Targeting Metabolic and Inflammatory Processes for Coronary Artery Disease Treatment and Prevention

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Abstract

There is a lack of understanding of how glucose–insulin–potassium (GIK), exert its benefits in subjects with coronary artery disease (CAD). There is an even greater gap in knowledge on the relationship between a unique metabolic biomarkers, adiponectin, and CAD. The aims of this project are: 1) To study the effect of GIK on C-reactive protein (CRP) levels in subjects with acute coronary syndrome (ACS), and the association between CRP and infarct size; 2) To investigate changes in adiponectin levels early in the course of ACS and its relation to outcomes; and 3) To evaluate current evidence on the association between adiponectin and primary and secondary CAD.

For the first two aims we analyzed data from the IMMEDIATE Trial on subjects with ACS. Three blood levels were drawn for biomarkers measurements. Data was collected on infarct size and one-year outcomes. For aim three we systematically reviewed studies reporting associations between adiponectin and CAD. We evaluated different associations between primary and secondary events, and between analyses that do and do not adjust for variables on the causal pathway between adiponectin and CAD (intermediate variables).

For Aim 1, 140 participants were included. High sensitivity CRP (hs-CRP) values at 12 hours were lower in GIK group compared to placebo (mean=0.65 mg/L in GIK, 0.84 mg/L in placebo, p=0.053). However, the rate of change in hs-CRP did not differ by treatment arm using mixed model analysis. The 12 hour hs-CRP levels were an independent predictor of infarct size. For aim 2, 120 participants were included. Adiponectin levels decreased by -0.005 μg/mL per hour (p=0.035). No association was found between adiponectin and outcomes. In aim 3, meta-analysis of models that did not incorporate intermediate variables, risk of primary CAD events decreased with higher

adiponectin levels (relative risk [RR] 0.73; 95% confidence interval [CI] 0.63–0.87). For secondary events maximum adjusted models showed that higher adiponectin levels were associated with higher risk of another CAD event (RR 1.27; 95% CI 1.16–1.39).

Results from this study suggests that GIK is less likely to exert its benefits in subjects with ACS through an inflammatory pathway. It also illustrates a modest response of adiponectin to the acute phase of ischemia. The findings from the largest systematic review to date, which incorporated novel approaches, show that while adiponectin was associated with less incidence CAD, it is paradoxically linked to an increased risk of subsequent events.

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Undertaking this PhD has been a truly life-changing experience for me and it would have not been possible to do without the support, help, and guidance that I received from many people.

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List of Abbreviations

ACS, acute coronary syndromes

BMI, body mass index

BNP, brain-type natriuretic peptide

CAD, coronary artery disease

CI, confidence interval

CRP, C-reactive protein

CVD, cardiovascular disease

ECG, echocardiogram

ED, emergency department

EMS, emergency medical services

FFA, free fatty acid

GIK, glucose-insulin-potassium

HbA1C, glycosylated hemoglobin

hs-CRP, high sensitivity C-reactive protein

HR, hazard ratio

IMMEDIATE, Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care

LV, left ventricular

MI, myocardial infarction

NSTEMI, non-ST elevation myocardial infarction

OR, odds ratio

PCI, percutaneous coronary intervention

RR, relative risk

STEMI, ST- elevation myocardial infarction

Chapter 1: **Introduction**

Despite advances in the prevention and management of cardiovascular disease (CVD), about one-third of deaths worldwide are attributed to CVD (1). Among those, coronary artery disease (CAD) is the leading cause of mortality in both men and women (1). Furthermore, CAD risk factors continue to increase in the US population (2-4). These include, advancing age as well as an increased prevalence of obesity, diabetes mellitus, and dyslipidemia (2-4). As a result, the prevalence of CAD will probably continue to increase (5). Over the past decade, considerable progress has been made to identify pathways that can be targeted for the prevention and treatment of CAD. One of those approaches is the modulation of the metabolic and inflammatory processes involved in the development and progressions of CAD.

Inflammation and CAD

Inflammation contributes to myocardial damage in ischemia, infarction, and reperfusion (6). The acute phase reactant C-reactive protein (CRP) is produced predominantly by the liver under the influence of cytokines such as interleukin-6 and tumor necrosis factor-alpha (6). C-reactive protein is released in response to inflammation in acute and chronic CAD (6). High CRP levels after acute myocardial infarction (MI) predict infarct expansion and plaque rupture (7-9). Whereas a reduction in CRP levels has been shown to reflect the efficacy of thrombolytic therapy and a patent infarct-related coronary artery (10; 11). Continued elevations in CRP portend increased risk of mortality, even in the presence of currently available therapies for CAD (12).

Metabolic Abnormalities and CAD

Together with inflammation, metabolic abnormalities play an important role before and after the development of myocardial damage and ischemia (13; 14). During the early stages of development of CAD, several metabolic processes contribute to

atherosclerosis, including insulin resistance, dyslipidemia, endothelial dysfunction, inflammation, and oxidative stress. During ischemia and reperfusion, free fatty acid (FFA) levels increase and become the dominant energy source for the heart, which can contribute to myocardial cell damage (14). After stress, recovery of energy metabolism is crucial for the healing and repair process (14). A unique biomarker for metabolic abnormalities is Adiponectin, which is a protein hormone produced solely by adipose tissue (15). Adiponectin modulating various metabolic processes and acts as a biomarker of metabolic abnormalities (15).

Targeting Metabolic and Inflammatory Processes in CAD

Treatment strategies that target metabolic and inflammatory processes, also known as metabolic therapy, are being considered for the prevention and management of CAD (13; 14; 16; 17). Those mainly involve the administration of a substance normally occurring in the body to correct disturbed levels, and to favorably influence a metabolic reaction occurring within the cell (13; 16; 18). Some have been under investigation for more than 20 years, such as glucose–insulin–potassium (GIK), while others are still in the pre-clinical research phase, such as modulation of adiponectin receptors. Generally, this form of therapy is under-utilized in CAD, partly due to inconsistent results in clinical trials and observational studies, and the limited knowledge on the behavior of several biomarkers at different stages of CAD state.

Metabolic Therapy with GIK in Subjects with Acute Coronary Syndromes (ACS)

Glucose -insulin-potassium infusion as metabolic therapy can reduce damage to myocardial cells in the setting of ischemia or infarction (13; 17-20). Since its introduction in the early 1960s (21), GIK treatment has been assessed in both animal models and human studies. However, the role of GIK in patients with acute MI remains controversial.

While several clinical trials show no benefit of GIK treatment in patients with acute MI (22-26), other studies have reported benefit (27-31). Most recently, the IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care) Trial, GIK was compared to placebo in patients with suspected ACS (31). While GIK did not reduce progression to, it was significantly associated with lower rates of the composite outcome of cardiac arrest or in-hospital mortality (32). Other favorable effects of GIK also included the significant reduction in infarct size (31). However, the mechanism of how GIK exerts these benefits is not agreed upon. GIK may work by reducing FFA levels, promoting glycolysis over lipolysis, and/or reducing inflammation (31; 33; 34).

The Effect of GIK on Inflammation in Subjects with ACS

Accumulating evidence shows a close relation between CRP and metabolic syndrome, making it a biomarker of both inflammation and metabolic abnormalities; therefore, one approach to studying the effects of metabolic interventions is to analyze GIK treatment response on CRP levels (6). Few trials have studied the impact of GIK on inflammation (34-36). One study showed significant reduction in CRP levels, while others showed no effect (34-36). However all of these trials were small, including only about 40-70 subjects. These trials also mostly compromised of subjects with ST-segment elevation MI (STEMI) (34-36); therefore the possible effect of GIK on CRP in the presence of ACS is yet to be understood. To investigate the possible anti-inflammatory effects of GIK on CRP can be done through using data from the IMMEDIATE Trial. This Trial is distinguished from other studies of GIK infusion, by the early administration of GIK prior to arrival at the hospital (31). In previous studies, GIK administration was delayed until hospital admission (22-26), with a median time of six hours from symptom onset to GIK initiation in one study (25). In addition, in the IMMEDIATE Trial infarct size was

measured at 30-days, giving a unique opportunity to assess its relationship with CRP levels following presentation with an ACS.

Adiponectin a Marker of Metabolic Abnormalities

The function of adipose tissue was originally thought to be limited to lipid storage (37). However, it is now well established that adipose tissue is an active endocrine organ that secrets bioactive molecules known as adipokines (38; 39). These molecules play a role in regulating energy and metabolic and inflammatory processes (39). Adiponectin, an adipokine, draws special attention due to its potentially anti-atherogenic effects (32; 40). Plasma adiponectin levels are lower in obese individuals and have a protective effect against the development of insulin resistance (41; 42). also genes that increase adiponectin levels can influence insulin sensitivity, and are protective against the development of type 2 diabetes (43). Women have higher concentrations of adiponectin than do men (44). In addition, adiponectin levels are inversely correlated with other cardiovascular risk factors, such as hyperlipidemia, hypertension, and CRP levels (45-47). Therefore adiponectin has become an attractive possible candidate therapy for the prevention of CAD (48; 49).

