Original Article

The High Molecular Weight Adiponectin Level is Associated with the Atherogenic Lipoprotein Profiles in Healthy Japanese Males

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Aim: Circulating adiponectin comprises high, medium and low molecular weight (HMW, MMW, and LMW) forms. Decreased adiponectin levels have been demonstrated to correlate with the atherogenic lipoprotein profile in patients with metabolic syndrome (MS). However, the associations of these isoforms with the atherogenic lipoprotein profiles in the healthy population remain unclear.

Methods: Apparently healthy male subjects were divided into non-MS ($n=132$) and MS ($n=63$) groups. We measured the total, HMW, MMW and LMW adiponectin levels by ELISA, and determined the detailed lipoprotein profiles by high-performance liquid chromatography.

Results: The total and HMW adiponectin levels in the MS group were significantly lower than those in the non-MS group (3.8 ± 1.9 vs. 4.9 ± 2.4 $\mu\text{g/mL}$, $p < 0.001$ and 1.6 ± 1.2 vs. 2.5 ± 1.9 $\mu\text{g/mL}$, $p < 0.001$, respectively), whereas the MMW and LMW levels did not differ significantly between the groups. The total and HMW adiponectin levels correlated with the atherogenic lipoprotein profiles, including the following: 1) increased cholesterol levels in the small LDL subclasses; 2) decreased cholesterol levels in the larger HDL subclasses; 3) increased triglycerides (TGs) in almost all VLDL and LDL subclasses and 4) decreased TGs in the large HDL and increased TGs in the small HDL subclasses. In addition, a multivariate analysis demonstrated that the HMW adiponectin level was an independent predictor of the small LDL and HDL levels ($p=0.02$ for both).

Conclusions: The HMW, but not MMW or LMW, adiponectin levels are associated with the typical atherogenic lipoprotein profiles, independent of MS components. Measurement of the HMW adiponectin levels could be useful for identifying the atherogenicity in apparently healthy males.

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Key words: High molecular weight adiponectin, Atherogenic lipoprotein, Metabolic syndrome, Small LDL, Small HDL

Introduction

Adiponectin has been identified as one of the adipocytokines with important metabolic effects¹⁻⁴. In addition, adiponectin is protective against the initiation and progression of atherosclerosis through various anti-inflammatory and antiatherogenic effects^{3, 4}. Adiponectin forms multimers in circulation, thus resulting in trimeric [low molecular weight (LMW)], hexa-

meric [medium molecular weight (MMW)] or oligomeric [high molecular weight (HMW)] forms⁵. HMW adiponectin is the active form of the protein that is involved in the development of insulin resistance, metabolic syndrome, diabetes mellitus and atherosclerosis⁶⁻¹⁴.

Metabolic syndrome, which is associated with visceral fat accumulation and a clustering of obesity-related disorders, is an independent risk factor for cardiovascular disease¹⁵⁻¹⁷. Metabolic syndrome has a typical atherogenic lipoprotein profile, which consists of increased small LDL particles, high triglyceride (TG) levels in large VLDL subclasses, and low cholesterol levels in large HDL subclasses^{18, 19}. Low serum adiponectin levels are associated with these athero-

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genic lipoprotein profiles^{20, 21}). A previous study in a small cohort of patients with insulin resistance and diabetes mellitus demonstrated that increased HMW adiponectin levels are associated with an increase in large HDL and small LDL subclasses²²). However, the associations between these adiponectin isoforms and the detailed atherogenic lipoprotein profiles (i.e., cholesterol and TG levels in specific lipoprotein subclasses) in the general (i.e., apparently healthy) population remain unclear.

In the present study, we systematically investigated the association between the total, HMW, MMW and LMW adiponectin levels and the detailed atherogenic lipoprotein profiles in healthy Japanese males. We provide clear evidence of the usefulness of the HMW levels for identifying the atherogenic lipoprotein profile in the general population.

