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Role of β1-adrenoceptor in increased lipolysis in cancer cachexia

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Increased production of hormone-sensitive lipase (HSL) protein has been demonstrated to be the major cause behind enhanced lipolysis in cancer cachexia. The mechanism governing this alteration is unknown and was presently investigated. This study was conducted to detect the expression of relevant receptors in the adipocytes of cancer cachexia patients, and to elucidate their implication in the increased lipolysis. Gene expressions of β1-adrenoceptor (ADRB1), β2-adrenoceptor (ADRB2), β3-adrenoceptor (ADRB3), α2C-adrenoceptor (ADRA2C), natriuretic peptide receptor A (NPRA), insulin receptor (INSR), and HSL were determined in adipose tissues of 34 patients by real-time PCR. Protein levels of ADRB1 and HSL were determined by western blot analysis. β1-Adrenoceptor (ADRB1) was also detected by immunofluorescence staining. mRNA expressions of both ADRB1 and HSL were approximately 50% elevated selectively in the cachexia group, whereas mRNA levels of the other receptors were unchanged. β1-Adrenoceptor (ADRB1) protein expression was 1.5-fold increased in cachexia as compared with the cancer controls, and 3-fold increased as compared with nonmalignant controls, and was confirmed as a membrane protein in adipocytes by immunofluorescence. Hormone-sensitive lipase (HSL) protein expression was 2–2.5-fold increased selectively in cachectic patients. There was a positive correlation between the protein expressions of ADRB1 and HSL. As much as approximately 50% of the variations in HSL protein expression could be explained by variations in ADRB1 protein expression. There was a link between ADRB1 protein level and lipolytic rate. Increased ADRB1 expression may account for some of the functional changes of HSL in patients with cancer cachexia. (*Cancer Sci* 2010; 101: 1639–1645)

Body fat depletion is a hallmark of cancer cachexia, a complex clinical syndrome associated with increased morbidity and mortality.[1–4] As the largest reservoir of energy stores and a major endocrine organ, white adipose tissue (WAT) plays a crucial metabolic role in regulating energy flux, plasma lipid levels, and glucose uptake. In cachectic patients, excessive fat consumption results in energy shortage and metabolic disturbances such as elevated serum levels of free fatty acids (FFAs) and glucose resistance, which interferes therapy against tumors.[5,6] Therefore, it is of value to understand the mechanisms behind fat loss in cancer cachexia. Antilipolysis treatment is especially meaningful for attenuating the progressive wasting, since fat deprivation often precedes and progresses faster than muscle atrophy in cancer cachexia.[7,8]

However, fat tissue wasting is not well established in cancer cachexia, as indicated by recent consensus.[9] At present, very little is known about the factors promoting loss of adipose tissue in cancer patients.[10–12] Although decreased lipogenesis may contribute, increased lipolysis has been revealed the primary cause.[13–16] In recent years, adipose lipolysis has been found to be under tight regulation by several lipolysis-regulating hormones.[15] The hormones regulate lipolysis through separate pathways that all converge at the rate-limiting enzymes hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL).[17] Recently, increased production of HSL protein has been established the major cause behind enhanced lipolysis in cancer cachexia.[18] Nevertheless, the mechanisms governing the altered HSL action remain a mystery. Since some recent studies have indicated that serum levels of relevant hormones are not significantly altered, alternations in signal transduction at the receptor and post-receptor level are putative mechanisms that stimulate HSL action.[16,18]

The present study was conducted to detect the expressions of relevant membrane receptors in the adipocytes of cancer cachexia patients, and to elucidate their implication in the increased lipolysis in this condition.

Materials and Methods

Patients. All patients scheduled for gastrointestinal cancer operation between June 2008 and November 2009 were evaluated for the study and all patients (i) who were fit in spite of their cancer disease, (ii) had not received prior anticancer treatment, and (iii) were willing to participate were included ($n = 34$). The exclusion criteria for the study were: age <18 years, body temperature exceeding 37.7°C, organic dysfunction, and previous treatment with chemotherapy or radiotherapy. The patients were divided into three groups based on diagnosis. All cancer patients had histologically documented cancers. Cancer stage was assessed according to the NCCN Clinical Practice Guidelines in Oncology V.2.2007. This study was approved by the Ethics Committee of Zhongshan Hospital and patients' informed consent was obtained.