Role of Adiponectin in CAD

Although the protective role of adiponectin in diabetes mellitus has been clearly established, this role is not as clear in CAD (43). Several observational studies have demonstrated an association between low adiponectin levels and stable CAD (50-53), while other studies have not been able to establish a link between serum levels of adiponectin and the development of CVD (54-56). Furthermore, studies in patients with ACS have shown that higher plasma adiponectin levels are associated with greater disease severity and with a higher risk of adverse outcomes (57-59). There is less

understanding on how adiponectin changes acutely after an acute coronary event, and even less understanding on whether early measurements of adiponectin would be related to infarct size as a surrogate marker for adverse cardiovascular outcomes. In most studies that have examined the relationship between adiponectin and CAD, adiponectin was measured in patients with stable CAD (60), or long after the onset of symptoms of myocardial ischemia (61). Animal studies indicate that a lack of adiponectin increases the size of an infarct following MI (62). However, to our knowledge, no human studies have examined the association between infarct size and serum adiponectin levels in patients with ACS.

Meta-Analyses on the Association between Adiponectin and CAD Risk

To add to the controversy around adiponectin, several meta-analyses have recently been published on the association between adiponectin and CAD, yielding different conclusions (63-66). One showed a beneficial association (64), others did not find an association (56; 63; 65), and some suggested that higher adiponectin levels are associated with increased risk of CAD events, particularly in subjects with established disease (66; 67). The reasons behind those discrepancies are unclear. However, we propose two main sources of bias that can potentially impact the results of previous meta-analyses. The basis of these inconsistencies is unknown; however, we have proposed two main sources of bias that could potentially impact the results of previous meta-analyses. Firstly, index event bias, arises when study inclusion is dependent on an index event (in this case, established CAD) and risk factors for recurrence are evaluated (56; 66; 68). This can lead to the potential for inversion of the relationship between a risk factor and recurrence (56; 66; 68). Secondly, over-adjustment bias, which occurs when intermediate variables on the causal pathway between a predictor and an outcome are included in multivariable models (e.g., adjusting for glucose in analyses of adiponectin

as a predictor of CAD) (69; 70). This can potentially mask some of the indirect effect of adiponectin on CAD, thereby biasing the results towards the null (69; 70). With the increased interest in the potential clinical use of adiponectin, there are several trials being conducted on drugs that can increase adiponectin levels or administration of recombinant adiponectin (71). This highlights the importance of first establishing whether endogenous adiponectin has a protective effect in CAD before introducing adiponectin to the market.

Study Aims

The overarching aim of this study is to better understand targeting inflammatory and metabolic processes for CAD prevention and management. The first aim is to investigate whether the benefits of metabolic therapy with GIK is exerted through an anti-inflammatory effect in subjects with ACS. The second aim is to determine whether adiponectin, a unique metabolic biomarker, responds to the acute phase of ischemia and correlates with clinical outcomes, to help determine whether it should be targeted in future studies for its potential role in ACS. Finally, our third aim is to evaluate currently available epidemiological data on the association between adiponectin and the risk of future primary and secondary CAD events, and to analyze the current discordance in the literature regarding adiponectin benefits with regard to CAD.

Therefore, the goals of this dissertation research are:

1) Compare the effect of early GIK administration versus placebo on serum CRP levels in patients with ACS within the first 12 hours of admission due to an acute event, using data from the IMMEDIATE trial. In addition, we aim to investigate the association between CRP levels in the first 12 hours and infarct size in patients with confirmed ACS. We hypothesize that CRP levels increase in

patients with ACS, in a manner related to infarct size. Early administration of GIK will exert an anti-inflammatory effect by decreasing CRP levels in this setting.

2) Describe the changes in adiponectin levels early in the course of ACS and assess the correlation between baseline adiponectin and clinical characteristics. Furthermore, we plan to investigate the association between adiponectin levels in ACS and 30-day infarct size, and one-year clinical outcomes. Our hypothesis is that adiponectin levels will change early in the course of ACS. We also hypothesize that higher adiponectin levels are associated with smaller infarct size and decreased incidence of adverse outcomes in patients with ACS.
3) Conduct a systematic review and meta-analysis of observational studies on the association between adiponectin and CAD events, stratifying them based on CAD status at baseline (to investigate the effect of index event bias) and the use of multivariable regression models with different degrees of adjustment (to assess the effect of intermediate variables on CAD risk estimates). We hypothesize that the relationship between adiponectin and CAD differs based by an individual's CAD status at the time of adiponectin measurement, and the use of intermediate variables as covariates in published models.

Chapter 2: **Methods and Results- C-Reactive Protein Reactions to Glucose-Insulin-Potassium Infusion and Relations to Infarct Size in Patients with Acute Coronary Syndromes**

Methods

Study Sample

This study analyzed data collected from participants enrolled in the IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care) Trial, the methodology of which has been published elsewhere (31). In brief, the IMMEDIATE Trial was a multicenter randomized, placebo-controlled, double-blind clinical effectiveness trial of glucose–insulin–potassium (GIK), from December 2006 through July 2011, in which paramedics, aided by electrocardiograph (ECG)-based decision support, randomized 911 participants aged ≥30 years with chest pain or other symptoms suggestive of acute coronary syndrome (ACS) of whom 871 were ultimately enrolled after providing written consent (31). Identification of ACS by paramedics was aided by the ECG-based Acute Cardiac Ischemia Time-Insensitive Predictive Instrument (ACI-TIPI) and Thrombolytic Predictive Instrument (TPI) decision support, using an ACI-TIPI threshold of 75% or higher predicted probability of having ACS, detection of suspected ST elevation myocardial infarction (STEMI) by the TPI, or both.

Participants enrolled in the trial were given either the GIK solution (30% glucose, 50 U/L of regular insulin, and 80 mEq of KCl/L) intravenously at 1.5 mL/kg/h for 12 hours, or an identical-appearing placebo 5% glucose solution. In the study, the median time from symptom onset to initiation of infusion was 90 minutes (31). This investigation was based on the results from the subset of IMMEDIATE Trial participants who also consented to be in the biological mechanism cohort (“biocohort”), conducted at six of the 13 sites of the parent trial. Biocohort participants all had confirmed ACS and were treated with study drug for at least eight hours.

Data Collection

During the 12-hour infusion of study drug blood samples were collected at three time points: 1) the initial measurement, which was drawn as soon as feasible after hospital arrival; 2) six hours after start of study drug; and 3) 12 hours after start of study drug, or upon discontinuation of the infusion. At 30-days, subjects returned for left ventricular (LV) function imaging studies and infarct size measurements by sestamibi SPECT imaging. The Trial's core laboratory interpreted the nuclear studies, and the core laboratory of Tufts Clinical and Translational Science Institute performed the biomarker measurements.

Biomarkers measured at the three time points listed above included serum levels of glucose, insulin, free fatty acids, and high sensitivity C-reactive protein (hs-CRP). Additional blood samples were collected at 30-days for the measurement of glycosylated hemoglobin (HbA1C) and brain-type natriuretic peptide (BNP). Other covariates measured in the biocohort include demographic data, vital signs, medical history, and medications used at home, in the hospital, or upon discharge. Additional details on exact timing of drug administration from symptom onset, and timing from drug administration to blood measurements were collected.

Data Analysis

Statistical analyses were performed using R, version 2.15.2, with nlme, and lme4 packages (72-74). Descriptive statistics were used to describe baseline characteristics. All tests were two-sided, using alpha <0.05 to determine statistical significance. Serum hs-CRP levels ranged widely and their distribution was highly skewed to lower levels; therefore hs-CRP concentrations were logarithmically (base 10) transformed in all further analyses. Linear regression models were used to assess the relationship between initial

hs-CRP measurements and baseline characteristics; variables significantly associated with initial hs-CRP levels were adjusted-for in subsequent random mixed model analyses. The independent sample t-test was used to detect unadjusted differences in cross-sectional hs-CRP levels between GIK and placebo at each study collection time point. In addition, using independent sample t-test, the differences between the initial and 12-hour hs-CRP measurements (delta hs-CRP) were used to assess differences between treatment arms.

For longitudinal data analysis a random effect mixed model was used to detect the differential effect of treatment on the rate of change in the initial, six-, and 12-hour hs-CRP levels. We used mixed models with fixed and random effects to account for the repeated measure by patients. Fixed effects were time, treatment, and time × treatment group interaction. Random effects were intercept and random slope. The P-values were calculated as a fixed-effects interaction term between treatment group and time, with subject-specific random effects for the intercept and time. Variables that were associated with baseline log hs-CRP values (i.e. initial log hs-CRP measurement) in univariable analysis at a p-value<0.1 were included in the model. None of those variables were time-varying covariates. The P-values were obtained by likelihood ratio tests comparing the model with random slope only to the full model with both random slope and intercept. Sensitivity analysis was performed using complete case analysis (i.e. excluding subjects with at least one missing hs-CRP value at any of the three measurement time points).

Univariable and multivariable linear regression models were used to study the association between hs-CRP levels at the three time points separately (as the predictors), in separate regression models, and infarct size (as the outcome), after adjustment for potential confounders including age, sex, and those correlated with infarct

size in univariate models at a p-value of <0.1. Because hs-CRP baseline levels were not measured prior to study infusion initiation, but rather, after hospital arrival, the time from when the drug started and time of the first hs-CRP measurement was also used as a covariate in multivariable regression models. Infarct sizes ranged from 0% to 59% of left ventricular mass, where 0% represent those who did not develop an infarct, and 59% was assigned to those who died (N=4) before the scheduled 30-day imaging. This imputed of 59% for patients who died was based on the observation that the largest infarct size measured in the study was 58% and death was considered a worse outcome. In addition, correlations between hs-CRP at the initial, six-, and 12-hour determinations and infarct size were assessed using Spearman's rank correlation and scatterplots of hs-CRP at each time point and infarct size created and reviewed.

