Review

Serum high-molecular-weight adiponectin as a marker for the evaluation and care of subjects with metabolic syndrome and related disorders

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In 1996, adiponectin was reported to be the most abundant transcript in adipose tissue. Animal studies revealed that administering adiponectin improves insulin resistance and blood glucose levels and inhibits atherosclerosis. In the present article, we review the significance of measuring serum high-molecular-weight (HMW) adiponectin levels in human subjects.

Our cross-sectional studies revealed that the serum HMW adiponectin concentration was 1.9 times higher in healthy Japanese females than males and had a strong positive correlation with HDL-cholesterol but a negative correlation with BMI and the homeostasis model assessment insulin resistance index (HOMA-IR). They also indicated that the serum HMW adiponectin concentration had a stronger association with HOMA-IR and metabolic syndrome than the total adiponectin concentration.

Our longitudinal study, a 6-year follow-up of Japanese men, suggested that a decreased level of HMW adiponectin is a predictor of progression to metabolic syndrome. In another intervention study, lifestyle modification for 3 months induced a decrease in BMI and waist circumference and an increase in serum HMW adiponectin but not the total adiponectin level in 16 Japanese males with metabolic syndrome.

Administering thiazolidinediones to diabetic patients increased the serum HMW adiponectin concentration 3 fold and improved glucose and lipid profiles and blood pressure. Some people may inherit a lower serum concentration of adiponectin, and have a higher risk of developing cardiovascular diseases.

It is suggested that HMW adiponectin is a useful marker for the evaluation and care of subjects with metabolic syndrome and related disorders.

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Key words; High-molecular-weight adiponectin, Metabolic syndrome, Insulin resistance, and Diabetes mellitus.

Introduction

The leading cause of death in Japan is cancer (malignant neoplasms 30.0%), followed by heart dis-

ease (15.9%) and cerebrovascular disease (11.1%). However, the rate of mortality for cardiovascular diseases (CVDs), which include heart and cerebrovascular diseases, is almost the same as that for cancer (Vital Statistics 2008 by Ministry of Health, Labour and Welfare, Japan). Insulin resistance is the decreased ability to respond to the effects of insulin, and is caused by heredity factors, environmental factors such as a high-fat diet and reduced physical activity, and “glucose toxicity” due to hyperglycemia, etc. Since the introduction of a westernized diet and reduced physi-

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Fig. 1. Roles of adipo (cyto) kines secreted from adipose tissue.

Ins. Res.: Insulin resistance; FFA: free fatty acid; TNF: tissue necrosis factor; BP: blood pressure; PAI-1: plasminogen activator inhibitor-1.

cal activity in Japan, as reflected by automation and motorcars, the prevalence of lifestyle-related diseases such as abdominal obesity, type 2 diabetes mellitus, hypertension, dyslipidemia, and atherosclerotic diseases has increased and become a social problem. Because these conditions often develop concurrently via visceral fat accumulation and/or insulin resistance¹⁾, they have been called syndrome X²⁾, the deadly quartet³⁾, insulin resistance syndrome⁴⁾, and visceral fat syndrome¹⁾. Recently, this syndrome has been called metabolic syndrome. The above conditions pertaining to metabolic syndrome are important risk factors for atherosclerosis and CVDs, and comorbidity is known to increase the risk of CVDs, not additively, but synergistically⁵⁾. Therefore, to prevent and control metabolic syndrome and/or CVDs, it is important to help people recognize the importance of health checkups and correct visceral-type obesity and insulin resistance.

It has long been considered that adipose tissue only regulates energy storage, but recent studies have revealed that the tissue is a site of synthesis and secretion of various bioactive substances (called adipokines, **Fig. 1**) such as free fatty acids (FFAs), TNF- α , resistin, leptin, adiponectin, PAI-1, and angiotensinogen¹⁾. **Fig. 2** shows the factors involved in the progression from obesity to atherosclerotic diseases, especially those involved in inducing insulin resistance and atherosclerosis.

In this article, we review the significance of measuring serum levels of high-molecular-weight (HMW) adiponectin for evaluating and caring for subjects with metabolic syndrome and related disorders.

