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Clinical and biological characterization of skeletal muscle tissue biopsies of surgical cancer patients

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Abstract

Background Researchers increasingly use intraoperative muscle biopsy to investigate mechanisms of skeletal muscle atrophy in patients with cancer. Muscles have been assessed for morphological, cellular, and biochemical features. The aim of this study was to conduct a state-of-the-science review of this literature and, secondly, to evaluate clinical and biological variation in biopsies of *rectus abdominis* (RA) muscle from a cohort of patients with malignancies.

Methods Literature was searched for reports on muscle biopsies from patients with a cancer diagnosis. Quality of reports and risk of bias were assessed. Data abstracted included patient characteristics and diagnoses, sample size, tissue collection and biobanking procedures, and results. A cohort of cancer patients (n = 190, 88% gastrointestinal malignancies), who underwent open abdominal surgery as part of their clinical care, consented to RA biopsy from the site of incision. Computed tomography (CT) scans were used to quantify total abdominal muscle and RA cross-sectional areas and radiodensity. Biopsies were assessed for muscle fibre area (μm^2), fibre types, myosin heavy chain isoforms, and expression of genes selected for their involvement in catabolic pathways of muscle.

Results Muscle biopsy occurred in 59 studies (total N = 1585 participants). RA was biopsied intraoperatively in 40 studies (67%), followed by quadriceps (26%; percutaneous biopsy) and other muscles (7%). Cancer site and stage, % of male participants, and age were highly variable between studies. Details regarding patient medical history and biopsy procedures were frequently absent. Lack of description of the population(s) sampled and low sample size contributed to low quality and risk of bias. Weight-losing cases were compared with weight stable cancer or healthy controls without considering a measure of muscle mass in 21 out of 44 studies. In the cohort of patients providing biopsy for this study, 78% of patients had preoperative CT scans and a high proportion (64%) met published criteria for sarcopenia. Fibre type distribution in RA was type I (46% ± 13), hybrid type I/IIA (1% ± 1), type IIA (36% ± 10), hybrid type IIA/D (15% ± 14), and type IID (2% ± 5). Sexual dimorphism was prominent in RA CT cross-sectional area, mean fibre cross-sectional area, and in expression of genes associated with muscle growth, apoptosis, and inflammation ($P < 0.05$). Medical history revealed multiple co-morbid conditions and medications.

Conclusions Continued collaboration between researchers and cancer surgeons enables a more complete understanding of mechanisms of cancer-associated muscle atrophy. Standardization of biobanking practices, tissue manipulation, patient characterization, and classification will enhance the consistency, reliability, and comparability of future studies.

Keywords Rectus abdominis; Skeletal muscle; Cancer; Biopsy; Sarcopenia

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Introduction

Several radiologically defined features of skeletal muscle have been associated with clinical outcomes in patients with cancer. Reduced muscle mass (i.e. sarcopenia), loss of muscle mass over time, and reduced muscle radiodensity are related to mortality, shorter progression—free survival, chemotherapy toxicity, and complications of cancer surger.[1–4] In light of the associations between muscle and outcomes, researchers are increasingly investigating the pathophysiology of muscle abnormalities[5–7] and attempting to relate the findings to the much broader base of knowledge that exists from research in animal models. Muscle may be obtained from cancer patients by percutaneous biopsy as well as intraoperatively during cancer surgery. Clinical data aligned with the biopsy provides a comprehensive approach to understand cancer cachexia from the vantage point of muscle wasting. Evaluation of human muscle contributes significantly to the understanding of molecular mechanisms in a variety of primary pathologies of skeletal muscle.[8,9]

Biopsy and tissue manipulation techniques can induce changes in the muscle that alter enzyme activity, metabolite concentrations, and protein metabolism.[10–12] Also, patient characteristics such as age, sex, cancer type, co-morbidities, and medications (including chemotherapy) taken at the time of biopsy collection are known factors that influence muscle metabolism.[13–17] These methodological issues pose limitations in the reliability, interpretation, and comparability of the findings on muscle biopsies in patients with cancer. Therefore, our first aim was to conduct a state-of-the-science review of the literature on muscle biopsy in cancer patients. This type of review retains many features of a systematic review except that studies are not excluded on the basis of a quality assessment and thus presents a broader search of the literature. An associated aim was to provide recommendations of components to consider when evaluating and reporting results of muscle biopsies from cancer patients.

The second aim of this study was to evaluate sources of variation in the muscle biopsy material to better understand the risk of sampling bias, to determine variance and effect size to enable sample size calculations, and to determine the possible consequences of sexual dimorphism and age as confounders using a relatively well-powered sample (n = 190). Our research group has experience in the radiological characterization of muscle[2,18,19] and skeletal muscle morphology, cell biology, and biochemistry.[7,20–22] Our collaborative effort with hepatopancreatobiliary cancer surgeons has enabled muscle biobanking and exploration of muscle biology within large populations. We have published studies on muscle expression of mRNA, microRNA, and alternative splice variants,[20,21,23] alongside specific and precise measures of muscle mass, radiodensity, and muscle loss.

Materials and methods

Literature review

A state-of-the-science review[24] is a broad search of the literature that includes all studies in a particular area. Our review protocol follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[25] guidelines to reduce bias (*Figure* 1). Articles indexed in SCOPUS from 1 January 1900 to 16 August 2018 were queried to capture reports on skeletal muscle biopsies from cancer patients. Search terms included adult humans, malignant disease [(cancer) OR (neoplasm) OR (carcinoma) OR (tumor) OR (malignant) OR (metastasis)], skeletal muscle [(skeletal muscle) OR (muscle mass) OR (lean body mass) OR (rectus abdominis) OR (cachexia) OR terms for other specific muscle], and biopsy. Review articles and studies on experimental models, laboratory animals, non-cancer populations, or those not employing muscle biopsies were excluded. Bibliographies of identified articles were hand searched to find additional relevant publications. There were no exclusion criteria regarding number of patients and type of study (retrospective, prospective, or cross sectional). Data were extracted from the result sections, tables, and figures of each article. As we did not aggregate the data, no additional data were contributed from the investigators.

Two reviewers independently assessed each of the included studies, and disagreements were resolved by consensus. A score for study quality was given using assessment tools provided by the National Heart, Lung and Blood Institute (NIH—U.S. Department of Health & Human Services) for cross-sectional, cohort, case-control, randomized control trials and before–after studies. The Newcastle–Ottawa scale modified for cross-sectional studies[26] was used to give a bias score based on the (i) representativeness, (ii) size, and (iii) non-respondent report.

Rectus abdominis biological characterization

Subjects and acquisition of muscle samples

The study was approved by the Health Research Ethics Board of Alberta-Cancer. Patients undergoing elective abdominal surgery were consecutively approached to participate in tumour and tissue banking at a hepatopancreatobiliary surgical service in Alberta, Canada. Three per cent of approached patients declined participation. Patients provided written informed consent for muscle biopsy and tissue banking. Release of n = 190 samples from the bank for analysis, as well as patient information (demographic, clinical, and operative data) from medical records, was performed under the auspices of Protocol ETH-21709: *The Molecular Profile of Cancer Cachexia*. Patients consent freely to muscle biopsy from the site of incision at the time of surgery, as this entails little if

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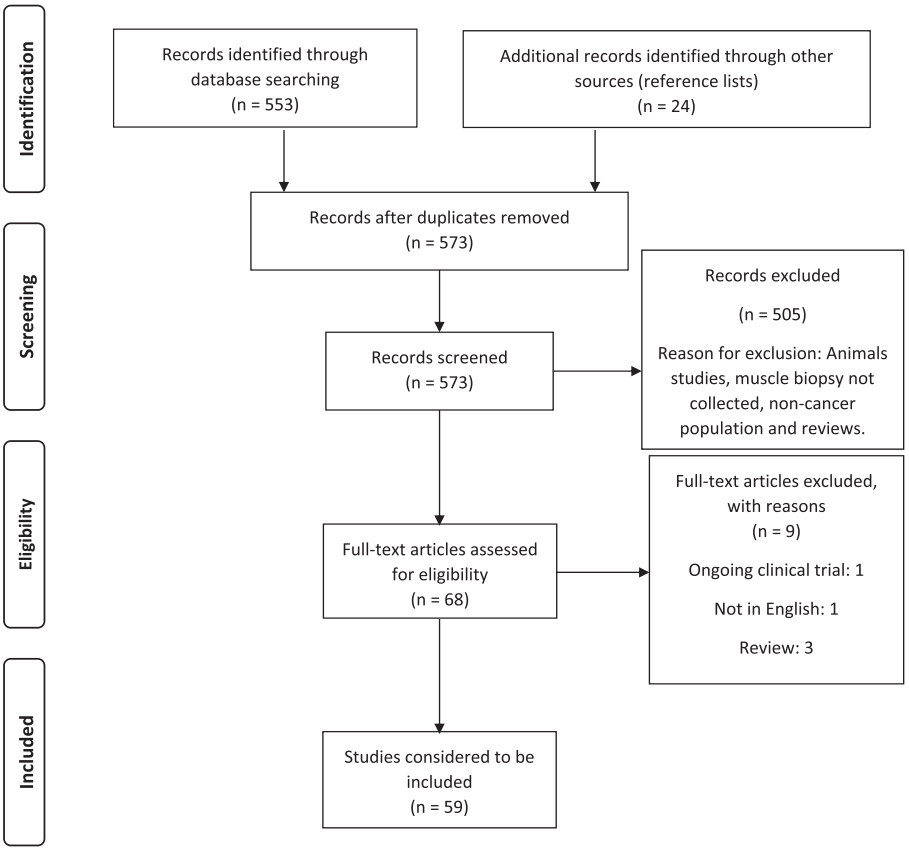
Figure 1 Flow chart of search. PRISMA diagram for the identification, screening, eligibility, and inclusion of papers (1 January 1990–16 August 2018) from SCOPUS. All articles included investigated cancer, skeletal muscle, and muscle biopsies. Excluded records: review articles and ongoing clinical trials.



any incremental discomfort or risk, as the surgery is inherently invasive. All patients were either diagnosed as having cancer or were suspected of having cancer due to their symptoms and radiological assessments such as computed tomography (CT) imaging.

The study cohort and conditions for acquisition of muscle samples have been described previously.[23] Briefly, *rectus abdominis* (0.5–3 g) samples were collected during open abdominal surgery scheduled as part of their clinical care. Upper abdominal transverse incision was performed, and muscle biopsy was obtained at opening by sharp dissection, without the use of electrocautery.

Computed tomography image analysis

Digital axial CT scans performed preoperatively and used to plan surgery were used to quantify skeletal muscle cross-sectional area (CSA, cm^2) as in our prior work.[18,27] Measures with CT have excellent precision (precision error values of ~1.5%).[28] Briefly, images at the 3rd lumbar vertebra (L3) were analysed for total L3-CSA within a specified Hounsfield unit (HU) range (−29 to +150) using Slice-O-Matic software (v.4.3, Tomovision, Magog, Canada). Muscle area was normalized for stature and reported as skeletal muscle index (SMI, cm^2/m^2). Mean radiodensity (HU) was also reported. Adipose tissue CSA at L3 was calculated in a HU range of −150 to −50 and −190 to −30, for visceral and subcutaneous adipose tissue, respectively.[28] The distribution of SMI of the patients providing biopsy for this study was compared with a previously described large cohort of oncology patients (n = 1473) to confirm that the population sampled is representative of muscle mass distribution and mean values for our population (*Figure* 2). Sarcopenia was classified according to previously reported[19,29] sex-specific and body mass index (BMI)-specific criteria: for BMI <30 kg/m^2, SMI <52.3 cm^2/m^2 for men and <38.6 cm^2/m^2 for women, and for BMI ≥ 30 kg/m^2, SMI <54.3 cm^2/m^2 for men and <46.6 cm^2/m^2 for women.

Processing of muscle biopsy

From each biopsy, several analysis were performed, each with specific preparation procedures. In the operating room, visible adipose and connective tissue was removed from the biopsy and it was cut into two pieces: one piece to be used

