Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 325 (2004) 151–156
BBRC
www.elsevier.com/locate/ybbrc

# Advanced glycation end products-modified proteins and oxidized LDL mediate down-regulation of leptin in mouse adipocytes via CD36

Yuka Unno[a], Masakazu Sakai[a], Yu-ichiro Sakamoto[a], Akihiko Kuniyasu[b], Hitoshi Nakayama[b], Ryoji Nagai[a], Seikoh Horiuchi[a,*]

[a] *Department of Medical Biochemistry, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan*
[b] *Department of Molecular Cell Function, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan*

Received 22 September 2004
Available online 22 October 2004

**Abstract**

Advanced glycation end products (AGE)-modified proteins as well as oxidized-LDL (Ox-LDL) undergo receptor-mediated endocytosis by CHO cells overexpressing CD36, a member of class B scavenger receptor family. The purpose of the present study was to examine the effects of glycolaldehyde-modified BSA (GA-BSA) as an AGE-ligand and Ox-LDL on leptin expression in adipocytes. GA-BSA decreased leptin expression at both protein and mRNA levels in 3T3-L1 adipocytes and mouse epididymal adipocytes. Ox-LDL showed a similar inhibitory effect on leptin expression in 3T3-L1 adipocytes, which effect was protected by *N*-acetylcysteine, a reactive oxygen species (ROS) inhibitor. Binding of $^{125}$I-GA-BSA or $^{125}$I-Ox-LDL to 3T3-L1 adipocytes and subsequent endocytic degradation were inhibited by a neutralizing anti-CD36 antibody. Furthermore, this antibody also suppressed Ox-LDL-induced leptin down-regulation. These results clarify that the interaction of GA-BSA and Ox-LDL with CD36 leads to down-regulation of leptin expression via ROS system(s) in 3T3-L1 adipocytes, suggesting that a potential link of AGE- and/or Ox-LDL-induced leptin down-regulation might be linked to insulin-sensitivity in metabolic syndrome.


*Keywords:* Advanced glycation end products; Oxidized LDL; Leptin; Adipocytes; CD36; Reactive oxygen species

The major functions of adipocytes are storage of triglycerides and release of free fatty acids and glycerol in response to energy demands. Recent studies emphasize the endocrine function of adipocytes in regulation of a wide variety of energy homeostases by adipocytokines such as leptin, adiponectin, resistin, tumor necrosis factor-α (TNF-α), and plasminogen activator inhibitor-1 (PAI-1) [1]. Leptin is a 16 kDa adipocyte-specific hormone [2,3]. A lack of leptin represented by the *ob*/*ob* mice shows obese and insulin resistance [4], and treatment of these mice with recombinant leptin results in a rapid reduction in body adiposity and the restoration of insulin sensitivity [2]. Moreover, when KK$A^y$ mice (a genetic model for obesity-diabetes syndrome) were cross-bred with transgenic skinny mice overexpressing leptin, their plasma leptin levels became significantly higher than those of KK$A^y$ mice under restricted food intake and their insulin-sensitivity as well as glucose tolerance were greatly ameliorated [5]. These lines of evidence strongly suggest that leptin is one of the key regulators for insulin-sensitivity and glucose tolerance particularly in metabolic syndrome.

CD36 was first shown to be the receptor for oxidized low density lipoprotein (Ox-LDL) in mouse macrophages [6]. The binding of Ox-LDL to human monocyte-derived macrophages and subsequent endocytic degradation were significantly reduced in CD36-deficient patients compared with those in normal individuals [7].

* Corresponding author. Fax: +81 96 364 6940.
*E-mail address:* horiuchi@gpo.kumamoto-u.ac.jp (S. Horiuchi).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.10.015

The subsequent study demonstrated that the expression of CD36 was up-regulated during the differentiation of preadipocytes to adipocytes and that Ox-LDL was subjected to receptor-mediated endocytosis by these adipocytes via CD36 in a fashion similar to macrophages [8]. In addition, Ohgami et al. [9] provided an interesting finding that advance glycation end products (AGE)-modified proteins (AGE-proteins) are recognized by CD36 as effective ligands. This finding led to demonstration that AGE-proteins are able to bind specifically to CD36 of adipocytes, followed by subsequent endocytic degradation by these cells [10]. These lines of evidence point to the significant expression of CD36 in adipocytes, suggesting the possibility that the ligand interaction with adipocytes via CD36 affects their cellular function, particularly synthesis and secretion of adipocytokines.

