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Research Article

Changes in the Distribution of Adiponectin Isoforms within Total Adiponectin in Japanese Subjects with Abdominal Obesity and their Relation to Insulin Resistance and Metabolic Syndrome

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Abstract

Changes in the distribution of adiponectin isoforms, especially middle- and low-molecular-weight (MMW, LMW) adiponectin (Ad), within total adiponectin (T-Ad) in subjects with abdominal obesity (AO) and how these changes might be related to insulin resistance (IR) and metabolic syndrome (MS) are not yet well-known. In the present study, we examined these changes and their relations to IR and MS. We compared the serum Ad-related parameters in a group of 283 subjects with AO and 485 subjects without AO (1st cohort). In addition, we also examined the associations between the Ad-related parameters and the HOMA-IR in a 2nd cohort, consisting of subjects of the 1st cohort except for those with diabetes. In the 1st cohort, in the subjects with AO as compared with those without AO, the distribution of Ad isoforms within T-Ad changed, with a lower percentage of high-molecular-weight (HMW)-Ad and higher percentage of MMW-Ad and LMW-Ad, resulting in higher MMW-Ad/HMW-Ad and LMW-Ad/HMW-Ad ratios (P values <0.01 for all). Among these changes, the increased LMW-Ad/T-Ad and LMW-Ad/HMW-Ad ratios tended to be positively associated with MS ($P = 0.07$). In the 2nd cohort, similar to the case for MS, these ratios were also significantly positively correlated with the HOMA-IR (P values <0.01 for all), independent of the BMI. The current investigation demonstrated changes in the distribution of the Ad isoforms, especially increase in the LMW-Ad/T-Ad and LMW-Ad/HMW-Ad ratios, in subjects with AO, and significant BMI-independent association of these changes with the severity of IR and risk of development of MS.

Keywords: Low-molecular-weight adiponectin; Insulin resistance; Metabolic syndrome

Introduction

Metabolic syndrome (MS) is a condition in which visceral fat accumulates, insulin resistance (IR) develops, and risk factors for atherosclerosis (high blood pressure (BP), dyslipidemia, and hyperglycemia) accumulate [1-3]. The prevalence of MS has been growing globally and individuals with MS are at a higher risk of developing type 2 diabetes (T2D) and cardiovascular disease [1-3]. Since many studies have demonstrated that the waist circumference (WC) bears a close relationship with the visceral fat area as assessed by computed tomography [4,5], visceral fat accumulation has also come to be called abdominal obesity (AO) [6]. AO is known to be associated with altered serum levels of adipokines (cytokines secreted by adipocytes), including decrease in the serum levels of adiponectin (Ad) and increases in the serum levels of TNF-α and IL-6 [7-9]. These changes are thought to predispose to IR, resulting in hyperglycemia, dyslipidemia due to abnormal lipoprotein lipase, and elevated BP due to accelerated renal sodium reabsorption in subjects with AO [10,11].

Adiponectin is known to exist in three isoforms in the blood: high-molecular-weight (HMW) oligomers, middle-molecular-weight (MMW) hexamers, and low-molecular- weight (LMW) trimers [12,13]. The three isoforms are collectively called total adiponectin (T-Ad), described above as "adiponectin". Among the three isoforms, HMW-Ad is considered as being the most biologically active isoform from the viewpoint of the maintenance of insulin sensitivity [14,15], and a cross-sectional study reported the decrease in its blood levels and its ratio to T-Ad in subjects with AO and MS [16]. Furthermore, according to a report from a longitudinal study, the serum level of HMW-Ad and serum HMW-Ad/T-Ad ratio are useful parameters for predicting the risk of MS [17]. On the other hand, a small number of reports have also shown that the levels of other isoforms, namely, MMW-Ad and LMW-Ad, are decreased in subjects with AO or obesity [18,19]. However, there are few reports on the changes in the MMW-Ad/T-Ad and LMW-Ad/T-Ad ratios in subjects with AO and the associations of these changes with the severity of IR and risk of MS.

Based on the above background, in the present study, we enrolled subjects with and without AO, and under a cross-sectional study design, examined the changes in the levels and percentage of MMW-Ad and LMW-Ad among the Ad isoforms in subjects with AO as compared to subjects without AO, and how these changes might be related to the severity of IR and risk of development of MS.