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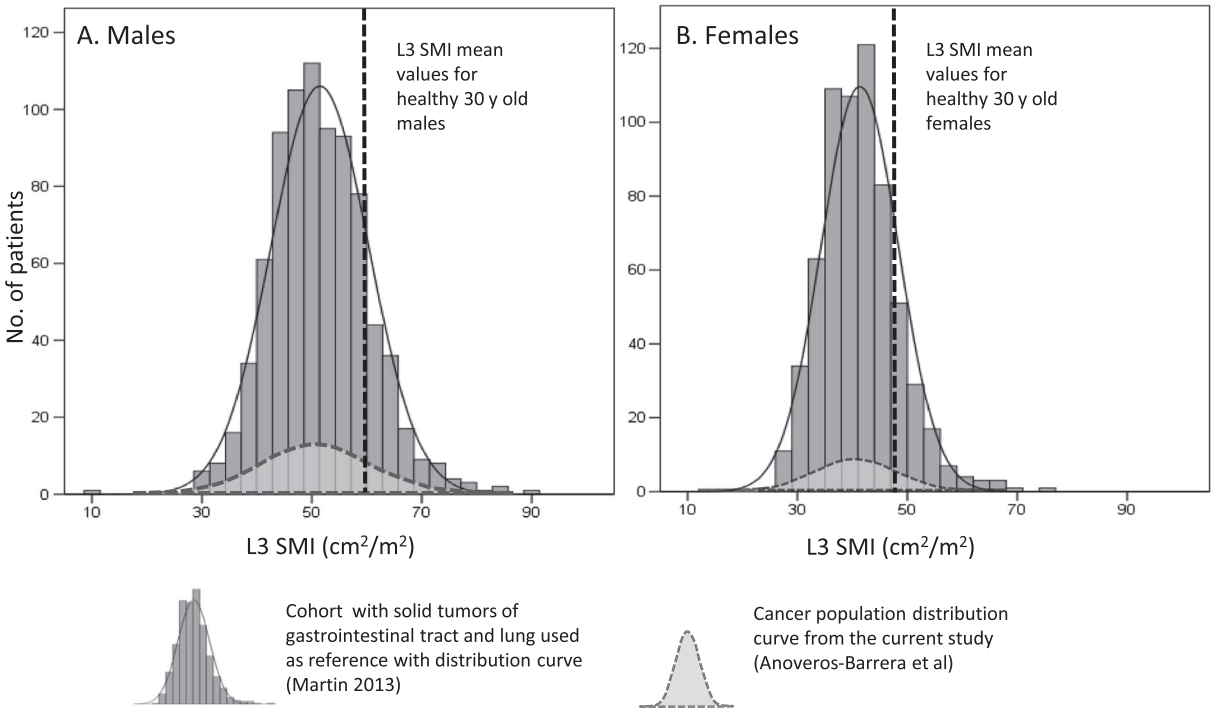
Figure 2 Figure represents overlap of L3 SMI distributions for male (A) and female (B) patients of current cancer population (small, light gray distribution) and a cancer cohort with solid tumours of gastrointestinal tract and lung (big, dark gray distribution).[1,2] (A) L3 SMI mean ± standard deviation values are 50.8 ± 8.3 and 51.5 ± 8.9 cm^2/m^2 for the current population and Martin *et al*. gastrointestinal and lung cancer cohort, respectively. (B) L3 SMI mean values are 39.8 ± 6 and 41.3 ± 7 cm^2/m^2 for the current population and Martin *et al*. gastrointestinal and lung cancer cohort, respectively. L3 SMI mean values for healthy 30-year-old kidney donor candidates (dotted line) are placed at 60.9 and 47.7 cm^2/m^2 for men and women, respectively.[15]



for analysis of gene expression, and myosin heavy chain (MyHC) by electrophoresis was immediately frozen in liquid nitrogen in the operating room prior to being transported to the lab for storage in liquid nitrogen until analysis. The other piece of the biopsy to be used for microscopy was transported on ice to the laboratory within 20 to 30 min. For morphological preservation, isopentane (2-methylbutane, C_5H_{12}) was cooled at −160°C in liquid nitrogen for 20 min or until the appearance of a thick frozen layer at the bottom of the container. A piece of muscle was oriented for transverse section and delicately placed on aluminum foil. Tissue was submerged in isopentane for 20 s, and aluminum foil was turned upside down to allow full exposure of the muscle section. After submersion, tissue was wrapped and left in liquid nitrogen for 5 min. Information about surgery date, time, and sample reception was documented.

Immunofluorescence: fibre types, laminin/dystrophin, and nuclear stain

Muscle serial sections (10 μm) were cryosectioned (cryostat Leica model CM300) transversely at −22°C and stored at −80°C until staining. MyHC I, IID, and IIA were determined as previously described.[30] Primary and secondary antibodies are described in Supporting Information, *Table* S1. After the secondary antibody application, a nuclear stain (4',6-diamidino-2-phenylindole) was added for 2 min and washed. Slides (Apex™ superior adhesive slides, Leica biosystems) were mounted, covered, and let dry for 12 h. Images for tissue sections were acquired using a 20X/0.85 oil lens with a spinning disk confocal microscope (Quorum Wave FX Spinning Disc Confocal System–Quorum technologies). Individual Z-stacked images were assembled to create a composite image of a whole tissue cross section. Tissue images were capture and analysed with Volocity 6.3 software (PerkinElmer, Waltham, MA, USA). A software script was established to identify muscle fibres types (I, I/IIA, IIA, IIA/D, and D) using intensity of the MyHC stains and quantified automatically by the software. Mean muscle fibre area (μm^2) was calculated by the detection of membrane (laminin/dystrophin antibody) fluorescence of muscle fibres in a cross section. Percentage of fibres with centralized nuclei was manually assessed by selecting muscle fibres with mispositioned nuclei (clearly separated from sarcolemma, equidistant, or not) in a tissue cross section.

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Electrophoretic analysis of myosin heavy chain isoform content

Semi-quantitative MyHC isoform analyses were completed on frozen rectus abdominis using western blotting as previously described.[30–32] All three of the adult MyHC isoforms (I, IIA, and IID) were clearly visible on all gels and reliably quantified in at least triplicate by integrated densitometry (Syngene ChemiGenius, GeneTools, Syngene).

Triglyceride content analysis

A piece of biopsy (50 mg) was ground using a frozen pestle and mortar without letting the tissue thaw. Ground tissue was homogenized in a 1.6 mL calcium chloride (CaCl2; 0.025%) solution with glass beads (0.5 mm diameter; FastPrep ®-24, MP Biomedicals, Santa Ana, CA, USA) in 20 s intervals for 1 min. Samples were placed on ice for 15 s between each homogenization interval. A modified Folch method was used to extract lipids using chloroform/methanol (2:1, vol/vol) as previously described.[33,34] The triglyceride (TG) fraction was isolated on G-plates and the TG band was identified and scraped. An internal standard C15:0 (10.2 mg/100 mL hexane) was added, followed by saponification and methylation. Samples were analysed using gas liquid chromatography (flame ionization detector) on a Varian 3900 (Varian Instruments, Georgetown, ON, Canada). Quantity of fatty acids within the TG fraction was calculated by comparison with the known concentration of the internal standard and sum of all fatty acids was reported as total TG.

Gene expression: microarray

Microarray was conducted as previously described.[23] The data have been deposited in the U.S. National Center for Biotechnology Information Gene Expression Omnibus25 and are accessible through GEO series accession number GSE41726.

Statistical analysis

Statistical analyses were conducted in IBM® SPSS ® software, version 24. A test for normal distribution was applied to the continuous variables. Descriptive statistics were reported as mean ± standard deviation. Comparisons between groups were conducted using independent *t*-test or Mann–Whitney *U* according to the variable normal distribution and χ^2 test for categorical variables. Statistical significance was considered at *P* values less than 0.05 (two-sided).

Results

Literature review

A total of 59 articles reporting analysis of skeletal muscle in cancer populations were reviewed. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses[25] flow diagram of our search strategy is shown in *Figure* 1.

Study quality and design

Table 1 includes all of the extracted data as well as scores for sampling bias (Newcastle–Ottawa scale) and study quality assessments (NIH). In general, the study quality rated as low for the majority of studies (*Table* 1). Applying the Newcastle–Ottawa criteria for sampling bias revealed the majority of studies had a high risk of sampling bias with 58% of studies lacking representativeness, 96% lacking sample size justification, and no study mentioned non-respondent rate (% of population approached who declined participation). Muscles biopsied were *rectus abdominis* (n = 40), quadriceps (n = 20), tibialis anterior (n = 1), gastrocnemius (n = 1), pectoralis major (n = 1), sternocleidomastoid (n = 1), serratus anterior (n = 1), diaphragm (n = 1), and latissimus dorsi (n = 1), and in seven studies, more than one muscle was collected. Four studies reported evaluation of rectus abdominis from cancer patients and quadriceps for non-cancer controls, and four studies reported biopsied muscle from two or three different muscles.

Gastrointestinal cancers were the most common diagnoses; 31/59 studies included patients of exclusively one cancer type: colorectal, pancreatic, gastric, breast, or prostate. Inclusion of patients with two or more cancer types was reported in 27/59 studies. Cancer stage or presence of metastasis was described in 39/59 studies. Combined data from two or more cancer stages were reported in 38/59 studies.

The majority of studies were cross sectional (Supporting Information, *Table* S2). For investigation of patients with cancer cachexia, weight loss was considered as the main reference for classification. In 36 studies, weight loss was graded with varying cut points (e.g. 5%, 10%, or 15%). Time frame of weight loss was not specified in 16 of these studies (*Table* 1). Percentage weight loss ranged from 5% to 22% in weight-losing groups (Supporting Information, *Table* S2). Measures of body composition were included in 25 studies; however, these measures were used to assess muscle mass or rate of muscle wasting over time in only seven studies (Supporting Information, *Table* S2).

Total sample size in each study was generally limited (mean, n = 26; median n = 18; and range 1–134). Seventy-six per cent of studies included $n \leq 30$ cancer patients; 48/59 studies included a non-cancer control group, sample size ranging from n = 3 to 41. Fifty-two studies included men and women, 5 studies only men, 1 study only women, and 2 studies did not

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Table 1 Original articles reporting muscle biopsy collection in patients with cancer: assessment of bias and study quality, population characteristics weight loss, or cancer cachexia classification criteria

						Cancer population		Control group		
Author	Bias[c]	Quality[d]	Muscle	Cancer site	Cancer Stage	*n* (% male)	Age (years) mean ± SD	*n* (% male)	Age (years) mean ± SD	Patient weight loss or cachexia criteria
Acharyya 2005[35]	1/3	3/12	RA	Gastric	NR	27 (NR)	NR	14 (NR)	NR	N/A
Agustsson 2011[36]	1/3	3/12	RA	Pancreas Other GI	NR	Pancreas 13 (30) Other GI 8 (37)	Pancreas: 70 ± 2 Other: 68 ± 3	Benign: 8 (37) Pancreatitis: 8 (63)	Benign: 53 ± 4 Pancreatitis 52 ± 3	NR
Aversa 2016[37]	1/3	6/12	RA	Colorectal pancreas gastric	1–4	All: 29 (59) WS = 14 WL = 15	68 ± 10.7	11 (63)	63 ± 13.2	5% WL (6 months)
Bonetto 2013[38]	1/3	3/12	RA	Gastric	1–4	16 (NR)	64 ± 11	6 (NR)	62 ± 17.4	>5% WL
Bossola 2006[39]	1/3	5/12	RA	Gastric	1–4	16 (50)	60.8 ± 11.2	5 (60)	65.6 ± 7.5	WL mild: 0–5%. WL moderate: 6–10%. WL severe: >10%.
Bossola 2001[40]	1/3	4/12	RA	Gastric	NR	20 (55)	61 ± 79.6	10 (60)	62 ± 45.8	WL mild: 0–5%. Moderate 6–10%. Severe: >10%.
Bossola 2003[41]	1/3	5/12	RA	Gastric	NR	23 (61)	59.5 ± 16.1	14 (64)	61.2 ± 12.3	>10% WL
Busquets 2007[42]	0/3	3/12	RA	Esophageal gastric pancreas	1–4	16 (NR)	66 ± 10	11 (NR)	66 ± 10.2	>5% WL (1 month)
DeJong 2005[43]	0/3	4/12	RA	Pancreas	1–4	16 (63)	66 ± 8	11 (81)	67 ± 13.2	N/A
D'Orlando 2014[44]	1/3	6/12	RA	Gastric	1–4	38 (66)	68.1 ± 11.6	12 (58)	64.2 ± 11.6	>5% WL (6 months)
Eley 2008[45]	1/3	3/12	RA	Esophageal gastric	1–4	15 (87)	66 (49–83)[a]	9 (10)	56 (41–86)[a]	N
Johns 2017[22]	2/3	9/12	RA	Esophageal gastric lung and other	1–4	134 (51)	65 ± 13	N/A	N/A	WL >5% >10% >15% and SMI with any degree of WL (>2%)
Johns 2014[46]	0/3	5/12	RA	Upper GI pancreas	NR	41 (73)	65 ± 12.8	N/A	N/A	>5% WL (6 months) and low muscularity with 2% WL
Khal 2005[47]	0/3	1/12	RA	Pancreas colorectal	NR	All: 18 (67) WS = 5 (60) WL = 13 (69)	WS: 79.8 ± 2.2 WL: 70.6 ± 8.2	10 (80)	69.6 ± 7.3	WL moderate: 1–11%. WL severe: >11%.
Lundholm 1976[48]	1/3	3/12	RA	Esophageal gastric pancreas colorectal kidney and others	NR	43 (44)	♂: 62 ± 13.1 ♀: 63 ± 9.7	55 (51)	56 ± 14.8	N/A
Marzetti 2017[49]	1/3	5/12	RA	Gastric	1–4	All: 18 (94) WS = 9 (100) WL = 9 (89)	WS: 70.6 ± 8.63 WL: 66.8 ± 12.5	9 (88)	57.4 ± 15.9	>5% WL (6 months)
Narasimhan 2017[21]	2/3	8/12	RA	Pancreas colorectal	1–4	22 (41)	64.9 ± 10	20 (45)	63.6 ± 7.9	>5% WL (6 months) or BMI of <20 with WL >2% and sarcopenia

(*Continues*)

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Table 1 (continued)