Clinical examination. Body height and weight were measured. Body composition including extracellular fluid (ECF, L) and intracellular fluid (ICF, L) were assessed by bioelectrical impedance analysis (Hydra ECF/ICF Bioimpedance Analyzer Model 4200; Xitron Technologies, San Diego, CA, USA). Fat mass (FM, kg) and fat free mass (FFM, kg) were further determined according to equations (1) and (2), respectively. Venous blood samples were obtained for the determination of FFA by the hospital's accredited routine chemistry laboratories, and glycerol was determined using the free glycerol kit (BioVision, Mountain View, CA, USA).

$$\mathrm{FFM} = 1.106 \times \mathrm{ECF} + 1.521 \times \mathrm{ICF} \quad (1)$$

$$\mathrm{FM} = \mathrm{BW} - \mathrm{FFM} \quad (2)$$

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Fat sample acquisition. Abdominal subcutaneous fat samples (1.5–2 g) were obtained during laparotomy and divided into 500 mg per piece. One piece of the collected adipose tissue was frozen in liquid nitrogen and kept at −80°C for later gene expression studies, one was used for protein secretion, and the other one was used for immunofluorescence staining. White adipose tissues (WAT) were obtained under general anesthesia, and it had previously been demonstrated that general anesthesia does not influence adipose tissue function.[19]

Real-time PCR. Total RNA was extracted from 500 mg of adipose tissue using Trizol (Invitrogen, Carlsbad, CA, USA) and the RNA concentration was determined from the absorbance at 260 nm. The RNA was reverse-transcribed with oligo(dT) primer using a SuperScript first-strand synthesis kit (Invitrogen) to generate the first-strand cDNA, followed by PCR to detect the expressions of β1-adrenoceptor (ADRB1), β2-adrenoceptor (ADRB2), β3-adrenoceptor (ADRB3), α2C-adrenoceptor (ADRA2C), natriuretic peptide receptor A (NPRA), insulin receptor (INSR), and HSL. The sequences of primers are shown in Table 1. For relative quantitation, the reactions were performed in mixtures containing 1.5 μL 10 × Taq reaction buffer, 1.125 μL deoxy-NTP mixture (2.5 mM each), 0.9 μL $MgCl_2$ stock (25 mM; Tiangen Biotech, Beijing, China), 0.75 μL EvaGreen (Biotium, Hayward, CA, USA), 0.09 μL fluorescein calibration dye stock (Bio-Rad, Hercules, CA, USA), 1 μL DNA (10 ng/μL), and 0.6 μL each primer (3 μmol) in a total volume of 15 μL. The PCR amplification and detection were carried out in an iCycler (Bio-Rad), each with 30 s at 94°C, 30 s at 56–60°C, and 1 min at 72°C for 40 cycles after the initial denaturing step for 5 min at 94°C. To exclude the presence of nonspecific products, a routine melting curve analysis was performed after finishing amplification. This was done by high-resolution data collection during an incremental temperature increase from 60 to 95°C. All real-time PCR procedures were performed three times. mRNA levels were determined by a comparative C_t method. The copy number of the target genes was normalized to actin as an endogenous reference. The fold change of controls was set at 1, and normalized fold change of genes was calculated.

Western blotting. Approximately 500 mg of abdominal subcutaneous WAT was crushed and lysed in protein lysis buffer (1% Triton-X 100, Tris-HCl [pH 7.6], and 150 mmol/L NaCl, 4°C), supplemented with protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride and Complete [Boehringer Mannheim, Mannheim, Germany]), and homogenized. The homogenate was centrifuged and the infranatant was collected and saved. Protein content was assayed using a BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA). One hundred micrograms of total protein was loaded on polyacrylamide gels and separated by standard 12% SDS-PAGE. Proteins were than transferred to the polyvinylidene difluoride membranes (Immobilon PVDF; Millipore, Billerica, MA, USA). The membranes were blocked for 1 h at room temperature in 5% nonfat dry milk in TBS containing 0.05% Tween 20, followed by an overnight incubation at 4°C with primary antibodies. The membranes were then incubated with horseradish peroxidase-labeled antirabbit secondary Ab (Chemicon, Temecula, CA, USA) for 1 h at room temperature. Peroxidase activity was detected via chemiluminescence (SuperSignal West Femto luminol substrate and peroxide buffer; Pierce). Primary antibodies used include anti-AR-β1 (SY-SC-567; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-HSL (SY-4107S; Cell Signaling Technology, Danvers, MA, USA), and anti-β-actin (Cat#4967; Cell Signaling Technology).

