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Green Tea Prevents Obesity by Increasing Expression of Insulin-like Growth Factor Binding Protein-1 in Adipose Tissue of High-Fat Diet-Fed Mice

Manabu Ueda and Hitoshi Ashida*

Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan

ABSTRACT: It is known that green tea has the ability to prevent obesity, but the underlying molecular mechanism is not fully understood to date. A preventive mechanism of green tea on obesity in C57BL/6 mice fed a high-fat (HF) diet was investigated by evaluating the expression levels of obesity-related proteins in mesenteric white adipose tissue by using protein array. An increase in the expression level of insulin-like growth factor binding protein (IGFBP)-1 by green tea was found in the white adipose tissues of both control and HF diet-fed mice by protein array and confirmed by Western blot. Moreover, the expression level was negatively correlated with adipose tissue weight. In 3T3-L1 adipocytes, treatment with green tea and its major polyphenol, (−)-epigallocatechin gallate, induced the expression of IGFBP-1 in a dose-dependent manner by Western blot. In conclusion, IGFBP-1 in adipose tissue is a novel molecule target for the prevention of obesity by green tea.

KEYWORDS: *green tea, obesity, insulin-like growth factor binding protein-1, adipose tissue*

INTRODUCTION

Adipose tissue is composed of adipocytes embedded in a loose connective tissue meshwork containing adipocyte precursors, fibroblasts, immune cells, and various other cell types. Adipose tissue is traditionally considered an energy storage depot with few interesting attributes. Recent studies demonstrate that adipose tissue is an active organ that releases a large number of bioactive mediators (adipokines) modulating blood pressure, lipid and glucose metabolism, and inflammation.[1] However, obesity induces adipocyte hypertrophy and disorder in secretions of adipokines from adipose tissue, resulting in diabetes and hyperglycemia. For example, leptin regulates food intake and energy homeostasis and improves peripheral insulin sensitivity.[2] Obesity-induced excessive secretion of leptin causes leptin resistance in the hypothalamus, resulting in elevation of blood insulin level and fat accrual.[2] It has been reported that the blood glucose level of retinol binding protein 4 (RBP4) is elevated in obesity and type 2 diabetes mellitus.[3] RBP4 increases the hepatic gluconeogenesis and attenuates insulin signaling in skeletal muscle, resulting in hyperglycemia.[3] Therefore, prevention of obesity-induced adipocyte hypertrophy is important in the improvement of hyperglycemia.

The insulin-like growth factor binding protein (IGFBP) superfamily consists of a total of 15 members of homologus proteins present in serum, other biological fluids, and tissue.[4] A family of six high-affinity IGFBPs (IGFBP-1−6) has been reported to serve as carriers of insulin-like growth factors (IGFs), to prolong IGF half-life in the circulation, and to modulate IGF activity at cell-surface receptors.[5] IGFBP-1 is produced mainly in the liver and kidney and slightly in other tissues, circulates in the serum, and modulates glucose metabolism and cell proliferation and differentiation.[5] The expression level of IGFBP-1 is reported to be correlated with obesity and hyperglycemia. Mogul et al.[6] reported that blood IGFBP-1 level was significantly decreased among obese postmenopausal women with hyperinsulinemia. In a rodent model, overexpression of IGFBP-1 in adipose tissue suppressed the development of insulin resistance[5] and reduced body weight, fat mass, and blood leptin level.[7] These findings suggest that IGFBP-1 prevents obesity through suppression of adipocyte hypertrophy.

Green tea is a beverage commonly consumed worldwide and has various beneficial activities such as antitumor, anti-inflammation, and antioxidative effects.[8,9] It has also been reported that green tea may help to prevent obesity and hyperglycemia.[10−12] Our previous reports showed that drinking green tea for 14 weeks prevented obesity and hyperglycemia by retaining the expression of glucose transporter 4 in muscle of high-fat (HF) diet-fed mice,[10] and in normal diet-fed rats, drinking green tea for 3 weeks decreased adipose tissue weight without affecting body weight and blood glucose level.[13] Chen et al.[11] showed that green tea lowered the body fat of rats through suppression of adipocyte differentiation. Choo et al.[12] showed that green tea reduced body fat accretion caused by a HF diet in rats through β-adrenorceptor activation of thermogenesis in brown adipose tissue. From these results, reduction of body fat is a common effect of green tea, and various mechanisms of green tea underlie the prevention of obesity and subsequent hyperglycemia. However, the underlying mechanism is not fully understood to date. Hence, in this study, we investigated the alternation of protein expression in