Materials and Methods

Participants and criteria for abdominal obesity and metabolic syndrome

We enrolled a study cohort conducted between 2008 and 2017; this cohort consisted of 1018 patients with T2D and 1003 non-diabetic control individuals (CON) who had participated in our previously performed genome-wide association study examining genetic loci associated with type 2 diabetes in the Japanese population [20,21]. Among the subjects of this cohort, we randomly measured the levels of the three Ad isoforms in 495 T2D patients and 374 CON subjects (Supplementary figure). Since it has been reported that thiazolidinediones (TZDs) such as pioglitazone can increase the serum Ad level by up to about twofold (as compared with the levels in subjects not taking TZDs) [22-24], we excluded 101 subjects with T2D who were receiving pioglitazone. Finally, a total of 768 subjects, including 394 T2D patients and 374 CON subjects, were recruited for the present study as the 1st cohort (Supplementary figure). The 768 subjects of the 1st cohort were then classified as having (AO group, n = 285) or not having (non-AO group, n = 483) AO according to the Japanese WC criteria for the diagnosis of MS (WC of 85 cm or more for men and 90 cm or more for women) [11], and the levels of the three Ad isoforms and their relative ratios to the levels of T-Ad and other isoforms were compared between the two groups (Supplementary figure). The clinical characteristics of the subjects in the AO-group and non-AO group (1st cohort) are shown in Table 1.

	AO group	Non-AO group	*P* value
n	285	483	
Sex (M/F)	193/ 92	220/ 263	<0.0001*
Age (years)	64.6±11.0	66.3±8.9	<0.05
BMI (kg/m^2)	26.6±3.2	21.9±2.2	<0.0001
Waist circumference (cm)	93.5±6.3	78.8±5.9	<0.0001
Presence of metabolic syndrome (%)	76.8	0	<0.0001*
S-Cre (mg/dL)	0.85±0.37	0.73±0.20	<0.0001
Systolic blood pressure (mmHg)	130.7±16.9	128.7±16.7	0.114
Diastolic blood pressure (mmHg)	77.4±11.5	75.7±11.2	<0.05
Use of antihypertensive agent (%)	60	38	<0.0001*
Presence of high blood pressure (%)	79.4	61.1	<0.0001*
HDL-cholesterol (mg/dl)	51.5±14.5	60.8±17.5	<0.0001
Triglyceride (mg/dl)	141.6±95.5	100.8±55.0	<0.0001
Use of lipid lowering drug (%)	40.9	31.2	<0.01
Presence of dyslipidemia (%)	67.4	42.7	<0.0001*
FPG (mg/dl)	123.8±37.3	112.8±28.6	<0.0001
HOMA-IR	2.38±1.83	1.27±0.88	<0.0001
HbA1c (NGSP value) (%)	6.99±1.60	6.34±1.32	<0.0001
Use of antidiabetic drug (%)	56.5	35.4	<0.0001*
Presence of hyperglycemia (%)	68.8	45.8	<0.0001*
Presence of diabetes (%)	64.6	43.5	<0.0001*

Data are means ± SD. * Pearson's chi-square test. BMI, body mass index; S-Cre, serum levels of creatinine; HDL-cholesterol, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulin resistance

Table 1: Clinical profiles of the study subjects in the 1st cohort.

Next, to evaluate the association of the adiponectin-related parameters with the homeostasis model assessment of IR (HOMA-IR), which is an index of IR, 394 patients with T2D in the 1st cohort were excluded since antidiabetic medications such as sulfonylureas (SU) and DPP4 inhibitors, etc. could affect the serum insulin levels. In addition, we also excluded 24 CON subjects whose serum insulin levels were unavailable. Finally, the above-mentioned associations were analyzed in 350 CON subjects (2nd cohort). The clinical characteristics of the subjects of the 2nd cohort are shown in Supplementary Table.

The inclusion criteria for CON and exclusion criteria for T2D have been described in our previous reports [20,21]. Diabetes was diagnosed based on the 1998 American Diabetes Association Criteria [25]. The study subjects were diagnosed as having MS, if in addition to having AO, they had two or more of the following three components according to the Japanese criteria [11]: (a) hyperglycemia: fasting plasma glucose (FPG) ≥110 mg/dl and/or receiving medication for diabetes; (b) high BP: systolic BP ≥130 mmHg, and/or diastolic BP ≥85 mmHg and/or taking antihypertensive medication; and c) dyslipidemia: serum high-density lipoprotein cholesterol (HDL-c) <40 mg/dl, and/or serum triglycerides (TG) ≥150 mg/dl, and/or taking lipid-lowering drugs. Then, we also examined the association of each of the adiponectin-related parameters with the odds ratio (OR) for MS in the 1st cohort.

All the study procedures were approved by the Ethics Committee of the University of Toyama, and written informed consent was obtained from all the study subjects.

