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Potential role for plasma placental growth factor in predicting coronary heart disease risk in women

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Abstract

Objective—To examine placental growth factor's (PlGF) predictive value in relation to coronary heart disease (CHD) risk in healthy women.

Methods and results—Among 32,826 women from the Nurses' Health Study who provided blood samples at baseline, 453 CHD events were documented during 14 years of follow-up. Controls were matched to cases (2:1) for age, smoking, fasting status, and date of blood sampling. PlGF was inversely correlated with HDL-cholesterol (HDL-C), and positively correlated with several coronary risk factors. In multivariate models, women in the highest versus lowest quintile of PlGF had a greater risk of CHD (RR:1.58;95%CI:1.03-2.41). Additional adjustment for many coronary risk factors did not substantively alter this relationship, but HDL-C attenuated the association (RR:1.25;95%CI:0.81-1.94). In exploratory time to event analysis, higher PlGF levels, measured > 10 years prior to CHD event, but not < 10 years pre-clinical event, were associated with increased risk of CHD, even after adjustment for co-morbid conditions and HDL-C levels (RR:2.79;95%CI:1.19-6.56).

Conclusions—Elevated prediagnostic PlGF levels were modestly associated with subsequent risk of CHD events and results were attenuated after controlling for HDL-C. PlGF may be most strongly associated with long term prediction of CHD, consistent with a potential role in early plaque formation and growth.

Keywords

PlGF; CHD; women

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INTRODUCTION

Placental growth factor (PlGF), a member of the vascular endothelial growth factor family, was originally discovered in the placenta, but is also present in a range of other tissues including the heart. PlGF plays an important role in a range of physiological and pathological conditions including preeclampsia, cardiovascular diseases (CVD), wound healing and tumour progression.[1] PlGF is a functional cytokine, which stimulates angiogenesis and atherogenic migration of monocytes/macrophages into the arterial wall.[2] Animal data suggest that PlGF may act as an early and primary inflammatory instigator of atherosclerotic plaque instability. Gene inactivation studies in mice show that loss of PlGF impairs both angiogenesis and arteriogenesis during pathological conditions [3] and result in a diminished and abbreviated inflammatory response[4]. Inhibition of PlGF, by blocking its receptor in an animal model, suppressed both atherosclerotic plaque growth and vulnerability by inhibiting inflammatory cell infiltration.[5, 6] In an animal knockout experiment, inhibition of both PlGF and Apo E decreased early atherosclerotic lesion size, but showed no difference in late lesions compared with ApoE knock out mice.[2]

Elevated PlGF concentrations are an independent biomarker of short term (30 days) and long-term (4 years) adverse outcome in patients with suspect acute coronary syndrome (ACS) [7,8], and an independent predictor of CVD morbidity and mortality in type 1 diabetics.[9] Although these data establish its pathological role, its potential physiological role in initiating and predicting CVD events in a healthy population is less clear. Therefore to examine the potential physiological role of PlGF in humans and determine its predictive value in relation to myocardial infarction (MI) risk, we conducted a nested case-control study within the Nurses' Health Study (NHS) to assess the association between baseline PlGF levels and risk of MI over a follow-up period of 14 years.

Materials and Methods

Study Population

The Nurses' Health Study (NHS) is a prospective cohort investigation involving 121,700 female U.S. registered nurses who were 30 to 55 years old at baseline in 1976. Information about health and disease is assessed biennially, and dietary information is obtained every four years through self-administered questionnaires.[10, 11] From 1989 to 1990, a blood sample was requested from all participants, and was provided by 32,826 women. Women who provided blood samples were similar to those who did not.[12] Among the women who provided a blood sample and were free of cardiovascular disease or cancer at the time of collection, we identified 453 women who had a nonfatal myocardial infarction or fatal coronary heart disease (CHD) between the date of blood drawing and June 2004. Using risk-set sampling [13], we randomly selected controls, in a 2:1 ratio who were matched to cases on age, smoking status, fasting status and date of blood sampling from the subgroup of participants who were free of diagnosed cardiovascular disease at the time coronarydisease was diagnosed in the cases.

