The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2021; 28: 483-490. http://doi.org/10.5551/jat.56218

Apolipoprotein A2 Isoforms in Relation to the Risk of Myocardial Infarction: A Nested Case-Control Analysis in the JPHC Study

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Aim: The fact that low concentrations of high-density lipoprotein cholesterol are associated with the risk of cardiovascular disease is well known, but high-density lipoprotein metabolism has not been fully understood. Apolipoprotein A2 (ApoA2) is the second-most dominant apolipoprotein of high-density lipoprotein. We tested the hypothesis that ApoA2 isoforms are inversely associated with myocardial infarction.

Methods: We measured the plasma levels of three ApoA2 isoforms (ApoA2-ATQ/ATQ, ApoA2-ATQ/AT, ApoA2-AT/AT) in nested case-control study samples of 1:2 from the Japan Public Health-Center-based Study (JPHC Study): 106 myocardial infarction incidence cases and 212 controls.

Results: ApoA2-AT/AT was inversely associated with risk of myocardial infarction, in a matched model (OR, 2.78; 95% CI, 1.26–6.09 for lowest compared with the highest quartile), but its association was attenuated after adjustment for smoking only (OR=2.13; 95% CI, 0.91–4.97) or drinking only (OR=2.11; 0.91–4.89), and the multivariable OR was 1.20 (95% CI, 0.41–3.57). Neither ApoA2-ATQ/ATQ nor ApoA2-ATQ/AT was associated with the risk of myocardial infarction.

Conclusions: Our nested case-control study did not show a significant association of ApoA2 isoforms with a risk of myocardial infarction.

See editorial vol. 28: 469-470

Key words: Apolipoprotein A-II, HDL, Coronary heart disease, Atherosclerosis, C-terminus

Introduction

High-density lipoprotein (HDL) is well known as a protective factor against atherosclerosis and coronary heart disease. Several prospective studies confirmed that HDL cholesterol was inversely associated with the risk of cardiovascular disease in the general population[1]. In the Japan Public Health Center-based Prospective Study (JPHC Study), HDL cholesterol concentrations were inversely associated with the risk of coronary heart disease[2]. However, a series of drug trials to increase plasma HDL cholesterol levels did not demonstrate a reduction in mortality from coronary heart disease in patients treated with statins[3-5].

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Received: March 4, 2020 Accepted for publication: June 17, 2020

These results suggest that no causal relationship might exist between HDL cholesterol and coronary heart disease. Therefore, more attention was paid to HDL metabolism, especially to the HDL proteome[6]. HDL consists mainly of apolipoproteins (apolipoprotein A1 [ApoA1], apolipoprotein A2 [ApoA2]), phospholipids, and cholesterol. ApoA1 is the most dominant of the apolipoproteins of HDL and exhibits anti-atherogenic properties according to animal and cell studies[7]. Conversely, ApoA2 is the second-most dominant apolipoprotein of HDL; however, its pathophysiological role has not been clarified. Many studies were conducted on HDL subtypes; LpA-I:A-II containing both ApoA1 and ApoA2, and LpA-I containing no ApoA2 but only ApoA1, which investigated the relationship between these subtypes and risk of cardiovascular events. However, a difference in the function between these subtypes has been unclear[8-10]. ApoA1 and ApoA2 were linked to HDL structure and function[11-13], and studies using transgenic animal models suggested that ApoA2 exhibits potent anti-inflammatory and antioxidant effects, which may contribute to protection from atherosclerosis[6, 13]. A nested case-control study in the European Prospective Investigation into Cancer and Nutrition-Norfolk cohort showed a significant inverse association between ApoA2 and risk of future coronary artery disease[8].

ApoA2 and ApoA1 exhibit a similar tandem array of amphipathic α-helices[6]. As for ApoA1, a recent *in vitro* study showed that the C-terminal cleavage of ApoA1 at Ser^{228}, positioned in α-helix 10 of ApoA1, was mediated by human monocyte-derived macrophages, reducing the anti-atherogenic properties of ApoA1. The authors also confirmed that the C-terminal cleavage of ApoA1 was also detected in human carotid plaque[14].