Collection of clinical information

The anthropometric measurements were conducted with the individuals wearing light clothing and not wearing shoes. The body height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively; the BMI was calculated from these measurements. The WC was measured at the end of normal expiration to the nearest 0.1 cm, using a flexible anthropometric tape at the level of the umbilicus. BP was measured twice using an automated device, with the subject in a sitting position after he/she had rested for at least 5 minutes. In addition, we obtained clinical information, including that related to the use of antidiabetic drugs, antihypertensive agents, and lipid-lowering drugs, from the medical records of the subjects and self-reported questionnaires. We also examined the fasting blood chemistry parameters (including FPG, HbA1c, plasma insulin, and serum TG, HDL-c, and creatinine). The HbA1c level was measured by a high-performance liquid chromatography method and expressed as the international standard value, i.e., HbA1c (1.02 × Japan Diabetes Society [JDS (%)] + 0.25%), as defined by the JDS [26]. The HOMA-IR was calculated as previously reported [27].

Adiponectin measurement

Serum levels of the three Ad isoforms (HMW-Ad, MMW-Ad, and LMW-Ad) were measured by enzyme-linked immunosorbent assay (ELISA) (SEKISUI Medical Co. Ltd., Japan) [13,23,24,28]. The dynamic range of this ELISA method was 0.075-4.8 ng/mL. The intra-assay coefficients of variation were 5.3% (for T-Ad), 4.1% (for MMW+HMW), and 3.3% (for HMW).

Statistical analysis

Categorical data are expressed as percentages, while continuous data are expressed as the means ± SD. The statistical analyses were performed using JMP for Windows, Version 14.0 (SAS Institute, Cary, NC, USA). The normality of distribution was checked using the skewed score, and variables with skewed distributions were logarithmically (naturally) transformed for the subsequent analyses. Differences in continuous variables pertaining to the clinical features and adiponectin-related parameters between the AO group and non-AO group were examined using the Student *t*-test and a multiple logistic regression analysis with adjustments for confounding factors (Tables 1 and 2). The ORs for MS or each component of MS according to the adiponectin-related parameters were calculated by logistic regression analysis with adjustments for confounding factors (Table 4 and 5). The associations of the various adiponectin-related parameters with the HOMA-IR were examined through calculating the β values using multiple linear regression analysis, with adjustments for related confounding factors (Table 3). As confounding factors, we mainly included age, sex, BMI, serum creatinine, and presence/absence of diabetes mellitus, because these factors have been shown to affect the risk of development of MS and/or the serum levels of the three Ad isoforms/ ratios of the levels of each isoform to the serum T-Ad, as previously reported [14,24,28-33]. Results with *P* values of <0.05 were considered as being statistically significant.

Results

In the 1st cohort, the percentage of male subjects, the mean BMI, and the mean WC were significantly higher in the AO group as compared with the non-AO group (Table 1). Furthermore, as compared with the non-AO group, the AO group showed a significantly higher diastolic BP, serum TG level, and FPG, significantly lower serum HDL-c, and a significantly higher percentage of subjects with high BP, dyslipidemia, and hyperglycemia. The percentages of subjects who were receiving medications for hypertension, dyslipidemia and/or diabetes, and percentage of subjects diagnosed as having diabetes were significantly higher in the AO group than in the non-AO group (Table 1). In addition, 76.8% (220 subjects) of subjects in the AO group met the diagnostic criteria for MS.

Next, we compared the various adiponectin-related parameters between the AO group and non-AO group (Table 2). The serum levels of HMW-Ad, MMW-Ad, and LMW-Ad were significantly lower in the AO group than in the non-AO group, even after adjustments for confounding factors (HMW-Ad: 2.2 ± 1.8 μg/mL vs. 3.5 ± 2.4 μg/mL, $P < 0.0001$; MMW-Ad: 1.2 ± 0.7 μg/mL vs. 1.6 ± 0.9 μg/mL, $P < 0.0001$; LMW-Ad: 1.7 ± 0.7 μg/mL vs. 2.2 ± 1.0 μg/mL; $P < 0.0001$) (Table 2). In regard to the ratios of each adiponectin isoform to the serum T-Ad or serum levels of other isoforms, the HMW-Ad/T-Ad (H/T) ratio was significantly lower, while the MMW-Ad/T-Ad (M/T), LMW-Ad/T-Ad (L/T), MMW-Ad/HMW-Ad (M/H), and LMW-Ad/HMW-Ad (L/H) ratios were significantly higher in the AO group as compared with the non-AO group, even after adjustments for confounding factors (H/T ratio: 0.38 ± 0.13 vs. 0.44 ± 0.13, $P < 0.0001$; M/T ratio: 0.24 ± 0.07 vs. 0.22 ± 0.07, $P < 0.01$; L/T ratio: 0.38 ± 0.12 vs. 0.33 ± 0.11, $P < 0.001$; M/H ratio : 0.79 ± 0.57 vs. 0.61 ± 0.49, $P < 0.001$; L/H ratio: 1.36 ± 1.29 vs. 0.95 ± 0.87, $P < 0.001$) (Table 2).

