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Adipose tissue-derived factors as potential biomarkers in cachectic cancer patients

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ABSTRACT

Cachexia, a paraneoplastic syndrome markedly associated with worsened prognosis in cancer patients, provokes profound wasting of both lean and adipose mass in an association with a state of metabolic "chaos". The white adipose tissue responds to cachexia with marked local inflammation and may be thus a relevant contributor to systemic inflammation. To address this hypothesis we examined the correlation between tissue expression of adipokines and plasma concentration in cachectic and stable weight patients with or without cancer. Adiponectin and liver-derived CRP concentration were significantly higher in the cachectic groups when compared with stable weight patients ($P < 0.01$). The concentration of plasma IL-6 was higher (11.4-fold) in the cancer cachectic group when compared with weight-stable controls, and presented a significant correlation with the presence of cancer ($P < 0.001$). A marked increase (5-fold) in IL-6 as a result of the interaction between the presence of cachexia and the presence of tumour was observed in the subcutaneous tissue of the patients, yet not in the visceral depot. Plasma adiponectin levels were higher in cachectic cancer patients, compared with stable weight cancer patients individually matched by age, sex, and BMI, and the subcutaneous depot was found to be the main contributing tissue, rather than the visceral pad. Based on the results we concluded that the subcutaneous adipose tissue is associated with plasma changes that may function as markers of cachexia.

1. Introduction

Cachexia, a wasting syndrome is associated with 22–40% of cancer deaths [1,2]. Progressive cachexia represents an independent prognostic factor concerning the response to antineoplastic therapy and survival [3], and has been recently defined as "a multifactor syndrome characterized by ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment" [4]. Although muscle wasting has been the main focus of cachexia-related research [5,6], studies show that fat loss occurs more rapidly and more precociously than the reduction of lean mass in cancer cachexia [7,8], and may extend up to 80% within a very short interval, especially in the immediate period preceding death [9].

The underlying molecular basis of cachexia is still poorly understood, yet the perspective of the syndrome as a chronic inflammatory state, in which the host's reaction to the presence of the tumour seems to be the main causal agent, is gaining crescent acceptance [10–12]. Inflammation may be a result of the production of mediators deriving both from the tumour and from the host's tissues, among which inflammatory cytokines appear as major contributors [6].

We have shown [13–15] that the white adipose tissue (WAT) actively expresses and secretes a plethora of pro-inflammatory factors in a rodent model of cancer cachexia and may thus be considered an important subscriber to the characteristic systemic inflammation. Furthermore, we hereby propose that factors deriving from this tissue may be adopted as markers for the diagnosis and grading of the syndrome, based on the aforementioned animal studies and on the results we now present with cancer patients.

WAT has been shown to largely subsidise systemic inflammation in a variety of diseases, as it has, over the last decades, been recognised to be a major endocrine organ, capable of actively synthesising and secreting a plethora of humoral factors, the adipokines, among which leptin, adiponectin, TNF-α, IL-6, IL-10, plasminogen activator inhibitor-1 and visfatin; all of which act locally in an autocrine/paracrine manner and/or as endocrine signals in the regulation of appetite, energy expenditure and a range of physiological processes including insulin sensitivity and the inflam-

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matory response [16]. Increased production of lipolytic factors by the adipose tissue and the tumour, such as interleukin 6 (IL-6), tumour necrosis factor α (TNF-α) or zinc-α2 glycoprotein (ZAG) contributes to the disrupted lipid metabolism and increased lipolysis in cancer cachexia [5,17]. Therefore, the white adipose tissue is both a victim and a sponsor of cachexia-related systemic inflammation.

In this study we present results with gastrointestinal cancer patients that may support the adoption of WAT-derived factors as markers of cachexia. The recently proposed grading of cachexia [2], envisaging three stages, namely; pre-cachexia, cachexia, and refractory cachexia, signals the necessity of establishment of tools allowing for unequivocal diagnosis aiming at precise intervention, according to the patient's actual disease stage and prevention of its aggravation.

2. Patients and methods

2.1. Patients

All patients were recruited between November 2008 and July 2011 at the ambulatory surgery division from the University Hospital of the University of São Paulo ($n = 235$). The inclusion criteria were; 1 – no prior anticancer treatment, 2 – not having clinical evidence of gastrointestinal obstruction, 3 – fitting into the logistical scheme for scientific studies before operation or preoperative treatment and 4 – willingness to participate ($n = 61$). The exclusion criteria were liver failure, renal failure, AIDS, inflammatory diseases of the bowel and autoimmune disorders. The study was approved by the Regional Ethics Committee (CEP-HU/USP 752/07, CAAE 0031.0.198.019-07). The investigation was explained in detail to each patient and a written informed consent was obtained. The patients were divided into four groups based on diagnosis after surgery. The cachectic groups were subdivided into two groups: cancer cachexia ($n = 31$), and control (non-cancer) cachexia ($n = 8$). Cancer cachexia was defined as prediagnosed gastrointestinal cancer with no evidence of gastrointestinal obstruction or anorexia with nausea and/or stomach pain, in combination with unintentional weight loss of >5% of the habitual weight during the last 3 months or >10% weight loss during the last 6 months. The same criteria were adopted to classify control cachexia, excluding cancer diagnosis. The weight-stable groups (no important weight change during last year and BMI <25) were divided into weight-stable cancer ($n = 12$) and weight-stable control (non-cancer) patients (control, $n = 10$). In the cancer groups, the tumour primary location was: colon ($n = 14$), stomach ($n = 13$), pancreas ($n = 3$), rectum ($n = 2$), and others ($n = 11$). The cachectic control group was composed by patients with: cirrhosis ($n = 3$), chronic pancreatitis ($n = 3$), and gastric or duodenal ulcers ($n = 2$). The weight-stable control group included patients undergoing surgery for incisional ($n = 7$), inguinal ($n = 2$) and umbilical ($n = 1$) hernia. The study was designed as "intention to compare"; therefore, all subjects were kept in the analyses despite a few missing values of the measurements. The remaining 174 patients were excluded because (a) although pre-diagnosed with gastrointestinal cancer they did not have malignancy according to final histological evaluations ($n = 41$) or (b) could not provide adequate amounts of adipose tissue, at least 0.9 g or (c) could not provide, for the same patient, samples from subcutaneous and omental adipose tissue depots and samples related with inflammatory and biochemical parameters, for a complete investigation. Table 1 presents the general characteristics of patients in each group.