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Table 1. Body Weight, Tissue Weight (Percent of Body Weight), and Glucose and Insulin Levels in Plasma of Mice Given Green Tea for 14 Weeks[a]

	C−W	C−T	HF−W	HF−T
body wt (g)	32.1 ± 1.4a	29.5 ± 1.0a	41.9 ± 1.2b	35.2 ± 2.2a
total WAT (% of body wt)	12.1 ± 1.2ac	7.8 ± 1.5a	25.6 ± 1.4b	14.5 ± 2.5c
mesenteric WAT	1.32 ± 0.11a	1.01 ± 0.12a	2.16 ± 0.28b	1.23 ± 0.22a
epididymal WAT	3.23 ± 0.29ac	2.03 ± 0.33a	5.62 ± 0.32b	3.86 ± 0.60c
retroperitoneal WAT	1.87 ± 0.21a	1.15 ± 0.24a	3.92 ± 0.26b	2.16 ± 0.63a
subcutaneous WAT	5.62 ± 0.65a	3.63 ± 0.82a	13.93 ± 1.13b	7.26 ± 1.49a
interscapular BAT	0.60 ± 0.04a	0.58 ± 0.07a	0.65 ± 0.08a	0.57 ± 0.04a
liver (% of body wt)	3.87 ± 0.07a	3.70 ± 0.16a	3.58 ± 0.16a	3.56 ± 0.12a
blood glucose (mg/dL)	110.3 ± 9.1a	93.5 ± 9.4a	168.1 ± 4.9b	109.5 ± 10.3a
blood insulin (ng/mL)	0.65 ± 0.14a	1.01 ± 0.21ac	5.21 ± 0.85b	2.84 ± 0.46c
HOMA-IR index	4.5 ± 01.0a	6.4 ± 1.8a	57.3 ± 10.1b	17.5 ± 4.5a

[a]Mice were fed a control diet (C) or high-fat diet (HF) with water (W) or green tea (T) for 14 weeks. Body weight, tissue weight as a percent of body weight, and glucose and insulin levels in plasma were measured. Data are shown as the mean ± SE (n = 8). Values with the same letters were not significantly different by the Tukey−Kramer multiple-comparison test ($p < 0.05$). WAT and BAT, white and brown adipose tissues, respectively.

white adipose tissue and found that IGFBP-1 was a novel molecular target of the prevention of obesity by green tea.

MATERIALS AND METHODS

Chemicals and Antibodies. Green tea extract (TF30A, a commercially available food material) was supplied from Ito En, Ltd. Composition of catechins and caffeine was determined by HPLC according to a previous study.[14] (−)-Epigallocatechin gallate (EGCg) was a gift from Dr. Suong-Hyu Hyon (Kyoto University, Japan). For the Western blot analysis, anti-IGFBP-1 goat IgG antibody was purchased from R&D Systems, Inc. (Minneapolis, MN, USA), anti-β-actin mouse IgG antibody from Sigma Chemical Co. (St. Louis, MO), and anti-goat IgG antibody from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). All other reagents used were of the highest grade available commercially.