To test this possibility, we examined the effect of AGE-ligands and Ox-LDL on leptin expression in adipocytes in the present study. The results indicate that down-regulation of leptin in mouse adipocytes is mediated by AGE-proteins and Ox-LDL via a CD36 pathway.

## Materials and methods

*Materials.* Mouse 3T3-L1 cells were purchased from the Health Science Research Resources Bank (Osaka, Japan). Fetal calf serum (FCS) was purchased from Invitrogen (Carlsbad, CA). Penicillin G and streptomycin were purchased from Life Technologies (New York, NY). Mouse anti-murine CD36 monoclonal antibody (clone 63) was purchased from Cascade Bioscience (Winchester, MA). Dulbecco's modified Eagle's medium (DMEM), collagenase (type II), and mouse IgA-κ (TEPC 15) were obtained from Sigma Chemical (St. Louis, MO). Sandwich enzyme-linked immunosorbent assay (ELISA) kit for leptin was purchased from Research and Diagnostics systems (Minneapolis, MN). Na[$^{125}$I] (74 MBq/ml) was purchased from Amersham Pharmacia Biotech (Beckinghamshire, UK). Troglitazone was obtained from Sankyo Pharmaceutical (Tokyo, Japan). *N*-Acetylcysteine (NAC) was from Nakalai Tesque (Kyoto, Japan). Fatty acid-free bovine serum albumin (BSA) was purchased from Wako (Osaka, Japan). All reagents used were of the best grade available from commercial sources.

*Cell culture.* Mouse 3T3-L1 cells ware plated and grown in DMEM supplemented with 25 mM glucose, 10% FCS, 20 U/ml penicillin, and 20 μg/ml streptomycin (medium A) in an atmosphere of 5% $CO_2$. These cells were cultured for 2 days with medium A containing 10 μg/ml insulin, 0.25 μg/ml dexamethasone, and 0.5 mM isobutylmethylxanthine, and 2 days with medium A containing 10 μg/ml insulin. These cells were cultured for 4 days in medium A in the presence or absence of 10 and 50 μg/ml glycolaldehyde-modified BSA (GA-BSA) or Ox-LDL, followed by assay for leptin synthesis and secretion by these cells.

*Primary cell culture*. Fibroblastic preadipocytes were isolated by incubating the epididymal fat pads of 8-week-old male ddY mice with collagenase (1 mg/ml) digestion for 1 h at 37 °C in a shaking water bath. The digest was filtered through sterile 250 μm nylon mesh and the filtrate was centrifuged at 200*g* for 10 min, and mature adipocytes floating in upper layer were removed by aspiration. For primary culture, the cells in the stromal-vascular fraction were adjusted to a density of $1.0 \times 10^5$ cells/ml in medium A and differentiated by 2 days' culture at 37 °C in medium A containing 10 μg/ml insulin, 0.25 μg/ml dexamethasone, and 0.5 mM isobutylmethylxanthine, and 2 days with medium A containing 10 μg/ml insulin in an atmosphere of 5% $CO_2$.

*Preparation of GA-BSA and Ox-LDL* .To prepare GA-BSA, 2 mg/ml of fatty acid-free BSA was incubated at 37 °C for 3 days with 33 mM glycolaldehyde in 0.05 mM sodium phosphate buffer (pH 7.4) and dialyzed against phosphate-buffered saline (PBS). HPLC analyses of basic amino acids (lysine, histidine, and arginine) modified by glycolaldehyde showed that the extent of lysine modified was 85%, whereas those of arginine and histidine modified were 13% and 29%. Amount of $N^{\varepsilon}$-(carboxymethyl)lysine (CML) contents of GA-BSA was determined to be 1.5 mol/mol BSA. LDL was isolated by sequential ultracentrifugation from the plasma of normolipidemic subjects after overnight fasting, and dialyzed against 0.15 M NaCl and 1 mM ethylenediaminetetraacetic acid (pH 7.4). Ox-LDL was prepared by incubating 0.1 mg/ml LDL with 5 μM $CuSO_4$ in PBS for 24 h at 37 °C as described previously [11]. GA-BSA was labeled with [$^{125}$I] by Iodo-Gen (Piece) [14], and Ox-LDL was labeled with [$^{125}$I] [12] to specific radioactivities of 400 and 800 cpm/ng, respectively.