2.2. Clinical parameters assessment

Height and weight were determined and approximately 10 mL of blood were collected after an overnight fasting, within the venous access procedure for anaesthesia during the surgery, allowing the measurement of plasma lipids, glucose, serum urea, and hemoglobin. Plasma and serum samples were immediately frozen at −80 °C until further analysis. Tumour staging was determined postoperatively according to the guidelines of the UICC TNM [18]. Cachexia staging, anorexia and inflammation were assessed by the Cachexia score [19].

2.3. Adipose tissue biopsies

Approximately 1 g of subcutaneous and omental white adipose tissue (by approximate anatomical site), were collected at 5 min intervals, similarly to described by [17]. Tissue samples were rapidly rinsed in saline, frozen in liquid nitrogen, and kept at −80 °C for further analyses. This procedure presented a minimal degree of risk, and did not interfere with surgery routine.

2.4. Plasma measurements

ELISA kits were employed for the measurements of TNF-α and IL6 (CytoSet™, Invitrogen); CRP (cat.# HCVD2-67BK-01), Adiponectin (cat.# HADK1-61K-A), Leptin (cat.#HENDO-65K-01), IL10 (cat.# MPXHCYTO-60K-02), (Genese Produtos Diagnósticos Ltda., Brasil) and Multiplex kits, for Luminex platform (R&D Systems, USA). Adipokine and cytokines concentration was determined in triplicate, and the mean was used for analysis. The inter-assay CV over all ranges was lower than 12%.

2.5. Gene expression analysis

Total RNA of the samples was isolated with TriPure Isolation Reagent (Roche®) following the recommendations of manufacturer and total RNA concentrations quantified by spectrophotometry (Nanodrop ND-1000). Complementary DNA synthesis was carried out with 13 μl assay mix containing 3 μg total RNA, 10U RNAse inhibitor, 2 μl random primers, 2 μl dNTP (10 nmol), 2 μl dithiothreitol, 10U M-MLV reverse transcriptase and 4 μl of 10× reaction buffer (100 mM TRIS–HCl, 500 mM KCl; 150 nM $MgCl_2$ in nuclease free water) (Invitrogen). The mRNA expression of the following genes was determined: IL-6 (NM_000600.3, Forward 5′TAC CCC CAG GAG AAG ATT CC3′, Reverse 5′AGG TTG TTT TCT GCC AGT GC3′), Adiponectin (NM_001177800.1, Forward 5′ATG ACC AGG AAA CCA CGA CT3′, Reverse 5′CAC CGA TGT CTC CCT TAG GA3′), TNF-α (NM_000594.2, Forward 5′ CTC TCT CCC CTG GAA AGG AC 3′, Reverse 5′ATC ACT CCA AAG TGC AGCA G3′), IL-10 (NM_000572.2, Forward 5′CCA AGC TGA GAA CCA AGA CC 3′, Reverse 5′AAG GCA TTC TTC ACC TGC TC 3′), and leptin (NM_000230.2, Forward 5′GGA GGG CAA GGG CCA TGC TG′3, Reverse 5CT GGC CAC AGC ACCA GCC TC′3). Five μL of cDNA (25 ng) were mixed with 2x SYBR Green PCR master mix (Applied Biosystems) and primers (Invitrogen). Quantitative real-time PCR was performed with an ABI 7300 Real Time Systems (Applied Biosystems). The mRNA levels were determined by comparative Ct method for each sample. A ΔCt value was obtained by subtracting 18S values from those of the gene of interest. The average ΔCt value of the control group was then subtracted from the sample to derive a $\Delta^{-\Delta Ct}$ value. The expression of each gene was evaluated by $2\text{-}(\Delta^{-\Delta Ct})$.

2.6. Statistical analysis

The statistical analysis was performed with the commercially available statistical package from SigmaStat (version 3.1, SigmaStat, SYSTAT, Point Richmond, CA). Data were expressed as means ± SE and analysed by 2-way ANOVA of $2 * 2$ design (cancer/non-cancer-control vs. stable weight/cachexia). Data were parti-

Table 1
Characteristics of study groups.

Measure	Weight-stable		Cachexia		ANOVA
	Control	Cancer	Control	Cancer	
n	10	12	8	31	
Gender (M/F)	6–4	8–4	5–3	19–12	
Age (year)	63 ± 8	66 ± 13	65 ± 8	57 ± 15	–
Weight (kg)	57 ± 3	59 ± 8	52 ± 10	56 ± 17	–
Height (m)	1.5 ± 0.8	1.6 ± 1	1.6 ± 1	1.6 ± 1	–
BMI (kg/m^2)	22 ± 1	23 ± 3	20 ± 4	21 ± 3	–
Weight loss (kg)	1.6 ± 1	1.5 ± 2	19 ± 11	13 ± 7	C., C. × T
Weight loss, relative (%)	3.0 ± 2	4.2 ± 2	26 ± 14	21 ± 8	C., C. × T
Tumour stage					
IA	1%	10%	0%	0%	
IB	0%	0%	1%	4%	
IIA	0%	0%	0%	0%	
IIB	1%	10%	3%	12%	
IIIA	2%	20%	7%	28%	
IIIB	2%	20%	2%	8%	
IV	2%	40%	12%	48%	
Adiponectin (μg/mL)	7.8 ± 2.7	11.1 ± 8.5	25.2 ± 17	19.1 ± 7.3	C.
Leptin (pM)	646 ± 158	309 ± 271	13.2 ± 12	221 ± 191	C.
Resistin (ng/mL)	15 0 + 26	11.3 ± 5.8	14.2 ± 10	15.7 ± 11	
CRP (μg/mL)	3.1 ± 1.8	14.9 ± 13	18.4 ± 15	24.9 ± 14	C.
TNF (pg mL)	11.1 ± 4.5	13.8 ± 4.3	10.3 ± 5.9	72.5 ± 29	C. × T
IL-6 (pg/mL)	12.9 ± 3.6	30.3 ± 8.2	36.9 ± 20	160 ± 58	T, C. × T
IL-10 (pg/mL)	2.4 ± 0.7	4.9 ± 3.6	6.0 ± 8.6	24.3 ± 19	
IL-6/IL-10	2.5 ± 0.8	6.0 ± 1.7	6.2 ± 2.5	24.8 ± 12	C. × T

Note: values are mean ± SD; *n*: number of patients; BMI: body mass index. Values that are significantly different by two-way ANOVA are indicated by two-way ANOVA, C. (stable weight vs. cachexia); T (tumor vs. non-tumor) and C × T (interaction between stable weight vs. cachexia and tumour vs. non-tumour).

tioned into main effects (cancer vs. non-cancer-control group effects, A; and stable weight vs. cachexia group effects, B). The interaction effects consisted of A ∗ B. When a significant *F* value was found by 2-way ANOVA, a Holm-Sidak *post hoc* test was performed to demonstrate all pairwise multiple comparisons between the means. Correlation analysis was conducted using Pearson correlation or Spearman rank correlation tests. Stepwise method of multiple regression analysis (with $p < 0.05$ as an entrance criterion and $p > 0.1$ as a removal criterion) was applied in order to identify variables independently associated with cachexia (% weight loss and BMI) and inflammatory (IL-6) parameters and to quantify the strength of the association. All calculated p values were two-sided and a $p < 0.05$ was considered significant.

