Exploring the role of mitochondria in the changes seen in overall skeletal muscle health associated with ageing and cancer.

Abstract

Purpose: Pathological and age-related declines in both physical fitness and muscle function are well established; however, the role mitochondrial function plays in these changes is less understood. With low skeletal muscle mass and function associated with poorer surgical outcomes, treatments and interventions that can limit the decrease in muscle mass and function seen in the elderly, known as sarcopenia, and in those with cancer, known as cancer cachexia, is vital.

Methods: A systematic review and meta-analysis was conducted to determine the impact of sarcopenia on overall and disease-free survival in patients with locally advanced rectal cancer. In addition, young and older healthy volunteers were recruited to determine links between advancing age and declines in global physical fitness and muscle function, as well as investigate if similar declines in mitochondrial function occur.

Results: Our systematic review and meta-analysis established that pre-existing sarcopenia was associated with shorter overall and disease-free survival in patients with locally advanced rectal cancer. Within our healthy volunteer cohort, age significantly impacted global physical function (HGS, 1-RM and VO_{2max}) and measures of muscle architecture, with reduced status in older adults. Conversely, mitochondrial function was not different between the age-groups.

Conclusions: There is clearly an age-related decline in global physical fitness and muscle function, however it remains unknown to what degree mitochondrial function is implicated in these changes. With sarcopenia and cachexia both having a negative impact on various prognostic outcomes, interventions such as exercise training regimes show promising results in improving cardiovascular fitness and muscle

mass/ function in both elderly and cancer patients undergoing surgery. Despite this, if or how these interventions may modify any mitochondrial dysfunction that may exist, especially in cancer patients undergoing neoadjuvant treatment prior to surgery is wholly unknown. More research is required to understand the complex relationship between mitochondrial function and the changes seen in the skeletal muscle of both the elderly and cancer patients.

Abbreviations

ADP – Adenosine diphosphate

ALM – Appendicular lean mass

ATP – Adenosine triphosphate

BIA – Bio-electrical impedance analysis

BMI – Body mass index

CPET – Cardiopulmonary exercise training

CRC – Colorectal cancer

CRF – Cardiorespiratory fitness

CRT – Chemoradiotherapy

CSA – Cross sectional area

CT – Computed tomography

DFS – Disease free survival

DRP – Dynamin related proteins

DXA – Dual-energy x-ray absorptiometry

ECG – Electrocardiogram

EET – Endurance exercise training

EWGSOP – European Working Group on Sarcopenia in Older People

FL – Fascicle length

GTP – Guanosine triphosphate

HGS – Hand grip strength

HIIT – High intensity interval training

HRR - High resolution respirometry

IFM – Interfibrillar mitochondria

LARC – Locally advanced rectal cancer

LOS – Length of stay

MPS – Muscle protein synthesis

MRI – Magnetic resonance imaging

MT – Muscle thickness

nCRT – Neoadjuvant chemoradiotherapy

NHS – National Health Service

NIBP – Non-invasive blood pressure

OS – Overall survival

OXPHOS – Oxidative phosphorylation

PA – Pennation angle

RET – Resistance exercise training

1-RM – One repetition maximum

ROS – Reactive oxygen species

RPM – Revolutions per minute

SEM – Standard error of the mean

SPPBT – Short physical performance battery test

SMI – Skeletal muscle index

SSM – Subsarcolemmal mitochondria

TCA – Tricarboxylic acid cycle

VL – *Vastus lateralis muscle*

Table of contents

Table of figures

Table of tables

1 Introduction

1.1 Chapter focus

This chapter will introduce the importance of skeletal muscle as an organ in the maintenance of whole-body health across the life-course and in the presence of a cancer. Skeletal muscle mitochondria will be afforded specific consideration, as the organelle is responsible for most of the cellular energy generation, as well as playing roles vital in preserving cellular health. To date mitochondrial function is a largely understudied topic in the context of its impact of physical function with ageing and disease. Finally, the growing evidence relating to the role of exercise training programs as a tool to prevent muscle loss and improve clinical outcomes in both ageing and disease will be presented.

1.2 Skeletal muscle

Skeletal muscle represents one of the major components of the human body, accounting for ~ 40% of total body mass in non-obese individuals (Romanello & Sandri, 2016). Skeletal muscle is a dynamic and plastic structure, containing 50 - 75% of all body protein and accounting for 30 – 50% of whole body protein turnover (Frontera & Ochala, 2015). By converting chemical energy, through the oxidation of metabolic substrates, to mechanical work at myosin cross bridges, skeletal muscle is able to generate force and power that maintains posture and produces movement that influences activity (Barclay, 2017). From a metabolic perspective, skeletal muscle contributes to basal energy metabolism, serves as a store for substrates such as amino acids and carbohydrates, generates heat to maintain core temperature and is the consumer of the majority of oxygen and fuel used in physical activity and exercise (Frontera & Ochala, 2015). Skeletal muscle also has well-established roles in maintaining whole body health in situations of obesity, ageing and chronic disease (Vanderveen et al., 2017).

1.2.1 Structure and types

The architecture of skeletal muscle is characterised by a very particular and well described arrangement of muscle fibres (myofibers) and associated connective tissue (Frontera & Ochala, 2015). Each myofiber is a single, multinucleated, long cylindrical cell contained within a cell membrane; the sarcolemma (Barrett et al., 2016). Individual muscle fibres are made up of thousands of myofibrils, which are composed of billions of myofilaments. The myofilaments contain several proteins that together make up the basic contractile machinery of the skeletal muscle, known as the sarcomere. Bundles of myofibers form the fascicles, and bundles of fascicles

form the muscle tissue, with each layer encapsulated by the extracellular matrix, and supported by the cytoskeletal network (Mukund & Subramaniam, 2020). The two most abundant myofilaments, comprising of 70 – 80% of total protein content of a single fibre, are actin and myosin (Frontera & Ochala, 2015) (Figure 1).

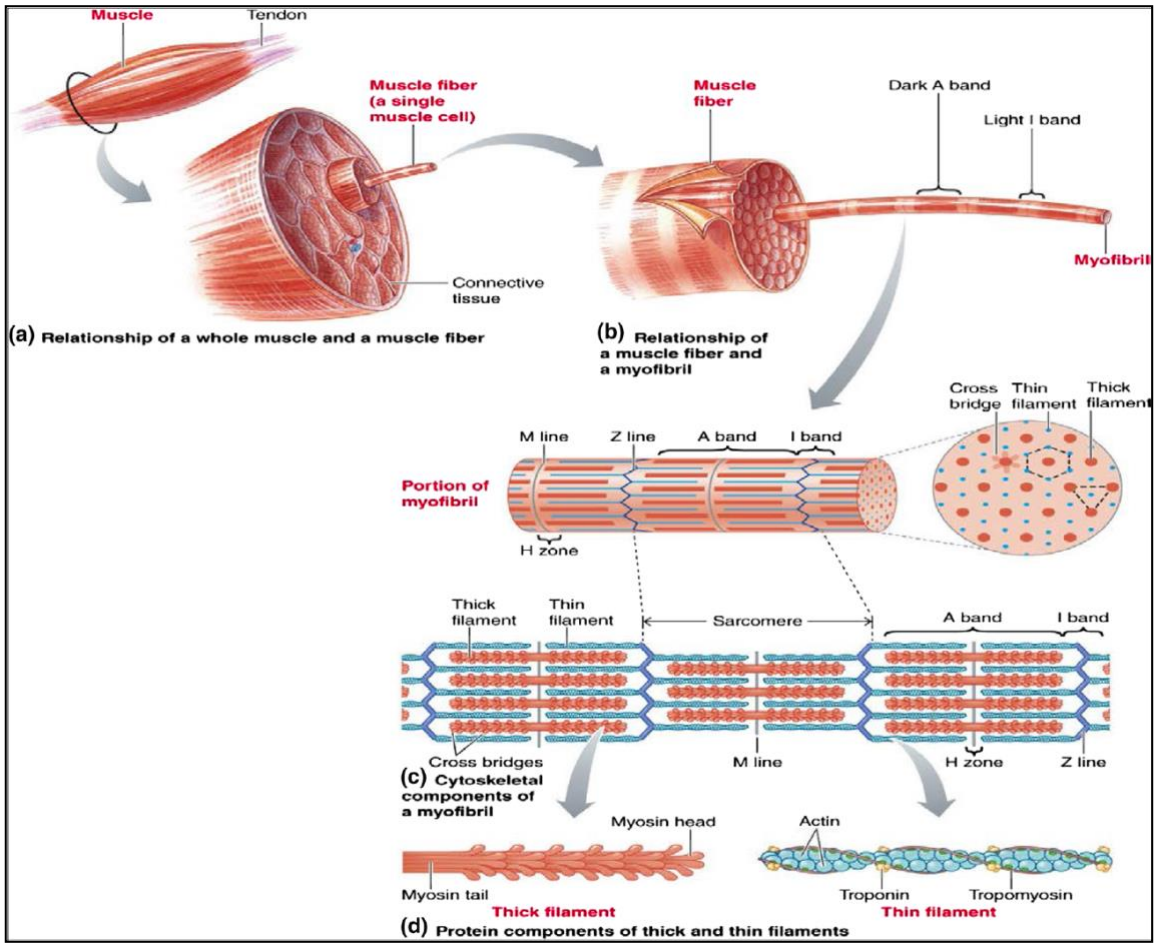


Figure 1 - Structure of skeletal muscle (Sherwood, 2010)

Other important cellular elements within the muscle fibre include the transverse tubular system (T tubules), the sarcoplasmic reticulum and the mitochondrial network. The T tubules and sarcoplasmic reticulum play important roles in muscle activation whilst the mitochondrial network provides much of the energy needed for muscle function. The specific role of the mitochondria is further outlined in section 1.3.

Human skeletal muscle is a heterogeneous tissue and is frequently classified into two main types: “slow” and “fast”. Different muscles are composed of varying amounts of three types of muscle fibres, which allow them to participate in activities of various metabolic and mechanical demands. Type I muscle is highly vascularised and saturated with mitochondria and myoglobin (Mukund & Subramaniam, 2020). They are fatigue resistant, contracting for long period of time but generating little force, and are found in abundance in elite endurance athletes (Carter et al., 2015). Type II fibres, which consist of IIa and IIx fibres, are larger, contain less mitochondria and are therefore more fatigable than type I fibres, however can generate more force and are seen in higher proportion in elite strength and power athletes (Carter et al., 2015).

1.2.2 Muscle contraction and force generation

Fundamentally, muscle contraction is the result of the thin actin myofilament sliding over the thick myosin myofilament within muscle cells. This process, known as the sliding filament theory, was first described in 1954 by Huxley et al,. (A. F. Huxley & Niedergerke, 1954; H. Huxley & Hanson, 1954) and remains the accepted explanation for how skeletal muscle generates force (Figure 2).

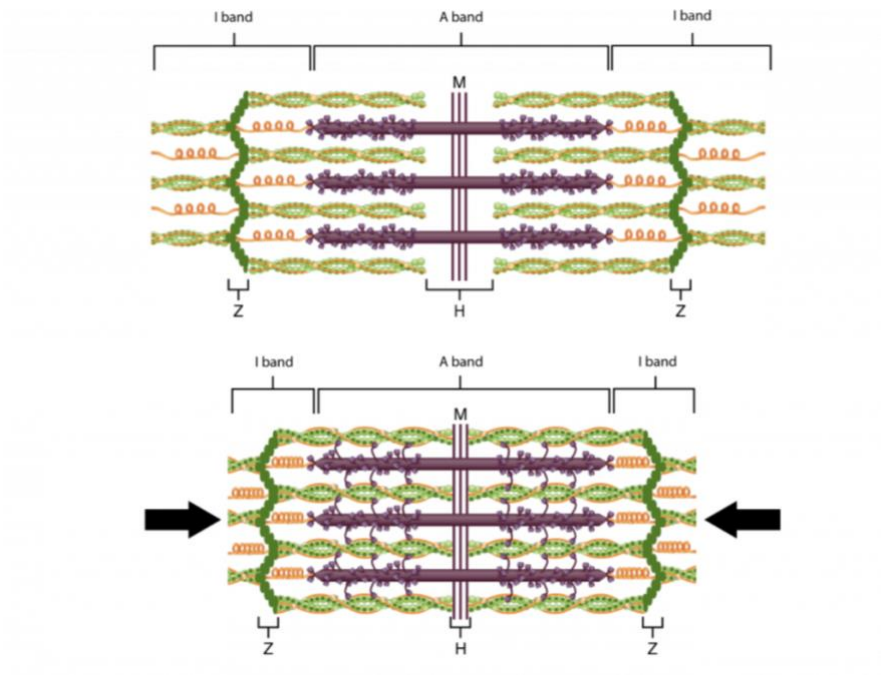


Figure 2 – The sliding filament model of muscle contraction theory (Biga & Dawson, 2010)

Contraction of muscle is initiated by a nerve action potential being transmitted via T tubules of a muscle fibre and leading to the release of calcium (Ca^{2+}) from the sarcoplasmic reticulum. The free Ca^{2+} then binds to the regulatory protein, troponin C, which, in the resting state is usually bound to the active site of actin. The binding of Ca^{2+} triggers a conformational change in troponin allowing the myosin head to bind to actin (Barrett et al., 2016). This creates a cross bridge between the myofilaments. Available ATP then binds to the myosin with the ATPase activity of myosin hydrolysing ATP to adenosine diphosphate (ADP) and free phosphate (Mukund & Subramaniam, 2020). This process allows myosin to move along the actin myofilament, resulting in an increased overlap within the muscle cell (Figure 2). The individual force generated from actin-myosin cross bridges is transmitted longitudinally and laterally within the fibre and produce movement when the forces reach the myotendinous junction, tendons and joints (Frontera & Ochala, 2015).

1.2.3 Energy production

All muscle actions require energy, and most activities involve different energy pathways at different points of time using a combination of carbohydrates and lipids (Barrett et al., 2016). The main energy source for cellular metabolism is glucose, which is catabolised in three subsequent processes; glycolysis, the tricarboxylic acid cycle (TCA), and oxidative phosphorylation (OXPHOS), to produce ATP (Bonora et al., 2012). Glycolysis involves two distinct phases in which glucose is cleaved into two pyruvate molecules (Chaudhry & Varacallo, 2018). The first phase requires the usage of two ATP molecules, with the second phase creating ATP and NADH molecules (Lenzen, 2014) Activities that last for just a few minutes can be sustained through the production of ATP via anaerobic glycolysis, however this pathway produces lactate and H^+ that impair muscle function and ultimately lead to fatigue (Frontera & Ochala, 2015). Energy required for any sustained duration is supplied by the other two main metabolic pathways, which occurs within the three-dimensional mitochondrial network of aerobic cells (Berg et al., 2002).

1.3 Mitochondria – the powerhouse of the cell

Literature reports that mitochondria arose ~2 billion years ago when a bacterium fused with an archaeal cell (Pollard et al., 2017). Mitochondria are highly dynamic organelles that provide 95% of cellular energy needs (Feng et al., 2018). The organelle comprise of two separate and functionally distinct outer and inner membranes that incapsulate the intermembrane space and matrix compartments (Nunnari & Suomalainen, 2012). Within the matrix is their own genome, which transcribes 1% of the organelle's proteins (Romanello & Sandri, 2016).

In skeletal muscle, mitochondria exist in two distinct morphologies: subsarcolemmal (SSM) and interfibrillar (IFM). This network of mitochondria forms a conductive pathway that can rapidly distribute energy to myofibrils, allowing muscles to immediately respond to changes in energy requirements (Romanello & Sandri, 2016). The most abundant subpopulation, accounting for 80% of total skeletal muscle mitochondria, are IFM which reside in between the myofilaments (Lundby & Jacobs, 2016). IFM are mostly responsible for the synthesis of ATP required for contractile activity and appear to be more affected than SSM by ageing and chronic age associated conditions, such as diabetes and cancer (Di Lisa & Scorrano, 2012). SSM are located beneath the sarcolemma and have been shown to utilise less oxygen than IFM (Lai et al., 2019) and are thought to be more involved in signalling processes (Wahwah et al., 2020).

1.3.1 Essential roles of mitochondrial network

One of the main roles of the mitochondria is the production of ATP. ATP is generated during the reactions of the TCA cycle, located in the matrix, and via OXPHOS, that occurs at the inner mitochondrial membrane (Bishop et al., 2014). OXPHOS is the main source of energy production in eukaryotic cells and is performed by means of four enzyme complexes known as the electron transport system. Through the utilisation of energy from the chemical bonds of glucose and fatty acids, the complexes generate an electrochemical proton gradient across the inner membrane that enables the fifth enzyme complex – ATP synthase to generate ATP from ADP and inorganic phosphate (Chaban et al., 2014; Pollard et al., 2017). The mitochondrial network is also involved in a number of other vital cellular processes including the synthesis of various amino and fatty acids (Spinelli & Haigis, 2018). It is

also the major intracellular site for producing reactive oxygen species (ROS) (Zhao et al., 2019), is involved in ionic homeostasis (O'Rourke et al., 2005), and has a role in the regulation of all modalities for cell death (Di Lisa & Scorrano, 2012).

Since the mitochondria is involved in multiple aspects of cellular function, it is unsurprising that mitochondrial networks are heavily integrated in cell signalling pathways (Tait & Green, 2012). A growing body of data suggests that mitochondrial signalling pathways are not just reactive but also actively provides signals back to the nucleus (Weinberg et al., 2015). In general, mitochondria can regulate cell signalling through two means; serving as a physical platform on which protein-protein signalling interactions occur, and by regulating the levels of key intracellular signalling molecules including Ca^{2+} and ROS (Tait & Green, 2012). Calcium ion concentration is a key regulator of various signalling pathways including nervous system excitability, the contraction of muscles and activation of enzymes (Song et al., 2019). Mitochondria can accumulate up to 20-fold higher concentrations than the cytosol (Anderson et al., 2019), and can therefore function as a buffering system to help restore homeostasis, as well as initiate signalling pathways by releasing these stores. Mitochondrial Ca^{2+} regulation is known to influence hormone secretion (Wiederkehr et al., 2011), tissue regeneration (Antony et al., 2016) and cytokine signalling associated with innate immunity (Cheng et al., 2016).

1.3.2 Maintaining mitochondrial homeostasis

In healthy skeletal muscle fibres, the mitochondrial content reflects that of the energy requirement (Devin & Rigoulet, 2007). Mitochondrial dynamics encompass processes associated with mitochondrial fission and fusion, the degree of biogenesis

and the induction of mitophagy (Senft & Ronai, 2016). It is these activities that determine the balance between energy production and cell death.

Mitochondrial biogenesis is a critical process for determining the number and function of the organelle and is influenced by several factors such as exercise training, oxidative stress, muscle regeneration and inflammation (van der Ende et al., 2018). Once activated, signalling cascades and transcriptional processes result in the synthesis of mitochondrial proteins and replication of the mitochondrial genome. This results in the expansion of mitochondrial structures and enlargement of the mitochondria (Pustylnikov et al., 2018). These enlarged mitochondria then undergo fission, leading to an increase in mitochondrial mass.

Fission, or division, of mitochondria produces one or more daughter mitochondria and fragments the network into unconnected, shorter organelles. It is required to transmit the organelles among dividing cells and when there is an increase in energy demands. Additionally, fission is a mechanism that segregates dysfunctional or damaged components of the mitochondria, allowing their removal from the network (Romanello & Sandri, 2016). Counterpart to fission is fusion, which unifies mitochondria, leading to elongated organelles with increased interconnectivity. This process allows the exchange of material between healthy mitochondria (Ni et al., 2015). Under conditions of high energy demand, fusion allows optimal function by increasing the bioenergetic capacity of the cell as well as protecting the mitochondria from mitophagy (Romanello & Sandri, 2016). This highly dynamic cycle of fusion and fission balances two competing processes. Fusion allows mitochondria to compensate for individual organelles defects by sharing components, thereby

helping to maintain energy output in conditions of increased cellular stress (Youle & Van Der Bliek, 2012). On the other hand, fission segregates the most seriously damaged mitochondria to preserve the health of the overall mitochondrial network.

Considerable progress has been made in identifying the molecular regulation of mitochondrial fusion and fission. Regulation is thought to be achieved mainly by mitochondrial shaping proteins known as dynamin related proteins (DRP) (R. Yu et al., 2020). Fusion is controlled by three key DRPs (mitofusin 1, mitofusin 2 and optic atriophy 1) that once activated, allows fusion to proceed in a step wise process. Firstly, the two mitochondria are tethered together and form a ring structure at the contact point between the outer membranes. Through a process triggered by guanosine triphosphate (GTP) hydrolysis, the two outer membranes fuse together, followed by a final fusion of two inner membranes (Brandt et al., 2016; Tilokani et al., 2018). The molecular regulation of mitochondrial fission is slightly less understood, however is known to involve specific DRPs being recruited to the mitochondrial surface triggering mitochondrial fission through GTPase activity (Otera et al., 2013). Recent data also shows that co-factors such as the endoplasmic reticulum and actin cytoskeleton also play a role in facilitating fission (R. Yu et al., 2020), with these factors suggested to wrap around the mitochondria and mark prospective sites for division.

Mitochondrial turnover is predominantly carried out by autophagy, a cellular housekeeping system that degrades mitochondria as well as other cellular components (Peterson et al., 2012). When a certain threshold of damage is reached, mitochondrial are removed through this system, referred to as mitophagy, a highly

selective process that removes organelles that are either damaged, long lived, or misfolded. Mitophagy, together with biogenesis, is essential in maintaining a healthy population of mitochondria (van der Ende et al., 2018).

A fine equilibrium of mitochondrial dynamics is required to preserve muscle mass and prevent muscle wasting. For example, loss of mitochondrial fusion results in muscle atrophy and reduced mitochondrial DNA, whilst failure in mitophagy leads to the accumulation of damaged organelles that can impact sensitivity to cell death, increase inflammatory cytokine production and possibly reduce antiviral response (Tait & Green, 2012; Vanderveen et al., 2017). More recently, studies have shown that a disturbance in the balance between fusion and fission is involved in the progression of several types of neoplasms (L. Chen et al., 2018).

1.3.3 Assessing mitochondrial function

Given the critical role skeletal muscle mitochondria play in several cellular processes both in health and disease, measuring their function is vital in furthering our understanding. There are several methods available, including non-invasive *in vivo* and *in vitro* techniques using fresh tissue samples.

Since the reduction of oxygen is a necessary precursor event to ATP synthesis, mitochondrial capacity can be assessed *in vivo* from the rate of oxygen consumption (Lanza & Nair, 2010). At a whole-body level, the maximal rate at which an individual consumes oxygen is referred to as VO_{2max}. As activity level increases, oxygen consumption by skeletal muscle consumes a larger fraction of the total body oxygen uptake. Therefore maximum oxygen consumption is postulated to provide an

estimate of muscle oxygen consumption and thus mitochondrial function (Liu & Marcinek, 2017). Although several studies have shown a linear relationship between VO_{2max} and muscle mitochondria content and capacity (Hoppeler, 1990; Van Der Zwaard et al., 2016), VO_{2max} is also a function of the oxygen carrying capacity of the blood, tissue perfusion and cardiac output. It is therefore impossible to gain any tissue specific information using this method (Lanza & Nair, 2010). This is why the measure of *in vitro* tissue is preferable to obtain an accurate assessment of mitochondrial capacity.

High resolution respirometry (HRR) has emerged as a powerful tool for *in vitro* measurement of isolated mitochondrial respiratory capacity (Lanza & Nair, 2010). The process involves permeabilised muscle fibres being added to a twin chambered instrument before sequentially titrating substrates, inhibitors and uncouplers to measure mitochondrial respiration at different stages. For instance, the maximum oxidative capacity of mitochondrial respiration can be determined following the addition of certain substrates, whilst the addition of an uncoupler can allow the maximum respiratory capacity of the mitochondria to be recorded (Porter et al., 2015).

The main advantage of HRR, which is now seen as the gold standard for *in vitro* assessment of mitochondrial function, is that multiple measurements can be gained from the same, very small sample of permeabilised muscle fibres. In addition, this method can provide mechanistic insight into the effects environmental interventions, such as exercise training and nutritional supplementation (Christensen et al., 2016), as well as the impact ageing (Greco et al., 2003; Hutter et al., 2004; Porter et al.,

2015) and chronic disease (Buck et al., 2017) can have on mitochondrial function. The main disadvantage of HRR is that, unless used with measurements of physical function (e.g., cardiorespiratory fitness or muscle function), the conclusions gained can be difficult to apply clinically.

1.4 The effect of ageing on muscle function

Growing old, or chronological ageing, is unavoidable and is defined as the progressive deteriorative changes seen during the adult period of life that underlie an increasing vulnerability to challenges and thereby decrease the ability of the organism to survive (Mosoro, 2017). Overall, the common denominator of ageing is an accumulation of damage to genetic material, which ultimately leads to the clinical effects seen in advancing age (Aunan et al., 2016).

1.4.1 Sarcopenia

One of the most distinctive features of ageing is the progressive loss of muscle mass, strength and physical function (Distefano et al., 2018). Muscle mass peaks around the middle of the 3rd decade of life, with a slow rate of decline then seen until the 5th decade. After this, the rate of muscle loss increases, ranging between 0.5% - 1.4% loss each year (Carter et al., 2015). As such, muscle mass is predicted to reduce between 30 – 50% between the ages of 30 – 80 years (Milanović et al., 2013). The implications of skeletal muscle loss are far reaching and can impact an individual's ability to perform activities of daily living (ADLs), as well as being a precipitating factor in a decline in physical function, increased risk of frailty, disability and death in the elderly (Joseph et al., 2016).

The term sarcopenia was used for the first time by Rosenberg when referring to progressive loss of lean body mass seen in ageing (Rosenberg, 1997). The definition has since been expanded to include the presence of lower muscle mass and weakness and/or impaired performance, with muscle strength the most reliable measure (Cruz-Jentoft et al., 2010, 2019). The aetiology of sarcopenia is multifactorial and includes alterations in variables such as nutritional status, physical activity levels and hormone concentrations (Joseph et al., 2016). It is estimated the prevalence of sarcopenia in the older population ranges between 4 – 27% depending on gender and country (Frontera & Ochala, 2015). Sarcopenia has financial implications to healthcare systems. For example, among older adults that are hospitalised, those with sarcopenia are 5 times more likely to generate higher hospital costs than those without (Cruz-Jentoft et al., 2019).

As individuals age, changes in skeletal mass and strength are dissociated, with muscle strength declining three times faster than mass (Peterson et al., 2012). This suggests that whilst an overall reduction in the muscle size is a contributor to sarcopenia, it is not the only change seen in ageing skeletal muscle. Architectural and molecular changes such as shifts in muscle fibre type distribution (Deschenes, 2004; Larsson & Karlsson, 1978; Lexell et al., 1988), changes in protein synthesis (Evans, 2010), myeosteatosis (Delmonico et al., 2009) and increased fibrosis are also seen with advancing age (Boengler et al., 2017). In addition, recent work has highlighted changes in neuromuscular communication with advancing age (Gonzalez-Freire et al., 2014). These changes indicate that a decrease in muscle quality also plays a role in the functional changes seen in sarcopenia.

1.4.2 Ageing and mitochondrial function

It is becoming increasingly clear that mitochondria play a critical role in the loss of skeletal muscle function seen in ageing and chronic disease (Liu & Marcinek, 2017). Ageing has been shown to be associated with an accumulation of abnormally enlarged and round mitochondria (Peterson et al., 2012; Sebastián et al., 2017). Additionally, there is an increased portion of mitochondria that are non-functional, something that is, in part, the result of an imbalance between efficient elimination of damaged mitochondria through mitophagy and appropriate mitochondrial biogenesis (Peterson et al., 2012). These changes result in the density of skeletal muscle mitochondria to drop substantially, something that has been shown with electron microscopy in the *vastus lateralis* muscle of people over 60 years old (Conley et al., 2000). There is also an accumulative damage to mitochondrial DNA, which reduces the ability of mitochondria to generate ATP, resulting in further loss of function (Mosoro, 2017). The mitochondrial dysfunction seen is ageing is closely related to loss of skeletal muscle mass and can lead to a decrease in physical activity and increased the risk of falls in the elderly (Seo et al., 2016) (Figure 3).

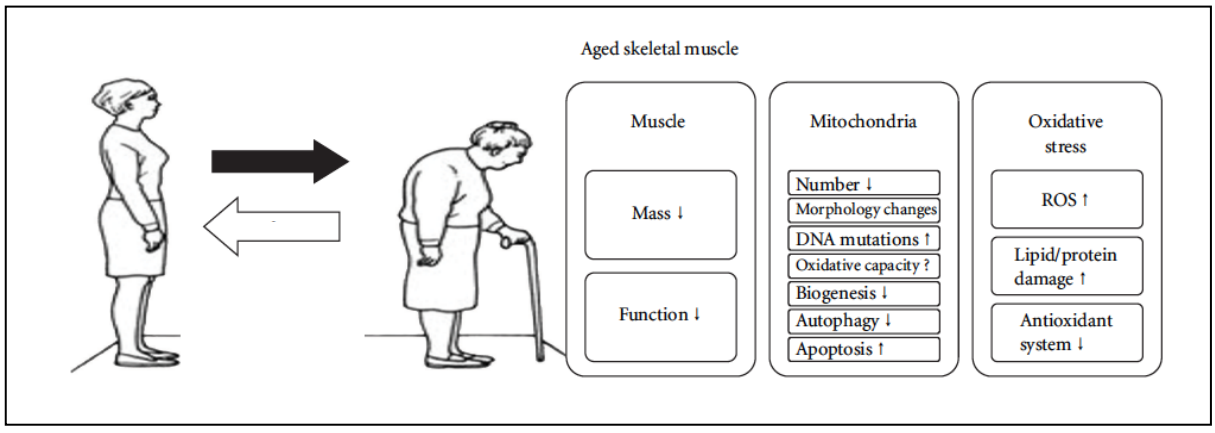


Figure 3 – Changes seen in skeletal muscle with ageing (Peterson et al., 2012)

Mitochondria constitute the main source of ROS, a by-product of the energy producing process, oxidative phosphorylation (OXPHOS). Related to this, the free radical theory of ageing, which was first proposed by Harman et al. in 1956, suggested that the progressive mitochondrial dysfunction seen in ageing is the result of an increased production of ROS, which in turn causes further mitochondrial deterioration and global cellular damage (Harman, 1956). In recent years, this theory has been re-evaluated as it is now thought that ROS function as signalling molecules, activating compensatory homeostatic responses and are therefore needed for normal cellular function (López-Otín et al., 2013). As we age however, the level of cellular stress and damage increases, with a subsequent paralleled increase in the ROS level to maintain survival. It is now thought that when ROS production goes above a certain threshold, they aggravate rather than alleviate the age-associated damage resulting in a reduced lifespan of the cell or cell death (Aunan et al., 2016).

Although the aetiology of sarcopenia is complex and multifunctional, the combined impairment of mitochondrial biogenesis and turnover contributes to the slow gradual loss of muscle mass seen in condition (Coen et al., 2019). One key area of ongoing research is whether the changes seen in mitochondrial function are an intrinsic property of ageing, or the result of reduced activity levels and as such, something that can be partly preventable (Liu & Marcinek, 2017).

1.4.3 The role of exercise in ageing

Even though a degree of age-related muscle loss is unavoidable, skeletal muscle maintains a high degree of plasticity throughout its life-course, and as such, age

associated decrements can be limited through modifiable lifestyle factors (Brook et al., 2016; Seo et al., 2016). Among these, physical activity is seen as the most effective intervention to attenuate the loss of muscle strength and mass seen with advancing age (Boengler et al., 2017).

Resistance exercise training (RET) in the elderly has been shown to increase the size of muscle fibres, thereby increasing overall mass (Mosoro, 2017), and continues to be the most effective intervention against sarcopenia (Yoo et al., 2018). There is also considerable evidence to support the theory that aged muscle retains the capacity to elicit biogenesis in a manner similar to that of young muscle when exercised (Carter et al., 2015). This is thought to be due to the fact cumulative bouts of exercise induces several molecular ‘stresses’ on skeletal muscle. Amongst these is an increase in ROS production. As mentioned previously, although excessive production of ROS is associated with the pathogenesis of numerous diseases and ageing, when produced in low to moderate amounts, they act as key intracellular signalling molecules regulating a host of physiological and biological processes (Tryfidou et al., 2020). These processes include upregulating skeletal muscle gene expression, protein synthesis and oxidative capacity (Peterson et al., 2012).

Whilst RET is primarily associated with skeletal muscle hypertrophy and strength gains, aerobic (or endurance) exercise training (EET) is predominantly associated with improvements in cardiorespiratory fitness (CRF) (Lin et al., 2015; Yoo et al., 2018) as well as being a potent stimulus for mitochondrial biogenesis, increasing both fission and fusion and regulating organelle renewal through mitophagy (Russell

et al., 2014). Each of these adaptions aid mitochondrial turnover and improve skeletal muscle efficacy (Vanderveen et al., 2017).

To date, there are conflicting opinions regarding the optimal exercise program that can be used to improve aspects of mitochondrial function, and whether mitochondrial content and function are altered the same with different forms of exercise (Bishop et al., 2014). Both human and animal models demonstrated increased mitochondrial enzyme activity and ATP production in response to an aerobic training program (Koltai et al., 2012; Uribe et al., 1992). More recently, animal models have shown that endurance training could stimulate the repair of mitochondrial DNA and allow for mitochondrial biogenesis (Safdar et al., 2016). It is important to note that although age-associated declines in mitochondrial function have been shown to be reduced in older adults who underwent EET, exercise training did not completely restore all aspects of mitochondrial function (Peterson et al., 2012). This suggests that although exercise can help attenuate age-associated changes in skeletal muscle mitochondria, it cannot completely prevent the onset of age-related mitochondrial decline (Carter et al., 2015). This indicates a need for further research to investigate these topics in aged individuals.

1.5 Cancer

Cancer has afflicted humanity for thousands of years. The first documented case of cancer was 2700 years ago, and cancerous growths have been found in Egyptian and Peruvian mummies dating back to 1500 BC (Faguet, 2015). Throughout history, the question of how cancer begins, and what are its causes, led to hypotheses and historic practices that have shaped our understanding and treatment of the disease

today. In the simplest form, cancer is a genetic disease. More precisely, it is a disease of gene expression, with different forms of cancer sharing common mechanisms governing uncontrolled cell proliferation and differentiation (Coleman, 2018). Despite this common origin of uncontrolled gene expression, there is estimated to be more than 277 different types of cancer disease (Hassanpour & Dehghani, 2017).

It is only in recent decades that the prevalence of cancer has markedly increased, largely due to a rapidly ageing population (White et al., 2014) and increasing 'risky' behaviour such as smoking, increased alcohol consumption and poor diet (Faguet, 2015). In 2014, the World Health Organisation declared that cancer had become the biggest cause of mortality worldwide (Costa et al., 2016), and the most recent estimates suggest that in 2016 there were 17 million new cases of cancer worldwide with 9.6 million cancer related deaths (Cancer Research, 2020)

1.5.1 Classification

Cancer is classified in two ways, firstly by the type of tissue the cancer originates from, known as the histological type. Most cancers fall into one of three main groups: i) carcinomas, ii) sarcomas and iii) leukaemia and lymphomas. Carcinoma refers to a cancer originating from the epithelial lining of internal or external organs, such as lung, skin, prostate, and colon. They are solid tumours that can be divided further into adenocarcinoma and squamous cell carcinoma (U.S National Institutes of Heal, 2020). Sarcomas are solid tumours of connective tissue such as muscle, bone, cartilage, and fibrous tissue. Finally, leukaemias and lymphomas arise from the blood forming cells and from cells of the immune system respectively (Cooper GM,

2000). Of these main groupings, carcinomas account for more than 85% of all new cases in the UK (Cancer Research, 2020).

Cancers can be further classified according to location, or primary site. In the UK prostate, lung, and colon cancer account for over half (53%) of new cases of cancer in men, with breast, lung and colon cancer being the most common in women (Cancer Research, 2020). To demonstrate the magnitude of this clinical burden, 42,300 new cases of colon cancer are diagnosed each year in the UK (Cancer Research, 2020).

1.5.2 Risk factors

The lifetime risk of being diagnosed with cancer in the UK is estimated to be 1 in 2 for individuals born after 1960 (Cancer Research, 2020). Increasing age is one of the most important risk factors for developing cancer, with three quarters of all cancers in the UK being diagnosed in those aged over 60, and a third diagnosed in those aged over 75 (Macdonald et al., 2018).

The link between cancer and ageing is perhaps unsurprising since both can be regarded as different manifestations of the same underlying process. As previously discussed, it is the time dependant accumulation of cellular damage that is considered to be the cause of chronological ageing and it is this cellular damage that, on occasions, can provide advantages to certain cells that eventually results in cancer (López-Otín et al., 2013). In under 10% of cancer patients, there is an inherited component that predisposes an individual to developing cancer, often at a much younger age than seen in non-inherited cancers (Gatalica et al., 2017). In

these cases, the individual has a mutation in a cancer predisposition gene that provides an early start in tumour development since the mutation is present in every cell (Gatalica et al., 2017). Examples of genetic mutations linked to inherited forms of cancer include BRCA, causing breast and ovarian cancer, and APC, resulting in familial adenomatous polyposis and colon cancer (Kupfer & Ellis, 2016).

Although growing older is unavoidable, the development of cancer is not an inevitable consequence of ageing, it is coincidentally associated with preventable chronic conditions, avoidable exposures and modifiable risk factors that are all linked to increasing the risk of developing cancer (White et al., 2014). It is estimated that 30% of cancers worldwide and 1 in 4 cancers in the UK are preventable (Cancer Research, 2020). Tobacco smoking is the leading cause of cancer in the UK and is linked to the development of multiple different forms of cancer including lung, oropharyngeal and bladder cancer (White et al., 2014). Other modifiable risk factors for cancer development include alcohol consumption (Thun et al., 1997), the presence of chronic health conditions (many of which are associated with advancing age, such as diabetes) (Epping-Jordan et al., 2005), obesity (Calle et al., 2003), excessive sun exposure (C. J. Stein & Colditz, 2004), infections such as HIV, hepatitis and HPV (Murthy & Mathew, 2004), and occupational and environmental factors, such as asbestos (Stewart & Wild, 2014).

1.5.3 Treatment options

Surgical resection has been the cornerstone in the treatment of solid cancer for more than 100 years (Reed, 2009) and is achieved through either an open incision, or by the use of minimally invasive techniques, such as laparoscopy or robotic assisted

surgery. The suitability of a patients with cancer having surgery is dependent on several factors. One of the most important considerations is the stage of the cancer, which relates to both the size and spread of the disease (Brierley et al., 2016). Briefly, cancer staging is based on the TNM (tumour, nodes and metastases) classification first developed in Paris in the 1940's by Pierre Denoix and the Union for International Cancer Control (Brierley et al., 2016). In the classification, T describes the extent of the primary tumour, either by size or depth of invasion, whilst N indicates the absence or presence of regional lymph node metastasis, and M indicates the presence or absence of distant metastasis (Brierley et al., 2016). By recording the stage of the cancer at presentation, members of the clinical team can apply evidence-based treatment guidelines in order to provide the most appropriate management for the patient. In general, the lower the staging the more appropriate the patient is for surgical resection. This is demonstrated by data from 2013-2014 showing that of the 45% of patients with solid tumours who underwent surgical resection, 69.7% had stage 1 disease compared to just 12.9% of those with the most advanced stage, stage 4 (NCRAS, 2020).

