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Title	Advancing cancer cachexia diagnosis with -omics technology and exercise as molecular medicine
Type	Article
URL	https://clock.uclan.ac.uk/id/eprint/49967/
DOI	https://doi.org/10.1016/j.smhs.2024.01.006
Date	2024
Citation	Hesketh, Stuart J (2024) Advancing cancer cachexia diagnosis with -omics technology and exercise as molecular medicine. Sports Medicine and Health Science, 6 (1). pp. 1-15.
Creators	Hesketh, Stuart J

It is advisable to refer to the publisher's version if you intend to cite from the work.
<https://doi.org/10.1016/j.smhs.2024.01.006>

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Review

Advancing cancer cachexia diagnosis with -omics technology and exercise as molecular medicine

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ARTICLE INFO

Keywords:

Cancer cachexia
Exercise
Muscle atrophy
Molecular medicine
Omics

ABSTRACT

Muscle atrophy exacerbates disease outcomes and increases mortality, whereas the preservation of skeletal muscle mass and function play pivotal roles in ensuring long-term health and overall quality-of-life. Muscle atrophy represents a significant clinical challenge, involving the continued loss of muscle mass and strength, which frequently accompany the development of numerous types of cancer. Cancer cachexia is a highly prevalent multifactorial syndrome, and although cachexia is one of the main causes of cancer-related deaths, there are still no approved management strategies for the disease. The etiology of this condition is based on the upregulation of systemic inflammation factors and catabolic stimuli, resulting in the inhibition of protein synthesis and enhancement of protein degradation. Numerous necessary cellular processes are disrupted by cachectic pathology, which mediate intracellular signalling pathways resulting in the net loss of muscle and organelles. However, the exact underpinning molecular mechanisms of how these changes are orchestrated are incompletely understood. Much work is still required, but structured exercise has the capacity to counteract numerous detrimental effects linked to cancer cachexia. Primarily through the stimulation of muscle protein synthesis, enhancement of mitochondrial function, and the release of myokines. As a result, muscle mass and strength increase, leading to improved mobility, and quality-of-life. This review summarises existing knowledge of the complex molecular networks that regulate cancer cachexia and exercise, highlighting the molecular interplay between the two for potential therapeutic intervention. Finally, the utility of mass spectrometry-based proteomics is considered as a way of establishing early diagnostic biomarkers of cachectic patients.

1. Introduction

Skeletal muscle mass and function are crucial to long-term health and quality-of-life, accounting for ~40% of the total body mass in healthy adults.^{1,2} Beyond its obvious involvement in force generation for movement, it also contributes to skeletal support, thermoregulation, and plays a fundamental role in metabolism.³ Therefore, pathological conditions that attenuate muscle development, maintenance, and function severely impact quality-of-life.

Cachexia is an incredibility complex and debilitating condition that describes muscle wasting and weight loss secondary to many chronic diseases, such as cancer,⁴ organ dysfunctions including renal or respiratory failure,⁵ and autoimmune disease.⁶ The agreed diagnostic criteria for cachexia are weight loss greater than 5% during the past 6 months in the absence of simple starvation, or weight loss greater than 2% in individuals with a body mass index (BMI) < 20 kg·m⁻² or sarcopenia.^{7,8} The clinical problem is significant with ~35% of cancer patients estimated to be affected by cachexia in the UK, and 50%–80% globally.⁹ Further, it is

responsible for more than 20% of all cancer related deaths and almost 1 million cancer survivors display a history of sarcopenia.¹⁰ Despite advancements in oncology treatment strategies, the prevalence of cancer cachexia remains alarmingly high. It is a common complication in the late stages of most cancers and can also develop early during others (e.g., pancreatic, gastroesophageal, lung), currently affecting up to 80% of all cancer patients at some point over the course of their disease.¹¹ Cachexia is a hypercatabolic state characterised by the progressive wasting of skeletal muscle and is strongly associated with reduced treatment tolerance leading to increased morbidity rates. Once the condition becomes clinically evident it is exceptionally difficult to reverse. However, there are currently no agreed early biomarkers to effectively detect cachexia, and at present there is a distinct paucity of high efficacy treatment strategies available.^{12,13} Thus, highlighting an urgent requirement for the development of effective therapies and early diagnosis tools to improve treatment responses, ultimately permitting intervention trials that mitigate disease progression and extend patient survival.

It is important to state that while there are some strategies that address cancer cachexia, there is no primary recognised intervention for

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<https://doi.org/10.1016/j.smhs.2024.01.006>

Received 21 September 2023; Received in revised form 15 January 2024; Accepted 20 January 2024

Available online 28 January 2024

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Abbreviation list:

3 MH	3-methylhistidine	IL-6	Interleukin-6
Ac	Acetylation	IL-6R	Interleukin 6 receptor
ACTRIIB	Activin type II receptor	MAFbx	Muscle atrophy F-box protein 1
AMP	Adenosine monophosphate	METS	Metabolic equivalents
AMPK	Adenosine monophosphate-activated protein kinase	MPS	Muscle protein synthesis
AngII	Angiotensin II	mRNA	Messenger ribonucleic acid
AT1R	Type 1 angiotensin II receptors	mTOR	Mammalian target of rapamycin
ATP	Adenosine triphosphate	MuRF1	Muscle ring finger protein 1
BMI	Body mass index	NF-κB	Nuclear factor kappa B
C26	Colon-26	P	Phosphorylation
Ca ²⁺	Calcium ion	p38 MAPK	p38 mitogen-activated protein kinase
CaM	Calmodulin	p53	Tumour protein p53
CaMKII	Calcium Calmodulin-dependent protein kinase II	p70 S6K	p70 ribosomal S6 kinase
CaMKK	Calcium Calmodulin-dependent protein kinase kinase	PCR	Polymerase chain reaction
CXCR4	C-X-C chemokine receptor type 4	PIF	Proteolysis inducing factors
DEG	protein degradation	PIFR	Proteolysis inducing factor receptor
EDL	Extensor digitorum longus	PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha
ERK 1/2	Extracellular signal-regulated kinase 1/2	PTMs	Post translational modifications
FAK	Focal adhesion kinase	RNA-seq	RNA sequencing
FOXO	Forkhead box transcription factor	ROS	Reactive oxygen species
FOXO1	Forkhead box protein O1	SDF1	Stromal cell-derived factor 1
JAK/STAT	Janus kinase/signal transducers and activators of transcription	SMAD 2/3	Suppressor of Mothers against Decapentaplegic 2 and 3
JNK	c-Jun N-terminal kinase	SWATH-MS	Sequential window acquisition of all theoretical mass spectra
IGF-1	Insulin-like growth factor	TNFα	Tumour necrosis factor alpha
IL-1 β	Interleukin 1-beta	TNF αR	Tumor necrosis factor alpha receptor
IL-1 βR	Interleukin 1-beta receptor	UPS	Ubiquitin proteasome system
		Yap	Yes-associated protein

treatment, and some approaches that are currently considered for its management can be inconsistent in their success. For example, oral and/or parenteral nutritional strategies include high calorie, high protein diets aimed at mitigating weight loss and muscle wasting.^{14–16} Pharmacological interventions including selective androgen modulators,^{17,18} non-steroidal anti-inflammatory drugs,^{19,20} ghrelin agonists,^{21,22} and omega-3-fatty acids,^{23,24} or more recently the use of cannabinoids²⁵ all of which focus on the promotion of healthy muscle mass and function, as well as improving skeletal muscle metabolic health. Multimodal approaches are often the most common management strategy where a combination of interventions can have synergistic affects in managing cachexia.^{12,26} However, the effectiveness of such an approach can vary widely among individuals and can highly depend on factors such as the type of cancer, stage of cachexia, overall health of the individual, and treatment regimen. Additionally, cachexia is a complex syndrome with multiple underlying mechanisms, making it difficult to establish a single universal solution. It is also important to note that current anti-cancer treatments (e.g., chemotherapy) add further complexity to identifying the underlying molecular signatures responsible for cachexia, and can often have a negative impact on muscle metabolism and wasting.^{27,28} However, amid the array of potential interventions, exercise and structured physical activity may prove to be a promising avenue for addressing cancer cachexia.²⁹ Historically, exercise was viewed with caution in cancer patients due to concerns regarding physical strain and potential exacerbation of cachexia-related symptoms. However, it is now well established that exercise interventions hold the potential to attenuate muscle wasting and enhance physical function, thus improving overall quality-of-life for cancer patients.^{29–31} More recently, there now shows promise, with growing clinical evidence to suggest that properly designed exercise interventions can exert positive influence on cachexia-related outcomes.^{32–35}

This review aims to bring together current knowledge regarding the interaction between cancer cachexia and the use of exercise as a

therapeutic intervention. Moreover, new perspective will be added by considering the novelty of emerging -omics technology in the field of exercise and cancer cachexia. By exploring the molecular, physiological, and clinical dimensions of this interaction, this review seeks to elucidate the potential mechanisms by which exercise may counteract the deleterious effects of cancer cachexia. Finally, recognising the paramount significance that new early detection methods would offer. The potential of cutting-edge discovery techniques, such as mass spectrometry-based proteomics are considered. Taken together, the understanding of the molecular interplay of exercise and cancer cachexia, along with the information gained by novel -omics approaches can potentially illuminate a path towards combining new robust diagnosis methods, and effective early treatment strategies, to ultimately combat disease progression and improve overall quality-of-life for those affected by this debilitating condition.

2. Cachexic mechanisms of muscle loss

Cancer cachexia is a multifactorial syndrome, of which the pathogenesis involves a complex interplay of factors. The development of cachexia is common during the progression of several types of malignant tumors, primarily upper gastrointestinal, pancreatic, and lung cancers.^{11,36} Cancer cachexia is divided into three consecutive clinical stages; pre-cachexia, cachexia, and refractory cachexia, though patients may not experience all three stages. The incidence and severity of cachexia are highly heterogeneous and depend on the type, location, and stage of the tumour. At present, there are no specific biomarkers for early stage cachexia identification. Staging is therefore entirely determined according to the clinical manifestations and characteristics of the patient. To date, the mechanism by which cancer cachexia induces muscle wasting has not been fully resolved. However, it is clear there is an imbalance between skeletal muscle protein synthesis and degradation during the progression of the disease.⁷ Emery et al.,³⁷ was one of the first

to suggest that muscle mass in cancer cachexia is regulated primarily by alterations in protein synthesis with changes in protein degradation likely to be secondary. The authors used L-leucine stable isotope infusion to measure whole body protein synthesis and observed synthetic rates of cachexia patients to be almost 4 %/d less than healthy controls. However, it is important to acknowledge that traditional amino acid tracer techniques may also be influenced by the metabolism and transport rates in particular tissues when taking whole body measures. Synthesis values from amino acid tracer studies also represent averages across the entire proteome i.e., data from mixtures containing many hundreds of proteins. Moreover, the calculation of degradation rate using this technique is less than ideal due to the rate of appearance of the labelled amino acids not being truly reflective of actual degradation rates. This is attributed to the problem of tracer 'label recycling' where tracer can be reincorporated into new protein.³⁸ Thus, providing end values that are potentially misleading. Another early study Lundholm et al.,³⁹ arrived at a similar conclusion to Emery et al., by measuring the release of 3-methylhistidine (3 MH) from leg tissue of cancer cachexia patients. Insignificant levels of 3 MH were measured for cachectic patients, whilst both acutely ill patients and healthy controls showed significant release. 3 MH is a post translationally methylated histidine found in myofibrillar proteins, commonly used as a marker of myofibrillar degradation because it cannot be further metabolised or recycled for use in protein synthesis.^{40,41} 3 MH methods are controversial⁴² and the routine measurement of 3 MH in urine means the degradation information is not specific to a specific muscle or muscle type. This positions the utility of this method with a

very limited scope, especially when trying to study pathologies or adaptive models where there is a changing protein mass such as in cancer cachexia. Despite lack of understanding of the relative contributions of synthesis and degradation in cachexia, there are obvious changes present. Which in part, can be attributed to the upregulation of inflammatory mediators,^{43–45} the activation of related transcription factors such as Forkhead box O,⁴⁶ and their associated signalling pathways.^{47–51} Systemic inflammation plays a significant role in the development of cachexia. However, exercise is known to acutely promote inflammatory signalling pathways that benefit muscle metabolic health and function. Whereas cachexia is associated with chronically elevated systemic inflammation which can negatively impact muscle health, and is linked to abnormalities in the expression of proteins such as Angiotensin II,⁵² insulin-like growth factor-1,⁵³ and protein kinases such as adenosine monophosphate-activated protein kinase (AMPK),^{54,55} as well as mitochondrial dysfunction.^{56–58}

Presently, there are three major pathways that are thought to solely govern skeletal muscle protein degradation. The ubiquitin proteasome system (UPS), the cell autophagy/lysosomal pathway, and the calcium (Ca^{2+})-activated degradation pathway.^{59–61} Among these pathways the principal mode of degradation is the UPS.⁶² In cancer cachexia the activation of these protein degradation pathways is frequently concomitant with the presence of inflammatory mediators (Fig. 1), including interleukin-1 β ,⁴⁵ interleukin-6 (IL-6),⁵⁵ and tumour necrosis factor alpha (TNF α).⁶³ Additionally, the presence of these factors in cachexia is often associated with the phosphorylation⁴⁸ or aberrant expression of key

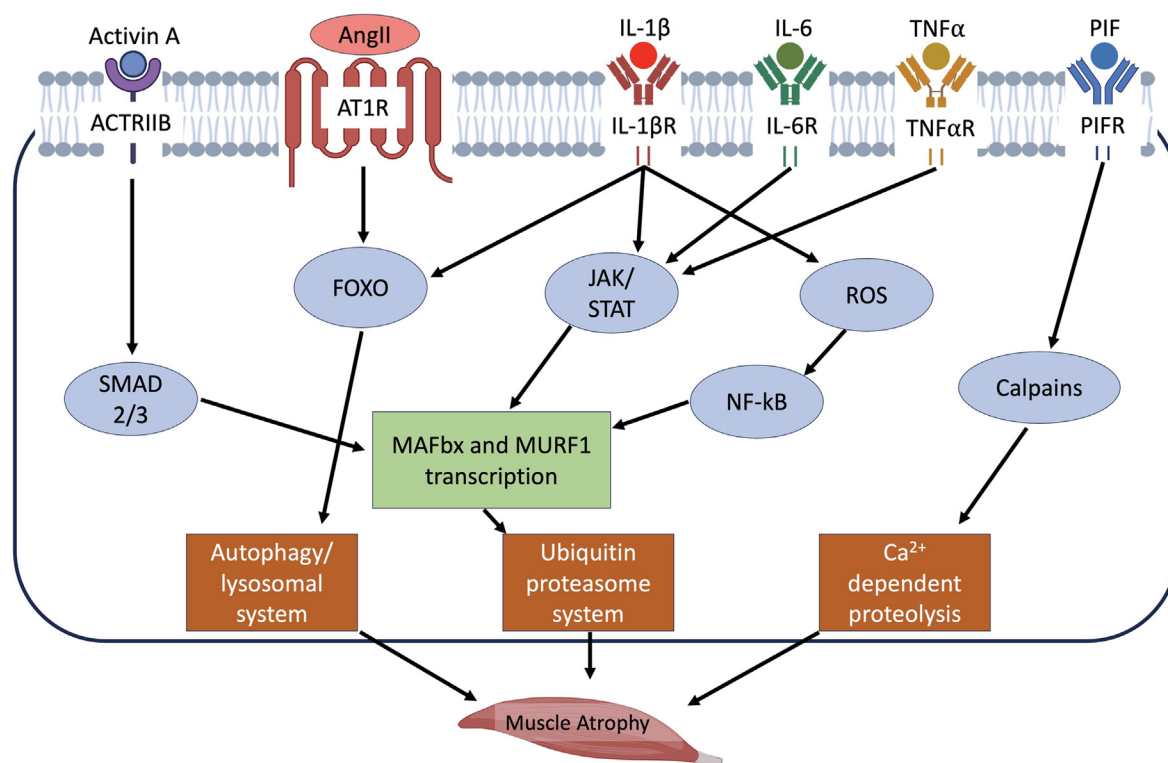


Fig. 1. Catabolic signaling involved in muscle wasting.

In cachexic conditions, inflammatory factors, and cytokines (e.g., IL-6, IL-1 β , TNF α) from tumors and immune cells induce activation of the transcription factors NF- κ B and FOXO through various signaling cascades, ultimately leading to the activation of the ubiquitin proteasome system and the autophagy/lysosomal system to result in muscle wasting. Activin A can also bind to ACTRIIB, in turn phosphorylating SMAD2/3, resulting in muscle atrophy through activation of the ubiquitin proteasome system. Glucocorticoids and AngII can also play a role in the activation of the ubiquitin proteasome system and the autophagy/lysosomal system, respectively, and lead to muscle wasting. Calcium dependent proteolysis is also activated by the calpains during cachexic conditions, contributing to the muscle wasting process.