Fig. 2. Progression from obesity to cardiovascular disease (CVD).

Vis-fat: visceral fat; SC-fat: subcutaneous fat; HMW: high-molecular weight; SNS: sympathetic nervous system; DM2: type 2 diabetes mellitus; DL: dyslipidemia; HT: hypertension.

What is Adiponectin?

Adiponectin was discovered by researchers at Osaka University in 1996 as the most abundant transcript in adipose tissue⁶⁾. It was found to be the same substance as a 28-kD gelatin-binding protein (GBP28) extracted from human serum by the Faculty of Pharmaceutical Sciences, Showa University in the same year⁷⁾.

Adiponectin inhibits signals triggering inflammation in vascular endothelial cells and the growth of vascular smooth muscle cells¹⁾. In animal experiments, adiponectin reportedly attached to injured blood vessel walls. Yamauchi *et al.*⁸⁾ and Berg *et al.*⁹⁾ found separately in studies using various types of obese mice and diabetic mice, that the administration of adiponectin improves both insulin resistance and blood glucose levels. Another paper reported that the concentration of adiponectin in blood decreased in rhesus monkeys with diabetes as insulin resistance worsened¹⁰⁾. Furthermore, studies using knockout mice^{11, 12)} indicated that a high-fat diet likely induces insulin resistance and atherosclerosis. Moreover, adiponectin was found to inhibit hepatic fibrosis¹³⁾ and the growth of cancer cells¹⁴⁾, and provide the survival benefits¹⁵⁾ in animal experiments.

Recent analyses by gel filtration have revealed that adiponectin is not present as a monomer in serum and that the HMW forms including the do-

decamer (4×3 mer) and octadecamer (6×3 mer) are more closely associated with the onset of coronary artery diseases (CADs) and weight reduction¹⁶⁾ and play the role of insulin sensitizers¹⁷⁾. Several studies *in vitro* have found that HMW adiponectin mainly activates AMP kinase¹⁸⁾, prevents apoptosis of vascular endothelial cells¹⁶⁾, and has cytostatic effects¹⁹⁾; thus, HMW adiponectin is an active form of adiponectin.

Clinical findings also confirmed that type 2 diabetic patients with CAD show a selective reduction in HMW adiponectin^{16, 17, 20)}. Furthermore, weight reduction¹⁶⁾ increases the HMW form of adiponectin but not the lower molecular complexes. Waki *et al.*²¹⁾ revealed that human adiponectin with rare missense mutations (G84R and G90S) does not form HMW multimers. These mutations were associated with insulin resistance and type 2 diabetes mellitus. They concluded that the proportion of each adiponectin oligomeric complex is important for the anti-diabetic and anti-atherogenic activities of this protein.

Several reports have shown HMW adiponectin to be more useful than total adiponectin. In particular, in type 2 diabetic patients receiving medications that include thiazolidinediones (TZDs), the HMW/total adiponectin ratio was reported to be more useful than the total serum adiponectin level. For example, Pajvani *et al.*¹⁷⁾ reported that the HMW/total adiponectin ratio was significantly more useful for monitoring improvements in insulin sensitivity in response to TZDs in type 2 diabetes mellitus. Hara *et al.*²²⁾ reported that the HMW/total adiponectin ratio had better power for predicting insulin resistance and metabolic syndrome than the total plasma adiponectin level. Aso *et al.*²⁰⁾ reported that the HMW/total adiponectin ratio was more useful for evaluating CAD in type 2 diabetic patients than simply measuring the total serum adiponectin level. We conducted a cross-sectional study in healthy Japanese males without any medication and found that HMW adiponectin is as effective as the HMW/total adiponectin ratio for predicting insulin resistance and/or metabolic syndrome²³⁾.

Recently, longitudinal data concerning HMW adiponectin have been reported. For example, a decreased HMW adiponectin level was found to be an independent risk factor for progression to type 2 diabetes in Japanese Americans during a 5.4-year follow-up study²⁴⁾, and Inoue *et al.* reported that the serum HMW adiponectin level was a predictor of cardiovascular events in patients with CAD during a 7-year follow-up study²⁵⁾.

