REVIEW

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Phenotypic features of cancer cachexia-related loss of skeletal muscle mass and function: lessons from human and animal studies

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Abstract

Cancer cachexia is a complex multi-organ catabolic syndrome that reduces mobility, increases fatigue, decreases the efficiency of therapeutic strategies, diminishes the quality of life, and increases the mortality of cancer patients. This review provides an exhaustive and comprehensive analysis of cancer cachexia-related phenotypic changes in skeletal muscle at both the cellular and subcellular levels in human cancer patients, as well as in animal models of cancer cachexia. Cancer cachexia is characterized by a major decrease in skeletal muscle mass in human and animals that depends on the severity of the disease/model and the localization of the tumour. It affects both type 1 and type 2 muscle fibres, even if some animal studies suggest that type 2 muscle fibres would be more prone to atrophy. Animal studies indicate an impairment in mitochondrial oxidative metabolism resulting from a decrease in mitochondrial content, an alteration in mitochondria morphology, and a reduction in mitochondrial metabolic fluxes. Immuno-histological analyses in human and animal models also suggest that a faulty mechanism of skeletal muscle repair would contribute to muscle mass loss. An increase in collagen deposit, an accumulation of fat depot outside and inside the muscle fibre, and a disrupted contractile machinery structure are also phenotypic features that have been consistently reported in cachectic skeletal muscle. Muscle function is also profoundly altered during cancer cachexia with a strong reduction in skeletal muscle force. Even though the loss of skeletal muscle mass largely contributes to the loss of muscle function, other factors such as muscle–nerve interaction and calcium handling are probably involved in the decrease in muscle force. Longitudinal analyses of skeletal muscle mass by imaging technics and skeletal muscle force in cancer patients, but also in animal models of cancer cachexia, are necessary to determine the respective kinetics and functional involvements of these factors. Our analysis also emphasizes that measuring skeletal muscle force through standardized tests could provide a simple and robust mean to early diagnose cachexia in cancer patients. That would be of great benefit to cancer patient's quality of life and health care systems.

Keywords Cancer cachexia; Fibre type; Fibrosis; Force; Metabolism; Skeletal muscle; Regeneration

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Introduction

Cancer cachexia is a complex catabolic syndrome characterized by an involuntary body mass loss essentially due to a severe depletion of skeletal muscle, with or without adipose tissue loss, while the non-muscle protein compartment is relatively preserved.[1] Cancer cachexia cannot be reversed by increasing conventional nutritional intake, thus highlighting the fact that the hypercatabolic state of cachectic patients is a critical determinant of the syndrome. The prevalence of

cachexia in cancer patients is quite variable, affecting 15–30% of prostate or breast cancer patients to 75% of pancreatic cancer patients,[2] with an increasing prevalence with the advanced stages of the disease.[2] It is thus estimated that the prevalence of cachexia can reach 90% in patients suffering from advanced colorectal cancer 100 days before death.[3] Furthermore, cancer cachexia can be also under-recognized by some confounding factors, such as obesity[4] or weight gain in the form of ascites or peripheral oedema.[5] Overall, it has been recently estimated that 15.8 subjects per 10 000 of the total general population in European Union in 2013 suffered from cancer cachexia.[2]

Cancer cachexia is one of the most debilitating and life-threatening aspects of cancer, profoundly affecting the patient's quality of life.[6–12] Cachexia increases surgical risks[13] and the susceptibility to the adverse effects of chemotherapy.[11,14,15] It also induces a progressive reduction in the body's functional capacities[16] leading to an increase in sedentarization[9,17] and a loss of autonomy, ultimately requiring institutional care of patients. Since the pioneering works of Dewys *et al*.[18] it has been consistently reported that the extent of cachexia is inversely correlated with the survival of cancer patients.[8–10,19–25] Even if probably underestimated, it is generally assumed, according to Warren,[26] that cachexia will be responsible for the death of approximately 20% of cancer patients with death typically occurring when weight loss reaches 30%, thus making it the leading cause of death in cancer.

During cancer cachexia, skeletal muscle represents the main site of protein loss.[27,28] The loss of muscle mass affects 5–89% of cancer patients depending on the cancer site and the method of measurement used.[29] Muscle mass loss is greater in weight-losing cancer patients when compared with weight-losing anorexia nervosa patients,[30] indicating that cancer-specific factors independently of undernutrition contribute to decrease skeletal muscle mass. As described for body mass, skeletal muscle mass loss in cancer patients is an independent factor that increases the risk of surgical complications,[31,32] decreases surgical efficiency,[24] and increases chemotherapy toxicity.[33–36] Skeletal muscle mass loss has therefore been consistently associated with a decreased survival rate of cancer patients.[20,24,31–33,35,37–40]

Cancer cachexia is a constantly developing area of great research interest.[41] Numerous clinical and experimental studies have thus been devoted to deciphering the molecular pathways involved in cancer cachexia, as well as developing strategies aimed at stopping or even reversing the loss of body mass and skeletal muscle mass loss (reviewed in literatures[5,42–49]). However, what is less known are the effects of cancer cachexia on skeletal muscle structure and function. To fully understand the aetiology of cancer cachexia, it is essential to identify its effects on skeletal muscle phenotype. In this review, we will consider the effects of cancer cachexia on skeletal muscle contractile and metabolic phenotypes both in cancer patients and in animal models. Because muscle structure cannot be dissociated from muscle function, we will also consider how and to what extent cancer cachexia affects skeletal muscle function.

Skeletal muscle atrophy during cancer cachexia

Skeletal muscle atrophy in human cancer patients

When studying cancer cachexia in human patients, it is essential to determine the extent of skeletal muscle atrophy, because body mass loss in cancer patients does not strictly reflect skeletal muscle mass loss.[50] This is particularly true in obese cachectic patients where high body mass index may mask the extent of skeletal muscle depletion.[20] Different techniques have been used to quantify the atrophy of skeletal muscle mass during the time course of cancer cachexia (for review, see previous studies[51,52]). Indirect assessments of skeletal muscle mass by the analysis of whole-body composition using neutron activation,[27] dual-energy X-ray absorptiometry,[53–56] or bioelectric impedance[10,25,57–60] indicate that lean body mass is reduced in cachectic cancer patients compared with healthy controls or non-cachectic cancer patients. When specifically looking at skeletal muscle by imaging techniques, studies also clearly evidence skeletal muscle atrophy. The *quadriceps* muscle area measured by magnetic resonance imaging is decreased by 10–33% in cachectic cancer patients when compared with healthy control subjects.[57,61,62] When compared with non-cachectic cancer patients, the *quadriceps* muscle area of cachectic cancer patients is either stable (female) or decreased (male) but to a lesser extent (14%),[61] indicating that even if cachexia is not clinically diagnosed (based on body mass analysis), skeletal muscle catabolism has already started. Lately, the use of computerized tomography scans has spread widely as these images may be available in the medical records of patients. Computerized tomography scan analysis at the third lumbar vertebrae level allows the quantification of the areas of *rectus abdominis*, *transversus abdominis*, *erector spinae*, *quadratus lumborum*, *psoas* minor and major, and internal and external abdominal oblique muscles,[63] which gives a good estimate of the whole-body muscle compartment.[64] By using this technique, the atrophy of skeletal muscle is also clearly evident and increases with body mass loss in cachectic cancer patients.[50] When skeletal muscle area is normalized to the height of patients (skeletal muscle index), similar results are obtained with a 4–13% decrease in skeletal muscle index in cachectic cancer patients compared with non-cachectic cancer patients.[10,12,59,65] Once again, skeletal muscle mass loss increases with the severity of the disease.