	AO group	Non-AO group	*P* value	*P* values* Multivariate
Total-Ad (μg/mL)[a]	5.10 ± 2.86	7.27± 3.66	<0.0001	<0.0001
HMW-Ad (μg/mL)[a]	2.19 ± 1.81	3.52 ± 2.41	<0.0001	<0.0001
MMW-Ad (μg/mL)[a]	1.18 ± 0.67	1.56 ± 0.86	<0.0001	<0.0001
LMW-Ad (μg/mL)[a]	1.74 ± 0.73	2.18 ± 1.00	<0.0001	<0.0001
H/T ratio	0.38 ± 0.13	0.44 ± 0.13	<0.0001	<0.0001
M/T ratio	0.24 ± 0.07	0.22 ± 0.07	<0.01	<0.01
L/T ratio	0.38 ± 0.12	0.33± 0.11	<0.0001	<0.001
M/H ratio[a]	0.79 ± 0.57	0.61 ± 0.49	<0.0001	<0.001
L/H ratio[a]	1.36 ± 1.29	0.95 ± 0.87	<0.0001	<0.001

[a] Parameters were transformed logarithmically when compared using the Student t-test and a multiple logistic regression analysis.
*Multivariate *P* values were adjusted for sex, age, serum creatinine level, and the presence or absence of diabetes mellitus.

Table 2: Comparison of the various adiponectin-related parameters between AO group and non-AO group in the 1st cohort.

We next investigated the associations of the various adiponectin-related parameters with the HOMA-IR in the 2nd cohort by multiple regression analysis with adjustments for confounding factors, including the BMI (Table 3). The serum levels of all the adiponectin isoforms and the H/T ratio were significantly and negatively associated with the HOMA-IR (β ln- HOMA-IR for HMW-Ad = -0.15, SE = 0.04, $P < 0.0001$; β ln- HOMA-IR for MMW-Ad = -0.18, SE = 0.05, $P < 0.001$; β ln- HOMA-IR for LMW-Ad = -0.12, SE = 0.06, $P < 0.05$; β ln- HOMA-IR for H/T ratio = -0.63, SE = 0.23, $P < 0.01$), while the L/T and L/H ratios were significantly and positively associated with the HOMA-IR (β ln- HOMA-IR for L/T ratio = 0.73, SE = 0.25, $P < 0.01$; β ln- HOMA-IR for L/H ratio = 0.12, SE = 0.04, $P < 0.01$) (Table 3). The M/T and M/H ratios were not associated with the HOMA-IR (Table 3).

Index-Level	β†	SE	*P* value
Total-Ad[a]	-0.28	0.06	<0.0001
HMW-Ad[a]	-0.15	0.04	<0.0001
MMW-Ad[a]	-0.18	0.05	<0.001
LMW-Ad[a]	-0.12	0.06	<0.05
Index-Ratio	β†	SE	*P* value
H/T ratio	-0.63	0.23	<0.01
M/T ratio	0.12	0.36	0.735

L/T ratio	0.73	0.25	<0.01
M/H ratio[a]	0.07	0.04	0.134
L/H ratio[a]	0.12	0.04	<0.01

[a] Parameters were transformed logarithmically before analysis.
† regression coefficient adjusted for age, sex, log BMI, serum creatinine level, and the presence of diabetes mellitus.

Table 3: Association of adiponectin-related parameters with HOMA-IR in the 2nd cohort.

Next, the associations of the various adiponectin-related parameters with the risk for MS were investigated by logistic regression analysis with adjustments for confounding factors, including the BMI (Table 4). The serum levels of all the adiponectin isoforms were significantly and negatively associated with MS (HMW-Ad: OR = 0.67 [95% CI, 0.51-0.88], $P < 0.01$; MMW-Ad: OR = 0.51 [95% CI, 0.33-0.78], $P < 0.01$; LMW-Ad: OR = 0.39 [95% CI, 0.21-0.71], $P < 0.01$). On the other hand, in regard to the associations of the aforementioned ratios with the risk for MS, although the H/T ratio tended to be negatively associated, and the L/T and L/H ratios tended to be positively associated with the risk for MS (H/T ratio: OR = 0.18 [95% CI, 0.03-1.02], $P = 0.054$; L/T ratio: OR = 5.78 [95% CI, 0.78-43.72], $P = 0.085$; L/H ratio: OR = 1.36 [95% CI, 0.97-1.91], $P = 0.070$) (Table 4), the associations did not reach statistical significance.

