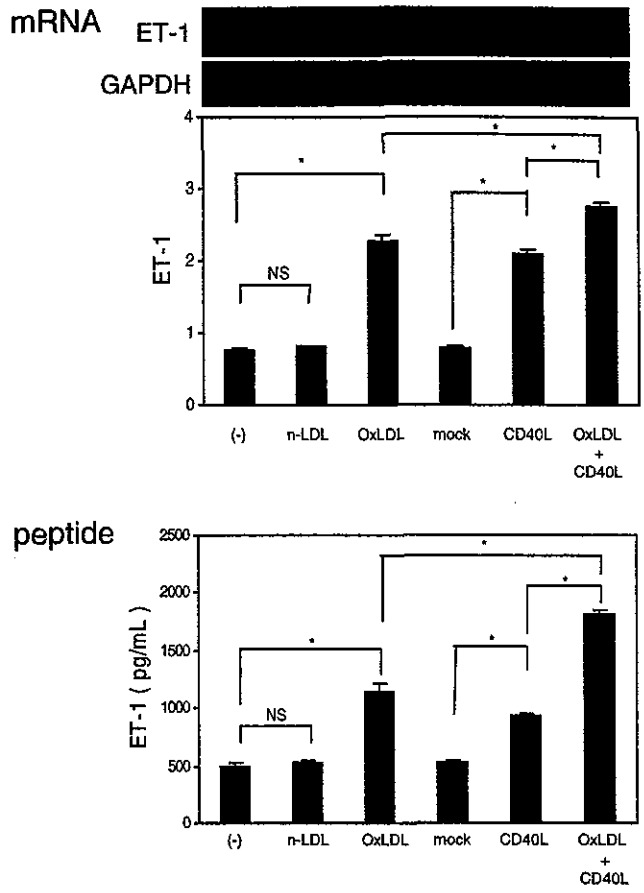

FIGURE 2. Induction of endothelin-1 (ET-1) production in bovine aortic endothelial cells (BAEC) in response to oxidatively modified low-density lipoprotein (OxLDL) and CD40L. BAEC were incubated for 8 hours at 37°C with OxLDL-expressing (30 μg/mL) and CD40L-expressing cells (1×10^5/mL). Induction of ET-1 production was assessed by reverse transcriptase-polymerase chain reaction (upper panel) and by enzyme immunoassay (lower panel). mRNA levels of ET-1 were expressed as the ratio to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Results are expressed as means ± SEM (n = 3); $*P < 0.01$. NS, no significant difference.

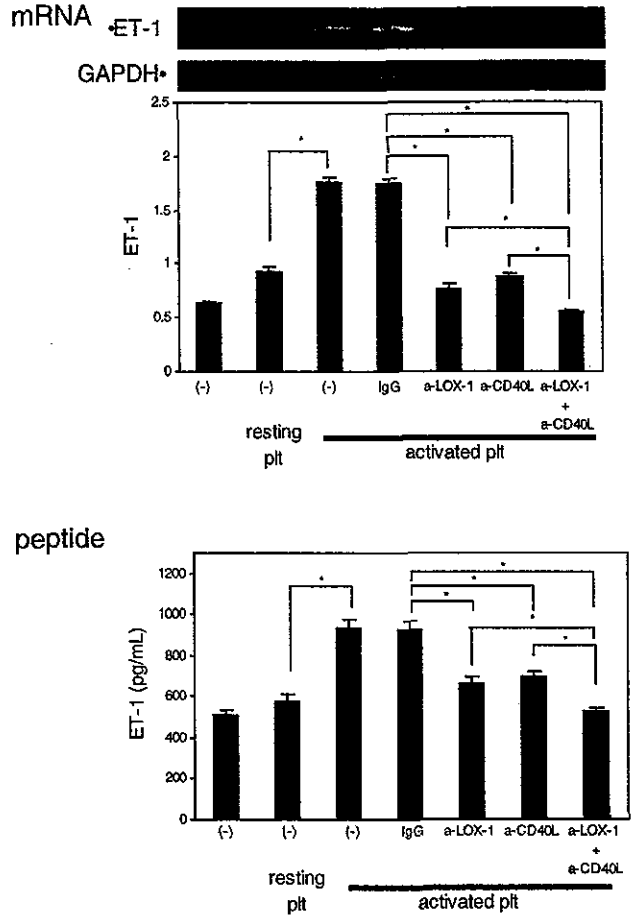

FIGURE 3. Induction of endothelin-1 (ET-1) production in bovine aortic endothelial cells in response to activated platelets (plt). BAEC were incubated with platelets for 3 h at 37°C, then unbound platelets were washed out and the cells were further incubated for 8 hours. Anti-LOX-1 (lectin-like oxidized low-density lipoprotein receptor-1) and anti-CD40L antibodies were added to the culture media prior to the addition of platelets and maintained throughout the experiments. mRNA and peptide levels of ET-1 were determined and expressed as in Figure 2. Results are expressed as means ± SEM (n = 3); $*P < 0.01$.

Stimulation of LOX-1 and CD40 Induce ET-1 Production from BAEC

In order to examine whether LOX-1 and CD40 co-operate, not merely in the binding of platelets, in functional activation of endothelial cells, the levels of ET-1 mRNA and ET-1 released from BAEC were measured as a marker of endothelial dysfunction.

Incubation of BAEC with OxLDL increased ET-1 expression levels, both mRNA and peptide, in BAEC ($P < 0.001$), whereas native LDL had no significant effects (Fig. 2). The induction was inhibited by anti-LOX-1 antibody (data not shown). Incubation of BAEC with hCD40L-expressing cells also increased ET-1 expression levels, both mRNA and peptide ($P < 0.001$), whereas mock transfectants showed no effects (Fig. 2). The increase was inhibited by anti-CD40L (data not shown). When OxLDL-expressing and hCD40L-expressing cells were put together into the culture medium of BAEC, the level of ET-1 induction further increased compared with that achieved by either OxLDL-expressing or hCD40L-expressing cells alone (Fig. 2). These results suggest that LOX-1 and CD40 activate endothelial cells and work synergistically.

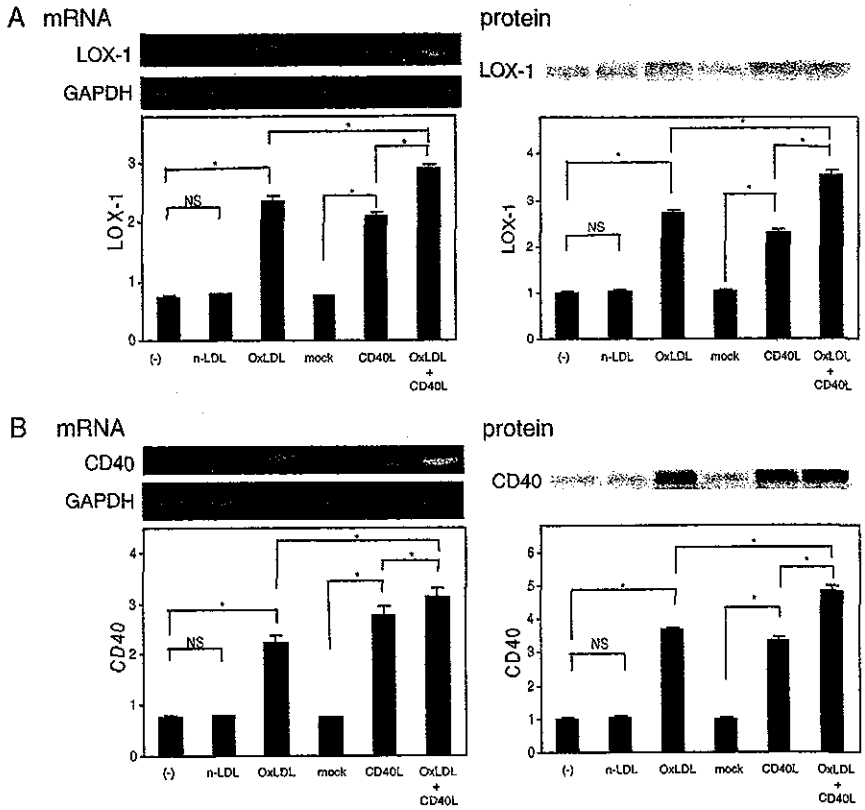

FIGURE 4. Auto-regulation and cross- regulation of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and CD40 expression in response to their ligands. Bovine aortic endothelial cells were incubated for 8 hours at 37°C with oxidatively modified low-density lipoprotein- (OxLDL; 30 μg/mL) and CD40L-expressing cells (1×10^5/mL). Induction of (A) LOX-1 and (B) CD40 expression was monitored by reverse transcriptase-polymerase chain reaction (left) and by Western blotting (right). mRNA levels were expressed as the ratio to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The protein levels were expressed as the ratio to that of untreated control. Results are expressed as means ± SEM (n = 3); *$P < 0.01$. NS, no significant difference.

The effect of platelets, which are a natural ligand for both LOX-1 and CD40 on endothelial cells, was then examined. Incubation of BAEC with activated platelets increased ET-1 mRNA and ET-1 production ($P < 0.001$), while resting platelets had little effect on the expression of ET-1 (Fig. 3). Both anti-LOX-1 and anti-CD40L inhibited the effects of activated platelets, and the effects of the antibodies were additive, supporting the possibility that LOX-1 and CD40 on endothelial cells might synergistically contribute to endothelial dysfunction.

LOX-1 and CD40 Stimulation Enhances their Expression of Each Other in BAEC

OxLDL induced the expressions of CD40 and LOX-1 on BAEC, while native LDL showed no significant effects (Fig. 4). The induction was inhibited by anti-LOX-1 (data not shown). The expression of CD40 and LOX-1 in BAEC was induced by hCD40L-expressing cells, while mock transfectants showed no significant effects (Fig. 4). The induction was inhibited by anti-CD40L (data not shown). These results suggest that LOX-1 and CD40 were not only auto-regulated by their ligands but also cross-regulate each other.

Activated platelets were again used to test whether the cross-regulation occurs upon binding of natural ligand. The expression of CD40 and LOX-1 on BAEC was induced by activated platelets (Fig. 5). Anti-LOX-1 and anti-CD40L significantly suppressed the induction of both LOX-1 and CD40, and simultaneous application of both antibodies further suppressed the induction of the proteins. These results appear to indicate that the expression of LOX-1 and CD40 is controlled by the cross-regulation between these molecules as well as by the auto-regulation.