Cross-sectional study in healthy subjects

Although adiponectin is secreted from adipocytes, adiponectin concentrations are reported to be lower in obese subjects²⁶⁾ and in patients with type 2 diabetes mellitus and much lower in those with a combination of type 2 diabetes mellitus and CAD²⁷⁾. Furthermore, a significant positive correlation was observed between plasma adiponectin concentrations and insulin sensitivity (effectiveness of insulin) in Pima Indians with severe obesity and diabetes²⁸⁾.

In our cross-sectional study, 945 healthy Japanese males and females aged 30-65 years who participated in regular health check-ups were enrolled as study subjects, in order to examine the relationship between the serum HMW adiponectin concentration and body mass index (BMI), blood pressure, lipid profile, plasma glucose, serum insulin level, etc. The serum adiponectin concentration was determined using a commercially available kit (Chugai Diagnostics Science Co., Ltd, Tokyo)²⁹⁻³²⁾ with IH7, the same antibody used in a novel enzyme-linked immunosorbent assay (ELISA) kit specific for HMW adiponectin (Fujirebio Inc., Tokyo)³³⁾. IH7 specifically identifies HMW adiponectin, rendering heat treatment unnecessary³³⁾. Therefore, we are sure that the concentration measured was that of HMW adiponectin. In the present review article, the serum adiponectin concentration, which was determined using the former kit²⁹⁻³²⁾, was converted to the data obtained by the latter kit and described as the HMW adiponectin concentration.

The serum HMW adiponectin concentration was 1.9 times higher in female subjects than in male subjects. The HMW adiponectin level negatively correlated with the homeostasis model assessment insulin resistance index (HOMA-IR) and positively correlated with HDL-cholesterol in both male and female subjects, even after adjusting for age and BMI (**Tables 1 and 2**). A significant negative correlation between the concentration and blood pressure was also observed, but the correlation was not significant after adjusting for age and BMI. A stepwise multiple regression analysis revealed that the serum HMW adiponectin concentration was independently associated with gender, HDL-cholesterol, HOMA-IR, and BMI (**Table 3**). The strongest correlation was between the concentration and HDL-cholesterol, in both male and female subjects, but details of the mechanisms remain unclear. The total adiponectin concentration was also measured using a commercially available kit (Otsuka Pharmaceutical Co., Ltd, Tokyo) in our cross-sectional study. The study results²³⁾ showed that the HMW adiponectin concentration had a stronger association

Table 1. Simple regressions with high-molecular-weight (HMW) and total adiponectin (ADPN) levels in 646 Japanese males aged 30-65 years

Parameter	Value $\pm$ SD		HMW ADPN		Total ADPN	
			<i>r</i>		<i>r</i>	
Age	47.4 $\pm$ 11.1	(years)	0.030	NS	0.012	NS
BMI	23.6 $\pm$ 3.0	(kg/m ²)	-0.351	**	-0.305	**
Systolic BP	124 $\pm$ 18	(mmHg)	-0.184	**	-0.152	**
Diastolic BP	77 $\pm$ 12	(mmHg)	-0.187	**	-0.152	**
Glucose	96 $\pm$ 16	(mg/dL)	-0.141	**	-0.135	**
Glycated Albumin	15.0 $\pm$ 2.0	(%)	0.079	*	0.098	*
Insulin	5.5 $\pm$ 4.1	(μ U/mL)	-0.373	**	-0.318	**
HOMA-IR	1.33 $\pm$ 1.09	(-)	-0.376	**	-0.325	**
Triglycerides	125 $\pm$ 98	(mg/dL)	-0.344	**	-0.330	**
HDL-cholesterol	58 $\pm$ 15	(mg/dL)	0.351	**	0.303	**
LDL-cholesterol	129 $\pm$ 30	(mg/dL)	-0.218	**	-0.206	**
Uric acid	6.4 $\pm$ 1.2	(mg/dL)	-0.208	**	-0.186	**

NS: not significant, * $p < 0.01$, ** $p < 0.001$. HMW: $3.2 \pm 2.1 \mu\text{g/mL}$ for males.
 BP: blood pressure; HOMA-IR: homeostasis model assessment insulin resistance index.