Subjects

We enrolled 195 consecutive apparently healthy male subjects in our study. Before enrollment, all subjects underwent a medical check-up. Subjects who had liver and/or renal dysfunction, inflammatory diseases or were taking certain medications, including lipid-lowering drugs, insulin and oral hypoglycemic agents, were excluded from the study. Patients previously diagnosed with diabetes mellitus or dyslipidemia were also excluded. The blood pressure (BP) was measured using a standard mercury sphygmomanometer after the subjects rested for at least five minutes. The height and weight were recorded, and the body mass index (BMI) was calculated as the ratio of weight in kilograms to the square of height in meters. The waist circumference was determined by measuring the circumference around the umbilical area following exhalation while the patient was standing in an upright position.

The study subjects were divided into an MS group and a non-MS group according to the definition of the International Diabetes Federation (IDF)²³). In brief, subjects with metabolic syndrome were defined as those having a waist circumference of ≥ 85 cm, with two or more of the following factors: 1) elevated concentration of TGs (>150 mg/dL), 2) a reduced concentration of high density lipoprotein cholesterol (HDL-C; <40 mg/dL or specific treatment for this lipid abnormality), 3) elevated blood pressure (BP; systolic BP >130 mmHg or diastolic BP >85 mmHg) or ongoing treatment for previously diagnosed hypertension and 4) an elevated fasting plasma glucose concentration (FPG; >100 mg/dL).

Blood Sampling

Whole blood samples were drawn after an overnight fast. The plasma glucose and serum insulin levels were measured by standard methods. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: fasting glucose (mg/dL) $\times$ fasting insulin (mU/L)/405²⁴). The high-sensitivity C-reactive protein (hs-CRP) levels were measured using a standard enzymatic method. The total, HMW, MMW and LMW adiponectin levels were measured using a commercially available enzyme-linked immunosorbent assay kit (Daiichi Pure Chemicals, Ibaraki, Japan)²⁵). Adiponectin multimers were selectively measured after sample pretreatment with two proteases that specifically digested the LMW forms or both the MMW and LMW forms. The total, sum of HMW and MMW, and HMW adiponectin levels were separately obtained. The LMW adiponectin levels were calculated by subtracting the sum of the HMW and MMW adiponectin levels from the total adiponectin levels. The MMW adiponectin levels were calculated by subtracting the HMW adiponectin levels from the sum of the HMW and MMW adiponectin levels. The intra- and inter-assay coefficients of variation were 5.3 and 5.0% (total adiponectin), 4.1 and 6.0% (sum of MMW and HMW adiponectin) and 3.3 and 5.7% (HMW adiponectin), respectively.

Analysis of the Lipoprotein Profiles

The cholesterol profiles in the plasma lipoproteins were analyzed by a dual detection high-performance liquid chromatography (HPLC) system with two tandem connected TSKgel LipopropakXL columns (300×7.8 mm; Tosoh, Japan), as described previously²⁶⁻²⁸). We obtained the cholesterol and TG levels in 20 lipoprotein subclasses divided according to the lipoprotein particle diameters. To simplify the data analysis, subclasses were grouped into one of the following 13 broad categories: chylomicrons (peak 1: >90 nm and peak 2: 75 nm), large very low-density lipoprotein (VLDL; peaks 3: 64 nm, 4: 53.6 nm and 5: 44.5 nm), medium VLDL (peak 6: 36.8 nm), small VLDL (peak 7: 31.3 nm), LDL (peak 8: 28.6 nm), medium LDL (peak 9: 25.5 nm), small LDL (peak 10: 23.0 nm), very small LDL (peaks 11: 20.7 nm, 12: 18.6 nm and 13: 16.7 nm), very large HDL (peaks 14: 15.0 nm and 15: 13.5 nm), large HDL (peak 16: 12.1 nm), medium HDL (peak 17: 10.9 nm), small HDL (peak 18: 9.8 nm) and very small HDL (peaks 19: 8.8 nm and 20: 7.6 nm). The average particle diameters (nm) of LDL and HDL were obtained from their peak times on HPLC chromatograms.