Superoxide Generation is Involved in the Mechanism of LOX-1-mediated Induction of ET-1 Release

The effect of SOD, which eliminates superoxide, was examined in order to address the possible involvement of

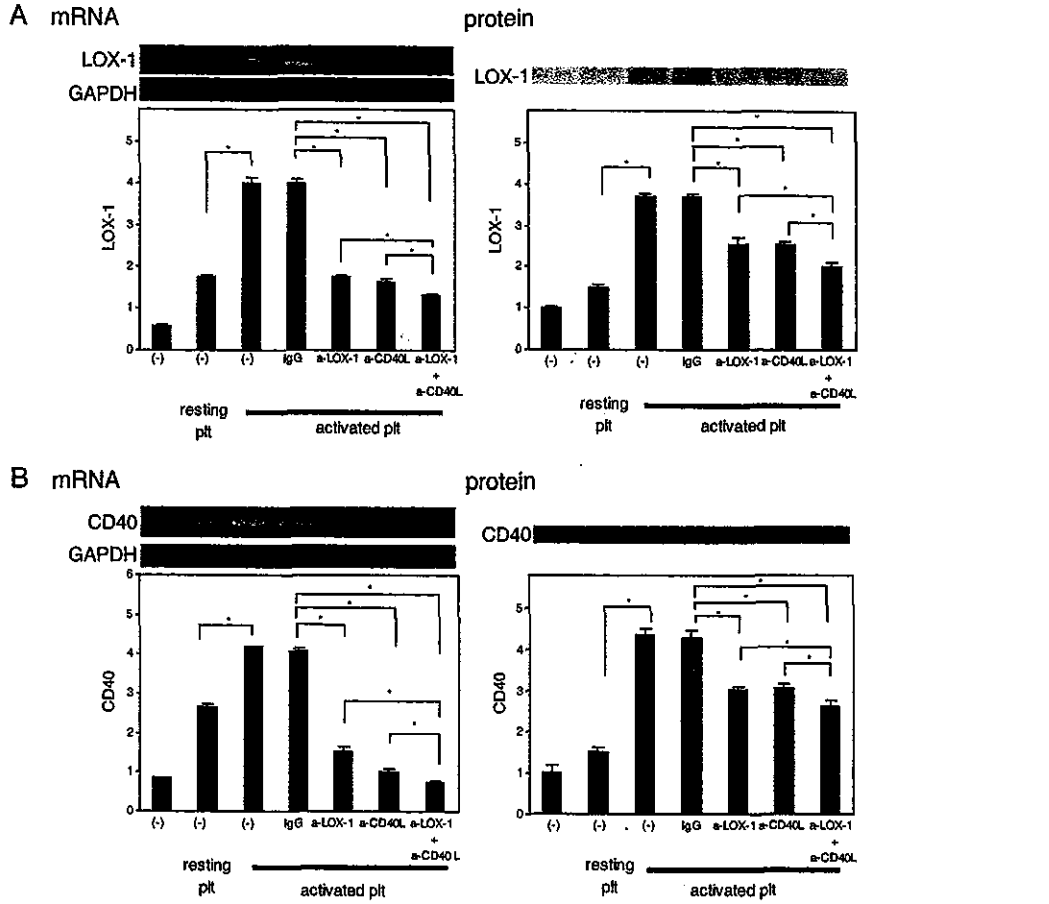

FIGURE 5. Regulation of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and CD40 expression by their common ligand platelets (plt). Bovine aortic endothelial cells were incubated with platelets under the same conditions as in Figure 3. Anti-LOX-1 and anti-CD40L antibodies were added to the culture medium prior to the addition of platelets and levels were maintained throughout the experiments. Induction of (A) LOX-1 and (B) CD40 expression was determined by reverse transcriptase-polymerase chain reaction (left) and by Western blotting (right). mRNA levels were expressed as the ratio to that of GAPDH. The protein levels were expressed as the ratio to that of untreated control. Results are expressed as means ± SEM (n = 3); $*P < 0.01$.

superoxide generation in the mechanism of upregulation of ET-1 by LOX-1 and CD40. The effect of OxLDL was almost reversed by the application of 50 U/mL of SOD and the release of ET-1 was suppressed to the basal level, while the effect of CD40L was not altered (Fig. 6). In accordance with these results, SOD suppressed more than half of the effect of activated platelets on the release of ET-1 (Fig. 6). These results suggest that LOX-1-mediated ET-1 induction takes a distinct pathway from that of CD40-mediated ET-1 induction.

DISCUSSION

It has been demonstrated that the CD40–CD40L system plays a pivotal role for inflammatory processes including atherosclerosis. A recent study demonstrated that platelet–endothelium interaction, via the CD40–CD40L system, induces the expression of proinflammatory cytokines.[16] Lectin-like oxidized low-density lipoprotein receptor-1 was originally identified as the major endothelial receptor for oxidized LDL, which might be of importance in bringing about endothelial dysfunction and consequently in atherogenesis. Subsequent studies showed that the expression of LOX-1 is induced by proinflammatory cytokines,[17] and LOX-1 acts as a leukocyte adhesion molecule,[18] suggesting the possibility that LOX-1 might play an important role in inflammation. The involvement of LOX-1 has been demonstrated in some models of inflammation, zymosan-induced arthritis,[19] and endotoxin-induced uveitis[18] in rat models. Lectin-like oxidized low density lipoprotein receptor-1 has also been reported to mediate platelet–endothelium interaction, as is the case with CD40.[13] Considering the situations where and when the two molecules work, this

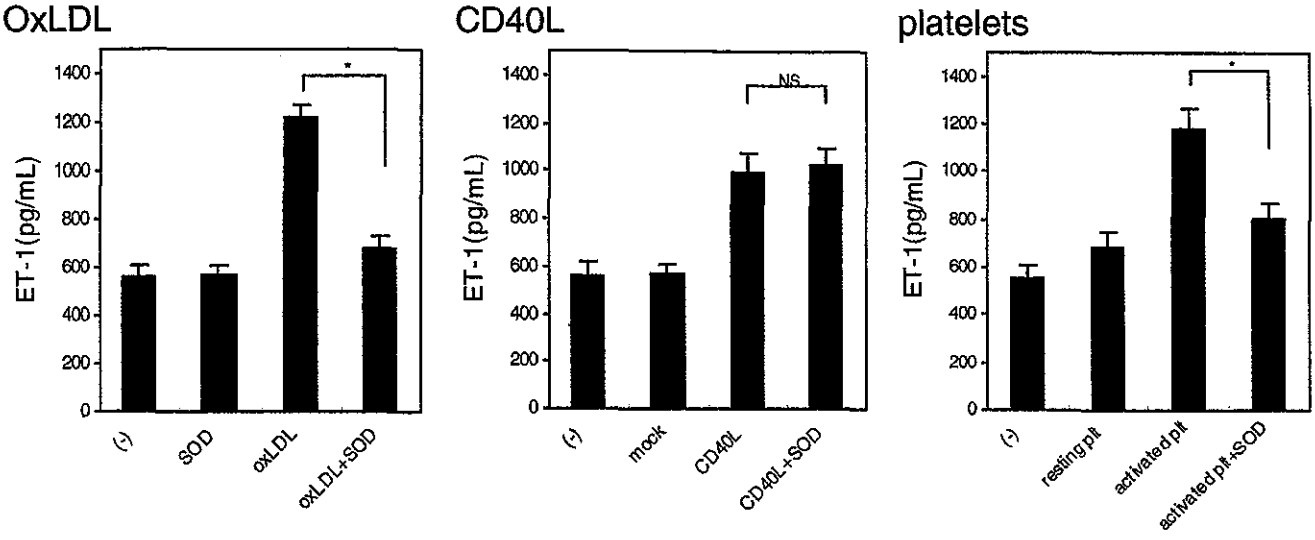

FIGURE 6. Sensitivity of endothelin-1 (ET-1) induction via lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and CD40 to superoxide dismutase. Bovine aortic endothelial cells were incubated with oxidatively modified low-density lipoprotein (OxLDL), CD40L-expressing cells, or platelets (plt) with or without superoxide dismutase (SOD; 50 U/mL). Released ET-1 level was determined by enzyme immunoassay. Results are expressed as means ± SEM ($n = 3$); *$P < 0.01$. NS, no significant difference.

study intended to reveal the close relationships between CD40 and the LOX-1 system. Although Li et al. have already reported that LOX-1-stimulation enhances CD40 expression in cultured human coronary endothelial cells,[20] this study further demonstrated that CD40 stimulation upregulates LOX-1 expression, and the CD40 system and LOX-1 system co-operate for the induction of platelet binding and ET-1 production. These results, in other words, may suggest that the two systems co-operatively might induce endothelial dysfunction.

The important point of the co-operative action between the LOX-1 and CD40 systems is that the expression of LOX-1 and CD40 is enhanced by the cross-regulation mechanism between these systems as well as by the auto-regulation mechanism. Activation of LOX-1 enhanced CD40 expression as well as LOX-1 expression, and vice versa. Under conditions where both OxLDL and CD40L stimulated endothelial cells, simultaneously increasing the expression of both LOX-1 and CD40 compared with either OxLDL or CD40L alone may accelerate endothelial dysfunction. Several reports have shown elevated levels of plasma OxLDL and soluble CD40L and up-regulation of LOX-1 and CD40, in endothelial cells under the hypercholesterolemic state,[7,21] suggesting that the mechanism revealed in the present study might occur in a particular pathological situation in vivo. Recent reports have demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) inhibit OxLDL-mediated LOX-1 and CD40 expression.[22,23] A cross-regulatory mechanism between the CD40 and LOX-1 systems might be involved in this kind of pleiotropic mechanism of statins. In addition, the binding of activated platelets, which work as the ligand for both CD40 and LOX-1, to endothelial cells induced LOX-1 and CD40 expression through cross-talk. Thrombus formation might be enhanced by CD40 and LOX-1, strengthening the platelet–endothelium interaction by physical co-operation at the endothelial surface and indirectly by enhancing their expression levels. It is known that hypercholesterolemia primes platelets for recruitment to atherosclerosis-prone sites, and platelets tether leukocytes.[24] The CD40 and LOX-1 systems might also be of importance in platelet-assisted leukocyte recruitment and the resultant inflammation, which are associated with hypercholesterolemia. Manipulation of the LOX-1 system, which would attenuate the CD40 and ET-1 pathways, might protect against inflammation, endothelial dysfunction and thrombus formation.

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REFERENCES

1. Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med.* 1999; 340:115–126.
2. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest.* 1991;88:1785–1792.
3. Sawamura T, Kume N, Aoyama T, et al. An endothelial receptor for oxidized low-density lipoprotein. *Nature.* 1997;386:73–77.
4. Chen M, Masaki T, Sawamura T. LOX-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: implications in endothelial dysfunction and atherosclerosis. *Pharmacol Ther.* 2002;95: 89–100.
5. Nagase M, Hirose S, Sawamura T, et al. Enhanced expression of endothelial oxidized low-density lipoprotein receptor (LOX-1) in hypertensive rats. *Biochem Biophys Res Commun.* 1997;237:496–498.
6. Chen M, Nagase M, Fujita T, et al. Diabetes enhances lectin-like oxidized LDL receptor-1 (LOX-1) expression in the vascular endothelium: possible role of LOX-1 ligand and AGE. *Biochem Biophys Res Commun.* 2001;287:962–968.
7. Chen M, Kakutani M, Minami M, et al. Increased expression of lectin-like oxidized low density lipoprotein receptor-1 in initial atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol.* 2000;20:1107–1115.
8. Chen H, Li D, Sawamura T, et al. Upregulation of LOX-1 expression in aorta of hypercholesterolemic rabbits: modulation by losartan. *Biochem Biophys Res Commun.* 2000;276:1100–1104.
9. Kataoka H, Kume N, Miyamoto S, et al. Expression of lectinlike oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation.* 1999;99:3110–3117.
10. Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. *Circulation.* 2000; 101:2889–2895.
11. Li D, Chen H, Romeo F, et al. Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: role of LOX-1. *J Pharmacol Exp Ther.* 2002; 302:601–605.
12. Cominacini L, Rigoni A, Pasini AF, et al. The binding of oxidized low density lipoprotein (ox-LDL) to ox-LDL receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells through an increased production of superoxide. *J Biol Chem.* 2001;276: 13750–13755.
13. Kakutani M, Masaki T, Sawamura T. A platelet-endothelium interaction mediated by lectin-like oxidized low-density lipoprotein receptor-1. *Proc Natl Acad Sci U.S.A.* 2000;97:360–364.
14. Cominacini L, Fratta Pasini A, Garbin U, et al. The platelet-endothelium interaction mediated by lectin-like oxidized low-density lipoprotein receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells. *J Am Coll Cardiol.* 2003;41:499–507.
15. Karmann K, Hughes CC, Schechner J, et al. CD40 on human endothelial cells: inducibility by cytokines and functional regulation of adhesion molecule expression. *Proc Natl Acad Sci U.S.A.* 1995;92: 4342–4346.
16. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature.* 1998; 391:591–594.
17. Kume N, Murase T, Moriwaki H, et al. Inducible expression of lectin-like oxidized LDL receptor-1 in vascular endothelial cells. *Circ Res.* 1998;83:322–327.
18. Honjo M, Nakamura K, Yamashiro K, et al. Lectin-like oxidized LDL receptor-1 is a cell-adhesion molecule involved in endotoxin-induced inflammation. *Proc Natl Acad Sci U.S.A.* 2003;100:1274–1279.
19. Nakagawa T, Akagi M, Hoshikawa H, et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates leukocyte infiltration and articular cartilage destruction in rat zymosan-induced arthritis. *Arthritis Rheum.* 2002;46:2486–2494.
20. Li D, Liu L, Chen H, et al. LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol.* 2003;23:816–821.
21. Cipollone F, Mezzetti A, Porreca E, et al. Association between enhanced soluble CD40L and prothrombotic state in hypercholesterolemia: effects of statin therapy. *Circulation.* 2002;106:399–402.
22. Li DY, Chen HJ, Mehta JL. Statins inhibit oxidized-LDL-mediated LOX-1 expression, uptake of oxidized-LDL and reduction in PKB phosphorylation. *Cardiovasc Res.* 2001;52:130–135.
23. Schonbeck U, Gerdes N, Varo N, et al. Oxidized low-density lipoprotein augments and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors limit CD40 and CD40L expression in human vascular cells. *Circulation.* 2002;106:2888–2893.
24. Theilmeier G, Michiels C, Spaepen E, et al. Endothelial von Willebrand factor recruits platelets to atherosclerosis-prone sites in response to hypercholesterolemia. *Blood.* 2002;99:4486–4493.

