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Original article

Myokines in treatment-naïve patients with cancer-associated cachexia

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SUMMARY

Cancer-associated cachexia is a complex metabolic syndrome characterized by weight loss and systemic inflammation. Muscle loss and fatty infiltration into muscle are associated with poor prognosis in cancer patients. Skeletal muscle secretes myokines, factors with autocrine, paracrine and/or endocrine action, which may be modified by or play a role in cachexia. This study examined myokine content in the plasma, skeletal muscle and tumor homogenates from treatment-naïve patients with gastric or colorectal stages I-IV cancer with cachexia (CC, N = 62), or not (weight stable cancer, WSC, N = 32). Myostatin, interleukin (IL) 15, follistatin-like protein 1 (FSTL-1), fatty acid binding protein 3 (FABP3), irisin and brain-derived neurotrophic factor (BDNF) protein content in samples was measured with Multiplex technology; body composition and muscle lipid infiltration were evaluated in computed tomography, and quantification of triacylglycerol (TAG) in the skeletal muscle. Cachectic patients presented lower muscle FSTL-1 expression ($p = 0.047$), higher FABP3 plasma content ($p = 0.0301$) and higher tumor tissue expression of FABP3 ($p = 0.0182$), IL-15 ($p = 0.007$) and irisin ($p = 0.0110$), compared to WSC. Neither muscle TAG content, nor muscle attenuation were different between weight stable and cachectic patients. Lumbar adipose tissue (AT) index, visceral AT index and subcutaneous AT index were lower in CC ($p = 0.0149$, $p = 0.0455$ and $p = 0.0087$, respectively), who also presented lower muscularity in the cohort (69.2% of patients; $p = 0.0301$), compared to WSC. The results indicate the myokine profile in skeletal muscle, plasma and tumor is impacted by cachexia. These findings show that myokines eventually affecting muscle wasting may not solely derive from the muscle itself (as the tumor also may

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contribute to the systemic scenario), and put forward new perspectives on cachexia treatment targeting myokines and associated receptors and pathways.

1. Introduction

Cachexia is a complex metabolic syndrome and a comorbidity of cancer, characterized by ongoing muscle loss with or without fat loss [1]. Cancer-associated cachexia occurs in around 80% of patients with advanced disease [2]. Muscle loss is markedly related to poor prognosis during cancer treatment, reduced quality of life, and decreased survival [3,4]. Systemic inflammation is a hallmark of cachexia and is hypothesized to be one of the driving forces inducing tissue wasting [5]. This chronic inflammatory profile is the result of the complex combination of tumor-secreted factors and the host's unbalanced immune/inflammatory response to the presence of the tumor [6,7].

Skeletal muscle corresponds to approximately 40% of the total body weight, and this tissue secretes several factors that act in autocrine, paracrine and/or endocrine manner regulating muscle physiology itself and that of remote organs [8]. These muscle-derived factors are known as myokines. Among them, myostatin, a member of the transforming growth factor β (TGF-β) superfamily, is postulated to be involved in the pathogenesis of cancer-associated cachexia, owing to its role in the negative regulation of muscle mass, through binding to type II activin receptors. Although several studies with animal models of cancer cachexia have shown that myostatin is implicated in skeletal muscle loss [9–11], a clinical trial studying patients with pancreatic cancer failed to show any benefit of antimyostatin antibody treatment in preserving muscle mass or improving patients' overall survival [12]. Moreover, plasma myostatin has been reported to be low in patients with colorectal or lung cancer cachexia [13]. We chose to investigate myokines previously described to be modulated by cachexia.

Interleukin (IL) 15 is a myokine involved in the regulation of skeletal muscle mass. IL-15 treatment decreased muscle loss in a rat model of cancer cachexia, diminishing protein breakdown when administered subcutaneously [14]. In a mouse model of breast cancer, overexpression of IL-15 attenuated skeletal muscle fatigue [15]. Furthermore, serum IL-15 has been reported to be inversely correlated with the percentage of body weight loss in patients with cancer cachexia [16]. In spite of that, systemic administration of IL-15 increases skeletal muscle apoptosis in rats [17]. Follistatin-like protein 1 (FSTL-1) is a myokine that stimulates angiogenesis and vascularization in the skeletal muscle [18]. FSTL-1 gene expression was found to be diminished in the skeletal muscle of cancer cachexia models [19], while little is known about FSTL-1 in the human form of the syndrome. Fatty acid binding protein 3 (FABP3) is a cytoplasmic fatty acid carrier that facilitates the transport of lipophilic substances from sarcolemma to cellular compartments. It has been shown that FABP3 is part of the secretome of the soleus and gastrocnemius rat muscles [20]. Physical exercise is able to increase FABP3 circulating levels [21] and its mRNA in skeletal muscle [22]. Moreover, circulating levels of FABP3 have been inversely associated with skeletal muscle attenuation (or skeletal muscle density) in patients with pancreatic cancer and cachexia [23], but a direct correlation with cachexia is still missing.

Other myokines, such as irisin and brain-derived neurotrophic factor (BDNF) have not been studied in the context of cancer cachexia. Irisin, a newly discovered myokine, seems to act as a pro-myogenic factor in skeletal muscle of mice, stimulating satellite cells activation and decreasing protein degradation, by inhibiting the protein expression of atrogin-1 and muscle RING-finger protein-1 [24]. Postmenopausal women with sarcopenia [25] and patients with colorectal [26] and breast [27] cancer show lower levels of irisin. The neurotrophin BDNF is ubiquitously expressed in the nervous system, where it participates in the survival and growth of neurons and in synaptogenesis [8]. Skeletal muscle has an abundant number of neurotrophin receptors and can express BDNF both in physiology and disease [8]. BDNF is also expressed in satellites cells, being thus implicated in skeletal muscle regeneration [8,28]. Finally, it has been shown that exercise increases the circulating levels of BDNF [29,30].

Considering the important roles of myokines for regulation of skeletal muscle and for the function of peripheral organs/tissues, as well as for central nervous system physiology our aim was to measure myostatin, FSTL-1, IL-15, FABP3, irisin, and BDNF content in the skeletal muscle, tumor and plasma of patients with cancer-associated cachexia, and to examine the possible association among myokines, body composition parameters and circulating inflammatory factors. Therefore, myokine quantification was carried out along computed tomography (CT) image analysis as to establish association patterns. Finally, we also evaluated the presence of myosteatosis, by studying CTs and the expression of proteins involved in muscle lipid storage and uptake, and by measuring triacylglycerol (TAG) in skeletal muscle samples.

2. Methods

2.1. Patients

All procedures adopted in this study were approved by the Ethics Committee on Research Involving Human Subjects of the Institute of Biomedical Sciences (CAAE nº 00475118.7.0000.5467 and 5493116.6.0000467), and by the University of Alberta Research Ethics Office (Pro00056656); and were performed following the principles of the Declaration of Helsinki. Patients with gastric or colorectal cancer of both sexes (ages 30–90 years) were recruited at the University Hospital of University of São Paulo, at the Santa Casa de Misericordia Hospital, and at the Instituto do Cancer Arnaldo Vieira de Carvalho (São Paulo, SP), after providing fully informed consent. Biopsies from the *rectus abdominis* muscle and from the tumor were collected during surgery. Height and weight were obtained by the hospital staff (using platform scales with height rod, while patients were wearing hospital gown and no shoes). Blood collection, weight change evaluation and obtainment of questionnaire answers occurred 18 h–2 h or immediately before the surgery. Exclusion criteria were: continuous use of anti-inflammatory treatment in the past six months, use of antibiotics in the previous month, engagement in regular physical exercise in the past six months, chemotherapy or radiotherapy in the past 5 years, acquired immunodeficiency syndrome, auto-immune disorders, liver or kidney failure, body mass index (BMI) > 29.9 kg/m^2, uncontrolled diabetes mellitus and any acute and/or chronic inflammation from other aetiologies apart from cachexia.