Skeletal muscle atrophy in animal models of cancer cachexia

Studies using different animal models of cancer cachexia also consistently report, when compared with control animals, a decrease in lean body mass,[66–69] as well as in the mass of skeletal muscles with different metabolic and contractile properties such as of *gastrocnemius*,[70–95] *soleus*,[69,73,77,78,80–82,86,88,90,93,96–98] *extensor digitorum longus*,[69,79,80,85,88,89,93,97–101] *tibialis anterior*,[69,70,73,76,79–81,84,85,88–95,97–99,101,102] *plantaris*,[80,82,85,86,99] *quadriceps*,[66,68,70,76,79,80,85,91,92,94,99] *triceps*,[66] *epitrochlearis*,[89] and diaphragm[86,96] muscles. When compared with non-cachectic cancer animals, the muscle mass of cachectic cancer animals is also lower.[71,77,79,102–106] Similarly to human cancer patients, the extent of skeletal muscle mass loss increases with the severity of the experimental model.[71,104,105,107] When normalized to body mass, skeletal muscle mass still remains lower in cachectic cancer animals,[72,79,80,85,103,108–118] indicating that skeletal muscle mass loss exceeds that of all other tissues and organs. Interestingly, many studies reported no difference in *soleus* muscle mass between cachectic cancer animals and healthy animals.[79,85,89,99,104,106,112,113] The postural/anti-gravitational function of the *soleus* muscle (enriched in type 1 muscle fibres[119]), which involves a tonic motor nerve activity, may explain its resistance to cancer cachexia, also suggesting that skeletal muscle phenotype, function, and pattern of neuronal innervation are critical in determining skeletal muscle sensitivity to cancer cachexia.

Skeletal muscle atrophy and metastasis

A higher incidence of metastasis (the dissemination of tumour cells from the primary site in distant organs) has been reported in pancreatic cachectic cancer patients compared with non-cachectic patients.[120] Similarly, lung cancer patients with metastases developed cachexia more often than patients without metastases.[121] It is thus generally considered that cachexia is a hallmark of metastatic cancer and the main feature of terminal metastatic cancer. However, if the presence of metastasis is associated with a higher incidence of cancer cachexia, it is not clear whether the extent of skeletal muscle depletion is increased in metastatic cancer patients. Animal studies provide important complementary information. Although largely underestimated (the vast majority of cancer cachexia studies in animals are not conducted in a metastatic context[122]), some recent studies covering this topic showed that skeletal muscle mass loss is exacerbated in metastatic compared with non-metastatic C26[123] or HCT116[124] tumor-bearing mice. Therefore, metastasis would aggravate the cachectic phenotype in animal models of cancer cachexia. This also illustrates the need to use *in vivo* metastatic models for the study of cancer cachexia to more closely mimic the diversity and complexity of cancer cachexia observed in human patients.

Skeletal muscle atrophy and chemotherapy

The depletion of the skeletal muscle compartment may also have important implications for anticancer drug toxicity. It has thus been shown that patients with depletion of skeletal muscle mass presented higher chemotherapy-related toxicity compared with patients with larger amounts of lean body mass.[11,14,15,33,36,125,126] Those patients are forced to reduce dosage or to delay the cycles of administration and ultimately have worsened outcomes.[125] Chemotherapy also triggers very debilitating side effects, including unintentional body weight loss that primarily affects skeletal muscle. Therefore, understanding the complex interplay between chemotherapy, skeletal muscle toxicities, and cachexia is an important issue. Some recent studies addressed this question by determining the effects of chemotherapy itself on skeletal muscle or by studying the combined effects of tumour growth and chemotherapy on skeletal muscle. Administration of FOLFOX (5-fluorouracil, leucovorin, oxaliplatin) or FOLFIRI (5-fluorouracil, leucovorin, CPT-11) in originally healthy mice triggers a loss of muscle mass and an increase in muscle weakness.[127–129] Similar observations have been reported using carboplatin,[130,131] cisplatin,[132–134] doxorubicin,[135–137] and 5-fluorouracil.[138] Injection of gemcitabine and cisplatin in mice induced an even greater decrease in skeletal muscle mass than an orthotopic injection of tumour cells alone.[139] One should note however that if some studies used similar dosages to that encountered in human patients,[127–129,132–134] others used higher dosages.[130,131,135,136,138] Furthermore, the drug is generally more frequently administered in animal studies compared with human patients leading to cumulative higher dosages. Overall, this may contribute to exacerbate the extent of skeletal muscle mass loss. In tumour-bearing mice, the combination of cancer cachexia and FOLFIRI administration resulted in a more severe depletion of quadriceps muscle mass compared with cancer cachexia or FOLFIRI alone.[94] Similar observations were also reported in tumour-bearing mice with the use of cisplatin[140] and doxorubicin.[141] Therefore, and while chemotherapy is obviously dispensable to combat tumour growth, these data from animal studies strongly suggest that chemotherapy potentially also plays a causative role in the occurrence of skeletal muscle loss and weakness during cancer cachexia. Developing strategies that preserve skeletal muscle mass would therefore counteract both chemotherapy-related toxicity and deleterious effects of chemotherapy on skeletal muscle.

Skeletal muscle fibre typology, size, and number during cancer cachexia

Skeletal muscle fibre type

Skeletal muscle fibres are heterogeneous with respect to their expression of myosin heavy chain isoforms conferring them distinct contractile properties.[142] Based on myosin heavy chain isoform expression, it is possible to distinguish type 1, 2A, 2X, and 2B (absent in human) fibres. The relative proportion of each fibre type inside a muscle differs according to species, muscle function, and innervation but also reflects dynamic adaptations to whole-body energy metabolism, neuromuscular activity, and muscle injuries.[142]

If one study reported a shift towards an increase in the proportion of fast myosin heavy chain isoform in cachectic cancer patients,[143] all other studies indicated that the distribution between type 1 and type 2 skeletal muscle fibres remained unchanged in cachectic cancer patients when compared with either non-cachectic cancer patients[54,144,145] or healthy control subjects[62,146] (*Table* 1). Similarly, in animal models of cancer cachexia, a large majority of studies did not find any difference in fibre type distribution in the *gastrocnemius*,[77,82,111,118,147–149] *plantaris*,[82] *tibialis anterior*,[80,99,111,150] diaphragm,[96,99,118,147–149] *extensor digitorum longus*,[111] *quadriceps*,[151] and *soleus*[111] muscles (*Table* 2). However, some animal studies reported an increase in the proportion of type 2A and 2B muscle fibres together with a concomitant decrease in the proportion of type 1 fibres in the slow-twitch oxidative *soleus* muscle, in cachectic cancer mice compared with control mice.[78,82,100] A transition from type 2A towards type 2B has also been reported in the *soleus*[100,152] and *tibialis anterior*[153] muscles of cachectic cancer mice. Differences between studies could be explained by the physiological function of the muscle (postural/antigravitational such as *soleus* vs. locomotor such as *gastrocnemius*) and the extent of cachexia. From a teleological point of view, a shift from slow to fast fibre type may lead to higher and faster force production (at the expense of greater fatigability), which may tentatively compensate for the whole decrease in muscle force due to muscle mass loss (see succeeding text). If the existence of a slow-to-fast fibre type shift needs to be further strengthened, such a shift could reflect an altered neuromuscular control, as the innervation pattern and neuromuscular junction integrity are essential for conferring contractile and metabolic characteristics to the muscle fibre.[154] This hypothesis is supported by observations showing that expression of denervation markers was increased in skeletal muscle of C26 tumour-bearing mice, which is consistent with the notion that muscle fibre denervation may contribute to muscle wasting during cachexia.[155] Although less convincing, fairly similar observations were also reported in skeletal muscle of cancer cachectic patients compared with non-weight-losing cancer patients and control subjects.[155] However, a recent study showed that neuromuscular junction morphology and structural integrity are conserved between control subjects, weight-stable cancer patients, and cachectic cancer patients.[156] Although a molecular characterization of denervation markers was not performed in this study, the absence of neuromuscular junction pathology is in contrast to what is found in the aforementioned study in cachectic C26 tumour-bearing mice.[155] Electromyographic studies would be helpful to provide complementary information about the functionality of motoneurons. Therefore, whether cancer cachexia is associated with alteration in the innervation pattern and neuromuscular junction fragmentation of the postsynaptic membrane remains to be further explored.

