Leptin:high molecular weight adiponectin ratio is independently correlated with carotid intima-media thickness in men, but not in women

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Abstract

Context: The leptin:adiponectin ratio (L:A ratio) is an independent predictor of carotid intima media thickness (CIMT).

Objective: To evaluate whether the leptin:high molecular weight adiponectin ratio (L:HA ratio) is associated with CIMT in the general population.

Methods: We investigated the relationship between the L:HA ratio and CIMT in 233 Japanese study participants (106 men and 127 women).

Results: After adjustment for confounding factors, CIMT was significantly correlated with the log L:HA ratio (β=0.11, p=0.024) in men, whereas no correlation was observed in women (β=0.02, p=0.50).

Conclusion: L:HA ratio is closely correlated with CIMT in men, but not in women.

Key words: Adiponectin; Carotid intima-media thickness; High molecular weight adiponectin

Introduction

Adipose tissue is a highly active endocrine organ, secreting a range of hormones which likely act as mediators between body fat distribution and insulin sensitivity. Adiponectin (A), an adipocyte-derived protein, likely modulates insulin sensitivity and plays a role in insulin resistance (Yang and Chuang 2006). In cross-sectional studies, plasma A levels have been negatively correlated with obesity, waist-to-hip ratio, insulin resistance, dyslipidemia, diabetes and cardiovascular diseases (CVD) (Arita et al. 1999; Hotta et al. 2000; Weyer et al. 2001; Matsubara et al. 2002; Nakamura et al. 2004; Rothenbacher et al. 2005). Additionally, it has been reported that, independent of traditional risk factors, elevated leptin (L) levels are predictors of cardiovascular events, post-coronary angiography restenosis and cerebral stroke (Wallace et al. 2001). In obese subjects, plasma A concentrations decreased and L concentrations were elevated. Consequently, it is speculated that L could accelerate, and A retard, atherosclerosis development.

The L:A ratio has been suggested as an atherosclerosis index in patients with type 2 diabetes, and a useful parameter for insulin resistance assessment in patients with and without diabetes (Kotani et al. 2005; Inoue et al. 2005). Furthermore, it has been

suggested that the L:A ratio is an independent predictor of carotid intima media thickness (CIMT), which is a strong predictor of CVD, and correlates with several anthropometric, metabolic and clinical parameters better than individual adipocytokines in healthy men (Wallace et al. 2001). This suggests that the L:A ratio could be a novel marker for the progression of atherosclerosis and future risk of CVD.

According to reports on the biological activity of A multimers, AMP-activated protein kinase activity varies among the multimeric forms of Ad (Kobayashi et al. 2004). It has been shown that high molecular weight A (HA) is an active form and its ratio to total A is closely related to insulin activity (Ebinuma et al. 2006). Recently, we showed that HA, as well as total A, were significantly correlated with body weight, BMI, HDL-cholesterol (HDL-C) and triglyceride (TG) in the general Japanese population (Ishibashi et al. 2007). Since HA is an active form among total Ad, we hypothesized that the L:HMW-A ratio (L:HA ratio), as well as L:A ratio is an accessible markers of the progression of atherosclerosis, and possibly for the future risk of CVD. In this study, we measured the L:HA ratio in the general population and investigated the relationship between CIMT and L:HA.

Materials and methods

Prior to this study, ethical approval was obtained from the special committee of Nagasaki University (project registration number 0 501 120 073). The study was conducted during a medical screening program for the general population over 40 years old, living in Goto City, Nagasaki Prefecture, Japan. Details of this screening program in Goto City have been described in elsewhere (Ishibashi et al. 2007; Kadota et al. 2008). The data was collected by the staff of Nagasaki University, in cooperation with the staff of Goto City. Participants who had evident past or present history of apparent diabetes mellitus (HbA_{1c}>7.0%), apparent dyslipidemia (LDL-C>2.5g/L and/or HDL-C<30g/L, and/or TG>300g/L), cerebral infarction or hemorrhage were excluded from the study. Finally, 233 Japanese participants (106 men and 127 women) were included in this study.