ACTRIIB, Activin type II receptor; AngII, Angiotensin II; AT1R, Type 1 angiotensin II receptors; IL-6, Interleukin 6; IL-6R, Interleukin 6 receptor; IL-1 β , Interleukin 1-beta; IL-1 β R, Interleukin 1-beta receptor; TNF α , Tumor necrosis factor alpha; TNF α R, Tumor necrosis factor alpha receptor; PIF, Proteolysis inducing factor; PIFR, Proteolysis inducing factor receptor; FOXO, Forkhead box transcription factor; JAK/STAT, Janus kinase/signal transducers and activators of transcription; ROS, Reactive oxygen species; SMAD 2/3, Suppressor of Mothers against Decapentaplegic 2 and 3; NF- κ B, Nuclear factor- κ B; MAFbx, Muscle-specific F-box protein; MURF1, Muscle ring-finger protein-1; Ca^{2+} , Calcium ion.

molecules involved in metabolism.⁴⁹ Furthermore, the abnormal catabolism that is observed in cachexia has previously been related to the dysfunction of several organelles; principally the endoplasmic reticulum⁵⁶ and the mitochondria.⁵⁷ Currently, there are two predominant E3 protein ligases that have been conclusively recognised for their pronounced involvement in the proteolysis underlying muscle atrophy.^{64–66} These ligases are the muscle atrophy F-box protein 1 (MAFbx), also known as atrogin-1, and the muscle ring finger protein 1 (MuRF1).⁶⁷ Their activity is closely regulated by several pathways associated with cachexia, encompassing nuclear factor kappa B (NF- κ B), IL-6, and p38 mitogen-activated protein kinase (p38 MAPK) signalling pathways.^{43,68–70} Remarkably, several investigations have identified the presence of these proinflammatory and transfer factors, alongside the activation of their associated pathways in skeletal muscle, consistently demonstrating their integral involvement to cachexia-induced muscle wasting. Noteworthy among these factors are TNF α ⁷¹ and Twist1,⁷² while significant pathways include the NF- κ B⁷³ and the p38 MAPK signalling pathway,⁷⁰ all upregulated in cancer cachexia.

The overexpression of proinflammatory factors, transcription factors, or constituents of associated signalling pathways, within the framework of cancer cachexia, all appear to culminate in a collective influence on the E3 ligases MuRF1 and MAFbx.^{74,75} This convergence instigates the stimulation of proteasome hydrolysis within the UPS, thereby initiating the process of skeletal muscle protein degradation to promote muscle loss.^{74,76} Some evidence would suggest that in cancer cachexia, the balance between synthesis of new proteins and the degradation of existing proteins is tipped towards the latter.^{7,77} For example, atrophy in myotubes can be induced by expressing ubiquitin ligase MAFbx in a rodent model. Consistent with this observation, mice deficient in either MAFbx or MuRF1 have been found to be resistant to atrophy.⁷⁸ Besides degradation of proteins via the UPS, the autophagy/lysosomal degradation pathway is similarly important in skeletal muscles.⁷⁹ Both protein degradation pathways are controlled by Forkhead-box protein O3,⁶⁶ and *in vivo*, and *in vitro* investigations have consistently demonstrated that increased expression of stromal cell-derived factor 1 (SDF1) or its receptor C-X-C chemokine receptor type 4 (CXCR4) offers a partial safeguard against muscle atrophy,^{80–82} both known to be reduced in cachexia.⁸³ Furthermore, Martinelli et al.,⁸⁰ revealed an inverse relationship between the expression levels of SDF1/CXCR4 in the rectus abdominis muscle of cancer patients and the expression of MAFbx and MuRF1 expression levels in muscle atrophy. Another study also illustrated that the administration of recombinant adeno-associated viral vectors promoted the overexpression of the *Smad7* gene in skeletal and cardiac muscle, reducing SMAD2/3 phosphorylation downstream of Activin A receptor type-2B, consequently inhibiting the expression levels of the muscle atrophy-associated ligases MuRF1 and MAFbx.⁸⁴ In contrast, a separate earlier study employed reverse transcription-quantitative PCR to analyse the expression of E3 ligases MuRF1 and MAFbx, and no significant correlation was established between mRNA expression levels and cancer-associated weight loss.⁸⁵ Nonetheless, the predominant body of evidence for the involvement of degradation pathways in cancer cachexia originates from animal models of muscle wastage. Thus, further clinical investigations necessitate a more extensive sample pool to explore the regulatory dynamics of protein degradation in human muscle loss, particularly in the context of cancer cachexia pathology.

Various proinflammatory factors have been demonstrated to play a pivotal role in the atrophic processes attributed to cancer cachexia.^{44,55,86,87} Notably, IL-6 is produced by macrophages⁸⁸ and fibroblasts,⁸⁹ and its secretion has been identified in tumour cells as well.⁹⁰ Interestingly, there have been several reports that have identified a correlation between profound weight loss resultant from cancer cachexia and elevated levels of circulating IL-6.^{91,92} Moreover, Op den Kemp et al.,⁹³ revealed that individuals afflicted with non-small cell lung cancer experiencing cachexia exhibit reduced muscle fibre cross-sectional area alongside significantly increased plasma IL-6 concentrations. The

IL-6/JAK/STAT3 signalling pathway has also been identified to have an essential role in the contribution of the progression of cancer cachexia through its regulatory capacity in the inflammatory response.^{44,87} Pin et al.,⁸³ conducted intraperitoneal injections of ES-2 human ovarian cancer cells into Nod-SCID γ mice to establish a cancer cachexia model. The experiment revealed a significant elevation in IL-6 and phosphorylated STAT3 levels within the plasma of treated mice compared to control animals. Similarly, ES-2-conditioned medium led to a marked increase in STAT3 phosphorylation in C₂C₁₂ myotubes and subsequently induced muscle atrophy in differentiated myotubes. However, the authors tried to rescue the muscle wasting with use of an IL-6/STAT3 signalling inhibitor and was able to successfully restore myotube size. A previous study using a similar cancer cachexia model investigated a dose-dependent inhibitory effect of IL-6 on mammalian target of rapamycin (mTOR) activity.⁵⁵ Importantly, this study discerned that the dampening effect of IL-6 on mTOR function was contingent upon the activation of AMPK, and independent of STAT signalling in myotubes.⁵⁵ In addition to alleviating suppression of anabolic signalling, AMPK inhibition concurrently attenuated IL-6 induced expression of MAFbx and ubiquitinated proteins. Therefore, there appears growing evidence to position IL-6 as an inhibitor to protein synthesis through the dampening of mTOR activity by AMPK activation. There is also further evidence to show that the activation of AMPK can also promote hydrolysis of muscle protein primarily by the UPS and autophagy.⁹⁴ However, in skeletal muscle AMPK is a master regulator of metabolism and plays an integral role in protein turnover. Accumulating data would suggest that modulation of activity of AMPK substrates will constitute good future candidates for muscle wasting therapy. In this context, it is important to understand the precise mechanisms that regulate protein degradation to develop and improve therapeutic strategies against conditions like cachexia. Among the signalling pathways that control proteolysis, AMPK may play a key role that should be further addressed, with attempts to evaluate the effects of physical exercise considered.

The cell autophagy/lysosomal and the Ca²⁺-dependent protein degradation pathways are two other processes that are currently regarded to be heavily involved in skeletal muscle protein degradation. Earlier research has underscored the significant involvement of the autophagy/lysosome system in the modulation of muscle mass.⁷⁹ Notably, various essential constituents of the autophagy mechanism have been found to undergo transcriptional upregulation during periods of muscle atrophy.^{95–97} Transgenic mouse models of cancer cachexia, for example colon-26 (C26), have previously been used to study the effects of autophagy inhibition (Beclin-1 knockout), or promotion (tumour protein p53 inducible nuclear protein 2 overexpression), during cancer-induced muscle loss.⁹⁵ The authors revealed that Beclin-1 knockout was ineffective in preventing muscle atrophy in tumour-bearing mice, whereas p53-mediated autophagy further exacerbated the muscle loss, suggesting that autophagy is not the primary mode of degradation but complimentary during cancer cachexia. Increases in autophagy have also been shown to have a direct relationship with a decrease in muscle mitochondrial function.⁹⁸ Recent findings suggest that activin A can regulate mitochondria.⁹⁹ Activin A is a receptor complex consisting of transmembrane serine/threonine kinases which can autophosphorylate, binding and activating SMAD transcriptional regulators.¹⁰⁰ However, Pettersen et al.,⁹⁵ revealed that activin A can also operate in an autocrine fashion to stimulate the synthesis and release of IL-6 from cancer cells. By inhibiting activin signalling, the production of IL-6 in cancer cells was diminished, along with the cancer cells capacity to induce accelerated autophagy in non-cancerous cells *in vivo*, resulting in the reversal of cachexia relates symptoms and decline in all assessed muscle groups. Energy and oxidative stress are elevated in cancer patients,^{101,102} with evidence existing for the functional role of MAP-kinase signalling in mediating oxidative stress through autophagy in cachectic muscle wasting.⁷⁰ In response to oxidative stress, an autophagy related gene, Atg7, expressed in the autophagy/lysosomal proteolytic pathway, as well as the E3

ligases MuRF1 and MAFbx have been identified to be temporally associated with the activation of the p38 MAPK pathway, independent of both NF- κ B and FOXO dependent transcriptional activation in cultured muscle cells.¹⁰³ However, there is recent evidence to now demonstrate that the hippo pathway noncanonically drives autophagy and cell survival in response to energy stress, largely through activation of the kinase MAP4K2, from the MAP kinase family, which phosphorylates LC3A at residue serine 87 promoting autophagy in cancer.¹⁰⁴

There is a strong association between cancer cachexia-induced muscle loss and mitochondrial dysfunction, with several studies now demonstrating a clear connection.^{105–108} To illustrate, Marzetti et al.,⁵⁵ examined the expression of key mediators in pathways related to mitochondrial quality control using muscle biopsies obtained from patients with gastric adenocarcinoma. Patients were split in to three groups ($n = 9$, in each), those diagnosed with cancer cachexia, those with cancer only and a control group. Notably, the patients with cachexia exhibited an upregulation in the expression levels of the mitotic protein Fis1, while a mitochondrial outer membrane GTPase protein, known to mediate mitochondrial clustering and fusion, Mitofusin-2, decreased in these patients. Consequently, these findings indicate an association between cachexia and alterations in mitochondrial dynamics in the muscle that reduce mitochondrial quality control. Interestingly, previous investigations have demonstrated that mitochondrial degeneration precedes muscle atrophy in the development of cancer cachexia in tumour-bearing mice, providing novel evidence to suggest that mitochondrial dysregulation can directly precede cachexia-induced muscle loss.¹⁰⁸ Neyroud et al.,¹⁰⁹ established a C26-induced cancer cachexia model in CD2F1 mice and observed the mitochondrial respiratory capacity and content of skeletal muscle. Indeed, skeletal muscle mitochondrial respiration, mitochondrial coupling and the mitochondrial content were all reduced, suggesting a link between mitochondrial dysregulation and cachexia. However, a better understanding of the mechanisms responsible for cancer-induced mitochondrial dysfunction are required, if future therapeutic treatments are to combat mitochondrial alterations in muscle atrophy.

3. The molecular response of exercise

It is beyond the scope of this review to discuss the molecular responses to exercise in its entirety. For this, readers are directed to an excellent comprehensive resource by Egan & Sharples,¹¹⁰ and an extensive account of current research and understanding of muscle hypertrophy by Roberts and colleagues.¹¹¹ Alternatively, this review will briefly highlight the importance of exercise adherence to long term health and discuss some of the molecular networks that overlap in cancer cachexia, and those known to be ‘switched on’ by exercise. Therefore, identifying important targets that can be exploited to potentiate or augment the benefits of exercise in potential therapeutic treatments for cachexia patients.

Current public health recommendations recognise regular exercise and physical activity as a cornerstone in the prevention, management, and treatment against the emergence of numerous chronic conditions.^{112–115} As such, the progression and preservation of functional capacity is achieved through a combination of aerobic fitness and skeletal muscle strength, which is central to healthy aging across the lifespan. In this regard, the American College of Sports Medicine recommends that adults engage in moderate-intensity cardiorespiratory exercise for 150 min per week, e.g., 30 min sessions 5 days per week. Alternatively, more vigorous-intensity exercise can be done in > 20 min sessions 3–4 days per week (i.e., ≥ 75 min/week). Various combinations of moderate and vigorous intensity exercise can be prescribed based on metabolic equivalents (METs), which relate the metabolic cost of an activity to the individual's resting $\text{VO}_{2\text{max}}$. For example, a mixed program of activities that achieves a total energy expenditure of 500–1 000 METs per week conveys significant health benefits.¹¹⁶ Skeletal muscle is an important component of exercise capacity, and in the context of disease and aging,

the maintenance of muscle mass and function becomes a key determinant of health span and quality-of-life.¹¹⁴ To maintain and enhance muscle mass and strength, resistance exercises at an intensity of 75%–85% of one repetition maximum, completed in 1–3 sets of 8–12 repetitions per session are recommended.¹¹⁶ These benefits are largely conferred by a comprehensive overhaul of skeletal muscle architecture due to the implementation of regular structured exercise training. The underlying mechanics of which are underpinned by a substantial body of research spanning nearly five decades, dedicated to unravelling the molecular responses of skeletal muscle in response to exercise training.^{110,111}

The onset of acute exercise presents a multitude of challenges for the maintenance of homeostasis. However, through the continuation of exercise, the resultant physiological stress leads to the alterations of key determinants associated with neuronal, mechanical, metabolic, and hormonal factors acting as primary messengers to form molecular signals for the initiation of signal transduction and other associated molecular responses to exercise (Fig. 2). These alterations include, in cell membrane electrolyte balance, changes in muscle cell and tissue volume, shifts in regulators governing energy producing pathways, and ATP turnover.¹¹⁷ For example, elevated intracellular calcium concentrations,¹¹⁸ metabolites linked to cytosolic phosphorylation potential, reactive oxygen species, and the redox state of mitochondria,¹¹⁹ as well as decreases in muscle glycogen levels, and intracellular partial pressure of oxygen and pH.¹²⁰ The above is by no means an exhaustive list of cooccurring responses originating from within the contracting muscle. However, there are also many external factors to the muscle that circulate systemically impacting upon the molecular responses to exercise. These include the prevailing hormonal environment, such as concentrations of catecholamines (e.g., epinephrine and norepinephrine), cytokines like tumour necrosis factor alpha (TNF α) and interleukin-6 (IL-6), hormones including insulin-like growth factor (IGF-1), and substrates such as glucose, amino acids and free fatty acids, as well as the possible involvement of a collection of these exercise-related factors acting in an autocrine or paracrine manner (e.g., IL-6, IGF-1).^{121,122} Nevertheless, the degree of alteration brought about by exercise to any given factor is predominately dictated by the intensity, duration, and specific type of exercise. Moreover, many acute responses continue in the hours after the cessation of exercise as part of the restoration of homeostasis, which include inflammatory and anti-inflammatory responses, restoration of fluid balance, and the replenishment of substrates utilised during exercise, as well as elevations in muscle protein synthesis.^{123–127}

In relation to cancer cachexia, exercise has profound molecular effects on the body, particularly in terms of improving muscle health, a critical factor for the elderly and individuals with muscle pathologies and comorbidities.^{30,128} These effects are driven by intricate signalling pathways and molecular interactions resulting in enhanced muscle function, mass and overall well-being.^{129–131} At the cellular level, exercise triggers a cascade of molecular events that stimulate muscle growth and repair.¹³² One key player in this process is IGF-1, a hormone produced during exercise activities which exerts influence on the mammalian target of rapamycin (mTOR) pathway, regulating protein synthesis, leading to muscle hypertrophy and improved muscle quality.^{133–135} Exercise also influences mitochondrial biogenesis, with regular physical activity enhancing mitochondrial function and content, improving energy production, and reducing oxidative stress.¹³⁶ This is particularly important for older individuals and those with muscle pathologies, as mitochondrial dysfunction is inextricably linked to muscle atrophy and weakness.^{57,108} The onset of exercise also includes an increase in the circulation of a diverse range of ‘exercise factors’, usually small peptides/proteins produced by muscle cells during contraction, but also include metabolites and several RNA species, which together have collectively been termed by the field as “myokines” or “exerkines”.^{122,137–140} Myokines have anti-inflammatory properties and can modulate metabolism contributing to improved muscle health and overall systemic effects. Notable myokines include irisin and IL-6, which play roles in fat metabolism¹⁴¹ and immune regulation.¹⁴²