Skeletal muscle fibre size

Skeletal muscle fibre cross-sectional area is decreased in cachectic cancer patients, when compared with healthy subjects,[55,57] non-cachectic cancer patients,[55,157] or atherosclerotic patients[158] (*Table* 1). A reduction in fibre cross-sectional area has also been consistently reported in cachectic cancer mice compared with control mice in the *quadriceps*,[76,141] *gastrocnemius* (−28% to −62%),[69,76,92,132,159–164] *tibialis anterior* (−22% to −40%),[79,90,94,95,97,98,101,114,117,150,152,153,165–175] *soleus* (−24% to −40%),[78,152,173,176] and *extensor digitorum longus* (−28%)[112,177] muscles (*Table* 3). Similar results have also been reported in cachectic cancer rats.[87,112]

The size of muscle fibres expressing type 1 myosin heavy chain (the slow contractile isoform) is decreased by about 26% in skeletal muscle of cachectic compared with non-cachectic cancer patients,[144] as well as between cancer patients and healthy control subjects.[54,178] Similarly, the cross-sectional area of type 1 fibres is also decreased by about 30% in the *gastrocnemius*,[118,147–149] diaphragm,[96,118,147–149] and *soleus*[152] muscles of cachectic cancer mice or rats compared with control animals. However, some studies did not report any change in type 1 fibre size in cachectic cancer patients when compared with either non-cachectic cancer patients[54] or healthy subjects.[62,146] Similar results have also been reported in cachectic cancer animals vs. control animals.[85,91,99,100,106,111,179,180] These discrepancies between studies regarding the extent of the decrease in type 1 fibre cross-sectional area could be explained by the tumour localization, the severity of cachexia, the typology and function of the analysed skeletal muscles, and also the criterion used to define cachexia. For instance, Johns *et al*.[144] showed that type 1 fibre cross-sectional area was significantly lower in cancer patients with cachexia compared with non-cachectic cancer patients only when considering low muscularity plus body

Table 1 Effect of cancer cachexia on muscle fibre cross-sectional area and muscle fibre type distribution in human cancer patients

Ref.	Skeletal muscle	Cancer type	Definition of cachexia	Control subjects	Fibre cross-sectional area			Fibre type distribution		
					1	2A	2X	1	2A	2X
Johns *et al.*[144]	*Rectus abdominis*	Gastrointestinal or pancreatic (n = 17)	Stature adjusted SMI consistent with low muscularity and weight loss >2% over past last 6 months	Gastrointestinal or pancreatic cancer age-paired patients without cachexia (n = 24)	−26%		−26%	=		=
Weber *et al.*[62]	*Vastus lateralis*	Gastric, pancreatic, colon, bronchogenic carcinoma, chronic lymphatic leukaemia (n = 11)	Weight loss >10% over past last 6 months	Age-paired, gender-paired, and body weight-paired healthy controls (n = 15)	=	=	−44%	=	=	=
Weber *et al.*[57]	*Vastus lateralis*	Gastrointestinal (n = 19)	Weight loss >10% over past last 6 months	Age-paired, gender-paired, and body height-paired healthy controls (n = 19)		−32%			Not reported	
Schmitt *et al.*[145]	*Rectus abdominis*	Pancreatic carcinoma (n = 8)	Weight loss >10% over past last 6 months before surgery	Pancreatic carcinoma or chronic pancreatitis age-paired patients without cachexia (n = 8)		Not reported		=		=
Zhang *et al.*[157]	*Rectus abdominis*	Gastric (n = 13)	Weight loss >5% over past last 6 months before surgery + SMI < cut-off values	Gastric cancer patients with weight loss <5% over past last 6 months before surgery + SMI > cut-off values (n = 10)		−72%			Not reported	
Op den Kamp *et al.*[54]	*Quadriceps*	Non-small cell lung (n = 16)	International consensus[1]	Non-small cell lung cancer patients without cachexia (n = 10)	−20%		=	=		=
				Healthy age-paired and gender-paired controls (n = 22)	−17%		−35%	=		=
Zampieri *et al.*[220]	*Rectus abdominis*	Colorectal (n = 10)	Cachectic patients even if the clinical criteria used for cachexia are not presented	Patients without neoplasia (n = 7)		=			Not reported	
Puig-Vilanova *et al.*[146]	*Vastus lateralis*	Lung cancer (n = 10)	International consensus	Healthy sedentary controls (n = 10)	=		−24%	=		=

Muscle fibre types were classified as type 1, type 2A, and type 2X according to the expression of myosin heavy chain isoforms. Ref., reference; SMI, skeletal muscle index.

Table 2 Cancer cachexia model

Cancer cachexia model		Muscle	Fiber type distribution			Reference
			1	2A	2B	
Tumor cell injection	Colon 26 cells	Diaphragm				96
		Extensor digitorum longus				100
		Gastrocnemius				82
		Plantaris				82
		Soleus				100
						82
		Tibialis anterior				153
						150
	Lewis lung carcinoma cells	Diaphragm				99
		Soleus				152
		Tibialis anterior				99
	Colorectal cancer cells	Tibialis anterior				80
	LP07 mouse pulmonary cancer cells	Diaphragm				147
						148
		Gastrocnemius				147
						148
	AH130 rat hepatocellular carcinoma cells	Diaphragm				118
		Extensor digitorum longus				111
		Gastrocnemius				118
						111
		Soleus				111
		Tibialis anterior				111
Chemically induced	$Apc^{Min/+}$	Gastrocnemius				77
						77*
		Soleus				78
	Urethane-induced cancer	Diaphragm				149
		Gastrocnemius				149

Increased
Unchanged
Decreased
Not investigated

For most of studies, comparisons were done with healthy animals. *Apc*, *adenomatous polyposis coli*.
[a]Indicates when comparisons were done with non-cachectic cancer animals.

mass loss as a criterion for cachexia, while no difference was observed when using either body mass loss only or low muscularity as a criterion of cachexia.

The cross-sectional area of type 2 muscle fibres (expressing either type 2A or type 2X myosin heavy chain isoforms) is smaller in the *quadriceps* muscle of cachectic cancer patients.[54] Similar observations have been also reported in the diaphragm[147–149] and *gastrocnemius*[147–149,179] muscles of cachectic cancer mice, as well as in the *extensor digitorum longus*,[112] diaphragm,[118] and *gastrocnemius*[111,118] muscles of cachectic cancer rats. If some studies show that muscle fibre atrophy is not fibre type dependent in both patients[54,144,178] and animals,[96,118,147–149,152,174,175] a preferential atrophy of type 2 muscle fibres has been reported in a biceps muscle biopsy from a cachectic cancer patient[181] and more recently in the *quadriceps* muscle of cachectic cancer patients that were compared with healthy patients.[146] Similarly, animal studies also indicate that type 2 muscle fibres are more prone to atrophy than type 1 muscle fibres.[85,100,111,179,180] Therefore, despite controversies, preferential atrophy of type 2 muscle fibres could occur during cancer cachexia, suggesting that the contractile and metabolic phenotypes of skeletal muscle fibre types may affect their sensibility to cancer cachexia. When specifically looking at the effect of cancer cachexia on type 2A and type 2X muscle fibres, human studies indicate that the decrease in the cross-sectional area of type 2 muscle fibres is similar in type 2A[144,178] and type 2X[62] muscle fibres of cachectic cancer patients compared with healthy controls. Animal studies also show a similar reduction in the cross-sectional area of type 2A muscle fibres[80,84,96,100,104,106,152] and type 2B[80,84,91,96,100,104,182] in cachectic cancer mice. Similar results have also been reported in skeletal muscle of cachectic cancer rats.[85] However, it should be noted though that some animal studies reported a greater decrease in fibre cross-sectional area of type 2B compared with type 2A muscle fibres,[91,104,106,150,153,182,183] suggesting greater sensitivity of type 2B muscle fibres to cachexia.