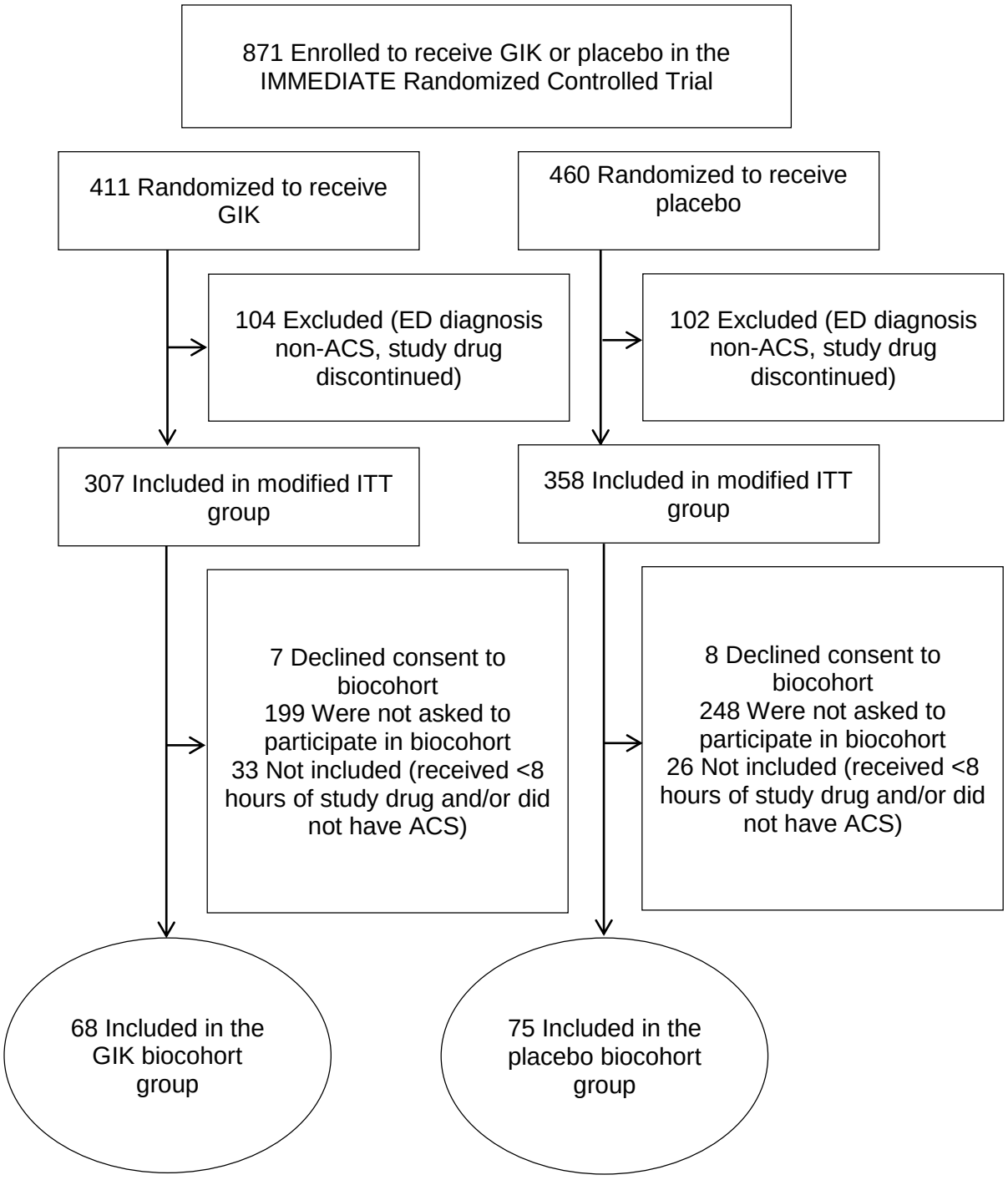
Sensitivity analyses were performed by removing participants who did not have an infarct (i.e. infarct size = 0%), to check if the association between hs-CRP and infarct size was in the same direction for those who had an infarct versus those who did not. Additional sensitivity analysis were done on participants in the placebo group only to test for the correlation between hs-CRP levels, at the three time points measured, and infarct size, without the effect of GIK.

Results

Characteristics of Study Population

A total of 143 participants met the inclusion criteria for the biocohort; 68 received GIK and 75 placebo. Not all individuals had complete hs-CRP measurements; participants with at least one hs-CRP measurement available were included in the analysis (N=143). Figure 2.1 illustrates how the biocohort participants were enrolled.

Figure 2.1. Participants included in the Biocohort from the IMMEDIATE Trial



Abbreviations: ACS, acute coronary syndrome; ED, emergency department; GIK; glucose-insulin-potassium; ITT, intent to treat analysis.

Demographic and clinical characteristics of the biocohort by treatment arm are shown in Table 2.1. The average age was 64 years in both groups; 77% of the GIK group and 70% of the placebo group were men. Chest pain was the chief symptom in over 86% of participants. The median time from start of study drug to the measurement of the initial hs-CRP values was 2.5 hours in the GIK group and 2.6 hours in the placebo group. The entry participant characteristics were well-balanced between groups.

Table 2.1. Baseline Demographics and Clinical Characteristics of Study Participants by Treatment Group in the Biocohort (N=143)*

	No. (%) [unless noted otherwise]	
Cohort Characteristics	**GIK (N=68)**	**Placebo (N=75)**
Age in years, mean (SD)	64.5 (12.9)	63.9 (12.8)
Men	52 (76.5)	52 (69.3)
White race	66 (97.1)	71 (94.7)
Chief complaint on presentation		
Chest pain	59 (86.8)	67 (89.3)
Shortness of breath	1 (1.5)	3 (4)
Other †	8 (11.7)	5 (6.7)
Medical history		
Diabetes mellitus	12 (17.6)	19 (25.3)
Heart failure	4 (5.9)	7 (9.3)
Acute MI	21 (30.9)	24 (32.0)
Medication history		
Statins	30 (44.1)	29 (38.7)
Aspirin	39 (57.3)	44 (58.7)
Minutes from symptom onset to study drug, median (25^{th} to 75^{th} percentile)	86 (51.5-160.5)	81 (53-123)

Minutes from symptom onset to study drug		
0-30	1 (1.5)	0 (0)
31-60	21 (30.9)	22 (29.3)
61-90	9 (13.2)	19 (25.3)
91-180	12 (17.7)	15 (20.0)
181-360	10 (14.7)	8 (10.7)
361-24 h	6 (8.8)	5 (6.7)
Within 24 h, unspecified	3 (4.4)	4 (5.3)
>24 h	6 (8.8)	2 (2.7)
Hospital reperfusion treatment		
Thrombolytic therapy	1 (1.5)	1 (1.3)
PCI	59 (86.8)	56 (74.7)
Coronary artery bypass graft	0 (0)	2 (2.7)
Confirmed diagnosis		
Acute MI	58 (85.3)	68 (90.7)
Any angina	10 (14.7)	7 (9.3)
Hours from study drug onset to biomarker measurement, median (25th to 75th percentile)		
Initial	2.5 (1.3-3.3)	2.6 (1.9-3.2)
6 hour	6.0 (6.0-6.3)	6.0 (6.0-6.2)
12 hour	12.0 (12.0-12.2)	12.1 (12.0-12.3)

Abbreviations: GIK; glucose-insulin-potassium; MI, myocardial infarction; PCI, percutaneous coronary intervention; and SD, standard deviation.

* No significant differences were noted between GIK and placebo groups.

† Abdominal pain, back pain, dizziness, heartburn, loss of consciousness, shoulder pain and weakness.

The clinical characteristics of participants in the biocohort were similar to the parent IMMEDIATE Trial participants by site of enrollment (Table 2.2). The diagnosis of ACS was more common in the biocohort, which was expected because a confirmed diagnosis of ACS was a requirement for enrollment into the biocohort.