Author	Bias[c]	Quality[d]	Muscle	Cancer site	Cancer Stage	Cancer population		Control group		Patient weight loss or cachexia criteria
						n (% male)	Age (years) mean ± SD	*n* (% male)	Age (years) mean ± SD	
Narasimhan 2018[20]	1/3	5/12	RA	Pancreas colorectal	1–4	All: 40 (43) WS = 19 (47) WL = 21 (40)	WS: 64 ± 8 WL: 66 ± 11	N/A	N/A	WL >5% >10% >15% and sarcopenic (SMI) with any degree of WL (>2%)
Noguchi 1998[50]	0/3	3/12	RA	Esophageal gastric colorectal	1–4	10 (90)	56 (50 to 63)[a]	N/A	N/A	N/A
Pessina 2010[51]	1/3	6/12	RA	Gastric	1–3	30 (57)	63.8 ± 2.8	8 (62)	64.2 ± 2.6	N/A
Prokopchuk 2016[52]	0/3	4/12	RA	Pancreas	1–4	All: 25 (32) NC = 13 (38) CC = 12 (25)	NC: 67 (36–87) CC: 70 (52–83)[a]	Benign = 15 (80) Pancreatitis = 9 (45)	Benign: 67 (32–73) Pancreatitis: 49.5 (40–75)[a]	>10% WL (6 months)
Ramage 2018[53]	1/3	3/12	RA	Esophageal gastric pancreas	1–4	32 (81)	64.5 (43–83)	N/A	N/A	>5% WL of pre-illness
Rhoads 2009[54]	1/3	6/12	RA	Gastric	1–4	All: 14 (57) WS = 6 (66) WL = 8 (50)	64.2 ± 3.8	10 (60)	63.9 ± 2.8	NR
Schmitt 2007[55]	0/3	2/12	RA	Pancreas	2, 4	All: 16 (63) NC = 8 (37) CC = 8 (88)	NC: 62 ± 8.5 CC: 53 ± 11.3	N/A	N/A	>10% WL (6 months)
Skorokhod 2012[56]	0/3	1/12	RA	Pancreas	2–4	All: 23 (61) WS = 13 (69) WL = 10 (50)	WS: 66 (51–69) WL: 65 (57–74)	N/A	N/A	>10% WL of pre-illness
Smith 2010[57]	0/3	4/12	RA	Gastric	1–4	15 (67)	66 ± 11.6	15 (80)	57 ± 19.3	>5% WL
Stephens 2011[58]	0/3	2/12	RA	Esophageal gastric pancreas rectal	2–4	19 (58)	67 ± 10	6 (33)	53 ± 8	>10% WL (6 months)
Stephens 2015[59]	0/3	3/12	RA	Esophageal gastric pancreas and other	1–4	All: 92 (72) NC = 41 (82) CC = 51(63)	All: 65 ± 10 NC: 68 ± 9 CC: 63 ± 9	15 (53)	56 ± 17	>5% WL
Stretch 2013[23]	0/3	4/12	RA	Liver bile duct GI tract pancreas and other	NR	134 (51)	♂: 59 ± 13 ♀: 63 ± 13	N/A	N/A	N/A
Sun 2012[60]	0/3	5/12	RA	Gastric	1–4	102 (71)	62.13 ± 6.54	29 (72)	61.8 ± 6.4	>10% WL
Taskin 2014[61]	0/3	1/12	RA	Colorectal pancreas gastric and other	NR	All: 14 (50) NC = 8 (37) CC = 6 (66)	NC: 68 ± 5 CC: 70 ± 15	5 (40)	77 ± 5	>10% WL (6 months) weight stable <5%
Williams 1999[62]	0/3	2/12	RA	Colorectal	NR	6 (66)	67 (53–76)[a]	6 (83)	54 (22–92)[a]	N/A
	0/3	5/12	RA		NR	30 (70)		N/A	N/A	>5 kg WL (3 months)

(*Continues*)

Table 1 (continued)

Author	Bias[c]	Quality[d]	Muscle	Cancer site	Cancer Stage	Cancer population		Control group		Patient weight loss or cachexia criteria
						n (% male)	Age (years) mean ± SD	*n* (% male)	Age (years) mean ± SD	
Zeiderman 1991[63]				Esophageal gastric colorectal pancreas			Hospital diet: 67 ± 9.5 3 days intervention: 72 ± 3.2 7 days intervention: 67 ± 6.3			
Zampieri 2010[64]	0/3	3/12	RA, QF	Colorectal	NR	14 (36)	65.1 ± 10.3	Myopathy: 13 (38) Healthy = 19 (NR)	Myopathy: 64.3 ± 6.3 Healthy: 30.1 ± 13.3	N/A
Zampieri 2009[65]	0/3	1/12	RA, QF	Colorectal	2–3	10 (30)	65.1 ± 10.3	10 (NR)	22.7 ± 2.6	N/A
Zampieri 2010[66]	1/3	3/12	RA, QF	Colorectal	2–3	11 (36)	65.1 ± 10.3	7 (0)	44.5 ± 18.3	N/A
Aversa 2012[67]	1/3	3/12	RA, SA	NSCLC gastric	1–4	39 (74)	Lung: 66 ± 9 Gastric: 65 ± 10	10 (50)	Abdominal: 63 ± 10 Thoracic: 65 ± 12	NR
MacDonald 2015[68]	0/3	2/12	RA, QF	Esophageal gastric	1–4	All: 14 (57) WS = 6 (66) WL = 8 (50)	WS: 62.5 (57.0–70.3)[b] WL: 63.4 (61.5–66.3)[b]	7 (42)	52.1 (51.5–53.1)[b]	>5% WL
Shaw 1991[69]	0/3	6/14	RA, SCM	Colorectal pancreas head & neck thyroid and other	NR	All: 43 (42) WS = 25 (48) WL = 18 (66)	WS: 61 ± 20 WL: 64 ± 12.7	18 (33)	57 ± 16.9	>15% WL of pre-illness
Stephens 2010[70]	1/3	3/12	RA, VL, DIAPH	Esophageal gastric pancreas	NR	18 (66) WL	67 ± 8.4	3 (66)	45 ± 3.4	>5% WL
Brzeszczynska 2016[71]	0/3	2/12	QF	Esophageal gastric pancreas	2–3	All: 28 (75) NC = 18 (72) CC = 10 (80)	NC: 67 ± 10.5 CC: 65 ± 8.1	Middle age 20 (60) Elderly: 21 (52)	Middle age: 61 ± 7 Elderly: 79 ± 3.6	>5% WL of pre-illness
Ebhardt 2017[72]	0/3	1/12	QF	Esophageal gastric pancreas	NR	All: 19 (79) NC = 14 (85) CC = 5 (60)	Non-CC: 66.3 ± 10.2 CC: 64 ± 4.1	Non-sarcopenic 10 (60) Sarcopenic 8 (50)	Non-sarcopenic: 77.4 ± 2.3 Sarcopenic: 80.3 ± 3.9	>5% WL of pre-illness
Gallagher 2012[73]	1/3	7/14	QF	Esophageal gastric pancreas	1–3	12 (83)	65	6 (66)	58	NR
Christensen 2016[74]	N/A	13/14	VL	Testicular germ cell	NR	8 (100)	33.4 ± 7.5	Control = 9 (100) Ref = 13 (100)	Control: 37.8 ± 7.6 Reference group: 32.1 ± 6.3	N/A
	N/A	13/14	VL	Testicular germ cell	NR	15 (100)		19 (100)	31.5 ± 6.0	N/A

(*Continues*)

Table 1 (continued)

Author	Bias[c]	Quality[d]	Muscle	Cancer site	Cancer Stage	Cancer population		Control group		Patient weight loss or cachexia criteria
						n (% male)	Age (years) mean ± SD	*n* (% male)	Age (years) mean ± SD	
Christensen 2014[75]							Intervention: 34.4 ± 7.6 Control: 35.8 ± 8.9			
Lamboley 2017[76]	1/3	3/12	VL	Prostate	2	8 (100)	68 ± 5.6	14 (100)	71 ± 3.7	N/A
Nilsen 2016[77]	N/A	9/14	VL	Prostate	NR	12 (100)	67 ± 7	11 (100)	64 ± 6	N/A
Op den Kamp 2015[78]	0/3	6/12	VL	NSCLC	3–4	All: 26 (65) Pre-CC = 10 (80) CC = 16 (56)	Pre-CC: 62.4 ± 10.4 CC: 59.8 ± 8.2	22 (59)	61.4 ± 7.0	>5% WL (6 months)
Op den Kamp 2012[79]	0/3	3/12	VL	NSCLC	1–3	16 (93)	65.9 ± 7.5	10 (70)	63.7 ± 5.6	10% WL (6 months)
Op den Kamp 2013[80]	0/3	5/12	VL	NSCLC	3–4	All: 26 (65) Pre-CC = 10 (80) CC = 16 (56)	Pre-CC: 62.4 ± 10.4 CC: 59.8 ± 8.2	22 (59)	61.4 ± 7.02	5% WL (6 months) 2% WL with BMI <20 or sarcopenia
Phillips 2013[81]	0/3	4/14	VL	Colorectal	Early	8 (50)	62.5 ± 23.4	8 (50)	70.7 ± 4.5	N/A
Puig-Vilanova 2014[82]	1/3	3/12	VL	Lung	1–4	10 (100)	65 ± 9	Healthy = 10 (100) COPD = 16 (100)	65 ± 11 64 ± 9	Fat free mass index: <18.5 kg/m^2
Weber 2007[83]	0/3	3/12	VL	Gastric pancreas leukemia	NR	17 (53)	52.5 ± 6.5	27 (52)	57.9 ± 12.4	>10% WL (6 months)
Weber 2009[84]	0/3	2/12	VL	GI tract (not defined)	NR	19 (52)	58 ± 9	19 (53)	56 ± 7	>10% WL (6 months)
Williams 2012[85]	0/3	5/12	VL	Colorectal	Early	13 (46)	66 ± 10.8	8 (50)	71 ± 5.6	N/A
Banduseela 2007[86]	N/A	N/A	TA	NSCLC	NR	1 (100)	63	6 (50)	Healthy: 49 ± 7 Myopathy: 60 ± 18	NR
Higuchi 2000[87]	N/A	N/A	Gastroc	Gastric	NR	1 (100)	54	N/A	N/A	N/A
Jagoe 2002[88]	0/3	1/12	LD	Lung	3–4	36 (75)	64.1 ± 9	10 (40)	51.3 ± 15.1	Any % WL (6 months)
Bohlen 2018[89]	0/3	4/12	PM	Breast	1–4	14 (0)	56.5 ± 17.2	6 (0)	44.2 ± 7.4	N/A

Values reported as mean ± standard deviation (SD) unless indicated otherwise. BMI, body mass index; DIAPH, diaphragm; Gastroc, gastrocnemius; GI, gastrointestinal; LD, latissimus dorsi; N/A, not applicable; NC, non-cachexia; NIH-NHLBI, National Heart Lung and Blood Institute; NSCLC, non-small cell lung carcinoma; NR, not reported; PM, pectoralis major; QF, quadriceps femoris; RA, rectus abdominis; TA, tibialis anterior; SA, serratus anterior; SCM, sternocleidomastoid; SMI, skeletal muscle index; VA, vastus lateralis; WL, weight loss; WS, weight stable.

[a]Median (range).

[b]Median (interquartile range).

[c]Modified Newcastle–Ottawa scale.

[d]Quality assessment score—high score means high quality.

specify the sex of their patients. For those studies including both sexes, 50 had an imbalance between treatment groups in the % of male and female patients, and only 3 studies matched the number of male and female participants. When reporting the results, almost all of the studies (98%) presented aggregate data from men and women.

When a non-cancer control group was employed in the study, the majority of studies included control groups that went under surgical procedures (i.e. cholelithiasis and cholecystitis, ovarian cyst, inguinal hernia, laparocele, abdominal aorta aneurysm, hemangioma of liver, gallstones, and chronic pancreatitis) or healthy volunteers (Supporting Information, *Table* S2). No study defined the criteria used to select healthy volunteers. *Table* 1 highlights the features of the cancer groups compared with control groups. More than 54% of the studies included cancer patients with an average age of ≥65 years, and for studies involving non-cancer patients as controls, 26% included patients with an average age of ≥65 years.

Most (33/59) reports failed to mention co-morbidities as a component of their exclusion criteria or patient's demographics. Commonly excluded diagnoses were diabetes, chronic obstructive pulmonary disease, liver failure, renal failure, chronic hepatitis, autoimmune diseases, and inflammatory bowel disease. Use of medications (e.g. corticosteroids, anabolic/catabolic agents, and/or beta blockers) was described in 17 studies as clinical characteristics or exclusion criteria. Prior exposure to antineoplastic drugs was reported in 14/59 studies. Inclusion of patients naïve to chemotherapy or radiotherapy was stated in 6/59 studies, two studies acknowledged the inclusion of some patients with one or fewer cycles of chemotherapy that concluded 4 weeks previous to biopsy collection.

Technical considerations

Biobanking protocol and tissue manipulation

Abdominal and thoracic muscle biopsies were collected during a surgical procedure in 43 studies, with collection at the start of surgery being explicitly stated in 31 studies (*Table* 2). Presence or absence of tissue cauterization was specified in 29/43 studies. Percutaneous procedure (needle biopsy) was the main method for collection of muscles of the lower limb (n = 19 studies), open muscle biopsy technique was reported in one study, and in one study, the collection method was unspecified. For both surgical and percutaneous biopsies, removal of blood traces and/or fat/fibrotic tissue after collection was mentioned in 7/59 studies (*Table* 2).

Information provided on biopsy manipulation was limited and mainly focused on freezing and storage procedures. In 43/59 studies, immediate freezing in liquid nitrogen was reported. In only one study was it explicitly stated that freezing was done in the operating room vs. a laboratory facility. The most common temperatures for sample storage were between −70°C and −80°C; storage details were not mentioned in 11/59 studies. Details on time between biopsy and transportation to laboratory facilities and waiting periods were not reported in any study.

Rectus abdominis biological characterization

Study population

Demographics and clinical data from 190 patients are provided in *Table* 3. Nearly all patients (97%) who were approached consented to intraoperative biopsy, as this entails little, if any, incremental discomfort as the surgery is inherently invasive. Therefore, there was no selection bias inherent in the cohort. Typical of hepato-pancreatic-biliary case load, 88% of cancers were gastrointestinal, with the largest proportions being colorectal and pancreatic cancer. Surgical procedures included hepatectomy, liver metastasectomy, pancreatectomy, Whipple procedure, bile duct resection, cholecystectomy, colectomy, and gastrectomy. Metastasis was present in 50% of the patients. Most of the patients were naïve to chemotherapeutic agents, 23% had exposure to chemotherapy within 2 to 4 weeks prior to the surgical procedure. The majority of patients were classified as overweight. Diabetes type II and hypertension were the most common co-morbidities. Most commonly used medications reported among the population were analgesics, anti-inflammatory, statins, glucose-lowering drugs, anti-hypertensives, anti-reflux, and thyroid hormone replacement (*Table* 4).

Computed tomography image analysis

Muscle L3-CSA, SMI, and muscle radiodensity of rectus abdominis and total muscle are shown in *Table* 5. Sarcopenia was present in 56% of the patients, 60% (n = 97) of men and 49% (n = 42) of women. Weight history was available for 45 patients. Fifty-six per cent of patients experienced weight loss (11 ± 12% in 5 ± 12 months), and 60% of weight-losing patients were sarcopenic. Out of 44% (n = 20) weight stable patients, 70% were sarcopenic.

Sex differences

In light of the fact that most of the papers in the literature review included samples of mixed sex of varying proportions, we examined all of the biopsy features for sex differences. Sexual dimorphism was prominent in L3-CSA total lumbar muscle and RA, muscle radiodensity of RA and total muscle (*Table* 5), mean fibre CSA (*Table* 6), and in expression of genes associated with muscle growth, apoptosis, and inflammation (*Table* 7). Proportions of fibre types using both quantification methods, MyHC isoforms and individual fibre types, were not different between male and female patients (*Table* 6).