The location of the cancer, age, general health of the patient, national treatment guidelines and patient preferences are all other considerations when deciding if surgery is indicated as a treatment option, with risk-benefit analysis forming the basis of these decisions. Although surgery can be used as definitive treatment, it is common practice for it to be used in combination with neo-adjuvant and adjuvant therapies, such as radiotherapy and chemotherapy, in order to improve survival outcomes (Drake et al., 2019; Iwashyna & Lamont, 2002). Figure 4 shows that the utility of surgical treatment (alone or in combination with other treatments) declines

with age whilst other care, such as targeting symptoms, being used instead. .This is often because the risk of surgery outweighs the benefits (G. L. Smith & Smith, 2014)

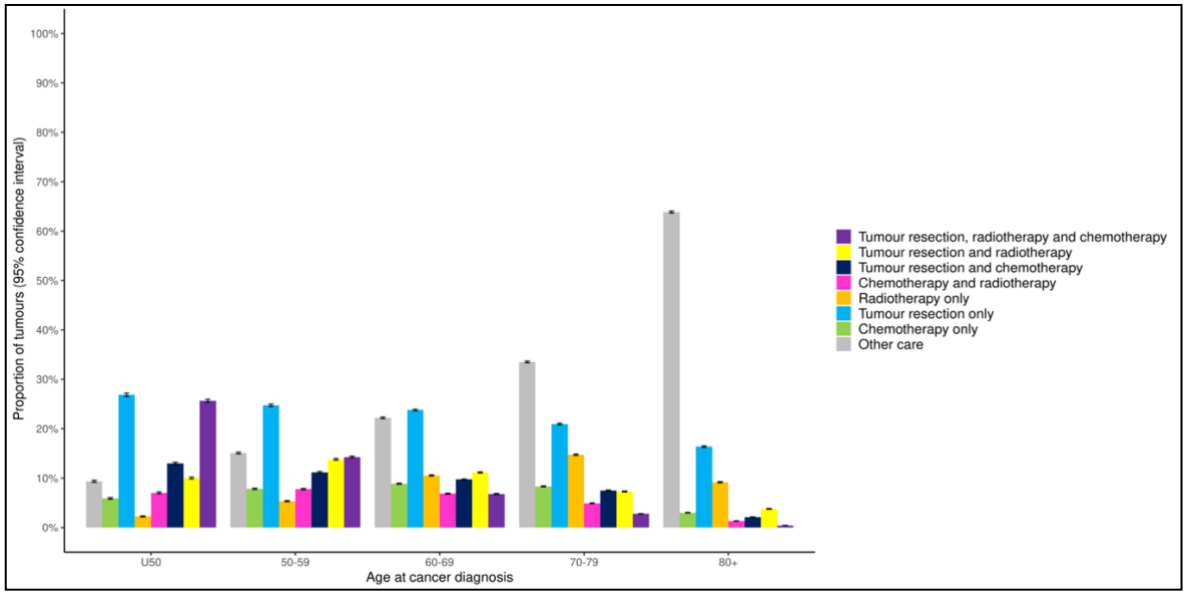


Figure 4 – Summary of differing treatment options and combinations for patients diagnosed with cancer between 2013 – 2016 based on age (NCRAS, 2020).

The therapeutic potential of radiation occurred shortly after the discovery of X-rays in 1895 (Gianfaldoni et al., 2017), with doctors initially using it to treat skin conditions such as lupus (Kassabian, 1908). It is now estimated that over half of all patients with cancer will have some form of radiotherapy included in their treatment (American Cancer Society, 2020). Radiotherapy works by using localised, high energy ionising radiation to damage the DNA within cells. Since rapidly proliferating cancer cells are more sensitive than normal cells to DNA damage, radiotherapy allows targeted treatment for solid tumours whilst minimising toxic effects on surrounding healthy tissue (Formenti & Demaria, 2009). Radiotherapy can be administered for several reasons. It can be used in conjunction with other treatment modalities where achieving curative intent is the aim, or as a palliative treatment in which relieving patient symptoms and pain is the focus (Gianfaldoni et al., 2017).

Radiotherapy and surgery formed the basis for solid tumour treatment well into the 1960's (Arruebo et al., 2011) however despite increasingly radical local treatments, cure rates plateaued at about 33% (DeVita & Chu, 2008). This was thought to be due to the inability of surgery or radiotherapy to address the distant spread, or metastasis, of the cancer. This limitation in the curative capability of these treatments paved the way for a greater use of a third type of treatment, chemotherapy.

The term chemotherapy was first coined by German chemist Paul Ehrlich in the early 1900's who defined it as '*the use of chemicals to treat disease*' (DeVita & Chu, 2008). Chemotherapy was introduced as a method to treat cancer in the 1940s with nitrogen mustards and antifolate drugs the first agents to be trialled (Chabner & Roberts, 2005). Chemotherapeutic agents prevent the division of rapidly dividing cells, such as cancer cells, by interrupting different parts of the cell cycle (Bagnyukova et al., 2010). Due to the drug being circulated in the bloodstream, it can target cancer cells almost anywhere in the body and has been shown to reduce incidence of both local and systemic recurrence along with improve overall survival (DeVita & Chu, 2008). Unfortunately, chemotherapy cannot differentiate between cancer cells and other rapidly dividing cells such as hair follicles, bone marrow and cells within the intestines. This can result in a number of side effects including fatigue, diarrhoea and constipation being reported by patients undergoing the treatment (Pearce et al., 2017).

Systemic treatment, such as chemotherapy, are considered most effective when used as an adjunct to local control measures, specifically surgery and radiotherapy

(Nicolaides et al., 2017). For example, downsizing of rectal tumours is seen in most patients who undergo neoadjuvant chemoradiotherapy, with complete response occurring in 15 – 20% (Dekker et al., 2019). The combination of treatment modalities, advances in technology and developments in research has seen survival from cancer steadily increase over the past 50 years, with the biggest observed changes being seen in colon, Non-Hodgkin's lymphoma and rectal cancer (Macmillan Cancer Support, 2012). This is illustrated by data from England and Wales showing that all cancer survival index at 1 year was 50% in 1970 yet had increased to 69% by 2011 (Quaresma et al., 2015).

1.5.4 Socioeconomic impact

Additional to the physical and psychological burden of cancer for sufferers and their support networks, cancer is financially expensive. The cost of cancer on the UK economy is thought to exceed £1.4 billion through lost productivity alone, with this figure increasing to £7.6 billion when mortality is taken into account (Hilhorst & Lockey, 2019). NHS England places annual expenditure on cancer diagnosis and treatment at £5.68 billion, amounting to 5% of total health expenditure (Jönsson & Hofmarcher, 2017). There are also significant financial implications to the individual and their families following diagnosis, with many having to adjust their working lives to fit around treatment. Research has showed that cancer patients were found to be, on average, £570 worse off per month following their diagnosis (Wind-Cowie & Salter, 2013), with this financial impact being more apparent in those diagnosed earlier and at retirement age (Figure 5).

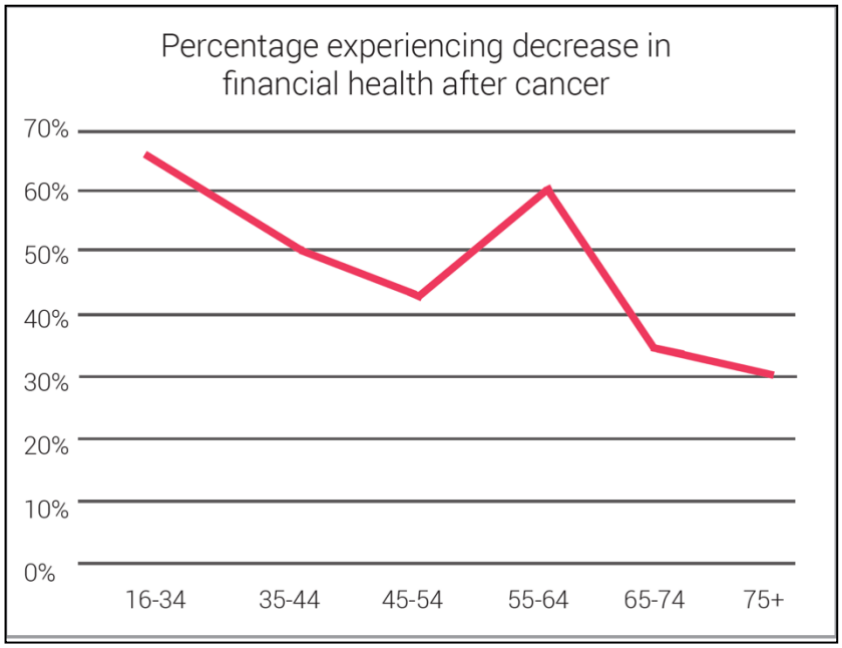


Figure 5 – The impact cancer has on financial health of individuals depending on the age at diagnosis (Hilhorst & Lockey, 2019)

1.5.5 Emotional impact

Regardless of the prognosis, a cancer diagnosis causes some degree of emotional distress, anxiety and depression for both the patient and their family (Woźniak & Izycki, 2014). In certain types of cancer, patients can also experience feelings of guilt associated with 'risky' behavioural traits such as smoking, which can add to the emotional distress (LoConte et al., 2008). The impact on mental well-being can result in patients finding it harder to cope with their treatment and any side effects (Sharma, 2020). Linked to this, depression has also been shown to result in higher rates of mortality, with patients who show even a few depressive symptoms being at a 25% increased risk (H. R. Smith, 2015).

A cancer diagnosis affects a multitude of people connected to an individual with cancer. More than three in four patients describe the impact of cancer on family life as very or moderately negative, and two thirds believe the diagnosis had had a negative impact on their family's social life (Hilhorst & Lockey, 2019). Additionally, with more and more people surviving cancer, long term side effects and disability can affect the mental wellbeing of individuals and their families for many years after treatments (K. D. Stein et al., 2008).

1.6 Cancer cachexia

Many patients with cancer experience muscle wasting and weakness (Morishita et al., 2017). This can be caused by sarcopenia or by cancer cachexia, a multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass, with or without loss of adipose tissue, that cannot be fully reversed with conventional nutritional support (Shum et al., 2018). Although cachexia and sarcopenia share some pathological mechanisms, cachexia is characterised by an energy imbalance, resulting from decreased nutritional intake and increased energy expenditure caused by a hypermetabolic state (Argilés et al., 2015). The often results in significant weight loss, a feature that is absent in in sarcopenia (Evans et al., 2008; Porporato, 2016). Although skeletal muscle is the main tissue involved, cachexia also affects multiple other organs including the brain, liver, gut and heart (Argilés et al., 2014).

The severity of cachexia is difficult to assess objectively, with cachexia being seen as a continuum through three clinically relevant stages as described in Figure 6. Not all patients transverse the entire spectrum, with the risk of progression dependent on

factors such as the cancer type and stage, degree of systemic inflammation, low food intake and lack of response to anticancer therapy (Fearon et al., 2011).

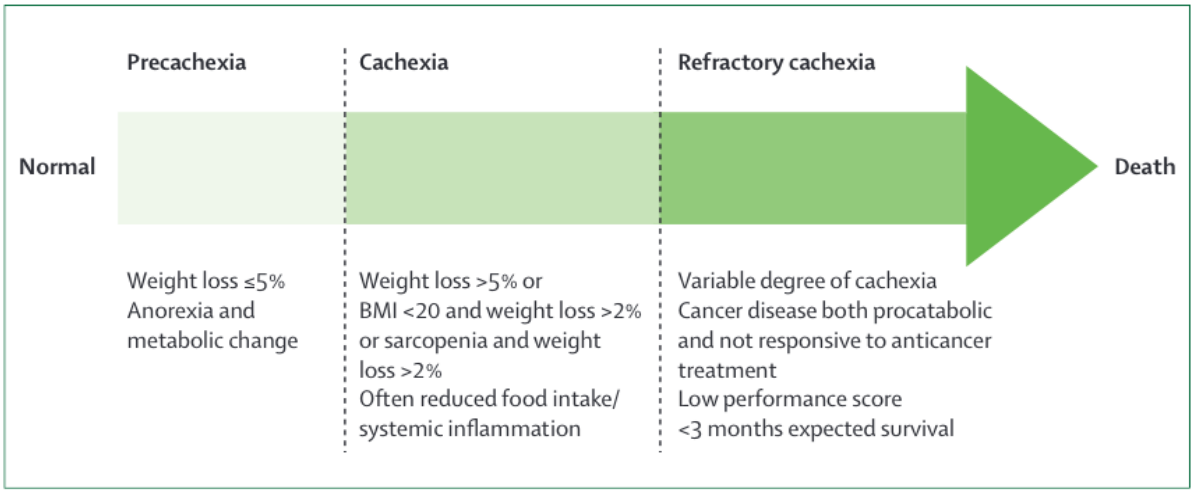


Figure 6 – Stages of cancer cachexia (Fearon et al., 2011)

1.6.1 Cachexia and cancer treatment

Cachexia is directly associated with cancer patient morbidity and mortality (Carson et al., 2016) and although not associated with all cancer types, cachexia is thought to affect 50 – 80% of cancer patients and is reported to be indirectly responsible for at least 20% of all cancer deaths (Penna et al., 2019). Patients that exhibit cancer cachexia have increased fatigue and decreased functional independence prior to and during treatment, and this has an impact on the success of the interventions used (Vanderveen et al., 2017).

Resectional surgery places profound metabolic and cardiorespiratory stresses on the body (Boereboom et al., 2016), and a number of studies have shown that patients with low skeletal muscle mass and density are at an increased risk of post-operative complications, mortality and increased length of hospital stay (M. H. Choi et al.,

2018; van Vugt et al., 2018). Similarly, in regards to the use of systemic treatment such as chemotherapy, treatment plans are often compromised by cachexia, with affected individuals requiring dose limitations and even therapy interruptions, resulting in a reduction in drug efficacy and patient survival odds (Penna et al., 2019).

Similar to the treatment of sarcopenia, physical exercise has been shown to be effective in improving physical function and quality of life (Morishita et al., 2017). Studies have shown that EET improves muscle wasting in cardiac and cancer patients (Alves et al., 2015), with RET being shown to attenuate muscle wasting associated with a variety of catabolic conditions including cancer (Al-Majid & Mccarthy, 2001). Additionally, the combination of EET and RET has been found to improve upper and lower body strength more effectively when compared to usual care in patients with cancer (Stene et al., 2013). Despite this however, and as seen with age related sarcopenia, no intervention has been found to completely reverse cachexia (V. E. Baracos et al., 2018).

1.6.2 Cachexia and skeletal muscle function

As with sarcopenia, the skeletal muscle phenotype in cancer cachexia is characterised by a reduced number of mitochondria and decreased expression of regulatory factors involved in mitochondrial biogenesis (Antunes et al., 2014). Similar to that seen in ageing, an increase in ROS levels could, in part, explain the changes seen in mitochondrial function (van der Ende et al., 2018). Additionally, pre-clinical animal models have demonstrated an upregulation in proteins responsible for

initiating mitophagy as well as negatively affecting biogenesis (van der Ende et al., 2018).

Systemic inflammation is recognised as a hallmark of cancer cachexia with circulating cytokines established initiators of skeletal muscle wasting as well as mitochondrial dysfunction (Carson et al., 2016; Vanderveen et al., 2017). Inflammatory mediators are released from both the tumour cells themselves, and from cells within the tumour microenvironment including the hosts own immune cells (V. E. Baracos et al., 2018). The presence of these mediators are thought to induce an imbalance between protein synthesis and degradation in muscle fibres (Antunes et al., 2014). Specific to cancer cachexia, three cytokines have been implicated. Firstly, IL-6 has been shown to effect skeletal muscle metabolism and is considered a prime regulator of the acute phase response seen in cachexia (Laine et al., 2013). Secondly, TNF has long been associated with muscle pathology and is thought to alter levels of circulating hormones required for muscle growth, as well as stimulate further catabolic cytokines that contribute to anorexia (Argilés et al., 2011). Finally myostatin, which is known to play a crucial role in the negative regulation of skeletal muscle growth and differentiation, has been found to be upregulated in cancer (Elkina et al., 2011)

To date, there is limited knowledge as to the underlying pathways responsible for the role mitochondrial dysfunction plays in cancer cachexia and whether it can be prevented with the similar proposed in the treatment of sarcopenia.

1.6.3 The use of exercise in cancer treatment

Exercise is traditionally classed as either endurance, aerobic/resistance, or strength training, with each modality associated with distinct physiological adaptations. EET is associated with an improved capacity for aerobic energy metabolism and fatigue resistance and is the most effective approach for improving CRF (Lin et al., 2015) whilst RET is linked to muscle hypertrophy and increased force generating capacity (Whyte, 2006). In recent years, there has been considerable interest surrounding high-intensity interval training (HIIT), with claims to induce health benefits of a similar, if not superior magnitude to moderate- intensity continuous exercise (Cassidy et al., 2017). Traditionally used in an elite athletic training setting, and performed at supra-maximal intensities (Gibala & Little, 2020), HIIT can be simply defined as intermittent periods of intense exercise with periods of recovery that can elicit adaptions resembling both endurance and strength training (MacInnis & Gibala, 2017). In addition, at a lower relative intensity than those used by athletic populations, HIIT has been shown to be a time efficient (Gibala et al., 2006), tolerable (Cassidy et al., 2016) and largely enjoyable (J. E. M. Blackwell et al., 2020) regime when used in a variety of clinical cohorts, including those with chronic disease and cancer (Cassidy et al., 2017; Loughney et al., 2015).

The preoperative status of the patient is now viewed as an increasingly important factor for improving postoperative outcomes. The practice of prehabilitation is becoming more prevalent in the preoperative workup of high risk patients, with the main aim directed at enhancing the patient's functional capacity before surgery (Banugo & Amoako, 2017). In broad terms, surgical prehabilitation is described as '*a strategy to address modifiable risk factors that impact treatment outcomes*', with the

theory behind it suggesting that by increasing the functional capacity prior to surgery, a prehabilitated patient will retain a higher level of functional ability and recovers more rapidly in the perioperative period compared with a non-prehabilitated patient (Francesco Carli et al., 2017; Franco Carli & Zavorsky, 2005) (Figure 7).

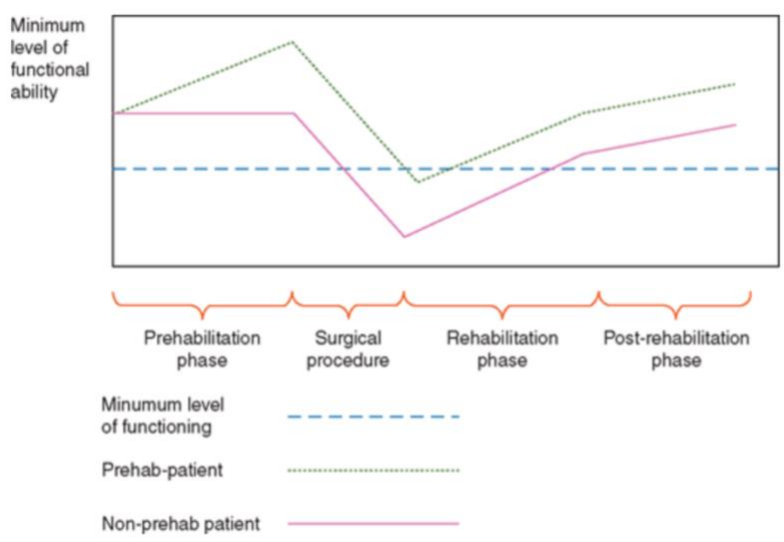


Figure 7 – Conceptual model depicting the theoretical benefits of prehabilitation

Over the past few years, several studies have evaluated the use of prehabilitation to improve physical function to overcome surgical stress and improve postoperative recovery time (Piraux et al., 2018). To date, prehabilitation regimes have had varying success, with some showing a meaningful benefit (Moran et al., 2016; Wang et al., 2016), while others have failed to elicit meaningful benefit in physiological parameters (Boereboom et al., 2019). In addition, the variation in prehabilitation regimes makes their efficacy difficult to conclude upon. For example, some regimes focused on a single target, such as nutrition (Braga et al., 2009), lifestyle and psychological support (Johnston & Vogele, 1993) or smoking cessation (Thomsen et al., 2014), while others were multi-modal (F. Carli et al., 2010; Li et al., 2013).

Exercise based prehabilitation programs have been shown to enhance muscle protein synthesis (MPS) (Smeuninx et al., 2021), improve mitochondrial function (Seo et al., 2016) and modulate the levels of inflammatory cytokines in patients with cancer (Antunes et al., 2014). The effects of prehabilitation in patients with cancer was first reviewed by Loughney et al., who found significant improvements in physical fitness but the effect on other outcomes, such as quality of life and fatigue were inconclusive (Loughney et al., 2016). Despite such positive findings, it is generally agreed that existing studies must be viewed with a degree of caution since pooling data is difficult given the heterogeneity of the study population (Banugo & Amoako, 2017).

The use of prehabilitation as part of the cancer treatment pathway faces many challenges. For example, in the UK, the National Cancer Action Team specifies that treatment should start within 31 days of the decision to treat (NCIN, 2010). As a result, many of the traditional exercise training programs fail to significantly improve CRF or other markers of physical function in that timeframe (Boereboom et al., 2019). Additionally, there are issues implementing a training program that can achieve adherence, and therefore positive results, in for a patient cohort that is prone to suffer high levels of fatigue (Eng et al., 2018; Fernandez et al., 2015).

Despite these challenges, studies have shown that an exercise training within this 31 day decision to treat program does have the potential to improve a patients CRF (Boereboom et al., 2016), and as such improve their post-surgical clinical outcomes (Moran et al., 2016). This finding does not appear to be uniform across all cancer types, with work from our group showing that a single HIIT protocol, shown to be

effective in healthy older adults, was able to improve the CRF of patients with prostate cancer (J. E. M. Blackwell et al., 2020), but not those with colorectal cancer (Boereboom et al., 2019) To date, there is little research exploring the relationship between CRF and mitochondrial content and function. A better understanding of this relationship in health, and in cancer, may help is understand the potential of prehabilitation in different cancer groups.

1.7 Research synopsis

Based on the available evidence presented in this chapter, we highlight the importance of skeletal muscle and its mitochondria for whole body health in both ageing and cancer. This thesis will firstly aim to systematically review the available literature exploring the link between pre-existing sarcopenia and clinical outcomes associated with individuals with locally advanced colorectal cancer. Following this, we will present data from a healthy volunteer study which seeks to determine the relationship between skeletal muscle mitochondrial activity and measures of physical function across the life course. Finally, we will put forward the rationale and experimental workplan for possible further research that could help establish differences in physical fitness and mitochondrial activity in those with different forms of cancer.

2 The prognostic significance of pre-existing sarcopenia in patients with locally advanced rectal cancer undergoing neoadjuvant chemotherapy prior to surgical resection: a systematic review and meta-analysis.

2.1 Chapter focus

In the previous chapter, we explored the link between sarcopenia and adverse health outcomes, such as post-operative complications and increased length of hospital stay. It is becoming increasingly apparent that the body composition and nutritional status of patients with cancer at the time of diagnosis plays a substantial role in predicting both short- and long-term clinical outcomes. In this chapter, we will review the prognostic significance sarcopenia has on the clinical outcomes of patients with locally advanced rectal cancer who undergo neoadjuvant chemotherapy prior to surgical resection.

2.2 Introduction

2.2.1 Background

Colorectal cancer (CRC) is the third leading cause of cancer deaths and fourth most commonly diagnosed cancer in the world (Rawla et al., 2019). In the UK, the largest proportion of bowel cancer cases occur in the rectum, accounting for 31.5% and 23.1% of cases in males and females respectively (Cancer Research, 2020). Surgery is the gold standard treatment for people with rectal cancer provided the tumour is deemed resectable (National Institute for Health and Care Excellence, 2020). In patients who have locally advanced rectal cancer, those that undergo neoadjuvant chemoradiotherapy (nCRT) have been found to have less local recurrence and better overall and disease free survival (National Institute for Health and Care Excellence, 2020). Other advantages of nCRT include decreasing tumour volume and enhancing the probability of anal sphincter preservation (Park et al., 2018). Treatment with nCRT also introduces the chance of complete pathological response, with a R0 resection being recorded in up to 15 - 20% of cases (Dekker et al., 2019).

It is becoming increasingly apparent that the body composition and nutritional status play an important role in predicting both short and long term outcomes in cancer patients (V. Baracos & Kazemi-Bajestani, 2013). In recent years, low skeletal muscle mass has been shown to be a negative prognostic indicator in malignant diseases of the lungs and gastrointestinal tract (Levolger et al., 2015; Martin et al., 2013). Specific to CRC, reduced muscle mass and increased visceral fat mass has been shown to be negative prognostic factors (Bardou et al., 2013; Gibson et al., 2015; C.

M. Prado et al., 2008). Similarly, an association between low skeletal muscle mass and short term outcomes such as length of stay (LOS) and infection rate has been shown in patients with CRC or colorectal liver metastasis (Lieffers et al., 2012; Peng et al., 2011; van Vugt et al., 2018).

Low skeletal muscle mass in cancer patients is multifactorial with both modifiable and non-modifiable features. Both chemotherapy and radiotherapy have well known side effects that include nausea, vomiting and anorexia, which can all result in a more rapid loss of muscle mass in cancer patients (Oflazoglu et al., 2020). Studies have also shown a relationship between the degree of muscle loss suffered by an individual and factors including the duration of treatment and the type of cancer (Oflazoglu et al., 2020; Yamaoka et al., 2015), however these are often in patients with advanced or metastatic disease. When looking at patients with resectable disease, Eriksson et al. showed that skeletal muscle mass decreased during nCRT for CRC liver metastasis, with those being sarcopenic preoperatively having a poorer overall survival (Eriksson et al., 2017). What is lacking in current literature is the impact neoadjuvant CRT (nCRT) has on the prognosis of patients with pre-existing sarcopenia.

2.2.2 Defining sarcopenia

Sarcopenia is characterised by the progressive and generalised loss of muscle mass and function. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) agreed a consensus definition of *'the loss of muscle mass with loss of muscle function (strength or physical performance), with measurements two Standard Deviations (SD) below the mean of a young reference population'* (Cruz-

Jentoft et al., 2010). Despite this, defining sarcopenia remains controversial and there are multiple definitions published within the literature.

The most commonly used cut off values to diagnose sarcopenia were first described by *Prado et al*. who used optimum stratification analysis between low muscle mass and mortality in a population of 250 obese Canadians with respiratory or GI malignancies (C. M. Prado et al., 2008). Although these values of 52.4 cm^2/m^2 for males, and 38.5 cm^2/m^2 for females considers the gender of the individual, they fail to account for other demographic factors such as ethnicity, body size, lifestyles and cultural background and this has led to differences in reported definitions (L. K. Chen et al., 2014).

More recently, due to an increased understanding of sarcopenia, the EWGSOP recommended that muscle strength, rather than mass, be the primary parameter of sarcopenia, due to it being shown to be better at predicting adverse outcomes (Cruz-Jentoft et al., 2019). This is because, although muscle mass and strength are related to one another, their trajectories of decline are not linear, with muscle strength declining much faster than mass (Goodpaster et al., 2006). There are several well validated tools for the assessment of muscle strength, such as hand grip strength (HGS) (Aadahl et al., 2011), lower limb muscle strength (Bohannon et al., 2012) and sit to stand test (Freire et al., 2012; Gómez Montes et al., 2013). Although these assessment tools are used in the research setting, their use in wider clinical practice is not always feasible with many suiting the secondary care setting rather than primary care (Beaudart et al., 2016). This limits any proposed definition of

sarcopenia being universally adopted, with the main parameter remaining body composition and muscle mass in the clinical setting.

2.2.3 Measuring body composition

A wide range of techniques can be used to quantify body composition with cost, availability and ease of use determining which technique is applied in specific settings. Anthropometric methods such as body mass index (BMI), calf circumference, mid upper arm circumference and skinfold thickness are all simple clinical tools which can estimate body composition (S. Yu et al., 2015). However, due to large predictor error and the lack of universally agreed cut off points, the validity of anthropometric methods on their own are of limited worth (Beaudart et al., 2016).

Dual-energy x-ray absorptiometry (DXA) is a well-established, low radiation technique used to assess body composition in both the clinical and research setting. Clinically, DXA is primarily used to diagnose osteoporosis, however it can also provide reproducible estimates of appendicular skeletal lean mass (ALM) by measuring the sum of the non-bone and non-fat mass of the four limbs (Levine et al., 2000). The ALM is then adjusted for body size to generate skeletal muscle index (SMI), which can be used to produce gender specific cut off points to diagnose sarcopenia (Beaudart et al., 2016). Limitations to DXA include its inability to assess other body components such as intramuscular fat, it’s failure to provide information on muscle quality and its inaccuracy when assessing muscle mass in people of varying age (Erlandson et al., 2016).

Bio-electrical impedance analysis (BIA) is another method used to estimate total or ALM. BIA does not measure muscle mass directly, instead provides an estimation of muscle mass based on whole body resistance to an electrical current (Cruz-Jentoft et al., 2019). Lean tissue supplies the least resistance to the current because of its high water content, with the speed of the current being converted to estimate body fat percentage and fat free mass (Esco et al., 2015). Although inexpensive and easy to use in clinical practice, the method is seen as less accurate than other methods available due to its dependence on the hydration status of the individual, and tends to overestimate muscle mass and underestimate fat mass (Esco et al., 2015; Reiss et al., 2016).

Computed tomography (CT) and magnetic resonance imaging (MRI) are considered to be the gold standard and most accurate imaging methods for quantifying muscle mass, muscle cross sectional area and muscle quality (Mitsiopoulos et al., 1998). MRI Is based on the magnetic properties of hydrogen protons, which constitute a large portion of body tissue. Unlike either DXA or CT, direct visualisation of MR images allows for quantification of muscle and fat volumes from body segments and individual muscle groups (Erlandson et al., 2016). The main disadvantage to MRI use is the limited access to the device and the requirement for highly trained individuals to perform and interpret the images. There are also several contraindications, such as pacemakers and other magnetic implants, along with the requirements for the individual to maintain a prolonged supine position (Erlandson et al., 2016). This can limit its use in the older population in which sarcopenia is more prevalent.

Cross sectional abdominal CT scans provide a precise measure of overall muscle mass through the assessment of core muscle size. Core muscle size can be measured as either the psoas area or total skeletal muscle at specific levels of the scan. Most researchers use consistent methods for CT image analysis focusing on 3rd lumbar vertebrae (L3) as the standard bony landmark (Kazemi-Bajestani et al., 2016). This is due to evidence suggesting the best correlation between core muscle size and total muscle mass was 5 cm above L4/L5 vertebral level (C. M. M. Prado et al., 2009; Shen et al., 2004). More recently, it has been suggested that calculation of psoas density may be a more accurate method of determining sarcopenia than psoas area as variable fat content in skeletal muscle may increase the cross sectional area, therefore confounding the measurement of lean muscle area (Herrod et al., 2019).

Since CT imaging is expensive, requires specialist interpretation and exposes the individual to a small amount of radiation, it is not recommended as a routine method for assessing body composition (Gibson et al., 2015). In the case of patients with cancer however, CT scans are used routinely in the diagnosis and staging process of their treatment. This has led to them being the most frequently used modality in research papers to analyse body composition in cancer patients.

2.2.4 Aim

The aim of this review is to examine the relationship between pre-existing sarcopenia and the prognostic outcomes in patients with locally advanced CRC who underwent nCRT prior to resectional surgery. The primary outcome was overall survival (OS), with disease free survival (DFS) being a secondary outcome.

2.3 Method

2.3.1 Study design

The systematic review was registered with PROSPERO prior to the literature search (registration number CRD42019157313) and carried out in accordance with the PRISMA statement (Moher et al., 2009). Any comparative study relating to the prognostic outcomes of sarcopenic versus non-sarcopenic patients diagnosed with LARC who received nCRT prior to resectable surgery were included. Exclusion criteria included any study in which the cohort included patients with irresectable, or advanced/metastatic colorectal cancer, combined outcomes of different types of cancer or treatment modalities, such as pre- and post-operative chemotherapy, or introduced interventions preventing muscle loss during the treatment pathway.

2.3.2 Literature search

A trained Clinical Research Librarian performed the literature search using MEDLINE and EMBASE databases. There was no language or date restriction, and the databases were searched from inception until 14th December 2020. The Cochrane library of systematic reviews was searched for previous reviews, whilst clinicaltrials.gov was searched for any unpublished studies. Systematic reviews of similar topics were searched for any relevant previous studies specific to locally advanced colorectal cancer.

Medical subject headings (MeSH) included the terms ‘COLORECTAL TUMOR’, ‘NEOADJUVANT THERAPY’, TREATMENT OUTCOME and ‘PROGNOSIS’. Free text words included ‘chemoradiotherapy’, ‘sarcopenia’ and ‘muscle mass’. Example

of the full search strategy can be found in Appendix A. Abstracts were independently screened by two authors (JH and TS) using the Rayyan systematic review software (2016, Qatar Computing Research Institute, Doha, Qatar) (Ouzzani et al., 2016). If either author deemed the abstract potentially relevant, it was then considered for full text review. Full text versions were independently screened against the inclusion and exclusion criteria by two authors (JH and TS). All discrepancies between studies were resolved by consensus.

2.3.3 Data extraction

Study characteristics including author, year of publication, mean/median age (years), percentage of male individuals, percentage defined as sarcopenic at baseline (%), Follow up (months), and gender specific cut of values used for defining sarcopenia (cm^2/m^2)) were extracted by one author (JH). Outcome data (OS and DFS) were independently extracted by two authors (JH and TS). When outcome data was not shown in the correct format, the corresponding author of the study was contacted via email and the data was requested. The Risk Of Bias In Non-randomised Studies – of Interventions (ROBINS-I) tool for assessing bias was used to measure risk of bias for included study. Risk of bias was performed independently by two authors (JH and EH), with any disagreement being resolved by consensus of a third-party author (TS).

2.3.4 Statistical analysis

We performed meta-analysis on reported hazard ratios (HR) and 95% confidence intervals (CI) using DerSimonian–Laird random-effects models. These were converted to logHR, and standard errors were calculated from 95% CI. Where possible, we used the multivariate HR where reported. We aimed to conduct

investigation of heterogeneity and publication bias although there were too few included studies. Statistical heterogeneity was assessed using the I^2 statistic, with values above 50% taken as evidence of statistical heterogenicity. GRADE was used to assess the certainty of evidence. All analyses were conducted on Stata Version 16.

2.4 Results

2.4.1 Search results

The primary search strategy produced a total of 1117 results including 87 duplicate studies. Of these, 1016 were excluded as not being relevant. This left 14 studies for full text review. From this, 5 studies were deemed suitable for inclusion for analysis. Figure 8 summarises the search results.

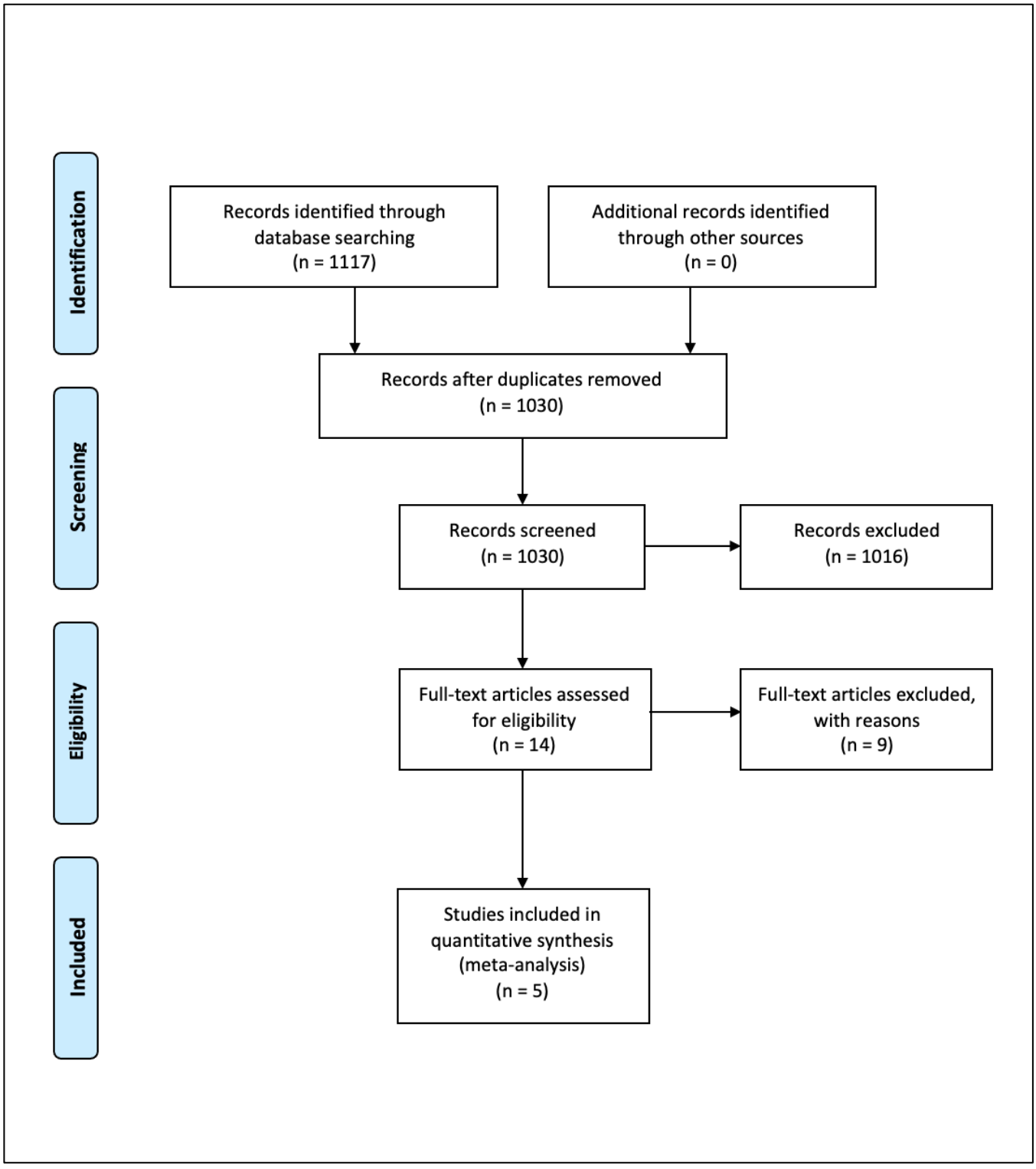


Figure 8 – PRISMA flowchart

2.4.2 Study characteristics

Characteristics of the included studies, which comprised of a total of 598 patients, can be found in Table 1. The earliest study that met the inclusion criteria was 2017, with the latest being 2020. All papers were published as journal articles in peer reviewed journals. All included studies were retrospective cohort studies.