Animal Experiment. Animal treatment was approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimentation Regulations (Permission 21-07-02). Male C57BL/6 mice (5 weeks old, 19−21 g; Japan SLC, Shizuoka, Japan) were housed in an air-conditioned room (25 ± 1 °C) under a 12 h light−dark cycle with free access to water and commercial chow. After acclimatization for 1 week, they were randomly divided into two groups of 16 and given a HF diet containing 30% lard or an AIN93M-based control diet. The compositions of the HF and control diets were the same as in our previous study.[15] The mice of each group were further divided at random into two subgroups of eight and given tap water (C−W and HF−W groups) or green tea (C−T and HF−T groups) for 14 weeks. Green tea was freshly prepared daily as follows: One gram of TF30A was dissolved in 200 mL of water. Concentrations of catechins and caffeine in green tea used were calculated from their composition of TF30A as follows: (+)-catechin, 15 mg/L; (−)-epicatechin, 85 mg/L; (−)-gallocatechin, 65 mg/L; (−)-epigallocatechin, 415 mg/L; (−)-catechin gallate, 15 mg/L; (−)-epicatechin gallate, 140 mg/L; (−)-gallocatechin gallate, 25 mg/L; EGCg, 825 mg/L; and caffeine, 315 mg/L. During the feeding period, food and water/tea intake and body weight were measured once a week. At the end of the feeding period, the blood of the mice was collected via cardiac puncture under anesthesia after 18 h of fasting, and then the mice were sacrificed. Mesenteric, epididymal, retroperitoneal, or subcutaneous white adipose tissue, interscapular brown adipose tissue, muscle, and liver were taken and weighed. Each tissue was washed with 1.15% (w/v) KCl and stored at −80 °C prior to use. Blood was collected from cardiac puncture, and plasma was prepared and subjected to measurements of the glucose and insulin levels as described previously.[10]

Protein Array and Western Blot Analysis. Preparation of the cell lysate fraction from mesenteric and epididymal white adipose tissues, liver, and muscle was performed according to our previous paper.[10] Obtained cell lysate from mesenteric white adipose tissue was subjected to a proteome profiler mouse obesity array kit (R&D Systems) according to the manufacturer's instructions. The cell lysate from white adipose tissues, liver, and muscle was subjected to Western blot analysis to detect the expression level of IGFBP-1. The density of specific bands was determined using Image J image analysis software.

Analysis of mRNA by Real-Time Quantitative PCR. Total RNA was isolated from an aliquot of 50 mg of adipose tissue by using an RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. After measurement of the quality and concentration of total RNA, the RNA samples were purified by digesting the residual DNA using DNase I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNase-treated RNA was subjected to One Step PrimeScript Plus RT-PCR Kit (Takara Bio, Shiga, Japan). The PCR primers used in this study were as follows: IGFBP-1 (forward, CCCAACAGAAAGCAGGAGAT; reverse, CATCCAGGGATGTCTCACAC); and β-actin (forward, GGTCATCACTATTGGCAACG; reverse, TCCATACCCAAGAAG-GAAGG). Reactions were run in a real-time PCR system (TaKaRa PCR Thermal Cycler Dice, Takara Bio). Relative gene expression level was calculated by the comparative CT method,[16] using the expression of the β-actin gene as an internal control. The results were expressed as a fold increase relative to the expression levels in mice of the C−W group.

Cell Culture and Treatment. 3T3-L1 preadipocytes were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% (v/v) cow serum, 100 μg/mL streptomycin, and 100 U/mL penicillin, at 37 °C in a 5% (v/v) CO_2 atmosphere. Adipocyte differentiation was induced as described previously.[17] Briefly, 2 days after reaching confluence, the cells were treated with 10% (v/v) fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, 10 μg/mL insulin, and 100 μM ascorbic acid phosphate for 3 days in DMEM. Then the cells were cultured in DMEM containing 10% (v/v) fetal bovine serum, 10 μg/mL insulin, and 100 μM ascorbic acid phosphate for another 2 days. These cells were treated with TF30A, EGCg, or caffeine at the indicated concentration in Figure 5 for 1 day in DMEM containing 10% (v/v) fetal bovine serum. The cells were harvested with RIPA buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) containing 0.5 mM dithiotheritol and commercial protease and phosphatase inhibitor cocktails (Roche Diagnostics K.K., Tokyo, Japan). The cell lysate was centrifuged at 18000g for 20 min, and the obtained supernatant was subjected to Western blot analysis to detect the expression level of IGFBP-1.

