Published in final edited form as:

Clin Endocrinol (Oxf). 2011 January ; 74(1): 44–50. doi:10.1111/j.1365-2265.2010.03879.x.

Gender Differences in the Association of C-Reactive Protein with Coronary Artery Calcium in Type-2 Diabetes

Nehal N. Mehta, MD MSCE^{*}, Caitlin St Clair, BS^{*}, Samira Farouk, BS^{*}, Seth Braunstein, MD[‡], Mark Schutta, MD[‡], Nayyar Iqbal, MD[‡], Daniel Rader, MD^{*,‡,§}, Muredach P. Reilly, MB MSCE^{*,‡,§}, Atif N. Qasim, MD^{*}, and Venkata Budharaju, MD[†]

^{*}Cardiovascular Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

[‡]The Institute of Diabetes Obesity and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

[§]The Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

[†]The Department of Endocrinology, Diabetes and Metabolism, University of Iowa Hospitals

Abstract

Objective—Plasma C-reactive protein (CRP) is associated with cardiovascular disease (CVD) but effects may vary by gender and degree of CVD risk. Whether CRP has value as a CVD risk marker in type-2 diabetes (T2DM) is unclear. We examined whether CRP has gender differences in the association of coronary artery calcium (CAC) in diabetic and non diabetic samples without clinical CVD.

Methods—We performed cross-sectional analyses of gender influence on CRP association with CAC in the Penn Diabetes Heart Study (N = 1299 with T2DM), the Study of Inherited Risk of Coronary Atherosclerosis (N = 860 non diabetic subjects), and a combined sample.

Results—Female gender was associated with higher plasma CRP in diabetic and non-diabetic samples after adjustment for covariates. There was a strong interaction by gender in the association of CRP with CAC (interaction $p < 0.001$). In diabetic women, CRP was associated with higher CAC even after further adjustment for age, race, medications, metabolic syndrome, Framingham risk score, and body mass index [Tobit ratio 1.60, 95% CI (1.03-2.47)]. Although this relationship was attenuated in non diabetic women, the combined sample maintained this association in fully adjusted models [1.44, 95% CI (1.13-1.83)]. There was no association of CRP with CAC in either diabetic or non diabetic men.

Conclusions—CRP may be a useful marker of cardiovascular risk in women, particularly in diabetic women who otherwise have no known CVD. Prospective studies are needed to better assess gender differences in CRP utility and the use of CRP in T2DM.

Key Terms

Coronary artery calcium; C-reactive protein; Diabetes; Gender

Address for Correspondence and for Reprint Requests: Muredach P. Reilly, Cardiovascular Institute, University of Pennsylvania Medical Center, 609 BRB 2/3, 421 Curie Blvd., Philadelphia, PA 19104-6160. Tel: 215-573-1214, Fax: 215-573-2094. muredach@mail.med.upenn.edu.

Disclosures: There are no conflicts of interest for this paper with respect to any of the authors.

Introduction

Inflammation plays a major role in the pathogenesis, progression, and acute manifestations of atherosclerosis¹. Extensive evidence suggests that C-reactive protein (CRP) may be a useful inflammatory biomarker of incident clinical cardiovascular disease (CVD)² although debate continues over its role as a causal factor³⁻⁵. Type-2 diabetes (T2DM) is a chronic metabolic disease associated with inflammation and CVD.⁶ While CRP has been associated with insulin resistance and the onset of T2DM^{7, 8}, there are few studies to date examining the role of CRP in atherosclerotic CVD in type-2 diabetic subjects.

Ridker et al found that CRP added value to screening lipids in prediction of CVD events in a study of 28,000 apparently healthy postmenopausal women⁹. Furthermore, several studies have shown that gender may actually influence CRP's utility in CVD risk prediction. For example, Cushman and colleagues found among 3971 men and women that CRP measurement was most helpful in intermediate-Framingham-risk men and high-Framingham-risk women¹⁰. In a study of 3,435 Caucasian men by Koenig et al, CRP was similarly most helpful in intermediate risk men¹¹. While diabetic individuals overall have been traditionally considered a CVD equivalent, none of these studies included a significant population of diabetics or focused on gender differences in CRP risk prediction in T2DM.

