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The roles of ubiquitin and 26S proteasome in human obesity

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Abstract

The ubiquitin-proteasome pathway is responsible for the degradation of most intracellular proteins in eukaryotes. It may also play a role in the modulation of inflammatory process and pathogenesis of cancer. Immunoglobulin levels are higher in cancer. Obesity is a risk factor for several common diseases, particularity type 2 diabetes mellitus, cardiovascular diseases, and tumors. The aim of this study was to study a possible correlation between plasma ubiquitin, 26S proteasome levels, and obesity. The body mass index (BMI), plasma ubiquitin levels, and 26S proteasome activity levels were determined and statistically analyzed in 31 volunteers, aged 19 to 58 years and including 9 men and 22 women, from the general population of Southern Taiwan. We also compared the immunoglobulin among the underweight, normal-weight, and overweight groups. We demonstrated that plasma ubiquitin is significantly decreased in obese individuals vs normal controls (65.2 ± 23.4 vs 159.5 ± 73.1 ng/mL). Plasma ubiquitin levels were found to be inversely correlated with the BMI of individuals ($r = -0.39$, $P < .001$). In addition, there was an inverse relationship between 20S proteasome levels in red blood cells and BMI ($r = -0.33$, $P < .001$), whereas 26S proteasome activity was found to be dependent quantitatively to S5a in erythrocytes ($r = 0.88$, $P < .001$). Immunoglobulin is significantly decreased in overweight individuals vs normal controls. Plasma ubiquitin and 20S proteasome levels are potential biomarkers for the risk assessment and possibly serve as one of the targeted studies for the development of human obesity.

1. Introduction

Chronic excess energy intake could cause obesity. Excessive body weight (EBW) has been shown to increase the prevalence of various diseases, particularity type 2 diabetes mellitus, cardiovascular diseases, and several common adult cancers including postmenopausal breast, colorectal, kidney, pancreatic, and aggressive prostate cancers [1-3]. Obesity is known to associate with metabolic syndrome with insulin resistance, hypertension, and proinflammatory state. Obesity increases the release of leptin, insulin-like growth factor, and tumor necrosis factor–α (TNF-α), and reduced adiponectin [4,5]. However, few studies address the possible role of ubiquitin-proteasome pathway (UPP) in obesity.

Ubiquitin-proteasome pathway is responsible for degrading most of the intracellular proteins in eukaryotes [6]. The pathway has diversified functions such as the sorting and distributing of proteins to their intracellular destinations. It also has functions related to cell signaling, cell division, gene transcription, and protein-protein interactions [7]. Ubiquitin is a 76–amino acid protein of 8.6-kd conserved small protein that exists in all eukaryotic cells. The conjugation of ubiquitin to target proteins is a multistep process that includes ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-ligating enzyme (E3). When polyubiquitin is attached to target proteins, tagged proteins are selected for destruction by cytoplasmic organelles called *proteasomes* [8,9]. The 26S proteasome (EC 3.4.99.46) is a large, multisubunit enzyme (molecular weight = 2 400 000) found in high concentration in all mammalian cells. The eukaryotic 26S proteasome is a proteolytic cellular apparatus that consists of 2 subunits: the 20S core particle [10] and the 19S regulatory particle (19S

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cap, PA700) [11,12]. The subunits of S5a in the 19S regulatory particle contain 2 ubiquitin-interacting motifs that recruit ubiquitinated proteins to the proteasome for their degradation [13]. The 20S core particle is a cylindrical structure composed of 4 stacked rings and a multicatalytic protease. The 2 outer rings (called α *rings*) complex with the 19S regulatory particles, forming a narrow channel through which only denatured proteins can pass. The catalytic chamber is formed by the 2 inner β rings, each of which contains 3 well-characterized peptidase activities: chymotrypsin-like, trypsin-like, and post–glutamyl peptide hydrolase–like hydrolytic active sites [14]. Proteins are degraded by the core particle in a progressive manner, generating peptides of 3 to 25 amino acids in length [15].