Assessment of Coronary Heart Disease

Diagnosis of CHD events was confirmed by the study physicians on the basis of the criteria of the World Health Organization (symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes). Deaths were identified from state records, the National Death Index or reported by the participant's next of kin or the postal system. Fatal coronary heart disease was confirmed by an examination of hospital or autopsy records, or by the recording of CHD as the underlying and most probable cause of death on the death certificate, and if evidence of previous CHD was available within the NHS. When

complete medical records could not be obtained, the case was considered probable (17% of cases). When analyses were restricted to confirmed cases only there was no change in results.

Assessment of Other Factors

Anthropometric, lifestyle, self reported blood pressure measurements and dietary data were derived from the questionnaire administered in 1990, with missing information substituted from previous questionnaires. Body-mass index was calculated as the weight in kilograms divided by the square of the height in meters. Average nutrient intake was computed with the use of a semi-quantitative food-frequency questionnaire. Physical activity was expressed in terms of metabolic equivalent (MET)–hours. The questionnaires and the validity and reproducibility of measurements have been described previously.[10, 11, 14]

Measurement of Biochemical Variables

Blood samples were collected in tubes treated with liquid sodium heparin, placed on ice packs, stored in Styrofoam containers, and returned to the laboratory by overnight courier, where they were centrifuged, and divided into aliquots for storage in liquid-nitrogen freezers (–130°C). Study samples were sent to the laboratory for analysis in randomly ordered batches, and the laboratory personnel were blinded to the participant's histories. Levels of PlGF were determined in plasma by enzyme-linked immunosorbent assay (R&D Systems, Wiesbaden, Germany). The minimal detectable dose for this assay is 7 ng/L and can therefore discriminate over the range of concentrations observed in our healthy population (10-40 ng/L). Total imprecision (expressed as coefficient of variation (CV)), based on blinded split samples provided by women from NHS was <6%. In a pilot study, where we assessed stability in plasma samples processed at 0, 24 and 48hrs, we observed an overall intraclass correlation coefficient of 0.51, with mean changes of <4% up to 48 hrs. These data suggest that PlGF concentrations remain stable if kept on ice for 48hr until processing, which agrees with previous data for the stability of a range of other biomarkers.[15]

Total, high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL) cholesterol and triglycerides (TRIG) were measured according to standard methods with the use of reagents from Roche Diagnostics and Genzyme with CVs of 1.7%, 1.8%, 2.5% and 3.1% respectively. Apolipoprotein B (ApoB), C-reactive protein (CRP) and HbA1c levels were quantified using an immunoturbidimetric technique on the Hitachi 911 analyzer, with a CV of 4.3%, 1.4% and 2.6% respectively. Total adiponectin levels were measured by competitive radioimmunoassay (Linco Research Inc, St Charles, Mo) with a CV of 3.4%. Lipid biomarkers and CRP assays were performed in the laboratory of Dr Nader Rifai (The Children's Hospital, Boston, Mass), which is certified by the NHLBI/CDC Lipid Standardization program.

The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

Statistical Analyses

Continuous variables are shown as means (standard deviations) or medians (interquartile range) and the unpaired t test or the Wilcoxon unpaired rank sum test was used to compare cases and controls. Proportions were compared using the χ^2 test. In cases and controls, associations between PLGF levels and selected cardiovascular risk factors were examined using an age-adjusted Spearman partial correlation coefficient.

PlGF levels were categorized into quintiles based on the distribution of levels in the control participants. We used conditional logistic regression to investigate the association between baseline PlGF concentrations and incidence of CHD. Models were conditioned on the matching variables (age + 2 years; smoking status (never, past, or current); the month of blood draw; and fasting status (yes/no). In our multivariable model, we further adjusted for parental history of MI before age 60 years (yes/no), nonsteroidal anti-inflammatory drug (NSAID) use (yes/no), postmenopausal hormone (PMH) (yes/no), physical activity (quintiles of MET), and alcohol intake and body mass index as continuous variables. For triglycerides, we did not have fasting values for 98 participants, and for other covariates, 3% or less of values were missing. Results were not altered when women with missing values were excluded or when results were restricted to fasting participants only. Tests for linear trend were conducted by assigning the median value for each quintile of PlGF and treating this new variable as continuous. We also modeled PlGF as a linear variable and have presented RR for approximately 2 standard deviation difference (5 ng/L). We also examined the association between length of time to CHD event from blood draw and type of CHD. Because of the design of our study, the odds ratio derived from conditional logistic regression directly estimates the incidence rate (hazard) ratio and, therefore, the relative risk (RR). [13, 16]