3. Results

3.1. Clinical findings

Baseline characteristics of the patients are shown in Table 1. The subjects in the four groups were of similar age and body mass index (BMI). In this regard, we have not evaluated lean body mass although groups were matched by BMI. The cancer cachexia group showed a marked decrease in body weight. Subjects in the cachectic groups (cachectic cancer and non-cancer patients) presented an accentuated reduction of relative body weight compared with weight-stable cancer (25.8 ± 14%) and control (20.6 ± 8.5%) within the period between cancer diagnosis and surgery ($p < 0.05$; 2-way ANOVA effect of cachexia). Plasma triglyceride levels were reduced in cachectic groups ($p < 0.01$; 2-way ANOVA effect of cachexia). There was no difference with respect to other biochemical parameters (i.e., plasma glucose, and serum urea, serum haemoglobin values), however, the haemoglobin values found in cachectic groups were lower than 12 g/dL, and plasma glucose showed a tendency to be different ($p = 0.076$; 2-way ANOVA effect of cachexia). There was no difference between the weight-stable cancer and cancer cachectic patients with respect to tumour stage.

3.2. Inflammatory cytokine plasma levels are increased and IL-6 positively correlates with tumour stage

The concentration of IL-6 was shown to be affected by both cancer ($p = 0.011$; 2-way ANOVA effect of cancer) and the interaction between the presence of cachexia and cancer ($p = 0.011$; 2-way ANOVA effect of interaction factors) (Table 1). Furthermore, there was an increase of IL-6 levels along the advancing disease stage (Table 2), as well as a correlation with the incidence of distant metastasis ($p = 0.035$, not shown), which was only related with IL-6. The levels of TNF-α were significantly higher in cachectic cancer patients ($p = 0.046$; 2-way ANOVA effect of interaction factors) compared with controls (weight stable and cachexia) (Table 1). There was no correlation between TNF-α plasma levels and cancer

Table 2
Association between cytokines and adipokines plasma levels and advancing cancer disease stage in cachectic cancer patients.

	Stages			
	I/II	III	IV	ANOVA
n	6	9	12	
Weight loss (%)	12.0 ± 1.7	19.7 ± 7.2	17.8 ± 5.3	0.077
Weight loss (A)	9.8 ± 1.8	14.0 ± 4.1	11.8 ± 4.1	0.160
BMI	22.9 ± 0.8	22.4 ± 3.7	21.3 ± 3.2	0.702
Adiponectin	9.9 ± 2.6	19.0 ± 7.9	13.5 ± 7.0	0.072
Leptin	276.2 ± 26.2	45.2 ± 27.0	222.7 ± 189.4	0.015
IL-6	37.4 ± 10.1	115.6 ± 56.9	415.7 ± 193.7	<0.001
CRP	23.1 ± 23.0	18.8 ± 20.5	26.8 ± 29.3	0.814
TNF	85.2 ± 56.1	57.1 ± 30.6	55.1 ± 51.7	0.823
IL-10	0.705 ± 0.3	32.5 ± 49	26.8 ± 42.8	0.382

BMI, body mass index; CRP, C-reactive protein; IL, interleukin; TNF, tumour necrosis factor.

stage (data not shown). CRP levels were increased in those patients with cachexia ($p = 0.047$ and $p = 0.015$; 2-way ANOVA effect of cachexia, respectively) with no other detectable effect of cancer and/or of interaction. IL-10 levels were not different among the groups. IL-6 to IL-10 ratio (IL-6/IL-10) was affected by the interaction between the presence of cachexia and cancer ($p = 0.005$; 2-way ANOVA effect of interaction factors) (Table 1).

3.3. Adipokine plasma levels are disrupted in cancer cachexia

The mean concentration of adiponectin was much higher in cachectic (cancer and non-cancer) patients ($p = 0.010$; 2-way ANOVA effect of cachexia) when compared with the weight stable groups (cancer and non-cancer) (Table 1). Once body weight loss is a hallmark of cachexia, we examined separately the percentage of weight loss and BMI as different parameters. The strongest association was found between adiponectin levels and these two variables (percentage of weight loss, $r = 0.67$, $p = 0.002$ and BMI, $r = -0.56$, $p = 0.02$) (Fig. 2).

3.4. IL-6 and adiponectin are more expressed in the subcutaneous vs. visceral adipose tissue

IL-6 and adiponectin mRNA expression was examined in two different adipose tissue pads (subcutaneous and visceral). IL-6 mRNA expression in the subcutaneous adipose tissue was affected by the presence of the tumour ($p = 0.001$; 2-way ANOVA effect of cancer), when compared with cachexia groups, with no effect of cachexia and/or interaction (Fig. 1A). This increase was not evident in samples from the visceral adipose tissue. The association between plasma levels ($r = 0.67$, $p = 0.002$) and mRNA expression of IL-6 ($r = -0.56$, $p = 0.02$) was observed just for the samples obtained from the subcutaneous depot (Fig. 1B). Adiponectin mRNA expression in the subcutaneous adipose tissue was affected by the presence of cachexia ($p = 0.005$; 2-way ANOVA effect of cachexia) and by the interaction between the presence of cachexia and tumour ($p = 0.036$; 2-way ANOVA effect of interaction factors), when compared with the weight stable controls (Fig. 2). Leptin and TNF-α mRNA expression were altered in the subcutaneous adipose tissue from cachectic (cancer and non-cancer) patients ($p = 0.005$ and $p = 0.009$; 2-way ANOVA effect of cachexia, respectively) when compared with the weight stable groups (cancer and non-cancer) (Fig. 3A and B). IL-10 was affected by the interaction between cancer vs. cachexia ($p = 0.046$; 2-way ANOVA effect of interaction factors) compared with controls (weight stable and cachexia) (Fig. 3C). No changes were detected in the visceral adipose tissue regarding the same genes.