Identification of cancer-associated cachexia followed the criteria proposed by Evans at al. (2008) [1]. Anorexia symptoms and quality

of life scores were obtained with validated questionnaires – Functional Assessment of Anorexia/Cachexia Therapy Anorexia/Cachexia Subscale/European Society for Clinical Nutrition and Metabolism (FAACT-ESPEN) [31] and European Organization for Research and Treatment of Cancer (EORTC QLQ-STO22) [32]. Patients with cancer-associated cachexia (CC group) presented > 5% of body weight loss in the last six months or BMI < 20 kg/m^2; metabolic alterations characterized by increased levels of serum C-reactive protein (CRP), lower serum albumin or blood hemoglobin; and presence of self-reported symptoms, such as anorexia, fatigue and impaired functional ability. Patients with cancer who did not meet these criteria were assigned to the weight stable cancer group (WSC).

2.2. Body composition

Computed tomography (CT) scans, obtained prior to abdominal surgery for tumour resection at the participating hospitals, were retrieved for the secondary purpose of analysis of body composition; no additional images were requested for the purpose of this study. CT scans were analysed at the Human Nutrition Research Unit (University of Alberta, Canada) by a trained researcher blinded to the Patient's group. A single axial CT image at the third lumbar vertebra was selected for body composition assessment, using SliceOmatic (v.5.0, Tomovision, Montreal, Canada). Skeletal muscle and adipose tissue (AT) were segmented using tissue-specific Hounsfield Units (HU; −29 to +150 for skeletal muscle, −190 to −30 for subcutaneous and intermuscular AT, and −150 to −50 for visceral AT) [33] and manually corrected as necessary. Muscle and AT cross-sectional area (square centimetres – cm^2) was calculated automatically as the sum of pixels multiplied by the pixel surface area [34]. Lumbar adiposity corresponds in this manner, to the sum of subcutaneous, visceral, and intermuscular AT area (all in cm^2). Skeletal muscle index, lumbar AT index, visceral AT index and subcutaneous AT index were calculated based on the cross-sectional area normalized by height square (m^2). Low skeletal muscle index was classified as stated in the ESPEN guidelines for nutrition in cancer patients [35] (men < 55 cm^2/m^2 and woman < 39 cm^2/m^2). Muscle radiodensity was also explored and reported as mean HU; reduced muscle attenuation is reflective of intermuscular AT or poor “quality” skeletal muscle mass.

2.3. Circulating parameters

Prior to surgery for tumor resection, blood samples were collected in tubes for plasma and serum separation. Tubes were centrifuged at 3000 rpm for 15 min at 4 °C and plasma and serum were placed in microtubes and stored at −80 °C for further analysis. Available commercial kits were used to obtain the serum concentration of CRP (ultrasensitive CRP Turbiquest plus, cat. nº 331, Labtest, Lagoa Santa, MG, Brazil), albumin (cat. nº 19, Labtest, Lagoa Santa, MG, Brazil), total cholesterol (cat. nº 76, Labtest, Lagoa Santa, MG, Brazil), LDL cholesterol (cat. nº 129, Labtest Lagoa Santa, MG, Brazil), HDL cholesterol (cat. nº 98, Labtest, Lagoa Santa, MG, Brazil) and TAG (cat. Nº 87, Labtest, Lagoa Santa, MG, Brazil) in an automatic analyser (LABMAX 240® equipment, Labtest, Lagoa Santa, MG, Brazil). Serum IL-6, IL-8, IL-15 and tumor necrosis factor (TNF) α, as well as circulating plasma myokines were measured using Multiplex technology with Magpix instrument (Life Technologies, Grand Island, NY, USA) with commercial kits (HCYTMAG-60 K-PX29 for IL-6, IL-8, TNFα and IL-15 and HMYOMAG-56 K for myokines, Merck-Millipore, St. Charles, MO, USA). IL-15 was not detected in plasma samples, but in serum. The intra and inter-assay coefficient of variation (CV) for CRP was 2.9% and 6.7%; for albumin was 1.1% and 1.6%; for total cholesterol 1.3% and 2.2%; for LDL cholesterol was 0.8% and 1.5%; for HDL cholesterol was 0.7% and 1.2%; for TAG was 1.1% and 1.8%; for IL-6 was 2.0% and 18.3%; for IL-8 was 1.9% and 3.5%; for TNFα was 2.6% and 13%; for serum IL-15 was 2.7% and 8.1% respectively, as stated by the manufacture. Plasma FABP3, FSTL-1 and BDNF intra and inter-assay CV was <10% and <15%, respectively, as stated by the manufacturer.

2.4. Skeletal muscle and tumor protein quantification

Biopsies of *rectus abdominis* and tumor were collected during surgery for tumor resection. Protein extraction from 50 mg of frozen tissue was performed using radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris base, 0.01 mM EDTA, 0.1 mM Sodium Chloride and 1% Triton X-100), with proteinase and phosphatase inhibitors (Roche, Mannheim, Germany), with a Polytron, on ice. The homogenate was centrifuged at 14,000×*g*, for 30 min, at 4 °C, the liquid upper phase was collected and transferred to a new tube. This centrifugation procedure was repeated, and the homogenate obtained from the second centrifugation was used to quantify total protein employing the Bradford protein assay (Bio-rad Laboratories; Hercules, CA, USA), and a standard curve of bovine serum albumin. Myokine quantification in skeletal muscle and tumor samples was performed with a commercial kit (HMYOMAG-56 K, Merck-Millipore, St. Charles, MO, USA), using Multiplex technology and Magpix instrument (Life Technologies, Grand Island, NY, USA). The intra and inter-assay CV for FABP3, FSTL-1, BDNF, IL-15, irisin and myostatin was <10% and <15%, respectively, as declared by the manufacturer.

2.5. Gene expression assessment

Total RNA from skeletal muscle samples was extracted using TRIzol® reagent (Trizol reagent—Invitrogen, Life Technologies, Carlsbad, CA, USA). RNA quality was verified in agarose gels and RNA concentration was quantified using a spectrophotometer (Fisher Scientific, Biotek, Winooski, USA). cDNA synthesis from mRNA was carried out using a reverse transcription kit (High Capacity cDNA Reverse Transcription Kit, Life Technologies). Sybr green master mix (Fast SYBR® Green Master Mix, Thermo Fisher Scientific, Vilnius, Lithuania) and specific primers for each gene (Invitrogen, Life Technologies, Carlsbad, CA, USA) were used to perform real time qPCR in a QuantStudio 12 K Flex Real-Time PCR System instrument (Applied Biosystems, Carlsbad, CA, USA). Relative gene expression was normalized against the housekeeping gene gyceraldehyde-3-phosphate dehydrogenase (GAPDH) and calculated by the comparative 2-ΔΔCT method. Sense (forward—F) and antisense (reverse—R) primers sequences used were: CD36 F – GATTAATGGTACAGATGCAGCCT, CD36 R – GGAAGAACAAATCTATACACAGGG; Perilipin 2 F – GGTGTTTGATTTCACCACCA, R – ATTCAACATCTTGCCCTGAG; Perilipin 5 F – CTTCCCTTTCTCCAGCAACC; Perilipin 5 R – CCAGTACAACATCCACAGCA; GAPDH F – CCTCTGACTTCAACAGCGAC, GAPDH R – CGTTGTCATACCAGGAAATGAG.

2.6. Muscle triacylglycerol content measurement

The total lipid content of the *rectus abdominis* biopsies was extracted following the method proposed by Folch et al. [36]. After lipid extraction, the chloroform solution was transferred to a clean glass tube dried under nitrogen flow. Lipids were resuspended in isopropanol and muscle TAG content was measured employing a commercial kit following the manufacturer's instructions (cat. nº 87, Labtest, Lagoa Santa, MG, Brazil). The intra and inter-assay CV for TAG was 1.1% and 1.8%, as stated by the manufacturer.

2.7. Statistical analysis

All data was tested for normality and homoscedasticity - D'Agostino & Pearson omnibus normality test, Shapiro–Wilk normality test and Kolmogorov–Smirnov normality test. Parametric data was compared using the unpaired Student T-test and are represented by mean ± standard error of mean. Non-parametric parameters were compared using Mann–Whitney test and are represented by median [interquartile range]. Chi-square test was employed to compare dichotomized parameters. Statistical analysis was performed using GraphPad Prism 6 and GNU R (version 4.0). Level of significance was set at a p value < 0.05 (two-tailed). G*Power software [37] was used to calculate the effect size and *post-hoc* power based on the variables mean, standard deviation and sample size of each variable for each group. Results of the *post-hoc* power analysis are presented in Supplemental Table 1.