Skeletal muscle fibre number

Skeletal muscle mass also depends on the number of muscle fibres. However, cancer cachexia does not seem to involve a decrease in muscle fibre number. The number of muscle fibres is similar in the *vastus lateralis* of cachectic cancer patients and healthy control subjects,[57] as well as the number of type 1 and type 2A muscle fibres in the *rectus abdominis* muscle of cachectic and non-cachectic cancer patients.[144] However, the count of muscle fibres was expressed per square millimetre,[57,144] which obviously did

Table 3 Muscle fibre cross-sectional area in mice and rat models of cancer cachexia

Cancer cachexia model		Muscle	Fiber size 1	Fiber size 2A	Fiber size 2B	Reference
Tumor cell injection	Colon 26 cells	Diaphragm	■	■	■	96
		Extensor digitorum longus	■	■	■	177
			▒	■	■	100
		Gastrocnemius	■	■	■	132
			■	■	■	159
			■	■	■	76
			■	■	■	92
			■	■	■	162
			□	■	■	179
		Quadriceps	■	■	■	141
			■	■	■	76
		Soleus	□	■	■	100
			□	■	■	180
		Tibialis anterior	■	■	■	167
			■	■	■	95
			■	■	■	172
			■	■	■	103
			■	■	■	103*
			■	■	■	171
			■	■	■	168
			■	■	■	79
			■	■	■	79*
			■	■	■	73
			■	■	■	170
			■	■	■	90
			■	■	■	94
			■	■	■	169
			■	■	■	98
			▒	□	■	150
			▒	□	■	153
	Lewis lung carcinoma cells	Diaphragm	□	□	□	99
		Gastrocnemius	■	■	■	164
		Soleus	■	■	■	173
			□	□	□	165
			■	■	▒	152
		Tibialis anterior	■	■	■	152
			■	■	■	173
			■	■	■	165
			■	■	■	101
			▒	□	■	183
			▒	□	□	99
	K7M2 mouse osteosarcoma cells	*Gastrocnemius*	■	■	■	160
	4T1 mouse breast cancer cells	*Tibialis anterior*	■	■	■	166
	C26m2 mouse colon carcinoma cells	*Tibialis anterior*	■	■	■	166
	Pan02 mouse PDAC cells	*Gastrocnemius*	■	■	■	161
	FC1242 mouse PDAC cells	*Gastrocnemius*	■	■	■	161
	Walker-256 rat breast cancer cells	*Soleus*	■	■	■	176
	Colorectal cancer cells	*Tibialis anterior*	▒	■	■	80
	LP07 mouse pulmonary cancer cells	Diaphragm	■	■	■	147
			■	■	■	148
		Gastrocnemius	■	■	■	147
			■	■	■	148
	PROb rat colon carcinoma cells	*Quadriceps*	□	■	■	85
	AH130 rat hepatocellular carcinoma cells	Diaphragm	■	■	■	118
		Extensor digitorum longus	□	□	□	111
		Gastrocnemius	■	■	■	118
			□	■	■	111
		Soleus	□	□	□	111
		Tibialis anterior	□	□	□	111
Genetically engineered mice	Inhibin-α KO	*Gastrocnemius*	■	■	■	163
			■	■	■	69
		Tibialis anterior	■	■	■	153
	Apc$^{Min/+}$	*Gastrocnemius*	▒	□	■	182
			▒	■	■	104
			▒	■	■	104*
		Soleus	■	■	■	78
		Tibialis anterior	■	■	■	101
			▒	■	■	84
	KL	*Extensor digitorum longus*	□	□	▒	106*
		Soleus	□	□	■	106*
	KPP	*Gastrocnemius*	□	□	■	91
Chemically induced	$C_8H_{12}N_2O_2$ induced carcinogenesis	*Gastrocnemius*	■	■	■	87
	Urethane-induced carcinogenesis	Diaphragm	■	■	■	149
		Gastrocnemius	■	■	■	149

□ Unchanged
■ Decreased
▒ Not investigated

For most of studies, comparisons were done with healthy animals. *Apc*, *adenomatous polyposis coli*; KL, *Kras*$^{G12D/+}$ *Lkb1*$^{f/f}$; KPP, *Kras*$^{G12D/+}$ *Ptf1a*$^{+/ER\text{-}Cre}$ *Pten*$^{f/f}$; PDAC, pancreatic ductal adenocarcinoma cancer.
[a]Indicates when comparisons were done with non-cachectic cancer animals.

not allow the determination of the absolute number of fibres in the muscle. In a mouse model of cancer cachexia, it has also been reported that the whole number of *tibialis anterior* muscle fibres was unchanged between control and cachectic cancer mice.[99] Therefore, the decrease in muscle mass during cancer cachexia would be mainly due to a decrease in muscle fibre volume rather than a decrease in muscle fibre number. If the number of muscle fibres seems to be unchanged during cancer cachexia, nuclear death by apoptosis may occur locally along the muscle fibre. Although not systematically observed,[184] the presence of extrafibre and intrafibre apoptotic nuclei has been reported in the muscle of cachectic cancer patients,[185,186] as well as in muscles of cachectic cancer mice and rats.[99,104,115,116,118,147–149] However, apoptosis does not seem to be high enough to decrease the whole number of muscle fibres, so that these apoptotic events may rather weaken the muscle fibre locally, rendering it more sensitive to micro-injuries (see succeeding text).

Skeletal muscle metabolic phenotype during cancer cachexia

Skeletal muscle metabolism in human cancer patients

The diversity between muscle fibres is not restricted to the expression of myofibrillar proteins but also extends to the energetic metabolic characteristics of the fibre. Only a limited number of studies have explored the metabolic phenotype of skeletal muscle in cancer patients. The balance between oxidative and glycolytic metabolisms seems to be maintained in skeletal muscle of cachectic cancer patients as indicated by a similar ratio of oxidative-to-glycolytic enzyme activities in the *quadriceps* muscle of cachectic cancer patients compared with that of patients without cachexia and healthy subjects.[54] Regarding mitochondrial content, studies provide contrasted results. Electron microscopy analyses showed unchanged mitochondrial density and diameter in skeletal muscle of cachectic cancer patients compared with healthy controls,[146] or increased intermyofibrillar mitochondrial area in skeletal muscle of cachectic cancer patients compared with non-cachectic patients.[186] This latter result may be explained by mitochondrial swelling and/or by a relative increase in mitochondrial density due to a faster and greater reduction in myofibrillar protein loss. In contrast, a pioneering case study[181] reported a decrease in the number of skeletal muscle mitochondria in a cachectic cancer patient. Finally, a biomolecular analysis indicated that the mitochondrial DNA copy number is unchanged in skeletal muscle of cachectic and non-cachectic cancer patients.[186] Regarding mitochondrial morphology, the presence of mitochondria with swollen appearance and the absence of cristae[157] have been reported, suggesting an impairment in mitochondrial function. Clearly, the number of studies aimed at exploring energetic metabolism and mitochondrial structure and function in skeletal muscle of human cachectic cancer patients is too limited to draw an accurate picture of the metabolic phenotypic alterations that occurs during cancer cachexia. An in-depth analysis of metabolism in skeletal muscle of cachectic cancer patients is therefore necessary.

Skeletal muscle metabolism in animal models of cancer cachexia

Animal studies provide important complementary information and allow us to draw a more precise picture of the regulation of energetic metabolism in skeletal muscle during cancer cachexia (*Figure* 1). The mitochondrial DNA-to-nuclear DNA ratio is lower in cachectic cancer mice compared with non-cachectic cancer mice,[77,148,187] suggesting a decrease in mitochondrial content. This reduction was associated with body mass loss.[77,187] A lower mitochondrial content has also been reported in skeletal muscle of cachectic cancer rats compared with control rats.[112] As observed in human patients, mitochondria also present an altered morphology[187] with a swollen appearance,[112,170,180,188,189] a smaller size,[187] and the presence of electron-lucent areas,[112,180,189] which all together indicate an alteration in the mitochondrial network and function. Biochemical analyses corroborate ultrastructural information. A reduction in the metabolic flux throughout the Krebs cycle,[188] which has also been confirmed by metabolomic approaches,[94,190,191] and a decrease in the activities of complexes I,[135,192] II,[80,99,135,170,192,193] III,[193] IV,[72,85,192–194] and V[72,87] have thus been consistently described. Accordingly, mitochondrial respiration rate is also reduced in isolated mitochondria from skeletal muscle of cachectic cancer mice compared with control mice.[192,195,196] Therefore, animal studies clearly indicate that the entire mitochondrial oxidative pathway is profoundly impaired by cancer cachexia.