4. Discussion

A novel finding of this study was that the expression of some molecules seems to be disrupted during cachexia while some other may present increased expression with the interaction between the presence of tumour and of cachexia development, notably IL-6. Moreover, an association between adiponectin with body weight loss, as well as a positive relationship between IL-6 and cancer T stage suggests an important role of these molecules as potential biomarkers of cancer cachexia. Due to the important role of adipose tissue as a source of inflammatory mediators, we also examined the fat depot-specific expression of these molecules in different WAT pads (VAT and SAT). We report depot-specific differences in adipose tissue mRNA expression of IL-6 and adiponectin,

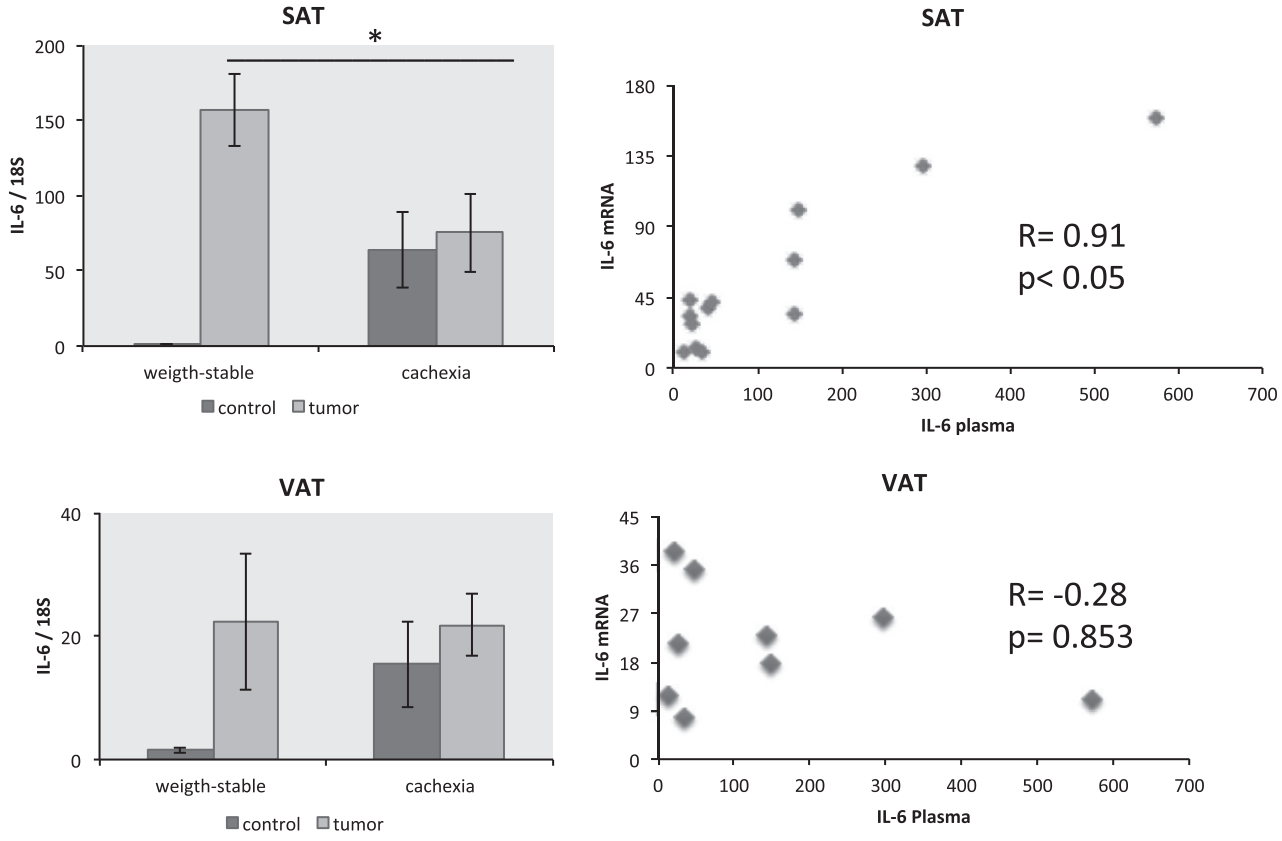

Fig. 1. IL-6 mRNA expression in subcutaneous (SAT) and visceral (VAT) adipose tissue in cancer cachexia, control cachexia, weight-stable with cancer and non-cancer patients (*right side*). Relationship between mRNA expression and plasma levels of IL-6 is shown in SAT (*top left*) and VAT (*bottom left*). ANOVA was employed in the right side graphs; otherwise linear regression was adopted. *$P = 0.001$; 2-way ANOVA effect of cancer.