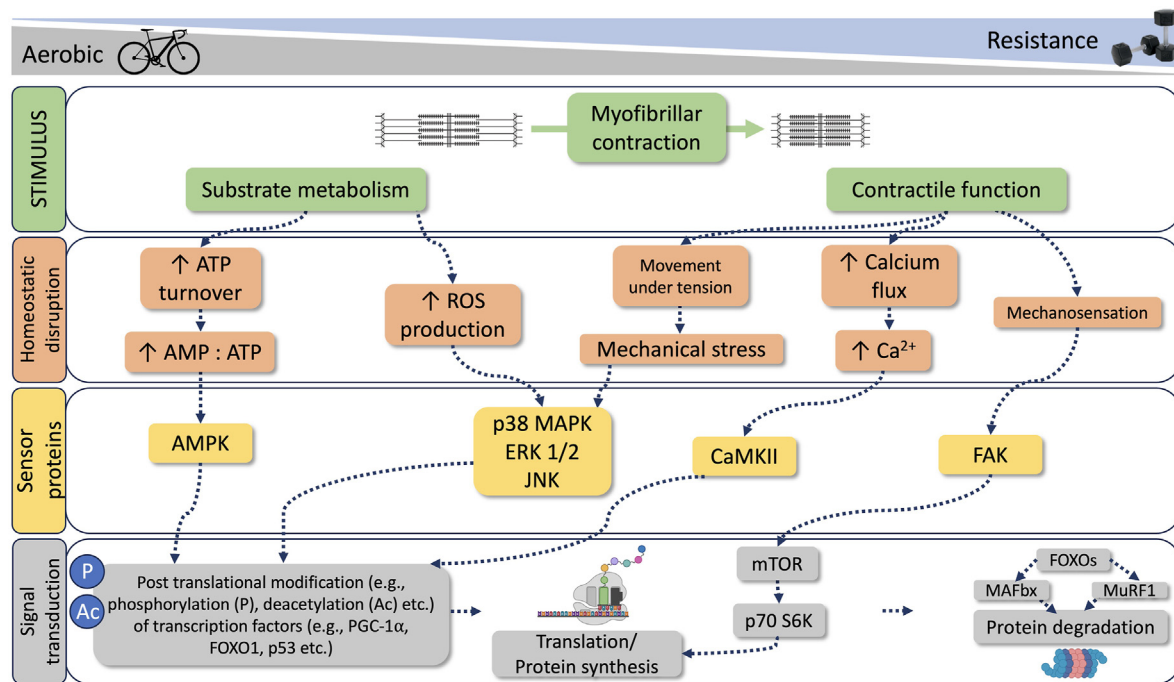


Fig. 2. Schematic of signal transduction-to-transcription coupling in skeletal muscle.

At the onset of exercise myofibrillar contractile activity results in multiple biochemical stimuli localised to the contracting muscles. These perturbations in skeletal muscle homeostasis lead to the activation of signaling molecule networks, including protein kinases, phosphatases, and deacetylases. All of which are integrated into physiological processes by downstream targets, including transcription factors and transcriptional coregulators. These events occur in a temporal manner, such that kinase activation and pre-transcriptional regulation occur rapidly during exercise and recovery, but transcript alterations resulting in translational events take time. The activation, contribution, and magnitude of the described pathways and downstream targets are dependent on the intensity, duration, and mode of the exercise stimulus. Here, linear pathways are depicted, but these pathways demonstrate some degree of dependence, crosstalk, interference, and redundancy in their regulation, making the exact contribution of each signaling pathway to measured changes in gene expression difficult to isolate.

ATP, Adenosine triphosphate; AMP, Adenosine monophosphate; ROS, Reactive oxygen species; Ca^{2+} , Calcium ion; AMPK, AMP-activated protein kinase; p38 MAPK, p38 mitogen-activated protein kinase; ERK 1/2, Extracellular signal-regulated kinase 1/2; JNK, Jun N-terminal kinase; CaMKII, Calmodulin-dependent protein kinase II; FAK, Focal adhesion kinase; P, Phosphorylation; Ac, Acetylation; PGC-1 α , Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; FOXO1, Forkhead box protein O1; p53, Tumour protein p53; mTOR, Mammalian target of rapamycin; p70 S6K, p70 ribosomal S6 kinase; MAFbx, Muscle-specific F-box protein; MuRF1, Muscle RING-finger protein-1.

The cytokine/myokine IL-6, which is secreted by both immune cells and myofibers, remains the best described in terms of kinetics of response to exercise and subsequent metabolic and/or molecular effects. During and soon after a single session of aerobic¹⁴³ or resistance¹⁴⁴ exercise, circulating IL-6 is robustly observed to increase (several-fold).¹⁴⁵ IL-6 is mostly derived from contracting skeletal muscle where it is transcribed, translated, and released from myofibers during exercise.^{146,147} It is also sensitive to nutritional status (i.e., exogenous carbohydrate ingestion and endogenous glycogen concentration),^{148,149} and exerts relevant metabolic effects during and after exercise, e.g., enhanced hepatic glucose output, adipose tissue lipolysis, and skeletal muscle insulin sensitivity.^{150–153} For most other exercise factors, beyond reports that indicate a change in circulating concentrations in response to acute exercise, the information available is severely lacking. However, there is growing evidence to suggest other small molecules such as metabolites,^{154,155} RNAs,^{156,157} and proteins^{140,158} respond to exercise in a similar manner and exert positive systemic effects. Despite sometimes inconsistent reports in relation to exerkines, there is strong speculation that these exercise factors could serve as the initiating signals for the adaptations that occur in response to repeated sessions of exercise, including local signals in skeletal muscle stimulated by contraction.¹⁵⁹

During skeletal muscle contraction, changes in calcium (Ca^{2+}) flux are crucial and involve the intermediate Ca^{2+} -binding protein Calmodulin (CaM). CaM is a versatile signal transducer that undergoes conformational alterations before activating other CaM-binding proteins; particularly downstream kinases, and the phosphatase calcineurin.^{119,160} In human skeletal muscle, Calcium Calmodulin-dependent protein kinase II (CaMKII) stands as the dominant isoform.^{161,162} The activation of this

multifunctional serine/threonine protein kinase is mediated by Ca^{2+} -dependent CaM binding, triggering autophosphorylation at Thr287 and activation of a Ca^{2+} /CaM-independent form.¹¹⁸ Phosphorylation grants CaMKII partial autonomy from Ca^{2+} /CaM; thus, following a transient cessation in Ca^{2+} release, the kinase maintains elevated activity above basal levels.¹⁶² This sustained CaMKII enzymatic activity enable continuous downstream substrate phosphorylation upon repeated stimulation i.e., multiple exercise efforts.¹⁶³ Aerobic exercise is known to amplify CaMKII phosphorylation in an intensity-dependent manner,^{161,164,165} potentially due to heightened Ca^{2+} release at greater force outputs from additional muscle fibre recruitment.¹⁶⁶ There is also a suggestion that Ca^{2+} signalling could be responsive to distinct exercise types (e.g., aerobic vs. resistance), given the differential amplitude and frequency of Ca^{2+} kinetics resulting from their respective contraction patterns.¹¹⁸ This theoretically leads to varying signal transduction patterns.¹⁶⁷ Nonetheless, despite increased phosphorylation following short-duration, maximally intense bouts of electrically stimulated contractile activity in rodent skeletal muscle.^{168–171} CaMKII phosphorylation often remains unchanged in human skeletal muscle after resistance exercise.^{172–174} Whether this distinction in response to aerobic versus resistance exercise holds significant implications for exercise types and its application to clinical scenarios is still awaiting clarification. Regrettably, many studies that compare signalling pathways in response to acute aerobic and resistance exercise, whether in parallel groups Camera et al.,¹⁷⁵ or a within-subject crossover design^{176–179} did not measure CaMKII phosphorylation. Notably, a correlation between increased resting CaMKII levels and hypertrophy in human vastus lateralis muscle has been previously reported,¹⁸⁰ prompting speculation regarding the

potential role of CaMKII in exercise-induced muscle hypertrophy. However, the investigation of CaM kinases in skeletal muscle glucose transport, lipid intake, oxidation, mitochondrial biogenesis, or remodelling primarily revolves around mechanistic studies employing a combination of Ca^{2+} -releasing agents and CaM kinase inhibitors.^{181–188} Therefore, the precise mechanism through which CaM kinases participate in skeletal muscle plasticity remains incompletely defined.

Ca^{2+} related signalling exemplifies the intricate nature of cross talk among signal transduction pathways. For instance, CaM kinases are recognised as an upstream regulator of AMP-activated protein kinase (AMPK), enabling phosphorylation at Thr172 and thereby triggering AMPK activation.^{189,190} Interestingly, AMPK activation has previously been shown to be directly activated from the initiation of Ca^{2+} signalling in myotubes, even in the absence of contraction.¹⁸² Additionally, there is existing evidence suggesting that p38 mitogen-activated protein kinase (MAPK) functions downstream of CaMKII, regulating peroxisome proliferator-activated receptor- γ coactivator 1- α (PGC-1 α) through a pathway initiated by Ca^{2+} flux.¹⁸⁶ Further studies have also established relationships between Ca^{2+} related signalling and pathways associated with protein kinase C, reactive oxygen species, as well as mechanosensory signal transduction in the context of skeletal muscle hypertrophy.¹⁹¹ MAPKs constitute a conserved family of serine/threonine kinases including extracellular signal-regulated kinase (ERK 1/2), c-Jun N-terminal kinase (JNK), and p38 MAPK. These kinases orchestrate signal transduction cascades pivotal in physiological processes like cell proliferation, differentiation, hypertrophy, inflammation, gene expression, and apoptosis.^{192–194} Their resultant activation responds to changes such as growth factors (e.g., IGF-1) and cytokines (e.g., IL-6).¹⁹⁴ Within an exercise context, the phosphorylation and activation of p38 MAPK are consistently observed in response to acute aerobic exercise,^{164,179,195–200} and high intensity exercise,^{165,195–197,199–202} with the caveat of, increases are sometimes observed to be of a greater magnitude in response to higher intensities or a greater metabolic stress of exercise,^{164,165} and lower within trained individuals.^{179,198} However, ERK 1/2 has been demonstrated to have a mechanistic role in the regulation of fatty acid oxidation in skeletal muscle during low-to moderate exercise intensities by modulating CD36 translocation to the plasma membrane for fatty acid uptake.^{203–205} Notably, experiments in a perfused rat hindlimb models reveal interactions between CaMKII, CaMKK signalling, involving both AMPK-dependent and -independent regulation, with ERK 1/2 signalling in both a time and intensity-dependant manner.²⁰⁴

MAPKs are also proposed to regulate protein translation and muscle hypertrophy through mTORC1-dependent and -independent pathways.^{206–208} More recently, there has been a focus on ERK1/2 and JNK activation after resistance exercise due to the purported roles of these kinases in mechanosensory signal transduction and muscle hypertrophy.^{191,209} However, several studies have reported no change in phosphorylation following acute resistance exercise.^{210–212} Holm et al.,²¹³ has suggested that their activation may be dependent on the intensity and/or volume of the resistance exercise session. However, the predominance of eccentric or concentric contractions have further been reported to influence the activation patterns of MAPKs,^{214,215} with eccentric contractions producing greater activation,^{216–218} perhaps having implications in a clinical setting. Notably, in a series of *in situ* experiments using rat plantaris muscle, ERK1/2 and JNK, but not p38 MAPK, were found to be phosphorylated in a tension-specific manner, with JNK being established as the most mechanosensitive of the three MAPKs in this context.²¹⁵ This is noteworthy because JNK has been proposed as a positive regulator of muscle hypertrophy through the inhibition of SMAD2-dependent myostatin activity, whereas muscle-specific JNK knock-out mice have a blunted hypertrophic response to two weeks of functional overload from synergistic ablation.²⁰⁸ These observations have led to the strong implication of a role for JNK in the mechanosensory regulation of muscle protein synthesis (MPS), translation, and muscle hypertrophy, with increased ERK1/2

phosphorylation being consistently observed in response to acute resistance exercise in human skeletal muscle.^{210,219–224} Greater ERK1/2 phosphorylation is observed with a higher number of muscle contractions,²¹¹ higher volume,²²⁵ and higher intensity.²¹³ Therefore, the cellular perception of mechanical cues is achieved through the stimulation of these mechanosensing proteins. The list of potential regulators of hypertrophy through mechanotransduction continues to expand and to date includes costamere-associated proteins such as focal adhesion kinase (FAK), Yes-associated protein (Yap) of the Hippo signalling pathway, integrins, titin, BAG family molecular chaperone regulator 3, filamin-C, and stretch-activated ion channels.¹³⁴ The activity of these proteins is the subject of much investigation for their role in mechanosensory signal transduction, especially in the context of resistance exercise and the regulation of MPS and hypertrophy. Thus, they are well positioned as candidates for potential therapeutic targets in clinical populations once research has defined their complete functional roles.

Proteostasis and the dynamics of protein turnover are central to phenotypic plasticity. As such, the phenotypic and functional consequences of exercise are entirely dependent on changes at the protein level. Be that in the form of altered abundance, or the turnover of the regulatory mechanisms that determine the activity or function of the muscle cell. In human skeletal muscle, proteins display global turnover rates of ~1.0%–1.5% per day in young healthy individuals.^{226,227} However, a dynamic equilibrium exists in that protein degradation (DEG) exceeds MPS in the fasted state and during exercise, whereas MPS exceeds DEG in the fed state and in the postexercise period.^{126,228} The net positive balance induced by exercise can be augmented by ingestion of protein to increase MPS.²²⁹ Thus, aerobic exercise and resistance exercise, either alone or combined with appropriate nutrition strategies influence protein turnover and skeletal muscle remodelling by acutely increasing the degree by which MPS exceeds DEG.^{176,230–233} Central to muscle hypertrophy in response to resistance exercise training is the contention that repeated transient increases in MPS through exercise, and appropriate nutrition intake result in the accumulation of predominantly myofibrillar proteins, which thereby increase the size of the exercise-trained muscle.¹²⁶ Recent advances in stable isotope protein labelling with deuterium oxide²³⁴ and dynamic proteome profiling²³⁵ have produced a number of studies detailing the contribution of synthesis and degradation to changes in protein abundance of individual proteins.^{236–240} Unsurprisingly, at the level of individual proteins, there is considerable variation in turnover rates and the balance between synthesis and degradation during changes in abundance in response to a stimulus.^{238,241} For example, Murphy et al.,²³³ investigated the response of skeletal muscle to 2 weeks of resistance exercise training under energy restriction in humans, they observed a notable 26% increase in whole-muscle MPS. However, at the individual protein level, 175 of the 190 identified proteins exhibited a significant increase in synthesis rate in exercised muscle, including mitochondrial and sarcoplasmic proteins. Interestingly, there was a broad range in the synthesis rates of these measured proteins, as low as 0.2%/day, to as high as 15%/day. During the adaptation to exercise there are changes in the abundance of individual muscle proteins which must be accounted for alongside the effects of exercise on MPS. In experiments where MPS are reported but protein abundance data are not investigated, it is not certain whether the reported changes to MPS result in gains to protein abundance or if they represent a change to the rate of protein turnover, therefore missing a crucial dimension to the information gained about changes to the proteome in response to exercise.