A logistic regression analysis was performed by calculating a binomial logistic model of the group classification for each myokine. Then we use the multiplicative coefficient of the logistic model, β_1 to compute the odds ratio (OR), given by e^{β_1}:

$$\ln\left(\frac{p(y)}{1-p(y)}\right) = \beta_0 + \beta_1 x$$

For statistically significant results, the OR values should be interpreted as follows: if $OR > 1$, a higher concentration of myokine is associated with greater odds of patients with cancer being affected by cachexia; if $OR < 1$, a higher concentration of myokine is associated with smaller odds of cachexia. For $OR \approx 1$, we can not assure a statistical relationship between the myokine concentration and the clinical condition. The OR value indicates how many times the odds increases when the explanatory variable ($x = \log_2(M)$) increases by unity, that is, when the myokine concentration (M) doubles:

$$OR = \frac{\text{odds}(x+1)}{\text{odds}(x)} = \frac{\text{odds}[\log_2(M)+1]}{\text{odds}[\log_2(M)]} = \frac{\text{odds}[\log_2(2 \cdot M)]}{\text{odds}[\log_2(M)]}$$

The p values were corrected for multiple tests using the method of Hommel (1988) [38]. The q values of false discovery rate (FDR) were calculated according to Benjamini and Hochberg (1995) [39].

Non-adjusted Spearman correlation and Spearman correlation adjusted by BMI were performed to test associations between myokines and anthropometrical and inflammatory parameters.

3. Results

3.1. Cachexia features in patients with cancer

Patients with cancer-associated cachexia (CC group, N = 62) presented lower body weight, lower BMI and higher weight loss in the last six months, compared with weight stable cancer patients (WSC group, N = 32), Table 1. Furthermore, CC patients showed higher scores for anorexia symptoms, in the FAACT-ESPEN questionnaire. The quality of life for cancer patients questionnaire showed that CC presented impaired function and increased disease-related symptoms, which included fatigue and lower appetite, as compared with WSC.

3.2. Circulating parameters and inflammatory markers

The comparison of the general clinical parameters of the two groups showed that CC presented anemia, lower levels of LDL cholesterol and of HDL cholesterol, in comparison to WSC, as shown in Table 1. Systemic inflammation was present in cachectic patients, as demonstrated by the higher levels of serum CRP, IL-6 and IL-8, in relation to WSC. Functional deficit and a symptomatological differences were revealed by the FAACT-ESPEN and functional and symptoms scale questionnaires.

3.3. Muscle, tumor and circulating levels of myokines

Skeletal muscle myokine concentration was measured in homogenates of *rectus abdominis* biopsies and are shown in Fig. 1A-F. FSTL-1 was significantly lower in the muscle of CC ($p = 0.047$, Fig. 1B), compared to WSC. The muscle content of FABP3, BDNF, IL-15, irisin and myostatin did not differ between WSC and CC ($p = 0.2989$, $p = 0.4045$, $p = 0.1072$, $p = 0.8683$ and $p = 0.2114$, respectively).

Circulating myokines presented a different pattern of alterations in cachexia, as compared to those reported for the muscle. FABP3 was higher in CC, compared to WSC. No differences were observed for FSTL-1 ($p = 0.7243$), BDNF ($p = 0.0865$) and IL-15 ($p = 0.2552$), as shown in Fig. 2A-D. Irisin and myostatin could not be detected in plasma samples.

The analysis of the same factors in tumor homogenates was then performed. CC showed higher FABP3, IL-15 and irisin protein amount in the homogenates compared to WSC, as shown in Fig. 3A, D and E, respectively. FSLT-1 ($p = 0.8609$, Fig. 3B), BDNF ($p = 0.3955$, Fig. 3C) and myostatin ($p = 0.1746$, Fig. 3F) content was not different between the groups. Addressing colon/rectum tumors separately from stomach tumors did not change the data significantly.

After myokines quantification, we performed a binomial logistic regression (Table 2) to evaluate the capacity of each myokine as a predictor for cancer cachexia. Each myokine has a specific number of observations, and, consequently, an independent logistic regression model estimate. The concentration of the myokine were transformed using the base 2 logarithm for enhancing the linear relationship between the *log odds* of the response variable required for logistic regression and for a clearer interpretation of the results. Tumor IL-15 and irisin presented odds ratio of 8.36 ($p = 0.0334$) and 5.55 ($p = 0.0375$), respectively, indicating that these tumor myokines could identify patients with cancer cachexia. However, after adjusting for multiple tests, the statistical significance was lost. In complement, we also computed the FDR to incorporate the sample size variability in our interpretation of the results [40]. Plasma BDNF, skeletal muscle FSTL-1, tumor FABP3, IL-15 and irisin presented a FDR of ≈20%, indicating that they are good candidates for future mechanistic studies. Thus, there are about four myokines which could be potential predictors of cachexia in cancer patients out of six, a valuable reduction on the amounts of myokines to be evaluated experimentally.

Patients with cachexia presented lower lumbar AT index, visceral AT index and subcutaneous AT index, compared to WSC patients (Table 3). As sex differences influence body composition, we performed analysis of WSC and CC also by gender. Female cachectic patients showed lower visceral AT index and a tendency of lower lumbar AT index compared to weight stable female patients. Skeletal muscle index categorization showed that CC patients demonstrated higher frequency of low muscle index compared to patients in the WSC group. A positive association was observed for lumbar AT index and BMI (Fig. 4A) and for lumbar AT index and muscle attenuation (Fig. 4B). Since CC showed reduced AT, we hypothesize that skeletal muscle from these patients might be actively uptaking and accumulating fatty acids released by AT.

No changes were observed for gene expression of CD36 ($p = 0.4133$, Fig. 5B), a membrane protein that facilitates fatty acid transport into skeletal and cardiac myocytes, adipocytes and enterocytes [41], neither for perilipin 2 and 5 ($p = 0.2697$ and

Table 1
General parameters of weight-stable cancer (WSC) and cancer-associated cachexia (CC) groups.

	WSC	n	CC	n	p Value
Sex (male/female)[b]	15/17		39/23		0.13641364
Tumor stage[a b]					
I-II	21		25		**0.0148**
III-IV	8		31		
Tumor location[b]					
Stomach	5		17		0.2006
Colon/rectum	27		45		
Age (year)	61.9 ± 1.7	32	61.2 ± 1.6	62	0.7945
Height (m)	1.64 ± 0.02	32	1.67 ± 0.01	62	0.1557
Weight previous to diagnosis (kg)	68.4 ± 2.0	32	73.2 ± 2.0	62	0.1286
Weight (kg)	68.0 ± 2.0	32	61.2 ± 1.6	62	**0.01**
Weight loss (kg)	0.0 [0.0; 0.0]	32	10.0 [7.0; 14.0]	62	<**0.0001**
Weight loss (%)	0.0 [0.0; 0.0]	32	15.1 [9.4; 19.4]	62	<**0.0001**
BMI (kg/m^2)	25.3 ± 0.5	32	21.8 ± 0.4	62	<**0.0001**
Questionnaires					
FAACT-ESPEN	37.00 [34.25; 39.00]	32	33.00 [22.00; 37.00]	59	<**0.0001**
Global Health Score	66.67 [66.67; 83.33]	31	66.67 [50.00; 83.33]	53	0.2860
Functional Scale	86.67 [73.33; 94.45]	29	71.11 [62.22; 86.67]	60	**0.0038**
Symptoms Scale	12.82 [7.69; 23.08]	29	23.08 [13.46; 35.90]	60	**0.0032**
Biochemical parameters					
Albumin (g/dl)	3.67 [3.12; 4.07]	31	3.30 [2.75; 4.05]	62	0.1349
Haemoglobin (mg/dl)	13.50 [11.70; 14.30]	29	11.25 [9.68; 12.00]	58	<**0.0001**
Cholesterol (mg/ml)	183.5 [156.0; 245.1]	30	163.5 [127.0; 211.9]	62	0.0843
HDL cholesterol (mg/ml)	44.32 ± 3.00	31	35.75 ± 1.63	61	**0.0075**
LDL cholesterol (mg/ml)	114.15 [73.25; 142.8]	30	81.00 [64.00; 120.0]	59	**0.0443**
Triacylglycerol (mg/dl)	142.00 [104.00; 181.0]	31	129.0 [77.25; 178.3]	62	0.1699
C-reactive protein (mg/l)	4.60 [1.07; 12.27]	31	10.69 [5.70; 12.31]	62	**0.0041**
IL-6 (pg/ml)	2.30 [0.50; 3.91]	20	7.16 [3.34; 24.86]	32	<**0.0001**
IL-8 (pg/ml)	10.61 [7.47; 15.60]	19	27.82 [12.94; 74.19]	31	**0.0007**
TNFα (pg/ml)	12.76 [8.64; 20.57]	19	15.20 [10.01; 27.13]	31	0.116

Data are expressed as mean ± standard error or as median [first quartile; third quartile]. Student's *t* test was used to compare means and Mann–Whitney test was used to compare median values between WSC and CC patients.
Bold: p value lower than 0.05.
[a] Missing tumor stage: 3 patients in the WSC and 6 patients in the CC.
[b] Chi-square test. WSC—weight-stable cancer patients; CC—cachectic cancer patients.