Index-Level	OR	95%CI	*P* value
Total-Ad [a]	0.48	(0.30-0.75)	<0.01
HMW-Ad [a]	0.67	(0.51-0.88)	<0.01
MMW-Ad [a]	0.51	(0.33-0.78)	<0.01
LMW-Ad [a]	0.39	(0.21-0.71)	<0.01
Index-Ratio	OR	95%CI	*P* value
H/T ratio	0.18	(0.03-1.03)	0.054
M/T ratio	3.77	(0.12-120.8)	0.453
L/T ratio	5.78	(0.78-43.7)	0.085
M/H ratio[a]	1.29	(0.88-1.89)	0.196
L/H ratio[a]	1.36	(0.97-1.91)	0.070

OR, odds ratio: CI, confidence interval
[a] Parameters were transformed logarithmically before analysis. Multivariate *P* values were adjusted for sex, age, log BMI, serum creatinine level, and the presence of diabetes mellitus.

Table 4: Odds ratios of adiponectin-related parameters for metabolic syndrome in the 1st cohort.

To examine the reason why the association with MS differed between the serum levels and serum ratios of the adiponectin-related parameters, we investigated the associations of the various adiponectin-related parameters with each component of MS by logistic regression analysis (Table 5). The results revealed that the serum levels of all the adiponectin isoforms and the H/T ratio were significantly and negatively associated, while the L/T ratio and L/H ratio were significantly and positively associated with hyperglycemia (Table 5). On the other hand, although the serum levels of all adiponectin isoforms were significantly and negatively associated with dyslipidemia, the ratios of the serum levels of the adiponectin isoforms to the serum T-Ad or levels of other isoforms were not significantly associated with dyslipidemia (Table 5). None of the adiponectin-related parameters examined was associated with high BP.

OR for hyperglycemia			
Index-Level	OR	95%CI	*P* value[#]
Total-Adiponectin [a]	0.26	(0.19-0.38)	<0.0001
HMW-Adiponectin [a]	0.52	(0.42-0.65)	<0.0001
MMW-Adiponectin [a]	0.39	(0.29-0.54)	<0.0001
LMW-Adiponectin [a]	0.26	(0.17-0.40)	<0.0001
Index-Ratio	OR	95%CI	*P* value[#]
HMW/Total	0.14	(0.04-0.51)	<0.01
MMW/Total	2.76	(0.28-27.6)	0.39
LMW/Total	8.09	(1.95-33.5)	<0.01
MMW/HMW[a]	1.47	(1.12-1.94)	<0.01
LMW/HMW[a]	1.45	(1.14-1.84)	<0.01
OR for dyslipidemia			
Index-Level	OR	95%CI	*P* value[†]
Total-Adiponectin [a]	0.42	(0.30-0.60)	<0.0001
HMW-Adiponectin [a]	0.66	(0.53-0.82)	<0.001
MMW-Adiponectin [a]	0.50	(0.36-0.68)	<0.0001
LMW-Adiponectin [a]	0.37	(0.24-0.56)	<0.0001
Index-Ratio	OR	95%CI	*P* value[†]
HMW/Total	0.36	(0.10-1.32)	0.12
MMW/Total	1.38	(0.13-14.2)	0.79
LMW/Total	3.01	(0.71-12.8)	0.13
MMW/HMW[a]	1.19	(0.90-1.56)	0.22
LMW/HMW[a]	1.21	(0.95-1.54)	0.12
OR for high blood pressure			
Index-Level	OR	95%CI	*P* value[†]
Total-Adiponectin [a]	0.90	(0.63-1.28)	0.55
HMW-Adiponectin [a]	0.96	(0.77-1.19)	0.70
MMW-Adiponectin [a]	0.77	(0.55-1.08)	0.13
LMW-Adiponectin [a]	0.86	(0.57-1.16)	0.48
Index-Ratio	OR	95%CI	*P* value[†]
HMW/Total	1.15	(0.30-4.47)	0.84

MMW/Total	0.24	(0.02-2.68)	0.24
LMW/Total	1.45	(0.31-6.73)	0.63
MMW/HMW[a]	0.89	(0.67-1.18)	0.42
LMW/HMW[a]	1.00	(0.78-1.29)	0.99

OR, odds ratio: CI, confidence interval
[a] Parameters were transformed logarithmically before analysis.
[#]Multivariate *P* values were adjusted for sex, age, log BMI, and serum creatinine level.
[†]Multivariate *P* values were adjusted for sex, age, log BMI, serum creatinine level, and the presence of diabetes mellitus.

Table 5: Odds ratios of adiponectin-related parameters for each component of metabolic syndrome in the 1st group.