For centralized nuclei assessment, the mean % of fibres with centralized nuclei was 12 ± 9% (4 to 36%) and 10 ± 8%

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Table 2 Biopsy collection and handling procedures across the studies

Author	Biopsy collection (collected in start or end of surgery)	Cauterized	Blood traces, fat, or connective tissue removed	Sample handling and storage conditions
Acharyya 2005[35]	NR	NR	NR	NR
Agustsson 2011[36]	Initial phase of surgery	NR	NR	Incubated in vitro
Aversa 2016[37]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Aversa 2012[67]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Banduseela 2007[86]	Percutaneous biopsy (local anaesthesia)	N/A	Yes (fat, connective tissue)	Immediately frozen, stored at −80°C
Bohlen 2018[89]	NR	N/A	NR	Stored in RNA stabilization solution at −4°C overnight and then stored at −80°C
Bonetto 2013[38]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Bossola 2006[39]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Bossola 2001[40]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Bossola 2003[41]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Brzeszczynska 2016[71]	Initial phase of surgery	No	Yes (blood)	Immediately frozen, stored at −80°C
Busquets 2007[42]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Christensen 2016[74]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immediately frozen, stored at −80°C
Christensen 2014[75]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immediately frozen, stored at −80°C
DeJong 2005[43]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
D'Orlando 2014[44]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Ebhardt 2017[72]	Percutaneous biopsy (local anaesthesia)	N/A	Yes (blood)	Immediately frozen, stored at −80°C
Eley 2008[45]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Gallagher 2012[73]	Percutaneous biopsy (local anaesthesia)	N/A	Yes (blood)	Immediately frozen, stored at −80°C
Higuchi 2000[87]	NR	N/A	NR	NR
Jagoe 2002[88]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Johns 2017[22]	Initial phase of surgery	No	NR	Immediately frozen, stored in liquid nitrogen
Johns 2014[46]	Initial phase of surgery	NR	Yes (blood)	Immediately frozen, stored at −80°C
Khal 2005[47]	NR	No	NR	Immediately frozen, stored at −70°C
Lamboley 2017[76]	Percutaneous biopsy (local anaesthesia)	N/A	Yes (blood)	Immediately and stored in liquid nitrogen
Lundholm 1976[48]	Initial phase of surgery	NR	NR	Muscle fibre isolation on fresh tissue
MacDonald 2015[68]	Initial phase of surgery and percutaneous biopsy (local anaesthesia)	NR	NR	Immediately frozen in liquid nitrogen, storage temperature NR
Marzetti 2017[49]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Narasimhan 2017[21]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Narasimhan 2018[20]	Initial phase of surgery	No	NR	Immediately frozen, stored at −80°C
Nilsen 2016[77]	Percutaneous biopsy (local anaesthesia)	N/A	Yes (fat)	Frozen by immersion in isopentane, stored at −80°C
Noguchi 1998[50]	Initial phase of surgery	NR	NR	Immediately frozen in situ, stored at −70°C
Op den Kamp 2015[78]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immediately frozen, stored at −70°C
Op den Kamp 2012[58]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immediately frozen, stored at −80°C
Op den Kamp 2013[80]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Frozen by immersion in isopentane, stored in −80°C
Pessina 2010[51]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Phillips 2013[81]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immunoblotting in fresh tissue
Prokopchuk 2016[52]	NR	NR	NR	Immediately frozen and stored at −80°C
Puig-Vilanova 2014[82]	Open muscle biopsy technique	N/A	NR	NR
Ramage 2018[53]	NR	NR	NR	Immediately frozen, stored at −80°C
Rhoads 2009[54]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Schmitt 2007[55]	NR	NR	NR	Immediately frozen in liquid nitrogen, storage temperature NR
Shaw 1991[69]	NR	NR	NR	Snap-frozen in liquid nitrogen, thawed after 48 h
Skorokhod 2012[56]	Initial phase of surgery	NR	NR	Immediately frozen in liquid nitrogen, storage temperature NR
Smith 2011[57]	Initial phase of surgery	No	NR	Immediately frozen and stored at −80°C

(*Continues*)

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Table 2 (continued)

Author	Biopsy collection (collected in start or end of surgery)	Cauterized	Blood traces, fat, or connective tissue removed	Sample handling and storage conditions
Stephens 2011[58]	Initial phase of surgery	NR	NR	Fixation for microscopy
Stephens 2010[70]	Rectus abdominis–NR Quadriceps–percutaneous biopsy (local anaesthesia)	NR	NR	Immediately frozen
Stephens 2015[59]	Initial phase of surgery	NR	Yes (blood)	Immediately frozen, stored at −80°C
Stretch 2013[23]	Initial phase of surgery	No	NR	Immediately frozen and stored in liquid nitrogen
Sun 2012[60]	Initial phase of surgery	NR	NR	Immediately frozen and stored at −80°C
Taskin 2014[61]	NR	NR	NR	Transferred to lab on ice cold buffer, stored at −20°C
Weber 2007[83]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immediately frozen, stored at −80°C
Weber 2009[84]	Percutaneous biopsy (local anaesthesia)	N/A	NR	Immediately frozen, stored at −70°C
Williams 2012[85]	Percutaneous biopsy (local anaesthesia)	N/A	NR	NR
Williams 1999[62]	Initial phase of surgery	No	NR	Immediately frozen, stored at −70°C
Zampieri 2010[66]	Rectus abdominis–NR Quadriceps–Percutaneous biopsy (local anaesthesia)	NR	NR	Immediately frozen and stored in liquid nitrogen
Zampieri 2009[65]	Rectus abdominis–NR Quadriceps–Percutaneous biopsy (local anaesthesia)	NR	NR	Immediately frozen and stored in liquid nitrogen
Zampieri 2010[64]	Rectus abdominis–NR Quadriceps–Percutaneous biopsy (local anaesthesia)	NR	NR	Immediately frozen and stored in liquid nitrogen
Zeiderman 1991[63]	Initial phase of surgery	NR	NR	Incubation

N/A: not applicable.

(3 to 27%) in men and women, respectively. No differences were found between men and women (p=0.39) with a combined mean value of 11 ± 8%.

Rectus abdominis: proportion of fibre types and muscle fibre area

Electrophoretic analysis of MyHC isoforms confirmed MyHC I and MyCH IIA to be present at similar proportions, while MyHC IID was less abundant (*Table* 6A). MyHC type IIA was the most abundant isotype, followed by MyHC type I and IID (*Table* 6B). In addition, 15.5% of the fibres were identified as hybrids, which is the sum of MyHC type I/IIA and IIA/D. For individual fibre types, type I fibres comprised the greatest proportion (46.4%) followed by fibre type IIA (36.1%) and hybrid type IIA/D (15%). Presence of fibre type IID, as well as hybrid type I/IIA, was minimal (1.8% and 0.7%). Mean muscle fibre area (μm^2) was calculated by the detection of membrane (laminin/dystrophin antibody) fluorescence on 1069 ± 771 muscle fibres per biopsy (*Table* 6C). Mean muscle fibre area was determined in total and per fibre type, which includes collective results of MyHC isoforms and individual fibre types (*Table* 6C). Mean fibre area of MyHC type I was smaller than MyHC type IIA and IID. For individual fibre types, type I and type I/IIA were smaller compared with type IIA, IIA/D, and IID. Type IID had the largest mean fibre area compared with the other individual fibre types.

Age effects

Comparison of older (74 ± 4 years, *n* = 13) and younger (50 ± 6 years, *n* = 13) men revealed no differences between groups with respect to mean muscle fibre area (total, individual fibre types, or MyHC isoforms), % of individual fibre types, or % of MyHC isoforms. Age effect was evaluated in men (*n* = 26) by comparing mean values of a younger group vs. an older group. No significant differences were found in relation to % of MyHC isoform content.

Skeletal muscle gene expression for genes associated with cancer cachexia

Differences in genes encoding proteins commonly explored in cancer-muscle wasting are summarized in *Table* 7 (also see Supporting Information, *Table* S3). Atrophy, autophagy, apoptosis, muscle growth, and inflammation genes were selected based on reviewed literature on muscle atrophy in cancer.[22,40,41,47,50,70,87,88,108] Sexual dimorphism exists in pathways related to skeletal muscle anabolism and catabolism illustrating the need for caution when generalizing results from only one sex or discussing results from a mixed group of cancer patients.

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Table 3 Patient characteristics

	Male (n = 122)	Female (n = 68)	P values
Age, mean years ± SD (Min–Max)	61 ± 12 (19–87)	65 ± 12 (21–87)	0.049
Tumour type, % (n)			0.006
Colorectal	45 (55)	26 (18)	
Pancreas	23 (28)	31 (21)	
Other gastrointestinal[a]	25 (31)	22 (15)	
Other[b]	6 (8)	20 (14)	
Presence of metastasis, % (n)	56 (68)	40 (27)	0.03
Chemotherapy exposure within 4 weeks prior to muscle biopsy, % (n)	23 (28)	22 (15)	0.9
Patients with weight lost, % (n)	56 (14)[c]	55 (11)[d]	0.9
Sarcopenia, % (n)	60 (61)[e]	50 (23)[f]	0.2
BMI (kg/m^2), mean ± SD	27 ± 5	28 ± 7	0.7
BMI classification, % (n)			0.1
Underweight	1 (1)	1 (1)	
Normal	26 (32)	26 (18)	
Overweight	39 (48)	28 (19)	
Obesity I	17 (21)	6 (4)	
Obesity II	5 (6)	10 (7)	
Obesity III	2 (2)	4 (3)	
Missing BMI	10 (12)	24 (16)	
Co-morbidities, % (n)			
Diabetes type II	12 (15)	18 (12)	0.3
Hypertension	24 (29)	29 (20)	0.4
Cardiovascular disease	15 (18)	7 (5)	0.1
Dyslipidemia	7 (9)	7 (5)	0.9
History of smoking habit, % (n)	28 (34)	24 (16)	0.3
Computed tomography, body composition analysis, mean ± SD			
Subcutaneous adipose tissue (cm^2)	166.4 ± 91.5[g]	251.1 ± 134.4[h]	<0.001
Visceral adipose tissue (cm^2)	174.8 ± 105.1[g]	111.9 ± 65.7[h]	<0.001
Muscle biopsy triglyceride content (μg/mg), mean ± SD	13.2 ± 14.8[i]	29.5 ± 21.7[j]	<0.001

Differences between men and women were analysed by independent t-test (continuous variables) and χ^2 test (categorical variables). BMI, body mass index.
[a]Other gastrointestinal: stomach, small intestine, liver, intrahepatic bile duct, gallbladder, biliary tract, and appendix.
[b]Other: adrenal gland, skin, kidney, mesothelium, lymphoma, melanoma, chronic lymphocytic leukemia, prostate, ovary, uterus, head, and neck.
[c]Patients with weight loss information: n = 25.
[d]Patients with weight loss information: n = 20.
[e]Patients with sarcopenia information: n = 102.
[f]Patients with sarcopenia information: n = 46.
[g]CT adipose tissue information: n = 98.
[h]CT adipose tissue information: n = 44.
[i]Patients with muscle biopsy triglyceride content: n = 69.
[j]Patients with muscle biopsy triglyceride content: n = 19.

Discussion

There is a perceived need to understand the human biology of cancer-associated muscle atrophy and to frame it in the context of our larger understanding of experimental findings.[6,22,109–111] The emergent literature on human muscle biopsies has been generated with that intent but has a number of substantial limitations within the study design as well as procedures for collection and preparation of the biopsy material. At the same time, there is substantial opportunity for collaboration between cancer surgeons and researchers to obtain intraoperative biopsies with a high rate of patient consent and the additional capability to describe the muscles of these patients with precise radiological metrics. Agreement to a set of standardized procedures and reporting will enhance the consistency, reliability, and comparability of future research in this area. Evaluation of human rectus abdominis muscle presents the expected variation in several measures that may be of interest for emerging studies in this area.

Study quality and design

The quality of the studies reporting on biopsy material to characterize varying features of muscle biology was uniformly low. Quality assessment tools revealed several inconsistencies in sample selection strategies, study design, data collection, and analysis in the existing literature. Bias assessment of sample selection exposed a clear absence of sample representativeness in 59% of studies and lack of sample size justification in 96% of studies. In 75% of the studies reviewed, samples from a relatively small number of participants (n =

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Table 4 Most common medications prescribed and potential effects on skeletal muscle

Class of drug	Medication	% (*n*)	Common use	Possible implications to skeletal muscle
Cyclooxygenase inhibitors	Aspirin and acetaminophen	15 (29)	Pain, fever, inflammation, and prevention of cardiovascular disease	Influence muscle prostaglandin synthesis, muscle protein metabolism, and cellular processes regulating muscle protein synthesis[90–93]
HMG-CoA reductase inhibitors	Rosuvastatin, simvastatin, and atorvastatin	13 (24)	Lipid lowering	Association with myalgia and related symptoms. Associated to mitochondrial oxidative stress[94,95]
Biguanide	Metformin	8 (16)	Type 2 diabetes, suppressor of hepatic gluconeogenesis	Mitochondrial dysfunction in skeletal muscle. Sensitizes muscle to insulin; increases glucose disposal in skeletal muscle[95–98]
Proton pump inhibitors	Omeprazole and pantoprazole	8 (16)	Gastroesophageal reflux and erosive esophagitis	Concomitant administration with atorvastatin and dexamethasone is associated to increase risk of myopathy[99]
Hormones	Levothyroxine	7 (13)	Thyroid hormone (T4) deficiency	Influences myogenesis, associated with sarcopenia and myopathy[15,100]
Angiotensin converting enzyme inhibitor	Ramipril	7 (13)	Hypertension and congestive heart failure	Associated with larger muscle cross sectional area and muscle remodeling, associated with cancer cachexia[99–104]
Thiazide diuretic	Hydrochloro-thiazide	6 (12)	Hypertension and diuretic by reducing sodium reabsorption	None reported or reviewed
Calcium channel blockers	Amlodipine	5 (9)	Hypertension and calcium channel blocker	None reported and reviewed[105]
Opioid	Oxycodone	3 (5)	Pain	Hypogonadism and testosterone depletion in men[106]
Alpha-adrenergic blocker	Tamsulosin	3 (5)	Muscle relaxer of prostate and bladder	None reported or reviewed
Xanthine oxidase inhibitor	Allopurinol	3 (5)	Gout prevention and decrease blood uric acid levels	Prevents skeletal muscle atrophy[107]
Anticoagulant	Warfarin	3 (5)	Anticoagulant	None reported or reviewed

Percentage of patients prescribed this medication out of a total of 190 patients who had a medical history available with information provided on current medication use.