Statistical Analysis. Statistical analyses were performed with factorial ANOVA with the Tukey−Kramer multiple-comparison test

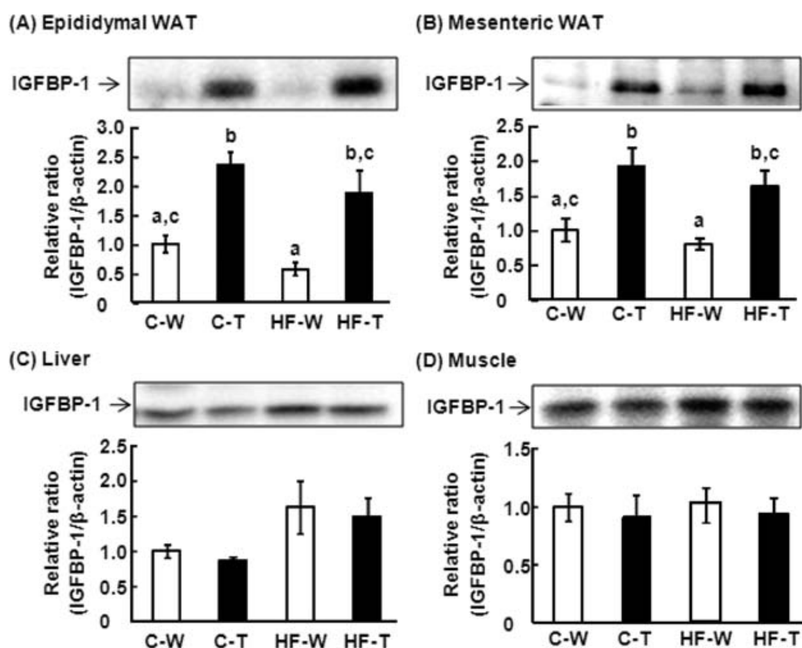

Figure 1. Effect of green tea on the protein expression level of IGFBP-1 in the white adipose tissues, liver, and muscle. Mice were fed a control diet (C) or high-fat diet (HF) with water (W) or green tea (T) for 14 weeks. The cell lysate was prepared from (A) epididymal WAT, (B) mesenteric WAT, (C) liver, and (D) muscle and subjected to Western blot analysis to detect IGFBP-1. Each upper panel shows representative data from four mice. Bottom panels show the density of each band, and values are the mean ± SE ($n = 8$). Different letters indicate significant differences between the groups ($p < 0.05$; Tukey−Kramer multiple-comparison test).

(Table 1; Figures 1 and 2) or Dunnett multiple-comparison test (Figures 4 and 5). The level of significance was defined as $p < 0.05$.

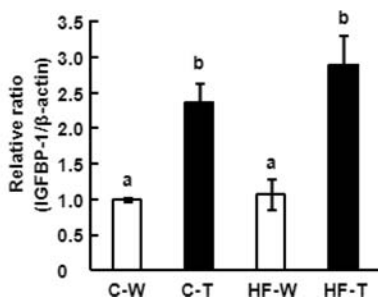

Figure 2. Effect of green tea on the mRNA expression level of IGFBP-1 in epididymal white adipose tissues. Mice were fed a control diet (C) or high-fat diet (HF) with water (W) or green tea (T) for 14 weeks. Total mRNA was isolated form epididymal white adipose tissue and subjected to real-time quantitative PCR as described under Materials and Methods. Data are shown as the mean ± SE ($n = 8$). Different letters indicate significant differences between the groups ($p < 0.05$; Tukey−Kramer multiple-comparison test).

■ RESULTS

Alternation of Protein Expression in Mesenteric White Adipose Tissue. In this study, we investigated the alteration of protein expression in white adipose tissue to clarify the preventive mechanism for obesity and hyperglycemia. First, we confirmed the antiobesity and antihyperglycemia effects of green tea in C57BL/6 mice fed the HF diet. In HF diet groups, green tea significantly decreased the body weight, white adipose tissue weight, and plasma glucose and insulin levels (Table 1). In control diet groups, green tea also decreased plasma glucose level but did not affect the body weight and white adipose tissue weight. In this experiment, the food and water intakes were not altered in each group, although the energy intake of the high-fat diet-fed mice was higher than that of the control diet-fed mice: Daily intake of food of the animals in the C−W, C−T, HF−W, and HF−T groups were 2.4 ± 0.12 g (8.35 ± 0.42 kcal), 2.7 ± 0.12 g (9.40 ± 0.42 kcal), 2.4 ± 0.07 g (12.43 ± 0.36 kcal), and 2.4 ± 0.07 g (12.43 ± 0.36 kcal), respectively; and intake of water in these groups were 2.8 ± 0.16, 2.9 ± 0.13, 2.8 ± 0.11 and 3.0 ± 0.16 mL, respectively. Moreover, abnormal behaviors and unhealthy status were not observed in mice. From these results, we confirmed that green tea prevents HF diet-induced obesity and hyperglycemia in C57BL/6 mice.