*Leptin mRNA expression*. Total RNA was prepared from these cells by Trizol reagent and leptin mRNA was quantitated by RT-PCR. The forward primer for mouse leptin sequence was 5′-ccaaaaccctcatcaa gacc-3′, and that of the reverse primer was 5′-gtccaactgttgaagaatgtccc-3′. The mouse β-actin sequence of the forward primer was 5′-tgac ccagatcatgtttgagagacc-3′, and that of the reverse primer was 5′-ccatac ccaagaaggaaggc-3′ [13]. After an initial denaturation for 3 min 94 °C, PCR was performed for 38 cycles for leptin detection and for 25 cycles for β-actin detection. PCR conditions were 94 °C for 45 s, 60 °C for 1 min, and 72 °C for 1.5 min. PCR products specific for leptin and β-actin were analyzed by electrophoresis on 1% agarose gel. DNA was visualized by ethidium bromide staining. The intensity of each band was evaluated by a LAS-1000plus (FUJIFILM, Tokyo, Japan).

*Leptin measurement*. Leptin concentrations in the culture medium were determined by a sandwich ELISA kit using recombinant mouse leptin as a standard according to the manufacturer's instruction. The minimal detectable level in this assay was 62.5 pg/ml.

*Binding and degradation experiments with $^{125}$I-GA-BSA and $^{125}$I-Ox-LDL*. For the binding assay, cells were incubated for 1.5 h at 4 °C with 0.5 ml DMEM supplemented with 25 mM glucose, 3% BSA, 20 U/ml penicillin, and 20 μg/ml streptomycin (medium B) containing 1.0–10 μg/ml of $^{125}$I-GA-BSA or $^{125}$I-Ox-LDL, followed by washing with PBS plus 3% BSA and determination of cell-bound radioactivity as described previously [14]. The non-specific binding was determined in the presence of a 50-fold excess of unlabeled GA-BSA or Ox-LDL. The specific binding was obtained by subtracting the non-specific binding from the total binding. For degradation assays, cells were incubated for 18 h at 37 °C with 5 μg/ml $^{125}$I-GA-BSA or $^{125}$I-Ox-LDL in medium B in the absence or presence of 10 μg/ml of a neutralizing anti-CD36 antibody or non-immune mouse IgA. An aliquot of each culture supernatant was determined for TCA-soluble radioactivity as described previously [14].

*Statistical analysis*. Data were expressed as means ±SD ($n = 3$). Differences between groups were evaluated by Student's *t* test. A value of $P < 0.05$ was regarded as statically significant.

## Results

### *Inhibitory effect of GA-BSA on leptin secretion from 3T3-L1 adipocytes*

To determine the effects of GA-BSA on leptin synthesis and secretion by 3T3-L1 adipocytes, the cells were incubated for 4 days without GA-BSA. A significant

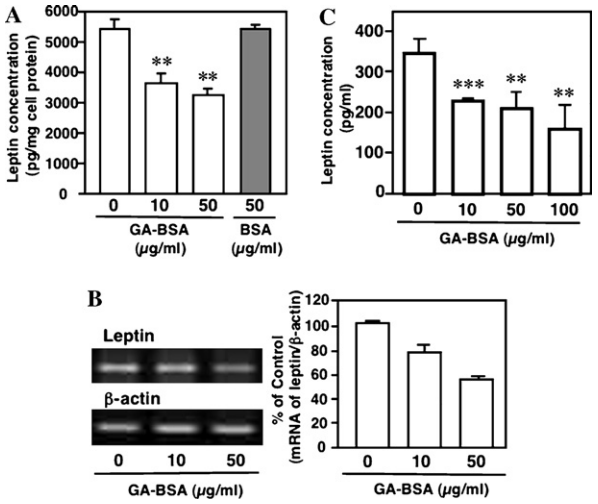


Fig. 1. Effects of GA-BSA on expression of leptin in 3T3-L1 adipocytes (A and B) and mouse epididymal adipocytes (C). Differentiated adipocytes were incubated for 4 days with GA-BSA (10 and 50 μg/ml) or BSA (50 μg/ml) and measured for leptin protein in the culture medium by ELISA (A) and leptin mRNA in these cells by RT-PCR (B, left panel) and their densitometric analyses (B, right panel) as described under Materials and methods. Similarly, differentiated epididymal adipocytes were incubated for 4 days with GA-BSA (10, 50, and 100 μg/ml) followed by determination of leptin proteins in the culture supernatant (C). All values are means ± SD (bars) obtained from three experiments. $^{**}p < 0.01$; $^{***}p < 0.005$ (compared without GA-BSA).