Remarkably, most studies of CRP and subclinical atherosclerosis, including coronary artery calcification (CAC) have focused predominantly on non diabetic individuals and have reported equivocal results¹²⁻¹⁴ or no association with CAC¹⁵⁻¹⁸. These studies were generally small and gender differences in CRP levels¹⁹⁻²¹ and atherosclerosis burden may have introduced heterogeneity, further limiting power. For example, in the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA), we reported no overall association of CRP with CAC; however, CRP tended to be associated with CAC in women, although this was attenuated in fully adjusted models.

Because, type-2 diabetic individuals have higher plasma CRP, greater subclinical atherosclerosis and higher CVD risk, we hypothesized that gender differences in CRP's relationship with atherosclerosis may be revealed in this setting. Therefore, we investigated the influence of gender on the association of CRP with CAC in 1299 participants in the Penn Diabetes Heart Study (PDHS), a cross sectional study of asymptomatic otherwise healthy type-2 diabetic subjects²². Further, we reanalyzed our SIRCA, non-diabetic, sample (N = 860) focusing on the influence of gender and then combined PDHS and SIRCA data to assess the influence of both gender and T2DM on the relationship of CRP with CAC.

Materials and Methods

Study Participants

Detailed descriptions of PDHS²² and SIRCA have been published^{23 24}. In brief, both are single center, cross-sectional studies of subjects, aged 35-75, without clinical evidence of CVD (defined as myocardial infarction, coronary revascularization, angiographic CVD, or positive stress test). Both PDHS and SIRCA are mutually exclusive and there is no overlap of participants. SIRCA subjects have been recruited based on a family history of premature CVD. PDHS subjects were recruited on the basis of clinical diagnosis of T2DM (defined as fasting blood glucose > 7.0mmol/L (126 mg/dl), 2-hr postprandial glucose > 11.1mmol/L (200 mg/dl), or use of oral hypoglycemic agents/insulin in a subject greater than 40 years of age). Exclusion criteria included clinical CVD, elevated creatinine and, in SIRCA, the presence of diabetes²⁴. The studies were approved by the University of Pennsylvania Institutional Review Board and informed consent was obtained from all study participants.

Evaluated Parameters

SIRCA and PDHS are single center studies using the same clinical research center, nursing staff, CT scanner and research laboratories. Briefly, participants were evaluated at the General Clinical Research Center at the University of Pennsylvania Medical Center after a 12-hour overnight fast. Standard lipid panels were measured in real-time in Penn's Centers for Disease Control-certified lipid laboratory using enzymatic assays (Hitachi 912, Roche Diagnostic Systems Inc., NJ, USA). Fasting LDL cholesterol (LDL-C) was measured directly in PDHS and calculated using the Friedewald formula in SIRCA. CRP levels were batch-assayed using the same high-sensitivity latex turbidimetric immunoassay in both studies (Wako Ltd., Osaka Japan) ²⁴. Laboratory test results were generated by personnel blinded to the clinical characteristics and CAC scores of research subjects.

Framingham risk scores (FRS), using total cholesterol, were calculated as described previously ²⁴. Participants in SIRCA and PDHS were classified as having the metabolic syndrome using the revised National Cholesterol Education Program (NCEP) ²⁵. For SIRCA the definition for the glucose cut-point used for metabolic syndrome was 5.55mmol/L (100 mg/dl)], whereas for PDHS, given that all participants had diabetes by entry criteria, all subjects were designated as meeting metabolic syndrome glucose criteria. Global Agatston CAC scores were measured at electron beam tomography (Imatron, San Francisco, CA).

Statistical Analysis

Data are reported as median and interquartile range (IQR) or mean $\pm$ standard deviation for continuous variables and as proportions for categorical variables. The correlation of CRP with lipid, metabolic and inflammatory parameters was examined by Spearman correlation. The K-Wallis statistic was used as a non parametric method to compare medians across non-normally distributed variables. In PDHS, SIRCA and both studies combined, multivariable analysis of CAC scores was performed using Tobit conditional regression of natural log (CAC+1) as CAC data has many zero scores but also a marked right skew ²⁴. Tobit combines a logistic regression of the presence of CAC (any CAC present vs. a CAC zero score) with a linear regression (of log-transformed CAC) when CAC is present to produce a single estimate for the relationship of risk factors with CAC data. In our models, for example a tobit ratio of 1.6 indicates a 60% increase in CAC score for every 1 natural log fold increase in CRP levels. The association of natural log-CRP with CAC was assessed in incremental Tobit models with increasing numbers of confounding CVD risk factors: Model 1: age and race; Model 2: age, race, medications, FRS, metabolic syndrome, Model 3: age, race, medications, FRS, metabolic syndrome, and body mass index (BMI). Interaction of CRP with gender, T2DM and race was tested by likelihood ratio testing (LRT) and stratified results are presented when appropriate (e.g. by gender). Statistical analyses were performed using Stata 10.0 software (Stata Corp, College Station, TX).