Table 2.2. Baseline Demographic and Clinical Characteristics of Study Participants by Treatment Group compared to the participants from the Parent IMMEDIATE Trial[*]

	No. (%) [unless noted otherwise]			
Cohort	**N=143 (Biocohort)**		**N=545[†] (Parent Trial)**	
Characteristics	**GIK (N=68)**	**Placebo(N=75)**	**GIK (N=256)**	**Placebo(N=272)**
Age, mean (SD), y	64.5 (12.9)	63.9 (12.8)	64.5 (13.6)	63.7 (13.9)
Men	52 (76.5)	52 (69.3)	186 (72.7)	194 (71.3)
White race	66 (97)	71 (95)	214 (83.6)	241(88.6)
Chief complaint on presentation				
Chest pain	59 (86.8)	67 (89.3)	227 (88.7)	232 (85.3)
Shortness of breath	1 (1.5)	3 (4.0)	6 (2.3)	12 (4.4)
Other [‡]	8 (11.7)	5 (6.7)	23 (9.0)	28 (10.3)
Medical history				
Diabetes mellitus	12 (17.6)	19 (25.3)	79 (29.8)	77 (28.3)
Heart failure	4 (5.9)	7 (9.3)	39 (14.7)	44 (16.2)
Acute MI	21 (30.9)	24 (32.0)	100 (37.7)	96 (35.3)
Medication history				
Statins	30 (44.1)	29 (38.7)	128 (50.0)	111(40.8)
Aspirin	39 (57.3)	44 (58.7)	147 (57.4)	148 (54.4)
Minutes from symptom onset to study drug, median (25th to 75th percentile)	86 (51.5-160.5)	81 (53.0-123.0)	85.5 (50.0-183.8)	82 (50.0-156.0)

Minutes from symptom onset to study drug, n (%)				
0-30	1 (1.5)	0 (0)	10 (4.0)	10 (3.7)
31-60	21 (30.9)	22 (29.3)	67 (26.7)	72 (26.8)
61-90	9 (13.2)	19 (25.3)	37 (14.7)	45 (16.7)
91-180	12 (17.7)	15 (20.0)	36 (14.3)	40 (14.9)
181-360	10 (14.7)	8 (10.7)	43 (17.1)	39 (14.5)
361-24 h	6 (8.8)	5 (6.7)	19 (7.6)	21 (7.8)
Within 24 h, unspecified	3 (4.4)	4 (5.3)	18 (7.2)	21 (7.8)
>24 h	6 (8.8)	2 (2.7)	21 (8.4)	21 (7.8)
Hospital reperfusion treatment				
Thrombolytic therapy	1 (1.5)	1 (1.3)	1 (0.4)	3 (1.1)
PCI	59 (86.8)	56 (74.7)	127 (49.6)	131 (48.2)
Coronary artery bypass graft	0 (0)	2 (2.7)	6 (2.3)	10 (3.7)
Confirmed diagnosis				
Acute MI	58 (85.3)	68 (90.7)	139 (54.3)	161 (59.2)
Any angina	10 (14.7)	7 (9.3)	33 (12.9)	32 (11.8)

Abbreviations: GIK, glucose-insulin-potassium; MI, myocardial infarction; PCI, percutaneous coronary intervention; SD, standard deviation.

* No significant differences were noted between biocohort and participants from the parent IMMEDIATE Trial.

† Participants enrolled in same centers as the biocohort.

‡ Abdominal pain, back pain, dizziness, heartburn, loss of consciousness, shoulder/arm pain and weakness.

Changes in hs-CRP Levels

The median initial, six hour, and 12 hour hs-CRP values, before log transformation) in the GIK group were 3.1, 3.4 and 4.5 mg/L respectively (Table 2.3). In comparison, the median initial, six hour, and 12 hour hs-CRP values (before log transformation) in the placebo group were 3.2, 4.2, and 5.9 mg/L respectively (Table 2.3). Log transformed hs-CRP values in both treatment arms can be found in Table 2.4.

Table 2.3. Hs-CRP Levels in the First 12 hours of ED Admission (N=143)

	GIK			Placebo		
Hs-CRP	**N**	**Mean (SD)**	**Median (IQR)**	**N**	**Mean (SD)**	**Median (IQR)**
Initial hs-CRP mg/L	59	8.1 (17.7)	3.1 (1.4-7.3)	61	12.3 (24.4)	3.2 (1.9-9.3)
6 hour hs-CRP mg/L	58	8.6 (17.0)	3.4 (1.8-8.8)	63	15.1 (30.7)	4.2 (2.0-12.4)
12 hour hs-CRP mg/L	57	9.2 (16.7)	4.5 (2.3-7.9)	64	17.6 (31.0)	5.9 (3.1-13.7)

Abbreviations: ED, emergency department; GIK, glucose-insulin-potassium; hs-CRP, high sensitivity C-reactive protein; IQR, interquartile range; SD, standard deviation.

Table 2.4. Hs-CRP Levels per Treatment Arm (N=143)[*]

hs-CRP, mg/L	GIK	Placebo	p-value
Initial, mean (SD)	0.51 (0.54) [N=59]	0.62 (0.60) [N=61]	0.29
6 hour, mean (SD)	0.57 (0.53) [N=58]	0.70 (0.62) [N=63]	0.22
12 hour, mean (SD)	0.65 (0.50) [N=57]	0.84 (0.58) [N=64]	0.053
Delta[†], mean (SD)	0.15 (0.25) [N=56]	0.20 (0.38) [N=57]	0.41

Abbreviations: GIK, glucose-insulin-potassium; hs-CRP, high sensitivity C-reactive protein.
[*] Data analyzed using logarithmically transformed hs-CRP values
[†] Difference between the initial hs-CRP and 12 hour hs-CRP measurements.

Linear regression models were performed to assess the association between the initial hs-CRP levels and baseline characteristics. They showed that older people, women and individuals with history of heart failure had higher hs-CRP levels upon admission (Table 2.5). Those associations remained significant after adjusting for the use of study drug. No other characteristics had a significant association with admission hs-CRP values.

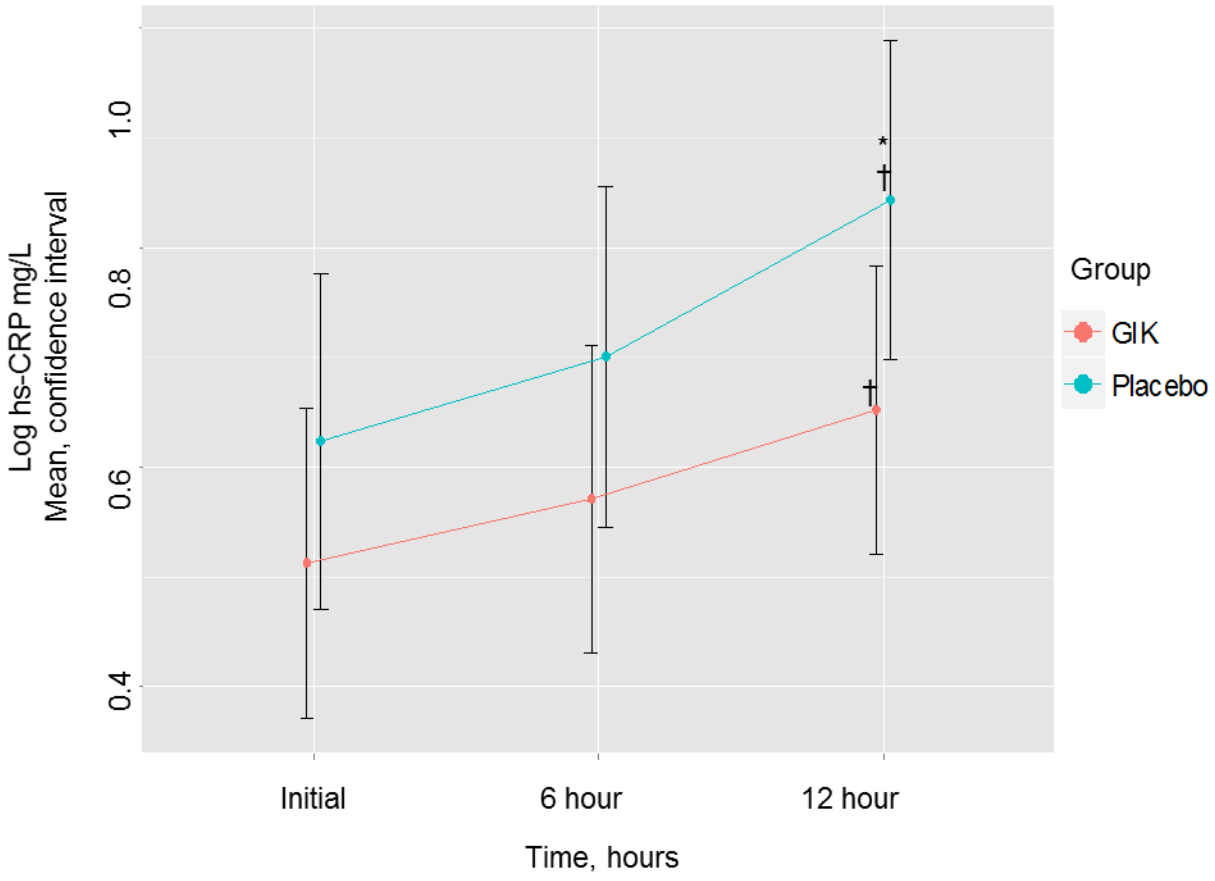
Table 2.5. Results of Regression Analysis between Initial hs-CRP Levels and Baseline Demographic and Clinical Characteristics*

	N=143	
Baseline Characteristics	**Beta Coefficient**	**p-value**
Age	0.01	0.05
Men	-0.27	0.02
History of Diabetes Mellitus	0.16	0.22
History of Heart Failure	0.57	0.01
History of Myocardial Infarction	0.11	0.32

Abbreviations: Hs-CRP, high sensitivity C-reactive protein.
* Data analyzed using logarithmically transformed hs-CRP values

The hs-CRP measurements increased significantly in both the control and the treatment groups by six and 12 hours compared with the initial measurement ($p<0.01$ for all intragroup comparisons, not controlling for multiple comparisons) (Figure 2.2). Hs-CRP values were not different for those who received GIK versus placebo at the initial and six hour measurements; however by 12 hours, the hs-CRP levels were slightly higher in placebo-treated versus GIK-treated participants ($p=0.053$) (Figure 2.2). When comparing delta hs-CRP, the differences between the initial and 12-hour hs-CRP measurements, there were no significant differences in those delta values between treatment arms.