Discussion

In the present study, not only were the serum levels of all three adiponectin isoforms significantly lower in the AO group as compared with the non-AO group, the distribution of adiponectin isoforms within T-Ad also changed, with a lower percentage of HMW-Ad and higher percentage of MMW-Ad and LMW-Ad, resulting in higher MMW-Ad/HMW-Ad and LMW-Ad/HMW-Ad ratios. Among these changes of the ratios, the decreased HMW-Ad/T-Ad ratio and increased LMW-Ad/T-Ad and LMW-Ad/HMW-Ad ratios were also significantly positively correlated with the HOMA-IR, independent of the BMI. In addition, similar to their associations with the HOMA-IR, the aforementioned ratios also tended to be associated with a higher OR for MS. These results indicate that AO could be associated not only with changes in the serum levels of MMW-Ad and LMW-Ad, but also with change in the ratios of LMW-Ad within T-Ad, i.e., increases in the serum LMW-Ad/ T-Ad and LMW-Ad/HMW-Ad ratios, and that these changes are strongly associated with the severity of insulin resistance and risk for MS.

Numerous reports have been published on the association of adiponectin with AO and MS [9,14,16,17,30,34,35]. Most of these studies have involved measurement of the T-Ad and HMW-Ad, as mentioned above in the Introduction section. A search of the literature to the best of our ability identified only a few reports of examination of the associations of other adiponectin isoforms, such as MMW-Ad and LMW-Ad, with AO [18,19]. In a study conducted in Japan on elementary school children aged an average of 9 years old, the effect of AO on the serum levels of all three adiponectin isoforms was examined. The results of that study showed significantly lower serum levels of all three adiponectin isoforms in the AO group as compared with the non-AO group [18]. In a Chinese study of normal-weight and obese young women, the serum levels of all three adiponectin isoforms were significantly lower in the obese group than in the normal-weight group [19]. Our results were consistent with the findings of both these aforementioned studies. However, these studies did not describe the changes in the distribution of each adiponectin isoforms within T-Ad. In addition, a study in obese and normal-weight populations of adolescents in Northern Ireland revealed an increase of the LMW-Ad/T-Ad ratio in addition to decrease of the HMW-Ad/T-Ad ratio in the obese group [36]; however, the relationship between increased LMW-Ad/T-Ad ratio and IR was not investigated in that study. We are the first to demonstrate in the present study that the increases in the LMW-Ad/T-Ad ratio and LMW-Ad/HMW-Ad ratios in the subjects with AO were independent of the BMI, were significantly and positively associated with IR, and also tended to be associated with an increased risk for MS.

Among the three isoforms, the functions of MMW-Ad and LMW-Ad have not yet been investigated to the same extent as those of HMW-Ad. MMW-Ad and LMW-Ad differ from HMW-Ad in that they can cross the blood-brain barrier and are reported to enter into the cerebrospinal fluid from circulation and play a major role in regulating feeding behavior in the central nervous system. However, there have been some controversies on whether MMW-Ad and LMW-Ad stimulates, suppresses, or has any role in food intake [37].

Although the mechanisms underlying the association of an increased LMW-Ad/HMW-Ad ratio with IR have not yet been elucidated, we speculated two possible mechanisms; the first is the possible existence of a competitive relationship between the HMW-Ad and LMW-Ad isoforms, as previously reported by Bouskila, et al. [15,38]; they speculated that LMW-Ad may act as an adiponectin antagonist by inhibiting the action of HMW-Ad at the receptor level. Another possibility is that LMW-Ad may act upstream of the adiponectin receptors to proteolytically cleave full-length HMW-Ad and inhibit upstream activators of ligands such as reductases and proteases that can generate truncated adiponectin [38]; the latter has the ability to activate downstream signaling cascades such as AMP- kinase signaling. From these previous reports, we speculate that the association of an increased LMW-Ad/HMW-Ad ratio with IR may be explained by the antagonistic effects of LMW-Ad on HMW-Ad at the ligand or receptor level to

suppress the insulin-sensitizing effects of HMW-Ad.

Significant changes in the distribution of the adiponectin isoforms within T-Ad were observed in the patients with AO in this study, i.e., the HMW-Ad/T-Ad ratio were decreased and the LMW-Ad/T-Ad and LMW-Ad/HMW-Ad ratios were increased, and these changes were significantly associated with an elevated severity of IR. Since IR is upstream of MS and has been reported to be associated with hyperglycemia, dyslipidemia, and high BP [10,11], which are components of MS, we expected that the significant changes in the aforementioned ratios would also be significantly related to the risk of development of MS. However, although the significant changes tended to be associated with an increase in the risk for MS, the associations were not significant. In this study, we also examined the associations of the changes in the aforementioned ratio with each component of MS, and found a significant association with hyperglycemia, but not with dyslipidemia or high BP. We speculated the reason for these findings as follows. The aforementioned three components of MS are often known to be associated with IR in patients with AO, but they may also occur in the absence of IR [10]. For example, excessive salt intake or age-related vascular stiffening can cause high BP. In the case of dyslipidemia, high TG levels can be caused by dietary preferences (excessive carbohydrate and alcohol intake) that do not lead to AO, and low HDL-c levels can be caused by smoking. Therefore, since factors other than IR can cause dyslipidemia and high BP, we speculated that while the changes in the distribution of the adiponectin isoforms in AO were related to the severity of IR, they were not significantly associated with the risk for MS.