Next, we carried out protein array analysis to investigate the alteration of protein expression in mesenteric white adipose tissue (Table 2). Interestingly, green tea increased the expression level of IGFBP-1 in both normal and HF diet groups, suggesting that the induction of IGFBP-1 expression is specific for green tea. HF diet increased the expression levels of agouti-related protein (AgRP), hepatocyte growth factor (HGF), fibroblast growth factor-21 (FGF-21), interleukin-11 (IL-11), leptin, and RBP4 and decreased the expression level of regulated upon activation normal T cell expressed and secreted (RANTES) compared with the control diet. The intake of green tea prevented HF diet-induced up- and down-regulation of these proteins compared with water. These results suggested that green tea suppressed HF diet-induced obesity and subsequent inflammation.

Green Tea Specifically Increased the Expression Level of IGFBP-1 in White Adipose Tissue. To confirm increased expression of IGFBP-1 by green tea in white adipose tissue, we carried out Western blot analysis of IGFBP-1 in mesenteric and epididymal white adipose tissue (Figure 1A, B). Intake of green tea significantly increased the expression level of IGFBP-1 in

Table 2. Effects of Green Tea on Obesity-Related Protein Expressions in Mesenteric Adipose Tissue[a]

protein	C−W	C−T	HF−W	HF−T
adiponectin	1.0	1.04	1.18	1.04
agouti-related protein	1.0	1.31	1.71	1.50
angioprotein-like 3	1.0	0.91	0.91	0.97
C-reactive protein	1.0	0.88	0.72	1.06
dipeptidyl peptidase IV	1.0	0.89	0.90	1.01
endocan	1.0	1.08	0.94	1.01
fetuin A	1.0	1.05	1.06	0.90
fibroblast growth factor acidic	1.0	1.03	1.04	0.97
fibroblast growth factor-21	1.0	0.76	1.52	0.87
hepatocyte growth factor	1.0	0.80	1.53	1.11
intercellular adhesion molecule-1	1.0	0.94	1.04	1.01
insulin-like growth factor-1	1.0	0.93	1.10	1.06
insulin-like growth factor-2	1.0	1.31	1.40	1.22
insulin-like growth factor binding protein-1	1.0	1.88	0.96	1.83
insulin-like growth factor binding protein-2	1.0	1.02	0.77	1.13
insulin-like growth factor binding protein-3	1.0	1.02	0.82	1.05
insulin-like growth factor binding protein-5	1.0	1.06	1.06	1.07
insulin-like growth factor binding protein-6	1.0	1.09	0.92	1.08
interleukin-6	1.0	1.10	1.24	0.78
interleukin-10	1.0	1.02	1.07	0.74
interleukin-11	1.0	0.89	1.56	1.13
leptin	1.0	1.09	1.66	1.23
leukemia inhibitory factor	1.0	1.15	1.42	1.13
lipocalin-2	1.0	0.79	0.94	0.98
macrophage chemoattractant protein-1	1.0	0.70	0.67	0.89
macrophage colony-stimulating factor	1.0	1.05	0.84	1.12
oncostatin M	1.0	1.01	1.26	1.18
pentraxin 2	1.0	0.89	1.22	1.18
pentraxin 3	1.0	0.86	1.05	0.98
preadipocyte factor-1	1.0	1.13	1.25	1.07
receptor for advanced glycation endproducts	1.0	0.63	0.53	0.40
regulated upon activation normal T cell expressed and secreted	1.0	0.63	0.37	0.73
retinol binding protein-4	1.0	1.02	1.50	1.09
resistin	1.0	0.96	1.05	1.01
plasminogen activator inhibitor-1	1.0	1.01	1.08	1.22
tumor necrosis factor-α	1.0	0.95	1.11	1.01
tissue inhibitors of metalloproteinase-1	1.0	1.01	1.41	1.01
vascular endothelial growth factor-1	1.0	1.15	0.94	1.02