Figure 2.2. Hs-CRP Levels per Treatment Arms



Time course of mean ± confidence interval hs-CRP mg/L values at initial, six hour and 12 hour per treatment arm.
Blue line (lower line) represents the GIK group, while the red line (upper line) represents the placebo group.
Abbreviations: GIK, glucose-insulin-potassium; hs-CRP, high sensitivity C-reactive protein; SEM, standard error of the mean.
* P=0.053 between groups at 12 hour (independent sample t-test), not adjusted for multiple comparisons.
† P<0.01 within group differences between initial and six hour, six hour and 12 hour, and initial and 12 hour (paired sample t-test) hs-CRP mg/L levels, not adjusted for multiple comparisons.
‡ Initial time represents the first hs-CRP measurement (median=2.5 hours).

Results from longitudinal data analysis using mixed models showed that hs-CRP levels increased significantly with time (p<0.001). However, the rate of change in hs-CRP levels did not differ between the GIK and placebo groups (p=0.27 for time*treatment interaction) (Table 2.6).

Table 2.6. Results of Random Mixed Model Analysis of Change in hs-CRP Levels*

Model Results[†, ‡]	**N=143**		
Fixed Effects	**β**	**SE**	**p-value**
Intercept	0.55	0.07	<0.001
Time[§], hours	0.02	0.004	<0.001
GIK treatment	-0.09	0.11	0.40
Time[§], GIK treatment interaction	-0.01	0.006	0.27

Abbreviations: GIK, glucose-insulin-potassium; hs-CRP, high sensitivity C-reactive protein; SE, standard error.

[*] Data analyzed using logarithmically transformed hs-CRP values

[†] Results of multivariable adjusted linear mixed models. Covariates adjusted for in the model included age, sex, and history of heart failure.

[‡] Model included terms for random slope and random intercept, together with fixed effect terms.

[§] Time from drug administration to hs-CRP measurement.

Likelihood ratio test showed that the model with both random intercept and slope is a better fit than a model with random intercept only (p<0.001). Therefore, we reported longitudinal data analysis using models with both random intercept and slope. When baseline clinical characteristics associated with the initial hs-CRP value, including age, sex, and history of heart failure, were added to the model, the results above did not change. In addition, when running the analysis on subjects with complete data (i.e. excluding subjects with missing hs-CRP values at any of the three time points) showed a similar pattern in results (Table 2.7).

Table 2.7. Results of Linear Mixed Model Analysis of Change in hs-CRP Levels, Excluding Subjects with Missing Data[*]

Model Results[†, ‡]	**N=106**		
Fixed Effects	**β**	**SE**	**p-value**
Intercept	0.56	0.09	<0.001
Time[§], hours	0.02	0.004	<0.001
GIK treatment	-0.09	0.13	0.46
Time[§], GIK treatment interaction	-0.01	0.006	0.38

Abbreviations: GIK, glucose-insulin-potassium; hs-CRP, high sensitivity C-reactive protein; SE, standard error.

[*] Data analyzed using logarithmically transformed hs-CRP values

[†] Results of multivariable adjusted linear mixed models. Covariates adjusted for in the model included age, sex, and history of heart failure.

[‡] Model included terms for both random slope and random intercept, together with fixed effect terms. An interaction term between GIK and time was also included in the model.

[§] Time from drug administration to hs-CRP measurement.

Association between hs-CRP and Infarct Size

First we assessed the univariable associations between infarct size and selected baseline characteristics (Table 2.8). Only age and randomization to GIK were associated with 30-day infarct size. This was demonstrated by an increase in infarct size with increasing age, and a decrease in infarct size in subjects who randomized to GIK compared to those randomized to placebo. Other variables including sex, race, chief complaint at presentation (i.e. chest pain versus other chief complains, medical history including history of diabetes mellitus, history of heart failure, and history of MI were not significantly associated with infarct size measured at 30-days. Therefore these variables were not included in multivariable regression models on the association between hs-CRP and infarct size.

Table 2.8. Results of Regression Analysis between 30-Day Infarct Size and Baseline Demographic and Clinical Characteristics

	N=143	
Baseline Characteristics	**Beta Coefficient**	**p-value**
Age	0.29	0.02
Men	-1.44	0.69
History of Diabetes Mellitus	1.63	0.67
History of Heart Failure	-2.47	0.73
History of MI	-2.29	0.53
GIK administration	-7.56	0.02

Abbreviations: GIK; glucose insulin potassium; MI, myocardial infarction.

In univariable models there were no significant associations between hs-CRP and infarct size at the initial and six-hour measurements. On the other hand the 12-hour measurement and delta hs-CRP values were significantly positively associated with infarct size (Table 2.9). This association remained significant in multivariable models, adjusting for age, sex, and GIK administration (β=6.8, p=0.04 for 12-hour hs-CRP, and β=13.9, p=0.02 for delta hs-CRP) (Table 2.9). Sensitivity analysis done by removing participants with no infarct (i.e. those with an infarct size of 0, or those who died and did not have infarct size measurement available) (N=31) showed the same association between hs-CRP levels and infarct size in univariable and multivariable regression models (Table 2.9). With initial and six-hour hs-CRP measurements showing no significant associations with infarct size, while the 12-hour hs-CRP and delta hs-CRP remained significantly associated with infarct size (β=10.6, p=0.02 for 12-hour hs-CRP, and β=23.1, p=0.01 for delta hs-CRP, using multivariable regression models) (Table 2.9).

Table 2.9. Association between hs-CRP levels and 30-day Infarct Size*

	All participants (N=143)			Participants with an infarct (N=70)		
Hs-CRP value	**N**	**β**	**p-value**	**N**	**β**	**p-value**
Univariable Analysis						
Initial hs-CRP mg/L	83	2.8	0.38	54	3.9	0.34
6 hour hs-CRP mg/L	85	4.6	0.11	56	6.5	0.10
12 hour hs-CRP mg/L	83	7.5	0.02	56	10.9	0.01
Delta hs-CRP mg/L†	78	13.9	0.02	51	20.5	0.03
Multivariable Analysis†						
Initial hs-CRP mg/L	83	2.3	0.46	54	3.8	0.35
6 hour hs-CRP mg/L	85	4.0	0.17	56	5.9	0.13
12 hour hs-CRP mg/L	83	6.8	0.04	56	10.6	0.02
Delta hs-CRP mg/L‡	78	13.9	0.02	51	23.1	0.01

Abbreviations: β, regression coefficient; GIK, glucose-insulin-potassium, hs-CRP high sensitivity C-reactive protein.

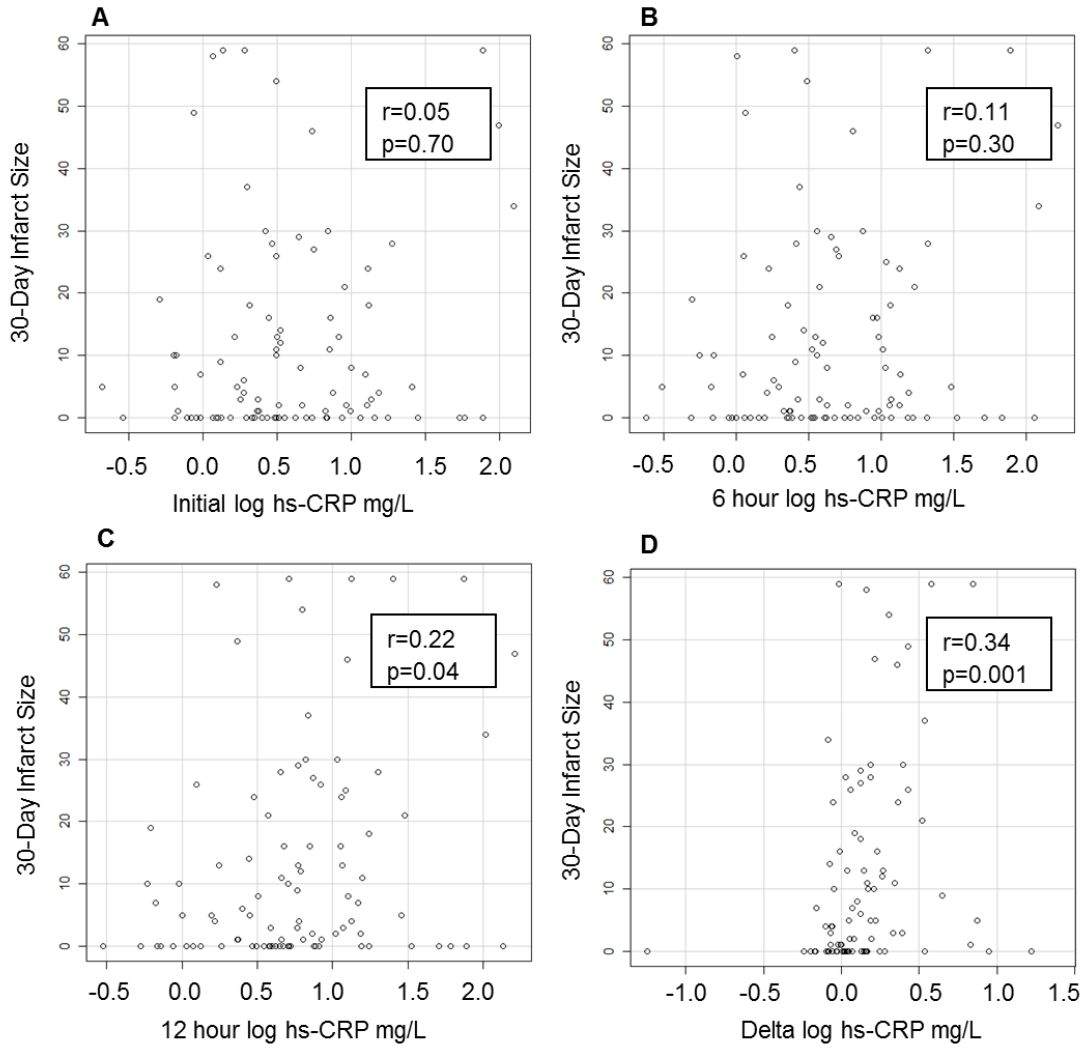
* Data analyzed using logarithmically transformed hs-CRP values.