Immunofluorescence staining. After fixing in paraformaldehyde for 36 h, adipose tissues were embedded in paraffin, and 6-μm sections were obtained. The sections were blocked with normal goat serum for 30 min at room temperature and were incubated with the primary antibody polyclonal rabbit anti-β1-AR (SY-SC-567, 1:200 dilution; Santa Cruz Biotechnology) at 4°C overnight, and subsequently in a fluorescein isothiocynate (FITC)-conjugated antirabbit secondary antibody (1:800 dilution) at room temperature for 2 h. Nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA) at room temperature for 20 min. Fluorescence was detected at an excitation wavelength of 490 nm and emission detection wavelengths of 525 nm (FITC) and 350 and 470 nm (DAPI) by confocal laser-scanning microscopy (TCS SP2; Leica, Mannheim, Germany) while the bright view was obtained without excitation and emission detection by confocal microscopy.

Statistical analysis. Results are presented as mean ± SD or median (range). The values with adipose tissue were considered to be normally distributed because retrospective analysis of previously investigated large cohorts using the same lipolysis and gene or protein expression methods showed a normal distribution of data.[20–22] Statistical analyses were performed using one-way ANOVA when appropriate, followed by Bonferroni post-hoc test. Pearson c^2-test was used to compare inter-group variations in gender. Bivariate correlation analysis was used to establish the relationship between relative HSL protein level and relative ADRB1 protein level, as well as the relationship between relative ADRB1 protein level and lipolytic rate. $P < 0.05$ was defined as statistically significant (two-sided). The statistical software package used was SPSS version 15.0 for Windows (SPSS, Chicago, IL, USA).

Table 1. Primer sequences

Gene	Accession no.	Sense/antisense primers (5′–3′)
ADRB1	NM_000684.2	CCTCGTCCGTAGTCTCCTTC
		GCAGCTGTCGATCTTCTTCA
ADRB2	NM_000024.5	AGAGCCTGCTGACCAAGAAT
		TAGCAGTTGATGGCTTCCTG
ADRB3	NM_000025.2	CTTCACTCTCTGCTGGTTGC
		AAGGCAGAATTGGCATAACC
ADRA2C	NM_000683.3	CCACAGAACCTCTTCCTGGT
		CCGAAGTACCAGTAGGCCAT
NPRA	NM_000906.3	AGAACAGCAGCAACATCCTG
		GAGGCAGGATCTGGTAGAGC
INSR	NM_000208.2	GAGACCTTGGAAATTGGGAA
		TCTGACAAGCAGAGTTTGGG
HSL	NM_005357.2	CTCCTCCTATTCCTAATCCTCC
		CACTTCCTCTTGGGTTTCACTC
Actin	NM_001101.3	GATCATTGCTCCTCCTGAGC
		ACTCCTGCTTGCTGATCCAC

ADRA2C, α2C-adrenoceptor; ADRB1, β1-adrenoceptor; HSL, hormone-sensitive lipase; INSR, insulin receptor; NPRA, natriuretic peptide receptor A.

Results

Basic characteristics of subjects. Twenty-five cancer patients and nine weight-losing nonmalignant controls were consecutively recruited in the study between June 2008 and November 2009 at Zhongshan Hospital. Patients were categorized as cancer cachexia (CC; $n = 12$) if they had unintentional weight loss of >5% of habitual weight during the last 3 months or >10% weight loss during the last 6 months.[23] One control group ($n = 13$) consisted of subjects with prediagnosed gastrointestinal cancer who reported no important weight change during the last year (weight-stable cancer controls, WS). The second control group ($n = 9$) was composed of subjects with prediagnosed gastrointestinal cancer who had nonmalignant diseases with significant weight loss (nonmalignant controls,

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NC). The cachexia group included five patients with gastric cancer, six with colorectal/rectal cancer, and one with pancreatic cancer; the cancer control group included five patients with gastric cancer, seven with colorectal/rectal cancer, and one with common bile duct cancer; the nonmalignant control group included four patients with cholelithiasis, one with inguinal hernia, one with gastric leiomyoma, one with pelvic leiomyoma, one with chronic inflammation in the duodenal mucosa, and one with pancreatic serous cystadenoma. Clinical details of the patients are summarized in Table 2. The three groups were well matched with respect to age and gender distribution. The cachexia group had lower adipose mass than the two control groups. Tumor severity was similar in the two cancer groups.

mRNA expression. In order to explore whether the expressions of relevant membrane receptors and HSL in human adipocytes are altered in cancer cachexia patients, gene expressions of *ADRB1*, *ADRB2*, *ADRB3*, *ADRA2C*, *NPRA*, and *INSR* in white fat were determined in cancer cachexia patients and two control groups (Table 3). mRNA levels of ADRB2, ADRB3, ADRA2C, NPRA, and INSR were unchanged in cancer cachexia patients compared with controls (both $P < 0.05$). β1-Adrenoceptor (ADRB1) mRNA expression was about 50% higher in the cachexia group, compared with the two control groups, which did not differ between each other (Fig. 1a). We also determined mRNA levels of HSL (Table 3). In the adipose tissue of cachectic patients, HSL mRNA expression was ~50% higher compared with the two control groups, which did not differ between each other (Fig. 1b).

