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Age and sex differences in serum adiponectin and its association with lipoprotein fractions

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Declarations

Conflict of interest

Professor Hiroshi Yoshida received honoraria for speaking activities from Astellas, Amgen, Bayer, Kowa, Mochida, MSD, Shionogi, Takeda.

Funding

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Ethical approval

This study was approved by the Ethics Committee of the Jikei University School of Medicine (24-100; 15-166).

Guarantor: Professor Hiroshi Yoshida, MD, PhD

Contributorship

Yoshiharu Tomono is in charge of data collection, data-analysis, data discussion, manuscript writing. Chika Hiraishi is in charge of data collection and data discussion. Hiroshi Yoshida is in charge of data-analysis, data discussion and manuscript writing.

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Abstract

Objective

The correlation of adiponectin with cholesterol level of fractionated lipoproteins has not been well investigated.

Methods

This study subjects were 174 persons (79 men and 95 women) without diabetes. The medical record data were investigated retrospectively. The study subjects with adiponectin< 8.3, than 8.3 but less 13.9, and ≥ 13.9 were classified into tertile groups: Group A (n= 59), B (n= 58) and C (n= 57), respectively.

Results

In women, age and HDL-C were higher in Group C than in Groups A and B, but BMI, TG, IDL-C, and VLDL-C were lower in Group C than in Groups A and B. In men, BMI was lower in Group C than in Groups A and B, and HDL-C was higher in Group C than in Groups A and B. In multiple stepwise regression analysis, BMI and HDL-C were significantly correlated with adiponectin in whole, male, and female subjects, but TG-rich lipoprotein cholesterol levels were not independently correlated.

Conclusions

HDL-C and BMI were independently correlated with adiponectin in non-diabetic men and women. These results suggest that high adiponectin may play a role on the increased HDL-C levels, implicated in the reduction of cardiovascular disease risk, in non-diabetic subjects.

Key words: adiponectin, age, BMI, HDL-C, lipoprotein fraction, sex-difference

Introduction

Metabolic syndrome relevant to visceral fat accumulation is a susceptible pathophysiologic state to atherosclerosis resulting from impaired metabolism of lipid and glucose, hypertension, obesity, and these complex risk factors (1, 2). Visceral fat accumulation is considered to be present upstream such a variety of metabolic disorders (1).

Adiponectin, one of adipocytokines, secreted from adipose tissue, and its concentrations in serum are inversely correlated with body mass index (BMI) and visceral fat accumulation (1, 3). Cell biology studies have demonstrated that adiponectin has diverse anti-atherosclerotic functions including anti-inflammatory actions and inhibitory effects of macrophage foam cell formation (1, 4, 5). In addition, adiponectin has been shown to increase insulin sensitivity (4, 6), and low adiponectin levels are found in subjects with obesity, type 2 diabetes mellitus, and dyslipidemia (4, 7).

In non-diabetic subjects, low serum adiponectin was associated with high serum triglyceride (TG) and low high density lipoprotein (HDL)-cholesterol, but not with low density lipoprotein (LDL)-cholesterol levels (8). Serum adiponectin was also reported to correlate positively with HDL-C and inversely with TG in patients with type 2 diabetes (9, 10).

Non-HDL cholesterol is able to account for total atherogenic burden by measuring the aggregate amount of cholesterol in apolipoprotein B-containing lipoproteins which are LDL, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL), chylomicron (CM) and CM remnant (11). Previous reports showed that cholesterol levels of both IDL and VLDL were associated with atherosclerotic cardiovascular disease risk (12-14). However, the relevance of adiponectin to dyslipidemia determined by means of measuring lipoproteins separated by ultracentrifugation or high-performance liquid chromatography (HPLC) remain incompletely understood. Recently, we have developed a novel HPLC method with anion exchange column containing a non-porous polymer-based gel to facilitate measurements of cholesterol levels in the fractionated serum lipoproteins [(HDL, LDL, IDL, VLDL, other faction (chylomicron, remnant lipoprotein, Lp(a)](15, 16). We have reported that low serum adiponectin was found in patients with Type 2 diabetes and type IIb hyperlipidemia, and adiponectin correlated inversely with VLDL-cholesterol determined by our developed HPLC method (17). However, the association of serum adiponectin with lipoprotein fractions in detail with the HPLC method in non-diabetic subjects has never been reported

Therefore, the present study was for the first time performed to investigate the association of adiponectin with cholesterol levels of lipoprotein fractions, determined by the HPLC method, in non-diabetic subjects.