Table 1. Characteristics of the study subjects

	Non-MS (<i>n</i> = 132)	MS (<i>n</i> = 63)	<i>p</i> value
Age (years)	50.0 ± 10.2	50.7 ± 8.5	NS
Body mass index (kg/m ²)	23.0 ± 2.6	26.1 ± 3.1	< 0.001
Waist circumference (cm)	82.8 ± 6.9	91.6 ± 6.5	< 0.001
Systolic blood pressure (mmHg)	126 ± 15	138 ± 15	< 0.001
Diastolic blood pressure (mmHg)	79 ± 10	88 ± 9	< 0.001
Current smokers	58 (44%)	27 (44%)	NS
Total cholesterol (mg/dL)	194 ± 34	192 ± 34	NS
Triglycerides (mg/dL)	106 ± 43	132 ± 38	< 0.001
HDL cholesterol (mg/dL)	59 ± 12	49 ± 12	< 0.001
LDL cholesterol (mg/dL)	77 ± 18	81 ± 17	NS
Fasting plasma glucose (mg/dL)	97 ± 10	108 ± 17	< 0.001
Insulin (mU/L)	4.4 ± 2.7	7.8 ± 5.8	< 0.001
HOMA-IR	1.1 ± 0.8	2.2 ± 1.8	< 0.001
High-sensitivity CRP (mg/dL)	0.086 ± 0.146	0.134 ± 0.180	0.05

MS = metabolic syndrome, HDL = high-density lipoprotein, LDL = low-density lipoprotein, HOMA-IR = homeostasis model assessment for insulin resistance, HOMA-IR = fasting glucose (mg/dL) × fasting insulin (mU/L)/405, CRP = C reactive protein, Data are the means ± SD.

Statistical Analysis

The data are presented as the means ± SD. The statistical significance of differences between the groups were analyzed using Student's *t*-test and the chi-square test. The correlations between two parameters were determined by a simple linear regression analysis. A stepwise multiple linear regression analysis was used to determine the associations between the total and HMW adiponectin levels and the LDL or HDL particle sizes, independent of other MS components. The statistical analysis was performed using the JMP software program (SAS Institute, Cary, NC, USA). *P* values < 0.05 were considered to be statistically significant.

Results

Characteristics of the Study Subjects

The characteristics of the subjects evaluated in the present study are shown in **Table 1**. There were no significant differences in age or in the prevalence of current smokers between the two groups. Compared with the non-MS group, the MS group had a significantly higher BMI (*p* < 0.001), waist circumference (*p* < 0.001), systolic BP (*p* < 0.001) and diastolic BP (*p* < 0.001). The MS group had significantly higher TG (*p* < 0.0001) and lower HDL-C (*p* < 0.0001) levels than the non-MS group, whereas the TC and LDL-C levels were not significantly different between the two groups. The FPG levels, fasting insulin levels

and HOMA-IR in the MS group were significantly higher than those in the non-MS group (*p* < 0.001, respectively). Subjects with metabolic syndrome had significantly higher hs-CRP levels than those without metabolic syndrome (*p* = 0.05).

The Total and HMW Adiponectin Levels Correlate with Metabolic Syndrome

As shown in **Fig. 1**, the total and HMW adiponectin levels in the MS group were significantly lower than those in the non-MS group (3.8 ± 1.9 vs. 4.9 ± 2.4 μg/mL, *p* < 0.001 and 1.6 ± 1.2 vs. 2.5 ± 1.9 μg/mL, *p* < 0.001, respectively), whereas no significant differences in the MMW or LMW adiponectin levels were found between the two groups (1.5 ± 1.0 vs. 1.7 ± 1.3 μg/mL and 0.8 ± 0.8 vs. 1.1 ± 1.0 μg/mL, respectively). The total and HMW adiponectin levels were significantly correlated with the waist circumference (*r* = −0.29, *p* < 0.001 and *r* = −0.26, *p* < 0.001, respectively), BMI (*r* = −0.34, *p* < 0.001 and *r* = −0.31, *p* < 0.001, respectively), fasting insulin level (*r* = −0.25, *p* < 0.001 and *r* = −0.27, *p* < 0.001, respectively) and HOMA-IR (*r* = −0.22, *p* = 0.002 and *r* = −0.25, *p* < 0.001, respectively). The LMW adiponectin levels were significantly associated with the BMI (*r* = −0.23, *p* = 0.001). All other relationships between the levels of each adiponectin form and classical risk factors were either relatively weak (*r* < 0.200) or not significant (*p* > 0.05).