Protein expression. Protein expressions of HSL and ADRB1 in white fat were determined in cancer cachexia patients and two control groups (Fig. 2). β1-Adrenoceptor (ADRB1) protein expression was 1.5-fold increased in cachexia as compared with the cancer control group (1.58 ± 0.66 *vs* 1.09 ± 0.40; $P < 0.001$), and 3-fold increased as compared with nonmalignant controls (1.58 ± 0.66 *vs* 0.48 ± 0.17; $P < 0.001$; Fig. 2a,b). Hormone-sensitive lipase (HSL) protein expression was 2–2.5-fold increased in cachexia as compared with the two control groups (0.88 ± 0.32 *vs* 0.42 ± 0.22 *vs* 0.32 ± 0.15; $P < 0.001$; Fig. 2c,d). When all protein data were compiled, there was a positive correlation between ADRB1 protein expression and HSL protein expression ($r = 0.474$; $P = 0.005$; Fig. 3). As much as approximately 50% of the variations in HSL protein expression could be explained by variations in ADRB1 protein expression.

Immunofluorescence staining. Adipocytes were analyzed by immunofluorescence for the localization of ADRB1 protein (Fig. 4). β1-Adrenoceptor (ADRB1) protein was confirmed as a membrane protein in adipocytes. Expression of ADRB1 was observed in all three groups.

Correlation between relative ADRB1 protein level and lipolytic rate. Glycerol/FM and FFA/FM are commonly used for the evaluation of lipolytic rate.[18] Glycerol/FM was higher in the cancer cachexia group than in the cancer controls (5.70 ± 2.22 *vs* 2.93 ± 0.89; $P = 0.001$). Free fatty acid (FFA)/FM was higher in the cancer cachexia group as compared with the cancer controls and nonmalignant controls (58.75 ± 17.47 *vs* 26.71 ± 13.91; $P < 0.001$; 58.75 ± 17.47 *vs* 39.60 ± 10.61; $P = 0.017$). Pearson correlation analysis showed a positive correlation between relative ADRB1 protein level and glycerol/FM and FFA/FM ($r = 0.406$; $P = 0.02$ for glycerol/FM and $r = 0.435$; $P = 0.01$ for FFA/FM) (Fig. 5).

Table 2. Characteristics of study groups

Measurement	Cancer cachexia ($n = 12$)	Cancer controls ($n = 13$)	Nonmalignant controls ($n = 9$)	*P*-value
Gender, M/F	10/2	8/5	4/5	0.174
Age, years	62 ± 7	57 ± 12	56 ± 8	0.368
BMI, kg/m^2	21.5 ± 2.4	24.2 ± 2.8	23.3 ± 3.1	0.057
BWL, %	13 ± 4	–	10 ± 3	0.062
Fat mass, kg	14.53 ± 3.47	24.11 ± 8.86	16.66 ± 2.82	0.001
Fat mass, %	28.42 ± 6.83	34.39 ± 7.93	27.67 ± 3.04	0.036
Tumor score, points	3 (2–4)	3 (1–4)	–	0.170
SBP, mmHg	116 ± 11	123 ± 15	113 ± 15	0.239
DBP, mmHg	72 ± 8	77 ± 9	68 ± 9	0.082
Heart rate, beats/min	78 ± 7	78 ± 4	76 ± 4	0.771
Glycerol/FM, μmol/L/kg	5.70 ± 2.22	2.93 ± 0.89	4.09 ± 1.82	0.001
FFA/FM, μmol/L/kg	58.75 ± 17.47	26.71 ± 13.91	39.60 ± 10.61	<0.001

BMI, body mass index; BWL, percent body weight loss over the previous 6 months; DBP, diastolic blood pressure; FFA, free fatty acid; FM, fat mass; SBP, systolic blood pressure.