Honda *et al.* previously reported that five circulating isoforms of ApoA2 were characterized by the truncation of varying numbers of amino acids from the C-terminus of the ApoA2 homodimer[15]. They are ApoA2-ATQ/ATQ (ApoA2-1, 17,380 Da), ApoA2-ATQ/AT (ApoA2-2, 17,252 Da), ApoA2-AT/AT (ApoA2-3, 17,124 Da), ApoA2-AT/A (ApoA2-4, 17,023 Da), and ApoA2-A/A (ApoA2-5, 16,922 Da). All five isoforms can be measured according to differences in molecular weight by using mass spectrometry-based proteomic analysis. That report[15] showed that ApoA2-ATQ/AT would be useful to diagnose early-stage pancreatic cancer. Moreover, novel sandwich ELISAs that provide for robust and rapid analysis of three ApoA2 isoforms (ApoA2-ATQ/ATQ, ApoA2-ATQ/AT, ApoA2-AT/AT)[16, 17] brought an expectation that it could be used as a screening test for pancreatic cancer. Although determinants of variations in blood levels of ApoA2 isoforms were not elucidated, they might also be associated with risk of cardiovascular disease since ApoA2 is a component of HDL, and pancreatic cancer and cardiovascular disease share common risk factors such as smoking and diabetes. Therefore, we conducted a nested case-control study to test the hypothesis that three major ApoA2 isoforms are inversely associated with myocardial infarction.

Methods

Study Population

The JPHC Study began in 1990 with Cohort I and followed in 1993 with Cohort II in 11 public health center areas throughout Japan. Details of the study design were reported previously[18]. Two public health center areas (Tokyo and Osaka) were excluded from the present study because data related to cardiovascular disease incidence were not available. The study population was defined as all residents (n=116,896) aged 40 to 59 years for Cohort I and 40 to 69 years for Cohort II at baseline. Of these, 220 were excluded because of non-Japanese nationality (n=51), late report of emigration occurring before the start of the follow-up period (n=166), and incorrect birth date (n=3), leaving 116,676 residents eligible for the study. Informed consent was considered to be implicitly provided by each participant at the time they filled in the baseline questionnaire, which described the aim of the study as well as its follow-up. The study was approved by the institutional review boards of the National Cancer Center, Tokyo and Osaka University, Osaka, Japan.

Baseline Surveys

A baseline self-administered questionnaire on various aspects of lifestyle was given to participants in 1990 for Cohort I and in 1993 and 1994 for Cohort II. The overall response rate was 82%, giving 95,374 respondents who were included in the JPHC Study cohort. A trained technician measured blood pressure in the right arm using a standard mercury sphygmomanometer, with the participant in the sitting position after resting for at least five minutes. Body mass index (BMI, kg/m^2) was calculated as weight divided by the square of height. The concentrations of total cholesterol, HDL cholesterol, triglycerides, and glucose in the blood were measured by conventional enzyme methods.

Among the study participants, 34,085 provided 10-ml samples of venous blood. The samples were collected into vacutainer tubes containing heparin at the time of the health examinations, conducted in the

same year as the baseline survey. They were divided into plasma and buffy layers and stored at −80℃ until analysis. Of all the blood samples, 41% were obtained 8 hours or more after the last meal.

Follow-Up Surveillance

We followed the study participants until December 31, 2002. Those who died or moved to other municipalities were identified annually through residential registries in their public health center area. Among the study participants, 9.9% moved away, and 0.2% were lost to follow-up during the study period.

The records of a total of 78 major hospitals located in the study communities and capable of treating patients with myocardial infarction were systematically surveyed for the occurrence of myocardial infarction. Physicians, blinded to the patient lifestyle data, reviewed the medical records at the admitting hospital.