Results

Baseline Characteristics and Risk Factor Correlations

Table 1 summarizes PDHS and, for comparison, SIRCA sample characteristics. As expected in PDHS, obesity was prevalent (average BMI of 33.5 and 31.1 in women and men respectively) and NCEP-defined metabolic syndrome was present in 78.9% of women and 69.6% of men. Plasma CRP levels differed by gender and diabetes status (K-Wallis $p < 0.001$) with highest levels in diabetic women and significantly higher levels in non-diabetic women compared to non-diabetic men (K-Wallis $p < 0.001$). Similarly, as expected, CAC differed by gender and diabetes status; men had higher CAC scores than women ($p < 0.001$) and scores were higher in diabetics than non-diabetics subjects ($p < 0.001$). CAC prevalence was consistent with greater sub-clinical atherosclerosis in asymptomatic T2DM patients;

over one third of men and almost 30% of women had CAC scores >75th percentile of age and gender adjusted in the general population. Framingham risk scores were predominantly intermediate-high risk (68% $\geq 10\%$ risk) in the PDHS sample, whereas most SIRCA participants had low-risk (82% <10% risk). Statin, aspirin and ace-inhibitor use was higher in T2DM patients.

Crude spearman correlations revealed modest associations of CRP with cardiovascular risk factors (Table 2). In general, correlation of CRP with lipid, metabolic and inflammatory parameters were stronger in women than men, regardless of diabetes status. Table 3 shows that, across increasing number of covariates, female gender was directly associated with CRP in both diabetics and non diabetics. This association was independent of demographic features, presence of metabolic syndrome, use of medications, and FRS and was modestly stronger in diabetics compared to non diabetics.

CRP is Associated with Coronary Calcification in Women but not Men

Table 4 shows the relationship of CRP with CAC. There was a significant interaction by gender in the association of CRP with CAC in type 2 diabetic subjects (interaction $p < 0.001$ for age and race adjusted model) and non-diabetic subjects (interaction $p < 0.001$), and therefore results are stratified by gender for each group. In contrast, there was no such interaction by diabetes status in men (interaction $p = 0.3$) or women (interaction $p = 0.6$) or by race (interaction $p = 0.6$ for men and 0.74 for women).

In women with T2DM, CRP had a significant association with CAC in age and race adjusted models even after adjustment for FRS, metabolic syndrome and BMI (Table 4). There was no significant association in men in any model tested. In non diabetic women but not men, CRP had a significant association with CAC in age and race adjusted models, which was no longer statistically significant after adjustment for FRS, metabolic syndrome and BMI. When PDHS and SIRCA were combined, there was a significant association with CAC in women in all models, including those with and without BMI (Figure 1). In the combined sample, there was no significant association of CRP with CAC in men (Figure 1) and the gender interaction was highly significant ($p < 0.001$ in all models). Results for men and women in each category were similar when logistic and linear regression was used instead of Tobit regression for analysis.

Further analysis in the combined sample showed no major differences in the association of CRP with CAC among those women who were on hormone replacement therapy [1.68 (1.10-2.54)] and those who were not [1.76, (1.36-2.28)]. In addition, there were similar relationships between CRP and CAC among women who were low risk [1.63 (1.24-2.14) for those with an FRS <10%] and intermediate risk [1.60, (1.06-2.43) for those with an FRS of 10-20%]. Finally, use of models that included individual risk factors as quantitative covariates (e.g., LDL-C, HDL-C, triglycerides, waist circumference) compared to models that used Framingham risk scores, metabolic syndrome, and body mass index had no impact on our findings (data not shown).

Discussion

CRP has been shown in numerous studies to have a positive association with clinical CVD^{26, 27}. Gender differences, however, may exist in CRP association with CVD¹⁹⁻²¹ and little is known of CRP as a predictor of CVD in T2DM, a chronic inflammatory state conferring increased CVD risk. In fact, CRP association with measures of subclinical atherosclerosis has been equivocal and such studies have largely lacked diabetic individuals. In the largest such study of CAC in T2DM subjects free of clinical CVD, we show that CRP was significantly associated with CAC in women, but not in men, even after controlling for

traditional risk factors, metabolic syndrome and BMI. Remarkably, this gender difference in CAC association was similar in a non-diabetic sample. There was no evidence of CRP-CAC association in men, either diabetic or non-diabetic. Our findings suggest that CRP may be a useful marker of cardiovascular risk in women particularly in diabetic women who otherwise have no known CVD.