Table 3. Assessment of mRNA levels of relevant receptors and HSL by real-time PCR

Gene	Mean ± SD			*P*-values			
	CC ($n = 12$)	WS ($n = 13$)	NC ($n = 9$)	Overall	CC *vs* WS	CC *vs* NC	WS *vs* NC
ADRB1	20.62 ± 8.65	13.48 ± 4.66	13.48 ± 3.90	0.012*	0.022*	0.043*	1.000
ADRB2	7.62 ± 4.33	4.84 ± 3.09	4.41 ± 2.93	0.081	0.179	0.146	1.000
ADRB3	3.85 ± 1.66	2.81 ± 1.7	2.60 ± 1.7	0.193	0.414	0.318	1.000
ADRA2C	18.98 ± 4.06	14.65 ± 6.2	14.73 ± 5.8	0.105	0.167	0.259	1.000
NPRA	2.99 ± 1.90	2.17 ± 1.39	2.20 ± 1.37	0.388	0.639	0.824	1.000
INSR	20.69 ± 5.55	18.82 ± 4.2	15.97 ± 3.03	0.075	0.925	0.072	0.467
HSL	9.53 ± 2.89	5.58 ± 3.45	6.31 ± 3.21	0.011*	0.013*	0.088	1.000

Asterisk (*) indicates significance at $P < 0.05$. ADRA2C, α2C-adrenoceptor; ADRB1, β1-adrenoceptor; CC, cancer cachexia; HSL, hormone-sensitive lipase; INSR, insulin receptor; NC, nonmalignant controls; NPRA, natriuretic peptide receptor A; WS, weight-stable cancer controls.

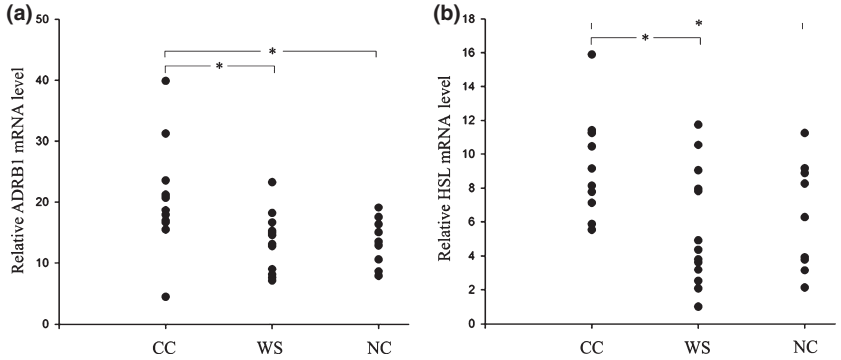

Fig. 1. Relative mRNA levels of β1-adrenoceptor (ADRB1) and hormone-sensitive lipase (HSL) in three groups. Quantitative real-time PCR was performed as described in the Materials and Methods, for ADRB1 and HSL, normalized to actin. Data bars, mean ± SE. Cancer cachexia (CC), $n = 12$; weight-stable cancer controls (WS), $n = 13$; nonmalignant controls (NC), $n = 9$. Asterisk (*) indicates significance at $P < 0.05$. Relative mRNA levels of ADRB1 (a) and HSL (b) were significantly increased in the cancer cachexia group when compared with the two control groups. No significant difference of relative mRNA level of ADRB1 or HSL was found in the two control groups.