† Difference between the initial hs-CRP and 12 hour hs-CRP measurements.

‡ Beta (β) coefficient for log transformed hs-CRP effect adjusted for age, sex, and GIK administration. In addition the time from when the drug started to the time of the first hs-CRP measurement was used as a covariate in multivariable regression models. The coefficient represents the fitted increase in infarct size per one unit change in log hs-CRP.

Results from spearman's rank correlation yielded similar associations between the 12 hour and delta hs-CRP values and 30-day infarct size (Figure 2.3). Both the initial hs-CRP measurement and six-hour values were not significantly correlated with 30-day infarct size. However, the 12-hour hs-CRP and delta hs-CRP levels were significantly correlated with infarct size, supporting the results seen above with univariable and multivariable regression models.

Figure 2.3. Correlation between hs-CRP Levels and 30-day Infarct Size*



Correlation was evaluated by Spearman's rank correlation method. A. Correlation between 30-day infarct size and initial hs-CRP. B. Correlation between 30-day infarct size and six hours hs-CRP. C. Correlation between 30-day infarct size and 12 hours hs-CRP. D. Correlation between 30-day infarct size and delta hs-CRP.
Abbreviations: Hs-CRP, high sensitivity C-reactive protein.
* Data analyzed using logarithmically transformed hs-CRP values.

When testing for the correlation between hs-CRP and infarct size in the placebo group only, we found that the 12-hour hs-CRP and the delta hs-CRP remained significantly correlated with infarct size (p-value = 0.022 and 0.049, for the 12 hour and delta hs-CRP respectively) (Table 2.10).

Table 2.10. Association between hs-CRP levels and 30-day Infarct Size in Subjects in the Placebo Group Only*

	(N=75)	
Hs-CRP value	**β**	**p-value**
Univariabl Analysis		
Initial hs-CRP mg/L	6.5	0.14
6 hour hs-CRP mg/L	7.7	0.05
12 hour hs-CRP mg/L	9.6	0.03
Delta hs-CRP mg/L†	8.4	0.26
Multivariable Analysis		
Initial hs-CRP mg/L	6.6	0.13
6 hour hs-CRP mg/L	7.4	0.05
12 hour hs-CRP mg/L	9.2	0.03
Delta hs-CRP mg/L‡	7.4	0.32

Abbreviations: β, regression coefficient; GIK, glucose-insulin-potassium, hs-CRP high sensitivity C-reactive protein.

* Data analyzed using logarithmically transformed hs-CRP values.

† Difference between the initial hs-CRP and 12 hour hs-CRP measurements.

‡ Beta (β) coefficient for log transformed hs-CRP effect adjusted for age, and sex. In addition the time from when the drug started to the time of the first hs-CRP measurement was used as a covariate in multivariable regression models. The coefficient represents the fitted increase in infarct size per one unit change in log hs-CRP.

Strengths and Weaknesses

This study has several limitations. First, although our sample is larger than previous studies on the effect of GIK on hs-CRP levels (34-36), the size of the IMMEDIATE Trial biocohort may have limited our power to detect treatment interactions. Second, hs-CRP levels prior to the onset of GIK infusion and after the 12 hour infusion were not available. As there was a modest difference in hs-CRP levels at the 12-hour measurement using unadjusted t-test in the GIK group compared to placebo, levels beyond that 12-hour may

be required to give a better reflection of the effect of GIK on hs-CRP. Finally, although CRP is commonly used as an inflammatory biomarker, it is somewhat nonspecific and other biomarkers have been considered as an alternative to CRP. For instance, serum amyloid A, interleukin-6, and adhesion molecules such as soluble intercellular adhesion molecule type 1, similar to CRP, are markers of inflammation that are produced by the liver (75). Therefore, if GIK exerts an anti-inflammatory effect it may be reflected through biomarkers other than CRP. Nevertheless this study has several strengths including serial hs-CRP measurements within 12 hours after GIK initiation, compared to other studies, in which the effect of GIK on CRP was assessed at 24-48 hours following treatment. Moreover, our data were collected in a randomized placebo-controlled trial, with both GIK and placebo participants having balanced characteristics.

Chapter 3: **Methods and Results- Serum Adiponectin Levels in Patients with Acute Coronary Syndromes: Serial Changes and Relation to Infarct Size**

Methods

Study Sample

This study analyzed data collected from participants enrolled in the IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care) Trial, the methodology of which has been published elsewhere (31). In brief, this was a randomized, placebo-controlled, double-blind, multi-center clinical effectiveness trial of glucose-insulin-potassium (GIK) that enrolled patients with suspected acute coronary syndrome (ACS) between 2006 and 2011. Paramedics, aided by electrocardiograph (ECG)-based decision support, randomized and enrolled patients aged ≥30 years with a high probability of ACS (31). Participants were given either GIK solution or an identical-appearing placebo. The present study was based on the IMMEDIATE Trial biological mechanism cohort ("biocohort"), which consisted of individuals who consented to participate in this biocohort, where confirmed as having ACS, and treated with GIK or placebo for at least eight hours. Enrollment in the biocohort began after the trial was started and only included six out of 13 study centers that were included in the parent IMMEDIATE Trial. We restricted this analysis to biocohort participants with at least one frozen blood sample available for measurement of adiponectin at any of the three examined time points, as described below.

Data Collection

During the 12-hour infusion of study drug in the IMMEDIATE Trial, blood levels were drawn at three time points: 1) initial measurement, as soon as feasible after hospital arrival; 2) six hours after the start of study drug; and 3) 12 hours after the start of study drug, or upon discontinuation of the infusion. Using time from acute symptom onset to study drug administration, and time for blood measurements, we re-calculated time from symptom onset to the first, second, and third measurements, using the exact time of the

blood drawing rather than the designation as being the initial, six-, or 12-hour samples. Participants returned for sestamibi perfusion and left ventricular (LV) function imaging studies at 30-days. Standardized interpretation of these studies was performed at the SPECT core laboratory at Tufts Medical Center. The core laboratory of Tufts Clinical and Translational Science Institute (CTSI) performed biomarker measurements.

Biomarkers measured included serum levels of glucose, insulin, free fatty acids (FFA), and high sensitivity CRP (hs-CRP). The measurement of adiponectin was not part of the IMMEDIATE Trial biocohort protocol. However, in 2013, we measured adiponectin levels from saved serum samples from biocohort participants that were stored promptly after collectionat -20°C. After thawing the samples, serum levels of adiponectin were measured by Human Adiponectin Platinum enzyme-linked immunosorbent assay (ELISA) kit (eBioscience Inc, San Diego, CA). Not all participants had adiponectin measurements at all three time points, but if at least one measurement was available, that participant was included in our data analyses. Additional blood samples were collected at 30-days for the measurement of glycosylated hemoglobin (HbA1C) and brain-type natriuretic peptide (BNP). Other covariates collected included patients' demographic characteristics, vital signs, medical history, and medications used at home, in the hospital, and upon discharge.

For one-year clinical outcomes we used the composite outcome of all-cause mortality or hospitalization for heart failure as adjudicated in the IMMEDIATE Trial (31; 76). For all re-hospitalizations during the follow-up period, source documents were provided for review by a Clinical Events Committee, to determine if the hospitalization was related to heart failure (76).

Data Analysis

Descriptive statistics were used to describe the baseline characteristics of the study population. Adiponectin and hs-CRP values were logarithmically transformed to obtain normal distributions. Although we did not anticipate any effect of GIK on adiponectin levels, we tested whether adiponectin levels were similar between the GIK and placebo groups before combining all subjects in one cohort for further analysis. For longitudinal data analysis, linear mixed models were used to examine the rate of change in log adiponectin levels across the three measurement time points. We used mixed models with fixed and random effects to account for the repeated measure by patients. Fixed effects were time, and treatment. Random effects were intercept and random slope. The P values were calculated as a fixed-effects for time variable with subject-specific random effects for the intercept. Variables that were associated with initial adiponectin levels in univariable analysis at a p-value<0.1 were included in the model. None of those variables were time-varying covariates. The p-values were obtained by likelihood ratio tests comparing the model with random slope only to the full model with both random slope and intercept.

Linear regression models were used to examine the relationship between baseline clinical characteristics (as the predictors) and the initial or first available measurement of adiponectin (as the outcome) for each participant. We chose the first available adiponectin value for each subject instead of the initial level in order to include all participants in our analysis, since not all subjects had an initial adiponectin value. Since adiponectin levels tend to be higher in women (77), all analyses were further stratified by sex. As a sensitivity analysis we ran descriptive statistics on adiponectin values excluding subjects with missing values at any of the three adiponectin measurements.