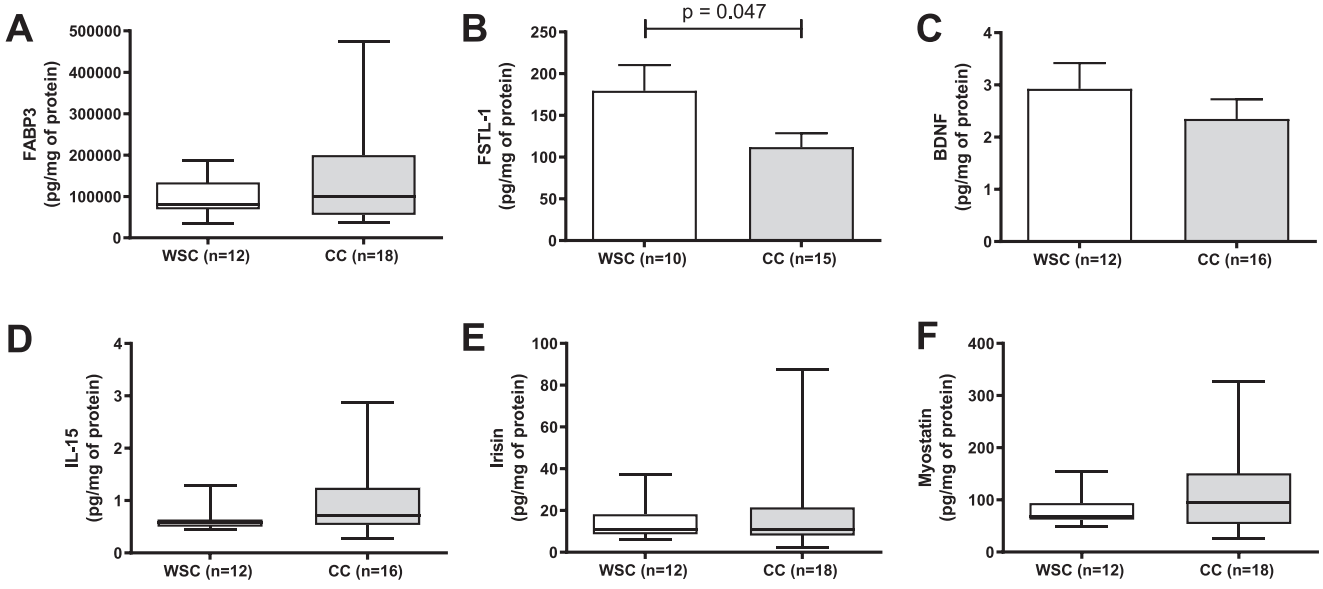

Fig. 1. Myokines in skeletal muscle homogenates from WSC and CC patients. Data are expressed as mean ± standard error or median and interquartile range. Student's *t* test was used to compare means values and Mann–Whitney test was used to compare median values between WSC and CC patients. FABP3 – fatty acid binding protein 3; FSTL-1 – follistatin-like protein 1; BDNF – brain-derived neurotrophic factor; IL-15 – interleukin 15; WSC – weight-stable cancer patients; CC – cachectic cancer patients.

p = 0.1821, Fig. 5C,D, respectively), structural proteins in the phospholipid monolayer involving lipid droplets. Moreover, TAG content did not differ between skeletal muscle of CC and WSC (p = 0.66, Fig. 5A). Still, muscle TAG was negatively associated with

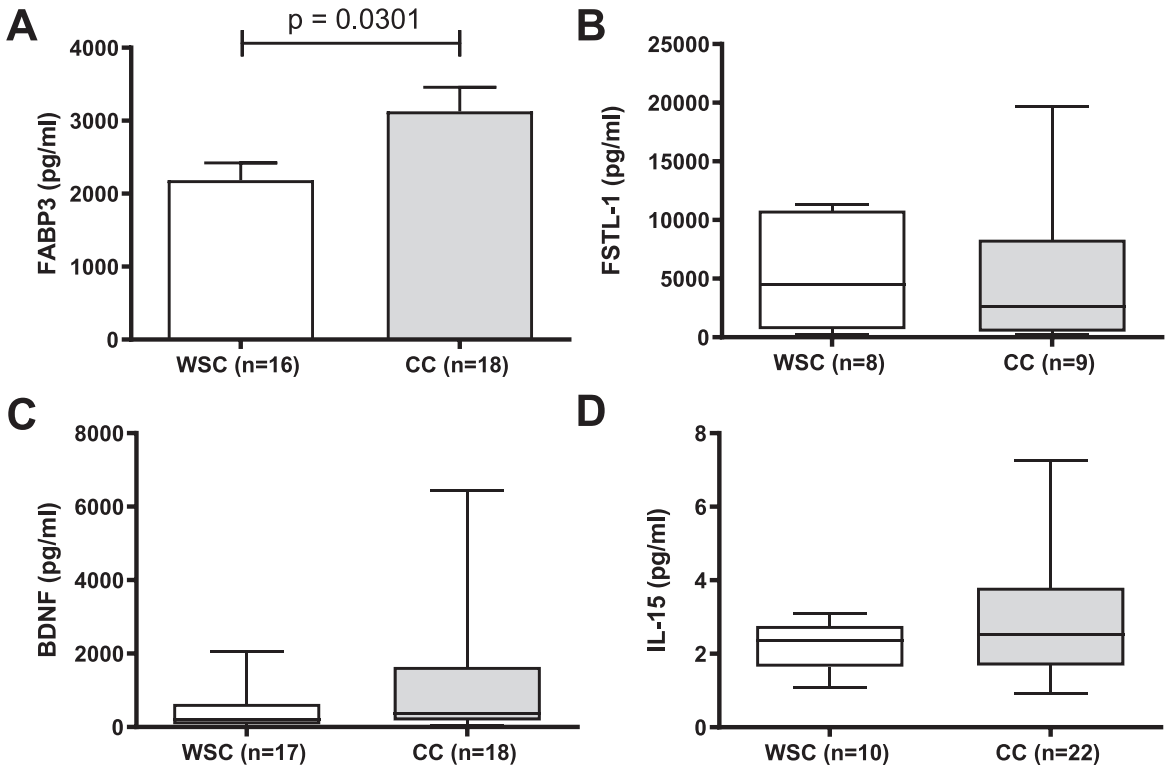

Fig. 2. Comparison of circulating myokines between WSC and CC groups. Data are expressed as mean ± standard error or median and interquartile range. Student's *t* test was used to compare means values and Mann–Whitney test was used to compare median values between WSC and CC patients. FABP3 – fatty acid binding protein 3; FSTL-1 – follistatin-like protein 1; BDNF – brain-derived neurotrophic factor; IL-15 – interleukin 15; WSC – weight-stable cancer patients; CC – cachectic cancer patients.

muscle attenuation (r_s = 0.60, p = 0.001, Fig. 5E) and positively associated with lumbar AT index (r_s = 0.55, p = 0.025, Fig. 5F). Skeletal muscle TAG content was inversely correlated with muscle radiodensity and muscular TAG was proportional to body adiposity in this cohort.