Body weight and height were measured, and body mass index (BMI; kg/m^2) was calculated. Percentage of fat mass (%Fat) was measured by the bio-impedance method. Systolic and diastolic blood pressure (SBP and DBP) was recorded at rest. Mean blood pressure (MBP) was calculated as (SBP-DBP)/3 + DBP.

After obtaining informed consent, blood samples were collected from the participants. Serum total A and high molecular weight A (HMW-A, more than octadecamer) were measured using a Human Adiponectin ELISA Kit for Total and Multimers® (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan). The inter-assay CV for total A and HMW-A was 5.0% at 7.7 mg/L and 5.7% at 3.5 mg/L, respectively. Serum total L concentration was measured using a Human Leptin RIA kit® (Cosmic Corporation, Tokyo, Japan). The inter-assay CV for L was 4.1% at 7.0 μg/L and 5.3% at 12.6 μg/L, respectively. Serum total cholesterol (TC), HDL-C and TG were measured by enzymatic methods, and LDL-cholesterol (LDL-C) was calculated by the Friedewald formula. HbA_{1c} was measured by the latex agglutination reaction.

The measurement of maximum CIMT by ultrasonography of carotid arteries was performed by two medical doctors (N.T. and M.N.) using a LOGIC Book XP with a 10-MHz linear array transducer (GE Medical Systems, Milwaukee, WI, USA). Averages of the left and right maximum CIMTs at the bifurcation position of carotid arteries were calculated and used in the analysis. Intra-observer variation of CIMT (N.T., n=32) was 0.91 ($p<0.01$) and inter-observer variation (N.T. *vs.* M.N., n=41) was 0.78 ($p<0.01$).

Results are expressed as mean ± SD or median (25^{th}-75^{th} quartiles). In order to confirm whether there is a significant correlation between CIMT and the L:A or L:HA ratios, independent of age, fat mass, circulation dynamics, and lipid and glucose metabolism, multivariate linear regression analysis was performed (adjusted for age, %Fat, MBP, HDL-C, and HbA_{1c}) on data obtained from men and women. Because the L:A and L:HA ratios were distributed in a skewed fashion, logarithmic transformations were performed for multivariate linear regression analyses. Probability values less than 0.05 were considered indicative of statistical significance. All statistical analyses were performed using SPSS v14.0 software (SPSS Japan, Tokyo, Japan).

Results

The characteristics of the study participants are shown in **Table 1**. The age ranges for men and women were 41 to 91 years and 41 to 89 years, respectively. DBP, TG and max CIMT were significantly higher in men than in women. However, TC and HDL-C were significantly higher in women than in men. As well, A and HA, L, and the L:A ratio were significantly higher in women than in men. There was no significant difference in the L:HA ratio of men and that of women.

Using simple linear regression analysis, CIMT was determined to be significantly correlated with age (r=0.26), SBP (r=0.25) and MBP (r=0.23) in men, and with age (r=0.51), %Fat (r=-0.23), SBP (r=0.30), DBP (r=0.20) and MBP (r=0.28) in women. (data not shown). On the other hand, CIMT was not significantly correlated with L:A ratio and L:HA ratio both in men and women, respectively (data not shown). When multivariate linear regression analysis was adjusted for confounding factors including log L:A ratio, CIMT was significantly correlated with age (β=0.004, p=0.009), HDL-C (β=-0.003, p=0.007) and the log L:A ratio (β=0.12, p=0.037) in men, whereas in women, it was significantly correlated only with age (β=0.005, p<0.001) and not with the log L:A ratio (β=0.02, p=0.52) (**Table 2**). Also, when multivariate linear regression analysis

was performed and adjusted for confounding factors including log L:HA ratio, CIMT significantly correlated with age (β=0.005, p<0.001), HDL-C (β=-0.002, p=0.025) and the log L:HA ratio (β=0.11, p=0.024) in men, whereas it was significantly correlated only with age (β=0.005, p<0.001) and not with the log L:A ratio (β=0.02, p=0.50) in women (**Table 3**). Furthermore, when multivariate linear regression analysis was performed and adjusted for confounding factors including log L:A ratio and log L:HA ratio, CIMT significantly correlated with log L:HA ratio (β=0.12, p=0.026) and relatively correlated with log L:A ratio (β=0.11, p=0.058) in men, whereas it was not with log L:A ratio (β=0.02, p=0.42) and with L:HA ratio (β=0.02, p=0.39) in women (data not shown).