2.4.3 Risk of Bias

Risk of bias was assessed using the ROBINS-I tool (Table 2). Four studies were considered moderate risk of bias regarding confounding factors since patients of different age, cancer stage and comorbidities were included. One study was considered high risk of bias in this category as it failed to perform multivariable statistical adjustment due to the small number of patients. Four studies were deemed moderate risk of bias in missing data since they excluded patients who, despite meeting the criteria for inclusion, did not have the appropriate imaging pre and post chemoradiotherapy. All five studies were judged to have moderate risk of bias in relation to measurement of outcomes since they were all retrospective, non-blinded studies. Two studies were deemed to be at moderate risk of bias in relation to reporting results, one reporting results of a subgroups of the total cohort whilst the other dividing the cohort into quartiles to report findings.

Overall, one study was deemed to be at severe risk of bias, with the remaining four studies considered at moderate risk.

Author	Year	Country	N	Age [a]	% male	Sarcopenia at baseline (%)	Follow up (months)	Sarcopenia cut off point, male [b]	Sarcopenia cut off point, female [b]
Choi et al.	2018	South Korea	188	61.3 (27 – 84)	62.2	39.34	52 (5 – 91)	≤52.4	≤38.5
Chung et al.	2019	South Korea	93	NR	64.5	51.6	NR	≤52.4	≤38.5
De Nardi et al.	2019	Italy	52	63 (32 – 79)	65	59.6	56 (32 – 83)	≤52	≤42
Levolger et al.	2017	Netherlands	121	61 (53 – 66.3)	58.2	NR	41 (26 – 62)	≤52.4	≤38.5
Takeda et al.	2018	Japan	144	62.5 (32 – 75)	70.8	25.7	67.6 (5.7 – 137.1)	≤45	≤33.8

Table 1 – Summary of patient characteristics

N – number, NR - not reported, [a] mean or median as published, [b] cm

Study	Confounding	Selection	Classification of Intervention	Deviation from intended intervention	Missing Data	Measurement of Outcomes	Reported Result	Overall
Choi et al. (2018)	Moderate	Low	Low	Low	Moderate	Moderate	Low	Moderate
Chung et al. (2019)	Moderate	Low	Low	Low	Moderate	Moderate	Moderate	Moderate
De Nardi et al. (2019)	Severe	Low	Low	Low	Low	Moderate	Moderate	Severe
Levolger et al. (2017)	Moderate	Low	Low	Low	Moderate	Moderate	Low	Moderate
Takeda et al. (2018)	Moderate	Low	Low	Low	Moderate	Moderate	Low	Moderate

Table 2 – Risk of bias of journals included in the review using the ROBINS-I tool

2.4.4 Data synthesis

Four studies reported OS, whilst 3 studies reported DFS within the paper itself. A further study was able to provide data relating to OS and DFS for patients with pre-existing sarcopenia following correspondence with the lead author.

2.4.5 Overall survival (OS)

A total of 598 patients from 5 studies were included in the analysis of hazard ratios for OS. All papers were comparative cohort studies. Hazard ratios were extracted from multivariate analysis in 2 studies and univariate analysis in 3 studies. Meta regression analysis, assessing the relationship between pre-existing sarcopenia and a poorer overall survival, showed a significant association (pooled HR 1.69, 95% CI: 1.15, 2.48) (Figure 9). There was low statistical heterogeneity (I^2 = 19.15%).

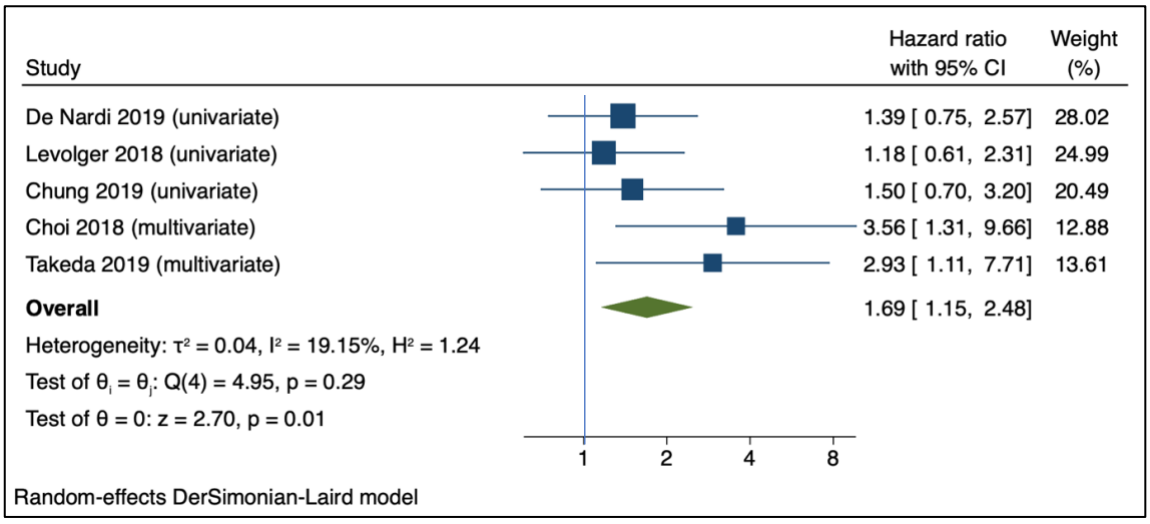


Figure 9 – Forest plot showing meta-analysis of the association between pre-existing sarcopenia and OS in patients with LARC.

2.4.6 Disease free survival (DFS)

A total of 4 studies comprising of 505 patients were available for analysis of HRs for DFS. Hazard ratios were extracted from multivariate analysis in 1 study and univariate analysis in 3 studies. All papers were comparative cohort studies. Meta regression analysis, assessing the relationship between pre-existing sarcopenia and a shorter DFS appeared to show an association, however this was not statistically significant (pooled HR 1.07, 95% CI 0.63, 1.82) (Figure 10). Heterogeneity between studies was moderate/high (I^2 = 56.05%).

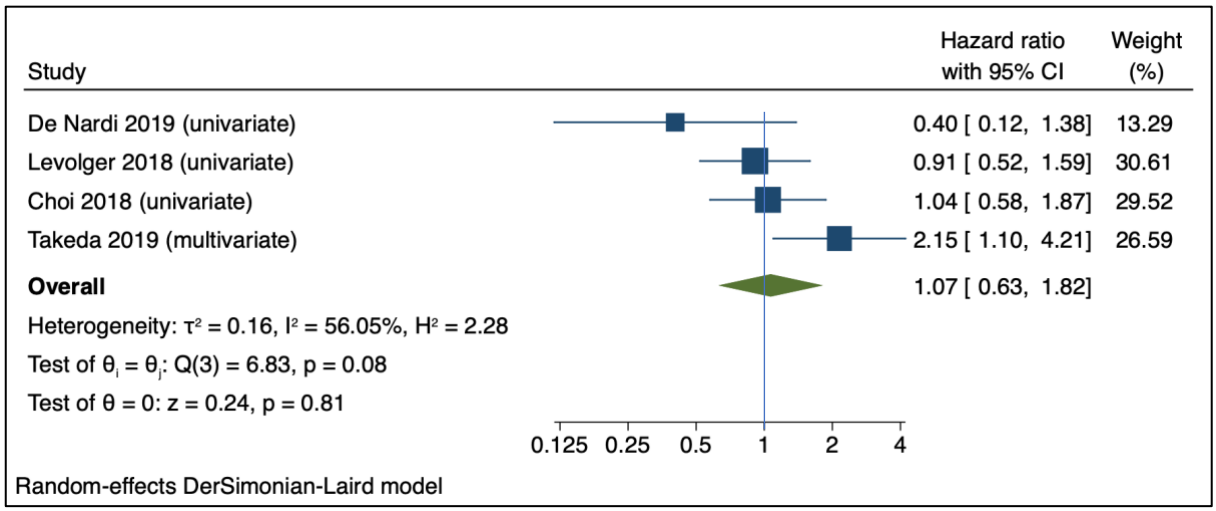


Figure 10 - Forest plot showing meta-analysis of the association between pre-existing sarcopenia and DFS in patients with LARC.

2.5 Discussion

Over the past decade, there has been significant intertest in the link between sarcopenia and prognostic outcomes in cancer treatment. To our knowledge this review is the first to evaluate the relationship between pre-existing sarcopenia and prognostic outcomes of patients with LARC who underwent both neoadjuvant chemotherapy and resectional surgery.

There was a significant association between pre-existing sarcopenia and poorer OS. This finding is in line with other studies that looked at patients who underwent curative resection of colorectal cancer and colorectal liver metastasis (Miyamoto et al., 2015; Peng et al., 2011), as well as in patients with various solid cancer types across different disease stages (Shachar et al., 2016). This indicates that a patient's body composition and muscle mass at the time of diagnosis is an important indicator for oncological outcomes.

The relationship between pre-existing sarcopenia and a worse DFS was less clear. Although an association was found, it was not significant and there was a high level of heterogeneity between the studies. The reasons for this are unclear but likely multifactorial. Firstly, cut off values used to define sarcopenia varied between studies and, as such patients classed as sarcopenic in one study may not have been included in another. Takeda et al. highlighted this by showing that, had they used the most common cut off values of 52.4 cm^2/m^2 for males and 38.5 cm^2/m^2 for females, 70.1% of patients in their study would have been classified as sarcopenic rather than the 25.7% that were when using their cut off values.

Another possible explanation for the high level of heterogeneity is related to the biologic response patients have to the neoadjuvant treatment. Previous studies have shown a significant association between the systemic inflammatory response caused by the tumour and the degree of muscle mass loss in patients with colorectal cancer (Malietzis et al., 2016; Richards et al., 2012). This theory was further supported by

Heus et al. who analysed the effect chemoradiation had on preoperative body composition of patients with LARC and found there was actually an increase in skeletal muscle area following chemoradiotherapy (Heus et al., 2016). It is therefore reasonable to suggest that pre-existing muscle loss resulting from the metabolic and inflammatory response caused by the tumour could be, in part, reversed because of successful neoadjuvant treatment. This was demonstrated by De Nardi et al who found 62.5% of patients who did not respond to nCRT suffered muscle loss of greater than 2%, whilst 68.2% of responders showed no muscle decrease. This could also explain why, of the 30 patients with pre-existing sarcopenia included in the De Nardi et al study, 6 gained muscle mass during neoadjuvant chemotherapy.

As previously mentioned, mitochondrial function plays a vital role in muscle health and function and dysfunction of the mitochondrial network is a suggested contributor in the development of sarcopenia. Animal models have shown that chemotherapy is responsible for the activation of pathways seen in the pathogenesis of sarcopenia and that this results in muscle mitochondrial dysfunction and depletion (Penna et al., 2010; Pin et al., 2018). Additionally, alterations of muscle mitochondrial homeostasis, as measured by markers of mitochondrial fusion, fission and biogenesis have been displayed in animal models affected by both cancer and exposure to chemotherapy (Barreto et al., 2016). This would suggest that cancer patients who have pre-existing sarcopenia could be more vulnerable to further losses of mitochondrial function, which may exacerbate reductions in muscle function. Cespedes Feliciano et al. found that lower muscle mass was associated with higher odds of early treatment discontinuation, treatment delay and dose reductions independent of age, sex and cancer stage (Cespedes Feliciano et al., 2017), all of which will have an impact on

the duration of DFS and, ultimately OS. Although this may in part be related to the pharmacokinetics and metabolism of chemotherapy agents, as patients with lower muscle mass have smaller tissue volume for distribution of certain chemotherapy agents resulting in reduced metabolism and clearance of the drug, leading to greater toxicity (Cespedes Feliciano et al., 2017), preserving muscle mitochondrial health and function may provide a target to prevent significant muscle loss during neoadjuvant treatment.

To date, few studies have looked at the impact a change in body composition during neoadjuvant treatment has on those with resectable colorectal cancer. It is now suggested that the degree of muscle mass loss during CRT is a more powerful prognostic factor than low skeletal muscle mass at a specific point. This was highlighted in many of the studies included in the review but since all were retrospective, the conclusions drawn were limited. De Nardi et al, Levoger et al and Chung et al all showed an association between the degree of skeletal muscle loss during neoadjuvant treatment and worse survival outcomes. These findings agree with others that looked at both oesophageal and non resectable colon cancer (Awad et al., 2012; Järvinen et al., 2018; Miyamoto et al., 2015; Okuno et al., 2019). This reenforces the idea that if significant changes in body composition could be prevented during neoadjuvant treatment, prognostic outcomes for this cohort of patients could be improved.

Despite the mounting evidence relating to poor outcomes and low skeletal muscle mass, there is no universally accepted therapy for improving and maintaining muscle

mass and in patients with cancer (Okuno et al., 2019). There are also currently no accepted nutritional recommendations for individuals with sarcopenia (Beaudart et al., 2016). Similarly, although there is an increasing number of studies that show physical exercise training programs before major surgery improve cardiorespiratory fitness (Berkel et al., 2018; Boereboom et al., 2016; O'Doherty et al., 2013), there is no robust evidence to show that prehabilitation increases muscle mass or function. This further highlights the complexity of treating sarcopenia and how it is likely that no single treatment will be successful in all patients.

2.5.1 Limitations

The study has several limitations. Firstly, due to no universally agreed values of SMI to define sarcopenia, multiple definitions were included in this study. The cut off used to define sarcopenia in females ranged from 33.5 – 42 cm^2/m^2 and in men from 45 – 52.4 cm^2/m^2. This variation when defining sarcopenia is likely to have played a part in the degree of heterogeneity seen in the results, especially that of DFS.

Secondly, since all the studies were retrospective, there is risk of bias due to confounding factors and absent data. All studies were single centred and had limited numbers of patients. This has often prevented multivariable analysis of data to be performed. Additionally, there was no measurement of muscle function included. With the general consensus suggesting that changes in muscle function play more of a role in diagnosing sarcopenia, the conclusions drawn from any retrospective study is limited.

Finally, with much of the data being collected between 2004 – 2014, most surgical resections were open rather than laparoscopic. It is now common practice to follow a laparoscopic approach as it is known to carry fewer complications and is associated with a shorter hospital stay and quicker return to oral diet (Devoto et al., 2017). When considering this in the elderly population, where any significant period of muscle inactivity results in muscle loss, the type of surgery could have played a role in decreasing OS and DFS.

2.6 Conclusion

Our meta-analysis shows a strong association between pre-existing sarcopenia and poorer OS in patients with LARC who undergo nCRT. Large scale, prospective studies looking at the presence of both low SMI and altered muscle function in individuals with CRC are required to gain a greater understanding. Additionally, more research is required to investigate potential preventative methods, such as nutritional support or exercise programs, is needed to improve outcomes for this cohort of patients.

3 Exploring the effect chronological age has on global physical function and measures of muscle function and mitochondrial activity.

3.1 Chapter focus

The changes seen in global physical function and measures of muscle function as humans age are well established, with these changes seen to have a detrimental effect on an individual's ability to remain healthy and independent. In this chapter. we aim to establish the degree of variation in physical and skeletal muscle function in a cohort of healthy young and old volunteers, before investigating if skeletal muscle mitochondrial respiratory function is affected in a similar manner.

3.2 Introduction

It is well established that the ageing process reduces both global physical performance and skeletal muscle function (Milanović et al., 2013). Due to the complex interplay between age associated reduction in physical activity and the intrinsic ageing processes, the aetiology behind this decline is still not fully understood.

As previously discussed in Chapter 1, loss of muscle mass is a well characterised, and easily measurable end point seen in the ageing process. Although muscle mass loss is associated with an increased risk of developing chronic disease and disability, an increase in muscle mass does not always translate into an improved level of physical performance in the elderly (C. Cooper et al., 2013). It is now thought that muscle 'health' is better predicted through assessment of muscle strength and power (Beaudart et al., 2019), with muscle function measures leading the latest definition of sarcopenia from the EWGSOP (Cruz-Jentoft et al., 2019).

Muscle strength refers to the maximal amount of force a muscle can produce with a single effort. A decline in muscle strength has been shown to be associated with loss of lean muscle mass in older adults, but importantly occurs much more rapidly, meaning changes in muscle function can be detected earlier than change in mass (C. Cooper et al., 2013). Muscle power, which refers to the ability to exert a maximal force in as short a time as possible (power = strength x velocity), appears better still at predicting functional status as it declines earlier and faster than both muscle mass and strength (Beaudart et al., 2019). From an assessment perspective, isokinetic

dynamometry is seen as the gold standard from measuring muscle strength (Bohannon et al., 2012) and is also used to measure muscle power. Despite this, the use of these measures is often limited in the clinical setting due to cost and availability of expensive, cumbersome, specialist equipment (Beaudart et al., 2016).

Global physical performance goes far beyond muscle function as it reflects not only cardiovascular adaptions to transport oxygen, but also the ability of skeletal muscle to utilise oxygen to meet the energy demands of physical activity (Tieland et al., 2018). Cardiorespiratory fitness (CRF) is an intermediate variable between physical activity behaviours and health outcomes that reflect the capacity of numerous bodily organs, such as the heart, lungs and muscles, to support energy production during physical activity and exercise (Lang et al., 2018). CRF is known to decline with advancing age, with decreases in aerobic capacity, as measured by maximum oxygen uptake (VO_{2max}), seen after the age of 30 and accelerating after the age of 50 (Roman et al., 2016). Not only has CRF been shown to be an independent predictor of mortality, patients with lower CRF have higher mortality and morbidity as well as longer hospital stays when undergoing major surgery (Snowden et al., 2013). As with the changes seen in muscle mass and function, the age associated decline in VO_{2max} is, in part, attributable to a reduction in physical activity seen in the elderly population (Roman et al., 2016). The decline is also the consequence of a drop in maximum cardiac output, as well as a reduction in skeletal muscles ability to extract oxygen (Roman et al., 2016; Weiss et al., 2006). In support of the notion that the decline in CRF is related to reduced physical activity, the speed of decline in aerobic capacity has been shown to be reduced if physical activity is maintained into older age, with a greater level of activity being associated with a greater VO_{2max} (Roman et

al., 2016). Additionally, the age related decline seen in aerobic capacity has been shown to be improved even with relatively short exercise training programmes (J. Blackwell et al., 2017).

Beyond the impact of reduced physical activity, the molecular and cellular disturbances responsible for the reduction in aerobic capacity seen in ageing are largely unknown. Although mitochondria have been implicated as a potential causative factor, the extent of mitochondrial decline and how this relates to poorer muscle quality and function, and subsequently global physical function, remain unresolved (Joseph et al., 2016). At a muscle fibre level, total mitochondrial content tends to be reduced in both type I and type II fibres (Roman et al., 2016) and cross sectional studies have shown age to be inversely related to *vastus lateralis* mitochondrial DNA and mRNA transcription (Short et al., 2005). This decline may result in lower muscle mitochondrial protein synthesis and thus changes in mitochondrial content and function. This would potentially explain why studies have shown that the reduced mitochondrial oxidative capacity seen after middle age is not fully reversed by endurance training (Santanasto et al., 2015).

The aim of this study is to establish if the decline in global physical and muscle function is, in part, related to a decline in mitochondrial quality and function as measured using a high-resolution respirometer. In addition, this study will seek to explore the relationship between muscle function and global physical function across young and older adults.

3.3 Experimental methods

3.3.1 Recruitment and screening

Participants were recruited by demographic targeted postal invitations and through presentations at local organisations. The study was approved by the University of Nottingham ethics committee (REF 407-1910) and complied with the Declaration of Helsinki (World Medical Association, 1964)

Following a full explanation of the study by a member of the research team and granting of consent, potential participants underwent a screening session conducted by a qualified medical doctor. The session lasted less than an hour and consisted of a cardiorespiratory examination, resting blood pressure and heart rate measurements, a 12-lead electrocardiogram (ECG) and routine haematological/biochemistry blood profiles. All results were checked by a qualified medical doctor.

3.3.2 Assessment of muscle function and physical performance

3.3.2.1 Hand-grip strength

Hand grip strength (HGS) is a good indicator of upper limb performance in everyday activity and has been shown to have prognostic value with respect to all-cause mortality, cardiovascular mortality and cardiovascular disease, along with playing an important part in the formation of frailty (Leong et al., 2016).

Maximum voluntary HGS contraction was assessed using an electric hand-grip dynamometer (Grip-D, takei, Japan). The participant was instructed to stand with their arm positioned at the side of the body, the elbow flexed at 90 degrees, before squeezing the dynamometer as hard as possible for 3 seconds (Leong et al., 2016). The assessment was repeated three times for both hands with at least 1 minute rest between each measurement. Both the maximum value and calculated mean were recorded.

3.3.2.2 Short Physical Performance Battery Test (SPPBT)

The SPPBT is a test calculated from three components:

1. the ability to stand for 10 seconds with feet positioned in three ways (together side by side, semi-tandem and tandem),
2. time to complete a 3m or 4m walk, and
3. the time to rise to a standing position from a chair five times (Treacy & Hassett, 2018).

The battery of tests focusses on lower extremity function since this has been shown to correlate with mobility, disability and patient outcomes including hospitalisation, institutionalisation and mortality (Beaudart et al., 2016). The SPPBT takes ~10 minutes to complete with the test scored to a maximum of 12 points. Participants scoring ≤ 8 points have been described as having “poor” physical performance (Cruz-Jentoft et al., 2010). Although a useful tool in monitoring the physical function of frail or ‘at risk’ older adults, it is less effective in high functioning younger adults (Treacy & Hassett, 2018).

3.3.2.3 1 repetition maximal strength (1-RM)

Due to the need for specialist equipment to perform isokinetic dynamometry, assessment of muscle strength is most often achieved using leg press or knee extension exercises. Maximal strength can be quantified through the one repetition maximum (1-RM) approach. 1-RM assessments determine the maximum single repetition that can be performed using free weights or traditional 'dual phase' (eccentric and concentric) resistance exercise training machine. In this study, unilateral knee extension was used.

The test was performed follow a 5-minute warm up period on a static bike. Participants then sat on the weight machine, with adjustments being made according to height and comfort (i.e., pivot point of machine aligned with mid knee joint). Following a rest of one minute, the resistance was set at 50% of the anticipated 1-RM weight and the participant was asked to complete 5 repetitions. If completed, the weight was increased to 70 – 75% of the anticipated 1-RM. Once the participant had rested for one minute, they were instructed to complete 5 repetitions at the new weight. If this was achieved and following another minute rest, a third set was performed at 85–90% of the predicted 1-RM and, as before, the participant should try to complete 5 repetitions. If the participant could only complete 1 repetition, this was deemed their 1-RM. If the participant could complete between 2 and 4 repetitions, a predicted 1-RM was calculated using the prediction coefficients shown in Table 3 (Brzycki, 1993).

Repetitions	Leg press coefficient
1	1.00
2	1.0475
3	1.13
4	1.1575

Table 3 – 1-RM prediction coefficients (Brzycki, 1993)

3.3.2.4 Cardiopulmonary exercise testing (CPET)

CPET provides a global assessment of the integrative exercise responses allowing evaluation of both submaximal and peak exercise responses (Weisman et al., 2003). All tests took place in the Clinical Physiology Exercise Suite, University of Nottingham, Royal Derby Hospital and were supervised by members of the research team with at least Immediate Life Support (ILS) training. Prior to commencement of each CPET, the metabolic cart (ZAN 680, nSpire Health, USA) requires calibration. Firstly, room ambient conditions (room temperature (degree Celsius), humidity (%) and atmospheric pressure (mbar)) are recorded. The flow sensor is calibrated with a 3 litres syringe attached to the cart. The inline gas analyser was calibrated against two precision gas mixtures (Weisman et al., 2003).

Prior to setting up, the CPET protocol, including safety and termination criteria, was fully explained to the patient. Following this, a 12 lead ECG (CardioCollect12S, USA), non-invasive blood pressure (NIBP) monitoring and pulse oximeter were fitted

to the participant. Both cardiac tracing via ECG and oxygen saturations via pulse oximeter were recorded continuously through the test, with the NIBP recording every 2 minutes. A silicone face mask was fitted to the participant using elasticated head gear (V2 mask, Hans Rudolph, USA). The flow sensor was then attached to the mask and the presence of any air leaks confirmed by occluding the end of the flow sensor whilst asking the participant to breathe out. Participants were then escorted to a cycle ergometer (Lode Corival, Lode, Netherlands) and the seat height and foot straps adjusted for comfort.

Each CPET comprised of different stages as described in the literature (Weisman et al., 2003). The test started with a 2-minute warm up period of unloaded cycling to allow physiological equilibrium. The workload was then added in a ramp wise manner as per the Bruce Ramp protocol (Bruce, 1971). The ramp protocol was selected upon the basis of clinical opinion of an experienced CPET operator who will set incremental increases of between 5 – 30 Watts/minute with the aim of reaching peak oxygen consumption (VO_{2PEAK}) in 8 – 12 minutes. During the test, participants were encouraged to maintain a cadence of 50 – 60 revolutions per minute (RPM) and continue cycling until exhaustion. The CPET was ended when the participant indicated they had reached maximal effort, they were unable to maintain a cadence above 50 RPM or there was clinical concern as outlined by the American Thoracic Society Statement of Cardiopulmonary Exercise Testing ((Weisman et al., 2003). There was then a 5-minute recovery period of low wattage (10W) cycling in which the participant was encouraged to maintain a cadence of 30 RPM.

In regard to this study, CPET analysis focused on the highest volume of oxygen utilised by the body, known as VO_{2max} (Kinnear & Blakey, 2014). Absolute VO_{2max} (L/min) achieved at CPET was determined as a 15 data point average, including the highest value attained, during the last 20 seconds of the test (Phillips et al., 2017). Tests were considered to have achieved VO_{2max} if 3 or more of the following criteria were achieved (J. Blackwell et al., 2017):

1) a plateau in the oxygen uptake curve (sustained flattening of VO_2 curve despite rising VCO_2).

2) a respiratory exchange ratio (RER) of >1.15.

3) HR over 85% age predicted maximum, and

4) a rating of perceived exertion (RPE); modified Borg scale (Borg, 1982) ≥9 immediately following the test

3.3.3 Assessment of muscle architecture

3.3.3.1 Ultrasonography

Muscle thickness (MT), pennation angle (PA), fascicle length (FL) and cross-sectional area (CSA) provide functional information regarding an individual's lean muscle bulk and can be measured non-invasively using ultrasonography. Images were acquired using B-mode ultrasonography (Mylab, Esaote Biomedica, Italy) with a 100mm, 4 - 13MHz, linear array probe for MT, PA and FL measurements and a 50mm, 4 – 13 MHz for CSA measurement. Participants were resting on an examination bed with knee in full extension as previously described (M. V. Franchi et al., 2018).

To ensure the same portion of muscle was being measured in each participant, anatomical landmarks as described (Perkisas et al., 2018) were first identified by the ultrasound operator. For *vastus lateralis* (VL), the greater trochanter and proximal border of patella formed the longitudinal measurements, with the medial and lateral borders of the muscle (assessed using ultrasound) formed the sagittal measurements. The ultrasound transducer was then placed longitudinally and aligned to the fascicle plane to clearly visualise the fascicles. For CSA, the transduce probe was changed and the participant moved to the edge of the examination bed. Care was taken by the ultrasound operator to apply as little pressure when placing the probe on the skin as excessive compression has been shown to distort and flatten muscles by up to 50% (Dupont et al., 2001).

Digital analysis of images was performed using ImageJ software (National Institutes for Health, USA). Muscle fascicle length was measured directly whenever possible, with linear extrapolation only being applied where the whole fascicle was not visible. The PA was measured at the fascicle intersection and deep tendon aponeurosis and MT was measured as the perpendicular distance between the superficial and deep aponeurosis. CSA was measured by tracing the outline of VL. An example of muscle architecture measurements can be seen in Figure 11. Three images from each ultrasound transducer were stored with each measurement repeated three times and the mean value calculated for statistical analysis.

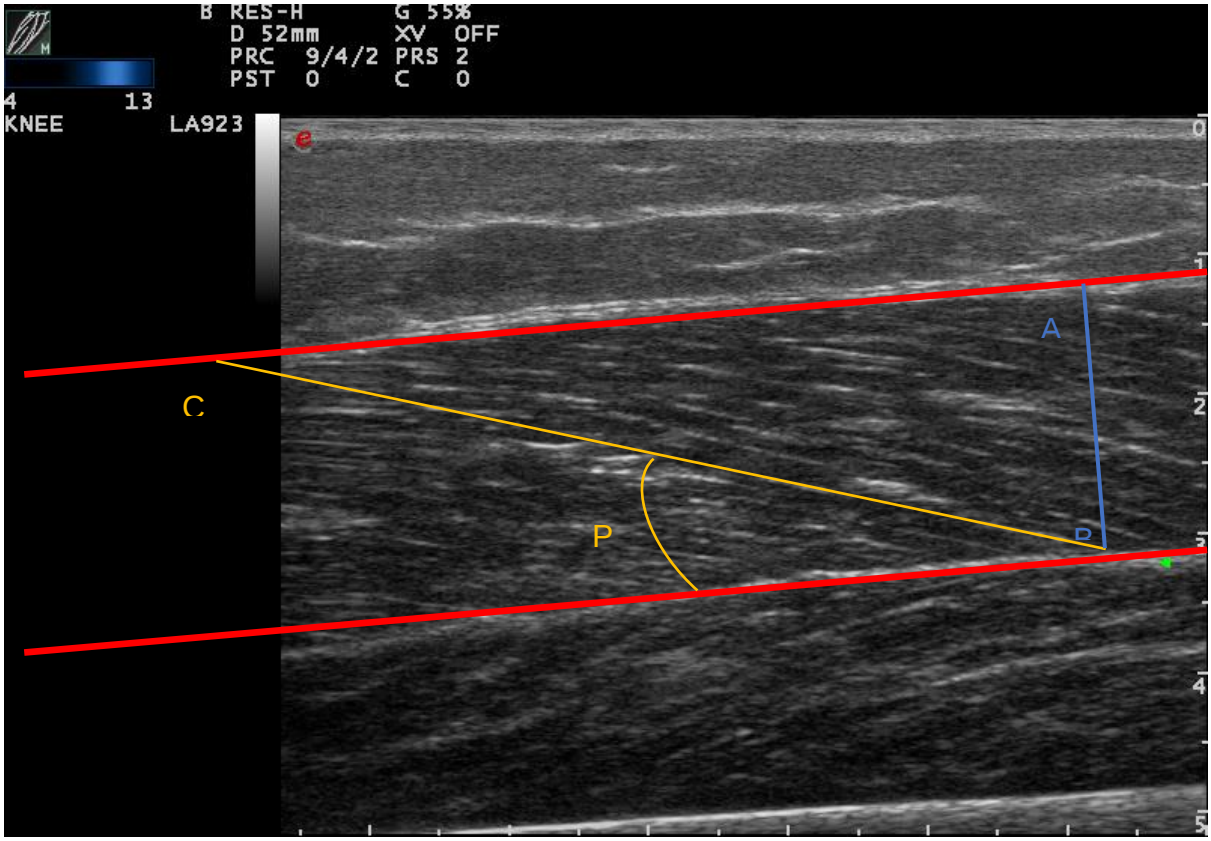


Figure 11 - Ultrasound image of the VL muscle of a 29-year-old participant. Annotations show parameters relating to the assessment of muscle architecture. The two facial planes (superficial and deep) are shown in red lines with the muscle thickness being the distance between A and B (blue line). The yellow line from B to C represents muscle fascicle length using linear extrapolation, with the pennation angle shown in yellow and marked by the letter P.

3.3.4 Assessment of body composition

3.3.4.1 Dual-energy x-ray absorptiometry (DXA)

Total mass, fat mass and lean tissue mass was assessed via dual-energy absorptiometry (DXA) scans on a GE Lunar Prodigy II scanner (GE Medical Systems, UK). DXA is non-invasive technique that generates low dose x-ray beams at two different energies, making use of the differential attenuation between the two to calculate body composition (Laskey, 1996).

DXA scans were performed by qualified research staff. Prior to the scan, participants remove all jewellery, ensure none of their clothing has any metal and not have a full bladder at the time of the scan. Participants are supine throughout the scan, with the duration of the scan varying between 6 – 11 minutes depending on their body mass. Along with whole body analysis, a customised region of interest (ROI) analysis was performed for the upper right leg (from greater trochanter to the midpoint of patella) (Figure 12)

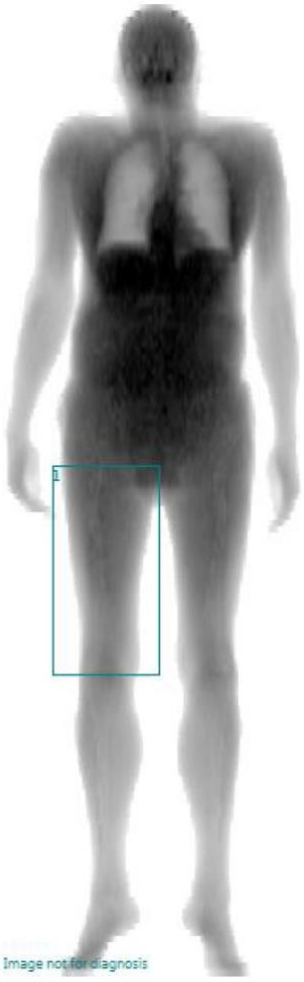


Figure 12 - DXA whole body with region of interest (ROI) measurements. Box 1 measures upper leg.

3.3.5 Assessment of mitochondrial activity

3.3.5.1 VL muscle biopsy

To assess mitochondrial activity, a small muscle biopsy was taken from the right VL of each participant. Percutaneous muscle biopsies are a well-established in both clinical and research practice, being deemed safe, effective and well tolerated technique (Baczynska et al., 2016). The procedure was explained, and consent confirmed prior to commencement. All biopsies were performed by qualified medical doctors with the samples being collected by a suitably trained member of the research team.

The patient was supine with their right thigh exposed. Due to previous measurements performed for ultrasonography, the mid-point of VL was already marked. Following gowning and gloving, the biopsy site was cleaned with aseptic skin preparation (0.5% chlorhexidine solution) and sterile adhesive aperture drape placed over the site. The skin and overlying fascia were infiltrated with 5 – 10ml of local anaesthetic (1% lidocaine) with a 23G needle. Any spare local anaesthetic was kept in case any discomfort during the procedure was reported. A small 5 – 10mm skin incision was made using an 11 bladed scalpel. The scalpel blade was then advanced within the subcutaneous fat down to the fascia where a small incision made. A Weil-Blakesley conchotome was then used to obtain the muscle biopsy. The conchotome is an instrument designed like forceps but with a sharp biting tip (Baczynska et al., 2016). The conchotome was inserted through the skin incision with the tip opened and closed within the tissue and rotated through 90 – 180^{o} to cut the muscle.

Once enough muscle had been collected, pressure was applied to the wound until haemostasis was confirmed. A single vertical mattress suture using 3/0 prolene (Ethicon) was placed across the incision, with the doctor performing the procedure ensuring the wound edges are appropriately approximated. Any further bleeding was controlled with direct pressure. For dressings, a semi-permeable dressing (Mepore Ultra) was placed over the wound, and a compression bandage (Pehaft) applied over it. Spare dressings and wound care advice was given to all participants, including advice to keep the wound dry and avoid vigorous physical activity for approximately 72 hours.

The amount of muscle obtained following repeated sampling can vary from 20mg to 290mg (Baczynska et al., 2016). The muscle samples were cleaned, weighed, and placed in a buffer solution on ice for further analysis with Oroboros 2k high resolution respirometer.

3.3.5.2 Oroboros 2k respiratory system

The Oxygraph-2K (O2k, OROBOROS INSTRUMENTS, Austria) is a closed, two chamber HRR that allows mitochondrial energy metabolism to be tested comprehensively, in real time, by titrating various substrates, uncouplers, inhibitors, and other substances during the experiment (Long et al., 2019). Once the muscle biopsy was taken from the participant it was placed in ice cold buffer solution (BIOPS) and transferred to the clinical physiology laboratory, University of Nottingham, Royal Derby Hospital. The biopsy remained on ice and used within 4 hours of collection.

Prior to performing the HRR, the appropriate substrate-uncoupler-inhibitor-titration (SUIT) protocol was selected on Open DatLab software (SUIT-008_O2_pfi_DO14). There are multiple different SUIT protocols, each offering sensitive diagnostic tests for intact cells, permeabilised cells and permeabilised muscle cells (Pesta & Gnaiger, 2012). We applied the SUIT 008 protocol in this study as it is an established and well validated protocol used in multiple other studies including those exploring the effects of immobilisation and ageing on muscle strength mitochondrial respiration (S. J. Edwards et al., 2020; Porter et al., 2015). The SUIT 008 protocol is specifically designed for use on permeabilised muscle fibres rather than isolated mitochondria and therefore provides a physiological relevant estimate of maximal mitochondrial respiration within that piece of muscle without the need for time consuming pre-analysis mitochondrial isolation steps (S. J. Edwards et al., 2020).

Both chambers of the Oxygraph-2K were rinsed with double distilled water (ddH_2O) twice before 2.5ml mitochondrial respiration medium (Mir05) was added and chambers closed for calibration. The stirrers within the chambers were switched on and the system was left for 30 minutes to allow oxygen (O_2) concentrations to stabilise.

During this period, the muscle biopsy was prepared for testing. Firstly, a 12-well culture plates was labelled containing the following:

- 1 x 1ml BIOPS
- 1 x 2ml BIOPS + 20µl saponin stock

- 3 x 1ml Mir05 (labelled Mir05, chamber A, chamber B)

The muscle biopsy was then transferred to a petri dish containing ice cold BIOPS and placed under a microscope. Using two 25G needles, any blood clots and connective tissue were cleared from the sample and the muscle was gently dissected into 2 – 5 mg bundles. The muscle bundles were then teased apart to expose individual fibres as shown in Figure 13. The individual muscle bundles were then transferred to the culture plate containing 1 ml BIOPS. The muscle biopsies were then transferred to the second well containing 2ml BIOPS + 20µl saponin stock and gently rocked on ice for 30 minutes. Following this, the muscle biopsies were transferred to the first well containing 1ml Mir05 and gently rocked on ice for a further 10 minutes. The first muscle bundle was dabbed dry using Kim tech tissue and then weighed on the fine balance before transferred to into the well containing 1ml Mir05 labelled chamber A. The weight of each bundle needs to be between 2 – 3 mg for processing in the Oxygraph-2K. The process was then repeated with the other muscle bundle and placed in chamber B.

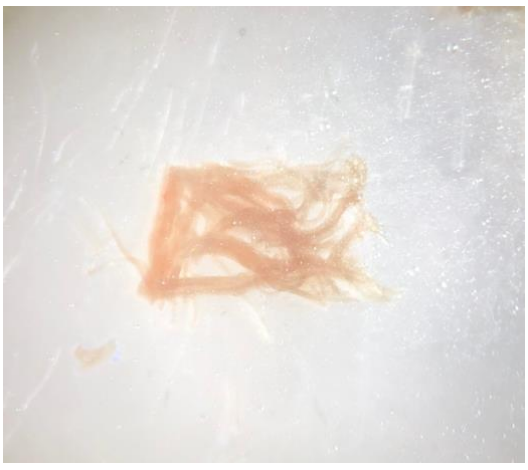


Figure 13 – An example of muscle fibres teased apart prior to transfer to culture plate.