The oxidative activity of a muscle fibre is also tightly associated with its capillary density,[197] which allows oxygen supply and substrate delivery to the muscle fibre. Although data are scarce, cancer cachexia does not seem to impact capillary density of cachectic cancer patients.[57] In animals, a study reported an increased number of blood vessels in skeletal muscle of cachectic cancer mice.[179] This would not be true angiogenesis but would rather reflect a relative increase in capillary density because of the reduction in muscle fibre cross-sectional area.

The decrease in oxidative activity is also associated with a decrease in skeletal muscle ATP content,[94,170,198] a decrease in creatine phosphate concentration,[94] a decrease in glucose concentration,[92,199] a decrease in glycogen store,[92] a decrease in glycolysis,[92,190,199] and a reduction in lactate concentration,[199] which together may contribute to reducing skeletal muscle to synthesize ATP during contraction.

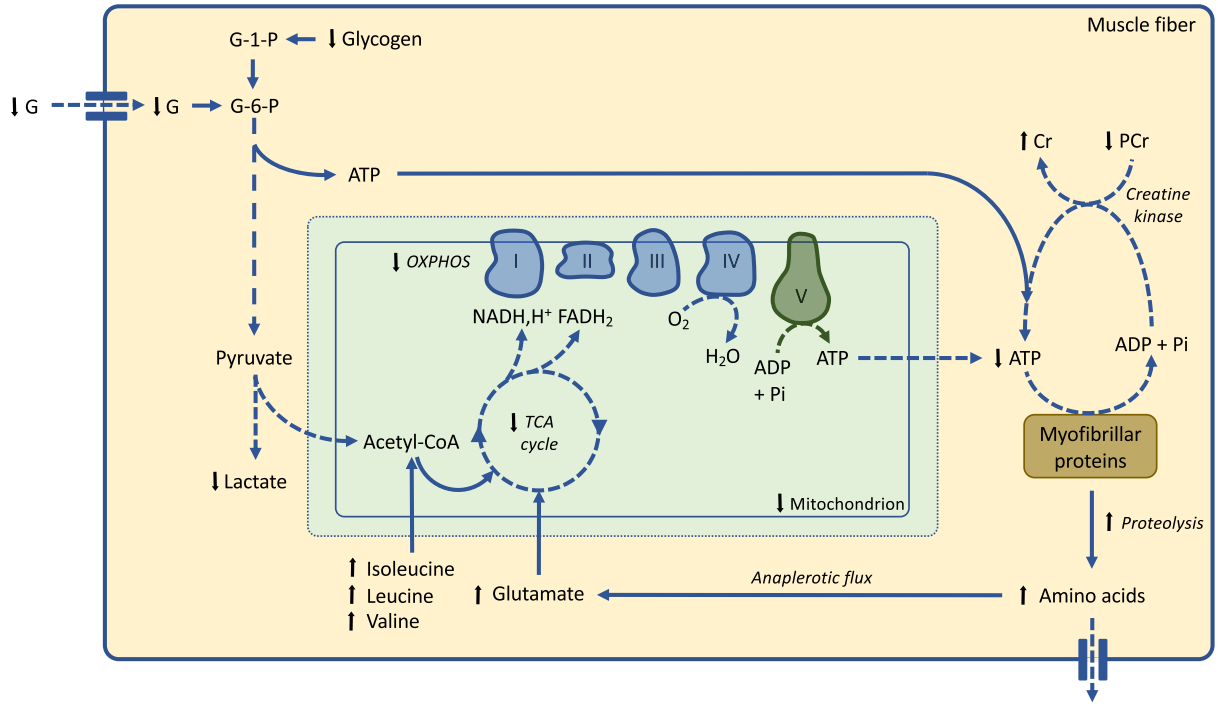

Figure 1 Effects of cancer cachexia on skeletal muscle energetic metabolism. This schematic representation is only based on animal studies. Reduced glycogen and glucose contents in skeletal muscle contribute to alter glycolysis flux. A decrease in mitochondrial content and mitochondrial oxidative phosphorylation, together with a decreased in ATP synthesis from phosphocreatine system, leads to a reduced ATP content in skeletal muscle. Skeletal muscle proteins are degraded and subsequently processed into amino acids. Individual amino acids can be either exported outside the muscle fibre or transported into the mitochondria (anaplerotic flux). Fatty acid metabolism is not represented because there is currently no study available. Black arrows indicate variations in metabolite concentration. Dashed arrow indicates experimentally demonstrated reduction in the corresponding metabolic pathway. Cr, creatine; G, glucose; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; OXPHOS, oxidative phosphorylation; PCr, phosphocreatine; TCA, tricarboxylic acid cycle.

Importantly, the decrease in skeletal muscle capacity to generate ATP[113,188] may also alter its capacity to perform other essential ATP-dependent cellular works involved in skeletal muscle homeostasis such as the maintenance of mineral balance.

Amino acid metabolism

The intense catabolism of skeletal muscle proteins during cancer cachexia[71,89,90,105,107,109,110,147,148,200–209] also raises the question of the metabolic fate of amino acids. Cancer cachexia is associated with an alteration in skeletal muscle amino acid profile,[151,199] as well as an altered concentration of circulating amino acids.[83,210] In cachectic skeletal muscle, increased provision of glutamate[199] may increase the anaplerotic flux to the Krebs cycle (α-cetoglutarate production). Similarly, branched chain amino acids may be converted to acetyl-CoA and then enter the tricarboxylic acid cycle.[199] Furthermore, a recent *in vivo* metabolomic study revealed that mitochondrial dysfunction in cachectic skeletal muscle tissue also seemed to influence amino acid metabolism.[151] Considering the whole decrease in mitochondrial activity that occurs during cancer cachexia, major adjustments of metabolic fluxes may occur, so that the muscle fibre may adapt skeletal muscle proteolysis.

Amino acids can be also released into the blood compartment, interconverted into gluconeogenic amino acids by the liver, and recycled into glucose through hepatic gluconeogenesis.[211] This may thus rewire amino acid metabolism to promote glucose supply for tumour growth.[47,212] Under stress conditions, hepatic protein synthesis also shifts from constitutive proteins (albumin, transferrin) to inflammatory acute-phase proteins, such as C-reactive protein, fibrinogen, or serum amyloid A1. How the acute-phase response is linked to the development of skeletal muscle atrophy during cancer cachexia is not clear, but it has been hypothesized that skeletal muscle can provide the amino acids required for the synthesis of hepatic positive acute-phase proteins.[210,213] Bonetto *et al.*[214] also showed that cancer cachexia in mice was associated with an increase in liver fibrinogen protein content but more surprisingly also by a strong elevation of fibrinogen and serum amyloid A1 protein content in skeletal muscle. Sustained synthesis of acute-phase proteins in liver

and skeletal muscle may thus represent a spillway for amino acids derived from skeletal muscle proteolysis.[210,213] A better knowledge of the contribution of skeletal muscle amino acid metabolism to whole-body metabolism during cancer cachexia is needed.

Mononucleated cell niche of skeletal muscle fibre during cancer cachexia

The micro-environment of skeletal muscle fibres contains different mononucleated cell types that are important for skeletal muscle repair and that contribute to skeletal muscle diversity. Upon muscle injury, quiescent resident satellite cells get activated, proliferate, and then fuse with pre-existing damaged muscle fibres to rebuild new functional fibres.[215] Activation of satellite cells, proliferation of myoblasts, and their differentiation into myotubes require the presence of pro-inflammatory macrophages (M1) and their transition into anti-inflammatory macrophages (M2), with M1 and M2 macrophages stimulating, respectively, the early and late phases of regeneration.[215] Fibro-adipogenic progenitors, fibroblasts, and endothelial cells are also important to achieve proper skeletal muscle regeneration.[215]

Pioneering works from Jewesbury and Topley at the beginning of the 20th century[216] and later by Marin and Denny-Brown[158] already indicated that skeletal muscle fibres from cachectic cancer patients had more nuclei in the vicinity of the sarcolemma even though the intrafibre or extrafibre localization of nuclei was not determined in these studies. Much more recently, cancer cachexia in pancreatic cancer patients was associated with a type of muscle damage characterized by the activation of both satellite cells and non-satellite muscle progenitor cells.[217] The extent of the activation correlated with body mass loss.[217] The presence of inflammatory cells,[146] macrophages, and fibro-adipogenic progenitors[218] was also reported in skeletal muscle of cachectic cancer patients. In animal models of cancer cachexia, skeletal muscle also contains a higher number of activated satellite cells and non-satellite muscle progenitor cells,[217] undifferentiated cells,[73,160] and inflammatory cells.[78,118,147–149,219] In contrast, it has been reported that the cachectic muscle of tumour-bearing mice was enriched in haematopoietic stem cells, but not in inflammatory cells.[167] Together, these data from human and animal studies suggest that an alteration in the mononuclear cell profile of skeletal muscle fibre micro-environment, and particularly the persistence of inflammatory cells, may contribute to skeletal muscle mass loss during cancer cachexia.