Myocardial infarction events were included in the study if they occurred after the date of return of the baseline questionnaire and before January 1, 2003. We confirmed myocardial infarction by medical records according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease project, which requires typical chest pain and evidence from an electrocardiogram, cardiac enzymes, or autopsy records[19]. For cases with typical prolonged chest pain (≥ 20 min) but not confirmed by electrocardiograms or cardiac enzymes, we diagnosed them as a possible myocardial infarction and included in the myocardial infarction cases.

Selection of Case and Control Participants

During the study period, 106 myocardial infarctions occurred among the 29,876 participants (10,334 men and 19,542 women) who returned the baseline questionnaire, reported no history of myocardial infarction, angina pectoris, stroke, or cancer, and provided blood samples. Two controls for each myocardial infarction case were selected by matching for sex, age (within two years), date of blood sampling (within three months), time since last meal (within 4 hours), and study location (public health center area).

Laboratory Assays

Honda et al. established sandwich ELISAs measuring ApoA2-ATQ and ApoA2-AT[15]. Measurements of ApoA2-ATQ/ATQ and ApoA2-AT/AT were performed by the use of an ApoA2i measurement kit (Human ApoA2 C-terminal ApoA2 ELISA Kit; Toray Industries, Inc., Tokyo, Japan), which uses antibodies specific for each of the homodimers, according to the instruction manual. Then the concentration of ApoA2-ATQ/AT heterodimers was calculated by the formula:

Equation-1: ApoA2-ATQ/AT = (ApoA2-ATQ* ApoA2-AT) ^1/2 [16, 17, 20].

We measured the plasma levels of three ApoA2 isoforms (ApoA2-ATQ/ATQ, ApoA2-ATQ/AT, and ApoA2-AT/AT) in our nested case-control study samples. We used sandwich ELISAs measuring ApoA2-ATQ and ApoA2-AT to quantify ApoA2-ATQ/ATQ, ApoA2-ATQ/AT, and ApoA2-AT/AT. The intra-assay and inter-assay coefficient of variance were both less than 15 % according to the data provided by the manufacturing company. All the laboratory personnel were blinded to case or control status.

Statistical Analyses

Pearson correlation coefficients between ApoA2 isoforms were calculated. ApoA2 isoforms were divided into quartiles according to plasma levels in the controls. We used a two-way analysis of variance tests to compare the baseline characteristics of the cases and controls. As for the comparison of baseline characteristics across quartiles of ApoA2 isoforms, one-way analysis of variance tests was used for comparing continuous variables, while chi-square tests and Fisher exact tests were used for comparing categorical variables. Because triglycerides were not normally distributed, we applied the Kruskal–Wallis test.

Conditional odds ratios (ORs) and 95% confidential intervals (95% CIs) were estimated using a conditional logistic regression model. The potential confounding variables included in the multivariable model were BMI (quartile), systolic blood pressure (quartile), antihypertensive medication use, diabetes mellitus (a fasting glucose level of ≥ 7.0 mmol/L, a non-fasting level of ≥ 11.1 mmol/L, or the use of medication for diabetes), smoking (never smoker, past smoker, and current smoker), and drinking (non-drinker, occasional drinker, and current drinkers of 1 to 149, 150 to 299, 300 to 449, and ≥ 450 g/week) (model 1), and we additionally adjusted for HDL cholesterol (quartile) and triglycerides (quartile) (model 2). BMI, systolic blood pressure, HDL cholesterol, and triglycerides were categorized into quartiles based on the distribution among the control participants. Missing data for any covariates in the analysis were treated as separate categories. All calculations were performed using SAS version 9.4 software (SAS Institute, Cary, NC, USA). All p values for the statistical tests were two-tailed, and p values <0.05 were considered significant.