3.4. Associations of myokines and anthropometrical and inflammatory parameters

Non-adjusted Spearman correlation and Spearman correlation adjusted by BMI for myokines and anthropometrical measurements

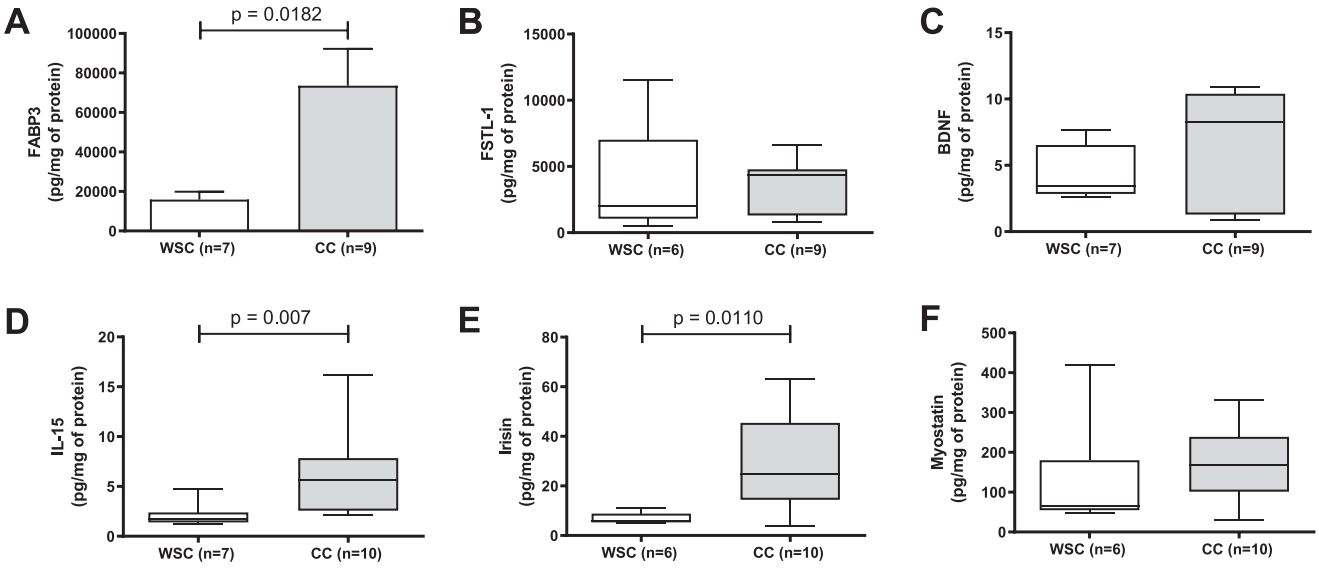

Fig. 3. Tumor protein content of FABP3, IL-15 and irisin are higher in patients with cancer-cachexia. Data are expressed as mean ± standard error or median and interquartile range. Student's *t* test was used to compare means values and Mann–Whitney test was used to compare median values between WSC and CC patients. FABP3 – fatty acid binding protein 3; FSTL-1 – follistatin-like protein 1; BDNF – brain-derived neurotrophic factor; IL-15 – interleukin 15; WSC – weight-stable cancer patients; CC – cachectic cancer patients.

Table 2
Binomial logistic regression to assess the ability of each myokine in predicting cancer cachexia.

	n	Odds Ratio	95% CI	p Value	Adjusted p Value	FDR q Value
Skeletal muscle myokines						
FABP3 (pg/mg of protein)	30	1.67	0.74–4.28	0.2398	0.8770	0.3837
FSTL-1 (pg/mg of protein)	25	0.40	0.12–0.99	0.0743	0.6689	0.2027
BDNF (pg/mg of protein)	28	0.69	0.31–1.36	0.3107	0.8770	0.4519
IL-15 (pg/mg of protein)	30	2.02	0.70–7.49	0.2293	0.8770	0.3837
Irisin (pg/mg of protein)	30	1.05	0.55–2.04	0.8770	0.8770	0.8770
Myostatin (pg/mg of protein)	30	1.46	0.60–3.98	0.4157	0.8770	0.5542
Circulating myokines						
FABP3 (pg/ml)	34	3.76	1.20–15.74	0.0386	0.4562	0.2027
FSTL-1 (pg/ml)	17	0.92	0.57–1.45	0.7086	0.8770	0.8098
BDNF (pg/ml)	33	1.42	0.99–2.19	0.0760	0.6843	0.2027
IL-15 (pg/ml)	32	2.07	0.71–7.37	0.2111	0.8770	0.3837
Tumor myokines						
FABP3 (pg/mg of protein)	17	5.40	1.64–93.18	0.0609	0.5996	0.2027
FSTL-1 (pg/mg of protein)	15	1.21	0.52–2.97	0.6535	0.8770	0.8043
BDNF (pg/mg of protein)	16	1.13	0.47–2.76	0.7787	0.8770	0.8306
IL-15 (pg/mg of protein)	17	8.36	1.82–128.94	0.0334	0.4008	0.2027
Irisin (pg/mg of protein)	16	5.55	1.61–53.72	0.0375	0.4494	0.2027
Myostatin (pg/mg of protein)	16	1.96	0.72–6.74	0.2198	0.8770	0.3837

FABP3 – fatty acid binding protein 3; FSTL-1 – follistatin-like protein 1; BDNF – brain-derived neurotrophic factor; IL-15 – interleukin 15; CI – confidence interval; FDR – false discovery rate.

Table 3
Body composition characteristics.

	WSC	n	CC	n	p Value
Skeletal muscle index (cm^2/m^2)	43.33 [40.87; 47.88]	16	45.37 [39.84; 53.25]	39	0.5316
Male	43.20 [41.10; 54.10]	9	49.80 [44.60; 55,40]	27	0.2444
Female	43.40 [38.50; 45.10]	7	38.05 [34.78; 40.78]	12	0.1148
Muscle attenuation (HU)	36.42 [30.75; 40.21]	16	39.20 [31.25; 43.78]	39	0.4731
Male	36.60 [29.95; 42.48]	9	39.90 [33.50; 45.75]	27	0.3027
Female	35.50 [31.60; 40.33]	7	36.12 [25.14; 40.96]	12	0.7051
Skeletal muscle index categorization[a] n (%)[b]					
Low	56.3	9	69.2	27	**0.0306**
Normal	43.7	7	30.8	12	
Lumbar AT index (cm^2/m^2)	101.9 ± 10.65	16	69.31 ± 7.06	39	**0.0149**
Male	88.87 ± 14.52	9	67.34 ± 8.26	27	0.2027
Female	118.7 ± 14.24	7	73.75 ± 13.94	12	0.0501
Visceral AT index (cm^2/m^2)	48.13 [23.33; 60.27]	16	27.46 [8.31; 45.34]	39	**0.0455**
Male	44.14 ± 6.93	9	36.09 ± 5.28	27	0.4270
Female	43.28 [21.11; 60.05]	7	9.43 [6.97; 35.94]	12	**0.0449**
Subcutaneous AT index (cm^2/m^2)	55.54 ± 7.18	16	35.05 ± 3.82	39	**0.0087**
Male	41.57 ± 8.04	9	28.83 ± 3.56	27	0.1065
Female	73.49 ± 9.39	7	49.05 ± 8.40	12	0.0806

[a] Sex-specific "ESPEN guidelines on nutrition in cancer patients" [35]. Data are expressed as mean ± standard error or as median [first quartile; third quartile]. Student's *t* test was used to compare means and Mann–Whitney test was used to compare median values between WSC and CC patients.
[b] Chi-square test. AT – adipose tissue; WSC — weight-stable cancer patients; CC — cachectic cancer patients.

and inflammatory markers was also performed for each group (Supplemental Figs. 1, 2, 3 and 4). In WSC, non-adjusted correlations showed associations between: skeletal muscle FABP3 and serum TNFα ($r_s = 0.82$, $p = 0.0108$); skeletal muscle BDNF and serum TNFα ($r_s = 0.72$, $p = 0.0369$); skeletal muscle irisin and serum CRP ($r_s = 0.70$, $p = 0.0145$); skeletal muscle irisin and serum TNFα ($r_s = 0.73$, $p = 0.0311$); plasma FABP3 and weight previous to diagnosis ($r_s = 0.53$, $p = 0.0346$); plasma FSTL-1 and weight previous to diagnosis ($r_s = 0.77$, $p = 0.0251$); plasma FSTL-1 and weight ($r_s = 0.73$, $p = 0.0378$); serum IL-15 and serum IL-8 ($r_s = 0.77$, $p = 0.0137$); tumor IL-15 and muscle attenuation ($r_s = 0.94$, $p = 0.0167$) and tumor myostatin and kg of weight loss ($r_s = 0.82$, $p = 0.0458$); tumor myostatin and % of weight loss ($r_s = 0.82$, $p = 0.0458$).