Fig. 1. A comparison of the total, high molecular weight (HMW), medium molecular weight (MMW) and low molecular weight (LMW) adiponectin levels between the metabolic syndrome (MS) and non-MS groups. The data are presented as the means $\pm$ SD.

The Correlations between the Adiponectin Forms and Detailed Lipoprotein Profiles

Table 2 shows the correlations between the total, HMW, MMW and LMW adiponectin levels and the detailed lipoprotein profiles measured by HPLC. The total, HMW and MMW adiponectin levels had significant negative correlations with the cholesterol levels in the small and very small LDL subclasses. The total and HMW adiponectin levels had significant positive correlations with the cholesterol levels in the very large, large and medium HDL subclasses. The LMW adiponectin levels were significantly correlated with the cholesterol levels in the medium HDL subclasses.

The total and HMW adiponectin levels had significant negative correlations with the TG levels in all VLDL and LDL subclasses (with the exception of the large LDL subclass for HMW). The total and HMW adiponectin levels had significant positive correlations with the TG levels in the large HDL subclasses, and negative correlations with the TG levels in small HDL subclasses. In addition, the total adiponectin levels negatively correlated with the TG levels in very small HDL subclasses. The other relationships between the levels of each adiponectin form and the lipoprotein profiles were either weak ($r < 0.200$) or not significant ($p > 0.05$). Taken together, these findings indicate that

the HMW, but not the MMW or LMW, adiponectin behaves like total adiponectin in terms of the relationships with the lipoprotein profiles.

The Correlations between the HMW Adiponectin Levels and LDL and HDL Particle Diameters

As shown in **Fig. 2**, the HMW adiponectin levels exhibited significant positive correlations with the LDL ($r = 0.30$, $p < 0.001$) and HDL ($r = 0.40$, $p < 0.001$) particle diameters as measured by HPLC. The total adiponectin levels were also correlated with the LDL ($r = 0.31$, $p < 0.001$) and HDL ($r = 0.43$, $p < 0.001$) particle diameters. In contrast, neither the MMW nor LMW adiponectin levels were significantly correlated with the LDL and HDL particle diameters. After adjusting for age, waist circumference, TG levels, HDL cholesterol levels, FPG levels and the BP, the HMW adiponectin levels, but not the total adiponectin, were still significantly correlated with decreased LDL and HDL particle diameters ($p = 0.02$ for both). The total adiponectin levels only showed a significant correlation with a decreased HDL diameter after adjustment for these confounding factors ($p = 0.04$).

Discussion

In the present study, we showed that both decreased

Table 2. Correlations of the total, HMW, MMW and LMW adiponectin levels with the detailed lipoprotein profiles measured by HPLC