[a]Mice were fed a control diet (C) or a high-fat diet (HF) with water (W) or green tea (T) for 14 weeks. At the end of the experiment, obesity-related protein expressions were measured in mesenteric adipose of mice by commercial protein array kit. The expression levels are expressed as a fold change to C−W group (=1.0). Underlined entries indicate that the expression levels of proteins were >50% increased or >50% decreased compared with those of the C−W group.

both mesenteric and epididymal white adipose tissues compared with water-drinking control. When the mRNA level of IGFBP-1 was measured, the intake of green tea also significantly increased mRNA level of IGFBP-1 in epididymal white adipose tissue (Figure 2). We also measured the protein expression level of IGFBP-1 in the liver and muscle and found that green tea did not affect the expression level in these tissues (Figure 1C, D). From these results, the intake of green tea specifically increased the expression of IGFBP-1 in white adipose tissue.

It has been reported that overexpression of IGFBP-1 in white adipose tissue of mice reduced adipose tissue weight.[7] To determine whether up-regulation of IGFBP-1 by green tea contributes to the reduction of adipose tissue weight, we calculated the correlation coefficient between adipose tissue weight and the expression level of IGFBP-1 (Figure 3). The correlation coefficient of green tea group was −0.71, whereas that of the water group was −0.50. This result indicates that significant negative correlation is observed between adipose tissue weight and the expression of IGFBP-1 in the green tea given mice.

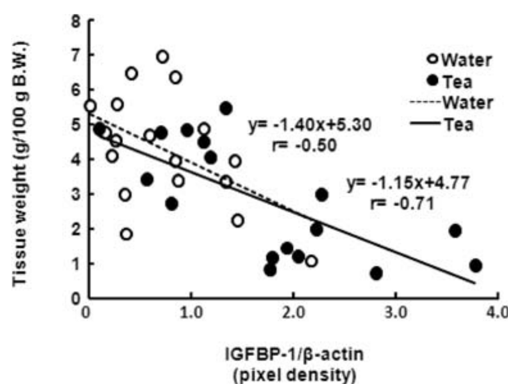

Figure 3. Correlation between the expression levels of IGFBP-1 and adipose tissue weight. The correlation coefficient was calculated from data in Figure 1A and Table 1. Open and solid circles represent the water group and the green tea group, respectively.

EGCg but Not Caffeine Induced the Expression of IGFBP-1 in 3T3-L1 Adipocytes. It has been reported that catechins, especially EGCg, and caffeine have potency to reduce the adipose tissue weight.[18] Therefore, we measured the expression level of IGFBP-1 in EGCg- or caffeine-treated 3T3-L1 adipocytes. As shown in Figure 4, the protein expression level of IGFBP-1 increased in differentiated 3T3-L1 adipocytes, and a significant increase was observed from 3 days after differentiation. In this study, we used the adipocytes 3 days

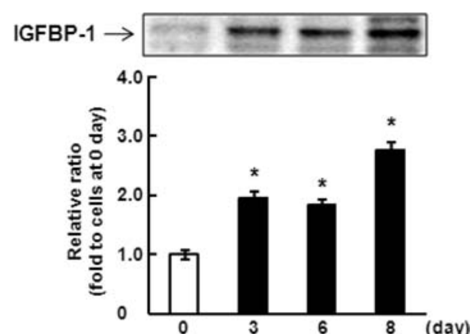

Figure 4. Protein expression level of IGFBP-1 during differentiation of 3T3-L1 adipocytes. The cells were harvested at indicated days after differentiation. Cell lysate was prepared and subjected to Western blot analysis to detect IGFBP-1. Upper panel shows representative data from independent triplicate analysis. Bottom panel shows the density of each band, and values are the mean ± SE (n = 3). ∗, significant difference from the DMSO-treated cells ($p < 0.05$; Dunnett multiple-comparison test).

after differentiation and treated with green tea or EGCg for 24 h. Both green tea and EGCg increased the protein expression level of IGFBP-1 dose-dependently, and a significant increase was obtained in the cells treated with green tea at 0.33 and 3.3 μg/mL and EGCg at 40 μM (Figure 5A). On the other hand,