Univariable and multivariable linear regression models were used to study the association between baseline or first available measurement of adiponectin (as the predictor) and 30-day infarct size (as the outcome). Adiponectin was examined continuously and in quartiles, with the first quartile as the referent. Variables in multivariable regression models included those determined to be clinically significant or statistically significant in univariable analysis at a $p<0.1$. Infarct sizes ranged from 0% to 59% of left ventricular mass, where 0% represented those who did not develop an infarct and 59% was assigned to those who died (N=4) before imaging, based on the fact that the largest infarct size measured in the study was 58%. In addition, Spearman rank correlations between the first available adiponectin and infarct size were estimated. Sensitivity analyses were performed by removing participants who did not develop an infarct.

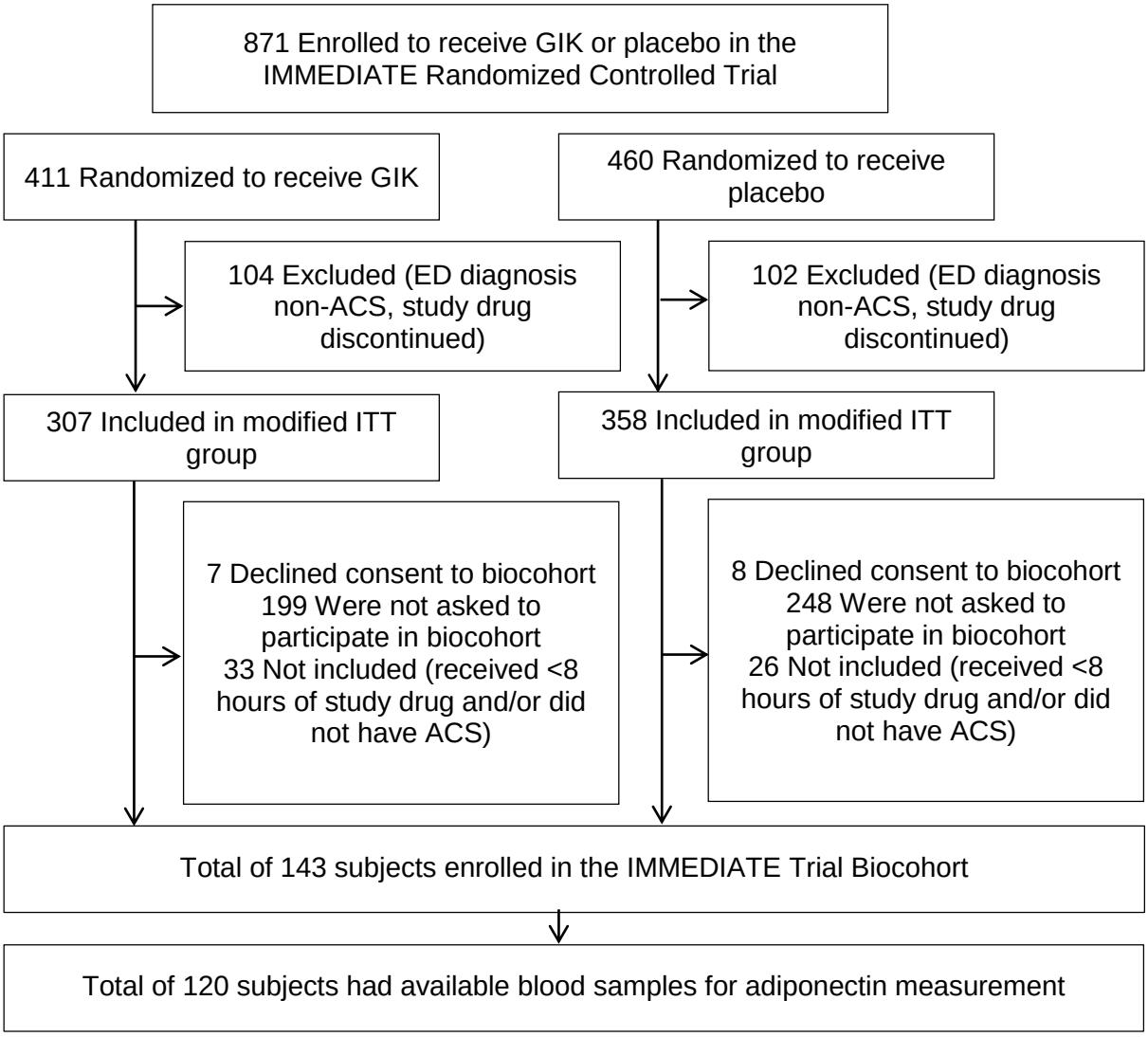
Cox proportional hazards models were used to estimate univariable hazard ratios (HR) for the association of one unit increase in adiponectin, using the first available measurement of adiponectin, with one-year composite outcome of all-cause mortality or hospitalization for heart failure. We also ran the analysis using quartiles of adiponectin. We checked the assumptions needed for proportional hazards analyses by plotting and testing Schoenfeld residuals as related to time. All analyses were done using R, version 3.0.2, with nlme, lme4 and ggplot2 packages (72-74; 78).

Results

Characteristics of Study Population

A total of 120 participants had at least one frozen blood sample available for adiponectin measurements, and 50 subjects had all three levels available. Figure 3.1 illustrates the enrollment of participants from the IMMEDIATE Trial biocohort.

Figure 3.1. Participants included in the Biocohort the IMMEDIATE Trial



Abbreviations: ACS, acute coronary syndrome; ED, emergency department; GIK; glucose-insulin-potassium; ITT, intent to treat analysis.

There were no significant differences in clinical characteristics, or in serum adiponectin levels, between the GIK and placebo groups, and therefore participants were combined into a single cohort for the purpose of these analyses (Tables 3.1 and 3.2). The clinical characteristics of participants in the biocohort, with available adiponectin measurements, were similar to the parent IMMEDIATE Trial participants by enrollment site (Table 3.1).

Table 3.1. Baseline Demographic and Clinical Characteristics of Study Participants Compared to the Participants from the IMMEDIATE Trial[*]

	No. (%) [unless noted otherwise]			
Cohort	**N=120 (Biocohort)**		**N=545[†] (Parent Trial)**	
Characteristics	**GIK (N=55)**	**Placebo(N=65)**	**GIK (N=256)**	**Placebo(N=272)**
Age, mean (SD), y	66.1 (12.7)	64.0 (12.2)	64.5 (13.6)	63.7 (13.9)
Men	40 (72.2)	44 (68.0)	186 (72.7)	194 (71.3)
White race	54 (98.2)	62 (95.4)	214 (83.6)	241(88.6)
Chief complaint on presentation				
Chest pain	47 (85.5)	67 (89.3)	227 (88.7)	232 (85.3)
Medical history				
Diabetes mellitus	10 (18.2)	19 (25.3)	79 (29.8)	77 (28.3)
Heart failure	4 (7.3)	6 (9.2)	39 (14.7)	44 (16.2)
Acute MI	16 (29.1)	21 (32.2)	100 (37.7)	96 (35.3)
PCI	47 (85.5)	50 (76.9)	127 (49.6)	131 (48.2)
Minutes from symptom onset to study drug, median (25th to 75th percentile)	86.0 (53.0-150.5)	81 (53.0-122.0)	85.5 (50.0-183.8)	82.0 (50.0-156.0)
Confirmed diagnosis				
Acute MI	46 (83.6)	60 (92.3)	139 (54.3)	161 (59.2)
Any angina	9 (16.4)	5 (7.7)	33 (12.9)	32 (11.8)

Abbreviations: GIK glucose-insulin-potassium; MI, myocardial infarction; PCI, percutaneous coronary intervention; SD, standard deviation.

[*] No significant differences were noted between biocohort and participants from the IMMEDIATE Trial.

[†] Participants enrolled in same centers as the biocohort.

[‡] Abdominal pain, back pain, dizziness, heartburn, loss of consciousness, shoulder/arm pain and weakness.

Table 3.2. Log Adiponectin Levels per Treatment Arm

All cohort (N=120)	GIK (N=55)	Placebo (N=65)	
Adiponectin measurement	**Mean/SD (N)**	**Mean/SD (N)**	**p-value**
Initial Adiponectin	1.85/0.80 (37)	1.64/0.80 (47)	0.22
6 hour Adiponectin	1.63/0.78 (39)	1.65/0.69 (48)	0.88
12 hour Adiponectin	1.71/0.67 (48)	1.69/0.77 (45)	0.88
Complete data (N=51)*	**Mean/SD (N)**	**Mean/SD (N)**	**p-value**
Initial Adiponectin	1.84/0.73 (22)	1.75/0.76 (29)	0.65
6 hour Adiponectin	1.81/0.75 (22)	1.69/0.71 (29)	0.57
12 hour Adiponectin	1.80/0.74 (22)	1.69/0.75 (29)	0.60

Abbreviations: GIK, glucose-insulin-potassium; SD, standard deviation.
* Analysis on subjects with adiponectin measurements at the three time points

The mean age of this study cohort was 64 years, 70% were men, 97% were white, and 23% had a history of diabetes mellitus (Table 3.3). The average body mass index (BMI) in this cohort was about 29 kg/m^2, and the majority of subjects had chest pain as the chief complaint on presentation. Approximately 88% of participants had a confirmed diagnosis of acute myocardial infarction (MI) and the remainders were diagnosed with unstable angina. Most subjects had PCI as their hospital reperfusion treatment. The median time from the onset of ischemic symptoms until the first, second and third blood measurements were approximately four, eight, and 14 hours respectively. Women were older than men and included higher percentages of persons with previously diagnosed diabetes mellitus and heart failure (Table 3.3). Other clinical characteristics, time from symptom onset to adiponectin measurements, and clinical outcomes were similar between men and women (Table 3.3).