Discussion

Our data indicated that the L:HA, as well as L:A ratios is a novel, independent predictors of CIMT (independent of age, fat mass, circulation dynamics, and lipid and glucose metabolism) in the general male population, but not in women. We assessed whether CIMT is associated with L or A *per se* using multivariate linear regression analysis, and found that both did not appear to be independent predictors of CIMT in men and women (data not shown). Since the measurements and calculations of L:A and L:HA ratios are relatively simple, these ratios have the possibility to be novel markers for the evaluation of atherosclerosis. Furthermore, since HA is an active form in total Ad, the L:HA ratio may become a more accurate predictor of atherosclerosis and future CVD events. When multivariate linear regression analysis was performed and adjusted for confounding factors including both log L:A ratio and log L:HA ratio, CIMT significantly correlated with log L:HA ratio (β=0.12, p=0.026), whereas it relatively but not significantly correlated with log L:A ratio (β=0.11, p=0.058) in men. These results may suggest that evaluation of L:HA ratio may reflect the progression of atherosclerosis more precisely.

Interestingly, we observed that both the L:A and L:HA ratios have significant associations with CIMT in men, but not in women. Inoue *et al.* screened Japanese adults without hyperglycemia and showed that the L:A ratio was significantly correlated with fat mass (FM) and TG in men, and FM and serum lipoprotein lipase in women (Inoue et al. 2006). Kotani et al. showed that L:A is a useful predictor of CIMT in women (Kotani *et al.* 2008). However, they showed such correlation only in the simple test, and the correlation attenuated to a non significant level after multivariate adjustment. On the other hand, Norata *et al.* showed that the L:A ratio is an independent predictor of CIMT in healthy subjects, however, only men were evaluated (Norata et al. 2007). Since it is well known that CIMT is strongly associated with sex and age (Takamura et al. 2007), the contribution of adipocytokines, such as L and A, to the progression of atherosclerosis may be different between men and women. Aging and sex differences are generally associated with various hormonal/metabolic alterations and the fat distribution change, with sex differences, that can affect the secretion of L and A. Further studies are needed to establish a suitable model for the evaluation of atherosclerosis in both sexes.

There are several limitations to our study. The sample size was relatively small, and we could not evaluate the relationship between the L:A and L:HA ratios, and insulin

and insulin sensitivity. Also, in our study, 141 of 233 (60.5 %) showed hypertension (SBP ≥ 140mmHg and/or DBP ≥ 90mmHg), since we included general community-dwelling elderly in this study, and could not exclude participants with hypertension, due to relatively high frequency of hypertension among them. Actually, when multivariate linear regression analysis was performed and adjusted for confounding factors only in men with hypertension, CIMT was significantly correlated with log L:A ratio (β= 0.76, p = 0.024) and with log L:HA ratio (β= 0.67, p = 0.009), whereas in men without hypertension, CIMT was relatively, but not significantly correlated with log L:A ratio (β= 0.24, p = 0.059) and with log L:HA ratio (β= 0.22, p = 0.064). Relatively high frequency of hypertension in study participants might affect current results. Also, we did not exclude patients with diabetes in this study. Further evaluation will be needed to clarify the applicability of the L:HA ratio.

Conclusions

We demonstrated that the L:HA ratio, as well as L:A ratio are novel independent predictors of CIMT in men, but not in women. Further study is required to establish reliable and novel markers for the early prediction of atherosclerosis.