In addition to its role in the removal of damaged proteins, recent experimental data showed that UPP is activated by obesity and other related diseases, chiefly cardiovascular disease, type 2 diabetes mellitus, and cancers [16-19]. In addition, extracellular ubiquitin has pleiotropic effects on host defense mechanisms or modulation on apoptosis, cytokine section, and growth regulation in vitro [20,21]. Ubiquitin-proteasome pathway provides potential therapeutic targets for the treatment of obesity and other disorders. The mechanisms that underlie the relationship between EBW and various diseases are yet to be fully understood, but it is believed that the ubiquitin-proteasome system may play a role in the pathogenesis of clinical obesity. Data to be presented support this hypothesis in human subjects.

2. Methods and procedures

2.1. Human subjects study protocol

Volunteers were recruited from the general population of Southern Taiwan. The institutional review board of the Kaohsiung Municipal Hsiao-Kang Hospital approved the protocol. All signed-up test subjects gave written informed consent. Eight-hour postfasting peripheral blood samples were collected from adults (men = 9, women = 22). Body mass index (BMI) ranked subjects in this study to 6 different group types: underweight, normal weight, mild overweight, overweight, obese, and severely obese. Subjects with history of medication of PS-341 (a proteasome inhibitor) in the previous 4 months were excluded from this study.

2.2. Blood sample collection and preparations

2.2.1. Blood collection

The 4.5-mL blood samples were collected with anticoagulant (sodium citrate). Plasma and white blood cells were removed from blood samples by centrifugation (500*g* for 10 minutes). Red blood cells (RBCs) were resuspended 1:1 (vol/vol) with cold phosphate-buffered saline and centrifuged at 3700*g* for 17 minutes at 4°C. The cell pellets were then frozen at −20°C before being used for the 26S proteasome analysis. During the procedure, the RBCs were lysed with Tris lyse buffer and centrifuged at 3700*g* for 90 minutes at 4°C twice. The blood plasma and supernatants of RBC lysate were then used for the following assays. All parameters were determined 3 times, and the means were recorded.

2.3. The enzyme-linked immunosorbent assay

The concentrations of ubiquitin in plasma were quantified by a competitive enzyme-linked immunosorbent assay in which biotinylated ubiquitin and ubiquitin in the test sample compete for a limited number of binding sites in the antiubiquitin antiserum. A horseradish peroxidase–labeled streptavidin was added for binding biotin. One hundred microliters of tetramethylbenzidine enzyme-linked immunosorbent assay solution (Sigma, St. Louis, MO) was added as a color substrate. The reaction was stopped by the addition of 100 μL 1 N HCl, and the optical densities were measured using a spectrophotometer at 450 nm. The correlation coefficients for each standard curve were 0.95 to 1. The lowest detection limit was 8 ng/mL. During blood sample collection, hemolysis was carefully avoided to prevent hemolysis because erythrocytes (RBC) contain high amounts of intracellular ubiquitin.

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blotting

The supernatants from above samples were evaluated by the Bradford assay. Equal amounts were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membrane and blotted with the relevant antibodies (S5a and α_1-subunit, 1:2000) (Calbiochem, Darmstadt, Germany); standard anti-rabbit alkaline phosphatase conjugate (1:2000) was then applied. The immunoglobulin (Ig) G level was detected by rabbit anti-human IgG conjugated with alkaline phosphatase (1:2000). Detection was achieved by using nitro-blue tetrazolium chloride/5-bromo-4-3'-indolyphosphate p-toluidine salt stock solution (Roche, Nutley, NJ). Images were scanned, and the density was determined by the ImageQuant software version 5.2 from Molecular Dynamics (Amersham Biosciences, Piscataway, NJ).