Table 1. Baseline characteristics of the case and control participants in the JPHC study

	Cases $n=106$	Controls $n=212$	P for difference
Age, y	57.4	57.2	-
Male, %	69.8	69.8	-
Body mass index, kg/m^2	24.4	23.7	0.06
Systolic blood pressure, mmHg	141.3	135.2	0.01
Diastolic blood pressure, mmHg	81.9	80.6	0.45
Total cholesterol, mmol/L	214.0	206.9	0.20
HDL cholesterol, mmol/L	49.3	54.5	0.02
Triglycerides, mmol/L	163.4	127.4	0.003
Antihypertensive medication use, %	30.8	18.7	0.01
Hypertension, %	66.3	53.0	0.04
Diabetes mellitus, %	21.1	9.6	0.005
Current smoker, %	49.1	29.7	<0.001
Alcohol intake ≥ 1 day/week, %	61.1	67.9	0.31
Alcohol intake ≥ 3 days/week, %	47.2	62.8	0.01
Sports during leisure time ≥ 1 time/week, %	26.0	19.5	0.20
Sports during leisure time ≥ 3 time/week, %	13.0	10.5	0.63

Values shown are means or proportions. P for difference was calculated using a two-way analysis of variance tests. Hypertension: systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication use. Diabetes mellitus: fasting glucose level of ≥ 7.0 mmol/L, non-fasting level of ≥ 11.1 mmol/L, or use of medication for diabetes.

Results

From 1990 to 2002, we identified 106 (74 men and 32 women) incident cases of myocardial infarction. Three ApoA2 isoforms (ApoA2-ATQ/ATQ, ApoA2-ATQ/AT, ApoA2-AT/AT) were measured in 106 cases and the matched 212 controls. ApoA2-ATQ/ATQ and ApoA2-AT/AT were negatively correlated ($r=-0.624$, $p<0.001$). No correlation was found between ApoA2-ATQ/ATQ and ApoA2-ATQ/AT ($r=0.100$, $p=0.074$), and a positive correlation was found between ApoA2-ATQ/AT and ApoA2-AT/AT ($r=0.586$, $p<0.001$).

Table 1 shows the risk characteristics of the cases and controls. The average age at the baseline survey was 57 years, and the proportion of men was 70% for both the cases and the controls. The prevalence of antihypertensive medication use, hypertension, diabetes mellitus, and current smoking were higher for myocardial infarction cases than for their controls, while the prevalence of current drinkers (alcohol intake ≥ 3 days per week) was lower for the cases than for the controls. The mean values of systolic blood pressure and triglycerides were higher for the cases than for their controls. The mean values of HDL cholesterol were lower for the cases than for their controls.

Table 2 and **Supplementary Tables 1 and 2** show the main characteristics of the controls divided into quartiles of ApoA2 isoforms. Generally, positive correlations with systolic blood pressure, total cholesterol, HDL cholesterol, and alcohol intake were observed for ApoA2-AT/AT and ApoA2-ATQ/AT, but not for ApoA2-ATQ/ATQ. BMI tended to be positively correlated with ApoA2-ATQ/ATQ.

Next, **Table 3** shows the matched and multivariable-adjusted conditional ORs and 95% confidence intervals for myocardial infarction according to ApoA2 isoforms. ApoA2-AT/AT was inversely associated with risk of myocardial infarction in the matched model, but not in the multivariable-adjusted model; the matched and multivariable ORs of myocardial infarction for the lowest-versus-highest quartiles of ApoA2-AT/AT were 2.78 (95% CI: 1.26–6.09) and 1.20 (95% CI: 0.41–3.57) (model 2), respectively. The association did not alter when confounding variables of HDL cholesterol and triglycerides were excluded from the model; the odds ratio was 1.30 (95% CI: 0.46–3.71) (model 1). Among the confounding variables, especially smoking and drinking exhibited a significant influence on the results. The inverse association between ApoA2-AT/AT and myocardial infarction was weakened after adjustment for smoking only (OR, 2.13; 95% CI, 0.91–4.97) or drinking only (OR, 2.11; 95% CI, 0.91–4.89). When adjusted for exercise, the results did not differ substantially (data not shown). ApoA2-ATQ/ATQ and ApoA2-ATQ/AT were not associated with the risk of myocardial infarction in either the matched or the multivariable-