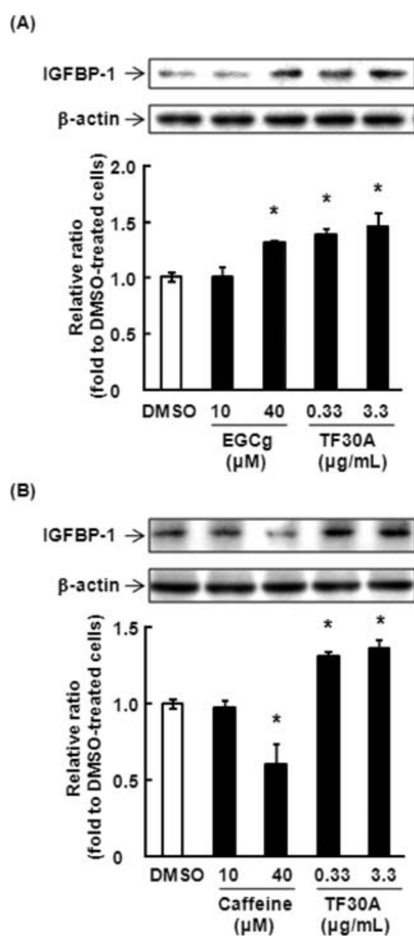

Figure 5. Effects of catechins and caffeine on the protein expression level of IGFBP-1 in 3T3-L1 adipocytes. The cells were differentiated for 3 days and treated with (A) indicated concentrations of EGCg or (B) caffeine for 24 h. TF30A at indicated concentrations was also used to treat the cells. The expression level of IGFBP-1 was detected by Western blot analysis. Each upper panel shows representative data from independent triplicate analysis. Bottom panels show the density of each band, and values are the mean ± SE (n = 3). *, significant difference from the DMSO-treated cells ($p < 0.05$; Dunnett multiple-comparison test).

treatment with caffeine decreased the expression level of IGFBP-1 in 3T3-L1 adipocytes dose-dependently, and a significant decrease was observed in the cells treated with caffeine at 40 μM (Figure 5B). From these results, green tea induced the expression of IGFBP-1 in adipose cells and EGCg is one of candidates for the active compound in green tea.

DISCUSSION

In this study, we have demonstrated that the induction of IGFBP-1 in white adipose tissue by green tea will be involved in the prevention of obesity and subsequent hyperglycemia. We observed a significant induction of IGFBP-1 in the mRNA and protein expression levels in the adipose tissue of mice given green tea for 14 weeks (Figures 1 and 2). We confirmed the induction of IGFBP-1 in 3T3-L1 adipocytes and found that EGCg is one of the active compounds in green tea (Figure 5), although the effective concentration of EGCg was higher than the physiological concentration reported previously.[19,20] Significant negative correlation was observed between the protein expression levels of IGFBP-1and adipose tissue weight in mice given green tea (Figure 3). To our knowledge, this is the first report that a food component regulates the expression of IGFBP-1in adipose cells. Moreover, the intake of green tea suppressed the HF diet-induced up-regulation of obesity-related proteins such as leptin and RBP4 in mesenteric white adipose tissue (Table 2). From these results, IGFBP-1, leptin, and RBP4 are involved in the preventive effect of green tea on obesity through the modulation of differentiation and proliferation in adipocytes.

In the present study, we propose that up-regulation of IGFBP-1 in white adipose tissue is a novel molecular target for green tea-induced prevention of obesity. Our finding supports a previous report that overexpression of IGFBP-1 in white adipose tissue suppressed sucrose-induced increase of body weight, adipose tissue weight, and serum leptin level.[7] In the same paper, overexpression of IGFBP-1 inhibited differentiation of adipocyte through the stimulation of IGF-1 but not of insulin.[7] Therefore, an increase of IGFBP-1 in white adipose tissue inhibits IGF-1-induced adipocyte differentiation. In preadipocytes, IGF-1 activated the cAMP-response element-binding protein through the IGF-1 receptor, resulting in up-regulation of C/EBPα and PPARγ.[21] In this study, we observed the protein expression level of PPARγ was decreased by treatment with green tea and EGCg in a dose-dependent manner (data not shown). This result is identical with our previous result that tea catechins including EGCg suppressed the expression of PPARγ and C/EBPα at the early period of the adipocyte differentiation in 3T3-L1 cells.[17] Our results together with previous ones suggest that green tea induces expression of IGFBP-1 in white adipose tissue, resulting in the inhibition of IGF-1-induced adipocyte differentiation.