2.5. Proteolysis measurement

Proteasome activity was assessed using synthetic peptide substrates linked to the fluorometric reporter aminomethylcoumarin (AMC). The peptide activity of the supernatant from the above blood sample toward the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC was measured by incubation in 30 mmol/L Tris-HCl (pH 8), 5 mmol/L $MgCl_2$, 1 mmol/L adenosine triphosphate, and 0.5 mmol/L dithiothreitol with 50 μmol/L Suc-Leu-Leu-Val-Tyr-AMC for 15 minutes at 37°C. The AMC hydrolyzed from the peptides was quantified in a BioTek (Winooski, VT) FL800 plate reader using 360-nm excitation and 460-nm emission wavelengths. Enzymatic activity was normalized for protein concentration and expressed as percentage of activity of control lysates.

2.6. Statistical analysis

Data are reported as mean ± SEM and analyzed by Pearson linear coefficient (r) regression method. Each sample represented the mean of 3 determinations. Significance was determined by Student t test with confidence level set at $P < .05$.

3. Results

3.1. Significantly decreased plasma ubiquitin in obese test subjects

The Taiwanese have lower prevalence of obesity than people from Western countries. Body mass index is an objective clinical criterion for obesity (BMI ≥27). In Taiwan, about 11% of women and 8% of men are qualified by BMI as obese (BMI >27; nonobese, BMI = 19.5 or <23). In this study, the BMI of 31 adults aged 19 to 58 years (male = 9, female = 22) was determined (Table 1). The ubiquitin levels in groups of normal weight and underweight (BMI <19) were compared with those of the obese group. It was found that the obese group had significantly lower concentrations of plasma ubiquitin than the nonobese groups (166.0 ± 68.0 vs 159.5 ± 73.1 ng/mL, $P < .05$). This observation extends to all 6 BMI scale groups (Table 1). Mean concentrations of plasma ubiquitin in mildly overweight (BMI = 23-25) and overweight groups (BMI = 25-30) were 121.4 ± 72.1 and 103.3 ± 71.0 ng/mL, respectively. It appears that plasma ubiquitin level is inversely correlated to BMI with a linear coefficient of −0.99 (Fig. 1A) if severely obese subjects were excluded.

Paradoxically, severely obese subjects (BMI >35) who were clinically complicated with hypertension and diabetes mellitus (3 patients) showed a statistically significant increase of plasma ubiquitin instead of a reduction (84.5 ± 48.9 ng/mL) if compared with obese subjects. The mechanism for this seemly contradictory finding is unclear at present, but it is possible that antihypertensive and diabetic medications that these subjects were administered may play a role in the observed increased ubiquitin instead of the expected decreased plasma ubiquitin level. This point however needs further study.

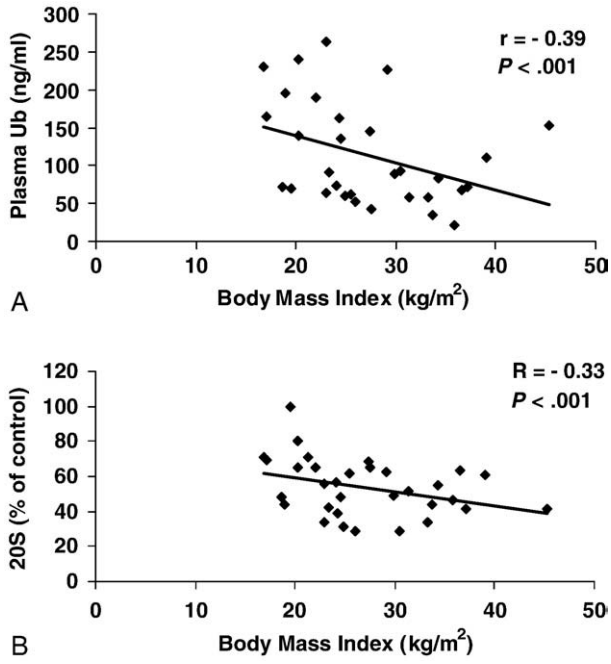

Fig. 1. Inverse relationship between (A) BMI and plasma ubiquitin and (B) BMI and 20S proteasome level. The plots show (A) a strong relationship of plasma ubiquitin and BMI and (B) a significant although modest correlation between BMI and 20S proteasome level. Percentage of control: percentage of the test values as a percentage of the highest 20S of study subject that was arbitrarily set as 100%.