4. Exercise in cachexia and application of -omics technologies

Exercise has been reported to increase insulin sensitivity, protein synthesis rates, and antioxidant enzyme activity,²⁴² with continuous training improving muscle strength, lean body mass, and attenuating inflammatory markers.^{243,244} Standard exercise testing methods are generally appropriate for patients with cancer who do not require

medical clearance or who have been medically cleared for exercise with awareness towards a patient's health history, any comorbid chronic diseases and health conditions, or any general exercise contraindications before commencing health-related fitness assessments or the designing of exercise prescriptions.³⁰ However, research regarding exercise interventions as a method of management for cancer cachexia is still in its infancy. Al-Majid et al.,²⁴⁵ was the first to directly examine the effect of resistance exercise training on cancer-related muscle wasting. The authors measured this effect on muscle mass and protein content in C-26 tumour bearing mice. The dorsiflexors and plantar flexors muscles were stimulated to contract eccentrically and concentrically by using an electrical stimulation protocol similar to.²⁴⁶ The protocol consisted of 10 sets of 6 repetitions per session and was used on alternate days for a total of 8 sessions. The mass and protein content of the stimulated EDL muscle was significantly higher (62% and 25%, respectively) than those of the non-stimulated EDL, and training did not have significant effects on the mass or protein content of other muscles in the tumour bearing mice, or control muscles. These results demonstrate that resistance training can significantly attenuate cancer-related wasting of the EDL muscle in C-26 mice. The lack of effect of the same training protocol on the EDL muscle in the control animals suggests that the volume and intensity of exercise training adequate to decrease muscle wasting is not sufficient to induce hypertrophy of healthy muscles. In contrast to resistance exercise, endurance exercise is very well tolerated by clinical patients, has no equipment constraints, and confers anti-inflammatory effects even at low intensities.²⁴⁷ In this regard, endurance exercise has also been investigated using pre-clinical models. For example, Deuster et al.,²⁴⁸ was among the first to use endurance exercise in attempt to modify tumour progression in cachexia. They randomly assigned Walker 256 tumour bearing rats to an exercise or sedentary group. Exercise consisted of treadmill running at 20 m·min⁻¹, 3 times per week for 100 min sessions for 7 weeks. Exercised animals were reported to have significantly greater (~22%) muscle: body mass ratios than their sedentary counterparts which demonstrated that exercise had contributed to the attenuation of muscle wasting. Muscle protein synthesis was also measured using 3H tyrosine incorporation into gastrocnemius muscle, and muscle protein degradation was estimated using urinary 3-methylhistidine excretion. In sedentary animals, synthesis was measured to be significantly depressed (~35%) throughout training when compared to exercised animals. However, degradation was observed to be significantly elevated (~10%) in sedentary animals but only until day 28 of training when the degradation rate for exercised animals became greater than that for sedentary animals. These data suggest that although exercise may have initially protected the animal from cancer-related muscle wasting, ultimately the energy cost of exercise may have accelerated a catabolic state. More recently, Lira et al.,²⁴⁹ studied the effect of submaximal prolonged exercise training on cachexia. Animals were subject to forced treadmill running at 60% VO_{2max} for 60 min/day, 5 days/week, for 8 weeks. The authors show that this training protocol is able to fully re-establish liver lipid metabolism (long-chain fatty acid oxidation, very low-density lipoprotein assembly and secretion, long-chain fatty acid re-esterification), markedly affected by cachexia. Interestingly, exercise training also caused tumour mass to decrease 10-fold, therefore, it is possible to conclude that endurance training not only promotes the re-establishment of lipid metabolism in cachectic tumour-bearing animals, especially in relation to very low-density lipoprotein secretion and assembly. But also regular structured exercise may be important for improving morbidity related with cachexia. However, much more work is required to appreciate the full impact exercise induced benefits to cachexia.

The existing literature has produced contrasting results, but together share the goal of mediating the key domains of cancer cachexia through exercise, including nutritional status, muscle mass, and physical function. For example, Uster et al.,³² examined the effects of a combined exercise and nutrition program on cancer cachexia patients. The patients in the intervention group received a minimum of three individual nutritional counselling sessions and participated in a 60 min exercise program twice

a week for 12 weeks. The results demonstrate that the intervention did not improve overall quality-of-life assessed by the European Organisation for Research and Treatment of Cancer Quality-of-Life Questionnaire. However, patients in the exercise group increased their protein intake and reported a decline in nausea and vomiting. Whilst clinical implications may be limited from these findings, there were no measures of muscle mass taken, the effects of aerobic exercise were not investigated, and the frequency of exercise did not meet minimum guidelines. However, these data do demonstrate that structured exercise can contribute to an adequate dietary intake and well-being, in patients that would otherwise struggle to meet dietary requirements. More recently, aerobic exercise through treadmill running has been shown to suppress cancer cachexia-induced muscle atrophy, *in vivo*, by activating adiponectin signalling.²⁵⁰ Furthermore, Wiskemann et al.,²⁹ conducted a randomised controlled trial for patients with pancreatic cancer. Here, patients were asked to perform resistance exercise twice a week for 24 weeks. Improvements in elbow and knee flexor/extensor muscles were observed, although there were no significant changes in patients body weight. There is much work to be done to define the optimal intersection between exercise and cachexia, but the above findings provide strong rationale for exploring structured exercise as a therapeutic intervention further in a more robust manner.

Omics technologies are utilised in clinical research principally for the identification of new biomarkers and/or to uncover predictors or novel pathways involved in the pathophysiology of different disease states. In the context of cachexia, previous pioneering work has found that multiple types of skeletal muscle atrophy involve a common program of gene expression. For example, the transcriptional induction of E3 ligases (i.e., MAFbx and MURF1) are considered a standard response in multiple types of skeletal muscle wasting, such as cancer cachexia, denervation, kidney failure, and infections.^{251–254} However, these early seminal studies were based on gene expression analysis via microarrays, which have technical limitations compared to current RNA sequencing (RNA-seq) technologies, such as decreased coverage and a lower quantitative range of detection for gene expression changes.^{255,256} In recent years, application of RNA-seq to the analysis of muscle wasting has indeed provided novel insight into the transcriptional changes associated with muscle atrophy and homeostasis.^{257–261} In addition to gene expression changes, muscle atrophy is also characterised by profound remodelling of the proteome due to changes in protein synthesis and degradation.^{262–265} However, the application of proteomics to study muscle atrophy is an approach that has been employed by only a few studies,^{266–272} some of which have limited proteome coverage due to technical constraints or focus on a single type of muscle atrophy.

Contrary to these previous data suggesting a common molecular signature underlies all types of muscle atrophy. Hunt et al.,²⁷³ used RNA-seq and quantitative mass spectrometry to determine the molecular changes that occur in mouse skeletal muscle during atrophy, induced by dexamethasone, cancer cachexia, or aging. The authors detected > 15 000 unique mRNAs and ~5 000 unique proteins for each model (overlap of ~13 000 mRNAs and ~3 000 proteins), uncovering a remarkable diversity in the mRNA and protein changes induced by distinct atrophic stimuli. By surveying the proteomic changes that characterise these different modes of atrophy, Hunt et al.,²⁷³ show that distinct catabolic stimuli induce muscle wasting via largely different molecular changes, including a significant disconnect between transcriptional and proteomic changes. On this basis, these integrated analyses indicate that muscle atrophy occurs via stimulus-specific protein changes molecularly differentiating types of muscle wasting that otherwise have remarkable phenotypic similarities. Together, these data provide a rich resource for data mining to help understand the specificity of muscle atrophy and provide potential leads that could be targeted in a clinical context to prevent muscle wasting. However, this study does not examine muscle wasting in human samples meaning that the clinical application of these data can only be postulated upon. Ebhardt et al.,²⁷⁴ used a range of omics approaches in clinical cohorts, quantifying the

soluble proteome of muscle biopsies from cancer cachexia patients, comparing them with regular cancer patients and healthy controls. Advanced high mass accuracy mass spectrometers in SWATH-MS acquisition mode were used, in combination with protein arrays to quantify phospho epitopes, and morphology was assessed using fluorescent microscopy. Comparing the proteomes of these cohorts, the authors quantified changes in muscle contractile myosin proteins and energy metabolism proteins allowing for a clear identification of cachexic patients. Despite the low patient cohort numbers and the conservative number of proteins quantified (~500), these data show promise in laying the foundation for the further understanding of cachexia-induced muscle wasting, as well as a way to potentially overcome the ambiguous weight loss measure used by clinicians for defining cachexia, instead replaced by a precise protein signature.

Most recently, Murgia et al.,²⁷⁵ applied mass spectrometry-based proteomics to measure plasma protein abundance changes in response to 10 days of bed rest in humans. To validate the correlation between muscle atrophy, in parallel, a cohort of cancer patients with or without cachexia and age-matched controls were analysed. The analysis resulted in the quantification of over 500 proteins, but just six proteins were identified to distinguish subjects from those developing unloading-mediated muscle atrophy from those who maintained their initial muscle mass. Taken together, these findings highlight that proteomic changes can be explored as potential biomarkers of muscle atrophy occurring under cancer cachexia conditions. However, precision medicine in this regard requires the association of molecular entities to clinical phenotypes such as disease stage and trajectory, or even drug responsiveness. Proteomic measurements of clinical specimens provide direct and informative insights into the biochemical and signalling state of the tested samples, of which, the proteins can be reliably measured in formalin-fixed paraffin-embedded tissues, blood, and body fluids. Furthermore, mass spectrometry-based proteomic measurements are robust because the measurement results are relatively insensitive to experimental variation. The proteomic characterisation of single cells is also now feasible with steadily increasing depth and throughput, as well as methods that have now been developed to access and quantify unique and functionally informative levels of the proteome, including proteoforms with PTMs, structural features, and the composition of complexes. Finally, targeted mass spectrometry also has the power to validate potential proteomic derived biomarkers which would help to reliably predict the molecular signatures of cancer cachexia-induced muscle wasting in humans.

5. Conclusions

It is well documented that muscle wasting exacerbates disease outcomes and increases mortality, whereas preserving skeletal muscle mass and function is protective.^{276–279} However, the mechanisms responsible for muscle atrophy are only in part understood, and currently there are limited therapies available for patients with co-occurring clinical conditions i.e., cancer cachexia. The pathogenesis of cancer cachexia is extremely complex and previous studies have produced inconsistent results when attempting to decode the molecular signatures responsible. Consequently, there is no single treatment that can effectively reverse cachexia, and there is no consensus in clinical guidelines for its management. Therefore, the lack of treatment options combined with the complicated pathogenesis necessitate the development of robust early detection and diagnosis methods, in combination with therapeutic interventions that activate multiple molecular pathways and targets for its effective management.

Regular structured exercise holds the potential to combat many of the adverse effects associated with cancer cachexia, primarily by stimulating muscle protein synthesis, enhancing mitochondrial function, and promoting the release of myokines. As a result, muscle mass and strength increase, leading to improved mobility, balance, and quality-of-life. To this aim, the understanding of the molecular responses to exercise can

assist in the fine-tuning of exercise training prescription and additional co-intervention strategies. More research is still required, but a better understanding of how these physiological processes and molecular networks manifest in cancer cachexia may facilitate the further development of personalised exercise medicine. To help achieve this, quantitative proteomics has become a robust high-throughput technology capable of generating massive datasets from minute amounts of biospecimens from clinical cohorts. Thus, in utilising the power of mass spectrometry-based proteomics there is the potential for far-reaching applications in the early detection of cancer cachexia, and for the resolution of the underlying molecular mechanisms. This understanding of the molecular landscape will then allow healthcare professionals to incorporate regular structured exercise into treatment plans to harness these molecular mechanisms, enhancing muscle function and ultimately attenuating the progression of the disease.

Submission statement

All authors have read and agree with manuscript content. The submission of this work implies that the work described has not been published previously, and is not under consideration for publication elsewhere, its final version is approved by the author and, and whilst the manuscript is being reviewed for this journal the manuscript will not be submitted elsewhere for review and publication.

Conflict of interest

SJH has no financial or personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

References

- McGregor RA, Cameron-Smith D, Poppitt SD. It is not just muscle mass: a review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. *Longev Heal*. 2014;3(1):9. <https://doi.org/10.1186/2046-2395-3-9>.
- Hanna L, Nguo K, Furness K, Porter J, Huggins CE. Association between skeletal muscle mass and quality of life in adults with cancer: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. 2022;13(2):839–857. <https://doi.org/10.1002/jcsm.12928>.
- Wolfe RR. The underappreciated role of muscle in health and disease. *Am J Clin Nutr*. 2006;84(3):475–482.
- Aversa Z, Costelli P, Muscaritoli M. Cancer-induced muscle wasting: latest findings in prevention and treatment. *Ther Adv Med Oncol*. 2017;9(5):369–382. <https://doi.org/10.1177/1758834017698643>.
- Puthucherry ZA, Rawal J, McPhail M, et al. Acute skeletal muscle wasting in critical illness. *JAMA*. 2013;310(15):1591–1600. <https://doi.org/10.1001/jama.2013.278481>.
- Schmidt J. Current classification and management of inflammatory myopathies. *J Neuromuscul Dis*. 2018;5(2):109–129. <https://doi.org/10.3233/JND-180308>.
- Fearon K, Strasser F, Anker SD, et al. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol*. 2011;12(2):489–495. <https://doi.org/10.1016/S1470>.
- Blum D, Stene GB, Solheim TS, et al. Validation of the consensus-definition for cancer cachexia and evaluation of a classification model—a study based on data from an international multicentre project (EPCRC-CSA). *Ann Oncol*. 2014;25(8):1635–1642. <https://doi.org/10.1093/annonc/mdu086>.
- von Haehling S, Anker SD. Prevalence, incidence and clinical impact of cachexia: facts and numbers—update 2014. *J Cachexia Sarcopenia Muscle*. 2014;5(4):261–263. <https://doi.org/10.1007/s13539-014-0164-8>.
- Sullivan ES, Daly LE, Power DG, Ryan AM. Epidemiology of cancer-related weight loss and sarcopenia in the UK and Ireland: incidence, prevalence, and clinical impact. *JCSM Rapid Commun*. 2020;3(2):91–102. <https://doi.org/10.1002/rco2.19>.
- von Haehling S, Anker MS, Anker SD. Prevalence and clinical impact of cachexia in chronic illness in Europe, USA, and Japan: facts and numbers update 2016. *J Cachexia Sarcopenia Muscle*. 2016;7(5):507–509. <https://doi.org/10.1002/jcsm.12167>.
- Roeland EJ, Bohlke K, Baracos VE, et al. Management of cancer cachexia. *ASCO guideline*. 2020;38(1):267–274. <https://doi.org/10.1200/JCO.20.00611>.
- Kadakia KC, Hamilton-Reeves JM, Baracos VE. Current therapeutic targets in cancer cachexia: a pathophysiologic approach. *Am Society of Clin Oncol*. 2023;43(3):1256–1267. https://doi.org/10.1200/edbk_389942.