Patients & Methods

Subjects, study protocol and measurements of parameters

This study subjects were 174 persons (79 men and 95 women, mean age: 67.9±11.3 years, mean body mass index (BMI): 22.8±3.0 kg/m^2) who underwent a medical checkup of the brain, including serum adiponectin, at the Jikei University Kashiwa hospital from April 2008 to March 2010. The medical record data were retrospectively investigated. The patients, who took medications for dyslipidemia, hypertension, and diabetes, were excluded from the present study. In addition, smokers and pre-menopausal women were not included. This study was approved by the Ethics Committee of the Jikei University School of Medicine.

Anthropometric measurements (height, weight) were performed in a standing position. Body mass index (BMI) was calculated as weight divided by the square of height in meters. Fasting blood samples were collected from all participants. Serum lipids, total cholesterol (TC) and triglyceride (TG), were measured by conventional enzymatic methods. Cholesterol levels of very low-density lipoprotein (VLDL), intermediate- density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), other fraction (chylomicron, chylomicron remnant, Lp(a)) were measured by the HPLC method as we reported previously (15, 16). Adiponectin was measured as total adiponectin by a latex particle-enhanced turbidimetric assay (Otsuka Pharmaceutical, Tokyo, Japan) (18). Glycohemoglobin A1c (HbA1c), plasma glucose, and serum insulin were measured by routine methods. The homeostasis model assessment of insulin resistance (HOMA-R), a surrogate marker for insulin resistance, was calculated as fasting insulin (mU/l) × plasma glucose (mg/dl)/ 405.

Statistical analysis

Continuous variables were expressed as Mean ± standard deviation (SD). One-way ANOVA with Fisher PLSD for multiple post hoc comparisons was used for analysis of differences between groups i. Simple correlations were estimated by Spearman's rank test. Multiple stepwise regression analysis was performed to identify any independent explanatory variables, including age and BMI, for serum adiponectin. A value of $p< 0.05$ was considered as statistically significant.

Results

The characteristics of whole study subjects were summarized in Table 1. Adiponectin, TC, non HDL-C, LDL-C, and HDL-C were significantly higher in women than in men, but age was significantly lower in women than in men. In the present study, a sex-difference in adiponectin levels was found in line with previous studies (1, 19, 20). The study subjects with adiponectin< 8.3, than 8.3 but less 13.9, and ≥ 13.9 were classified into tertile groups: Group A (n= 59), B (n= 58) and C (n= 57), respectively.

In the whole subjects, age and HDL-C were significantly higher in Group C than in Groups A and B (Table 2). BMI, TG, IDL-C, VLDL, and lipoprotein other fraction were significantly lower in Group C than in Groups A and B. HOMA-R and fasting insulin were higher in Group A than in Groups B and C. Next, the differences in parameters among the tertile groups were tested by sex. In men, BMI was lower in Group C than in Groups A and B, and HDL-C was higher in Group C than in Groups A and B (Table 3). TG and lipoprotein other fraction were lower in Groups C than in Group B. In women, age and HDL-C were higher in Group C than in Groups A and B (Table 4). However, BMI, TG, IDL-C, and VLDL-C were lower in Group C than in Groups A and B. HOMA-R and fasting insulin were higher in Group A than in Group C. The results of female subjects were similar with the results of whole subjects. By contrast, fasting plasma glucose and HbA1c were not relevant to adiponectin levels in non-diabetic subjects.

Then, using HOMA-R, HDL-C, LDL-C, IDL-C, VLDL-C, lipoprotein other fraction and universal confounding factors (age and BMI) as independent explanatory variables, multivariate stepwise-regression analysis was performed to determine significant explanatory factors for adiponectin, a dependent objective variable (Table 5). In whole subjects and women, age, BMI and HDL-C were significantly correlated with adiponectin. In men, BMI and HDL-C were significantly correlated with adiponectin, but the correlations of adiponectin with HDL-C were presumably greater in women than in men.

Discussion

The present study shows that HDL-C and BMI were independently and significantly with adiponectin in both male and female non-diabetic subjects. As for the sex-difference in the present study subjects, the higher levels of TC, non HDL-C, LDL-C, and HDL-C and lower levels of age were found in women than in men. Previous studies reported that serum adiponectin concentrations tended to rise with age (19, 21). In the present investigations by sex, age was independently correlated with adiponectin only in women. The subjects with high levels of adiponectin live

long (1, 19, 21), and consequently elderly people could have high levels of adiponectin although the reasons why serum adiponectin may rise with age are not well defined. However, the independent correlation of age with adiponectin in men was not found in the present study. This sex-difference might be associated with the difference in average life expectancy between men and women, but it remains to be cleared. However, the independent correlations of BMI with adiponectin were found in whole, male, and female subjects as reported previously (1, 3)

The sex-difference in serum adiponectin levels has been reported (19, 20). The distribution of serum adiponectin concentrations was lower in men than in women, but the difference in adiponectin concentrations between pre- and post-menopausal women were not found. In addition, testosterone treatment reduced adiponectin secretion into the culture media from 3T3-L1 adipocytes. Therefore, the sex-difference in serum adiponectin concentrations may be attributable in part to androgens mediated inhibition of adiponectin secretion from adipocytes.