3.2. Relationship of 20S proteasome level in RBCs and BMI

Quantitative Western blotting was used to evaluate the relationship between BMI scales and 20S in RBCs. For comparison purpose, the highest 20S, S5a levels, 26S activity, and Ig of study subject were arbitrarily set as

Table 1
Characteristics of patients, clinical conditions, and medications if applied

Diagnosis	No. of patients	Average of age	Average of BMI	Average of plasma ubiquitin	Average of 20S proteasome	Drug treatment
		Mean ± SD	Mean ± SD (kg/m^2)	Mean ± SD (ng/mL)	Mean ± SD % of control	
Underweight: BMI <19.5	4	38.5 ± 14.8	17.9 ± 1.1	166.0 ± 68.0	57.9 ± 13.9	No
Normal weight: 19.5 ≤ BMI < 23	4	37.2 ± 15.8	20.5 ± 1.0	159.5 ± 73.1	77.4 ± 16.6	No
Mild overweight: 23 ≤ BMI < 25	7	51.8 ± 6.9	23.9 ± 0.8	121.4 ± 72.1	43.8 ± 10.2*	No
Overweight: 25 ≤ BMI < 30	6	46.8 ± 14.8	27.6 ± 1.7	103.3 ± 71.0	55.9 ± 14.7	No
Obese: 30 ≤ BMI < 35	5	46.5 ± 14.5	32.6 ± 1.6	65.2 ± 23.4*,†	42.4 ± 11.3*	No
Severely (or morbidly) obese: BMI ≥35	5	35.6 ± 16.4	38.9 ± 3.8	84.5 ± 48.9	50.7 ± 10.7*	Yes (DM and HT)

Values are expressed as mean ± SEM. DM indicates diabetes mellitus; HT, hypertension.

* Comparison with normal weight (19.6 ≤ BMI < 23).

† Value for underweight (BMI <19.6). Student t test, $P < .05$.