We assessed effect modification by several cardiovascular risk factors: BMI (<25, 25-29.9, ≥30 kg/m^2), CRP (<1, 1-3, >3 mg/dl), HDL (tertiles: <51, 51-65, >65) age (<60, ≥60) and presence of the metabolic syndrome (<3, ≥3 risk factors). To test formally for interaction, we created a product term of categorized CVD risk factor, as stated above, and the PlGF concentration (continuous) and used a likelihood ratio test, comparing the model with and without the interaction term. All P values presented are 2-tailed; P<.05 was considered statistically significant. All analyses were performed using SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

Results

PlGF levels were significantly higher in the cases (n=453) compared to the controls (n=895) (Table 1). As expected lipoproteins, inflammatory markers, history of hypertension, hypercholesterolemia and diabetes as well as other CHD risk factors were significantly different in cases compared to controls.

In the controls, there was a modest positive correlation between PlGF concentrations and age (r=0.19, p<0.001), triglyceride concentrations (r=0.21, p<0.001), and an inverse correlation with HDL-C levels (r=-0.19, p<0.001) (Table 2).

In models conditioned on matching factors and adjusted for BMI, parental history of MI, alcohol use, exercise, NSAID and PMH use, participants in the highest (≥17.9 ng/L) versus lowest (≤14.1 ng/L) quintile of PlGF had a higher risk of MI (RR, 1.58; 95% confidence interval (CI), 1.03-2.41; RR per 5 ng/L increase: 1.50; 95% CI 1.07-2.10) (Table 3). Addition of HDL-C to the model significantly attenuated the association (RR per 5 ng/L increase: 1.23; 95% CI 0.87-1.75). Addition of further cardiovascular biomarkers to the model, which included CRP, TRIG, ApoB, HbA1c, adiponectin, systolic and diastolic blood pressure, did not appreciably alter the association (data not shown). Further adjustment for history of comorbid conditions, including hypertension, diabetes and high cholesterol attenuated the result (RR per 5 ng/L increase: 1.11; 95% CI (0.77-1.60).

To examine a potential threshold of CHD risk at higher levels, as previous clinical data observed effects only at levels >27ng/L, we further split quintile 5 to look at the top 10% concentrations as only 5 women in our population had PlGF levels exceeding 27 ng/L. Even

within the range of PlGF levels in these healthy women (13.2 – 19.2 ng/L median range for quintiles 1 to 5) the observed linear trend over the quintiles of PlGF levels was further apparent when we looked at the top 10% concentrations of PlGF. The multivariate RR (95% CI) for CHD before and after adjustment for HDL-C, comparing the top 10% of PlGF concentrations (median value 20.2 ng/L) with the lowest quintile were 1.76 (1.09-2.84) and 1.39 (0.85-2.28) respectively.

To further explore the potential role of PlGF as an acute or long term marker of CHD risk we conducted an analysis of time to event from blood draw. In contrast to our *a priori* expectations, we found no significant short-term relationship between PlGF concentrations and CHD risk after initial blood draw (Table 4). However we observed that baseline PlGF levels were a significant predictor of CHD events that occurred >10 years from follow-up; women in the highest PlGF quintile had a more than 2-fold increased risk of CHD (RR, 3.48;95% CI 1.53-7.88) compared to those in the lowest quintile. This relationship remained significant even after adjustment for HDL-C and history of other comorbid conditions (RR 2.79;95% CI 1.19-6.56). We did not observe a similar pattern over time with known established biomarkers of CVD risk. For example, for a 10 mg/dL increase in HDL-C, relative risks were RR, 0.72; 95% CI 0.58-0.90 for < 5 years, RR, 0.80; 95% CI 0.67-0.97 5-10 years and RR, 0.83; 95% CI 0.67-1.02 >10 years from blood draw. This relationship was not explained by differences in median PlGF values in the cases which were similar at all three time-points (1st 5 years: 16.1 ng/L; 5-10 years 16.2 ng/L; 10+ years: 16.3 ng/L).

There was no evidence for effect modification between PlGF and CRP, BMI, HDL-C, age and metabolic syndrome risk factors (data not shown).

Discussion

In this prospective “nested” case control study, we found a modest association between plasma PlGF levels and subsequent risk of CHD in women, a result that was attenuated after controlling for HDL-C. Our results suggest that PlGF may be a long-term biomarker of CHD risk, as PlGF levels predicted risk only after 10 years of follow-up.