In WSC, Spearman correlations adjusted by BMI indicated the following associations: skeletal muscle IL-15 and lumbar AT index ($r_s = 0.95$, $p = 0.0011$); skeletal muscle irisin and serum TNFα ($r_s = 0.75$, $p = 0.0255$); skeletal muscle myostatin and serum TNFα ($r_s = 0.72$, $p = 0.0369$); plasma FABP3 and weight previous to diagnosis ($r_s = 0.54$, $p = 0.0338$); plasma FABP3 and weight ($r_s = 0.53$, $p = 0.0385$); plasma FSTL-1 and weight previous to diagnosis ($r_s = 0.76$, $p = 0.0368$); plasma FSTL-1 and weight ($r_s = 0.74$, $p = 0.0458$); serum IL-15 and kg of weight loss ($r_s = 0.79$, $p = 0.0098$) and serum IL-15 and % of weight loss ($r_s = 0.79$, $p = 0.0098$).

CC non-adjusted correlations indicated the following associations: tumor FABP3 and CRP ($r_s = 0.71$, $p = 0.0227$); tumor FABP3 and IL-8 ($r_s = 0.76$, $p = 0.0368$); tumor BDNF and visceral AT index ($r_s = 0.89$, $p = 0.0333$); tumor BDNF and serum IL-8 ($r_s = 0.79$, $p = 0.0480$); tumor IL-15 and subcutaneous AT index ($r_s = 0.94$, $p = 0.0167$); tumor IL-15 and serum IL-6 ($r_s = 0.83$, $p = 0.0154$). Spearman correlations adjusted by BMI performed among CC group parameters showed associations between: skeletal muscle BDNF and visceral AT index ($r_s = -0.74$, $p = 0.0458$); tumor FSTL-1 and CRP/albumin ratio ($r_s = 0.70$, $p = 0.0433$); tumor BDNF and serum IL-8 ($r_s = 0.82$, $p = 0.0341$); tumor IL-15 and serum IL-8 ($r_s = 0.90$, $p = 0.0046$) and tumor myostatin and serum IL-8 ($r_s = 0.74$, $p = 0.0458$). The adjusted correlations indicated that BMI is not an

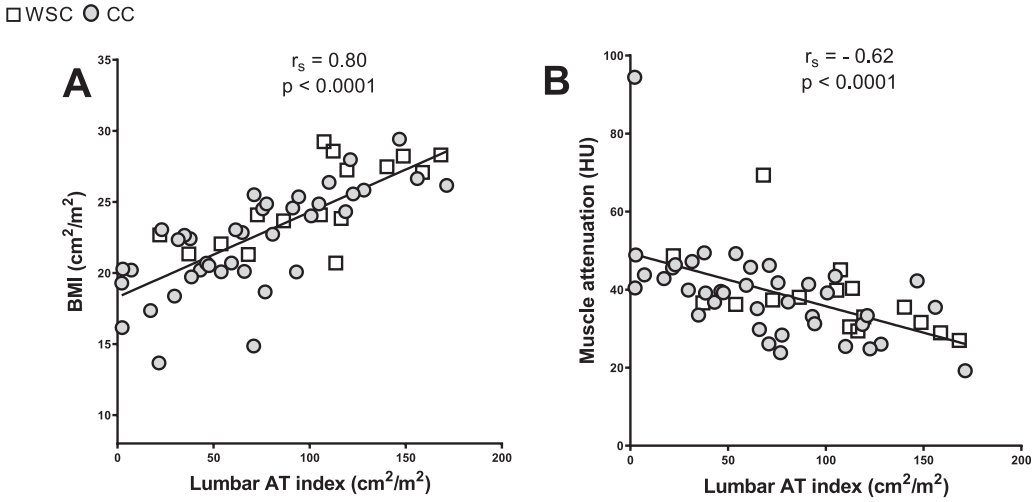

Fig. 4. Lumbar adipose tissue index is associated with A) body mass index, and B) muscle attenuation. Nonparametric Spearman correlation was used to determine associations between body compartments analyzed by computed tomography. BMI – body mass index; AT – adipose tissue; WSC—weight-stable cancer patients; CC—cachectic cancer patients.

important confounding factor to the associations between myokines and inflammatory markers.

Correlations analysis between skeletal muscle and tumor myokines of the WSC and CC groups is shown in Supplemental Tables 2 and 3, respectively. In the WSC, tumor FSLT-1 was negatively associated with skeletal muscle irisin ($r_s = -0.89$, $p = 0.0333$, $n = 6$). CC presented a positive association between tumor BDNF and skeletal muscle FABP3 ($r_s = 0.74$, $p = 0.0458$, $n = 8$). We could not perform correlations between circulating and tissues myokines due to the low number of matching samples.

4. Discussion

Skeletal muscle wasting in cancer has been substantially studied due to its relevance to patient's prognosis and quality of life [3]. However, few human studies have evaluated the role of skeletal muscle as a player in tissue crosstalk in cancer cachexia. Animal models of cachexia have demonstrated that myokines are able to regulate muscle mass in pathological conditions [9–11]. Nevertheless, we failed to observe robust differences in myokine content in the skeletal muscle of patients with cancer cachexia. Thus, we hypothesized that the tumor might contribute in cachexia with synthesis and secretion of these factors, subsequently affecting skeletal muscle biology and taking part in wasting. We have shown for the first time, that patients with cancer-associated cachexia present lower content of skeletal muscle FSTL-1, higher levels of plasma FABP3 and higher tumor content of FABP3, IL-15 and irisin, in comparison to patients with cancer who do not develop the syndrome. A relevant finding was that myokines were differently expressed in tumors from CC and WSC.

Muscle FSTL-1 expression was lower in CC in relation to WSC. This protein is primarily produced by cells with mesenchymal origin [42], including skeletal [43] and cardiac [44] fibers. Fontes-Oliveira reported that FSTL-1 gene expression is diminished in extensor digitorum longus muscle in cachectic rats bearing AH-130 Yoshida ascites hepatoma cells, as compared to control animals [19]. In muscle tissue, FSLT-1 synthesis seems to be modulated by inflammatory stimuli, as myotubes present increased FSTL-1 secretion into culture media after treatment with IL-1β and interferon gamma [43]. FSTL-1 can act as a hormone [45]. Furthermore, FSTL-1 expression is higher in myotubes compared to myoblasts and its synthesis increases along cell differentiation, suggesting a role in muscle homeostasis [43]. Changes in FSTL-1 during muscle differentiation are regulated by MyoD, a transcription factor with a central role in myoblast differentiation [46]. MyoD increases the expression of microRNA (miR) 206 which degrades FSTL-1 mRNA [46]. Intense exercise results in increased serum FSLT-1 concentration, compared to basal state in trained humans [43]. FSTL-1 is also extensively studied in the context of cardiac and vascular disease, in which circulating levels of FSTL-1 are increased [47], although likely displaying a protective role, since FSTL-1 seems to have anti-apoptotic effects in cardiac myocites [48]. Skeletal muscle vascularization is important for both muscle performance and growth. Ouchi et al. [18] showed that specific skeletal muscle transgenic Akt1 overexpression enhanced FSTL-1 in both gastrocnemius muscle and in the plasma and increased capillary vessel formation in rats [18]. Moreover, in a mice model of vascular insufficiency, the use of adenoviral vectors expressing FSTL-1 increased FSTL-1 protein content in the ischemic tissue, enhanced capillary density and flow recovery, in comparison with control mice, on the 14th postoperative day [18]. In endothelial cells, FSTL-1 increases endothelial nitric oxide synthase and Akt phosphorylation, as well as endothelial cell differentiation and migration, concomitant with a decrease in endothelial cells apoptosis. These findings indicate that FSTL-1 is a myokine playing a pivotal part for skeletal muscle vascularization [18]. Thus, reduced skeletal muscle content of FSTL-1 in patients with cancer cachexia may be causative to the reduced muscle performance (e.g. fatigue) [49] and impaired muscle regeneration, commonly reported [50].

