimuli to the small intestine to release the hormones. The opposite effect, rapid gastric emptying into the ileum following bariatric surgery if thought to account for raised GLP-1 levels seen in this setting [347]. However, the time to peak is the same in anorectic patients to both non-anorectic and healthy volunteers, which therefore does not support this hypothesis. Additionally, GLP-1 is release from L cells, predominantly located in the ileum, which is also the main site of release of PYY, which in anorectic patients demonstrated raised levels. It may be therefore, that altered CNS signalling in anorectic patients could account for these changes.

On a literature review I was able to find one other study demonstrating low insulin and glucose levels in patients with gastric cancer [348], but there is limited data around insulin responses in cancer patients in the literature to date. In this study both insulin and glucose levels were lower in cancer patients, however in my cohort glucose levels were within normal limits at baseline, with no significant differences between groups during the study time period. Anorectic patients had a slightly shorter mean fast duration than non-anorectic patients (12.29 hours vs 13.3 hours), and all participants had the same meal. These findings are in contrast to those of patients with small bowel Crohn's disease, who had very similar GIP and GLP-1 levels to patients with large bowel Crohn's [336], suggesting this is not an effect due to local inflammation.

Given that GIP, GLP-1, and insulin have anorexigenic effects, it does not seem that these changes would account for reduced appetite in these patients.

3.10.4 Pancreatic polypeptide

Pancreatic polypeptide levels were lower in anorectic patients than in non-anorectic and healthy controls, this did not reach statistical significance. Levels demonstrated a blunted peak at 30 minutes. Blunted PP levels have been reported in children with Prader-Willi syndrome, chronic pancreatitis, and distal pancreatectomy [349-351]. In patients with insulin resistance PP response to a test meal was raised [352]. I was unable to identify any data about PP responses in gut inflammatory conditions or cancer states, though reported raised levels with pancreatic neuroendocrine tumours are noted [353, 354].

Pancreatic polypeptide is released from the pancreas, so less likely to be impacted by delayed gastric emptying, its' secretion is in response to vagal signalling and so mechanical changes to normal gut motility may have an impact. Pancreatic polypeptide is an anorexigenic hormone so reduced levels would be expected to increase appetite rather than reduce it.

3.10.5 Peptide YY

A clear difference in median values for PYY at each time point was noted for anorectic patients compared to patients with normal appetite. This did not reach statistical significance on ANOVA testing, likely due to the small sample size and missing data for this hormone. Unfortunately baseline values for PYY were only reported for 13/20 patients, 26 values were below detectable range (19% of total values), these were noted across multiple patients and multiple time-points, with no clear pattern.

All samples were analysed in duplicate, and the mean value taken. On assessment of the sample data, where results were not available for PYY, both results were undetectable. There was no clear pattern of results that have failed analysis indicating an issue with the ELISA process, suggesting that this a sample issue. Therefore, the most likely reason for the failed analysis is haemolysis within the samples.

The AUC for anorectic patients was higher than for those with normal appetite and healthy volunteers, even within context of missing values, suggesting that, though it did not reach statistical significance, this is a true exposure difference between groups.

For patients with normal appetite and healthy volunteers PYY levels showed a small peak at 45 minutes and then levels reduced (though a small second peak was seen in healthy volunteers).

For anorectic patients there was a larger peak at 15 minutes, rising to a much higher peak than the other cohorts at 60 minutes. In a recent study of healthy volunteers PYY levels peaked at 30 minutes post meal and remained raised at 45 and 60 minutes before gradually falling back to baseline [335]. It is possible that delayed entrance of stomach contents into the ileum could account for the delayed, later peak in anorectic patients. Though, as discussed above this does not fit with the results seen for GLP-1, which is also released from ileal cells.

These results reflect those reported in patients with inflammatory bowel disease [164] where PYY levels were markedly raised in patients with small bowel Crohn's disease, but not in those with large bowel Crohn's disease. However, the degree of elevation in this cohort is not as significant as that seen in patients with Crohn's, where PYY values for patients with small bowel Crohn's disease were 3x higher than patients with large bowel Crohn's disease. In contrast to my results, a study by Garcia et al [163] found equivalent levels of PYY between patients with cancer cachexia and those without. However, in this cohort cachexia was defined by unintentional weight loss >5% within 6 months, and not by presence of anorexia or inflammation. Patients with unintentional weight loss may have normal appetite. This study excluded patients with cancers of the upper GI tract. It is therefore possible that this effect is due predominantly to mechanical disturbance or local inflammation, and less mediated by systemic inflammation. Equally, the cohort of patients with cachexia in the study by Garcia et al. had no significant difference in TNF-α levels compared to non-cachexic patients and this result may predominantly be due to patient selection.

3.10.6 TNF-α

TNF-α levels were higher in anorectic patients than in other groups, though again this did not reach statistical significance. This is likely due to small numbers within the groups, though does reflect the results reported by Garcia et al. [163] (though as noted their comparative groups were cachexia defined by 5% weight loss without inflammatory marker or anorexia status selection). There was no significant change in

any group across time points. As an inflammatory cytokine which wouldn't be dependent on nutrient or gut motility dependent this is an expected finding.

TNF-α levels strongly correlated with PYY levels and suggest that this increase may well be an inflammatory mediated response. TNF-α levels also correlated with PP, insulin and GLP-1 levels which would fit with an inflammatory mediated insulin resistance picture.

3.10.7 Limitations

The primary limitation of this work is the small numbers included. Due to reduced recruitment it was challenging to recruit specifically anorexic patients. These patients were more unwell and some initially consented to trial became too unwell to take part.

This data is therefore underpowered. However, it was designed to be signal finding work and power calculations for expansion work have been undertaken.

As discussed due to issues with storage and supply of reagents we were unable to analyse ghrelin and active GLP-1 which has limited the breadth of the analysis.

3.10.8 Conclusions and future directions

In summary, this data demonstrates a signal that abnormal enteroendocrine function appears to be present in patients with anorexia and gastro-oesophageal junction or gastric cancer. It is not possible to tell from this data whether the raised PYY levels seen are a result of local inflammation, altered gut motility or abnormal CNS signalling. It is important to note that within normal participant samples there are significantly wide ranges of values seen for all gut hormones [335]. Despite this, my data does suggest a trend to a significant difference, despite the small sample size.

Future research would aim to validate these results in a larger population, as this could theoretically identify a potential future target for treating anorexia.

To assess sample size for an expansion cohort I performed power calculations using the peak difference values for PYY of T45 where values for the anorectic cohort were 58% higher than non-anorectic patients. If we assume a type 1 error rate of 5%, calculations

suggest a sample size of 76 (with a 50:50 anorectic: non-anorectic split) to detect this size difference with 80% power as shown below.

$$k = \frac{n_2}{n_1} = 1$$

$$n1 = \frac{\left(\sigma_1^2 + \frac{\sigma_2^2}{K}\right)\left(z_1 - \frac{\alpha}{2} + z1_1 - \beta\right)^2}{\Delta^2}$$

$$n1 = \frac{\left(139^2 + \frac{139^2}{1}\right)(1.96 + 0.84)^2}{89^2}$$

n_1=38 n_2=K∗n_1=38

Further work will also focus on a wider panel of inflammatory cytokines with a view to further delineating the interaction between inflammatory cytokines and gut hormones.

It will be important to understand the impact of reduced gut motility in this process, and correlate gut hormone results with gastric transit times. This could be via gastric scintigraphy or barium meal testing. If delayed gastric emptying correlated with raised PYY levels, then it may be that increased use of pro-kinetics in this patient group could be of benefit.

If gastric emptying does not appear to be significantly different between groups, a potential next step could be to investigate hypothalamic neural signalling using functional MRI.

Understanding the significance and impact of blunted insulin response in these patients will also be relevant. It may be useful to use a more carbohydrate rich test-meal in the expansion phase of this study. Another method could involve short term use of continuous glucose monitoring alongside food diaries or test meals in patients. Whilst it is unlikely that altered insulin metabolism contributes to anorexia in this patient group, there is some epidemiological evidence suggesting that insulin resistance is associated

with poorer outcomes [338] in patients with cancer. However, this hasn’t been investigated at an individual patient level.

4. The association of sarcopenia with frailty and treatment outcomes

4.1 Introduction

Sarcopenia has become a recent focus of interest in oncology research due to the development of criteria for CT-measured muscle mass and the ready availability of CT imaging for oncology patients. Machine learning software exists which can quickly and easily read CT scans to provide measures of muscle mass. However, sarcopenia and body composition changes have long been a focus of elderly medicine research due to the fact that muscle mass decreases with age [19] and some evidence that sarcopenia is associated with frailty and poor outcomes [41]. Frailty is associated with increased morbidity, loss of independence and increased rates of mortality from cardiovascular, respiratory diseases, cancer, and all-cause mortality [41]. There is a significant amount of data for these two fields, but limited research has investigated the overlap, despite a high proportion of cancer patients being older [5].

The association between frailty and sarcopenia is most clearly evident at the end of the scale, but less clearly delineated in intermediate categories of frailty. In a large cross-sectional study sarcopenia was present in some patients without frailty, but no-one had frailty or pre-frailty without co-existent sarcopenia [40]. Much of the research around sarcopenia in older adults has been performed using BIA which has inferior sensitivity to other methods [186]. There is limited data about the association of sarcopenia measured using CT and frailty [355].

Frail older patients with cancer may experience significant toxicity from treatment. A common finding is that with the use of frailty screening and comprehensive geriatric assessment (CGA) more older patients get aggressive treatment, and more patients get no anti-cancer treatment at all [224-226]. Whilst frailty is associated with poorer outcomes, fit older patients may tolerate treatment as well as younger patients [227, 228].

The measures used in these studies are predominantly frailty screening tools which usually do not include an objective physical function measure. Recommendations are

that sarcopenia be diagnosed by a functional measure such as grip strength [356], however this is rarely undertaken in most oncology sarcopenia papers.

The Rockwood clinical frailty scale (CFS) has been introduced at our institution to assess frailty in older patients. The Rockwood scale has been demonstrated to correlate well with CT-measured sarcopenia in a non-cancer population [357] but there is limited data about this in cancer populations. The CFS is shown in figure table 4.1.

Table 4.1: Rockwood clinical frailty scale	
1	Very Fit – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.
2	Well – People who have no active disease symptoms but are less fit than category 1. Often, they exercise or are very active occasionally, e.g. seasonally.
3	Managing Well – People whose medical problems are well controlled, but are not regularly active beyond routine walking.
4	Vulnerable – While not dependent on others for daily help, often symptoms limit activities. A common complaint is being “slowed up”, and/or being tired during the day.
5	Mildly Frail – These people often have more evident slowing, and need help in high order IADLs (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.
6	Moderately Frail – People need help with all outside activities and with keeping house. Inside, they often have problems with stairs and need help with bathing and might need minimal (cuing, standby) with dressing.
7	Severely Frail – Completely dependent for personal care, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).
8	Very Severely Frail – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.
9	Terminally Ill - Approaching the end of life. This category applies to people with a life expectancy <6 months who are not otherwise evidently frail.

Category number 9 is problematic for oncology patients, given that many patients could be considered to have this life expectancy, and it otherwise gives no indication to the physical state of the patient.

The prevalence of sarcopenia varies in the general older population, in part because of heterogenous definitions and methods of assessment used for it. When assessing the same population using different criteria it may be present in between 0-15% of healthy adults aged >65 [42].

In patients with cancer, sarcopenia has been demonstrated to be associated with poorer outcomes [194], and in some cases increased treatment toxicity but there is significant heterogeneity of association. Rates of sarcopenia vary according to cut-offs used with much of the existing data coming from small, retrospective studies. The commonly used definition criteria are shown in table 4.2. The populations used in the Martin [43] and Prado [358] studies were patients with cancers of the lung and GI tract across a variety of stages. These cancers predominantly present in older adults (50% of cases within the UK are diagnosed in patients aged over 75 [5]) and the mean age in Martin et al. was 64 years. Therefore, in view of age, malignancy, and co-morbidities, they may be expected to have a lower than population average level of muscle mass. This could mean that these cut-offs do not detect sarcopenia that could be of significance for younger patients. They are also likely to over-identify sarcopenia in older patients and may only have limited applicability here.

This may not be a significant issue, due to the overlap of frailty and sarcopenia, and poor outcomes, but since the relationship between sarcopenia and frailty hasn't been fully elucidated, there remains uncertainty about the utility of the measure.

Table 4.2: sarcopenia definition criteria (skeletal muscle index, SMI) from CT measures

Criteria	Female cut-off for sarcopaenia (cm^2/m^2)	Male cut-off for sarcopaenia (cm^2/m^2)
Martin et al [43].	42.0	43.0
Prado et al [358].	38.5	52.4
Derstine et al [192].	34.4	45.4
EWGSOP2 [190]	39	55

Additionally, there is some evidence that cross-sectional area and muscle strength are not directly correlated [188], potentially due to fat infiltration of muscle. Prior to losing mass, muscles undergo fatty infiltration and so lose density prior to losing mass. Skeletal muscle density (SMD) measured by Hounsfield units (HU) may therefore represent a more accurate marker of muscle strength than cross-sectional area.

In chapter 2, I reported that weight loss ≥5% weight loss at baseline correlated with survival, but no individual nutritional state marker correlated with treatment toxicity. Predicting treatment toxicity to guide management for patients could avoid significant treatment-associated impacts on quality of life. Therefore, it would be helpful to know whether sarcopenia and frailty are independent or dependent biomarkers predictive of toxicity.

If frailty does not correlate well with sarcopenia, could the addition of sarcopenia to frailty provide better sensitivity to outcomes?

4.2 Aims

The aims of this chapter are to:

- Investigate the association between the Rockwood CFS and CT-measured sarcopenia in this cohort of patients with advanced upper GI cancer
- Investigate the relationship between the Rockwood CFS, CT-measured sarcopenia and hand grip strength and markers of malnutrition
- Investigate the relationship between sarcopenia, CFS and PS with survival and treatment toxicity

4.3 Methodology

Patients were recruited as detailed in chapter 2. Assessments of height, weight, body mass index (BMI), mid-upper arm circumference, and hand grip strength (HGS) were taken at first oncology assessment. Muscle mass was measured on diagnostic or most recent to baseline CT scan (if multiple pre-treatment scans were available).

4.3.2 Measuring muscle mass

Body composition was assessed on CT imaging performed as standard of care at baseline, mid-treatment, and end of treatment, using validated technology developed in-house.

All scans were downloaded from the picture archiving and communication system (PACS) for image analysis. Images were then packed using Worldmatch 4-way 3D match viewer software (version 8.17b). I undertook identification of the CT slice showing relevant bony landmark (L3). An example of CT segmentation is shown figure 4.1, in this skeletal muscle is outlined in a mask of purple and bone in red. Bone is delineated on initial segmentation to correct muscle segmentation but then removed for quantification to account for partial volume effect.

Slices were segmented to the level of the 3rd lumbar vertebra and psoas muscle mass was outlined using a neural learning software that has been developed in house and validated against manual delineation (considered gold standard as described in the paper by Martin et al. [43]). Initial training for the UNet convolutional neural network was performed on a set of 201 CT images that had undergone manual delineation. Of these

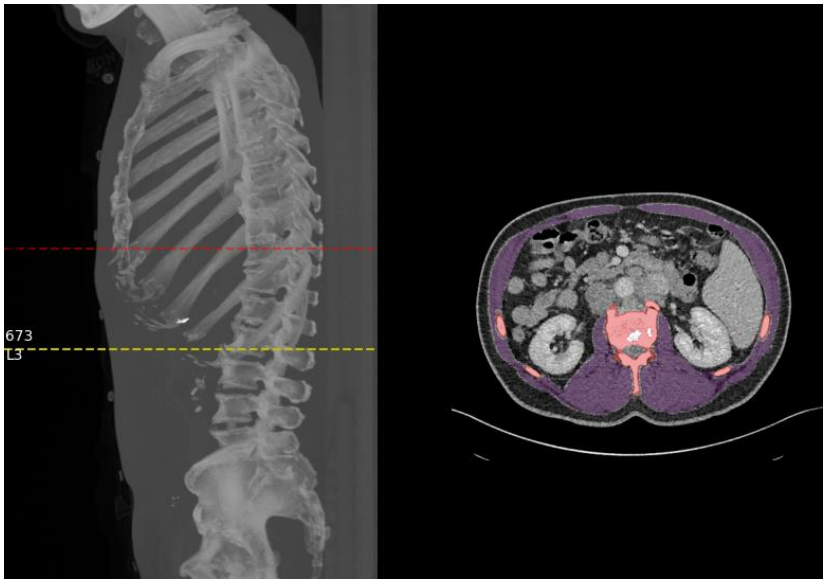

Figure 4.1: example of CT mask showing muscle mass delineated in purple at L3 level, and bone delineated in red.

160 images were used as a training set and 41 used as validation set. Segmentation was validated using a cross validation process and distance to agreement calculated for each volume. All segmentations were reviewed by me and validated for quantification with any inaccurate segmentations removed. With the data provided by the software of skeletal muscle area (SMA, cm^2) I was then able to normalise this for height (cm^2/m^2) to give skeletal muscle index (SMI). Muscle density was calculated as mean Hounsfield Units (HU) within the mask.

Reduced muscle density was counted as a mean HU of < 41 in patients with a BMI < 25 and <33 for those with a BMI of ≥ 25 as defined in the study by Martin et al [43].

4.3.2 Statistical methods

Statistical analysis was undertaken using SPSS (IBM, version 25, 2017). Proportions were analysed with descriptive statistics, differences between groups with χ^2, Fisher's exact test or non-parametric tests as appropriate.

Survival was analysed using Kaplan-Meier analysis and cox regression. Associations between sarcopenia and binary outcomes were assessed using logistic regression and correlations using Pearson's co-efficient**.** Cut-off for survival was taken on September 17th, 2022.

Sarcopenia was investigated using the Prado, Martin and Derstine criteria detailed in table 4.2.

4.4 Results

There were 158 patients recruited in total, The first 60 patients were prospectively recruited between January 2021 and July 2021 under an application to the ukCAT database which allowed for some baseline data collection and CT scan analysis of these patients and the whole ANCHOR cohort (full details available in chapter 2). Following confirmation of additional ethics approval for the wider ANCHOR study a further 98 patients were recruited with additional quality of life and anthropomorphic data.

Final analysis was undertaken on all patients 158 patients with a median follow-up of 12 months, all but 2 patients had had sufficient time to complete 1st line chemotherapy

at point of analysis. CT muscle mass measures were available for 131 patients of the overall cohort (missing data due to imaging not available for transfer for analysis).

4.4.1 Demographics

Patient demographics are shown in table 4.3. Scan data was available for 131 patients.

Mean SMI for men was 44.74 cm^2/m^2 (range 24.4-64.1) and 36.7 cm^2/m^2 for women (range 23.9-52.6). Mean for the cohort overall was 42.6 cm^2/m^2. Mean SMD was 42.6 HU (-5.1 to +42.8).

Sarcopenia according to Martin criteria was present in 60% of older patients compared to 46.5% of younger patients, p 0.09.

Table 4.3: patient demographics

		N	%
Aged	≥70	60	45.8
	<70	71	54.2
PS	0-1	120	76
	2-3	31	24
CFS	1-2	74	56.5
	3-4	40	30.5
	4-5	11	8
	NA	6	5
Presence of sarcopenia according to different criteria	Martin	69	52.7
	Prado	101	77.1
	Derstine	66	50.4
Presence of reduced muscle density		109	83.2

4.4.2 Relationship between frailty, performance status and CT measures of sarcopenia

Clinical frailty scale was assessed in 151/158 (96%) patients, 125/131 with CT data available. Patients who were assessed as PS 0 were all assessed as CFS 1-2, however for PS 1 upwards the range of CFS scores was much more varied as demonstrated in figure 4.2.

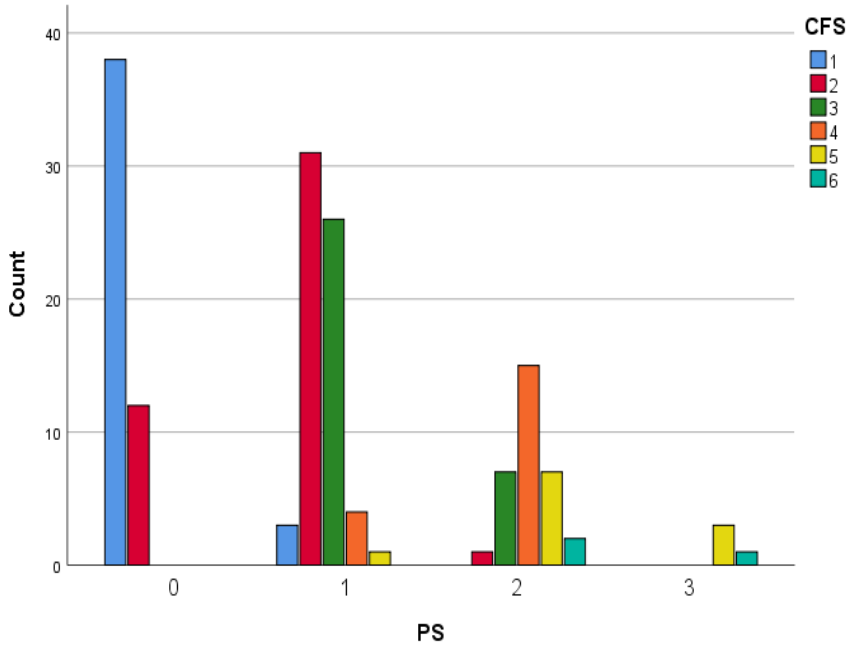

Figure 4.2: distribution of CFS by PS 2. CFS = Clinical Frailty Score, PS = Performance Status

The range of noted SMI values for each CFS score is shown in figure 4.3. There was a wide range of SMI values for each individual CFS category.

There was no significant correlation between SMI and CFS, r -0.14, p 0.12 or PS r -0.14, p 0.12. There was also no significant correlation between SMD and CFS (r-0.08, 0 0.39) or PS (r -0.08, p 0.51).

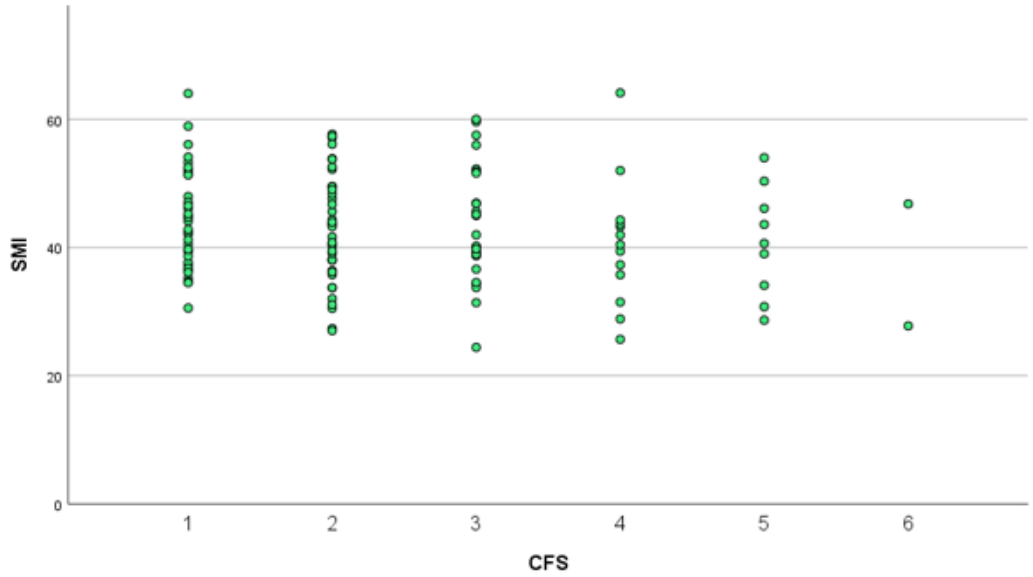

Figure 4.3 Scatter plot of clinical frailty scale against skeletal muscle index

SMI and SMD correlated with HGS, r 0.48 and 0.40 respectively, both p <0.001.

Delineated by the presence or absence of sarcopenia, there was a fairly even split of sarcopenia (Martin criteria)/normal muscle mass across the CFS scores 1-3, but sarcopenia became more prevalent at CFS 4 upwards.

For PS the split of sarcopenic/non-sarcopenic was almost 50:50 except for PS 3. The distribution of SMI values for different PS and CFS categories are shown in figures 4.4 and 4.5.

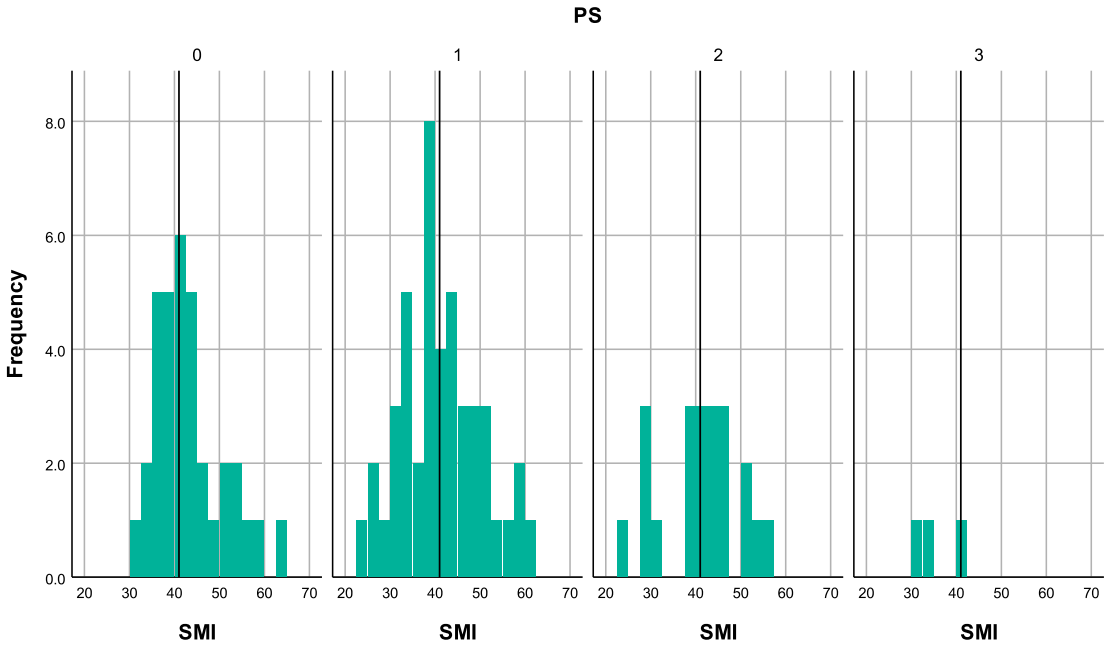

Figure 4.4 Clustered histogram of SMI values according to PS status with marker bar at mean value

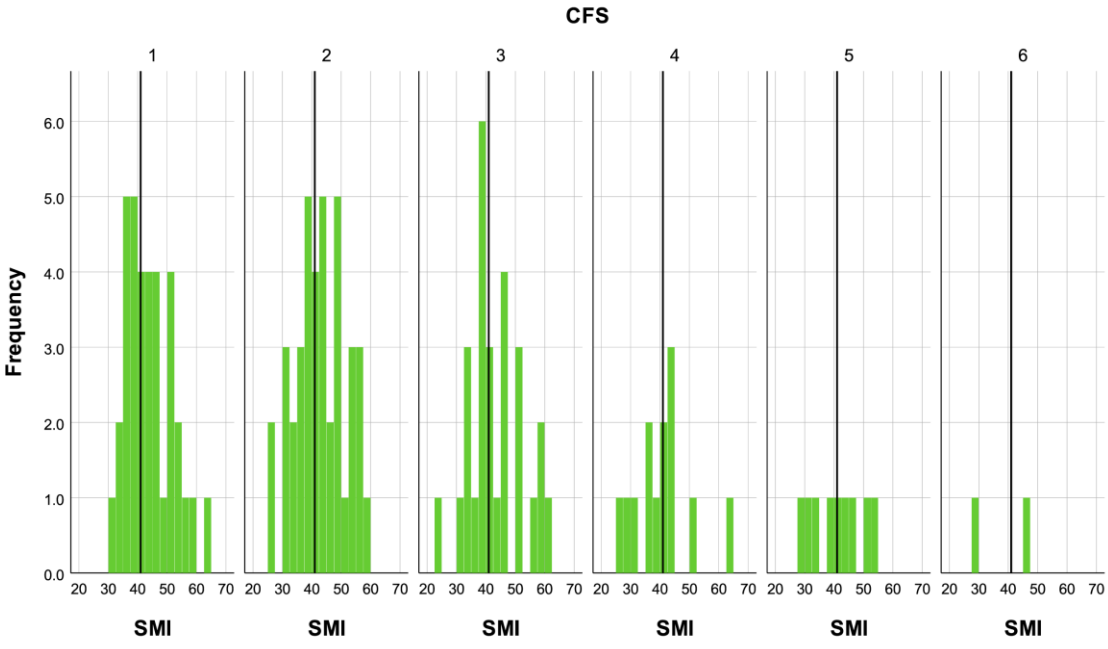

Figure 4.5 Clustered histogram of SMI values according to CFS status with marker bar at mean value

4.4.3 Association of fitness factors with survival and treatment outcomes

A performance status of ≥2 was associated with poorer overall survival HR 3.21 (95% CI 1.78-5.79, p <0.01), and a CFS score >3 was associated with poorer PFS and OS, HR for OS 2.78 (95% CI 1.49-5.10, p 0.001).

Survival according to CFS score is shown in figure 4.6. Interestingly survival according to CFS score appears to fall into 3 distinct groups with patients with CFS scores 1 and 2 having a median OS of 15 months, patients with a score of 3 or 4 having a mOS of 8 and 7 months and patients with a CFS of 5 or 6 having an mOS of just 1 month. Comparatively, patients with a PS of 0 or 1 had a mOS of 12 and 14 months respectively, compared to just 2 and 1 months for PS score of 2 and 3 respectively. This suggests that CFS scores of 0-1 and 5 and 6 reflect PS values, but scores of 3-4 may select an intermediate prognosis group.

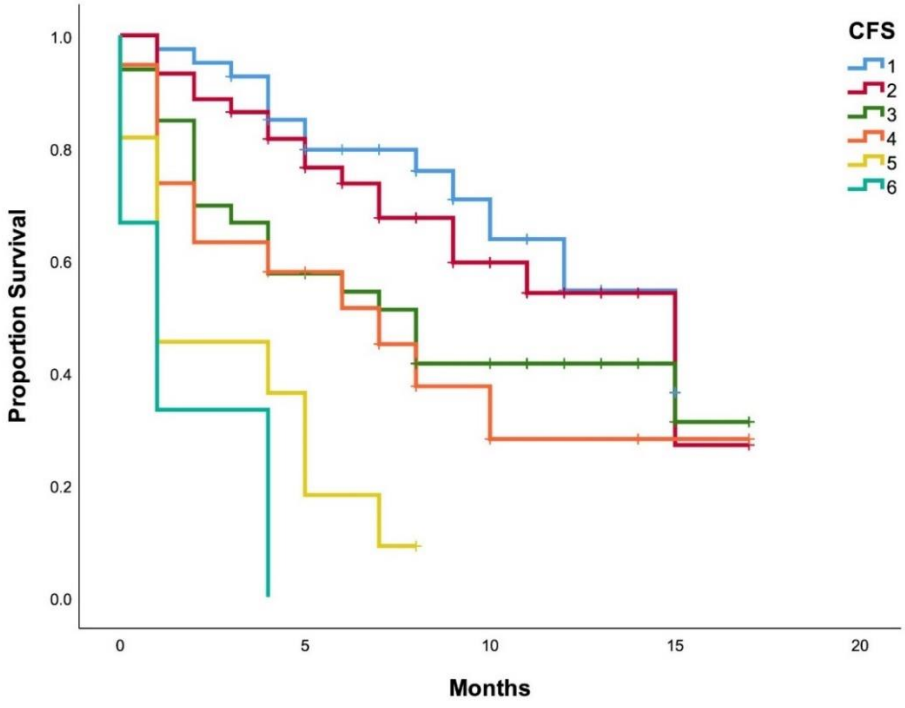

Figure 4.6: overall survival according to CFS score

There was no clear difference between proportions of patients with each co-morbidity score value for each PS group, p 0.08 across all groups. There was no association between ACE comorbidity score and PFS but an ACE score of 1 was associated with poorer OS, HR 1.82 (95% CI 1.07-3.07, p 0.03).

There was no association between CFS scores and any of the following toxicity outcomes; dose delays (HR for a CFS ≥3 1.4, p 0.43), dose reductions (HR 0.89, p 0.78) or hospital admission related to toxicity (HR 1.47, p 0.38).

A significant number of patients with a higher CFS score had a dose reduction at baseline, 63.2%, 72.7% and 100% for scores of 4, 5, and 6 respectively. For the cohort overall patients with a baseline dose reduction were significantly less likely to have a toxicity outcome than those who didn't; 24.5% vs 50.0%, p 0.009. This may therefore have counteracted the negative effect on toxicity outcomes in these frailer patients.

Neither Martin criteria SMI or SMD were significantly associated with overall survival on cox regression (HR 1.12, 95% CI 0.69-1.81, p 0.65 and HR 1.60, 95% CI 0.79-3.23, p 0.19 respectively). SMI and SMD analysed as a continuous variable did not correlate with OS. HR for SMI 0.99 (95% CI 0.96-1.02, p 0.44), HR for SMD 0.99 (95% CI 0.98-1.01, p 0.32).

The presence of sarcopenia by any criteria was not significantly related to survival by any of the criteria assessed. This is shown in figure 4.7.

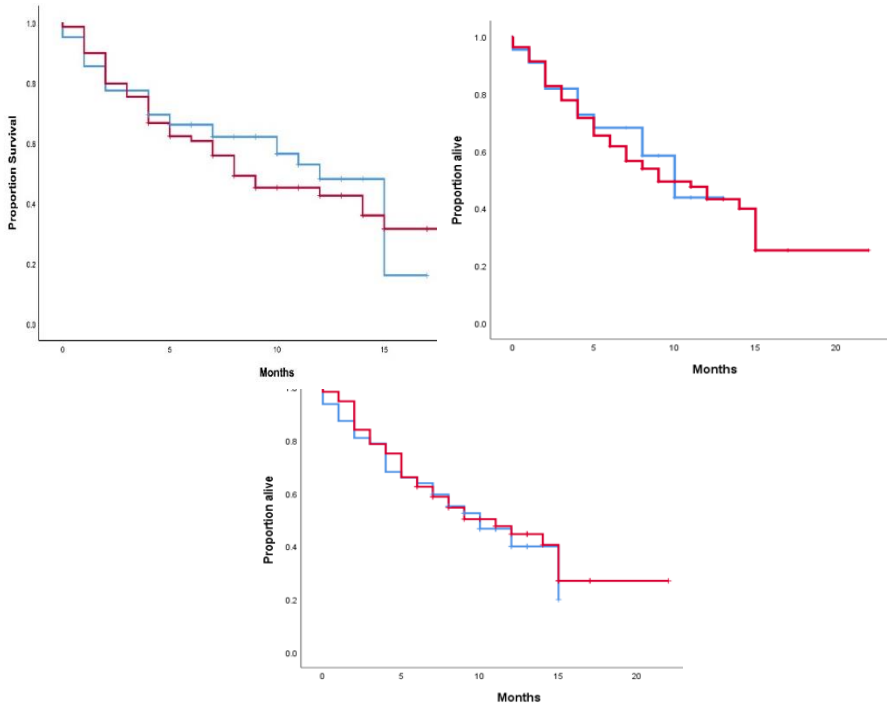

Figure 4.7 Kaplan-Meier curves for the presence/absence of sarcopenia according to Martin (top left), Prado (top right) and Derstine (bottom) criteria. Red = sarcopenic, blue = no sarcopenia

There was a trend towards poorer survival for patients with lower muscle density as shown in figure 4.8. This did not reach significance on log-rank analysis, p 0.10.

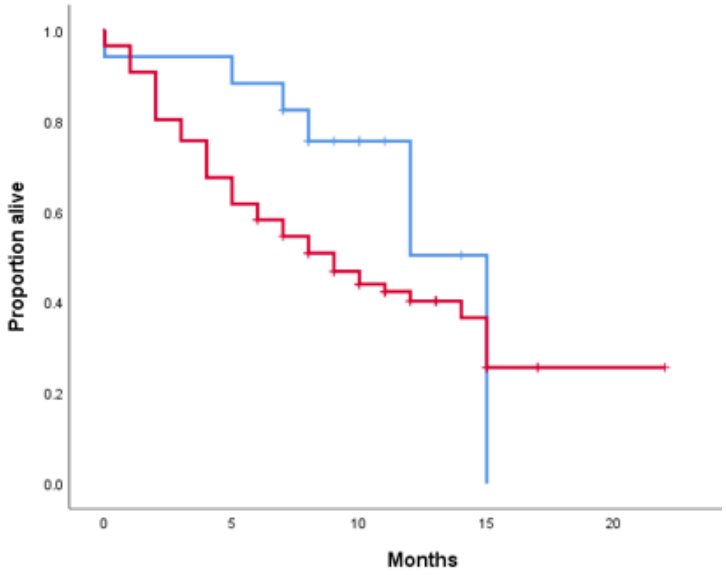

Figure 4.8 Kaplan-Meier curve for survival according to presence of reduced muscle density (Martin criteria). Red = reduced density, blue = normal density

On logistic regression analysis there was no statistically significant association between the presence of sarcopenia (Martin criteria) and dose reductions (HR 1.17, 95% CI 0.47-2.93, p 0.73), delays (HR 0.95, 95% CI 0.44-2.03 P 0.89), or cessation due to toxicity (HR 0.83, 95% CI 0.27-2.52, p 0.74). Sarcopenia was significantly related to toxicity-related hospital admissions (HR 2.36, 95% CI 1.08-5.14, p 0.03). Patients with sarcopenia were not significantly more likely to receive a dose reduction at baseline (38.5% vs 33.3%, p 0.58).

Reduced skeletal muscle density (Martin criteria) was not associated significantly with dose reductions (HR 0.92, 95% CI 0.35-2.38, p 0.86), dose delays (HR 1.66, 95% CI 0.66-4.21, p 0.28), cessation of treatment due to toxicity (HR 1.47, 95% CI 0.30-7.05, p 0.63) or admissions related to toxicity (HR 2.28, 95% CI 0.78-6.65, p 0.13).

4.4.4 Association of sarcopenia with cachexia

Of patients who met the definition of cachexia (5% weight loss and inflammation represented by NLR >3) 60.3% of patients with cachexia were sarcopenic according to Martin criteria compared to 45.6% of non-cachexic patients. This did not reach significance on χ^2 analysis, p 0.09.

I identified patients who were sarcopenic and cachexic (N=38) and compared the survival of these patients to those with cachexia and not sarcopenia (N=25), and those with no cachexia (N=68). Median OS was slightly longer for cachexic and sarcopenic patients, 7 months compared to 5 months for cachexic but non-sarcopenic patients. Both were shorter than non-cachexic patients (mOS 12 months) and this was non-significant on log-rank analysis, p 0.09.

4.4.5 Combining sarcopenia and frailty

In order to assess whether sarcopenia had an additional prognostic value to frailty scoring I grouped patients who had both CFS score and sarcopenia data available (N=124) according to CFS score; not frail score 1-4, frail score ≥5 and presence of sarcopenia according to Martin criteria.

The Kaplan-Meier curve for overall survival for these groups is shown below in figure 4.9. Median survival for non-frail, non-sarcopenic patients (N=56) was 15 months, compared to 9 months for non-frail but sarcopenic patients (N =58). Patients with frailty but not sarcopenia (N=3) had a mOS of 1 month and patients with sarcopenia and frailty (N=7) had a mOS of 4 months. These differences were significant on log-rank analysis p <0.001.

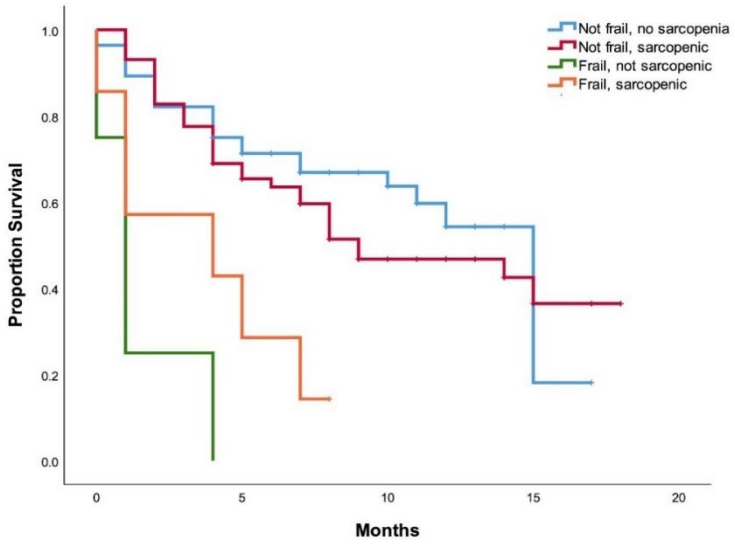

Figure 4.9: Kaplan-Meier curve of overall survival for patients grouped according to frailty status (CFS 1-4 = not frail, CFS 5-8 frail) and sarcopenia status as defined by Martin criteria

4.5 Discussion

In this prospectively collected cohort, sarcopenia measured at L3 and defined by all the commonly used criteria, does not correlate with survival or toxicity outcomes, with the exception of hospital admissions related to treatment toxicity. There was a trend towards an association with poorer survival for patients with lower muscle density, but SMD did also not correlate with toxicity outcomes except for hospital related admissions. There was a moderately strong correlation between muscle mass and density and muscle strength as measured by HGS in this cohort, supporting the utility of this simple clinical tool.

There was a wide range of skeletal muscle indexes across each PFS and CFS score, with approximately half of patients having a non-sarcopenic score at each level, with the exception of the highest scores. The rates of sarcopenia were higher at highest CFS scores, which seemed to better delineate this. However, the correlation between sarcopenia and CFS score was not significant. This is in contrast to some existing data [355], but consistent with other studies [235], though both these studies used different definitions of frailty.