≤30) were evaluated without accounting for age or sex variation.

The majority of published studies use weight loss (vs. weight stability) to define cachexia. This approach is limited by not accounting for the characteristics of muscle (muscle mass or change in muscle over time), which are the clinically relevant features related to cancer outcomes. Indeed, weight stable patients may well be losing muscle over time[112] and they can also be profoundly sarcopenic.[2,27] Weight loss was the most commonly used criteria for cancer cachexia assessment; however, application of this measure alone poses major concerns in misclassification and unintended exclusion of cachectic patients. Many studies were published prior to the widespread use of CT images to quantify muscle, as well as prior to the publication of the international cachexia consensus, which defines muscle mass as a diagnostic criterion for cachexia.[35,36,39–42,45,47,48,50,54,55,57,63–66,69,70,83,84,86–88,108] The premise of using weight loss when muscle is being evaluated is erroneous. Muscle wasting can be experienced by patients with less than 5% weight loss.[112] Also, the arbitrary selection of weight loss percentage and time frame in different studies complicates the comparison of results between studies. In the cohort of patients we evaluated, 70% of weight stable patients and 60% of weight-losing patients were sarcopenic. Therefore, assessment of muscle mass is essential, and this can be easily achieved through the secondary analysis of CT images used to plan the surgery.[18,19,29]

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Table 5 Computed tomography defined muscle composition at L3 for *rectus abdominis* and total skeletal muscle in cancer patients, stratified by sex and age decade

Sex	Age stratum	*N*	Rectus abdominis	Total lumbar muscle	Lumbar skeletal muscle index	Rectus abdominis	Total lumbar muscle
			L3-CSA (cm^2)		cm^2/m^2	Radiodensity (Hounsfield units)	
Male	<50	17	15.9 ± 3.8 (9.8–23.4)	188.7 ± 29.1 (123.6–238.2)	58.2 ± 8.9 (42.8–73.3)	36.2 ± 12.3 (7.6–54.8)	39.6 ± 10.5 (15.4–55.3)
	50–60	34	13.6 ± 3.9 (6.6–24.5)	156.2 ± 27.5 (107.2–228.9)	50.6 ± 8.2 (37.1–66.5)	30.9 ± 12.2 (4.4–50.0)	36.5 ± 8.9 (13.8–50.5)
	60–70	23	13.3 ± 3.3 (5.7–19.4)	158.4 ± 20.7 (109.0–192.5)	50.8 ± 6.6 (36.4–60.8)	28.0 ± 12.3 (−10.8–44.3)	33.8 ± 10.1 (7.1–54.4)
	70–80	23	11.5 ± 2.6 (6.0–17.6)	141.4 ± 23.0 (94.6–187.2)	46.6 ± 6.0 (35.6–59.1)	20.0 ± 11.3 (−2.0–44.6)	28.9 ± 7.7 (10.0–42.6)
	>80	4	9.8 ± 4.2 (6.2–15.2)	139.0 ± 16.4 (122.8–160.9)	46.1 ± 7.1 (40.1–56.3)	21.5 ± 8.3 (12.3–30.4)	27.5 ± 3.0 (24.8–31.5)
Female	<50	3	9.3 ± 3.2 (5.9–12.2)	114.9 ± 14.8 (97.8–124.4)	43.8 ± 1.6 (42.9–45.7)	32.0 ± 5.7 (26.6–38.0)	45.1 ± 5.3 (40.5–50.9)
	50–60	11	7.0 ± 2.4 (3.8–10.9)	101.5 ± 16.8 (67.5–125.4)	38.3 ± 6.8 (23.9–46.4)	22.7 ± 13 (4.2–41.1)	35.4 ± 7.6 (20.9–46.1)
	60–70	15	8.7 ± 3.7 (2.8–16.9)	102 ± 16.6 (66.2–122.7)	39.2 ± 7.0 (27.7–52.8)	19.1 ± 10.3 (2.5–39.1)	29.0 ± 7.1 (18.2–39.6)
	70–80	16	6.7 ± 2.3 (1.4–10.9)	101.0 ± 13.8 (79.0–127.3)	40.5 ± 4.8 (33.8–49.7)	13.1 ± 10.0 (−7.7–30.9)	28.9 ± 7.0 (15.0–38.9)
	>80	3	7.7 ± 3.1 (4.2–10.0)	92.8 ± 14.8 (77.9–107.5)	41.1 ± 8.1 (32.9–49.1)	12.2 ± 19.8 (−10.1–27.6)	22.9 ± 4.1 (18.2−25.3)
Total male		101	13.6 ± 3.8 (5.7–24.5)	158.2 ± 29 (94.6–238.2)	50.8 ± 8.3 (35.6–73.3)	28.2 ± 12.9 (−10.8–54.8)	34.3 ± 9.7 (7.1–55.3)
Total female		48	7.6 ± 2.9 (1.4–16.9)	101.7 ± 15.4 (66.2–127.3)	39.8 ± 6 (23.9–52.8)	18.2 ± 12 (−10.1–41.1)	31 ± 8.3 (15–50.9)

Values reported in mean ± SD (range). CSA, cross-seccional area; L3, 3rd Lumbar vertebra.

Table 6 Rectus abdominis myosin heavy chain content and mean muscle fibre area of cancer patients

	All	Male	Female	*P* value
A. MyHc content by electrophoresis[a] (% ± SD *N* = 40 M/*n* = 8 F)				
MyHC I (%)	39.3 ± 11.1	39.1 ± 10.3	40.6 ± 15.6	0.73
MyHC IIA (%)	38.4 ± 11.1	37.5 ± 10.0	42.6 ± 15.7	0.24
MyHC IID (%)	22.3 ± 8.9	23.4 ± 8.6	16.8 ± 9.1	0.06
B. MyHc content by immunohistochemistry[a] (% ± SD *N* = 20 M/*n* = 10 F)				
MyHC isoforms (%)				
MyHC type I	47.1 ± 13.0	47.0 ± 12.6	47.3 ± 14.6	0.91
MyHC type IIA	51.8 ± 13.4	52.4 ± 12.6	50.5 ± 15.6	0.53
MyHC type IID	16.7 ± 14.3	19.2 ± 13.7	11.8 ± 15.1	0.19
All Hybrids[b]	15.5 ± 13.5	18.5 ± 13.5	9.6 ± 12.2	0.08
Individual fibre types (%)				
Fibre type I	46.4 ± 12.9	48.9 ± 9.4	46.2 ± 14.2	0.32
Fibre type I/IIA	0.7 ± 1.0	0.6 ± 0.9	1.2 ± 1.6	0.15
Fibre type IIA	36.1 ± 9.5	35.7 ± 9.4	40.7 ± 9.6	0.71
Fibre type IIA/D	15.0 ± 13.7	13.1 ± 12.4	8.5 ± 12.9	0.39
Fibre type IID	1.8 ± 4.6	1.7 ± 3.7	3.4 ± 7.3	0.32
C. Mean muscle fibre area (μm^2) (% ± SD *N* = 20 M/*n* = 10 F)				
All fibres	3236 ± 1390	3784 ± 1285	2139 ± 854	<0.05
MyHC isoforms (μm^2)				
MyHC type I	2323 ± 944	2591 ± 970	1786 ± 635	<0.05
MyHC type IIA	4009 ± 1937	4848 ± 1725	2331 ± 1054	<0.05
MyHC type IID	4026 ± 2060	4722 ± 1895	2461 ± 1546	<0.05
Individual fibre types (μm^2)				
Fibre type I	2325 ± 941	2591 ± 970	1795 ± 633	<0.05
Fibre type I/IIA	2253 ± 1209	2726.6 ± 1181	1306 ± 528	<0.05
Fibre type IIA	3940 ± 1970	4760 ± 1820	2299 ± 1012	<0.05
Fibre type IIA/D	4012 ± 2055	4833.5 ± 1841	2266 ± 1268	<0.05
Fibre type IID	5243 ± 2407	5323 ± 2553	4729 ± 1524	0.75

MyHC: myosin heavy chain.
[a]There were no differences in age, BMI, metastasis, chemotherapy exposure, co-morbidities, nor smoking history between men and women.
[b]All hybrids refer to fibres of mixed myosin heavy chain isoforms MyHC type I/IIA and MyHC type I.

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Table 7 Skeletal muscle gene expression for genes associated with cancer cachexia in cancer patients[a]

Biological function	Gene symbol	Gene name	Agilent transcript ID [Refseq RNA ID]	Female (n = 64)	Male (n = 69)	P value
Atrophy	FOXO1	Forkhead box O1	A_24_P22079	1.53 ± 1.04	1.11 ± 0.68	0.005
Autophagy	BECN1	Beclin 1	A_23_P433071 [NM_003766]	0.91 ± 0.27	1.03 ± 0.3	0.05
			A_23_P89410 [NM_003766]	1.00 ± 0.27	1.11 ± 0.33	0.05
	CTSL2	Cathepsin L2	A_23_P146456 [NM_001333]	1.31 ± 0.57	0.99 ± 0.44	<0.0001
Apoptosis	CASP8	Caspase 8	A_23_P209389 [NM_033355]	0.97 ± 0.32	1.09 ± 0.38	0.08
	CASP9	Caspase 9	A_23_P97309 [NM_001229]	0.95 ± 0.19	1.06 ± 0.25	0.008
			A_24_P111342 [NM_001229]	0.97 ± 0.22	1.08 ± 0.31	0.03
Muscle growth	AKT1	V-Akt murine thymoma viral oncogene homolog 1	A_23_P2960 [NM_005163]	1.23 ± 0.52	1.04 ± 0.35	0.03
	DMD	Dystrophin	A_24_P342388 [NM_004019]	1.34 ± 0.67	0.94 ± 0.29	<0.0001
			A_24_P185854 [NM_004010]	1.11 ± 0.27	0.94 ± 0.23	<0.0001
			A_24_P34186 [NM_004010]	1.19 ± 0.55	0.97 ± 0.39	0.01
			A_32_P199796 [NM_004023]	1.27 ± 0.66	0.98 ± 0.42	0.005
	MSTN	Myostatin	A_23_P165727 [NM_005259]	1.71 ± 2.43	2.74 ± 3.74	0.02
	PAX7	Paired box 7	A_23_P126225 [NM_013945]	0.99 ± 0.49	1.08 ± 0.39	0.05
			A_23_P500985 [NM_013945]	0.96 ± 0.45	1.03 ± 0.33	0.09
	PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	A_24_P303052 [NM_013261]	1.22 ± 0.77	1.00 ± 0.51	0.07
	SMAD3	SMAD family member 3	A_23_P48936 [NM_005902]	1.14 ± 0.42	1.00 ± 0.28	0.07
	TGFB1	Transforming growth factor, beta 1	A_24_P79054 [NM_000660]	1.42 ± 1.47	1.06 ± 0.54	0.01
Inflammation	JAK1	Janus kinase 1	A_24_P410678 [NM_002227]	0.92 ± 0.37	1.15 ± 0.43	0.001
	JAK2	Janus kinase 2	A_23_P123608 [NM_004972]	1.21 ± 0.48	1.06 ± 0.45	0.03
	JAK3	Janus kinase 3	A_23_P329112 [NM_000215]	1.03 ± 0.46	1.19 ± 0.57	0.09
	STAT3	Signal transducer and activator of transcription 3	A_23_P107206[NM_213662]	1.21 ± 1.02	0.53 ± 0.35	0.02
	STAT5A	Signal transducer and activator of transcription 5A	A_23_P207367 [NM_003152]	1.12 ± 1.01	0.32 ± 0.34	0.03
			A_24_P173088 [NM_003152]	1.19 ± 1.00	0.47 ± 0.45	0.005
	TNF	Tumor necrosis factor	A_24_P50759 [NM_000594]	0.99 ± 0.35	1.15 ± 0.44	0.03

Values (unitless) reported as mean ± standard deviation.
[a]Cancer type (0.003) and metastasis presence (0.002) were different between men and women. There were no differences in age, BMI, chemotherapy exposure, co-morbidities, nor smoking history between men and women.

Some authors reported mortality-defined cutpoints to define sarcopenia according to age and sex of a reference population[27,113] and these have been secondarily used by other authors.[114] Caution should be used in applying these cutpoints to define sarcopenia in patients undergoing muscle biopsy, and these may not necessarily reflect the population from which biopsies are evaluated.[114] Here, we suggest to use CT to quantify muscle features for the overall population from which the biopsy sampling is done. In this way, patients providing biopsy for our study are clearly representative of the entire L3 SMI distribution of our regional population (Alberta, Canada) (*Figure* 2). This representation eliminates the possibility of sampling bias. It also allows each patients' SMI to be ranked within the population distribution overall as well as compared with values available for healthy young individuals.[115]

Age and sex differences exist at the level of muscle function, biochemistry/metabolism, and mass.[14,17,116] The majority of studies reported combined data from both sexes without acknowledging sexual dimorphisms. Age was generally not accounted for. In the first 40 years of life, muscle mass is relatively stable in both men and women, and then it begins to decline; however, the rate of loss is slower in women than in men.[62] In our sample, differences between men and women were observed for muscle fibre area, SMI, and muscle radiodensity. Sexual dimorphism in gene expression was not limited to a particular pathway or function but was identified in growth (AKT1, FOXO1, MSTN, PAX7, and TGFα1), apoptosis (CASP9), and inflammation (TNF and STAT3). In relation to the age effect, we did not find any significant differences in mean muscle fibre area and proportion of fibre types when comparing young vs. old male cancer patients; this could be potentially explained by the narrow age range in our study. Differences between young (18 to 48 years) and older (66 to 99 years) participants[117] have been reported for fibre type distribution in rectus abdominis and vastus lateralis. Therefore, age differences and sexual dimorphism must be acknowledged when comparing, reporting, and interpreting muscle characteristics.