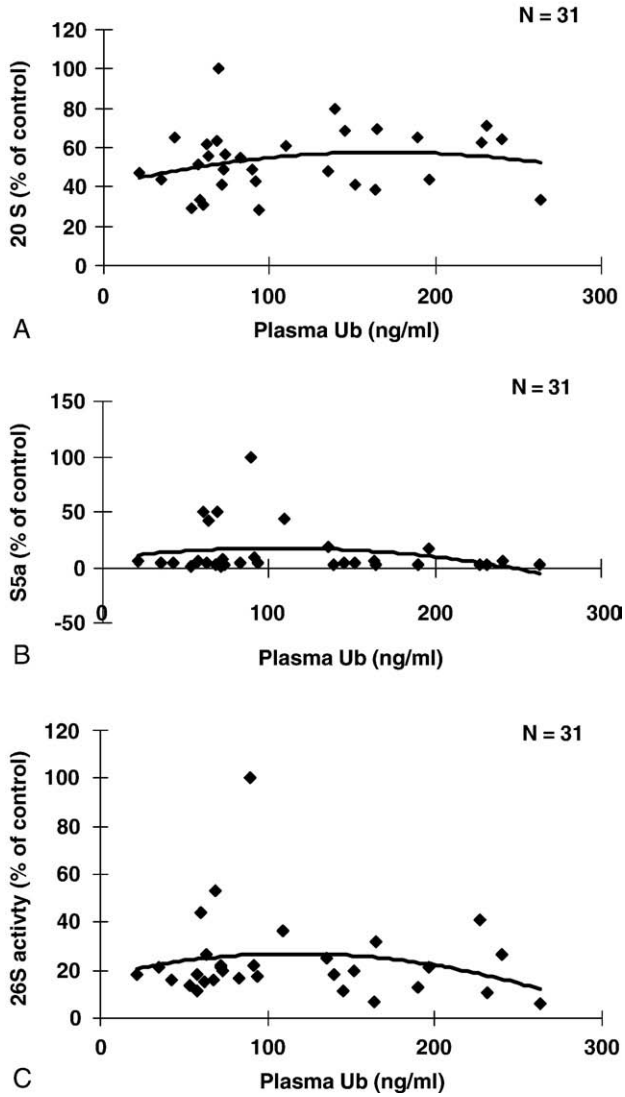

Fig. 2. Relationship between (A) plasma ubiquitin and 20S, (B) plasma ubiquitin and S5a, and (C) plasma ubiquitin and 26S activity. Weak linear correlation between (A) plasma ubiquitin and 20S and inverse relationships between (B) plasma ubiquitin and S5a and (C) plasma ubiquitin and 26S activity are observed. For details, see "Methods and procedures." Percentage of control: percentage of the test values as a percentage of the highest S5a levels and 26S activity of study subject that were arbitrarily set as 100%. Data are the means of 3 determinations.

100% to compare the relative value of each BMI group. The 20S levels in subjects with normal weight (n = 4) showed 77.4% ± 16.6% of control; mildly overweight (n = 7), 43.8% ± 10.2% of control; obese (n = 5), 42.4% ± 11.3% of control; and severely obese subjects (n = 5), 50.7% ± 10.7% of control (all are means ± SD); or 20S levels decreased by the order of 43%, 45%, and 35% in these defined BMI groups (Table 1). An inverse linear relationship between the number of 20S and the individual's BMI was evidently noted (Fig. 1B). However, we found that there was no correlative relationship between S5a vs BMI or proteasome activity vs BMI in these tests subjects (data not shown).

3.3. Relationships among plasma ubiquitin and 20S, S5a, and 26S activity

There are 20S, 26S, and hybrid proteasomes in eukaryotic cells [22]. 26S is the most abundant in most cells, and the term *26S* is often used to refer to this complex. In cross-sectional studies, our data indicate that the (*a*) concentration of ubiquitin increased in plasma when the α1 of 20S of RBC was mildly increased (r = 0.15) (Fig. 2A), (*b*) plasma ubiquitin levels increased when S5a of 19S of RBC was slightly decreased (r = −0.18) (Fig. 2B), and (*c*) plasma ubiquitin levels increased when the chymotrypsin-like 26S activity was slightly decreased (r = −0.12) (Fig. 2C). The severely obese but not medicated test subjects had the lowest level of plasma ubiquitin, whereas the α1 of 20S levels were slightly reduced. In addition, the chymotrypsin-like activity of 26S and the S5a in their RBCs were mildly increased. There were no differences on measuring chymotrypsin-like activity of 26S in the presence of specific proteasome inhibitor MG132 on chymotrypsin-like activity of all samples.

3.4. Chymotrypsin-like activity of 26S correlates with the amounts of S5a in erythrocytes

S5a is one of the subunits of 19S proteasome. The ubiquitin-interacting motif 1 of S5a is able to accept polyubiquitin chains and bring ubiquitinated proteins into 20S proteasome. 26S activity and S5a showed a strong linear correlation (r = 0.88, P < .001) (Fig. 3). Most of the donors had less than 50% S5a level, same as the 26S activity when compared with controls. The levels of 20S in our study subjects, however, showed no consistent correlation with S5a levels and 26S proteasome activity.