It could be that the CFS is not a sufficiently sensitive definition of frailty. The CFS could be viewed as a more detailed PS, and this criticism could be warranted based on the grouping of survival that occurred with CFS categories in this cohort. Equally it

could be that sarcopenia is not associated with frailty, though this seems less likely based on other existing data [41]. Furthermore, scoring was performed on these patients by oncologists in the clinic, who did not have specific training, and therefore there could be some inaccuracy on this basis.

The finding of no association with survival is not consistent with a significant amount of existing data [194] though consistent with other studies of patients with oesophago-gastric cancer [203]. It should be noted that much of this previous data is retrospectively collected. There is data suggesting that muscle density is a stronger biomarker of survival, and this would be consistent with my findings, though it still did not reach statistical significance.

The association between sarcopenia and cachexia was also non-significant, with 40% of patients who were cachexic not being sarcopenic. Sarcopenia is included in many definitions of cachexia [359], and therefore this is an interesting finding. It could be explained by the fact that mean BMI for this cohort was 26.36, but many patients had lost significant amounts of weight, and would therefore have had an overweight or obese BMI to start with. It is recognised that overweight people tend to have higher than average muscle mass [360] and so they may have remained above the sarcopenic threshold despite significant weight loss. It could also be that they had lost predominantly fat mass, but this cannot be confirmed without pre-morbid imaging to compare. It also seems unlikely given that it is recognised that even healthy people will loss muscle mass if they do not eat sufficient calories for their requirements [361, 362]. However, consistent with the lack of association with survival in the cohort overall, patients with cachexia and sarcopenia did not have poorer survival than those without, again arguing against the utility of sarcopenia as a prognostic biomarker in patients with upper GI cancers.

Rates of sarcopenia within this cohort were broadly consistent or slightly higher, than those reported in other cancer cohorts 50% for Martin criteria compared with 40% in their original study [43], and therefore the lack of association is unlikely to be due to variance within this cohort compared to other cancer populations. It could be therefore that the existing definitions for sarcopenia are not sufficiently sensitive. As discussed

previously the Martin and Prado criteria have been taken from cancer populations and do not account for age-related sarcopenia.

In this cohort, sarcopenia only correlated with toxicity-related hospital admissions. It should be noted that toxicity was very common in this cohort, with nearly half of patients experiencing a dose delay or reduction during treatment. Specific toxicity data, or grade was not collected, but this could suggest an association between sarcopenia and more severe toxicities. Existing data around the relationship between sarcopenia and treatment toxicity in patients with advanced oesophago-gastric cancer is conflicting. One study demonstrated an association between reduced muscle density and more severe toxicity [203] but no association with muscle mass, whilst another retrospective study found an association between sarcopenia and neutropaenia and mucositis [363]. In contrast a meta-analysis of studies of patients with a range of cancer types and settings did report an association between reduced muscle mass and systemic cancer treatment toxicity [364].

Limitations of this data are that the cohort is fairly small and follow-up is short for some of the cohort. Any sub-group analyses are therefore of limited power. Furthermore, toxicity was not routinely graded and therefore it cannot be assessed whether sarcopenia or reduced density predicts more severe toxicity, though the significant correlation with toxicity-related hospital admissions suggests this may be the case.

However, from this data, sarcopenia cannot be used as a useful prognostic or predictive biomarker for patients with advanced oesophago-gastric cancers. Nor does it seem to add to the sensitivity of cachexia as a prognostic biomarker.

As the ANCHOR trial continues to recruit, and data matures a more significant relationship may develop. As we move forward more detailed toxicity grading could allow better understanding of the ability of sarcopenia to detect severe treatment toxicity. Understanding more about the combination of reduced muscle mass and strength will be of significance, as will investigating longitudinal changes in muscle mass on treatment.

5.0 Pharmacological interventions for sarcopenia with reference to patients with cancer: A scoping review

5.1 Introduction

A clear and consistent association of sarcopenia with poor survival outcomes is demonstrated in patients with cancer, across all stages of disease [194, 365]. Less consistent data exists demonstrating a possible association with treatment toxicity. Outcomes appear to be particularly poor with patients who have sarcopenic obesity, that is sarcopenia in the presence of obesity[191].

Theoretically, if reduced muscle mass is a poor prognostic biomarker, and predictive of treatment toxicity, then improving muscle mass may improve outcomes in terms of treatment toxicity and survival. However, effective treatments to improve muscle mass in patients with cancer are limited.

Early studies of drugs to ameliorate cancer associated cachexia focussed on weight gain only. As techniques for assessing body composition developed it became clear that in some cases these drugs cause fluid gain, rather than improvements in fat or muscle [305, 306]. Therefore, body composition measures are likely to provide more meaningful data on the impact of a drug on muscle anabolism. The most commonly used methods have historically been dual x-ray energy absorptiometry (DXA), ultrasound (USS), magnetic resonance imaging (MRI) and bio-electrical impedance analysis (BIA), though more modern studies have tended to focus on CT measure of muscle mass.

It is often assumed that patients with cancer are sarcopenic because of their disease – often termed pre-cachexia. However, patients may be sarcopenic for different reasons, including other co-morbid conditions, which cannot necessarily be interpreted from a radiological assessment. It is known that sarcopenia is a feature of normal ageing, with a reduction of skeletal muscle mass in the order of 1-2% per year from the 5th decade of life [19]. Cancer is predominantly a disease of the elderly [193] and therefore many patients may be sarcopenic due to their age. Sarcopenia in older age is associated with reduced physical function, frailty, and increased morbidity [41, 366], and thus it would

be desirable to treat sarcopenia of ageing in a patient with cancer, just as much as sarcopenia related to disease.

Because cancer patients may have sarcopenia for different reasons, there may be lessons for oncologists to learn from studies where medications have been trialled in older adults, or where agents have been trialled for patients with loss of muscle mass due to other disease.

Existing narrative reviews have discussed possible treatments for improving cancer cachexia [367], and similarly systematic reviews exist of individual treatments for improving muscle mass [368-371]. Since no review exists covering all settings and all agents it was felt that this review could add something to the existing literature. A scoping review requires the same systematic and rigorous searching methods as a systematic review but allows coverage of a wider field of data [372].

5.1.2 Nutritional supplements

This review covers pharmaceutical agents whether used alone or in combination with diet and exercise. Nutritional interventions have been reviewed widely. In reference to patients with cancer a recent systematic review considered patients with cachexia, defined as those with advanced cancer and weight loss >5% [373]. The review found articles showing trends towards increases in lean body mass with protein supplementation. The supplementation was given in various forms, including individual amino acids as well as dietary forms of protein. The authors did note a paucity of high-quality research of nutritional supplements. Trials were included in this review that included medications for appetite stimulation alongside diet but not those that trialled protein supplements alone. There was not strong evidence to support other nutritional supplements from this review.

For older adults the evidence supporting protein supplementation for treating sarcopenia is less clear. At least 6 separate systematic reviews with meta-analysis investigating protein or amino acid supplementation were published between 2012 and 2019. Of these, 4 meta-analyses suggested a benefit to protein in older adults [374-377], whilst 2 suggested there was no benefit [378, 379]. In combination with exercise, 2 meta-

analyses suggested no benefit to protein or amino acid supplementation over exercise alone [380, 381], whilst a further study did show some additional benefit [382].

5.1.3 Exercise

Data around the role of exercise as an intervention alone appears more limited. A Cochrane review found no trials suitable assessing exercise for cancer cachexia [383]. A separate review and meta-analysis assessing exercise interventions for patients with cancer undergoing multimodal treatment found that exercise interventions appeared to be safe, and there was some evidence supporting a moderate effect on physical fitness and quality of life [384] but did not comment on its' ability to improve sarcopenia in these patients.

In older adults meta-analyses have shown a benefit in physical function in older adults, which therefore may have benefits in terms of independence and mobility, however no improvement was found in muscle mass or quality of life [385, 386].

5.2 Methodology

5.2.1 Search strategy

Databases searched included Cumulative Index to Nursing and Allied Health Literature - CINAHL (year of inception 1961), CENTRAL (Cochrane controlled register of trials), Ovid MEDLINE (year of inception 1946) and EMBASE (year of inception 1980) from database inception up to November 2020. Manual searches were undertaken of references from relevant systematic reviews.

5.2.2 Search criteria

Search terms are included in table 5.1.

Table 5.1: search terms
1. (aged or Old* or elder* or frail* or functional* impair* or cache* or sarcop*).mp
2. ("muscle mass" or "muscle strength" or "body composition").mp
4. (drug or medication or pharmaco*).mp
4. 1 and 2 and 3
5. limit 4 to (english language and humans, and adults, and (adaptive clinical trial or clinical study or clinical trial, all or clinical trial, phase I or clinical trial, phase ii or clinical trial, phase iii or clinical trial, phase iv or clinical trial or comparative study or controlled clinical trial or observational study or pragmatic clinical trial or randomized controlled trial or "systematic review"))
mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms

5.2.3 Study inclusion and exclusion criteria

Inclusion criteria were trials investigating the impact of a pharmacological intervention on body composition including a measure of muscle mass, for example using DXA, BIA, CT, USS or MRI as an end-point and conducted in adult humans. The muscle mass measures included are those recommended in EWGSOP2 guidelines [190], though it is noted that BIA has sensitivity issues. Total body potassium (TBK) was included, having demonstrated similar sensitivity to DXA, but whole-body air displacement plethysmography [387], and hydrostatic weighing were excluded due to lower reported sensitivity [388].

Trials from any date were included. Both randomised and non-randomised trials were included but observational population database studies were excluded. Trials looking at pharmacological interventions in combination with nutrition and exercise were included. In these studies, if a functional measure was included, it was considered a positive result if the pharmacological agent showed a gain above that seen with exercise alone.

Studies that did not include a measure of muscle mass or only included anthropomorphic measures, did not include an intervention, included a nutritional or exercise only intervention, included children or teenagers, animal models or in vitro studies were also excluded. Studies investigating a treatment with the aim of overall weight loss were excluded if they were combined with a hypocaloric diet.

Studies investigating treatments for condition with an underlying autoimmune or neurological cause such amyotrophic lateral sclerosis or myasthenia gravis were reviewed but only included if it was the opinion of a co-reviewer and myself that they could have transferable efficacy. Studies investigating treatments for single muscles or muscle groups were excluded (for example surgical studies of knee replacement) or only respiratory muscles.

The European Medicines Agency (EMA) regulatory powers covers medications, therefore substances such as amino acid capsules and omega-3 fatty acids are usually considered a dietary supplement. However, the US Food and Drug administration (FDA) regulates food and medications, and some preparations of omega-3 fatty acids fall under medications and so these studies were included.

Conference abstracts, theses, commentaries, books, reviews, and case reports were excluded.

5.2.4 Study selection

Abstracts were reviewed by the myself, with secondary review by one of my supervisors (JMW). If any disagreement about inclusion was present this was resolved by my primary supervisor (WM).

5.2.5 Data extraction

Following initial screening data was extracted from the selected studies including the following points.

- study population
- number of participants
- study duration

- agent used
- dose of agent used
- method(s) of assessing muscle mass
- method(s) of assessing muscle function – if present
- whether the study is randomised
- whether the study is blinded
- outcome of muscle mass (gain/stability vs loss)
- outcome of muscle function (if present)

Different methods of assessment provide different measures of muscle mass, such as lean body mass (LBM), fat-free mass (FFM) or body cell mass (BCM). For the purpose of this review the term muscle mass (MM) is used to represent all measures. Given that progressive loss of muscle mass is seen with both disease and aging, the preservation of muscle mass was also taken as a positive effect of an agent.

5.3 Results

5.3.1 search results

Because of the inherently wide nature of the search a large number of total results were returned.

These were reviewed in a 3-stage process. Initial title and abstract review of Medline search results was able to exclude a large number of studies due to being a) focussed on nutrition or exercise alone, b) reviews c) animal or pre-clinical studies d) studies of children or e) otherwise not relevant.

A second pass then reviewed 758 of the Medline results, further excluding 188 results which were nutritional only or systematic reviews. At this stage all results from CINAHL and EBSCO host were also reviewed, with title and abstract review of 2588 papers.

Following removal of duplicates final review was undertaken on 570 papers from Medline, plus a further 46 from EBSCO host and CINAHL. Final exclusion of these papers left 350 for data extraction. These results are shown in the consort diagram, figure 5.1.

The number of papers reviewed for each pharmacological agent group is shown in table 5.2. The most studies groups were hormonal agents, with 91 papers investigating testosterone and other androgenic hormones, and 74 of growth hormone.

A text summary of results is included below, and full patient tables are included in appendix 3.

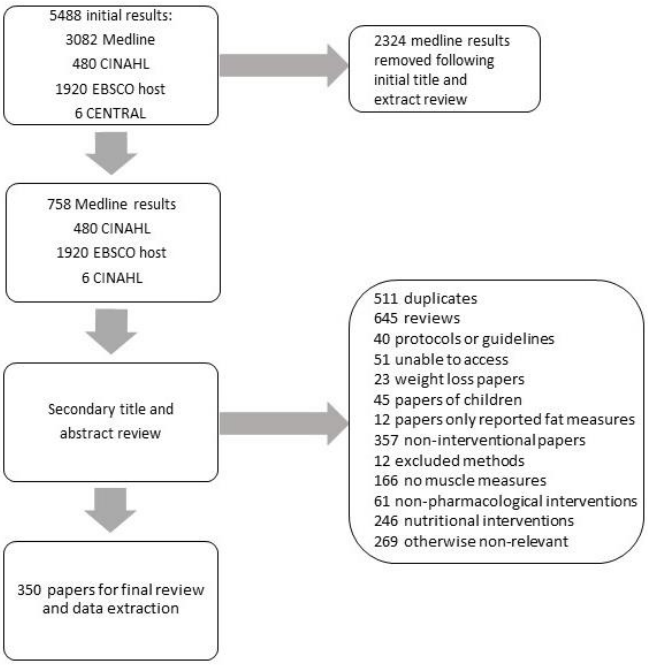

Figure 5.1 Consort diagram of study selection

5.3.2 Testosterone and other androgens.

5.3.2.1 Testosterone

As may be expected, only 2 papers out of 56 (3.5%) [389, 390] investigating the effects of testosterone specifically included women and these were at much lower doses than in men (physiological doses). It was however generally well tolerated in women with the predominant reported toxicity being acne.

Older men were studied in 22 papers (see appendix 3, table 5.3.4) [316, 391-411], with 2580 total participants. Of these studies 8 (36%) included patients with low testosterone levels, 3 (13%) didn't report baseline testosterone levels and the remaining 11 (48%) included patients with normal, or low-normal levels.

There was significant heterogeneity of preparation and dose of testosterone replacement used, and duration of study (average 9.3 months, range 1.5-36). The method of assessment for muscle mass was BIA in 2 studies, CT in 1 study and DXA in the remainder. The impact of testosterone alone was studied in most of the studies, with 2 studies combining testosterone and exercise, and 3 cross-sectional studies that investigated testosterone plus exercise, compared with testosterone alone exercise alone or placebo alone. An objective measure of physical function was included in 17/23 (74%) studies, most commonly individual muscle strength measures.

An increase in MM was seen in 21/22 (95%) studies and an increase in physical function seen in 8/16 (50%) studies. However, of the 5 studies that investigated exercise alongside testosterone, an increase in physical function compared to that achieved with exercise alone was only seen in 2/5 (40%) and these were small absolute gains. Gains in MM were frequently dose dependent and only seen at higher doses where multiple dose levels were trialled.

Younger, and middle-aged men (aged < 60) were studied in 10 trials (appendix table 5.3.5) [412-419]. All studies investigated the effect of drug alone. Baseline testosterone levels were normal in 6 (60%) of the studies, normal or low in 1 (10%), low in 1 (10%) and purposefully suppressed in 2 (20%). Again, there was marked heterogeneity in preparation, dose, and duration of study.

Three studies included obese patients but without diet restriction. Changes in muscle mass were not reported in 2 of the 10 studies (although the methodology included a measurement) and a gain was seen in 4/8 (50%) studies that did report muscle mass. Functional measures were included in 4 studies and function was increased in 3/4 (75%), of these, one reported an increase in function at highest T doses, and the other only in one muscle measure.

Table 5.2 Types of agents investigated and number of included papers	
Agent class	Number
Testosterone & other androgens	92
Oestrogens and progesterones (excluding megestrol acetate)	29
Growth hormone, IGF-1	71
Megestrol Acetate	6
Vitamin D	18
Omega-3 fatty acids	23
Bisphosphonates	2
Anti-inflammatories	19
Anti-diabetic agents	18
ACE inhibitors	3
Statins	1
Miscellaneous	13
Anti-muscle catabolism agents	12
Ghrelin and ghrelin receptor modulators	9
combinations	34
Total	350

Patients with androgen deficiency were studied in 12 trials (appendix table 5.3.6) [390, 420-430]. As with other studies there was heterogeneity of dose and duration, but all investigated the effect of drug alone and all but one used DXA as the method of assessment. All 12 studies demonstrated an increase in MM, and 2/3 studies which included a functional measure demonstrated an increase in physical function.

Testosterone in the disease state

Twelve studies, with a total of 543 patients investigated the impact of testosterone in patients with chronic diseases (appendix table 5.3.7) [389, 431-441]. These included 2 studies of patients with heart failure, 2 of patients with chronic obstructive pulmonary disease (COPD), 3 studies of patients with Human Immunodeficiency Virus (HIV) associated wasting, and one each of myotonic dystrophy, hypopituitarism, cancer-

related weight loss and complete spinal cord injury. These are heterogenous conditions, with disuse atrophy the likely cause for sarcopenia in some, and inflammatory wasting more significant in others. This group included the only two studies of women, one, women with HIV wasting, and the other women with hypopituitarism. Four of the twelve studies investigated the impact of testosterone combined with exercise. As with other groups there was heterogeneity of dose and duration, 8 of 12 studies included a functional measure.

An increase in MM was shown in 9/12 (75%) studies, and an increase in function in 4/8 (50%).

5.3.2.2 Synthetic androgens

The synthetic androgens oxandrolone and nandrolone, metenelone, oxymetholone and stanozolol were investigated in 20 studies. Of these, 9 studies were older adults with 159 participants (appendix table 5.3.8) [442-450], and 11 were disease state (appendix table 5.3.9) [451-461] with a total of 635 participants. There was heterogeneity of dose and duration, and none assessed baseline androgen levels.

Older adults

All of the studies of older adults were randomised and blinded. Drug alone was investigated of 7/9 studies, drug plus protein supplementation in 1 and drug with resistance exercise in 1. DXA was the method of analysis in 6 studies, DXA and MRI in 2 and MRI alone in 1.

An increase in MM was seen in 8/9 (89%) of studies. A functional measure (1RM, 1 repetition max muscle strength) was assessed in 5 studies, of these 3 (60%) showed a gain.

Androgens in the disease state

Studies investigating the use of synthetic androgens in disease states included 8 randomised (1 cross-over), and 3 non-randomised trials. The disease states included stroke rehabilitation or paralysis (2), COPD (1), HIV wasting (5), severe burns (1) and haemodialysis (2). Methods of analysis were BIA (4), DXA (3), CT (1), MRI (1) and

MRI and DXA (2). All 11 studies demonstrated an increase in lean body mass and 6/9 studies included a functional measure, of these, 4/6 (66%) showed an improvement in physical function.

The synthetic androgens oxandrolone, which was then switched to intramuscular (IM) nandrolone were compared with testosterone in a small, randomised, blinded study of 30 obese adult men using CT to measure MM. This demonstrated increases in MM for oxandrolone and testosterone but not nandrolone, where MM decreased.

The impact of androgens in patients with HIV and weight loss was assessed in a meta-analysis. They reported a small but significant impact on both weight and MM in patients receiving androgens compared with placebo.

5.3.2.3 DHEA

Dehydroepiandrosterone (DHEA) is a steroid hormone pre-cursor, produced in the adrenal glands naturally, it has a partial androgen, partial oestrogenic effect. It was investigated in 16 papers. Of these, 11 studied older adults (946 participants) (appendix table 5.3.8) [264, 462-471] and 5 studied patients in a disease state (192 patients appendix table 5.3.9) [472-476]. All but two studies investigated DHEA at the dose of 50mg per day. Six studies included patients with low DHEA levels, and 3 with low-normal, and 1 not reported. All studies but one were randomised and blinded.

Drug alone was investigated in 8 studies and with exercise in 3. Five studies included a functional measure. There was only an increase in MM in 5/11 (45%) studies and an increase in physical function in 3/5 (60%) studies.

DHEA was studied in the disease state in 5 papers, of which all but one were randomised and blinded, with diseases including kidney disease requiring haemodialysis (1) and adrenal failure (4).

There was an increase in MM in 4/5 papers (80%), and an increase in physical function in 1/3 (33%).

DHEA was compared to testosterone in one study of 24 older adults. Women received DHEA, men received DHEA or T. The study used DXA, CPET and knee extension

force as measures of MM and function respectively. There was a gain in MM seen only in men receiving T and no improvement in function.

In summary, there is good evidence to support the fact that androgens, both natural and synthetic, increase muscle mass, though there is significant heterogeneity in the data. The data to support an improvement in function is less clear.

5.3.3 Oestrogens and progesterones

For the purpose of this section, megestrol acetate will be discussed separately.

The effect of oestrogens on body composition was investigated in 29 eligible studies. Of these, all but 3 studies investigated post-menopausal or peri-menopausal women.

Post-menopausal women

Almost all of the 26 studies (appendix table 5.3.10) [260, 477-501] investigating post-menopausal women used DXA as the method of assessment, 4 used BIA and 2 used CT.

There was heterogeneity of drug type, preparation and dose, and duration of study (mean duration 24 months), all but 2 studies investigated the impact of drug alone. In total 7551 patients were included. Eight of the papers were non-randomised or blinded.

An increase or maintenance of MM was seen in 13/26 (50%), of these, 3 studies were comparing tibolone to other oestrogenic preparations and a benefit was only seen in those receiving tibolone. A functional measure was included in 4 studies, with 1 of these showing benefit.

The impact of oestrogens in post-menopausal women was assessed in a meta-analysis, which demonstrated a small, but significant reduction in muscle loss in users of HRT [502]. Another meta-analysis assessing strength only, demonstrated a small benefit in users of HRT [503].

Younger women

The impact of oestrogens and progesterones in the form of hormonal contraceptives was investigated in 3 studies of younger women (appendix table 5.3.11) [504-506], with a total of 302 participants and durations of 12 or 24 months.

All 3 studies investigated drug alone, though one specifically selected women who regularly exercised. Two studies used DXA and one used BIA. None of the studies investigated function.

Two of three studies [504, 505, 507] showed a benefit in MM, one of these studies also showed a gain in overall body mass.

Combinations

Tibolone was compared with the selective oestrogen receptor modulator raloxifene in a randomised, blinded study of 290 frail post-menopausal women [508]. The study used DXA and HGS to measure MM and function and showed a gain in MM with tibolone and raloxifene compared to placebo, but neither was superior and there was no functional improvement.

A combination of HRT with tibolone, or oestrogen and medroxyprogesterone with vitamin D, was compared to vitamin D alone in 1 study of 155 post-menopausal women in a randomised but unblinded study using DXA [509]. Gain in MM was seen in the tibolone arm only.

5.3.4 Androgen or oestrogen combinations

Androgen combination studies are shown in appendix table 5.3.12. Testosterone has been investigated in combination with a 5-alpha reductase inhibitor in 3 studies, 2 of older men [510, 511], and 1 of men aged <50 [512]. Both studied were randomised, blinded, and assessed MM using DXA. They investigated drug alone, included no functional measure and both showed a gain in MM.

A combination of androgens and vitamin D was assessed in two studies of older men [513, 514] with testosterone and one study of older women [515] with nandrolone. Both studies of men were randomised and blinded and used DXA and a functional measure.

One study of older men showed a gain in MM but not function [513], whereas the other showed a gain in both [514]. The study of post-menopausal women showed a gain in MM, with no assessment of function.

Androgens combined with glucocorticoids was assessed in two small, randomised, and blinded studies using DXA of patients with disease requiring glucocorticoid use [516, 517]. Both showed a gain in MM, whereas those receiving glucocorticoids alone gained only fat. One study assessed function and showed improvement [516].

A combination of oestrogens and androgens was investigated in 8 studies, with a total of 317 participants, and mean durations 9.4 months (range 2-24 moths) [518-525]. All the studies were randomised and blinded. Post-menopausal women were investigated in 6/8 studies. MRI was used in 1 study and DXA in 6.

There was an increase in MM in 6/7 studies, and an increase in function in 2/4 studies which included a functional measure.

Where combinations were trialled compared to either agent alone, the combination of oestrogen and androgen showed a larger gain in MM than either agent alone.

5.3.5 Megestrol

Megestrol acetate was investigated in 6 studies that met inclusion criteria [304, 305, 526-529] (appendix 3, table 5.3.13), with a total of 496 patients included. One study included older adults and the remainder investigated megestrol in the disease state. Of these, 1 study included patients on dialysis, 3 included patients with HIV related weight loss and one, patients with COPD and weight loss. Five of six studies were randomised and blinded. Two included a functional measure.

Four studies used BIA as method of assessment, 1 used DXA and 1 used CT plus whole-body air displacement plethysmography. Two studies included a functional measure.

Four of seven studies investigated a dose of 800mg per day. All of the studies were under 6 months duration, and 4/7 were 3 months or under.

Two studies (33%) demonstrated a gain in LBM. Of the others, some showed a gain in weight but not MM. Neither of the 2 studies including a functional measure showed an increase in function.

Megestrol combinations:

Megestrol was investigated in comparison to or combination with androgens in 3 studies, 2 of patients with HIV[530, 531] and one of older men[532] (appendix 3, table 5.3.12). Two studies used BIA and one DXA, all 3 were randomised and blinded. All 3 studies showed a gain in MM and did not assess function.

Megestrol was combined with formoterol in a small, unrandomized, study of 13 patients with advanced cancer and weight loss[314]. The duration was 2 months, and MM was assessed with MRI, and function with HGS. There was an increase in MM seen and a non-significant improvement in function.

Megestrol was investigated in 124 women with gynaecological malignancies alone, compared with celecoxib and protein supplementation in a randomised and blinded study[533]. MM was assessed using DXA and a gain was reported.

Megestrol was compared to omega-3 fatty acids, thalidomide and a dietary amino acid supplement or a combination of all 4 agents in a randomised, unblinded study of 332 adults with advanced malignancy [534]. The omega-3 fatty acid arm was withdrawn after interim analysis due to inferiority.

The combination arm showed a small but significant increase in MM, whereas the other investigational arms showed a decrease.

5.3.6 Growth Hormone

The impact of growth hormone on MM has been investigated in 58 studies that met inclusion criteria, with a total of 4502 participants. Of these 39 studies were in growth hormone (GH) deficient adults. Nineteen studies were of adults, including obese adults, or older adults and 13 studies included adults with a co-morbid disease.

Adults with GH deficiency

Studies of adults with GH deficiency included a total of 2584 participants (appendix 3, table 5.3.14) [535-572]. All studies investigated the impact of drug alone, though one included dietary advice. There was significant heterogeneity of dose and preparation of drug used, though the majority included subcutaneous recombinant human growth hormone. Mean study duration was 14 months, range 0.5-120.

The method of assessment was DXA alone in 21 studies, DXA and either BIA, TBK or CT in 8 studies, BIA in 3 and CT in 4. Twenty studies were randomised, and 17 blinded. Functional measures were included in 11 studies. An increase in MM was seen in 37/38 studies. An increase in function was seen in 7/11 studies, of these, some studies demonstrated an increase in exercise capacity but not individual muscle strength.

Older and obese adults

Growth hormone was investigated in 13 studies of older adults [317, 573-584], and 6 studies of obese adults[585-590] (appendix 3, tables 5.3.15 and 5.3.16), with a total of 885 patients, including one large study of 395 older adults. All but 2 studies were randomised and blinded, mean duration was 5.51 months, range 1.5-18 months.

The method of assessment was DXA in 12 studies, DXA plus MRI or CT in 8 and TBK in 1 study.

An increase in MM was seen in 8/13 (62%) studies of older adults [574-578, 591-593] and an increase in function was seen in 6/13 studies which included a functional measure. Only 1 study which included exercise showed a benefit to GH above exercise alone.

All studies of obese adults investigated drug alone and none included a functional measure. Five of 6 (83%) studies showed an increase in MM with associated loss of fat mass[585, 587-590].

Growth hormone in the disease state

Thirteen studies investigated growth hormone in the disease state, with a total of 942 participants (appendix 3, table 5.3.17) [594-607], though the majority of these came through two large studies, with 10/13 studies having fewer than 30 participants.

Conditions studied included: HIV (5) [595, 597, 598, 604, 605], Dialysis (1) [596], muscular atrophy (1) [607], short bowel (3) [599, 601, 606], Crohn's disease (1) [594] malnutrition (1) [600] and injury induced muscle wasting (1) [608]. Methods of MM measurements were BIA (4), MRI (2), and DXA (8).

As with other groups, there was significant heterogeneity of dose and preparation, and all studies were 6 months or shorter.

Nutritional support was given in the 3 studies of patients with short bowel, and the study of patients with malnutrition. Rehabilitation exercise was investigated in one study, the rest investigated drug alone. Four studies included a functional measure.

An increase in MM was reported in 13/14 (93%) studies, and all 4 studies that included a functional measure showed an improvement, including the study which included rehabilitation exercise.

Growth hormone combinations

Growth hormone in combination with androgens or oestrogens was investigated in 8 randomised and blinded studies [609-616] (Appendix 3 table 5.1.18). One study included patients with HIV [616], one with hypopituitarism [614] and the remainder in older adults. Method of assessment was DXA in 7 studies and BIA in 1.

An increase in MM was seen in all studies, and in strength in 5/6 studies. The increase in MM was seen for androgens and GH alone in all but 2 studies, and the increase in strength only seen in combination arms in 3 studies [609, 617, 618].

Meta-analyses reviewed the impact of GH on MM in older adults[619]. A measure of lean mass was reported in 14 studies that met inclusion, with a mean gain of 2.13 kg across the studies. However, they noted that there was no benefit seen over lifestyle interventions (exercise). A small, non-significant increase in VO_2 max on

cardiopulmonary exercise testing was reported. A further meta-analysis by Liu et al. reported on 27 studies of young adults investigating the impact of GH on athletic performance [620]. A similar mean gain in MM of 2.10 kg was seen, but no impact on athletic performance.

5.3.7 Vitamin D

The impact of vitamin D has been assessed in 18 included trials, with a total of 2465 participants and a mean duration of 7 months (range 1.5-24), appendix 3, table 5.3.19 [621-638]. Fourteen of eighteen studies were of older adults, three were of adults with vitamin D deficiency and one of healthy adults. Vitamin D has not been studied in the disease state in any eligible studies.

There was significant heterogeneity of dosing. Drug alone was investigated in 8/18 studies [622, 624-626, 629-632, 634, 635, 637], 3 studies investigated vitamin D and protein diets [621, 623, 636] and 3 the concomitant impact of exercise [627, 633, 638, 639].

Vitamin D levels were low in 10/18 studies, and patients were not selected according to vitamin D levels in the remainder. The most common method of assessment was DXA, used in 11 studies, then BIA (5), CT (1), US (1) were used in the remainder. Functional measures were assessed in 12/18 studies. All studies were randomised, 3 were open-label and the remainder blinded. A gain or maintenance in MM was seen in 6 of 18 studies [622, 623, 628, 632, 633, 636]. Of these one compared 2 doses of vitamin D alongside physical exercise (Nordic walking) at different intensities, improvements were only seen in MM in at higher doses and in the moderate intensity group. This study showed an improvement in function, but it should be noted that all groups were receiving exercise training, and there was no placebo arm [633]. Three studies investigating drug alone showed benefit, of these, two of the 3 included patients with low baseline vitamin D, only two of these included a functional measure and only one showed improvement in patients unselected for baseline vitamin D. Two further studies showed an increase in vitamin D, which also included a protein enhanced diet. Of these, one small study of older adults showed an increase in MM but reported higher mean vitamin D levels in the control group (receiving protein only) [623]. The other, a large study of older adults compared a protein and vitamin D enriched nutritional supplement,

with a non-protein enriched nutritional supplement and no vitamin D [621], therefore it can be confirmed that it was not the impact of protein rather than vitamin D which had effect here. This study showed a benefit in function in one measure only; sit to stand test.

In summary, although some studies have shown a benefit to muscle mass, confounding factors mean that a definitive opinion cannot be drawn from the available literature.

Meta-analyses [640-646] have shown conflicting results about the impact of vitamin D on muscle strength and no impact on mass.

Vitamin D combinations

Vitamin D has been trialled in one small study in combination with the bisphosphonate drug alendronate[647]. The population was post-menopausal women with osteopenia. The study was non-randomised and used DXA and HGS for assessment. There was no improvement in MM but a small gain in HGS was seen.

Vitamin D trialled in combination with omega-3 fatty acids will be discussed below.

5.3.8 Omega 3 fatty acids

Omega-3 fatty acids were investigated in 23 included studies. The populations were adults in 2 studies, older adults in 7 (appendix 3, table 5.3.20) [648-656] and disease states in 14 [657-659], of these 11 studies investigated cancer populations [660-670]. Drug alone was investigated in 12 of the studies, with dietary support in 8 of the studies and exercise in 3.

Studies of adults and older adults investigated a total of 393 participants in small studies with a mean duration of 3.8 months (range 3-6 months). All studies were randomised and blinded. Methods of assessment were BIA (4), DXA (3) and MRI (2) and 7/9 studies included a functional measure.

An improvement in MM was seen in 4/9 (44%) studies [651, 652, 654, 655] and an improvement in function in 5/7 (71%) [648, 651, 653-655].

In the disease state, 3 studies investigated adults with heart disease and 11 patients with cancer. Apart from one large study of 518 patients [662], all studies were relatively small, and all had a duration of 3 months or less. Six of eleven studies were randomised and blinded. One study used DXA and 1 used CT, with the remainder using BIA. Only 1 study, of males with coronary artery disease, included a functional measure [659]. An improvement in MM was seen in 9/14 (65%) studies, [659-661, 663, 665-667, 669] and there was no functional benefit seen in the one study that investigated this.

In summary, there is some evidence to support the use of polyunsaturated fatty acids (PUFAs) in the disease state, which is less convincing in older adults. However, almost all of these studies used BIA which is the least sensitive of the included methods of assessment, and more research in this area would be of benefit.

Polyunsaturated fatty acids combinations

A small study of healthy adults after a period of immobilisation investigated eicosapentaenoic acid (EPA) and vitamin D in combination [671]. It was randomised and blinded and used DXA as method of MM assessment. The study was just 2 weeks long and showed a non-significant trend towards improved MM.

A small, 6-week, randomised and blinded study of 22 patients with NSCLC investigated PUFAs in combination with the anti-inflammatory agent celecoxib, or placebo [672]. The study used BIA and HGS as assessment methods and showed a benefit to the combination over PUFAs alone.

A meta-analysis of the use of PUFA in older adults reported a small, but significant mean effect in increasing muscle mass with larger effects seen in higher doses and longer duration [673]. A meta-analysis of PUFA in healthy adults did not demonstrate a significant effect on MM [674]. A meta-analysis of the omega-3 fatty acid eicosapentaenoic acid (EPA) for cancer associated cachexia, looking at an outcome of weight gain, rather than muscle gain specifically, was unable to recommend EPA for the treatment of cancer [675].

5.3.9 Bisphosphonates

Two included studies of older adults investigated the effect of bisphosphonates on MM as well as on bone mass, with a total of 127 participants[676, 677]. Both studies were 12 weeks long, used DXA as method of assessment and included a functional measure. Both were randomised but not blinded, they used different bisphosphonates and doses. One study included a dietary supplement, the other compared the drug with exercise, compared to exercise alone or drug alone or placebo. Neither study showed an improvement in MM or function.

5.3.10 Anti-inflammatories

Thirteen studies of anti-inflammatories [310, 312, 678-688], and 6 studies of anti-TNF-α agents met inclusion criteria [689-694].

Of the 13 studies, 5 investigated patient in the disease state [312, 685, 687, 688], 4 patients with cancer and 1 adults with active infection [686], 2 studies investigated adults and the remaining 6 investigated older adults.

The agent used was ibuprofen in 8 of the studies, celecoxib in 3 studies and other non-steroidal anti-inflammatories (NSAIDs) in 2.

Studies of adults and older adults

Mean duration of the studies was 4.5 months (range 1.5-9), with a total of 502 participants. All studies were randomised and blinded, and drugs were trialled in combination with, or against, exercise. Ibuprofen was the agent used in 7/8 studies at varying doses, and paracetamol was used in the 8th trial. Four studies used DXA and 4 used MRI as method of assessment. Five of eight studies included a functional measure.

None of the studies showed a significant increase in MM from the addition of anti-inflammatories to exercise, in fact it appeared to attenuate gains seen from exercise in one study [310]. An improvement in function was seen in 1/5 (20%) studies [683], this was the study of paracetamol, which was undertaken in older adults with osteoarthritis. It could therefore be that the analgesic effect of the drug increased the amount of training participants were able to achieve rather than a direct drug effect.

Studies in the disease state

Two studies were randomised and blinded, one study randomised open label and two non-randomised trials. Four studies investigated adults with cancer, of these 3 used celecoxib (2 studies dosed at 300mg/d and one at 200mg twice daily) [312, 687, 688] and one ibuprofen (400mg three times daily) [685]. Three studies investigated drug alone and one celecoxib plus exercise and a nutritional supplement containing omega-3 fatty acids. One study investigated patients with acute infection. All studies were of short duration, between 0.75 and 4 months. There was a range of assessment methods included, 2 studies used BIA and DXA, one DXA alone, one TBK and one CT.

One small study of patients with advanced cancer showed small but significant gains in MM and strength [688] and one showed maintenance of MM. All of the other trials were negative.

5.3.11 Anti-TNF-α agents

Anti-TNF-α agents were trialled in 6 studies, of which 5 included patients with conditions treated by anti-TNF-α agents such as psoriasis or spondyloarthropathies [689-692, 694] and one patients with metabolic syndrome [693], with a total of 266 participants. All studies investigated drug alone, with a mean duration of 9 months (range 1-24 months). Only 1 study was randomised, all were open label DXA as method of assessment, with one study using MRI, and only 1 study including a functional measure. An improvement in MM was seen in 3/6 studies [690-692], of which one also showed an increase in function.

5.3.12 Anti-diabetic agents

Eighteen studies of anti-diabetic agents met criteria for inclusion (appendix 3, table 5.2.23), with a total of 1150 participants. Of these 12 studies were of adults with diabetes [695-705], 1 of healthy older adults[706] and 5 of patients in the disease state (cancer, HIV and renal disease requiring dialysis) [707-711].

Patients with diabetes

A range of agents and doses were included in the studies with a total of 747 participants and mean duration 7 months (range 2-12 months). Seven of 14 trials were randomised,

of these two were blinded and all investigated drug alone. Method of assessment was DXA in 9 studies and BIA in 5. An improvement in MM was seen in 5/14 studies. Two studies included a functional measure, of which one showed a benefit.

Anti-diabetic agents in the disease state

A small non-randomised study of 21 patients investigated a GLP-1 agonists against a DPP-4 inhibitor in patients requiring haemodialysis (assessment method BIA) [707]. A small randomised, open-label study investigated metformin in 25 patients with HIV associated lipodystrophy (assessment method CT) [710]. An open-label study of patients with non-alcoholic steatohepatitis investigated a combination of anti-diabetic agents (DXA) [709] and a randomised, double-blind study of 104 patients with cardiovascular disease investigated rosiglitazone [708]. A randomised, open-label study of 138 patients with advanced cancer investigated the impact of insulin alongside "maximum supportive care" which included nutritional support, indomethacin, and erythropoietin (assessment method DXA) [710].

None of the studies showed an improvement in MM or function. Anti-diabetic agents led to a loss of muscle mass in the study of patients undergoing dialysis and the study of patients with HIV.

5.3.13 Anti-hypertensives and cholesterol lowering agents

Three studies of anti-hypertensives and one study of statins met inclusion criteria (Appendix 3, table 5.3.24). This included two studies of older adults [712, 713], one, a large observational cohort study comparing users of ACE inhibitors with non-users [713]. The other was a small, randomised, and blinded study of ACE-inhibitor alone, ACE-inhibitor plus exercise and placebo plus exercise.

The large observational cohort, by nature, included variable doses and duration of treatment. Patients were assessed using DXA and the timed-up and go test (TUG). No significant differences in MM or strength were noted between the two cohorts.

The randomised study demonstrated an increase in MM and function in the losartan/ exercise and placebo/exercise groups compared with the sedentary group, but no benefit was seen to losartan over placebo in the exercise groups.

A trial of patients requiring haemodialysis for renal disease investigated the impact of angiotensin II receptor blockers (AIIRBs) [714]. The study included a control group not on drug and a single time-point assessment only. Using BIA and HGS as methods of assessment they identified a small but significant increase in HGS in the group receiving AIIRBs.

No meta-analysis of the effect of anti-hypertensives on MM exists, a meta-analysis of their impact upon physical function showed no benefit [715].

One trial of patients with HIV receiving highly active anti-retroviral treatment and statins met inclusion criteria [716]. In this randomised, blinded trial of 147 patients, patients received either rosuvastatin 10mg daily or placebo for 24 months and MM was assessed by DXA. An association with increased MM was noted on multivariate analysis only.

5.3.14 Anti-muscle catabolism agents

Anti-muscle catabolism agents are new agents, targeting cytokines within the muscle catabolism pathway, most commonly myostatin and activin II. Twelve studies of anti-muscle catabolism agents met inclusion criteria (Appendix 3, table 5.3.25) [272, 717-727], with a total of 1563 participants.

Three classes of agent were included, anti-myostatin antibodies (3), anti-follistatin antibodies (1) and activin II receptor (AIIR) antibodies (8). Three studies which were dose escalation were non-randomised and open label, the remainder were randomised and blinded.

Activin receptor antibodies

Seven studies investigated AIIR antibodies in 3 studies of older adults [720, 721, 727], 1 of healthy adults [722] and 3 in the disease state [717-719], Five of eight studies investigating AIIR antibodies used DXA and MRI as method of assessment, 2/8 used DXA alone and 1 used MRI alone. A functional measure was included in 6/8 studies. Mean duration was 6 months. Dose used was bimagrumab 30mg/kg but at different dosing schedules in 4/8 studies, with different or escalating doses in the other studies. One study included dietary advice and exercise, the remainder investigated drug alone.

One small study of healthy adults showed a gain in MM, but no functional improvement was seen.