Leptin-to-Adiponectin Ratio as a Potential Atherogenic Index in Obese Type 2 Diabetic Patients

NORIKO SATOH, MD, PHD[1]
MITSUHIDE NARUSE, MD, PHD[1]
TAKESHI USUI, MD, PHD[1]
TETSUYA TAGAMI, MD, PHD[1]
TAKAYOSHI SUGANAMI, MD, PHD[2]
KAZUNORI YAMADA, MD, PHD[3]
HIDESHI KUZUYA, MD, PHD[3]
AKIRA SHIMATSU, MD, PHD[1]
YOSHIHIRO OGAWA, MD, PHD[2,4]

Obesity promotes the progression of atherosclerosis by inducing multiple cardiovascular and metabolic derangements such as diabetes, hypertension, and dyslipidemia, all of which have high atherogenic potential. Adipose tissue has been considered an important endocrine organ that secretes many biologically active substances, collectively known as adipocytokines (1). Two major adipocytokines, leptin and adiponectin, are thought to play important roles in the regulation of cardiovascular and metabolic homeostasis. Leptin acts directly on the hypothalamus, thereby regulating food intake and energy expenditure (2). Plasma leptin concentrations are significantly elevated in obese subjects in proportion to the degree of adiposity (3), suggesting that hyperleptinemia may play a role in the pathogenesis of obesity-related complications. On the other hand, adiponectin increases tissue fat oxidation, leading to reduced levels of fatty acids and tissue triglyceride content, thus increasing insulin sensitivity (4). Paradoxically, plasma adiponectin concentrations are decreased in obese subjects (5), suggesting that hypoadiponectinemia is involved in the pathophysiology of obesity. Two recent studies have demonstrated that vascular remodeling and neointimal formation are markedly attenuated in leptin-deficient *ob/ob* mice and *db/db* mice with leptin receptor mutation (6,7), suggesting that leptin may accelerate the development of vascular injury. Conversely, studies with adiponectin-deficient mice have revealed that adiponectin plays a protective role in the development of atherosclerosis (8,9). In obese type 2 diabetic patients who are susceptible to atherosclerosis, plasma concentrations of leptin are increased, whereas those of adiponectin are decreased. We, therefore, hypothesize that the leptin-to-adiponectin ratio serves as an atherogenic index superior to leptin or adiponectin alone. This study was designed to assess the potential of the leptin-to-adiponectin ratio as a biomarker for atherosclerosis in obese type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

A total of 158 Japanese type 2 diabetic patients (69 men and 89 women, mean age 59.8 years) were recruited in our outpatient clinics from April 2003 to May 2004 (Table 1). The study protocol was approved by the ethics committee on human research in Kyoto Medical Center, and all participants gave written informed consent. The patients had stable and relatively high blood glucose and HbA_{1c} levels ($7.0\% \leq HbA_{1c} \leq 9.0\%$). They were classified by BMI (nonobese BMI <25.0 and obese BMI ≥ 25.0 kg/m^2, according to the criteria of the Japan Society for the Study of Obesity) (10); 98 and 60 were defined as nonobese and obese subjects, respectively. There were 39 men and 59 women in the nonobese group and 30 men and 30 women in the obese group. None were receiving insulin, metformin, or thiazolidinediones. Fifty-one nonobese and 25 obese subjects had been treated with sulfonylureas, whereas 47 and 35, respectively, had only been treated with diet.

Fasting plasma glucose, HbA_{1c}, total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels were measured according to standard procedures. Plasma insulin concentration was measured by enzyme immunoassay using a commercially available kit (Tosoh, Tokyo, Japan). Insulin resistance index was assessed by homeostasis model assessment (11). Plasma concentrations of adiponectin and leptin were determined using the respective radioimmunoassay kits (Linco Research, St. Charles, MO). Systolic and diastolic blood pressures were measured twice with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Komaki, Japan). Pulse wave velocity (PWV) (12) was determined by an automated multiple pulse wave measurement (ABI-form model: BP-203RPE; Nippon Colin). In this study, PWV was calculated as the mean of the left and right brachial-ankle PWVs, as previously described (13).

Statistical analysis

All statistical analyses were performed using the StatView program version 5.0 for Windows (SAS Institute, Cary, NC). Data are presented as means ± SE, and $P < 0.05$ was considered statistically significant. Differences among the nonobese and obese subjects were assessed by a two-tailed Student's *t* test. Significance of

From the [1]Clinical Research Institute for Endocrine Metabolic Disease, Kyoto Medical Center, Fushimi-ku, Kyoto, Japan; the [2]Department of Molecular Medicine and Metabolism, Medical Research Institute, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo, Japan; the [3]Diabetes Center, National Hospital Organization, Kyoto Medical Center, Fushimi-ku, Kyoto, Japan; and the [4]Center of Excellence Program for Frontier Research on Molecular Destruction and Reconstitution of Tooth and Bone, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo, Japan.

Address correspondence and reprint requests to Yoshihiro Ogawa, MD, PhD, Department of Molecular Medicine and Metabolism, Medical Research Institute, Tokyo Medical and Dental University, 2-3-10 Kanda-surugadai, Chiyoda-ku, Tokyo 101-0062, Japan. E-mail: ogawa.mmm@mri.tmd.ac.jp.

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Abbreviations: PWV, pulse wave velocity.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Baseline characteristics of the study subjects and Spearman's rank correlation*

Variables	Nonobese (n = 98)		Obese (n = 60)		P values	
	Men	Women	Men	Women	Men	Women
n	39	59	30	30		
Age (years)	61.7 ± 1.41	60.9 ± 1.04	58.0 ± 2.38	57.7 ± 2.35	NS	NS
BMI (kg/m^2)	22.4 ± 0.27	21.3 ± 0.29	26.6 ± 0.26	27.4 ± 0.35	<0.0001	<0.0001
PWV (cm/s)	1667 ± 40	1700 ± 34	1628 ± 37	1634 ± 42	NS	NS
Leptin (ng/ml)	3.70 ± 0.25	5.83 ± 0.33	8.90 ± 0.35	11.9 ± 0.59	<0.0001	<0.0001
Adiponectin (mg/ml)	4.84 ± 0.25	8.25 ± 0.28	3.44 ± 0.16	5.09 ± 0.30	0.0003	<0.0001
Leptin/adiponectin	0.85 ± 0.08	0.79 ± 0.06	2.81 ± 0.17	2.59 ± 0.20	<0.0001	<0.0001
Spearman's rank correlation (P values)						
Leptin vs. PWV	−0.060 (NS)	0.053 (NS)	0.281 (NS)	0.315 (NS)		
Adiponectin vs. PWV	0.079 (NS)	0.090 (NS)	−0.309 (NS)	−0.122 (NS)		
Leptin/adiponectin vs. PWV	−0.061 (NS)	0.013 (NS)	0.378 (P = 0.0420)	0.390 (P = 0.0356)		

Data are means ± SE. NS, not significant. Two-tailed Student's t test was used.

correlations for leptin, adiponectin, or leptin-to-adiponectin ratio with PWV was assessed by Spearman's rank correlation analysis.

RESULTS— There were significant differences in BMI, plasma insulin concentration, and the homeostasis model assessment of insulin resistance between the nonobese and obese subjects (P < 0.0001), with no significant differences in systolic blood pressure, diastolic blood pressure, fasting plasma glucose, HbA_{1c}, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. In all of the study subjects, leptin, adiponectin, and leptin-to-adiponectin ratio showed no significant correlation with PWV (leptin: $\rho = -0.046$ and $P = 0.5676$, adiponectin: $\rho = 0.094$ and $P = 0.241$, leptin-to-adiponectin ratio: $\rho = -0.066$ and $P = 0.4086$). In the lean subjects (BMI <25 kg/m^2, n = 98), when divided into men and women, there were no significant correlations of leptin, adiponectin, and leptin-to-adiponectin ratio with PWV (leptin: $\rho = 0.062$ and $P = 0.5399$, adiponectin: $\rho = 0.112$ and $P = 0.2715$, leptin-to-adiponectin ratio: $\rho = -0.022$ and $P = 0.8297$). However, in the obese subjects (BMI ≥25 kg/m^2, n = 60), leptin-to-adiponectin ratio was positively correlated with PWV ($\rho = 0.308$ and $P = 0.0182$), whereas leptin or adiponectin alone had no correlation (leptin: $\rho = 0.255$ and $P = 0.0505$, adiponectin: $\rho = -0.130$ and $P = 0.3188$). In the male (n = 30) and female (n = 30) patients in the obese group, leptin-to-adiponectin ratios were positively correlated with PWV (male patients: $\rho = 0.378$ and $P = 0.0420$, female patients: $\rho = 0.390$ and $P = 0.0356$), whereas leptin or adiponectin alone had no correlation in the male and female patients.

CONCLUSIONS— This study represents the first demonstration that in obese type 2 diabetic patients, leptin-to-adiponectin ratio is more strongly correlated with PWV than leptin or adiponectin alone. Since atherosclerosis at necropsy correlates closely with arterial stiffness assessed noninvasively in human and animal studies (12), the data of this study suggest that leptin-to-adiponectin ratio may serve as a potential atherogenic index in obese type 2 diabetic patients. With no significant correlation between leptin-to-adiponectin ratio and PWV in the nonobese subjects, we speculate that the vascular injury seen in these nonobese subjects may be related more strongly with other mechanisms than dysregulated production of adipocytokines. Evidence has suggested that leptin and adiponectin, both of which occur in the adipose tissue, act directly on vascular cells as proatherogenic and antiatherogenic factors, respectively (14,15), implying that they are important mediators linking adiposity and atherosclerosis in the adipovascular axis (9). Collectively, these observations suggest that the leptin-to-adiponectin ratio may serve as a potential atherogenic index in obese type 2 diabetic patients.