3.5. Significantly decreased Ig in overweight test subjects

Much of previously reported research efforts showed that Ig level related to EBW [23]. Here we try to address whether

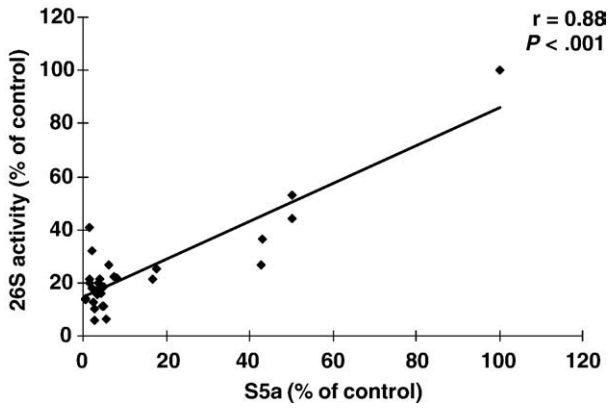

Fig. 3. Strong linear relationships between 26S activity and the amounts of S5a. The plots show a strong correlation between 26S activity and the amounts of S5a. The highest value for each measured activity was assigned as 100% control. Data of other test subjects are expressed as percentage of control. The 26S and S5a activities for each sample are the means of 3 determinations.

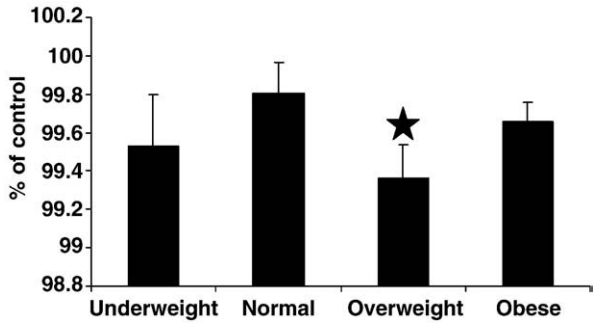

Fig. 4. Comparison of the percentage of underweight, normal-weight, overweight, and obese groups. The figure shows that the overweight group has significantly lower concentrations of plasma IgG than those of the normal group. The highest IgG levels of studied subjects are arbitrarily set as 100% to compare the relative value of each BMI group. Data are presented as mean ± SEM. Each data point is the mean of 3 determinations. *Values where difference from control value is statistically significant at $P < .005$.

there is a difference on IgG level in the normal-weight group and the obese group. It was found that the overweight group had significantly lower concentrations of plasma IgG than the normal group (Fig. 4). However, no statistical difference was found between the normal and obese groups in these test subjects.

4. Discussion

Chronic excess energy intake is a major factor contributing to obesity. Obesity has been identified as a risk factor of various cancers. We collected 8-hour postfasting morning peripheral blood samples for this study because ubiquitin level is quantitatively stable at this point. This study showed that plasma ubiquitin was significantly decreased in obese populations (Table 1), with an inverse linear relationship with BMI (Fig. 1A). Previous reported data suggested that when exogenous ubiquitin was added to plasma, an inhibitory effect on TNF-α and interleukin-6 responses was demonstrable in normal blood samples [24]. Tumor necrosis factor–α is regarded as a good biomarker for low-grade chronic inflammation of adipose tissue in obese patients [25,26].

The mean plasma ubiquitin levels of the diabetic group were previously reported as higher than those of the control group [25]. Our data showed that the severely obese group (BMI ≥35) with hypertension and diabetes has the highest mean plasma ubiquitin level as compared with the obese group without hypertension and diabetes (Table 1). Our data apparently agree with reported findings, although we have found that obese subjects without diabetes and hypertension had decreased plasma ubiquitin.