In 3 studies of older adults there was an improvement in MM in all 3 studies. A functional measure was included in 2/3 studies and there was an improvement in both of these, though only in gait speed and not grip strength within one study.

Two studies investigated AIIR antibodies in patients with neuromuscular diseases, and one studied patients with COPD and weight loss. All were randomised and blinded, investigated drug alone and all used DXA or DXA with MRI and included a functional measure. There was heterogeneity of dosing and study duration.

All studies showed an improvement in MM, one small study in neuromuscular diseases showed an improvement in function, but a much larger study in patients with inclusion body myositis did not. The study of patients with COPD did not show an improvement in function.

Other muscle catabolism agents

Anti-myostatin antibodies have been investigated in two studies of older adults [272, 726], one study of healthy volunteers [723] and one study of patients with pancreas cancer receiving chemotherapy [724]. All 4 studies were randomised, blinded and used DXA, with 2/4 including a functional measure.

Different dosing schedules were used in the three studies. Three of 4 studies showed an improvement in MM and 2/2 showed a functional improvement (the study of patients with cancer showed a functional benefit but not a benefit to MM).

Finally, a small, non-randomised and open-label phase 1 trial of a follistatin inhibitor [725], in healthy volunteers met criteria for inclusion. Muscle mass was assessed with MRI and function with dynametry over 3 months. There was an improvement in MM but not function.

In summary, anti-muscle catabolism agents show promise, in both disease states and older adults for increasing MM but have not yet demonstrated that they improve physical function.

5.3.15 Ghrelin, and Ghrelin receptor agonists

Ghrelin has been investigated in 6 studies [311, 728-732], and the ghrelin receptor agonist anamorelin [733-735] in 3 studies which met inclusion criteria (appendix 3, Table 5.3.26).

Ghrelin or ghrelin mimetics were investigated in a total of 383 participants, in 1 study of healthy older adult[732]s, 3 studies of adults with COPD[728-730], 1 study of patients with congestive cardiac failure[731] and 1 of adults with advanced cancer[311]. With the exception of the study of healthy adults, all studies were of short duration, <3 months. All studies except 2 (1 of cardiac failure patients and 1 of patients with COPD) were randomised and blinded. An improvement in MM was seen in 4/6 studies, with no benefit seen in one small study of patients with COPD[730]. A functional benefit was seen in 2/5 studies, it should be noted these were both non-randomised open label studies.

Anamorelin

Two studies of anamorelin in patients NSCLC [313, 734] and two of patients with advanced cancer and weight loss [733, 735] met inclusion criteria, with a total of 1227 participants. Three of four studies were randomised and blinded, all used DXA as method of assessment and 2/4 included a functional measure. The dose was 100mg/d in 3 studies and 50mg/d in the study of advanced cancer patients and all were 3 months duration. All studies investigated the impact of drug alone.

All 4 studies demonstrated a gain in MM, and neither of the 2 which included a functional measure showed a gain.

It has been widely discussed that anamorelin did not gain a licence in the European Union or US due to a limited benefit in terms of MM and no functional improvement, or improvement in quality of life [307].

5.3.16 Miscellaneous agents

Thirteen studies of agents that did not fall into any of the previous categories met criteria for inclusion (appendix 3, Table 5.3.267).

The agents were levothyroxine [736, 737] in patients with thyroid disease, melatonin [738, 739] in patients with advanced cancer and post-menopausal women, tadalafil [740], an antihistamine in patients with HIV [741], an anti-viral agent [742] in patients with hepatitis B, erythropoietin [743] in patients with hip fracture, beta-2 agonists [744, 745] in patients with cardiac failure and patients with muscular dystrophy, a novel peptide-nucleic acid [746] in patients with advanced cancer and thalidomide [747] in patients with advanced cancer.

All these studies included small patient numbers, and most were of short duration.

Positive results were seen in the following studies: an open label study of tadalafil in men with erectile dysfunction[740], a study of melatonin in post-menopausal women[738] (a study of melatonin in advanced cancer patients was negative). A study of thalidomide in patients with advanced cancer [747], a study of the anti-histamine ketotifen in patients with HIV [741], a study of erythropoietin in older adults with sarcopenia [743], which also showed an improvement in physical function and a study of the beta-2 agonist albuterol in patients with neuromuscular disease [744] which also showed an improvement in function.

Beta-2 agonists and erythropoietin are well recognised drugs of abuse in elite sports, but there is insufficient evidence from these limited studies to support their use in the cancer setting.

A summary of results is shown in table 5.3a and 5.3b.

Table 5.3a Summary of results

Agent & population	Proportion studies showing gain in MM		Proportion of studies showing gain in function	
	N	%	N	%
Testosterone - older adults	22/23	96	9/17	53
Testosterone - disease	8/12	66	4/8	50
Other androgens - older adults	8/9	89	3/5	60
Other androgens disease	11/11	100	4/6	66

Table 5.3b Summary of results continued				
	N	%	N	%
DHEA – older adults	3/11	27	3/5	60
DHEA - Disease	3/5	60	1/3	33
Oestrogens and progestogens– older women	14/26	54	1/6	16
Oestrogens and progestogens - disease	NA	NA	NA	NA
Megestrol – older adults	0/1	0	0/1	0
Megestrol - disease	2/5	40	0/2	0
Growth hormone – older adults	8/13	62	5/12	42
Growth hormone - disease	12/13	93	4/4	100
Vitamin D – older adults	6/18	33	5/12	42
Omega-3 fatty acids – older adults	4/9	44	5/7	71
Omega-3 fatty acids – disease	9/14	65	0/1	0
Bisphosphonates older adults	0/2	0	0/2	0
Bisphosphonates – disease	NA	NA	NA	NA
Anti-inflammatories older adults	1/6	16	2/5	40
Anti-inflammatories – disease	2/5	20	1/3	33
Anti-TNF - disease	3/6	50	1/1	100
Anti-diabetic agents – older adults	0/1	0	0/1	0
Anti-diabetic agents – disease	0/5	0	0/3	0
Anti-hypertensives – older adults	0/2	0	0/2	0
Anti-hypertensives – disease	0/1	0	1/1	100
Anti-muscle catabolism agents - older adults	5/5	100	2/3	66

A summary of results of trials of patients with cancer is shown in table 5.4

Table 5.4 Trial of patients with cancer				
Agent	N studies	Population	Total N	Impact

Testosterone	1	Men aged < 55 who'd received chemo	35	No effect
Aromatase inhibitors or oestrogen receptor modulators	6	women treated for early breast cancer		3/6 studies showed gain in mass, none tested function
Anti-inflammatories	4	Advanced lung and GI cancers and patients with weight loss	97	1/4 studies showed gain in mass and function (only one testing function)
Omega-3 fatty acids	11	Various cancers	960	8/11 studies showed gain in mass. None tested function
Ghrelin/Ghrelin Analogues	5	Advanced lung and GI cancers and patients with weight loss	1308	4/5 studies showed gain in mass. 0/2 showed gain in function
anti-myostatin antibody	1	advanced cancer	125	Gain in mass, not function
thalidomide	1	Patients with oesophageal cancer	10	Gain in mass, not function
OHR118 peptide nucleic acid	1	Patients with advanced cancer	21	No effect
melatonin	1	Patients with advanced cancer & weight loss >5%	73	No effect
Indomethacin vs Indomethacin plus erythropoietin	1	Patients with advanced cancer and weight loss	108	No impact mass but gain in function
Celecoxib + megestrol acetate vs. megestrol alone	1	Women with gynaecological tumours and cachexia	124	Gain in mass, function not tested
EPA + celecoxib	1	Patients with NSCLC	22	Gain mass and function
Formoterol + Megestrol	1	Patients with advanced cancer and weight loss	13	Gain in mass, not function
Megestrol/MPA, EPA, L-carnitine, all of the above	1	Adults with advanced cancer and weight loss	322	Gain for EPA only

5.4 Discussion

5.4.1 summary

The aim of this review was to consider the current evidence of pharmacological agents' ability to increase muscle mass, to provide an evidence base for future trials aiming to improve the physical condition of patients with cancer. In doing so a wide variety of data in both older adults and different disease states associated with sarcopenia has been incorporated, as these could have applicability for patients with cancer. In summary, gains in muscle mass are consistently seen in trials of androgens, growth hormone and agents targeting muscle catabolism. However, data regarding improvements in function are much less consistent, particularly for androgens and growth hormone. There is less consistent data to support a gain in muscle mass and function for omega-3 fatty acids and ghrelin or ghrelin analogues but these data are present for patients with advanced cancer. Gains in MM were on average around 1kg, and it's difficult to know how significant an effect this would have for patients who may have lost significant weight and muscle mass.

Functional measures were less commonly studied and less commonly showed a benefit. No single class of agent has consistently shown a benefit in physical function. Where a pharmacological agent was studied compared with exercise, they have very rarely showed a benefit over exercise alone.

Androgens and growth hormone have been widely studied, whilst more recently developed agents such as ghrelin analogues, and agents targeting muscle catabolism have more limited data to support their use. Trials of these agents are ongoing.

In considering the use of pharmacological agents for patients with cancer the side-effect profile of the agents must be considered, but also the potential impact on overall body composition and muscle function.

5.4.2 Side effects of relevance to patients with cancer

Androgens, in addition to routinely increasing muscle mass, frequently demonstrated corresponding reductions in fat mass, and improvements in total cholesterol levels [748]. Despite these potential benefits on cardiovascular risk factors, some evidence suggests increased cardiovascular disease risk with testosterone replacement, leading to

warnings from the FDA, though not the EMA [749]. Chemotherapy compounds, particularly platinum agents and fluorouracil are associated with cardiovascular toxicity and so some caution may be required here. Clearly, in the case of prostate cancer, where androgen deprivation therapy is the hallmark of treatment, androgen treatment could not be safely used. Similarly, oestrogens and progestogens may not be appropriate for hormone sensitive cancers including breast, endometrial and ovarian cancer. Case series have reported an increased risk of venous thromboembolism (VTE) and cardiovascular events with androgens [408, 750, 751]. Since both cancer and cancer treatment are well recognised to increase the risk of VTE the use of androgens therefore must be treated with some caution. The risk of VTE has been considered a potential limiter in the use of medroxyprogesterone or megestrol acetate, though a recent trial comparing megestrol acetate to corticosteroids for appetite improvement did not demonstrate increased rates of VTE [752].

Growth hormone is associated with fluid retention [753] which may be undesirable in some patients with cancer already struggling with this troublesome symptom.

Ghrelin and omega-3 fatty acids appear to be generally well tolerated, and the side effect profile of most muscle-catabolism targeting agents appears acceptable, with muscle spasm and diarrhoea most commonly reported [272, 720, 754].

It has long been considered that anti-inflammatory agents would represent an important part of treating cancer cachexia, given the inflammatory state seen in this disease. However, the risk of side-effects, particularly GI toxicity may limit their safe use in patients with cancers of the GI tract.

5.4.3 Impact on function

A frequent feature of studies of pharmacological agents is an increase in muscle mass, but with no corresponding increase in function. It may be that an increase in function requires triggering of the neuromuscular junction, which cannot be achieved with pharmaceutical agents, but is by exercise [248, 315]. Exercise may be difficult for patients with cancer to achieve, due to physical or psychological effects of malignancy, or cancer treatment-related fatigue.

Much of the scientific work into pharmacological agents to improve muscle mass and function has been preceded by illegal use of these agents in both amateur and professional sports. The continued use of these agents suggest they may have some effect on physical function. The reason this has not translated into consistent results in clinical studies could be for a number of reasons, firstly, that in sports doping the pharmacological agent is routinely combined with exercise. Secondly, athletes who have admitted to doping previously have discussed taking a combination of multiple agents [753] and finally, very minimal gains in elite sports may be of significance whereas they may not have a noticeable effect in patients.

Whether a significant impact on function is required in patients with cancer remains unclear. If our overall aim of treatment were to improve performance status, then this may be more necessary. However, if a change in muscle mass could have a positive impact on quality of life or treatment toxicity, despite no significant change in function, then this could still be of benefit to patients receiving cancer treatment.

As cancer-associated muscle wasting is thought to be secondary to inflammation, significant work has been put into the study of anti-inflammatory drugs, though with limited benefit from the studies included within this scoping review. However, in one study in young adults, use of ibuprofen actually attenuated gains in muscle mass from exercise [310] and did not show benefit in studies of older adults. Therefore, for some patients with cancer it is possible that an anti-inflammatory could have a negative effect on muscle mass.

5.4.4 Limitations

The major limitation of this scoping review is the considerable heterogeneity within it, including both populations, doses and durations of study and methods of assessment. Given the age of the studies few include CT as the main measure of assessment, but CT is rapidly becoming the standard measure of sarcopenia in patients with cancer. Any results therefore cannot be directly extrapolated across without further confirmation.

That said, the aim of this review was to summarise a wide breadth of data, and therefore heterogeneity was to be expected and some clear patterns have become evident despite this.

5.4.5 Future directions

Given the complexities of patients with cancer, and their treatment it seems unlikely that a one-size-fits-all pharmacological agent for improving muscle mass will be developed. This review could provide a basis for future studies investigating pharmacological agents for treating sarcopenia, but it emphasises the importance of understanding why a patient is sarcopenic. However, currently no easily available biomarker of wasting vs age-related atrophy exists. Understanding how to identify this difference will be key for future studies, as the treatment for sarcopenia is predominantly age related, and for sarcopenia due to disease-related wasting are likely to be different. Change in muscle mass over time would give some idea, but prior imaging will not be available for all patients. Furthermore, the development of muscle mass cut-offs based on large population studies with age-separated values would be of significant benefit. It may be that for older patients two agents may be necessary, or that combinations of agents to impact on disease-related inflammation, and something to impact on age related sarcopenia are necessary.

It remains unclear whether a pharmacological agent would be of benefit without combination with exercise and how achievable for cancer patients this would be. Studies of exercise in patients with cancer have demonstrated that it is feasible, and some have demonstrated improvements in function, though these have primarily investigated a prehabilitation or adjuvant treatment setting, rather than patients with advanced disease [303, 383, 384, 755-757]. Therefore, more data about the feasibility and efficacy of exercise in patients with advanced cancer would be helpful, before further investigating the addition of pharmacological agents.

As exercise may be challenging for patients with advanced cancer, any agent that can maximise gains from what exercise a patient can achieve may be of clinical benefit. But muscle mass increases metabolic rate which may be a negative for patients in a nutritionally depleted state and so this requires further elucidation. Any future studies in patients with cancer would need to have careful management of nutritional and metabolic states and dietary support.

Future studies for pharmacological agents in this setting are warranted. But what is primarily needed in patients with advanced cancer is high-quality, randomised,

controlled, blinded studies, with careful patient selection. Whilst pragmatically, some heterogeneity of cancer sub-type may be necessary to ensure recruitment, studies should aim to stratify patients according to body composition measures, age, nutritional state, and inflammatory status. Studies should aim to be multi-modal, with regulated protein-enhanced dietary support and exercise included as a baseline measure for all.

6.0 Discussion

The aim of this work was to characterise prevalence and patterns of anorexia in patients with advanced upper GI cancers, investigate the potential role of gut hormones in the pathophysiology of gastric cancer and investigate the relationship between sarcopenia and frailty.

This work represents the initial data from an ongoing project and will help to guide the future directions of this work.

6.1 Anorexia and weight loss

Anorexia and weight loss are highly prevalent in patients in advanced upper GI cancers. However, the patterns of these nutritional changes and their impact on outcomes remains less clear. Dysphagia was also highly prevalent within the cohort, as might be expected, and there was significant overlap between dysphagia and anorexia. The relationship between mechanical obstruction and appetite warrants further investigation. Mechanical obstruction experienced as dysphagia did not account for all anorexia seen within the cohort, and this was predominantly seen in patients with gastric cancer. Altered motility due to delayed gastric outlet obstruction could be contributing to anorexia here, or other mechanisms may be at play. The presence of raised anorexigenic hormone PYY levels points to a role for altered enteroendocrine signalling in this patient group.

From this initial data baseline weight loss and anorexia only impact on survival at the more severe ends of the relative scales. This may well be due to the impact of dietician support, limiting the negative impact of early malnutrition as patients proceed through treatment. It was not possible to directly, comparatively, investigate the impact of this support, as it would not have been ethical to omit nutritional support to patients identified as needing it, but existing comparative trials showing the impact of nutritional support suggest this may be having an impact [294, 295].

Patterns of weight change varied significantly within the cohort, some patients gain significant weight, some patients remain stable, and some patients continue to lose weight. However, the relationship between weight change and disease response to therapy was very variable and there was no clear relationship with treatment toxicity.

The longer term of this work is to characterise which patients may require enhanced support, with a view to improving outcomes, but this initial data does not yet provide a clear path to this. Baseline, early and ongoing weight loss does appear to be a biomarker of disease activity but given this heterogeneity it does not appear that it can select out these patients with necessary sensitivity. Ongoing weight loss, whilst suggestive of disease activity, may also be the result of poor adherence to nutritional support, or treatment toxicity, and this cannot be identified from this data set.

Similarly, cachexia at baseline, based on current definitions did not have sufficient sensitivity to select out only the patients with poorest prognosis. These patients were predominantly represented within the cohort, but many other patients with cachexia had outcomes comparable to those without.

This is likely to be due to two things; either the cut-offs used to select the group are insufficient or, the impact of disease biology is the over-riding factor. Binary cut-offs for definitions are popular due to simplicity but may lack the required sensitivity to select out the relevant patients. In this cohort, the patients with the poorest prognosis had mean weight loss ≥5% at baseline, and mean neutrophil: lymphocyte ratio of 6.13 suggesting that higher cut-offs may be necessary. However, other work has suggested that much lower weight-loss cut-offs are associated with poor outcomes, and so it may be that these higher cut-offs rank the highest risk patients rather than select those for intervention.

Equally, the impact of disease biology is clearly significant. It is well recognised that currently the only effective treatment for cancer cachexia is treatment of the cancer itself. Despite significant cachexia some patients gained good response to chemotherapy and improvement in clinical condition, whereas others did not. So, for patients early in their cancer journey, who have potentially effective treatments available to them, even severe cachexia should not prevent a patient receiving treatment if they are deemed fit enough to receive it.

The long search for effective treatments for cachexia has proved fruitless to date, and this may in part be due to the patient selection in trials used. For patients who have exhausted effective cancer treatment, no anti-inflammatory (for example) is going to have a significant impact on their clinical course, but for patients at the start of their

cancer journey, could a treatment act as a bridge to allow them to remain well enough to receive cancer treatment? This could involve treatments to dampen the inflammatory response, or potentially more active management of symptoms impacting nutritional state and quality of life, such as stenting, prokinetics or appetite stimulants.

The next steps to investigating this are firstly, continued data collection within the ANCHOR trial, to increase sub-group numbers and allow data maturation. Secondly, to further investigate the impact of symptoms such as dysphagia and their relationship to appetite and then moving onto to investigate early active symptom management in more detail in this cohort.

6.2 Enteroendocrine function

The results of the analysis of gut hormones open up two relevant areas of future investigation. The altered GIP, GLP-1 and insulin responses warrant further investigation, initially to confirm this response and then to investigate any correlations with nutritional state and outcomes.

The elevation of PYY levels also suggests a possible pathophysiological mechanism of anorexia in these patients, and this requires confirmation with an expansion cohort. In patients with Crohn's disease raised PYY levels were seen in patients with active disease within the small bowel, but not the large, possibly suggesting the raised levels of PYY are in response to local inflammation rather than systemic. In our patients the site of likely local inflammation was the GOJ and stomach, and it is not possible to know if local inflammation was present in the small intestine (though it would appear unlikely). This would suggest that systemic inflammation signals are the mediator of effect, but the mechanism of this remains unclear.

As well as confirmation of effect, it would be interesting to repeat samples in patients who had demonstrated disease response to see if any change in levels were seen and whether these correlate to improvements in appetite.

6.3 Sarcopenia

In my introduction I discussed that in order to become a biomarker with practical utility for patients with advanced cancer, sarcopenia needs to able to be used to either a) guide

treatment decisions, that is, whether or not to treat b) help guide the amount of treatment; modified choice of treatment or dose reductions or c) identify patients who need an intervention to improve their muscle mass. My data from this prospectively collected cohort of patients with advanced OG cancer suggests with current cut-offs, sarcopenia is not able to do this. It does suggest that the survival for patients with the poorest CFS is so poor that it could guide selection of patients who should not be receiving treatment. Whereas, patients with good CFS scores had good overall survival for the cohort. The overlap between CFS and PS is variable, if properly applied they assess different things: frailty vs cancer symptomology, and so for older patients who are minimally symptomatic of their cancer, the CFS could represent a more reliable assessment tool. Currently there was a trend towards poorer survival for patients with lower muscle density, and it could be that as data matures this becomes significant.

None of the assessments used in this study; CFS, PS, HGS, SMI or SMD were well able to predict toxicity, this may in part be because toxicity was common. Equally, patients with higher CFS scores mostly received baseline dose reductions. This is a strategy known to reduce treatment toxicity for older and frailer patients [758]. There was a significant association between SMI and SMD and toxicity related hospital admission which could be considered more severe toxicity. It could be that sarcopenia has some use to predict the most severe toxicity. Future work expanding this cohort should look at formal toxicity grading to help elucidate this. Other toxicity prediction tools in the frailty arena such as the CARG tool [233] may be better placed to assess toxicity risk, especially in older patients. These tools are detailed and require significant time input, and so the temptation to use a simpler biomarker such as PS or CFS will remain.

All of the risk predictors investigated, SMI, SMD, CFS, PS, and cachexia seem to have good sensitivity for the sickest patients, but I'm not convinced this adds much to a good clinical assessment alone. The hope for sarcopenia was that it might help identify patients who otherwise seem well but are actually at higher risk of negative treatment outcomes but based on current evidence this does not seem possible.

6.4 Scoping review

Whilst in this cohort sarcopenia did not correlate with prognosis, there is a wealth of evidence from both cancer and non-cancer populations to suggest that it is related to

mortality. Therefore, the investigation of medications that could support muscle mass remains of relevance.

The scoping review raises several key points; what is a meaningful muscle mass gain for a patient with advanced cancer? Does muscle mass have more of an impact on survival than fat gain? In expanding the sarcopenia data within the ANCHOR cohort looking at longitudinal changes, and the impact of fat vs muscle will be helpful in answering this question.

Furthermore, with limited good quality of life data available for these studies, it is difficult to know what improvement may be achieved with a gain in muscle mass. There are agents which show considerable promise and future, carefully selected and well-designed studies will be necessary to address if there is a clinically useful benefit to be had from pharmaceutical agents.

It is likely that the biggest benefit will come from multi-modal treatment, with nutritional support, exercise, and potentially pharmaceutical support.

6.5 Conclusion

Anorexia and sarcopenia are highly prevalent for patients with advanced oesphagogastric tract cancers. Unfortunately, many of these patients have a very short survival, living just a few short weeks after initial oncology review, and 1 in 5 patients received either no or just 1 cycle of anti-cancer therapy. Furthermore, these symptoms are distressing for patients and their carers, and there remains a significant unmet need for effective treatments to manage them. Anorexia and sarcopenia overlap significantly and non-linearly with other symptoms, and the syndromes of frailty and cachexia. There is some association with poorer survival, but there does not seem to be a clear correlation between these biomarkers and systemic anti-cancer therapy toxicity in this cohort based on current data.

Despite these uncertainties, what this work does demonstrate is that for a number of patients with advanced upper GI cancers and appropriate dietician support, significant weight and strength gain is feasible.

Further work is needed to identify the optimal way to manage these patients, but this work provides a deep baseline characterisation which will act as a strong foundation for future research. With completion of the ANCHOR trial we will have one of the largest, deepest, prospectively collected cohorts of real-world patients with advanced OG cancer. Using this biomarker led approach we will aim to optimally characterise patients according to the frailty, cachexia and nutrition needs. This could lead on to a phase 2 trial of stratified patient optimisation using existing or new investigational agents alongside nutritional and psychological support. For example, stratifying patients according to frailty, inflammation status or anorexia driven malnutrition with different treatments for each group. This approach, with careful biomarker led patient selection will be key in the future for improving patient outcomes.

References

1. Ferlay, J., et al., *Cancer statistics for the year 2020: An overview.* Int J Cancer, 2021.
2. Dewys, W.D., et al., *Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group.* Am J Med, 1980. **69**(4): p. 491-7.
3. Argilés, J.M., et al., *Cancer cachexia: understanding the molecular basis.* Nat Rev Cancer, 2014. **14**(11): p. 754-62.
4. Sung, H., et al., *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.* CA: A Cancer Journal for Clinicians, 2021. **71**(3): p. 209-249.
5. UK, C.R. *Cancer statistics*. 2022; Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics-for-the-uk.
6. Edgren, G., et al., *A global assessment of the oesophageal adenocarcinoma epidemic.* Gut, 2013. **62**(10): p. 1406-14.
7. Nelson, K.A., et al., *Assessment of upper gastrointestinal motility in the cancer-associated dyspepsia syndrome.* J Palliat Care, 1993. **9**(1): p. 27-31.
8. Kang, H.W. and S.G. Kim, *Upper Gastrointestinal Stent Insertion in Malignant and Benign Disorders.* Clin Endosc, 2015. **48**(3): p. 187-93.
9. Cunningham, D., et al., *Capecitabine and Oxaliplatin for Advanced Esophagogastric Cancer.* New England Journal of Medicine, 2008. **358**(1): p. 36-46.
10. Dijksterhuis, W.P.M., et al., *Heterogeneity of first-line palliative systemic treatment in synchronous metastatic esophagogastric cancer patients: A real-world evidence study.* International Journal of Cancer, 2020. **146**(7): p. 1889-1901.
11. Bang, Y.J., et al., *Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial.* Lancet, 2010. **376**(9742): p. 687-97.
12. Sun, J.M., et al., *Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study.* Lancet, 2021. **398**(10302): p. 759-771.
13. Janjigian, Y.Y., et al., *First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial.* Lancet, 2021. **398**(10294): p. 27-40.
14. Barajas Galindo, D.E., et al., *Appetite disorders in cancer patients: Impact on nutritional status and quality of life.* Appetite, 2017. **114**: p. 23-27.
15. Arends, J., et al., *ESPEN guidelines on nutrition in cancer patients.* Clin Nutr, 2017. **36**(1): p. 11-48.

16. Cruz-Jentoft, A.J., et al., *Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People.* Age Ageing, 2010. **39**(4): p. 412-23.

17. Arends, J., et al., *Cancer cachexia in adult patients: ESMO Clinical Practice Guidelines(☆).* ESMO Open, 2021. **6**(3): p. 100092.

18. Muscaritoli, M., et al., *Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics".* Clin Nutr, 2010. **29**(2): p. 154-9.

19. Keller, K. and M. Engelhardt, *Strength and muscle mass loss with aging process. Age and strength loss.* Muscles Ligaments Tendons J, 2013. **3**(4): p. 346-50.

20. Mitchell, W.K., et al., *Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review.* Front Physiol, 2012. **3**: p. 260.

21. Baumgartner, R.N., et al., *Cross-sectional age differences in body composition in persons 60+ years of age.* The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 1995. **50**(6): p. M307-M316.

22. Sjöblom, S., et al., *Relationship between postmenopausal osteoporosis and the components of clinical sarcopenia.* Maturitas, 2013. **75**(2): p. 175-80.

23. Collins, J., et al., *The assessment and impact of sarcopenia in lung cancer: a systematic literature review.* BMJ Open, 2014. **4**(1): p. e003697.

24. Deng, C.Y., et al., *Progressive Sarcopenia in Patients With Colorectal Cancer Predicts Survival.* AJR Am J Roentgenol, 2018. **210**(3): p. 526-532.

25. Imai, K., et al., *Sarcopenia Impairs Prognosis of Patients with Hepatocellular Carcinoma: The Role of Liver Functional Reserve and Tumor-Related Factors in Loss of Skeletal Muscle Volume.* Nutrients, 2017. **9**(10).

26. Rutten, I.J., et al., *Loss of skeletal muscle during neoadjuvant chemotherapy is related to decreased survival in ovarian cancer patients.* J Cachexia Sarcopenia Muscle, 2016. **7**(4): p. 458-66.

27. Prado, C.M., et al., *Sarcopenia as a determinant of chemotherapy toxicity and time to tumor progression in metastatic breast cancer patients receiving capecitabine treatment.* Clin Cancer Res, 2009. **15**(8): p. 2920-6.

28. Fearon, K., et al., *Definition and classification of cancer cachexia: an international consensus.* Lancet Oncol, 2011. **12**(5): p. 489-95.

29. Arends, J., et al., *Cancer cachexia in adult patients: ESMO Clinical Practice Guidelines().* ESMO Open, 2021. **6**(3): p. 100092.

30. Barker, T., et al., *An elevated neutrophil-to-lymphocyte ratio associates with weight loss and cachexia in cancer.* Sci Rep, 2020. **10**(1): p. 7535.

31. Grenader, T., et al., *Prognostic value of neutrophil-to-lymphocyte ratio in advanced oesophago-gastric cancer: exploratory analysis of the REAL-2 trial.* Ann Oncol, 2016. **27**(4): p. 687-92.

32. Arends, J., et al., *ESPEN expert group recommendations for action against cancer-related malnutrition.* Clin Nutr, 2017. **36**(5): p. 1187-1196.

33. Warren, S., *THE IMMEDIATE CAUSES OF DEATH IN CANCER.* The American Journal of the Medical Sciences., 1932. **184**(5): p. 610-615.

34. Ambrus, J.L., et al., *Causes of death in cancer patients.* J Med, 1975. **6**(1): p. 61-4.

35. Sullivan, E.S., et al., *Epidemiology of cancer-related weight loss and sarcopenia in the UK and Ireland: incidence, prevalence, and clinical impact.* JCSM Rapid Communications, 2020. **3**(2): p. 91-102.

36. Muscaritoli, M., et al., *Prevalence of malnutrition in patients at first medical oncology visit: the PreMiO study.* Oncotarget, 2017. **8**(45): p. 79884-79896.

37. Grond, S., et al., *Prevalence and pattern of symptoms in patients with cancer pain: A prospective evaluation of 1635 cancer patients referred to a pain clinic.* Journal of Pain and Symptom Management, 1994. **9**(6): p. 372-382.

38. LeBlanc, T.W., et al., *Validation and real-world assessment of the Functional Assessment of Anorexia-Cachexia Therapy (FAACT) scale in patients with advanced non-small cell lung cancer and the cancer anorexia-cachexia syndrome (CACS).* Support Care Cancer, 2015. **23**(8): p. 2341-7.

39. McKernan, M., et al., *The relationship between quality of life (EORTC QLQ-C30) and survival in patients with gastro-oesophageal cancer.* Br J Cancer, 2008. **98**(5): p. 888-93.

40. Petermann-Rocha, F., et al., *Factors associated with sarcopenia: A cross-sectional analysis using UK Biobank.* Maturitas, 2020. **133**: p. 60-67.

41. Petermann-Rocha, F., et al., *The joint association of sarcopenia and frailty with incidence and mortality health outcomes: A prospective study.* Clin Nutr, 2020.

42. Reijnierse, E.M., et al., *The Association between Parameters of Malnutrition and Diagnostic Measures of Sarcopenia in Geriatric Outpatients.* PLoS One, 2015. **10**(8): p. e0135933.

43. Martin, L., et al., *Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index.* J Clin Oncol, 2013. **31**(12): p. 1539-47.

44. Johnson, A.K. and P.M. Gross, *Sensory circumventricular organs and brain homeostatic pathways.* The FASEB Journal, 1993. **7**(8): p. 678-686.

45. Beck, B., *Neuropeptide Y in normal eating and in genetic and dietary-induced obesity.* Philos Trans R Soc Lond B Biol Sci, 2006. **361**(1471): p. 1159-85.

46. Krashes, M.J., et al., *Rapid, reversible activation of AgRP neurons drives feeding behavior in mice.* The Journal of Clinical Investigation, 2011. **121**(4): p. 1424-1428.

47. Mizuno, T.M., et al., *Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin.* Diabetes, 1998. **47**(2): p. 294-7.

48. Mizuno, T.M., et al., *Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin.* Endocrinology, 1999. **140**(10): p. 4551-7.

49. Gee, C.E., et al., *Identification of proopiomelanocortin neurones in rat hypothalamus by in situ cDNA-mRNA hybridization.* Nature, 1983. **306**(5941): p. 374-376.

50. Perry, B. and Y. Wang, *Appetite regulation and weight control: the role of gut hormones.* Nutr Diabetes, 2012. **2**(1): p. e26.

51. Sergeyev, V., et al., *Effect of 2-mercaptoacetate and 2-deoxy-D-glucose administration on the expression of NPY, AGRP, POMC, MCH and hypocretin/orexin in the rat hypothalamus.* Neuroreport, 2000. **11**(1): p. 117-21.

52. Oomura, Y., et al., *Glucose and osmosensitive neurones of the rat hypothalamus.* Nature, 1969. **222**(5190): p. 282-4.

53. Woods, S.C., et al., *Suppression of food intake by intravenous nutrients and insulin in the baboon.* Am J Physiol, 1984. **247**(2 Pt 2): p. R393-401.

54. Gietzen, D.W., *Neural mechanisms in the responses to amino acid deficiency.* J Nutr, 1993. **123**(4): p. 610-25.

55. Niijima, A. and M.M. Meguid, *An electrophysiological study on amino acid sensors in the hepato-portal system in the rat.* Obes Res, 1995. **3 Suppl 5**: p. 741S-745S.

56. Rogers, P.J. and J.E. Blundell, *Reanalysis of the effects of phenylalanine, alanine, and aspartame on food intake in human subjects.* Physiol Behav, 1994. **56**(2): p. 247-50.

57. Bagnol, D., et al., *Anatomy of an endogenous antagonist: relationship between Agouti-related protein and proopiomelanocortin in brain.* J Neurosci, 1999. **19**(18): p. Rc26.

58. Giraudo, S.Q., C.J. Billington, and A.S. Levine, *Feeding effects of hypothalamic injection of melanocortin 4 receptor ligands.* Brain Res, 1998. **809**(2): p. 302-6.

59. Loos, R.J., et al., *Common variants near MC4R are associated with fat mass, weight and risk of obesity.* Nat Genet, 2008. **40**(6): p. 768-75.

60. Bai, L., et al., *Genetic Identification of Vagal Sensory Neurons That Control Feeding.* Cell, 2019. **179**(5): p. 1129-1143.e23.

61. Crooks, B., N.S. Stamataki, and J.T. McLaughlin, *Appetite, the enteroendocrine system, gastrointestinal disease and obesity.* Proc Nutr Soc, 2020: p. 1-9.

62. Date, Y., et al., *The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats.* Gastroenterology, 2002. **123**(4): p. 1120-8.

63. Nakazato, M., et al., *A role for ghrelin in the central regulation of feeding.* Nature, 2001. **409**(6817): p. 194-8.

64. Phillips, R.J. and T.L. Powley, *Gastric volume rather than nutrient content inhibits food intake.* Am J Physiol, 1996. **271**(3 Pt 2): p. R766-9.

65. Kaplan, J.M., A.C. Spector, and H.J. Grill, *Dynamics of gastric emptying during and after stomach fill.* Am J Physiol, 1992. **263**(4 Pt 2): p. R813-9.

66. Maljaars, P.W., et al., *Ileal brake: a sensible food target for appetite control. A review.* Physiol Behav, 2008. **95**(3): p. 271-81.

67. Batterham, R.L., et al., *Gut hormone PYY(3-36) physiologically inhibits food intake.* Nature, 2002. **418**(6898): p. 650-4.

68. Batterham, R.L., et al., *PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans.* Nature, 2007. **450**(7166): p. 106-9.

69. Dufresne, M., C. Seva, and D. Fourmy, *Cholecystokinin and gastrin receptors.* Physiol Rev, 2006. **86**(3): p. 805-47.

70. Verdich, C., et al., *A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans.* J Clin Endocrinol Metab, 2001. **86**(9): p. 4382-9.

71. Adrian, T.E., et al., *Human distribution and release of a putative new gut hormone, peptide YY.* Gastroenterology, 1985. **89**(5): p. 1070-7.

72. Gasbjerg, L.S., et al., *GIP and GLP-1 Receptor Antagonism During a Meal in Healthy Individuals.* J Clin Endocrinol Metab, 2020. **105**(3).

73. Sandoval, D.A., et al., *Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake.* Diabetes, 2008. **57**(8): p. 2046-54.

74. Anini, Y., et al., *Oxyntomodulin inhibits pancreatic secretion through the nervous system in rats.* Pancreas, 2000. **20**(4): p. 348-60.

75. Le Quellec, A., et al., *Oxyntomodulin-like immunoreactivity: diurnal profile of a new potential enterogastrone.* J Clin Endocrinol Metab, 1992. **74**(6): p. 1405-9.

76. Lonovics, J., et al., *Action of pancreatic polypeptide on exocrine pancreas and on release of cholecystokinin and secretin.* Endocrinology, 1981. **108**(5): p. 1925-30.

77. Verschueren, S., et al., *Effect of pancreatic polypeptide on gastric accommodation and gastric emptying in conscious rats.* Am J Physiol Gastrointest Liver Physiol, 2014. **307**(1): p. G122-8.

78. Gibbs, J., R.C. Young, and G.P. Smith, *Cholecystokinin elicits satiety in rats with open gastric fistulas.* Nature, 1973. **245**(5424): p. 323-5.

79. Lieverse, R.J., et al., *Satiety effects of a physiological dose of cholecystokinin in humans.* Gut, 1995. **36**(2): p. 176-9.

80. Lumb, K.J., et al., *Novel selective neuropeptide Y2 receptor PEGylated peptide agonists reduce food intake and body weight in mice.* Journal of medicinal chemistry, 2007. **50**(9): p. 2264-8.

81. Näslund, E., et al., *Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men.* Int J Obes Relat Metab Disord, 1999. **23**(3): p. 304-11.

82. Wynne, K., et al., *Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial.* Diabetes, 2005. **54**(8): p. 2390-5.

83. Cohen, M.A., et al., *Oxyntomodulin Suppresses Appetite and Reduces Food Intake in Humans.* The Journal of Clinical Endocrinology & Metabolism, 2003. **88**(10): p. 4696-4701.

84. Arafat, A.M., et al., *The impact of insulin-independent, glucagon-induced suppression of total ghrelin on satiety in obesity and type 1 diabetes mellitus.* J Clin Endocrinol Metab, 2013. **98**(10): p. 4133-42.

85. Austin, J. and D. Marks, *Hormonal regulators of appetite.* Int J Pediatr Endocrinol, 2009. **2009**: p. 141753.

86. Woods, S.C., et al., *Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons.* Nature, 1979. **282**(5738): p. 503-505.

87. Geary, N., et al., *Individual, but not simultaneous, glucagon and cholecystokinin infusions inhibit feeding in men.* Am J Physiol, 1992. **262**(6 Pt 2): p. R975-80.

88. Kristensen, P., et al., *Hypothalamic CART is a new anorectic peptide regulated by leptin.* Nature, 1998. **393**(6680): p. 72-6.

89. Considine, R.V., et al., *Serum immunoreactive-leptin concentrations in normal-weight and obese humans.* N Engl J Med, 1996. **334**(5): p. 292-5.

90. Anini, Y. and P.L. Brubaker, *Role of leptin in the regulation of glucagon-like peptide-1 secretion.* Diabetes, 2003. **52**(2): p. 252-9.

91. Peters, J.H., et al., *Cooperative activation of cultured vagal afferent neurons by leptin and cholecystokinin.* Endocrinology, 2004. **145**(8): p. 3652-7.

92. Acosta, A., et al., *Salivary PYY: a putative bypass to satiety.* PLoS One, 2011. **6**(10): p. e26137.

93. Cushing, H., *THE HYPOPHYSIS CEREBRI CLINICAL ASPECTS OF HYPERPITUITARISM AND OF HYPOPITUITARISM.* 1909. **LIII**(4): p. 249.

94. Savontaus, E., I.M. Conwell, and S.L. Wardlaw, *Effects of adrenalectomy on AGRP, POMC, NPY and CART gene expression in the basal hypothalamus of fed and fasted rats.* Brain Res, 2002. **958**(1): p. 130-8.

95. Uddén, J., et al., *Effects of glucocorticoids on leptin levels and eating behaviour in women.* Journal of Internal Medicine, 2003. **253**(2): p. 225-231.

96. Tataranni, P.A., et al., *Effects of glucocorticoids on energy metabolism and food intake in humans.* Am J Physiol, 1996. **271**(2 Pt 1): p. E317-25.

97. Miller, S., et al., *Use of Corticosteroids for Anorexia in Palliative Medicine: A Systematic Review.* Journal of Palliative Medicine, 2014. **17**(4): p. 482-485.

98. Dagogo-Jack, S., et al., *Robust leptin secretory responses to dexamethasone in obese subjects.* J Clin Endocrinol Metab, 1997. **82**(10): p. 3230-3.

99. Miell, J.P., P. Englaro, and W.F. Blum, *Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects.* Horm Metab Res, 1996. **28**(12): p. 704-7.

100. Newcomer, J.W., et al., *Dose-dependent cortisol-induced increases in plasma leptin concentration in healthy humans.* Arch Gen Psychiatry, 1998. **55**(11): p. 995-1000.

101. Thompson, D.A. and R.G. Campbell, *Hunger in humans induced by 2-deoxy-D-glucose: glucoprivic control of taste preference and food intake.* Science, 1977. **198**(4321): p. 1065-8.

102. Novin, D., et al., *Is there a role for the liver in the control of food intake?* Am J Clin Nutr, 1985. **42**(5 Suppl): p. 1050-62.

103. Grill, H.J. and R. Norgren, *Chronically decerebrate rats demonstrate satiation but not bait shyness.* Science, 1978. **201**(4352): p. 267-9.

104. Aleman, M.R., et al., *Leptin role in advanced lung cancer. A mediator of the acute phase response or a marker of the status of nutrition?* Cytokine, 2002. **19**(1): p. 21-6.

105. Mantovani, G., et al., *Serum levels of leptin and proinflammatory cytokines in patients with advanced-stage cancer at different sites.* J Mol Med (Berl), 2000. **78**(10): p. 554-61.

106. Dwarkasing, J.T., et al., *Increased hypothalamic serotonin turnover in inflammation-induced anorexia.* BMC Neurosci, 2016. **17**(1): p. 26.

107. Plata-Salaman, C.R., S.E. Ilyin, and D. Gayle, *Brain cytokine mRNAs in anorectic rats bearing prostate adenocarcinoma tumor cells.* Am J Physiol, 1998. **275**(2): p. R566-73.