Following the transfer of the muscle bundles into their respective chambers, sample information (experiment code, participant ID and biopsy number), along with the weight of the muscle bundles were entered into the software. Since the permeabilised muscle fibres are sensitive to oxygen supply, approximately 2ml of O_2 was injected to oxygenate each chamber. Once the trace is stable, there is a systematic addition of substrates, uncoupler and inhibitors to each chamber in order to assess mitochondrial respiration (Table 4).

Step	Substrate added	Action
1PM	15µl P/M solution (30µl pyruvate (P), 15µl malate (M))	Obtains complex I (CI)-linked LEAK respiration
2D	20µl ADP	Induces CI-linked OXOPHOS
2c	5µl CytC	Tests integrity of mitochondrial outer membrane
3G	10µl Glutamate	Allows observation of CI + II-linked OXPHOS
4S	20µl Succinate	Allows observation of CI + II-linked OXPHOS
5U	1µl of 1mM FCCP additions until max flux achieved	Maximum noncoupled respiration fuelled by complex II
6Rot	2µl Rotenone	Inhibits complex I and measures C II-linked OXOPHOS
7Ama	1µl Antimycin A	Inhibits C III

Table 4 - Summary of the substrates added to each chamber and the action they elicit on the mitochondria

The point of adding each substrate was marked on the trace for subsequent analysis and the traces left to stabilise prior to the addition of the next substrate (see figure 14). The O_2 concentration was continuously monitored, with further O_2 being injected into the chambers if the O_2 concentration dropped below a certain level. In the event of this happening, the trace was left to stabilise before continuation of the run. Once completed, the contents of the chambers were aspirated and the lids and chambers were washed three times with ddH_2O and increasing concentrations of ethanol (70%, 100%). This ensured that all inhibitors are completely removed for next use.

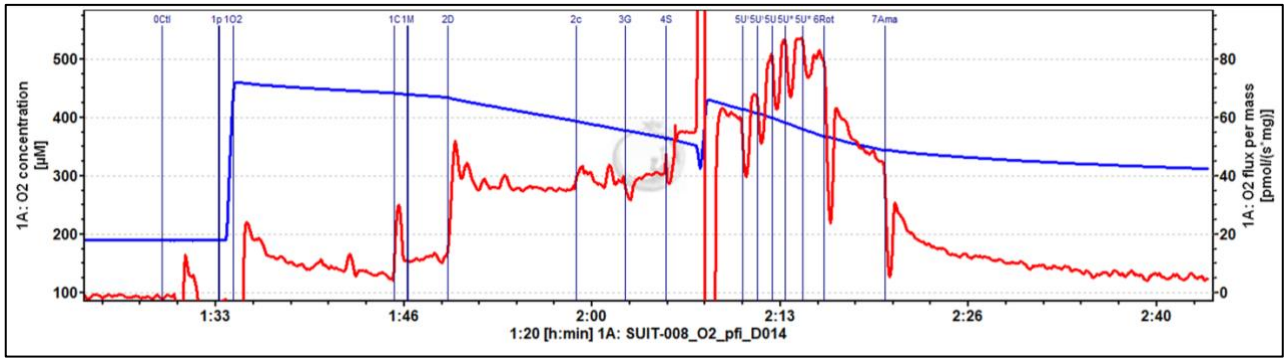


Figure 14 - An example trace using protocol SUIT-008_O2_pfi_DO14. The thick blue line represent O2 concentration whilst the thick red line represent mitochondrial activity. Each substrate addition is identified by a thin, vertical blue line

Digital analysis was carried out using the Open DatLab software. Between the points in which the various substrates were added, a mark was drawn manually over a section of time where the trace was most stable, as shown in Figure 15. The marks relate to each substrate addition (1PM, 2D,2c, 3G, 4S, 5U, 6Rot, 7Ama) and generated a range of data which was then exported for analysis. When assessing the mitochondrial response to the uncoupling agent, the maximum flux was marked to signify maximum respiration. Where possible, the time sections used were similar

in both chambers. The trace of both chambers was analysed, with data relating to specific flux (pmol/sec) and flux control ratio extracted and combined to form a median of both values.

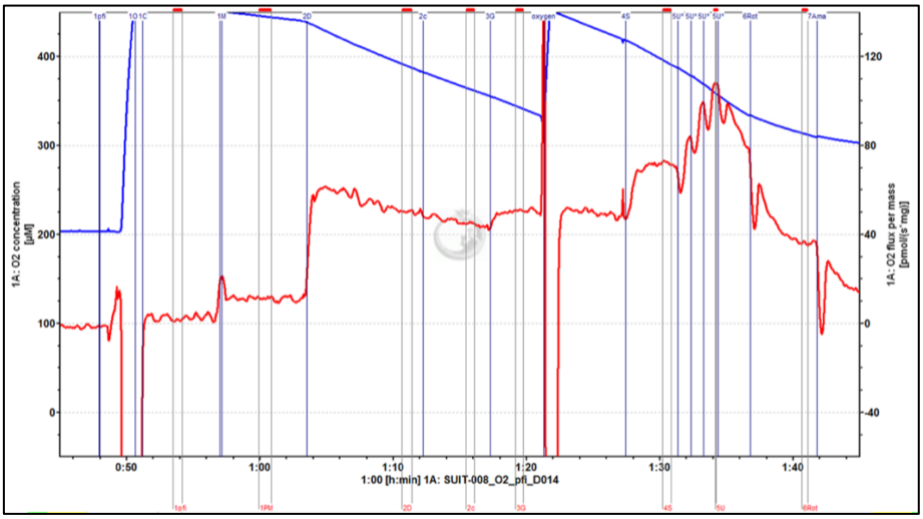


Figure 15 - An example of the analysis carried out on each of the traces. Between the addition of each substrate and prior to addition of a further substrate (thin, vertical blue lines), an area of the trace deemed most stable is marked for analysis (thin, vertical grey lines).

3.3.6 Statistical analysis

Descriptive data are presented as mean +/- standard error. Statistical significance was set at $p < 0.05$. Unpaired t tests were used for group analysis and Pearson's correlation analysis was performed to establish the relationship between measures of global physical, muscle and cellular function and age. We report the correlation coefficient (r) with a corresponding p value for the model. All analysis was conducted on GraphPad Prism version 9.

3.4 Results

3.4.1 Subject baseline characteristics

The baseline characteristics are shown in table 5. A total of 11 participants were recruited to the study and divided into young (18 – 40 years old) and old (>65 years

old) groups. There were no adverse events during the study and all subjects completed all aspects of the study day apart from the DXA scan. Due to a technical issue with the DXA scanner, 3 of the older participants were not able to have a scan. As such, due to low numbers available for comparison, DXA-derived analysis of body composition was not performed on the older participants.

Sex	**Age [a]**	**Height [b]**	**Weight [c]**	**BMI [d]**
Young participants				
Male	29	181	85.7	26.2
Male	33	193	79.9	21.5
Female	31	169.5	62.7	21.8
Male	40	175	94.9	31
Male	34	191	90.3	24.8
Male	32	186.5	91.5	26.3
	33.17 (± 3.76)	182.67 (± 9.23)	84.17 (± 11.73)	25.27 (± 3.30)
Old participants				
Male	78	182	83	25.1
Female	65	164	71	26.7
Female	68	168	64.2	22.7
Female	67	160	62	24.2
Male	72	181.5	100.8	30.6
	70 (± 5.15)	171.1 (± 10.13)	76.2 (± 16.0)	25.86 (± 3.02)
P value	<0.0001	0.788	0.365	0.773

Table 5 - Summary of patient characteristics. Descriptive data represented as mean (SD)

[a] years [b] centimetres, [c] kilograms, [d] kg/m^2.

3.4.2 Assessment of global physical function

For each of the three assessments of physical function, the older group recorded significantly lower measurements than the young (1-RM: 49.6kg vs. 25.62kg, $p<0.03$; HGS: 46.5kg vs. 31.6kg, $p<0.03$; VO_{2max}: 44.72ml/kg*min vs. 24.56 ml/kg*min, $p<0.01$), indicating an age-associated decline in global physical function. The difference between means (+/- SEM) were -23.98kg +/- 8.190, $p<0.05$ for 1-RM, -14.90kg +/- 5.906, $p < 0.05$ for HGS, and -20.16 +/- 5.541 ml/kg*min, $p<0.01$ for VO_{2max} when comparing old against young (Figure 16 a-c, respectively).

In relation to SPPB tests, all volunteers scored the maximum score of 12 and although it took the older participants longer to perform sit to stand, it was not statistically significant (9.61 sec vs. 10.13 sec, $p=0.65$).

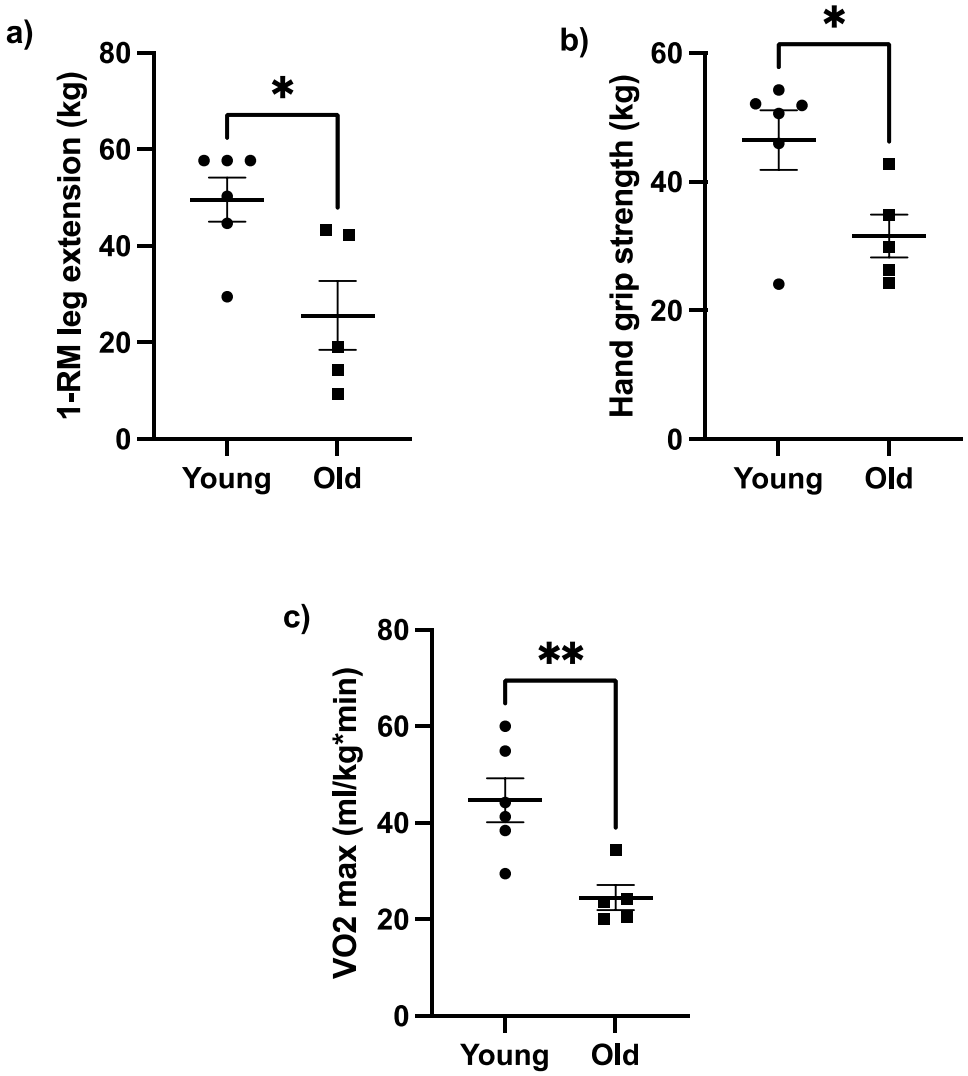


*Figure 16 - Measures of global physical fitness in young and old groups (a) 1-RM, b) HGS, c) VO2max). Graphs depict mean+/- SEM along with individual values. Analysis via unpaired students t-test. *=p<0.05, **=p<0.01.*

When assessing the relationship between different aspects of global physical function amongst all participants, with the exception of the young group HGS vs VO_{2max}, there was a significant association between HGS and both 1-RM and VO_{2max} in all groups (Figure 17 a-b respectively).

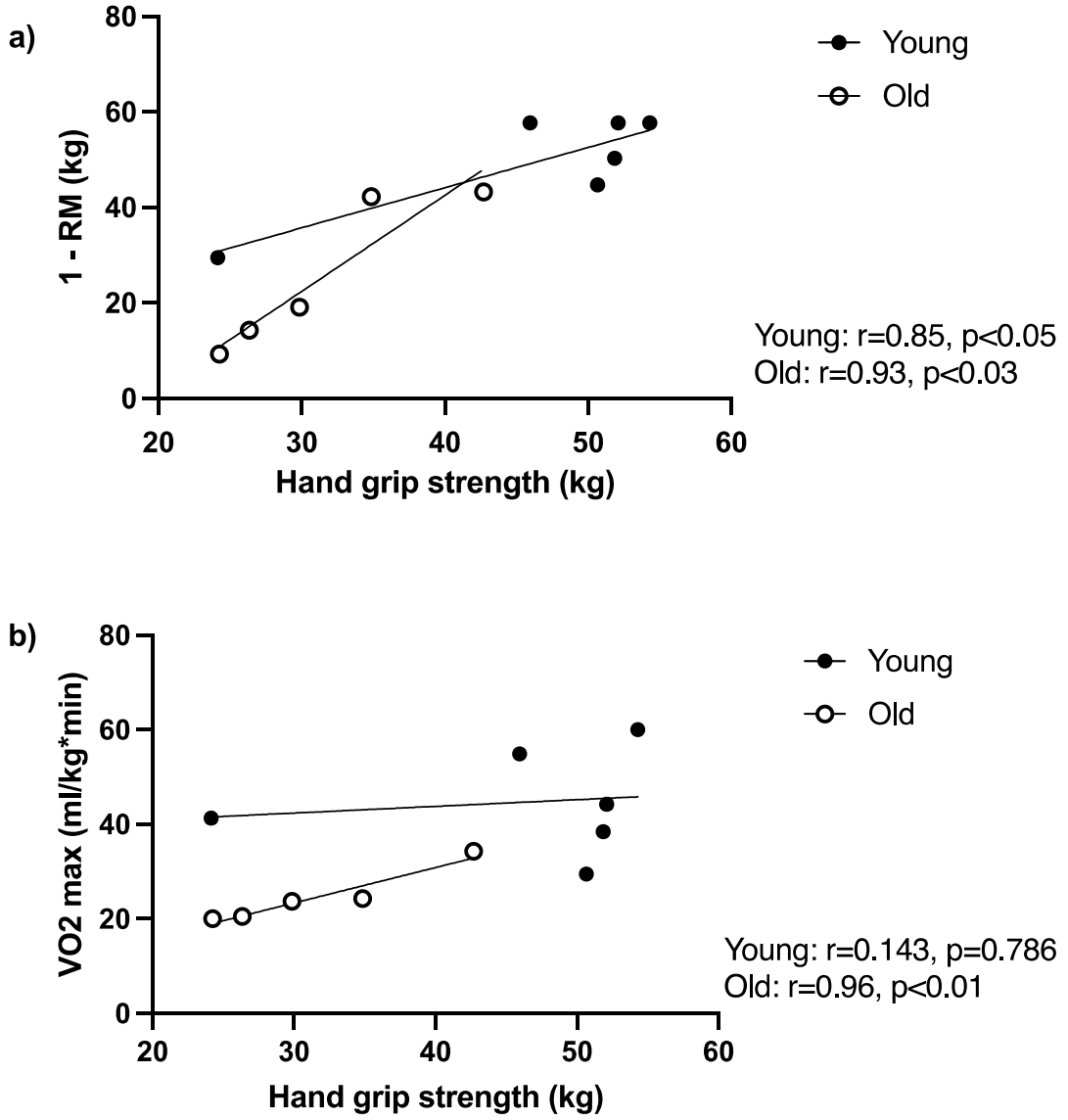


Figure 17 - Correlation between different aspects of global functional fitness (a) HGS and 1-RM and (b) HGS and VO_{2max} for young group (•) and old group (○). Line represents simple linear regression.

3.4.3 Assessment of skeletal muscle architecture

There was no significant difference between the two age groups MT, FL and CSA. Regarding PA, the younger groups' pennation angle was significantly larger than the older groups (17.23° vs 15.44°, $p < 0.05$) (Figure 18 a-d respectively).

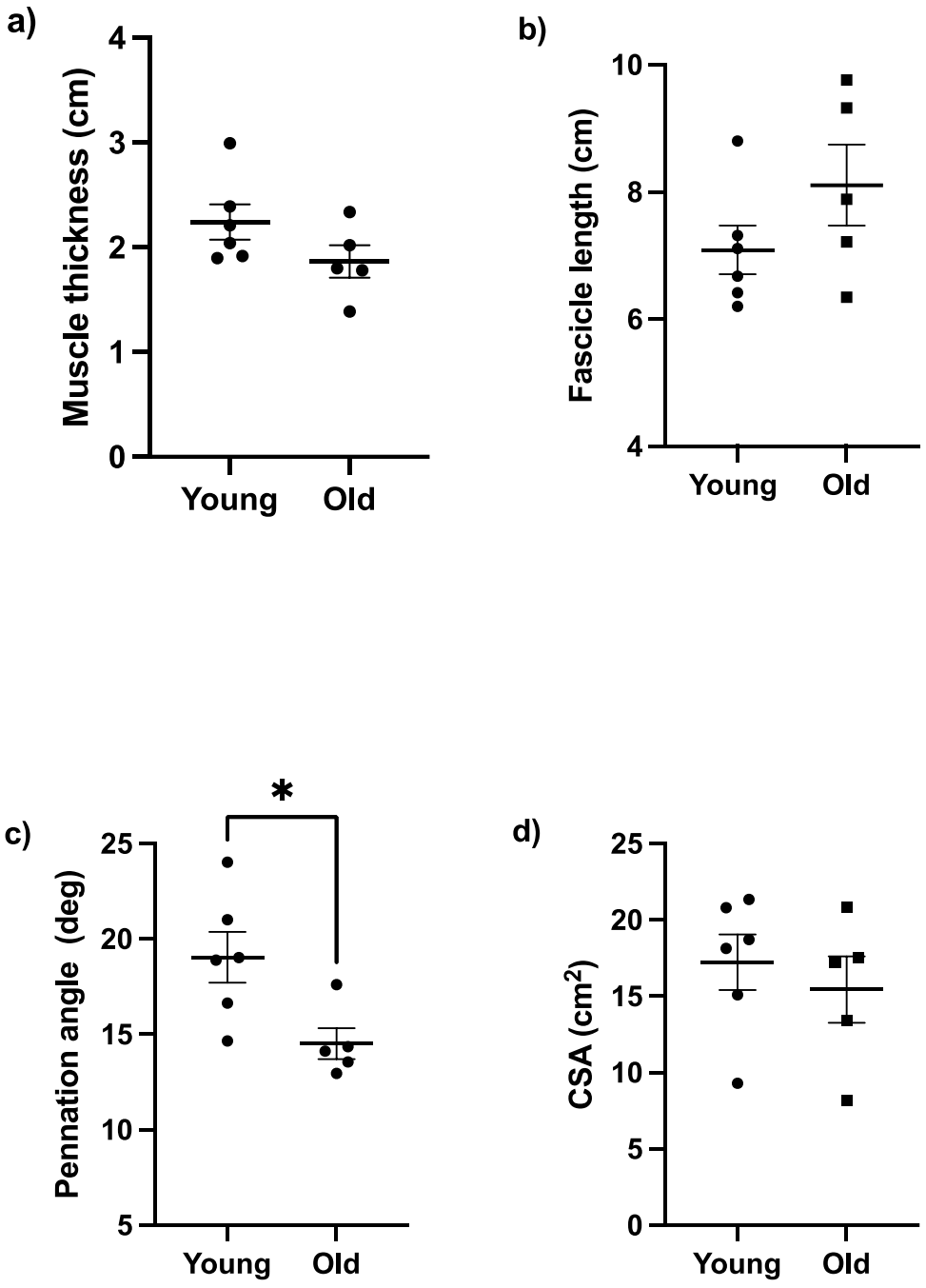


Figure 18 - Measures of muscle architecture in young and old groups. (a) Muscle thickness, b) Fascicle length, c) Pennation angle, d) CSA). Graphs depict mean+/- SEM along with individual values. Analysis via unpaired students t-test.

Since one of our aims was to assess the relationship between muscle mass and both global physical and cellular function, a measurement of muscle mass that was obtained for both groups was required. Unfortunately, due to DXA analysis not being

available for the older age group, the relationship between DXA derived lean leg mass and MT and CSA in the younger group was analysed in order to establish which measurement correlated best. Although there was a positive numerical correlation between DXA-derived lean leg mass and both CSA (Figure 19) and MT (Figure 20), neither reached significance. Despite this, because of the closer correlation, it was decided that CSA would be used as the measure for muscle mass that would be used in further analysis of results for both groups.

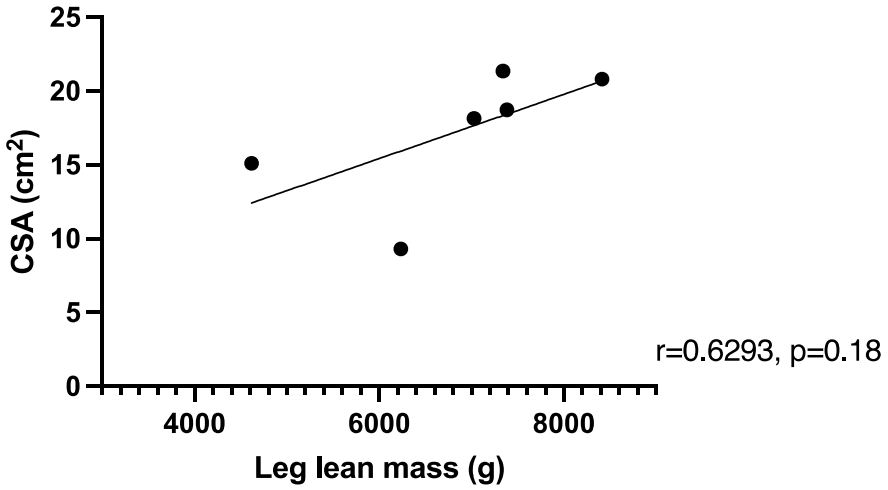


Figure 19 - Correlation between CSA and leg lean mass as measured by DXA. Line represents simple linear regression.

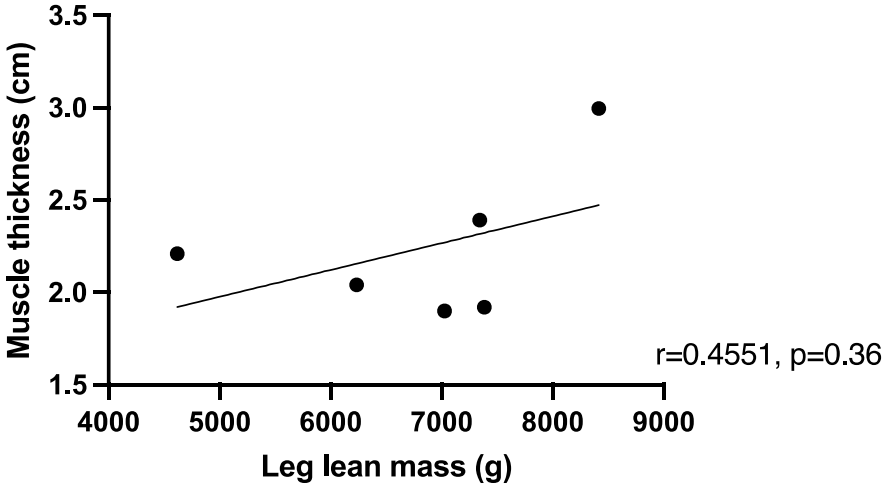


Figure 20 – Correlation between muscle thickness and leg lean mass as measured by DXA. Line represents simple linear regression.

3.4.4 Relationship between muscle architecture and global physical fitness

A numerical positive correlation was seen between CSA, our measurement of muscle mass, and 1-RM, HGS and VO_{2max} in both the young and old groups. However, no relationship reached statistical significance. (Figure 21, a-c respectively).

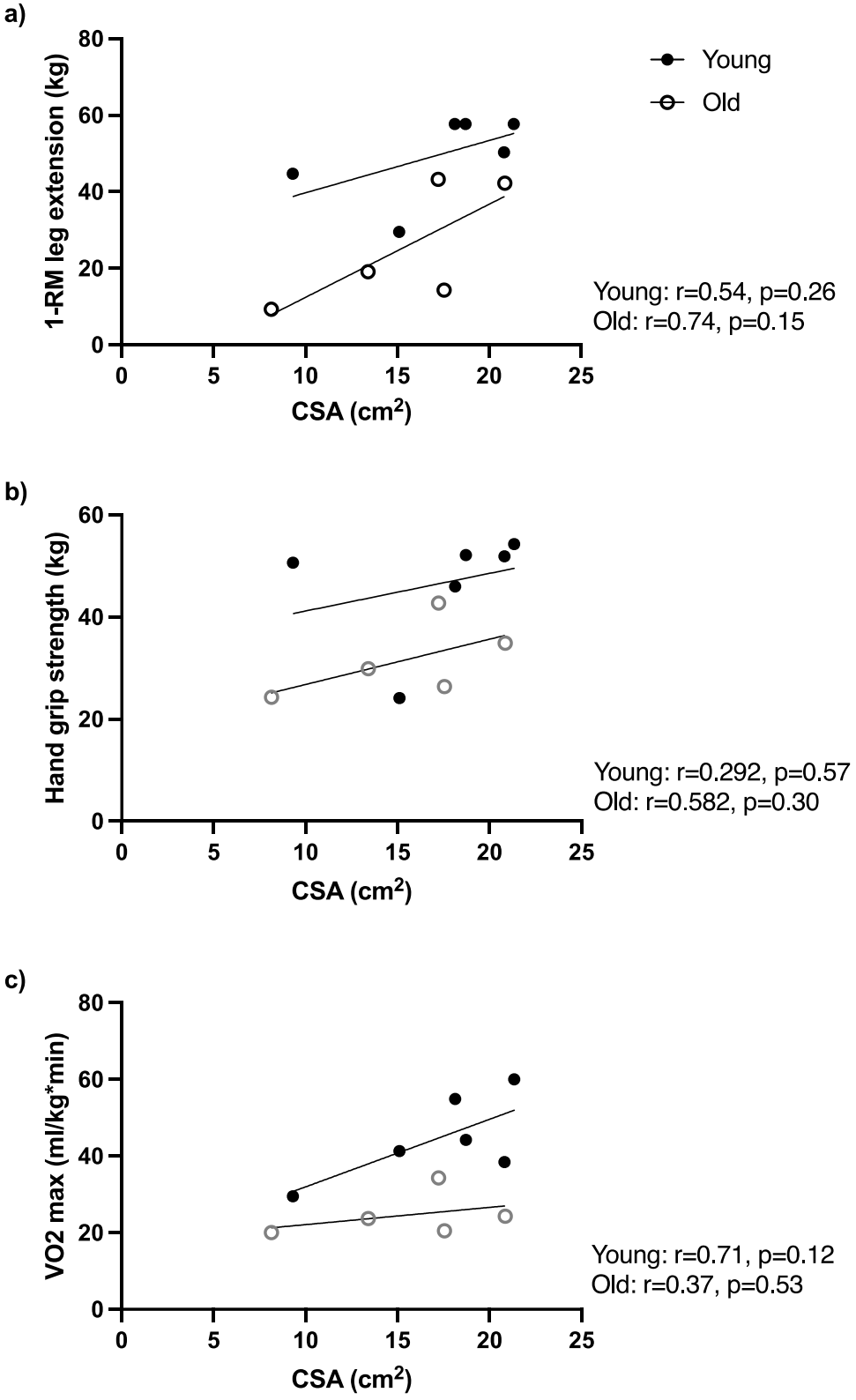


Figure 21 - Correlation between measure of muscle mass (CSA) against measures of global physical fitness. (a) 1-RM, b) HGS, c) VO_{2max}). Lines represent simple linear regression for young (•) and old (○) groups. Pearson's correlation and p values for each group and combined shown.

3.4.5 Assessment of changes in mitochondrial function in ageing

There was no significant difference between young and old groups mitochondrial response to each substrate addition (Figure 21). The mitochondrial response of the two groups were then analysed according to their respiratory response relating specific stages of the mitochondrial respiratory chain (enzyme complex I (CI) and II (CII) activity, the maximum oxidative phosphorylation capacity (mO) and the maximum uncoupled capacity (mU)). Again, there was no significant difference between the two groups (Figure 22).

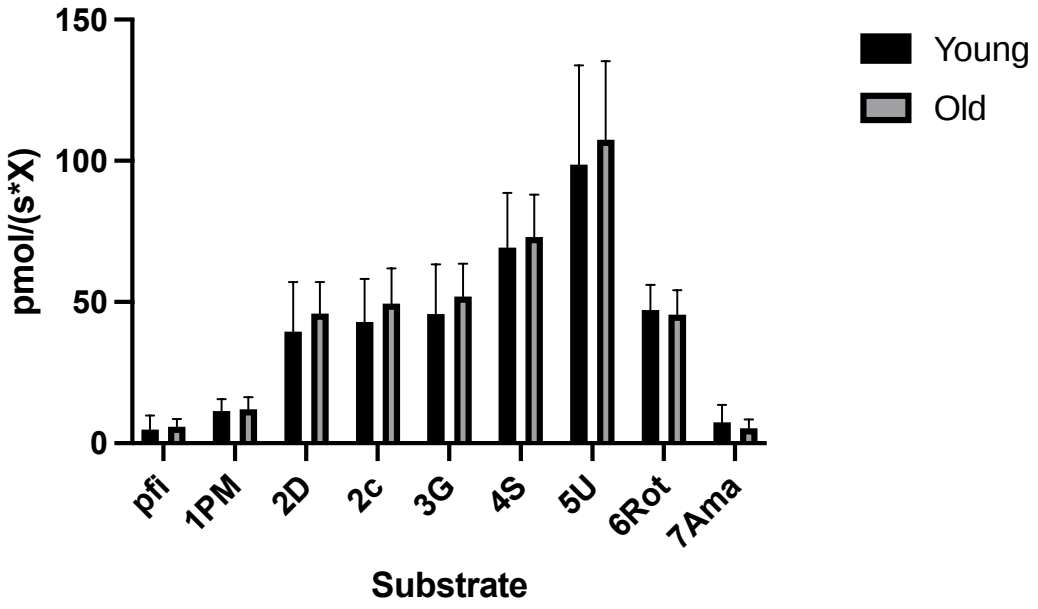


Figure 22 –Mean mitochondrial response to substrate addition as measure by O2k two chambered respirometer. Graph depicts mean +/- SEM for both young and old groups substrate following pooling of individual values with x axis indicating the cumulative substrate addition and y axis showing oxygen consumption.

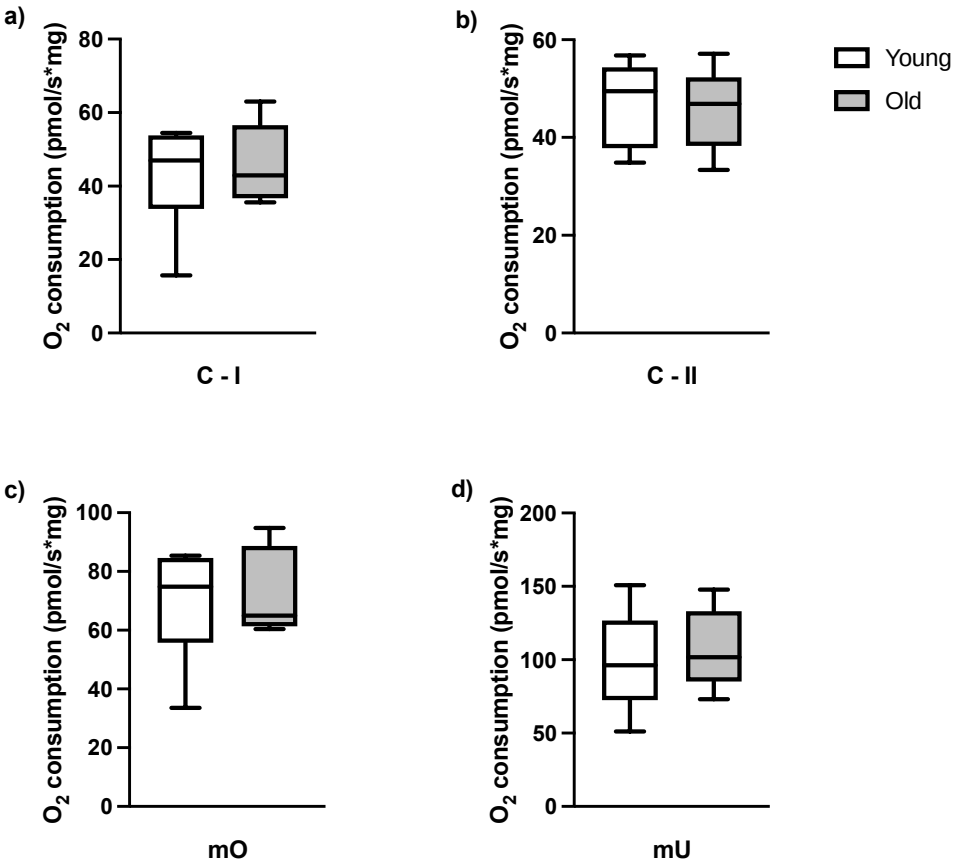


Figure 23 – Difference in mitochondrial respiratory function of a) Complex I activity, b) Complex II activity, c) Maximum oxidative phosphorylation capacity (mO) and d) Maximum uncoupled capacity (mU). Graph depicts mean +/- SEM for young and old groups. Analysis via 2-way ANOVA.

3.4.6 Relationship between mitochondrial activity and global physical function

There was no significant relationship between mitochondrial activity, measured as maximum oxidative phosphorylation capacity (mO) and maximum uncoupled capacity (mU), with measures of global fitness (1-RM, HGS and VO_{2max}). Interestingly, although not significant, a negative relationship was found in all but 1-RM in the young group (r=0.056) when mO was used as the measure of mitochondrial activity (Figure 23 a-c respectively), and in all groups when using mU as the measure of mitochondrial activity (Figure 24 a-c respectively).

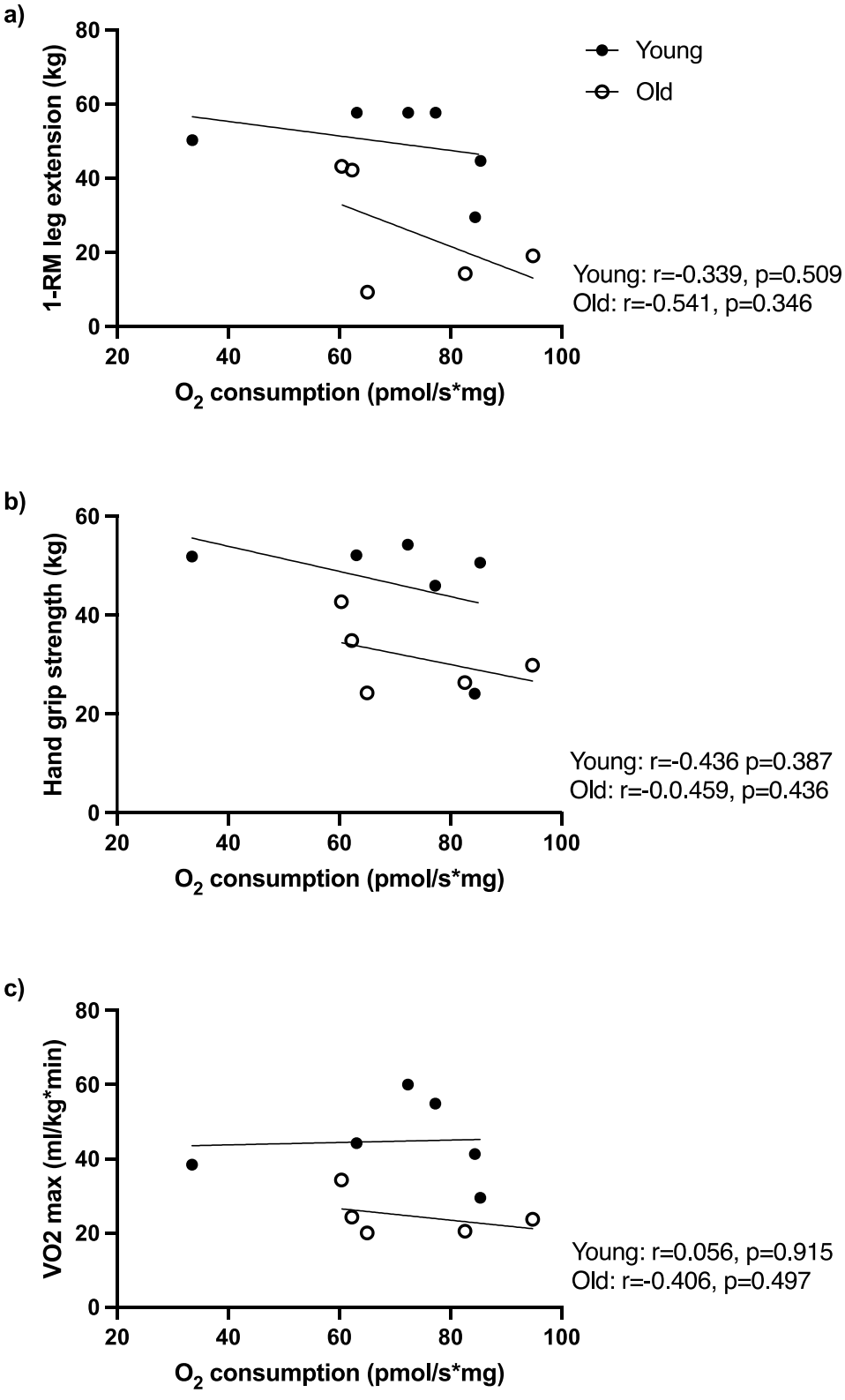


Figure 24 – Correlation between mitochondrial maximum oxidative capacity (mO) and measures of global physical fitness (a) 1-RM, b) HGS, c) VO_{2max}). Lines represent simple linear regression for young (•) and old (♀)groups.