14. Balstad TR, Solheim TS, Strasser F, Kaasa S, Bye A. Dietary treatment of weight loss in patients with advanced cancer and cachexia: a systematic literature review. *Crit Rev Oncol Hematol*. 2014;91(2):210–221. <https://doi.org/10.1016/j.critrevonc.2014.02.005>.
15. de van der Schueren MAE, Laviano A, Blanchard H, Jourdan M, Arends J, Baracos VE. Systematic review and meta-analysis of the evidence for oral nutritional intervention on nutritional and clinical outcomes during chemo(radio) therapy: current evidence and guidance for design of future trials. *Ann Oncol*. 2018; 29(5):1141–1153. <https://doi.org/10.1093/annonc/ndy114>.
16. Tobberup R, Thoresen L, Falkmer UG, Yilmaz MK, Solheim TS, Balstad TR. Effects of current parenteral nutrition treatment on health-related quality of life, physical function, nutritional status, survival and adverse events exclusively in patients with advanced cancer: a systematic literature review. *Crit Rev Oncol Hematol*. 2019; 139(4):96–107. <https://doi.org/10.1016/j.critrevonc.2019.04.014>.
17. Wright TJ, Dillon EL, Durham WJ, et al. A randomized trial of adjunct testosterone for cancer-related muscle loss in men and women. *J Cachexia Sarcopenia Muscle*. 2018;9(3):482–496. <https://doi.org/10.1002/jcsm.12295>.
18. Advani SM, Advani PG, Vonville HM, Jafri SH. Pharmacological management of cachexia in adult cancer patients: a systematic review of clinical trials. *BMC Cancer*. 2018;18(1):388–394. <https://doi.org/10.1186/s12885-018-5080-4>.
19. Reid J, Hughes CM, Murray LJ, Parsons C, Cantwell MM. Non-steroidal anti-inflammatory drugs for the treatment of cancer cachexia: a systematic review. *Palliat Med*. 2013;27(4):295–303. <https://doi.org/10.1177/0269216312441382>.
20. Solheim TS, Fearon KCH, Blum D, Kaasa S. Non-steroidal anti-inflammatory treatment in cancer cachexia: a systematic literature review. *Acta Oncol*. 2013; 52(1):6–17. <https://doi.org/10.3109/0284186X.2012.724536>.
21. Temel JS, Abernethy AP, Currow DC, et al. Anamorelin in patients with non-small-cell lung cancer and cachexia (ROMANA 1 and ROMANA 2): results from two randomised, double-blind, phase 3 trials. *Lancet Oncol*. 2016;17(4):519–531. [https://doi.org/10.1016/S1470-2045\(15\)00558-6](https://doi.org/10.1016/S1470-2045(15)00558-6).
22. Bai Y, Hu Y, Zhao Y, et al. Anamorelin for cancer anorexia-cachexia syndrome: a systematic review and meta-analysis. *Support Care Cancer*. 2017;25(5):1651–1659. <https://doi.org/10.1007/s00520-016-3560-0>.
23. Ries A, Trottenberg P, Elsner F, et al. A systematic review on the role of fish oil for the treatment of cachexia in advanced cancer: an EPCRC cachexia guidelines project. *Palliat Med*. 2012;26(4):294–304. <https://doi.org/10.1177/0269216311418709>.
24. Ma YJ, Yu J, Xiao J, Cao BW. The consumption of omega-3 polyunsaturated fatty acids improves clinical outcomes and prognosis in pancreatic cancer patients: a systematic evaluation. *Nutr Cancer*. 2015;67(1):112–118. <https://doi.org/10.1080/01635581.2015.976315>.
25. Seymour-Jackson E, Laird BJA, Sayers J, Fallon M, Solheim TS, Skipworth R. Cannabinoids in the treatment of cancer anorexia and cachexia: where have we been, where are we going? *Asia Pac J Oncol Nurs*. 2023;10(Suppl 1):100292. <https://doi.org/10.1016/j.apjon.2023.100292>.
26. Solheim TS, Laird BJA, Balstad TR, et al. A randomized phase II feasibility trial of a multimodal intervention for the management of cachexia in lung and pancreatic cancer. *J Cachexia Sarcopenia Muscle*. 2017;8(5):778–788. <https://doi.org/10.1002/jcsm.12201>.
27. Amrute-Nayak M, Pegoli G, Holler T, Lopez-Davila AJ, Lanzaolo C, Nayak A. Chemotherapy triggers cachexia by deregulating synergetic function of histone-modifying enzymes. *J Cachexia Sarcopenia Muscle*. 2021;12(1):159–176. <https://doi.org/10.1002/jcsm.12645>.
28. Pin F, Barreto R, Couch ME, Bonetto A, O'Connell TM. Cachexia induced by cancer and chemotherapy yield distinct perturbations to energy metabolism. *J Cachexia Sarcopenia Muscle*. 2019;10(1):140–154. <https://doi.org/10.1002/jcsm.12360>.
29. Grande AJ, Silva V, Riera R, et al. Exercise for cancer cachexia in adults. *Cochrane Database Syst Rev*. 2014;11:CD010804. <https://doi.org/10.1002/14651858.CD010804.pub2>.
30. Campbell KL, Winters-Stone KM, Wiskemann J, et al. Exercise guidelines for cancer survivors: consensus statement from international multidisciplinary roundtable. *Med Sci Sports Exerc*. 2019;51(11):2375–2390. <https://doi.org/10.1249/MSS.00000000000002116>.
31. Rajarajeswaran P, Vishnupriya R. Exercise in cancer. *Indian J Med Paediatr Oncol*. 2009;30(2):61–70. <https://doi.org/10.4103/0971-5851.60050>.
32. Wiskemann J, Clauss D, Tjaden C, et al. Progressive resistance training to impact physical fitness and body weight in pancreatic cancer patients: a randomized controlled trial. *Pancreas*. 2019;48(2):257–266. <https://doi.org/10.1097/MPA.0000000000001221>.
33. Storck LJ, Ruehlin M, Gaeumann S, et al. Effect of a leucine-rich supplement in combination with nutrition and physical exercise in advanced cancer patients: a randomized controlled intervention trial. *Clin Nutr*. 2020;39(12):3637–3644. <https://doi.org/10.1016/j.clnu.2020.04.008>.
34. Schink K, Herrmann HJ, Schwappacher R, et al. Effects of whole-body electromyostimulation combined with individualized nutritional support on body composition in patients with advanced cancer: a controlled pilot trial. *BMC Cancer*. 2018;18(1):886. <https://doi.org/10.1186/s12885-018-4790-y>.
35. Uster A, Ruehlin M, Mey S, et al. Effects of nutrition and physical exercise intervention in palliative cancer patients: a randomized controlled trial. *Clin Nutr*. 2018;37(4):1202–1209. <https://doi.org/10.1016/j.clnu.2017.05.027>.
36. Dewys WD, Begg C, Lavin PT, et al. Prognostic effect of weight loss prior to chemotherapy in cancer Patients. *Am J Med*. 1980;69(3):201–211. [https://doi.org/10.1016/s0149-2918\(05\)80001-3](https://doi.org/10.1016/s0149-2918(05)80001-3).
37. Emery PW, Edwards T, Rennie MJ, Souhami RL, Halliday D. Protein synthesis in muscle measured in vivo in cachectic patients with cancer. *Br Med J*. 1984;29(2):123–133. <https://doi.org/10.1136/bmj.289.6445.584>.
38. Nair S, Halliday D, Griggs RC, Sreekumar K. Leucine incorporation into mixed skeletal protein in humans. *Am J Physiol*. 1988;254(2):208–213. <https://doi.org/10.1152/ajpendo.1988.254.2.E208>.
39. Lundholm K, Bennegård K, Edåen E, Svaninger G, Emery PW, Rennie MJ. Efflux of 3-methylhistidine from the leg in cancer patients who experience weight loss. *Cancer Res*. 1982;42(11):4807–4811.
40. Vesali RF, Klaude M, Thunblad L, Rooyackers OE, Wernerman J. Contractile protein breakdown in human leg skeletal muscle as estimated by [2H3]-3-methylhistidine: a new method. *Metabolism*. 2004;53(8):1076–1080. <https://doi.org/10.1016/j.metabol.2004.02.017>.
41. Holm L, Ebenstein D, Toth MJ, et al. Determination of steady-state protein breakdown rate in vivo by the disappearance of protein-bound tracer-labeled amino acids: a method applicable in humans. *Am J Physiol Endocrinol Metab*. 2013;304(4): 895–907. <https://doi.org/10.1152/ajpendo.00579.2012-A>.
42. Rennie MJ, Phillips S, Smith K. Reliability of results and interpretation of measures of 3-methylhistidine in muscle interstitium as marker of muscle proteolysis [published correction appears in J Appl Physiol. 2009;106(2):749]. *J Appl Physiol*. 2009;106(2):749. <https://doi.org/10.1152/jappphysiol.90782.2008>.
43. Li YP, Chen Y, John J, et al. TNF- α acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *Faseb J*. 2005;19(3):362–370. <https://doi.org/10.1096/fj.04-2364com>.
44. Bonetto A, Aydogdu T, Jin X, et al. JAK/STAT3 pathway inhibition blocks skeletal muscle wasting downstream of IL-6 and in experimental cancer cachexia. *Am J Physiol Endocrinol Metab*. 2012;303(4):410–421. <https://doi.org/10.1152/ajpendo.00039.2012-Cachexia>.
45. Braun TP, Zhu X, Szumowski M, et al. Central nervous system inflammation induces muscle atrophy via activation of the hypothalamic-pituitary-adrenal axis. *J Exp Med*. 2011;208(12):2449–2463. <https://doi.org/10.1084/jem.20111020>.
46. Judge SM, Wu CL, Beharry AW, et al. Genome-wide identification of FoxO-dependent gene networks in skeletal muscle during C26 cancer cachexia. *BMC Cancer*. 2014;14(1):711–720. <https://doi.org/10.1186/1471-2407-14-997>.
47. Schmitt TL, Martignoni ME, Bachmann J, et al. Activity of the Akt-dependent anabolic and catabolic pathways in muscle and liver samples in cancer-related cachexia. *J Mol Med*. 2007;85(6):647–654. <https://doi.org/10.1007/s00109-007-0177-2>.
48. Silva KAS, Dong J, Dong Y, et al. Inhibition of Stat3 activation suppresses caspase-3 and the ubiquitin-proteasome system, leading to preservation of muscle mass in cancer cachexia. *J Biol Chem*. 2015;290(17):11177–11187. <https://doi.org/10.1074/jbc.M115.641514>.
49. Murtton AJ, Maddocks M, Stephens FB, Marimuthu K, England R, Wilcock A. Consequences of late-stage non-small-cell lung cancer cachexia on muscle metabolic processes. *Clin Lung Cancer*. 2017;18(1):e1–e11. <https://doi.org/10.1016/j.clcc.2016.06.003>.
50. Pin F, Minerio VG, Penna F, et al. Interference with Ca²⁺-dependent proteolysis does not alter the course of muscle wasting in experimental cancer cachexia. *Front Physiol*. 2017;8(4):35–41. <https://doi.org/10.3389/fphys.2017.00213>.
51. Yang J, Zhang Z, Zhang Y, et al. ZIP4 promotes muscle wasting and cachexia in mice with orthotopic pancreatic tumors by stimulating RAB27B-regulated release of extracellular vesicles from cancer cells. *Gastroenterology*. 2019;156(3):722–734.e6. <https://doi.org/10.1053/j.gastro.2018.10.026>.
52. Sugiyama M, Yamaki A, Furuya M, et al. Ghrelin improves body weight loss and skeletal muscle catabolism associated with angiotensin II-induced cachexia in mice. *Regul Pept*. 2012;178(1):21–28. <https://doi.org/10.1016/j.regpep.2012.06.003>.
53. Costelli P, Muscaritoli M, Bossola M, et al. IGF-1 is downregulated in experimental cancer cachexia. *Am J Physiol Regul Integr Comp Physiol*. 2006;291(3):111–121. <https://doi.org/10.1152/ajpregu.00104.2006>.
54. Raun SH, Ali MS, Han X, et al. Adenosine monophosphate-activated protein kinase is elevated in human cachectic muscle and prevents cancer-induced metabolic dysfunction in mice. *J Cachexia Sarcopenia Muscle*. 2023;59(4):211–221. <https://doi.org/10.1002/jcsm.13238>.
55. White JP, Puppa MJ, Gao S, Sato S, Welle SL, Carson JA. Muscle mTORC1 suppression by IL-6 during cancer cachexia: a role for AMPK. *Am J Physiol Endocrinol Metab*. 2013;304:1042–1052. <https://doi.org/10.1152/ajpendo.00410.2012>.
56. Bohnert KR, Gallot YS, Sato S, Xiong G, Hindi SM, Kumar A. Inhibition of ER stress and unfolding protein response pathways causes skeletal muscle wasting during cancer cachexia. *Faseb J*. 2016;30(9):3053–3068. <https://doi.org/10.1096/fj.201600250RR>.
57. Fontes-Oliveira CC, Busquets S, Toledo M, et al. Mitochondrial and sarcoplasmic reticulum abnormalities in cancer cachexia: altered energetic efficiency? *Biochim Biophys Acta Gen Subj*. 2013;1830(3):2770–2778. <https://doi.org/10.1016/j.bbagen.2012.11.009>.
58. Marzetti E, Lorenzi M, Landi F, et al. Altered mitochondrial quality control signaling in muscle of old gastric cancer patients with cachexia. *Exp Gerontol*. 2017; 87(5):92–99. <https://doi.org/10.1016/j.exger.2016.10.003>.
59. Williams Arthur, Sun Xiaoyan, Fischer Josef E, Per-Olof Hasselgren. The expression of genes in the ubiquitin-proteasome proteolytic pathway is increased in skeletal muscle from patients with cancer. *Surgery*. 1999;126(4):744–750.
60. Doyle A, Zhang G, Fattah EAA, Eissa NT, Li Y. Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. *Faseb J*. 2011;25(1): 99–110. <https://doi.org/10.1096/fj.10-164152>.
61. Furuno K, Goldberg AL. The activation of protein degradation in muscle by Ca²⁺ or muscle injury does not involve a lysosomal mechanism. *Biochem J*. 1986;237(3): 114–121. <https://doi.org/10.1042/bj2370859>.