Indeed, a greater fragility of skeletal muscle to micro-injuries and injuries in the cachectic muscle may lead to episodes of degeneration/regeneration, thus allowing the activation of satellite cells. The existence of ongoing episodes of regeneration in cachectic skeletal muscle is supported by the observation that skeletal muscle of cachectic cancer patients[218,220,221] and cachectic cancer mice or rats[78,118,147–149] displays a higher number of muscle fibres with centralized nuclei, indicating the presence of regenerating muscle fibres. Interestingly, internally located nuclei were predominantly found in type 2 muscle fibres,[220] which is consistent with the notion that these fibres would be more prone to cachexia. Importantly, satellite cells of cachectic cancer mice are able to commit to the myogenic program, but not to completely differentiate, as indicated by the persistent expression of Pax7.[217] Together with the deficiency of cancer cachectic skeletal muscle to regenerate after freeze clamping-induced[70,217] or cardiotoxin-induced[103] muscle injury, these data collectively indicate that an accumulation of unresolved/incomplete episodes of skeletal muscle repair could contribute to the loss of skeletal muscle mass and function during cancer cachexia. Finally, this response could also indicate the existence of a vain compensatory mechanism to limit the extent of skeletal muscle mass loss during cancer cachexia.

Other histological features of skeletal muscle during cancer cachexia

Endomysial space

Skeletal muscle from cachectic cancer patients displays an increased area occupied by collagen that positively correlates with weight loss and poor survival.[218] This increase in fibrosis is in agreement with the increase in endomysial space observed in skeletal muscle of cachectic cancer patients,[178,218] but not in non-cachectic cancer patients.[218] This is also consistent with decreased mRNA level for matrix metalloproteinase 3, an enzyme involved in extracellular matrix protein breakdown.[222] A similar increase in collagen deposition was also reported in skeletal muscle of cachectic cancer mice,[160,223] together with an increased area of non-contractile tissue that may reflect disrupted extracellular matrix remodelling.[84] The progressive development of fibrosis may be the long-term consequence of an increase in the expansion and differentiation of fibro-adipogenic progenitor in the cachectic muscle.[218] Surprisingly, a down-regulation of extracellular matrix gene expression has been also reported in skeletal muscle of cancer cachectic patients[91] and animal models of cancer cachexia[91,166,172,214,223–228] with *Col1a1*, *Col3a1*, *Col5a1*, and *Col5a2*, the most frequently down-regulated genes. Anyhow, it remains clear that extracellular matrix is markedly disorganized in cachectic skeletal muscle, which may likely alter the mechanical properties of skeletal muscle and may also contribute to render muscle fibres more susceptible to injuries.

Fat depot

Computerized tomography analyses of the third lumbar vertebra level show an increase in fat infiltration in skeletal muscle of cachectic cancer patients[12,65,229] that was not correlated with muscle mass loss.[230] An increase in the size and the number of lipid droplets[231] and an increase in the lipid content[218] have also been reported in skeletal muscle of cachectic cancer patients. This increase in lipid content in skeletal muscle of cachectic cancer patients is due to both an intramyocellular and an extramyocellular accumulation of triglycerides.[232] The expansion and differentiation of fibro-adipogenic progenitors, which occur in skeletal muscle of cachectic cancer patients,[218] may explain the accumulation of extramyocellular lipids. It has also been proposed that impaired lipid oxidation due to altered mitochondrial function may contribute to the accumulation of intramyocellular lipid droplets in skeletal muscle fibres of cachectic cancer patients.[230] It may be noted that one study reported a decreased lipid content in the slow-twitch muscle fibres of cachectic patients with late-stage non-small-cell lung cancer.[233] In animals, lipid accumulation in the skeletal muscle fibres of cachectic cancer mice has been also reported.[188] Consequently, an increase in fat depot appears to be a common histological feature of cachectic skeletal muscle in both patients and animal models, but its consequence on skeletal muscle function has to be further explored.

Altered myofibrillar structure

Sarcolemmal alterations,[146,157,218] which heighten with body mass loss,[217] an increase in the number of damaged[218] and shrunken[178] fibres, a loss of normal cross-striation pattern,[158,178,181,218] disrupted triads,[186] and a dilated sarcoplasmic reticulum,[181] have been observed in skeletal muscle of cachectic cancer patients. Similarly, a disorganization of the sarcolemma[76,179] and basement membrane,[97,179,224] an alteration of sarcomere structure,[97,180,188,189] disrupted triads,[170] and a dilated sarcoplasmic reticulum[112] have been also reported in skeletal muscle of animal models of cancer cachexia. These structural alterations may impact membrane excitability, calcium transient frequency, and cross-bridge kinetics, which are major determinants of myofibre contractile performance (see succeeding text).

Figure 2 sums up the most important cellular and subcellular phenotypic changes in skeletal muscle during cancer cachexia.

Skeletal muscle function during cancer cachexia

The main function of skeletal muscle is to generate force to maintain posture and produce movement. Any alteration in skeletal muscle mass, metabolism, structure, and organization may profoundly alter its capacity to generate force. The data reported earlier in human patients and in animal models of cancer cachexia highlighting marked alterations in skeletal muscle mass and metabolism indicate that the function of cachectic skeletal muscle must be profoundly modified.

Decrease in skeletal muscle force

Cachectic cancer patients have a lower handgrip force (−7% to −31%) than cancer patients without cachexia.[7,10,12,25,32,53] Absolute isometric muscle force of knee extensors[55,57,61,146] and knee flexors[55] has also been consistently reported to be lowered in cachectic cancer patients compared with healthy subjects, as well as compared with non-cachectic cancer patients.[55] The isokinetic force of knee extensors and knee flexors is also reduced in cachectic cancer patients when compared with healthy subjects[54,57] or non-cachectic cancer patients.[54] Accordingly, the speed of contraction is also lower in men, but interestingly not in women, cachectic cancer patients, when compared with gender-paired healthy control subjects.[61]

In murine models of cancer cachexia, a decrease in grip force (−9% to −40%) has also been observed in cachectic cancer mice or rats when compared with control animals.[69,73,74,79,80,92,94,95,101,108,150,152,161,162,166,170,177,192,196,207,234–241] We can note that this decrease was not observed when cachectic cancer mice were compared with mild-cachectic mice,[79] indicating that moderate cachexia is already associated with functional alterations of skeletal muscle. Maximal contraction force of the *extensor digitorum longus*,[67,81,91,97,100,242] *soleus*,[67,97,100,135] *tibialis anterior*,[79,99,102,153] and diaphragm[96] muscles are also decreased in cachectic cancer mice compared with control mice, as well as in cachectic cancer mice compared with non-cachectic cancer mice.[102] Interestingly, skeletal muscles of cachectic cancer mice with a majority of fast fibres, such as *extensor digitorum longus*, are more prone to a decrease in force,[67] further strengthening the notion that type 2 fibres are more impacted by cachexia. Although some authors did not find any difference,[79,242] the speed of muscle contraction and relaxation in response to a single twitch stimulation is also reduced in the *tibialis anterior*[102] and *extensor digitorum longus*[81,100] muscles of cachectic cancer mice when compared with control mice. Similar results have been obtained when the comparison was done with non-cachectic cancer mice.[102] nterestingly, the extent of the decrease in speed contraction correlated with body mass loss.[102]