The major strength of this study was that we compared not only the serum levels of adiponectin isoforms other than HMW-Ad, such as MMW-A and LMW-A, but also the ratios of these adiponectin isoforms within the T-Ad between the AO and non-AO groups. A significant association of an increased LMW-Ad/T-Ad ratio and LMW-Ad/HMW-Ad ratio with IR, as well as a tendency towards associations of these parameters with the risk for MS were observed in the AO group, irrespective of the BMI. These findings are novel. Nevertheless, some limitations of this study must be acknowledged. First, regarding the analysis of the association between AO and adiponectin, we could not examine the association in the age-, sex- and percentage of having diabetes-matched subjects between the AO group and non-AO group. The mean age and percentage of female subjects were significantly higher, and the percentage of cases with diabetes was significantly lower in the non-AO group than in the AO group. Since previous reports have indicated increase in the serum levels of all adiponectin isoforms associated with aging, female gender, and absence of diabetes mellitus [24,28,31-33], the differences in the characteristics of the subjects between the AO group and non-AO groups might have influenced our findings. However, in the present study, we evaluated the relationship between AO and adiponectin-related parameters using not only the Student *t*-test, but also multiple logistic regression analysis with adjustments for confounding factors including the age, sex, and presence/absence of diabetes, and all of the analyses revealed the existence of significant relationships. Second, we could not obtain any information regarding the alcohol and smoking habits of the subjects, which are known to affect the levels of the three adiponectin isoforms [32,33]. Therefore, lack of this information could have influenced our results. Third, modification of adiponectin-related parameters by administration of various drugs should also be taken into consideration. In the present study, the percentages of subjects who took diabetic medications, lipid-lowering agents and antihypertensive drugs were higher in the AO group than in the non-AO group. In addition to TZDs (23, 24), it has been reported that other antidiabetic medications (including insulin, SU, and metformin, etc.), antihypertensive agents (angiotensin II type 1 receptor blockers, etc.), and lipid-lowering drugs (fibrates) can also affect the serum levels of T-Ad [39]. However, since these agents have only been reported to possibly increase, but not decrease the serum T-Ad level [40,41], we think that modification of the serum adiponectin levels by various drugs may not have affected our finding of reduced levels of all adiponectin isoforms in the AO group. Fourth, to measure the three adiponectin isoforms, we used the SEKISUI ELISA kit (SEKISUI Medical Co. Ltd., Japan) [13], as mentioned in the Materials and Methods section. In this kit, the LMW-Ad values are calculated by subtracting the HMW-Ad plus MMW-Ad values from the T-Ad value. Therefore, with this kit, it can be expected that unless the MMW-Ad/T-Ad ratio changes, the LMW-Ad/T-Ad ratio is higher in situations where the HMW-Ad/T-Ad ratio is lower. However, in this study, since we observed only a slightly significant difference ($P < 0.05$) in the MMW-Ad/T-Ad ratio between the AO group and the non-AO group, we think that the higher LMW-Ad/T-Ad ratio in the AO group may not simply be a reflection of the lower HMW-Ad/T-Ad ratio. Finally, since this study was a cross-sectional analysis, we could not establish the temporal relationships of the higher LMW-Ad/T-Ad and higher LMW-Ad/HMW-Ad ratios with increased IR and risk of development of MS.

Conclusion

In the present study, we demonstrated that AO was associated not only with changes in the serum levels of MMW-Ad and LMW-Ad, but also with change in the distribution of adiponectin isoforms, especially, increase of the LMW-Ad/T-Ad and LMW-Ad/HMW-Ad ratios, and that these change in ratios are strongly associated with the severity of insulin resistance and risk for MS. These finding suggest that the changes in the distribution of adiponectin isoforms as well as those in the level of its isoforms, in the subjects with AO, may be also important in the pathogenesis of MS.

Disclosure

Author Contributions: Conceptualization, M.I. and K.T.; methodology, M.I.; formal analysis, M.I.; investigation, M.I. and K.T.; data curation, K.O., Y.K, A.T., H.K., S.M., and K. H.; writing—original draft preparation, M.I.; writing—review and editing, K.T.; supervision, K.T.

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Data Availability Statement: The data underlying this study cannot be publicly released because they contain sensitive information. When we obtained informed consent from the participants in this study, we explained that we would not provide their de-identified data to any institution other than our university, Tokyo University (Tokyo, Japan), and the RIKEN Center for Genomic Medicine (Yokohama, Kanagawa, Japan). Data accession requests can be directed to the Ethics Committee of the University of Toyama using the following email address: ethics@med.u-toyama.ac.jp.

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Conflicts of Interest: None.