Induction of IGFBP-1 by green tea was specific in white adipose tissue, and the induction was not observed in the liver and muscle (Figure 1). These inconsistent results may be due to the mode of action of green tea for inducing *IGFBP-1* gene transduction in white adipose tissue being different from that in the liver and muscle. Induction of IGFBP-1 is regulated in the following three ways: an increase of cAMP level, suppression of insulin level, and activation of PPARs. Glucocorticoids and glucagon induce IGFBP-1 expression through the increase of cAMP level, and a cAMP-responsive element has been identified in the human IGFBP-1 promoter.[22] Insulin suppresses *IGFBP-1* gene transcription through the activation of PI3K and Akt pathways followed by the activation of forkhead box class O (FOXO).[23] The activated FOXO acts as a repressor of the transcription step.[23] PPARγ agonist, rosiglitazone-induced IGFBP-1 production in HepG2 cells, and PPAR responsive elements are located in the promoter region of the *IGFBP-1* gene.[24] Of these induction pathways of IGFBP-1, catechins will regulate the suppressive action of insulin. Our previous paper demonstrated that intake of green tea increased glucose uptake in muscle while decreasing it in white adipose tissue.[13] These inconsistent effects were also observed in the cultured cell system using L6 myotubes and 3T3-L1 adipocytes.[25,26] These results strongly suggest that catechins mimic the insulin action in skeletal muscle, whereas it inhibited the insulin action in white adipose tissue. Taken

together, these results suggest that green tea induces *IGFBP-1* gene transcription by inhibition of insulin action in white adipose tissue.

Our results from the protein array indicated that green tea suppressed the HF diet-induced expression of leptin, AgRP, HGF, FGF-21, RBP4, and IL-11 and increased down-expression of RANTES (Table 2). Leptin is known to regulate food intake and energy homeostasis.[27] Our previous paper showed that the long-term intake of green tea suppressed plasma leptin level.[10] It is reported that AgRP increases food intake and its expression is elevated by fasting and leptin resistance.[27] HGF is mainly produced in adipocytes, and the circulating HGF level in serum is elevated in obese individuals.[28] The serum FGF-21 and RBP4 levels are also increased in obesity and type 2 diabetes mellitus.[3,29] As to IL-11, this protein plays a role in the regulation of both transcription and protein synthesis in mature adipocyte.[30] RANTES increases survival of adipose tissue from macrophage-induced inflammatory.[31] Regulation of these protein expressions by green tea is probably involved in the prevention of obesity and hyperglycemia. Further study is needed to clarify molecular mechanisms of catechins on these protein expressions.

In this study, we found that IGFBP-1 in white adipose tissue is a novel molecular target of green tea and EGCg for the prevention of obesity and subsequent hyperglycemia. Induction of IGFBP-1 by green tea will lead to suppression of adipocyte differentiation and hypertrophy, resulting in the reduction of adipose tissue weight and inflammatory adipokine levels. These sequential molecular mechanisms will be involved in the prevention effects of green tea on obesity and diabetes mellitus.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +81 78 803 5878. E-mail: ashida@kobe-u.ac.jp.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AgRP, Agouti-related protein; C/EBPα, CCAAT/enhancer-binding protein α; DMEM, Dulbecco's Modified Eagle's medium; EGCg, (−)-epigallocatechin gallate; FGF-21, fibroblast growth factor-21; FOXO, forkhead box crass O; HF, high-fat; HGF, hepatocyte growth factor; IGFBP-1, insulin-like growth factor binding protein-1; IGFs, insulin-like growth factors; IL-11, interleukin-11; PPAR, peroxisome proliferator-activated receptor; RANTES, regulated upon activation normal T cell expressed and secreted; RBP4, retinol binding protein 4

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