To our knowledge this is the first evidence to suggest that PlGF levels may predict MI events years in advance in a population without diagnosed CHD. Animal data support a potential role for PlGF in early plaque formation and growth [2, 5] and provide biological plausibility to our findings. PlGF is upregulated in early atherosclerotic lesions in apolipoprotein E deficient (ApoE $^{-/-}$) mice[2] , and in double knock out mice for ApoE $^{-/-}$ and PlGF both the size and macrophage content of early atherosclerotic plaques are significantly reduced.[2] PlGF also directly promotes atherogenic intimal thickening and intimal macrophage recruitment, suggesting that its production is required for macrophage migration into early atherosclerotic lesions.[2]

No previous studies have examined the potential role of PlGF among healthy populations, although in a cohort of 190 type 1 diabetic patients with diabetic nephropathy, elevated PlGF concentrations predicted higher risk of CVD after 10 years of follow-up, independent of kidney function and established CHD risk biomarkers.[9] However, a growing body of evidence supports its prognostic value in relation to short and long term outcome in patients with acute coronary syndromes (ACS).[7, 8] Elevated PlGF concentrations have emerged as an important independent biomarker of short term adverse outcomes in patients with acute chest pain and known or suspected ACS.[7] In addition, during long term follow-up of patients with ACS, elevated plasma PlGF concentrations are associated with adverse clinical outcomes.[8] In another study they determined the clinical significance of PlGF cardiac expression and showed that it is rapidly produced in the infarct myocardium, mainly by

endothelial cells during the acute phase of MI. [17] PlGF production post MI is in part determined by the amount of injured myocardium and plasma levels on day 3 post MI were positively correlated with circulating monocyte counts during the acute phase and the degree of improvement in left ventricular function during the chronic phase of MI. These data suggest that cardiac production of PlGF may have a beneficial effect on wound healing, possibly by inducing peripheral mobilization of mononuclear cells and enhancing angiogenesis.[17]

Together the animal and clinical data show the divergent pathological and physiological roles PLGF plays. The biological roles of both PlGF and its specific receptor, Flt-1 (VEGFR-1) are currently unclear, with different mechanisms involved in explaining physiological and pathophysiological effects. Evidence suggests its pathophysiological role relates more to vascular inflammation than to angiogenesis [5], while its effects in healthy individuals relate to its role in the promotion of macrophage infiltration and/or mobilization of bone marrow-derived progenitors for macrophages or plaque neovessels in early plaque formation and growth.[2] PlGF has other known mechanisms of action including stimulation of vascular smooth muscle growth, recruitment of macrophages into atherosclerotic lesions and up regulation of production of TNFα and MCP1 by macrophages.[5, 18-20] In cholesterol fed rabbits, targeted adenoviral PlGF overexpression promoted a spectrum of atherogenic processes including formation of macrophage-rich neointimal lesions.[2] It also plays a role in vascular development [3, 21, 22] by stimulating arteriogenesis and angiogenesis, enhancing capillary and collateral formation in ischemic tissues [3], and promoting mobilization of Flt-1 positive haematopietic stem cells that are involved in regeneration of vessels and myocardium from bone marrow to the peripheral circulation.[21, 22]

In patients with ACS, a diagnostic threshold of >27 ng/L maximized the predictive value of PlGF.[7, 8] In our study of healthy participants, the range of PlGF levels were much lower, as expected (Table 2). Over this more narrow range of PlGF concentrations we observed only a modest increase in risk of CHD especially after adjustment for HDL-C. When we split the highest quintile to look at the top 10% of the PlGF distribution, risk was further elevated compared to the bottom quintile. While not statistically significant, the RR of CHD was higher in women in the top decile of PlGF (≥19.2ng/L) even after controlling for HDL-C and CVD risk factors (RR, 1.39:95% CI 0.85-2.28). It is plausible that PlGF at higher concentrations than detected in our population may be a better predictor of CHD risk.

In our overall analysis, the risk of CHD was attenuated after controlling for HDL-C and CVD related conditions. Our data cannot determine if these factors are confounders or potential mediators of the observed associations between PlGF and CHD risk but which factors explain the time to event analysis remains unclear.