We have previously reported that VLDL-C independently and inversely correlated with adiponectin in patients with type 2 diabetes (17). Adiponectin might decrease serum TG and VLDL-C, in part by inhibiting VLDL and TG production or by stimulating lipolysis of TG by lipoprotein lipase (1, 17, 23). It would be considered that the increased levels of serum TG and VLDL-C are found in patients with type 2 diabetes partially because of hypoadiponectinemia. Serum TG levels in the highest tertile of adiponectin (Group C) were lower than other Groups in whole, male, and female subjects, but TG levels were not so high probably because of non-diabetic subjects. Consequently, the independent correlations between adiponectin and TG rich lipoprotein, including VLDL, might not be found. By contrast, low HDL-C usually is found in hypoadiponectinemia in part resulting from effects of adiponectin on cholesterol efflux from macrophages and reverse cholesterol transport (24, 25). Namely, adiponectin could increase HDL-C irrespective of TG levels, although the positive and inverse correlations of adiponectin with HDL-C and TG, respectively, have been reported (26, 27). Therefore, the independent correlations of adiponectin with HDL-C were found in whole, male, and female subjects in the present study.

This study has several limitations. First, the present study was a retrospective study, and BMI data were found, but waist circumference data were not included. Consequently, the diagnosis of metabolic syndrome could not be conducted by Japanese guideline (28). However, mean BMI was 22.8 kg/m^2 and defined obese subjects might be excluded. Second, the study subjects were relatively elderly, and the present results may not be extrapolated to the general population.

In conclusion, the present study shows that HDL-C and BMI were independently and significantly with adiponectin in non-diabetic men and women, and that simple correlations of TG and TG-rich lipoprotein cholesterol to adiponectin were found but not independently. These results suggest that high adiponectin may have some kind of role in the increased HDL-C levels, implicated in the reduction of cardiovascular disease risk, in non-diabetic subjects.

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Declaration of Conflicting Interests

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Table1. The characteristics of whole study subjects.

	Whole (n= 174)	Male (n= 79)	Female (n= 95)	P Value
Adiponectin (μg/mL)	13.2 ±8.8	11.6 ±8.2	14.5 ±9	0.003
Age	67.9 ±11.3	69.8 ±11.2	66.3 ±11.2	0.0201
Body mass index (kg/m^2)	22.8 ±3.0	23.0 ±3.1	22.7 ±2.9	0.3621
Fasting plasma glucose (mg/dL)	103 ±24.9	108 ±33.1	100 ±14.1	0.1346
Hemoglobin A1c (%)	5.5 ±0.5	5.6 ±0.6	5.5 ±0.4	0.9506
Insulin (μ U/mL)	7.3 ±7.2	7.9 ±7.1	6.8 ±7.2	0.1672
Insulin Resistance (HOMA-R)	2.0 ±2.4	2.3 ±2.3	1.8 ±2.4	0.0841
Serum lipid data (mg/dL)				
Total cholesterol	205 ±33.5	191 ±28.7	216 ±33.2	<.0001
Triglyceride	126 ±63.7	127 ±67.1	126 ±61.1	0.9735
Non-HDL cholesterol	144 ±30.2	135 ±27.8	150 ±30.5	0.0013
Lipoprotein cholesterol measured by anion-exchange liquid chromatography (mg/dL)				
HDL	61.13 ±17.4	55.23 ±15.7	66.0 ±17.3	<.0001
LDL	119 ±28.4	112 ±26.8	124 ±28.8	0.0135
IDL	5.9 ±2.8	5.4 ±2.5	6.3 ±3.0	0.0721
VLDL	9.9 ±6.6	9.4 ±6.6	10.2 ±6.6	0.3215
Others	5.5 ±3.8	5.1 ±3.5	5.8 ±4.1	0.1984

AVERAGE±SD

HOMA-R, homeostasis model assessment of insulin resistance; others means cholesterol of chylomicron, chylomicron remnant and lipoprotein (a).