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References

1. Matsuzawa Y, Funahashi T, Nakamura T: Molecular mechanism of metabolic syndrome X: contribution of adipocytokines, adipocyte-derived bioactive substances. *Ann N Y Acad Sci* 892:146–154, 1999
2. Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998
3. Considine RV, Subha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
4. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I: Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 24:29–33, 2004
5. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
6. Stephenson K, Tunstead J, Tsai A, Gordon

R, Henderson S, Dansky HM: Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice. *Arterioscler Thromb Vasc Biol* 23: 2027–2033, 2004
7. Schäfer K, Halle M, Goeschen C, Dellas C, Pynn M, Loskutoff DJ, Konstantinides S: Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Biol* 24:112–117, 2004
8. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Frougel P, Nagai R, Kimura S, Kadowaki T, Noda T: Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 277:25863–25866, 2002
9. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y: Role of adiponectin in preventing vascular stenosis: the missing link of adipo-vascular axis. *J Biol Chem* 277:37487–37491, 2002
10. The Examination Committee of Criteria for "Obesity Disease" in Japan: Japan Society for the Study of Obesity: new criteria for "obesity disease" in Japan. *Circ J* 66: 987–992, 2002
11. Haffner SM, Kennedy E, Gonzalez C, Stern MP, Miettinen H: A prospective analysis of the HOMA model: the Mexico City Diabetes Study. *Diabetes Care* 19: 1138–1141, 1996
12. Lehmann ED: Clinical value of aortic pulse-wave velocity measurement. *Lancet* 354:528–529, 1999
13. Satoh N, Ogawa Y, Usui T, Tagami T, Kohno S, Uesugi H, Sugiyama H, Sugawara A, Yamada K, Shimatsu A, Kuzuya H, Nakao K: Antiatherogenic effect of pioglitazone in type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. *Diabetes Care* 26: 2493–2499, 2003
14. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzman M, Brownlee M: Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem* 276:25096–25100, 2001
15. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 100: 2473–2476, 1999

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Adiponectin in Anorexia Nervosa and Bulimia Nervosa

TETSUYA TAGAMI, NORIKO SATOH, TAKESHI USUI, KAZUNORI YAMADA, AKIRA SHIMATSU, AND HIDESHI KUZUYA

Clinical Research Institute, Center for Endocrine and Metabolic Diseases, Kyoto National Hospital, Kyoto 612-8555, Japan

To study the role of adiponectin, a novel adipocyte-specific secreted protein, on the pathophysiology of eating disorders, circulating levels of fasting adiponectin, leptin, insulin, and glucose were measured in 31 female patients with anorexia nervosa (AN) and in 11 with bulimia nervosa. Hormone levels were compared with 16 age-matched, normal body weight controls, six healthy constitutionally thin subjects, and nine obese subjects. Moreover, changes in levels were reevaluated after nutritional treatment and weight gain in 13 patients with AN. Serum adiponectin concentrations in AN and bulimia nervosa were significantly lower than those in normal-weight controls. These results were unexpected because the levels were high in constitutionally thin subjects and low in obese subjects, which provide a negative correlation with body mass index (BMI) and body fat mass. In contrast, serum leptin levels correlated very well with BMI and fat mass among all the patients and controls. The insulin resistance was significantly low in AN and high in obese subjects. The concentrations of adiponectin after weight recovery increased to the normal level despite a relatively small increase in BMI. These findings suggest that abnormal feeding behavior in the patients with eating disorders may reduce circulating adiponectin level, and weight recovery can restore it. (*J Clin Endocrinol Metab* 89: 1833–1837, 2004)

ADIPONECTIN IS A new member of adipocytokines with structural resemblance to complement factor C1q, which regulates energy homeostasis and glucose and lipid metabolism. It is produced solely in adipocytes and secreted into serum (1). The plasma levels are decreased in obese humans (2), and low levels are associated with insulin resistance and hyperinsulinemia (3), in contrast to other secreted proteins from adipose tissue. Administration of this 30-kDa protein to obese or diabetic mice reduced circulating free fatty acid levels by enhanced skeletal muscle fat oxidation (4) and reduced glucose excursions and enhanced insulin sensitivity (5–7). Thiazolidinediones, currently used as insulin sensitizers in the treatment of patients with type 2 diabetes (8), have been shown to enhance the expression of adiponectin mRNA and plasma levels in human subjects (9, 10) and animal models of insulin resistance and type 2 diabetes (11–13). Because body weight reduction increased circulating levels of adiponectin in obese rats and humans (14, 15), it seems that adiponectin is negatively correlated with body fat mass unlike other adipocytokines. However, it is also true that adiponectin level is strongly reduced in patients with generalized lipodystrophy who exhibit marked adipose tissue depletion (16).

Leptin, another adipocytokine encoded by the *ob* gene, regulates food intake, body weight, energy expenditure and neuroendocrine function (17, 18). In humans, serum leptin levels are high in obese subjects (19) and are correlated positively with body fat mass (20). Leptin-deficient *ob/ob* mice and leptin receptor-deficient *db/db* mice exhibit severe insulin resistance, which is reversed in the *ob/ob* mice by leptin administration (21, 22). Leptin levels are low in mice that are severely insulin resistant due to lack of adipose tissue, and leptin treatment restored insulin sensitivity in these models (23, 24).

Anorexia nervosa (AN) is an eating disorder characterized by decreased caloric intake, low weight, and reduced body fat. AN is diagnosed by weight loss with refusal to maintain body weight, intense fear of gaining weight or becoming fat, self-evaluation unduly influenced by body shape and weight, and amenorrhea. Bulimia nervosa (BN) is a related disorder that is diagnosed by recurrent episodes of binge eating, recurrent inappropriate compensatory behavior to prevent weight gain, self-evaluation unduly influenced by body shape and weight. Multiple abnormalities of the neuroendocrine system are often observed in these patients (25), including activation of the hypothalamic-pituitary-adrenal axis and suppression of the thyroid and gonadal axes. In the GH-IGF-I axis, pituitary GH secretion is increased and liver IGF-I production is suppressed (26, 27). Recently identified ghrelin, an endogenous ligand for the GH secretagogue receptor, is also elevated in the patients with AN (28). In the present work, we assessed serum adiponectin level in the patients with AN before and after weight recovery and in the patients with BN, and compared with levels in control, obese, and constitutionally thin subjects. The relationship between serum level of leptin or adiponectin and insulin sensitivity was also investigated.

Subjects and Methods

Subjects

Five groups of women (19–48 yr old) participated in the study. Forty-two women were recruited from the Outpatient Program for eating disorders of Kyoto National Hospital. All these patients met the criteria of the Diagnostic and Statistical Manual of Mental Disorders (29), and 31 women were diagnosed with AN and 11 with BN. They are all clinically stable and took no medication known to affect body composition. Thirteen of the 31 subjects with AN who recovered weight [de-

Abbreviations: AN, Anorexia nervosa; BMI, body mass index; BN, bulimia nervosa; HOMA, homeostasis model assessment; HOMA-IR, HOMA for insulin resistance.

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fined as a 10% increase in body mass index (BMI)] were studied again at weight recovery. Subjects demonstrated good adherence to recommendations of their outpatient treatment programs. Blood sampling was performed at the initiation of this study and after weight recovery (time range of the refeeding period, 2–16months). Normal volunteers (31 age-matched healthy women) with no apparent medical illness and with regular menstrual cycles were recruited from the staff and the nurses of the Section of Endocrine and Metabolic Diseases of Kyoto National Hospital. They were divided into three groups according to their BMI: normal-weight controls, 18.0–25.0; constitutionally thin subjects, less than 18.0; and obese subjects, more than 25.0. All patients and subjects gave their informed consent for the study.

Methods

Blood samples were collected from each subject in the morning after an overnight fast, and serum and plasma were frozen until analysis. Serum adiponectin concentrations were measured using human adiponectin RIA kit (Linco Research, St. Charles, MO; intra- and interassay coefficients of variations, 1.8–6.2 and 6.9–9.3%, respectively; sensitivity, 1 ng/ml). Serum leptin concentrations were measured using human leptin RIA kit (Linco Research; intra- and interassay coefficients of variations, 3.4–8.3 and 3.0–6.2%, respectively; sensitivity, 0.5 ng/ml). Fasting plasma glucose and serum insulin were assessed by standard laboratory methods. Insulin resistance was estimated using the homeostasis model assessment (HOMA) value for insulin resistance (HOMA-IR) with the following formula: HOMA-IR = fasting glucose (mM) × fasting insulin (mU/ml)/22.5 (30). The body fat mass was (in percentage of total body weight) was estimated by bioelectrical impedance analysis using body fat analyzer TBF-110 (Tanita, Tokyo, Japan).

Statistical analysis

Data are expressed as the mean ± SD. Independent Student's *t* tests were used to compare age, BMI, body fat mass, leptin, adiponectin, glucose, insulin, and HOMA-IR between controls and other groups. Comparisons between the patients with AN at the two time points were performed using matched pair *t* tests. Correlations of leptin, adiponectin, and HOMA-IR with BMI and body fat mass were determined by using regression analyses.

Results

There were no significant differences in age among five groups in this study (Table 1). The BMI and body fat mass were significantly low in the patients with AN and in constitutionally thin subjects and high in obese subjects, compared with normal body weight controls. The mean serum leptin was significantly decreased in the patients with AN (2.2 ± 1.4 ng/ml; range, 0.9–6.1, $P < 0.0001$) and increased in obese subjects (15.1 ± 6.2 ng/ml; range, 9.4–27.8, $P < 0.05$), compared with normal-weight controls (8.6 ± 5.1 ng/ml; range, 2.2–22.5). The mean serum adiponectin was significantly decreased in the patients with AN (11.0 ± 7.8 μg/ml; range, 0.9–33.7, $P < 0.01$) and BN (11.5 ± 6.2 μg/ml; range, 4.8–21.3, $P < 0.05$) as well as in obese subjects (5.7 ± 2.0 μg/ml; range, 2.1–9.1, $P < 0.001$), compared with normal-weight controls (18.3 ± 9.8 μg/ml; range, 5.7–40.7). Fasting glucose and insulin were significantly low in the patients with AN, and fasting insulin was high in obese subjects, compared with normal-weight controls, such that HOMA-IR was lower in the patients with AN (1.0 ± 1.2, $P < 0.05$) and higher in obese subjects (6.1 ± 4.8, $P < 0.01$) than normal-weight controls (2.0 ± 1.0).

The relationships of leptin to BMI and body fat mass are shown in Fig. 1. Consistent with the previous reports (19–20, 31), serum leptin levels were high in obese subjects and low in anorectic patients and were correlated positively with BMI

TABLE 1. Characteristics of constitutionally thin, control, and obese subjects and patients with AN and BN

	Constitutionally thin subjects	Control subjects	Obese subjects	AN patients	BN patients
Age (yr)	27.5 ± 4.2 (6)	25.7 ± 2.9 (16)	27.0 ± 6.8 (9)	25.5 ± 8.1 (31)	23.5 ± 3.9 (11)
BMI (kg/m^2)	17.7 ± 0.5^e (6)	20.3 ± 1.5 (16)	30.3 ± 5.6^f (9)	14.0 ± 2.5^f (31)	20.5 ± 1.8 (11)
Body fat mass (% BW)	17.0 ± 2.6^e (6)	23.4 ± 2.7 (13)	42.2 ± 13.5^f (8)	7.1 ± 4.7^f (25)	19.2 ± 5.9^a (6)
Leptin (ng/ml)	4.5 ± 1.2 (6)	8.6 ± 5.1 (16)	15.1 ± 6.2^a (9)	2.2 ± 1.4^f (31)	7.6 ± 5.6 (11)
Adiponectin (μg/ml)	20.1 ± 7.6 (6)	18.3 ± 9.8 (16)	5.7 ± 2.0^d (9)	11.0 ± 7.8^b (31)	11.5 ± 6.2^a (11)
Fasting glucose (mM)	4.94 ± 0.62 (4)	4.97 ± 0.37 (13)	5.35 ± 0.80 (8)	4.18 ± 0.67^e (31)	4.50 ± 0.69 (11)
Fasting insulin (μU/ml)	9.1 ± 4.4 (4)	9.2 ± 5.4 (13)	25.7 ± 19.5^b (8)	5.0 ± 5.6^a (31)	13.6 ± 10.0 (11)
HOMA-IR	2.1 ± 1.2 (4)	2.0 ± 1.0 (13)	6.1 ± 4.8^b (8)	1.0 ± 1.2^a (31)	2.6 ± 1.4 (11)

The data are expressed as mean ± SD, and the number of subjects is indicated in *parentheses*. BW, Body weight. Statistically different from controls: $^a P < 0.05$; $^b P < 0.01$; $^c P < 0.005$; $^d P < 0.001$; $^e P < 0.0005$; $^f P < 0.0001$.

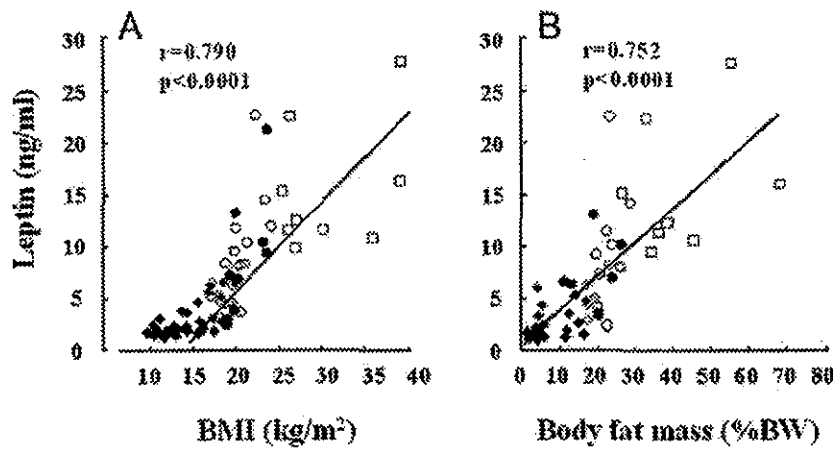

FIG. 1. Relationships between circulating leptin levels and BMI (A) or body fat mass (B) in patients with AN (◆) and BN (●) and constitutionally thin (◇), normal body weight control (○), and obese subjects (□). Correlation analysis was performed in the entire population.