Table 2. Simple regressions with high-molecular-weight (HMW) and total adiponectin (ADPN) level in 299 Japanese female subjects aged 30-65 years

Parameter	Value $\pm$ SD		HMW ADPN		Total ADPN	
			<i>r</i>		<i>r</i>	
Age	38.3 $\pm$ 9.9	(years)	0.069	NS	0.089	NS
BMI	20.6 $\pm$ 2.9	(kg/m ²)	-0.216	**	-0.187	**
Systolic BP	109 $\pm$ 16	(mmHg)	-0.069	NS	-0.010	NS
Diastolic BP	65 $\pm$ 10	(mmHg)	-0.023	NS	-0.089	NS
Glucose	87 $\pm$ 12	(mg/dL)	-0.236	**	-0.247	**
Glycated Albumin	15.3 $\pm$ 1.5	(%)	0.062	NS	0.118	NS
Insulin	3.9 $\pm$ 2.4	(μ U/mL)	-0.256	**	-0.221	**
HOMA-IR	0.85 $\pm$ 0.55	(-)	-0.292	**	-0.260	**
Triglycerides	65 $\pm$ 36	(mg/dL)	-0.246	**	-0.206	**
HDL-cholesterol	71 $\pm$ 15	(mg/dL)	0.378	**	0.352	**
LDL-cholesterol	110 $\pm$ 29	(mg/dL)	-0.111	NS	-0.093	NS
Uric acid	4.4 $\pm$ 0.9	(mg/dL)	-0.120	*	-0.056	NS

NS: not significant, * $p < 0.01$, ** $p < 0.001$. HMW: $6.0 \pm 3.5 \mu\text{g/mL}$ for females.
 BP: blood pressure; HOMA-IR: homeostasis model assessment insulin resistance index.

Table 3. Stepwise multiple regression with high-molecular-weight (HMW) adiponectin as a dependent variable in 938 Japanese subjects

Variable	Standardized regression coefficient	<i>F</i>	<i>p</i>
Sex	0.257	75.8	< 0.001
HDL-cholesterol	0.241	62.0	< 0.001
HOMA-IR	-0.188	33.5	< 0.001
BMI	-0.124	12.8	< 0.001

Age and mean blood pressure were also permitted to enter the regression mode.
 HOMA-IR: homeostasis model assessment insulin resistance index; BMI: body mass index.
 $R^2 = (0.606)^2 = 0.367$

Fig. 3. Relationship of subject numbers with metabolic syndrome (MetS) and high-molecular-weight (HMW) (left panel) or total (right panel) adiponectin levels in 637 Japanese males divided into 4 groups according to each value.

with HOMA-IR and metabolic syndrome than the total adiponectin concentration (**Fig. 3**).

Longitudinal studies related to the serum adiponectin level

Although we could not identify the relationship between the HMW adiponectin concentration at baseline and changes in the BMI and lipid profile over 2 years, a significant negative correlation between the HMW adiponectin concentration at baseline and changes in insulin and HOMA-IR was observed³¹. These associations were also significant after adjusting for changes in BMI. Thus, insulin resistance did not worsen much over 2 years when the serum HMW adiponectin concentration was high. In contrast, when the concentration was low, the insulin resistance worsened in 2 years.

A longitudinal study conducted by Daimon et al. in Funagata, Yamagata Prefecture, Japan, indicated hypoadiponectinemia to be a risk factor for the development of type 2 diabetes mellitus³⁴. Another study conducted in 18,225 American men aged 40–75 years found that nonfatal myocardial infarction or fatal coronary artery diseases developed in 226 subjects during the 6-year follow-up period and indicated a low blood concentration of adiponectin to be one of the predictors of myocardial infarction³⁵. Even after adjusting for age, study enrollment time, smoking, and other factors, the incidence of myocardial infarction in the lowest quartile of the adiponectin concentration was 2.5 times higher than that in the top quartile of the concentration ($p < 0.001$)³⁵.