108. Strassmann, G., et al., *Mechanisms of experimental cancer cachexia. Interaction between mononuclear phagocytes and colon-26 carcinoma and its relevance to IL-6-mediated cancer cachexia.* J Immunol, 1992. **148**(11): p. 3674-8.

109. Langhans, W. and B. Hrupka, *Interleukins and tumor necrosis factor as inhibitors of food intake.* Neuropeptides, 1999. **33**(5): p. 415-24.

110. Lawrence, C.B. and N.J. Rothwell, *Anorexic but not pyrogenic actions of interleukin-1 are modulated by central melanocortin-3/4 receptors in the rat.* J Neuroendocrinol, 2001. **13**(6): p. 490-5.

111. Lonnroth, C., et al., *Tumor necrosis factor-alpha and interleukin-1 alpha production in cachectic, tumor-bearing mice.* Int J Cancer, 1990. **46**(5): p. 889-96.

112. Sonti, G., S.E. Ilyin, and C.R. Plata-Salaman, *Neuropeptide Y blocks and reverses interleukin-1 beta-induced anorexia in rats.* Peptides, 1996. **17**(3): p. 517-20.

113. Whitaker, K.W. and T.M. Reyes, *Central blockade of melanocortin receptors attenuates the metabolic and locomotor responses to peripheral interleukin-1beta administration.* Neuropharmacology, 2008. **54**(3): p. 509-20.

114. Yang, Z.J., et al., *Interleukin-1alpha injection into ventromedial hypothalamic nucleus of normal rats depresses food intake and increases release of dopamine and serotonin.* Pharmacol Biochem Behav, 1999. **62**(1): p. 61-5.

115. Bernstein, I.L., *Neutral mediation of food aversions and anorexia induced by tumor necrosis factor and tumors.* Neurosci Biobehav Rev, 1996. **20**(1): p. 177-81.

116. Bodnar, R.J., et al., *Mediation of anorexia by human recombinant tumor necrosis factor through a peripheral action in the rat.* Cancer Res, 1989. **49**(22): p. 6280-4.

117. Arruda, A.P., et al., *Hypothalamic Actions of Tumor Necrosis Factor α Provide the Thermogenic Core for the Wastage Syndrome in Cachexia.* Endocrinology, 2010. **151**(2): p. 683-694.

118. Cahlin, C., et al., *Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia.* Cancer Res, 2000. **60**(19): p. 5488-93.

119. Laviano, A., et al., *Effects of intra-VMN mianserin and IL-1ra on meal number in anorectic tumor-bearing rats.* J Investig Med, 2000. **48**(1): p. 40-8.

120. Tas, F., et al., *Serum levels of leptin and proinflammatory cytokines in advanced-stage non-small cell lung cancer.* Med Oncol, 2005. **22**(4): p. 353-8.

121. Deans, D.A., et al., *Elevated tumour interleukin-1beta is associated with systemic inflammation: A marker of reduced survival in gastro-oesophageal cancer.* Br J Cancer, 2006. **95**(11): p. 1568-75.

122. Karayiannakis, A.J., et al., *Serum levels of tumor necrosis factor-alpha and nutritional status in pancreatic cancer patients.* Anticancer Res, 2001. **21**(2B): p. 1355-8.

123. Moradi, M.M., et al., *Serum and ascitic fluid levels of interleukin-1, interleukin-6, and tumor necrosis factor-alpha in patients with ovarian epithelial cancer.* Cancer, 1993. **72**(8): p. 2433-40.

124. Paulsen, O., et al., *The relationship between pro-inflammatory cytokines and pain, appetite and fatigue in patients with advanced cancer.* PLoS One, 2017. **12**(5): p. e0177620.

125. Jatoi, A., et al., *A placebo-controlled double blind trial of etanercept for the cancer anorexia/weight loss syndrome: results from N00C1 from the North Central Cancer Treatment Group.* Cancer, 2007. **110**(6): p. 1396-403.

126. Rigas, J.R., et al., *Efect of ALD518, a humanized anti-IL-6 antibody, on lean body mass loss and symptoms in patients with advanced non-small cell lung cancer (NSCLC): Results of a phase II randomized, double-blind safety and efficacy trial.* Journal of Clinical Oncology, 2010. **28**(15_suppl): p. 7622-7622.

127. Scarlett, J.M., et al., *Regulation of central melanocortin signaling by interleukin-1 beta.* Endocrinology, 2007. **148**(9): p. 4217-25.

128. Sergeyev, V., C. Broberger, and T. Hokfelt, *Effect of LPS administration on the expression of POMC, NPY, galanin, CART and MCH mRNAs in the rat hypothalamus.* Brain Res Mol Brain Res, 2001. **90**(2): p. 93-100.

129. Gayle, D., S.E. Ilyin, and C.R. Plata-Salaman, *Central nervous system IL-1 beta system and neuropeptide Y mRNAs during IL-1 beta-induced anorexia in rats.* Brain Res Bull, 1997. **44**(3): p. 311-7.

130. Chance, W.T., et al., *Possible role of neuropeptide Y in experimental cancer anorexia.* Adv Exp Med Biol, 1994. **354**: p. 185-201.

131. Makarenko, I.G., et al., *Normalization of hypothalamic serotonin (5-HT 1B) receptor and NPY in cancer anorexia after tumor resection: an immunocytochemical study.* Neurosci Lett, 2005. **383**(3): p. 322-7.

132. Chance, W.T., et al., *Assessment of feeding response of tumor-bearing rats to hypothalamic injection and infusion of neuropeptide Y.* Peptides, 1996. **17**(5): p. 797-801.

133. Joppa, M.A., et al., *Central infusion of the melanocortin receptor antagonist agouti-related peptide (AgRP(83-132)) prevents cachexia-related symptoms induced by radiation and colon-26 tumors in mice.* Peptides, 2007. **28**(3): p. 636-42.

134. Jatoi, A., et al., *Neuropeptide Y, leptin, and cholecystokinin 8 in patients with advanced cancer and anorexia: a North Central Cancer Treatment Group exploratory investigation.* Cancer, 2001. **92**(3): p. 629-33.

135. McCarthy, H.D., et al., *Megestrol acetate stimulates food and water intake in the rat: effects on regional hypothalamic neuropeptide Y concentrations.* Eur J Pharmacol, 1994. **265**(1-2): p. 99-102.

136. Sato, T., et al., *Involvement of plasma leptin, insulin and free tryptophan in cytokine-induced anorexia.* Clin Nutr, 2003. **22**(2): p. 139-46.

137. Heisler, L.K., et al., *Serotonin reciprocally regulates melanocortin neurons to modulate food intake.* Neuron, 2006. **51**(2): p. 239-49.

138. Dwarkasing, J.T., et al., *Differences in food intake of tumour-bearing cachectic mice are associated with hypothalamic serotonin signalling.* J Cachexia Sarcopenia Muscle, 2015. **6**(1): p. 84-94.

139. Schwartzberg, L., et al., *Pooled analysis of phase III clinical studies of palonosetron versus ondansetron, dolasetron, and granisetron in the prevention of chemotherapy-induced nausea and vomiting (CINV).* Support Care Cancer, 2014. **22**(2): p. 469-77.

140. Marks, D.L., N. Ling, and R.D. Cone, *Role of the central melanocortin system in cachexia.* Cancer Res, 2001. **61**(4): p. 1432-8.

141. Burfeind, K.G., et al., *TRIF is a key inflammatory mediator of acute sickness behavior and cancer cachexia.* Brain Behav Immun, 2018. **73**: p. 364-374.

142. Ogimoto, K., M.K. Harris, Jr., and B.E. Wisse, *MyD88 is a key mediator of anorexia, but not weight loss, induced by lipopolysaccharide and interleukin-1 beta.* Endocrinology, 2006. **147**(9): p. 4445-53.

143. Wisse, B.E., et al., *Evidence that lipopolysaccharide-induced anorexia depends upon central, rather than peripheral, inflammatory signals.* Endocrinology, 2007. **148**(11): p. 5230-7.

144. Zhu, X., et al., *MyD88 signalling is critical in the development of pancreatic cancer cachexia.* J Cachexia Sarcopenia Muscle, 2019. **10**(2): p. 378-390.

145. Daniel, J.A., et al., *Leukemia inhibitory factor as a mediator of lipopolysaccharide effects on appetite and selected hormones and metabolites.* J Anim Sci, 2016. **94**(7): p. 2789-97.

146. Grossberg, A.J., et al., *Arcuate nucleus proopiomelanocortin neurons mediate the acute anorectic actions of leukemia inhibitory factor via gp130.* Endocrinology, 2010. **151**(2): p. 606-16.

147. Mullican, S.E., et al., *GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates.* Nat Med, 2017. **23**(10): p. 1150-1157.

148. Worth, A.A., et al., *The cytokine GDF15 signals through a population of brainstem cholecystokinin neurons to mediate anorectic signalling.* Elife, 2020. **9**.

149. Suriben, R., et al., *Antibody-mediated inhibition of GDF15–GFRAL activity reverses cancer cachexia in mice.* Nature Medicine, 2020. **26**(8): p. 1264-1270.

150. Lerner, L., et al., *Plasma growth differentiation factor 15 is associated with weight loss and mortality in cancer patients.* J Cachexia Sarcopenia Muscle, 2015. **6**(4): p. 317-24.

151. Wang, W., et al., *Effects of ghrelin on anorexia in tumor-bearing mice with eicosanoid-related cachexia.* Int J Oncol, 2006. **28**(6): p. 1393-400.

152. Hanada, T., et al., *Upregulation of ghrelin expression in cachectic nude mice bearing human melanoma cells.* Metabolism, 2004. **53**(1): p. 84-8.

153. Hanada, T., et al., *Anti-cachectic effect of ghrelin in nude mice bearing human melanoma cells.* Biochem Biophys Res Commun, 2003. **301**(2): p. 275-9.

154. Shimizu, Y., et al., *Increased plasma ghrelin level in lung cancer cachexia.* Clin Cancer Res, 2003. **9**(2): p. 774-8.

155. Nagaya, N., et al., *Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors.* Circulation, 2001. **104**(17): p. 2034-8.

156. Moschovi, M., et al., *Serial plasma concentrations of PYY and ghrelin during chemotherapy in children with acute lymphoblastic leukemia.* J Pediatr Hematol Oncol, 2008. **30**(10): p. 733-7.

157. Fujitsuka, N., et al., *Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival.* Transl Psychiatry, 2011. **1**(7): p. e23.

158. Asakawa, A., et al., *Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin.* Gastroenterology, 2001. **120**(2): p. 337-45.

159. Janik, J.E., et al., *Interleukin 1 alpha increases serum leptin concentrations in humans.* J Clin Endocrinol Metab, 1997. **82**(9): p. 3084-6.

160. Zumbach, M.S., et al., *Tumor necrosis factor increases serum leptin levels in humans.* J Clin Endocrinol Metab, 1997. **82**(12): p. 4080-2.

161. Lopez-Soriano, J., et al., *Leptin and tumor growth in rats.* Int J Cancer, 1999. **81**(5): p. 726-9.

162. Molfino, A., et al., *Cancer anorexia: hypothalamic activity and its association with inflammation and appetite-regulating peptides in lung cancer.* J Cachexia Sarcopenia Muscle, 2017. **8**(1): p. 40-47.

163. Garcia, J.M., et al., *Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia.* J Clin Endocrinol Metab, 2005. **90**(5): p. 2920-6.

164. Moran, G.W., F.C. Leslie, and J.T. McLaughlin, *Crohn's disease affecting the small bowel is associated with reduced appetite and elevated levels of circulating gut peptides.* Clin Nutr, 2013. **32**(3): p. 404-11.

165. Besterman, H.S., et al., *Gut hormones in tropical malabsorption.* Br Med J, 1979. **2**(6200): p. 1252-5.

166. Leslie, F.C., et al., *Plasma cholecystokinin concentrations are elevated in acute upper gastrointestinal infections.* QJM, 2003. **96**(11): p. 870-1.

167. Worthington, J.J., et al., *Adaptive immunity alters distinct host feeding pathways during nematode induced inflammation, a novel mechanism in parasite expulsion.* PLoS Pathog, 2013. **9**(1): p. e1003122.

168. McDermott, J.R., et al., *Immune control of food intake: enteroendocrine cells are regulated by CD4+ T lymphocytes during small intestinal inflammation.* Gut, 2006. **55**(4): p. 492-7.

169. Sakai, H., et al., *Role of peptide YY in 5-fluorouracil-induced reduction of dietary intake.* Clin Exp Pharmacol Physiol, 2016. **43**(8): p. 753-9.

170. Sanchez-Lara, K., et al., *Brain activity correlated with food preferences: a functional study comparing advanced non-small cell lung cancer patients with and without anorexia.* Nutrition, 2013. **29**(7-8): p. 1013-9.

171. I, I.J., et al., *Changes in taste and smell function, dietary intake, food preference, and body composition in testicular cancer patients treated with cisplatin-based chemotherapy.* Clin Nutr, 2017. **36**(6): p. 1642-1648.

172. Amano, K., et al., *Eating-related distress and need for nutritional support of families of advanced cancer patients: a nationwide survey of bereaved family members.* J Cachexia Sarcopenia Muscle, 2016. **7**(5): p. 527-534.

173. Massie, M.J., *Prevalence of Depression in Patients With Cancer.* JNCI Monographs, 2004. **2004**(32): p. 57-71.

174. Fang, F.M., et al., *Quality of life as a survival predictor for esophageal squamous cell carcinoma treated with radiotherapy.* Int J Radiat Oncol Biol Phys, 2004. **58**(5): p. 1394-404.

175. van Kleef, J.J., et al., *Prognostic value of patient-reported quality of life for survival in oesophagogastric cancer: analysis from the population-based POCOP study.* Gastric Cancer, 2021. **24**(6): p. 1203-1212.

176. Abraham, M., et al., *Early recognition of anorexia through patient-generated assessment predicts survival in patients with oesophagogastric cancer.* PLoS One, 2019. **14**(11): p. e0224540.

177. Schiessel, D.L., et al., *Clinical and nutritional characteristics on overall survival impact in patients with gastrointestinal cancer.* Clin Nutr ESPEN, 2022. **48**: p. 336-341.

178. Kaźmierczak-Siedlecka, K., et al., *Influence of malnutrition stage according to GLIM 2019 criteria and SGA on the quality of life of patients with advanced cancer.* Nutr Hosp, 2020. **37**(6): p. 1179-1185.

179. Guo, Z.Q., et al., *Survey and analysis of the nutritional status in hospitalized patients with malignant gastric tumors and its influence on the quality of life.* Support Care Cancer, 2020. **28**(1): p. 373-380.

180. Karabulut, S., et al., *Does nutritional status affect treatment tolerability, chemotherapy response and survival in metastatic gastric cancer patients? Results of a prospective multicenter study in Turkey.* J Oncol Pharm Pract, 2022. **28**(1): p. 127-134.

181. Bicakli, D.H., et al., *The effect of chemotherapy on nutritional status and weakness in geriatric gastrointestinal system cancer patients.* Nutrition, 2018. **47**: p. 39-42.

182. Li, Y.F., et al., *Prognostic Value of the Nutritional Risk Screening 2002 Scale in Metastatic Gastric Cancer: A Large-Scale Cohort Study.* J Cancer, 2019. **10**(1): p. 112-119.

183. Takayoshi, K., et al., *Weight Loss During Initial Chemotherapy Predicts Survival in Patients With Advanced Gastric Cancer.* Nutr Cancer, 2017. **69**(3): p. 408-415.

184. Mansoor, W., et al., *Early Weight Loss as a Prognostic Factor in Patients with Advanced Gastric Cancer: Analyses from REGARD, RAINBOW, and RAINFALL Phase III Studies.* Oncologist, 2021. **26**(9): p. e1538-e1547.

185. Kyle, U.G., et al., *Bioelectrical impedance analysis--part I: review of principles and methods.* Clin Nutr, 2004. **23**(5): p. 1226-43.

186. Mourtzakis, M., et al., *A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care.* Appl Physiol Nutr Metab, 2008. **33**(5): p. 997-1006.

187. Shen, W., et al., *Visceral adipose tissue: relations between single-slice areas and total volume.* Am J Clin Nutr, 2004. **80**(2): p. 271-8.

188. Yoshida, D., et al., *Using two different algorithms to determine the prevalence of sarcopenia.* Geriatr Gerontol Int, 2014. **14 Suppl 1**: p. 46-51.

189. Fielding, R.A., et al., *Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia.* J Am Med Dir Assoc, 2011. **12**(4): p. 249-56.

190. Cruz-Jentoft, A.J., et al., *Sarcopenia: revised European consensus on definition and diagnosis.* Age and Ageing, 2019. **48**(1): p. 16-31.

191. Prado, C.M., et al., *Sarcopenia and cachexia in the era of obesity: clinical and nutritional impact.* Proc Nutr Soc, 2016. **75**(2): p. 188-98.

192. Derstine, B.A., et al., *Skeletal muscle cutoff values for sarcopenia diagnosis using T10 to L5 measurements in a healthy US population.* Sci Rep, 2018. **8**(1): p. 11369.

193. UK, C.R. *Cancer incidence by age*. [cited 2022 18/01/2022]; Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/incidence/age.

194. Shachar, S.S., et al., *Prognostic value of sarcopenia in adults with solid tumours: A meta-analysis and systematic review.* Eur J Cancer, 2016. **57**: p. 58-67.

195. Lee, C.M. and J. Kang, *Prognostic impact of myosteatosis in patients with colorectal cancer: a systematic review and meta-analysis.* J Cachexia Sarcopenia Muscle, 2020. **11**(5): p. 1270-1282.

196. Looijaard, S., et al., *Do older individuals who are diagnosed with cancer have worse physical performance prior to diagnosis compared to matched controls? A longitudinal cohort study.* BMC Geriatr, 2018. **18**(1): p. 166.

197. Williams, G.R., et al., *Assessment of Sarcopenia Measures, Survival, and Disability in Older Adults Before and After Diagnosis With Cancer.* JAMA Netw Open, 2020. **3**(5): p. e204783.

198. Deng, C.-Y., et al., *Progressive Sarcopenia in Patients With Colorectal Cancer Predicts Survival.* American Journal of Roentgenology, 2018. **210**(3): p. 526-532.

199. Baker, S.D., et al., *Role of Body Surface Area in Dosing of Investigational Anticancer Agents in Adults, 1991–2001.* JNCI: Journal of the National Cancer Institute, 2002. **94**(24): p. 1883-1888.

200. Nguyen, L., et al., *Population pharmacokinetics of total and unbound etoposide.* Cancer Chemother Pharmacol, 1998. **41**(2): p. 125-32.

201. Prado, C.M., et al., *Body composition as an independent determinant of 5-fluorouracil-based chemotherapy toxicity.* Clin Cancer Res, 2007. **13**(11): p. 3264-8.

202. Ali, R., et al., *Lean body mass as an independent determinant of dose-limiting toxicity and neuropathy in patients with colon cancer treated with FOLFOX regimens.* Cancer medicine, 2016. **5**(4): p. 607-616.

203. Dijksterhuis, W.P.M., et al., *Association between body composition, survival, and toxicity in advanced esophagogastric cancer patients receiving palliative chemotherapy.* Journal of cachexia, sarcopenia and muscle, 2019. **10**(1): p. 199-206.

204. Shachar, S.S., et al., *Skeletal Muscle Measures as Predictors of Toxicity, Hospitalization, and Survival in Patients with Metastatic Breast Cancer Receiving Taxane-Based Chemotherapy.* Clin Cancer Res, 2017. **23**(3): p. 658-665.

205. Shachar, S.S., et al., *Body Composition as a Predictor of Toxicity in Patients Receiving Anthracycline and Taxane-Based Chemotherapy for Early-Stage Breast Cancer.* Clin Cancer Res, 2017. **23**(14): p. 3537-3543.

206. Freckelton, J., et al., *Body Composition Adjusted Dosing of Gemcitabine-Nab-Paclitaxel in Pancreatic Cancer Does Not Predict Toxicity Compared to Body Surface Area Dosing.* Nutr Cancer, 2019. **71**(4): p. 624-628.

207. Antoun, S., et al., *Association of skeletal muscle wasting with treatment with sorafenib in patients with advanced renal cell carcinoma: results from a placebo-controlled study.* J Clin Oncol, 2010. **28**(6): p. 1054-60.

208. Antoun, S., et al., *Low body mass index and sarcopenia associated with dose-limiting toxicity of sorafenib in patients with renal cell carcinoma.* Ann Oncol, 2010. **21**(8): p. 1594-8.

209. Wang, J., L. Cao, and S. Xu, *Sarcopenia affects clinical efficacy of immune checkpoint inhibitors in non-small cell lung cancer patients: A systematic review and meta-analysis.* Int Immunopharmacol, 2020. **88**: p. 106907.

210. Williams, G.R., et al., *The impact of skeletal muscle on the pharmacokinetics and toxicity of 5-fluorouracil in colorectal cancer.* Cancer Chemother Pharmacol, 2018. **81**(2): p. 413-417.

211. Vega, M.C., A. Laviano, and G.D. Pimentel, *Sarcopenia and chemotherapy-mediated toxicity.* Einstein (Sao Paulo), 2016. **14**(4): p. 580-584.

212. Veasey Rodrigues, H., et al., *Body composition and survival in the early clinical trials setting.* Eur J Cancer, 2013. **49**(15): p. 3068-75.

213. Cousin, S., et al., *Low skeletal muscle is associated with toxicity in patients included in phase I trials.* Invest New Drugs, 2014. **32**(2): p. 382-7.

214. Oken, M.M., et al., *Toxicity and response criteria of the Eastern Cooperative Oncology Group.* Am J Clin Oncol, 1982. **5**(6): p. 649-55.

215. Yates, J.W., B. Chalmer, and F.P. McKegney, *Evaluation of patients with advanced cancer using the Karnofsky performance status.* Cancer, 1980. **45**(8): p. 2220-4.

216. Kelly, C.M. and A. Shahrokni, *Moving beyond Karnofsky and ECOG Performance Status Assessments with New Technologies.* J Oncol, 2016. **2016**: p. 6186543.

217. Ando, M., et al., *Prognostic value of performance status assessed by patients themselves, nurses, and oncologists in advanced non-small cell lung cancer.* British Journal of Cancer, 2001. **85**(11): p. 1634-1639.

218. Talarico, L., G. Chen, and R. Pazdur, *Enrollment of elderly patients in clinical trials for cancer drug registration: a 7-year experience by the US Food and Drug Administration.* J Clin Oncol, 2004. **22**(22): p. 4626-31.

219. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2019.* CA Cancer J Clin, 2019. **69**(1): p. 7-34.

220. Bellera, C.A., et al., *Screening older cancer patients: first evaluation of the G-8 geriatric screening tool.* Ann Oncol, 2012. **23**(8): p. 2166-2172.

221. Rockwood, K., et al., *A global clinical measure of fitness and frailty in elderly people.* CMAJ, 2005. **173**(5): p. 489-95.

222. Fried, L.P., et al., *Frailty in older adults: evidence for a phenotype.* J Gerontol A Biol Sci Med Sci, 2001. **56**(3): p. M146-56.

223. Hamaker, M.E., et al., *Frailty screening methods for predicting outcome of a comprehensive geriatric assessment in elderly patients with cancer: a systematic review.* Lancet Oncol, 2012. **13**(10): p. e437-44.

224. Hamaker, M.E., et al., *Baseline comprehensive geriatric assessment is associated with toxicity and survival in elderly metastatic breast cancer patients receiving single-agent chemotherapy: results from the OMEGA study of the Dutch breast cancer trialists' group.* Breast, 2014. **23**(1): p. 81-7.

225. Kenis, C., et al., *Adherence to geriatric assessment-based recommendations in older patients with cancer: a multicenter prospective cohort study in Belgium.* Ann Oncol, 2018. **29**(9): p. 1987-1994.

226. Corre, R., et al., *Use of a Comprehensive Geriatric Assessment for the Management of Elderly Patients With Advanced Non-Small-Cell Lung Cancer: The Phase III Randomized ESOGIA-GFPC-GECP 08-02 Study.* J Clin Oncol, 2016. **34**(13): p. 1476-83.

227. Handforth, C., et al., *The prevalence and outcomes of frailty in older cancer patients: a systematic review.* Annals of Oncology, 2015. **26**(6): p. 1091-1101.

228. Lewis, A.R., et al., *Outcomes in patients ≥ 80 years with a diagnosis of a hepatopancreaticobiliary (HPB) malignancy.* Med Oncol, 2019. **36**(10): p. 85.

229. Guralnik, J.M., et al., *Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery.* The journals of gerontology. Series A, Biological sciences and medical sciences, 2000. **55**(4): p. M221-M231.

230. Ellis, G., et al., *Comprehensive geriatric assessment for older adults admitted to hospital.* Cochrane Database Syst Rev, 2017. **9**(9): p. Cd006211.

231. Versteeg, K.S., et al., *Predicting outcome in older patients with cancer: Comprehensive geriatric assessment and clinical judgment.* Journal of Geriatric Oncology, 2020.

232. Hurria, A., et al., *Validation of a Prediction Tool for Chemotherapy Toxicity in Older Adults With Cancer.* J Clin Oncol, 2016. **34**(20): p. 2366-71.

233. Ortland, I., et al., *Comparing the performance of the CARG and the CRASH score for predicting toxicity in older patients with cancer.* J Geriatr Oncol, 2020.

234. Zwart, A.T., et al., *CT-measured skeletal muscle mass used to assess frailty in patients with head and neck cancer.* J Cachexia Sarcopenia Muscle, 2019.

235. Williams, G.R., et al., *Frailty and skeletal muscle in older adults with cancer.* Journal of Geriatric Oncology, 2018. **9**(1): p. 68-73.

236. Lexell, J., et al., *Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections.* Muscle Nerve, 1983. **6**(8): p. 588-95.

237. Lindle, R.S., et al., *Age and gender comparisons of muscle strength in 654 women and men aged 20–93 yr.* Journal of applied physiology, 1997. **83**(5): p. 1581-1587.

238. Picard, M., et al., *Alterations in intrinsic mitochondrial function with aging are fiber type-specific and do not explain differential atrophy between muscles.* Aging Cell, 2011. **10**(6): p. 1047-55.

239. Mendell, J.R. and W.K. Engel, *The fine structure of type II muscle fiber atrophy.* Neurology, 1971. **21**(4): p. 358.

240. Wang, H., et al., *Apoptosis in capillary endothelial cells in ageing skeletal muscle.* Aging Cell, 2014. **13**(2): p. 254-262.

241. Deschenes, M.R., et al., *Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers.* Exp Gerontol, 2010. **45**(5): p. 389-93.

242. Rooyackers, O.E., et al., *Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle.* Proc Natl Acad Sci U S A, 1996. **93**(26): p. 15364-9.

243. Dodd, S.L., et al., *Ros-mediated activation of NF-kappaB and Foxo during muscle disuse.* Muscle Nerve, 2010. **41**(1): p. 110-3.

244. Balagopal, P., et al., *Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans.* American Journal of Physiology-Endocrinology And Metabolism, 1997. **273**(4): p. E790-E800.

245. Grgic, J., et al., *Effects of Resistance Training on Muscle Size and Strength in Very Elderly Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.* Sports Med, 2020. **50**(11): p. 1983-1999.

246. Giuliano, C., et al., *The effects of resistance training on muscle strength, quality of life and aerobic capacity in patients with chronic heart failure — A meta-analysis.* International Journal of Cardiology, 2017. **227**: p. 413-423.

247. Roeder, L., et al., *Effects of Resistance Training on Measures of Muscular Strength in People with Parkinson's Disease: A Systematic Review and Meta-Analysis.* PLOS ONE, 2015. **10**(7): p. e0132135.

248. Deschenes, M.R., *Adaptations of the neuromuscular junction to exercise training.* Current Opinion in Physiology, 2019. **10**: p. 10-16.

249. Deschenes, M.R., et al., *Effects of exercise training on neuromuscular junction morphology and pre- to post-synaptic coupling in young and aged rats.* Neuroscience, 2016. **316**: p. 167-77.

250. Baumgartner, R.N. and K.M. Koehler, *Gallagher. Epidemiology of sarcopenia in elderly people in New Mexico.* Am J Epidemiol, 1998. **147**: p. 744-763.

251. Fiatarone, M.A., et al., *Exercise training and nutritional supplementation for physical frailty in very elderly people.* N Engl J Med, 1994. **330**(25): p. 1769-75.

252. Rasmussen, B.B., R.R. Wolfe, and E. Volpi, *Oral and intravenously administered amino acids produce similar effects on muscle protein synthesis in the elderly.* J Nutr Health Aging, 2002. **6**(6): p. 358-62.

253. Paddon-Jones, D., et al., *Amino acid ingestion improves muscle protein synthesis in the young and elderly.* Am J Physiol Endocrinol Metab, 2004. **286**(3): p. E321-8.

254. Moore, D.R., et al., *Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men.* The Journals of Gerontology: Series A, 2015. **70**(1): p. 57-62.

255. Morley, J.E., et al., *Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men.* Metabolism, 1997. **46**(4): p. 410-3.

256. Perry, H.M., et al., *Testosterone and leptin in older African-American men: relationship to age, strength, function, and season.* Metabolism-Clinical and Experimental, 2000. **49**(8): p. 1085-1091.

257. Brodsky, I.G., P. Balagopal, and K.S. Nair, *Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men--a clinical research center study.* The Journal of Clinical Endocrinology & Metabolism, 1996. **81**(10): p. 3469-3475.

258. Urban, R.J., et al., *Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis.* American Journal of Physiology-Endocrinology And Metabolism, 1995. **269**(5): p. E820-E826.

259. Hackett, G., et al., *UK policy statements on testosterone deficiency.* Int J Clin Pract, 2017. **71**(3-4).

260. Sørensen, M.B., et al., *Obesity and sarcopenia after menopause are reversed by sex hormone replacement therapy.* Obes Res, 2001. **9**(10): p. 622-6.

261. Benbassat, C.A., K.C. Maki, and T.G. Unterman, *Circulating levels of insulin-like growth factor (IGF) binding protein-1 and -3 in aging men: relationships to insulin, glucose, IGF, and dehydroepiandrosterone sulfate levels and anthropometric measures.* J Clin Endocrinol Metab, 1997. **82**(5): p. 1484-91.

262. Chu, L.W., et al., *A randomized controlled trial of low-dose recombinant human growth hormone in the treatment of malnourished elderly medical patients.* J Clin Endocrinol Metab, 2001. **86**(5): p. 1913-20.

263. Rudman, D., et al., *Effects of human growth hormone in men over 60 years old.* N Engl J Med, 1990. **323**(1): p. 1-6.

264. Flynn, M.A., et al., *Dehydroepiandrosterone replacement in aging humans.* J Clin Endocrinol Metab, 1999. **84**(5): p. 1527-33.

265. Roubenoff, R., et al., *Monocyte cytokine production in an elderly population: effect of age and inflammation.* The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 1998. **53**(1): p. M20-M26.

266. Wåhlin-Larsson, B., et al., *Mechanistic Links Underlying the Impact of C-Reactive Protein on Muscle Mass in Elderly.* Cellular Physiology and Biochemistry, 2017. **44**(1): p. 267-278.

267. Ribeiro, A.S., et al., *Effect of resistance training on C-reactive protein, blood glucose and lipid profile in older women with differing levels of RT experience.* Age (Dordr), 2015. **37**(6): p. 109.

268. Sardeli, A.V., et al., *Effect of resistance training on inflammatory markers of older adults: A meta-analysis.* Experimental Gerontology, 2018. **111**: p. 188-196.

269. Hofmann, M., et al., *Serum concentrations of insulin-like growth factor-1, members of the TGF-beta superfamily and follistatin do not reflect different stages of dynapenia and sarcopenia in elderly women.* Exp Gerontol, 2015. **64**: p. 35-45.

270. Bodine, S.C., et al., *Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo.* Nat Cell Biol, 2001. **3**(11): p. 1014-9.

271. Ryan, A.S., M.C. Serra, and O. Addison, *THE ROLE OF SKELETAL MUSCLE MYOSTATIN IN SARCOPENIA IN OLDER ADULTS.* Innov Aging, 2017. **1**(Suppl 1): p. 361.

272. Becker, C., et al., *Myostatin antibody (LY2495655) in older weak fallers: a proof-of-concept, randomised, phase 2 trial.* Lancet Diabetes Endocrinol, 2015. **3**(12): p. 948-57.

273. Assoian, R.K., et al., *Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization.* J Biol Chem, 1983. **258**(11): p. 7155-60.

274. Lorite, M.J., P. Cariuk, and M.J. Tisdale, *Induction of muscle protein degradation by a tumour factor.* Br J Cancer, 1997. **76**(8): p. 1035-40.

275. Smith, H.J. and M.J. Tisdale, *Signal transduction pathways involved in proteolysis-inducing factor induced proteasome expression in murine myotubes.* Br J Cancer, 2003. **89**(9): p. 1783-8.

276. Whitehouse, A.S. and M.J. Tisdale, *Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-kappaB.* Br J Cancer, 2003. **89**(6): p. 1116-22.

277. Wieland, B.M., et al., *Is there a human homologue to the murine proteolysis-inducing factor?* Clin Cancer Res, 2007. **13**(17): p. 4984-92.

278. Bodine, S.C., et al., *Identification of ubiquitin ligases required for skeletal muscle atrophy.* Science, 2001. **294**(5547): p. 1704-8.

279. Clarke, B.A., et al., *The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle.* Cell Metab, 2007. **6**(5): p. 376-85.

280. Cohen, S., et al., *During muscle atrophy, thick, but not thin, filament components are degraded by MuRF1-dependent ubiquitylation.* J Cell Biol, 2009. **185**(6): p. 1083-95.

281. Bossola, M., et al., *Increased muscle ubiquitin mRNA levels in gastric cancer patients.* Am J Physiol Regul Integr Comp Physiol, 2001. **280**(5): p. R1518-23.

282. Williams, A., et al., *The expression of genes in the ubiquitin-proteasome proteolytic pathway is increased in skeletal muscle from patients with cancer.* Surgery, 1999. **126**(4): p. 744-9; discussion 749-50.

283. Penna, F., et al., *Effect of the specific proteasome inhibitor bortezomib on cancer-related muscle wasting.* Journal of Cachexia, Sarcopenia and Muscle, 2016. **7**(3): p. 345-354.

284. McPherron, A.C., A.M. Lawler, and S.J. Lee, *Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member.* Nature, 1997. **387**(6628): p. 83-90.

285. McPherron, A.C. and S.J. Lee, *Double muscling in cattle due to mutations in the myostatin gene.* Proc Natl Acad Sci U S A, 1997. **94**(23): p. 12457-61.

286. Chen, J.L., et al., *Elevated expression of activins promotes muscle wasting and cachexia.* The FASEB Journal, 2014. **28**(4): p. 1711-1723.

287. Zhou, X., et al., *Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival.* Cell, 2010. **142**(4): p. 531-43.

288. Chen, Y., et al., *Tumour necrosis factor-alpha stimulates human neutrophils to release preformed activin A.* Immunol Cell Biol, 2011. **89**(8): p. 889-96.

289. Sishi, B.J.N. and A.-M. Engelbrecht, *Tumor necrosis factor alpha (TNF-α) inactivates the PI3-kinase/PKB pathway and induces atrophy and apoptosis in L6 myotubes.* Cytokine, 2011. **54**(2): p. 173-184.

290. Loumaye, A., et al., *Role of Activin A and Myostatin in Human Cancer Cachexia.* The Journal of Clinical Endocrinology & Metabolism, 2015. **100**(5): p. 2030-2038.

291. Paajanen, J., et al., *Elevated circulating activin A levels in malignant pleural mesothelioma patients are related to cancer cachexia and poor response to platinum-based chemotherapy.* European Respiratory Journal, 2019. **54**(suppl 63): p. OA3795.

292. Bargetzi, L., et al., *Nutritional support during the hospital stay reduces mortality in patients with different types of cancers: secondary analysis of a prospective randomized trial.* Annals of Oncology, 2021. **32**(8): p. 1025-1033.

293. Kaegi-Braun, N., et al., *Association of Nutritional Support With Clinical Outcomes in Malnourished Cancer Patients: A Population-Based Matched Cohort Study.* Frontiers in Nutrition, 2021. **7**: p. 244.

294. Lu, Z., et al., *Early Interdisciplinary Supportive Care in Patients With Previously Untreated Metastatic Esophagogastric Cancer: A Phase III Randomized Controlled Trial.* Journal of Clinical Oncology, 2021. **39**(7): p. 748-756.

295. van der Werf, A., et al., *The effect of nutritional counseling on muscle mass and treatment outcome in patients with metastatic colorectal cancer undergoing chemotherapy: A randomized controlled trial.* Clin Nutr, 2020. **39**(10): p. 3005-3013.

296. Zanetti, M., et al., *The Impact of Protein Supplementation Targeted at Improving Muscle Mass on Strength in Cancer Patients: A Scoping Review.* Nutrients, 2020. **12**(7).

297. Greenlee, H., et al., *Association between Body Mass Index and Cancer Survival in a Pooled Analysis of 22 Clinical Trials.* Cancer Epidemiology Biomarkers & Prevention, 2017. **26**(1): p. 21.

298. Petrelli, F., et al., *Association of Obesity With Survival Outcomes in Patients With Cancer: A Systematic Review and Meta-analysis.* JAMA Network Open, 2021. **4**(3): p. e213520-e213520.

299. Yoshimura, Y., et al., *Interventions for Treating Sarcopenia: A Systematic Review and Meta-Analysis of Randomized Controlled Studies.* J Am Med Dir Assoc, 2017. **18**(6): p. 553.e1-553.e16.

300. West, M.A., et al., *Effect of prehabilitation on objectively measured physical fitness after neoadjuvant treatment in preoperative rectal cancer patients: a blinded interventional pilot study.* Br J Anaesth, 2015. **114**(2): p. 244-51.

301. Sebio Garcia, R., et al., *Functional and postoperative outcomes after preoperative exercise training in patients with lung cancer: a systematic review and meta-analysis.* Interact Cardiovasc Thorac Surg, 2016. **23**(3): p. 486-97.

302. Dittus, K.L., R.E. Gramling, and P.A. Ades, *Exercise interventions for individuals with advanced cancer: A systematic review.* Preventive Medicine, 2017. **104**: p. 124-132.

303. Peddle-McIntyre, C.J., et al., *Exercise training for advanced lung cancer.* Cochrane Database Syst Rev, 2019. **2**(2): p. Cd012685.

304. Weisberg, J., et al., *Megestrol acetate stimulates weight gain and ventilation in underweight COPD patients.* Chest, 2002. **121**(4): p. 1070-8.

305. Oster, M.H., et al., *Megestrol acetate in patients with AIDS and cachexia.* Ann Intern Med, 1994. **121**(6): p. 400-8.

306. Yavuzsen, T., et al., *Systematic Review of the Treatment of Cancer-Associated Anorexia and Weight Loss.* Journal of Clinical Oncology, 2005. **23**(33): p. 8500-8511.

307. (CHMP), C.f.M.P.f.H.U. *Assessment report - Adlumiz* 2017 30/11/2017 [cited 2022 18th January]; Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/adlumiz.

308. Baracos, V.E., et al., *Cancer-associated cachexia.* Nat Rev Dis Primers, 2018. **4**: p. 17105.

309. Braun, T.P. and D.L. Marks, *Pathophysiology and treatment of inflammatory anorexia in chronic disease.* J Cachexia Sarcopenia Muscle, 2010. **1**(2): p. 135-145.

310. Lilja, M., et al., *High doses of anti-inflammatory drugs compromise muscle strength and hypertrophic adaptations to resistance training in young adults.* Acta Physiol (Oxf), 2018. **222**(2).

311. Lundholm, K., et al., *Effects by daily long term provision of ghrelin to unselected weight-losing cancer patients: A randomized double-blind study.* Cancer, 2010. **116**(8): p. 2044-2052.

312. Solheim, T.S., et al., *A randomized phase II feasibility trial of a multimodal intervention for the management of cachexia in lung and pancreatic cancer.* Journal of Cachexia, Sarcopenia and Muscle, 2017. **8**(5): p. 778-788.

313. Katakami, N., et al., *Anamorelin (ONO-7643) for the treatment of patients with non–small cell lung cancer and cachexia: Results from a randomized, double-blind, placebo-controlled, multicenter study of Japanese patients (ONO-7643-04).* Cancer, 2018. **124**(3): p. 606-616.

314. Greig, C.A., et al., *Phase I/II trial of formoterol fumarate combined with megestrol acetate in cachectic patients with advanced malignancy.* Supportive Care in Cancer, 2014. **22**(5): p. 1269-1275.

315. Deschenes, M.R., et al., *Effects of resistance training on neuromuscular junction morphology.* Muscle & Nerve, 2000. **23**(10): p. 1576-1581.

316. Hildreth, K.L., et al., *Effects of testosterone and progressive resistance exercise in healthy, highly functioning older men with low-normal testosterone levels.* J Clin Endocrinol Metab, 2013. **98**(5): p. 1891-900.

317. Hennessey, J.V., et al., *Growth hormone administration and exercise effects on muscle fiber type and diameter in moderately frail older people.* J Am Geriatr Soc, 2001. **49**(7): p. 852-8.

318. Setakornnukul, J., et al., *Cutoff point of neutrophil-to-lymphocyte ratio for predicting survival in nasopharyngeal carcinoma.* Medicine (Baltimore), 2021. **100**(34): p. e27095.

319. Pirozzolo, G., et al., *Neutrophil-to-lymphocyte ratio as prognostic marker in esophageal cancer: a systematic review and meta-analysis.* J Thorac Dis, 2019. **11**(7): p. 3136-3145.

320. Cederholm, T., et al., *GLIM criteria for the diagnosis of malnutrition - A consensus report from the global clinical nutrition community.* J Cachexia Sarcopenia Muscle, 2019. **10**(1): p. 207-217.

321. Bowrey, D.J., et al., *Use of alarm symptoms to select dyspeptics for endoscopy causes patients with curable esophagogastric cancer to be overlooked.* Surg Endosc, 2006. **20**(11): p. 1725-8.

322. Maconi, G., et al., *Gastric cancer in young patients with no alarm symptoms: focus on delay in diagnosis, stage of neoplasm and survival.* Scand J Gastroenterol, 2003. **38**(12): p. 1249-55.