(r = 0.790, $P < 0.0001$) and body fat mass (r = 0.752, $P < 0.0001$) for the entire group taken together. The serum leptin levels were also correlated positively with BMI for the AN group (r = 0.613, $P = 0.0002$) and the BN group (r = 0.714, $P = 0.0136$). In contrast, although an exponential negative correlation was observed between serum adiponectin level and BMI (r = −0.604, $P < 0.001$) or adiponectin and body fat mass (r = −0.581, $P = 0.002$) for three control groups considered as a whole, these levels were not elevated in the patients with AN and BN, despite the fact that their BMI and fat mass were relatively low (Fig. 2). The relationships of insulin resistance index to BMI and body fat mass are shown in Fig. 3. The HOMA-IR was correlated very well with BMI (r = 0.722, $P < 0.0001$) and fat mass (r = 0.653, $P < 0.0001$) for the entire group. The HOMA-IR was also correlated positively with BMI for the AN group (r = 0.524, $P = 0.0025$).

Thirteen of the 31 patients with AN who recovered weight after nutritional treatment were studied again at weight recovery. There were significant increases in the concentrations of leptin (2.2 ± 1.4 to 4.1 ± 3.5 ng/ml; $P < 0.05$) and adiponectin (10.9 ± 8.5 to 19.9 ± 9.3 μg/ml; $P < 0.005$) (Table 2). Fasting glucose, insulin, and HOMA-IR were significantly increased after weight gain. The relationships of adiponectin to BMI and body fat mass before and after weight gain are shown in Fig. 4. The adiponectin levels of the patients with AN increased associated with their weight recovery.

Discussion

The adiponectin levels were significantly low in the patients with AN and BN, compared with normal-weight control subjects, and hypoadiponectinemia was reversed by weight recovery in the patients with AN. These results were surprising because circulating adiponectin level is reported to be down-regulated in obesity (2), and weight reduction increases this adipocytokine in obese rats and humans (14, 15), suggesting that adiponectin may correlate negatively with BMI. Indeed, we have shown that adiponectin levels are negatively correlated with BMI and body fat mass among normal body weight controls and obese and constitutionally thin subjects in this study. Among the majority of secreted proteins from adipose tissue such as leptin, TNFα, resistin,

TABLE 2. The effects of nutritional treatment on parameters of patients with AN (n = 13)

	Before nutrition	After nutrition	*P*
BMI (kg/m²)	13.8 ± 2.4	15.9 ± 2.8	<0.0001
Body fat mass (% BW)	7.6 ± 5.1	13.2 ± 7.0	0.0014
Leptin (ng/ml)	2.2 ± 1.4	4.1 ± 3.5	0.0483
Adiponectin (μg/ml)	10.9 ± 8.5	19.9 ± 9.3	0.0014
Fasting glucose (mM)	3.97 ± 0.67	4.48 ± 0.77	0.0003
Fasting insulin (μU/ml)	4.9 ± 4.3	10.4 ± 8.2	0.0114
HOMA-IR	0.9 ± 0.9	2.2 ± 2.0	0.0117

BW, Body weight.

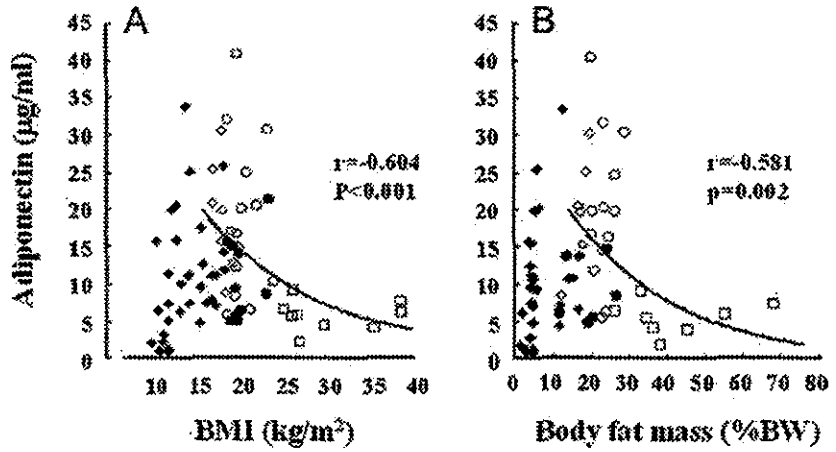

FIG. 2. Relationships between circulating adiponectin levels and BMI (A) or body fat mass (B) in patients with AN (♦) and BN (●) and constitutionally thin (◇), normal body weight control (○), and obese subjects (□). Correlation analysis was performed in the control population (constitutionally thin, normal body weight control, and obese subjects).

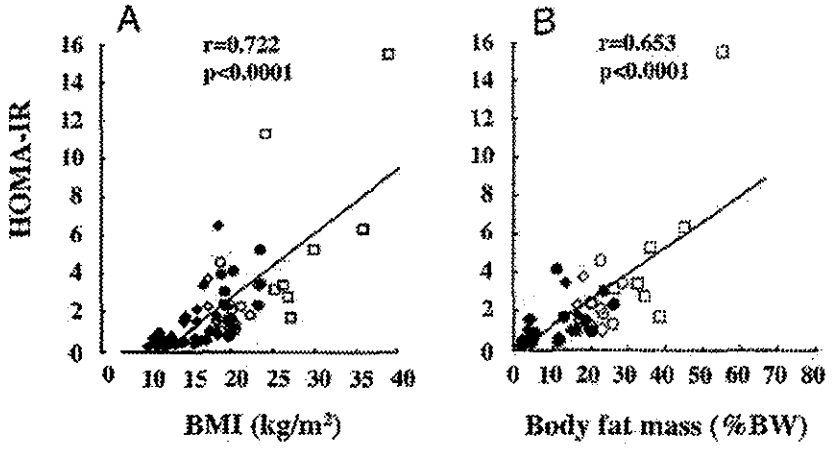

FIG. 3. Relationships between HOMA-IR and BMI (A) or body fat mass (B) in patients with AN (♦) and BN (●) and constitutionally thin (◇), normal body weight control (○), and obese subjects (□). Correlation analysis was performed in the entire population.

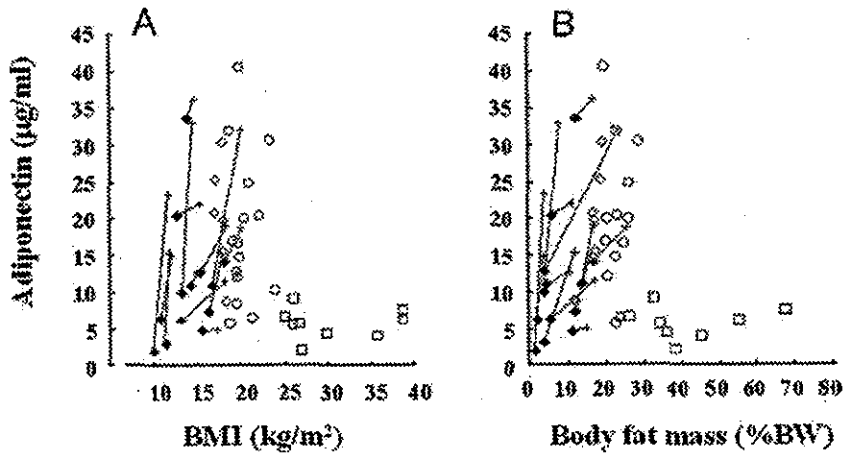

FIG. 4. Relationships between circulating adiponectin levels and BMI (A) or body fat mass (B) in patients with AN before (♦) and after (×) nutritional treatment and constitutionally thin (◇), normal body weight control (○), and obese subjects (□). The points were connected for individual patients with AN at baseline and at weight recovery.

IL-6, which are elevated in obesity and in general proportional to overall adipose mass (32), it is still unclear why only the adiponectin level is decreased in obesity. However, in patients with lipodystrophies who have variable loss of body fat, adiponectin levels are reported to be quite low (16). Although the assay system they used (ELISA) is different from the current study (RIA) and age and sex compositions are heterogeneous in their study, the values were 1.5 μg/ml (range, 0.4–7.5) for congenital generalized type, 3.2 μg/ml (range, 0.6–7.7) for acquired generalized type, 6.4 μg/ml (range, 1.9–23.2) for familial partial type, and 7.9 μg/ml (range, 3.1–13.3) for acquired partial type.

Together with our current results, these studies suggest that there might be an optimal fat mass for adiponectin secretion. Here it seems that standard volume of body fat mass gives highest circulating level of adiponectin, as indicated by Figs. 2B and 4B. Although the mechanisms involved in adiponectin regulation have not yet been elucidated, there might be other causes in the reduction of this adipocytokine in eating disorders. Because prolonged administration of adiponectin reduced body weight (4) and increased thermogenesis (5) in mice, hypoadiponectinemia in the patients with AN might be normal physiologic response to starvation, similar to the suppression of thyroid and gonadal axes (25) with the fact of reduced basal metabolic rate in the patients with AN (31, 33). Alternatively, abnormal feeding behavior may affect some intrinsic factors because adiponectin levels were equally low in the patients with AN and BN, regardless of their BMI. For instance, the factor(s) might be up-regulated in these disorders, and it may directly down-regulate the adiponectin. The circulating concentration of TNFα is reported to be increased in untreated patients with AN (34). Because this proinflammatory cytokine is increased in obesity (35) as mentioned above, the paradoxical increase of TNFα in the patients with AN may contribute to the reduction in their adiponectin level. Supporting this hypothesis, TNFα reduced the expression of adiponectin in adipocytes by suppressing its promoter activity (9). However, the elevated TNFα concentration did not decrease at weight recovery of the patients with AN (34). This is rather surprising because the majority of neuroendocrine disturbances are restored by weight recovery of the patients with AN (25).

Hypoadiponectinemia in obesity and type 2 diabetes is closely associated with insulin resistance and hyperinsulinemia (3). In the case of lipodystrophies, hypoadiponectinemia is strongly associated with severe insulin resistance (16). In addition, administration of adiponectin reversed insulin resistance in animal models of obesity and lipoatrophy (5, 6). Synthetic peroxisome proliferator-activated receptor-γ ligands, thiazolidinediones, reversed insulin resistance of glucose-intolerant, or type 2 diabetes accompanied with increased circulating adiponectin levels (9, 10, 15). Thus, adiponectin has been thought to closely correlate with insulin sensitivity. In this study, however, this concept does not appear to apply to the case of eating disorders. In the patients with AN, both insulin resistance indices and adiponectin levels were significantly reduced, compared with normal-weight controls and both increased associated with weight recovery. For instance, in an AN patient with the most reduced BMI of 9.9 kg/m^2 and 1.6% body weight of fat mass who is still physically stable and has no infective illness, the levels of adiponectin, leptin, and HOMA-IR were quite low (1.9 μg/ml, 1.3 ng/ml, and 0.2, respectively). In contrast, insulin resistance indices in the patients with BN were not different from the controls despite the fact that they had hypoadiponectinemia. It is likely that insulin resistance is determined not only by adiponectin but also by other cytokines such as leptin, TNFα, and resistin, among others (32).