	Total adiponectin		HMW adiponectin		MMW adiponectin		LMW adiponectin	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
CM cholesterol	-0.138	0.054	-0.125	0.082	0.006	0.935	-0.126	0.078
VLDL cholesterol								
Large VLDL	-0.041	0.566	-0.007	0.928	-0.057	0.429	-0.022	0.756
Medium VLDL	-0.033	0.646	0.033	0.648	-0.073	0.314	0.009	0.895
Small VLDL	0.015	0.837	0.101	0.161	-0.055	0.443	0.035	0.628
LDL cholesterol								
Large LDL	0.064	0.374	0.074	0.307	-0.073	0.308	0.043	0.550
Medium LDL	-0.129	0.072	-0.111	0.123	-0.181	0.011	0.014	0.846
Small LDL	-0.281	<0.001	-0.242	<0.001	-0.230	0.001	-0.047	0.517
Very small LDL	-0.282	<0.001	-0.211	0.003	-0.203	0.005	-0.092	0.203
HDL cholesterol								
Very large HDL	0.356	<0.001	0.365	<0.001	0.159	0.026	0.131	0.067
Large HDL	0.426	<0.001	0.380	<0.001	0.156	0.030	0.224	0.002
Medium HDL	0.279	<0.001	0.246	<0.001	0.024	0.735	0.177	0.013
Small HDL	-0.012	0.871	-0.010	0.891	-0.152	0.034	0.077	0.285
Very small HDL	0.095	0.187	0.150	0.037	-0.026	0.721	0.034	0.642
CM triglycerides	-0.194	0.007	-0.160	0.025	-0.035	0.626	-0.141	0.050
VLDL triglycerides								
Large VLDL	-0.295	<0.001	-0.238	<0.001	-0.164	0.022	-0.125	0.081
Medium VLDL	-0.358	<0.001	-0.261	<0.001	-0.182	0.011	-0.157	0.029
Small VLDL	-0.296	<0.001	-0.200	0.005	-0.172	0.016	-0.152	0.033
LDL triglycerides								
Large LDL	-0.243	<0.001	-0.197	0.006	-0.170	0.017	-0.118	0.100
Medium LDL	-0.295	<0.001	-0.266	<0.001	-0.169	0.018	-0.128	0.075
Small LDL	-0.287	<0.001	-0.258	<0.001	-0.129	0.072	-0.136	0.058
Very small LDL	-0.259	<0.001	-0.213	0.003	-0.091	0.208	-0.166	0.021
HDL triglycerides								
Very large HDL	-0.028	0.699	0.005	0.945	0.006	0.929	-0.085	0.236
Large HDL	0.280	<0.001	0.257	<0.001	0.129	0.072	0.116	0.106
Medium HDL	-0.130	0.071	-0.107	0.136	-0.046	0.522	-0.101	0.160
Small HDL	-0.233	0.001	-0.228	0.001	-0.100	0.164	-0.113	0.116
Very small HDL	-0.231	0.001	-0.187	0.009	-0.061	0.400	-0.155	0.031

HMW=high molecular weight, MMW=medium molecular weight, LMW=low molecular weight, CM=Chylomicron, VLDL=Very low-density lipoprotein, HDL=High-density lipoprotein, LDL=Low-density lipoprotein.

total and HMW, but not MMW and LMW, adiponectin levels correlated with the atherogenic lipoprotein profiles in healthy Japanese males, including the following: 1) increased cholesterol levels in small LDL subclasses; 2) decreased cholesterol levels in larger HDL subclasses; 3) increased TGs in almost all VLDL and LDL subclasses; 4) decreased TGs in large HDL classes and increased TGs in small HDL subclasses. In addition, using a multivariate analysis, we demonstrated that the HMW adiponectin level is an independent predictor of the small LDL and HDL levels

in the males in the general population.

Metabolic syndrome is characterized by a typical atherogenic lipoprotein profile, which includes increased small LDL particles, high TG levels in large VLDL subclasses and low cholesterol levels in large HDL subclasses^{18, 19}. A previous study in a small cohort of patients demonstrated that increased adiponectin levels (both total and HMW) were associated with large HDL and small LDL in patients with insulin resistance and diabetes mellitus²². Under hyperinsulinemic conditions, the total adiponectin levels decrease, pri-

Fig. 2. The correlation of the high molecular weight (HMW) adiponectin levels with the LDL particle diameter (left panel) and HDL particle diameter (right panel).

marily due to a reduction in the HMW form, whereas the LMW forms are not significantly affected¹¹). Therefore, the decreased HMW adiponectin levels caused by hyperinsulinemia were associated with atherogenic lipoprotein profiles in patients with metabolic syndrome. On the other hand, the HMW adiponectin levels were associated with the LDL and HDL particle diameters, independent of metabolic syndrome, suggesting that different mechanism(s) link the adiponectin levels to the atherogenic lipoprotein profile.