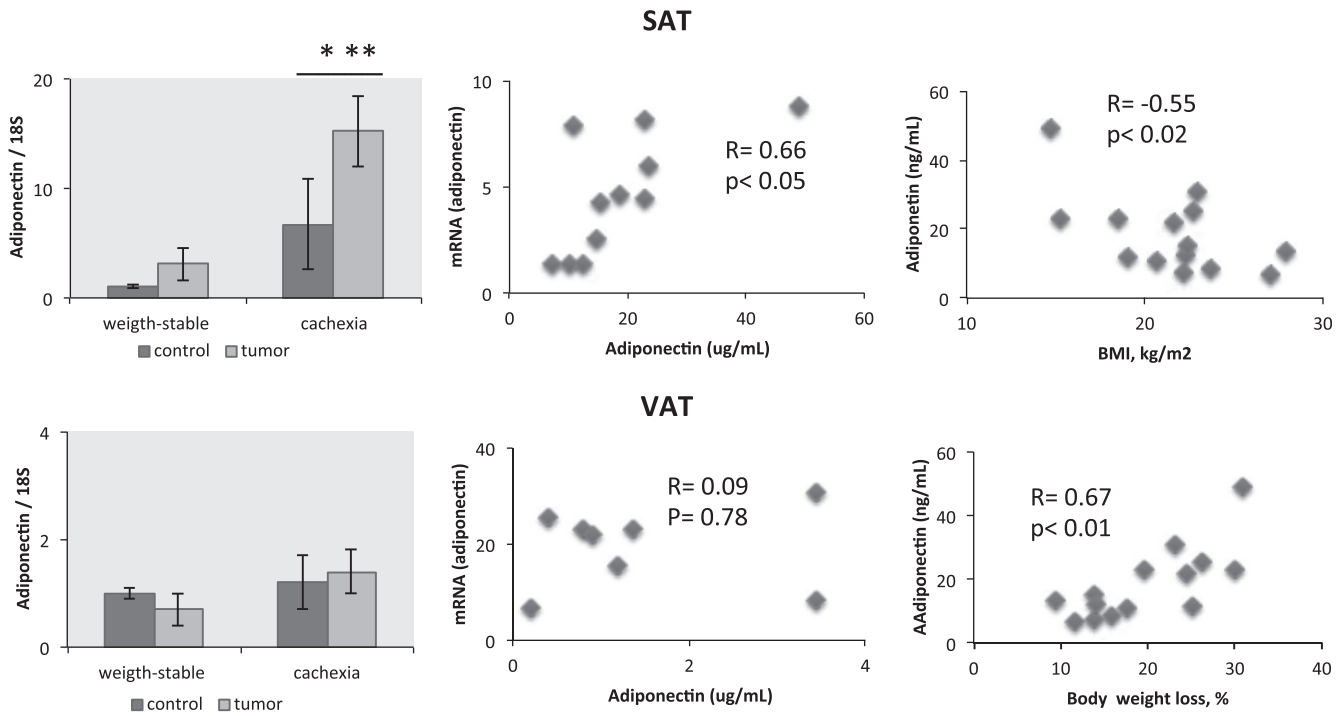

Fig. 2. Adiponectin mRNA expression in subcutaneous (SAT) and visceral (VAT) adipose tissue in cancer cachexia, control cachexia, weight-stable with cancer and non-cancer patients (*right side*). Relationship between mRNA expression and plasma levels from adiponectin is shown from SAT (*top central*) and VAT (*bottom central*). ANOVA was employed in the right side graphs; otherwise linear regression was adopted. Relationship between adiponectin plasma levels with BMI (*top right*) and body weight loss (*bottom right*). $^{*}P = 0.005$; 2-way ANOVA effect of cachexia and $^{**}P = 0.036$; 2-way ANOVA effect of interaction factors.

increased in SAT of cancer cachexia patients while VAT mRNA expression was unaffected.

It is clear nowadays that adipose tissue wasting precedes lean mass degradation both in cancer patients and experimental models of cachexia, and such symptom may be detected even before any changes in food consumption are noticed [20,21]. In addition to that, it is becoming increasingly clear that chronic systemic inflammation is the hallmark of the syndrome, and that it may be adopted for assessing not only the occurrence of cachexia, but in addition, its depth [22,23]. The WAT contributes in a significant manner to systemic inflammation, as a potent source of inflammatory factors [11]. Our results show an accentuated modification in the gene expression, as well as in plasma concentration of adipokines and pro-inflammatory molecules in cachectic gastrointestinal cancer patients, in relation to the non-cachectic counterparts. These include adiponectin, leptin, TNF-α, IL-6, and IL-10. Moreover, we have compared the contribution of the visceral and abdominal subcutaneous pads, having found that the changes affecting the latter may contribute for the development and progression of cachexia.

With respect to TNF-α, cachectic cancer patients demonstrate a 6.5-fold increase in plasma concentration, as compared with the stable weight controls, while subcutaneous adipose pad gene expression is concomitantly increased by around 70-fold. The magnitude of this alteration provides an early indication of the presence of marked local inflammation with systemic consequences in the following steps of cachexia progression. Similarly, the reduction in SAT gene expression of leptin is more pronounced (around 6.5-fold) than that observed in plasma concentration (2.9-fold), and adiponectin tissue expression (enhanced by 13.8-fold), follows the same pattern, with plasma concentration varying 2.4-fold, solely, as compared to control stable-weight patients (non-cancer).

Increased TNF-α concentration, along with augmented levels of IL-6 and IL-10 have been frequently reported in cachectic cancer patients, indicating that these cytokines seems to respond both to the presence of the tumour and to the occurrence of cachexia: marked alteration of cytokine circulating levels has recently been reported in esophageal squamous cell carcinoma [24] as well as in gastric adenocarcinoma [25,26], and in gastroesophageal cancer patients [27,28]. It has been postulated that up-regulation of pro-inflammatory cytokines is a common feature in the syndrome [29], while reduction in food intake does not induce a detectable increase in the circulating levels of these factors [30]. Therefore, early alterations of higher magnitude of these well-established markers may indeed contribute to precocious diagnosis.

Adipose tissue derived hormones, on the other hand, have been claimed not to be correlated with inflammation in cachexia [31]. This assumption is based on the premise that they rather reflect adipose tissue wasting, rather than actively contribute to the cachexia-associated alterations. For instance, inconsistent results show increased adiponectin levels and reduced BMI in cachectic patients (positive correlation) [32,33], low levels in patients with weight-loss in advanced lung cancer (negative correlation) [34], or still, absence of correlation in cachexia patients [35]. For leptin, this also stands true [36]. This inconsistence is partly due to the absence of individual controls matched by age, sex, BMI, and cancer TNM stage, as well as presence of inflammatory disease related to low grade inflammation of control groups [37].

Nevertheless, the studies examining human adipokines and cachexia have focused on the plasma content of these factors, which is the final result of the different contributions of the many adipose depots. We have previously shown in an animal model of cachexia, that there is marked zonal heterogeneity of response to cachexia among fat pads [38]. In addition, increased lipolysis in cancer cachexia has been suggested as key factor in triggering the loss of adipose tissue in these patients, notably in relation to subcutaneous pads [17,39]. The present results show that clear peculiarities are associated with the anatomical localization of the depot, with