Very recently other studies examining adiponectin levels in the patients with AN have been published (36, 37). In contrast to our findings, hyperadiponectinemia was observed in the patients with AN. Although it is not clear why opposite results were obtained, there might be several possibilities. First, the AN population they recruited may be somehow heterogeneous because the patients were medically stable and had no concurrent medical illness (36) or weight-stable for at least 3 months before the study as assessed by clinical report (37), suggesting that they were not always untreated. This might be important because, in our study, adiponectin levels were restored by weight recovery, even with little increase of BMI. Second, we studied in relatively large number of patients and values were distributed over a wide range (0.9–33.7 μg/ml). Because there is a tendency that constitutionally thin subjects have higher adiponectin levels reflecting their lower BMI, feeding behavior may determine the degree of reduction of this adipocytokine

as they otherwise might have had hyperadiponectinemia which is regulated by BMI.

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Address all correspondence and requests for reprints to: Tetsuya Tagami, M.D., Ph.D., Clinical Research Institute, Center for Endocrine and Metabolic Diseases, Kyoto National Hospital, 1-1 Mukaihata-cho, Fukakusa, Fushimi-ku, Kyoto, 612-8555, Japan. E-mail: ttagami@kyotolan.hosp.go.jp.

References

1. **Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF** 1995 A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270:26746–26749
2. **Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y** 1999 Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79–83
3. **Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA** 2001 Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86:1930–1935
4. **Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MRS, Yen FT, Bihain BE, Lodish HF** 2001 Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 98:2005–2010
5. **Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Frogual P, Kadowaki T** 2001 The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941–946
6. **Berg AH, Combs TP, Du XL, Brownlee M, Scherer PE** 2001 The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 7:947–953
7. **Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L** 2001 Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 108:1875–1881
8. **Goldstein BJ** 1999 Current views on the mechanism of action of thiazolidinedione insulin sensitizers. Diabetes Technol Ther 1:267–275
9. **Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y** 2001 PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 50:2094–2099
10. **Satoh N, Ogawa Y, Usui T, Tagami T, Kohno S, Uesugi H, Sugiyama H, Sugawara A, Yamada K, Shimatsu A, Kuzuya H, Nakao K** 2003 Antiatherogenic effect of pioglitazone in type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. Diabetes Care 26:2493–2499
11. **Moore GB, Chapman H, Holder JC, Lister CA, Piercy V, Smith SA, Clapham JC** 2001 Differential regulation of adipocytokine mRNAs by rosiglitazone in db/db mice. Biochem Biophys Res Commun 286:735–741
12. **Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T** 2001 The mechanisms by which both heterozygous peroxisome proliferator-activated receptor γ (PPARγ) deficiency and PPARγ agonist improve insulin resistance. J Biol Chem 276:41245–41254
13. **Combs TP, Wagner JA, Berger J, Doebber T, Wang WJ, Zhang BB, Tanen M, Berg AH, O'Rahilly S, Savage DB, Chatterjee K, Weiss S, Larson PJ, Gottesdiener KM, Gertz BJ, Charron MJ, Scherer PE, Moller DE** 2002 Induction of adipocyte complement-related protein of 30 kilodaltons by PPARγ agonists: a potential mechanism of insulin sensitization. Endocrinology 143:998–1007
14. **Milan G, Granzotto M, Scarda A, Calcagno A, Pagano C, Federspil G, Vettor R** 2002 Regional adipose tissue differences of resistin and adiponectin expression in genetically obese rats: effect of weight loss. Obese Res 10:1095–1103
15. **Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM** 2001 Weight reduction increases plasma levels of adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 86:3815–3819
16. **Haque WA, Shimomura I, Matsuzawa Y, Garg A** 2002 Serum adiponectin and leptin levels in patients with lipodystrophies. J Clin Endocrinol Metab 87:2395–2398
17. **Friedman JM, Halaas JL** 1998 Leptin and the regulation of body weight in mammals. Nature 395:763–770
18. **Flier JS** 1998 What's in a name? In search of leptin's physiologic role. J Clin Endocrinol Metab 83:1407–1413
19. **Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al** 1996 Serum immunoreactive-leptin concentrations in normal weight and obese humans. N Engl J Med 334:292–295
20. **Maffei M, Halaas JL, Ravussin E Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S** 1995 Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155–1161
21. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowith D, Lallone RL, Burley SK, Friedman JM** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546
22. **Campfield LA, Smith FJ, Quisez Y, Devos R, Burn P** 1996 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546–549
23. **Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein LJ** 1999 Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature 401:73–76
24. **Gavrilova O, Marcus-Samuels B, Leon LR, Vinson C, Reitman ML** 2000 Leptin and diabetes in lipoatrophic mice. Nature 403:850
25. **Rubin RT, Kaye WH** 2001 Anorexia nervosa and other eating disorders. In: DeGroot LJ, Jameson JL, Burger HG, Loriaux DL, Marshall JC, Melmed S, Odell WD, Potts Jr JT, Rubenstein AH, eds. Endocrinology. 4th ed. New York: WB Saunders; 631–641
26. **Counts DR, Gwirtsman H, Carlsson LMS, Lesem M, Cutler Jr GB** 1992 The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs) and the IGF-binding proteins. J Clin Endocrinol Metab 75:762–767
27. **LeRoith D** 1997 Insulin-like growth factors. N Engl J Med 336:533–640
28. **Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyoda K, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K** 2001 Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity in humans. J Clin Endocrinol Metab 86:4753–4758
29. **American Psychiatric Association** 1994 Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Press
30. **Mathews DR, Hosher JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
31. **Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, Ma Z, Vignati L, Bowsher R, Herzog D, Klibanski A** 1996 Serum leptin levels in women with anorexia nervosa. J Clin Endocrinol Metab 81:3861–3863
32. **Steppan CM, Lazar MA** 2002 Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 13:18–23
33. **Mantzoros C, Flier JS, Lesem MD, Brewerton TD, Jimerson DC** 1997 Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. J Clin Endocrinol Metab 82:1845–1851
34. **Nakai Y, Hamagaki S, Takagi R, Taniguchi A, Kurimoto F** 1999 Plasma concentrations of tumor necrosis factor-α (TNF-α) and soluble TNF receptors in patients with anorexia nervosa. J Clin Endocrinol Metab 84:1226–1228
35. **Moller DE** 2000 Potential role of TNF-α in the pathogenesis of insulin resistance and type 2 diabetes. Trends Endocrinol Metab 11:212–217
36. **Delporte ML, Brichard SM, Hermans MP, Beguin C, Lambert M** 2003 Hyperadiponectinemia in anorexia nervosa. Clin Endocrinol (Oxf) 58:22–29
37. **Pannacciulli N, Vetter R, Milan G, Granzotto M, Catucci A, Federspil G, Giacomo PD, Giorgino R, Pergola GD** 2003 Anorexia nervosa is characterized by increased adiponectin plasma levels and reduced nonoxidative glucose metabolism. J Clin Endocrinol Metab 88:1748–1752

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レプチン抵抗性の発現機序とレプチンの臨床応用

佐藤　哲子[*1]　小川　佳宏[*2]

はじめに

肥満は，環境因子と遺伝素因の複雑な相互作用により発症する代表的な多因子疾患であり，従来，分子レベルのアプローチが困難であった．1994年末に遺伝性肥満 *ob/ob* マウスの病因遺伝子として肥満遺伝子産物レプチンが同定されて以来，肥満研究は大きく進歩してきた．レプチンは，主に視床下部を介して強力な摂食抑制およびエネルギー消費亢進をもたらす代表的な脂肪細胞由来ホルモンであり，肥満や体重増加の制御に関与する．このため，新しい抗肥満薬としてのレプチンの可能性が注目されている．しかし，多くの肥満患者では血中のレプチン濃度が上昇しているにも関わらず，肥満が是正されない状態，いわゆる「レプチン抵抗性」を呈していることが問題となってきた．現在，世界中でレプチンの作用経路を明らかにしレプチン抵抗性の発症メカニズムを解明するための研究が進められている．本稿では，肥満におけるレプチン抵抗性の発症メカニズムとして考えられる現在の仮説と抗肥満薬としてのレプチンの臨床応用の現況と展望について概説する．

[*1] 国立病院機構京都医療センター臨床研究センター代謝研究部　臨床代謝栄養室長
[*2] 東京医科歯科大学難治疾患研究所分子代謝医学分野
Noriko Satoh and Yoshihiro Ogawa : Mechanism of leptin resistance and clinical application of leptin.
[*1] Kyoto Medical Center, Clinical Research Institute for Endocrine & Metabolic Disease.
[*2] Department of Molecular Medicine and Metabolism, Medical Research Institute, Tokyo Medical and Dental University.

1 レプチン・レプチン受容体系

1）レプチン

レプチンは1994年，高度な肥満を呈する2型糖尿病モデルマウスである *ob/ob* マウスよりFriedmanらによりポジショナルクローニングされた遺伝子である[1]．ヒトのレプチン遺伝子は，N末端部に21アミノ酸からなるシグナルペプチドを有する167アミノ酸のレプチン前駆体蛋白質をコードし，循環血液中にはシグナルペプチドが除去された，146アミノ酸からなるレプチンとして存在している．

2）肥満におけるレプチン遺伝子発現

レプチン遺伝子発現は，マウス，ラット，ヒトのいずれにおいても脂肪組織に特異的に認められる．種々の肥満モデル動物あるいはヒト肥満において著しく亢進している[2]．レプチンの血中濃度も *ob/ob* マウスやレプチン遺伝子変異により発症する肥満症患者以外の肥満では上昇しており，体格指数（BMI）や体脂肪率と良好な正相関を示すことが知られている（**図1**）[3]．したがって，レプチンは体脂肪量や全身の栄養状態を反映する診断薬として使用されている．以上より，肥満者では，血中レプチン濃度が上昇するにもかかわらず肥満の程度が改善しないため，レプチンの作用障害，すなわち「レプチン抵抗性」があると想定されている．一方，「高レプチン血症」によるレプ

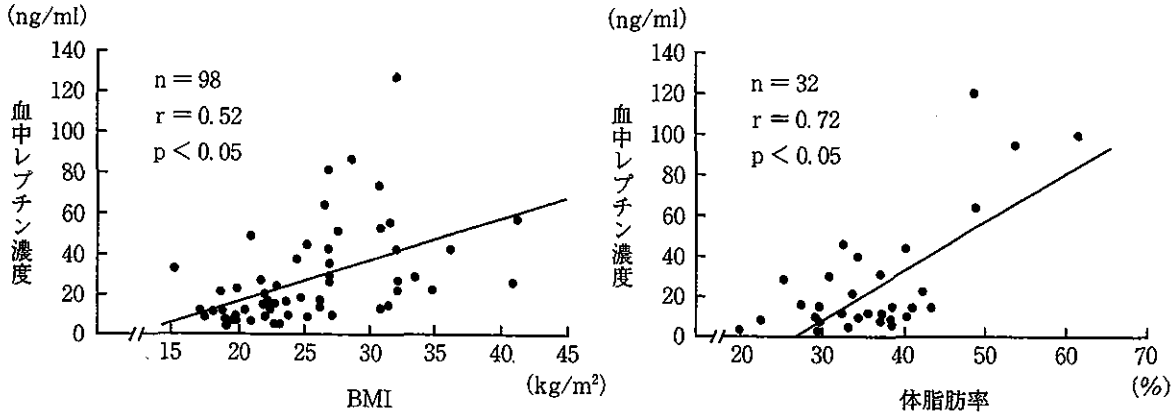

図1　血中レプチン濃度とBMI（body mass index）および体脂肪率との相関[3]
n＝個体数，r＝相関指数

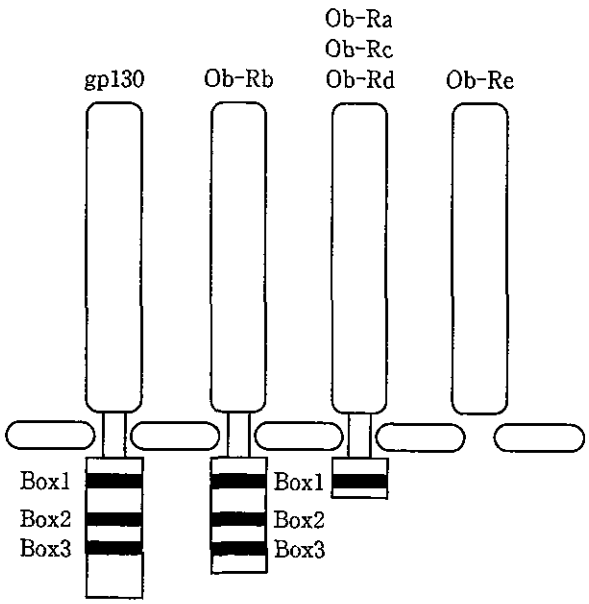

図2　レプチン受容体（Ob-R）アイソフォーム（Ob-Ra～e）の構造[5]