We also investigated whether a decreased HMW adiponectin concentration is a predictor of progres-

sion to metabolic syndrome during a 6-year follow-up period in Japanese men³⁶. This study included 416 men without metabolic syndrome, aged 30–59 years at baseline, who had participated in annual health check-ups in both 2000 and 2006. The study found that a low concentration of HMW adiponectin ($\leq 2.65 \mu\text{g/mL}$) was associated with a substantially higher hazard ratio of the progression to metabolic syndrome after adjusting for age and BMI (hazard ratio 1.561, 95% confidence interval 1.051–2.292, $p = 0.028$). The number of subjects who progressed to metabolic syndrome in each tertile based on the baseline HMW adiponectin concentration was significantly different among the 3 groups (HMW adiponectin, $\chi^2 = 7.473$, $p = 0.0238$; total adiponectin, $\chi^2 = 4.477$, $p = 0.1066$; HMW/total ratio, $\chi^2 = 1.676$, $p = 0.4325$).

In another intervention study, 16 Japanese males with metabolic syndrome aged 54.8 ± 7.2 years were enrolled in our program. Lifestyle modifications increasing physical activity and decreasing calories, were taught to each subject by physician nurses and by a nutritionist at our institute (Health Center, Keio University). Exercise training was performed by these subjects. Lifestyle modifications for 3 months induced decreases in BMI (though NS), waist circumference, and serum TG and post-prandial insulin levels (**Table 4**) and an increase in the serum HMW adiponectin level but not the total adiponectin level in these subjects (**Table 5**). We also found that the change in the HMW adiponectin level was most strongly correlated with the change in BMI ($r = -0.656$, $p = 0.0046$) but not significantly with the change in waist circumference ($r = -0.085$, $p = 0.7586$). Although the reason for this discrepancy was not clear, there is a possibility that the BMI reflected more precisely the reduction in body fat. In contrast waist circumference was not measured by one examiner in this study.

Furthermore, some papers have reported that the serum HMW adiponectin concentration increased slightly when angiotensin receptor blockers (ARBs), statins, and insulin secretagogues were administered. Losartan, an ARB, is widely used as an anti-hypertensive agent, and its metabolite is reported to have partial peroxisome proliferator-activated receptor gamma (PPAR γ) activity *in vitro*³⁷. Several studies have demonstrated that losartan treatment increases the serum HMW-adiponectin level^{38, 39}, although the effects of losartan on metabolic parameters in clinical use are controversial^{40–42}. On the other hand, treatment with diuretics, including hydrochlorothiazide (HCTZ) and indapamide, was reported to decrease the adiponectin level^{43–45}. We recently reported that changing the treatment to losartan/HCTZ from a usual dosage of

Table 4. Beneficial effects of a 3-month lifestyle modification (mainly exercise) on metabolic parameters in 16 Japanese males with metabolic syndrome aged 54.8 ± 7.2 years

	Baseline	3 Months	<i>p</i> value
BMI (kg/m ²)	26.8 ± 2.6	26.6 ± 2.5	NS
Waist circumference (cm)	91.9 ± 6.4	90.3 ± 6.2	0.0687
Systolic blood pressure (mmHg)	135 ± 19	133 ± 20	NS
Diastolic blood pressure (mmHg)	84 ± 14	85 ± 13	NS
Fasting plasma glucose (mg/dL)	119 ± 23	117 ± 19	NS
Post prandial (2h) glucose (mg/dL)	146 ± 52	142 ± 50	NS
Fasting serum insulin (μU/mL)	10.7 ± 4.2	10.4 ± 4.3	NS
Post-prandial (2h) insulin (μU/mL)	44.9 ± 18.1	35.8 ± 17.1	0.0032
HOMA-IR	3.1 ± 1.3	3.0 ± 1.4	NS
Total cholesterol (mg/dL)	214 ± 46	210 ± 47	NS
Fasting serum TG (mg/dL)	224 ± 251	153 ± 95	NS
Post-prandial (2h) TG (mg/dL)	269 ± 255	212 ± 107	NS
HDL cholesterol (mg/dL)	54 ± 17	53 ± 14	NS
LDL-cholesterol (mg/dL)	123 ± 35	132 ± 40	NS

Values are means ± SDs. By the Wilcoxon test.