Here, we present many characteristics of human rectus abdominis muscle. We obtained a detailed analysis of its radiological features, for the first time. Our analysis of fibre

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type is multidimensional and confirms the mixed fibre distribution of the rectus abdominis. A prior study in cancer patients with upper gastrointestinal malignancies reported mean values of 48% and 55% for MyHC type I and IIa, respectively[46]. Muscle gene expression and TG content levels as presented here are new information about rectus abdominis. Future work on rectus abdominis can be usefully planned, using this base of information. The majority of evidence to date (*Table* 1) on muscle from cancer patients is coming from rectus abdominis. Due to the unique characteristics of each muscle type, we suggest that future researchers identify candidate muscles for intensive research using the principle that the muscle(s) most often transected in cancer surgeries would be the greatest resource. This can be decided in function of the common surgical approaches. Thus, over time, a large base of evidence may be obtained from *latissimus dorsi*, *serratus anterior*, or intercostal muscle (e.g.) from thoracic cancer surgeries.

A key component of case-control studies is to provide details of the control group relative to the research question. However, this is rarely done in the literature that we reviewed.[20,21] Detailed clinical characterization of non-cancer controls is usually missing, and assumption of a healthier status of the control group when compared with cancer patients is common. In many cases, the comparator group is a non-cancer surgical patient population; however, there is no documentation provided around diagnosis or medications. Presumably, healthy volunteers could have underlying co-morbid conditions or be taking medications that impact skeletal muscle. Co-morbidities and use of medications were not generally mentioned either for patients undergoing non-cancer surgery or 'healthy' volunteers recruited outside the clinical setting. Approximately 60% of people diagnosed with malignancy are 65 years and older.[13] Prevalence of co-morbidity in cancer population ranges from 30% to 50% depending on type of cancer[19] and a patient with history of cancer has on average three co-morbidities.[118,119] Diabetes and hypertension were the most common conditions in our patient population, but cardiovascular disorders and mental health problems are also prevalent in the cancer population.[13,19] These chronic conditions and medications taken to control them can independently affect muscle physiology[15,106,120–128] (*Table* 4). COX inhibitors, statins, biguanides, proton pump inhibitors, and thyroid hormones were the most common medications prescribed in our patient population apart from those prescribed during cancer treatment. These classes of drugs have known effects on muscle protein synthesis[90–92,129] and catabolism,[130–133] atrophy pathways,[134] insulin sensitivity,[96] and mitochondria function.[97] Therefore, it is important that for both the cancer group and 'control' groups have a detailed medical history that captures diagnosis of other conditions and medications. In addition to drugs prescribed for management of co-morbid conditions, antineoplastic treatment previous to tissue biopsy is also a relevant event that may impact interpretation of results as the long-lasting effects in the muscle are unknown.[135]

Technical considerations

We suggest recommendations for minimum procedures to follow in biobanking practices, tissue manipulation, and patient characterization to enhance the consistency, reliability, and comparability of future research (*Table* 8). Acknowledgement of differences between muscle groups is essential when comparing and interpreting results. RA is commonly collected in patients with gastrointestinal disease due to its practicality in relation to the surgical incision while maintaining patient burden to the essential minimum. Its broad extension in the abdominal area enables for collection of muscle tissue from a variety of locations[136]; however, no one has demonstrated how homogeneous the RA is in relation to the biopsy site. On the other hand,

Table 8 Summary of recommendations for muscle biopsy processing and population characterization

(A) Biobanking protocols and tissue manipulation
- · For intraoperative muscle biopsies, collect at the start of the surgical procedure and avoid cauterization.
- · Avoid or report the use of foreign substances (e.g. use of saline-moistened gauzes).
- · Report waiting periods between surgical/needle removal, transportation to other facilities, and freezing; include the use or not of crushed ice during the waiting process.
- · Report any removal of blood traces or unrelated tissue from the muscle biopsy.
- · If muscle is 'immediately frozen,' clarify the location, time, and other relevant details (e.g. RNA stabilizer solution) of this action after the surgical removal.
- · Sample storage recommended $\leq -70°C$; however, the temperature selection will depend on the molecules of interest and/or experimental techniques.

(B) Cancer population characterization
- · Clearly state the patient selection method and possible limitations.
- · Report information on metastatic status or tumour classification.
- · Report co-morbidities and medications.
- · Report past or current exposure of antineoplastic treatments.

(C) Inclusion of control groups
- · Provide a clear characterization of the control group.
- · Report co-morbidities and medications.
- · Match age and sex with study population. Provide justification for case-matching criteria.
- · Collect same muscle in control and study populations.

(D) Classification and results
- · Avoid mixing the results of two or more muscle groups or comparing one muscle group with a different muscle group (e.g. rectus abdominis vs. quadriceps).
- · Acknowledge sexual dimorphism in skeletal muscle by reporting results based in men and women, include mean and standard deviation.
- · Classification of cancer cachexia should include both, body composition analysis (muscle mass values) and weight loss.

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quadriceps or tibialis anterior are collected in healthy volunteers serving as controls as there is no justification for surgical intervention. Importantly, physiological variations between muscle groups exist,[137,138] which strongly suggest that studies collecting different muscles must avoid comparing or combining data of more than one muscle.

Most researchers did not report on surgical procedures and muscle biopsy collection, transport, and processing of the samples, each of which can impact on the morphological and molecular profile of the biopsy.[10,139,140] Collecting abdominal muscle biopsies at the start of the surgical procedure and avoidance of electrocautery is strongly recommended to reduce variations associated with the surgical trauma, variable duration of surgery, and intraoperative effect of anaesthetics.[10,11,141–144] Skeletal muscle collected at the start and end of a surgery expresses differences in genes associated with inflammation, growth differentiation, and transcription factors.[142] For percutaneous biopsies, the Bergstrom protocol is a well-developed method with several adjustments to improve the quality of the muscle biopsies.[145,146] Procedures followed after biopsy collection must also be detailed as sample preservation and storage impacts on muscle integrity and potentially interpretation of the results. Lastly, the numbers of medical conditions and drugs taken by patients in this sample are important and all of these and their different combinations may have an impact on specific aspects of muscle biology. As much as possible, we recommend to annotate the presence of co-morbidities and medications in patients consenting to biopsy.

Overall, the literature review reveals a high risk of sampling bias and poorly characterized patient populations. These features make reliable comparison between studies and aggregation of data challenging. Muscle biopsy preparation and biobanking practices are also variable between studies. Data from an unbiased sample of 190 patients present a variety of measures of interest on rectus abdominis to provide a point of reference for researchers exploring biological characteristics of this muscle. Continued collaboration between researchers and cancer surgeons would enable a more complete understanding of mechanisms of cancer-associated muscle atrophy.

Author contributions

Ms. Anoveros-Barrera and Mr. Bhullar, who each contributed equally to data analyses, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. A.A. and A.S.B. contributed to conceptualization, design, analysis, writing, and interpretation. C.S. contributed to the gene array data analysis and interpretation. N.E. contributed with data collection and analysis. A.R.D. contributed with CT image analysis and experimental optimization. K.J.B.M. contributed to experimental optimization and image analysis. D.B., T.M., R.G.K., and O.F.B. contributed in patient recruitment, biopsy, and clinical data collection. S.D., R.J.S., and C.T.P. contributed interpretation and editing. V.C.M. and V.E.B contributed to conceptualization, design, analysis, interpretation, and editing. All authors of this research paper have approved the final version submitted.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Antibody information used for immunofluorescence experiments: muscle fiber types, laminin/dystrophin and nuclear stain.
Table S2. Complete extraction table of the reviewed articles in relevance of muscle biopsy collection in cancer patients
Table S3. Skeletal muscle gene expression for genes associated with cancer cachexia in cancer patients

Conflict of interest

No authors declare a conflict of interest.

References

1. Kazemi-Bajestani SMR, Mazurak VC, Baracos V. Computed tomography-defined muscle and fat wasting are associated with cancer clinical outcomes. *Semin Cell Dev Biol* 2016;**54**:2–10.
2. Martin L, Birdsell L, MacDonald N, Reiman T, Clandinin MT, Mccargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J Clin Oncol* 2013;**31**:1539–1547.
3. Prado CMM, Baracos VE, McCargar LJ, Reiman T, Mourtzakis M, Tonkin K, et al. Sarcopenia as a determinant of chemotherapy toxicity and time to tumor progression in metastatic breast cancer patients receiving capecitabine treatment. *Clin Cancer Res* 2009;**15**:2920–2926.
4. Lieffers JR, Bathe OF, Fassbender K, Winget M, Baracos VE. Sarcopenia is associated with postoperative infection and delayed recovery from colorectal cancer resection surgery. *Br J Cancer* 2012;**107**:931–936.
5. Miyamoto Y, Hanna DL, Zhang W, Baba H, Lenz H-J. Molecular Pathways: Cachexia signaling—a targeted approach to cancer treatment. *Clin Cancer Res* 2016;**22**:3999–4004.
6. Mueller TC, Bachmann J, Prokopchuk O, Friess H, Martignoni ME. Molecular pathways leading to loss of skeletal muscle mass in cancer cachexia—can findings from animal models be translated to humans? *BMC Cancer* 2016;**16**:1–14.
7. Stretch C, Aubin JM, Mickiewicz B, Leugner D, Al-manasra T, Tobola E, et al. Sarcopenia and myosteatosis are accompanied by distinct biological profiles in patients with pancreatic and periampullary adenocarcinomas. *PLoS ONE* 2018;**13**:e0196235:1–17.
8. Lacomis D. The utility of muscle biopsy. *Curr Neurol Neurosci Rep* 2004;**4**:81–86.
9. Joyce NC, Oskarsson B, Jin L-W. Muscle biopsy evaluation in neuromuscular disorders. *Phys Med Rehabil Clin N Am* 2012;**23**:609–631.
10. Chatterjee S. Artefacts in histopathology. *J Oral Maxillofac Pathol* 2014;**18**:S111–S116.
11. Varadhan KK, Constantin-Teodosiu D, Constantin D, Greenhaff PL, Lobo DN. Inflammation-mediated muscle metabolic dysregulation local and remote to the site of major abdominal surgery. *Clin Nutr* 2018;**37**:2178–2185.
12. Hers HG, van Hoof F. Enzymes of glycogen degradation in biopsy material. *Methods Enzymol* 1966;**8**:525–532.
13. Edwards BK, Noone A-M, Mariotto AB, Simard EP, Boscoe FP, Henley SJ, et al. Annual report to the nation on the status of cancer, 1975-2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. *Cancer* 2014;**120**:1290–1314.
14. Stephens NA, Gray C, MacDonald AJ, Tan BH, Gallagher IJ, Skipworth RJE, et al. Sexual dimorphism modulates the impact of cancer cachexia on lower limb muscle mass and function. *Clin Nutr* 2012;**31**:499–505.
15. Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle—new insights and potential implications. *Nat Rev Endocrinol* 2014;**10**:206–214.
16. Batchelor TT, Taylor LP, Thaler HT, Posner JB, DeAngelis LM. Steroid myopathy in cancer patients. *Neurology* 1997;**48**:1234–1238.
17. Jackson W, Alexander N, Schipper M, Fig L, Feng F, Jolly S. Characterization of changes in total body composition for patients with head and neck cancer undergoing chemoradiotherapy using dual-energy x-ray absorptiometry. *Head Neck* 2014;**36**:1356–1362.
18. Mourtzakis M, Prado CMM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Appl Physiol Nutr Metab* 2008;**33**:997–1006.
19. Xiao J, Caan BJ, Weltzien E, Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, et al. Associations of pre-existing comorbidities with skeletal muscle mass and radiodensity in patients with non-metastatic colorectal cancer. *J Cachexia Sarcopenia Muscle* 2018;**9**:654–663.
20. Narasimhan A, Greiner R, Bathe OF, Baracos V, Damaraju S. Differentially expressed alternatively spliced genes in skeletal muscle from cancer patients with cachexia. *J Cachexia Sarcopenia Muscle* 2018;**9**:60–70.
21. Narasimhan A, Ghosh S, Stretch C, Greiner R, Bathe OF, Baracos V, et al. Small RNAome profiling from human skeletal muscle: novel miRNAs and their targets associated with cancer cachexia. *J Cachexia Sarcopenia Muscle* 2017;**8**:405–416.
22. Johns N, Stretch C, Tan BHL, Solheim TS, Sørhaug S, Stephens NA, et al. New genetic signatures associated with cancer cachexia as defined by low skeletal muscle index and weight loss. *J Cachexia Sarcopenia Muscle* 2017;**8**:122–130.
23. Stretch C, Khan S, Asgarian N, Eisner R, Vaisipour S, Damaraju S, et al. Effects of sample size on differential gene expression, rank order and prediction accuracy of a gene signature. *PLoS ONE* 2013;**8**:1–6.
24. Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. *Health Info Libr J* 2009;**26**:91–108.
25. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;**339**:b2700–b2700.
26. Modesti PA, Reboldi G, Cappuccio FP, Agyemang C, Remuzzi G, Rapi S, et al. Panethnic differences in blood pressure in Europe: a systematic review and meta-analysis. *PLoS ONE* 2016;**11**:1–21.
27. Prado CMM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol* 2008;**9**:629–635.
28. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *J Appl Physiol* 1998;**85**:115–122.
29. Caan BJ, Meyerhardt JA, Kroenke CH, Alexeeff S, Xiao J, Weltzien E, et al. Explaining the obesity paradox: the association between body composition and colorectal cancer survival (C-SCANS Study). *Cancer Epidemiol Biomarkers Prev* 2017;**26**:1008–1015.
30. Gallo M, Gordon T, Syrotuik D, Shu Y, Tyreman N, MacLean I, et al. Effects of long-term creatine feeding and running on isometric functional measures and myosin heavy chain content of rat skeletal muscles. *Pflügers Arch - Eur J Physiol* 2006;**452**:744–755.
31. Putman CT, Martins KJB, Gallo ME, Lopaschuk GD, Pearcey JA, MacLean IM, et al. α-Catalytic subunits of 5′AMP-activated protein kinase display fiber-specific expression and are upregulated by chronic low-frequency stimulation in rat muscle. *Am J Physiol Integr Comp Physiol* 2007;**293**:R1325–R1334.
32. Martins KJB, St-Louis M, Murdoch GK, MacLean IM, McDonald P, Dixon WT, et al. Nitric oxide synthase inhibition prevents activity-induced calcineurin-NFATc1 signalling and fast-to-slow skeletal muscle fibre type conversions. *J Physiol* 2012;**590**:1427–1442.
33. Murphy RA, Mourtzakis M, Chu QS, Reiman T, Mazurak VC. Skeletal muscle depletion is associated with reduced plasma (n-3) fatty acids in non-small cell lung cancer patients 1–3. *J Nutr* 2010;**140**:1602–1606.
34. Pratt VC, Tredget EE, Clandinin MT, Field CJ. Fatty acid content of plasma lipids and erythrocyte phospholipids are altered following burn injury. *Lipids* 2001;**36**:675–682.
35. Acharyya S, Butchbach MER, Sahenk Z, Wang H, Saji M, Carathers M, et al. Dystrophin glycoprotein complex dysfunction: a regulatory link between muscular dystrophy and cancer cachexia. *Cancer Cell* 2005;**8**:421–432.