Gender differences in plasma CRP are well established with higher circulating levels in women¹⁹⁻²¹. This difference is incompletely understood but may relate to gender differences in both visceral and subcutaneous fat, a strong determinant of CRP levels²¹, or to differences in estrogen, which is known to increase levels of CRP²⁸. However, studies which adjusted for measures of adiposity, menopausal status and hormone replacement therapy or that looked only at post menopausal women still found higher levels of CRP in women. Similarly, we found that women had higher CRP values than men independent of other demographic and cardiometabolic risk factors. Although CRP levels were higher in T2DM subjects compared to non diabetics, levels were higher in women than men independent of T2DM status. Furthermore, we and others²¹ report gender differences in the association of CRP with cardiovascular risk factors, particularly adipose and metabolic syndrome related parameters.

One difficulty in interpreting gender-differences in CRP and the association of CRP with risk factors and disease relates to the uncertainty of whether the relationship of CRP with CVD is causal or simply due to complex confounding with CVD risk factors. While a few animal models support CRP's role in atherothrombosis⁴, this has been harder to show in humans. Several lines of evidence to the contrary include recent large studies of genetic variations in CRP that affect CRP levels but have no association with atherosclerotic CVD^{3, 5}. Nonetheless, even if CRP is not causal in its relationship to atherothrombosis, it remains an important correlative biomarker that captures aspects of CVD risk that are not fully captured by established risk strategies.

Gender differences in circulating CRP and the correlation of CRP with cardiometabolic risk factors may have a confounding influence on the relationship of CRP with T2DM and atherosclerotic CVD. Conversely, higher CRP in women may reflect gender differences in the inflammatory environment caused by metabolic stress and thus reflect a true increase in risk of T2DM and CVD risk. Indeed, several studies support this notion. In 923 middle aged Caucasians, CRP was significantly higher in women with metabolic syndrome than in men²⁹. Hu et al found in a cross-sectional population-based Finnish study of over 12,000 subjects that higher baseline levels of CRP was more strongly associated with development of diabetes in women compared to men³⁰. Remarkably, Erbel and colleagues recently reported that CRP was more important in re-classifying higher CVD risk women than men³¹. Our findings of CRP association with CAC in women but not men with T2DM is novel and consistent with the concept that CRP may be a more useful CVD risk predictor in women than men at increased cardiometabolic risk.

The association of CRP with CAC has been controversial to date, perhaps because of limited sample size and failure to account appropriately for the heterogeneous influence of demographic factors including gender and race. Initial studies by Hunt and colleagues looked at healthy male army personnel and found no association between CRP and CAC¹⁵. Redberg et al also showed no association among 172 non diabetic postmenopausal women¹⁶. Wang et al published a study of 321 individuals from the Framingham Heart Study without clinical CVD and found a positive association between CRP and CAC in men and women by spearman correlation which only remained significant in men after an adjustment for BMI¹². Khera et al looked at 3373 subjects from the Dallas Heart Study found a trend for increasing CRP levels with CAC in men but not women. This association was lost in

multivariate analysis and, notably, this study did not include many diabetic patients¹³. We previously reported a lack of association of CRP with CAC in fully adjusted models in non diabetic participants in the SIRCA study³². However, there was a trend toward gender differences - CRP was positively associated with CAC in women, not men, in models that did not include BMI.