HOMA-IR: homeostasis model assessment insulin resistance index; TG: triglycerides, NS: $p > 0.15$.

Table 5. Beneficial effects of a 3-month lifestyle modification (mainly exercise) on serum adiponectin levels in 16 Japanese males with metabolic syndrome

	Baseline	3 Months	<i>p</i> value
Fasting			
HMW adiponectin	2.5 ± 1.8	3.4 ± 2.6	0.0045
Total adiponectin	4.1 ± 1.7	4.3 ± 2.1	NS
HMW/Total	0.56 ± 0.15	0.71 ± 0.24	0.0016
2 hr after meal test (541 kcal with 28.3g fat)			
HMW adiponectin	2.6 ± 2.1	3.3 ± 2.7	0.0115
Total adiponectin	4.3 ± 1.8	4.2 ± 2.1	NS
HMW/Total	0.57 ± 0.17	0.71 ± 0.24	0.0023

Values are means ± SDs. By the Wilcoxon test.

HMW: high-molecular-weight, NS: $p > 0.15$.

ARB, provided good blood pressure control, while the HMW-adiponectin level remained unchanged in male subjects with hypertension⁴⁶⁾. We have found that administering telmisartan, which also has partial PPAR γ activity *in vitro*⁴⁷⁻⁵⁰⁾, increased the serum HMW adiponectin concentration in Japanese males with hypertension and abdominal obesity⁵¹⁾.

Studies in subjects with type 2 diabetes mellitus

Our previous study⁵²⁾ and others have shown that when troglitazone, an insulin sensitizer, was administered to type 2 diabetic patients for 3 to 6 months (400 mg/day), body weight and skinfold thickness increased but intra-abdominal fat decreased.

Since intra-abdominal fat is a well-known risk factor for insulin resistance and atherosclerosis¹⁾, reducing intra-abdominal fat is important to prevent atherosclerosis.

We studied the effect of a 3-month administration of pioglitazone (30 mg/day) in 10 Japanese males with type 2 diabetes mellitus (57.7 ± 7.4 years)³⁰⁾, using the same protocol as for troglitazone. The results indicated that the pioglitazone significantly decreased the subjects' fasting plasma glucose, insulin, blood pressure, and HbA_{1c} levels while significantly increasing BMI, LDL-cholesterol, and leptin levels. To our great surprise at that time, the mean serum HMW adiponectin concentration increased 3 fold in all cases ($3.2 \pm 1.1 \rightarrow 9.6 \pm 1.4 \mu\text{g/mL}$). A CT scan revealed that

Table 6. Effects of a 3-month treatment with pioglitazone (30 mg/day) on the high-molecular-weight (HMW) adiponectin level and liver function of 10 Japanese males with type 2 diabetes mellitus

	Baseline	3 months	
Number	10	10	
HMW adiponectin ($\mu\text{g/mL}$)	3.2 ± 1.1	9.6 ± 1.4	**
AST (IU/L)	27.9 ± 13.2	23.5 ± 7.2	NS
ALT (IU/L)	27.9 ± 15.6	22.9 ± 9.4	NS
γGTP (IU/L)	61.5 ± 44.6	47.9 ± 40.1	*

Data are means $\pm$ SDs. * $p < 0.05$ and ** $p < 0.01$ by the Wilcoxon test.
NS: $p > 0.10$.

the subcutaneous fat area (S) significantly increased from 155 ± 69 to $179 \pm 81\text{cm}^2$ and the visceral fat area (V) tended to increase (from 165 ± 38 to $180 \pm 46\text{cm}^2$, and the V/S ratio from 1.2 ± 0.3 to 1.1 ± 0.3 , NS for both), unlike with the troglitazone administration. Both troglitazone and pioglitazone activate PPAR γ , a transcription factor present mainly in subcutaneous fat tissue; consequently, many reports indicate that body weight, subcutaneous fat, and leptin (mainly secreted from subcutaneous fat) increase with troglitazone and pioglitazone administration. However, the effects of troglitazone and pioglitazone on visceral fat were different. Although the precise mechanism is unknown, troglitazone contains vitamin E and so itself could have an effect on intra-abdominal fat. The above-mentioned study did not target patients with fatty liver, but an improvement in AST, ALT, and γGTP levels was also observed (Table 6). To summarize this study, the administration of pioglitazone to type 2 diabetic patients improved their plasma glucose level and blood pressure and increased the serum HMW adiponectin concentration, suggesting pioglitazone to be useful for preventing the onset and progression of atherosclerosis in type 2 diabetic patients, as shown in the PROactive Study⁵³.