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Declaration of interest

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Table 1. Characteristics of study participants in men and women

Characteristics	Men (n=106)	Women (n=127)	All (n=233)
Age, years	67.0 ± 11.5	66.0 ± 8.9	65.1±11.5
BMI, kg/m^2	24.1 ± 2.5	23.7 ± 2.6	24.0 ± 2.6
%Fat, percentage	23.2 ±5.6	29.7 ±5.4	26.7 ±6.4†
SBP, mmHg	146.0 ± 18.9	142.0 ± 20.3	144.0 ± 19.6
DBP, mmHg	87.0 ±12.0	83.0 ± 9.5	85.0 ± 10.8*
MBP, mmHg	105.6±13.2	103.4±12.0	104.5±12.6
Creatinine, mg/L	9.9±3.3	7.5±1.7	8.6±2.8†
TC, g/L	1.9 ± 0.4	2.2 ± 0.4	2.1 ± 0.4†
HDL-C, g/L	0.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.2†
LDL-C, g/L	1.2 ± 0.3	1.4 ± 0.1	1.3 ± 0.1
TG, g/L	1.2 (0.8-1.8)	1.1 (0.8-1.4)	1.1 (0.8-1.6)*
HbA_{1c} , %	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4
Total A, mg/L	4.8 (3.5-6.3)	7.6 (5.3-10.7)	6.0 (4.3-9.0)†
HA, mg/L	2.0 (1.2-3.0)	3.9 (2.4-6.1)	2.8 (1.7-4.9)†
L, μg/L	1.4 (0.7-2.8)	1.7 (0.8-3.3)	1.5 (0.7-3.1)†
L:A ratio	0.55 (0.31-0.97)	0.77 (0.44-1.65)	0.71 (0.36-1.31)*
L:HA ratio	1.4 (0.7-2.8)	1.7 (0.8-3.3)	1.5 (0.7-3.1)
CIMT (mm)	0.93 (0.81-1.16)	0.88 (0.78-0.99)	0.91 (0.80-1.06)*
Hypertension	61 (57.5%)	73 (57.5%)	134 (57.5%)

*$p<0.05$ and †$p<0.001$ between men and women

Table 2: Multivariate linear regression analysis for CIMT adjusted for confounding factors including L:A Ratio in men and women.

	Men			Women		
	$\beta^{\S}$	95%CI	p	β	95%CI	p
Age	0.011	0;0.022	0.059	0.010	0.006;0.015	<0.001
%Fat	-0.008	-0.032;0.016	0.52	-0.003	-0.014;0.009	0.66
MBP	0.007	-0.002;0.016	0.14	0.001	-0.002;0.006	0.25
Creatinine	0.31	-0.063;0.677	0.77	0.18	-0.13;0.49	0.24
HbA_{1c}	-0.053	-0.41;0.30	0.77	-0.11	-0.23;0.19	0.095
HDL-C	-0.013	-0.0020;-0.006	0.001	0	-0.004;0.002	0.57
Log L:A ratio	0.41	0.001;0.231	0.042	0.016	-0.14;0.18	0.85

[§]regression coefficient

Table 3: Multivariate linear regression analysis for CIMT adjusted for confounding factors including L:HA Ratio in men and women.

	Men			Women		
	β	95%CI	p	β	95%CI	p
Age	0.011	0;0.023	0.042	0.010	0.006;0.015	<0.001
%Fat	-0.006	-0.029;0.017	0.59	-0.002	-0.014;0.009	0.67
MBP	0.007	-0.002;0.016	0.14	0.001	-0.002;0.006	0.25
Creatinine	0.31	-0.05;0.68	0.09	0.18	-0.12;0.49	0.24
HbA_{1c}	-0.06	-0.41;0.29	0.74	-0.11	-0.23;0.02	0.095
HDL-C	-0.014	-0.021;-0.006	<0.001	0	-0.004;0.002	0.57
Log L:HA ratio	0.37	0.03;0.72	0.014	0.01	-0.17;0.86	0.86