FABP3 is an intracellular lipid transporter highly expressed in cardiac and skeletal muscles. Hormones, nutritional state and physical exercise are known metabolic stimuli able to modulate FABP3 content in the skeletal muscle [51]. Although no differences were observed in this tissue, FABP3 content were increased in both the plasma and the tumor of CC, as compared to WSC. In mice cardiomyocites, miR-1 negatively regulates FABP3 expression [52] and, since miR-1 downregulates cardiac hypertrophy [53], increase in FABP3 levels may be indicative of the opposite phenomenon, i.e. underlying striated muscle atrophy. In agreement, previous literature demonstrated that circulating FABP3 may be indicative of muscle damage, as its concentration increases after heart ischemic injury [54,55] and after skeletal muscle injuries [56–58]. In polymyositis, serum FABP3 levels are negatively associated with muscle

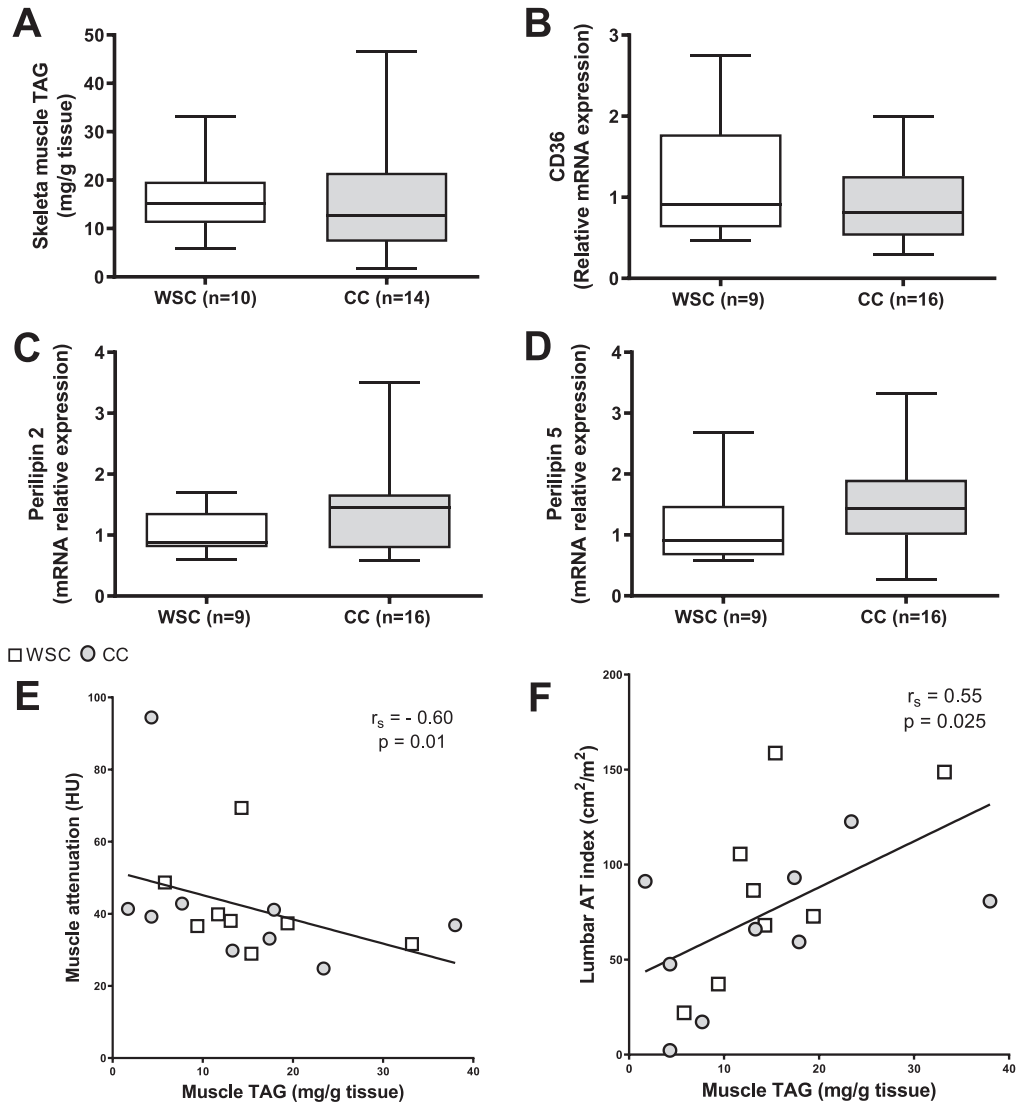

Fig. 5. Skeletal muscle gene expression of proteins related to lipid metabolism and total content of muscle TAG. Data are expressed as median and interquartile range. RT-PCR was used to assess mRNA expression levels (WSC, n = 9–10; CC, n = 16). Mann–Whitney test was used to compare medians between WSC and CC patients. Nonparametric Spearman correlation was used to verify a correlation between muscle TAG (mg/g tissue) and muscle attenuation (HU) and muscle TAG and lumbar AT index (cm^2/m^2). TAG – triacylglycerol; AT – adipose tissue; WSC – weight-stable cancer patients; CC – cachectic cancer patients.

strength [58]. In view of that and based on our results, we suggest that quantification of circulating FABP3 may also be employed as a biomarker of cancer-associated cachexia. The increased amount of tumor FABP3 seems to be associated with poor prognosis, as non-small cell lung cancer biopsies show augmented mRNA and protein content of FABP3 and FABP4, when compared with paired non-cancerous tissue [59]. Similarly, both proteins are positively associated with advanced tumor node metastasis and worse overall survival [59]. Moreover, FABP3 expression in gastric carcinoma is associated with invasion, metastasis and lower survival [60]. Nevertheless, FABP3 is downregulated in breast [61,62] and embryonic tumors [63], suggesting the existence of a complex regulation of FABP3 and possibly, heterogeneity among tumor types. We show here that in cancer cachexia, FABP3 is increased in the plasma and in tumor homogenates; and also, that tumor FABP3 is positively associated with the circulating levels of CRP and IL-8 in CC. Based on these findings, we propose that higher FABP3 in the circulation may be related to systemic inflammation in patients with gastrointestinal cancer.

Tumor homogenate IL-15 was higher in CC compared to WSC. This cytokine has been shown to increase the immune response against the tumor in mice [64,65] and is present in the tumor microenvironment as its complexed and soluble forms – IL-15-IL-15 receptor α (IL15Rα). In this scenario it regulates tumor-infiltrating lymphocytes, as seen in mice bearing the B16 melanoma [64]. Treatment of mice with lung cancer with intraperitoneally-injected IL-15 decreases the number of tumor nodules, while increasing the expression of immune system negative regulatory checkpoint, the programmed death-1 (PD-1). At the same time, it augments the secretion of the immunosuppressive cytokine IL-10 by $CD8^+$ T cell [65]. Positive staining for IL-15 in tumor biopsies is higher in patients with mucosal hyperplasia in colon cancer, as compared with those without this alteration [66]. Tissue growth factor β expression is concurrent in several IL-15-producing colon cancers [66]. Interleukin-15 was found to have antiapoptotic effects and to increase expression of proliferation and angiogenesis markers in IEC6 intestinal epithelial cells, which was prevented by anti-IL-15 Ab or anti-IL-15 Rα Ab treatment [66].

Although IL-15 acts as a chemoattractant for natural killer (NK) cells, in metastatic colon cancer NK cells infiltrating tumors do not correlate with IL-15 concentration and were fewer, in comparison to the numbers observed in nonmetastatic tumors [66]. Our previous work showed that tumor homogenates from CC patients with colon cancer presents increased protein content for TGF-β and higher presence of angiogenic markers, together with higher deposition of collagen fibers [67], reduced percentage of M2 macrophages and increased content of proinflammatory cytokines, in relation to WSC [7]. Therefore, IL-15 expression seems to be correlated with tumor growth and metastatic potential, being a likely player in inducing increased tumor inflammation observed in cachexia.