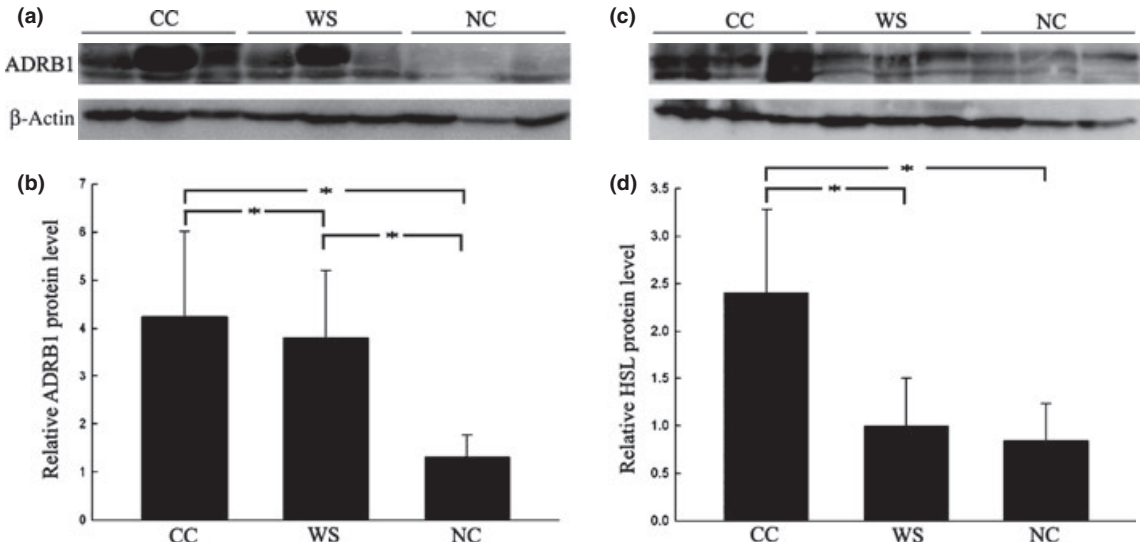

Fig. 2. Western blotting for relative protein levels of β1-adrenoceptor (ADRB1) and hormone-sensitive lipase (HSL) in three groups. Approximately 500 mg of abdominal subcutaneous adipose tissue was crushed, lysed, and homogenized. The homogenate was centrifuged and the infranatant was collected. One hundred micrograms of total protein was loaded on polyacrylamide gels and separated by 12% SDS-PAGE, and then transferred to membrane. The membranes were blocked and incubated with primary antibodies including anti-AR-β1, anti-HSL, and anti-β-actin. (a,c) Typical result for nine independent samples (top); (b,d) results from all cases (bottom). The protein levels of ADRB1 and HSL were normalized to β-actin. Data bars, mean ± SE. Cancer cachexia (CC), $n = 12$; weight-stable cancer controls (WS), $n = 13$; nonmalignant controls (NC), $n = 9$. Asterisk (*) indicates significance at $P < 0.05$. Relative protein levels of ADRB1 (a,b) and HSL (c,d) were significantly increased in the cancer cachexia group when compared with the two control groups. Relative protein level of ADRB1 in the weight stable cancer controls was significantly increased when compared with the nonmalignant controls (b). No significant difference of relative mRNA level of HSL was found in the two control groups (d).

Discussion

Loss of adipose tissue in cancer cachexia results from an imbalance between lipid hydrolysis and synthesis.[24] Although decreased lipogenesis may be implicated, increased lipolysis has been demonstrated the primary cause.[13–16] Little is known, however, regarding the enhanced lipolysis in cancer cachexia. Decades ago, increased β-adrenergic activity was found to be potentially important to the stimulation of lipolysis in cancer cachexia.[13] No substantial progress, however, has been made until recently; enhanced HSL action, the final step in lipolysis activation, has been determined a primary role.[11,18,25] However, the mechanisms governing the altered HSL action remain unexplored.

The present study found an increased expression of ADRB1 in the cachexia group. β1-Adrenoceptor (ADRB1) mRNA expression was about 50% higher selectively in the cachexia group. β1-Adrenoceptor (ADRB1) protein expression was 1.5-fold increased in cachexia as compared with the cancer controls, and 3-fold increased as compared with the nonmalignant weight-losing controls. Immunohistochemical detection confirmed ADRB1 protein as a membrane protein in adipocytes. Since ADRB1 expression was detected on adipocytes from nonmalignant controls at a low level, elevated ADRB1 expression in cancer cachexia is probably due to enhanced expression on individual adopocytes, not an increase in ADRB1-positive adipocytes. In accordance with previous observations,[18,26] expressions of HSL mRNA and protein in our cachexia group

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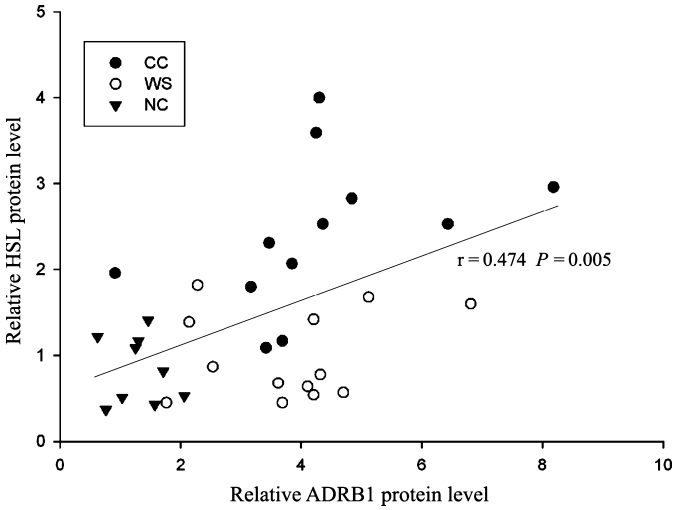

Fig. 3. Correlation analysis for relative β1-adrenoceptor (ADRB1) protein level and relative hormone-sensitive lipase (HSL) protein level. Bivariate correlation analysis showed a positive correlation between relative ADRB1 and relative HSL protein levels ($r = 0.474$; $P = 0.005$). As much as approximately 50% of the variations in HSL protein expression could be explained by variations in ADRB1 protein expression.