Table2. Comparison among tertile groups classified by adiponectin concentration in whole subjects

	A Group (adiponectin < 8.3) (n = 59)	B Group (8.3≦ adiponectin < 13.9) n = 78	C Group (13.9 ≦ adiponectin) n = 57	ANOVA P Value	AvsB	AvsC	BvsC
Adiponectin (μg/mL)	6.3 ±1.5	10.6 ±1.5	22.9 ±9.1	<.0001	<.0001	<.0001	<.0001
Age	65.8 ±12.5	66.7 ±11.5	71.3 ±8.9	0.0198	0.6761	0.009	0.0279
Body mass index (kg/m^2)	23.6 ±2.6	23.5 ±3.3	21.3 ±2.5	<.0001	0.7093	<.0001	<.0001
Fasting plasma glucose(mg/dL)	105.3 ±20.6	100.2 ±21.3	104.6 ±31.6	0.4898	0.2699	0.8816	0.3441
Hemoglobin A1c (%)	5.6 ±0.6	5.5 ±0.4	5.5 ±0.4	0.4497	0.2499	0.3071	0.9009
Insulin (μU/mL)	9.6 ±8.9	6.6 ±6.9	5.8 ±4.4	0.0099	0.0215	0.0041	0.5536
HOMA-R	2.8 ±3.1	1.7 ±2.1	1.5 ±1.4	0.0095	0.0161	0.0047	0.6598
Serum lipid data (mg/dL)							
Total cholesterol	198.8 ±35.6	207.3 ±33.0	208.5 ±31.4	0.2353	0.1717	0.1201	0.8456
Triglyceride	142.9 ±72.1	136.9 ±66.9	98.1 ±37.3	0.0002	0.5928	0.0001	0.0008
Non-HDL cholesterol	144.8 ±32.8	147.0 ±30.6	138.7 ±26.6	0.3078	0.6975	0.2711	0.1393
Lipoprotein cholesterol measured by anion-exchange liquid chromatography (mg/dL)							
HDL	53.1 ±14.8	60.1 ±15.9	70.5 ±17.1	<.0001	0.0191	<.0001	0.0006
LDL	121.1 ±29.0	120.2 ±30.3	115.6 ±26.0	0.5347	0.868	0.2969	0.3818
IDL	6.3 ±2.8	6.7 ±3.2	4.7 ±2.0	0.0004	0.4046	0.0028	0.0002
VLDL	11.3 ±8.0	10.8 ±6.4	7.4 ±4.0	0.0022	0.6288	0.0011	0.0054
Others	5.9 ±4.0	6.1 ±4.7	4.4 ±2.1	0.038	0.7859	0.0373	0.0194

AVERAGE±SD

Table3. Comparison among tertile groups classified by adiponectin concentration in men

	A Group (adiponectin < 7.4) (n = 59)	B Group (7.4≦ adiponectin < 11.2) n = 78	C Group (11.2 ≦ adiponectin) n = 57	ANOVA P Value	AvsB	AvsC	BvsC
Adiponectin (μg/mL)	5.7 ±1.2	9.0 ±1.3	19.9 ±9.8	<.0001	0.0368	<.0001	<.0001
Age	65.6 ±15.7	71.6 ±7.9	71.9 ±7.9	0.0771	0.0508	0.0461	0.9359
Body mass index (kg/m^2)	23.8 ±3.2	23.6 ±2.9	21.5 ±2.9	0.0112	0.8465	0.0077	0.0107
Fasting plasma glucose (mg/dL)	104.6 ±23.7	110.1 ±27.5	108.0 ±45.3	0.8349	0.5517	0.7149	0.8211
Hemoglobin A1c (%)	5.6 ±0.7	5.6 ±0.5	5.5 ±0.6	0.8705	0.9432	0.6834	0.6233
Insulin (μU/mL)	9.8 ±7.7	8.0 ±7.9	6.0 ±5.0	0.1697	0.3571	0.0607	0.3099
HOMA-R	2.8 ±2.7	2.3 ±2.4	1.7 ±1.6	0.2277	0.3838	0.0864	0.3702
Serum lipid data (mg/dL)							
Total cholesterol	182.7 ±27.2	196.9 ±31.6	193.6 ±25.7	0.1775	0.0736	0.1762	0.6693
Triglyceride	130.2 ±51.5	148.4 ±88.1	99.8 ±42.9	0.0256	0.3118	0.0977	0.0074
Non-HDL cholesterol	132.2 ±25.8	143.2 ±33.3	129.9 ±21.7	0.1695	0.1502	0.7649	0.0796
Lipoprotein cholesterol measured by anion-exchange liquid chromatography (mg/dL)							
HDL	50.1 ±15.2	52.2 ±14.1	63.4 ±15	0.0034	0.5998	0.0018	0.0065
LDL	113.4 ±24.6	115.1 ±32.2	108.8 ±22.7	0.6742	0.8152	0.5422	0.3888
IDL	5.3 ±2.4	6.0 ±2.8	5.0 ±2.2	0.3066	0.3425	0.5856	0.1306
VLDL	8.9 ±4.8	11.4 ±8.8	7.9 ±4.7	0.1313	0.1632	0.5951	0.0523
Others	4.9 ±2.5	6.4 ±4.9	3.9 ±1.2	0.0282	0.1094	0.2998	0.0083