Accumulation of ubiquitin has been documented in various cancers such as colon cancer, breast cancer, and leukemia, whereas previous data suggested that both free ubiquitin and multiubiquitin chain levels are up-regulated in colorectal cancer [27]. Tumor cells appear to have higher proteasome activity and proteasome levels than nonmalignant cells, as demonstrated in K562 human myelogenous leukemia cells [28]. Although an association of increased BMI and several cancers is well documented, the exact mechanism is not yet precisely defined. Interestingly, our data show that the α_1 subunits of 20S in the erythrocytes decreased as individuals' BMI increased (Fig. 1B). Our data also indicate that α_1 subunits of 20S were significantly decreased in the mild-overweight, obese, and severely obese groups (Table 1), which was not previously described. However, in a recent report, the 20S levels of C-26 in murine colon adenocarcinoma cells were not changed by exogenous murine TNF [29]. Furthermore, in contrast to previous data, our result shows that plasma ubiquitin levels are slightly decreased when α_1 subunits of 20S are decreased ($r = 0.15$) (Fig. 2A). Our observation shows that the severely obese group (BMI ≥35) with hypertension and diabetes had an increased mean 20S levels as compared with the obese subgroup. The mechanism of this observation is uncertain, but medications administered could possibly play a role. Plasma ubiquitin levels were found to be mildly decreased when chymotrypsin-like activity of 26S increased ($r = -0.12$, Fig. 2C). This was consistent with the previous data, which showed that the chymotrypsin-like activity of proteasomes measured in C-26 cell lysates was induced by murine TNF [29].

Our data demonstrate that plasma ubiquitin level correlates inversely with S5a of 19S; that is, when ubiquitin increases, the S5a of 19S decreases. In addition, our data demonstrate that there is a strong linear relationship between 26S activity and S5a (Fig. 3). These findings support previous report that S5a is an essential factor for 26S proteasome to facilitate polyubiquitinated proteins into the 26S for proteolytic degradation [30].

Myeloma cells secrete a large amount of Ig, which causes defective ribosomal products and increases other unfolded proteins. These proteins normally become ubiquitinylated and then degraded by 26S proteasome to ensure the cell survival. This is a mechanism postulated for 26S participation to the induction of antiapoptotic factor [23]. Our data also show that the overweight group has significantly lower concentrations of plasma IgG than the normal group (Fig. 4). The average of IgG in the obese group was higher than that in the overweight group. However, there was no statistical relationship for IgG level between the normal and obese groups. Although obesity is known to play a role in the promotion of proinflammatory state, our data suggest that obesity may not play a role in the induction of antiapoptotic factor by UPP.

This study showed that plasma ubiquitin plays an important role in obesity. Excess body weight reduced the production of plasma ubiquitin. Lower plasma ubiquitin levels may induce an increased TNF-α production in obese individuals. When high concentration of TNF-α is bound to its receptors, the chymotrypsin-like activity of proteasomes and the S5a in RBCs are increased; and these cascading events may contribute to the observed causative relationship

between obesity and higher prevalence of neoplasms. Furthermore, plasma ubiquitin may be used as a quantifiable factor to study the risk of obesity and possibly a potential target for therapeutic management of clinical obesity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.05.020.