Few studies to date have included a significant number of diabetic subjects in assessing the relationship of CAC with CRP. Colhoun and colleagues examined 199 type-1 diabetics and 201 non diabetics and found a positive association of CRP with CAC in men but not in women after adjustment for other risk factors.¹⁴ However, this was a small study with only 94 diabetic women and subjects were younger and had type-1 diabetics. A recent paper in press from the MESA cohort study of 6783 subjects (53% female) of varying race including ~14% diabetic subjects found a weak association of CRP with CAC that was attenuated in fully adjusted models³³. However, stratified analyses in women and diabetics were not reported. The largest study prior to ours of diabetic patients was reported by Bowden and colleagues and included 551 diabetic patients (59% women, 83% with T2DM) in the family-based Diabetes Heart Study¹⁸. They found no significant association with the presence or extent of CAC in fully adjusted models. Compared to our findings in PDHS, potential reasons for conflicting results in this study are sample size, family-based design, inclusion of type-1 diabetes mellitus and prevalent CVD, and differences in the analytic approach to CAC data. The study of CRP in diabetics for the prediction of clinical CVD has been limited, in part, because T2DM has been considered a CVD risk equivalent. Yet among diabetics, without established CVD or multiple CVD risk factors, additional discrimination of those with highest CVD risk has implications for therapeutic goals and strategies^{34, 35}. Of a few studies of CRP in CVD risk prediction in T2DM, Soinio and colleagues found that CRP values were independent predictors of cardiovascular death over a 7-year follow-up, even after adjusting for traditional risk factors and BMI in 1059 T2DM subjects³⁶. Our findings for CRP-CAC associations in diabetic women are broadly consistent with this clinical finding at least for women. Overall, plasma CRP may be a hallmark of the chronic inflammation that is a feature of adipose dysfunction and insulin resistance in T2DM, particularly in women, and thereby reflects this mechanism of increased CVD risk in T2DM.

Limitations of our study include cross-sectional analysis preventing causal inference. While we evaluated two study samples whose demographics differed somewhat, they were contemporary and derived from the same community, using similar lab assays and protocols. We used the surrogate, non-clinical endpoint of CAC, which is an estimate, and not a direct measure of coronary atherosclerosis. While CAC scores do not detect all types of coronary atherosclerotic plaques, they are clinically relevant because they are strong, independent predictors of CVD including in diabetic subjects³⁷. Furthermore, since our non diabetic subjects were recruited on the basis of a family history of premature CVD, our results may not be generalizable to all non diabetic individuals. Replication is needed in more diverse populations to assess for the relevance of clinical CVD outcomes.

In conclusion, CRP may be particularly useful as a marker of atherosclerotic CVD in women. While current guidelines make no recommendation for measurement of CRP in diabetic subjects, our study may suggest otherwise, particularly in diabetic women who otherwise have no known clinical CVD. Further prospective studies are needed to better assess gender differences in CRP utility in CVD risk prediction and its application in T2DM where it might impact novel therapeutic strategies and targets.

Acknowledgments

This work was supported by a Clinical and Translational Science Award (UL1RR024134) from the National Center for Research Resources (NCRR) and a Diabetes and Endocrine Research Center (P20-DK 019525) award, both to the University of Pennsylvania. M.P.R. is also supported by RO1 HL-073278 and P50 HL-083799-SCCOR from the National Institutes of Health.

References

1. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002; 105:1135–1143. [PubMed: 11877368]
2. Musunuru K, Kral BG, Blumenthal RS, Fuster V, Campbell CY, Gluckman TJ, Lange RA, Topol EJ, Willerson JT, Desai MY, Davidson MH, Mora S. The use of high-sensitivity assays for C-reactive protein in clinical practice. *Nat Clin Pract Cardiovasc Med*. 2008; 5:621–635. [PubMed: 18711404]
3. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert JC, Bennett D, Coin L, Ashby D, Tzoulaki I, Brown IJ, Mt-Isa S, McCarthy MI, Peltonen L, Freimer NB, Farrall M, Ruukonen A, Hamsten A, Lim N, Froguel P, Waterworth DM, Vollenweider P, Waeber G, Jarvelin MR, Mooser V, Scott J, Hall AS, Schunkert H, Anand SS, Collins R, Samani NJ, Watkins H, Kooner JS. Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA*. 2009; 302:37–48. [PubMed: 19567438]
4. Devaraj S, Singh U, Jialal I. The evolving role of C-reactive protein in atherothrombosis. *Clin Chem*. 2009; 55:229–238. [PubMed: 19095731]
5. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med*. 2008; 359:1897–1908. [PubMed: 18971492]
6. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet*. 2008; 371:1800–1809. [PubMed: 18502305]
7. Thorand B, Lowel H, Schneider A, Kolb H, Meisinger C, Frohlich M, Koenig W. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984–1998. *Arch Intern Med*. 2003; 163:93–99. [PubMed: 12523922]
8. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, Packard CJ, Sattar N. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes*. 2002; 51:1596–1600. [PubMed: 11978661]
9. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000; 342:836–843. [PubMed: 10733371]
10. Cushman M, Arnold AM, Psaty BM, Manolio TA, Kuller LH, Burke GL, Polak JF, Tracy RP. C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: the cardiovascular health study. *Circulation*. 2005; 112:25–31. [PubMed: 15983251]
11. Koenig W, Lowel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. *Circulation*. 2004; 109:1349–1353. [PubMed: 15023871]
12. Wang TJ, Larson MG, Levy D, Benjamin EJ, Kupka MJ, Manning WJ, Clouse ME, D'Agostino RB, Wilson PW, O'Donnell CJ. C-reactive protein is associated with subclinical epicardial coronary calcification in men and women: the Framingham Heart Study. *Circulation*. 2002; 106:1189–1191. [PubMed: 12208790]
13. Khera A, de Lemos JA, Peshock RM, Lo HS, Stanek HG, Murphy SA, Wians FH Jr, Grundy SM, McGuire DK. Relationship between C-reactive protein and subclinical atherosclerosis: the Dallas Heart Study. *Circulation*. 2006; 113:38–43. [PubMed: 16380546]
14. Colhoun HM, Schalkwijk C, Rubens MB, Stehouwer CD. C-reactive protein in type 1 diabetes and its relationship to coronary artery calcification. *Diabetes Care*. 2002; 25:1813–1817. [PubMed: 12351483]