Table4. Comparison among tertile groups classified by adiponectin concentration in women

	A Group (adiponectin < 9.5) (n = 32)	B Group (9.5≦ adiponectin < 15.4) (n = 32)	C Group (15.4 ≦ adiponectin) (n = 31)	ANOVA P Value	AvsB	AvsC	BvsC
Adiponectin (μg/mL)	7.0 ±1.7	12.0 ±1.8	24.9 ±8.5	<.0001	0.0002	<.0001	<.0001
Age	61.8 ±9.5	65.1 ±12.6	72.1 ±8.7	0.0006	0.2106	0.0002	0.009
BMI(kg/m^2)	23.5 ±2.7	23.2 ±3.1	21.2 ±2.3	0.0017	0.7332	0.0011	0.0032
FPG (mg/dL)	101.6 ±18.3	98.5 ±11.9	99.5 ±11.3	0.6646	0.377	0.5517	0.7777
Hemoglobin A1c (%)	5.5 ±0.5	5.5 ±0.3	5.4 ±0.3	0.759	0.8481	0.4738	0.5983
Insulin (μU/mL)	9.3 ±10.4	6.1 ±4.7	5.1 ±4.4	0.0532	0.0791	0.0203	0.55
Insulin Resistance(HOMA-R)	2.7 ±3.6	1.6 ±1.5	1.2 ±1.2	0.0524	0.0766	0.0203	0.5593
Serum lipid data (mg/dL)							
Total cholesterol	214.0 ±35.8	215.3 ±33.1	218.9 ±31.5	0.833	0.8788	0.5605	0.6664
Triglyceride	149.4 ±70.6	136.5 ±59.5	90.5 ±30.1	0.0002	0.36	<.0001	0.0016
Non-HDL cholesterol	154.7 ±33.4	153.0 ±28.6	143.2 ±28.9	0.2785	0.8215	0.138	0.2066
Lipoprotein cholesterol measured by anion-exchange liquid chromatography (mg/dL)							
HDL	58.6 ±13.4	62.7 ±16.1	77.2 ±16.7	<.0001	0.3	<.0001	0.0003
LDL	129.1 ±30.7	125.4 ±27.3	118.5 ±28.2	0.3388	0.6092	0.1478	0.3442
IDL	7.5 ±3.2	7.0 ±3.1	4.3 ±1.5	<.0001	0.4479	<.0001	0.0002
VLDL	13.2 ±8.3	10.8 ±5.9	6.5 ±2.3	0.0002	0.12	<.0001	0.0065
Others	6.4 ±4.3	6.3 ±5	4.6 ±2.2	0.1361	0.9262	0.0756	0.0915

Table 5 Results of multiple regression analysis using adiponectin as a confounding factor.

	Whole		Male		Famale	
	t Value	(P Value)	t Value	(P Value)	t Value	(P Value)
Age	3.452	(0.0007)	1.25	(0.2154)	3.41	(0.001)
BMI	-3.379	(0.0009)	-2.565	(0.0125)	-2.688	(0.0086)
HOMA-R	-0.554	(0.5805)	0.855	(0.3956)	-1.265	(0.2092)
HDL-C	4.758	(<.0001)	2.015	(0.0477)	3.048	(0.0031)
LDL-C	-1.062	(0.2897)	-0.045	(0.9643)	-1.812	(0.0736)
IDL-C	-1.29	(0.1988)	-0.125	(0.9007)	-1.585	(0.1166)
VLDL-C	-1.093	(0.2761)	-0.582	(0.5626)	-0.844	(0.401)
Others	0.701	(0.4841)	-0.028	(0.978)	0.814	(0.418)

HOMA-R, homeostasis model assessment of insulin resistance; BMI, body mass index; others means cholesterol of chylomicron, chylomicron remnant and lipoprotein (a).

Cholesterol levels of HDL, LDL, IDL, VLDL, and others were measured by anion-exchange liquid chromatography.