62. Lecker SH, Solomon V, Mitch WE, Goldberg AL. Clinical trials for the treatment of secondary wasting and cachexia muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. *J Nutr*. 1999;1(2): 134–145. <https://doi.org/10.1093/jn/129.1.227S>.
63. Li Y, Schwartz RJ, Waddell ID, Holloway BR, Reid MB. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF- κ B activation in response to tumor necrosis factor. *Faseb J*. 1998;12(10):871–880. <https://doi.org/10.1096/fasebj.12.10.971>.
64. Sandri M. Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. *Int J Biochem Cell Biol*. 2013;45(10):2121–2129. <https://doi.org/10.1016/j.biocel.2013.04.023>.
65. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell*. 2004;117(3):11–20. <https://doi.org/10.1016/j.jbsmb.2011.07.002>. Identification.
66. Mammucari C, Milan G, Romanello V, et al. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab*. 2007;6(6):458–471. <https://doi.org/10.1016/j.cmet.2007.11.001>.
67. Rom O, Reznick AZ. The role of E3 ubiquitin-ligases MuRF-1 and MAFbx in loss of skeletal muscle mass. *Free Radic Biol Med*. 2016;98(2):218–230. <https://doi.org/10.1016/j.freeradbiomed.2015.12.031>.
68. Cai D, Daniel Frantz J, Tawa NE, et al. IKK/NF-B activation causes severe muscle wasting in mice improved by NF-B inhibition in MISR mice, consistent with a critical role for NF-B in the pathology of muscle wasting and establishing it as an important clinical target for the treatment of muscle atrophy. *Cell*. 2004;119(3): 145–152. <https://doi.org/10.1016/j.cell.2004.09.027>.
69. Li W, Moylan JS, Chambers MA, Smith J, Reid MB. Interleukin-1 stimulates catabolism in C2C12 myotubes. *Am J Physiol Cell Physiol*. 2009;297(1):706–714. <https://doi.org/10.1152/ajpcell.00626.2008-Interleukin-1>.
70. McClung JM, Judge AR, Powers SK, Yan Z. p38 MAPK links oxidative stress to autophagy-related gene expression in cachectic muscle wasting. *Am J Physiol Cell Physiol*. 2010;298(3):C542–C549. <https://doi.org/10.1152/ajpcell.00192.2009>.
71. Patel HJ, Patel BM. TNF- α and cancer cachexia: molecular insights and clinical implications. *Life Sci*. 2017;170(3):56–63. <https://doi.org/10.1016/j.lfs.2016.11.033>.
72. Parajuli P, Kumar S, Loumaye A, et al. Twist1 activation in muscle progenitor cells causes muscle loss akin to cancer cachexia. *Dev Cell*. 2018;45(6):712–725.e6. <https://doi.org/10.1016/j.devcel.2018.05.026>.
73. Fry CS, Nayeem SZ, Dillon EL, et al. Glucocorticoids increase skeletal muscle NF- κ B inducing kinase (NIK): links to muscle atrophy. *Phys Rep*. 2016;4(21):e13014. <https://doi.org/10.14814/phy2.13014>.
74. Gallot YS, Durieux AC, Castells J, et al. Myostatin gene inactivation prevents skeletal muscle wasting in cancer. *Cancer Res*. 2014;74(24):7344–7356. <https://doi.org/10.1158/0008-5472.CAN-14-0057>.
75. Bédard N, Jammoul S, Moore T, et al. Inactivation of the ubiquitin-specific protease 19 deubiquitinating enzyme protects against muscle wasting. *Faseb J*. 2015;29(9): 3889–3898. <https://doi.org/10.1096/fj.15-270579>.
76. Chen L, Yang Q, Zhang H, et al. Cryptotanshinone prevents muscle wasting in CT26-induced cancer cachexia through inhibiting STAT3 signaling pathway. *J Ethnopharmacol*. 2020;260(4):371–380. <https://doi.org/10.1016/j.jep.2020.113066>.
77. Jagoe RT, Goldberg AL. What do we really know about the ubiquitin proteasome pathway in muscle atrophy? *Curr Opin Clin Nutr Metab Care*. 2001;23(2):967–975. <https://doi.org/10.1097/00075197-200105000-00003>.
78. Bodine SC, Latres E, Baumhueter S, et al. Identification of ubiquitin required for skeletal atrophy. *Science*. 2001;199(4):201–211. <https://doi.org/10.1126/science.1065874>.
79. Masiero E, Agatea L, Mammucari C, et al. Autophagy is required to maintain muscle mass. *Cell Metab*. 2009;10(6):507–515. <https://doi.org/10.1016/j.cmet.2009.10.008>.
80. Chong SW, Nguyen LM, Jiang YJ, Korzh V. The chemokine Sdf-1 and its receptor Cxcr4 are required for formation of muscle in zebrafish. *BMC Dev Biol*. 2007;21(7): 267–273. <https://doi.org/10.1186/1471-213X-7-54>.
81. Melchionna R, Di Carlo A, De Mori R, et al. Induction of myogenic differentiation by SDF-1 via CXCR4 and CXCR7 receptors. *Muscle Nerve*. 2010;41(6):828–835. <https://doi.org/10.1002/mus.21611>.
82. Bobadilla M, Sainz N, Abizanda G, et al. The CXCR4/SDF1 axis improves muscle regeneration through MMP-10 activity. *Stem Cell Dev*. 2014;23(12):1417–1427. <https://doi.org/10.1089/scd.2013.0491>.
83. Martinelli GB, Olivari D, Re Cecconi AD, et al. Activation of the SDF1/CXCR4 pathway retards muscle atrophy during cancer cachexia. *Oncogene*. 2016;35(48): 6212–6222. <https://doi.org/10.1038/onc.2016.153>.
84. Winbanks CE, Murphy KT, Bernardo BC, et al. Smad7 gene delivery prevents muscle wasting associated with cancer cachexia in mice. *Sci Transl Med*. 2016;33(1): 355–367. <https://doi.org/10.1126/scitranslmed.aac4976>.
85. Stephens IJG, Rooyackers O, Skipworth RJ, Fearon KC, Timmons JA. Using transcriptomics to identify and validate novel biomarkers of human skeletal muscle cancer cachexia. *Genome Med*. 2010;11(2):1174–1185. <https://doi.org/10.1186/gm122>.
86. Pin F, Barreto R, Kitase Y, et al. Growth of ovarian cancer xenografts causes loss of muscle and bone mass: a new model for the study of cancer cachexia. *J Cachexia Sarcopenia Muscle*. 2018;9(4):685–700. <https://doi.org/10.1002/jcsm.12311>.
87. Eskiler G, Bezdegumeli E, Ozman Z, et al. IL-6 mediated JAK/STAT3 signaling pathway in cancer patients with cachexia. *Bratislava Med J*. 2019;120(11):819–826. https://doi.org/10.4149/BLM_2019_136.
88. Grabiec AM, Korchynskiy O, Tak PP, Reedquist KA. Histone deacetylase inhibitors suppress rheumatoid arthritis fibroblast-like synoviocyte and macrophage IL-6 production by accelerating mRNA decay. *Ann Rheum Dis*. 2012;71(3):424–431. <https://doi.org/10.1136/ard.2011.154211>.
89. Ma F, Li Y, Jia L, et al. Macrophage-stimulated cardiac fibroblast production of IL-6 is essential for TGF β /Smad activation and cardiac fibrosis induced by angiotensin II. *PLoS One*. 2012;7(5):74–83. <https://doi.org/10.1371/journal.pone.0035144>.
90. Miki S, Iwano M, Miki Y, et al. Interleukin-6 (IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas. *FEBS Lett*. 1989;250(2):607–610. [https://doi.org/10.1016/0014-5793\(89\)80805-1](https://doi.org/10.1016/0014-5793(89)80805-1).
91. White JP, Baltgalvis KA, Puppa MJ, Sato S, Baynes JW, Carson JA. Muscle oxidative capacity during IL-6-dependent cancer cachexia. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(4):201–211. <https://doi.org/10.1152/ajpregu.00300.2010-Many>.
92. Fujimoto-Ouchi K, Onuma E, Shirane M, Mori K, Tanaka Y. Capecitabine improves cancer cachexia and normalizes IL-6 and PTHrP levels in mouse cancer cachexia models. *Cancer Chemother Pharmacol*. 2007;59(6):807–815. <https://doi.org/10.1007/s00280-006-0338-y>.
93. Op den Kamp CM, Gosker HR, Lagarde S, et al. Preserved muscle oxidative metabolic phenotype in newly diagnosed non-small cell lung cancer cachexia. *J Cachexia Sarcopenia Muscle*. 2015;16(2):164–173. <https://doi.org/10.1002/jcsm.12007>.
94. Sanchez AMJ, Candau RB, Csibi A, Pagano AF, Raibon A, Bernardi H. The role of AMP-activated protein kinase in the coordination of skeletal muscle turnover and energy homeostasis. *Am J Physiol Cell Physiol*. 2012;303(3):475–485. <https://doi.org/10.1152/ajpcell.00125.2012>.
95. Pettersen K, Andersen S, Degen S, et al. Cancer cachexia associates with a systemic autophagy-inducing activity mimicked by cancer cell-derived IL-6 trans-signaling. *Sci Rep*. 2017;7(1):23–36. <https://doi.org/10.1038/s41598-017-02088-2>.
96. Bargiela A, Cerro-Herreros E, Fernandez-Costa JM, Vilchez JJ, Llamusi B, Artero R. Increased autophagy and apoptosis contribute to muscle atrophy in a myotonic dystrophy type 1 Drosophila model. *DMM Dis Mod and Mech*. 2015;8(7):679–690. <https://doi.org/10.1242/dmm.018127>.
97. Sandri M. Autophagy in health and disease. Involvement of autophagy in muscle atrophy. *Am J Physiol Cell Physiol*. 2010;298(2):1291–1297. <https://doi.org/10.1152/ajpcell.00531.2009>.
98. Penna F, Ballaró R, Martínez-Cristóbal P, et al. Autophagy exacerbates muscle wasting in cancer cachexia and impairs mitochondrial function. *J Mol Biol*. 2019; 431(15):2674–2686. <https://doi.org/10.1016/j.jmb.2019.05.032>.
99. Sun Q, Fang L, Tang X, et al. TGF- β upregulated mitochondrial mass through the SMAD2/3–C/EBP β –PRMT1 signal pathway in primary human lung fibroblasts. *J Immunol*. 2019;202(1):37–47. <https://doi.org/10.4049/jimmunol.1800782>.
100. Renlund N, O'Neill FH, Zhang LH, Sidis Y, Teixeira J. Activin receptor-like kinase-2 inhibits activin signaling by blocking the binding of activin to its type II receptor. *J Endocrinol*. 2007;195(1):95–103. <https://doi.org/10.1677/JOE-07-0281>.
101. Pettersen K, Andersen S, van der Veen A, et al. Autocrine activin A signalling in ovarian cancer cells regulates secretion of interleukin 6, autophagy, and cachexia. *J Cachexia Sarcopenia Muscle*. 2020;11(1):195–207. <https://doi.org/10.1002/jcsm.12489>.
102. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med*. 2010;49(11):1603–1616. <https://doi.org/10.1016/j.freeradbiomed.2010.09.006>.
103. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in cancer. *Cancer Cell*. 2020;38(2):167–197. <https://doi.org/10.1016/j.ccell.2020.06.001>.
104. Seo G, Yu C, Han H, et al. The Hippo pathway noncanonically drives autophagy and cell survival in response to energy stress. *Mol Cell*. 2023;17(2):233–241. <https://doi.org/10.1016/j.molcel.2023.07.019>.
105. van der Ende M, Grefte S, Plas R, et al. Mitochondrial dynamics in cancer-induced cachexia. *Biochim Biophys Acta Rev Cancer*. 2018;1870(2):137–150. <https://doi.org/10.1016/j.bbcan.2018.07.008>.
106. White JP, Puppa MJ, Sato S, et al. IL-6 regulation on skeletal muscle mitochondrial remodeling during cancer cachexia in the ApcMin/+ mouse. *Skeletal Muscle*. 2012; 2:14. <https://doi.org/10.1186/2044-5040-2-14>.
107. Vanderveen BN, Fix DK, Carson JA. Disrupted skeletal muscle mitochondrial dynamics, mitophagy, and biogenesis during cancer cachexia: a role for inflammation. *Oxid Med Cell Longev*. 2017;11(2):311–323. <https://doi.org/10.1155/2017/3292087>.
108. Brown JL, Rosa-Caldwell ME, Lee DE, et al. Mitochondrial degeneration precedes the development of muscle atrophy in progression of cancer cachexia in tumour-bearing mice. *J Cachexia Sarcopenia Muscle*. 2017;8(6):926–938. <https://doi.org/10.1002/jcsm.12232>.
109. Neyroud D, Nosacka RL, Judge AR, Hepple RT. Colon 26 adenocarcinoma (C26)-induced cancer cachexia impairs skeletal muscle mitochondrial function and content. *J Muscle Res Cell Motil*. 2019;23(2):123–132. <https://doi.org/10.1007/s10974-019-09510-4>.
110. Egan B, Sharples AP. Molecular responses to acute exercise and their relevance for adaptations in skeletal muscle to exercise training. *Physiol Rev*. 2023;103(3): 2057–2170. <https://doi.org/10.1152/physrev.00054.2021>.
111. Roberts MD, McCarthy JJ, Hornberger TA, et al. Mechanisms of mechanical overload-induced skeletal muscle hypertrophy: current understanding and future directions. *Physiol Rev*. 2023;103(4):2679–2757. <https://doi.org/10.1152/physrev.00039.2022>.
112. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. *Compr Physiol*. 2012;2(2):1143–1211. <https://doi.org/10.1002/cphy.c110025>.

113. McLeod JC, Stokes T, Phillips SM. Resistance exercise training as a primary countermeasure to age-related chronic disease. *Front Physiol.* 2019;10:654. <https://doi.org/10.3389/fphys.2019.00645>.
114. Carlee GD, Hepple RT, Bammam MM, Zierath JR. Exercise promotes healthy aging of skeletal muscle. *Cell Metab.* 2016;23(6):1034–1047. <https://doi.org/10.1016/j.cmet.2016.05.007>.
115. Hardee JP, Counts BR, Carson JA. Understanding the role of exercise in cancer cachexia therapy. *Am J Lifestyle Med.* 2019;13(1):46–60. <https://doi.org/10.1177/1559827617725283>.
116. Garber CE, Blissmer B, Deschenes MR, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc.* 2011;43(7):1334–1359. <https://doi.org/10.1249/MSS.0B013E318213FEFB>.
117. Kjøbsted R, Hingst JR, Fentz J, et al. AMPK in skeletal muscle function and metabolism. *Faseb J.* 2018;32(4):1741–1777. <https://doi.org/10.1096/fj.201700442R>.
118. Chin ER. Intracellular Ca²⁺ signaling in skeletal muscle: decoding a complex message. *Exerc Sport Sci Rev.* 2010;38(2):76–85. <https://doi.org/10.1097/JES.0b013e3181d495d2>.
119. Nogueiras R, Habegger KM, Chaudhary N, et al. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. *Physiol Rev.* 2012;92(3):1479–1514. <https://doi.org/10.1152/physrev.00022.2011>.
120. Lindholm ME, Rundqvist H. Skeletal muscle hypoxia-inducible factor-1 and exercise. *Exp Physiol.* 2016;101(1):28–32. <https://doi.org/10.1113/EP085318>.
121. Chow LS, Gerszten RE, Taylor JM, et al. Exerkines in health, resilience and disease. *Nat Rev Endocrinol.* 2022;18(5):273–289. <https://doi.org/10.1038/s41574-022-00641-2>.
122. Murphy RM, Watt MJ, Febbraio MA. Metabolic communication during exercise. *Nat Metab.* 2020;2(9):805–816. <https://doi.org/10.1038/s42255-020-0258-x>.
123. Moniz SC, Islam H, Hazell TJ. Mechanistic and methodological perspectives on the impact of intense interval training on post-exercise metabolism. *Scand J Med Sci Sports.* 2020;30(4):638–651. <https://doi.org/10.1111/sms.13610>.
124. Burke LM, Van Loon LJC, Hawley JA. Postexercise muscle glycogen resynthesis in humans. *J Appl Physiol* (1985). 2017;122(5):1055–1067. <https://doi.org/10.1152/jappphysiol.00860.2016>.
125. Jentjens R, Jeukendrup AE. Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Med.* 2003;33(2):117–144. <https://doi.org/10.2165/00007256-200333020-00004>.
126. McGlory C, van Vliet S, Stokes T, Mittendorfer B, Phillips SM. The impact of exercise and nutrition on the regulation of skeletal muscle mass. *J Physiol.* 2019;597(5):1251–1258. <https://doi.org/10.1113/JP275443>.
127. Lundsgaard AM, Fritzen AM, Kiens B. The importance of fatty acids as nutrients during post-exercise recovery. *Nutrients.* 2020;12(2):280. <https://doi.org/10.3390/nu12020280>.
128. Langhammer B, Bergland A, Rydwick E. The importance of physical activity exercise among older people. *BioMed Res Int.* 2018;2018:7856823. <https://doi.org/10.1155/2018/7856823>.
129. Russell AP. Molecular regulation of skeletal muscle mass. *Clin Exp Pharmacol Physiol.* 2010;37(3):378–384. <https://doi.org/10.1111/j.1440-1681.2009.05265.x>.
130. Phillip SM. Physiologic and molecular bases of muscle hypertrophy and atrophy: impact of resistance exercise on human skeletal muscle (protein and exercise dose effects). *Appl Physiol Nutr Metab.* 2009;34(3):403–410. <https://doi.org/10.1139/H09-042>.
131. Distefano G, Goodpaster BH. Effects of exercise and aging on skeletal muscle. *Cold Spring Harb Perspect Med.* 2018;8:a029785. <https://doi.org/10.1101/cshperspect.a029785>.
132. Rossetti ML, Steiner JL, Gordon BS. Androgen-mediated regulation of skeletal muscle protein balance. *Mol Cell Endocrinol.* 2017;447(2):35–44. <https://doi.org/10.1016/j.mce.2017.02.031>.
133. Cairns SP, Borroni F. β -Adrenergic modulation of skeletal muscle contraction: key role of excitation-contraction coupling. *J Physiol.* 2015;593(21):4713–4727. <https://doi.org/10.1113/JP270909>.
134. Wackerhage H, Schoenfeld BJ, Hamilton DL, Lehti M, Hulmi JJ. Stimuli and sensors that initiate skeletal muscle hypertrophy following resistance exercise. *J Appl Physiol* (1985). 2019;126(1):30–43. <https://doi.org/10.1152/jappphysiol.00685.2018>.
135. Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skeletal Muscle.* 2011;1(1):4. <https://doi.org/10.1186/2044-5040-1-4>.
136. Hood DA. Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle. *Appl Physiol Nutr Metab.* 2009;34(3):465–472. <https://doi.org/10.1139/H09-045>.
137. Severinsen MCK, Pedersen BK. Muscle–organ crosstalk: the emerging roles of myokines. *Endocr Rev.* 2020;41(4):594–609. <https://doi.org/10.1210/ENDREV/BNA016>.
138. Whitham M, Parker BL, Friedrichsen M, et al. Extracellular vesicles provide a means for tissue crosstalk during Exercise. *Cell Metab.* 2018;27(1):237–251.e4. <https://doi.org/10.1016/j.cmet.2017.12.001>.
139. Morville T, Sahl RE, Moritz T, Helge JW, Clemmensen C. Plasma metabolome profiling of resistance exercise and endurance exercise in humans. *Cell Rep.* 2020;33(13):108554. <https://doi.org/10.1016/j.celrep.2020.108554>.
140. Safdar A, Tarnopolsky MA. Exosomes as mediators of the systemic adaptations to endurance exercise. *Cold Spring Harb Perspect Med.* 2018;8(3):a029827. <https://doi.org/10.1101/cshperspect.a029827>.
141. Jiang S, Bae JH, Wang Y, Song W. The potential roles of myokines in adipose tissue metabolism with exercise and cold exposure. *Int J Mol Sci.* 2022;23(19):11523. <https://doi.org/10.3390/ijms231911523>.
142. Bay ML, Pedersen BK. Muscle–organ crosstalk: focus on immunometabolism. *Front Physiol.* 2020;11(2):567881. <https://doi.org/10.3389/fphys.2020.567881>.
143. Steensberg A, van Hall G, Osada T, Sacchetti BS, Pedersen BK. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol.* 2000;529(Pt 1):237–242. <https://doi.org/10.1111/j.1469-7793.2000.00237.x> Pt 1.
144. Phillips MD, Mitchell JB, Currie-Elof LM, Yellott RC, Hubing KA. Influence of commonly employed resistance exercise protocols on circulating IL-6 and indices of insulin sensitivity. *J Strength Condit Res.* 2010;24(4):1091–1101. <https://doi.org/10.1519/JSC.0b013e3181cc2212>.
145. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived Interleukin-6. *Physiol Rev.* 2008;88(4):1379–1406. <https://doi.org/10.1152/physrev.90100.2007>.
146. Hiscock N, Chan MHS, Bisucci T, Darby IA, Febbraio MA. Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *Faseb J.* 2004;18(9):992–994. <https://doi.org/10.1096/fj.03-1259fje>.
147. Whitham M, Chan MHS, Pal M, et al. Contraction-induced interleukin-6 gene transcription in skeletal muscle is regulated by c-Jun terminal kinase/activator protein-1. *J Biol Chem.* 2012;287(14):10771–10779. <https://doi.org/10.1074/jbc.M111.310581>.
148. Keller C, Steensberg A, Pilegaard H, et al. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *Faseb J.* 2001;15(14):2748–2750. <https://doi.org/10.1096/fj.01-0507fje>.
149. Starkie RL, Arkinstall MJ, Koukoulas I, Hawley JA, Febbraio MA. Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *J Physiol.* 2001;533(Pt 2):585–591. <https://doi.org/10.1111/j.1469-7793.2001.0585a.x>.
150. Van Hall G, Steensberg A, Sacchetti M, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab.* 2003;88(7):3005–3010. <https://doi.org/10.1210/jc.2002-021687>.
151. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes.* 2004;53(7):1643–1648. <https://doi.org/10.2337/diabetes.53.7.1643>.
152. Glund S, Deshmukh A, Yun CL, et al. Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. *Diabetes.* 2007;56(6):1630–1637. <https://doi.org/10.2337/db06-1733>.
153. Ellingsgaard H, Hauselmann I, Schuler B, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med.* 2011;17(11):1481–1489. <https://doi.org/10.1038/nm.2513>.
154. Sakaguchi CA, Nieman DC, Signini EF, Abreu RM, Catai AM. Metabolomics-based studies assessing exercise-induced alterations of the human metabolome: a systematic review. *Metabolites.* 2019;9(8):164. <https://doi.org/10.3390/metabo9080164>.
155. Kelly RS, Kelly MP, Kelly P. Metabolomics, physical activity, exercise and health: a review of the current evidence. *Biochim Biophys Acta, Mol Basis Dis.* 2020;1866(12):165936. <https://doi.org/10.1016/j.bbadis.2020.165936>.
156. Sapp RM, Shill DD, Roth SM, Hagberg JM. Circulating microRNAs in acute and chronic exercise: more than mere biomarkers. *J Appl Physiol* (1985). 2017;122(3):702–717. <https://doi.org/10.1152/jappphysiol.00982.2016>.
157. Fernández-Sanjurjo M, De Gonzalo-Calvo D, Fernández-García B, et al. Circulating microRNA as emerging biomarkers of exercise. *Exerc Sport Sci Rev.* 2018;46(3):160–171. <https://doi.org/10.1249/JES.0000000000000148>.
158. Hoffmann C, Weigert C. Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations. *Cold Spring Harb Perspect Med.* 2017;7(11):a029793. <https://doi.org/10.1101/cshperspect.a029793>.
159. Leuchtmann AB, Adak V, Dilbaz S, Handschin C. The role of the skeletal muscle secretome in mediating endurance and resistance training adaptations. *Front Physiol.* 2021;12:709807. <https://doi.org/10.3389/fphys.2021.709807>.
160. Chin ER. Role of Ca²⁺/calmodulin-dependent kinases in skeletal muscle plasticity. *J Appl Physiol* (1985). 2005;99(2):414–423. <https://doi.org/10.1152/jappphysiol.00015.2005>.
161. Rose AJ, Kiens B, Richter EA. Ca²⁺-calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. *J Physiol.* 2006;574(3):889–903. <https://doi.org/10.1113/jphysiol.2006.111757>.
162. Hudmon A, Schulman H. Structure-function of the multifunctional Ca²⁺/calmodulin-dependent protein kinase II. *Biochem J.* 2002;364(Pt 3):593–611. <https://doi.org/10.1042/BJ20020228>.
163. Tavi P, Westerblad H. The role of in vivo Ca²⁺ signals acting on Ca²⁺-calmodulin-dependent proteins for skeletal muscle plasticity. *J Physiol.* 2011;589(21):5021–5031. <https://doi.org/10.1113/jphysiol.2011.212860>.
164. Egan B, Carson BP, Garcia-Roves PM, et al. Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor γ coactivator-1 α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *J Physiol.* 2010;588(10):1779–1790. <https://doi.org/10.1113/jphysiol.2010.188011>.
165. Fiorenza N, Gunnarsson TP, Hostrup M, et al. Metabolic stress-dependent regulation of the mitochondrial biogenic molecular response to high-intensity exercise in human skeletal muscle. *J Physiol.* 2018;596(14):2823–2840. <https://doi.org/10.1113/JP275972>.
166. Godin R, Asch A, Daussin FN. Intensity-dependent activation of intracellular signalling pathways in skeletal muscle: role of fibre type recruitment during