Our study has several limitations. As with any observational study design no causal associations can be made and there is the possibility of unmeasured confounding, although we controlled for a range of known cardiovascular risk factors. Plasma PlGF levels were based on a single blood sample collected in 1990 which may not represent average PlGF concentrations. On the basis of the current data, it is unclear if PlGF concentrations change over time. Time series blood samples would determine if higher levels are sustained throughout life, increase over time, are transiently high or undergo age related shifts. We do not have data to determine if these findings are generalizable to men or other ethnicities. The assay's minimal detectable dose is 7 ng/L thus the method discriminated over the range of concentrations observed in our healthy population (10-40 ng/L) and the development of a more sensitive assay would potentially provide a higher predictive value for PlGF in future studies.

Our study suggests that elevated PlGF levels among women without known CHD may be modestly associated with subsequent risk of CHD. We found PlGF to be a long term predictor of CHD risk, a finding that was not observed for other traditional biomarkers of CHD risk. This finding is supported by the animal data which suggest a role for PlGF in the promotion of macrophage infiltration and early plaque formation and growth.[2] This finding therefore warrants further investigation and replication to substantiate these novel observations, and further understand PlGF's importance in atheroma formation and plaque destabilization, and to determine its utility as a long term risk biomarker.

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Table 1

Baseline characteristics of women with incident CHD and matched controls during 14 years of follow-up in the Nurses' Health Study

	Cases (n = 453)	Controls (n=895)	p-value
PLGF ng/L			
Mean (SD)	16.6 (2.9)	16.1 (2.6)	<0.01
Median (Interquartile Range)	16.2 (14.7, 17.8)	15.8 (14.5, 17.3)	<0.01
Age, years	60 (6.5)	60 (6.5)	--
Current smoking, %	26	26	--
BMI, kg/m^2	26.7 (5.3)	25.1 (4.2)	<0.001
Postmenopausal Hormone use, %	35	39	0.22
Family history of MI, %	21	13	<0.001
History of hypertension, %	51	27	<0.001
History of hypercholesterolemia, %	53	41	<0.001
History of diabetes, %	15	6	<0.001
Cholesterol (mg/dl), Mean (SD)			
Total	234 (38)	227 (39)	<0.01
LDL	144 (36)	135 (37)	<0.001
HDL	52.1 (14.9)	59.8 (16.8)	<0.001
Triglycerides (mg/dl), median (IQR) *	125 (87, 177)	105 (76, 146)	<0.001
Apolipoprotein B (mg/dl), mean (SD)	117 (34)	105 (28)	<0.001
HbA1c (%), median (IQR)	5.6 (5.3, 6.0)	5.4 (5.2, 5.7)	<0.001
CRP (mg/dl), median (IQR)	0.27 (0.12-0.58)	0.20 (0.08, 1.39)	<0.001
Adiponectin (ng/mL), median (IQR)	7524 (5349, 10088)	8678 (6521, 11427)	<0.001
Physical activity (MET-hr/wk), median (IQR)	11.0 (3.9, 25.7)	12.1 (5.2, 25.2)	0.08
Alcohol (g/day), median (IQR)	1.0 (0, 5.3)	1.8 (0, 8.3)	<0.01

* 425 cases and 825 controls for fasting Trig

Table 2

Spearman correlation coefficients between plasma PLGF concentrations and selected cardiovascular risk factors among the 895 controls (age adjusted) from the Nurses' Health Study

Characteristics	r	p-value
Age, years	0.19	<0.001
Cholesterol (mg/dl)		
Total	0.01	0.80
LDL	0.03	0.37
HDL	−0.19	<0.001
Triglycerides (mg/dl) *	0.21	<0.001
Apolipoprotein B (mg/dl)	0.07	0.04
CRP (mg/dl)	0.09	0.02
HbA1c (%)	0.10	0.003
Adiponectin (ng/mL)	−0.11	0.001
BMI (kg/m^2)	0.08	0.02
Systolic Blood Pressure (mm Hg)	0.10	<0.01
Diastolic Blood Pressure (mm Hg)	0.11	0.001
Alcohol (g/day)	−0.07	0.04
Physical activity (MET-hr/wk)	−0.04	0.29

* N = 825 for fasting triglycerides

Table 3

Relative risks (95% confidence intervals) of CHD during 14 years of follow-up according to baseline PLGF levels