In keeping with the results of previous studies that reported an association between TZDs and the serum adiponectin concentration, the administration of troglitazone⁵⁴, pioglitazone³⁰, or rosiglitazone⁵⁵ increased the total adiponectin concentration approximately 2 fold and the serum HMW adiponectin concentration 3 fold. "Good adiponectin" and "bad TNF- α " are thought to inhibit each other's secretion and effects on muscles, suggesting that TZDs may inhibit TNF- α via an increase in the adiponectin concentration in order to induce beneficial effects. Further, many reports indicate that these TZDs actually increase LDL cholesterol levels. Nevertheless, adiponectin has been shown to inhibit atherosclerosis *in vitro*

and *in vivo*, suggesting that it might prevent the onset and progression of CVDs in diabetic patients and also in patients with metabolic syndrome.

Effects of genetic factors on serum adiponectin concentration

A genetic study of French caucasions revealed that the long arm of chromosome 3 (3q27), which harbors the adiponectin gene, is strongly involved in type 2 diabetes mellitus⁵⁶. Furthermore, a linkage analysis in white families indicated that the 3q27 region is strongly associated with metabolic syndrome⁵⁷.

Hara *et al.* studied the single nucleotide polymorphisms (SNPs) of the adiponectin gene⁵⁸ and reported that the GG genotype of SNP45 and the GG genotype of SNP276 were frequently observed in diabetic patients. In particular, obese subjects with the GG genotype of SNP276 had a lower serum adiponectin concentration and higher HOMA-IR. Kondo *et al.* reported that a change of the 164th amino acid of adiponectin from isoleucine to threonine (Thr), which is quite a rare mutation, was related to a lower adiponectin concentration, and that all 9 subjects with the Thr-allele had at least one symptom of metabolic syndrome as a complication, such as diabetes mellitus, hypertension, dyslipidemia, and CVDs⁵⁹.

We also investigated SNP45, SNP276, and SNP349 in the adiponectin gene and the SNP whose 12th amino acid (proline) in the PPAR γ gene was substituted with alanine (Ala) by using the TaqMan PCR method³². There was no difference in the serum HMW adiponectin concentration between the 3 SNPs of the adiponectin gene in our healthy population. On the other hand, the serum HMW adiponectin concentration was significantly lower in patients with the Ala-allele of the PPAR γ gene in both males and females, which suggested that the concentration depends on genetic factors³². With regard to the mecha-

Fig. 4. Possible mechanism by which the subjects with the Ala12 allele in the PPAR γ gene have lower adiponectin promoter activity, resulting in a lower adiponectin level in circulation.

nism, experiments *in vitro* have revealed that human PPAR γ 2 with Ala12 has reduced transactivation activity⁽⁶⁰⁾ and that the promoter activity of adiponectin is markedly enhanced by PPAR γ ligands in 3T3L1 adipocytes. Taking these findings into account, it is possible that subjects with the Ala12 allele have lower adiponectin promoter activity, resulting in a lower serum adiponectin level (**Fig. 4**). These findings suggest that the serum adiponectin level is decreased due to obesity and genetic predispositions and is involved in glucose and/or lipid metabolism.

CONCLUSION

Previous studies *in vitro* and *in vivo* have indicated that adiponectin inhibits atherosclerosis, suggesting that adiponectin might prevent the onset and progression of cardiovascular diseases in patients with metabolic syndrome and in diabetic patients. It is suggested that HMW adiponectin is a useful marker for the evaluation and care of subjects with metabolic syndrome and related disorders.

Acknowledgments

The studies described here were conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject or patient after a full explanation of the purpose, nature, and risk of all procedures used. The protocols were approved by the ethical review committees of the Health Center and the Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan.

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