323. Li, W.-H., et al., *Role of the initial degree of anaemia and treatment model in the prognosis of gastric cancer patients treated by chemotherapy: a retrospective analysis.* BMC Cancer, 2020. **20**(1): p. 414.

324. Lu, Z., et al., *Early Interdisciplinary Supportive Care in Patients With Previously Untreated Metastatic Esophagogastric Cancer: A Phase III Randomized Controlled Trial.* J Clin Oncol, 2021. **39**(7): p. 748-756.

325. Mwachiro, M., et al., *Predictors of adverse events and early mortality after esophageal stent placement in a low resource setting: a series of 3823 patients in Kenya.* Endosc Int Open, 2022. **10**(4): p. E479-e487.

326. Kim, J.Y., et al., *Clinical outcomes of esophageal stents in patients with malignant esophageal obstruction according to palliative additional treatment.* J Dig Dis, 2015. **16**(10): p. 575-84.

327. Schauer, C., et al., *Quality of life after oesophageal stenting in patients with palliative oesophageal cancer.* The New Zealand Medical Journal (Online), 2021. **134**(1545): p. 115-128.

328. Laviano, A., M.M. Meguid, and F. Rossi-Fanelli, *Cancer anorexia: clinical implications, pathogenesis, and therapeutic strategies.* Lancet Oncol, 2003. **4**(11): p. 686-94.

329. Laviano, A., et al., *Neural control of the anorexia-cachexia syndrome.* Am J Physiol Endocrinol Metab, 2008. **295**(5): p. E1000-8.

330. Zietek, T. and E. Rath, *Inflammation Meets Metabolic Disease: Gut Feeling Mediated by GLP-1.* Front Immunol, 2016. **7**: p. 154.

331. DeBoer, M.D., *Ghrelin and cachexia: Will treatment with GHSR-1a agonists make a difference for patients suffering from chronic wasting syndromes?* Molecular and cellular endocrinology, 2011. **340**(1): p. 97-105.

332. Deans, C. and S.J. Wigmore, *Systemic inflammation, cachexia and prognosis in patients with cancer.* Curr Opin Clin Nutr Metab Care, 2005. **8**(3): p. 265-9.

333. Delano, M.J. and L.L. Moldawer, *The origins of cachexia in acute and chronic inflammatory diseases.* Nutr Clin Pract, 2006. **21**(1): p. 68-81.

334. Ellacott, K.L.J. and R.D. Cone, *The role of the central melanocortin system in the regulation of food intake and energy homeostasis: lessons from mouse models.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2006. **361**(1471): p. 1265-1274.

335. Meek, C.L., et al., *Expected values for gastrointestinal and pancreatic hormone concentrations in healthy volunteers in the fasting and postprandial state.* Ann Clin Biochem, 2021. **58**(2): p. 108-116.

336. Moran, G.W., *Enteroendocrine peptides in intestinal inflammation*, in *School of Translational Medicine, Faculty of Medical and Human Sciences*. 2011, University of Manchester: Manchester, UK. p. 243.

337. Mineur, Y.S., et al., *Nicotine decreases food intake through activation of POMC neurons.* Science, 2011. **332**(6035): p. 1330-2.

338. Pisani, P., *Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies.* Archives of Physiology and Biochemistry, 2008. **114**(1): p. 63-70.

339. Winter, A., J. MacAdams, and S. Chevalier, *Normal protein anabolic response to hyperaminoacidemia in insulin-resistant patients with lung cancer cachexia.* Clin Nutr, 2012. **31**(5): p. 765-73.

340. Copeland, G.P., et al., *Insulin resistance in patients with colorectal cancer.* Journal of British Surgery, 1987. **74**(11): p. 1031-1035.

341. Makino, T., et al., *Circulating interleukin 6 concentrations and insulin resistance in patients with cancer.* BJS (British Journal of Surgery), 1998. **85**(12): p. 1658-1662.

342. Muoio, D.M. and C.B. Newgard, *Mechanisms of disease:Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes.* Nat Rev Mol Cell Biol, 2008. **9**(3): p. 193-205.

343. Hotamisligil, G.S., N.S. Shargill, and B.M. Spiegelman, *Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance.* Science, 1993. **259**(5091): p. 87-91.

344. Plomgaard, P., et al., *Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation.* Diabetes, 2005. **54**(10): p. 2939-45.

345. Ellingsgaard, H., et al., *GLP-1 secretion is regulated by IL-6 signalling: a randomised, placebo-controlled study.* Diabetologia, 2020. **63**(2): p. 362-373.

346. Kahles, F., et al., *GLP-1 Secretion Is Increased by Inflammatory Stimuli in an IL-6–Dependent Manner, Leading to Hyperinsulinemia and Blood Glucose Lowering.* Diabetes, 2014. **63**(10): p. 3221-3229.

347. Müller, T.D., et al., *Glucagon-like peptide 1 (GLP-1).* Mol Metab, 2019. **30**: p. 72-130.

348. Begenik, H., et al., *Serum leptin levels in gastric cancer patients and the relationship with insulin resistance.* Arch Med Sci, 2015. **11**(2): p. 346-52.

349. Brunicardi, F.C., et al., *Pancreatic polypeptide administration improves abnormal glucose metabolism in patients with chronic pancreatitis.* J Clin Endocrinol Metab, 1996. **81**(10): p. 3566-72.

350. Zipf, W.B., et al., *Blunted pancreatic polypeptide responses in children with obesity of Prader-Willi syndrome.* J Clin Endocrinol Metab, 1981. **52**(6): p. 1264-6.

351. Fink, A.S., et al., *Meal-induced pancreatic polypeptide release in a validated pancreatic denervation model: a role for the distal pancreas?* Pancreas, 1990. **5**(3): p. 323-9.

352. Glaser, B., et al., *Pancreatic polypeptide response to secretin in obesity: effects of glucose intolerance.* Horm Metab Res, 1988. **20**(5): p. 288-92.

353. Panzuto, F., et al., *Utility of combined use of plasma levels of chromogranin A and pancreatic polypeptide in the diagnosis of gastrointestinal and pancreatic endocrine tumors.* J Endocrinol Invest, 2004. **27**(1): p. 6-11.

354. Adrian, T.E., et al., *Secretion of pancreatic polypeptide in patients with pancreatic endocrine tumors.* N Engl J Med, 1986. **315**(5): p. 287-91.

355. Zwart, A.T., et al., *CT-measured skeletal muscle mass used to assess frailty in patients with head and neck cancer.* J Cachexia Sarcopenia Muscle, 2019. **10**(5): p. 1060-1069.

356. Bauer, J., et al., *Sarcopenia: A Time for Action. An SCWD Position Paper.* J Cachexia Sarcopenia Muscle, 2019. **10**(5): p. 956-961.

357. Brown, K., et al., *Association of SARC-F Score and Rockwood Clinical Frailty Scale with CT-Derived Muscle Mass in Patients with Aortic Aneurysms.* J Nutr Health Aging, 2022. **26**(8): p. 792-798.

358. Prado, C.M., et al., *Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study.* Lancet Oncol, 2008. **9**(7): p. 629-35.

359. Baracos, V.E., V.C. Mazurak, and A.S. Bhullar, *Cancer cachexia is defined by an ongoing loss of skeletal muscle mass.* Ann Palliat Med, 2019. **8**(1): p. 3-12.

360. Abramowitz, M.K., et al., *Muscle mass, BMI, and mortality among adults in the United States: A population-based cohort study.* PLoS One, 2018. **13**(4): p. e0194697.

361. Gallagher, D., et al., *Changes in skeletal muscle and organ size after a weight-loss intervention in overweight and obese type 2 diabetic patients.* Am J Clin Nutr, 2017. **105**(1): p. 78-84.

362. Weiss, E.P., et al., *Effects of Weight Loss on Lean Mass, Strength, Bone, and Aerobic Capacity.* Med Sci Sports Exerc, 2017. **49**(1): p. 206-217.

363. Catanese, S., et al., *Role of Baseline Computed-Tomography-Evaluated Body Composition in Predicting Outcome and Toxicity from First-Line Therapy in Advanced Gastric Cancer Patients.* J Clin Med, 2021. **10**(5).

364. Surov, A., et al., *Low skeletal muscle mass is a predictor of treatment related toxicity in oncologic patients. A meta-analysis.* Clin Nutr, 2021. **40**(10): p. 5298-5310.

365. Au, P.C., et al., *Sarcopenia and mortality in cancer: A meta-analysis.* Osteoporos Sarcopenia, 2021. **7**(Suppl 1): p. S28-s33.

366. Chen, L., et al., *Relationship between muscle mass and muscle strength, and the impact of comorbidities: a population-based, cross-sectional study of older adults in the United States.* BMC Geriatrics, 2013. **13**(1): p. 74.

367. Advani, S.M., et al., *Pharmacological management of cachexia in adult cancer patients: A systematic review of clinical trials.* BMC Cancer, 2018. **18**(1).

368. Ruiz-Garcia, V., et al., *Megestrol acetate for cachexia-anorexia syndrome. A systematic review.* J Cachexia Sarcopenia Muscle, 2018. **9**(3): p. 444-452.

369. Reid, J., et al., *Non-steroidal anti-inflammatory drugs for the treatment of cancer cachexia: A systematic review.* Palliative Medicine, 2013. **27**(4): p. 295-303.

370. Reid, J., et al., *Thalidomide for managing cancer cachexia.* Cochrane Database Syst Rev, 2012(4): p. CD008664.

371. Maltoni, M., et al., *High-dose progestins for the treatment of cancer anorexia-cachexia syndrome: a systematic review of randomised clinical trials.* Ann Oncol, 2001. **12**(3): p. 289-300.

372. Munn, Z., et al., *Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach.* BMC Med Res Methodol, 2018. **18**(1): p. 143.

373. Mochamat, et al., *A systematic review on the role of vitamins, minerals, proteins, and other supplements for the treatment of cachexia in cancer: a European Palliative Care Research Centre cachexia project.* J Cachexia Sarcopenia Muscle, 2017. **8**(1): p. 25-39.

374. Cheng, H., et al., *Systematic review and meta-analysis of the effect of protein and amino acid supplements in older adults with acute or chronic conditions.* British Journal of Nutrition, 2018. **119**(5): p. 527-542.

375. Dewansingh, P., et al., *Supplemental protein from dairy products increases body weight and vitamin D improves physical performance in older adults: a systematic review and meta-analysis.* Nutr Res, 2018. **49**: p. 1-22.

376. Hanach, N.I., F. McCullough, and A. Avery, *The Impact of Dairy Protein Intake on Muscle Mass, Muscle Strength, and Physical Performance in Middle-Aged to Older Adults with or without Existing Sarcopenia: A Systematic Review and Meta-Analysis.* Advances in Nutrition, 2019. **10**(1): p. 59-69.

377. Komar, B., L. Schwingshackl, and G. Hoffmann, *Effects of leucine-rich protein supplements on anthropometric parameter and muscle strength in the elderly: A systematic review and meta-analysis.* Journal of Nutrition, Health and Aging, 2015. **19**(4): p. 437-446.

378. Ten Haaf, D.S.M., et al., *Effects of protein supplementation on lean body mass, muscle strength, and physical performance in nonfrail community-dwelling older adults: A systematic review and meta-analysis.* American Journal of Clinical Nutrition, 2018. **108**(5): p. 1043-1059.

379. Xu, Z.R., et al., *Clinical effectiveness of protein and amino acid supplementation on building muscle mass in elderly people: a meta-analysis.* PLoS One, 2014. **9**(9): p. e109141.

380. Courel-Ibanez, J., et al., *Health Benefits of beta-Hydroxy-beta-Methylbutyrate (HMB) Supplementation in Addition to Physical Exercise in Older Adults: A Systematic Review with Meta-Analysis.* Nutrients, 2019. **11**(9).

381. Finger, D., et al., *Effects of Protein Supplementation in Older Adults Undergoing Resistance Training: A Systematic Review and Meta-Analysis.* Sports Medicine, 2015. **45**(2): p. 245-255.

382. Liao, C.D., et al., *Effects of protein supplementation combined with resistance exercise on body composition and physical function in older adults: A systematic review and meta-analysis.* American Journal of Clinical Nutrition, 2017. **106**(4): p. 1078-1091.

383. Grande, A.J., et al., *Exercise for cancer cachexia in adults.* Cochrane Database of Systematic Reviews, 2021. **2021**(3).

384. Loughney, L.A., et al., *Exercise interventions for people undergoing multimodal cancer treatment that includes surgery.* Cochrane Database Syst Rev, 2018. **12**: p. CD012280.

385. Zhang, Y., et al., *Exercise interventions for improving physical function, daily living activities and quality of life in community-dwelling frail older adults: A systematic review and meta-analysis of randomized controlled trials.* Geriatr Nurs, 2020. **41**(3): p. 261-273.

386. Chou, C.H., C.L. Hwang, and Y.T. Wu, *Effect of exercise on physical function, daily living activities, and quality of life in the frail older adults: a meta-analysis.* Arch Phys Med Rehabil, 2012. **93**(2): p. 237-44.

387. Lowry, D.W. and A.J. Tomiyama, *Air displacement plethysmography versus dual-energy x-ray absorptiometry in underweight, normal-weight, and overweight/obese individuals.* PLoS One, 2015. **10**(1): p. e0115086.

388. Houtkooper, L.B., et al., *Comparison of methods for assessing body-composition changes over 1 y in postmenopausal women.* The American Journal of Clinical Nutrition, 2000. **72**(2): p. 401-406.

389. Choi, H.H., et al., *Effects of testosterone replacement in human immunodeficiency virus-infected women with weight loss.* J Clin Endocrinol Metab, 2005. **90**(3): p. 1531-41.

390. Miller, K.K., et al., *Effects of testosterone replacement in androgen-deficient women with hypopituitarism: a randomized, double-blind, placebo-controlled study.* J Clin Endocrinol Metab, 2006. **91**(5): p. 1683-90.

391. Behre, H.M., et al., *A randomized, double-blind, placebo-controlled trial of testosterone gel on body composition and health-related quality-of-life in men with hypogonadal to low-normal levels of serum testosterone and symptoms of androgen deficiency over 6 months with 12 months open-label follow-up.* Aging Male, 2012. **15**(4): p. 198-207.

392. Bouloux, P.M., et al., *Effects of oral testosterone undecanoate therapy on bone mineral density and body composition in 322 aging men with symptomatic testosterone deficiency: a 1-year, randomized, placebo-controlled, dose-ranging study.* Aging Male, 2013. **16**(2): p. 38-47.

393. Ferrando, A.A., et al., *Differential anabolic effects of testosterone and amino acid feeding in older men.* J Clin Endocrinol Metab, 2003. **88**(1): p. 358-62.

394. Frederiksen, L., et al., *Testosterone therapy increased muscle mass and lipid oxidation in aging men.* Age (Dordr), 2012. **34**(1): p. 145-56.

395. Gharahdaghi, N., et al., *Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men.* Journal of Cachexia, Sarcopenia and Muscle, 2019. **10**(6): p. 1276-1294.

396. Idan, A., et al., *Long-term effects of dihydrotestosterone treatment on prostate growth in healthy, middle-aged men without prostate disease: a randomized, placebo-controlled trial.* Ann Intern Med, 2010. **153**(10): p. 621-32.

397. Katznelson, L., et al., *Effects of modest testosterone supplementation and exercise for 12 weeks on body composition and quality of life in elderly men.* Eur J Endocrinol, 2006. **155**(6): p. 867-75.

398. Kvorning, T., et al., *Mechanical muscle function and lean body mass during supervised strength training and testosterone therapy in aging men with low-normal testosterone levels.* J Am Geriatr Soc, 2013. **61**(6): p. 957-62.

399. Liu, P.Y., et al., *The short-term effects of high-dose testosterone on sleep, breathing, and function in older men.* J Clin Endocrinol Metab, 2003. **88**(8): p. 3605-13.

400. Ly, L.P. and D.J. Handelsman, *Muscle strength and ageing: methodological aspects of isokinetic dynamometry and androgen administration.* Clin Exp Pharmacol Physiol, 2002. **29**(1-2): p. 37-47.

401. Magnussen, L.V., *Testosterone therapy of men with type 2 diabetes mellitus: A randomized, double-blinded, placebo-controlled study.* Danish Medical Journal, 2017. **64**(7).

402. O'Connell, M.D., et al., *Do the effects of testosterone on muscle strength, physical function, body composition, and quality of life persist six months after treatment in intermediate-frail and frail elderly men?* J Clin Endocrinol Metab, 2011. **96**(2): p. 454-8.

403. Sheffield-Moore, M., et al., *A randomized pilot study of monthly cycled testosterone replacement or continuous testosterone replacement versus placebo in older men.* J Clin Endocrinol Metab, 2011. **96**(11): p. E1831-7.

404. Snyder, P.J., et al., *Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age.* J Clin Endocrinol Metab, 1999. **84**(8): p. 2647-53.

405. Storer, T.W., et al., *Effects of Testosterone Supplementation for 3 Years on Muscle Performance and Physical Function in Older Men.* J Clin Endocrinol Metab, 2017. **102**(2): p. 583-593.

406. Sullivan, D.H., et al., *Effects of muscle strength training and testosterone in frail elderly males.* Med Sci Sports Exerc, 2005. **37**(10): p. 1664-72.

407. Svartberg, J., et al., *Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip.* Int J Impot Res, 2008. **20**(4): p. 378-87.

408. Travison, T.G., et al., *Clinical meaningfulness of the changes in muscle performance and physical function associated with testosterone administration in older men with mobility limitation.* J Gerontol A Biol Sci Med Sci, 2011. **66**(10): p. 1090-9.

409. Wang, C., et al., *Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men.* J Clin Endocrinol Metab, 2004. **89**(5): p. 2085-98.

410. Wittert, G.A., et al., *Oral testosterone supplementation increases muscle and decreases fat mass in healthy elderly males with low-normal gonadal status.* Journals of Gerontology - Series A Biological Sciences and Medical Sciences, 2003. **58**(7): p. 618-625.

411. Emmelot-Vonk, M.H., et al., *Effect of testosterone supplementation on functional mobility, cognition, and other parameters in older men: a randomized controlled trial.* JAMA, 2008. **299**(1): p. 39-52.

412. Allan, C.A., et al., *Testosterone therapy prevents gain in visceral adipose tissue and loss of skeletal muscle in nonobese aging men.* J Clin Endocrinol Metab, 2008. **93**(1): p. 139-46.

413. Merza, Z., et al., *Double-blind placebo-controlled study of testosterone patch therapy on bone turnover in men with borderline hypogonadism.* Int J Androl, 2006. **29**(3): p. 381-91.

414. Marin, P., et al., *Androgen treatment of abdominally obese men.* Obes Res, 1993. **1**(4): p. 245-51.

415. Marin, P., et al., *The effects of testosterone treatment on body composition and metabolism in middle-aged obese men.* Int J Obes Relat Metab Disord, 1992. **16**(12): p. 991-7.

416. Storer, T.W., et al., *Testosterone dose-dependently increases maximal voluntary strength and leg power, but does not affect fatigability or specific tension.* J Clin Endocrinol Metab, 2003. **88**(4): p. 1478-85.

417. Young, N.R., et al., *Body composition and muscle strength in healthy men receiving testosterone enanthate for contraception.* J Clin Endocrinol Metab, 1993. **77**(4): p. 1028-32.

418. Zachwieja, J.J., et al., *Testosterone administration preserves protein balance but not muscle strength during 28 days of bed rest.* J Clin Endocrinol Metab, 1999. **84**(1): p. 207-12.

419. Bhasin, S., et al., *Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle.* J Clin Endocrinol Metab, 2005. **90**(2): p. 678-88.

420. Aguirre, L.E., et al., *Bone and body composition response to testosterone therapy vary according to polymorphisms in the CYP19A1 gene.* Endocrine, 2019. **65**(3): p. 692-706.

421. Basaria, S., et al., *Effects of testosterone replacement in men with opioid-induced androgen deficiency: a randomized controlled trial.* PAIN, 2015. **156**(2): p. 280-288.

422. Gianatti, E.J., et al., *Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial.* Diabetes Care, 2014. **37**(8): p. 2098-107.

423. Leifke, E., et al., *Effects of testosterone replacement therapy on cortical and trabecular bone mineral density, vertebral body area and paraspinal muscle area in hypogonadal men.* Eur J Endocrinol, 1998. **138**(1): p. 51-8.

424. McNicholas, T.A., et al., *A novel testosterone gel formulation normalizes androgen levels in hypogonadal men, with improvements in body composition and sexual function.* BJU Int, 2003. **91**(1): p. 69-74.

425. Rodriguez-Tolra, J., et al., *Effects of testosterone treatment on body composition in males with testosterone deficiency syndrome.* Aging Male, 2013. **16**(4): p. 184-90.

426. Steidle, C., et al., *AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function.* J Clin Endocrinol Metab, 2003. **88**(6): p. 2673-81.

427. Thirumalai, A., et al., *Dose-response effects of sex hormone concentrations on body composition and adipokines in medically castrated healthy men administered graded doses of testosterone gel.* Clin Endocrinol (Oxf), 2017. **87**(1): p. 59-67.

428. Wang, C., et al., *Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men--a clinical research center study.* J Clin Endocrinol Metab, 1996. **81**(10): p. 3654-62.

429. Wang, C., et al., *Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men.* J Clin Endocrinol Metab, 2000. **85**(8): p. 2839-53.

430. Glintborg, D., et al., *Testosterone replacement therapy of opioid-induced male hypogonadism improved body composition but not pain perception: a double-blind, randomized, and placebo-controlled trial.* Eur J Endocrinol, 2020. **182**(6): p. 539-548.

431. Bhasin, S., *The dose-dependent effects of testosterone on sexual function and on muscle mass and function.* Mayo Clinic Proceedings, 2000. **75**(SUPPL.): p. S70-S76.

432. Bhasin, S., et al., *Effects of testosterone supplementation on whole body and regional fat mass and distribution in human immunodeficiency virus-infected men with abdominal obesity.* J Clin Endocrinol Metab, 2007. **92**(3): p. 1049-57.

433. Casaburi, R., et al., *Effects of testosterone and resistance training in men with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2004. **170**(8): p. 870-8.

434. Dos Santos, M.R., et al., *Effect of Exercise Training and Testosterone Replacement on Skeletal Muscle Wasting in Patients With Heart Failure With Testosterone Deficiency.* Mayo Clin Proc, 2016. **91**(5): p. 575-86.

435. Gorgey, A.S., et al., *Low-Dose Testosterone and Evoked Resistance Exercise after Spinal Cord Injury on Cardio-Metabolic Risk Factors: An Open-Label Randomized Clinical Trial.* J Neurotrauma, 2019. **36**(18): p. 2631-2645.

436. Griggs, R.C., et al., *Myotonic dystrophy: Effect of testosterone on total body potassium and on creatinine excretion.* Neurology, 1985. **35**(7): p. 1035-1040.

437. Grinspoon, S., et al., *Effects of testosterone and progressive resistance training in eugonadal men with AIDS wasting. A randomized, controlled trial.* Ann Intern Med, 2000. **133**(5): p. 348-55.

438. Howell, S.J., et al., *Randomized placebo-controlled trial of testosterone replacement in men with mild Leydig cell insufficiency following cytotoxic chemotherapy.* Clin Endocrinol (Oxf), 2001. **55**(3): p. 315-24.

439. Svartberg, J., et al., *Testosterone treatment improves body composition and sexual function in men with COPD, in a 6-month randomized controlled trial.* Respir Med, 2004. **98**(9): p. 906-13.

440. Wright, T.J., et al., *A randomized trial of adjunct testosterone for cancer-related muscle loss in men and women.* Journal of Cachexia, Sarcopenia and Muscle, 2018. **9**(3): p. 482-496.

441. Malkin, C.J., et al., *Testosterone therapy in men with moderate severity heart failure: a double-blind randomized placebo controlled trial.* Eur Heart J, 2006. **27**(1): p. 57-64.

442. Mavros, Y., et al., *Oxandrolone Augmentation of Resistance Training in Older Women: A Randomized Trial.* Medicine & Science in Sports & Exercise, 2015. **47**(11): p. 2257-2267.

443. Schroeder, E.T., M. Terk, and F.R. Sattler, *Androgen therapy improves muscle mass and strength but not muscle quality: results from two studies.* Am J Physiol Endocrinol Metab, 2003. **285**(1): p. E16-24.

444. Schroeder, E.T., et al., *Six-week improvements in muscle mass and strength during androgen therapy in older men.* J Gerontol A Biol Sci Med Sci, 2005. **60**(12): p. 1586-92.

445. Schroeder, E.T., et al., *Treatment with oxandrolone and the durability of effects in older men.* J Appl Physiol (1985), 2004. **96**(3): p. 1055-62.

446. Frisoli, A., Jr., et al., *The effect of nandrolone decanoate on bone mineral density, muscle mass, and hemoglobin levels in elderly women with osteoporosis: a double-blind, randomized, placebo-controlled clinical trial.* J Gerontol A Biol Sci Med Sci, 2005. **60**(5): p. 648-53.

447. Hamdy, R.C., et al., *Nandrolone decanoate for men with osteoporosis.* Am J Ther, 1998. **5**(2): p. 89-95.

448. Hassager, C., et al., *Changes in soft tissue body composition and plasma lipid metabolism during nandrolone decanoate therapy in postmenopausal osteoporotic women.* Metabolism, 1989. **38**(3): p. 238-42.

449. Tidermark, J., et al., *Effects of protein-rich supplementation and nandrolone in lean elderly women with femoral neck fractures.* Clin Nutr, 2004. **23**(4): p. 587-96.

450. Schroeder, E.T., et al., *Effects of an oral androgen on muscle and metabolism in older, community-dwelling men.* American Journal of Physiology - Endocrinology and Metabolism, 2003. **284**(1 47-1): p. E120-E128.

451. Demling, R.H. and L. DeSanti, *Oxandrolone induced lean mass gain during recovery from severe burns is maintained after discontinuation of the anabolic steroid.* Burns, 2003. **29**(8): p. 793-7.

452. Earthman, C.P., et al., *Body cell mass repletion and improved quality of life in HIV-infected individuals receiving oxandrolone.* Journal of Parenteral and Enteral Nutrition, 2002. **26**(6): p. 357-365.

453. Grunfeld, C., et al., *Oxandrolone in the treatment of HIV-associated weight loss in men: A randomized, double-blind, placebo-controlled study.* Journal of Acquired Immune Deficiency Syndromes, 2006. **41**(3): p. 304-314.

454. Gold, J., et al., *Safety and efficacy of nandrolone decanoate for treatment of wasting in patients with HIV infection.* AIDS, 1996. **10**(7): p. 745-52.

455. Johansen, K.L., K. Mulligan, and M. Schambelan, *Anabolic effects of nandrolone decanoate in patients receiving dialysis: A randomized controlled trial.* Journal of the American Medical Association, 1999. **281**(14): p. 1275-1281.

456. Sattler, F.R., et al., *Effects of pharmacological doses of nandrolone decanoate and progressive resistance training in immunodeficient patients infected with human immunodeficiency virus.* J Clin Endocrinol Metab, 1999. **84**(4): p. 1268-76.

457. Johansen, K.L., et al.

458. Okamoto, S., et al., *Change in thigh muscle cross-sectional area through administration of an anabolic steroid during routine stroke rehabilitation in hemiplegic patients.* Am J Phys Med Rehabil, 2011. **90**(2): p. 106-11.

459. Ferreira, I.M., et al., *The influence of 6 months of oral anabolic steroids on body mass and respiratory muscles in undernourished COPD patients.* Chest, 1998. **114**(1): p. 19-28.

460. Hengge, U.R., et al., *Double-blind, randomized, placebo-controlled phase III trial of oxymetholone for the treatment of HIV wasting.* AIDS, 2003. **17**(5): p. 699-710.

461. Halstead, L.S., et al., *The effects of an anabolic agent on body composition and pulmonary function in tetraplegia: a pilot study.* Spinal Cord, 2010. **48**(1): p. 55-9.

462. Casson, P.R., et al., *Postmenopausal dehydroepiandrosterone administration increases free insulin-like growth factor-I and decreases high-density lipoprotein: a six-month trial.* Fertil Steril, 1998. **70**(1): p. 107-10.

463. Igwebuike, A., et al., *Lack of dehydroepiandrosterone effect on a combined endurance and resistance exercise program in postmenopausal women.* J Clin Endocrinol Metab, 2008. **93**(2): p. 534-8.

464. Jankowski, C.M., et al., *Effects of dehydroepiandrosterone replacement therapy on bone mineral density in older adults: a randomized, controlled trial.* J Clin Endocrinol Metab, 2006. **91**(8): p. 2986-93.

465. Jedrzejuk, D., et al., *Dehydroepiandrosterone replacement in healthy men with age-related decline of DHEA-S: effects on fat distribution, insulin sensitivity and lipid metabolism.* Aging Male, 2003. **6**(3): p. 151-6.

466. Kenny, A.M., et al., *Dehydroepiandrosterone combined with exercise improves muscle strength and physical function in frail older women.* J Am Geriatr Soc, 2010. **58**(9): p. 1707-14.

467. Morales, A.J., et al., *The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women.* Clin Endocrinol (Oxf), 1998. **49**(4): p. 421-32.

468. Villareal, D.T. and J.O. Holloszy, *DHEA enhances effects of weight training on muscle mass and strength in elderly women and men.* Am J Physiol Endocrinol Metab, 2006. **291**(5): p. E1003-8.

469. Villareal, D.T., J.O. Holloszy, and W.M. Kohrt, *Effects of DHEA replacement on bone mineral density and body composition in elderly women and men.* Clin Endocrinol (Oxf), 2000. **53**(5): p. 561-8.

470. von Muhlen, D., et al., *Effect of dehydroepiandrosterone supplementation on bone mineral density, bone markers, and body composition in older adults: the DAWN trial.* Osteoporos Int, 2008. **19**(5): p. 699-707.

471. Percheron, G., et al., *Effect of 1-year oral administration of dehydroepiandrosterone to 60- to 80-year-old individuals on muscle function and cross-sectional area: A double-blind placebo-controlled trial.* Archives of Internal Medicine, 2003. **163**(6): p. 720-727.

472. Callies, F., et al., *Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity.* J Clin Endocrinol Metab, 2001. **86**(5): p. 1968-72.

473. Christiansen, J.J., et al., *Long-term DHEA substitution in female adrenocortical failure, body composition, muscle function, and bone metabolism: a randomized trial.* Eur J Endocrinol, 2011. **165**(2): p. 293-300.

474. Gebre-Medhin, G., et al., *Oral dehydroepiandrosterone (DHEA) replacement therapy in women with Addison's disease.* Clin Endocrinol (Oxf), 2000. **52**(6): p. 775-80.

475. Gurnell, E.M., et al., *Long-term DHEA replacement in primary adrenal insufficiency: a randomized, controlled trial.* J Clin Endocrinol Metab, 2008. **93**(2): p. 400-9.

476. Supasyndh, O., et al., *Effect of oral anabolic steroid on muscle strength and muscle growth in hemodialysis patients.* Clinical Journal of the American Society of Nephrology, 2013. **8**(2): p. 271-279.

477. Di Carlo, C., et al., *Serum leptin levels and body composition in postmenopausal women: effects of hormone therapy.* Menopause, 2004. **11**(4): p. 466-73.

478. Jensen, J., C. Christiansen, and P. Rødbro, *Oestrogen-progestogen replacement therapy changes body composition in early post-menopausal women.* Maturitas, 1986. **8**(3): p. 209-216.

479. Kenny, A.M., et al., *Effects of ultra-low-dose estrogen therapy on muscle and physical function in older women.* Journal of the American Geriatrics Society, 2005. **53**(11): p. 1973-1977.

480. Aloia, J.F., et al., *The influence of menopause and hormonal replacement therapy on body cell mass and body fat mass.* Am J Obstet Gynecol, 1995. **172**(3): p. 896-900.

481. Arabi, A., et al., *Changes in body composition during post-menopausal hormone therapy: a 2 year prospective study.* Hum Reprod, 2003. **18**(8): p. 1747-52.

482. Bea, J.W., et al., *Effect of hormone therapy on lean body mass, falls, and fractures: 6-year results from the Women's Health Initiative hormone trials.* Menopause, 2011. **18**(1): p. 44-52.

483. Cagnacci, A., et al., *Influence of transdermal estradiol in the regulation of leptin levels of postmenopausal women: a double-blind, placebo-controlled study.* Menopause, 2002. **9**(1): p. 65-71.

484. Chen, Z., et al., *Postmenopausal hormone therapy and body composition -- a substudy of the estrogen plus progestin trial of the Women's Health Initiative.* American Journal of Clinical Nutrition, 2005. **82**(3): p. 651-656.

485. Gower, B.A. and L. Nyman, *Associations among Oral Estrogen Use, Free Testosterone Concentration, and Lean Body Mass among Postmenopausal Women1.* The Journal of Clinical Endocrinology & Metabolism, 2000. **85**(12): p. 4476-4480.

486. Hansen, R.D., et al., *Effects of 20-mg oestradiol implant therapy on bone mineral density, fat distribution and muscle mass in postmenopausal women.* Acta Diabetol, 2003. **40 Suppl 1**: p. S191-5.

487. Napolitano, A., et al., *Body composition and resting metabolic rate of perimenopausal women using continuous progestogen contraception.* Eur J Contracept Reprod Health Care, 2016. **21**(2): p. 168-75.

488. Papadakis, G.E., et al., *Menopausal Hormone Therapy Is Associated With Reduced Total and Visceral Adiposity: The OsteoLaus Cohort.* Journal of Clinical Endocrinology & Metabolism, 2018: p. N.PAG-N.PAG.

489. Ronkainen, P.H., et al., *Postmenopausal hormone replacement therapy modifies skeletal muscle composition and function: a study with monozygotic twin pairs.* J Appl Physiol (1985), 2009. **107**(1): p. 25-33.

490. Taaffe, D.R., et al., *Estrogen replacement, muscle composition, and physical function: The Health ABC Study.* Med Sci Sports Exerc, 2005. **37**(10): p. 1741-7.

491. Tanko, L.B., et al., *The effect of hormone replacement therapy on appendicular lean tissue mass in early postmenopausal women.* Menopause, 2002. **9**(2): p. 117-21.

492. Villareal, D.T., et al., *Effects of exercise training added to ongoing hormone replacement therapy on bone mineral density in frail elderly women.* J Am Geriatr Soc, 2003. **51**(7): p. 985-90.

493. Walker, R.J., et al., *The effects of sequential combined oral 17beta-estradiol norethisterone acetate on insulin sensitivity and body composition in healthy postmenopausal women: a randomized single blind placebo-controlled study.* Menopause, 2001. **8**(1): p. 27-32.

494. Thorneycroft, I.H., R. Lindsay, and J.H. Pickar.

495. Aubertin-Leheudre, M., et al., *HRT provides no additional beneficial effect on sarcopenia in physically active postmenopausal women: A cross-sectional, observational study.* Maturitas, 2005. **51**(2): p. 140-145.

496. Meeuwsen, I.B., et al., *The effect of tibolone on fat mass, fat-free mass, and total body water in postmenopausal women.* Endocrinology, 2001. **142**(11): p. 4813-7.

497. Tommaselli, G.A., et al., *Serum leptin levels and body composition in postmenopausal women treated with tibolone and raloxifene.* Menopause, 2006. **13**(4): p. 660-8.

498. Dittmar, M., *Comparison of soft tissue body composition in postmenopausal women with or without hormone replacement therapy considering the influence of reproductive history and lifestyle.* Ann Hum Biol, 2001. **28**(2): p. 207-21.

499. Skelton, D.A., et al., *Hormone replacement therapy increases isometric muscle strength of adductor pollicis in post-menopausal women.* Clin Sci (Lond), 1999. **96**(4): p. 357-64.

500. Dedeoğlu, E.N., M. Erenus, and P. Yörük, *Effects of hormone therapy and tibolone on body composition and serum leptin levels in postmenopausal women.* Fertil Steril, 2009. **91**(2): p. 425-31.

501. Hänggi, W., et al., *Differential impact of conventional oral or transdermal hormone replacement therapy or tibolone on body composition in postmenopausal women.* Clin Endocrinol (Oxf), 1998. **48**(6): p. 691-9.

502. Javed, A.A., et al., *Association Between Hormone Therapy and Muscle Mass in Postmenopausal Women: A Systematic Review and Meta-analysis.* JAMA Netw Open, 2019. **2**(8): p. e1910154.

503. Greising, S.M., et al., *Hormone therapy and skeletal muscle strength: a meta-analysis.* J Gerontol A Biol Sci Med Sci, 2009. **64**(10): p. 1071-81.

504. Procter-Gray, E., et al., *Effect of oral contraceptives on weight and body composition in young female runners.* Med Sci Sports Exerc, 2008. **40**(7): p. 1205-12.

505. Quintino-Moro, A., et al., *Thyroid function during the first year of use of the injectable contraceptive depot medroxyprogesterone acetate.* European Journal of Contraception & Reproductive Health Care, 2019. **24**(2): p. 102-108.

506. Franchini, M., et al., *Evaluation of body composition during low-dose estrogen oral contraceptives treatment.* Acta Eur Fertil, 1995. **26**(2): p. 69-73.

507. Aleixo, G.F.P., et al., *The association of body composition parameters and adverse events in women receiving chemotherapy for early breast cancer.* Breast Cancer Research and Treatment, 2020. **182**(3): p. 631-642.

508. Jacobsen, D.E., et al., *Raloxifene and tibolone in elderly women: a randomized, double-blind, double-dummy, placebo-controlled trial.* J Am Med Dir Assoc, 2012. **13**(2): p. 189 e1-7.

509. Ziaei, S., M. Moaya, and S. Faghihzadeh, *Comparative effects of continuous combined hormone therapy and tibolone on body composition in postmenopausal women.* Climacteric, 2010. **13**(3): p. 249-53.

510. Bhasin, S., et al., *Effect of testosterone supplementation with and without a dual 5α-reductase inhibitor on fat-free mass in men with suppressed testosterone production: a randomized controlled trial.* Jama, 2012. **307**(9): p. 931-9.

511. Page, S.T., et al., *Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T.* J Clin Endocrinol Metab, 2005. **90**(3): p. 1502-10.

512. Borst, S.E., et al., *Musculoskeletal and prostate effects of combined testosterone and finasteride administration in older hypogonadal men: a randomized, controlled trial.* Am J Physiol Endocrinol Metab, 2014. **306**(4): p. E433-42.

513. Kenny, A.M., et al., *Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty.* J Am Geriatr Soc, 2010. **58**(6): p. 1134-43.

514. Kenny, A.M., et al., *Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels.* J Gerontol A Biol Sci Med Sci, 2001. **56**(5): p. M266-72.

515. Hedström, M., et al., *Positive effects of anabolic steroids, vitamin D and calcium on muscle mass, bone mineral density and clinical function after a hip fracture.* The Journal of Bone and Joint Surgery. British volume, 2002. **84-B**(4): p. 497-503.

516. Crawford, B.A.L., et al., 2003.

517. Reid, I.R., et al., *Testosterone therapy in glucocorticoid-treated men.* Arch Intern Med, 1996. **156**(11): p. 1173-7.

518. Nair, K.S., et al., *DHEA in Elderly Women and DHEA or Testosterone in Elderly Men.* New England Journal of Medicine, 2006. **355**(16): p. 1647-1659.

519. Zang, H., et al., *Effects of treatment with testosterone alone or in combination with estrogen on insulin sensitivity in postmenopausal women.* Fertil Steril, 2006. **86**(1): p. 136-44.

520. Herbst, K.L., et al., *The male contraceptive regimen of testosterone and levonorgestrel significantly increases lean mass in healthy young men in 4 weeks, but attenuates a decrease in fat mass induced by testosterone alone.* J Clin Endocrinol Metab, 2003. **88**(3): p. 1167-73.

521. Dobs, A.S., et al., *Differential effects of oral estrogen versus oral estrogen-androgen replacement therapy on body composition in postmenopausal women.* J Clin Endocrinol Metab, 2002. **87**(4): p. 1509-16.

522. Davis, S.R., et al., *Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality.* Maturitas, 1995. **21**(3): p. 227-36.

523. Davis, S.R., K.Z. Walker, and B.J.G. Strauss.

524. Huang, G., et al., *Testosterone dose-response relationships in hysterectomized women with or without oophorectomy: effects on sexual function, body composition, muscle performance and physical function in a randomized trial.* Menopause, 2014. **21**(6): p. 612-23.

525. Dayal, M., et al., *Supplementation with DHEA: Effect on Muscle Size, Strength, Quality of Life, and Lipids.* Journal of Women's Health, 2005. **14**(5): p. 391-400.

526. De Oteyza, C.P., et al., *Megastrol in the treatment of AIDS associated cachexia. Valuation by bioelectric impedance analysis of body composition.* Anales de Medicina Interna, 1998. **15**(5): p. 255-258.

527. Sullivan, D.H., et al., *Effects of muscle strength training and megestrol acetate on strength, muscle mass, and function in frail older people.* Journal of the American Geriatrics Society, 2007. **55**(1): p. 20-28.

528. Von Roenn, J.H., et al., *Megestrol acetate in patients with AIDS-related cachexia.* Annals of Internal Medicine, 1994. **121**(6): p. 393-399.

529. Yeh, S.S., et al., *Report of a pilot, double-blind, placebo-controlled study of megestrol acetate in elderly dialysis patients with cachexia.* J Ren Nutr, 2010. **20**(1): p. 52-62.

530. Batterham, M.J. and R. Garsia, *A comparison of megestrol acetate, nandrolone decanoate and dietary counselling for HIV associated weight loss.* Int J Androl, 2001. **24**(4): p. 232-40.

531. Mulligan, K., et al., *Testosterone supplementation of megestrol therapy does not enhance lean tissue accrual in men with human immunodeficiency virus-associated weight loss: a randomized, double-blind, placebo-controlled, multicenter trial.* J Clin Endocrinol Metab, 2007. **92**(2): p. 563-70.

532. Lambert, C.P., et al., *Effects of Testosterone Replacement and/or Resistance Exercise on the Composition of Megestrol Acetate Stimulated Weight Gain in Elderly Men: A Randomized Controlled Trial.* The Journal of Clinical Endocrinology & Metabolism, 2002. **87**(5): p. 2100-2106.

533. Maccio, A., et al., *A randomized phase III clinical trial of a combined treatment for cachexia in patients with gynecological cancers: evaluating the impact on metabolic and inflammatory profiles and quality of life.* Gynecol Oncol, 2012. **124**(3): p. 417-25.