15. Hunt ME, O'Malley PG, Vernalis MN, Feuerstein IM, Taylor AJ. C-reactive protein is not associated with the presence or extent of calcified subclinical atherosclerosis. *Am Heart J.* 2001; 141:206–210. [PubMed: 11174333]
16. Redberg RF, Rifai N, Gee L, Ridker PM. Lack of association of C-reactive protein and coronary calcium by electron beam computed tomography in postmenopausal women: implications for coronary artery disease screening. *J Am Coll Cardiol.* 2000; 36:39–43. [PubMed: 10898410]
17. Lorenz MW, Karbstein P, Markus HS, Sitzer M. High-sensitivity C-reactive protein is not associated with carotid intima-media progression: the carotid atherosclerosis progression study. *Stroke.* 2007; 38:1774–1779. [PubMed: 17446427]
18. Bowden DW, Lange LA, Langefeld CD, Brosnihan KB, Freedman BI, Carr JJ, Wagenknecht LE, Herrington DM. The relationship between C-reactive protein and subclinical cardiovascular disease in the Diabetes Heart Study (DHS). *Am Heart J.* 2005; 150:1032–1038. [PubMed: 16290992]
19. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH Jr, Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. *J Am Coll Cardiol.* 2005; 46:464–469. [PubMed: 16053959]
20. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB Jr, Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. *Am Heart J.* 2006; 152:593–598. [PubMed: 16923436]
21. Cartier A, Cote M, Lemieux I, Perusse L, Tremblay A, Bouchard C, Despres JP. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? *Am J Clin Nutr.* 2009; 89:1307–1314. [PubMed: 19297456]
22. Reilly MP, Iqbal N, Schutta M, Wolfe ML, Scally M, Localio AR, Rader DJ, Kimmel SE. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. *J Clin Endocrinol Metab.* 2004; 89:3872–3878. [PubMed: 15292320]
23. Valdes AM, Wolfe ML, Tate HC, Geffer W, Rut A, Rader DJ. Association of traditional risk factors with coronary calcification in persons with a family history of premature coronary heart disease: the study of the inherited risk of coronary atherosclerosis. *J Investig Med.* 2001; 49:353–361.
24. Qasim A, Mehta NN, Tadesse MG, Wolfe ML, Rhodes T, Girman C, Reilly MP. Adipokines, insulin resistance, and coronary artery calcification. *J Am Coll Cardiol.* 2008; 52:231–236. [PubMed: 18617073]
25. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Executive summary. *Cardiol Rev.* 2005; 13:322–327. [PubMed: 16708441]
26. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med.* 2004; 350:1387–1397. [PubMed: 15070788]
27. Mora S, Musunuru K, Blumenthal RS. The clinical utility of high-sensitivity C-reactive protein in cardiovascular disease and the potential implication of JUPITER on current practice guidelines. *Clin Chem.* 2009; 55:219–228. [PubMed: 19095730]
28. Kwok S, Canoy D, Ashton WD, Lowe GD, Wood D, Humphries SE, Charlton-Menys V, Durrington PN. Increased C-reactive protein levels in overweight and obese women taking exogenous hormones: the United Kingdom Women's Heart Study (UKWHS). *Clin Endocrinol (Oxf).* 2009
29. Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Gender differences in C-reactive protein, interleukin-1 receptor antagonist and adiponectin levels in the metabolic syndrome: a population-based study. *Diabet Med.* 2008; 25:747–750. [PubMed: 18435780]
30. Hu G, Jousilahti P, Tuomilehto J, Antikainen R, Sundvall J, Salomaa V. Association of serum C-reactive protein level with sex-specific type 2 diabetes risk: a prospective finnish study. *J Clin Endocrinol Metab.* 2009; 94:2099–2105. [PubMed: 19318443]
31. Erbel R, Mohlenkamp S, Lehmann N, Schmermund A, Moebus S, Stang A, Gronemeyer D, Seibel R, Mann K, Volbracht L, Dragano N, Siegrist J, Jockel KH. Sex related cardiovascular risk