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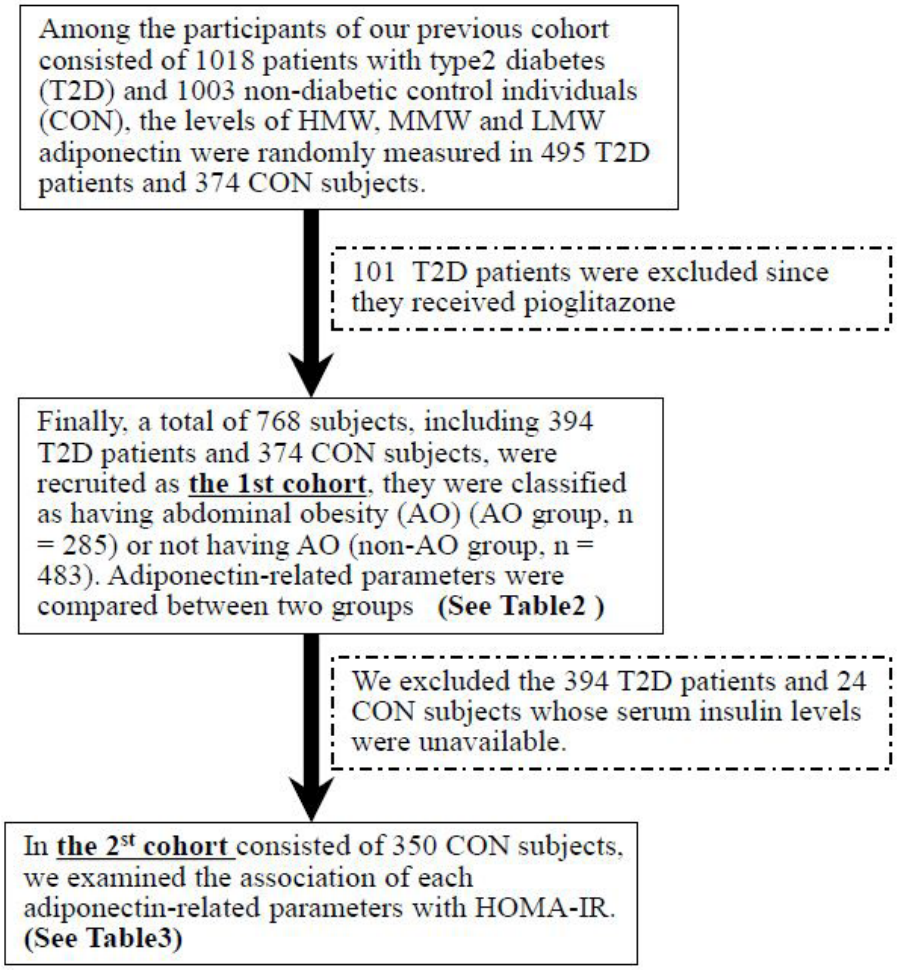

Supplementary figure. Selection of study subjects and analyses in the 1st and 2nd cohorts.

N	350
Sex (M/F)	151/ 199
Age (years)	67.2±8.1
BMI (kg/m^2)	23.1±3.0
Waist circumference (cm) (male)	84.0±8.2
Waist circumference (cm) (female)	81.9±9.0
Presence of abodminal obesity (%)	27.7
Presence of metabolic syndrome (%)	12.6
S-Cre (mg/dL)	0.75±0.17
FPG (mg/dl)	96.6±8.5
HbA1c (NGSP value) (%)	5.48±0.27
HOMA-IR	1.22±0.73
Presence of hyperglycemia (%)[a]	6.6
Systolic blood pressure (mmHg)	130.1±17.1
Diastolic blood pressure (mmHg)	78.4±11.4
Use of antihypertensive agent (%)	35.5
Presence of high blood pressure (%)[b]	60.3
HDL-cholesterol (mg/dl)	61.5±16.5
Triglyderide (mg/dl)	104.9±67.3
Use of lipid lowering drug (%)	22.1
Presence of dyslipidemia (%)[c]	37.4
Total-Adiponectin (μg/mL)	7.75 ± 3.68
HMW-Adiponectin (μg/mL)	3.74 ± 2.48
MMW-Adiponectin (μg/mL)	1.68 ± 0.89
LMW-Adiponectin (μg/mL)	2.33 ± 1.08
HMW/Total	0.44 ± 0.13
MMW/Total	0.23 ± 0.08
LMW/Total	0.33 ± 0.11
MMW/HMW	0.62 ± 0.46
LMW/HMW	0.95 ± 0.87

Data are means ± SD. BMI, body mass index; SCre, serum levels of creatinine; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulinresistance; HDL-cholesterol, high-density

Supplementary Table. Clinical profiles of the study subjects in the 2nd cohort