534. Mantovani, G., *Randomised phase III clinical trial of 5 different arms of treatment on 332 patients with cancer cachexia.* European Review for Medical and Pharmacological Sciences, 2010. **14**(4): p. 292-301.

535. Abdi, L., et al., *A monocentric experience of growth hormone replacement therapy in adult patients.* Annales d'Endocrinologie, 2014. **75**(3): p. 176-183.

536. Ahmad, A.M., et al., *Body composition and quality of life in adults with growth hormone deficiency; effects of low-dose growth hormone replacement.* Clin Endocrinol (Oxf), 2001. **54**(6): p. 709-17.

537. Attanasio, A.F., et al., *Human growth hormone replacement in adult hypopituitary patients: long-term effects on body composition and lipid status--3-year results from the HypoCCS Database.* J Clin Endocrinol Metab, 2002. **87**(4): p. 1600-6.

538. Baum, H.B., et al., *Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial.* Ann Intern Med, 1996. **125**(11): p. 883-90.

539. Beauregard, C., et al., *Growth hormone decreases visceral fat and improves cardiovascular risk markers in women with hypopituitarism: a randomized, placebo-controlled study.* J Clin Endocrinol Metab, 2008. **93**(6): p. 2063-71.

540. Biller, B.M., et al., *Effects of once-weekly sustained-release growth hormone: a double-blind, placebo-controlled study in adult growth hormone deficiency.* J Clin Endocrinol Metab, 2011. **96**(6): p. 1718-26.

541. Biller, B.M., et al., *Withdrawal of long-term physiological growth hormone (GH) administration: differential effects on bone density and body composition in men with adult-onset GH deficiency.* J Clin Endocrinol Metab, 2000. **85**(3): p. 970-6.

542. Burt, M.G., et al., *Relationship between GH-induced metabolic changes and changes in body composition: a dose and time course study in GH-deficient adults.* Growth Horm IGF Res, 2008. **18**(1): p. 55-64.

543. Chihara, K., et al., *Efficacy and safety of growth hormone (GH) in the treatment of adult Japanese patients with GH deficiency: A randomised, placebo-controlled study.* Growth Hormone and IGF Research, 2006. **16**(2): p. 132-142.

544. Chrisoulidou, A., et al., *Effects of 7 years of growth hormone replacement therapy in hypopituitary adults.* J Clin Endocrinol Metab, 2000. **85**(10): p. 3762-9.

545. Cuneo, R.C., et al., *Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength.* J Appl Physiol (1985), 1991. **70**(2): p. 688-94.

546. Daugaard, J.R., et al., *Effect of 6 months of GH treatment on myosin heavy chain composition in GH-deficient patients.* Eur J Endocrinol, 1999. **141**(4): p. 342-9.

547. Elbornsson, M., et al., *Baseline characteristics and effects of ten years of growth hormone (GH) replacement therapy in adults previously treated with pituitary irradiation.* Growth Hormone and IGF Research, 2013. **23**(6): p. 249-255.

548. Fernholm, R., et al., *Growth hormone replacement therapy improves body composition and increases bone metabolism in elderly patients with pituitary disease.* Journal of Clinical Endocrinology and Metabolism, 2000. **85**(11): p. 4104-4112.

549. Fideleff, H.L., et al., *Comparative results of a 4-year study on cardiovascular parameters, lipid metabolism, body composition and bone mass between untreated and treated adult growth hormone deficient patients.* Growth Horm IGF Res, 2008. **18**(4): p. 318-24.

550. Franco, C., et al., *Baseline characteristics and effects of growth hormone therapy over two years in younger and elderly adults with adult onset GH deficiency.* Journal of Clinical Endocrinology and Metabolism, 2006. **91**(11): p. 4408-4414.

551. Gibney, J., et al., *The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients.* J Clin Endocrinol Metab, 1999. **84**(8): p. 2596-602.

552. Gillberg, P., et al., *Commencing growth hormone replacement in adults with a fixed low dose. Effects on serum lipoproteins, glucose metabolism, body composition, and cardiovascular function.* Growth Horm IGF Res, 2001. **11**(5): p. 273-81.

553. Hana, V., et al., *The effects of GH replacement in adult GH-deficient patients: changes in body composition without concomitant changes in the adipokines and insulin resistance.* Clin Endocrinol (Oxf), 2004. **60**(4): p. 442-50.

554. Hayakawa, M., et al., *Metabolic effects of 20-kilodalton human growth hormone (20K-hGH) for adults with growth hormone deficiency: Results of an exploratory uncontrolled multicenter clinical trial of 20K-hGH.* Journal of Clinical Endocrinology and Metabolism, 2004. **89**(4): p. 1562-1571.

555. Hoffman, A.R., et al., *Growth Hormone (GH) Replacement Therapy in Adult-Onset GH Deficiency: Effects on Body Composition in Men and Women in a Double-Blind,*

Randomized, Placebo-Controlled Trial. Journal of Clinical Endocrinology and Metabolism, 2004. **89**(5): p. 2048-2056.

556. Johansson, J.O., et al., *Long-term treatment with growth hormone decreases plasminogen activator inhibitor-1 and tissue plasminogen activator in growth hormone-deficient adults.* Thromb Haemost, 1996. **76**(3): p. 422-8.

557. Jorgensen, J.O., et al., *Growth hormone versus placebo treatment for one year in growth hormone deficient adults: increase in exercise capacity and normalization of body composition.* Clin Endocrinol (Oxf), 1996. **45**(6): p. 681-8.

558. Koranyi, J., et al., *Body composition during GH replacement in adults - methodological variations with respect to gender.* Eur J Endocrinol, 2006. **154**(4): p. 545-53.

559. Koranyi, J., et al., *Baseline characteristics and the effects of five years of GH replacement therapy in adults with GH deficiency of childhood or adulthood onset: a comparative, prospective study.* J Clin Endocrinol Metab, 2001. **86**(10): p. 4693-9.

560. Laursen, T., et al., *Long-term effects of continuous subcutaneous infusion versus daily subcutaneous injections of growth hormone (GH) on the insulin-like growth factor system, insulin sensitivity, body composition, and bone and lipoprotein metabolism in GH-deficient adults.* Journal of Clinical Endocrinology and Metabolism, 2001. **86**(3): p. 1222-1228.

561. Whitehead, H.M., et al., *Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study.* Clin Endocrinol (Oxf), 1992. **36**(1): p. 45-52.

562. Johannsson, G., K.S. Sunnerhagen, and J. Svensson.

563. Verhelst, J., et al., *Baseline characteristics and response to 2 years of growth hormone (GH) replacement of hypopituitary patients with GH deficiency due to adult-onset craniopharyngioma in comparison with patients with nonfunctioning pituitary adenoma: data from KIMS (Pfizer International Metabolic Database).* J Clin Endocrinol Metab, 2005. **90**(8): p. 4636-43.

564. Rodríguez, A., et al.

565. Burman, P., et al.

566. Ai-Shoumer, K.A.S., et al.

567. Beshyah, S.A., et al.

568. Chung, Y.S., et al.

569. Orme, S.M., et al.

570. Gotherstrom, G., et al.

571. Hansen, T.B., et al., *Whole body and regional soft tissue changes in growth hormone deficient adults after one year of growth hormone treatment: a double-blind, randomized, placebo-controlled study.* Clin Endocrinol (Oxf), 1995. **43**(6): p. 689-96.

572. Ezzat, S., et al., *Gender-specific responses of lean body composition and non-gender-specific cardiac function improvement after GH replacement in GH-deficient adults.* Journal of Clinical Endocrinology and Metabolism, 2002. **87**(6): p. 2725-2733.

573. Friedlander, A.L., et al., *One year of insulin-like growth factor I treatment does not affect bone density, body composition, or psychological measures in postmenopausal women.* J Clin Endocrinol Metab, 2001. **86**(4): p. 1496-503.

574. Papadakis, M.A., et al., *Growth Hormone Replacement in Healthy Older Men Improves Body Composition but Not Functional Ability.* Annals of Internal Medicine, 1996. **124**(8): p. 708-716.

575. White, H.K., et al., *Effects of an oral growth hormone secretagogue in older adults.* J Clin Endocrinol Metab, 2009. **94**(4): p. 1198-206.

576. Welle, S., et al., *Growth hormone increases muscle mass and strength but does not rejuvenate myofibrillar protein synthesis in healthy subjects over 60 years old.* J Clin Endocrinol Metab, 1996. **81**(9): p. 3239-43.

577. Weissberger, A.J., et al., *Recombinant human growth hormone treatment in elderly patients undergoing elective total hip replacement.* Clin Endocrinol (Oxf), 2003. **58**(1): p. 99-107.

578. Lange, K.H., et al., *Growth hormone enhances effects of endurance training on oxidative muscle metabolism in elderly women.* Am J Physiol Endocrinol Metab, 2000. **279**(5): p. E989-96.

579. Boesen, A.P., et al., *Effect of growth hormone on aging connective tissue in muscle and tendon: gene expression, morphology, and function following immobilization and rehabilitation.* J Appl Physiol (1985), 2014. **116**(2): p. 192-203.

580. Lange, K.H., et al., *GH administration changes myosin heavy chain isoforms in skeletal muscle but does not augment muscle strength or hypertrophy, either alone or combined with resistance exercise training in healthy elderly men.* J Clin Endocrinol Metab, 2002. **87**(2): p. 513-23.

581. Saaf, M., et al., *Growth hormone treatment of osteoporotic postmenopausal women - a one-year placebo-controlled study.* Eur J Endocrinol, 1999. **140**(5): p. 390-9.

582. Vittone, J., et al., *Effects of single nightly injections of growth hormone-releasing hormone (GHRH 1-29) in healthy elderly men.* Metabolism, 1997. **46**(1): p. 89-96.

583. Yarasheski, K.E., J.A. Campbell, and W.M. Kohrt, *Effect of resistance exercise and growth hormone on bone density in older men.* Clin Endocrinol (Oxf), 1997. **47**(2): p. 223-9.

584. Taaffe, D.R., B. Lewis, and R. Marcus, *Quantifying the effect of hand preference on upper limb bone mineral and soft tissue composition in young and elderly women by dual-energy X-ray absorptiometry.* Clin Physiol, 1994. **14**(4): p. 393-404.

585. Svensson, J., et al., *Two-month treatment of obese subjects with the oral growth hormone (GH) secretagogue MK-677 increases GH secretion, fat-free mass, and energy expenditure.* J Clin Endocrinol Metab, 1998. **83**(2): p. 362-9.

586. Bredella, M.A., et al., *Effects of GH on body composition and cardiovascular risk markers in young men with abdominal obesity.* J Clin Endocrinol Metab, 2013. **98**(9): p. 3864-72.

587. Bredella, M.A., et al.

588. Veldhuis, J.D., et al., *Sustained growth hormone (GH) and insulin-like growth factor I responses to prolonged high-dose twice-daily GH-releasing hormone stimulation in middle-aged and older men.* J Clin Endocrinol Metab, 2004. **89**(12): p. 6325-30.

589. Pasarica, M., et al., *Effect of growth hormone on body composition and visceral adiposity in middle-aged men with visceral obesity.* J Clin Endocrinol Metab, 2007. **92**(11): p. 4265-70.

590. Richelsen, B., et al., *Growth hormone treatment of obese women for 5 wk: effect on body composition and adipose tissue LPL activity.* Am J Physiol, 1994. **266**(2 Pt 1): p. E211-6.

591. Taaffe, D.R., et al.

592. Boesen, A.P., et al.

593. Hennessey, J.V., et al.

594. Ellegård, L., et al., *Low-dose recombinant human growth hormone increases body weight and lean body mass in patients with short bowel syndrome.* Ann Surg, 1997. **225**(1): p. 88-96.

595. Ellis, K.J., et al., *Body composition of HIV/AIDS males: Effects of treatment with Insulin-like Growth Factor (IGF-I) and Growth Hormone (GH).* Applied Radiation and Isotopes, 1998. **49**(5): p. 653-655.

596. Hansen, T.B., et al., *Influence of growth hormone on whole body and regional soft tissue composition in adult patients on hemodialysis. A double-blind, randomized, placebo-controlled study.* Clin Nephrol, 2000. **53**(2): p. 99-107.

597. Moyle, G.J., et al., *Growth hormone improves lean body mass, physical performance, and quality of life in subjects with HIV-associated weight loss or wasting on highly active antiretroviral therapy.* J Acquir Immune Defic Syndr, 2004. **35**(4): p. 367-75.

598. Schambelan, M., et al., *Recombinant Human Growth Hormone in Patients with HIV-Associated Wasting.* Annals of Internal Medicine, 1996. **125**(11): p. 873-882.

599. Seguy, D., et al.

600. Chu, L.-W., et al., *A Randomized Controlled Trial of Low-Dose Recombinant Human Growth Hormone in the Treatment of Malnourished Elderly Medical Patients 1.* Journal of clinical endocrinology & metabolism., 2001. **86**(5): p. 1913-1920.

601. P B Jeppesen, J.S.C.E.

602. Scolapio, J.S.

603. Mendias, C.L., et al., *The Use of Recombinant Human Growth Hormone to Protect Against Muscle Weakness in Patients Undergoing Anterior Cruciate Ligament Reconstruction: A Pilot, Randomized Placebo-Controlled Trial.* The American Journal of Sports Medicine, 2020. **48**(8): p. 1916-1928.

604. Jager, H., et al., *Treatment of HIV-associated wasting with recombinant human growth hormone: monitoring of body composition changes by bioelectrical impedance analysis (BIA).* Eur J Med Res, 2002. **7**(3): p. 103-8.

605. Lo, J.C., et al., *The effects of recombinant human growth hormone on body composition and glucose metabolism in HIV-infected patients with fat accumulation.* J Clin Endocrinol Metab, 2001. **86**(8): p. 3480-7.

606. Scolapio, J.S., *Effect of growth hormone, glutamine, and diet on body composition in short bowel syndrome: a randomized, controlled study.* JPEN J Parenter Enteral Nutr, 1999. **23**(6): p. 309-12; discussion 312-3.

607. Vlachopapadopoulou, E., et al., *Metabolic and clinical response to recombinant human insulin-like growth factor I in myotonic dystrophy--a clinical research center study.* J Clin Endocrinol Metab, 1995. **80**(12): p. 3715-23.

608. Mendias, C.L., et al., *The Use of Recombinant Human Growth Hormone to Protect Against Muscle Weakness in Patients Undergoing Anterior Cruciate Ligament Reconstruction: A Pilot, Randomized Placebo-Controlled Trial.* Am J Sports Med, 2020. **48**(8): p. 1916-1928.

609. Giannoulis, M.G., et al., *The effects of growth hormone and/or testosterone in healthy elderly men: a randomized controlled trial.* J Clin Endocrinol Metab, 2006. **91**(2): p. 477-84.

610. Sattler, F.R., et al., *Testosterone and growth hormone improve body composition and muscle performance in older men.* Journal of Clinical Endocrinology and Metabolism, 2009. **94**(6): p. 1991-2001.

611. Schroeder, E.T., et al., *Value of measuring muscle performance to assess changes in lean mass with testosterone and growth hormone supplementation.* Eur J Appl Physiol, 2012. **112**(3): p. 1123-31.

612. Zajac, A., et al., *Effects of growth hormone and testosterone therapy on aerobic and anaerobic fitness, body composition and lipoprotein profile in middle-aged men.* Ann Agric Environ Med, 2014. **21**(1): p. 156-60.

613. Harman, S.M. and M.R. Blackman, *The Effects of Growth Hormone and Sex Steroid on Lean Body Mass, Fat Mass, Muscle Strength, Cardiovascular Endurance and Adverse Events in Healthy Elderly Women and Men.* Hormone Research in Paediatrics, 2003. **60(suppl 1)**(Suppl. 1): p. 121-124.

614. Birzniece, V., et al.

615. Ragnarsson, O., et al., *Effect of short-term GH and testosterone administration on body composition and glucose homoeostasis in men receiving chronic glucocorticoid therapy.* European journal of endocrinology / European Federation of Endocrine Societies, 2013. **168**(2): p. 243-251.

616. Storer, T.W., et al., *A randomized, placebo-controlled trial of nandrolone decanoate in human immunodeficiency virus-infected men with mild to moderate weight loss with recombinant human growth hormone as active reference treatment.* Journal of Clinical Endocrinology and Metabolism, 2005. **90**(8): p. 4474-4482.

617. Harman, S.M. and M.R. Blackman.

618. Sattler, F.R., et al., *Durability of the effects of testosterone and growth hormone supplementation in older community-dwelling men: the HORMA Trial.* Clin Endocrinol (Oxf), 2011. **75**(1): p. 103-11.

619. Liu, H., *Systematic Review: The Safety and Efficacy of Growth Hormone in the Elderly* Annals of Internal Medicine, 2007. **146**: p. 104-115.

620. Liu, H., *Systematic Review: The Effects of Growth Hormone on Athletic Performance.* Annals of Internal Medicine. **148**: p. 747-758.

621. Bo, Y., et al., *A high whey protein, vitamin D and E supplement preserves muscle mass, strength, and quality of life in sarcopenic older adults: A double-blind randomized controlled trial.* Clinical Nutrition, 2019. **38**(1): p. 159-164.

622. Cangussu, L.M., et al., *Effect of vitamin D supplementation alone on muscle function in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial.* Osteoporosis International, 2015. **26**(10): p. 2413-2421.

623. Chanet, A., et al., *Supplementing breakfast with a vitamin D and leucine-enriched whey protein medical nutrition drink enhances postprandial muscle protein synthesis and muscle mass in healthy older men.* Journal of Nutrition, 2017. **147**(12): p. 2262-2271.

624. Cuellar, W.A., et al., *Vitamin D supplements for trunk muscle morphology in older adults: secondary analysis of a randomized controlled trial.* Journal of Cachexia, Sarcopenia and Muscle, 2019. **10**(1): p. 177-187.

625. Lerchbaum, E., et al., *Vitamin D and Testosterone in Healthy Men: A Randomized Controlled Trial.* J Clin Endocrinol Metab, 2017. **102**(11): p. 4292-4302.

626. Suebthawinkul, C., et al., *The effect of vitamin D2 supplementation on muscle strength in early postmenopausal women: a randomized, double-blind, placebo-controlled trial.* Climacteric, 2018. **21**(5): p. 491-497.

627. Verschueren, S.M., et al., *The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: A 6-month randomized, controlled trial.* Journal of Bone and Mineral Research, 2011. **26**(1): p. 42-49.

628. Ito, S., et al., *Use of alfacalcidol in osteoporotic patients with low muscle mass might increase muscle mass: An investigation using a patient database.* Geriatrics and Gerontology International, 2014. **14**(SUPPL.1): p. 122-128.

629. Lerchbaum, E., et al., *Effects of Vitamin D Supplementation on Body Composition and Metabolic Risk Factors in Men: A Randomized Controlled Trial.* Nutrients, 2019. **11**(8).

630. Sadiya, A., et al., *Vitamin D3 supplementation and body composition in persons with obesity and type 2 diabetes in the UAE: A randomized controlled double-blinded clinical trial.* Clin Nutr, 2016. **35**(1): p. 77-82.

631. Shea, M.K., R.A. Fielding, and B. Dawson-Hughes, *The effect of vitamin D supplementation on lower-extremity power and function in older adults: a randomized controlled trial.* Am J Clin Nutr, 2019. **109**(2): p. 369-379.

632. El Hajj, C., et al., *Vitamin D supplementation and muscle strength in pre-sarcopenic elderly Lebanese people: a randomized controlled trial.* Arch Osteoporos, 2018. **14**(1): p. 4.

633. Mieszkowski, J., et al., *The Effect of Nordic Walking Training Combined with Vitamin D Supplementation on Postural Control and Muscle Strength in Elderly People-A Randomized Controlled Trial.* Int J Environ Res Public Health, 2018. **15**(9).

634. Vaes, A.M.M., et al., *Cholecalciferol or 25-Hydroxycholecalciferol Supplementation Does Not Affect Muscle Strength and Physical Performance in Prefrail and Frail Older Adults.* J Nutr, 2018. **148**(5): p. 712-720.

635. Bislev, L.S., et al., *Effects of Vitamin D3 Supplementation on Muscle Strength, Mass, and Physical Performance in Women with Vitamin D Insufficiency: A Randomized Placebo-Controlled Trial.* Calcif Tissue Int, 2018. **103**(5): p. 483-493.

636. Bauer, J.M., et al., *Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial.* J Am Med Dir Assoc, 2015. **16**(9): p. 740-7.

637. Lagari, V., O. Gomez-Marin, and S. Levis, *The role of vitamin D in improving physical performance in the elderly.* J Bone Miner Res, 2013. **28**(10): p. 2194-201.

638. Kukuljan, S., et al., *Effects of a multi-component exercise program and calcium-vitamin-D3-fortified milk on bone mineral density in older men: a randomised controlled trial.* Osteoporos Int, 2009. **20**(7): p. 1241-51.

639. Abe, S., O. Ezaki, and M. Suzuki, *Medium-chain triglycerides in combination with leucine and vitamin D increase muscle strength and function in frail elderly adults in a randomized controlled trial.* Journal of Nutrition, 2016. **146**(5): p. 1017-1026.

640. Annweiler, C., et al., *Vitamin D-related changes in physical performance: a systematic review.* J Nutr Health Aging, 2009. **13**(10): p. 893-8.

641. Beaudart, C., et al., *The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials.* J Clin Endocrinol Metab, 2014. **99**(11): p. 4336-45.

642. CM, C., *EFFECTS OF VITAMIN D SUPPLEMENTATION ON muscle in atheletes sytematic review.* Journal ofStrength and Conditioning Research. **31**(2): p. 566–574.

643. Muir, S.W. and M. Montero-Odasso, *Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis.* J Am Geriatr Soc, 2011. **59**(12): p. 2291-300.

644. Pathak, K., et al., *Vitamin D supplementation and body weight status: a systematic review and meta-analysis of randomized controlled trials.* Obes Rev, 2014. **15**(6): p. 528-37.

645. Stockton, K.A., et al., *Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis.* Osteoporos Int, 2011. **22**(3): p. 859-71.

646. Tomlinson, P.B., C. Joseph, and M. Angioi, *Effects of vitamin D supplementation on upper and lower body muscle strength levels in healthy individuals. A systematic review with meta-analysis.* J Sci Med Sport, 2015. **18**(5): p. 575-80.

647. Park, J.H., et al., *Concomitant increase in muscle strength and bone mineral density with decreasing IL-6 levels after combination therapy with alendronate and calcitriol in postmenopausal women.* Menopause, 2013. **20**(7): p. 747-53.

648. Boutry-Regard, C., et al., *Supplementation with Whey Protein, Omega-3 Fatty Acids and Polyphenols Combined with Electrical Muscle Stimulation Increases Muscle Strength in Elderly Adults with Limited Mobility: A Randomized Controlled Trial.* Nutrients, 2020. **12**(6).

649. Cornish, S.M., et al., *Omega-3 supplementation with resistance training does not improve body composition or lower biomarkers of inflammation more so than resistance training alone in older men.* Nutrition Research, 2018. **60**: p. 87-95.

650. Krzymińska-Siemaszko, R., et al., *The Effect of a 12-week omega-3 supplementation on body composition, muscle strength and physical performance in elderly individuals with decreased muscle mass.* International Journal of Environmental Research and Public Health, 2015. **12**(9): p. 10558-10574.

651. Logan, S.L. and L.L. Spriet, *Omega-3 Fatty Acid Supplementation for 12 Weeks Increases Resting and Exercise Metabolic Rate in Healthy Community-Dwelling Older Females.* PLoS One, 2015. **10**(12): p. e0144828.

652. Sneddon, A.A., et al., *Effect of a conjugated linoleic acid and omega-3 fatty acid mixture on body composition and adiponectin.* Obesity (Silver Spring), 2008. **16**(5): p. 1019-24.

653. Stavrinou, P.S., et al., *The Effects of a 6-Month High Dose Omega-3 and Omega-6 Polyunsaturated Fatty Acids and Antioxidant Vitamins Supplementation on Cognitive Function and Functional Capacity in Older Adults with Mild Cognitive Impairment.* Nutrients, 2020. **12**(2).

654. Da Boit, M., et al., *Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: A randomized controlled trial.* American Journal of Clinical Nutrition, 2017. **105**(1): p. 151-158.

655. Smith, G.I., et al., *Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults.* American Journal of Clinical Nutrition, 2015. **102**(1): p. 115-122.

656. Hill, A.M., et al., *Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors.* Am J Clin Nutr, 2007. **85**(5): p. 1267-74.

657. Jafari Salim, S., et al., *Effect of Omega-3 Polyunsaturated Fatty Acids Supplementation on Body Composition and Circulating Levels of Follistatin-Like 1 in Males With Coronary Artery Disease: A Randomized Double-Blind Clinical Trial.* Am J Mens Health, 2017. **11**(6): p. 1758-1764.

658. Mansoori, A., et al., *Docosahexaenoic Acid-Rich Fish Oil Supplementation Improves Body Composition without Influence of the PPARgamma Pro12Ala Polymorphism in Patients with Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial.* J Nutrigenet Nutrigenomics, 2015. **8**(4-6): p. 195-204.

659. Wu, C., et al., *Supplementation of l-Alanyl-l-Glutamine and Fish Oil Improves Body Composition and Quality of Life in Patients With Chronic Heart Failure.* Circulation: Heart Failure, 2015. **8**(6): p. 1077-1087.

660. Abe, K., et al., *Effects of ω-3 fatty acid supplementation in patients with bile duct or pancreatic cancer undergoing chemotherapy.* Anticancer Research, 2018. **38**(4): p. 2369-2375.

661. Barber, M.D., et al., *The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer.* Br J Cancer, 1999. **81**(1): p. 80-6.

662. Fearon, K.C.H., et al., *Double-blind, placebo-controlled, randomized study of eicosapentaenoic acid diester in patients with cancer cachexia.* Journal of Clinical Oncology, 2006. **24**(21): p. 3401-3407.

663. Murphy, R.A., et al., *Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy.* Cancer, 2011. **117**(8): p. 1775-1782.

664. Paixao, E., et al., *The effects of EPA and DHA enriched fish oil on nutritional and immunological markers of treatment naive breast cancer patients: a randomized double-blind controlled trial.* Nutr J, 2017. **16**(1): p. 71.

665. Read, J.A., et al., *Nutrition intervention using an eicosapentaenoic acid (EPA)-containing supplement in patients with advanced colorectal cancer. Effects on nutritional and inflammatory status: a phase II trial.* Support Care Cancer, 2007. **15**(3): p. 301-7.

666. Ryan, A.M., et al., *Enteral nutrition enriched with eicosapentaenoic acid (EPA) preserves lean body mass following esophageal cancer surgery: results of a double-blinded randomized controlled trial.* Ann Surg, 2009. **249**(3): p. 355-63.

667. Solís-Martínez, O., et al., *Effect of Eicosapentaenoic Acid on Body Composition and Inflammation Markers in Patients with Head and Neck Squamous Cell Cancer from a Public Hospital in Mexico.* Nutrition and Cancer, 2018. **70**(4): p. 663-670.

668. Wigmore, S.J., et al., *Effect of oral Eicosapentaenoic acid on weight loss in patients with pancreatic cancer.* Nutrition and Cancer, 2000. **36**(2): p. 177-184.

669. Sanchez-Lara, K., et al., *Effects of an oral nutritional supplement containing eicosapentaenoic acid on nutritional and clinical outcomes in patients with advanced non-small cell lung cancer: randomised trial.* Clin Nutr, 2014. **33**(6): p. 1017-23.

670. Feijo, P.M., et al., *Effects of omega-3 supplementation on the nutritional status, immune, and inflammatory profiles of gastric cancer patients: A randomized controlled trial.* Nutrition, 2019. **61**: p. 125-131.

671. Bostock, E.L., et al., *Omega-3 Fatty Acids and Vitamin D in Immobilisation: Part A-Modulation of Appendicular Mass Content, Composition and Structure.* J Nutr Health Aging, 2017. **21**(1): p. 51-58.

672. Cerchietti, L.C.A., A.H. Navigante, and M.A. Castro, *Effects of eicosapentaenoic and docosahexaenoic n-3 fatty acids from fish oil and preferential cox-2 inhibition on systemic syndromes in patients with advanced lung cancer.* Nutrition & Cancer, 2007. **59**(1): p. 14-20.

673. Huang, Y.H., et al., *Effects of Omega-3 Fatty Acids on Muscle Mass, Muscle Strength and Muscle Performance among the Elderly: A Meta-Analysis.* Nutrients, 2020. **12**(12).

674. Delpino, F.M. and L.M. Figueiredo, *Supplementation with omega-3 and lean body mass in the general population: A systematic review and meta-analysis.* Clinical Nutrition ESPEN, 2021. **44**: p. 105-113.

675. Dewey, A., et al., *Eicosapentaenoic acid (EPA, an omega-3 fatty acid from fish oils) for the treatment of cancer cachexia.* Cochrane Database Syst Rev, 2007(1): p. CD004597.

676. Flodin, L., et al., *Effects of protein-rich nutritional supplementation and bisphosphonates on body composition, handgrip strength and health-related quality of life after hip fracture: A 12-month randomized controlled study Public health, nutrition and epidemiology.* BMC Geriatrics, 2015. **15**(1).

677. Chilibeck, P.D., et al., *The effect of strength training combined with bisphosphonate (etidronate) therapy on bone mineral, lean tissue, and fat mass in postmenopausal women.* Canadian Journal of Physiology and Pharmacology, 2002. **80**(10): p. 941-950.

678. Candow, D.G., et al., *Ingestion of low-dose ibuprofen following resistance exercise in postmenopausal women.* Journal of Cachexia, Sarcopenia and Muscle, 2013. **4**(1): p. 41-46.

679. Dideriksen, K., et al., *Skeletal muscle adaptation to immobilization and subsequent retraining in elderly men: No effect of anti-inflammatory medication.* Exp Gerontol, 2016. **82**: p. 8-18.

680. Duff, W.R., et al., *Effects of Ibuprofen and Resistance Training on Bone and Muscle: A Randomized Controlled Trial in Older Women.* Med Sci Sports Exerc, 2017. **49**(4): p. 633-640.

681. Jankowski, C.M., et al., *Timing of ibuprofen use and musculoskeletal adaptations to exercise training in older adults.* Bone Reports, 2015. **1**: p. 1-8.

682. Kohrt, W.M., et al., *Timing of ibuprofen use and bone mineral density adaptations to exercise training.* Journal of Bone and Mineral Research, 2010. **25**(6): p. 1415-1422.

683. Petersen, S.G., et al., *Nonsteroidal anti-inflammatory drug or glucosamine reduced pain and improved muscle strength with resistance training in a randomized controlled trial of knee osteoarthritis patients.* Arch Phys Med Rehabil, 2011. **92**(8): p. 1185-93.

684. Trappe, T.A., et al., *Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults.* Am J Physiol Regul Integr Comp Physiol, 2011. **300**(3): p. R655-62.

685. Wigmore, S.J., et al., *Ibuprofen reduces energy expenditure and acute-phase protein production compared with placebo in pancreatic cancer patients.* Br J Cancer, 1995. **72**(1): p. 185-8.

686. Beyer, I., et al., *Effects on muscle performance of NSAID treatment with Piroxicam versus placebo in geriatric patients with acute infection-induced inflammation. a double blind randomized controlled trial.* BMC Musculoskeletal Disorders, 2011. **12**(1): p. 292.

687. Lai, V., et al., *Results of a pilot study of the effects of celecoxib on cancer cachexia in patients with cancer of the head, neck, and gastrointestinal tract.* Head and Neck, 2008. **30**(1): p. 67-74.

688. Mantovani, G., et al., *Phase II nonrandomized study of the efficacy and safety of COX-2 inhibitor celecoxib on patients with cancer cachexia.* J Mol Med (Berl), 2010. **88**(1): p. 85-92.

689. Serelis, J., et al., *Effect of anti-TNF treatment on body composition and serum adiponectin levels of women with rheumatoid arthritis.* Clin Rheumatol, 2008. **27**(6): p. 795-7.

690. Subramaniam, K., et al., *Infliximab reverses inflammatory muscle wasting (sarcopenia) in Crohn's disease.* Alimentary Pharmacology and Therapeutics, 2015. **41**(5): p. 419-428.

691. Renzo, L.D., et al., *Prospective assessment of body weight and body composition changes in patients with psoriasis receiving anti-TNF-α treatment.* Dermatologic Therapy, 2011. **24**(4): p. 446-451.

692. Briot, K., et al., *Prospective assessment of body weight, body composition, and bone density changes in patients with spondyloarthropathy receiving anti-tumor necrosis factor-α treatment.* Journal of Rheumatology, 2008. **35**(5): p. 855-861.

693. Lo, J., et al., *Effects of TNF-alpha neutralization on adipocytokines and skeletal muscle adiposity in the metabolic syndrome.* Am J Physiol Endocrinol Metab, 2007. **293**(1): p. E102-9.

694. Marcora, S.M., et al., *Randomized phase 2 trial of anti-tumor necrosis factor therapy for cachexia in patients with early rheumatoid arthritis.* Am J Clin Nutr, 2006. **84**(6): p. 1463-72.

695. Bouchi, R., et al., *Dipeptidyl peptidase 4 inhibitors attenuates the decline of skeletal muscle mass in patients with type 2 diabetes.* Diabetes/Metabolism Research and Reviews, 2018. **34**(2).

696. Bunck, M.C., et al., *Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition.* Diabetes Care, 2010. **33**(8): p. 1734-7.

697. Harder, H., et al., *The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes.* Diabetes Care, 2004. **27**(8): p. 1915-21.

698. Hirose, S., et al., *Impact of the 8-week administration of tofogliflozin for glycemic control and body composition in Japanese patients with type 2 diabetes mellitus.* Internal Medicine, 2016. **55**(22): p. 3239-3245.

699. Kamei, S., et al., *Effect of Tofogliflozin on Body Composition and Glycemic Control in Japanese Subjects with Type 2 Diabetes Mellitus.* Journal of Diabetes Research, 2018. **2018**.

700. Li, C.-J., et al., *Changes in liraglutide-induced body composition are related to modifications in plasma cardiac natriuretic peptides levels in obese type 2 diabetic patients.* Cardiovascular Diabetology, 2014. **13**(1): p. 36.

701. Rizzo, M.R., et al., *Sarcopenia in Elderly Diabetic Patients: Role of Dipeptidyl Peptidase 4 Inhibitors.* Journal of the American Medical Directors Association, 2016. **17**(10): p. 896-901.

702. Sugiyama, S., et al., *Dapagliflozin reduces fat mass without affecting muscle mass in type 2 diabetes.* Journal of Atherosclerosis and Thrombosis, 2018. **25**(6): p. 467-476.

703. Yamakage, H., et al., *Effects of dapagliflozin on the serum levels of fibroblast growth factor 21 and myokines and muscle mass in Japanese patients with type 2 diabetes: A randomized, controlled trial.* J Diabetes Investig, 2020. **11**(3): p. 653-661.

704. Inoue, H., et al., *Ipragliflozin, a sodium-glucose cotransporter 2 inhibitor, reduces bodyweight and fat mass, but not muscle mass, in Japanese type 2 diabetes patients treated with insulin: A randomized clinical trial.* J Diabetes Investig, 2019. **10**(4): p. 1012-1021.

705. Perna, S., et al., *Liraglutide and obesity in elderly: efficacy in fat loss and safety in order to prevent sarcopenia. A perspective case series study.* Aging Clinical and Experimental Research, 2016. **28**(6): p. 1251-1257.

706. Walton, R.G., et al., *Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in older adults: A randomized, double-blind, placebo-controlled, multicenter trial: The MASTERS trial.* Aging Cell, 2019. **18**(6): p. e13039.

707. Yajima, T., et al., *The effect of dulaglutide on body composition in type 2 diabetes mellitus patients on hemodialysis.* Journal of Diabetes and its Complications, 2018. **32**(8): p. 759-763.

708. Bastien, M., et al., *Effect of PPARgamma agonist on aerobic exercise capacity in relation to body fat distribution in men with type 2 diabetes mellitus and coronary artery disease: a 1-yr randomized study.* Am J Physiol Endocrinol Metab, 2019. **317**(1): p. E65-E73.

709. Feng, W.H., et al., *Effects of liraglutide, metformin and gliclazide on body composition in patients with both type 2 diabetes and non-alcoholic fatty liver disease: A randomized trial.* J Diabetes Investig, 2019. **10**(2): p. 399-407.

710. SD, D., *Effects of exercise training and metformin on body*

composition and cardiovascular indices in HIV-infected patients. AIDS. **18**: p. 465–473.

711. Lundholm, K., et al., *Insulin treatment in cancer cachexia: effects on survival, metabolism, and physical functioning.* Clin Cancer Res, 2007. **13**(9): p. 2699-706.

712. Heisterberg, M.F., et al., *Losartan has no additive effect on the response to heavy-resistance exercise in human elderly skeletal muscle.* J Appl Physiol (1985), 2018. **125**(5): p. 1536-1554.

713. Spira, D., et al., *Angiotensin-Converting Enzyme Inhibitors and Parameters of Sarcopenia: Relation to Muscle Mass, Strength and Function: Data from the Berlin Aging Study-II (BASE-II).* Drugs & Aging, 2016. **33**(11): p. 829-837.

714. Lin, Y.L., et al., *Angiotensin II receptor blockade is associated with preserved muscle strength in chronic hemodialysis patients.* BMC Nephrology, 2019. **20**(1).

715. Caulfield, L., et al., *Effect of Angiotensin System Inhibitors on Physical Performance in Older People - A Systematic Review and Meta-Analysis.* J Am Med Dir Assoc, 2020.

716. Erlandson, K.M., et al., *Effects of 96 Weeks of Rosuvastatin on Bone, Muscle, and Fat in HIV-Infected Adults on Effective Antiretroviral Therapy.* AIDS Res Hum Retroviruses, 2016. **32**(4): p. 311-6.

717. Amato, A.A., et al., *Treatment of sporadic inclusion body myositis with bimagrumab.* Neurology, 2014. **83**(24): p. 2239.

718. Hanna, M.G., et al., *Safety and efficacy of intravenous bimagrumab in inclusion body myositis (RESILIENT): a randomised, double-blind, placebo-controlled phase 2b trial.* The Lancet Neurology, 2019. **18**(9): p. 834-844.

719. Polkey, M.I., et al., *Activin Type II Receptor Blockade for Treatment of Muscle Depletion in Chronic Obstructive Pulmonary Disease. A Randomized Trial.* Am J Respir Crit Care Med, 2019. **199**(3): p. 313-320.

720. Rooks, D., et al., *Treatment of Sarcopenia with Bimagrumab: Results from a Phase II, Randomized, Controlled, Proof-of-Concept Study.* Journal of the American Geriatrics Society, 2017. **65**(9): p. 1988-1995.

721. Rooks, D., et al., *Bimagrumab vs Optimized Standard of Care for Treatment of Sarcopenia in Community-Dwelling Older Adults: A Randomized Clinical Trial.* JAMA Netw Open, 2020. **3**(10): p. e2020836.

722. Rooks, D.S., et al., *Effect of bimagrumab on thigh muscle volume and composition in men with casting-induced atrophy.* J Cachexia Sarcopenia Muscle, 2017. **8**(5): p. 727-734.

723. Bhattacharya, I., et al., *Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Domagrozumab (PF-06252616), an Antimyostatin Monoclonal Antibody, in Healthy Subjects.* Clin Pharmacol Drug Dev, 2018. **7**(5): p. 484-497.

724. Golan, T., et al., *LY2495655, an antimyostatin antibody, in pancreatic cancer: a randomized, phase 2 trial.* Journal of Cachexia, Sarcopenia and Muscle, 2018. **9**(5): p. 871-879.

725. Glasser, C.E., et al., *Locally acting ACE-083 increases muscle volume in healthy volunteers.* Muscle Nerve, 2018. **57**(6): p. 921-926.

726. Woodhouse, L., et al., *A Phase 2 Randomized Study Investigating the Efficacy and Safety of Myostatin Antibody LY2495655 versus Placebo in Patients Undergoing Elective Total Hip Arthroplasty.* J Frailty Aging, 2016. **5**(1): p. 62-70.

727. Attie, K.M., et al., *A single ascending-dose study of muscle regulator ACE-031 in healthy volunteers.* Muscle Nerve, 2013. **47**(3): p. 416-23.

728. Levinson, B. and J. Gertner, *Randomized study of the efficacy and safety of SUN11031 (synthetic human ghrelin) in cachexia associated with chronic obstructive pulmonary disease.* e-SPEN Journal, 2012. **7**(5): p. e171-e175.

729. Matsumoto, N., et al., *Ghrelin Administration for Chronic Respiratory Failure: A Randomized Dose-Comparison Trial.* Lung, 2015. **193**(2): p. 239-247.

730. Miki, K., et al., *Ghrelin treatment of cachectic patients with chronic obstructive pulmonary disease: a multicenter, randomized, double-blind, placebo-controlled trial.* PLoS One, 2012. **7**(5): p. e35708.

731. Nagaya, N., et al., *Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure.* Circulation, 2004. **110**(24): p. 3674-9.

732. Nass, R., et al., *Effects of an Oral Ghrelin Mimetic on Body Composition and Clinical Outcomes in Healthy Older Adults.* Annals of Internal Medicine, 2008. **149**(9): p. 601-611.

733. Garcia, J.M., et al., *Anamorelin for patients with cancer cachexia: an integrated analysis of two phase 2, randomised, placebo-controlled, double-blind trials.* Lancet Oncology, 2015. **16 1077-4114 (Print)**(1): p. 108-116.

734. Temel, J.S., et al., *Anamorelin in patients with non-small-cell lung cancer and cachexia (ROMANA 1 and ROMANA 2): results from two randomised, double-blind, phase 3 trials.* The Lancet Oncology, 2016. **17**(4): p. 519-531.

735. Hamauchi, S., et al., *A multicenter, open-label, single-arm study of anamorelin (ONO-7643) in advanced gastrointestinal cancer patients with cancer cachexia.* Cancer, 2019. **125**(23): p. 4294-4302.

736. Dubois, S., et al., *Thyroxine Therapy in Euthyroid Patients Does Not Affect Body Composition or Muscular Function.* Thyroid, 2007. **18**(1): p. 13-19.

737. Samuels, M.H., et al., *Effects of levothyroxine replacement or suppressive therapy on energy expenditure and body composition.* Thyroid, 2016. **26**(3): p. 347-355.