- exercise. *J Physiol.* 2010;588(21):4073–4074. <https://doi.org/10.1113/jphysiol.2010.195925>.
167. Dolmetsch RE, Lewist RS, Goodnowt CC, Healyt JJ. Differential activation of transcription factors by Ca²⁺ response and duration. *Nature.* 1997;386(2):855–858. <https://doi.org/10.1038/386855a0>.
 168. Iwanaka N, Egawa T, Satoubu N, et al. Leucine modulates contraction- and insulin-stimulated glucose transport and upstream signaling events in rat skeletal muscle. *J Appl Physiol*(1985). 2010;108(2):274–282. <https://doi.org/10.1152/japplphysiol.00420.2009>.
 169. Steinert ND, Potts GK, Wilson GM, et al. Mapping of the contraction-induced phosphoproteome identifies TRIM28 as a significant regulator of skeletal muscle size and function. *Cell Rep.* 2021;34(9):108796. <https://doi.org/10.1016/j.celrep.2021.108796>.
 170. Kido K, Ato S, Yokokawa T, Makanae Y, Sato K, Fujita S. Acute resistance exercise-induced IGF1 expression and subsequent GLUT4 translocation. *Phys Rep.* 2016; 4(16):e12907. <https://doi.org/10.14814/phy2.12907>.
 171. Potts GK, McNally RM, Blanco R, et al. A map of the phosphoproteomic alterations that occur after a bout of maximal-intensity contractions. *J Physiol.* 2017;595(15): 5209–5226. <https://doi.org/10.1113/JP273904>.
 172. Ahtiainen JP, Walker S, Silvennoinen M, et al. Exercise type and volume alter signaling pathways regulating skeletal muscle glucose uptake and protein synthesis. *Eur J Appl Physiol.* 2015;115(9):1835–1845. <https://doi.org/10.1007/s00421-015-3155-3>.
 173. Apró W, Wang L, Pontén M, Blomstrand E, Sahlin K. Resistance exercise induced mTORC1 signaling is not impaired by subsequent endurance exercise in human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2013;305(4):22–32. <https://doi.org/10.1152/ajpendo.00091.2013>.
 174. Groenendaek T, Jespersen NR, Jakobsgaard JE, et al. Skeletal muscle mitochondrial protein synthesis and respiration increase with low-load blood flow restricted as well as high-load resistance training. *Front Physiol.* 2018;9:1796. <https://doi.org/10.3389/fphys.2018.01796>.
 175. Camera DM, Edge J, Short MJ, Hawley JA, Coffey VG. Early time course of akt phosphorylation after endurance and resistance exercise. *Med Sci Sports Exerc.* 2010; 42(10):1843–1852. <https://doi.org/10.1249/MSS.0b013e3181d964e4>.
 176. Wilkinson SB, Phillips SM, Atherton PJ, et al. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J Physiol.* 2008;586(15):3701–3717. <https://doi.org/10.1113/jphysiol.2008.153916>.
 177. Donges CE, Burd NA, Duffield R, et al. Concurrent resistance and aerobic exercise stimulates both myofibrillar and mitochondrial protein synthesis in sedentary middle-aged men. *J Appl Physiol*(1985). 2012;112(12):1992–2001. <https://doi.org/10.1152/japplphysiol.00166.2012>.
 178. Mazo CE, D'Lugos AC, Sweeney KR, et al. The effects of acute aerobic and resistance exercise on mTOR signaling and autophagy markers in untrained human skeletal muscle. *Eur J Appl Physiol.* 2021;121(10):2913–2924. <https://doi.org/10.1007/s00421-021-04758-6>.
 179. Coffey VG, Zhong Z, Shield A, et al. Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. *Faseb J.* 2006;20(1):190–192. <https://doi.org/10.1096/fj.05-4809fje>.
 180. Martinez-Canton M, Gallego-Selles A, Gelabert-Rebato M, et al. Role of CaMKII and sarcolipin in muscle adaptations to strength training with different levels of fatigue in the set. *Scand J Med Sci Sports.* 2021;31(1):91–103. <https://doi.org/10.1111/sms.13828>.
 181. Small L, Altıntaş A, Laker RC, et al. Contraction influences Per2 gene expression in skeletal muscle through a calcium-dependent pathway. *J Physiol.* 2020;598(24): 5739–5752. <https://doi.org/10.1113/JP280428>.
 182. Freysenet D, Irrcher I, Connor MK, Carlo M Di, Hood DA. Calcium-regulated changes in mitochondrial phenotype in skeletal muscle cells. *Am J Physiol Cell Physiol.* 2004;286(5):1053–1061. <https://doi.org/10.1152/ajpcell.00418.2003>.
 183. Witczak CA, Fujii N, Hirshman MF, Goodyear LJ. Ca²⁺/calmodulin-dependent protein kinase kinase- α regulates skeletal muscle glucose uptake independent of AMP-activated protein kinase and Akt activation. *Diabetes.* 2007;56(5):1403–1409. <https://doi.org/10.2337/db06-1230>.
 184. Witczak CA, Jessen N, Warro DM, et al. CaMKII regulates contraction-but not insulin-induced glucose uptake in mouse skeletal muscle. *Am J Physiol Endocrinol Metab.* 2010;298(2):1150–1160. <https://doi.org/10.1152/ajpendo.00659.2009>.
 185. Wright DC, Hucker KA, Holloszy JO, Ho Han D. Ca²⁺ and AMPK both mediate stimulation of glucose transport by muscle contractions. *Diabetes.* 2004;12(3): 330–335. <https://doi.org/10.2337/diabetes.53.2.330>.
 186. Wright DC, Geiger PC, Han DH, Jones TE, Holloszy JO. Calcium induces increases in peroxisome proliferator-activated receptor γ coactivator-1 α and mitochondrial biogenesis by a pathway leading to p38 mitogen-activated protein kinase activation. *J Biol Chem.* 2007;282(26):18793–18799. <https://doi.org/10.1074/jbc.M611252200>.
 187. Ojuka EO, Jones TE, Han D, Chen M, Holloszy JO. Raising Ca²⁺ in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle. *Faseb J.* 2003; 17(6):675–681. <https://doi.org/10.1096/fj.02-0951com>.
 188. Ojuka EO, Jones TE, Han DH, et al. Intermittent increases in cytosolic Ca²⁺ stimulate mitochondrial biogenesis in muscle cells. *Am J Physiol Endocrinol Metab.* 2002;11(2):E1040–E1045. <https://doi.org/10.1152/ajpendo.00242.2002>.
 189. Hurley RL, Anderson KA, Franzoni JM, Kemp BE, Means AR, Witters LA. The Ca²⁺ +/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem.* 2005;280(32):29060–29066. <https://doi.org/10.1074/jbc.M503824200>.
 190. Hawley SA, Pan DA, Mustard KJ, et al. Calmodulin-dependent protein kinase kinase- β is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab.* 2005;2(1):9–19. <https://doi.org/10.1016/j.cmet.2005.05.009>.
 191. Attwaters M, Hughes SM. Cellular and molecular pathways controlling muscle size in response to exercise. *FEBS J.* 2022;289(6):1428–1456. <https://doi.org/10.1111/febs.15820>.
 192. Kramer HF, Goodyear LJ. Exercise, MAPK, and NF- κ B signaling in skeletal muscle. *J Appl Physiol*(1985). 2007;103(1):388–395. <https://doi.org/10.1152/japplphysiol.00085>.
 193. Long Y, Widegren U, Zierath JR. Exercise-induced mitogen-activated protein kinase signalling in skeletal muscle. *Proc Natl Acad Sci USA.* 2004;63(2):227–232. <https://doi.org/10.1079/pns2004346>.
 194. Morrison DK. MAP kinase pathways. *Cold Spring Harbor Perspect Biol.* 2012;4(11): E1040–E1045. <https://doi.org/10.1101/cshperspect.a011254>.
 195. Little JP, Safdar A, Cermak N, Tarnopolsky MA, Gibala MJ. Acute endurance exercise increases the nuclear abundance of PGC-1 in trained human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol.* 2010;298(2):912–917. <https://doi.org/10.1152/ajpregu.00409.2009>.
 196. Cochran AJR, Percival ME, Tricarico S, et al. Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations. *Exp Physiol.* 2014;99(5):782–791. <https://doi.org/10.1113/expphysiol.2013.077453>.
 197. Parker L, Trewin A, Levinger I, Shaw CS, Stepto NK. The effect of exercise-intensity on skeletal muscle stress kinase and insulin protein signaling. *PLoS One.* 2017; 12(2):e0171613. <https://doi.org/10.1371/journal.pone.0171613>.
 198. Yu M, Stepto NK, Chibalin AV, et al. Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise. *J Physiol.* 2003;546(2): 327–335. <https://doi.org/10.1113/jphysiol.2002.034223>.
 199. Bartlett JD, Joo CH, Jeong TS, et al. Matched work high-intensity interval and continuous running induce similar increases in PGC-1 α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. *J Appl Physiol*(1985). 2012;112(7): 1135–1143. <https://doi.org/10.1152/japplphysiol.01040.2011>.
 200. Brandt N, Gunnarsson TP, Hostrup M, et al. Impact of adrenaline and metabolic stress on exercise-induced intracellular signaling and PGC-1 α mRNA response in human skeletal muscle. *Phys Rep.* 2016;4(14):e12844. <https://doi.org/10.14814/phy2.12844>.
 201. Combes A, Dekerle J, Webborn N, Watt P, Bougault V, Daussin FN. Exercise-induced metabolic fluctuations influence AMPK, p38-MAPK and CaMKII phosphorylation in human skeletal muscle. *Phys Rep.* 2015;3(9):e12462. <https://doi.org/10.14814/phy2.12462>.
 202. Gibala MJ, Mcgee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1 in human skeletal muscle. *J Appl Physiol*(1985). 2009;106(1): 929–934. <https://doi.org/10.1152/japplphysiol.90880.2008>.
 203. Turcotte LP, Raney MA, Todd MK. ERK1/2 inhibition prevents contraction-induced increase in plasma membrane FAT/CD36 content and FA uptake in rodent muscle. *Acta Physiol Scand.* 2005;184(2):131–139. <https://doi.org/10.1111/j.1365-201X.2005.01445.x>.
 204. Raney MA, Turcotte LP. Evidence for the regulation of contraction-induced fatty acid oxidation via extracellular signal-regulated kinase 1/2 activation independent of changes in fatty acid uptake. *Metabolism.* 2007;56(9):1192–1200. <https://doi.org/10.1016/j.metabol.2007.04.014>.
 205. Raney MA, Turcotte LP. Regulation of contraction-induced FA uptake and oxidation by AMPK and ERK1/2 is intensity dependent in rodent muscle. *Am J Physiol Endocrinol Metab.* 2006;291(3):1220–1227. <https://doi.org/10.1152/ajpendo.00155.2006>.
 206. Miyazaki M, McCarthy JJ, Fedele MJ, Esser KA. Early activation of mTORC1 signalling in response to mechanical overload is independent of phosphoinositide 3-kinase/Akt signalling. *J Physiol.* 2011;589(7):1831–1846. <https://doi.org/10.1113/jphysiol.2011.205658>.
 207. Martin TD, Dennis MD, Gordon BS, Kimball SR, Jefferson LS. mTORC1 and JNK coordinate phosphorylation of the p70S6K1 autoinhibitory domain in skeletal muscle following functional overloading. *Am J Physiol Endocrinol Metab.* 2014; 306(5):1397–1405. <https://doi.org/10.1152/ajpendo.00064.2014>.
 208. Lessard SJ, MacDonald TL, Pathak P, et al. JNK regulates muscle remodeling via myostatin/SMAD inhibition. *Nat Commun.* 2018;9(1):3030. <https://doi.org/10.1038/s41467-018-05439-3>.
 209. Olsen LA, Nicoll JX, Fry AC. The skeletal muscle fiber: a mechanically sensitive cell. *Eur J Appl Physiol.* 2019;119(2):333–349. <https://doi.org/10.1007/s00421-018-04061-x>.
 210. Williamson D, Gallagher P, Harber M, Hollon C, Trappe S. Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. *J Physiol.* 2003;547(3):977–987. <https://doi.org/10.1113/jphysiol.2002.036673>.
 211. Burd NA, Holwerda AM, Selby KC, et al. Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *J Physiol.* 2010;588(16):3119–3130. <https://doi.org/10.1113/jphysiol.2010.192856>.
 212. Willkomm L, Gehlert S, Jacko D, Schiffer T, Bloch W. P38 MAPK activation and H3K4 trimethylation is decreased by lactate in vitro and high intensity resistance training in human skeletal muscle. *PLoS One.* 2017;12(5):e0176609. <https://doi.org/10.1371/journal.pone.0176609>.
 213. Holm L, Van Hall G, Rose AJ, et al. Contraction intensity and feeding affect collagen and myofibrillar protein synthesis rates differently in human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2010;298(2):257–269. <https://doi.org/10.1152/ajpendo.00609.2009-Exercise>.