Table 2. Baseline characteristics of controls divided into quartiles of ApoA2-AT/AT concentrations

	Quartile of ApoA2-AT/AT				
Range of ApoA2-AT/AT	Q1 10.5–58.4	Q2 58.5–79.8	Q3 79.9–120	Q4 121–230	*P* for difference
Age, y	56.4	55.7	58.4	58.5	0.15
Men, %	76.9	58.5	68.5	75.5	0.15
Body mass index, kg/m^2	24.5	23.9	23.3	23.3	0.15
Systolic blood pressure, mmHg	131.6	136.8	135.9	136.5	0.39
Diastolic blood pressure, mmHg	80.6	82.1	79.0	80.8	0.49
Total cholesterol, mmol/L	197.8	203.0	197.3	229.2	<0.001
HDL cholesterol, mmol/L	52.4	54.1	50.3	61.4	0.004
Triglycerides, mmol/L	112.8	117.6	126.0	151.0	0.24[†]
Antihypertensive medication use, %	23.5	15.7	18.5	17.0	0.75
Hypertension, %	50.0	53.1	56.0	52.9	0.95
Diabetes mellitus, %	9.1	11.6	8.1	9.5	0.97[‡]
Current smoker, %	32.7	28.3	27.8	30.2	0.95
Alcohol intake ≥ 1 day/week, %	61.5	53.8	72.2	83.3	0.03
Alcohol intake ≥ 3 days/week, %	53.8	51.3	63.9	81.0	0.02
Sports during leisure time ≥ 1 time/week, %	25.0	22.4	18.9	12.0	0.39
Sports during leisure time ≥ 3 time/week, %	10.4	16.3	9.4	6.0	0.41

Values shown are means or proportions. *P* for difference was calculated using a one-way analysis of variance tests or the chi-square test. Hypertension: systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication use. Diabetes mellitus: fasting glucose level of ≥ 7.0 mmol/L, non-fasting level of ≥ 11.1 mmol/L, or use of medication for diabetes.
[†]Kruskal–Wallis test. [‡]Fisher exact test.

Table 3. Conditional odds ratios (ORs) and 95% confidence intervals (95% CIs) of incident myocardial infarction associated with ApoA2 isoforms

	Number cases/controls	Matched OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
ApoA2-ATQ/ATQ				
Quartile 1	19/55	0.66 (0.32–1.36)	1.08 (0.44–2.65)	0.91 (0.35–2.37)
Quartile 2	31/54	1.07 (0.55–2.11)	1.71 (0.71–4.10)	1.44 (0.58–3.56)
Quartile 3	29/52	1.08 (0.54–2.13)	1.57 (0.66–3.75)	1.34 (0.54–3.29)
Quartile 4	27/51	1	1	1
P for trend		0.246	0.940	0.902
ApoA2-ATQ/AT				
Quartile 1	36/54	1.93 (0.95–3.92)	1.54 (0.61–3.88)	1.60 (0.59–4.35)
Quartile 2	33/52	1.77 (0.88–3.59)	1.15 (0.47–2.83)	1.06 (0.41–2.76)
Quartile 3	17/53	0.91 (0.43–1.89)	0.90 (0.36–2.23)	0.85 (0.32–2.27)
Quartile 4	20/53	1	1	1
P for trend		0.016	0.420	0.419
ApoA2-AT/AT				
Quartile 1	39/52	2.78 (1.26–6.09)	1.30 (0.46–3.71)	1.20 (0.41–3.57)
Quartile 2	20/53	1.30 (0.60–2.81)	0.62 (0.24–1.62)	0.57 (0.21–1.59)
Quartile 3	29/54	1.87 (0.89–3.93)	1.19 (0.47–2.97)	0.98 (0.36–2.68)
Quartile 4	18/53	1	1	1
P for trend		0.017	0.801	0.970

Matched for age, sex, date of blood sampling, time since last meal, and study location. Model 1: adjusted for body mass index, systolic blood pressure, antihypertensive medication use, diabetes mellitus, smoking, and drinking. Model 2: model 1 + HDL cholesterol and triglycerides.

adjusted model.