increased by 50% and 2–2.5-fold, respectively. There was a positive correlation between ADRB1 protein expression and HSL protein expression. As much as approximately 50% of the variations in HSL protein expression could be explained by variations in ADRB1 protein expression. Since overexpression of HSL leads to a marked increase in adipocyte lipase activity, elevated ADRB1 expression probably induces functional changes of HSL by enhancing the expression of this enzyme.[18,27]

In a previous study, a 2-fold increase in lipolytic activity was observed in cancer cachexia, and the expression levels of HSL mRNA and protein strongly correlated with lipolytic stimulation.[18] The current study established a positive correlation between ADRB1 protein expression and lipolytic rate. As much as approximately 40% of the variations in glycerol/FM and FFA/FM could be explained by variations in ADRB1 protein expression. Compared with other regulation means of receptor activity such as affinity change, altered expression is the most frequent and significant mechanism applied in organisms. In contrast, other receptors including ADRB2, ADRB3, ADRA2C, NPRA, and INSR were unaltered in the cachexia group. As ADRB1 is the most massive expressed in human adipocytes, it is especially influential to lipolysis, and is probably implicated in the increased HSL action in such patients.

In this study, major determinants including age, gender distribution, body mass index (BMI), and tumor severity were comparable to provide an evident conclusion. None of our patients was extremely lean, since BMI of subjects were more than 18.4 kg/m^2 except for one which was 16.7 kg/m^2, indicating that we might have studied patients in an early cachexia phase when there is predominantly loss of adipose tissue. It is very difficult to recruit cachectic cancer patients to this type of investigation. However, an adequate population was recruited in this study for statistical significance, as verified in the previous studies.[16,18]

In recent years, adipose lipolysis has been demonstrated under tight hormonal regulation.[15,28,29] Hormones combine with relevant membrane receptors and regulate lipolysis by altering actin of rate-limiting enzymes HSL or ATGL, through intracellular signal transduction mediated by either cAMP or cGMP.[30] Hormone-sensitive lipase (HSL) and ATGL are at the final step in lipolysis activation where separate mechanisms of hormonal regulation converge.[17] As the only hormone-sensitive enzyme, HSL plays a major role for the altered lipolysis in cachectic patients.[18,30] Recently, serum levels of lipolysis-inducing hormones have been shown unchanged, which implied that cellular mechanisms for increased lipolysis in cachexia are probably due to enhanced lipolytic signaling of the hormone systems at receptor or post-receptor levels. The present study indicated that increased expression of ADRB1 activated HSL, and enhanced the sensitivity of the lipolysis pathway. Since the hormones are always present in the circulation, lipolysis is continuously activated because the antilipolytic action of insulin is not altered.[18]

Putative mechanisms underlying the increased ADRB1 expression remain an open issue. It is reasonably presumed that this results from a feedback regulation of the altered nervous-endocrine system. In our study, blood pressure and pulse rate were not different among groups, which suggested no evidence