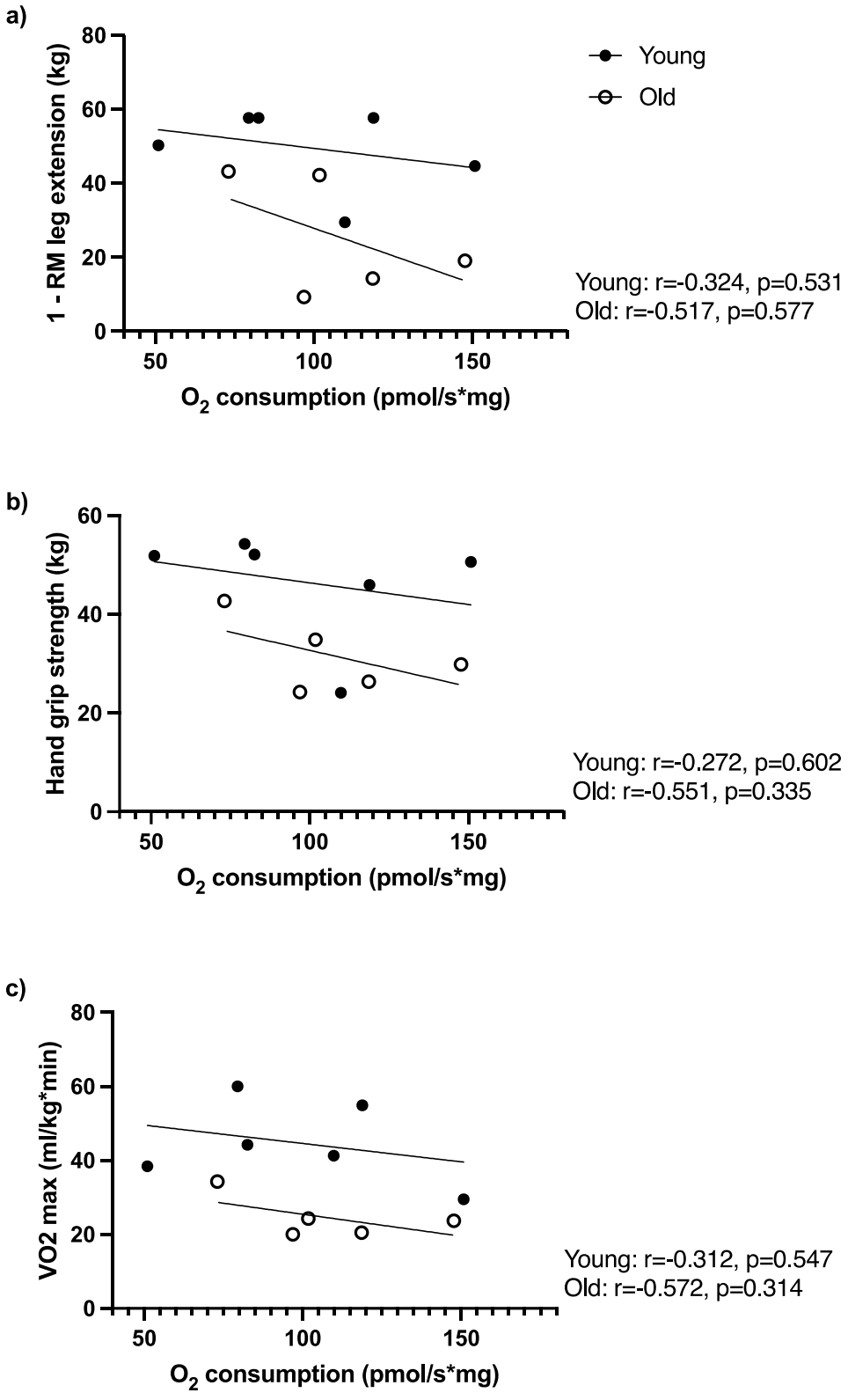


Figure 25 – Scatter plots demonstrating the relationship between mitochondrial maximum uncoupled capacity (mU) and measures of global physical fitness (a) 1-RM, b) HGS, c) VO_{2max}). Lines represent simple linear regression for young (•) and old (♀) groups.

3.5 Discussion

As expected, this study showed a significant decline in global physical fitness with advancing age, even in independent community-dwelling older adults. A reduction in CRF with advancing age has been established by a significant evidence base ((Fleg et al., 2005; Proctor & Joyner, 1997)) and as such, this aspect of our findings is largely confirmatory. As previously mentioned, some of the decline in physical fitness is related to physical inactivity (Roman et al., 2016). Since the participants in our older group all reported recreationally active lives, we postulate that the age associated reduction in global physical fitness is not solely the result of disuse, rather the intrinsic effect of ageing. This suggestion agrees with previously published studies that implicate intrinsic mechanisms, such as structural and functional changes in skeletal muscle (Distefano & Goodpaster, 2018), cardiorespiratory changes including reduced cardiac output and muscle blood flow (Milanović et al., 2013), and neuromuscular junction function loss (Joseph et al., 2016).

Of potential clinical relevance, we saw an age-associated reduction in 1-RM of knee extension and HGS which, as alluded to above, is unlikely explained by reduced physical activity. Muscle strength has been shown to be a predictor of numerous health outcomes such as mortality, future disability, post-operative complications, and resource usage (Bohannon, 2008; R. Cooper et al., 2010) with both isometric measures having been shown to correlate with one another (Bohannon et al., 2012) as a reflection of overall limb muscle strength. Additionally, with the wealth of literature relating CRF to all-cause mortality (M. K. Edwards & Loprinzi, 2016; Snowden et al., 2013), it was promising that our study found positive correlation

between HGS with the assessment of both knee extensor strength and CRF. Based on these findings, HGS could be utilised as a cheap, easy method for predicting CRF in various clinical settings.

It is well accepted that there is an age related reduction in muscle mass (C. Cooper et al., 2013). When assessing the effect of age on muscle architecture, we found a significant reduction in PA only, with MT and CSA numerically, but not significantly, lower in the older age group. The direction of change represented by these results is in agreement with much of the published literature, which shows a degree of alteration in these measures as we age (M. V. Narici et al., 2003; Ticinesi et al., 2017). That we were unable to observe significant differences in MT and CSA is likely related to our small sample size since previous studies have shown age related differences in muscle architecture via ultrasound, with CSA of quadricep muscles being 25 – 33% different between young and older participants (M. V. Narici et al., 2003).

Along with MT, FL and PA represent the spatial arrangement of the muscle fibres within the muscle. PA represents muscle fibre arrangement in parallel, and has been shown to be linked with muscle strength by influencing mechanical efficacy of force transmission at the tendons and aponeurosis (Mpampoulis et al., 2021). FL represents muscle fibre arrangement in series and is more linked with power performance, with longer fascicles allowing faster contractions speeds. Both have been found to decrease with age (Marco V. Narici et al., 2021). Although not significant, the difference in FL observed between our young and older groups

suggested the older group had longer fascicles, a result that was not expected, nor can it be explained. Previous studies have shown that a reduction in both PA and FL could contribute to the reduced CSA seen in ageing (M. V. Narici et al., 2003). As such, this finding must be interpreted with caution, especially as no significant difference was found and a small sample size used. In addition, the role both PA and FL play in terms of clinical relevance has been questioned, with several studies suggesting they are not significantly related to functional muscle measurements and suggesting the use of MT or CSA instead (Kuyumcu et al., 2016; Strasser et al., 2013).

Muscle ultrasound measurements, such as MT and CSA, have been shown to have high concordance with both DXA and MRI-derived predicted values of muscle mass (Martino V. Franchi et al., 2020; Ticinesi et al., 2017). This suggests that ultrasonography could be a useful tool for the assessment of muscle mass, especially in the absence of research facilities. In agreement with existing research, our comparison of DXA values for lean muscle mass in the young group positively correlated with both MT and CSA values obtained using ultrasound. Although we failed to reach significance for either of these correlations, likely the result of the small sample size, the findings need to be further investigated in the future including data from older adults. Furthermore, by using an ultrasound-based measure of CSA, we were able to show a positive correlation between muscle mass and HGS, 1-RM and VO_{2max} in both the young and older groups. Again although these correlations failed to reach statistical significance, the findings in agreement with other studies that have shown CSA to be associated with measures of global physical function (C. Cooper et al., 2013).

Contrary to the findings relating to the assessments of physical function, we did not find any significant difference in mitochondrial respiratory capacity in response to substrate addition between the age groups. This differs from prior research, in which a substantial fall in several pathways of ATP synthesis, including anaerobic glycolysis and oxidative phosphorylation, have been shown to be impaired in ageing skeletal muscle (Tieland et al., 2018; Trounce et al., 1989). Furthermore, our results also found no link between a mitochondrial respiration capacity and measures of global physical function, with our results showing that participants with the highest measures of global physical fitness had some of the lowest mitochondrial respiration responses. These conclusions must be taken with caution due to our low sample size and lack of statistically significant results. The results do however highlight challenges related to researching the true impact of ageing on mitochondrial dysfunction, with some previous studies supporting a decrease in mitochondrial capacity (Porter et al., 2015; Tonkonogi et al., 2003; Trounce et al., 1989), whilst others have failed to find such association (Gouspillou et al., 2014; Hütter et al., 2007; Larsen et al., 2012).

That we report no relationship between mitochondrial respiratory capacity and global physical fitness or age may be, in part, related to our selection of physically active, healthy older participants. Recent studies have suggested that maintaining physical activity into old age mitigates the negative effects usually seen with ageing, with physically active young and old groups having similar protein markers of mitochondrial content and respiration (S. Choi et al., 2016; St-Jean-Pelletier et al., 2017). Despite this, VO_{2peak} has been shown to be lower in older groups, even with

similar mitochondrial energetics (Beere et al., 1999), suggesting the effect age has on other physiological determinants, such as cardiac output and muscle perfusion, cannot be fully reversed by physical activity.

3.5.1 Limitations

There are several limitations to this study which should be acknowledged, the main being the low sample size resulting in statistically underpowered results. Despite significance being reached in demonstrating age-related declines in global physical fitness and a relationship between different measures of global physical function, we failed to show a significant association between global physical fitness and muscle mitochondrial function, or any age-associated difference in mitochondrial function. For any firm conclusions to be made, a larger sample size is needed.

Although self-selection bias can occur in all age-groups when individuals volunteer for research, health problems and functional limitations increase with age (Golomb et al., 2012). As such, older age research participants are less likely to represent their age group than in younger cohorts. This is particularly relevant in a study involving physical function tests as it may deter more sedentary individuals. To mitigate this limitation, we would again need to increase the number of participants and purposefully seek older adults who are representative of their age-group (i.e., in respect to comorbidities, polypharmacy and functional status).

Finally, in the OROBOROS method used, we added substrate quantities more than those seen *in vivo* conditions. Additionally, the use of isolated mitochondria has been shown to exaggerate the observed deficit in mitochondrial function (Distefano et al.,

2018). As such, these results do not necessarily reflect what occurs in the human body

3.6 Conclusion

The study shows that there is clearly a decline in global physical function associated with advancing age that is unlikely linked to physical inactivity. Unfortunately, we failed to show the anticipated links between the physical function and both muscle and mitochondrial function, although this is likely due to a small sample size. Further work is required, using a greater number of participants of varying physical fitness status, to gain a greater understanding of the impact not only age, but also level of activity has on muscle and mitochondrial function.

4 Discussion

With the worldwide population living longer, the importance of preserving health and quality of life with advancing age is vital. It is well established that there is a progressive change in muscle mass, metabolism and functional capacity in ageing, which is associated with loss of independence and increased total mortality (Shur et al., 2021). Despite this, the complex mechanisms underpinning sarcopenia and the associated detrimental impacts are not yet fully understood. With many of the physiological features seen in ageing also being associated with inactivity, increasing the physical activity of older individuals is now seen as a crucial preventative target in maintaining muscle mass and function, and subsequent overall health. One area that remains poorly understood is the role mitochondrial dysfunction plays in skeletal muscle ageing – the focus of this thesis.

Our aim for the healthy volunteer study presented in Chapter 3 of this thesis was to build on existing evidence demonstrating the differences in global physical and muscle functions with ageing, as well as attempt to gain more knowledge regarding possible changes in mitochondrial respiration with advancing age. Our results agreed with other studies reporting a significant difference between global physical function measures between young and older participants. We also showed a numerical, but not statistically significant, difference in measurements of muscle architecture (with statistical analysis likely limited by a small sample size), and a positive correlation between muscle CSA and measures of global fitness. These correlations suggest a clear relationship between skeletal muscle mass and function, however given the fact muscle strength is lost much more rapidly than muscle mass (Mitchell et al., 2012), it is clear that other factors, such as mitochondrial function, play an important role.

The concept of age-related mitochondrial dysfunction remains under considerable debate, and as such so does its implication in age-associated reduction in physical function. Our healthy volunteer study, although underpowered for this analysis, appeared to show what recent studies have also indicated - that physical activity, rather than chronological age, influences mitochondrial function (Distefano et al., 2018). Since our older participants all led active lifestyles, our results are in line with two recent reports showing that physically active young and old groups had similar mitochondrial respiration (Gouspillou et al., 2014; St-Jean-Pelletier et al., 2017). This contrasts other work by both Trounce et al and Porter et al., which showed significant negative correlation between mitochondrial respiratory capacity and age (Porter et al., 2015; Trounce et al., 1989). These differing opinions highlight the complexity when trying to draw firm conclusions regarding the true impact ageing has on mitochondrial function, mainly due to the multiple covariates (including physical activity, cardiovascular fitness and adiposity) that also affect mitochondrial function (Distefano et al., 2018). A future study with a greater number of participants, with varying levels of habitual physical activity, would be needed to gain a greater insight into possible changes seen with age and confirm the seemingly greater influence of physical activity status.

Moving on to consider the impact of age-associated disease on skeletal muscle mass and function, our systematic review and meta-analysis performed in Chapter 2 of the thesis showed that pre-existing sarcopenia in patients with LARC resulted in a significantly shortened OS, as well as an association with shorter DFS. Since the underlying aetiology of sarcopenia is multifactorial and complex, especially on a background of disease, there remains very few therapeutic options. This has led to an increasing focus on preventing muscle loss in the older population to improve

clinical outcomes. Although it has been shown that physical exercise training prior to major surgery can improve cardiorespiratory fitness (Berkel et al., 2018; Boereboom et al., 2016), there is less robust evidence demonstrating that prehabilitation increase muscle mass or improves mitochondrial function. Additionally, very little is known about the impact of nCRT has on skeletal muscle mitochondria.

The findings presented in this thesis illustrate a large scope for potential future research that can be applied in the clinical setting. Our research group has previously shown that patients with colorectal cancer demonstrate reduced muscle protein synthesis (MPS) and trend towards increased muscle protein breakdown (MPB), both of which were reversed following surgical resection (Williams et al., 2012). This, in combination with studies showing a mixed response to preoperative exercise programs in individuals with various cancers (J. E. M. Blackwell et al., 2020; Boereboom et al., 2019), suggests that the pathological and systemic effects of various forms of cancers effect global physical fitness and muscle function differently. What has so far been poorly investigated is the impact of cancer on mitochondrial function, quantity and its relationship with overall skeletal muscle mass and global physical fitness. In animal studies, the presence of cancer has been shown to reduce mitochondrial content in skeletal muscle. Further to this, animals treated with chemotherapy agents have shown long term impairment in mitochondrial respiration and increase ROS within skeletal muscle (Gouspillou et al., 2015). The interest in this field is growing in human studies, with study groups in both Sweden and Taiwan registering clinical trials investigating the effects of different forms of exercise programs have on skeletal muscle mitochondrial function in patients receiving neo-adjuvant chemotherapy prior to surgical resection (Clinicaltrials.gov, 2021).

No study has yet investigated the mitochondrial function of patients with various cancer types, who all undergo chemotherapy prior to surgical resection. A study of this nature would build on our understanding of why certain cancer types appear to respond better to exercise programs than others and determine the impact of cancer and its treatment has on mitochondrial function. In addition, by following these patients throughout their treatment pathway, the results gained could provide enhanced understanding at to what other changes occur to skeletal muscle, both structurally and on a cellular level. A full study design for this proposed future work in the form of a research ethics application can be found in Appendix B

In conclusion, this thesis builds on existing knowledge relating to the impact of age has on skeletal muscle mass and function, as well as providing preliminary data on the impact ageing has on mitochondrial function using a contemporary analysis method that has not been used by our research group previously. Finally, based on the findings in systematic review and meta-analysis, along with the healthy volunteer study outlined in the chapters of this thesis, a future study design that could help answer questions relating to the relationship of mitochondrial dysfunction in age and cancer is presented in Appendix B.

5 Appendix

5.1 Appendix A

Search Strategy HDAS Medline

NEOPLASMS/ OR exp NEOPLASMS/ OR exp NEOPLASMS/su OR exp "ANUS NEOPLASMS"/ OR exp "BILIARY TRACT NEOPLASMS"/ OR exp "BILE DUCT NEOPLASMS"/ OR exp "CECAL NEOPLASMS"/ OR exp "COLONIC NEOPLASMS"/ OR exp "COLORECTAL NEOPLASMS"/ OR exp "DIGESTIVE SYSTEM NEOPLASMS"/ OR exp "GASTROINTESTINAL NEOPLASMS"/ OR exp "INTESTINAL NEOPLASMS"/ OR exp "LIVER NEOPLASMS"/ OR exp "PANCREATIC NEOPLASMS"/ OR exp "PELVIC NEOPLASMS"/ OR exp "PROSTATIC NEOPLASMS"/ OR exp "RECTAL NEOPLASMS"/ OR "SIGMOID NEOPLASMS"/ OR exp "STOMACH NEOPLASMS"/ OR exp "UROGENITAL NEOPLASMS"/ OR exp "UROLOGIC NEOPLASMS"/ OR exp "NEOPLASM METASTASIS"/ OR exp "ABDOMINAL NEOPLASMS"/ OR (abdo* OR cancer OR metas* OR malign*).ti,ab

AND

"NEOADJUVANT THERAPY"/ OR exp "COMBINED MODALITY THERAPY"/ OR (neoadjuvant ADJ2 therapy).ti,ab OR (chemorad*).ti,ab OR ("neodjuvant chemotherapy").ti,ab OR (neodjuvant chemotherapy).ti,ab

AND

exp COLECTOMY/ OR exp PROCTECTOMY/ OR exp "SURGICAL PROCEDURES, OPERATIVE"/ OR (excis* OR resect* OR surg*).ti,ab

AND
((musc*).ti,ab AND (function* OR strength OR mass OR loss OR wast* OR power OR deplet*).ti,ab) OR ((skeletal).ti,ab AND (function* OR strength OR mass OR loss OR wast* OR power OR deplet*).ti,ab) OR (sarcopenia).ti,ab OR SARCOPENIA/ OR exp "MUSCULAR ATROPHY"/ OR exp "MUSCLE CONTRACTION"/ OR exp "MUSCLE RELAXATION"/ OR exp "MUSCLE STRENGTH"/ OR exp "MUSCULOSKELETAL AND NEURAL PHYSIOLOGICAL PHENOMENA"/

AND
(prognosis OR surviv*).ti,ab OR exp "TREATMENT OUTCOME"/ OR exp PROGNOSIS/ OR "DISEASE-FREE SURVIVAL"/ OR "PROGRESSION-FREE SURVIVAL"/ OR "REMISSION INDUCTION"/

AND

exp "CASE-CONTROL STUDIES"/ OR (observational study).ti,ab,pt OR (case-control stud*).ti,ab,pt OR (randomized controlled trial OR randomised controlled trial).ti,ab,pt OR ("retrospective stud*").ti,ab,pt OR ("systematic review").ti,ab,pt OR ("review").ti,ab,pt OR ("meta analysis").ti,ab,pt OR ("cohort stud*").ti,ab,pt OR "OBSERVATIONAL STUDY"/ OR exp "RANDOMIZED CONTROLLED TRIAL"/ OR

"SYSTEMATIC REVIEW"/ OR exp REVIEW/ OR "META-ANALYSIS"/ OR "RETROSPECTIVE STUDIES"/ OR "COHORT STUDIES"/

Results: 760

#	Database	Search term	Results
1	Medline	NEOPLASMS/	428375
2	Medline	exp NEOPLASMS/	3380357
3	Medline	exp NEOPLASMS/su	539586
4	Medline	exp "ANUS NEOPLASMS"/	6413
5	Medline	exp "BILIARY TRACT NEOPLASMS"/ OR exp "BILE DUCT NEOPLASMS"/	28742
6	Medline	exp "CECAL NEOPLASMS"/	5610
7	Medline	exp "COLONIC NEOPLASMS"/	75179
8	Medline	exp "COLORECTAL NEOPLASMS"/	203568
9	Medline	exp "DIGESTIVE SYSTEM NEOPLASMS"/	626437
10	Medline	exp "GASTROINTESTINAL NEOPLASMS"/	382767
11	Medline	exp "INTESTINAL NEOPLASMS"/	229773
12	Medline	exp "LIVER NEOPLASMS"/	166987
13	Medline	exp "PANCREATIC NEOPLASMS"/	76847
14	Medline	exp "PELVIC NEOPLASMS"/	7099
15	Medline	exp "PROSTATIC NEOPLASMS"/	129563
16	Medline	exp "RECTAL NEOPLASMS"/	47680
17	Medline	"SIGMOID NEOPLASMS"/	4607
18	Medline	exp "STOMACH NEOPLASMS"/	97137

19	Medline	exp "UROGENITAL NEOPLASMS"/	515660
20	Medline	exp "UROLOGIC NEOPLASMS"/	135583
21	Medline	exp "NEOPLASM METASTASIS"/	205276
22	Medline	exp "ABDOMINAL NEOPLASMS"/	34008
23	Medline	(abdo* OR cancer OR metas* OR malign*).ti,ab	2557432
24	Medline	(1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23)	4266580
25	Medline	"NEOADJUVANT THERAPY"/ OR exp "COMBINED MODALITY THERAPY"/	266022
26	Medline	(neoadjuvant ADJ2 therapy).ti,ab	8897
27	Medline	(chemorad*).ti,ab	84
28	Medline	("neodjuvant chemotherapy").ti,ab	9
29	Medline	(neodjuvant chemotherapy).ti,ab	18
30	Medline	(25 OR 26 OR 27 OR 28 OR 29)	269792
31	Medline	exp COLECTOMY/	20948
32	Medline	exp PROCTECTOMY/	3978
33	Medline	exp "SURGICAL PROCEDURES, OPERATIVE"/	3197525
34	Medline	(excis* OR resect* OR surg*).ti,ab	2154938
35	Medline	(31 OR 32 OR 33 OR 34)	4306359
36	Medline	(musc*).ti,ab	788042
37	Medline	(function* OR strength OR mass OR loss OR wast* OR power OR deplet*).ti,ab	5744176
38	Medline	(skeletal).ti,ab	200614

39	Medline	(36 AND 37)	278716
40	Medline	(37 AND 38)	73804
41	Medline	(sarcopenia).ti,ab	8148
42	Medline	SARCOPENIA/	4684
43	Medline	exp "MUSCULAR ATROPHY"/	15635
44	Medline	exp "MUSCLE CONTRACTION"/	200184
45	Medline	exp "MUSCLE RELAXATION"/	29854
46	Medline	exp "MUSCLE STRENGTH"/	34575
47	Medline	exp "MUSCULOSKELETAL AND NEURAL PHYSIOLOGICAL PHENOMENA"/	2400378
48	Medline	(39 OR 40 OR 41 OR 42 OR 43 OR 44 OR 45 OR 46 OR 47)	2603263
49	Medline	(prognosis OR surviv*).ti,ab	1419450
50	Medline	exp "TREATMENT OUTCOME"/ OR exp PROGNOSIS/	1693647
51	Medline	"DISEASE-FREE SURVIVAL"/ OR "PROGRESSION-FREE SURVIVAL"/ OR "REMISSION INDUCTION"/	116419
52	Medline	exp "CASE-CONTROL STUDIES"/	1115615
53	Medline	(observational study).ti,ab,pt	191316
54	Medline	(case-control stud*).ti,ab,pt	124608
55	Medline	(randomized controlled trial OR randomised controlled trial).ti,ab,pt	586810
56	Medline	("retrospective stud*").ti,ab,pt	164298
57	Medline	("systematic review").ti,ab,pt	193115
58	Medline	("review").ti,ab,pt	3343679

59	Medline	("meta analysis").ti,ab,pt	186839
60	Medline	("cohort stud*").ti,ab,pt	218707
61	Medline	"OBSERVATIONAL STUDY"/	0
62	Medline	(49 OR 50 OR 51)	2699616
63	Medline	exp "RANDOMIZED CONTROLLED TRIAL"/	0
64	Medline	"SYSTEMATIC REVIEW"/	0
65	Medline	exp REVIEW/	0
66	Medline	exp REVIEW/	0
67	Medline	"META-ANALYSIS"/	0
68	Medline	"RETROSPECTIVE STUDIES"/ OR "COHORT STUDIES"/	1057894
69	Medline	(52 OR 53 OR 54 OR 55 OR 56 OR 57 OR 58 OR 59 OR 60 OR 61 OR 63 OR 64 OR 65 OR 66 OR 67 OR 68)	5358100
70	Medline	(24 AND 30 AND 35 AND 48 AND 62)	1469
71	Medline	(69 AND 70)	760

Search Strategy HDAS Embase

exp NEOPLASM/ OR exp "ANUS TUMOR"/ OR exp "BILIARY TRACT TUMOR"/ OR exp "CECUM TUMOR"/ OR exp "COLON TUMOR"/ OR exp "COLORECTAL TUMOR"/ OR exp "DIGESTIVE SYSTEM TUMOR"/ OR exp "GASTROINTESTINAL TUMOR"/ OR exp "INTESTINE TUMOR"/ OR exp "LIVER TUMOR"/ OR exp "PANCREAS TUMOR"/ OR exp "PELVIS TUMOR"/ OR exp "PROSTATE TUMOR"/ OR exp "RECTUM TUMOR"/ OR exp "STOMACH TUMOR"/ OR exp "UROGENITAL TRACT TUMOR"/ OR exp "URINARY TRACT TUMOR"/ OR exp "LARGE INTESTINE TUMOR"/ exp "INTESTINE TUMOR"/ OR exp METASTASIS/ OR exp "ABDOMINAL TUMOR"/ OR (abdo* OR cancer OR metas* OR malign*).ti,ab

AND

exp "NEOADJUVANT THERAPY"/ OR "MULTIMODALITY CANCER THERAPY"/ OR (neoadjuvant ADJ2 therapy).ti,ab OR (chemorad*).ti,ab OR ("neodjuvant chemotherapy").ti,ab OR (neodjuvant chemotherapy).ti,ab

AND
exp "COLON RESECTION"/ OR exp "RECTUM RESECTION"/ OR exp SURGERY/ OR (excis* OR resect* OR surg*).ti,ab

AND
((musc*).ti,ab AND (function* OR strength OR mass OR loss OR wast* OR power OR deplet*).ti,ab) OR ((skeletal).ti,ab AND (function* OR strength OR mass OR loss OR wast* OR power OR deplet*).ti,ab) OR (sarcopenia).ti,ab OR SARCOPENIA/ OR exp "MUSCULAR ATROPHY"/ OR exp "MUSCLE CONTRACTION"/ OR exp "MUSCLE RELAXATION"/ OR exp "MUSCLE STRENGTH"/

AND
(prognosis OR surviv*).ti,ab OR exp "TREATMENT OUTCOME"/ OR exp PROGNOSIS/ OR "DISEASE COURSE"/ OR exp SURVIVAL/ OR exp "DISEASE FREE SURVIVAL"/ OR exp REMISSION/

AND
exp "CASE CONTROL STUDY"/ OR (observational study).ti,ab,pt OR (case-control stud*).ti,ab,pt OR (randomized controlled trial OR randomised controlled trial).ti,ab,pt OR ("retrospective stud*").ti,ab,pt OR ("systematic review").ti,ab,pt OR ("review").ti,ab,pt OR ("meta analysis").ti,ab,pt OR ("cohort stud*").ti,ab,pt OR exp "OBSERVATIONAL STUDY"/ OR exp "RANDOMIZED CONTROLLED TRIAL"/ OR "SYSTEMATIC REVIEW"/ OR exp REVIEW/ OR exp "META ANALYSIS"/ OR "RETROSPECTIVE STUDY"/ OR "COHORT ANALYSIS"/

Results: 357

#	Database	Search term	Results
1	EMBASE	exp NEOPLASM/	4574377

2	EMBASE	exp "ANUS TUMOR"/	9927
3	EMBASE	exp "BILIARY TRACT TUMOR"/	50699
4	EMBASE	exp "CECUM TUMOR"/	2276
5	EMBASE	exp "COLON TUMOR"/	331718
6	EMBASE	exp "COLORECTAL TUMOR"/	30069
7	EMBASE	exp "DIGESTIVE SYSTEM TUMOR"/	1023354
8	EMBASE	exp "GASTROINTESTINAL TUMOR"/	27209
9	EMBASE	exp "INTESTINE TUMOR"/	414447
10	EMBASE	exp "LIVER TUMOR"/	287123
11	EMBASE	exp "PANCREAS TUMOR"/	149983
12	EMBASE	exp "PELVIS TUMOR"/	7899
13	EMBASE	exp "PROSTATE TUMOR"/	249156
14	EMBASE	exp "RECTUM TUMOR"/	264815
15	EMBASE	exp "STOMACH TUMOR"/	152114
16	EMBASE	exp "UROGENITAL TRACT TUMOR"/	819348
17	EMBASE	exp "URINARY TRACT TUMOR"/	230790
18	EMBASE	exp "LARGE INTESTINE TUMOR"/ OR exp "INTESTINE TUMOR"/	414447
19	EMBASE	exp METASTASIS/	647645
20	EMBASE	exp "ABDOMINAL TUMOR"/	43903
21	EMBASE	(abdo* OR cancer OR metas* OR malign*).ti,ab	3652022
22	EMBASE	(1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR	5558679

		16 OR 17 OR 18 OR 19 OR 20 OR 21)	
23	EMBASE	exp "NEOADJUVANT THERAPY"/	25938
24	EMBASE	"MULTIMODALITY CANCER THERAPY"/	70822
25	EMBASE	(neoadjuvant ADJ2 therapy).ti,ab	15554
26	EMBASE	(chemorad*).ti,ab	50575
27	EMBASE	("neodjuvant chemotherapy").ti,ab	32
28	EMBASE	(neodjuvant chemotherapy).ti,ab	32
29	EMBASE	(23 OR 24 OR 25 OR 26 OR 27 OR 28)	146465
30	EMBASE	exp "COLON RESECTION"/	44510
31	EMBASE	exp "RECTUM RESECTION"/	18722
32	EMBASE	exp SURGERY/	4928835
33	EMBASE	(excis* OR resect* OR surg*).ti,ab	2944649
34	EMBASE	(30 OR 31 OR 32 OR 33)	5746455
35	EMBASE	(musc*).ti,ab	1084502
36	EMBASE	(function* OR strength OR mass OR loss OR wast* OR power OR deplet*).ti,ab	7352294
37	EMBASE	(35 AND 36)	411819
38	EMBASE	(skeletal).ti,ab	248873
39	EMBASE	(36 AND 38)	99770
40	EMBASE	(sarcopenia).ti,ab	13372
41	EMBASE	SARCOPENIA/ OR exp "MUSCLE ATROPHY"/	43886
42	EMBASE	exp "MUSCLE CONTRACTION"/	120152
43	EMBASE	exp "MUSCLE RELAXATION"/	23897

44	EMBASE	exp "MUSCLE STRENGTH"/	65572
45	EMBASE	(37 OR 39 OR 40 OR 41 OR 42 OR 43 OR 44)	585737
46	EMBASE	(prognosis OR surviv*).ti,ab	2086043
47	EMBASE	exp "TREATMENT OUTCOME"/	1725481
48	EMBASE	exp PROGNOSIS/	748026
49	EMBASE	"DISEASE COURSE"/	460533
50	EMBASE	exp "DISEASE FREE SURVIVAL"/	87528
51	EMBASE	exp SURVIVAL/	1156866
52	EMBASE	exp REMISSION/	205418
53	EMBASE	(46 OR 47 OR 48 OR 49 OR 50 OR 51 OR 52)	4360504
54	EMBASE	exp "CASE CONTROL STUDY"/	183174
55	EMBASE	(observational study).ti,ab,pt	138068
56	EMBASE	(case-control stud*).ti,ab,pt	139244
57	EMBASE	(randomized controlled trial OR randomised controlled trial).ti,ab,pt	135233
58	EMBASE	("retrospective stud*").ti,ab,pt	263338
59	EMBASE	("systematic review").ti,ab,pt	212667
60	EMBASE	("review").ti,ab,pt	3785725
61	EMBASE	("meta analysis").ti,ab,pt	209737
62	EMBASE	("cohort stud*").ti,ab,pt	321782
63	EMBASE	exp "OBSERVATIONAL STUDY"/	215825
64	EMBASE	exp "RANDOMIZED CONTROLLED TRIAL"/	635724
65	EMBASE	"SYSTEMATIC REVIEW"/	273959

66	EMBASE	exp REVIEW/	2702744
67	EMBASE	exp "META ANALYSIS"/	203612
68	EMBASE	"RETROSPECTIVE STUDY"/	1001216
69	EMBASE	"COHORT ANALYSIS"/	644864
70	EMBASE	(54 OR 55 OR 56 OR 57 OR 58 OR 59 OR 60 OR 61 OR 62 OR 63 OR 64 OR 65 OR 66 OR 67 OR 68 OR 69)	6225288
71	EMBASE	(22 AND 29 AND 34 AND 45 AND 53 AND 70)	357

5.2 Appendix B

The impact of cancer, chemotherapy, and resistance exercise training (RET) on mitochondrial activity.

Protocol 1.0
21.09.2020

Short title:	Impact of cancer, chemotherapy, and exercise on mitochondria.
Acronym:	ICCE-Mito
Trial Registration:	NCT04558398
ISRCTN:	if appropriate
IRAS Project ID:	275264
Trial Sponsor:	University of Nottingham
Sponsor reference:	20019
Funding Source:	Research funds of Mr Jon Lund

TRIAL / STUDY PERSONNEL AND CONTACT DETAILS

Sponsor: University of Nottingham
Contact name: Ms Angela Shone
Head of Research Governance
Research and Graduate Services
King's Meadow Campus
Lenton Lane
Nottingham
NG7 2NR
Email: sponsor@nottingham.ac.uk

Chief investigator:
Mr Jon Lund DM, FRCS
Clinical Associate Professor and Consultant colorectal surgeon
Phone: 01332 724641
Email: jon.lund@nottingham.ac.uk

Co-investigators:
Dr Bethan Phillips, BSc (Hons), PhD, Research Fellow, Division of Graduate Entry Medicine and Health, School of Medicine, University of Nottingham.
Email: beth.phillips@nottingham.ac.uk

Mr Jacob Hatt, Clinical Research Fellow, MBChB, BSc (Hons), MRCSEd. (PhD Student) Division of Graduate Entry Medicine and Health, School of Medicine, University of Nottingham
Email: jacob.hatt@nhs.net / mzxjh2@nottingham.ac.uk

Mr Thomas Smart, Clinical Research Fellow, MBBS, BSc (Hons), MRCSEd. (PhD Student) Division of Graduate Entry Medicine and Health, School of Medicine, University of Nottingham
Email: thomas.smart2@nhs.net / mzxts@nottingham.ac.uk

Trial / Study Statistician:
Dr Brett Doleman. Clinical Fellow, Royal Derby Hospital, Derby.
Email: dr.doleman@gmail.com

Trial / Study Coordinating Centre:
University of Nottingham, Royal Derby Hospital, School of Medicine, Royal Derby Hospital,
Uttoxeter Road, Derby, DE22 3NE

SYNOPSIS

Title	The impact of cancer, chemotherapy, and resistance exercise training (RET) on mitochondrial activity.
Acronym	ICCE-Mito
Short title	Cancer and mitochondrial activity
Chief Investigator	Mr Jon Lund
Objectives	Primary Objectives: To assess the impact of cancer and neoadjuvant chemotherapy have on mitochondrial activity. To assess the impact of cancer and neoadjuvant chemotherapy on body composition and muscle function. To investigate if preoperative home RET programme can improve mitochondrial activity, body composition and muscle function in patients awaiting surgery for intra-abdominal cancer. Secondary Objectives: To investigate myokine levels in different types of cancer and the impact neo adjuvant treatment has on these markers. To establish if physiological responses to home RET differ between cancer types.
Trial Configuration	Single centre pilot study.
Setting	Royal Derby Hospital, Secondary Referral Centre (NHS) and University of Nottingham.
Sample size estimate	A formal sample size calculation for this study is not possible due to the absence of comparable data for our primary endpoint. As such this study will be conducted as a single centre pilot study. Despite this, sample sizes like that proposed herein have been used to demonstrate changes in mitochondrial activity in response to alternative interventions and we have previously reported changes in body composition and function as a result of RET in samples groups of this size.
Number of participants	64 participants will be required to complete the study in the following groups: n = 8 breast cancer patients n = 16 oesophageal cancer patients (8 will require neoadjuvant chemotherapy) n = 16 prostate cancer patients (8 will undergo home RET) n = 24 colorectal cancer patients (8 will undergo home RET, 8 will require neoadjuvant chemotherapy).

	Based on past experience of recruiting cancer patients for exercise training studies, up to 68 participants will be recruited to allow for an anticipated 20% drop out rate.
Eligibility criteria	Adult patients 50 years or over diagnosed with oesophageal, breast, prostate or colorectal cancer that are deemed eligible to undergo resectional surgery and are physically capable of performing RET (prostate and colorectal cancer patients only).
Description of interventions	An intervention will only be delivered to the RET arm in each of prostate and colorectal cancer patients' groups. All other participants will have physiological investigations at different times within their normal treatment pathway. After they have provided informed consent and attended an initial screening session to ensure eligibility for the study. All participants will undergo a study session on the morning of their planned surgery day consisting of: A muscle (*m. vastus lateralis* (VL)) biopsy to investigate mitochondrial activity using an OROBOROS respirometer. (2) An ultrasound (U/S) scan of VL to determine muscle thickness and architecture. (2) A selection of muscle function assessments. (2) Bloods samples to determine myokine and hormone levels. (2) A short subjective questionnaire on health perception. These measures will have been carried out on those participants allocated to the RET arm prior to the commencement of the home training program. The maximum number of repeats are in parentheses above. In addition to the measures outlined above, rates of muscle protein synthesis (MPS) will also be assessed in all participants at baseline and throughout the RET for those allocated to that intervention. To facilitate this, all participants will be asked to consume a "heavy water" (D_2O) tracer 24-hours prior to their baseline study visit. For those in the RET groups, they will also be asked to consume smaller doses of this tracer each week during their RET programme. A saliva sample will be collected before and 2-hours after each tracer ingestion. Participants selected to undergo the RET programme will undergo a maximum of 3 sessions per week for up to 4 weeks (minimum 9 sessions). Following an original teaching session, these RET sessions are performed in participants own home, with weekly contact made by a member of the research team to maximise participant engagement and ensure correct technique. All baseline measurements will take place at the University of Nottingham, Royal Derby Hospital in the week following recruitment at a time mutually convenient to the patient and the research team.
Duration of study	Study estimated to last a total of 3-years Individual participants will be required to be involved within the study from the date they consent to be involved in the study until completion of the study. In accordance with national guidelines the preoperative stage should be no longer than 31 days from MDT decision to treat. A postal

	questionnaire will be sent to the participant at 28 days post-surgery. This leads us to believe that a single participant would be unlikely to be involved with the study for more than 8 weeks for those not requiring neoadjuvant chemotherapy and 24 weeks for those who do.
Randomisation and blinding	Randomisation will occur only for the colorectal and prostate cancer patients to determine if they will complete RET. Blinding to an exercise intervention is not possible.
Outcome measures	*Primary:* Changes related to cancer and neoadjuvant chemotherapy on; Muscle mitochondria function and quantity Muscle structure and function Body composition The relationship between muscle mitochondria function and quantity and the other parameters outlined above. Changes in the above parameters following completion of a 4-week home RET regime. *Secondary:* Changes in myokine and hormone responses (IL-6, TNFα, and TGFβ) to cancer. Subjective questionnaire data. Acceptability of the RET protocol in the pre-operative period. Safety: Adverse event monitoring during RET.
Statistical methods	Data will be analysed using Graphpad Prism by the research team, led by the study statistician. After testing for normality, data will be assessed using appropriate post-hoc tests to determine differences between the cancer groups (one-way ANOVA) and group x time interactions in the prostate and colorectal cancer groups. Correlative analysis will be used to explore relationships between mitochondrial parameters and other physiological data sets.