References

[1] Renehan AG, Howell A. Preventing cancer, cardiovascular disease, and diabetes. Lancet 2005;365:1449-51.
[2] Vainio H, Bianchini F. International Agency for Research in Cancer. Weight control and physical activity. Lyon, France: IARC Press; 2002.
[3] Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579-91.
[4] Renehan AG, Frystyk J, Flyvbjerg A. Obesity and cancer risk: the role of the insulin-IGF axis. Trends Endocrinol Metab 2006;17:328-36.
[5] Maison P, Balkau B, Souberbielle JC, et al, Study Group. Evidence for distinct effects of GH and IGF-I in the metabolic syndrome. Diabet Med 2007;24:1012-8.
[6] Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 2002; 82:373-428.
[7] Marx J. Cell biology. Ubiquitin lives up to its name. Science 2002;297: 1792-4.
[8] Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998;67:425-79.
[9] Haas AL, Siepmann TJ. Pathways of ubiquitin conjugation. FASEB J 1997;11:1257-68.
[10] Groll M, Ditzel L, Lowe J, et al. Structure of 20S proteasome from yeast at 2.4 A resolution. Nature 1997;386:463-71.
[11] Peters JM. Proteasomes: protein degradation machines of the cell. Trends Biochem Sci 1994;19:377-82.
[12] Peters JM, Franke WW, Kleinschmidt JA. Distinct 19 S and 20 S subcomplexes of the 26 S proteasome and their distribution in the nucleus and the cytoplasm. J Biol Chem 1994;69:7709-18.
[13] Wang Q, Young P, Walters KJ. Structure of S5a bound to monoubiquitin provides a model for polyubiquitin recognition. J Mol Biol 2005;348:727-39.
[14] Groll M, Koguchi Y, Huber R, et al. Crystal structure of the 20 S proteasome:TMC-95A complex: a non-covalent proteasome inhibitor. J Mol Biol 2001;311:543-8.
[15] Nussbaum AK, Dick TP, Keilholz W, et al. Cleavage motifs of the yeast 20S proteasome beta subunits deduced from digests of enolase 1. Proc Natl Acad Sci USA 1998;95:12504-9.
[16] Adams J, Palombella VJ, Sausville EA, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 1999; 59:2615-22.
[17] Cooke PS, Holsberger DR, Cimafranca MA, et al. The F box protein S phase kinase–associated protein 2 regulates adipose mass and adipocyte number in vivo. Obesity (Silver Spring) 2007;15:1400-8.
[18] Neels JG, Olefsky JM. Cell signaling. A new way to burn fat. Science 2006;312:1756-8.
[19] Medina EA, Afsari RR, Ravid T, et al. Tumor necrosis factor–{alpha} decreases Akt protein levels in 3T3-L1 adipocytes via the caspase-dependent ubiquitination of Akt. Endocrinology 2005;146:2726-35.
[20] Daino H, Matsumura I, Takada K, et al. Induction of apoptosis by extracellular ubiquitin in human hematopoietic cells: possible involvement of STAT3 degradation by proteasome pathway in interleukin 6–dependent hematopoietic cells. Blood 2000;95: 2577-85.
[21] Akarsu E, Pirim I, Capoglu I, et al. Relationship between electro-neurographic changes and serum ubiquitin levels in patients with type 2 diabetes. Diabetes Care 2001;24:100-3.
[22] Tanahashi N, Murakami Y, Minami Y, et al. Hybrid proteasomes. Induction by interferon-gamma and contribution to ATP-dependent proteolysis. J Biol Chem 2000;275:14336-45.
[23] Meister S, Schubert U, Neubert K, Herrmann K, Burger R, Gramatzki M, et al. Extensive immunoglobulin production sensitizes myeloma cells for proteasome inhibition. Cancer Res 2007;67:1783-92.
[24] Patel MB, Proctor KG, Majetschak M. Extracellular ubiquitin increases in packed red blood cell units during storage. J Surg Res 2006;135:226-32.
[25] Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. Trends Endocrinol Metab 2000;11: 212-7.
[26] Kern PA, Ranganathan S, Li C, et al. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001;280:E745-51.
[27] Ishibashi Y, Hanyu N, Suzuki Y, et al. Quantitative analysis of free ubiquitin and multi-ubiquitin chain in colorectal cancer. Cancer Lett 2004;211:111-7.
[28] Kumatori A, Tanaka K, Inamura N, et al. Abnormally high expression of proteasomes in human leukemic cells. Proc Natl Acad Sci USA 1990;87:7071-5.
[29] Nowis D, McConnell EJ, Dierlam L, et al. TNF potentiates anticancer activity of bortezomib (Velcade) through reduced expression of proteasome subunits and dysregulation of unfolded protein response. Int J Cancer 2007;121:431-41.
[30] Kang Y, Chen X, Lary JW, et al. Defining how ubiquitin receptors hHR23a and S5a bind polyubiquitin. J Mol Biol 2007;369:168-76.