In this study, the total and HMW adiponectin levels were negatively associated with the TGs in the VLDL and LDL subclasses. Because TGs from the core of large VLDL are exchanged with the cholesterol ester in the core of LDL by a cholesterol ester transport protein, the LDL particle becomes depleted of cholesterol and enriched with TGs. Such a large LDL particle may finally be transformed into a smaller, denser LDL particle by hydrolysis of its core TG²⁹). Adiponectin-overexpressing mice exhibit decreased large and medium VLDL particle levels, with elevated post-heparin plasma lipoprotein lipase (LPL) activity. Adiponectin also increases the LDL and VLDL receptor expression in skeletal muscle³⁰). Furthermore, increased adiponectin levels in patients with and without diabetes mellitus were demonstrated to be inversely associated with the post-heparin plasma hepatic lipase activity, which produces small, dense LDL by hydrolysis of the core TG³¹). Therefore, the total and HMW adiponectin may induce low TG levels in VLDL and

decrease the small, dense LDL through increased VLDL-TG catabolism and decreased hepatic lipase activity.

Another important finding of our study is that both the total and HMW adiponectin levels were positively correlated with the cholesterol levels in the larger HDL subclass, which has been identified in several studies as the most cardioprotective HDL component^{18, 19, 32}). The cholesterol ester transport protein can mediate an exchange of cholesterol ester on HDL for the VLDL-TG^{27, 29}). Large amounts of VLDL-TG with decreased adiponectin levels increased the TG and decreased the cholesterol levels on HDL, which is more likely to be taken up and catabolized by the kidneys³³). In addition, adiponectin upregulates the expression of ATP-binding cassette transporter 1 in human macrophages, and also upregulates the apolipoprotein A1-mediated cholesterol efflux from macrophages by the activation of the liver X receptor α and peroxisome proliferator-activated receptor γ ^{34, 35}). HMW adiponectin was demonstrated to reduce the cholesterol accumulation in THP-1 foam cells compared with LMW adiponectin in a previous study³⁵). These biological activities of adiponectin may contribute to increased cholesterol levels on larger HDL subclasses.

Limitations

Because this was a cross-sectional study, we could not completely evaluate the mechanistic links between HMW adiponectin and the atherogenic lipoprotein

profiles. We also did not directly examine the activity of enzymes that may correlate with the lipoprotein profiles, including LPL, hepatic lipase and cholesterol ester transport protein. Future studies are needed to clarify the effects of each adiponectin isoform on the specific lipoprotein profiles and enzymes by intervening with various risk reduction therapies. In this study, we enrolled only males to avoid a potential confounding factor, because the serum adiponectin levels in females are generally higher than those in males²⁰. Therefore, future studies are needed to clarify the association between adiponectin isoforms and lipoprotein profiles in females.

Conclusion

Among the three isoforms of adiponectin, the HMW levels were primarily associated with the atherogenic lipoprotein profiles, which were consistent with the association with the total adiponectin level, in healthy Japanese male subjects. In addition, increased HMW adiponectin levels were positively correlated with small LDL and HDL particles, independent of other known MS components. In conclusion, the plasma HMW adiponectin levels appear to be useful as a biomarker for identifying the atherogenic lipoprotein profiles in apparently healthy males.

Conflicts of Interest

None.