ABBREVIATIONS

Add to / amend accordingly (please ensure ALL abbreviations used in the protocol are listed here)

AE Adverse Event

CI Chief Investigator overall

CRF Case Report Form

DMC Data Monitoring Committee

GCP Good Clinical Practice

HIIT High intensity interval training

ICF Informed Consent Form

IPAQ International Physical Activity Questionnaire

NHS National Health Service

PI Principal Investigator at a local centre

PIS Participant Information Sheet

RDH Royal Derby Hospital

REC Research Ethics Committee

RET Resistance Exercise Training

R&D Research and Development department

SAE Serious Adverse Event

USS Ultrasound scan

UoN University of Nottingham

VL *m. Vastus Lateralis*

TABLE OF CONTENTS

TRIAL / STUDY BACKGROUND INFORMATION AND RATIONALE

Each year, there are around 363,000 new cancer cases in the UK, with almost half of the patients going on to have resectional surgery (Cancer Research, 2020). Improving survival and quality of life are inherent goals for successful treatment of cancer patients and, as such, research is focussing on optimising patient's health prior to, and during, their cancer treatment.

Skeletal muscle represents one of the major compartments of the human body, with its function necessary for a variety of biological processes including movement and respiration (Porporato, 2016). A tight balance exists between muscle protein synthesis (MPS) and breakdown that maintains muscle homeostasis and any disruption in this results in skeletal muscle depletion. Although there is a largely unavoidable age-related decline in muscle mass and function, known as sarcopenia, patients with conditions such as cancer appear to suffer more rapid and extensive loss. This is often referred to as cachexia, a multifactorial syndrome characterised by progressive loss in body weight, adipose depletion and metabolic abnormalities (Penna et al., 2019). Cachexia is thought to affect 50-80% of patients with cancer and directly accounts for 20% of cancer deaths. It is also a known factor affecting patient physical performance, causing functional impairment and increasing short term postoperative morbidity (Eriksson et al., 2017).

Mitochondria are essential organelles necessary for normal cellular activity and play a crucial role in metabolic homeostasis, including the metabolic processes required for the maintenance of muscle mass (Pustylnikov et al., 2018). In addition, skeletal muscle is highly reliant on mitochondria to meet the energy requirements for contraction, therefore any alteration in mitochondrial homeostasis directly impacts muscle function (Antunes et al., 2014). In animal studies, the presence of cancer has been shown to reduce mitochondrial content in skeletal muscle and, as a result led to muscle atrophy. The impact cancer has on mitochondrial function, quantity, and its relationship with overall skeletal muscle mass in humans remains poorly investigated.

With the established link between cancer cachexia and post-operative morbidity and mortality, improving perioperative cardiorespiratory fitness and body composition is of increasing clinical importance. In recent years, the term 'prehabilitation' has been adopted to define a group of interventions, either performed under supervision or at home, that are integrated into the clinical pathway prior to surgery. To date these consist mainly of combined endurance and resistance exercise. Endurance exercise training is most effective for improving cardiorespiratory fitness while resistance training (RET) increases muscle mass and function, whilst also promoting improved metabolic function (Piraux et al., 2018). These interventions are aimed at both reducing imminent patient risk and promoting lasting beneficial effects on perioperative recovery and outcome (Weston et al., 2016).

Since mitochondrial turnover and function are both altered in sarcopenic and cachectic muscles, it is hypothesised that pre-operative exercise in cancer patients could act as a good counter measure to improve mitochondrial quantity and quality and resultant muscle function (Romanello & Sandri, 2016). A number of studies, including those from our own research group, have demonstrated an improvement in preoperative cardiorespiratory fitness following supervised or home based short term exercise prehabilitation (Boereboom et al., 2016; Licker et al., 2017; Weston et al., 2016), however few examine the direct effect prehabilitation has on mitochondrial function of cancer patient.

The rationale behind this study is to gain a greater level of understanding of the impact cancer and its treatments have on overall muscle function, both at a cellular and whole-body level. The study will also assess if any positive changes seen with a regime of home

exercise prehabilitation are acceptable and feasible to both the patient and clinician within the time restraints of the clinical environment.

TRIAL / STUDY OBJECTIVES AND PURPOSE

PURPOSE

To investigate the impact cancer and the various treatment options have on mitochondrial quantity and quality, in addition to muscle mass, function and body composition, and whether this can be modified through exercise prehabilitation.

PRIMARY OBJECTIVE

To assess the impact cancer and chemotherapy have on mitochondrial activity.

To assess the impact cancer and chemotherapy have on body composition and muscle mass and function.

To investigate if a preoperative exercise program can improve mitochondrial activity, body composition, muscle mass and function in patients awaiting surgery for intra-abdominal cancer.

SECONDARY OBJECTIVES

To investigate myokine and hormone levels in the different types of cancer and the impact neoadjuvant chemotherapy have on these.

To establish whether there are different responses to exercise training between different cancer types.

DETAILS OF PRODUCT(S)

Description

No product will be as intervention. The only product to be given is D_2O (95% deuterium oxide), commonly referred to as “heavy water”. This product will be given to participants undergoing muscle biopsies to enable us to quantify rates of muscle protein synthesis (MPS). The product is purchased from Sigma-Aldrich, Poole, UK. It is premixed and stored in locked medical storage cupboard within the University of Nottingham Clinical Physiology Unit. D_2O is water containing one “heavy” deuterium atom in the place of a hydrogen atom. This method has been used successfully in several previous studies in our laboratory including those in pre-operative cancer patients, to quantify protein turnover without the need for radioactive labelling or invasive monitoring of participants. D_2O is incorporated as a tracer into amino acids such as alanine. The fractional synthetic rate of D_2O bound to, for example, alanine in the muscle vs. the total alanine body pool can then be measured to give rates of muscle protein synthesis. It can be assumed that pre-study D_2O levels are zero, this will be confirmed using plasma protein from the blood sample on the screening visit. Participants will then be ‘primed’ with 3mls/kg of D_2O 24 hours prior to their baseline study visit.

Manufacture

Sigma-Aldrich, Poole, UK

Storage, dispensing and return

It is premixed and stored in locked medical storage cupboard within the University of Nottingham Clinical Physiology Unit (Royal Derby Hospital). Each participant will be provided with the priming dose of D_2O at the time of the screening visit and instructed to ingest the

D_2O tracer 24 hours before their baseline study visit. If there is any reason the participant is illegible to participate in the study following the screening visit, one of the research team will contact them and instruct them to dispose of the D_2O.

Known Side Effects

D_2O is very well tolerated, and most people have no side effects at all. Self-limiting dizziness is the commonest side effect which resolves spontaneously; however, we have previously minimised the incidence of this by splitting the initial priming dose into two smaller doses which are taken at spaced intervals. This method will be applied in this study. The side effect profile is thought to be dose dependent (total body pool enrichment) and we will aim for an enrichment level of 0.4% which is well within safe limits as shown previously.

TRIAL / STUDY DESIGN

TRIAL / STUDY CONFIGURATION

Single centre pilot study.

Primary endpoints

Variations related to cancer and neoadjuvant chemotherapy on;
Muscle mitochondria function and quantity
Muscle structure and metabolism
Body composition
Cardiorespiratory fitness and muscle function

The relationship between muscle mitochondria function and quantity and the other parameters outlined above.

Variations in muscle mitochondria function and quantity and the other parameters outlined above following completion of a 4-week exercise training regime.

Secondary endpoints

Changes in myokine (IL-6, TNFα, and TGFβ) and hormone responses preoperatively.

Subjective questionnaire data related to overall health status.

Acceptability of the exercise training protocol in the pre-operative period.

Safety endpoints

Safety variables:
Screening results: physical examination by a qualified doctor, vital signs (pulse rate, BP, O_2 saturations), 12-lead ECG and blood results (FBC, U&E's, LFT, coagulation screen and lipid profile).

Whole study - AEs spontaneously reported during the study, discontinuations due to AEs.

Please see exclusion criteria below for medical conditions inhibiting initial or continued inclusion in the study:
Abnormal results of screening blood tests as detailed below,
Abnormal screening ECG as detailed below,
Abnormal clinical measurements e.g., blood pressure,
Side effects of D_2O ingestion.

Stopping rules and discontinuation

Any participant may withdraw from the study at any point, without giving reason.
Screening tests that indicate renal failure, liver failure, significant anaemia or abnormal coagulation would inhibit inclusion.
An abnormal screening ECG showing any abnormal arrhythmia, 2nd or 3rd degree heart block would inhibit inclusion.
A screening blood pressure of greater than 160/100mmHg would inhibit inclusion.

Exercise training monitoring will comply with the divisional policy on research participation set by Dr John Williams, Consultant Anaesthetist. For those participating in the home RET training regime, information regarding any adverse reactions to exercise will be explained. These include:

- Chest pain or tightness,
- Faintness,
- Sudden pallor,
- Loss of co-ordination, confusion, dizziness,
- Signs of respiratory failure
- Palpitations

If any such adverse reactions are experienced, the participant would be advised to stop the exercise immediately and seek medical advice.

RANDOMIZATION AND BLINDING

For participants within the study with a diagnosis of breast, oesophageal or colorectal (requiring neoadjuvant chemotherapy) cancer, there will be no randomisation taking place as the effect of routine treatment will be assessed.

For participants with a diagnosis of prostate or colorectal (not requiring neoadjuvant chemotherapy) they will be randomly allocated to either the home resistance exercise training (RET) 4-week program or to a control (routine treatment) study arm. This will be done using Sealed Envelope, Sealed Envelope Ltd London. This software will randomly allocated participants to either group. A member of the research team who is not involved in the data collection or recruitment of participants will generate the randomisation codes and keep these securely. Block randomisation using random permuted block sizes of two and four will be used. Participants will be stratified according to age and sex to ensure a higher likelihood of equal baseline characteristics. Allocation concealment will be ensured by using opaque, sealed envelopes with participant group allocation performed on the first visit. Due to the nature of the intervention, patients will not be blinded to the intervention or control group. However, data collectors will be blinded to the participant's group assignment.

Independent reviewers of muscle USS will be blinded to both group and pre / post results.

TRIAL/STUDY MANAGEMENT

Data will be inputted to a secure database with encryption. Consent forms and hard copies of data will be kept securely under lock and key in a locked office with access only to named researchers on this project.

Mr Jacob Hatt and Mr Thomas Smart (Clinical Research Fellows/ PhD students) will be responsible for recruitment and overall conduct of the study including screening, physiological and exercise training, data collection in both the University and clinical environments and the taking of muscle biopsies.

The Chief Investigator has overall responsibility for the study and shall oversee all study management.

The data custodian will be the Chief Investigator.

DURATION OF THE TRIAL / STUDY AND PARTICIPANT INVOLVEMENT

Study Duration: Enrolment will begin as soon as ethical approval has been granted and will run until enough participants have been recruited. We anticipate that this project may take up to three years to complete in total (Oct 2020 - Mar 2023).

Participant Duration: Individual participants will be required to be involved within the study from the date they consent to be involved in the study until completion of the study. In accordance with national guidelines the preoperative stage should be no longer than 31 days from MDT decision to treat. A postal questionnaire will be sent to the participant at 28 days post-surgery. This leads us to believe that a single participant would be unlikely to be involved with the study for more than 8 weeks for those who do not require neoadjuvant chemotherapy and 24 weeks for those that do.

End of the Trial:

The end of the study will be the return of the 28-day questionnaire from the final participant, or 6 weeks after this was sent in the case of no questionnaire return.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Potential participants, where there is sufficient evidence confirming suspected cancer (breast, oesophageal, prostate, and colorectal), who are eligible for curative treatment (surgical resection with or without neoadjuvant chemotherapy) will be identified at weekly cancer multi-disciplinary team (MDT) meetings. These individuals will have already been informed by a member of their clinical team (either doctor or cancer specialist nurses) of the suspicion of the cancer diagnosis and the plan for treatment.

A member of the clinical team will inform potential participants of the study, and provide a participant information sheet, giving them chance to read about the study. Contact details for the research team will be provided along with the PIS, to allow potential participants to contact the research team directly to arrange their screening visit. At the screening visit, a member of the research team will go through the patient information sheet and answer any questions with the potential participant, prior to obtaining written consent. If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms, but the consent forms and information sheets will not be printed in other languages.

All participants will give written informed consent immediately once they agree to enter the study. This will be prior to the randomisation process (specific to prostate and colorectal patients only). We will then allow a minimum of 24 hours before contacting them via telephone to answer any further questions about the trial and confirm they are still willing to participate. For those participants who could undergo a training regime prior to their resectional surgery, the intervention period is severely time limited, and we would aim to initiate any training as soon as reasonably possible after the consent and screening process is completed.

It will be explained that entry into the trial is entirely voluntary and that their treatment and care would not be affected by their decision to participate or not. It will also be explained that they can withdraw at any time, but the data collected up to that point would not be erased and we would seek consent to use the data in the final analyses where appropriate.

All participants will have the results of the histological specimens performed as part of their NHS treatment reviewed by a member of the research team following their surgery. All participants will be followed up even if their disease is revealed to be benign, however further recruitment will be carried out to ensure the numbers assessed with the disease is correct. The participants will then be offered to continue in the study if they wish to, however the data collected would be excluded from final analysis with explanation provided in the write up.

Eligibility criteria

Inclusion criteria

1. Aged 50 years and over (no upper age limit)
2. Histologically confirmed malignancy, radiologically or direct visualisation leading to high clinical suspicion of cancer with planned surgical resection as treatment option (breast, oesophageal, prostate, or colorectal).
3. Sufficient mobility to be able to complete resistance exercise (prostate and colorectal only).
4. Capacity to give informed and written consent
5. Ability to travel to the RDH to complete the assessment session

Exclusion criteria

1. Current participation in a formal exercise regime
2. Inability to complete exercise training
3. A BMI <16.5 or >35 kg/m^2
4. Active cardiovascular disease:
    - Uncontrolled hypertension (BP > 160/100)
    - Angina
    - Heart failure (class III/IV)
    - Significant arrhythmia
    - Right to left cardiac shunt
    - Recent cardiac event
5. Taking beta-adrenergic blocking agents
6. Cerebrovascular disease:
    - Previous stroke
    - Aneurysm (large vessel or intracranial)
    - Epilepsy
7. Respiratory disease including:
    - Pulmonary hypertension
    - Significant COPD
    - Uncontrolled asthma
8. Clotting dysfunction or current use of anticoagulants (e.g., Warfarin/Clopidogrel/ Rivaroxaban)
9. Significant musculoskeletal or neurological disorders

Removal of participants from therapy or assessments/Participant Withdrawal

Patients will be withdrawn from the study if they lose capacity for consent or if they wish to voluntarily withdraw consent.

Patients will be withdrawn from the study if they suffer any adverse effects of exercise training. Patients will be withdrawn from the study if they suffer any adverse effects which are attributed to the ingestion of deuterium oxide (D_2O) (e.g., nausea, vomiting or abdominal pain). This is very unlikely given that D_2O is usually well tolerated and has been used

extensively in our research group without adverse events. Should this occur, we will refer the patient to hospital for further tests and treatment.

Patients recruited into the resistance training arm of the study will be withdrawn from the study if they fail to complete a minimum of 8 exercise sessions prior to operation date. If the participant wishes to remain in the study, they can be included in the follow up aspect of the study following surgery.

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

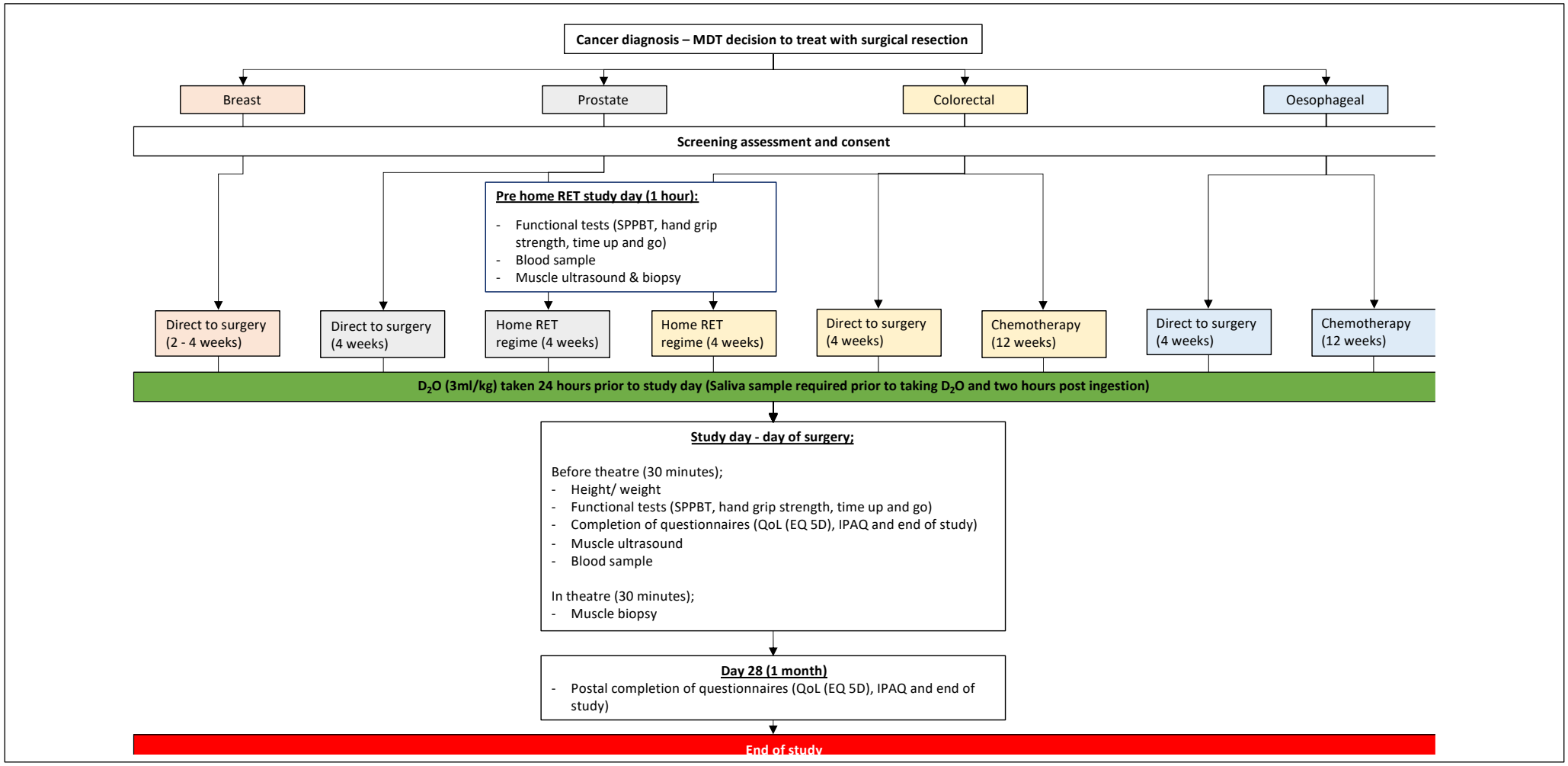
Informed consent

All participants will be provided with the details of the trial in depth and given a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. Once the participant is satisfied with the study, written informed consent will then be obtained by the investigator. The Informed Consent Form will be signed and dated by the participant before they enter the trial. The Investigator will answer any questions that the participant has concerning study participation.

Informed consent will be collected from each participant before they undergo any interventions related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the patient's hospital records.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended Consent Form which will be signed by the participant

TRIAL / STUDY TREATMENT AND REGIMEN



Key for schematic diagram: Bloods (FBC, UE, LFT, lipid profile, TFT, CRP, HBA1c, glucose), RET = resistance exercise training, SPPBT = short performance physical battery tests, QoL = Quality of Life

Participants will be recruited from surgical outpatient clinics following initial introduction to the trial by their clinical team. At this point, they will be provided with a participant information sheet. Contact details will be provided on the information sheet to enable potential participants to arrange a screening visit. The screening visit would entail a detailed description of all study elements and addressing any questions prior to gaining written consent. Once the patient provides written informed consent, they will undergo a full cardiovascular examination by a medically qualified research team member and will have a set of screening bloods taken.

Eligible participants for the home resistance exercise training (RET) program (colorectal and prostate cancer patients with no planned neoadjuvant chemotherapy) will be randomly allocated to either the training arm of the study or to routine care at this point.

Pre-Exercise study day – For participants within the Home-RET study arm
Participants randomly allocated to the home-RET study group will attend the University of Nottingham Graduate Entry Medical School at the Royal Derby hospital for an assessment visit prior to the commencement of their training protocol. Participants will be asked to arrive starved from 03:00 of the day. On arrival they will undergo a limited number of physical function assessments (including time up and go, hand grip strength, and the short performance physical battery test). Following this an ultrasound scan of the thigh to assess muscle architecture will be performed.
A muscle biopsy will be taken from the mid belly of *m. vastus lateralis* using a conchotome biopsy technique. Biopsies will be taken by medically qualified doctors trained and experienced in this procedure. The procedure will be performed under aseptic conditions. After local anaesthetic, a 5-10mm incision will be made in the skin and underlying fascia and a sample of muscle will be taken (approximately 100mg). Direct pressure will be applied to achieve haemostasis. A single non-absorbable suture will be placed to allow skin apposition and healing. A sterile dressing and compression bandage will be applied to remain in place for the duration of the day. The suture will be removed around 5-7 days after the biopsy was taken. The biopsy sample will be assessed using an Oroboros respirometer for the primary endpoint of mitochondrial activity, analysed by western blotting and PCR to determine cell signalling and gene expression.
Participants will then be provided with a complimentary breakfast, while the training regimen is demonstrated to them with time allowed for questions regarding the training regimen. This will conclude this study day.

Training program:
All exercise training will take place in the participants own home. With a documentation pack provided to participants to guide exercise and for participants to log their workouts.
Participants will be asked to perform 3 workouts per week over the 4-week period (12 workouts in total) with each workout estimated to take 30 minutes maximum.
Participants will be provided with a set of TheraBand resistance bands to provide resistance to their workout.
As well as the education session provided at the pre-exercise study day, participants will be contacted weekly via telephone or video call to encourage participation, to answer any difficulties participants maybe experiencing and to ensure no adverse symptoms are being experienced. A video will be recorded of a member of the research team performing the exercise regimen to enable participants to watch from home.

Each session will include:
- 2 min warm-up jogging on the spot
- 2 sets of 12-15 repetitions of:
    - Squats

    - Hip flexion
    - Hip extension
    - Hip abduction
    - Seated row
    - Bench press
    - Lateral raises
- 2 min jogging on the spot cooldown.

Each exercise will be separated by 1-minute rest, with participants encouraged to work out to mild fatigue on their final repetition in each set. If able to perform more than the allocated maximum of 15 repetitions prior to fatigue, then participants will be informed to increase the resistance by using a tougher TheraBand.

Our research group has conducted many similar exercise regimes of this intensity to subjects in the anticipated age-range without incident. Participants will be advised to terminate exercise training immediately if experiencing:

- Chest pain or tightness
- Faintness
- Sudden pallor
- Loss of co-ordination
- Confusion
- Dizziness
- Palpitations
- Sudden breathlessness

Should any participant experience these effects during any session, they will be advised to seek urgent medical attention and will be withdrawn from the exercise arm of the study for safety purposes.

Day of surgery study day – All study participants undertake

24 hours prior to the study day, participants will be asked to consume a stable isotope tracer drink (D_2O) at a 3 ml/kg priming dose at home. Participants will be asked to provide saliva samples immediately prior to D_2O consumption and a further sample 2-hours post consumption. Participants will be provided with written instructions, the tracer drinks and collection receptacles at their screening visit or via postal delivery for them to collect these samples at home.

All participants will attend for their study day fasted from 03:00 of the day, in line with their starving instructions for their planned surgical procedure. Participants will first have an ultrasound scan of the thigh to assess muscle architecture. Following this, participants will be asked to complete a quality-of-life questionnaire (EQ-5D) and a self-administered short-form international physical activity questionnaire (IPAQ). Venepuncture will be performed to gather samples for analysis of myokine expression, and the sputum samples collected at home received from the participants. Participants will then perform physical function assessments to determine muscle function, including the timed-up-and-go test, and the short performance physical battery test.

Once the participant has been anesthetized for their planned surgical procedure, a member of the research team will then perform a single muscle biopsy. The muscle biopsy will be taken from the mid belly of *m. vastus lateralis* using the conchotome biopsy technique as previously described.

Day 21-28 post-surgery

Participants will be contacted by postal survey, and asked to complete a further EQ-5D and IPAQ questionnaire, along with a study feedback questionnaire to ascertain participants views on the study

As detailed above, in addition to their normal treatments, patients will potentially undergo the following invasive or potentially harmful tests according to their limb of study (risks outlined in Risk section): two blood tests and a maximum of 2 muscle biopsies.

Compliance

Compliance with the protocol will be monitored by the member of the research team present at every session.

Acceptable compliance for the exercise arm of the study will be defined as completion of a minimum of 8 sessions as logged by the participants. Lower than this would be expected to result in minimal adaptation and therefore the participant would be excluded from analysis of the data but would be welcomed to complete the study.

Criteria for terminating trial

The study will only be terminated should there be a complete lack of recruitment to the study/willing participants. Terminating the whole study may be because of a formal or informal interim analysis (data to be analysed after 6 participants) or based on new overwhelming evidence of efficacy/inefficacy, major safety concerns, new information, or issues with trial conduct (e.g., poor recruitment, loss of resources).

In the event of trial termination, any research data already collected would be kept as outlined in the archiving section below, the participants are consented for data storage at the beginning of the trial.

RADIATION EXPOSURE

There is no anticipated additional radiation exposure with participants involvement in the study

TRANSPORT AND STORAGE OF THE TISSUES

Each participant will be assigned a trial identity code number with the prefix ‘IC-Mi’ for use on CRF’s (Case report forms), other trial documents, the electronic database, and the subject identification log in the trial master file. The documents and database will also use the study acronym, initials, and sequential study number e.g.: IC-MiAB_01. Back up of electronic files will exist in a password protected program on a password protected computer within the chief investigator’s office.

Any blood samples sent to NHS pathology laboratory will be labelled in accordance with local procedure.

Only the Clinical Research Fellows (Jacob Hatt and Thomas Smart) and Chief Investigator (Jon Lund) will have access to personal information about the participant, this will be securely stored in encrypted files away from any samples or other study data.

Human tissue and storage location will be recorded in an anonymised tissue sample log stored securely on the local network on a document that is registered with the local HTMG in accordance with the human tissue act for compliance and audit purposes.

Muscle biopsies: half of the sample (estimated 50mg) will be instantly frozen in liquid nitrogen, labelled and stored in a -80-degree freezer. The other half will be placed immediately in biopsy preservation solution (BIOPS) prior to being analysed immediately within the laboratory. All laboratory analysis will be performed by a suitably trained member of the research group within the Clinical Physiology department.

Blood samples will be centrifuged to separate plasma and buffy coat from the samples which will be labelled and stored in a -80-degree freezer.

Samples stored for future research will be stored at the research tissue bank at the MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research housed within the University of Nottingham, Division of Medical Sciences and Graduate Entry Medicine, only if participants are agreeable and sign the optional clause on the consent form.

Where participants do not agree to the future use of their samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

The analysis of samples will take place at the University of Nottingham Royal Derby Hospital Centre.

LABORATORY ANALYSES

Blood samples will be sent to chemical pathology within Royal Derby Hospital to be analysed for routine health measures (FBC, UE, LFT, Coagulation screen, TFT) and other markers of improved health (lipid profile).

Labelling of body water with D_2O will be assessed by extraction of the water from the saliva samples and analysed using direct liquid injection TC/EA-IRMS. Muscle samples will be derivatised to separate out; myofibrillar, sarcoplasmic, lipid, satellite cell and DNA fractions according to established procedures in our lab. These fractions will then be analysed via GC-pyrolysis-IRMS and GC-MS for deuterium labelling; this combined with the body water labelling will allow the calculation of rates of synthesis for each fraction using the standard precursor-product model:

$$\text{Fractional Synthesis Rate (\%/day)} = [(APE_{Ala})] / [(APE_P) \times t] \times 100$$

Where APE_{Ala} = deuterium enrichment of protein-bound alanine, APE_P = mean precursor enrichment over the time period, and t is the time between samples.

For additional insight into the mechanisms regulating control of muscle mass and muscle protein metabolism we will measure several target proteins within muscle by western blotting and real-time PCR.

The muscle sample stored in BIOPS buffer solution will be analysed using a high-resolution respirometer to measure cellular oxygen consumption in a closed chamber system (Oroboros O2k- FluoRespirometer, Oroboros Instruments) according to an established substrate-uncoupler-inhibitor titration (SUIT) protocol developed to assess mitochondrial function within permeabilised muscle fibres.

As well as the above blood tests taken for medical screening a venous blood sample will be taken for plasma analysis via ELISA for hormone and myokine expression.

STATISTICS

Methods

Objective measures of mitochondrial oxidative phosphorylation (OXPHOS) capacity will be analysed for differences between cancer diagnosis groups, and changes seen throughout routine cancer treatment as the primary outcome.

Upon study completion data will be analysed using GraphPad Prism, v5.0, (La Jolla, Calif. US) and SPSS version 19 (IBM, US), with interim analysis planned after the completion of 6 participants in each group to ensure correct sample size selection. Data will be analysed in house by members of the research team with statistical oversight provided by the study statistician.

Distribution of the data will be tested using the Kolmogorov-Smirnov test, with normally distributed data expressed as mean (SD) and non-normally distributed data as median +/- interquartile range.

The comparison of mitochondrial OXPHOS activity between cancer types will tested against the null hypothesis: There is no difference in the mitochondrial OXPHOS activity between cancer types. It is anticipated that it will require analysis via a Kruskal-Wallis test (two-tailed, multi-variable, un-paired, non-parametric continuous data).

The comparison of mitochondrial OXPHOS activity between the resistance exercise and control groups (RET v Con) prior to surgery, this will be tested against the null hypothesis: Mitochondrial OXPHOS activity will not be changed by RET when compared to control. This is anticipated to require analysis via ANOVA (two-tailed, non-paired, non-parametric continuous data with a single variable)

For all data, SPSS v19 software will be used and the significance level will be set at 0.05. Analysis of data will be on University of Nottingham computers and backed up to the University's servers.

Sample size and justification

As this is a pilot study, no power calculation has been performed. This is due to limited published evidence on the changes in muscle mitochondrial activity between cancer-types. This is also true for the change in muscle mitochondrial activity throughout cancer treatment.

An interim analysis will be performed after the recruitment of 6 participants in each group to ensure that sample size selection is correct.

Assessment of efficacy

Primary efficacy endpoint:
The difference in muscle mitochondrial OXPHOS capacity between four cancer types.

Secondary efficacy endpoints:

Mean differences in baseline clinical measures (Such as BP, resting HR, blood chemistry) across cancer types
Differences in body composition, and muscle function between cancers, and its relationship with mitochondrial activity.
The effect of an exercise 'prehabilitation' program (4-weeks of resistance-based exercise) on muscle mitochondrial activity and muscle function.
The effect of cancer types on myokine and hormone expression.

See study regime above for timings.

Assessment of safety

Due to the current global pandemic situation, several adjustments have been made in the protocol to minimise contact as far as possible. When possible, a 2-metre distance will be maintained between researchers and participants, and when this is not possible the appropriate level of personal protective equipment will be worn by both parties to minimise the risk of transmission. Hand sanitiser will also be available for use regularly during periods of contact.
All blood samples and biopsies will be taken by medically qualified, trained individuals. Any complication that may occur will be dealt with in accordance with standard NHS practice.
Procedures for missing, unused, and spurious data: Participants will be removed from the study if significant amounts of data are missing e.g., greater than 4 exercise sessions missed.

Definition of populations analysed

Safety set: Participants who attend for at least their day of surgery assessment study day.
Full Analysis set: All participants, who participated in a pre-exercise assessment study day, day of surgery assessments, and post-operative postal survey. For participants enrolled in an exercise program to complete at least 8 exercise sessions.
Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

Mr Jacob Hatt or Mr Thomas Smart will screen participants for violations of eligibility criteria during the consenting procedure. Deviations from the protocol will be assessed by members of the research team, primarily by Mr Jacob Hatt or Mr Thomas Smart as adherence and compliance to the protocol is monitored by attendance to the sessions.

ADVERSE EVENTS

The occurrence of an adverse event because of participation within this study is not expected and no adverse event data will be collected

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), the respective National Health Service (NHS) Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible, and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996, the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasise to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Case Report Forms

All case report forms will be kept in the chief investigators file. This file will be locked within a cupboard in a locked office on site.

Each participant will be assigned a trial identity code number with the prefix 'IC-Mi', allocated at randomisation if appropriate, for use on CRFs other trial documents and the electronic database. Each participant will be assigned a trial identity code number for use on CRF's (Case report forms), other trial documents and the electronic database. The documents and database will also use the study acronym, 2 randomly chosen letters and sequential study number e.g.: IC-MiCaAB01. Back up electronic file will exist in a password protected program on a password protected computer within the chief investigator's office.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial, in accordance with regulatory requirements and for follow-up as required. CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled, and dated.
The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Sample Labelling

Each participant will be assigned a trial identity code number for use on the samples, consent forms and other study documents and the electronic database. The documents and

database will also use the study acronym (IC-Mi, initials (of first and last names separated by a hyphen or a middle name initial when available) and study enrolment number.

Samples for NHS pathology analysis will be labelled in accordance with local NHS procedures.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents
The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be always available for review by the Chief Investigator, Sponsor's designee, and inspection by relevant regulatory authorities (e.g., DH, Human Tissue Authority).

DATA PROTECTION

Paper records will be filed and placed into locked cupboards within a locked office in the department of Clinical Physiology, University of Nottingham, Royal Derby Hospital. Electronic data will be placed on password protected databases (secure network) on a Nottingham University password protected computer within a locked office in the same department.

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above). Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one-way encryption method).

Information about the trial in the participants medical records / hospital notes will be treated confidentially in the same was as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Trial conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of trial materials and equipment calibration logs.

TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the trial database will be checked. Where corrections are required, these will carry a full audit trail and justification.

Trial data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained because of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated because of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments, and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

This study will form part of the research fellow's thesis. No identifiable information will be used at all. The results will be published within a peer reviewed journal and international presentations will be made.

USER AND PUBLIC INVOLVEMENT

A recent study undertaken by our unit assessed the effectiveness of an exercise training program in an elderly, healthy volunteer group. Following the study all volunteers completed a training feedback form. This was designed to assess the acceptably of the intervention to the volunteers and provide a mechanism for making the training more acceptable if necessary. The volunteers said that the program was enjoyable, it was not a significant time burden, it did not interfere significantly with other aspects of their life, and they would recommend it to others.

We will use the same training feedback form to assess the acceptability of the training to the cancer patients, as well as the other questionnaires as outlined in the protocol.

STUDY FINANCES

Funding source

This study is funded by research funds of Mr Jon Lund.

Participant stipends and payments

Participants will not be paid to participate in the trial; however, a small inconvenience allowance will be made available (£10) to contribute towards travel expenses outside of their usual care visits. In addition, free car parking will be made available for all research visits.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name)_____

Signature: _____

Date: _____

Co- investigator: (name) _____

Signature: _____

Date: _____

6 References

Aadahl, M., Beyer, N., Linneberg, A., Thuesen, B. H., & Jørgensen, T. (2011). Grip strength and lower limb extension power in 19-72-year-old Danish men and women: The Health2006 study. *BMJ Open*, *1*(2). https://doi.org/10.1136/bmjopen-2011-000192

Al-Majid, S., & Mccarthy, D. O. (2001). Cancer-Induced Fatigue and Skeletal Muscle Wasting: The Role of Exercise. *Biological Research For Nursing*, *2*(3). https://doi.org/10.1177/109980040100200304

Alves, C. R. R., Da Cunha, T. F., Da Paixão, N. A., & Brum, P. C. (2015). Aerobic exercise training as therapy for cardiac and cancer cachexia. In *Life Sciences* (Vol. 125). https://doi.org/10.1016/j.lfs.2014.11.029

American Cancer Society. (2020). *Radiation Therapy*. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/radiation.html

Anderson, A. J., Jackson, T. D., Stroud, D. A., & Stojanovski, D. (2019). Mitochondria—hubs for regulating cellular biochemistry: Emerging concepts and networks. In *Open Biology* (Vol. 9, Issue 8). https://doi.org/10.1098/rsob.190126

Antony, A. N., Paillard, M., Moffat, C., Juskeviciute, E., Correnti, J., Bolon, B., Rubin, E., Csordás, G., Seifert, E. L., Hoek, J. B., & Hajnóczky, G. (2016). MICU1 regulation of mitochondrial Ca 2+ uptake dictates survival and tissue regeneration. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms10955

Antunes, D., Padrão, A. I., Maciel, E., Santinha, D., Oliveira, P., Vitorino, R., Moreira-Gonçalves, D., Colaço, B., Pires, M. J., Nunes, C., Santos, L. L., Amado, F., Duarte, J. A., Domingues, M. R., & Ferreira, R. (2014). Molecular insights into mitochondrial dysfunction in cancer-related muscle wasting. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1841*(6), 896–905. https://doi.org/10.1016/j.bbalip.2014.03.004

Argilés, J. M., Busquets, S., & López-Soriano, F. J. (2011). Anti-inflammatory therapies in cancer cachexia. *European Journal of Pharmacology*, *668*(SUPPL. 1). https://doi.org/10.1016/j.ejphar.2011.07.007

Argilés, J. M., Busquets, S., Stemmler, B., & López-Soriano, F. J. (2014). Cancer cachexia: Understanding the molecular basis. In *Nature Reviews Cancer*. https://doi.org/10.1038/nrc3829

Argilés, J. M., López-Soriano, F. J., & Busquets, S. (2015). Muscle wasting in cancer: The role of mitochondria. In *Current Opinion in Clinical Nutrition and Metabolic Care* (Vol. 18, Issue 3). https://doi.org/10.1097/MCO.0000000000000164

Arruebo, M., Vilaboa, N., Sáez-Gutierrez, B., Lambea, J., Tres, A., Valladares, M., & González-Fernández, Á. (2011). Assessment of the evolution of cancer treatment therapies. In *Cancers*. https://doi.org/10.3390/cancers3033279

Aunan, J. R., Watson, M. M., Hagland, H. R., & Søreide, K. (2016). Molecular and biological hallmarks of ageing. In *British Journal of Surgery* (Vol. 103, Issue 2). https://doi.org/10.1002/bjs.10053

Awad, S., Tan, B. H., Cui, H., Bhalla, A., Fearon, K. C. H., Parsons, S. L., Catton, J. A., & Lobo, D. N. (2012). Marked changes in body composition following neoadjuvant chemotherapy for oesophagogastric cancer. *Clinical Nutrition*. https://doi.org/10.1016/j.clnu.2011.08.008

Baczynska, A. M., Shaw, S., Roberts, H. C., Cooper, C., Sayer, A. A., & Patel, H. P. (2016). Human vastus lateralis skeletal muscle biopsy using the Weil-Blakesley conchotome. *Journal of Visualized Experiments*. https://doi.org/10.3791/53075

Bagnyukova, T., Serebriiskii, I. G., Zhou, Y., Hopper-Borge, E. A., Golemis, E. A., & Astsaturov, I. (2010). Chemotherapy and signaling: How can targeted therapies supercharge cytotoxic agents? In *Cancer Biology and Therapy* (Vol. 10, Issue 9). https://doi.org/10.4161/cbt.10.9.13738

Banugo, P., & Amoako, D. (2017). Prehabilitation. *BJA Education*, *17*(12), 401–405.