36. Agustsson T, D'souza MA, Nowak G, Isaksson B. Mechanisms for skeletal muscle insulin resistance in patients with pancreatic ductal adenocarcinoma. *Nutrition* 2011;**27**:796–801.
37. Aversa Z, Pin F, Lucia S, Penna F, Verzaro R, Fazi M, et al. Autophagy is induced in the skeletal muscle of cachectic cancer patients. *Sci Rep* 2016;**6**:1–11.
38. Bonetto A, Penna F, Aversa Z, Mercantini P, Baccino FM, Costelli P, et al. Early changes of muscle insulin-like growth factor-1 and myostatin gene expression in gastric cancer patients. *Muscle Nerve* 2013;**48**:387–392.
39. Bossola M, Mirabella M, Ricci E, Costelli P, Pacelli F, Tortorelli AP, et al. Skeletal muscle apoptosis is not increased in gastric cancer patients with mild-moderate weight loss. *Int J Biochem Cell Biol* 2006; **38**:1561–1570.
40. Bossola M, Muscaritoli M, Costelli P, Bellantone R, Pacelli F, Busquets S, et al. Increased muscle ubiquitin mRNA levels in gastric cancer patients. *Am J Physiol Regul Integr Comp Physiol* 2001;**280**: R1518–R1523.
41. Bossola M, Muscaritoli M, Costelli P, Grieco G, Bonelli G, Pacelli F, et al. Increased muscle proteasome activity correlates with disease severity in gastric cancer patients. *Ann Surg* 2003;**237**:384–389.
42. Busquets S, Deans C, Figueras M, Moore-Carrasco R, López-Soriano FJ, Fearon KCH, et al. Apoptosis is present in skeletal muscle of cachectic gastro-intestinal cancer patients. *Clin Nutr* 2007;**26**:614–618.
43. DeJong CHC, Busquets S, Moses AGW, Schrauwen P, Ross JA, Argiles JM, et al. Systemic inflammation correlates with increased expression of skeletal muscle ubiquitin but not uncoupling proteins in cancer cachexia. *Oncol Rep* 2005;**14**: 257–263.
44. D'Orlando C, Marzetti E, François S, Lorenzi M, Conti V, di Stasio E, et al. Gastric cancer does not affect the expression of atrophy-related genes in human skeletal muscle. *Muscle Nerve* 2014;**49**: 528–533.
45. Eley HL, Skipworth RJE, Deans DAC, Fearon KCH, Tisdale MJ. Increased expression of phosphorylated forms of RNA-dependent protein kinase and eukaryotic initiation factor 2α may signal skeletal muscle atrophy in weight-losing cancer patients. *Br J Cancer* 2008;**98**: 443–449.
46. Johns N, Hatakeyama S, Stephens NA, Degen M, Degen S, Frieauff W, et al. Clinical classification of cancer cachexia: phenotypic correlates in human skeletal muscle. *PLoS ONE* 2014;**9**:1–13.
47. Khal J, Hine AV, Fearon KCH, Dejong CHC, Tisdale MJ. Increased expression of proteasome subunits in skeletal muscle of cancer patients with weight loss. *Int J Biochem Cell Biol* 2005;**37**:2196–2206.
48. Lundholm K, Bylund A, Holm J, Schersten T. Skeletal muscle metabolism in patients with malignant tumor. *Eur J Cancer* 1976;**12**:465–473.
49. Marzetti E, Lorenzi M, Landi F, Picca A, Rosa F, Tanganelli F, et al. Altered mitochondrial quality control signaling in muscle of old gastric cancer patients with cachexia. *Exp Gerontol* 2017;**87**:92–99.
50. Noguchi Y, Yoshikawa T, Marat D, Doi C, Makino T, Fukuzawa K, et al. Insulin resistance in cancer patients is associated with enhanced tumor necrosis factor-alpha expression in skeletal muscle. *Biochem Biophys Res Commun* 1998;**253**:887–892.
51. Pessina P, Conti V, Pacelli F, Rosa F, Doglietto GB, Brunelli S, et al. Skeletal muscle of gastric cancer patients expresses genes involved in muscle regeneration. *Oncol Rep* 2010;**24**:741–745.
52. Prokopchuk O, Steinacker JM, Nitsche U, Otto S, Bachmann J, Schubert EC, et al. IL-4 mRNA is downregulated in the liver of pancreatic cancer patients suffering from cachexia. *Nutr Cancer* 2017;**69**:84–91.
53. Ramage MI, Johns N, Deans CDA, Ross JA, Preston T, Skipworth RJE, et al. The relationship between muscle protein content and CT-derived muscle radio-density in patients with upper GI cancer. *Clin Nutr* 2018;**37**:752–754.
54. Rhoads MG, Kandarian SC, Pacelli F, Doglietto GB, Bossola M. Expression of NF-κB and IκB proteins in skeletal muscle of gastric cancer patients. *Eur J Cancer* 2010;**46**:191–197.
55. Schmitt TL, Martignoni ME, Bachmann J, Fechtner K, Friess H, Kinscherf R, 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**:647–654.
56. Skorokhod A, Bachmann J, Giese NA, Martignoni ME, Krakowski-Roosen H. Real-imaging cDNA-AFLP transcript profiling of pancreatic cancer patients: Egr-1 as a potential key regulator of muscle cachexia. *BMC Cancer* 2012;**12**:265.
57. Smith IJ, Aversa Z, Hasselgren P-O, Pacelli F, Rosa F, Doglietto GB, et al. CALPAIN activity is increased in skeletal muscle from gastric cancer patients with no or minimal weight loss. *Muscle Nerve* 2011;**43**: 410–414.
58. Stephens NA, Skipworth RJE, MacDonald AJ, Greig CA, Ross JA, Fearon KCH. Intramyocellular lipid droplets increase with progression of cachexia in cancer patients. *J Cachexia Sarcopenia Muscle* 2011;**2**:111–117.
59. Stephens NA, Skipworth RJE, Gallagher IJ, Greig CA, Guttridge DC, Ross JA, et al. Evaluating potential biomarkers of cachexia and survival in skeletal muscle of upper gastrointestinal cancer patients. *J Cachexia Sarcopenia Muscle* 2015;**6**: 53–61.
60. Sun YS, Ye ZY, Qian ZY, Xu XD, Hu JF. Expression of TRAF6 and ubiquitin mRNA in skeletal muscle of gastric cancer patients. *J Exp Clin Cancer Res* 2012;**31**:81.
61. Taskin S, Stumpf VI, Bachmann J, Weber C, Martignoni ME, Friedrich O. Motor protein function in skeletal abdominal muscle of cachectic cancer patients. *J Cell Mol Med* 2014;**18**:69–79.
62. Smith GI, Mittendorfer B. Sexual dimorphism in skeletal muscle protein turnover. *J Appl Physiol* 2016;**120**:674–682.
63. Zeiderman MR, Gowland G, Peel B, McMahon MJ. The influence of short-term preoperative intravenous nutrition upon anthropometric variables, protein-synthesis and immunological indexes in patients with gastrointestinal cancer. *Clin Nutr* 1991;**10**:213–221.
64. Zampieri S, Valente M, Adami N, Biral D, Ghirardello A, Rampudda ME, et al. Polymyositis, dermatomyositis and malignancy: a further intriguing link. *Autoimmun Rev* 2010;**9**:449–453.
65. Zampieri S, Valente M, Adami N, Corbianco S, Doria A, Biral D, et al. Subclinical myopathy in patients affected with early stage colorectal cancer at disease onset: no evidence of inflammatory cells infiltration in the skeletal muscle biopsies harvested during diagnostic laparoscopy Immunohistochemical analysis. *Basic Appl Myol* 2009;**19**:253–257.
66. Zampieri S, Doria A, Adami N, Biral D, Vecchiato M, Savastano S, et al. Subclinical myopathy in patients affected with newly diagnosed colorectal cancer at clinical onset of disease: evidence from skeletal muscle biopsies. *Neurol Res* 2010;**32**: 20–25.
67. Aversa Z, Bonetto A, Penna F, Costelli P, Di Rienzo G, Lacitignola A, et al. Changes in myostatin signaling in non-weight-losing cancer patients. *Ann Surg Oncol* 2012;**19**:1350–1356.
68. MacDonald AJ, Johns N, Stephens N, Greig C, Ross JA, Small AC, et al. Habitual myofibrillar protein synthesis is normalin patients with upper GI cancer cachexia. *Clin Cancer Res* 2015;**21**:1734–1740.
69. Shaw JH, Humberstone DA, Douglas RG, Koea J. Leucine kinetics in patients with benign disease, non-weight-losing cancer, and cancer cachexia: studies at the whole-body and tissue level and the response to nutritional support. *Surgery* 1991;**109**:37–50.
70. Stephens NA, Gallagher IJ, Rooyackers O, Skipworth RJ, Tan BH, Marstrand T, et al. Using transcriptomics to identify and validate novel biomarkers of human skeletal muscle cancer cachexia. *Genome Med* 2010;**2**:12.
71. Brzeszczynska J, Johns N, Schilb A, Degen S, Degen M, Langen R, et al. Loss of oxidative defense and potential blockade of satellite cell maturation in the skeletal muscle of patients with cancer but not in the healthy elderly. *Aging (Albany NY)* 2016;**8**:1690–1702.
72. Ebhardt HA, Degen S, Tadini V, Schilb A, Johns N, Greig CA, et al. Comprehensive proteome analysis of human skeletal muscle in cachexia and sarcopenia: a pilot study. *J Cachexia Sarcopenia Muscle* 2017;**8**:567–582.
73. Gallagher IJ, Stephens NA, MacDonald AJ, Skipworth RJE, Husi H, Greig CA, et al. Suppression of skeletal muscle turnover in cancer cachexia: evidence from the transcriptome in sequential human