チンの過剰な作用が，種々の肥満の合併症の発症に関与する可能性がある．

（1）レプチン受容体

レプチン受容体（Ob-R）はIL-6，G-CSF，LIFなどのサイトカイン受容体とヘテロダイマーを形成する信号伝達遺伝子であるgp130と相同性を有する，膜一回貫通型のⅠ型サイトカイン受容体である．レプチン受容体には選択的スプライシングによって生じる数種類のアイソフォームが存在する（**図2**）[4]．このうち細胞内ドメインが最も長いアイソフォームであるOb-Rbは1,162アミノ酸からなり，細胞内ドメインには，細胞内シグナル伝達に関与するJanus activated kinases（JAKs）と転写因子であるsignal transducers and activators of transcription（STATs）の結合に関与するBox1，Box2およびBox3のモチーフが存在する．Ob-Ra，Ob-Rc，Ob-Rdの細胞内ドメインにはBox1モチーフのみが存在する．Ob-Reには膜貫通領域が欠如しており，分泌型のアイソフォームと考えられている．

*db/db*マウスは，Ob-Rbのみを選択的に欠如する動物であることが明らかとなり，各種アイソフォームの中でも，Ob-Rbが重要な役割を果たすことが示された[4]．また，Ob-Rbは視床下部に高濃度に発現しており，レプチンの末梢投与により視床下部のみでSTAT3のチロシン残基のリン酸化（活性化）が認められることから，レプチン作用のシグナル伝達には，視床下部のOb-Rbが重要であると考えられている[5]．

（2）レプチンの生理作用とそのメカニズム—視床下部ニューロネットワークへの調節

レプチンは，視床下部を介して摂食抑制作用のみならず，エネルギー消費亢進作用（運動量の増加，体温上昇，交感神経活動亢進，酸素消費量の増加）を発揮して体重を調節することが明らかに

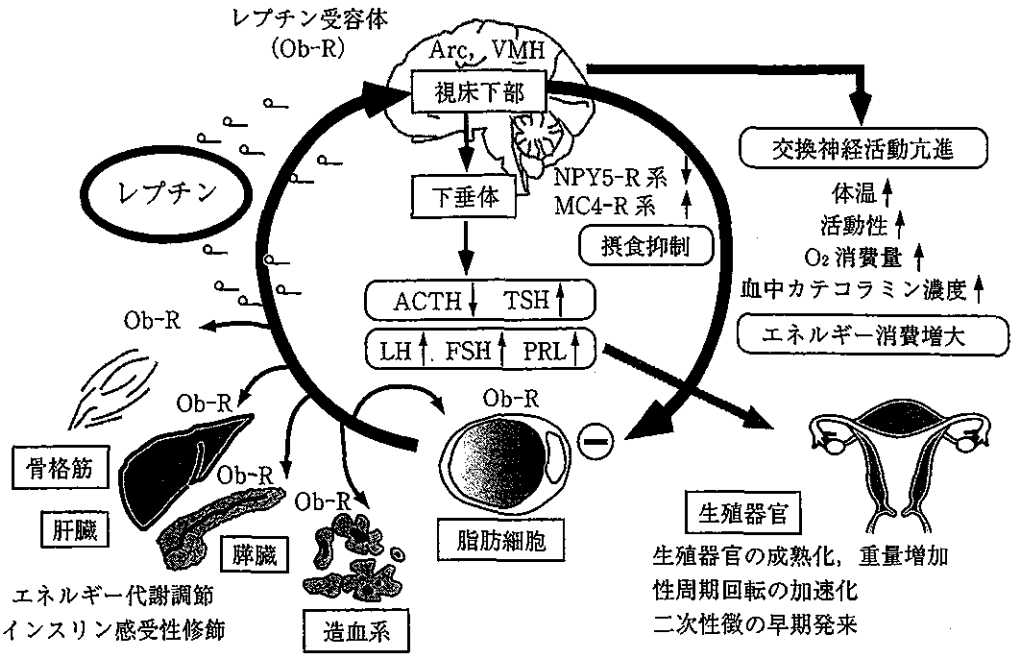

図3 レプチンの多彩な生理作用

なった（図3）．我々は，ラットの側脳室内や視床下部各神経核にレプチンを直接投与することにより，レプチンの作用部位を検討した．レプチンの摂食抑制作用および体重減少作用は，弓状核（Arc）＞腹内側核（VMH）＞外側核（LH）の順に直接作用が発揮された[6]．一方，レプチンによるカテコラミン上昇や褐色脂肪細胞におけるUCP-1の発現亢進作用は，Arcに直接投与しても発揮されず，VMHに直接投与したときに発揮された[7]．またVMH破壊ラットでは，レプチンによる上記の交感神経活動亢進作用が認められなかったことより，レプチンの摂食抑制作用は主にArcを，交感神経活動亢進作用は主にVMHを介している可能性が示唆された[7]．Arcには食行動を調節する2つのニューロン系，つまり摂食亢進系でありレプチンにより負に調節されるneuropeptide Y（NPY）/agouti related protein（AGRP）ニューロンと，摂食抑制系でありレプチンにより正に調節されるproopiomelanocortin（POMC）/cocaine- and amphetamine-regulated transcript（CART）ニューロンが存在する．我々は，レプチンの作用が視床下部のメラノコルチン系を介することをαMSHの拮抗薬SHU9119を用いて証明した[8]．最近，ArcにおけるNPYニューロンやPOMCニューロンへのシナプス支配に関するレプチンの制御についても報告されている[9]．

3）レプチン過剰発現トランスジェニックマウスより得られた知見

生体におけるレプチンの持続作用を明らかにするために，我々の研究室では，肝臓においてレプチンを過剰発現させることにより血中レプチン濃度が高度肥満者と同程度に上昇する，レプチン過剰発現トランスジェニックスマウス（LepTgマウス）を作製した[10]．LepTgマウの摂食量と体重は，対照マウスの約70％に減少し，全身の脂肪組織を消失するほどの著しい痩せが認められた．一方，このマウスでは正常な血糖値を示すものの，対照マウスと比較して明らかな低インスリン血症が認められ，糖負荷試験あるいはインスリン負荷試験により糖代謝とインスリン感受性の亢進が証明された[11]．さらに，骨格筋および肝臓におけるインスリン受容体シグナリングの亢進が認められた[11]．以上より，抗肥満薬あるいは抗糖尿病薬としてのレプチンの臨床応用が示唆された．

4）レプチンおよびレプチン受容体遺伝子異常症

ヒトのレプチン遺伝子は第七染色体長腕(7q31.3)に約20kbにわたって存在し，3個のエクソンと2個のイントロンから構成される．1997年にパキスタンのパンジャブ地方の肥満家系において，レプチン遺伝子にフレームシフトを生じる1塩基欠失が認められ，正常のレプチンが産出されない症例が見出された[12]．その後，トルコの1家系においてレプチン遺伝子変異により肥満と視床下部性性腺機能低下症を来す家系も報告されている[13]．以上より，レプチン遺伝子はヒト肥満の病因遺伝子になることが証明された．レプチン受容体遺伝子異常症については北アフリカのカビル人の1家系が報告されている[14]．確かに高度肥満者の中にはレプチン遺伝子，あるいはレプチン受容体遺伝子異常症が存在することは明らかとなったが，ヒトの肥満症例においてレプチンあるいはレプチン受容体そのものの異常が原因である割合はきわめて低く，多くの場合，主要な原因はレプチンに対する反応性の低下，すなわち‘レプチン抵抗性’が関与していると考えられる．

2 レプチン抵抗性の発症メカニズム

末梢の脂肪組織より生合成されるレプチンは循環血液中に分泌され，血液脳関門を通過して視床下部に到達し，視床下部のレプチン受容体に結合することにより，細胞内情報伝達物質を介してPOMCやNPYをはじめとする摂食・エネルギー代謝調節因子を制御し，抗肥満作用を発揮すると考えられている（図4）．したがって，レプチン抵抗性の原因としては，①レプチンの血液脳関門を介した脳脊髄液への移行性，②レプチン受容体以降の細胞内情報伝達系，③レプチン受容体発現ニューロン以降の中枢経路，以上の3点が考えられる．ここに現在考えられているレプチン抵抗性の発症メカニズムについての仮説を紹介する．

1）脳脊髄液移行性

ヒトにおいて脳脊髄液中のレプチン濃度は，レプチン血中濃度と同様に，BMIや体脂肪率と良好な正の相関を示すことが知られている．しかし，血中および脳脊髄液中のレプチン濃度を同時に測定した場合，脳脊髄液中のレプチン濃度は血中レプチン濃度と正の相関を示すものの，脳脊髄液血中レプチン比は血中レプチン濃度の上昇に従い低