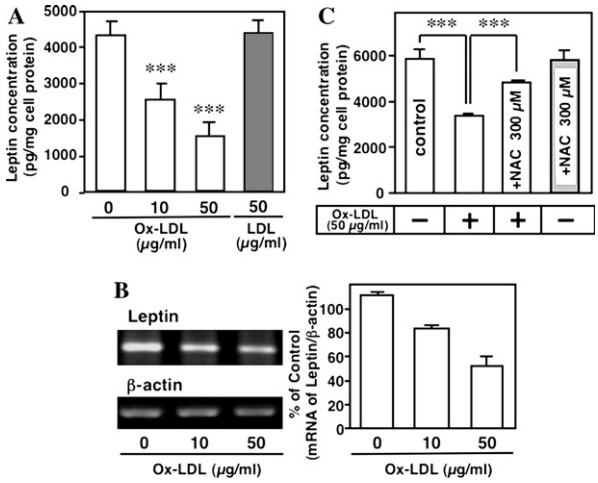


Fig. 2. Effect of Ox-LDL on expression of leptin in 3T3-L1 adipocytes (A and B). Differentiated adipocytes were cultured for 4 days with Ox-LDL (10 and 50 μg/ml) or LDL (50 μg/ml) and determined for leptin proteins in the culture supernatant by an ELISA (A) and leptin mRNA in these cells by RT-PCR (B, left panel) and their densitometric analyses (B, right panel) as described under Materials and methods. Effects of NAC on Ox-LDL-induced leptin down-regulation in 3T3-L1 adipocytes (C). Differentiated adipocytes were incubated for 4 days with 50 μg/ml Ox-LDL in the presence or absence of 300 μM NAC. Leptin concentrations in culture supernatant were measured by ELISA. All values are means ± SD (bars) obtained from three experiments. $^{***}p < 0.005$ (compared without Ox-LDL).

amount of leptin was secreted into the culture medium, giving a basal level of 5400 pg of leptin/mg cell protein. Incubation with 10 and 50 μg/ml GA-BSA resulted in 33% and 41% reduction, respectively, of leptin secretion whereas unmodified BSA had no effect (Fig. 1A). Leptin mRNA was also reduced by GA-BSA (Fig. 1B). GA-BSA showed a similar inhibitory effect on leptin secretion by mouse epididymal adipocytes, with 52% inhibition being achieved at 100 μg/ml GA-BSA (Fig. 1C).

### *Inhibitory effect of Ox-LDL on leptin secretion from 3T3-L1 adipocytes*

Effects of Ox-LDL on leptin production by 3T3-L1 adipocytes were determined under identical conditions. Amounts of leptin secreted from the cells were inhibited by Ox-LDL in a dose-dependent manner with 60% reduction at 50 μg/ml Ox-LDL, whereas LDL had no effect (Fig. 2A). Amounts of leptin mRNA were similarly reduced by Ox-LDL (Fig. 2B). These data taken together indicate that GA-BSA and Ox-LDL are able to inhibit leptin synthesis at a mRNA level, leading to reduction in leptin secretion from these adipocytes. Under identical conditions, Ox-LDL-induced leptin down-regulation was effectively protected by 300 μM NAC (Fig. 2C), a reactive oxygen species (ROS) inhibitor, indicating the involvement of a ROS system(s) in this phenomenon.

### *Contribution of CD36 to cellular binding of $^{125}$I-GA-BSA or $^{125}$I-Ox-LDL to, and endocytic degradation by 3T3-L1 adipocytes*

Binding experiments demonstrated that 3T3-L1 adipocytes possess a high affinity binding site for $^{125}$I-GA-BSA with an apparent $K_d$ of 5.5 μg/ml and maximal surface binding of 403 ng $^{125}$I-GA-BSA/mg of cell protein (data not shown). The total cellular binding of $^{125}$I-GA-BSA to these adipocytes was effectively inhibited by unlabeled GA-BSA, Ox-LDL, and the anti-CD36 antibody, whereas the non-immune IgA had no effect (Fig. 3A). The anti-CD36 antibody also showed a significant inhibition for the endocytic degradation of $^{125}$I-GA-BSA by these cells (Fig. 3B). In cross-competitive experiments, the cellular binding of $^{125}$I-Ox-LDL was inhibited effectively by unlabeled Ox-LDL and partially by GA-BSA (Fig. 3C). The anti-CD36 antibody effectively inhibited not only the binding process of $^{125}$I-Ox-LDL (Fig. 3C), but also the subsequent endocytic degradation of $^{125}$I-Ox-LDL by these cells (Fig. 3D), whereas non-immune IgA had no effect. These results strongly suggested that CD36 plays a major role in both cellular binding of GA-BSA and