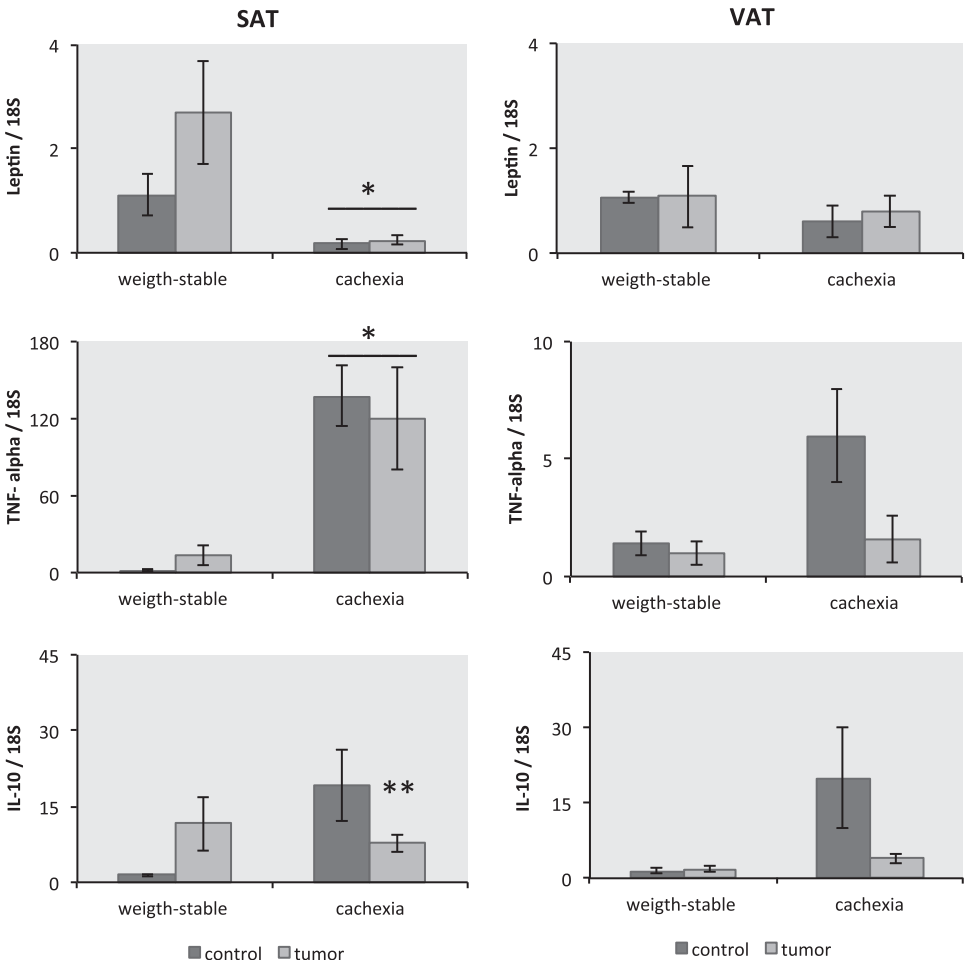

Fig. 3. Leptin (A), TNF-α (B) and IL-10 (C) mRNA expression in subcutaneous (SAT) and visceral (VAT) adipose tissue in cancer cachexia, control cachexia, weight-stable with cancer and non-cancer patients (*right side*). ANOVA was employed in the right side graphs; otherwise linear regression was adopted. $^{*}P = 0.005$; 2-way ANOVA effect of cachexia and $^{**}P = 0.036$; 2-way ANOVA effect of interaction factors.

more pronounced adjustments in the subcutaneous pad. Bearing this in mind, it is possible to regard tissue expression differences (13.8-fold for adiponectin and decreased 65.8% for leptin expression between control stable-weight patients and cancer cachexia groups) as early markers of cachexia-related adipose wasting.

The reported changes in adiponectin from patients were found to be markedly correlated with weight loss in the previous 6 months. Through BMI matching, we isolated the confounding effect of the inverse association between BMI and adiponectin. To the best of our knowledge, no previous study has compared adiponectin levels in BMI-matched cachectic and non-cachectic patients, with or without gastrointestinal cancer. Therefore we may suggest, corroborating [33], that the higher capacity for adiponectin production in cachexia may contribute to the wasting process, as adiponectin administration in experimental animal models has been demonstrated to increase energy expenditure followed by body weight lost [40].

Still regarding adiponectin, the higher levels herein reported may have been influenced by differences in the characteristics of our patients. The cancer cachectic group showed more advanced tumour stage classification and higher CRP values. The association of adiponectin with tumour stage classification in colorectal adenoma-bearing patients has been recently shown Nakajima [41], however, we found no clear relationship between the degree of cachexia and tumour staging. Indeed, it has been shown that patients bearing very small tumour burdens may present profound cachexia and worsened prognosis than those bearing large tumour masses [42]. Furthermore, the cachectic cancer patients show intense inflammatory activation (as demonstrated by high CRP), accentuated body weight lost (about 20%), and lower haemoglobin levels. Although these symptoms may simply reflect cachexia severity, since cancer cachexia is a characteristic of advanced/end-stage disease, a clear correlation between these variables with adiponectin has never been reported.

The controversial results on leptin and cancer cachexia in the literature [36] may also be related with the fact that the most of the studies investigate the plasma levels of the hormone, rather than adipose tissue expression. Tessitore et al. [43] described decreased leptin mRNA levels in the white adipose tissue of breast and gynecological cancer patients, a result that is similar to that we now report for the cachectic gastrointestinal cancer patients. We may once again, imply that tissue modifications are more appropriate for the precise detection of pathological changes. Another interesting finding still regarding the heterogeneous response of white adipose tissue pads to cachexia, is the subcutaneous tissue-specific increase of IL-6 expression. IL-6 is considered to be an adipose tissue-derived lipolytic factor, and thus plays an important role in promoting adipose wasting in cancer patients [39]. In addition, a marked positive relationship between subcutaneous pad IL-6 mRNA expression and plasma levels of this cytokine was found, indicating that subcutaneous depots may be an important source of IL-6 in cancer cachexia. Therefore, although it is clear that plasma IL-6 is increased in cancer cachexia, both in patients [28,39] and rodents [44], the source(s)

of the cytokine has/have not been established. We thus propose that SAT contributes in a relevant way to this parameter.

Up-regulation of IL-6 seems conspicuous in cachectic cancer subjects [39,45], a result we also report. In advanced cancer patients, increased circulating levels of IL-6 are negatively related to impairment of physical and cognitive functions [45] and associated with functional disability of daily living [46]. We additionally show that up-regulation of IL-6 is also present in weight stable cancer patients. Furthermore, the high IL-6 levels related with tumour staging suggest that the primary tumour is an important source of pro-cachectic cytokines in gastrointestinal cancer patients.