Discussion

Among ApoA2 isoforms, only ApoA2-AT/AT was associated inversely with the risk of myocardial infarction before adjustment for confounding variables, but that association was no longer significant after further adjustments. Originally, ApoA2 isoforms were found in the process of developing a biomarker for early detection of pancreatic cancer and was not studied for its association with cardiovascular diseases. The present study suggested that ApoA2 isoforms were not a predictor for the risk of myocardial infarction, independent of conventional risk factors.

Nonetheless, the fact that the ORs were different among ApoA2 isoforms is noteworthy; that is, only ApoA2-AT/AT was associated with risk of myocardial infarction in the unadjusted model. ApoA2 isoforms are determined by the cleaved pattern of the C-terminal amino acids of the ApoA2 homodimer. ApoA2-AT/AT is a light isoform, and its C-terminal amino acids are shorter than those of ApoA2-ATQ/ATQ and ApoA2-ATQ/AT. Whether ApoA2 isoforms demonstrate different anti-atherogenic or pro-atherogenic functions is uncertain, nor whether lifestyles, such as smoking and drinking, affect their metabolisms.

Since we, unfortunately, did not measure total ApoA2, the association between total ApoA2 and the risk of myocardial infarction was unknown in this population. A previous study showed a negative association between total ApoA2 and the risk of coronary artery disease[8]. Concordantly, the present study showed inverse trends for the risk of myocardial infarction with ApoA2-ATQ/AT and ApoA2-AT/AT isoforms in the matched models, which may partly explain the association with total ApoA2. However, the composition of each isoform may not necessarily proportionally reflect total ApoA2, under a negative correlation between ApoA2-ATQ/ATQ and ApoA2-AT/AT.

This prospective nested case-control study was the first to report the association between ApoA2 isoforms and the risk of myocardial infarction. However, this study demonstrated several limitations. First, we did not measure ApoA2 concentrations *per se* or minor ApoA2 isoforms (ApoA2-AT/A and ApoA2-A/A), and their association with myocardial infarction could not be analyzed in the present study. ApoA2-AT/A and ApoA2-A/A were not measured in this study because they could not be measured using ELISA. Second, the nested case-control study design and its limited sample size might be one of the reasons for the absence of significant associations between ApoA2 isoforms and myocardial infarction. In the present study, all multivariable-adjusted associations were 1.60 or less, which did not reach statistical significance, probably owing to the limited statistical power. Third, the samples were stored at –80°C for more than 10 years, and the repeatability of the ApoA2 measurements over a long preservation time was unknown, although the preservation time was almost the same among samples.

Conclusions

This is the first analysis of the association between ApoA2 isoforms and the risk of incident myocardial infarction in a prospective nested case-control study, and all three ApoA2 isoforms did not show any significant associations. Only ApoA2-AT/AT was associated with incident myocardial infarction in the matched model, but its association, if any, may be less clinically significant than that of HDL cholesterol.

Acknowledgements

The authors thank Ms. Flaminia Miyamasu, University of Tsukuba, for editorial assistance. JPHC members are listed at the following site (as of April 2019): https://epi.ncc.go.jp/en/jphc/781/8390.html

Notice of Grant Support

This study was supported by a National Cancer Center Research and Development Fund (since 2011) and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan (from 1989 to 2010).

Conflict of Interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript. Kazufumi Honda obtained research funding from Toray Co., outside the submitted work.