Taken together, all these data emphasize that skeletal muscle force could be an important criterion to diagnose cachexia. In this context, it is essential to know the kinetic of skeletal muscle force decrease, because if the loss in muscle force occurs before the loss in muscle mass during

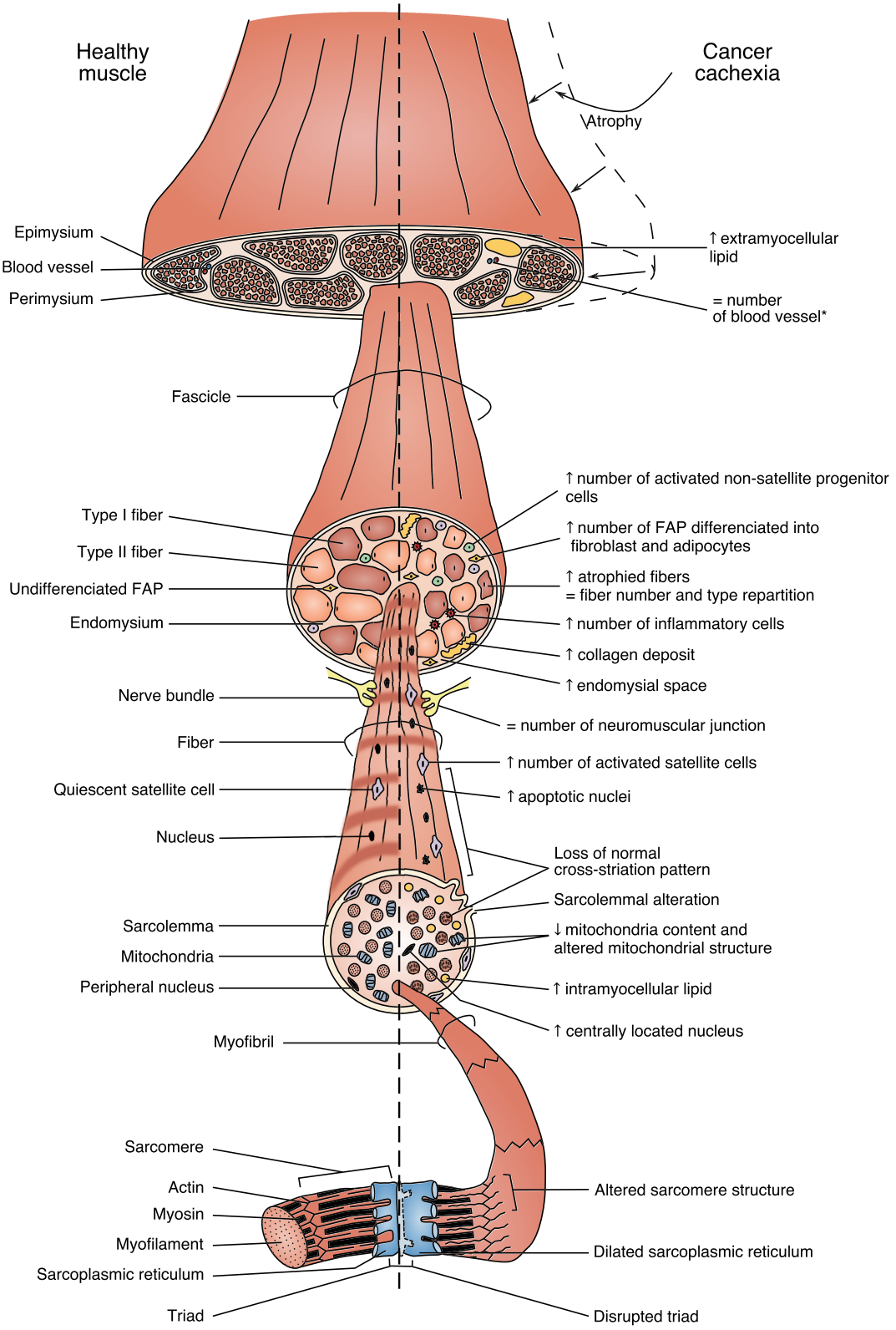

Figure 2 Schematic overview of the main cellular and subcellular phenotypic changes that occur in cachectic cancer skeletal muscle. This schematic representation is based on the analysis of both human and animal studies. Arrows indicate either an increase or a decrease for the corresponding parameters during cancer cachexia. =, the parameter is unchanged with cancer cachexia. * denotes a phenotypic change that has only been reported in animal models of cancer cachexia. FAP, fibro-adipogenic precursor.

cancer cachexia, as it is observed during the geriatric muscle mass loss,[243] the measurement of muscle force would be a precocious and easily measurable predicting factor of cancer cachexia in human cancer patients. However, there is limited information on this topic. An 8 week follow-up study reported that isometric *quadriceps* and hamstring muscle force, as well as handgrip force, were stable in cachectic cancer patients, but skeletal muscle mass was not determined in this study.[244] More studies are necessary to provide comparative analyses of the time-course changes in the loss of skeletal muscle mass and function in human cancer patients. Finally, an increase in muscle fatigue may also contribute to decrease muscle force in cancer cachectic patients. Some studies reported an increase in muscle fatigue that was attributed to an increased central fatigue,[245–247] but no information about the cachectic status of the cancer patients was reported. Therefore, studies aimed at exploring muscle fatigue would be very helpful to further understand the aetiology of the decrease in skeletal muscle force in cancer cachectic patients. In animal models of cancer cachexia, muscle fatigue has been consistently reported to increase.[79,80,97,100,102,150,221,242] Importantly, muscle fatigue also correlated with body mass loss.[80]

Potential factors involved in muscle force decrease

Beyond the description remains the question of the mechanisms involved in the loss of skeletal muscle force production in the cachectic muscle. A decrease in muscle mass can obviously contribute to explain the decrease in muscle force in human cancer patients.[57] However, when muscle force is normalized to muscle cross-sectional area or body mass, difference in muscle force between cachectic cancer patients and control subjects still persists.[61] Similarly, in mice models of cancer cachexia, if a decrease in muscle force can be attributed to a loss of body mass[74] or muscle mass[242] or a decrease in skeletal muscle cross-sectional area,[79,91,97,99,135] the loss in muscle force persists even after normalization by body mass,[79] muscle mass,[81] or muscle fibre cross-sectional area[96,100,102] or both.[67] Collectively, these data indicate that muscle mass loss does not account entirely for the decrease in force and support the conclusion that cancer cachexia is also associated with intrinsic contractile dysfunctions (i.e. diminished function per unit muscle size).

Factors required for co-ordinated muscle contractile function involve neuromuscular junction integrity, excitation–contraction coupling, calcium handling, sarcomere structure, and energetic metabolism.[142] Previous studies indicate that the decrease in muscle force would not involve an alteration in neuromuscular junction as cancer cachexia seems to affect neither muscular nor intramuscular nerve bundles in patients,[158,181] nor neuromuscular junction integrity,[156] or the number of neuromuscular junctions in the muscle of cachectic cancer mice.[167] However, this concept has recently been questioned at least in C26 tumour-bearing mice.[155] Anyhow, an in-depth analysis of skeletal muscle junction and neuromuscular coupling is necessary for cancer patients and animal models of cancer cachexia. Loss in muscle force can also result from impairment in calcium handling. Unexpectedly, isolated muscle fibres from cachectic cancer patients have an increased calcium sensitivity,[143] which could be explained by a shift from slow to fast myosin isoform expression, as type 2 muscle fibres are more calcium sensitive.[248] In contrast, muscle fibre calcium sensitivity is reduced in cachectic cancer mice compared with control mice.[96] Moreover, the calcium-activated force and cross-bridge kinetics are reduced in cachectic cancer mice compared with control mice,[96] strongly suggesting that the loss of skeletal muscle force can be due to an alteration of calcium handling. A recent study by Judge *et al*. also described an increase in calcium deposition in skeletal muscle of cachectic pancreatic cancer patients.[218] Calcium overload within skeletal muscle fibre may exert deleterious effects leading to muscle damage via the activation of calcium-activated proteases (calpains) and the disruption of sarcolemma integrity.[249] Furthermore, calcium overload can be also sensed by mitochondria, which may further contribute to alter mitochondrial metabolism and worsen cellular damages.[249] Together, this may profoundly alter the capacity of the fibre to generate force. Therefore, and although several evidences suggest that calcium handling may be involved in the loss of muscle force, more investigations are required to clearly establish the role of altered calcium handling.