738. Amstrup, A.K., et al., *Reduced fat mass and increased lean mass in response to 1 year of melatonin treatment in postmenopausal women: A randomized placebo-controlled trial.* Clin Endocrinol (Oxf), 2016. **84**(3): p. 342-7.

739. Del Fabbro, E., et al., *Effects of melatonin on appetite and other symptoms in patients with advanced cancer and cachexia: A double-blind placebo-controlled trial.* Journal of Clinical Oncology, 2013. **31**(10): p. 1271-1276.

740. Aversa, A., et al., *Tadalafil improves lean mass and endothelial function in nonobese men with mild ED/LUTS: in vivo and in vitro characterization.* Endocrine, 2017. **56**(3): p. 639-648.

741. Ockenga, J., et al., *Ketotifen in HIV-infected patients: effects on body weight and release of TNF-alpha.* Eur J Clin Pharmacol, 1996. **50**(3): p. 167-70.

742. Iwasa, M., et al., *Change in skeletal muscle mass after administering entecavir in patients with hepatitis B.* Nutrition, 2015. **31**(9): p. 1173-1174.

743. Zhang, Y., et al., *Intervention with erythropoietin in sarcopenic patients with femoral intertrochanteric fracture and its potential effects on postoperative rehabilitation.* Geriatrics and Gerontology International, 2020. **20**(2): p. 150-155.

744. Kissel, J.T., et al., *Randomized, double-blind, placebo-controlled trial of albuterol in facioscapulohumeral dystrophy.* Neurology, 2001. **57**(8): p. 1434.

745. Harrington, D., T.P. Chua, and A.J.S. Coats, *The effect of salbutamol on skeletal muscle in chronic heart failure.* International Journal of Cardiology, 2000. **73**(3): p. 257-265.

746. Chasen, M., S.Z. Hirschman, and R. Bhargava, *Phase ii study of the novel peptide-nucleic acid ohr118 in the management of cancer-related anorexia/cachexia.* Journal of the American Medical Directors Association, 2011. **12**(1): p. 62-67.

747. Khan, Z.H., et al., *Oesophageal cancer and cachexia: The effect of short-term treatment with thalidomide on weight loss and lean body mass.* Alimentary Pharmacology and Therapeutics, 2003. **17**(5): p. 677-682.

748. Isidori, A.M., et al., *Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: A meta-analysis.* Clinical Endocrinology, 2005. **63**(3): p. 280-293.

749. Corona, G., et al., *European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males.* Andrology, 2020. **8**(5): p. 970-987.

750. Glueck, C.J. and P. Wang, *Testosterone therapy, thrombosis, thrombophilia, cardiovascular events.* Metabolism, 2014. **63**(8): p. 989-94.

751. Vorona, E. and E. Nieschlag, *Adverse effects of doping with anabolic androgenic steroids in competitive athletics, recreational sports and bodybuilding.* Minerva Endocrinol, 2018. **43**(4): p. 476-488.

752. Currow, D.C., et al., *A randomised, double blind, placebo-controlled trial of megestrol acetate or dexamethasone in treating symptomatic anorexia in people with advanced cancer.* Sci Rep, 2021. **11**(1): p. 2421.

753. Holt, R.I.G. and K.K.Y. Ho, *The Use and Abuse of Growth Hormone in Sports.* Endocrine Reviews, 2019. **40**(4): p. 1163-1185.

754. Heymsfield, S.B., et al., *Effect of Bimagrumab vs Placebo on Body Fat Mass Among Adults With Type 2 Diabetes and Obesity: A Phase 2 Randomized Clinical Trial.* JAMA Netw Open, 2021. **4**(1): p. e2033457.

755. Antunes, J.M.M., R.M.P. Ferreira, and D. Moreira-Goncalves, *Exercise Training as Therapy for Cancer-Induced Cardiac Cachexia.* Trends Mol Med, 2018. **24**(8): p. 709-727.

756. Hojan, K., M. Molinska-Glura, and P. Milecki, *Physical activity and body composition, body physique, and quality of life in premenopausal breast cancer patients during endocrine therapy--a feasibility study.* Acta Oncol, 2013. **52**(2): p. 319-26.

757. Mijwel, S., et al., *Exercise training during chemotherapy preserves skeletal muscle fiber area, capillarization, and mitochondrial content in patients with breast cancer.* Faseb j, 2018. **32**(10): p. 5495-5505.

758. Hall, P.S., et al., *Efficacy of Reduced-Intensity Chemotherapy With Oxaliplatin and Capecitabine on Quality of Life and Cancer Control Among Older and Frail Patients With Advanced Gastroesophageal Cancer: The GO2 Phase 3 Randomized Clinical Trial.* JAMA Oncol, 2021. **7**(6): p. 869-877.

Appendix 1.

Table 2.3. Treatments planned – whole group		
Treatment	**N**	**%**
FLOT (fluorouracil oxaliplatin, docetaxel)	3	1.9
Paclitaxel	5	3.2
CXH, CarboXH	26	16.5
Capecitabine irinotecan	1	0.6
HER-2 directed therapy in trial	2	1.3
Platinum 5FU immunotherapy in trial	8	5.1
Platinum 5FU +/- claudin inhibitor	4	2.5
Oxaliplatin Capecitabine (OX)	100	63.3
Oxaliplatin Capecitabine + Immunotherapy	9	5.7

Appendix 2.

Table 3.9: Group comparisons Insulin							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% CI Lower Bound	95% CI Upper Bound
Insulin T0	A	NA	-0.49	0.17	**0.04**	-0.95	-0.02
		HV	-0.40	0.16	0.19	-1.05	0.25
Insulin T15	A	NA	-0.48	0.16	**0.02**	-0.91	-0.06
		HV	-0.61	0.23	0.18	-1.59	0.37
Insulin T30	A	NA	-0.30	0.17	0.27	-0.77	0.16
		HV	-0.41	0.16	0.13	-0.93	0.12
Insulin T45	A	NA	-0.41	0.13	**0.03**	-0.78	-0.03
		HV	-0.34	0.17	0.41	-1.35	0.66
Insulin T60	A	NA	-0.35	0.15	0.09	-0.74	0.05
		HV	-0.32	0.20	0.55	-1.50	0.86
Insulin T90	A	NA	-0.27	0.15	0.26	-0.69	0.15
		HV	-0.30	0.27	0.74	-2.22	1.61
Insulin T120	A	NA	-0.33	0.18	0.26	-0.84	0.18
		HV	-0.23	0.13	0.36	-0.71	0.25

Table 3.10 GLP-1 group comparisons							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
GLP T0	A	NA	0.00	0.15	1.00	-0.42	0.42
		HV	0.06	0.23	0.99	-1.00	1.12
GLP T15	A	NA	-0.08	0.17	0.96	-0.53	0.37
		HV	-0.06	0.17	0.99	-0.62	0.51
GLP T30	A	NA	-0.10	0.16	0.90	-0.54	0.34
		HV	-0.09	0.18	0.95	-0.68	0.50
GLP T45	A	NA	-0.10	0.16	0.90	-0.56	0.35
		HV	-0.09	0.17	0.94	-0.62	0.43
GLP T60	A	NA	-0.06	0.18	0.98	-0.57	0.45
		HV	-0.14	0.18	0.85	-0.69	0.41
GLP T90	A	NA	0.02	0.15	1.00	-0.42	0.45
		HV	-0.13	0.14	0.76	-0.56	0.30
GLP T120	A	NA	-0.03	0.14	0.99	-0.41	0.35
		HV	0.04	0.13	0.99	-0.36	0.44

Table 3.11: GIP group comparisons							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
GIP T0	A	NA	-0.10	0.22	0.96	-0.75	0.54
		HV	-0.14	0.20	0.90	-0.78	0.51
GIP T15	A	NA	-0.21	0.20	0.67	-0.75	0.33
		HV	-0.29	0.18	0.38	-0.82	0.25
GIP T30	A	NA	0.10	0.26	0.98	-0.61	0.80
		HV	-0.25	0.12	0.23	-0.63	0.14
GIP T45	A	NA	-0.11	0.17	0.89	-0.56	0.34
		HV	-0.19	0.10	0.29	-0.53	0.14
GIP T60	A	NA	-0.03	0.20	1.00	-0.57	0.51
		HV	-0.17	0.15	0.66	-0.62	0.29
GIP T90	A	NA	-0.08	0.17	0.95	-0.56	0.39
		HV	-0.22	0.14	0.41	-0.68	0.23
GIP T120	A	NA	-0.08	0.15	0.95	-0.51	0.35

Table 3.12: PP Group comparisons							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PP T0	A	NA	-0.28	0.26	0.65	-0.99	0.43
		HV	-0.25	0.22	0.64	-0.93	0.42
PP T15	A	NA	-0.44	0.20	0.15	-1.01	0.14
		HV	-0.28	0.22	0.54	-0.94	0.37
PP T30	A	NA	-0.29	0.19	0.38	-0.80	0.23
		HV	-0.33	0.21	0.42	-1.01	0.35
PP T45	A	NA	-0.23	0.22	0.70	-0.85	0.39
		HV	-0.29	0.23	0.58	-1.02	0.43
PP T60	A	NA	-0.18	0.24	0.83	-0.83	0.47
		HV	-0.21	0.24	0.78	-0.94	0.51
PP T90	A	NA	-0.15	0.22	0.88	-0.75	0.45
		HV	-0.22	0.21	0.71	-0.87	0.43
PP T120	A	NA	-0.24	0.24	0.70	-0.88	0.40

Table 3.13: PYY group comparisons							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PYY T0	A	NA	0.11	0.19	0.93	-0.44	0.65
		HV	0.10	0.14	0.87	-0.38	0.58
PYY T15	A	NA	0.13	0.17	0.87	-0.40	0.66
		HV	0.18	0.16	0.67	-0.36	0.72
PYY T30	A	NA	0.22	0.21	0.67	-0.35	0.79
		HV	0.10	0.17	0.93	-0.43	0.63
PYY T45	A	NA	-0.01	0.19	1.00	-0.58	0.56
		HV	0.00	0.18	1.00	-0.59	0.58
PYY T60	A	NA	0.23	0.16	0.43	-0.22	0.68
		HV	0.22	0.15	0.48	-0.27	0.70
PYY T90	A	NA	0.18	0.19	0.74	-0.35	0.72
		HV	0.09	0.14	0.90	-0.35	0.54
PYY T120	A	NA	0.15	0.21	0.87	-0.45	0.74
		HV	0.13	0.15	0.81	-0.41	0.68

Table 3.14: TNF-α group comparisons							
	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TNF T0	A	NA	0.00	3.08	1.00	-8.28	8.28
		HV	2.21	3.17	0.89	-8.93	13.36
TNF T15	A	NA	0.47	2.90	1.00	-7.34	8.28
		HV	1.74	3.37	0.95	-12.70	16.18
TNF T30	A	NA	1.38	2.84	0.95	-6.26	9.02
		HV	1.57	4.17	0.98	-17.69	20.83
TNF T45	A	NA	-0.19	4.24	1.00	-18.75	18.37
		HV	-1.57	4.17	0.98	-20.83	17.69
TNF T60	A	NA	1.78	2.35	0.84	-4.54	8.11
		HV	0.90	2.66	0.99	-9.23	11.02
TNF T90	A	NA	2.13	2.61	0.81	-4.89	9.15
		HV	0.64	4.58	1.00	-24.86	26.13
TNF T120	A	NA	2.59	2.57	0.70	-4.32	9.50
		HV	1.54	5.75	0.99	-34.63	37.70

Appendix 3. Scoping review tables

DXA = dual x-ray absorptiometry, USS = ultrasound, BIA = bioelectrical impedance analysis, CT = computer tomography, NA = not applicable

Table 5.3.4: testosterone in older adults

Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Dose	Duration (months)	Baseline T	Measure of muscle	Functional measure	Effect on muscle mass	Effect on function
Gharahdaghi N	2019	older adults	18	randomized, double-blinded & placebo-controlled	exercise	250mg SC bi weekly	1.5	normal	DXA, USS	Knee extensor force test	Gain	Gain
Magnussen LV	2017	Men 50-70 with type 2 diabetes	39	randomized, double-blinded & placebo-controlled	drug alone	T gel 5g/d	6	low	DXA	Leg Rig (extension power) & gait speed	Gain	No effect
Storer TW	2017	Healthy older males	203	randomized, double-blinded & placebo-controlled	drug alone	7.5 g of 1% T	36	low /low normal	DXA	leg press, chest press, stair climb	Gain	Gain
Kvorning T	2013	Older men	49	randomized, double-blinded & placebo-controlled	drug alone vs drug plus exercise	50-100 mg/d	6	low	DXA	cycle test	Gain	No effect
Bouloux PM	2013	Older men	322	randomized, double-blinded & placebo-controlled	drug alone	80 mg/d, 160 mg/d, or 240 mg/d	12	low	DXA	NA	Gain	NA

Hildreth KL	2013	Healthy older males	167	randomized, double-blinded & placebo-controlled trial (not blinded to exercise)	drug alone vs drug plus exercise vs placebo + Ex	5g gel daily then titrating	12	low-normal	DXA	1RM strength reps	Gain	No effect
Behre HM	2012	Men aged 50-80	362	randomized, double-blinded & placebo-controlled	drug alone	1% T gel	18	low-low normal	DXA	NA	Gain	NA
Frederiksen L	2012	Older men	38	randomized, double-blinded & placebo-controlled	drug alone	5g gel daily	6	low-normal	DXA	NA	Gain	NA
Sheffield-Moore M	2011	Older men	24	randomized, double-blinded & placebo-controlled	drug alone	100mg OD continuous vs alt monthly	5	low-normal	DXA	1RM arm and leg reps	Gain	Gain
Travison TG	2011	Older men with mobility limitation	165	parallel group, placebo-controlled, double-blind randomized	drug alone	gel 5g daily	6	low	DXA	1RM, stair climb	Gain	Gain
O'Connell MD	2011	frail older men	274	randomized, double-blinded & placebo-controlled	drug alone	25-75 mg daily	6	low	DXA	knee extension torque	Gain	Gain
Idan A	2010	Healthy men aged >50	114	parallel group, placebo-controlled, double-blind randomized trial	Drug alone	Gel 70mg/d	24	NA	DXA	NA	Gain	NA
Svartberg J	2008	men aged 60-80	38	randomized, double-blinded & placebo-controlled	drug alone	IM 1000mg @ 6, 16, 28 and 40 weeks	12	low-normal	DXA	Knee & grip strength	Gain	No effect

Emmelot-Vonk MH	2008	older men	207	randomized, double-blinded & placebo-controlled	drug alone	80mg /d	6	normal	DXA	NA	Gain	No effect
Katznelson L	2006	Older men	70	randomized, double-blinded & placebo-controlled trial (not blinded to exercise)	drug v placebo plus resistance ex or no ex	5mg gel daily	3	normal	DXA	Self-reported QoL physical functioning	No effect	Gain
Sullivan DH	2005	older men	61	randomized, double-blinded & placebo-controlled trial (not blinded to exercise)	drug v placebo + resistance exercise or no ex	100mg IM weekly	3	low	CT	1RM, sit-stand, stair climb, gait	Gain	No effect
Wang C	2004	Older men	123	parallel group, placebo-controlled, double-blind randomized trial	drug alone	5, 7.5, or 10 g	42	low	DXA	1RM leg press & chest press	Gain	No effect
Liu PY	2003	Older men	17	randomized, double-blinded & placebo-controlled trial - crossover	drug alone	500 mg, 250 mg, and 250 mg	2	NA	BIA	NA	Gain	NA
Wittert GA	2003	Older men	76	randomized, double-blinded & placebo-controlled trial	drug alone	80mg BD	12	low-normal	DXA	Calf & quad peak torque	Gain	No effect
Ly LP	2002	older men	35	randomized, double-blinded & placebo-controlled trial	drug alone	DHT gel; 70 mg/day	3	low-normal	BIA	dynametry	Gain	Gain

Ferrando AA;	2002	Older men	12	randomized, double-blinded & placebo-controlled trial	drug alone	Weekly, then biweekly IM T	6	low-normal	MRI & DXA	1RM leg press & bicep and tricep	Gain	Gain
Snyder PJ	1999	Men Aged >65	96	randomized, double-blinded & placebo-controlled trial	drug alone	6mg/day patch	36	low	DXA	dynametry	Gain	No effect

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Table 5.3.5: Testosterone adult males												
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Dose	Duration (months)	Baseline T	Measure of muscle	Functional measure	Effect on muscle mass	Effect on function
Allan CA	2008	Healthy, non-obese, middle aged men	60	Placebo controlled randomised double blinded trial	drug alone	Patch	12	? Low	DXA	NA	Gain	NA
Bhasin S	2005	Healthy older men vs younger	112	non placebo controlled randomised double blinded	drug alone	25, 50, 125, 300, or 600 mg weekly	5	suppressed	DXA	1RM leg press	Gain	Gain
Storer TW	2003	Healthy men aged 18-35	54	non placebo controlled randomised double blinded	drug alone	25, 50, 125, 300, or 600 mg	5	supressed	MRI	1RM leg press	NA	No effect
Zachwieja JJ	1999	Healthy men, forced bed rest	10	Non-randomised, non-controlled study	Drug alone	200mg/wk	1	normal	DXA	dynametry	Mainte-nance	No effect
Young NR	1993	Healthy men	13	Case control study	drug alone	200mg/wk	6	normal	DXA	dynametry	Gain	Gain
Marin P	1992	middle aged obese men	23	Placebo controlled randomised double blinded	drug alone	UK	8	normal	CT	NA	No effect	NA

Marin P	1993	middle aged obese men	31	Placebo controlled randomised double blinded	drug alone	5g gel daily	9	Low-normal	CT	NA	No effect	NA
Merza Z	2006	Men aged >40 with sexual dysfunction	39	Placebo controlled randomised double blinded	drug alone	5 mg/day patch v placebo	12	low-normal	DXA	NA	Gain	NA

Table 5.3.6: Testosterone in androgen deficient patients											
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Dose	Duration (months)	Baseline T	Measure of muscle	Functional measure	Effect on muscle mass
Aguirre LE	2019	Hypogonadal men	105	Open label cohort study	drug alone	200mg IM alt weeks	19	low	DXA	NA	Gain
Glintborg D	2020	Males with opioid treated chronic pain and androgen deficiency	41	Placebo controlled randomised double blinded	drug alone	bi monthly	6	low	DXA	NA	Gain
Thirumalai A	2017	medically castrated healthy men	48	Placebo controlled randomised double blinded	drug alone	1.25 g, 2.5 g, 5.0 g, 10 g or 15 g) /d or double placebo (injections and gel	3	suppressed	DXA	NA	Gain
Basaria S	2015	men with androgen deficiency	65	Placebo controlled randomised double blinded	drug alone	5g gel daily	3.5	low	?	NA	Gain
Rodriguez-Tolra J	2013	men with androgen deficiency	50	Non-randomised cohort study	drug alone	50mg gel OD	24	low	DXA	NA	Gain
Miller KK	2006	Women with hypopituitarism	51	Placebo controlled randomised double blinded	Drug alone	300mcg OD patch	12	low	DXA & CT	NA	Gain
Steidle C	2003	Men with androgen deficiency	406	Multi dose level, non placebo controlled randomised double blinded	Drug alone	50mg,100mg gel, 5mg patch	3	low	DXA	NA	Gain

McNicholas TA	2003	Men, 31-80 with low T	208	Multi dose level, non placebo controlled randomised double blinded	drug alone	T Gel 50 & 100 mg (OD dose of 5 mg & 10 mg), vs patch 2x 2.5 mg	3	Low	DXA	NA	Gain
Wang C	2000	Men with androgen deficiency	227	Non-randomised cohort study	drug alone	1% T gel, 50mg or 100mg/d vs patch 5mg/day	6	low	DXA	leg press	Gain
Leifke E	1998	Men on T replacement	32	Non-randomised cohort study	drug alone	varying	varying	low	CT	NA	Gain
Wang C	1996	Men with androgen deficiency	67	Non-randomised cohort study	drug alone	5mg TDS sublingual	6	low	DXA	1RM leg and chest press	Gain
Giannati EJ	2014	Men with T2DM and androgen deficiency	88	Placebo controlled randomised double blinded	drug alone	1000mg IM 0, 6, 18, and 30 weeks	9	low	DXA	NA	Gain

Table 5.3.7: Testosterone disease												
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Dose	Duration (months)	Baseline T	Measure of muscle	Functional measure	Effect on muscle mass	Effect on muscle mass
Gorgey AS	2019	Patients with complete spinal cord injury, age 18-50	22	Randomised open-label trial	resistance training exercise vs drug alone	Patch 2-6mg/day	4	NA	MRI & DXA	NA	Gain	NA
Wright TJ	2018	Patients with cancer related wasting, HNSCC	21	Placebo controlled randomised double blinded trial	drug alone	100mg IM weekly	1.8	NA	DXA	Leg extension power & SPPB	Gain	No effect
Dos Santos MR	2016	adults with heart failure and testosterone deficiency	39	Randomised open-label trial	Exercise (cardio) v drug alone	1000mg depot	4	low	DXA	CPET	Reduction LBM	No effect
Bhasin S	2007	Men with HIV receiving HAART	88	Placebo controlled randomised double blinded trial	drug alone	10g gel	6	NA	DXA and CT	NA	Gain	NA
Choi HH	2005	Women with HIV	52	Placebo controlled randomised double blinded trial	drug alone	300mcg/24h	6	low	DXA	Leg strength	No effect	No effect
Svartberg J	2004	Patients with COPD	29	Placebo controlled randomised double blinded trial	drug alone	250mg IM 4weekly	6.5	NA	DXA	NA	Gain	NA
Casaburi R	2004	Men with COPD	47	Placebo controlled randomised double blinded trial	Drug alone v Drug plus resistance training	100mg IM weekly	2.5	NA	DXA	1RM leg press	Gain	Gain
Howell SJ	2001	Men <55 yrs who'd received previous chemo	35	Placebo controlled randomised double blinded trial	drug alone	2.5mg patch increased to 5mg	12	low or low-normal	DXA	NA	No effect	NA

Bhasin S	2000	Men with HIV and weight loss	49	Placebo controlled randomised double blinded trial	plus resistance exercise	100mg/week IM	4	low	DXA	leg, bench and chest press	Gain	Gain
Grinspoon S	1998	men with HIV wasting	51	Placebo controlled randomised double blinded trial	drug alone	300mg IM weekly	6	low	DXA, BIA	6MWT	Gain	No effect
Griggs RC	1989	Men with myotonic dystrophy	40	Placebo controlled randomised double blinded trial	drug alone	3mg/kg/wk	12	NA	40K method	dynametry	Gain	No effect
Malkin CJ	2006	Men with cardiac failure	76	Placebo controlled randomised double blinded trial	drug alone	5 mg/day patch v placebo	12	NA	CT	shuttle walk, hand grip,	No effect	Gain

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Table 5.3.8: synthetic androgens older adults													
Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Baseline androgens	Measure MM	Measure function	Effect muscle mass	Effect function
Mavros Y	2015	older adults	29	Double blind, placebo-controlled randomised	oxandrolone	Resistance exercise	10mg OD	3	NA	DXA	leg press, chest press, gait speed, stair climb	No effect	No effect
Schroeder ET	2005	older men	32	Randomised, placebo-controlled double blinded trial	oxandrolone	drug alone	10mg BD	3	NA	MRI & DXA	1RM leg, chest and lat press, gait speed	Gain	No effect
Hamdy RC	1998	Men with osteopor-osis	21	Randomised, non-blinded	Nandrolone	drug alone	50mg IM weekly	12	NA	DXA	NA	Gain	NA
Hassager C	1989	Post-meno-pausal women	22	Open label randomised, placebo-controlled	nandrolone	drug alone	50mg 3-4 weekly	12	NA	DXA	NA	Gain	NA
Schroeder ET	2003	older men	31	Randomised, placebo-controlled double blinded trial	oxymetholone	drug alone	50mg or 100mg	3	NA	DXA	1RM	Gain	Gain
Tidermark J	2004	older women	60	randomised blinded trial	Nandrolone	protein	25 mg i.m./3 weeks	6	NA	DXA	NA	Maintenance	NA
Schroeder ET	2004	Older men	32	Randomised, placebo-controlled double blinded trial	Oxandrolone	drug alone	20 mg oxandrolone/day	3	NA	MRI & DXA	1RM	Gain	Gain

Frisoli A Jr	2005	older women with osteo-porosis	65	double-blind, randomized, placebo-controlled trial.	nandrolone	drug alone	50mg 3/wkly	24	NA	DXA	NA	Gain	NA
Schroeder ET	2003	older men	30	Randomised, double blind, placebo-controlled trial	oxandrolone	drug alone vs plus exercise	20mg OD	3	NA	MRI	1RM leg press	Gain in both arms, larger with exercise	Gain in both arms, larger with exercise
Igwebuike A	2008	Postmeno-pausal women	31	Double blind, placebo-controlled randomised trial	DHEA	resistance exercise	50mg OD	3	assume low	DXA	1RM	No effect	No effect
von Muhlen D	2008	older adults	225	Double blind, placebo-controlled randomised trial	DHEA	drug alone	50mg OD	12	low	DXA	NA	No effect	NA
Kenny AM	2010	Frail older women	87	Double blind, placebo-controlled randomised trial	DHEA	Exercise	50mg OD	6	low	DXA	1RM	No effect	Gain
Villareal DT	2006	older adults	64	Double blind, placebo-controlled randomised trial	DHEA	alone then with resistance exercise	50mg OD	10	age normal	MRI	1RM	Gain with exercise	gain
Jankowski CM	2006	older adults	140	Double blind, placebo-controlled randomised trial	DHEA	drug alone	50mg OD	12	low	DXA	NA	No effect	NA
Jedrzejuk D	2003	older men	12	Crossover randomised placebo-	DHEA	drug alone	50mg OD	3	low	DXA	NA	No effect	NA

				controlled double blinded trial									
Percheron G	2003	older adults	280	Double blind, placebo-controlled randomised trial	DHEA	drug alone	50mg OD	12	age normal	MRI	1RM knee, handgrip	No effect	No effect
Villareal DT	2000	older adults	36	open randomised, controlled trial	DHEA	drug alone	50mg OD	6	age normal	DXA	NA	Gain	NA
Morales AJ	1998	older adults	19	Crossover randomised placebo-controlled double blinded trial	DHEA	drug alone	100mg	6	low	DXA	1RM	Gain (men only)	Gain (men only)
Casson PR	1998	Postmenopausal women	13	Double blind, placebo-controlled randomised trial	DHEA	drug alone	25mg OD	6	age normal	DXA	NA	No effect	NA
Flynn MA	1999	older men	39	Double blind, placebo-controlled randomised trial	DHEA	drug alone	100 mg	9	NA	K40 method	NA	No effect	NA

Table 5.3.9: synthetic androgens disease													
Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Base-line androgens	Measure MM	Measure function	Effect muscle mass	Effect function
Demling RH;	2003	severe burns patients	45	Open, randomised, controlled trial	Oxandrol-one	Nutrition plus drug vs nutrition alone	20mg OD	variable	NA	BIA	NA	Gain	NA
Okamoto S	2011	Patients receiving stroke rehab	26	Open, randomised controlled trial	Metenel-one	Rehab but not specific exercise	100mg IM weekly for 6 weeks	1.5	NA	CT	NA	Gain	NA
Hengge UR	2003	Patients with HIV wasting	89	double-blind, randomized, placebo-controlled clinical trial	Oxymeth olone	drug alone	50mg BD or TDS	4	NA	BIA	NA	Gain	NA
Ferreira IM	1998	Men with COPD	23	double-blind, randomized, placebo-controlled clinical trial	Testoster-one + Stanozolol	Resp rehab	250mg IM T stat plus 50mg stanozolol OD	7	NA	DXA	CPEX	Gain	No effect
Gold J	1996	Patients with HIV wasting non-respon-	17	Open, non-randomised trial	Nandrol-one	drug alone	100mg/ml bi-weekly	4	NA	BIA	NA	Gain	NA

		sive to nutrition											
Halstead LS	2010	Men with tetraplegia	10	Open, non-randomised	Oxand-rolone	drug alone	20mg OD	2	NA	DXA	PFTs	Gain	Gain
Sattler	1999	Men with HIV	30	Non placebo-controlled, open label, randomized	Nandrol-one	resistance exercise vs drug alone	600mg IM weekly N	3	NA	MRI	1RM leg press	Gain	Gain
Johansen	1996	Patients on haemodialysis	29	double-blind, randomized, placebo-controlled	Nandrol-one	drug alone	100mg weekly	6	NA	DXA	gait speed, stair climb, HGS	Gain	Gain
Johansen KL	2006	Patients receiving haemodialysis	79	2x2 double-blind, randomized, placebo-controlled	Nandrol-one	resistance exercise	100 mg for women; 200 mg for men	3	NA	MRI & DXA	Knee extensor 1RM	Gain	Gain
Earthman	2002	Adults with HIV & weight loss	25	Open, non-randomised	Oxandrol-one	"nutrition management"	20mg/d	5	NA	DXA	NA	Gain	NA
Grunfeld	2006	Adults with HIV & weight loss	262	double-blind, randomized, placebo-controlled	Oxandrol-one	drug alone	20, 40 or 80mg/d	3	NA	BIA	treadmill test	Gain	No effect
Supasyndh O	2013	Patients receiving	43	double-blind, randomized,	DHEA	drug alone	50mg BD	6	NA	DXA	grip strength	Gain	Gain

		haemodialysis		placebo-controlled									
Christiansen JJ	2011	Women with adrenal failure	10	double-blind, randomized, placebo-controlled	DHEA	drug alone	50mg OD	6	Low	DXA	dynametry biceps and quads	Gain	No effect
Gurnell EM	2008	Patients with Addison's disease	106	double-blind, randomized, placebo-controlled	DHEA	drug alone	50mg OD	12	low	DXA	NA	Gain	NA
Callies F	2001	Women with adrenal failure	24	Crossover double-blind, randomized, placebo-controlled	DHEA	drug alone	50mg OD	4	low	BIA	cycle test	No effect	No effect
Gebre-Medhin G	2000	Women with adrenal failure	9	Open, non-randomised	DHEA	drug alone	50mg or 200mg	3	Low	DXA	NA	No effect	NA

Table 5.3.10: oestrogens older women												
First author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Measure MM	Functional measure	Effect muscle mass	Effect function
Bea JW;	2011	postmenopausal women	1467	Randomised blinded	oestrogen alone vs combined with MPA v placebo	drug alone	0.625 mg/d O, 2.5mg/d MPA	66	DXA	NA	Gain	No effect
Kenny AM	2005	older women	167	Randomised blinded	17-beta estradiol	drug alone	0.25 mg /d	36	DXA	PASE	No effect	No effect
Di Carlo C	2004	Post-menopausal women	44	Non-randomised, open-label	transdermal 17beta-estradiol & nomegestrol or no treatment	drug alone	(50 mcg/day N 5mg/d 12/28 days	12	DXA	NA	Gain	NA
Hansen RD	2003	Post-menopausal women	20	Randomised open-label	oestradiol implant	drug alone	20-mg 4 monthly	16	DXA	NA	Gain	NA
Arabi A	2003	Post-menopausal women	109	Non-randomised, open label	tibolone 2.5 mg or 1.25 mg or estradiol 2 mg + norethisterone 1 mg (E2 + NETA)	drug alone	tibolone 2.5 mg or 1.25 mg or estradiol 2 mg + norethisterone 1 mg (E2 + NETA)	24	DXA	NA	Gain	NA

Villareal DT	2003	frail older post-menopausal women	28	Randomised open label	various	Exercise + HRT vs drug alone	various	9	DXA	NA	Gain	NA
Jensen LB	2003	Post-menopausal women	2016	Randomised open label	various	drug alone	various	60	DXA	NA	No effect	NA
Tanko LB	2002	Post menopausal women	48	Randomised blinded	Estradiol + cyproterone or levonorgestrel	drug alone	2 mg E continuously w 1 mg C or sequentially with 75 mcg L (days 17-28), or placebo	36	DXA	NA	No effect	NA
Cagnacci A	2002	Post menopausal women	40	Randomised blinded	transdermal patch placebo or estradiol	drug alone	50	2	BIA	NA	No effect	NA
Dittmar M.	2001	Post-menopausal women	64	Non-randomised, open label	various	drug alone	various	60	BIA	NA	No effect	NA
Walker RJ	2001	Post-menopausal women	30	Randomised blinded	17beta-estradiol norethisterone	drug alone	2 mg of E d 1-12, 2 mg of E and 1 mg N 10d, and 1 mg E 6d	6	DXA	NA	No effect	NA
Gower BA	2000	Post-menopausal women	70	Non-randomised, open label	various	drug alone	various	0	DXA	NA	No effect	NA

Skelton DA	1999	Post-menopausal women	.	Randomised, open label	Prempak C 0.625	drug alone	unclear	12	DXA	thumb strength	No effect	Gain
Aloia JF	1995	Post-menopausal women	118	Randomised, open label	HRT vs calcium vs placebo	drug alone	6.25mg EE + MPA 10mg	36	DXA	NA	No effect	NA
Sorensen MB	2001	Post-menopausal women	16	Randomised, blinded	17beta estradiol plus cyclic norethisterone acetate	drug alone	unclear	2.5	DXA	NA	Gain	NA
Ronkainen	2009	Post-menopausal twins	30	Non-randomised, open label	various	drug alone	various	84	CT	1RM knee and grip plus jump height	Gain	No effect
Taaffe DR	2005	Post-menopausal women	51	Randomised, blinded	oestradiol & noretisterone acetate	vs resistance training, or combo	Oestrogen 2mg, Noresiterone 1mg	12	CT	1RM knee ext, jumping height	Gain	No effect
Napolitano A	2016	peri-menopausal women	110	Non-randomised, open label	DHS pill vs LNS implant	drug alone	unclear	12	BIA	NA	No effect	NA
Meeuwsen IB	2001	Post-menopausal women	85	Randomised, blinded	Tibolone	drug alone	2.5mg	12	BIA	NA	Gain	NA
Chen	2005	Post-menopausal women	835	Randomised, blinded	Oestrogen and Progesterone vs placebo	drug alone	unclear	36	DXA	NA	Gain	No effect

Papadakis`	2018	Post-menopausal women	1053	Non-randomised, open label	current users vs past users vs never users	drug alone	unclear	Single measurement	DXA	NA	No effect	NA
Aubertin	2005	Post-menopausal women	40	Non-randomised, open label	HRT	drug alone	various	Single measurement	DXA	NA	No effect	NA
Thorneycroft IH	2007	Post-menopausal women	822	Randomised, blinded	E vs E + MPA	drug alone	various	24	DXA	NA	Gain	NA
Dedeoglu EN	2009	Post-menopausal women	120	Randomised, open label	Tibolone vs E + MPA vs Nil	drug alone	T 2.5, E 0.0625mg, MPA 2.5mg/d	6	DXA	NA	Gain	NA
Tommaselli GA	2006	Post-menopausal women	68	Randomised, open label	Tibolone vs raloxifene vs placebo	drug alone	T2.5, R 60mg/d	12	DXA	NA	Gain	NA
Hanggi W;	1998	Postmenopausal women	100	Randomised, open label	drug alone	tibolone vs Oral E + DHE vs transder-mal E, vs control	O E 2mg/d, transdermal E, 50mcg/d D 10mg/d, Tib 2.5mg/d	6	DXA	NA	Gain	NA
E = oestrogen, MPA = medroxyprogesterone, HRT = hormone replacement therapy, DHE = dihydroesterone												

Table 5.3.11: oestrogens in younger women												
First author	**Year**	**Population**	**N**	**Drug**	**Diet, exercise or drug alone?**	**Dose**	**Duration (months)**	**Baseline Androgens**	**Measure of muscle**	**Functional measure**	**Inc LBM**	**Inc Function**
Procter-Gray E	2008	female runners	150	30 mcg of ethinyl estradiol and 0.3 mg of norgestrel	drug alone though all runners	30 mcg of ethinyl estradiol & 0.3 mg of norgestrel	24	Normal	DXA	NA	Gain	NA
Franchini M	1995	Women using hormonal contraceptives	100	EE/desogestrel or EE/gestodene, 20 pts progesterone Iintrauterine device control	drug alone	20 mcg ethinyl estradiol + 150 mcg desogestrel or 30 mcg EE + 75 mcg gestodene	12	normal	BIA	NA	No effect	NA
Quintino-Moro	2019	Women using hormonal contraception	52	Mirena - medroxyprogesterone vs copper coil	drug alone		12	normal	DXA	NA	Gain	NA

Table 5.3.12: Androgen combinations												
First author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Drugs	Dose	duration (months)	Measure of LBM	Function measure	Effect muscle mass	Effect function
Borst SE	2014	Men aged > 60 with low T	60	Randomised blinded	drug alone	T + 5ari	125 mg/wk _5mg OD dutasteride	12	DXA	NA	Gain	NA
Page ST	2005	Older men	70	randomized, double-blinded & placebo-controlled	drug alone	T + 5ari	1) 200mg im Q2w placebo/d 2) 200mg q2w + 5 mg 5Ari od or 3) placebo	36	DXA	Low leg & grip strength	Gain	Gain
Bhasin S	2012	healthy adult males <50	102	Randomised blinded	drug alone	T + 5ARi or placebo	unclear	5	DXA	NA	Gain	NA
Kenny AM	2010	Older men with androgen deficiency and history of fracture	131	Randomised blinded	drug alone	Testosterone vs placebo + calcium and vit D	5mg/D T, Vit D 1000 iu/D	24	DXA	1RM leg, SPPB, TUG	Gain	No effect

Ziaei S	2010	Post-menopausal women	155	Randomised open label	drug alone	Tibolone + Vit D vs E & MPA + vit D, vs Vit D alone	Vit D 200IU/d, T 2.5mg/d, E 0.625 mg & MPA 2.5mg/d	9		NA	Gain	NA
Mulligan K	2007	Men with HIV & weight loss	79	Randomised blinded	drug alone	Megestrol Acetate & Testosterone	800mg MA OD + T 200mg Biweekly or placebo	3	BIA	NA	Gain	NA
Nair KS	2006	Older adults	24	Randomised blinded	Drug alone	DHEA female, T or DHEA men or placebo	75mg OD DHEA, T 5mg patch	24	DXA	CPET, knee extension	Gain for T only	No effect
Zang H	2006	Post-menopausal women	63	Randomised open label	drug alone	estradiol, T or combo	E 2md OD, T 40mg alt days	3	DXA	NA	Gain	NA
Crawford BA	2003	adults requiring steroids	51	Randomised blinded	drug alone	T or Nandrolone + glucocorticoids	200mg IM fortnightly	12	DXA	Dyname-try	Gain	Gain
Herbst KL	2003	Healthy young men	37	Randomised blinded	drug alone	T + Progestin, levonorgestrel	100 mg T im, weekly plus 125 mcg LNG, Vs T alone; vs LNG alone both with placebo vs placebo	2	DXA	NA	Gain	NA

Hedstrom M	2002	Older women post hip fracture	63	Randomised open label	drug alone	Nandrolone + Vit D vs calcium alone	N 25mg, Alfacalcidol 0.25 mcg	12	CT thigh, DXA	NA	Gain	NA
Lambert CP	2002	Older men	30	Randomised blinded	exercise	Megestrol acetate, + T or placebo	100mg/wk T	3	CT	NA	Gain	NA
Dobs AS	2002	Post-menopausal women	40	Randomised blinded	drug alone	Estrogen + T vs E alone	0.25 mg e + 2.5 mg T/d	4	DXA	1RM	Gain	Gain
Kenny AM	2001	Older men with androgen deficiency	44	Randomised blinded	drug alone	T + calcium and vit D	5mg patch and vit D 400 iuD	12	DXA	1RM	Gain	Gain
Davis SR	2000	Post-menopausal women	33	Randomised blinded	drug alone	E + T vs E alone	50mg E, 50mg T every 3/12	24	DXA	NA	Gain	NA
Reid IR	1996	Men with asthma on long term steroids	15	Randomised blinded	drug alone	T in men receiving long term prednisolone	30 mg or 60mgproprionate, 100 mg decanoate (250-mg/mo intramuscular depot injection)	12	DXA	NA	Gain	NA

Davis SR	1995	Post-menopausal women	32	Randomised blinded	drug alone	HRT - oestrogen and cyclical progresterones if had a uterus. Plus T	50mg T, 50mg estradiol	24	DXA	NA	1	.
Batterham MG	1997	adults with HIV and weight loss	15	Randomised open label	Diet one arm	Nandrolone vs Megestrol vs diet	N 100 mg/2 wk, MA 400mg/d	3	BIA	NA	1	.
Huang G	2014	Post-menopausal women with hysterectomy	62	Randomised blinded	drug alone	T + E	3,6,12 or 24mg weekly IM	6	DXA	Chest & leg press	1	1
Dayal M	2005	Post-menopausal women	50	Randomised blinded	drug alone	Oestrogens & DHEA vs each alone vs placebo	DHEA 50mg OD, conjugated equine estrogen 0.625 mg OD	3	MRI	dynametry plantar flexors	0	0
Jacobsen DE	2010	frail older women	290	Randomised blinded		Raloxifene (SERM) and tibolone	raloxifene 60 mg, tibolone 1.25 mg, or placebo.	24	BIA, DXA	HGS	1	0

E= oestradiol, T = testosterone, LNG = levonorgesterol, MA = megestrol acetate, SERM = selective oestrogen receptor modulator, iu = international units, 5ari = 5-alpha reductase inhibitor, MPA = medroxyprogresterone, DHEA = Dehydroepiandrosterone, 1RM = 1 repetition max, TUG = timed up and go, SPPB = short performance battery, CPET = cardiopulmonary exercise test, HGS = hand grip strength, IM = intramuscular, OD or /d = once daily

Table 5.3.13: Megestrol											
Author	Year	Population	N	Diet, exercise or drug alone?	Trial design	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Yeh SS	2010	Male Patients on haemodialysis	9	resistance exercise	Randomised double-blind, placebo-controlled	800mg OD	5	BIA	NA	Gain	NA
Sullivan DH	2007	older adults with functional decline	29	Resistance ex high vs low intensity	2x2 Randomised double-blind, placebo-controlled	800mg OD	3	CT	STS, gait speed, stair climb	Sligh reduction	No effect
Weisberg	2002	Patients with COPD and weight loss	128	drug alone	Randomised double-blind, placebo-controlled	800mg/d	2	DXA	6MWD	No effect	No effect
Oster MH;	1994	Patients with AIDS and weight loss	100	drug alone	Randomised double-blind, placebo-controlled	800mg/d	3	BIA	NA	No effect	NA
Von Roenn JH	1994	Patients with AIDS and weight loss	195	drug alone	Randomised double-blind, placebo-controlled	100mg/ 400mg, 800mg/d or placebo	3	BIA	NA	No effect	NA
De Oteyza	1998	Adults with HIV & weight loss	25	drug alone	Open label cohort study	320 mg/day	3	BIA	NA	Gain	NA