We found that irisin protein expression was higher in the tumor samples from CC, in relation to WSC. Lung [68], liver [69] and other gastrointestinal tumors [70] also were shown to express a high content of irisin. In spite of this increase amount of irisin within tumors, lower serum concentration of irisin has been reported in patients with colorectal [26] and breast cancer [27], compared to healthy subjects. In the non-small cell lung carcinoma, irisin was detected in the cytoplasm of cancer cells and in the cytoplasm of tumor stromal cells, but not in normal lung parenchyma [68]. The increased content in tumor cells may be due to the higher glycolytic rate in such cells, as in muscle cells irisin seems to increase glucose uptake by enhancing p38 mitogen-activated protein kinase activation, through AMPKα2 and GLUT4 translocation to the plasma membrane [71]. Furthermore, irisin has been related to browning of white AT, as it can increase Uncoupling Protein 1 expression in adipocytes mitochondria [72]. To the best of our knowledge, this is the first study to report higher irisin content in tumors of cachectic patients, when compared to those of weight stable counterparts. This strongly suggests a new role for this myokine in cancer cachexia.

It is also primordial to evaluate muscle fat infiltration beyond assessing muscle mass, as myosteatosis is related to shorter survival in patients with cancer cachexia [4,73]. Thus, we evaluated skeletal muscle fat infiltration as an indicator of muscle quality, by measuring muscle TAG and the gene expression of proteins involved with fatty acid uptake and storing. In our cohort of patients with cancer cachexia submitted only to tumor resection as primary treatment at the time of tissue collection, we did not observe increase in muscle fat infiltration. However, the cachectic patients in the study showed decreased lumbar AT index, visceral AT index and subcutaneous AT index, all of which have also been linked to decreased survival [74,75]. A negative association of muscle TAG content and muscle attenuation was found in this study, and previously observed by Bhullar et al. [76].

In addition, lumbar AT index was positively associated with BMI and negatively associated with muscle attenuation, showing that patients with lower adiposity also present lower BMI and muscle fat infiltration. Moreover, 56% of the WSC patients, even in the absence of weight loss, also showed low skeletal muscle index. In a longitudinal study that evaluated consecutive CTs from patients with gastrointestinal cancer, skeletal muscle mass was stable in 45% of the time analyzed, and the percentage of patients who lost muscle and AT increased closer to death [77]. In another study, patients with pancreatic cancer that presented AT wasting showed the same overall media survival as patients with pancreatic cancer, with both AT and muscle wasting [74]. Furthermore, higher skeletal muscle and AT wasting, simultaneous to increase in tumor burden were observed in patients with colorectal cancer, suggesting that catabolism of both tissues may occur along disease progression [78]. Despite the lower lumbar AT index in CC, we failed to detect changes in the gene expression of proteins related to skeletal muscle metabolism and TAG content. Nevertheless, skeletal muscle TAG was positively associated with the lumbar AT index measured by CT analysis, showing that increased adiposity was associated with muscle lipid content. Myosteatosis has been observed in patients with colorectal cancer and is positively associated with BMI, total adiposity and AT depots evaluated by CT analysis [73].

We have previously demonstrated that CC patients present disrupted mitochondrial morphology, increased apoptosis and dysfunctional autophagy in the skeletal muscle [79], alterations that are driven by increased sustained systemic inflammation [80–82] and correspond to the initial phases of muscle loss [83,84]. Previous work showed that in subcutaneous AT, the consequences of cancer cachexia are related to an increased presence of inflammatory cells in this tissue [85], a higher content of pro-inflammatory factors [7] and tissue remodeling [86]. In the present study, increased systemic inflammation was demonstrated by the observed higher levels of circulating CRP, IL-6 and IL-8 in patients with CC. Cachexia was also associated with increased self-related symptoms and lower functional capacity. Patients with cancer cachexia presented lower serum HDL and LDL cholesterol, which may be part of the natural course of cachexia. Previous research has shown that patients with pancreatic ductal adenocarcinoma also showed a decrease in serum lipids, concomitant with weight loss, and decreased AT and muscle mass 18 to 6 months before cancer diagnosis [87].

The description of lower muscle FSTL-1 and consequent possible compromised skeletal muscle vascularization; the higher content of FABP3, a circulating muscle damage marker; and the higher tumor FABP3, IL-15 and irisin, (proteins related to increased tumor metabolic activity and tumor growth); in addition to lower body adiposity, systemic inflammation and more advanced tumor stages consists on the main contribution of this study for the understanding of human cachexia. Correlation analysis showed that inflammatory parameters were associated with myokines in circulation, skeletal muscle and tumor. It has been shown that proteins secreted by L6 skeletal muscle cells are modulated by TNFα treatment [88] and that C2C12 myotubes show increased secretion of activin after treatment with tumor-conditioned media [89]. Furthermore, inverse association of tumor FSTL-1 and muscle irisin in WSC group may indicate a possible role of modulation of the skeletal muscle in the prevention of tumor progression, as tumor FSTL-1 is related to tumor aggressiveness [90], and that skeletal muscle irisin content responds to physical activity [72]. In the CC group, a positive association between tumor BDNF and skeletal muscle FABP3, may be associated with the more advanced tumor stage and more profound skeletal muscle damage in this group, as tumor BDNF influences tumor progression [91], and skeletal muscle FABP3 is increased in patients with muscle inflammation and muscle weakness [58].

So far, there is no stablished treatment to cachexia. Physical activity is able reduce the risk of developing several types of cancer [92] and reduces mortality after colon cancer diagnosis [93]. These effects may be mediated by skeletal muscle-secreted myokines [94]. In an animal model, physical exercise blunted tumor growth through epinephrine-dependent mobilization of NK cells and higher immune cell infiltration into tumors [95]. Furthermore, inflammation and insulin-related pathways are regulated by physical exercise in patients with cancer [96] suggesting that physical exercise is a promising therapy for patients with cancer cachexia.

One of the limitations of this study is that the quantity of tissue available for biopsies was frequently small, as a consequence, it was not always possible to perform all measurements with the same sample number and with the tissues from the same patient. That also prevented the correlation analysis between circulating and tissues myokines. A healthy control group is missing, however,

when comparing two groups of patients with tumors we may affirm that the differences were caused by cachexia. The small sample size is a limitation that influenced the effect size and also precluded matching groups on gender, age and date of diagnosis. However, these parameters were found not to differ statistically between groups. Finally, tissue irisin quantification by antibody-based methods, although being a common method employed in the literature, does not equal mass spectrometry analysis [97].

This study provided an overview of the structural and metabolic changes of the muscle mass, but we could not relate these with functional measures (i.e., handgrip strength and gait speed). The number of females was lower than male patients and the number of patients per group was also different. Despite these limitations, we believe this to be the first study to present the cachexia-related alterations of the myokines in *rectus abdominis* and tumor biopsies from cancer patients.

In conclusion, the different myokine content in skeletal muscle, plasma and tumor from patients with cancer-associated cachexia may have a role in tumor evasion, inflammation and tissue remodeling. These changes may be implicated in the decreased capacity for skeletal muscle regeneration, increased muscle breakdown and tumor aggressiveness associated with cachexia. It is also noteworthy that these results demonstrate that reports on animal models of cancer cachexia not always mirror findings in humans.

Author contributions

G.S.C. acquired the data, performed statistical analysis, data interpretation and article writing. G.S.C., J.C.L, E.S., R.G.F.C., S.P.G., K.R., contributed to patient recruitment, interviews, blood collection and tissue handling. F.C.B., F.B.F., L.G.L.G, R.P.S, L.H.A.N., M.F., C.C.C., F.T, P.S.M.A. and J.P.O. contributed to tissue collection during surgical procedures and acquisition of patients' medical record information. C.E.O., J.X. and C.M.P. were responsible for body composition analysis through computed tomography. J.C.L., E.S., S.P.G., D.C.G., R.G.F.C., K.R., U.L. and A.E.T. contribute to data acquisition. L.R.G. and A.F.R. performed the correlations and logistic regression analysis and contributed to data interpretation and article writing. J.P.O., A.L., D.C., C.E.O., J.X., V.C.M. and C.M.P. contributed to data interpretation and article writing. M.S. designed the research, contributed to data interpretation and article writing. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.10.050.

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