- stratification based on quantification of atherosclerosis and inflammation. *Atherosclerosis*. 2008; 197:662–672. [PubMed: 17386928]
32. Reilly MP, Wolfe ML, Localio AR, Rader DJ. C-reactive protein and coronary artery calcification: The Study of Inherited Risk of Coronary Atherosclerosis (SIRCA). *Arterioscler Thromb Vasc Biol*. 2003; 23:1851–1856. [PubMed: 12933535]
33. Jenny NS, Brown ER, Detrano R, Folsom AR, Saad MF, Shea S, Szklo M, Herrington DM, Jacobs DR Jr. Associations of inflammatory markers with coronary artery calcification: Results from the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis*. 2009
34. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation*. 2004; 110:227–239. [PubMed: 15249516]
35. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, Witztum JL. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J Am Coll Cardiol*. 2008; 51:1512–1524. [PubMed: 18402913]
36. Soinio M, Marniemi J, Laakso M, Lehto S, Ronnema T. High-sensitivity C-reactive protein and coronary heart disease mortality in patients with type 2 diabetes: a 7-year follow-up study. *Diabetes Care*. 2006; 29:329–333. [PubMed: 16443882]
37. Elkeles RS, Godsland IF, Feher MD, Rubens MB, Roughton M, Nugara F, Humphries SE, Richmond W, Flather MD. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. *Eur Heart J*. 2008; 29:2244–2251. [PubMed: 18573867]

Figure 1. Association of C-Reactive Protein with Coronary Artery Calcification in Diabetics and non Diabetics Combined

FRS = Framingham Risk Score; MetSyn = metabolic syndrome; BMI = body mass index; Meds = medications. Medications include aspirin, statins and angiotensin converting enzyme inhibitors. The gender interaction was $p < 0.01$ in all models.

Table 1
Characteristics of the Study Samples

	Type-2 Diabetic Men (n = 819)	Type-2 Diabetic Women (n = 480)	Non diabetic Men (n = 457)	Non diabetic Women (n = 403)
Age, years (range)	60 (34-77)	57 (35-76)	46 (20-71)	50 (31-73)
Race: White, %	69.0	51.7	95	95
Black, %	25.1	44.1	2	3.5
Total cholesterol, mmol/L	4.4 (3.8-5.1)	4.6(4.1-5.3)	5.2 (4.8-5.8)	5.5 (4.7-6.1)
HDL-cholesterol, mmol/L	1.1 (0.9-1.3)	1.4 (1.2-1.7)	1.1 (0.93-1.3)	1.5 (1.2-1.8)
Triglycerides, mmol/L	1.4 (1.0-2.1)	1.2 (0.9-1.7)	1.4 (1.0-2.0)	1.3 (0.9-1.7)
LDL-cholesterol, mmol/L	2.5 (2.0-3.0)	2.6 (2.1-3.1)	3.3 (2.7-3.9)	3.3 (2.7-3.8)
Glucose, mmol/L	6.7 (5.7-8.1)	6.2 (5.2-7.8)	5.3 (4.9-5.7)	5.1 (4.8-5.4)
Hemoglobin A1c, %	6.7(6.2-7.7)	6.8 (6.2-7.8)	-	-
Blood Pressure, mm Hg				
Systolic	131 (122-141.5)	130 (120.5-143)	127 (119.5-136.5)	123.5 (113-134.5)
Diastolic	77 (72-82.5)	74 (70-80)	79 (74.5-84.5)	75 (68-82)
Metabolic Syndrome, %	73.7	80.1	34.4	24.9
Body Mass Index	31.2 (28.1-34.6)	33.8 (29.4-38.4)	27.5 (25.2-30.3)	25.6 (22.8-30.2)
Waist Circumference, cm	107 (99-117)	104 (94-117)	95 (89-104)	81 (74-91)
Current Tobacco Use, %	11.5	10.1	12.1	10.8
Framingham Risk Score	13 (8-20)	10 (7-13)	7 (4-8)	4 (2-6)
C-Reactive Protein, mg/dl	1.4 (0.7-2.9)	2.8 (1.4-6.6)	1.1 (0.5-2.1)	1.4 (0.6-3.7)
HRT Use, %	--	37	--	27
Statin Use, %	58.7	52.2	17.5	10.2
Niacin Use, %	5.8	1	2.9	2.7
ACE Inhibitor Use, %	65.7	60.3	6.5	5
Aspirin Use, %	48.4	42.1	18.2	11.2
Mean CAC ($\pm$ SD)	418.5 $\pm$ 751	96.1 $\pm$ 261.7	123.5 $\pm$ 329	41.1 $\pm$ 133.1
Median CAC	107.5 (0-467)	0 (0-57)	7 (1-82)	1 (0-14)