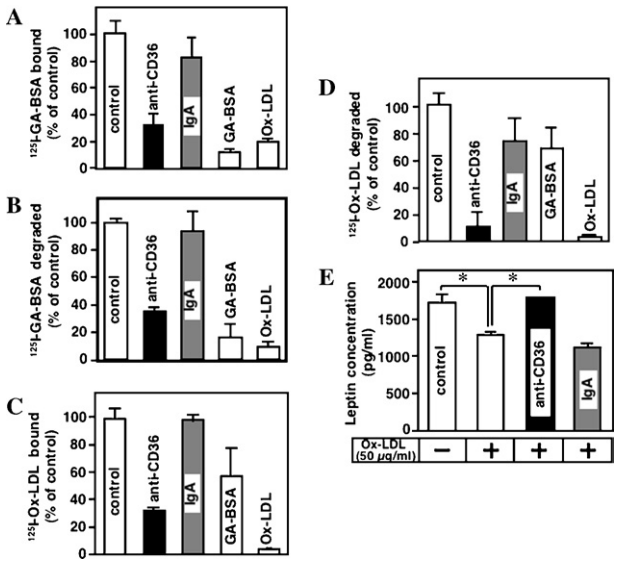


Fig. 3. Effects of the anti-CD36 antibody on cellar binding of $^{125}$I-GA-BSA to 3T3-L1 adipocytes (A) and subsequent endocytic degradation (B). Cells were incubated at 4 °C for 90 min with 5 μg/ml $^{125}$I-GA-BSA in the absence or presence of the anti-CD36 antibody, control IgA, 125 μg/ml unlabeled GA-BSA or unlabeled Ox-LDL. The cells were determined for the cell-bound radioactivity (A). The cells were incubated at 37 °C for 18 h with 5 μg/ml $^{125}$I-GA-BSA in the absence or presence of the antibody, unlabeled GA-BSA or unlabeled Ox-LDL, followed by determination of amounts of $^{125}$I-GA-BSA degraded by these cells (B). The 100% value for binding was 0.14 μg/mg cell protein and that for degradation was 0.12 μg/mg cell protein/18 h. Effects of the anti-CD36 antibody on cellar binding of $^{125}$I-Ox-LDL to 3T3-L1 adipocytes (C) and subsequent endocytic degradation (D). Amount of binding and endcytic degradation of $^{125}$I-Ox-LDL were determined in the same way as described above. The 100% values for binding and that for degradation were 0.4 μg/mg of cell protein and 0.4 μg/mg of cell protein/18 h, respectively. Effects of the anti-CD36 antibody on Ox-LDL-induced down-regulation of leptin in 3T3-L1 adipocytes (E). The cells were incubated for 4 days with 50 μg/ml Ox-LDL in the absence or presence of the anti-CD36 antibody or control IgA to determine amounts of leptin in the culture supernatant. All values shown in this figure are means ± SD (bars) obtained from three experiments. *$p < 0.05$.

Ox-LDL to these 3T3-L1 adipocytes and subsequent endocytic degradation.

### *Contribution of CD36 to Ox-LDL-induced down-regulation of leptin in 3T3-L1 adipocytes*

Finally, we tested the effect of the anti-CD36 antibody on Ox-LDL-induced leptin down-regulation. The amount of leptin secreted into the medium was significantly reduced, whereas reduction was suppressed by the presence of the anti-CD36 antibody (Fig. 3E). The non-immune antibody had no such effect (Fig. 3E). Neither the antibody nor non-immune antibody affected synthesis and secretion of leptin by these cells (data not shown). These results suggest that the cellular binding of Ox-LDL to CD36 of these adipocytes may be critically involved in Ox-LDL-induced leptin down-regulation.

## Discussion

The novel finding in the present study is that the interaction of AGE-ligands or Ox-LDL with 3T3-L1-adipocytes via CD36 induces ROS as an intracellular signal, which leads to inhibition of leptin expression in these adipocytes.

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a ligand-activated transcription factor and functions as a heterodimer with a retinoid X receptor (RXR). Incubation with HX531, a RXR antagonist with 3T3-L1 adipocytes, enhanced the leptin production [15], whereas troglitazone, a PPARγ activator, inhibited leptin production by 3T3-L1 adipocytes [16]. In fact, leptin production was inhibited by 41% by 20 μM troglitazone by 3T3-L1 adipocytes under the present conditions (data not shown). These data strongly suggest the involvement of PPAR-γ in leptin production by these adipocytes. This notion is also supported by the in vivo finding that leptin is overproduced in hetero-knockout mice of PPARγ (+/−) [17].