References

- 1) Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to c1q, produced exclusively in adipocytes. *J Biol Chem*, 1995; 270: 26746-26749
- 2) Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K: Cdna cloning and expression of a novel adipose specific collagen-like factor, apm1 (adipose most abundant gene transcript 1). *Biochem Biophys Res Commun*, 1996; 221: 286-289
- 3) Shimada K, Miyazaki T, Daida H: Adiponectin and atherosclerotic disease. *Clin Chim Acta*, 2004; 344: 1-12
- 4) Lara-Castro C, Fu Y, Chung BH, Garvey WT: Adiponectin and the metabolic syndrome: Mechanisms mediating risk for metabolic and cardiovascular disease. *Curr Opin Lipidol*, 2007; 18: 263-270
- 5) Wang Y, Lam KS, Chan L, Chan KW, Lam JB, Lam MC, Hoo RC, Mak WW, Cooper GJ, Xu A: Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. *J Biol Chem*, 2006; 281: 16391-16400
- 6) Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T: Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care*, 2006; 29: 1357-1362
- 7) Inoue T, Kotooka N, Morooka T, Komoda H, Uchida T, Aso Y, Inukai T, Okuno T, Node K: High molecular weight adiponectin as a predictor of long-term clinical outcome in patients with coronary artery disease. *Am J Cardiol*, 2007; 100: 569-574
- 8) Liu Y, Retnakaran R, Hanley A, Tungtrongchit R, Shaw C, Sweeney G: Total and high molecular weight (hmw) but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in thai subjects. *J Clin Endocrinol Metab*, 2007
- 9) Seino Y, Hirose H, Saito I, Itoh H: High molecular weight multimer form of adiponectin as a useful marker to evaluate insulin resistance and metabolic syndrome in japanese men. *Metabolism*, 2007; 56: 1493-1499
- 10) Hirose H, Yamamoto Y, Seino-Yoshihara Y, Kawabe H, Saito I: Serum high-molecular-weight adiponectin as a marker for the evaluation and care of subjects with metabolic syndrome and related disorders. *J Atheroscler Thromb*, 2010; 17: 1201-1211
- 11) Basu R, Pajvani UB, Rizza RA, Scherer PE: Selective downregulation of the high molecular weight form of adiponectin in hyperinsulinemia and in type 2 diabetes: Differential regulation from nondiabetic subjects. *Diabetes*, 2007; 56: 2174-2177
- 12) Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, Okuno T, Inoue T, Node K, Tobe T, Inukai T, Nakano Y: Comparison of serum high-molecular weight (hmw) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect hmw adiponectin. *Diabetes*, 2006; 55: 1954-1960
- 13) Saito I, Yamagishi K, Chei CL, Cui R, Ohira T, Kitamura A, Kiyama M, Imano H, Okada T, Kato T, Hitsumoto S, Ishikawa Y, Tanigawa T, Iso H: Total and high molecular weight adiponectin levels and risk of cardiovascular disease in individuals with high blood glucose levels. *Atherosclerosis*, 2013; 229: 222-227
- 14) Pischon T, Hu FB, Girman CJ, Rifai N, Manson JE, Rexrode KM, Rimm EB: Plasma total and high molecular weight adiponectin levels and risk of coronary heart disease in women. *Atherosclerosis*, 2011; 219: 322-329
- 15) Alexander CM, Landsman PB, Teutsch SM, Haffner SM: Ncep-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among nhanes iii participants age 50 years and older. *Diabetes*, 2003; 52: 1210-1214
- 16) Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J: Metabolic syndrome with and without c-reactive protein as a predictor of coronary heart disease and diabetes in the west of scotland coronary prevention study. *Circulation*, 2003; 108: 414-419
- 17) Kasai T, Miyauchi K, Kurata T, Ohta H, Okazaki S,