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Supplementary Table 1. Baseline characteristics of controls divided into quartiles of ApoA2-ATQ/ATQ concentrations

	Quartile of ApoA2-ATQ/ATQ				
Range of ApoA2-ATQ/ATQ	Q1 3.9–32.5	Q2 32.6–44.8	Q3 44.9–61.3	Q4 61.4–105	*P* for difference
Age, y	60.7	57.0	55.0	56.0	<0.001
Men, %	65.5	72.2	65.4	76.5	0.53
Body mass index, kg/m^2	22.8	23.9	24.1	24.2	0.053
Systolic blood pressure, mmHg	134.5	137.8	132.3	136.3	0.42
Diastolic blood pressure, mmHg	78.7	81.6	79.5	83.0	0.14
Total cholesterol, mmol/L	205.1	201.3	208.2	213.3	0.63
HDL cholesterol, mmol/L	54.8	54.2	51.2	58.1	0.25
Triglycerides, mmol/L	137.5	125.7	120.5	122.5	0.51[†]
Antihypertensive medication use, %	18.5	18.5	21.6	16.0	0.91
Hypertension, %	44.4	58.0	57.1	53.2	0.49
Diabetes mellitus, %	9.5	9.3	4.9	15.0	0.52[‡]
Current smoker, %	18.2	27.8	40.4	33.3	0.08
Alcohol intake ≥ 1 day/week, %	71.4	77.3	52.8	68.3	0.12
Alcohol intake ≥ 3 days/week, %	71.4	68.2	44.4	65.9	0.07
Sports during leisure time ≥ 1 time/week, %	24.0	9.4	27.5	17.4	0.10
Sports during leisure time ≥ 3 time/week, %	14.0	3.8	19.6	4.3	0.02

Values shown are means or proportions. *P* for difference was calculated using a one-way analysis of variance tests or the chi-square test. Hypertension: systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication use. Diabetes mellitus: fasting glucose level of ≥ 7.0 mmol/L, non-fasting level of ≥ 11.1 mmol/L, or use of medication for diabetes.
[†]Kruskal-Wallis test. [‡]Fisher exact test.

Supplementary Table 2. Baseline characteristics of controls divided into quartiles of ApoA2-ATQ/AT concentrations

	Quartile of ApoA2-ATQ/AT				
Range of ApoA2-ATQ/AT	Q1 21.6–50.6	Q2 50.7–58.5	Q3 58.6–66.6	Q4 66.7–116	*P* for difference
Age, y	58.4	57.1	56.0	57.4	0.44
Men, %	74.1	61.5	73.6	69.8	0.47
Body mass index, kg/m^2	23.5	23.9	24.0	23.6	0.83
Systolic blood pressure, mmHg	130.9	137.7	132.8	139.6	0.04
Diastolic blood pressure, mmHg	78.7	80.8	80.3	82.8	0.24
Total cholesterol, mmol/L	188.5	198.7	212.5	229.4	<0.001
HDL cholesterol, mmol/L	51.0	52.5	52.6	63.0	<0.001
Triglycerides, mmol/L	115.9	126.1	119.0	149.4	0.48[†]
Antihypertensive medication use, %	19.2	19.2	13.2	23.1	0.63
Hypertension, %	43.1	58.8	54.0	56.3	0.41
Diabetes mellitus, %	4.8	11.4	7.5	15.0	0.43[‡]
Current smoker, %	27.8	25.0	30.2	35.8	0.66
Alcohol intake ≥ 1 day/week, %	61.5	63.6	67.4	78.0	0.40
Alcohol intake ≥ 3 days/week, %	53.8	54.5	65.1	75.6	0.15
Sports during leisure time ≥ 1 time/week, %	29.2	13.5	23.1	12.5	0.11
Sports during leisure time ≥ 3 time/week, %	16.7	7.7	11.5	6.3	0.34

Values shown are means or proportions. *P* for difference was calculated using a one-way analysis of variance tests or the chi-square test. Hypertension: systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication use. Diabetes mellitus: fasting glucose level of ≥ 7.0 mmol/L, non-fasting level of ≥ 11.1 mmol/L, or use of medication for diabetes.
[†]Kruskal-Wallis test. [‡]Fisher exact test.