Leptin down-regulation by Ox-LDL was prevented by ROS inhibitor (Fig. 2C), while leptin down-regulation was induced by hydrogen peroxide alone (data not shown), suggesting some ROS system(s) might play some roles. It is possible that ROS generated from Ox-LDL in the culture medium might get into cytoplasm of these cells, which leads to down-regulation of leptin. However, since Ox-LDL-induced down-regulation of leptin by these adipocytes was effectively protected by the anti-CD36 antibody (Fig. 3E), the binding of Ox-LDL to CD36 and/or subsequent endocytic degradation might activate an intracellular ROS system(s). No reports have been available about a functional link of PPARγ with a ROS system(s). Therefore, it would be safe to propose that a ROS system(s) in addition to PPARγ might play a crucial role in Ox-LDL-induced leptin down-regulation in adipocytes.

AGE-induced biological phenomena are believed to be mediated by the AGE-receptors such as receptor for AGE (RAGE) [18], galectin-3 [19], SR-A [20], CD36 [21], SR-BI [22], LOX-1 [23], and FEEL-1/2 [24]. Uchida et al. [25] have recently provided interesting finding that AGE-modified BSA (AGE-BSA) increased PAI-1 expression in rat adipocytes in which activation of intracellular ROS and NF-κB might be involved in up-regulation of PAI-1 mRNA. However, the authors did not clarify an AGE-receptor(s) involved in AGE-BSA-enhanced PAI-1 expression although RAGE was discussed as a potential AGE-receptor. In this connection, the present study clarified that interaction of GA-BSA or Ox-LDL with CD36 of these adipocytes was crucial for leptin down-regulation, because: (i) the cellular binding of GA-BSA or Ox-LDL and their endocytic degradation by 3T3-L1 adipocytes were

effectively inhibited by the neutralizing anti-CD36 antibody (Figs. 3A–D), (ii) Ox-LDL-induced leptin down-regulation by these cells was significantly prevented by the same antibody (Fig. 3E), and (iii) CD36 was highly expressed by 3T3-L1 adipocytes [8] and rat and human adipocytes [21,26].

Glycolaldehyde is generated from the Schiff base in the Maillard reaction [27]. In addition, production of glycolaldehyde is mediated by a pathway involving both enzymatic and nonenzymatic reactions during inflammation. Leucocytes are activated to secrete myeloperoxidase, which mediates the enzymatic formation of hypochlorous acid (HOCl) from hydrogen peroxide and chloride [28]. HOCl then reacts with L-serine to form glycolaldehyde which is involved in the generation of glycolaldehyde-modified proteins. Myeloperoxidase was detected in lipid-rich human atherosclerotic lesions, and the active myoperoxidase was purified from atherosclerotic arteries [29]. Human atherosclerotic lesions were also shown to be stained by the monoclonal antibody against HOCl-modified proteins [30]. In addition, our recent study identified the new chemical structure from glycolaldehyde-modified proteins and named it as GA-pyridine, and foam cells in human atherosclerotic lesions were positively stained by the anti-GA-pyridine antibody [28]. Moreover, Takeuchi et al. [31] recently identified two proteins (200 and 0.85 kDa) in human serum by the antibody specific for glycolaldehyde-modified proteins. These lines of evidence support the notion that glycolaldehyde contributes to protein modification in vivo.

Concentrations of the AGE-ligand and Ox-LDL used in the present study may not reflect in vivo conditions. However, plasma levels of AGE-proteins and Ox-LDL are reported to increase in patients with diabetes [31], renal failure [32] or myocardial infarction [33]. Although levels of AGE-proteins or Ox-LDL are not known in adipose tissues, the recent finding that the myeloperoxidase activity in the ischemic myocardium is significantly higher in cholesterol-fed Zucker diabetic fatty rats than cholesterol-fed lean rats [34] suggests the possibility that down-regulation of leptin induced by AGE-ligands or Ox-LDL in adipocytes might be related to insulin-sensitivity and glucose tolerance in metabolic syndrome.

## Acknowledgments

We are grateful for helpful discussions to Miss Maki Satoh of our laboratory, and Drs. Takaaki Akaike and Teruo Akuta at the Department of Microbiology, Kumamoto University Graduate School of Medical and Pharmaceutical sciences. This work was supported, in part, by Grants-in-Aid for Scientific Research (B) (2) (13470228 to S.H.) from Japan Society for the Promotion of Science.