214. Wretman C, Lionikas A, Widegren U, Lännergren J, Westerblad H, Henriksson J. Effects of concentric and eccentric contractions on phosphorylation of MAPK ϵ 1/2 and MAPK δ 38 rat skeletal muscle. *J Physiol*. 2001;535(1):155–164. <https://doi.org/10.1111/j.1469-7793.2001.00155.x>.
215. Martineau LC, Gardiner PF. Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension. *J Appl Physiol*(1985). 2001; 91(2):693–702. <https://doi.org/10.1152/jappl.1999.87.5.1668>.
216. Boppard MD, Aronson D, Gibson L, et al. Eccentric exercise markedly increases C-Jun NH 2-terminal kinase activity in human skeletal muscle. *J Appl Physiol*(1985). 1999;87(5):1668–1673. <https://doi.org/10.1152/jappl.1999.87.5.1668>.
217. Franchi MV, Atherton PJ, Reeves ND, et al. Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta Physiol*. 2014;210(3):642–654. <https://doi.org/10.1111/apha.12225>.
218. Gehlert S, Suhr F, Gutsche K, et al. High force development augments skeletal muscle signalling in resistance exercise modes equalized for time under tension. *Pflügers Archiv*. 2015;467(6):1343–1356. <https://doi.org/10.1007/s00424-014-1579-y>.
219. R Karlsson HK, Nilsson PA, Nilsson J, et al. Branched-chain amino acids increase p70 S6k phosphorylation in human skeletal muscle after resistance exercise. *Am J Physiol Endocrinol Metab*. 2004;13(6):487–499. <https://doi.org/10.1152/ajpendo.00430.2003>.
220. Blazev R, Carl CS, Ng YK, et al. Phosphoproteomics of three exercise modalities identifies canonical signaling and C18ORF25 as an AMPK substrate regulating skeletal muscle function. *Cell Metab*. 2022;34(10):1561–1577.e9. <https://doi.org/10.1016/j.cmet.2022.07.003>.
221. Davids CJ, Naess TC, Moen M, et al. Acute cellular and molecular responses and chronic adaptations to low-load blood flow restriction and high-load resistance exercise in trained individuals. *J Appl Physiol*(1985). 2021;131(6):1731–1749. <https://doi.org/10.1152/japplphysiol.00464.2021>.
222. Creer A, Gallagher P, Slivka D, et al. Influence of muscle glycogen availability on ERK1/2 and Akt signaling after resistance exercise in human skeletal muscle. *J Appl Physiol*(1985). 2005;99(3):950–956. <https://doi.org/10.1152/japplphysiol.00110.2005>.
223. Moore DR, Atherton PJ, Rennie MJ, Tarnopolsky MA, Phillips SM. Resistance exercise enhances mTOR and MAPK signalling in human muscle over that seen at rest after bolus protein ingestion. *Acta Physiol*. 2011;201(3):365–372. <https://doi.org/10.1111/j.1748-1716.2010.02187.x>.
224. Salvador AF, Askow AT, McKenna CF, et al. Resistance exercise-induced regulation of muscle protein synthesis to intratester rest. *Med Sci Sports Exerc*. 2020;52(5): 1022–1030. <https://doi.org/10.1249/MSS.0000000000002213>.
225. Hulmi JJ, Walker S, Ahtiainen JP, Nyman K, Kraemer WJ, Häkkinen K. Molecular signaling in muscle is affected by the specificity of resistance exercise protocol. *Scand J Med Sci Sports*. 2012;22(2):240–248. <https://doi.org/10.1111/j.1600-0838.2010.01198.x>.
226. Wilkinson DJ, Franchi MV, Brook MS, et al. A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *Am J Physiol Endocrinol Metab*. 2014; 306(5):E571–E579. <https://doi.org/10.1152/ajpendo.00650.2013>.
227. Brook MS, Wilkinson DJ, Mitchell WK, et al. Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. *Faseb J*. 2015;29(11): 4485–4496. <https://doi.org/10.1096/fj.15-273755>.
228. Vandr  X, Figueiredo C. Revisiting the roles of protein synthesis during skeletal muscle hypertrophy induced by exercise. *Am J Physiol Regul Integr Comp Physiol*. 2019;317(3):709–718. <https://doi.org/10.1152/ajpregu>.
229. Morton RW, McGlory C, Phillips SM. Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Front Physiol*. 2015;6(9): 1–9. <https://doi.org/10.3389/fphys.2015.00245>.
230. Moro T, Brightwell CR, Deer RR, et al. Muscle protein anabolic resistance to essential amino acids does not occur in healthy older adults before or after resistance exercise training. *J Nutr*. 2018;148(6):900–909. <https://doi.org/10.1093/jn/nxy064>.
231. Balagopal P, Schimke JC, Ades P, et al. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. *Am J Physiol Endocrinol Metab*. 2001;280(2):203–208. <https://doi.org/10.1152/ajpendo.2001.280.2.E203>.
232. Reidy PT, Borack MS, Markofski MM, et al. Post-absorptive muscle protein turnover affects resistance training hypertrophy. *Eur J Appl Physiol*. 2017;117(5):853–866. <https://doi.org/10.1007/s00421-017-3566-4>.
233. Kim PL, Staron RS, Phillips SM. Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *J Physiol*. 2005;568(1):283–290. <https://doi.org/10.1113/jphysiol.2005.093708>.
234. Miller B.F., Reid J.J., Price J.C., Lin H.J.L., Atherton P.J., Smith K. CORP: the use of deuterated water for the measurement of protein synthesis. *J Appl Physiol*(1985). 2020;128(5):1163–1176. doi:<https://doi.org/10.1152/japplphysiol.00855.2019>.
235. Burniston JG, Chen YW. *Omics Approaches to Understanding Muscle Biology*. Springer Press; 2019. <https://doi.org/10.1007/978-1-4939-9802-9>.
236. Camera DM, Burniston JG, Pogson MA, Smiles WJ, Hawley JA. Dynamic proteome profiling of individual proteins in human skeletal muscle after a high-fat diet and resistance exercise. *Faseb J*. 2017;12. <https://doi.org/10.1096/fj.201700531R>.
237. Murphy CH, Shankaran M, Churchward-Venne TA, et al. Effect of resistance training and protein intake pattern on myofibrillar protein synthesis and proteome kinetics in older men in energy restriction. *J Physiol*. 2018;596(11):2091–2120. <https://doi.org/10.1113/JP275246>.
238. Hesketh SJ, Sutherland H, Lisboa PJ, Jarvis JC, Burniston JG. Adaptation of rat fast-twitch muscle to endurance activity is underpinned by changes to protein degradation as well as protein synthesis. *Faseb J*. 2020;34(8):10398–10417. <https://doi.org/10.1096/fj.202000668RR>.
239. Holwerda AM, Bouwman FG, Nabben M, Wang P. Endurance-type exercise increases bulk and individual mitochondrial protein synthesis rates in rats. *Int J Sport Nutr Exerc Metabol*. 2020;23(2):1–12. <https://doi.org/10.1123/ijnsnem.2019-0281>.
240. Shankaran M, King CL, Angel TE, et al. Circulating protein synthesis rates reveal skeletal muscle proteome dynamics. *J Clin Invest*. 2015;126(1):1–15. <https://doi.org/10.1172/JCI79639>.
241. Stead CA, Hesketh SJ, Bennett S, et al. Fractional synthesis rates of individual proteins in rat soleus and plantaris muscles. *Proteomes*. 2020;8(2):10. <https://doi.org/10.3390/proteomes8020010>.
242. Aoyagi T, Terracina KP, Raza A, Matsubara H, Takabe K. Cancer cachexia, mechanism and treatment. *J Gastrointest Oncol*. 2015;7(4):17–29. <https://doi.org/10.4251/wjgo.v7.i4.17>.
243. Little JP, Phillips SM. Resistance exercise and nutrition to counteract muscle wasting. *Appl Physiol Nutr Metabol*. 2009;34(5):817–828. <https://doi.org/10.1139/H09-093>.
244. Al-Majid S, McCarthy DO. Cancer-induced fatigue and skeletal muscle wasting: the role of exercise. *Biol Res Nurs*. 2001;12(4):555–563. <https://doi.org/10.1177/109980040100200304>.
245. Al-Majid S, McCarthy DO. Resistance exercise training attenuates wasting of the extensor digitorum longus muscle in mice bearing the colon-26 adenocarcinoma. *Biol Res Nurs*. 2001;8(1):566–570. <https://doi.org/10.1177/109980040100200301>.
246. Baar K, Esser K. Phosphorylation of p70 S6k correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol Cell Physiol*. 1999;276(1): 120–127. <https://doi.org/10.1152/ajpcell.1999.276.1.C120>.
247. Lira FS, Neto JCR, Seelaender M. Exercise training as treatment in cancer cachexia. *Appl Physiol Nutr Metabol*. 2014;39(6):679–686. <https://doi.org/10.1139/apnm-2013-0554>.
248. Deuster P, Morrison S, Ahrens R. Endurance exercise modifies cachexia of tumor growth in rats. *Med Sci Sports Exerc*. 1985;17(3):385–392.
249. Lira FS, Tavares FL, Yamashita AS, et al. Effect of endurance training upon lipid metabolism in the liver of cachectic tumour-bearing rats. *Cell Biochem Funct*. 2008; 26(6):701–708. <https://doi.org/10.1002/cbf.1495>.
250. Morinaga M, Sako N, Isobe M, Lee-Hotta S, Sugiura H, Kametaka S. Aerobic exercise ameliorates cancer cachexia-induced muscle wasting through adiponectin signaling. *Int J Mol Sci*. 2021;22(6):3110. <https://doi.org/10.3390/ijms22063110>.
251. Gomes MD, Lecker SH, Thomas Jagoe R, Navon A, Goldberg AL, Israel B. Atrogin-1, a muscle-specific P-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci U S A*. 2001;98(25):14440–14445. <https://doi.org/10.1073/pnas.251541198>.
252. Jagoe RT, Lecker SH, Gomes M, Goldberg AL. Patterns of gene expression in atrophying skeletal muscles: response to food deprivation. *Faseb J*. 2002;16(13): 1697–1712. <https://doi.org/10.1096/fj.02-0312com>.
253. Lecker SH, Jagoe RT, Gilbert A, et al. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *Faseb J*. 2004;18(1): 39–51. <https://doi.org/10.1096/fj.03-0610com>.
254. Sacheck JM, Hyatt JK, Raffaello A, et al. Rapid disuse and denervation atrophy involve transcriptional changes similar to those of muscle wasting during systemic diseases. *Faseb J*. 2007;21(1):140–155. <https://doi.org/10.1096/fj.06-6604com>.
255. Rao MS, Van Vleet TR, Ciurlionis R, et al. Comparison of RNA-Seq and microarray gene expression platforms for the toxicogenomic evaluation of liver from short-term rat toxicity studies. *Front Genet*. 2019;9:636. <https://doi.org/10.3389/fgene.2018.00636>.
256. Wang C, Gong B, Bushel PR, et al. The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance. *Nat Biotechnol*. 2014;32(9):926–932. <https://doi.org/10.1038/nbt.3001>.
257. Chapman MA, Arif M, Emanuelsson EB, et al. Skeletal muscle transcriptomic comparison between long-term trained and untrained men and women. *Cell Rep*. 2020;31(12):107808. <https://doi.org/10.1016/j.celrep.2020.107808>.
258. Kunkel SD, Suneja M, Ebert SM, et al. mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. *Cell Metab*. 2011;13(6):627–638. <https://doi.org/10.1016/j.cmet.2011.03.020>.
259. Llano-Diez M, Fury W, Okamoto H, Bai Y, Gromada J, Larsson L. RNA-sequencing reveals altered skeletal muscle contraction, E3 ligases, autophagy, apoptosis, and chaperone expression in patients with critical illness myopathy. *Skeletal Muscle*. 2019;9(1):254–261. <https://doi.org/10.1186/s13395-019-0194-1>.
260. Mahmassani ZS, Reidy PT, McKenzie AI, Stubben C, Howard MT, Drummond MJ. Age-dependent skeletal muscle transcriptome response to bed rest-induced atrophy. *J Appl Physiol*(1985). 2019;126(4):894–902. <https://doi.org/10.1152/japplphysiol.00811.2018>.
261. Terry EE, Zhang X, Hoffmann C, et al. Transcriptional profiling reveals extraordinary diversity among skeletal muscle tissues. *Elife*. 2018;11(4):476–482. <https://doi.org/10.7554/eLife.34613.001>.
262. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. *DMM Dis Mod and Mech*. 2013;6(1):25–39. <https://doi.org/10.1242/dmm.010389>.
263. Demontis F, Piccirillo R, Goldberg AL, Perrimon N. Mechanisms of skeletal muscle aging: insights from drosophila and mammalian models. *DMM Dis Mod and Mech*. 2013;6(6):1339–1352. <https://doi.org/10.1242/dmm.012559>.
264. Piccirillo R, Demontis F, Perrimon N, Goldberg AL. Mechanisms of muscle growth and atrophy in mammals and drosophila. *Dev Dynam*. 2014;243(2):201–215. <https://doi.org/10.1002/dvdy.24036>.

265. Reid MB, Judge AR, Bodine SC. CrossTalk opposing view: the dominant mechanism causing disuse muscle atrophy is proteolysis. *J Physiol.* 2014;592(24):5345–5347. <https://doi.org/10.1113/jphysiol.2014.279406>.
266. Shum AMY, Poljak A, Bentley NL, Turner N, Tan TC, Polly P. Proteomic profiling of skeletal and cardiac muscle in cancer cachexia: alterations in sarcomeric and mitochondrial protein expression. *Oncotarget.* 2018;31:22001–22022. <https://doi.org/10.18632/oncotarget.25146>.
267. Mugahid DA, Sengul TG, You X, et al. Proteomic and transcriptomic changes in hibernating grizzly bears reveal metabolic and signaling pathways that protect against muscle atrophy. *Sci Rep.* 2019;9(1):19976. <https://doi.org/10.1038/s41598-019-56007-8>.
268. Ubaida-Mohien C, Lyashkov A, Gonzalez-Freire M, et al. Discovery proteomics in aging human skeletal muscle finds change in spliceosome, immunity, proteostasis and mitochondria. *Elife.* 2019;12(3):267–278. <https://doi.org/10.7554/eLife.49874.001>.
269. Lang F, Khaghani S, Türk C, et al. Single muscle fiber proteomics reveals distinct protein changes in slow and fast fibers during muscle atrophy. *J Proteome Res.* 2018;17(10):3333–3347. <https://doi.org/10.1021/acs.jproteome.8b00093>.
270. Ibebunjo C, Chick JM, Kendall T, et al. Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia. *Mol Cell Biol.* 2013;33(2):194–212. <https://doi.org/10.1128/mcb.01036-12>.
271. Lang F, Aravamudhan S, Nolte H, et al. Dynamic changes in the mouse skeletal muscle proteome during denervation-induced atrophy. *DMM Dis Mod and Mech.* 2017;10(7):881–896. <https://doi.org/10.1242/dmm.028910>.
272. Fuller HR, Gillingwater TH, Wishart TM. Commonality amid diversity: multi-study proteomic identification of conserved disease mechanisms in spinal muscular atrophy. *Neuromuscul Disord.* 2016;26(9):560–569. <https://doi.org/10.1016/j.nmd.2016.06.004>.
273. Hunt LC, Graca FA, Pagala V, et al. Integrated genomic and proteomic analyses identify stimulus-dependent molecular changes associated with distinct modes of skeletal muscle atrophy. *Cell Rep.* 2021;37(6):109971. <https://doi.org/10.1016/j.celrep.2021.109971>.
274. Ebhardt HA, Degen S, Tadini V, et al. Comprehensive proteome analysis of human skeletal muscle in cachexia and sarcopenia: a pilot study. *J Cachexia Sarcopenia Muscle.* 2017;8(4):567–582. <https://doi.org/10.1002/jcsm.12188>.
275. Murgia M, Brocca L, Monti E, et al. Plasma proteome profiling of healthy subjects undergoing bed rest reveals unloading-dependent changes linked to muscle atrophy. *J Cachexia Sarcopenia Muscle.* 2023;14(1):439–451. <https://doi.org/10.1002/jcsm.13146>.
276. Johnston AJ, Murphy KT, Jenkinson L, et al. Targeting of Fn14 prevents cancer-induced cachexia and prolongs survival. *Cell.* 2015;162(6):1365–1378. <https://doi.org/10.1016/j.cell.2015.08.031>.
277. Zhou X, Wang JL, Lu J, et al. Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. *Cell.* 2010;142(4):531–543. <https://doi.org/10.1016/j.cell.2010.07.011>.
278. Tisdale MJ. Reversing cachexia. *Cell.* 2010;142(4):511–512. <https://doi.org/10.1016/j.cell.2010.08.004>.
279. Shavlakadze T, Grounds M. Of bears, frogs, meat, mice and men: complexity of factors affecting skeletal muscle mass and fat. *Bioessays.* 2006;28(10):994–1009. <https://doi.org/10.1002/bies.20479>.