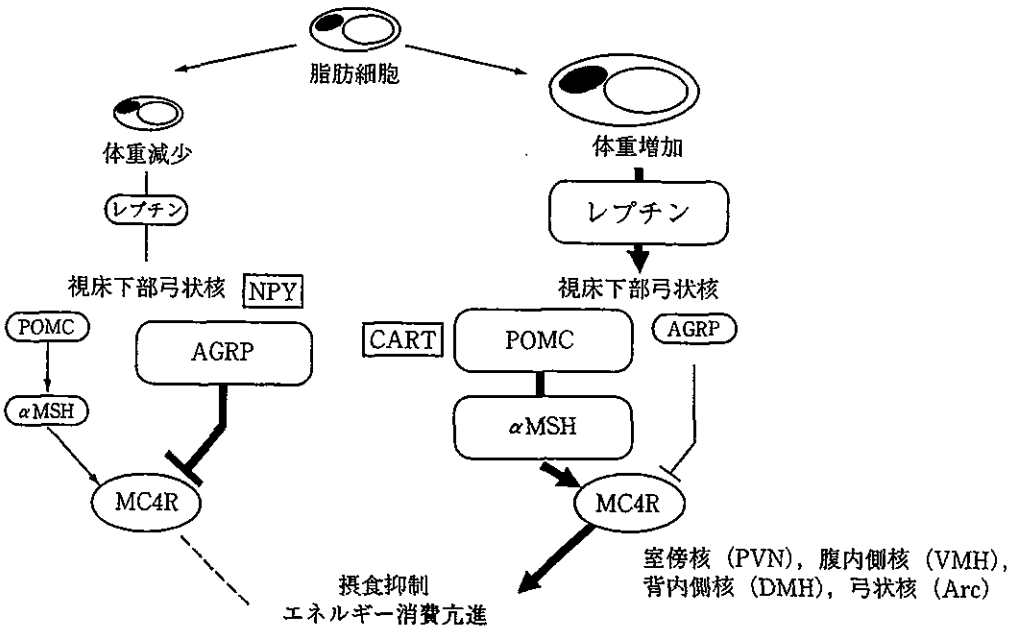

図4　レプチンによる中枢性エネルギー代謝調節機構

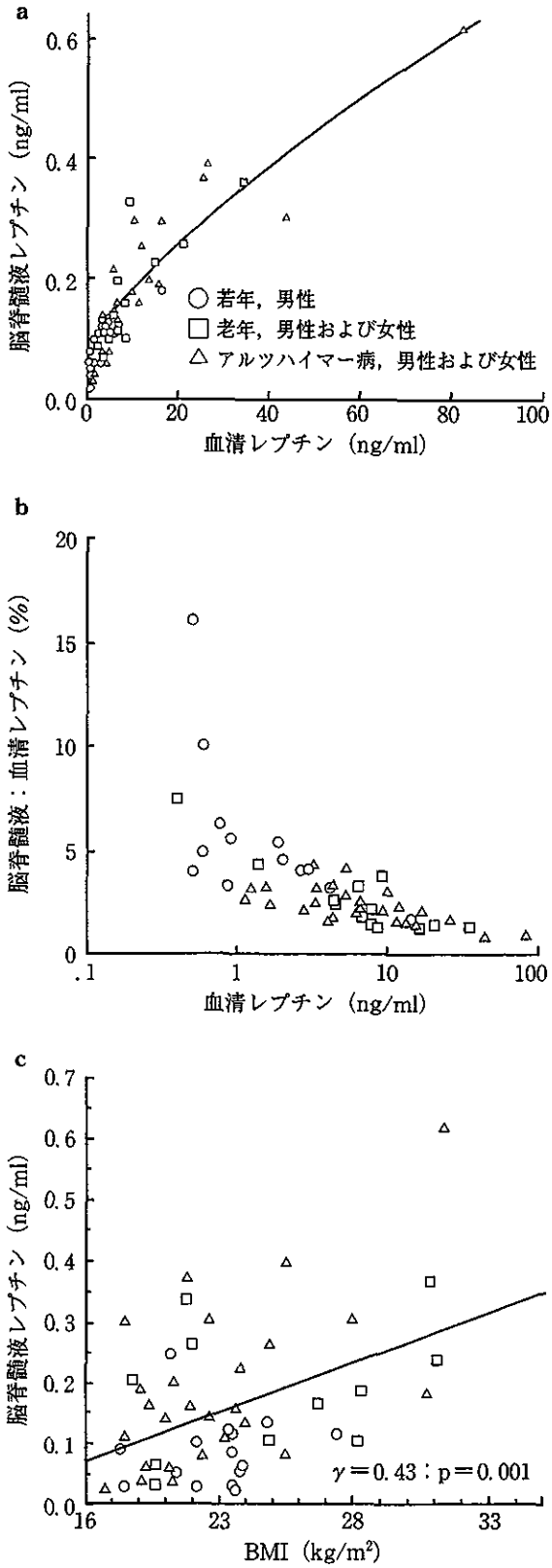

図5 脳脊髄液レプチン濃度と血清レプチン濃度の相関（a），脳脊髄液：血清レプチン濃度比と血清レプチン濃度の相関（b），脳脊髄液レプチン濃度とBMIの相関（c）（γ：相関指数）[15]

下することが知られている（図5）．更に脳脊髄液血中レプチン比はBMIの増加に従って低下し，正常体重者と比較して肥満者では脳脊髄液血中レプチン比が約1/5に低下しているとの報告がある[15]．これらの成績は，高レプチン血症を伴う肥満者においてレプチンの脳脊髄液移行性が低下していることを意味し，レプチン抵抗性発症の原因の一つと考えられている．

実際に，高脂肪食を負荷して肥満を誘導したdiet-induced obesity（DIO）マウスに，末梢からレプチンを投与しても摂食抑制効果は認められずSTAT3の活性化も誘導されないが，中枢性にレプチンを投与すると摂食抑制効果が認められSTAT3の活性化も誘導されることから，肥満者においてレプチンの脳脊髄液移行性が低下しレプチン抵抗性が生じている可能性が示唆される[16]．しかし，普通食を摂取している動物に中枢性にレプチンを投与した場合と比べて，高脂肪食を負荷した動物に中枢性にレプチンを投与した場合では，STAT3の活性化が減弱していることから脳脊髄液移行性のみならず細胞内の情報伝達経路が障害されている可能性も示唆されている[16]．

レプチン受容体のうち細胞内領域に短いアイソフォームOb-Raは脳脈絡叢に高濃度に発現しており，レプチンを血液中から脳脊髄液中へ運ぶトランスポーターとしての機能が示唆されている[17]．DIOマウスやNew Zealand Obeseマウスでは，BBBでのレプチンの輸送が低下していること[18]，またLPSの単回投与により，LPSそのものではなく血中に増加したレプチン自身や中性脂肪によりBBBでのレプチンの移行性が低下する[19]ことなどが報告されているが，いまだに肥満に伴うレプチンの脳脊髄液移行性の低下の詳細なメカニズムは解明されていない．

また，レプチン受容体のうち分泌型受容体であると推定されるOb-Reは，他のサイトカイン受容体の分泌型アイソフォームと同様に，レプチン結合蛋白として血液中に存在し，レプチンの作用を修飾している可能性が示唆されている．つま

り，Ob-Reをはじめとするレプチン結合蛋白の増加はレプチンの脳脊髄液移行性に影響を与える可能性が考えられる．しかし，これらのレプチン結合蛋白と肥満症との関連は不明である[20]．

2）レプチン受容体による細胞内情報伝達

レプチンの作用発現に最も重要であると考えられているOb-Rbはgp130と高い相関性を有し，細胞内における情報伝達機構も共通するところが多い．実際，レプチン受容体はレプチンの刺激により細胞内領域のチロシン残基がリン酸化を受け，更にレプチン受容体の細胞内領域に結合したJAKおよびSTATがリン酸化されることにより活性化することが知られている．リン酸化を受けたSTATは二量体を形成して核内に移行し遺伝子の転写を促進すると考えられている．

最初にレプチン抵抗性に関わる分子として注目されたのがSH2-containing protein tyrosine phosphatase-2（SHP-2）である．レプチンによりOb-Rが活性されるとOb-Rの細胞内ドメインの986番目のTyrがSHP-2をチロシンリン酸化し，それによりOb-RとSHP-2と結合する．この986番目のTyrに変異を起こしてPheにすると，SHP-2をチロシンリン酸化とOb-Rへの結合が消失し，STAT3による遺伝子発現が増強するという結果が認められ，SHP-2はレプチン抵抗性のkey moleculeと考えられた[21]．

最近，サイトカインにより発現が誘導される情報伝達阻害因子として，SOCS（Suppressor of cytokine signaling）ファミリーが発見された．SOCSファミリーにはCIS（cytokine-inducible sequence）やSOCS-1，SOCS-2，SOCS-3が含まれる．CISあるいはSOCSは中央部にSH2ドメインとC末梢に約40アミノ酸からなるSOCS boxを有する比較的小さな蛋白質である．SOCSのSH2ドメインはリン酸化したJAKに結合し，SOCS boxはSOCS蛋白の崩壊を防いでいると考えられているレプチンやIL-6をはじめとするサイトカインスーパーファミリーは，一つあるいは複数のSOCSファミリー蛋白の発現を誘導し，誘導されたCISあるいはSOCSはサイトカインによる情報伝達および生物作用を阻害することが報告されている．すなわち，CISおよびSOCSはサイトカインの細胞内情報伝達に対する負の調節因子であると考えられる．

レプチンは視床下部において特にSOCS-3の遺伝子発現を誘導し，培養細胞においてSOCS-3の強制発現がレプチン受容体を介したJAK2のリン酸化を阻害することが報告されている[22]．またレプチンで前処置した培養細胞では，SOCS-3の発現が誘導され数時間後にレプチンを添加しても，レプチンによる細胞内シグナルは減弱したままであることなどが報告されている[23]．これらの成績はSOCS-3がレプチンによる細胞内情報伝達における負の調節因子であるのみならず，高レプチン血症が持続する肥満においてSOCS-3がレプチン抵抗性の原因となりうることを示唆している．今年，脳神経特異的なSOCS-3欠損マウス[24]とSOCS-3ヘテロノックアウトマウス[25]の成績が報告された．いずれのマウスにおいても，脳内でのSOCS-3が欠如しているとレプチン感受性が上昇し，DIOなどの肥満によるレプチン抵抗性が解除されることが示されており，SOCS-3の発現レベルがレプチン感受性の決定因子であり，脳内のSOCS-3が肥満のレプチン抵抗性の治療の大きなターゲットになる可能性が提唱された．

またProtein-tyrosine phosphatase 1B（PTP 1B）もレプチン抵抗性のkey moleculeのひとつである．PTP1Bはレプチンに関与する視床下部ニューロンに存在し，Jak2を介してレプチン受容体を脱リン酸化する[26]．PTP1Bノックアウトマウスでは，レプチン感受性が極めて良好で，レプチンに誘導されるSTAT3のリン酸化が亢進しており，レプチンが欠損する*Ob/Ob*マウスとの交配実験では，*Ob/Ob*マウスの肥満をも是正した[27]．

妊娠中はレプチン血中濃度が上昇するにも関わらず，摂食量が増強しておりひとつの“レプチン抵抗性”状態を呈していると考えられる．妊娠中

のラットにおいて，視床下部のArcやVMHにおいてのみ，レプチンによるSTAT3のリン酸化が低下しておりこのことが妊娠中のレプチン抵抗性に関与している可能性が提唱されている[28]．

以上より，レプチン抵抗性には脳内のJAK2-STAT3 systemに関わる分子が重要であり，更に各因子のレプチン抵抗性における病態生理的意義の解明が急がれるところである．

3）レプチン受容体発現ニューロン以降の中枢経路

現在，レプチン受容体発現ニューロン以降の中枢経路として注目されているのが，POMC（α-MSH）/メラノコルチン4受容体（MC4-R）系である[8]．遺伝性肥満モデル動物であるAgouti（A^y/a）マウスは，本来毛包にのみ発現するagouti蛋白質が，その遺伝子のプロモーター領域の異常により異所性に過剰発現することによって黄色い毛並みと遅発性の肥満を発症する．その後の研究より，agouti蛋白質はMC1-RとMC4-Rのアンタゴニストであることが示された．現在，A^y/aマウスの肥満発症の原因として注目されている仮説は，中枢神経系に異所性に発現したagouti蛋白質が，MC4-Rをブロックするために肥満を発症するという仮説がある．実際に，MC4-Rノックアウト（KO）マウスは肥満を発症すること，ヒト肥満症例においてMC4-R遺伝子異常症が比較的高頻度に存在することが明らかとなっている[29~31]．また，MC4-Rのアゴニストであるα-MSHをコードしているPOMCを欠損するPOMC KOマウスやヒトPOMC遺伝子異常症も，肥満と副腎不全を呈することが報告されている．レプチン作用の中枢経路の下流に位置するMC4-Rの障害はレプチンの作用を減弱させるため，レプチン抵抗性の原因の一つと考えられている[32,33]．しかし単純性肥満の多くではこれらの遺伝子異常は見出されていない．

最近，agouti蛋白質と高い相同性を有し，Arcに高発現するAGRPが発見された[34]．AGRPはagouti蛋白質と同様にMC4-Rとの結合においてα-MSHに対し拮抗的に作用し，内因性の摂食調節因子である．レプチンはAGRPの遺伝子発現を抑制することが報告されており，レプチンの視床下部メラノコルチン系を介する摂食調節作用は，MC4-Rのアゴニストであるα-MSHとアンタゴニストであるAGRPのバランスの上に成立しているといえる[35]．ヒトにおけるAGRPの遺伝子異常はこれまでのところ発見されていないが，AGRPの発現異常はレプチン抵抗性の原因の可能性がある．実際にAGRPを過剰発現するトランスジェニックマウスは肥満を呈し，レプチン抵抗性が認められている[34]．

3 選択的レプチン抵抗性

レプチンにはLep-Tgマウスの血圧が高値であったことからも，血圧上昇作用を有している．高レプチン血症を伴うA^yマウスやLep-TgマウスとA^yマウスを交配することで得られるLep-Tg/A^yマウスにおける血圧上昇は，肥満個体，すなわち“抗肥満作用におけるレプチン抵抗性”が存在する個体においても血圧上昇作用におけるレプチン感受性は保持されていることを意味しており（選択的レプチン抵抗性），肥満における高血圧の発症・進展におけるレプチンの重要性を物語っている．実際に，A^yマウスにレプチンを投与したときの，摂食抑制作用は減弱していたが（レプチン抵抗性あり），交感神経活動亢進作用は保持されていたとの報告もある（選択的レプチン抵抗性）[36]．

このレプチン抵抗性が選択的であるという事実から，レプチンの摂食抑制作用と交感神経活動亢進作用が異なるシグナル伝達系を介する可能性が推察される．Lep-Tgマウスに視床下部メラノコルチン系阻害剤を投与すると，減少していた摂食量は元に戻るが，血圧は上昇したままである．前述のレプチンの摂食抑制作用は主にArcに，交感神経活動亢進作用は主にVMHに効果が強く，作用部位が異なっていたこととも通じる．A^yマウスが視床下部メラノコルチン系が阻害されたマウ