In animal models of cachexia [14,44,47], the involvement of IL-6, TNF-α, IL-1 and IL-10 seems to be well established, while clinical studies on humans not always manage to confirm the importance of TNF-α and IL-6 [48]. Moreover, the participation of certain cytokines in the pathomechanisms of weight loss in cancer-related cachexia appears to depend on several factors, such as tumour type [5], cancer TNM stage and experimental designing; individual controls matched by age, sex, BMI. In this aspect, up-regulation of IL-6 has been a consistent finding in several studies, notably in cachectic patients, independently of the etiology of the syndrome [28]. IL-6 has relatively the longest half-life, which may explain why IL-6 but not TNF-α and IL-1, is frequently found to be augmented in cancer, and associated with weight loss [28,49].

We suggest, based on the results of the present study, showing plasma IL-6 to be robustly increased during the syndrome, that this may represent an interesting tool for assessing cachexia. Despite the fact that IL-10 plasma levels were not changed due to cachexia and/or cancer, IL6/IL-10 ratio is a valuable tool for the determination of inflammatory and anti-inflammatory cytokine balance [50]. Increased IL-10 plasma concentration has been found to be associated with the process of chronic inflammation and worsened outcome in several diseases [11]. Although this is not the case of the cachectic cancer patients herein studied, at least considering IL-10 changes, IL6/IL-10 ratio seems to be specifically increased by cachexia and cancer association. Nevertheless, a more detailed study on the adequacy of IL6/IL-10 ratio in the setting of cancer cachexia is required.

Limitations of this study should be acknowledged. We have not evaluated lean body mass among the groups, although they were matched by BMI. In this aspect, the cohort of patient enrolled in the current study may be not representative of a typical cancer population from Europe or North America and some differences such as; total fat mass from cancer groups and overweight and obesity prevalence should be considered. Cachectic groups showed values ranging from 11% to 25% of body weight loss in the previous 6 months and CRP higher than 5 μg/mL, indicating that we studied patients staging between cachexia to refractory cachexia. Cancer cachexia may have several etiologies, including malnutrition, anorexia and tumour-specific effects. Our study was performed with patients with gastrointestinal adenocarcinoma, predominantly located on colon and stomach, which are associated with significant weight loss. The current study was addressed to assess the effect of cachexia and tumour *per se* but was not empowered to detect differences between selective cancer forms. In this aspect, larger cohorts with further grouping of patients according to tumour phenotype could have resulted in other differences.

5. Concluding remarks

In conclusion, our study shows systemic inflammation in cachectic cancer patients, a characteristic that is important for both the development of cancer and of cachexia. IL-6 plasma levels were increased in both weight-stable and cachexia cancer patients, and this change showed a positive relationship with IL-6 and cancer T stage, suggesting an important role for this cytokine as a biomarker of cancer cachexia. Plasma adiponectin was higher in patients with cancer cachexia compared with cancer controls individually matched by BMI. Furthermore, we also demonstrated a role for the subcutaneous depot as a possible systemic source of such molecules.

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References

[1] Warren ER. Position of wood in beaver dams. Science 1932;75:194–5.
[2] Blum D, Omlin A, Fearon K, Baracos V, Radbruch L, Kaasa S, et al. Evolving classification systems for cancer cachexia: ready for clinical practice? Support Care Cancer 2010;18:273–9.
[3] Davis MP, Dickerson D. Cachexia and anorexia: cancer's covert killer. Support Care Cancer 2000;8:180–7.
[4] Fearon KC. The 2011 ESPEN Arvid Wretlind lecture: cancer cachexia: the potential impact of translational research on patient-focused outcomes. Clin Nutr 2012.
[5] Tisdale MJ. Molecular pathways leading to cancer cachexia. Physiology (Bethesda) 2005;20:340–8.
[6] Argiles JM, Busquets S, Toledo M, Lopez-Soriano FJ. The role of cytokines in cancer cachexia. Curr Opin Support Palliat Care 2009;3:263–8.
[7] Fouladiun M, Korner U, Bosaeus I, Daneryd P, Hyltander A, Lundholm KG. Body composition and time course changes in regional distribution of fat and lean tissue in unselected cancer patients on palliative care-correlations with food intake, metabolism, exercise capacity, and hormones. Cancer 2005;103:2189–98.
[8] Arner P. Medicine. Lipases in cachexia. Science 2011;333:163–4.
[9] Lieffers JR, Mourtzakis M, Hall KD, McCargar LJ, Prado CM, Baracos VE. A viscerally driven cachexia syndrome in patients with advanced colorectal cancer: contributions of organ and tumor mass to whole-body energy demands. Am. J. Clin. Nutr. 2009;89:1173–9.
[10] Lundholm K, Daneryd P, Korner U, Hyltander A, Bosaeus I. Evidence that long-term COX-treatment improves energy homeostasis and body composition in cancer patients with progressive cachexia. Int. J. Oncol. 2004;24:505–12.
[11] Seelaender M, Batista MJ, Lira F, Silverio R, Rossi-Fanelli F. Inflammation in cancer cachexia: to resolve or not to resolve (is that the question?). Clin Nutr 2012.
[12] Skipworth RJ, Stewart GD, Dejong CH, Preston T, Fearon KC. Pathophysiology of cancer cachexia: much more than host–tumour interaction? Clin Nutr 2007;26:667–76.
[13] Batista Jr ML, Peres SB, McDonald ME, Alcantara PS, Olivan M, Otoch JP, et al. Adipose tissue inflammation and cancer cachexia: possible role of nuclear transcription factors. Cytokine 2012;57:9–16.
[14] Machado AP, Costa Rosa LF, Seelaender MC. Adipose tissue in Walker 256 tumour-induced cachexia: possible association between decreased leptin concentration and mononuclear cell infiltration. Cell Tissue Res. 2004;318:503–14.
[15] Batista Jr ML, Neves RX, Peres SB, Yamashita AS, Shida CS, Farmer SR, et al. Heterogeneous time-dependent response of adipose tissue during the development of cancer cachexia. J Endocrinol 2012;215:363–73.
[16] Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br. J. Nutr. 2004;92:347–55.
[17] Agustsson T, Ryden M, Hoffstedt J, van Harmelen V, Dicker A, Laurencikiene J, et al. Mechanism of increased lipolysis in cancer cachexia. Cancer Res. 2007;67:5531–7.
[18] Kwon SJ. Evaluation of the 7th UICC TNM Staging System of Gastric Cancer. J Gastric Cancer 2011;11:78–85.
[19] Argiles JM, Lopez-Soriano FJ, Toledo M, Betancourt A, Serpe R, Busquets S. The cachexia score (CASCO): a new tool for staging cachectic cancer patients. J Cachexia Sarcopenia Muscle 2011;2:87–93.
[20] Bing C. Lipid mobilization in cachexia: mechanisms and mediators. Curr Opin Support Palliat Care 2011;5:356–60.
[21] Murphy RA, Wilke MS, Perrine M, Pawlowicz M, Mourtzakis M, Lieffers JR, et al. Loss of adipose tissue and plasma phospholipids: relationship to survival in advanced cancer patients. Clin Nutr 2010;29:482–7.
[22] McMillan DC. Systemic inflammation, nutritional status and survival in patients with cancer. Curr Opin Clin Nutr Metab Care 2009;12:223–6.
[23] Fearon KC. Cancer cachexia and fat-muscle physiology. New Engl. J. Med. 2011;365:565–7.
[24] Ren Y, Cao B, Law S, Xie Y, Lee PY, Cheung L, et al. Hepatocyte growth factor promotes cancer cell migration and angiogenic factors expression: a