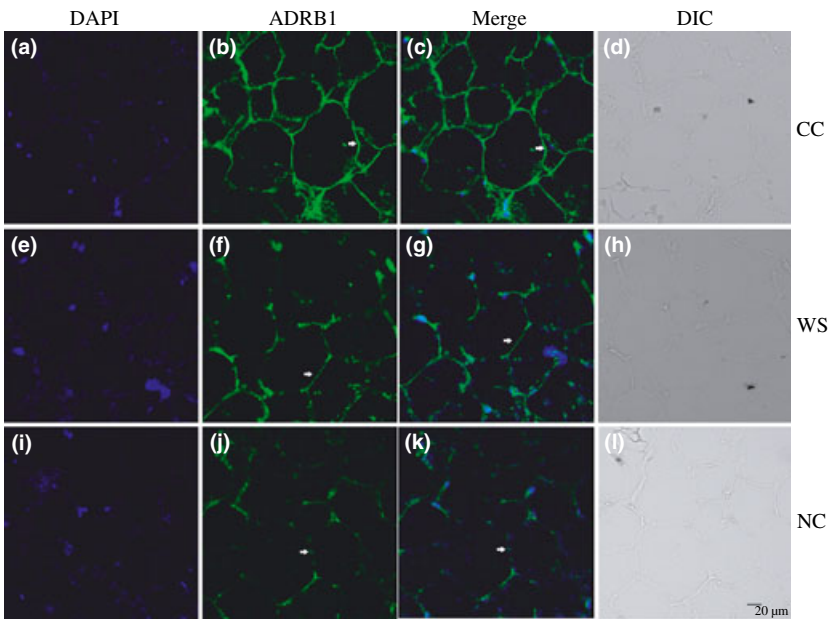

Fig. 4. Immunofluorescence staining for β1-adrenoceptor (ADRB1) (magnification: ×200). β1-Adrenoceptor (ADRB1) protein was stained with anti-β1-AR antibody (SY-SC-567; Santa Cruz Biotechnology). The primary antibodies were visualized by a fluorescein isothiocynate (FITC)-conjugated antirabbit secondary antibody. The nuclei were counter stained with DAPI. DIC, differential interference contrast. β1-Adrenoceptor (ADRB1) was confirmed as a membrane protein in human adipocytes. Bars = 20 μm. Expression of ADRB1 was detected in all three groups (arrow). Results are representative of the three groups.

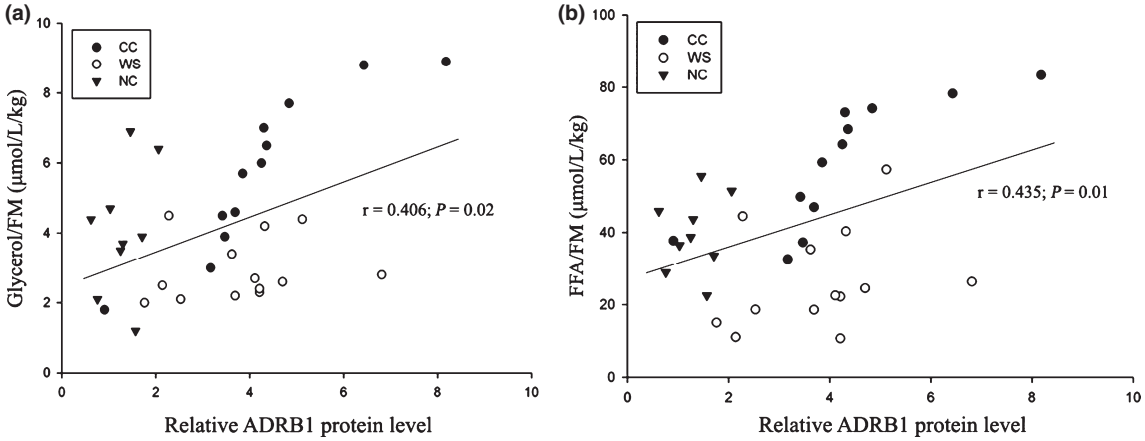

Fig. 5. Correlation analysis for relative β1-adrenoceptor (ADRB1) protein level and lipolytic rate including Glycerol/fat mass (FM) and free fatty acid (FFA)/FM. Pearson correlation analysis showed a positive correlation between relative ADRB1 protein level and Glycerol/FM (r = 0.406; P = 0.02), and a positive correlation between relative ADRB1 protein level and FFA/FM (r = 0.435; P = 0.01).

of increased sympathetic nervous activity. Nevertheless, as a participant of hypothalamic–pituitary–adrenal axis, the alternation of ADRB1 possibly stems from endocrine derangement or activation of nerve innervations.

In animal models, Zinc-α2-glycoprotein (ZAG), a lipolytic factor derived from adipose tissue and tumor, was shown to sensitize adipose tissue to lipolytic stimuli in cancer cachexia with systemic and local effects.[31,32] In rodents, ZAG was proved to stimulate lipolysis by promoting HSL action through the ADRB3 pathway.[33] This route, however, is hardly applicable since the expression of ADRB3 is rare in human adipocytes. Since ADRB1 is predominant in human fat cells,[34,35] it is possible that ZAG-induced lipolysis is enhanced in cachectic patients because of increased expression and action of ADRB1.

Cancer cachexia is regarded as an inflammatory condition.[36] Previously, inflammatory mediators such as tumor necrosis factor-α (TNF-α) and inerleukin-6 (IL-6) were suggested not to be involved in the increased lipolysis in cancer cachexia.[18] This study, however, proposed a possibility that the factors could influence lipolysis through stimulating ADRB1 expression.

Further studies should be conducted for detailed functional assessment of ADRB1 in lipolysis in cancer cachexia. It is notable that cellular research is particularly helpful for substantiating the involvement of ADRB1, since animal experiments are limited by large species-specific differences between humans and rodents.

In conclusion, we propose that increased expression of ADRB1 accounts for some of the functional changes of HSL in patients with cancer cachexia. This enhances the stimulatory effect of lipolytic hormones and possibly of specific cachexia factors such as ZAG. The clinical value of ADRB1 in the attenuation of fat loss in cancer cachexia deserves further exploration.

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Disclosure Statement

The authors have no conflict of interest.

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