Median values with interquartile ranges are shown unless otherwise specified. HDL = high density lipoprotein, LDL = low density lipoprotein, HRT = hormone replacement therapy, ACE = angiotensin converting enzyme, CAC = coronary artery calcium.

Table 2
Spearman Correlations of C-Reactive Protein with Cardiovascular Risk Factors

	Type-2 Diabetic Men	Type-2 Diabetic Women	Non diabetic Men	Non diabetic Women
Age	-0.02	-0.11 [‡]	0.03	0.21 [*]
Systolic Blood Pressure	0.06	0.04	0.12 [‡]	0.29 [*]
HDL-Cholesterol	-0.14 [*]	-0.27 [*]	-0.17 [*]	-0.01
Triglycerides	0.10 [‡]	0.18 [*]	0.13 [‡]	0.42 [*]
LDL-Cholesterol	0.04	0.19 [*]	0.1 [‡]	0.14 [‡]
Body Mass Index	0.25 [*]	0.42 [*]	0.33 [*]	0.49 [*]
Glucose	0.08 [‡]	0.13 [‡]	0.08	0.24 [*]
Waist circumference	0.24 [*]	0.41 [*]	0.40 [*]	0.51 [*]
Framingham Risk Score	0.073	0.13 [‡]	0.18 [*]	0.34 [*]

*
 $p < 0.001$

[‡]
 $p < 0.01$

[‡]
 $p < 0.05$. HDL = high density lipoprotein; LDL = low density lipoprotein.

Table 3
Association of Female Gender with Plasma C-Reactive Protein

Model Adjusted for:	Type-2 Diabetic Subjects Beta (95% CI)*	Non Diabetic Subjects Beta (95% CI)*
Age and Race	0.67 (0.52-0.81)	0.35 (0.20-0.50)
Age, Race, FRS and Meds	0.76 (0.60-0.61)	0.51 (0.34-0.67)
Age, Race, FRS, Meds, MetSyn, BMI	0.52 (0.37-0.68)	0.48 (0.33-0.63)

* p values for all Betas are < 0.001. Beta values represent the increase in the log value of CRP for women compared to men. Covariates: FRS = Framingham Risk Score, MetSyn= metabolic syndrome; Meds = medications. Medications include aspirin, statins and angiotensin converting enzyme inhibitors. BMI = body mass index.

Table 4
Association of C-Reactive Protein with Coronary Artery Calcification by Diabetes Status

	Type-2 Diabetic Subjects	Non Diabetic Subjects
Model Adjusted for:	Tobit Ratio (95% CI)	Tobit Ratio (95% CI)
Age and Race		
Men	0.92 (0.75-1.13)	1.03 (0.84-1.25)
Women	1.67 (1.11-2.51)	1.67 (1.30-2.15)
Age, Race, Meds, FRS, MetSyn, BMI		
Men	0.83 (0.67-1.02)	0.82 (0.67-1.01)
Women	1.60 (1.03-2.47)	1.29 (0.98-1.69)

Results of Tobit regression are presented as the ratio of increase in coronary artery calcification score for a natural log fold increase in CRP levels. FRS = Framingham Risk Score; MetSyn = metabolic syndrome; BMI = body mass index; Meds = medications. Medications include aspirin, statins and angiotensin converting enzyme inhibitors.