Baracos, V. E., Martin, L., Korc, M., Guttridge, D. C., & Fearon, K. C. H. (2018). Cancer-associated cachexia. In *Nature Reviews Disease Primers*. https://doi.org/10.1038/nrdp.2017.105

Baracos, V., & Kazemi-Bajestani, S. M. R. (2013). Clinical outcomes related to muscle mass in humans with cancer and catabolic illnesses. In *International Journal of Biochemistry and Cell Biology*.

https://doi.org/10.1016/j.biocel.2013.06.016

Barclay, C. J. (2017). The basis of differences in thermodynamic efficiency among skeletal muscles. *Clinical and Experimental Pharmacology and Physiology*, *44*(12). https://doi.org/10.1111/1440-1681.12850

Bardou, M., Barkun, A. N., & Martel, M. (2013). Obesity and colorectal cancer. *Gut*. https://doi.org/10.1136/gutjnl-2013-304701

Barreto, R., Mandili, G., Witzmann, F. A., Novelli, F., Zimmers, T. A., & Bonetto, A. (2016). Cancer and chemotherapy contribute to muscle loss by activating common signaling pathways. *Frontiers in Physiology*, *7*(OCT). https://doi.org/10.3389/fphys.2016.00472

Barrett, K., Barman, S., Boitano, S., & Brooks, H. (2016). *Ganong's Review of Medical Physiology* (25th Editi). McGraw-Hill Education.

Beaudart, C., McCloskey, E., Bruyère, O., Cesari, M., Rolland, Y., Rizzoli, R., Araujo de Carvalho, I., Amuthavalli Thiyagarajan, J., Bautmans, I., Bertière, M. C., Brandi, M. L., Al-Daghri, N. M., Burlet, N., Cavalier, E., Cerreta, F., Cherubini, A., Fielding, R., Gielen, E., Landi, F., … Cooper, C. (2016). Sarcopenia in daily practice: assessment and management. *BMC Geriatrics*. https://doi.org/10.1186/s12877-016-0349-4

Beaudart, C., Rolland, Y., Cruz-Jentoft, A. J., Bauer, J. M., Sieber, C., Cooper, C., Al-Daghri, N., Araujo de Carvalho, I., Bautmans, I., Bernabei, R., Bruyère, O., Cesari, M., Cherubini, A., Dawson-Hughes, B., Kanis, J. A., Kaufman, J. M., Landi, F., Maggi, S., McCloskey, E., … Fielding, R. A. (2019). Assessment of Muscle Function and Physical Performance in Daily Clinical Practice: A position paper endorsed by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). *Calcified Tissue International*, *105*(1), 1–14. https://doi.org/10.1007/s00223-019-00545-w

Beere, P. A., Russell, S. D., Morey, M. C., Kitzman, D. W., & Higginbotham, M. B. (1999). Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. *Circulation*, *100*(10). https://doi.org/10.1161/01.CIR.100.10.1085

Berg, J., Tymoczko, J., & Stryer, L. (2002). Biochemistry, 5th edition. In

Biochemistry.

Berkel, A. E. M., Bongers, B. C., van Kamp, M. J. S., Kotte, H., Weltevreden, P., de Jongh, F. H. C., Eijsvogel, M. M. M., Wymenga, A. N. M., Bigirwamungu-Bargeman, M., van der Palen, J., van Det, M. J., van Meeteren, N. L. U., & Klaase, J. M. (2018). The effects of prehabilitation versus usual care to reduce postoperative complications in high-risk patients with colorectal cancer or dysplasia scheduled for elective colorectal resection: Study protocol of a randomized controlled trial. *BMC Gastroenterology*, *18*(1). https://doi.org/10.1186/s12876-018-0754-6

Biga, L. ., & Dawson, S. (2010). *Anatomy & Physiology*.

Bishop, D. J., Granata, C., & Eynon, N. (2014). Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content? In *Biochimica et Biophysica Acta - General Subjects*. https://doi.org/10.1016/j.bbagen.2013.10.012

Blackwell, J., Atherton, P. J., Smith, K., Doleman, B., Williams, J. P., Lund, J. N., & Phillips, B. E. (2017). The efficacy of unsupervised home-based exercise regimens in comparison to supervised laboratory-based exercise training upon cardio-respiratory health facets. *Physiological Reports*, *5*(17). https://doi.org/10.14814/phy2.13390

Blackwell, J. E. M., Doleman, B., Boereboom, C. L., Morton, A., Williams, S., Atherton, P., Smith, K., Williams, J. P., Phillips, B. E., & Lund, J. N. (2020). High-intensity interval training produces a significant improvement in fitness in less than 31 days before surgery for urological cancer: a randomised control trial. *Prostate Cancer and Prostatic Diseases*, *23*(4). https://doi.org/10.1038/s41391-020-0219-1

Boengler, K., Kosiol, M., Mayr, M., Schulz, R., & Rohrbach, S. (2017). Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. In *Journal of Cachexia, Sarcopenia and Muscle* (Vol. 8, Issue 3, pp. 349–369). Wiley Blackwell. https://doi.org/10.1002/jcsm.12178

Boereboom, C. L., Blackwell, J. E. M., Williams, J. P., Phillips, B. E., & Lund, J. N. (2019). Short-term pre-operative high-intensity interval training does not improve fitness of colorectal cancer patients. *Scandinavian Journal of Medicine and*

Science in Sports, *29*(9). https://doi.org/10.1111/sms.13460

Boereboom, C. L., Phillips, B. E., Williams, J. P., & Lund, J. N. (2016). A 31-day time to surgery compliant exercise training programme improves aerobic health in the elderly. *Techniques in Coloproctology*, *20*(6), 375–382. https://doi.org/10.1007/s10151-016-1455-1

Bohannon, R. W. (2008). Hand-grip dynamometry predicts future outcomes in aging adults. *Journal of Geriatric Physical Therapy*, *31*(1). https://doi.org/10.1519/00139143-200831010-00002

Bohannon, R. W., Magasi, S. R., Bubela, D. J., Wang, Y. C., & Gershon, R. C. (2012). Grip and Knee extension muscle strength reflect a common construct among adults. *Muscle and Nerve*, *46*(4). https://doi.org/10.1002/mus.23350

Bonora, M., Patergnani, S., Rimessi, A., de Marchi, E., Suski, J. M., Bononi, A., Giorgi, C., Marchi, S., Missiroli, S., Poletti, F., Wieckowski, M. R., & Pinton, P. (2012). ATP synthesis and storage. In *Purinergic Signalling* (Vol. 8, Issue 3). https://doi.org/10.1007/s11302-012-9305-8

Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, *14*(5). https://doi.org/10.1249/00005768-198205000-00012

Braga, M., Ljungqvist, O., Soeters, P., Fearon, K., Weimann, A., & Bozzetti, F. (2009). ESPEN Guidelines on Parenteral Nutrition: Surgery. *Clinical Nutrition*, *28*(4). https://doi.org/10.1016/j.clnu.2009.04.002

Brandt, T., Cavellini, L., Kühlbrandt, W., & Cohen, M. M. (2016). A mitofusin-dependent docking ring complex triggers mitochondrial fusion in vitro. *ELife*, *5*(JUNE2016). https://doi.org/10.7554/eLife.14618

Brierley, J., Gospodarowicz, M., & O'Sulivan, B. (2016). The principles of cancer staging. *Ecancermedicalscience*, *10*. https://doi.org/10.3332/ecancer.2016.ed61

Brook, M. S., Wilkinson, D. J., Phillips, B. E., Perez-Schindler, J., Philp, A., Smith, K., & Atherton, P. J. (2016). Skeletal muscle homeostasis and plasticity in youth and ageing: Impact of nutrition and exercise. In *Acta Physiologica* (Vol. 216, Issue 1). https://doi.org/10.1111/apha.12532

Bruce, R. A. (1971). Exercise testing of patients with coronary heart disease.

Principles and normal standards for evaluation. *Annals of Clinical Research*.

Brzycki, M. (1993). Strength Testing—Predicting a One-Rep Max from Reps-to-Fatigue. *Journal of Physical Education, Recreation & Dance*, *64*(1). https://doi.org/10.1080/07303084.1993.10606684

Buck, E., Zügel, M., Schumann, U., Merz, T., Gumpp, A. M., Witting, A., Steinacker, J. M., Landwehrmeyer, G. B., Weydt, P., Calzia, E., & Lindenberg, K. S. (2017). High-resolution respirometry of fine-needle muscle biopsies in pre-manifest Huntington's disease expansion mutation carriers shows normal mitochondrial respiratory function. *PLoS ONE*, *12*(4). https://doi.org/10.1371/journal.pone.0175248

Calle, E. E., Rodriguez, C., Walker-Thurmond, K., & Thun, M. J. (2003). Overweight, Obesity, and Mortality from Cancer in a Prospectively Studied Cohort of U.S. Adults. *New England Journal of Medicine*, *348*(17). https://doi.org/10.1056/nejmoa021423

Cancer Research. (2020). *Cancer statistics for the UK*. Cancer Research UK. http://www.cancerresearchuk.org/health-professional/cancer-statistics

Carli, F., Charlebois, P., Stein, B., Feldman, L., Zavorsky, G., Kim, D. J., Scott, S., & Mayo, N. E. (2010). Randomized clinical trial of prehabilitation in colorectal surgery. *British Journal of Surgery*, *97*(8). https://doi.org/10.1002/bjs.7102

Carli, Francesco, Silver, J. K., Feldman, L. S., McKee, A., Gilman, S., Gillis, C., Scheede-Bergdahl, C., Gamsa, A., Stout, N., & Hirsch, B. (2017). Surgical Prehabilitation in Patients with Cancer. *Physical Medicine and Rehabilitation Clinics of North America*, *28*(1). https://doi.org/10.1016/j.pmr.2016.09.002

Carli, Franco, & Zavorsky, G. S. (2005). Optimizing functional exercise capacity in the elderly surgical population. In *Current Opinion in Clinical Nutrition and Metabolic Care* (Vol. 8, Issue 1). https://doi.org/10.1097/00075197-200501000-00005

Carson, J. A., Hardee, J. P., & VanderVeen, B. N. (2016). The emerging role of skeletal muscle oxidative metabolism as a biological target and cellular regulator of cancer-induced muscle wasting. In *Seminars in Cell and Developmental Biology* (Vol. 54). https://doi.org/10.1016/j.semcdb.2015.11.005

Carter, H. N., Chen, C. C. W., & Hood, D. A. (2015). Mitochondria, muscle health, and exercise with advancing age. *Physiology*, *30*(3), 208–223. https://doi.org/10.1152/physiol.00039.2014

Cassidy, S., Thoma, C., Hallsworth, K., Parikh, J., Hollingsworth, K. G., Taylor, R., Jakovljevic, D. G., & Trenell, M. I. (2016). High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia*, *59*(1). https://doi.org/10.1007/s00125-015-3741-2

Cassidy, S., Thoma, C., Houghton, D., & Trenell, M. I. (2017). High-intensity interval training: a review of its impact on glucose control and cardiometabolic health. In *Diabetologia* (Vol. 60, Issue 1). https://doi.org/10.1007/s00125-016-4106-1

Cespedes Feliciano, E. M., Lee, V. S., Prado, C. M., Meyerhardt, J. A., Alexeeff, S., Kroenke, C. H., Xiao, J., Castillo, A. L., & Caan, B. J. (2017). Muscle mass at the time of diagnosis of nonmetastatic colon cancer and early discontinuation of chemotherapy, delays, and dose reductions on adjuvant FOLFOX: The C-SCANS study. *Cancer*, *123*(24). https://doi.org/10.1002/cncr.30950

Chaban, Y., Boekema, E. J., & Dudkina, N. V. (2014). Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation. In *Biochimica et Biophysica Acta - Bioenergetics* (Vol. 1837, Issue 4). https://doi.org/10.1016/j.bbabio.2013.10.004

Chabner, B. A., & Roberts, T. G. (2005). Chemotherapy and the war on cancer. In *Nature Reviews Cancer* (Vol. 5, Issue 1, pp. 65–72). https://doi.org/10.1038/nrc1529

Chaudhry, R., & Varacallo, M. (2018). Biochemistry, Glycolysis. In *StatPearls*.

Chen, L. K., Liu, L. K., Woo, J., Assantachai, P., Auyeung, T. W., Bahyah, K. S., Chou, M. Y., Chen, L. Y., Hsu, P. S., Krairit, O., Lee, J. S. W., Lee, W. J., Lee, Y., Liang, C. K., Limpawattana, P., Lin, C. S., Peng, L. N., Satake, S., Suzuki, T., … Arai, H. (2014). Sarcopenia in Asia: Consensus report of the Asian working group for sarcopenia. In *Journal of the American Medical Directors Association*. https://doi.org/10.1016/j.jamda.2013.11.025

Chen, L., Zhang, J., Lyu, Z., Chen, Y., Ji, X., Cao, H., Jin, M., Zhu, J., Yang, J., Ling,

R., Xing, J., Ren, T., & Lyu, Y. (2018). Positive feedback loop between mitochondrial fission and Notch signaling promotes survivin-mediated survival of TNBC cells. *Cell Death and Disease*, *9*(11). https://doi.org/10.1038/s41419-018-1083-y

Cheng, J., Liao, Y., Zhou, L., Peng, S., Chen, H., & Yuan, Z. (2016). Amplified RLR signaling activation through an interferon-stimulated gene-endoplasmic reticulum stress-mitochondrial calcium uniporter protein loop. *Scientific Reports*, *6*. https://doi.org/10.1038/srep20158

Choi, M. H., Oh, S. N., Lee, I. K., Oh, S. T., & Won, D. D. (2018). Sarcopenia is negatively associated with long-term outcomes in locally advanced rectal cancer. *Journal of Cachexia, Sarcopenia and Muscle*. https://doi.org/10.1002/jcsm.12234

Choi, S., Reiter, D. A., Shardell, M., Simonsick, E. M., Studenski, S., Spencer, R. G., Fishbein, K. W., & Ferrucci, L. (2016). 31P magnetic resonance spectroscopy assessment of muscle bioenergetics as a predictor of gait speed in the Baltimore longitudinal study of aging. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *71*(12). https://doi.org/10.1093/gerona/glw059

Christensen, P. M., Jacobs, R. A., Bonne, T., Flöck, D., Bangsbo, J., & Lundby, C. (2016). A short period of high-intensity interval training improves skeletal muscle mitochondrial function and pulmonary oxygen uptake kinetics. *Journal of Applied Physiology*, *120*(11). https://doi.org/10.1152/japplphysiol.00115.2015

Clinicaltrials.gov. (2021). *Clinicaltrials.gov*. https://doi.org/https://clinicaltrials.gov/ct2/results?term=Chemotherapy%2C+mitochondria&cond=Cancer

Coen, P. M., Musci, R. V., Hinkley, J. M., & Miller, B. F. (2019). Mitochondria as a target for mitigating sarcopenia. In *Frontiers in Physiology* (Vol. 10, Issue JAN). https://doi.org/10.3389/fphys.2018.01883

Coleman, W. B. (2018). Neoplasia. In *Molecular Pathology: The Molecular Basis of Human Disease* (pp. 71–97). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802761-5.00004-3

Conley, K. E., Jubrias, S. A., & Esselman, P. C. (2000). Oxidative capacity and ageing in human muscle. *Journal of Physiology*, *526*(1). https://doi.org/10.1111/j.1469-7793.2000.t01-1-00203.x

Cooper, C., Fielding, R., Visser, M., Van Loon, L. J., Rolland, Y., Orwoll, E., Reid, K., Boonen, S., Dere, W., Epstein, S., Mitlak, B., Tsouderos, Y., Sayer, A. A., Rizzoli, R., Reginster, J. Y., & Kanis, J. A. (2013). Tools in the assessment of sarcopenia. *Calcified Tissue International*, *93*(3), 201–210. https://doi.org/10.1007/s00223-013-9757-z

Cooper GM. (2000). The Development and Causes of Cancer. In *The Cell: A Molecular Approach. 2nd Edition.*

Cooper, R., Kuh, D., & Hardy, R. (2010). Objectively measured physical capability levels and mortality: Systematic review and meta-analysis. In *BMJ (Online)* (Vol. 341, Issue 7774). https://doi.org/10.1136/bmj.c4467

Costa, D. S. J., Mercieca-Bebber, R., Rutherford, C., Gabb, L., & King, M. T. (2016). The Impact of Cancer on Psychological and Social Outcomes. *Australian Psychologist*, *51*(2), 89–99. https://doi.org/10.1111/ap.12165

Cruz-Jentoft, A. J., Baeyens, J. P., Bauer, J. M., Boirie, Y., Cederholm, T., Landi, F., Martin, F. C., Michel, J. P., Rolland, Y., Schneider, S. M., Topinková, E., Vandewoude, M., & Zamboni, M. (2010). Sarcopenia: European consensus on definition and diagnosis. *Age and Ageing*, *39*(4), 412–423. https://doi.org/10.1093/ageing/afq034

Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A. A., Schneider, S. M., Sieber, C. C., Topinkova, E., Vandewoude, M., Visser, M., Zamboni, M., Bautmans, I., Baeyens, J. P., Cesari, M., … Schols, J. (2019). Sarcopenia: Revised European consensus on definition and diagnosis. *Age and Ageing*, *48*(1), 16–31. https://doi.org/10.1093/ageing/afy169

Dekker, E., Tanis, P. J., Vleugels, J. L. A., Kasi, P. M., & Wallace, M. B. (2019). Colorectal cancer. In *The Lancet*. https://doi.org/10.1016/S0140-6736(19)32319-0

Delmonico, M. J., Harris, T. B., Visser, M., Park, S. W., Conroy, M. B., Velasquez-

Mieyer, P., Boudreau, R., Manini, T. M., Nevitt, M., Newman, A. B., & Goodpaster, B. H. (2009). Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *American Journal of Clinical Nutrition*, *90*(6). https://doi.org/10.3945/ajcn.2009.28047

Deschenes, M. R. (2004). Effects of aging on muscle fibre type and size. In *Sports Medicine* (Vol. 34, Issue 12). https://doi.org/10.2165/00007256-200434120-00002

Devin, A., & Rigoulet, M. (2007). Mechanisms of mitochondrial response to variations in energy demand in eukaryotic cells. In *American Journal of Physiology - Cell Physiology* (Vol. 292, Issue 1). https://doi.org/10.1152/ajpcell.00208.2006

DeVita, V. T., & Chu, E. (2008). A history of cancer chemotherapy. In *Cancer Research* (Vol. 68, Issue 21, pp. 8643–8653). https://doi.org/10.1158/0008-5472.CAN-07-6611

Devoto, L., Celentano, V., Cohen, R., Khan, J., & Chand, M. (2017). Colorectal cancer surgery in the very elderly patient: a systematic review of laparoscopic versus open colorectal resection. In *International Journal of Colorectal Disease* (Vol. 32, Issue 9). https://doi.org/10.1007/s00384-017-2848-y

Di Lisa, F., & Scorrano, L. (2012). *Muscle*. Elsevier Inc.

Distefano, G., & Goodpaster, B. H. (2018). Effects of exercise and aging on skeletal muscle. *Cold Spring Harbor Perspectives in Medicine*, *8*(3). https://doi.org/10.1101/cshperspect.a029785

Distefano, G., Standley, R. A., Zhang, X., Carnero, E. A., Yi, F., Cornnell, H. H., & Coen, P. M. (2018). Physical activity unveils the relationship between mitochondrial energetics, muscle quality, and physical function in older adults. *Journal of Cachexia, Sarcopenia and Muscle*, *9*(2). https://doi.org/10.1002/jcsm.12272

Drake, J., Tauer, K., Portnoy, D., & Weksler, B. (2019). Adjuvant chemotherapy is associated with improved survival in patients with nodal metastases after neoadjuvant therapy and esophagectomy. *Journal of Thoracic Disease*, *11*(6). https://doi.org/10.21037/jtd.2019.05.66

Dupont, A. C., Sauerbrei, E. E., Fenton, P. V., Shragge, P. C., Loeb, G. E., & Richmond, F. J. R. (2001). Real-time sonography to estimate muscle thickness: Comparison with MRI and CT. *Journal of Clinical Ultrasound*. https://doi.org/10.1002/jcu.1025

Edwards, M. K., & Loprinzi, P. D. (2016). All-cause mortality risk as a function of sedentary behavior, moderate-to-vigorous physical activity and cardiorespiratory fitness. *Physician and Sportsmedicine*, *44*(3). https://doi.org/10.1080/00913847.2016.1221751

Edwards, S. J., Smeuninx, B., McKendry, J., Nishimura, Y., Luo, D., Marshall, R. N., Perkins, M., Ramsay, J., Joanisse, S., Philp, A., & Breen, L. (2020). High-dose leucine supplementation does not prevent muscle atrophy or strength loss over 7 days of immobilization in healthy young males. *American Journal of Clinical Nutrition*, *112*(5). https://doi.org/10.1093/ajcn/nqaa229

Elkina, Y., von Haehling, S., Anker, S. D., & Springer, J. (2011). The role of myostatin in muscle wasting: An overview. In *Journal of Cachexia, Sarcopenia and Muscle* (Vol. 2, Issue 3). https://doi.org/10.1007/s13539-011-0035-5

Eng, L., Pringle, D., Su, J., Shen, X. W., Mahler, M., Niu, C., Charow, R., Tiessen, K., Lam, C., Halytskyy, O., Naik, H., Hon, H., Irwin, M., Pat, V., Gonos, C., Chan, C., Villeneuve, J., Harland, L., Shani, R. M., … Jones, J. M. (2018). Patterns, perceptions, and perceived barriers to physical activity in adult cancer survivors. *Supportive Care in Cancer*, *26*(11). https://doi.org/10.1007/s00520-018-4239-5

Epping-Jordan, J. A. E., Galea, G., Tukuitonga, C., & Beaglehole, R. (2005). Preventing chronic diseases: Taking stepwise action. In *Lancet* (Vol. 366, Issue 9497). https://doi.org/10.1016/S0140-6736(05)67342-4

Eriksson, S., Nilsson, J. H., Strandberg Holka, P., Eberhard, J., Keussen, I., & Sturesson, C. (2017). The impact of neoadjuvant chemotherapy on skeletal muscle depletion and preoperative sarcopenia in patients with resectable colorectal liver metastases. *Hpb*, *19*(4), 331–337. https://doi.org/10.1016/j.hpb.2016.11.009

Erlandson, M. C., Lorbergs, A. L., Mathur, S., & Cheung, A. M. (2016). Muscle analysis using pQCT, DXA and MRI. *European Journal of Radiology*, *85*(8),

1505–1511. https://doi.org/10.1016/j.ejrad.2016.03.001

Esco, M. R., Snarr, R. L., Leatherwood, M. D., Chamberlain, N. A., Redding, M. L., Flatt, A. A., Moon, J. R., & Williford, H. N. (2015). Comparison of total and segmental body composition using DXA and multifrequency bioimpedance in collegiate female athletes. *Journal of Strength and Conditioning Research*, *29*(4). https://doi.org/10.1519/JSC.0000000000000732

Evans, W. J. (2010). Skeletal muscle loss: Cachexia, sarcopenia, and inactivity. In *American Journal of Clinical Nutrition* (Vol. 91, Issue 4). https://doi.org/10.3945/ajcn.2010.28608A

Evans, W. J., Morley, J. E., Argilés, J., Bales, C., Baracos, V., Guttridge, D., Jatoi, A., Kalantar-Zadeh, K., Lochs, H., Mantovani, G., Marks, D., Mitch, W. E., Muscaritoli, M., Najand, A., Ponikowski, P., Rossi Fanelli, F., Schambelan, M., Schols, A., Schuster, M., … Anker, S. D. (2008). Cachexia: A new definition. *Clinical Nutrition*, *27*(6), 793–799. https://doi.org/10.1016/j.clnu.2008.06.013

Faguet, G. B. (2015). A brief history of cancer: Age-old milestones underlying our current knowledge database. *International Journal of Cancer*, *136*(9), 2022–2036. https://doi.org/10.1002/ijc.29134

Fearon, K., Strasser, F., Anker, S. D., Bosaeus, I., Bruera, E., Fainsinger, R. L., Jatoi, A., Loprinzi, C., MacDonald, N., Mantovani, G., Davis, M., Muscaritoli, M., Ottery, F., Radbruch, L., Ravasco, P., Walsh, D., Wilcock, A., Kaasa, S., & Baracos, V. E. (2011). Definition and classification of cancer cachexia: An international consensus. In *The Lancet Oncology*. https://doi.org/10.1016/S1470-2045(10)70218-7

Feng, L. R., Nguyen, Q., Ross, A., & Saligan, L. N. (2018). Evaluating the Role of Mitochondrial Function in Cancer-related Fatigue. *Journal of Visualized Experiments*, *135*, 1–8. https://doi.org/10.3791/57736

Fernandez, S., Franklin, J., Amlani, N., DeMilleVille, C., Lawson, D., & Smith, J. (2015). Physical activity and cancer: A cross-sectional study on the barriers and facilitators to exercise during cancer treatment. *Canadian Oncology Nursing Journal = Revue Canadienne de Nursing Oncologique*, *25*(1). https://doi.org/10.5737/236880762513742

Fleg, J. L., Morrell, C. H., Bos, A. G., Brant, L. J., Talbot, L. A., Wright, J. G., & Lakatta, E. G. (2005). Accelerated longitudinal decline of aerobic capacity in healthy older adults. *Circulation*, *112*(5). https://doi.org/10.1161/CIRCULATIONAHA.105.545459

Formenti, S. C., & Demaria, S. (2009). Systemic effects of local radiotherapy. In *The Lancet Oncology* (Vol. 10, Issue 7, pp. 718–726). https://doi.org/10.1016/S1470-2045(09)70082-8

Franchi, M. V., Longo, S., Mallinson, J., Quinlan, J. I., Taylor, T., Greenhaff, P. L., & Narici, M. V. (2018). Muscle thickness correlates to muscle cross-sectional area in the assessment of strength training-induced hypertrophy. *Scandinavian Journal of Medicine and Science in Sports*. https://doi.org/10.1111/sms.12961

Franchi, Martino V., Fitze, D. P., Hanimann, J., Sarto, F., & Spörri, J. (2020). Panoramic ultrasound vs. MRI for the assessment of hamstrings cross-sectional area and volume in a large athletic cohort. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-71123-6

Freire, A. N., Guerra, R. O., Alvarado, B., Guralnik, J. M., & Zunzunegui, M. V. (2012). Validity and reliability of the short physical performance battery in two diverse older adult populations in Quebec and Brazil. *Journal of Aging and Health*, *24*(5). https://doi.org/10.1177/0898264312438551

Frontera, W. R., & Ochala, J. (2015). Skeletal Muscle: A Brief Review of Structure and Function. *Behavior Genetics*, *45*(2), 183–195. https://doi.org/10.1007/s00223-014-9915-y

Gatalica, Z., Ghazalpour, A., Lilleberg, S. L., Swensen, J., & Lynch, H. T. (2017). Hereditary Cancer. In W. B. Coleman & G. J. Tsongalis (Eds.), *The Molecular Basis of Human Cancer* (pp. 335–353). Springer New York. https://doi.org/10.1007/978-1-59745-458-2_22

Gianfaldoni, S., Gianfaldoni, R., Wollina, U., Lotti, J., Tchernev, G., & Lotti, T. (2017). An overview on radiotherapy: From its history to its current applications in dermatology. *Open Access Macedonian Journal of Medical Sciences*, *5*(4 Special Issue GlobalDermatology). https://doi.org/10.3889/oamjms.2017.122

Gibala, M. J., & Little, J. P. (2020). Physiological basis of brief vigorous exercise to

improve health. In *Journal of Physiology* (Vol. 598, Issue 1). https://doi.org/10.1113/JP276849

Gibala, M. J., Little, J. P., van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., Raha, S., & Tarnopolsky, M. A. (2006). Short-term sprint interval versus traditional endurance training: Similar initial adaptations in human skeletal muscle and exercise performance. *Journal of Physiology*, *575*(3). https://doi.org/10.1113/jphysiol.2006.112094

Gibson, D. J., Burden, S. T., Strauss, B. J., Todd, C., & Lal, S. (2015). The role of computed tomography in evaluating body composition and the influence of reduced muscle mass on clinical outcome in abdominal malignancy: A systematic review. In *European Journal of Clinical Nutrition*. https://doi.org/10.1038/ejcn.2015.32

Golomb, B. A., Chan, V. T., Evans, M. A., Koperski, S., White, H. L., & Criqui, M. H. (2012). The older the better: Are elderly study participants more non-representative? A cross-sectional analysis of clinical trial and observational study samples. *BMJ Open*, *2*(6). https://doi.org/10.1136/bmjopen-2012-000833

Gómez Montes, J. F., Curcio, C. L., Alvarado, B., Zunzunegui, M. V., & Guralnik, J. (2013). Validity and reliability of the Short Physical Performance Battery (SPPB): A pilot study on mobility in the Colombian Andes. *Colombia Medica*, *44*(3). https://doi.org/10.25100/cm.v44i3.1181

Gonzalez-Freire, M., de Cabo, R., Studenski, S. A., & Ferrucci, L. (2014). The neuromuscular junction: Aging at the crossroad between nerves and muscle. In *Frontiers in Aging Neuroscience* (Vol. 6, Issue AUG). https://doi.org/10.3389/fnagi.2014.00208

Goodpaster, B. H., Park, S. W., Harris, T. B., Kritchevsky, S. B., Nevitt, M., Schwartz, A. V., Simonsick, E. M., Tylavsky, F. A., Visser, M., & Newman, A. B. (2006). The loss of skeletal muscle strength, mass, and quality in older adults: The Health, Aging and Body Composition Study. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*. https://doi.org/10.1093/gerona/61.10.1059

Gouspillou, G., Scheede-Bergdahl, C., Spendiff, S., Vuda, M., Meehan, B., Mlynarski, H., Archer-Lahlou, E., Sgarioto, N., Purves-Smith, F. M., Konokhova,

Y., Rak, J., Chevalier, S., Taivassalo, T., Hepple, R. T., & Jagoe, R. T. (2015). Anthracycline-containing chemotherapy causes long-term impairment of mitochondrial respiration and increased reactive oxygen species release in skeletal muscle. *Scientific Reports*, *5*. https://doi.org/10.1038/srep08717

Gouspillou, G., Sgarioto, N., Kapchinsky, S., Purves-Smith, F., Norris, B., Pion, C. H., Barbat-Artigas, S., Lemieux, F., Taivassalo, T., Morais, J. A., Aubertin-Leheudre, M., & Hepple, R. T. (2014). Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans. *FASEB Journal*, *28*(4). https://doi.org/10.1096/fj.13-242750

Greco, M., Villani, G., Mazzucchelli, F., Bresolin, N., Papa, S., & Attardi, G. (2003). Marked aging-related decline in efficiency of oxidative phosphorylation in human skin fibroblasts. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *17*(12). https://doi.org/10.1096/fj.02-1009fje

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology*, *11*(3), 298–300. https://doi.org/10.1093/geronj/11.3.298

Hassanpour, S. H., & Dehghani, M. (2017). Review of cancer from perspective of molecular. *Journal of Cancer Research and Practice*, *4*(4), 127–129. https://doi.org/10.1016/j.jcrpr.2017.07.001

Herrod, P. J. J., Boyd-Carson, H., Doleman, B., Trotter, J., Schlichtemeier, S., Sathanapally, G., Somerville, J., Williams, J. P., & Lund, J. N. (2019). Quick and simple; psoas density measurement is an independent predictor of anastomotic leak and other complications after colorectal resection. *Techniques in Coloproctology*, *23*(2). https://doi.org/10.1007/s10151-019-1928-0

Heus, C., Cakir, H., Lak, A., Doodeman, H. J., & Houdijk, A. P. J. (2016). Visceral obesity, muscle mass and outcome in rectal cancer surgery after neo-adjuvant chemo-radiation. *International Journal of Surgery*, *29*. https://doi.org/10.1016/j.ijsu.2016.03.066

Hilhorst, S., & Lockey, A. (2019). *Cancer Costs A "ripple effect" analysis of cancer's wider impact*. www.demos.co.uk

Hoppeler, H. (1990). The different relationship of V̇O2 to muscle mitochondria in humans and quadrupedal animals. *Respiration Physiology*, *80*(2–3), 137–145. https://doi.org/10.1016/0034-5687(90)90077-C

Hutter, E., Renner, K., Pfister, G., Stöckl, P., Jansen-Dürr, P., & Gnaiger, E. (2004). Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. *Biochemical Journal*, *380*(3). https://doi.org/10.1042/BJ20040095

Hütter, E., Skovbro, M., Lener, B., Prats, C., Rabøl, R., Dela, F., & Jansen-Dürr, P. (2007). Oxidative stress and mitochondrial impairment can be separated from lipofuscin accumulation in aged human skeletal muscle. *Aging Cell*, *6*(2). https://doi.org/10.1111/j.1474-9726.2007.00282.x

Huxley, A. F., & Niedergerke, R. (1954). Structural changes in muscle during contraction: Interference microscopy of living muscle fibres. *Nature*. https://doi.org/10.1038/173971a0

Huxley, H., & Hanson, J. (1954). Changes in the Cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature*. https://doi.org/10.1038/173973a0

Iwashyna, T. J., & Lamont, E. B. (2002). Effectiveness of adjuvant fluorouracil in clinical practice: A population-based cohort study of elderly patients with stage III colon cancer. *Journal of Clinical Oncology*, *20*(19). https://doi.org/10.1200/JCO.2002.03.083

Järvinen, T., Ilonen, I., Kauppi, J., Salo, J., & Räsänen, J. (2018). Loss of skeletal muscle mass during neoadjuvant treatments correlates with worse prognosis in esophageal cancer: A retrospective cohort study. *World Journal of Surgical Oncology*, *16*(1). https://doi.org/10.1186/s12957-018-1327-4

Johnston, M., & Vogele, C. (1993). Benefits of psychological preparation for surgery: A meta-analysis. In *Annals of Behavioral Medicine* (Vol. 15, Issue 4). https://doi.org/10.1093/abm/15.4.245

Jönsson, B., & Hofmarcher, T. (2017). *COMPARATOR REPORT ON PATIENT ACCESS TO CANCER MEDICINES IN – A UK PERSPECTIVE*.

Joseph, A. M., Adhihetty, P. J., & Leeuwenburgh, C. (2016). Beneficial effects of

exercise on age-related mitochondrial dysfunction and oxidative stress in skeletal muscle. *Journal of Physiology*, *594*(18). https://doi.org/10.1113/JP270659

Kassabian, M. K. (1908). Roentgen Rays and Electro-Therapeutics, with Chapters on Radium and Photo-Therapy. *Archives of The Roentgen Ray*, *12*(8). https://doi.org/10.1259/arr.1908.0108

Kazemi-Bajestani, S. M. R., Mazurak, V. C., & Baracos, V. (2016). Computed tomography-defined muscle and fat wasting are associated with cancer clinical outcomes. In *Seminars in Cell and Developmental Biology*. https://doi.org/10.1016/j.semcdb.2015.09.001

Kinnear, W., & Blakey, J. (2014). A Practical Guide to the Interpretation of Cardio-Pulmonary Exercise Tests. In *A Practical Guide to the Interpretation of Cardio-Pulmonary Exercise Tests*. https://doi.org/10.1093/med/9780198702467.001.0001

Koltai, E., Hart, N., Taylor, A. W., Goto, S., Ngo, J. K., Davies, K. J. A., & Radak, Z. (2012). Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *303*(2). https://doi.org/10.1152/ajpregu.00337.2011

Kupfer, S. S., & Ellis, N. A. (2016). Hereditary colorectal cancer. In *The Molecular Basis of Human Cancer* (pp. 381–400). Springer New York. https://doi.org/10.1007/978-1-59745-458-2_25

Kuyumcu, M. E., Halil, M., Kara, Ö., Çuni, B., Çağlayan, G., Güven, S., Yeşil, Y., Arik, G., Yavuz, B. B., Cankurtaran, M., & Özçakar, L. (2016). Ultrasonographic evaluation of the calf muscle mass and architecture in elderly patients with and without sarcopenia. *Archives of Gerontology and Geriatrics*, *65*. https://doi.org/10.1016/j.archger.2016.04.004

Lai, N., M. Kummitha, C., Rosca, M. G., Fujioka, H., Tandler, B., & Hoppel, C. L. (2019). Isolation of mitochondrial subpopulations from skeletal muscle: Optimizing recovery and preserving integrity. *Acta Physiologica*, *225*(2). https://doi.org/10.1111/apha.13182

Laine, A., Iyengar, P., & Pandita, T. K. (2013). The role of inflammatory pathways in cancer-associated cachexia and radiation resistance. In *Molecular Cancer Research* (Vol. 11, Issue 9). https://doi.org/10.1158/1541-7786.MCR-13-0189

Lang, J. J., Phillips, E. W., Orpana, H. M., Tremblay, M. S., Ross, R., Ortega, F. B., Silva, D. A. S., & Tomkinson, G. R. (2018). Field-based measurement of cardiorespiratory fitness to evaluate physical activity interventions. In *Bulletin of the World Health Organization* (Vol. 96, Issue 11). https://doi.org/10.2471/BLT.18.213728

Lanza, I. R., & Nair, K. S. (2010). Mitochondrial metabolic function assessed in vivo and in vitro. In *Current Opinion in Clinical Nutrition and Metabolic Care*. https://doi.org/10.1097/MCO.0b013e32833cc93d

Larsen, S., Hey-Mogensen, M., Rabøl, R., Stride, N., Helge, J. W., & Dela, F. (2012). The influence of age and aerobic fitness: Effects on mitochondrial respiration in skeletal muscle. *Acta Physiologica*, *205*(3). https://doi.org/10.1111/j.1748-1716.2012.02408.x

Larsson, L., & Karlsson, J. (1978). Isometric and dynamic endurance as a function of age and skeletal muscle characteristics. *Acta Physiologica Scandinavica*, *104*(2). https://doi.org/10.1111/j.1748-1716.1978.tb06259.x

Laskey, M. A. (1996). Dual-energy X-ray absorptiometry and body composition. In *Nutrition*. https://doi.org/10.1016/0899-9007(95)00017-8

Lenzen, S. (2014). A fresh view of glycolysis and glucokinase regulation: History and current status. In *Journal of Biological Chemistry* (Vol. 289, Issue 18). https://doi.org/10.1074/jbc.R114.557314

Leong, D. P., Teo, K. K., Rangarajan, S., Kutty, V. R., Lanas, F., Hui, C., Quanyong, X., Zhenzhen, Q., Jinhua, T., Noorhassim, I., AlHabib, K. F., Moss, S. J., Rosengren, A., Akalin, A. A., Rahman, O., Chifamba, J., Orlandini, A., Kumar, R., Yeates, K., … Yusuf, S. (2016). Reference ranges of handgrip strength from 125,462 healthy adults in 21 countries: a prospective urban rural epidemiologic (PURE) study. *Journal of Cachexia, Sarcopenia and Muscle*. https://doi.org/10.1002/jcsm.12112

Levine, J. A., Abboud, L., Barry, M., Reed, J. E., Sheedy, P. F., & Jensen, M. D.