## References

[1] Y. Matsuzawa, T. Funahashi, S. Kihara, I. Shimomura, Adiponectin and metabolic syndrome, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 29–33.
[2] J.L. Halaas, K.S. Gajiwala, M. Maffei, S.L. Cohen, B.T. Chait, D. Rabinowitz, R.T. Lallone, S.K. Burley, J.M. Friedman, Weight-reducing effects of the plasma protein encoded by the obese gene, Science 269 (1995) 543–546.
[3] D.A. Sandoval, S.N. Davis, Leptin: metabolic control and regulation, J. Diabetes Complications 17 (2003) 108–113.
[4] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.
[5] H. Masuzaki, Y. Ogawa, M. Aizawa-Abe, K. Hosoda, J. Suga, K. Ebihara, N. Satoh, H. Iwai, G. Inoue, H. Nishimura, Y. Yoshimasa, K. Nakao, Glucose metabolism and insulin sensitivity in transgenic mice overexpressing leptin with lethal yellow agouti mutation: usefulness of leptin for the treatment of obesity-associated diabetes, Diabetes 48 (1999) 1615–1622.
[6] A.C. Nicholson, S. Frieda, A. Pearce, R.L. Silverstein, Oxidized LDL binds to CD36 on human monocyte-derived macrophages and transfected cell lines. Evidence implicating the lipid moiety of the lipoprotein as the binding site, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 269–275.
[7] S. Nozaki, H. Kashiwagi, S. Yamashita, T. Nakagawa, B. Kostner, Y. Tomiyama, A. Nakata, M. Ishigami, J. Miyagawa, K. Kameda-Takemura, et al., Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36-deficient subjects, J. Clin. Invest. 96 (1995) 1859–1865.
[8] A. Kuniyasu, S. Hayashi, H. Nakayama, Adipocytes recognize and degrade oxidized low density lipoprotein through CD36, Biochem. Biophys. Res. Commun. 295 (2002) 319–323.
[9] N. Ohgami, R. Nagai, M. Ikemoto, H. Arai, A. Kuniyasu, S. Horiuchi, H. Nakayama, Cd36, a member of the class b scavenger receptor family, as a receptor for advanced glycation end products, J. Biol. Chem. 276 (2001) 3195–3202.
[10] A. Kuniyasu, N. Ohgami, S. Hayashi, A. Miyazaki, S. Horiuchi, H. Nakayama, CD36-mediated endocytic uptake of advanced glycation end products (AGE) in mouse 3T3-L1 and human subcutaneous adipocytes, FEBS Lett. 537 (2003) 85–90.
[11] T. Biwa, H. Hakamata, M. Sakai, A. Miyazaki, H. Suzuki, T. Kodama, M. Shichiri, S. Horiuchi, Induction of murine macrophage growth by oxidized low density lipoprotein is mediated by granulocyte macrophage colony-stimulating factor, J. Biol. Chem. 273 (1998) 28305–28313.
[12] A.S. Mcfarlane, Efficient trace-labelling of proteins with iodine, Nature 182 (1958) 53.
[13] K.M. Morton, V. Emilsson, Y.L. Liu, M.A. Cawthorne, Leptin action in intestinal cells, J. Biol. Chem. 273 (1998) 26194–26201.
[14] R. Nagai, K. Matsumoto, X. Ling, H. Suzuki, T. Araki, S. Horiuchi, Glycolaldehyde, a reactive intermediate for advanced glycation end products, plays an important role in the generation of an active ligand for the macrophage scavenger receptor, Diabetes 49 (2000) 1714–1723.
[15] C.B. Kallen, M.A. Lazar, Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes, Proc. Natl. Acad. Sci. USA 93 (1996) 5793–5796.
[16] D. Sinha, S. Addya, E. Murer, G. Boden, 15-Deoxy-delta (12,14) prostaglandin J2: a putative endogenous promoter of adipogenesis suppresses the *ob* gene, Metabolism 48 (1999) 786–791.
[17] N. Kubota, Y. Terauchi, H. Miki, H. Tamemoto, T. Yamauchi, K. Komeda, S. Satoh, R. Nakano, C. Ishii, T. Sugiyama, K. Eto, Y. Tsubamoto, A. Okuno, K. Murakami, H. Sekihara, G. Hasegawa, M. Naito, Y. Toyoshima, S. Tanaka, K. Shiota, T. Kitamura, T. Fujita, O. Ezaki, S. Aizawa, T. Kadowaki, et al.,

PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance, Mol. Cell 4 (1999) 597–609.
[18] M. Neeper, A.M. Schmidt, J. Brett, S.D. Yan, F. Wang, Y.C. Pan, K. Elliston, D. Stern, A. Shaw, Cloning and expression of RAGE: a cell surface receptor for advanced glycation endproducts of proteins, J. Biol. Chem. 267 (1992) 14998–15004.
[19] H. Vlassara, Y.M. Li, F. Imani, D. Wojciechowicz, Z. Yang, F.T. Liu, A. Cerami, Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGEreceptor complex, Mol. Med. 1 (1995) 634–646.
[20] T. Kodama, M. Freeman, L. Rohrer, J. Zabrecky, P. Matsudaira, M. Krieger, Type I macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils, Nature 343 (1990) 531–535.
[21] G. Endemann, L.W. Stanton, K.S. Madden, C.M. Bryant, R.T. White, A.A. Protter, CD36 is a receptor for oxidized low density lipoprotein, J. Biol. Chem. 268 (1993) 11811–11816.
[22] S. Acton, A. Rigotti, K.T. Landschulz, S. Xu, H.H. Hobbs, M. Krieger, Identification of scavenger receptor SR-BI as a high density lipoprotein receptor, Science 271 (1996) 518–520.
[23] T. Sawamura, N. Kume, T. Aoyama, H. Moriwaki, H. Hoshikawa, Y. Aiba, T. Tanaka, S. Miwa, Y. Katsura, T. Kita, T. Masaki, An endothelial receptor for oxidized low-density lipoprotein, Nature 386 (1997) 73–77.
[24] H. Adachi, M. Tsujimoto, FEEL-1, a novel scavenger receptor with in vitro bacteria-binding and angiogenesis-modulating activities, J. Biol. Chem. 277 (2002) 34264–34270.
[25] Y. Uchida, K. Ohba, T. Yoshioka, K. Irie, T. Muraki, Y. Maru, Cellular carbonyl stress enhances the expression of plasminogen activator inhibitor-1 in rat white adipocytes via reactive oxygen species-dependent pathway, J. Biol. Chem. 279 (2004) 4075–4083.
[26] M. Febbraio, N.A. Abumrad, D.P. Hajjar, K. Sharma, W. Cheng, S.F. Pearce, R.L. Silverstein, A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism, J. Biol. Chem. 274 (1999) 19055–19062.
[27] M.A. Glomb, V.M. Monnier, Mechanism of protein modification by glyoxal and glycolaldehyde, reactive intermediates of the Maillard reaction, J. Biol. Chem. 270 (1995) 10017–10026.
[28] R. Nagai, C.M. Hayashi, L. Xia, M. Takeya, S. Horiuchi, Identification in human atherosclerotic lesions of GA-pyridine, a novel structure derived from glycolaldehyde-modified proteins, J. Biol. Chem. 277 (2002) 48905–48912.
[29] A. Daugherty, J.L. Dunn, D.L. Rateri, J.W. Heinecke, Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions, J. Clin. Invest. 94 (1994) 437–444.
[30] L.J. Hazell, L. Arnold, D. Flowers, G. Waeg, E. Malle, R. Stocker, Presence of hypochlorite-modified proteins in human atherosclerotic lesions, J. Clin. Invest. 97 (1996) 1535–1544.
[31] M. Takeuchi, Z. Makita, R. Bucala, T. Suzuki, T. Koike, Y. Kaneda, Immunological evidence that non-carboxymethyllysine advanced glycation endproducts are produced from short chain sugars and dicarbonyl compounds in vivo, Mol. Med. (2000) 114–125.
[32] S. Sugiyama, T. Miyata, Y. Ueda, H. Tanaka, K. Maeda, S. Kawashima, C. VanYpersele de Strihou, K. Kurokawa, Plasma levels of pentosidine in diabetic patients: an advanced glycation end product, J. Am. Soc. Nephrol. 9 (1998) 1681–1688.
[33] S. Ehara, M. Ueda, T. Naruko, K. Haze, A. Itoh, M. Otsuka, R. Komatsu, T. Matsuo, H. Itabe, T. Takano, Y. Tsukamoto, M. Yoshiyama, K. Takeuchi, J. Yoshikawa, A.E. Becker, Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes, Circulation 103 (2001) 1955–1960.
[34] S. Hoshida, N. Yamashita, K. Otsu, T. Kuzuya, M. Hori, Cholesterol feeding exacerbates myocardial injury in Zucker diabetic fatty rats, Am. J. Physiol. Heart. Circ. Physiol. 278 (2000) 256–262.