- Miyazaki T, Kajimoto K, Kubota N, Daida H: Prognostic value of the metabolic syndrome for long-term outcomes in patients undergoing percutaneous coronary intervention. *Circ J*, 2006; 70: 1531-1537
- 18) Otvos JD, Jeyarajah EJ, Cromwell WC: Measurement issues related to lipoprotein heterogeneity. *Am J Cardiol*, 2002; 90: 22i-29i
- 19) Miyazaki T, Shimada K, Sato O, Kotani K, Kume A, Sumiyoshi K, Sato Y, Ohmura H, Watanabe Y, Mokuno H, Daida H: Circulating malondialdehyde-modified ldl and atherogenic lipoprotein profiles measured by nuclear magnetic resonance spectroscopy in patients with coronary artery disease. *Atherosclerosis*, 2005; 179: 139-145
- 20) Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE: Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: Evidence for independent roles of age and sex. *Diabetologia*, 2003; 46: 459-469
- 21) Okada T, Saito E, Kuromori Y, Miyashita M, Iwata F, Hara M, Harada K: Relationship between serum adiponectin level and lipid composition in each lipoprotein fraction in adolescent children. *Atherosclerosis*, 2006; 188: 179-183
- 22) Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT: Adiponectin multimeric complexes and the metabolic syndrome trait cluster. *Diabetes*, 2006; 55: 249-259
- 23) Alberti KG, Zimmer P, Shaw J: The metabolic syndrome--a new worldwide definition. *Lancet*, 2005; 366: 1059-1062
- 24) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 1985; 28: 412-419
- 25) Katsuki A, Suematsu M, Gabazza EC, Murashima S, Nakatani K, Togashi K, Yano Y, Sumida Y: Decreased high-molecular weight adiponectin-to-total adiponectin ratio in sera is associated with insulin resistance in Japanese metabolically obese, normal-weight men with normal glucose tolerance. *Diabetes Care*, 2006; 29: 2327-2328
- 26) Usui S, Hara Y, Hosaki S, Okazaki M: A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by hplc. *J Lipid Res*, 2002; 43: 805-814
- 27) Miyazaki T, Shimada K, Miyauchi K, Kume A, Tanimoto K, Kiyanagi T, Sumiyoshi K, Hiki M, Mokuno H, Okazaki S, Sato H, Kurata T, Daida H: Effects of fenofibrate on lipid profiles, cholesterol ester transfer activity, and intimal hyperplasia in patients after elective coronary stenting. *Lipids Health Dis*, 2010; 9: 122
- 28) Matsumori R, Miyazaki T, Shimada K, Kume A, Kitamura Y, Oshida K, Yanagisawa N, Kiyanagi T, Hiki M, Fukao K, Hirose K, Ohsaka H, Mokuno H, Daida H: High levels of very long-chain saturated fatty acid in erythrocytes correlates with atherogenic lipoprotein profiles in subjects with metabolic syndrome. *Diabetes Res Clin Pract*, 2013; 99: 12-18
- 29) Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR: Cholesteryl ester transfer protein: A novel target for raising hdl and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol*, 2003; 23: 160-167
- 30) Qiao L, Zou C, van der Westhuyzen DR, Shao J: Adiponectin reduces plasma triglyceride by increasing vldl triglyceride catabolism. *Diabetes*, 2008; 57: 1824-1833
- 31) Schneider JG, von Eynatten M, Schiekofer S, Nawroth PP, Dugi KA: Low plasma adiponectin levels are associated with increased hepatic lipase activity in vivo. *Diabetes Care*, 2005; 28: 2181-2186
- 32) Soedamah-Muthu SS, Colhoun HM, Thomason MJ, Betteridge DJ, Durrington PN, Hitman GA, Fuller JH, Julier K, Mackness MI, Neil HA, Investigators C: The effect of atorvastatin on serum lipids, lipoproteins and nmr spectroscopy defined lipoprotein subclasses in type 2 diabetic patients with ischaemic heart disease. *Atherosclerosis*, 2003; 167: 243-255
- 33) Kwiterovich PO Jr: Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity. *Am J Cardiol*, 2002; 90: 30i-47i
- 34) Tsubakio-Yamamoto K, Matsuura F, Koseki M, Oku H, Sandoval JC, Inagaki M, Nakatani K, Nakaoka H, Kawase R, Yuasa-Kawase M, Masuda D, Ohama T, Maeda N, Nakagawa-Toyama Y, Ishigami M, et al: Adiponectin prevents atherosclerosis by increasing cholesterol efflux from macrophages. *Biochem Biophys Res Commun*, 2008; 375: 390-394
- 35) Tian L, Luo N, Klein RL, Chung BH, Garvey WT, Fu Y: Adiponectin reduces lipid accumulation in macrophage foam cells. *Atherosclerosis*, 2009; 202: 152-161