Further insights into the mechanisms involved in the loss of skeletal muscle force have been also provided by *ex vivo* analysis of the contractile properties of skeletal muscle fibres[178] or muscle fibre bundles[143] from cachectic cancer patients. The absolute[143] and specific[178] maximal force of isolated fibres has thus been shown to decrease in cachectic cancer patients. Furthermore, specific maximal force correlates with myosin-to-actin ratio,[178] indicating that the loss of contractile machinery is a factor contributing to decreased muscle force. However, one may remind that measuring the actin-to-myosin ratio is strongly dependent on the extraction conditions and may thus lead to misinterpretations.[250] Another study showed that single fibre isometric tension was reduced in type 2A fibres from non-cachectic and cachectic cancer patients, which was explained by a reduction in the number of strongly bound cross-bridges.[251] Myosin–actin cross-bridge kinetics were also reduced in type 1 fibres from non-cachectic and cachectic cancer patients.[251] Therefore, a reduction in myofilament protein function may be a potential molecular

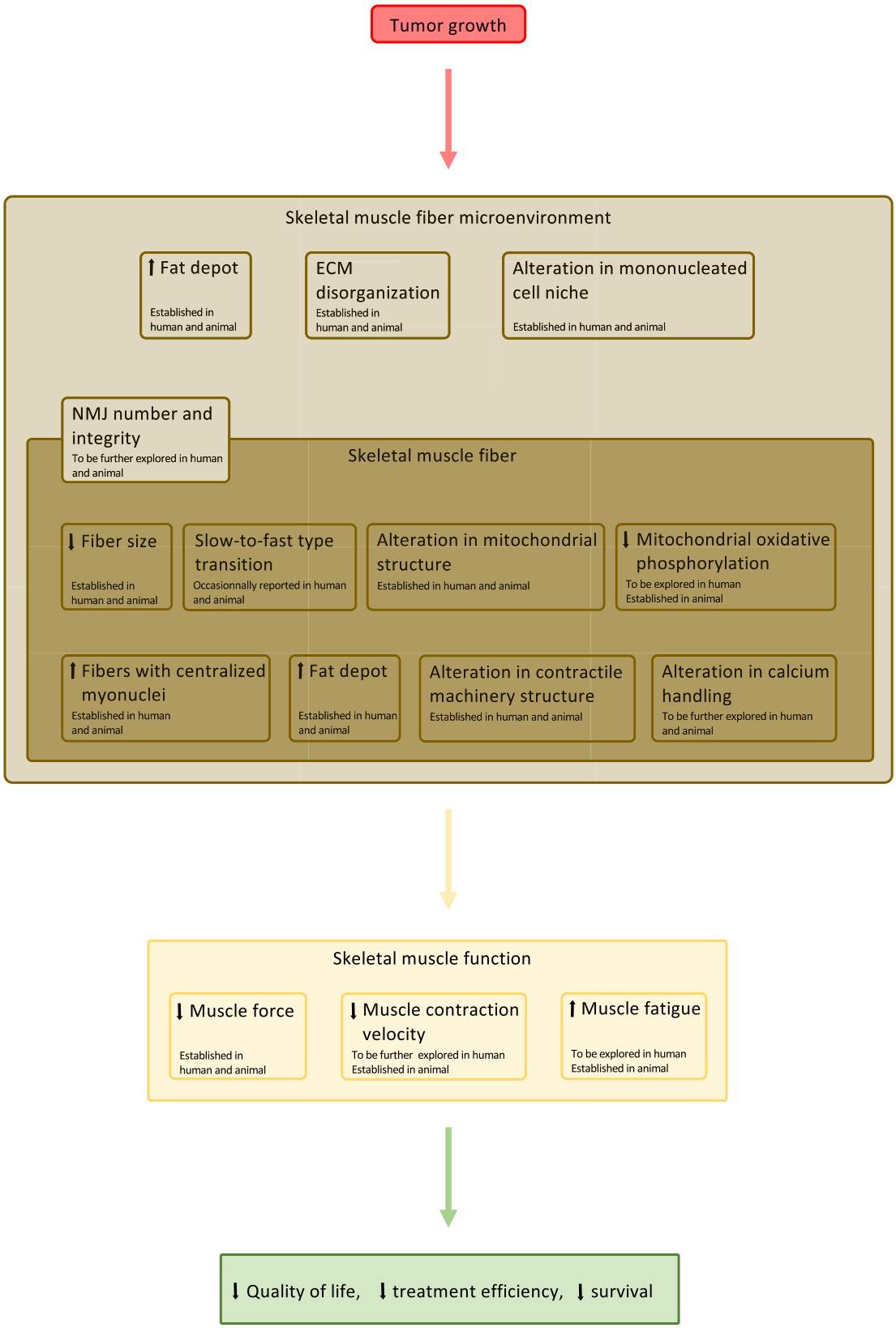

Figure 3 Functional relationship between skeletal muscle fibre phenotypic changes, skeletal muscle function, and patient's quality of life during cancer cachexia. ECM, extracellular matrix; NMJ, neuromuscular junction.

mechanism contributing to muscle weakness in human cancer patients. Finally, a decrease in the capacity of skeletal muscle to sustain ATP production by mitochondrial oxidative phosphorylation during contraction (see earlier discussion) could also contribute to decrease muscle force and increase muscle fatigue.

Summary and future perspectives

The purpose of this review was to specifically focus on the structure and function of cachectic skeletal muscle in human cancer patients and animal models of cancer cachexia. The extent of skeletal muscle mass loss has largely been described, and the consequences of cachexia on skeletal muscle function are now getting more and more documented. The loss of muscle mass is of course an important factor to consider when studying cancer cachexia, but qualitative factors such as changes in skeletal muscle metabolism, muscle fibre micro-environment, fibrosis, neuromuscular junction, and sarcomere integrity, as well as calcium handling, are certainly involved in the impaired muscle function and need to be explored in details (*Figure* 3). A comparative analysis of the time-course changes of these qualitative factors and skeletal muscle mass during cancer cachexia is also necessary.

Our review also underlines important methodological aspects that may explain contrasted results between studies. For instance, the choice of the control group is quite heterogeneous between clinical studies (due to obvious constraints related to the recruitment of the subjects). Healthy control subjects, non-cachectic cancer patients, and cachectic non-cancer patients have thus been used. A careful analysis of the reference group is therefore necessary. Longitudinal analyses of the kinetic of the loss in skeletal muscle mass and function in cancer patients should be also preferred. This implicates that the detection of cachexia must be carefully and regularly performed with the use of solid image-based analyses[4,37] and standardized functional tests. This also applies to animal models of cancer cachexia where time point analyses are essential to delineate the time-course changes in the loss of skeletal muscle mass and function occurring during cancer cachexia. Non-invasive measurement of skeletal muscle force, together with the use of image-based analysis of skeletal muscle mass, such as computerized tomography or magnetic resonance imaging, should be therefore encouraged whenever it is possible. The analysis of a gender effect in the development of cancer cachexia has also to be explored in both human and animal studies. Differences in skeletal muscle physiology between species[142,252,253] must be also kept in mind when analysing and translating animal data to human patients, as animal models of cancer cachexia remain very different from human cancer cachexia.[239,254] The physiological status of the animal species is also very important to consider. Many studies, especially those using tumour-bearing models of cancer cachexia, use young growing animals (2 to 3 months old). The strong stimulation of protein synthesis that supports skeletal muscle growth during the post-natal period may mask/modify numerous cellular events. Non-growing adult animals should be thus preferentially used when using tumour-bearing animal models of cancer cachexia. Finally, our analysis emphasizes that measuring skeletal muscle force on a large epidemiologic scale by standardized functional tests is clinically fundamental to have a simple and robust mean to early diagnose cachexia in cancer patients. This could lead to proposing specific physical activity programs that may slow down the progression of cachexia and improve patient's quality of life.

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Conflict of interest

A.M. and D.F. declare that they have no conflict of interest.

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