	Quintiles of PLGF concentration (ng/L)						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**	**5ng/L increase**
Range	10.4-14.1	14.2-15.2	15.3-16.3	16.4-17.8	17.9-40.4		
Median	13.3	14.8	15.8	17.0	19.2		
Cases	75	75	90	101	112		
Controls	179	179	178	180	179		
Multivariate model	1.0 (ref)	1.05 (0.67-1.46)	1.25 (0.84-1.88)	1.33 (0.88-2.01)	1.58 (1.03-2.41)	0.02	1.50 (1.07-2.10)
Multivariate model + HDL	1.0 (ref)	0.99 (0.62-1.38)	1.17 (0.78-1.77)	1.21 (0.79-1.85)	1.25 (0.81-1.94)	0.24	1.23 (0.87-1.75)
Multivariate model + HDL and history of comorbid conditions *	1.0 (ref)	0.94 (0.58-1.34)	1.22 (0.80-1.86)	1.08 (0.70-1.67)	1.11 (0.71-1.77)	0.57	1.11 (0.77-1.60)

RR estimated by conditional logistic regression, conditioned on the matching factors (smoking, age, month of blood draw, fasting status).

Multivariate Model adjusted for BMI, alcohol, exercise, family history of MI, NSAID use, PMH use

* history of hypertension, diabetes or hypercholesterolemia

Table 4

RR of CHD by length of time since blood draw in 453 CHD cases and matched controls in the Nurses' Health Study

	Quintiles of PLGF concentration (ng/L)						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**		
Median	**13.3**	**14.8**	**15.8**	**17.0**	**19.2**	**p-trend**	**5 ng/L increase**
1st 5 years (29%)							
Cases	24	21	28	28	32		
Controls	51	53	59	50	47		
Multivariate model	1.0 *(ref)*	0.81 (0.37-1.78)	1.02 (0.49-2.12)	1.19 (0.54-2.62)	1.29 (0.61-2.75)	0.28	1.38 (0.76-2.49)
Multivariate model and HDL-C	1.0 *(ref)*	0.82 (0.36-1.85)	1.02 (0.47-2.18)	1.10 (0.49-2.46)	1.03 (0.47-2.26)	0.75	1.10 (0.60-2.04)
Multivariate model + history of comorbid conditions *	1.0 *(ref)*	0.63 (0.26-1.54)	1.21 (0.53-2.75)	0.86 (0.36-2.06)	0.80 (0.34-1.93)	0.80	0.92 (0.47-1.81)
5-10 years (38%)							
Cases	28	29	33	35	46		
Controls	59	64	58	70	88		
Multivariate model	1.0 *(ref)*	0.94 (0.50-1.80)	1.13 (0.57-2.24)	0.94 (0.47-1.88)	1.04 (0.52-2.09)	0.93	1.03 (0.59-1.78)
Multivariate model and HDL-C	1.0 *(ref)*	0.90 (0.47-1.73)	1.05 (0.53-2.11)	0.86 (0.42-1.76)	0.82 (0.40-1.67)	0.53	0.83 (0.47-1.47)
Multivariate model + history of comorbid conditions *	1.0 *(ref)*	0.90 (0.45-1.80)	1.10 (0.54-2.25)	0.74 (0.35-1.56)	0.70 (0.33-148)	0.27	0.72 (0.39-1.30)
10+ years (33%)							
Cases	23	25	29	38	34		
Controls	69	62	61	60	44		
Multivariate model	1.0 *(ref)*	1.46 (0.73-2.94)	1.71 (0.85-3.47)	2.19 (1.07-4.51)	3.48 (1.53-7.88)	0.02	2.83 (1.46-5.50)
Multivariate model and HDL-C	1.0 *(ref)*	1.34 (0.66-2.71)	1.55 (0.76-3.16)	1.96 (0.95-4.07)	2.86 (1.24-6.59)	0.01	2.43 (1.23-4.80)
Multivariate model + history of comorbid conditions *	1.0 *(ref)*	1.30 (0.63-2.68)	1.51 (0.73-3.09)	1.83 (0.87-3.86)	2.79 (1.19-6.56)	0.01	2.37 (1.19-4.75)

* Multivariate Model adjusted for BMI, alcohol, exercise, family history of MI, NSAID use, PMH use, HDL-cholesterol, history of hypertension, diabetes or hypercholesterolemia

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