muscle biopsies. *Clin Cancer Res* 2012;**18**:2817–2827.
74. Christensen JF, Schjerling P, Andersen JL, Daugaard G, Rørth M, Mackey AL. Muscle satellite cell content and mRNA signaling in germ cell cancer patients—effects of chemotherapy and resistance training. *Acta Oncol (Madr)* 2016;**55**:1246–1250.
75. Christensen JF, Jones LW, Tolver A, Jørgensen LW, Andersen JL, Adamsen L, et al. Safety and efficacy of resistance training in germ cell cancer patients undergoing chemotherapy: a randomized controlled trial. *Br J Cancer* 2014;**111**:8–16.
76. Lamboley CR, Xu H, Dutka TL, Hanson ED, Hayes A, Violet JA, et al. Effect of androgen deprivation therapy on the contractile properties of type I and type II skeletal muscle fibres in men with non-metastatic prostate cancer. *Clin Exp Pharmacol Physiol* 2017;146–154.
77. Nilsen TS, Thorsen L, Fosså SD, Wiig M, Kirkegaard C, Skovlund E, et al. Effects of strength training on muscle cellular outcomes in prostate cancer patients on androgen deprivation therapy. *Scand J Med Sci Sports* 2016;**26**:1026–1035.
78. Op den Kamp CM, Gosker HR, Lagarde S, Tan DY, Snepvangers FJ, Dingemans AMC, et al. Preserved muscle oxidative metabolic phenotype in newly diagnosed non-small cell lung cancer cachexia. *J Cachexia Sarcopenia Muscle* 2015;**6**:164–173.
79. Op den Kamp CM, Langen RC, Minnaard R, Kelders MC, Snepvangers FJ, Hesselink MK, et al. Pre-cachexia in patients with stages I-III non-small cell lung cancer: systemic inflammation and functional impairment without activation of skeletal muscle ubiquitin proteasome system. *Lung Cancer* 2012;**76**:112–117.
80. Op den Kamp CM, Langen RC, Snepvangers FJ, de Theije CC, Schellekens JM, Laugs F, et al. Nuclear transcription factor k B activation and protein turnover adaptations in skeletal muscle of patients with progressive stages of lung cancer cachexia. *Am J Clin Nutr* 2013;**98**:738–748.
81. Phillips BE, Smith K, Liptrot S, Atherton PJ, Varadhan K, Rennie MJ, et al. Effect of colon cancer and surgical resection on skeletal muscle mitochondrial enzyme activity in colon cancer patients: a pilot study. *J Cachexia Sarcopenia Muscle* 2013;**4**:71–77.
82. Puig-Vilanova E, Rodriguez DA, Lloreta J, Ausin P, Pascual-Guardia S, Broquetas J, et al. Oxidative stress, redox signaling pathways, and autophagy in cachectic muscles of male patients with advanced COPD and lung cancer. *Free Radic Biol Med* 2015;**79**:91–108.
83. Weber MA, Kinscherf R, Krakowski-Roosen H, Aulmann M, Renk H, Künkele A, et al. Myoglobin plasma level related to muscle mass and fiber composition—a clinical marker of muscle wasting? *J Mol Med* 2007;**85**:887–896.
84. Weber MA, Krakowski-Roosen H, Schröder L, Kinscherf R, Krix M, Kopp-Schneider A, et al. Morphology, metabolism, microcirculation, and strength of skeletal muscles in cancer-related cachexia. *Acta Oncol (Madr)* 2009;**48**:116–124.
85. Williams JP, Phillips BE, Smith K, Atherton PJ, Rankin D, Selby AL, et al. Effect of tumor burden and subsequent surgical resection on skeletal muscle mass and protein turnover in colorectal cancer patients. *Am J Clin Nutr* 2012;**96**:1064–1071.
86. Banduseela V, Ochala J, Lamberg K, Kalimo H, Larsson L. Muscle paralysis and myosin loss in a patient with cancer cachexia. *Acta Myol* 2007;**26**:136–144.
87. Higuchi I, Niiyama T, Uchida Y, Inose M, Hu J, Nakagawa M, et al. Microvascular endothelial abnormality in skeletal muscle from a patient with gastric cancer without dermatomyositis. *Acta Neuropathol* 2000;**100**:718–722.
88. Jagoe RT, Redfern CPF, Roberts RG, Gibson GJ, Goodship THJ. Skeletal muscle mRNA levels for cathepsin B, but not components of the ubiquitin–proteasome pathway, are increased in patients with lung cancer referred for thoracotomy. *Clin Sci* 2002;**102**:353–361.
89. Bohlen J, McLaughlin SL, Hazard-Jenkins H, Infante AM, Montgomery C, Davis M, et al. Dysregulation of metabolic-associated pathways in muscle of breast cancer patients: preclinical evaluation of interleukin-15 targeting fatigue. *J Cachexia Sarcopenia Muscle* 2018;**9**:701–714.
90. Burd NA, Dickinson JM, Lemoine JK, Carroll CC, Sullivan BE, Haus JM, et al. Effect of a cyclooxygenase-2 inhibitor on postexercise muscle protein synthesis in humans. *Am J Physiol Endocrinol Metab* 2010;**298**:E354–E361.
91. Standley RA, Liu SZ, Jemiolo B, Trappe SW, Trappe TA. Prostaglandin E2 induces transcription of skeletal muscle mass regulators interleukin-6 and muscle RING finger-1 in humans. *Prostaglandins Leukot Essent Fatty Acids* 2013;**88**:361–364.
92. Trappe TA, Liu SZ. Effects of prostaglandins and COX-inhibiting drugs on skeletal muscle adaptations to exercise. *J Appl Physiol* 2013;**115**:909–919.
93. Liu SZ, Jemiolo B, Lavin KM, Lester BE, Trappe SW, Trappe TA. Prostaglandin E2/cyclooxygenase pathway in human skeletal muscle: influence of muscle fiber type and age. *J Appl Physiol* 2016;**120**:546–551.
94. Bouitbir J, Singh F, Charles A-L, Schlagowski A-I, Bonifacio A, Echaniz-Laguna A, et al. Statins trigger mitochondrial reactive oxygen species-induced apoptosis in glycolytic skeletal muscle. *Antioxid Redox Signal* 2016;**24**:84–98.
95. Diaz EC, Herndon DN, Porter C, Sidossis LS, Suman OE, Børsheim E. Effects of pharmacological interventions on muscle protein synthesis and breakdown in recovery from burns. *Burns* 2015;**41**:649–657.
96. Malin SK, Kashyap SR. Effects of metformin on weight loss. *Curr Opin Endocrinol Diabetes Obes* 2014;**21**:323–329.
97. Wessels B, Ciapaite J, van den Broek NMA, Nicolay K, Prompers JJ. Metformin impairs mitochondrial function in skeletal muscle of both lean and diabetic rats in a dose-dependent manner. *PLoS ONE* 2014;**9**:e100525.
98. Elsaid O, Taylor B, Zaleski A, Panza G, Thompson PD. Rationale for investigating metformin as a protectant against statin-associated muscle symptoms. *J Clin Lipidol* 2017;**11**:1145–1151.
99. Elazzazy S, Eziada SS, Zaidan M. Rhabdomyolysis secondary to drug interaction between atorvastatin, omeprazole, and dexamethasone. *Int Med Case Rep J* 2012;**5**:59–61.
100. Bloise FF, Oliveira TS, Cordeiro A, Ortiga-Carvalho TM. Thyroid hormones play role in sarcopenia and myopathies. *Front Physiol* 2018;**9**:560.
101. Di Bari M, Van De Poll-Franse LV, Onder G, Kritchevsky SB, Newman A, Harris TB, et al. Antihypertensive medications and differences in muscle mass in older persons: the health, aging and body composition study. *J Am Geriatr Soc* 2004;**52**:961–966.
102. Burks TN, Andres-Mateos E, Marx R, Mejias R, Van Erp C, Simmers JL, et al. Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. *Sci Transl Med* 2011;**3**:82ra37–82ra37.
103. Delafontaine P, Yoshida T. The renin-angiotensin system and the biology of skeletal muscle: mechanisms of muscle wasting in chronic disease states. *Trans Am Clin Climatol Assoc* 2016;**127**:245–258.
104. Penafuerte CA, Gagnon B, Sirois J, Murphy J, MacDonald N, Tremblay ML. Identification of neutrophil-derived proteases and angiotensin II as biomarkers of cancer cachexia. *Br J Cancer* 2016;**114**:680–687.
105. Godfraind T. Discovery and development of calcium channel blockers. *Front Pharmacol* 2017;**8**:286.
106. Vuong C, Van Uum SHM, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010;**31**:98–132.
107. Derbre F, Ferrando B, Gomez-Cabrera MC, Sanchis-Gomar F, Martinez-Bello VE, Olaso-Gonzalez G, et al. Inhibition of xanthine oxidase by allopurinol prevents skeletal muscle atrophy: role of p38 MAPKinase and E3 ubiquitin ligases. *PLoS ONE* 2012;**7**:e46668.
108. Williams A, Sun X, Fischer JE, Hasselgren PO. The expression of genes in the ubiquitin-proteasome proteolytic pathway is increased in skeletal muscle from patients with cancer. *Surgery* 1999;**126**:744–750.
109. Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia: understanding the molecular basis. *Nat Rev Cancer* 2014;**14**:754–762.
110. Baracos VE, Martin L, Korc M, Guttridge DC, Fearon KCH. Cancer-associated cachexia. *Nat Rev Dis Primers* 2018;**4**:17105.

111. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. *Crit Rev Biochem Mol Biol* 2014;**49**:59–68.
112. Roeland EJ, Ma JD, Nelson SH, Seibert T, Heavey S, Revta C, et al. Weight loss versus muscle loss: re-evaluating inclusion criteria for future cancer cachexia interventional trials. *Support Care Cancer* 2017;**25**:365–369.
113. Martin L, Senesse P, Gioulbasanis I, Antoun S, Bozzetti F, Deans C, et al. Diagnostic criteria for the classification of cancer-associated weight loss. *J Clin Oncol* 2015;**33**:90–99.
114. Rier HN, Jager A, Sleijfer S, Maier AB, Levin M-D. The prevalence and prognostic value of low muscle mass in cancer patients: a review of the literature. *Oncologist* 2016;**21**:1396–1409.
115. Derstine BA, Holcombe SA, Ross BE, Wang NC, Su GL, Wang SC. Skeletal muscle cutoff values for sarcopenia diagnosis using T10 to L5 measurements in a healthy US population. *Sci Rep* 2018;**8**:11369.
116. Esfandiari N, Ghosh S, Prado CMM, Martin L, Mazurak V, Baracos VE. Age, obesity, sarcopenia, and proximity to death explain reduced mean muscle attenuation in patients with advanced cancer. *J Frailty Aging* 2014;**3**:3–8.
117. Marzani B, Felzani G, Bellomo RG, Vecchiet J, Marzatico F. Human muscle aging: ROS-mediated alterations in rectus abdominis and vastus lateralis muscles. *Exp Gerontol* 2005;**40**:959–965.
118. Seo PH, Pieper CF, Cohen HJ. Effects of cancer history and comorbid conditions on mortality and healthcare use among older cancer survivors. *Cancer* 2004;**101**:2276–2284.
119. Garman KS, Pieper CF, Seo P, Cohen HJ. Function in elderly cancer survivors depends on comorbidities. *J Gerontol A Biol Sci Med Sci* 2003;**58**:M1119–M1124.
120. Marquis K, Debigaré R, Lacasse Y, LeBlanc P, Jobin J, Carrier G, et al. Midthigh muscle cross-sectional area is a better predictor of mortality than body mass index in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;**166**:809–813.
121. Wüst RCI, Degens H. Factors contributing to muscle wasting and dysfunction in COPD patients. *Int J Chron Obstruct Pulmon Dis* 2007;**2**:289–300.
122. Langen RCJ, Gosker HR, Remels AHV, Schols AMWJ. Triggers and mechanisms of skeletal muscle wasting in chronic obstructive pulmonary disease. *Int J Biochem Cell Biol* 2013;**45**:2245–2256.
123. D'Souza DM, Al-Sajee D, Hawke TJ. Diabetic myopathy: impact of diabetes mellitus on skeletal muscle progenitor cells. *Front Physiol* 2013;**4**:379.
124. Leenders M, Verdijk LB, van der Hoeven L, Adam JJ, van Kranenburg J, Nilwik R, et al. Patients with type 2 diabetes show a greater decline in muscle mass, muscle strength, and functional capacity with aging. *J Am Med Dir Assoc* 2013;**14**:585–592.
125. Bouchi R, Fukuda T, Takeuchi T, Nakano Y, Murakami M, Minami I, et al. Insulin treatment attenuates decline of muscle mass in Japanese patients with type 2 diabetes. *Calcif Tissue Int* 2017;**101**:1–8.
126. Wang T, Feng X, Zhou J, Gong H, Xia S, Wei Q, et al. Type 2 diabetes mellitus is associated with increased risks of sarcopenia and pre-sarcopenia in Chinese elderly. *Sci Rep* 2016;**6**:38937.
127. Larsen BA, Wassel CL, Kritchevsky SB, Strotmeyer ES, Criqui MH, Kanaya AM, et al. Association of muscle mass, area, and strength with incident diabetes in older adults: the health ABC study. *J Clin Endocrinol Metab* 2016;**101**:1847–1855.
128. Henriksen TI, Davidsen PK, Pedersen M, Schultz HS, Hansen NS, Larsen TJ, et al. Dysregulation of a novel miR-23b/27b-p53 axis impairs muscle stem cell differentiation of humans with type 2 diabetes. *Mol Metab* 2017;**6**:770–779.
129. Trappe TA, White F, Lambert CP, Cesar D, Hellerstein M, Evans WJ. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. *Am J Physiol Metab* 2002;**282**:E551–E556.
130. Bodine SC, Furlow JD. Glucocorticoids and skeletal muscle. *Adv Exp Med Biol* 2015;**872**:145–176.
131. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 2004;**117**:399–412.
132. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell* 2004;**14**:395–403.
133. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, et al. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab* 2007;**6**:458–471.
134. Cetrone M, Mele A, Tricarico D. Effects of the antidiabetic drugs on the age-related atrophy and sarcopenia associated with diabetes type II. *Curr Diabetes Rev* 2014;**10**:231–237.
135. Schiessel DL, Baracos VE. Barriers to cancer nutrition therapy: excess catabolism of muscle and adipose tissues induced by tumour products and chemotherapy. *Proc Nutr Soc* 2018;**77**:1–9.
136. Pedersen J, Song DH, Selber JC. Robotic, intraperitoneal harvest of the rectus abdominis muscle. *Plast Reconstr Surg* 2014;**134**:1057–1063.
137. Miller AEJ, MacDougall JD, Tarnopolsky MA, Sale DG. Gender differences in strength and muscle fiber characteristics. *Eur J Appl Physiol Occup Physiol* 1993;**66**:254–262.
138. Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 1973;**18**:111–129.
139. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol* 2002;**161**:1961–1971.
140. Truong M, Liang L, Kukreja J, O'Brien J, Jean-Gilles J, Messing E. Cautery artifact understages urothelial cancer at initial transurethral resection of large bladder tumours. *Can Urol Assoc J* 2017;**11**:E203–E206.
141. Essen P, McNurlan MA, Wernerman J, Vinnars E, Garlick PJ. Uncomplicated surgery, but not general anesthesia, decreases muscle protein synthesis. *Am J Physiol Metab* 1992;**262**:E253–E260.
142. Ruel M, Bianchi C, Khan TA, Xu S, Liddicoat JR, Voisine P, et al. Gene expression profile after cardiopulmonary bypass and cardioplegic arrest. *J Thorac Cardiovasc Surg* 2003;**126**:1521–1530.
143. Lattermann R, Carli F, Wykes L, Schricker T. Epidural blockade modifies perioperative glucose production without affecting protein catabolism. *Anesthesiology* 2002;**97**:374–381.
144. Luo J-L, Hammarqvist F, Andersson K, Wernerman J. Surgical trauma decreases glutathione synthetic capacity in human skeletal muscle tissue. *Am J Physiol Metab* 1998;**275**:E359–E365.
145. Tarnopolsky MA, Pearce E, Smith K, Lach B. Suction-modified Bergström muscle biopsy technique: experience with 13,500 procedures. *Muscle Nerve* 2011;**43**:716–725.
146. Shanely RA, Zwetsloot KA, Triplett NT, Meaney MP, Farris GE, Nieman DC. Human skeletal muscle biopsy procedures using the modified Bergström technique. *J Vis Exp* 2014;**91**:51812, https://doi.org/10.3791/51812.
147. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2017. *J Cachexia Sarcopenia Muscle* 2017;**8**:1081–1083.