(2000). Measuring leg muscle and fat mass in humans: Comparison of CT and dual- energy X-ray absorptiometry. *Journal of Applied Physiology*. https://doi.org/10.1152/jappl.2000.88.2.452

Levolger, S., Van Vugt, J. L. A., De Bruin, R. W. F., & IJzermans, J. N. M. (2015). Systematic review of sarcopenia in patients operated on for gastrointestinal and hepatopancreatobiliary malignancies. In *British Journal of Surgery*. https://doi.org/10.1002/bjs.9893

Lexell, J., Taylor, C. C., & Sjöström, M. (1988). What is the cause of the ageing atrophy?. Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *Journal of the Neurological Sciences*, *84*(2–3). https://doi.org/10.1016/0022-510X(88)90132-3

Li, C., Carli, F., Lee, L., Charlebois, P., Stein, B., Liberman, A. S., Kaneva, P., Augustin, B., Wongyingsinn, M., Gamsa, A., Kim, D. J., Vassiliou, M. C., & Feldman, L. S. (2013). Impact of a trimodal prehabilitation program on functional recovery after colorectal cancer surgery: A pilot study. *Surgical Endoscopy*, *27*(4). https://doi.org/10.1007/s00464-012-2560-5

Licker, M., Karenovics, W., Diaper, J., Frésard, I., Triponez, F., Ellenberger, C., Schorer, R., Kayser, B., & Bridevaux, P. O. (2017). Short-Term Preoperative High-Intensity Interval Training in Patients Awaiting Lung Cancer Surgery: A Randomized Controlled Trial. *Journal of Thoracic Oncology*, *12*(2), 323–333. https://doi.org/10.1016/j.jtho.2016.09.125

Lieffers, J. R., Bathe, O. F., Fassbender, K., Winget, M., & Baracos, V. E. (2012). Sarcopenia is associated with postoperative infection and delayed recovery from colorectal cancer resection surgery. *British Journal of Cancer*. https://doi.org/10.1038/bjc.2012.350

Lin, X., Zhang, X., Guo, J., Roberts, C. K., McKenzie, S., Wu, W. C., Liu, S., & Song, Y. (2015). Effects of exercise training on cardiorespiratory fitness and biomarkers of cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials. *Journal of the American Heart Association*, *4*(7). https://doi.org/10.1161/JAHA.115.002014

Liu, S. Z., & Marcinek, D. J. (2017). Skeletal muscle bioenergetics in aging and heart failure. *Heart Failure Reviews*. https://doi.org/10.1007/s10741-016-9586-z

LoConte, N. K., Else-Quest, N. M., Eickhoff, J., Hyde, J., & Schiller, J. H. (2008). Assessment of guilt and shame in patients with non-small-cell lung cancer compared with patients with breast and prostate cancer. *Clinical Lung Cancer*, *9*(3). https://doi.org/10.3816/CLC.2008.n.026

Long, Q., Huang, L., Huang, K., & Yang, Q. (2019). Assessing mitochondrial bioenergetics in isolated mitochondria from mouse heart tissues using oroboros 2k-oxygraph. In *Methods in Molecular Biology*. https://doi.org/10.1007/978-1-4939-9195-2_19

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194. https://doi.org/10.1016/j.cell.2013.05.039

Loughney, L., West, M. A., Kemp, G. J., Grocott, M. P. W., & Jack, S. (2015). Exercise intervention in people with cancer undergoing adjuvant cancer treatment following surgery: A systematic review. *European Journal of Surgical Oncology*, *41*(12), 1590–1602. https://doi.org/10.1016/j.ejso.2015.08.153

Loughney, L., West, M. A., Kemp, G. J., Grocott, M. P. W., & Jack, S. (2016). Exercise intervention in people with cancer undergoing neoadjuvant cancer treatment and surgery: A systematic review. In *European Journal of Surgical Oncology* (Vol. 42, Issue 1). https://doi.org/10.1016/j.ejso.2015.09.027

Lundby, C., & Jacobs, R. A. (2016). Adaptations of skeletal muscle mitochondria to exercise training. *Experimental Physiology*, *101*(1). https://doi.org/10.1113/EP085319

Macdonald, S., Cunningham, Y., Patterson, C., Robb, K., Macleod, U., Anker, T., & Hilton, S. (2018). Mass media and risk factors for cancer: the under-representation of age. *BMC Public Health*, *18*(1), 490. https://doi.org/10.1186/s12889-018-5341-9

MacInnis, M. J., & Gibala, M. J. (2017). Physiological adaptations to interval training and the role of exercise intensity. In *Journal of Physiology*. https://doi.org/10.1113/JP273196

Macmillan Cancer Support. (2012). *A research briefing paper by Macmillan Cancer Support*.

Malietzis, G., Johns, N., Al-Hassi, H. O., Knight, S. C., Kennedy, R. H., Fearon, K. C.

H., Aziz, O., & Jenkins, J. T. (2016). Low muscularity and myosteatosis is related to the host systemic inflammator y response in patients undergoing surger y for colorectal cancer. *Annals of Surgery*, *263*(2). https://doi.org/10.1097/SLA.0000000000001113

Martin, L., Birdsell, L., MacDonald, N., Reiman, T., Clandinin, M. T., McCargar, L. J., Murphy, R., Ghosh, S., Sawyer, M. B., & Baracos, V. E. (2013). Cancer cachexia in the age of obesity: Skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *Journal of Clinical Oncology*. https://doi.org/10.1200/JCO.2012.45.2722

Milanović, Z., Pantelić, S., Trajković, N., Sporiš, G., Kostić, R., & James, N. (2013). Age-related decrease in physical activity and functional fitness among elderly men and women. *Clinical Interventions in Aging*, *8*. https://doi.org/10.2147/CIA.S44112

Mitchell, W. K., Williams, J., Atherton, P., Larvin, M., Lund, J., & Narici, M. (2012). Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. In *Frontiers in Physiology: Vol. 3 JUL*. https://doi.org/10.3389/fphys.2012.00260

Mitsiopoulos, N., Baumgartner, R. N., Heymsfield, S. B., Lyons, W., Gallagher, D., & Ross, R. (1998). Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *Journal of Applied Physiology*. https://doi.org/10.1152/jappl.1998.85.1.115

Miyamoto, Y., Baba, Y., Sakamoto, Y., Ohuchi, M., Tokunaga, R., Kurashige, J., Hiyoshi, Y., Iwagami, S., Yoshida, N., Yoshida, M., Watanabe, M., & Baba, H. (2015). Sarcopenia is a Negative Prognostic Factor After Curative Resection of Colorectal Cancer. *Annals of Surgical Oncology*. https://doi.org/10.1245/s10434-014-4281-6

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., Altman, D., Antes, G., Atkins, D., Barbour, V., Barrowman, N., Berlin, J. A., Clark, J., Clarke, M., Cook, D., D'Amico, R., Deeks, J. J., Devereaux, P. J., Dickersin, K., Egger, M., Ernst, E., … Tugwell, P. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. In *PLoS Medicine*. https://doi.org/10.1371/journal.pmed.1000097

Moran, J., Guinan, E., McCormick, P., Larkin, J., Mockler, D., Hussey, J., Moriarty, J., & Wilson, F. (2016). The ability of prehabilitation to influence postoperative outcome after intra-abdominal operation: A systematic review and meta-analysis. *Surgery (United States)*, *160*(5). https://doi.org/10.1016/j.surg.2016.05.014

Morishita, S., Tsubaki, A., & Fu, J. B. (2017). Understanding Cachexia, Sarcopenia, and Physical Exercise in Patients with Cancer. In *Frailty and Sarcopenia - Onset, Development and Clinical Challenges*. IntechOpen. https://doi.org/10.5772/67628

Mosoro, E. (2017). *Medial Physiology* (3rd ed.). Elsevier Inc.

Mpampoulis, T., Methenitis, S., Papadopoulos, C., Papadimas, G., Spiliopoulou, P., Stasinaki, A.-N., Bogdanis, G. C., Karampatsos, G., & Terzis, G. (2021). Weak Association between Vastus Lateralis Muscle Fiber Composition and Fascicle Length in Young Untrained Females. *Sports*, *9*(5). https://doi.org/10.3390/sports9050056

Mukund, K., & Subramaniam, S. (2020). Skeletal muscle: A review of molecular structure and function, in health and disease. In *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* (Vol. 12, Issue 1). https://doi.org/10.1002/wsbm.1462

Murthy, N. S., & Mathew, A. (2004). Cancer epidemiology, prevention and control. In *Current Science* (Vol. 86, Issue 4).

Narici, M. V., Maganaris, C. N., Reeves, N. D., & Capodaglio, P. (2003). Effect of aging on human muscle architecture. *Journal of Applied Physiology*, *95*(6), 2229–2234. https://doi.org/10.1152/japplphysiol.00433.2003

Narici, Marco V., McPhee, J., Conte, M., & Franchi, M. V. (2021). Age-related alterations in muscle architecture are asignature of sarcopenia: the ultrasound sarcopeniaindex. *Journal of Cachexia, Sarcopenia and Muscle*. https://doi.org/10.1002/jcsm.12720

National Institute for Health and Care Excellence. (2020). *Colorectal cancer*. www.nice.org.uk/guidance/ng151

NCIN. (2010). *Cancer Waiting Times (CWT)*. Cancer Waiting Times (CWT).

http://www.ncin.org.uk/collecting_and_using_data/data_collection/gfocw

NCRAS. (2020). *National Cancer Registration and Analysis Service and Cancer Research UK*. Chemotherapy, Radiotherapy and Tumour Resection in England: 2013 -2014.

Ni, H. M., Williams, J. A., & Ding, W. X. (2015). Mitochondrial dynamics and mitochondrial quality control. In *Redox Biology* (Vol. 4, pp. 6–13). Elsevier B.V. https://doi.org/10.1016/j.redox.2014.11.006

Nicolaides, T., Horn, B., & Banerjee, A. (2017). Chemotherapy. In *Pediatric Oncology* (Issue 9783319307879, pp. 317–342). Springer Verlag. https://doi.org/10.1007/978-3-319-30789-3_15

Nunnari, J., & Suomalainen, A. (2012). Mitochondria: In sickness and in health. In *Cell* (Vol. 148, Issue 6, pp. 1145–1159). https://doi.org/10.1016/j.cell.2012.02.035

O'Doherty, A. F., West, M., Jack, S., & Grocott, M. P. W. (2013). Preoperative aerobic exercise training in elective intra-cavity surgery: A systematic review. In *British Journal of Anaesthesia* (Vol. 110, Issue 5). https://doi.org/10.1093/bja/aes514

O'Rourke, B., Cortassa, S., & Aon, M. A. (2005). Mitochondrial ion channels: Gatekeepers of life and death. In *Physiology* (Vol. 20, Issue 5). https://doi.org/10.1152/physiol.00020.2005

Oflazoglu, U., Alacacioglu, A., Varol, U., Kucukzeybek, Y., Salman, T., Taskaynatan, H., Yildiz, Y., Saray, S., & Tarhan, M. O. (2020). Chemotherapy-induced sarcopenia in newly diagnosed cancer patients: Izmir Oncology Group (IZOG) study. *Supportive Care in Cancer*. https://doi.org/10.1007/s00520-019-05165-6

Okuno, M., Goumard, C., Kopetz, S., Vega, E. A., Joechle, K., Mizuno, T., Tzeng, C. W. D., Chun, Y. S., Lee, J. E., Vauthey, J. N., Aloia, T. A., & Conrad, C. (2019). Loss of muscle mass during preoperative chemotherapy as a prognosticator for poor survival in patients with colorectal liver metastases. *Surgery (United States)*, *165*(2). https://doi.org/10.1016/j.surg.2018.07.031

Otera, H., Ishihara, N., & Mihara, K. (2013). New insights into the function and regulation of mitochondrial fission. In *Biochimica et Biophysica Acta - Molecular*

Cell Research (Vol. 1833, Issue 5). https://doi.org/10.1016/j.bbamcr.2013.02.002

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan-a web and mobile app for systematic reviews. *Systematic Reviews*. https://doi.org/10.1186/s13643-016-0384-4

Park, S. E., Hwang, I. G., Hwan, C., Kang, H., Kim, B. G., Park, B. K., Cha, S. J., Jang, J. S., & Choi, J. H. (2018). Sarcopenia is poor prognostic factor in older patients with locally advanced rectal cancer who received preoperative or postoperative chemoradiotherapy. *Medicine (United States)*, *97*(48). https://doi.org/10.1097/MD.0000000000013363

Pearce, A., Haas, M., Viney, R., Pearson, S. A., Haywood, P., Brown, C., & Ward, R. (2017). Incidence and severity of self-reported chemotherapy side effects in routine care: A prospective cohort study. *PLoS ONE*, *12*(10). https://doi.org/10.1371/journal.pone.0184360

Peng, P. D., Van Vledder, M. G., Tsai, S., De Jong, M. C., Makary, M., Ng, J., Edil, B. H., Wolfgang, C. L., Schulick, R. D., Choti, M. A., Kamel, I., & Pawlik, T. M. (2011). Sarcopenia negatively impacts short-term outcomes in patients undergoing hepatic resection for colorectal liver metastasis. *HPB*. https://doi.org/10.1111/j.1477-2574.2011.00301.x

Penna, F., Ballarò, R., Beltrà, M., De Lucia, S., Castillo, L. G., & Costelli, P. (2019). The skeletal muscle as an active player against cancer cachexia. *Frontiers in Physiology*, *10*(FEB), 1–15. https://doi.org/10.3389/fphys.2019.00041

Penna, F., Costamagna, D., Fanzani, A., Bonelli, G., Baccino, F. M., & Costelli, P. (2010). Muscle wasting and impaired Myogenesis in tumor bearing mice are prevented by ERK inhibition. *PLoS ONE*, *5*(10). https://doi.org/10.1371/journal.pone.0013604

Perkisas, S., Baudry, S., Bauer, J., Beckwée, D., De Cock, A. M., Hobbelen, H., Jager-Wittenaar, H., Kasiukiewicz, A., Landi, F., Marco, E., Merello, A., Piotrowicz, K., Sanchez, E., Sanchez-Rodriguez, D., Scafoglieri, A., Cruz-Jentoft, A., & Vandewoude, M. (2018). Application of ultrasound for muscle assessment in sarcopenia: towards standardized measurements. *European Geriatric Medicine*. https://doi.org/10.1007/s41999-018-0104-9

Pesta, D., & Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods in Molecular Biology*, *810*. https://doi.org/10.1007/978-1-61779-382-0_3

Peterson, C. M., Johannsen, D. L., & Ravussin, E. (2012). Skeletal muscle mitochondria and aging: A review. In *Journal of Aging Research* (Vol. 2012). https://doi.org/10.1155/2012/194821

Phillips, B. E., Kelly, B. M., Lilja, M., Ponce-González, J. G., Brogan, R. J., Morris, D. L., Gustafsson, T., Kraus, W. E., Atherton, P. J., Vollaard, N. B. J., Rooyackers, O., & Timmons, J. A. (2017). A practical and time-efficient high-intensity interval training program modifies cardio-metabolic risk factors in adults with risk factors for type II diabetes. *Frontiers in Endocrinology*, *8*(SEP). https://doi.org/10.3389/fendo.2017.00229

Pin, F., Couch, M. E., & Bonetto, A. (2018). Preservation of muscle mass as a strategy to reduce the toxic effects of cancer chemotherapy on body composition. In *Current Opinion in Supportive and Palliative Care* (Vol. 12, Issue 4). https://doi.org/10.1097/SPC.0000000000000382

Piraux, E., Caty, G., & Reychler, G. (2018). Effects of preoperative combined aerobic and resistance exercise training in cancer patients undergoing tumour resection surgery: A systematic review of randomised trials. *Surgical Oncology*, *27*(3), 584–594. https://doi.org/10.1016/j.suronc.2018.07.007

Pollard, T., Earnshaw, W., Lippincott-Schwatz, J., & Johnson, G. (2017). *Cell Biology* (3rd ed.). Elsevier Inc.

Porporato, P. E. (2016). Understanding cachexia as a cancer metabolism syndrome. *Oncogenesis*, *5*(2), 1–10. https://doi.org/10.1038/oncsis.2016.3

Porter, C., Hurren, N. M., Cotter, M. V, Bhattarai, N., Reidy, P. T., Dillon, E. L., Durham, W. J., Tuvdendorj, D., Sheffield-moore, M., Volpi, E., Sidossis, L. S., Rasmussen, B. B., Børsheim, E., Wj, D., Tuvdendorj, D., & Volpi, E. (2015). *Mitochondrial respiratory capacity and coupling control decline with age in human skeletal muscle*. 224–232. https://doi.org/10.1152/ajpendo.00125.2015

Prado, C. M., Lieffers, J. R., McCargar, L. J., Reiman, T., Sawyer, M. B., Martin, L.,

& Baracos, V. E. (2008). Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *The Lancet Oncology*, *9*(7), 629–635. https://doi.org/10.1016/S1470-2045(08)70153-0

Prado, C. M. M., Birdsell, L. A., & Baracos, V. E. (2009). The emerging role of computerized tomography in assessing cancer cachexia. *Current Opinion in Supportive and Palliative Care*. https://doi.org/10.1097/SPC.0b013e328331124a

Proctor, D. N., & Joyner, M. J. (1997). Skeletal muscle mass and the reduction of V̇O2(max) in trained older subjects. *Journal of Applied Physiology*, *82*(5), 1411–1415. https://doi.org/10.1152/jappl.1997.82.5.1411

Pustylnikov, S., Costabile, F., Beghi, S., & Facciabene, A. (2018). Targeting mitochondria in cancer: current concepts and immunotherapy approaches. *Translational Research*, *202*, 35–51. https://doi.org/10.1016/j.trsl.2018.07.013

Quaresma, M., Coleman, M. P., & Rachet, B. (2015). 40-year trends in an index of survival for all cancers combined and survival adjusted for age and sex for each cancer in England and Wales, 1971-2011: A population-based study. *The Lancet*, *385*(9974), 1206–1218. https://doi.org/10.1016/S0140-6736(14)61396-9

Rawla, P., Sunkara, T., & Barsouk, A. (2019). Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. In *Przeglad Gastroenterologiczny* (Vol. 14, Issue 2). https://doi.org/10.5114/pg.2018.81072

Reed, M. (2009). Principles of cancer treatment by surgery. In *Surgery* (Vol. 27, Issue 4, pp. 178–181). https://doi.org/10.1016/j.mpsur.2009.01.003

Reiss, J., Iglseder, B., Kreutzer, M., Weilbuchner, I., Treschnitzer, W., Kässmann, H., Pirich, C., & Reiter, R. (2016). Case finding for sarcopenia in geriatric inpatients: Performance of bioimpedance analysis in comparison to dual X-ray absorptiometry. *BMC Geriatrics*. https://doi.org/10.1186/s12877-016-0228-z

Richards, C. H., Roxburgh, C. S. D., MacMillan, M. T., Isswiasi, S., Robertson, E. G., Guthrie, G. K., Horgan, P. G., & McMillan, D. C. (2012). The relationships between body composition and the systemic inflammatory response in patients with primary operable colorectal cancer. *PLoS ONE*, *7*(8). https://doi.org/10.1371/journal.pone.0041883

Roman, M. A., Rossiter, H. B., & Casaburi, R. (2016). Exercise, ageing and the lung. In *European Respiratory Journal* (Vol. 48, Issue 5). https://doi.org/10.1183/13993003.00347-2016

Romanello, V., & Sandri, M. (2016). Mitochondrial quality control and muscle mass maintenance. *Frontiers in Physiology*, *6*(JAN), 1–21. https://doi.org/10.3389/fphys.2015.00422

Rosenberg, I. H. (1997). Symposium: Sarcopenia: Diagnosis and Mechanisms Sarcopenia: Origins and Clinical Relevance 1. *J. Nutr*, *127*, 990–991.

Russell, A. P., Foletta, V. C., Snow, R. J., & Wadley, G. D. (2014). Skeletal muscle mitochondria: A major player in exercise, health and disease. In *Biochimica et Biophysica Acta - General Subjects*. https://doi.org/10.1016/j.bbagen.2013.11.016

Safdar, A., Khrapko, K., Flynn, J. M., Saleem, A., De Lisio, M., Johnston, A. P. W., Kratysberg, Y., Samjoo, I. A., Kitaoka, Y., Ogborn, D. I., Little, J. P., Raha, S., Parise, G., Akhtar, M., Hettinga, B. P., Rowe, G. C., Arany, Z., Prolla, T. A., & Tarnopolsky, M. A. (2016). Exercise-induced mitochondrial p53 repairs mtDNA mutations in mutator mice. In *Skeletal Muscle* (Vol. 6, Issue 1). https://doi.org/10.1186/s13395-016-0075-9

Santanasto, A. J., Glynn, N. W., Jubrias, S. A., Conley, K. E., Boudreau, R. M., Amati, F., Mackey, D. C., Simonsick, E. M., Strotmeyer, E. S., Coen, P. M., Goodpaster, B. H., & Newman, A. B. (2015). Skeletal Muscle Mitochondrial Function and Fatigability in Older Adults. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *70*(11). https://doi.org/10.1093/gerona/glu134

Sebastián, D., Palacín, M., & Zorzano, A. (2017). Mitochondrial Dynamics: Coupling Mitochondrial Fitness with Healthy Aging. In *Trends in Molecular Medicine*. https://doi.org/10.1016/j.molmed.2017.01.003

Senft, D., & Ronai, Z. A. (2016). Regulators of mitochondrial dynamics in cancer. *Current Opinion in Cell Biology*, *39*(Table 1), 43–52. https://doi.org/10.1016/j.ceb.2016.02.001

Seo, D. Y., Lee, S. R., Kim, N., Ko, K. S., Rhee, B. D., & Han, J. (2016). Age-related

changes in skeletal muscle mitochondria: the role of exercise. *Integrative Medicine Research*, *5*(3). https://doi.org/10.1016/j.imr.2016.07.003

Shachar, S. S., Williams, G. R., Muss, H. B., & Nishijima, T. F. (2016). Prognostic value of sarcopenia in adults with solid tumours: A meta-analysis and systematic review. In *European Journal of Cancer*. https://doi.org/10.1016/j.ejca.2015.12.030

Sharma, K. (2020). Psycho Oncology. *Acta Scientific Cancer Biology*, *4*(5), 7–8.

Shen, W., Punyanitya, M., Wang, Z. M., Gallagher, D., St.-Onge, M. P., Albu, J., Heymsfield, S. B., & Heshka, S. (2004). Total body skeletal muscle and adipose tissue volumes: Estimation from a single abdominal cross-sectional image. *Journal of Applied Physiology*. https://doi.org/10.1152/japplphysiol.00744.2004

Sherwood, L. (2010). *Human physiology* (7th Editio). Brooks/Cole-Cengage Learning.

Short, K. R., Bigelow, M. L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., & Nair, K. S. (2005). Decline in skeletal muscle mitochondrial function with aging in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(15). https://doi.org/10.1073/pnas.0501559102

Shum, A. M. Y., Poljak, A., Bentley, N. L., Turner, N., Tan, T. C., & Polly, P. (2018). Proteomic profiling of skeletal and cardiac muscle in cancer cachexia: Alterations in sarcomeric and mitochondrial protein expression. *Oncotarget*, *9*(31). https://doi.org/10.18632/oncotarget.25146

Shur, N. F., Creedon, L., Skirrow, S., Atherton, P. J., MacDonald, I. A., Lund, J., & Greenhaff, P. L. (2021). Age-related changes in muscle architecture and metabolism in humans: The likely contribution of physical inactivity to age-related functional decline. In *Ageing Research Reviews* (Vol. 68). https://doi.org/10.1016/j.arr.2021.101344

Smeuninx, B., Elhassan, Y. S., Manolopoulos, K. N., Sapey, E., Rushton, A. B., Edwards, S. J., Morgan, P. T., Philp, A., Brook, M. S., Gharahdaghi, N., Smith, K., Atherton, P. J., & Breen, L. (2021). The effect of short-term exercise prehabilitation on skeletal muscle protein synthesis and atrophy during bed rest

in older men. *Journal of Cachexia, Sarcopenia and Muscle*, *12*(1). https://doi.org/10.1002/jcsm.12661

Smith, G. L., & Smith, B. D. (2014). Radiation treatment in older patients: A framework for clinical decision making. In *Journal of Clinical Oncology* (Vol. 32, Issue 24). https://doi.org/10.1200/JCO.2014.55.1168

Smith, H. R. (2015). Depression in cancer patients: Pathogenesis, implications and treatment (review). *Oncology Letters*, *9*(4), 1509–1514. https://doi.org/10.3892/ol.2015.2944

Snowden, C. P., Prentis, J., Jacques, B., Anderson, H., Manas, D., Jones, D., & Trenell, M. (2013). Cardiorespiratory fitness predicts mortality and hospital length of stay after major elective surgery in older people. *Annals of Surgery*, *257*(6). https://doi.org/10.1097/SLA.0b013e31828dbac2

Song, Z., Wang, Y., Zhang, F., Yao, F., & Sun, C. (2019). Calcium signaling pathways: Key pathways in the regulation of obesity. In *International Journal of Molecular Sciences* (Vol. 20, Issue 11). https://doi.org/10.3390/ijms20112768

Spinelli, J. B., & Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. In *Nature Cell Biology* (Vol. 20, Issue 7). https://doi.org/10.1038/s41556-018-0124-1

St-Jean-Pelletier, F., Pion, C. H., Leduc-Gaudet, J. P., Sgarioto, N., Zovilé, I., Barbat-Artigas, S., Reynaud, O., Alkaterji, F., Lemieux, F. C., Grenon, A., Gaudreau, P., Hepple, R. T., Chevalier, S., Belanger, M., Morais, J. A., Aubertin-Leheudre, M., & Gouspillou, G. (2017). The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men. *Journal of Cachexia, Sarcopenia and Muscle*, *8*(2). https://doi.org/10.1002/jcsm.12139

Stein, C. J., & Colditz, G. A. (2004). Modifiable risk factors for cancer. *British Journal of Cancer*, *90*(2), 299–303. https://doi.org/10.1038/sj.bjc.6601509

Stein, K. D., Syrjala, K. L., & Andrykowski, M. A. (2008). Physical and psychological long-term and late effects of cancer. In *Cancer* (Vol. 112, Issue 11 SUPPL.). https://doi.org/10.1002/cncr.23448

Stene, G. B., Helbostad, J. L., Balstad, T. R., Riphagen, I. I., Kaasa, S., & Oldervoll,

L. M. (2013). Effect of physical exercise on muscle mass and strength in cancer patients during treatment-A systematic review. In *Critical Reviews in Oncology/Hematology* (Vol. 88, Issue 3). https://doi.org/10.1016/j.critrevonc.2013.07.001

Stewart, B., & Wild, C. (2014). World Cancer Report 2014. *International Agency for Research on Cancer*, *22*(1).

Strasser, E. M., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6). https://doi.org/10.1007/s11357-013-9517-z

Tait, S. W. G., & Green, D. R. (2012). Mitochondria and cell signalling. In *Journal of Cell Science* (Vol. 125, Issue 4). https://doi.org/10.1242/jcs.099234

Thomsen, T., Villebro, N., & Møller, A. M. (2014). Interventions for preoperative smoking cessation. In *Cochrane Database of Systematic Reviews* (Vol. 2014, Issue 3). https://doi.org/10.1002/14651858.CD002294.pub4

Thun, M. J., Peto, R., Lopez, A. D., Monaco, J. H., Henley, S. J., Heath, C. W., & Doll, R. (1997). Alcohol Consumption and Mortality among Middle-Aged and Elderly U.S. Adults. *New England Journal of Medicine*, *337*(24). https://doi.org/10.1056/nejm199712113372401

Ticinesi, A., Meschi, T., Narici, M. V., Lauretani, F., & Maggio, M. (2017). Muscle Ultrasound and Sarcopenia in Older Individuals: A Clinical Perspective. In *Journal of the American Medical Directors Association* (Vol. 18, Issue 4). https://doi.org/10.1016/j.jamda.2016.11.013

Tieland, M., Trouwborst, I., & Clark, B. C. (2018). Skeletal muscle performance and ageing. In *Journal of Cachexia, Sarcopenia and Muscle* (Vol. 9, Issue 1). https://doi.org/10.1002/jcsm.12238

Tilokani, L., Nagashima, S., Paupe, V., & Prudent, J. (2018). Mitochondrial dynamics: Overview of molecular mechanisms. In *Essays in Biochemistry* (Vol. 62, Issue 3). https://doi.org/10.1042/EBC20170104

Tonkonogi, M., Fernström, M., Walsh, B., Ji, L. L., Rooyackers, O., Hammarqvist, F., Wernerman, J., & Sahlin, K. (2003). Reduced oxidative power but unchanged

antioxidative capacity in skeletal muscle from aged humans. *Pflugers Archiv European Journal of Physiology*, *446*(2). https://doi.org/10.1007/s00424-003-1044-9

Treacy, D., & Hassett, L. (2018). The Short Physical Performance Battery. In *Journal of Physiotherapy*. https://doi.org/10.1016/j.jphys.2017.04.002

Trounce, I., Byrne, E., & Marzuki, S. (1989). DECLINE IN SKELETAL MUSCLE MITOCHONDRIAL RESPIRATORY CHAIN FUNCTION: POSSIBLE FACTOR IN AGEING. *The Lancet*, *333*(8639). https://doi.org/10.1016/S0140-6736(89)92143-0

Tryfidou, D. V., McClean, C., Nikolaidis, M. G., & Davison, G. W. (2020). DNA Damage Following Acute Aerobic Exercise: A Systematic Review and Meta-analysis. In *Sports Medicine*. https://doi.org/10.1007/s40279-019-01181-y

U.S National Institutes of Heal. (2020). *SEER Training Modules, Cancer Classifications*. https://training.seer.cancer.gov/

Uribe, J. M., Stump, C. S., Tipton, C. M., & Fregosi, R. F. (1992). Influence of exercise training on the oxidative capacity of rat abdominal muscles. *Respiration Physiology*, *88*(1–2). https://doi.org/10.1016/0034-5687(92)90038-X

van der Ende, M., Grefte, S., Plas, R., Meijerink, J., Witkamp, R. F., Keijer, J., & van Norren, K. (2018). Mitochondrial dynamics in cancer-induced cachexia. In *Biochimica et Biophysica Acta - Reviews on Cancer* (Vol. 1870, Issue 2). https://doi.org/10.1016/j.bbcan.2018.07.008

Van Der Zwaard, X. S., De Ruiter, C. J., Noordhof, D. A., Sterrenburg, R., Bloemers, F. W., De Koning, J. J., Jaspers, R. T., & Van Der Laarse, W. J. (2016). Maximal oxygen uptake is proportional to muscle fiber oxidative capacity, from chronic heart failure patients to professional cyclists. *Journal of Applied Physiology*, *121*(3). https://doi.org/10.1152/japplphysiol.00355.2016

van Vugt, J. L. A., Coebergh van den Braak, R. R. J., Lalmahomed, Z. S., Vrijland, W. W., Dekker, J. W. T., Zimmerman, D. D. E., Vles, W. J., Coene, P. P. L. O., & IJzermans, J. N. M. (2018). Impact of low skeletal muscle mass and density on short and long-term outcome after resection of stage I-III colorectal cancer. *European Journal of Surgical Oncology*, *44*(9), 1354–1360.

https://doi.org/10.1016/j.ejso.2018.05.029

Vanderveen, B. N., Fix, D. K., & Carson, J. A. (2017). Disrupted Skeletal Muscle Mitochondrial Dynamics, Mitophagy, and Biogenesis during Cancer Cachexia: A Role for Inflammation. *Oxidative Medicine and Cellular Longevity*, *2017*, 24–27. https://doi.org/10.1155/2017/3292087

Wahwah, N., Kras, K. A., Roust, L. R., & Katsanos, C. S. (2020). Subpopulation-specific differences in skeletal muscle mitochondria in humans with obesity: Insights from studies employing acute nutritional and exercise stimuli. In *American Journal of Physiology - Endocrinology and Metabolism* (Vol. 318, Issue 4). https://doi.org/10.1152/ajpendo.00463.2019

Wang, L., Lee, M., Zhang, Z., Moodie, J., Cheng, D., & Martin, J. (2016). Does preoperative rehabilitation for patients planning to undergo joint replacement surgery improve outcomes? A systematic review and meta-analysis of randomised controlled trials. In *BMJ Open* (Vol. 6, Issue 2). https://doi.org/10.1136/bmjopen-2015-009857

Weinberg, S. E., Sena, L. A., & Chandel, N. S. (2015). Mitochondria in the regulation of innate and adaptive immunity. In *Immunity* (Vol. 42, Issue 3). https://doi.org/10.1016/j.immuni.2015.02.002

Weisman, I. M., Weisman, I. M., Marciniuk, D., Martinez, F. J., Sciurba, F., Sue, D., Myers, J., Casaburi, R., Marciniuk, D., Beck, K., Zeballos, J., Swanson, G., Myers, J., Sciurba, F., Johnson, B., Whipp, B., Zeballos, J., Weisman, I. M., Beck, K., … Sietsema, K. (2003). ATS/ACCP Statement on cardiopulmonary exercise testing. *American Journal of Respiratory and Critical Care Medicine*, *167*(2), 211–277. https://doi.org/10.1164/rccm.167.2.211

Weiss, E. P., Spina, R. J., Holloszy, J. O., & Ehsani, A. A. (2006). Gender differences in the decline in aerobic capacity and its physiological determinants during the later decades of life. *Journal of Applied Physiology*, *101*(3). https://doi.org/10.1152/japplphysiol.01398.2005

Weston, M., Weston, K. L., Prentis, J. M., & Snowden, C. P. (2016). High-intensity interval training (HIT) for effective and time-efficient pre-surgical exercise interventions. *Perioperative Medicine*, *5*(1), 1–9. https://doi.org/10.1186/s13741-015-0026-8

White, M. C., Holman, D. M., Boehm, J. E., Peipins, L. A., Grossman, M., & Jane Henley, S. (2014). Age and cancer risk: A potentially modifiable relationship. *American Journal of Preventive Medicine*, *46*(3 SUPPL. 1). https://doi.org/10.1016/j.amepre.2013.10.029

Whyte, G. (2006). *The Physiology of Training: Advances in Sport and Exercise Science series*. Churchill Livingstone.

Wiederkehr, A., Szanda, G., Akhmedov, D., Mataki, C., Heizmann, C. W., Schoonjans, K., Pozzan, T., Spät, A., & Wollheim, C. B. (2011). Mitochondrial matrix calcium is an activating signal for hormone secretion. *Cell Metabolism*, *13*(5). https://doi.org/10.1016/j.cmet.2011.03.015

Williams, J. P., Phillips, B. E., Smith, K., Atherton, P. J., Rankin, D., Selby, A. L., Liptrot, S., Lund, J., Larvin, M., & Rennie, M. J. (2012). Effect of tumor burden and subsequent surgical resection on skeletal muscle mass and protein turnover in colorectal cancer patients. *American Journal of Clinical Nutrition*, *96*(5). https://doi.org/10.3945/ajcn.112.045708

Wind-Cowie & Salter. (2013). *"Getting to grips with the financial consequences of cancer…."*

World Medical Association. (1964). *WMA Declaration of Helsinki - Ethical Principles For Medical Research Involving Human Subjects*. WMA Declaration of Helsinki - Ethical Principles For Medical Research Involving Human Subjects. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

Woźniak, K., & Izycki, D. (2014). Cancer: A family at risk. In *Przeglad Menopauzalny* (Vol. 13, Issue 4). https://doi.org/10.5114/pm.2014.45002

Yamaoka, Y., Fujitani, K., Tsujinaka, T., Yamamoto, K., Hirao, M., & Sekimoto, M. (2015). Skeletal muscle loss after total gastrectomy, exacerbated by adjuvant chemotherapy. *Gastric Cancer*. https://doi.org/10.1007/s10120-014-0365-z

Yoo, S. Z., No, M. H., Heo, J. W., Park, D. H., Kang, J. H., Kim, S. H., & Kwak, H. B. (2018). Role of exercise in age-related sarcopenia. In *Journal of Exercise Rehabilitation* (Vol. 14, Issue 4). https://doi.org/10.12965/jer.1836268.134

Youle, R. J., & Van Der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress.

In *Science* (Vol. 337, Issue 6098). https://doi.org/10.1126/science.1219855

Yu, R., Lendahl, U., Nistér, M., & Zhao, J. (2020). Regulation of Mammalian Mitochondrial Dynamics: Opportunities and Challenges. In *Frontiers in Endocrinology* (Vol. 11). https://doi.org/10.3389/fendo.2020.00374

Yu, S., Appleton, S., Chapman, I., Adams, R., Wittert, G., Visvanathan, T., & Visvanathan, R. (2015). An Anthropometric Prediction Equation for Appendicular Skeletal Muscle Mass in Combination With a Measure of Muscle Function to Screen for Sarcopenia in Primary and Aged Care. *Journal of the American Medical Directors Association*. https://doi.org/10.1016/j.jamda.2014.06.018

Zhao, R. Z., Jiang, S., Zhang, L., & Yu, Z. Bin. (2019). Mitochondrial electron transport chain, ROS generation and uncoupling (Review). In *International Journal of Molecular Medicine* (Vol. 44, Issue 1). https://doi.org/10.3892/ijmm.2019.4188