prognostic marker of human esophageal squamous cell carcinomas. Clin. Cancer Res. 2005;11:6190–7.
[25] Macri A, Versaci A, Loddo S, Scuderi G, Travagliante M, Trimarchi G, et al. Serum levels of interleukin 1beta, interleukin 8 and tumour necrosis factor alpha as markers of gastric cancer. Biomarkers 2006;11:184–93.
[26] Deans DA, Wigmore SJ, Gilmour H, Paterson-Brown S, Ross JA, Fearon KC. Elevated tumour interleukin-1beta is associated with systemic inflammation: a marker of reduced survival in gastro-oesophageal cancer. Br. J. Cancer 2006;95:1568–75.
[27] Krzystek-Korpacka M, Matusiewicz M, Diakowska D, Grabowski K, Blachut K, Kustrzeba-Wojcicka I, et al. Impact of weight loss on circulating IL-1, IL-6, IL-8, TNF-alpha, VEGF-A, VEGF-C and midkine in gastroesophageal cancer patients. Clin. Biochem. 2007;40:1353–60.
[28] Kemik O, Kemik AS, Begenik H, Erdur FM, Emre H, Sumer A, et al. The relationship among acute-phase response proteins, cytokines, and hormones in various gastrointestinal cancer types patients with cachectic. Hum. Exp. Toxicol. 2011.
[29] Acharyya S, Butchbach ME, 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;8:421–32.
[30] Tisdale MJ. Mechanisms of cancer cachexia. Physiol. Rev. 2009;89:381–410.
[31] Gulen ST, Karadag F, Karul AB, Kilicarslan N, Ceylan E, Kuman NK, et al. Adipokines and systemic inflammation in weight-losing lung cancer patients. Lung 2012.
[32] Kerem M, Ferahkose Z, Yilmaz UT, Pasaoglu H, Ofluoglu E, Bedirli A, et al. Adipokines and ghrelin in gastric cancer cachexia. World J. Gastroenterol. 2008;14:3633–41.
[33] Araujo JP, Lourenco P, Rocha-Goncalves F, Ferreira A, Bettencourt P. Adiponectin is increased in cardiac cachexia irrespective of body mass index. Eur. J. Heart Fail. 2009;11:567–72.
[34] Kondo M, Shibata R, Miura R, Shimano M, Kondo K, Li P, et al. Caloric restriction stimulates revascularization in response to ischemia via adiponectin-mediated activation of endothelial nitric-oxide synthase. J. Biol. Chem. 2009;284:1718–24.
[35] Wolf I, Sadetzki S, Kanety H, Kundel Y, Pariente C, Epstein N, et al. Adiponectin, ghrelin, and leptin in cancer cachexia in breast and colon cancer patients. Cancer 2006;106:966–73.
[36] Patra SK, Arora S. Integrative role of neuropeptides and cytokines in cancer anorexia–cachexia syndrome. Clin. Chim. Acta 2012.
[37] Fantuzzi G. Adiponectin and inflammation: consensus and controversy. J Allergy Clin Immunol 2008;121:326–30.
[38] Bertevello PS, Seelaender MC. Heterogeneous response of adipose tissue to cancer cachexia. Braz. J. Med. Biol. Res. 2001;34:1161–7.
[39] Rydén M, Agustsson T, Laurencikiene J, Britton T, Sjölin E, Isaksson B, et al. Lipolysis-not inflammation, cell death, or lipogenesis is involved in adipose tissue loss in cancer cachexia. Cancer 2008;113:1695–704.
[40] Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001;98:2005–10.
[41] Nakajima TE, Yamada Y, Hamano T, Furuta K, Matsuda T, Fujita S, et al. Adipocytokines as new promising markers of colorectal tumors: adiponectin for colorectal adenoma, and resistin and visfatin for colorectal cancer. Cancer Sci. 2010;101:1286–91.
[42] Glare P. Clinical predictors of survival in advanced cancer. J Support Oncol 2005;3:331–9.
[43] Tessitore L, Vizio B, Pesola D, Cecchini F, Mussa A, Argiles JM, et al. Adipocyte expression and circulating levels of leptin increase in both gynaecological and breast cancer patients. Int J Oncol 2004;24:1529–35.
[44] Bonetto A, Aydogdu T, Kunzevitzky N, Guttridge DC, Khuri S, Koniaris LG, et al. STAT3 activation in skeletal muscle links muscle wasting and the acute phase response in cancer cachexia. PLoS ONE 2011;6:e22538.
[45] Ishikawa T, Kokura S, Sakamoto N, Okajima M, Matsuyama T, Sakai H, et al. Relationship between circulating cytokine levels and physical or psychological functioning in patients with advanced cancer. Clin Biochem 2012;45:207–11.
[46] Costanzo ES, Lutgendorf SK, Sood AK, Anderson B, Sorosky J, Lubaroff DM. Psychosocial factors and interleukin-6 among women with advanced ovarian cancer. Cancer 2005;104:305–13.
[47] Lira FS, Yamashita AS, Rosa JC, Koyama CH, Caperuto EC, Batista Jr ML, et al. Exercise training decreases adipose tissue inflammation in cachectic rats. Horm Metab Res 2012;44:91–8.
[48] Tisdale MJ. New cachexic factors. Curr Opin Clin Nutr Metab Care 1998;1:253–6.
[49] Kotler DP. Cachexia. Ann Intern Med 2000;133:622–34.
[50] Antvorskov JC, Fundova P, Buschard K, Funda DP. Dietary gluten alters the balance of proinflammatory and anti-inflammatory cytokines in T cells of BALB/c mice. Immunology 2012.