Table 5.3.14: Growth Hormone in adults with deficiency												
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Agent	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Biller BM	2011	adults with GH deficiency	152	Double blind, randomised placebo controlled	drug alone	sustained release GH	2-3mg /week	6	DXA	NA	Gain	NA
Chihara K;	2010	adults with GH deficiency	96	Double blind, randomised placebo controlled	drug alone	rhGH	0.06mg/kg/d vs 0.12mg/kg/d	6	DXA	NA	Gain	NA
Beauregard C	2008	Women with GH deficiency	43	Double blind, randomised placebo controlled	drug alone	rhGH	0.67 mg	6	DXA	NA	No effect	NA
Fideleff HL	2008	adults with GH deficiency	71	Open, controlled prospective cohort	drug alone	GH	0.1 mg/day	48	DXA	NA	Gain	NA
Burt MG	2008	adults with GH deficiency	16	Open, randomised controlled study	drug alone	GH	3 or 6 microg/kg/d	3	DXA	NA	Gain	NA
Koranyi J	2006	adults with growth hormone deficiency	88	Prospective cohort study	drug alone	rhGH	variable	6	TBK DXA & BIA	NA	Gain	NA
Verhelst J	2005	craniopharyngioma vs adenoma pts	721	Retrospective cohort study	drug alone	rhGH	variiable	24	DXA or BIA	NA	Gain	NA
Hoffman AR	2004	adults with GH deficiency	166	Double blind, randomised placebo controlled	drug alone	GH	0.00625 mg/kg.d - 0.025mg	12	DXA	NA	Gain	NA

Johannsson G	2004	adults with GH deficiency	30	open non randomised	drug alone	GH	various	24	DXA	NA	Gain	NA
Hayakawa M	2004	adults with GH deficiency	54	Open label, multi-dose level comparative trial	drug alone	20K-hGH	(0.006, 0.012, and 0.024 mg/kg.	4	CT	NA	Gain	NA
Hana V	2004	adults with GH deficiency	17	Prospective observational cohort	drug alone	rhGH	various	12	DXA	NA	Gain	NA
Attanasio AF	2002	adults with GH deficiency	242	Prospective observational cohort	drug alone	rhGH	various	36	BIA or DXA	NA	Gain	NA
Gillberg P	2001	adults with GH deficiency	53	Open, controlled prospective cohort	drug alone	rhGH	0.17 mg/day	3	DXA	cycle ergono-metry	Gain	No effect
Koranyi J	2001	adults with GH deficiency	42	Prospective observational cohort	drug alone	rhGH	various	60	DXA	HGS and knee extensor	Gain	Gain
Ahmad AM	2001	Adults with adult-onset GH deficiency	46	Open, prospective cohort study	drug alone	rhGH	0.4-0.5 IU	3	BIA	NA	Gain	NA
Fernholm R	2000	older adults with GH deficiency	31	Double blind, randomised placebo controlled	drug alone	rhGH	0.05 IU/kg x wk 1/12, then 0.1 IU/kg x week daily divided doses	12	DXA	NA	Gain	NA
Chrisoulidou A	2000	adults with GH deficiency	33	Open, controlled prospective cohort study	drug alone	rhGH	variable	84	BIA, DXA	NA	Gain	NA

Biller BM	2000	adult men with GH deficiency	40	randomised, single blind, placebo-controlled	drug alone	rhGH	10 mcg/kg/d, 4 mcg/kg/d	18	DXA	NA	Gain	NA
Daugaard JR	1999	adults with GH deficiency	22	Double blind, randomised placebo controlled	drug alone	rhGH	0.125IU/m2 /wk	6	BIA	cycle ergometry	Gain	No effect
Rodriguez-Arnao J	1999	adults with GH deficiency	35	Double blind, randomised placebo controlled	drug alone	rhGH	0.25 IU/Kg/wk	12	DXA	Treadmill walk and 1RM quads	Gain	Gain
Gibney J;	1999	adults with GH deficiency	22	Open label, non-randomised controlled trial	drug alone	rhGH	various	120	TBK & CT thigh	NA	Gain	NA
Burman P	1997	adults with GH deficiency	36	Double blind, randomised placebo controlled	drug alone	rhGH	0.5 U/m2 inc to 2u/m2	9	DXA	NA	Gain	NA
Jorgensen JO	1996	adults with GH deficiency	29	Double blind, randomised placebo controlled	drug alone	rhGH	2 IU/m2 per day	12	DXA & BIA & CT	1RM & cycle ergometry	Gain	Gain
Al-Shoumer KA;	1996	adults with GH deficiency	13	Open, prospective cohort study	drug alone	rhGH	various	48	TBK, DXA, BIA	NA	Gain	NA
Baum HB	1996	adult males with GH deficiency	32	Double blind, randomised placebo controlled	drug alone	rhGH	10 mcg/kg	18	DXA	NA	Gain	NA
Johansson JO	1996	adult men with GH deficiency	9	Open, prospective cohort study	drug alone	rhGH	0.25 U/kg/wk	0.5	BIA	NA	Gain	NA
Beshyah SA;	1995	adults with GH deficiency	40	Double blind, randomised placebo controlled	drug alone	rhGH	0.04 (0.02-0.05) IU/kg	6	TBK	dynametry	Gain	No effect

Chung YS	1994	adults with GH deficiency	28	Open label randomised placebo-controlled	Dietary advice	rhGH	0.06U/kg 3x/wk vs daily vs placebo	6	CT	HGS	Gain	Gain
Whitehead HM	1992	adults with growth hormone deficiency	14	Double blind, randomised placebo controlled	drug alone	biosynthetic GH	various	13	CT	1RM	Gain	No effect
Orme SM;	1992	adults with growth hormone deficiency	8	Open, prospective cohort study	drug alone	Biosynth-etic GH	4U x3/wk	2	DXA, BIA, CT, TBK	Exercise bike, HGS	Gain	Gain*
Cuneo RC	1991	adults with growth hormone deficiency	24	Double blind, randomised placebo-controlled trial	drug alone	rhGH	0.07 U/kg	6	CT	dynametry exercise bike	Gain	Gain in girdle strength and exercise capacity
Gotherstrom G	2005	older adults with GH deficiency	26	Open, prospective cohort study	drug alone	rhGH	11·9 µg/kg/day (0·25 IU/kg/week	60	DXA	dynametry	Gain	Gain
Hansen TB	1995	adults with GH deficiency	29	Double blind, randomised placebo-controlled trial	drug alone	Biosynth-etic Gh	2.0 IU/m2	12	DXA	NA	Gain	NA
Abdi	2014	adults with GH deficiency	81	Retrospective cohort study	drug alone	rhGH	various	various	DXA	NA	Gain	NA
Elbornsson	2013	adults with GH deficiency after XRT	18	Open, prospective cohort study	drug alone	rhGH	11.9µg/kg/d (0.25 IU/kg/week	120	DXA	NA	Gain	NA
Ezzat	2002	adults with GH deficiency	115	Double blind, randomised	drug alone	rhGH	0.005 mg/kg/d for 1/12, then	6	DXA & BIA	NA	Gain	NA

				placebo-controlled trial			0.010 mg/kg/d for 5/12					
Franco	2006	adults and older adults with GH deficiency	48	Open, prospective cohort study	drug alone	rhGH	0.31 mg/d	24	DXA	NA	Gain	NA
Laursen	2001	adults with GH deficiency	14	Open label, randomised multi-dose level	drug alone	rhGH	various	6	DXA	NA	Gain	NA
rhGH = recombinant human growth hormone, 1RM= 1 repetition max, HGS = hand grip strength												

Table 5.3.15: Growth hormone older adults												
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Agent	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Boesen AP	2014	older men, post immobilisation	12	Double blind, randomised placebo-controlled trial	rehab	rhGH	33.3 g/kg/d	.	MRI & DXA	1RM	Gain	Gain
Friedlander AL	2001	postmenopausal women	16	Double blind, randomised placebo-controlled trial	drug alone	rhGH	15mcg/kg BD	12	DXA	1RM	No effect	No effect
Taaffe DR	1994	Healthy older men	18	Double blind, randomised placebo-controlled trial	exercise	rhGH	0.02 mg/kg	2.5	DXA	1RM	Gain	No effect
White HK;	2009	older adults with mild functional impairment	395	Double blind, randomised placebo-controlled trial	drug alone	pralmorelin	10 mg 2x/wk, vs 3 mg BD, vs 10 mg ON, vs 10 mg BD	12	DXA	tandem walk, stair climb, HGS	Gain	Gain
Weissberger AJ	2003	older adults after hip replacement	33	Double blind, randomised placebo-controlled trial	drug alone	rhGH	target GH dose 0.04 U/kg/day	4	DXA & MRI	1RM	Gain	Gain
Lange KH	2002	healthy older men	31	2x2 Double/single blind, randomised placebo-controlled trial	drug alone vs RT alone vs GH + RT vs placebo	rhGH	7.2 +/- 0.8 mcg/kd/d	3	DXA & MRI	Quads strength	No effect	No effect

Hennessey JV;	2001	older adults	31	Double blind, randomised placebo-controlled trial	drug alone vs RT alone vs GH + RT vs placebo	rhGH	0.5 IUm2; then 1.5IU/m2	6	DXA & MRI	Dyn-ametry	Gain	No effect
Lange KH	2000	older women	16	Double blind, randomised placebo controlled trial	endurance training	rhGH	1.5 IU/m2	3	DXA	cycle ergono-metry	Gain	No effect
Saaf M	1999	older women with osteoporosis	16	Double blind, randomised placebo-controlled trial	drug alone	rhGH	up to 3u/d	12	DXA	NA	No effect	NA
Yarasheski KE	1997	older men	18	Double blind, randomised placebo-controlled trial	resistance exercise	GH	12.5 or 18 mcg/kg/d	4	DXA	1RM	No effect	No effect
Vittone J	1997	older men	11	Non-randomised cohort study	drug alone	GHrH	2 mg ON	1.5	DXA	1RM	No effect	Gain
Welle S	1996	Healthy older adults	10	Double blind, randomised placebo-controlled trial	drug alone	rhGH	0.03 mg/kg.sc	3	TBK	Knee 1RM	Gain	Gain
Papadakis MA	1996	Healthy older men with low IGF-1	52	Double blind, randomised placebo controlled trial	drug alone	rhGH	0.03 mg/kg x3/wk	3	DXA	HGS and Knee 1RM	Gain	No effect
rhGH= recombinant human growth hormone, BD = twice daily, ON = at night, U = units, TBK = total body potassium, 1RM = 1 repetition max												

Table 5.3.16: Growth hormone in Adults												
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Agent	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Svensson J	1998	Obese males	24	Double blind, randomised placebo-controlled trial	drug alone	MK-677 - GH secretagogue	25mg	2	DXA	NA	Gain	NA
Richelsen B	1994	Obese women	9	Double blind, randomised placebo-controlled trial	drug alone	rhGH	0.03 mg/kg IBW/d	1.25	DXA & CT	NA	Gain	NA
Pasarica M	2007	Adult men with central obesity	30	Double blind, randomised placebo-controlled trial	drug alone	GH	0.95 mg/d	6	DXA & CT	NA	Gain	NA
Veldhuis JD	2004	Healthy adult males	22	Randomised parallel cohort double blind trial	drug alone	rhGH	1mg or 4mg BD	3	DXA	Stair climb, 1RM lower limb	Gain	Gain
Bredella MA	2013	obese adult males	62	Double blind, randomised placebo-controlled trial	drug alone	rhGH	2 µg/kg/d	6	CT & DXA	NA	No effect	NA
Bredella MA	2012	pre-menopausal women	79	Double blind, randomised placebo-controlled trial	drug alone	rhGH	4 mg/kg per day	6	CT & DXA	NA	Gain	NA

Table 5.3.17: Growth hormone in disease											
Author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Agent	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Mendias CL	2020	Adults with ACL injury undergoing surgery	19	Double blind, randomised placebo-controlled trial	rehab	GH	0.5 mg/m2 BD	1.5	MRI	Dyna-metry	No effect	Gain
Moyle GJ	2004	Adults with HIV associated wasting on HAART	555	Double blind, randomised placebo-controlled trial	drug alone	rhGH	0.1 mg/kg	3	BIA	NA	Gain	NA
Seguy D	2014	adults with short bowel	8	Double blind, randomised placebo controlled, crossover trial	Nutrition-al support	rhGH	0.05mg/kg/d	1.5	BIA	NA	Gain	NA
Jager H	2002	Patients with HIV, on HAART with weight loss	27	Open-label, multi-dose level randomised trial.	drug alone	rhGH	6mg OD or QOD	3	BIA	NA	Gain	NA
Lo JC	2001	Men with HIV associated fat accumulation	8	Prospective, open-label cohort study	drug alone	rhGH	3 mg/d	6	DXA	NA	Gain	NA

Chu LW	2001	older adults with malnutrition	19	Double blind, randomised placebo-controlled trial	dietician support	rhGH	0.09 IU/kg x3.wk	1	BIA	5m walk time	Gain	Gain
Jeppesen PB	2001	Adults with short bowel	8	Double blind, randomised placebo-controlled trial	glutamine	rhGH	0.12 mg/kg/day	1	DXA	NA	Gain	NA
Hansen TB	2000	Adults on haemodialysis	20	Double blind, randomised placebo-controlled trial	drug alone	rhGH	4 IU/m2/d	6	DXA	NA	Gain	NA
Scolapio JS.	1999	adults with short bowel	8	Double blind, randomised placebo-controlled trial	glutamine and high carb diet	rhGH	0.14mg/kg/d	1.5	DXA	NA	Gain	NA
Ellegard L	1997	adults with Crohn's disease	10	Double blind, randomised placebo controlled, crossover trial	drug alone	rhGH	0.5 IU/kg/wk = 0.024 mg/kg/d	2	DXA & BIA	NA	Gain	NA
Schambelan M	1996	Adults with HIV associated wasting on HAART	178	Double blind, randomised placebo-controlled trial	drug alone	rhGH	0.1 mg/kg/d	3	DXA	treadmill walk	Gain	Gain
Vlachopapadopoulou E;	1995	adults with myotonic dystrophy	16	Double blind, randomised placebo-controlled trial	drug alone	drug alone	5 mg	4	DXA	1RM	Gain	Gain
Ellis KJ	1998	men with HIV	66	Double blind, randomised placebo-controlled trial	drug alone	GH + IGF-1	0.34 mg bid +5mg BID	3	DXA	NA	Gain	NA

.**Table 5.3.18 Growth hormone combinations**												
First author	Year	Population	N	Trial design	Diet, exercise or drug alone?	Drugs	Dose	duration (months)	Measure of MM	Function measure	Effect MM	Effect function
Zajac A	2014	middle aged men with low T		randomised, blinded	Drug alone	GH + T	30mcg/kg GH +IM T 100mg weekly	3	BIA	Bike exercise test	Gain	Gain
Ragnarsson O	2013	Patients receiving steroids (Glucocorticoids)	12	randomised, open label	drug alone	GH VS T vs GH + T		35	DXA	NA	Gain	NA
Birzniece V	2012	Adults women with hypopituitarism	16	randomised, open label	drug alone	GH + oestrogen vs GH + SERM	unclear	24	DXA	NA	Gain	NA
Schroeder ET	2012	older males		randomised, blinded	drug alone	T +GH	T at 5 or 10 g/day & rhGH at 0, 3.0 or 5.0 mug/kg/day f	4	DXA	1RM of major groups	Gain	Gain

Sattler FR	2009	Older men with androgen deficiency	122	randomised, blinded	drug alone	T +GH	various	4	DXA	1RM	Gain	Gain
Giannoulis MG	2006	Older men	80	randomised, blinded	drug alone	GH alone, T alone, combo or placebo	GH titrated to IGF-1 levels, 5mg T	6	DXA and CT	CPET, knee ext and hand grip peak torque	Gain	Gain
Harman SM	2003	older adults	131	randomised, blinded	drug alone	HRT/T, rhGH, HRT + GH or placebo	NA	6	DXA	1RM & treadmill test	Gain	Gain
Storer TW	2005	Men with HIV	69	Randomised placebo controlled	drug alone	Nandrolone/ placebo + GH	N 150 mg im biweekly, GH SC weekly	3	DXA	1RM leg and chest press	Gain	No effect
1RM = 1 repetition max, CPET = cardiopulmonary exercise test												

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Table 5.3.19: Vitamin D													
Author	Year	Population	N	Diet, exercise or drug alone?	Trial design	Agent	Dose	Duration (months)	Baseline vitamin D	Measure MM	Measure function	Effect muscle mass	Effect function
Bo Y	2019	sarcopenic older adults	60	protein diet in experimental arm only	RCT	D3	702IU OD equivalent to 20,000 IU monthly	6	NA	BIA	TUG 6MWT hand grip	No effect	Gain in HGS, not other measures
Lerchbaum E	2019	Adult men with low vit D	192	drug alone	RCT	D3	20,000 IU vitamin D	3	<75 nmol/L	DXA	NA	No effect	NA
Cuellar WA	2019	older adults with OA	186	drug alone	RCT	D3	50.000 IU monthly	24	low	US	NA	No effect	NA
Sadiya A	2016	adults with T2DM and obesity	87	drug alone	randomized, double-blind, placebo-controlled trial	D3	6000 IU/d for 3/12 then 3000IU/d3/12	6	low	BIA	NA	No effect	NA
Shea MK	2019	older adults	97	drug alone	Open label randomised controlled	D3	800IU /d up to 1600iu/d if levels still low at 4 mo	12	low	DXA	1RM leg extensor and SPPB	Slight decrease	No effect
El Hajj	2018	pre-sarcopenic older adults	115	drug alone	randomized, double-blind, placebo-controlled	D3	10,000 x3/week = 120000IU monthly	6	low	BIA	HGS	Gain	No effect

Mieszkowski	2018	older women	42	exercise: Nordic walking high vs mod intensity	randomized, double-blind, controlled	D3	4000IU/D vs 800IU/d	3	unselected	BIA	Dyname-try knee & elbow	Gain, low doses vit D and HIIT or walking, high doses with walking	Gain
Suebtha-winkul C	2018	post-menopausal women	87	drug alone	randomized, double-blind, placebo-controlled trial	D3	20,000IU weekly vs placebo	3	low	BIA	HGS	No effect	Gain
Vaes AMM	2018	frail older adults	78	drug alone	randomized, double-blind, placebo-controlled trial	D3	10mcg, 20mcg or placebo	6	low	DXA	Knee extensor and HGS	No effect	No effect
Bislev LS	2018	vitamin D deficient adults	91	drug alone	randomized, double-blind, placebo-controlled trial	D3	70 microg (2800 IU)/day vs placebo	3	low	DXA	HGS, knee ext, TUG	No effect	Reduced
Lerchbaum E	2017	Healthy adults with normal T and low vit D	98	drug alone	randomized, double-blind, placebo-controlled trial	D3	20000 IU weekly	NA	low (normal T)	DXA	NA	Reductio n	NA

Chanet A	2017	healthy older adults	24	Protein	randomized, double-blind, placebo-controlled trial	D3	800IU/D vs no protein	1.5	unselected	DXA	NA	Gain	NA
Bauer JM	2015	Sarcopenic Older adults	380	Protein diet	randomized, double-blind, placebo-controlled trial	D3	800 IU vitamin D,	3	variable	DXA	HGS, STS	Gain	Gain in STS but not HGS
Cangussu LM	2015	post-menopausal women	160	Drug alone	randomized, double-blind, placebo-controlled trial	D3	1000 IU/d	9	unselected	DXA	HGS, STS	Maintenance	Gain
Lagari V	2013	older adults	86	drug alone	randomized, double-blind, controlled trial	D3	400IU or 2000IU daily	6	unselected, mostly normal	DXA	HGS, STS, 4MWT	No effect	No effect
Verschueren SM	2011	older women in residential care	113	whole body vibration training vs none	randomized, open label to exercise, controlled trial	D3	880IU vs 1600IU/d	6	unselected	CT	dynametry	No effect	No effect
Kukuljan S	2009	community dwelling older men	180	Ex alone vs drug protein alone vs combo vs neither	randomized, double-blind, controlled trial	D3	800iu 12g protein /d	12	Unselected – mostly normal	DXA	1RM	No effect	No effect

Ito	2014	adults receiving treatment for osteoporosis	389	Drug alone	Retrospective cohort study	alfacalcidol	various	12	normal or low	DXA	NA	Gain	NA
IU = international units, D3 = vitamin D3, HGS = hand grip strength, STS = sit to stand test, 4MWT = 4 minute walk test, TUG = timed up and go test, SPPB = short performance battery, 1RM = 1 repetition max													

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Table 5.3.20: Omega-3 fatty acids Older adults and adults											
Author	Year	Population	N	Diet, exercise or drug alone?	Trial design	Agent & Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Da Boit M	2017	older adults	50	Resistance exercise	randomised double blind trial	long-chain n-3 PUFA3 g fish oil/d	4.5	MRI	max torque & SPPB	Gain	Gain women only
Krzyminska-Siemaszko R	2015	older adults with sarcopenia	53	drug alone	randomised double blind trial	1.3 g of PUFA and 10 mg of vitamin E vs Vit E alone	3	BIA	Grip strength and TUG	No effect	No effect
Logan SL	2015	Older women	24	drug alone	randomised double blind trial	3g/d of EPA and DHA or a placebo	3	BIA	Handgrip, TUG	Gain	Gain
Cornish SM	2018	Older men	23	Resistance exercise	randomised double blind trial	3g/d of EPA and DHA or a placebo	3	DXA	1RM leg and chest press, TUG	No effect	No effect
Boutry regard	2020	older adults with reduced mobility	37	Plus protein vs protein alone vs placebo	randomised double blind trial	1.5 g/day fish oil type that provided 18% (EPA) and 7% DHA and 500 mg/day curcumin with 95% curcuminoids	3	USS thigh, BIA	knee extensor and gait speed	No effect	Gain

Stavrinou	2020	Older adults with mild cognitive impairment	36	vitamins	randomised double blind trial	810 mg EPA 4140 mg DHA and omega-6 fatty acids (1800 mg gamma-Linolenic acid and 3150 mg Linoleic acid	6	BIA	STS, TUG, 6MWT	No effect	Gain
Smith GI	2015	older adults	44	drug alone	randomised double blind trial	n-3 PUFA [four 1-g pills/d	6	MRI thigh	HGS & 1RM	Gain	Gain
Sneddon AA	2008	Men, lean and obese	61	crossover	randomised double blind trial	6 g/day control fat or 3 g/day CLA (50:50 cis-9, trans-11:trans-10, cis-12) and 3 g/day n-3 LC-PUFA	3	DXA	NA	Gain	NA
Hill AM	2007	overweight adults	65	Drug alone, vs plus ex (light cardio)	randomised double blind trial	6 g tuna FO/d (≈1.9 g n−3 FA vs placebo	3	DXA	NA	No effect	.NA
HGS = hand grip strength, STS = sit to stand test, 4MWT = 4-minute walk test, TUG = timed up and go test, SPPB = short performance battery, 1RM = 1 repetition max											

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Table 5.3.21: Omega-3 fatty acids disease states											
Author	Year	Population	N	Diet, exercise or drug alone?	Trial design	Agent & Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Solis-Martinez O	2018	Patients with HNSCC	64	supplemented diet vs diet alone	Random-ised double blind trial	2g/day	1.5	BIA	NA	Maintenance	NA
Abe K	2018	HPB cancer patients	27	Drug alone	Non-randomised non-blinded single arm trial	(200 kcal/300 mg of OFA 300mg PUFAs per pack, 2-4 packs /d	2	BIA	NA	Gain	NA
Paixao EMDS	2017	early breast cancer patients	45	drug alone	randomised double blind trial	2 g/ day of FO concentrate containing 1.8 g of n-3 fatty acid	1	BIA	NA	No effect	NA
Jafari Salim S	2017	males with coronary artery disease	48	drug alone	randomised double blind trial	four soft gels of ω-3 PUFA, (2 BD), containing 480 mg DHA & 720 mg EPA	2	BIA	NA	No effect	NA
Feijo, Patricia M	2019	gastric cancer patients	68	diet	RCT	3.2 g/d of v-3 EPA/DHA enriched nutrition vs non--enriched	1	BIA	NA	Maintenance	NA

Mansoori A	2015	adults with type 2 diabetes	68	drug alone	randomised double-blind placebo-controlled trial	DHA-rich fish oil DHA 1,450 mg and EPA 400 mg, vs placebo	2	BIA	NA	No effect	NA
Sanchez-Lara K	2014	adults with NSCLC receiving chemo	84	drug alone	randomised double blind trial	nutritional supplement containing EPA but within isocaloric diet so matched arms	2	BIA	NA	Gain	NA
Murphy RA	2011	Patients with NSCLC	40	drug alone	Non-randomised non-blinded	2.2 g of EPA/day	3	CT	NA	Gain	NA
Ryan AM	2009	Patients undergoing oesophagec-tomy	53	nutritional support both group	randomised double blind trial	2.2 g EPA/d for 5 days preop, PO & 21 days postop via jejunostomy	1	BIA	NA	Maintenance	NA
Fearon KC	2006	Advanced lung and GI cancer patients	518	drug alone	randomised double-blind placebo-controlled trial	EPA 2 g or 4 g daily or placebo	2	BIA	NA	No effect	NA

Wigmore SJ	2000	patients with advanced pancreas cancer	26	drug alone	Single arm study	EPA 1g/d escalating to 6g/day	3	BIA	NA	No effect	NA
Barber MD	1999	patients with advanced pancreas cancer	20	Protein rich nutritional supplement	Single arm study	.09 g EPA, 2 cans/d	2	BIA	NA	Maintenance	NA
Read JA	2007	Patients with advanced CRC receiving 2nd line chemotherapy	15	drug alone	Non-randomised single arm study	480 ml of EPA containing supplement	3	BIA	NA	Gain	NA
Wu C	2015	adults with heart failure	31	Amino acid supplements	Single arm study	PUFA (6.5 g/d)	3	DXA	HGS, 6MWD, CPET	Gain	No effect

OFA = omega-3 fatty acid, PUFA = polyunsaturated fatty acid, EPA =eicosapentaenoic acid, DHA= docosahexaenoic acid, HNSCC = head and neck squamous cell carcinoma, HPB = hepatobiliary, CRC = colorectal cancer, NSCLC= non-small cell lung cancer

Table 5.3.22: Anti-inflammatory agents												
Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Kohrt	2010	Healthy pre-menopausal women	73	randomized, double-blind, placebo-controlled	Ibuprofen pre vs post vs placebo	resistance exercise	400mg/D	9	DXA	NA	No effect	NA
Lilja M	2018	young adults	31	randomized, singe blind, controlled trial	Ibuprofen vs aspirin	Resistance training	1200mg/d vs 75mg/d	2	MRI	1RM	Smaller gains	No effect
Duff WR	2017	older women	90	randomized, double-blind, placebo-controlled	ibuprofen vs placebo	Resistance training or placebo	400mg OD	9	DXA	NA	No effect	NA
Dideriksen	2016	older men	19	randomized, double-blind, placebo-controlled	Ibuprofen	Resistance training, whey protein	1200mg QD	1.5	MRI	MVC	No effect	No effect
Petersen	2011	Older adults with osteoarthritis	36	randomized, double-blind, placebo-controlled	Ibuprofen vs glucose-mine vs placebo	Resistance training and protein	600mg BD	3	MRI	5RM	No effect	Gain
Trappe TA	2011	older adults	36	randomized, double-blind, placebo-controlled	Acetomino-phen vs ibuprofen	Resistance exercise	4g/d	3	MRI	1RM	Gain	gain
Candow	2013	post-menopausal women	28	randomized, double-blind, placebo-controlled	ibuprofen	resistance exercise	400mg/D	2	DXA, USS	1RM	No effect	No effect
Jankowski	2015	older adults	189	randomized, double-blind, placebo-controlled	Ibuprofen pre vs post vs placebo	resistance exercise, calcium vit D	400mg/D	7	DXA	NA	No effect	NA
Lai V	2008	Patients with HNSCC, GI cancers	11	randomized, double-blind, placebo-controlled	Celecoxib	drug alone	200mg BD	0.75	BIA, DXA	NA	No effect	NA

Beyer I	2011	Older adults with acute infection	30	Randomised, placebo-controlled, double-blind trial	Piroxicam	drug alone	10mg OD	0.75	TBK	grip strength	No effect	No effect
Wigmore	1995	patients with pancreas cancer	16	Randomised, placebo controlled single-blind trial	ibuprofen	drug alone	400mg TDS	variable	BIA	NA	No effect	NA
Mantovani	2010	Patients with advanced cancer and weight loss	24	Prospective, single arm trial	Celecoxib	drug alone	300mg/d	4	BIA, DXA	dynameter	Gain	Gain
Solheim TS	2017	Patients with lung and pancreas cancer	46	Randomised, open label controlled, crossover design	Celecoxib	exercise, EPA containing nutritional supplements vs usual care,	300mg OD	1.5	CT	grip strength	Maintenance	No effect
Subramaniam K	2015	adults with Crohn's disease and wasting	19	Prospective cohort study	Infliximab	drug alone	5mg/kg at wks 4,6,10,18	6	MRI	Quads strength	Gain	Gain
Renzo LD	2011	Patients with psoriasis	40	Prospective cohort study	Infliximab	drug alone		6	DXA	NA	Gain	NA
Briot K	2008	Patients with spondylo-arthropathy	106	Prospective, randomised, open label comparative study	Infliximab or etanercept	drug alone	3 or 5mg/kg every 6-8 weeks or E 25mg twice weekly	24	DXA	NA	Gain	NA
Serelis J	2008	Women with rheumatoid arthritis	12	Prospective cohort study	infliximab	drug alone	3mg/kg 0,2,6 weeks then 8 weekly	12	DXA	NA	No effect	NA

Lo J	2007	adults with metabolic syndrome	56	randomized, double-blind, placebo-controlled trial	etanercept	drug alone	50mM weekly	1	CT & DXA	NA	No effect	NA
Marcora SM	2006	Patients with RA	26	Prospective, randomised, open label comparative study	etanercept vs methotrexate	drug alone	7.5mg up to 20mg/wk	6	DXA	NA	No effect	NA
1RM = 1 repetition max, MVC = maximum voluntary contraction												

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Table 5.3.23: Anti-diabetic agents												
Author	Year	Population	N	Agent	Trial design	Diet, exercise or drug alone?	Dose	Duration (months)	Measure of muscle	Function-al measure	Effect muscle mass	Effect funct-ion
Bouchi R	2018	adults with T2DM	105	Gliptins	Retrospective cohort study	drug alone	variable	12	DXA	NA	Maintenance	NA
Li CJ	2014	adults with T2DM	31	liraglutide in addition to other oral agents	Prospective cohort study	drug alone	1.2mg OD	3	DXA	NA	Loss	NA
Bunck MC	2010	Adults with T2DM	69	Exenatide or insulin in addition to metformin	open-label, prospective, randomized controlled trial	drug alone	NA	12	DXA	NA	maintenance	NA
Inoue H;	2019	Adults with type 2 diabetes	49	Ipragliflozin or placebo in addition to insulin	open-label, prospective, randomized controlled trial	drug alone	variable	6	DXA & BIA	NA	Maintained	NA
Harder H	2004	Obese adults with T2DM	33	Liraglutide	randomized, double-blind, placebo-controlled	drug alone	0.6mg OD	2	DXA	NA	No effect	NA
Sinha A;	1996	Adults with T1 vs T2DM	24	Insulin	Prospective, open label study	drug alone	0.6iu/kg/d	6	DXA	NA	Gain	NA
Yamakage	2020	Adults with diabetes	54	dapgliflozin	randomized, open-label, active controlled,	drug alone	5 mg/day	6	BIA	NA	No effect	NA

					blinded end-point trial							
Hirose	2016	adults with diabetes	17	tofogliflozin	Prospective cohort study	drug alone	20mg OD	2	BIA	NA	Loss	NA
Kamei	2018	adults with diabetes	37	tofogliflozin	Retrospective cohort study	drug alone	20mg OD	3	BIA	NA	Maintenance	NA
Walton RG	2020	Healthy Older adults	94	Metformin	randomized, double-blind, placebo-controlled	Resistance training	1700mg/day	4	CT, DXA	1RM	No effect	No effect
Rizzo	2016	older adults with diabetes	80	DPIV inhibitors	Case control study	drug alone	various	24	BIA	Dyna-metry	Gain	Gain
Perna	2016	overweight older adults with T2DM	9	liraglutide, in addition to metformin	Prospective cohort study	drug alone	various up to 3mg/d	6	DXA	NA	Gain	NA
Sugiyama	2018	overweight older adults with T2DM	50	dapgliflozin vs "other meds"	Prospective cohort study	drug alone	5mg/d	6	BIA & CT	NA	maintenance	NA
Yajima	2018	patients undergoing haemodialysis (HD).	21	dulaglutide vs teneligliptin and all insulin	open-label, prospective, randomized trial	drug alone	various	6	BIA	NA	Loss	NA
Bastien M;	2019	Men with diabetes and CVD	104	Rosiglitazone	randomized, double-blind, placebo-controlled	drug alone	max 8mg BD	12	DXA, CT	Treadmill capacity test	No effect	No effect

Feng WH	2019	adults with T2DM and NASH	85	liraglutide, metformin and gliclazide	open-label, prospective, randomized trial	drug alone	1.mg OD, 1000mg BD and up to 120mg OD	6	DXA	NA	No effect	NA
Driscoll SD;	2004	Patients with HIV and lipodystrophy	25	Metformin	open-label, prospective, randomized controlled trial	drug alone vs drug plus exercise	850mg BD	3	CT thigh muscle	1RM	Loss of muscle	mixed
Lundholm K	2007	Patients with advanced malignancies (mostly GI)	138	Insulin + max supportive care vs supportive care alone	open-label, prospective, randomized controlled trial	nutritional support as needed, including parenteral nutrition	0.11 ± 0.05 units/kg/d	6	DXA	maximal exercise test	No effect	No effect

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Table 5.3.24: Statins and anti-hypertensives												
Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Measure of muscle	Functional measure	Effect on muscle mass	Effect on function
Heisterberg MF	2018	older adults	71	randomized, double-blinded placebo-controlled	Losartan	Resistance exercise	100mg OD	4	MRI	1RM	No effect	No effect
Spira	2016	older adults	838	Cross-sectional observational study	various	drug alone	various	single measurement	DXA	TUG	No effect	No effect
Lin YL	2019	patients receiving haemodialysis	120	Cross-sectional observational study	various	drug alone	various	single measurement	BIA	HGS	No effect	gain
Erlandson KM	2016	Adults with HIV on HAART	147	randomized, double-blinded placebo-controlled	Rosuvastatin	drug alone	10mg OD	24	DXA	NA	gain	.

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Table 5.3.25: anti-muscle catabolism agents												
Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Rooks DS	2017	young men with casting induced atrophy	24	Randomised, double-blind, placebo controlled	Bimagru-mab	drug alone	30 mg/kg	12	MRI	Knee extension 1RM	Gain	No increase speed return of function
Polkey MI	2019	patients with COPD and reduced muscle mass	67	Randomised, double-blind, placebo controlled trial	Activin receptor blocker	drug alone	30 mg/kg IV, two dose 8wk apart	6	MRI & DXA	6MWT, 1RM leg press	Gain	No effect
Rooks D	2017	older adults with slow gait speed	32	Randomised, double-blind, placebo controlled trial	Bimagrumab	drug alone	30,g/kg	6	MRI & DXA	Gait speed, grip strength and 6MWT	Gain	Gain
Amato AA	2014	Patients with neuromuscu lar disease	50	Randomised, double-blind, placebo controlled trial	Bimagrumab	drug alone	30mg/kg	2	MRI & DXA	6MWT	Gain	Gain
Hanna MG	2019	patients with inclusion body myositis	251	Randomised, double-blind, placebo controlled trial	Bimagrumab	drug alone	10 mg/kg, 3 mg/kg, or 1 mg/kg	12	DXA	6MWT	Gain	No effect
Attie KM	2013	Healthy post-menopausal women	48	Randomised, double-blind, dose finding placebo controlled trial	Activin receptor IIB	drug alone	escalating	6	MRI & DXA	NA	Gain	NA

Rooks DS	2020	older adults	159	Randomised, double-blind, placebo controlled trial	Bimagrumab	Plus diet and exercise	700mg/d	6	DXA	6MWT, gait speed	Gain	No effect
Bhattacharya I	2018	Healthy volunteers	73	Randomised, double-blind, dose finding placebo controlled trial	anti-myostatin antibody domogrozu-mab	drug alone	ascending	4	MRI & DXA	NA	Gain	NA
Golan T	2018	Pts with pancreas cancer receiving SOC chemo	125	Randomised, double-blind, placebo controlled trial	anti-myostatin antibody	drug alone	300 mg LY2495655, 100 mg LY2495655, IV q14d or placebo	2	CT & DXA	6MWT	No effect	Gain
Woodhouse L	2016	patients undergoing hip replacement	400	Randomised, double-blind, placebo controlled trial	myostatin antibody	drug alone	LY2495655 (35 mg, 105 mg, or 315 mg) q4w for 4 doses	6	DXA	NA	Gain	NA
Becker C	2015	older adults who fall	201	Randomised, double-blind, placebo controlled trial	myostatin antibody	drug alone	315mg 4 weekly	6	DXA	SPPB	Gain	Gain
Glasser CE	2018	Healthy volunteers	58	Randomised, double-blind, dose finding placebo controlled trial	Anti-follistatin	drug alone	50-200 mg 1 or 2 doses IM	3	MRI	Dynametry	Gain	No effect

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Table 5.3.26: Ghrelin and ghrelin receptor agonists

Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Nass R	2008	Healthy older adults	65	double-blind, randomized, placebo-controlled, crossover	Ghrelin Mimetic	drug alone	25mg/d	24	DXA	Dynametry	Gain	No effect
Lundholm K	2010	Patients with GI cancer losing weight	31	Randomised, double-blind controlled trial	Ghrelin	drug alone	0.7mcg/kg or 13 mcg/kg	2	DXA	NA	No effect	NA
Nagaya N	2004	Patients with congestive heart failure	18	Prospective open-label controlled study	Ghrelin	drug alone	2mcg/kg BD	0.75	DXA	CPET & 6MWD	Gain	Gain
Katakami N	2018	NSCLC cachectic patients	174	double-blind, randomized, placebo-controlled, clinical trial	Anamorelin	drug alone	100mg/d	3	DXA	HGS, 6MWT	Gain	No effect
Miki K	2012	patients with COPD and cachexia	33	double-blind, randomized, placebo-controlled, clinical trial	Ghrelin	drug alone	2 mcg/kg	0.75	DXA	6MWD	No effect	No effect
Temel JS	2016	NSCLC cachectic patients	979	double-blind, randomized, placebo-controlled, clinical trial	Anamorelin	drug alone	100mg/d	3	DXA	HGS	Gain	No effect

Garcia JM	2015	Patients with advanced cancer and >5% weight loss	74	double-blind, randomized, placebo-controlled trial	Anamorelin	drug alone	50mg/D	3	DXA	NA	Gain	NA
Levinson	2012	adults with COPD	192	double-blind, randomized, placebo-controlled, clinical trial	synthetic human ghrelin	drug alone	40 μg/kg bid	3	DXA	6MWT & SPPB	Gain	No effect
Matsumoto	2015	adults with COPD	44	Randomised, double blind, multi-dose level	synthetic human ghrelin	drug alone	1 or 2 μg/kg	0.75	DXA	6MWT	Gain	Gain
Hamauchi, S	2019	patients with advanced GI cancer	50	Prospective, single-arm trial	Anamorelin	drug alone	100mg/d	3	DXA	NA	Gain	NA

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Table 5.3.27: miscellaneous agents

Author	Year	Population	N	Trial design	Agent	Diet, exercise or drug alone?	Dose	Duration (months)	Measure MM	Measure function	Effect muscle mass	Effect function
Khan ZH	2003	Patients with oesophageal cancer	10	Open label, non-randomised	thalidomide	drug alone	200mg/d	1	DXA	NA	Gain	NA
Ockenga J;	1996	Adults with HIV	6	Open label, non-randomised	Ketotifen (anti-histamine)	drug alone	4mg/d	3	BIA	NA	Gain	NA
Iwasa	2015	Patients with hepatitis B	30	Open label, non-randomised	Entecavir	drug alone	variable	Variable, median 39	CT	NA	no effect	NA
Dubois S	2008	women with thyroid nodules	37	Randomised non-blinded	Levothyroxine	drug alone	various	12	DXA, BIA	NA	No effect	NA
Samuels	2016	Women with hypothyroidism	122	Open label, non-randomised	levothyroxine	drug alone	variable	single assessment	DXA	NA	No effect	NA
Zhang Y	2020	Older adults with hip fracture and sarcopenia	141	Randomised, non-blinded	Erythropoietin	drug alone	variable	1	DXA	HGS	Gain	Gain HGS
Kissel JT	2001	patients with facioscapulohumeral dystrophy	90	Randomised, double blind placebo controlled	albuterol	drug alone	8mg or 16mg BD vs placebo	12	DXA	1RM & HGS	Gain at 16mg dose	Gain in HGS

Harrington D;	2000	Adults with heart failure	15	randomised, blinded.	salbutamol	drug alone	8mg BD	0.75	CT thigh	1RM	No effect	Gain in resp muscle strength
Aversa A;	2017	healthy weight men with ED/LUTS	43	Randomised open label	Tadalafil	drug alone	5 mg/d or 20 mg PRN	2	DXA	NA	Gain	NA
Del Fabbro	2013	Patients with advanced cancer & weight loss >5%	73	Randomised, double blind placebo controlled	Melatonin	drug alone	20mg/d	1	BIA	NA	No effect	NA
Amstrup AK	2016	postmenopausal women	81	Randomised, double blind placebo controlled	melatonin	drug alone	1mg or 3mg ON or placebo	12	DXA	dynametry	Gain	No effect
Chasen M	2011	Patients with advanced cancer	21	Open label, non-randomised	OHR118 peptide nucleic acid	drug alone	4.0 mL sc/d	1	BIA	STS	unclear	unclear