Table 3.3. Baseline Characteristics of Adiponectin Cohort

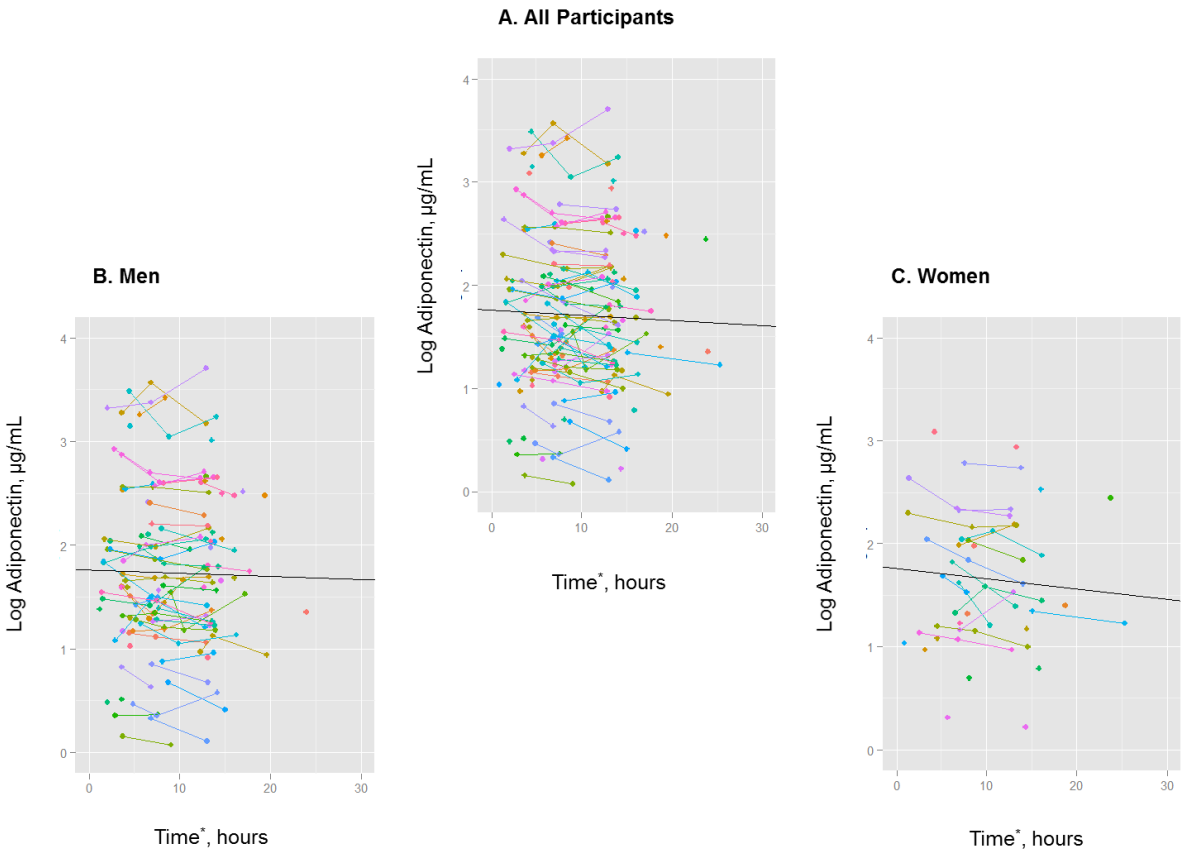
	No. (%) [unless noted otherwise]		
Clinical Characteristics	**All participants (N=120)**	**Men (N=84)**	**Women (N=36)**
Age, mean (SD), y	64.1 (12.4)	62.7 (11.7)	70.0 (12.7)
White race	116 (96.7)	83 (98.8)	33 (91.7)
BMI, mean (SD), kg/m^2	28.9 (7.4)	28.9 (7.6)	28.8 (6.8)
Chest pain on presentation	105 (87.5)	74 (88.1)	31 (86.1)
Medical history			
Diabetes mellitus	27 (22.5)	15 (17.9)	12 (33.3)
Heart failure	10 (8.3)	5 (6.0)	5 (13.9)
Hypertension	76 (63.3)	51 (60.7)	25 (69.4)
Acute MI	37 (30.8)	27 (32.1)	10 (27.8)
Hospital reperfusion treatment			
PCI	97 (80.8)	69 (82.1)	28 (77.8)
Coronary artery bypass graft	1 (0.8)	1 (1.2)	0 (0)
Confirmed acute MI diagnosis	106 (88.3)	73 (86.9)	33 (91.7)
Hours from symptom onset to adiponectin measurement, median (25th to 75th percentile)			
First measurement	4.0 (3.2-5.7)	3.9 (3.3-5.2)	4.5 (2.9-7.1)
Second measurement	7.5 (7.0-8.5)	7.5 (6.9-8.3)	7.8 (7.1-8.9)
Third measurement	13.5 (13.0-14.6)	13.5 (12.9-14.2)	13.9 (13.1-15.1)
Randomized to GIK	55 (45.8)	40 (47.6)	15 (41.7)
One-year outcomes			
All-cause mortality	10 (8.3)	4 (4.8)	6 (16.7)
Hospitalization due to heart failure	9 (7.5)	3 (3.6)	6 (16.7)

Abbreviations: BMI, body mass index; GIK, glucose-insulin-potassium; MI, myocardial infarction; PCI, percutaneous coronary intervention; SD, standard deviation.

Changes in Serum Adiponectin Levels

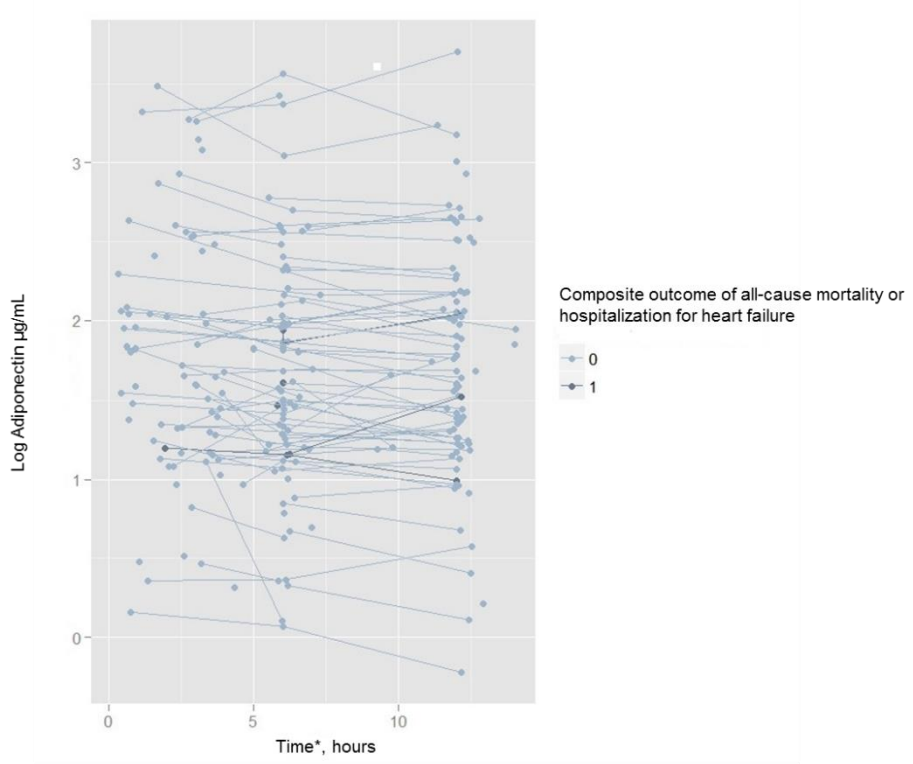
The mean and standard deviation (SD) values of the first, second, and third adiponectin levels before log transformation were 7.62 (6.58) μg/mL, 6.79 (6.08) μg/mL, and 7.12 (5.99) μg/mL respectively. Figure 3.2 shows a scatter plot illustrating the individual changes in adiponectin levels over time for all participants and for men and women. This figure shows a wide range of starting values of adiponectin and relatively small changes overtime. This was also performed by stratifying subjects to those with and without a composite outcome, and in subjects with large and small infarct sizes divided at the median of infarct size values (Figures 3.3 and 3.4). Similarly to the results shown in Figure 3.2, there were relatively small changes in adiponectin levels over time.

Figure 3.2. Individual Changes in Adiponectin Levels†



* Time from symptom onset to adiponectin measurement.
† Data analyzed using logarithmically transformed adiponectin values.
Data points without lines represent those participants with only one adiponectin measure available.
Regression line was obtained using linear mixed models.

Figure 3.3. Individual Changes in Adiponectin Levels in Subjects with and without a Composite Outcome†



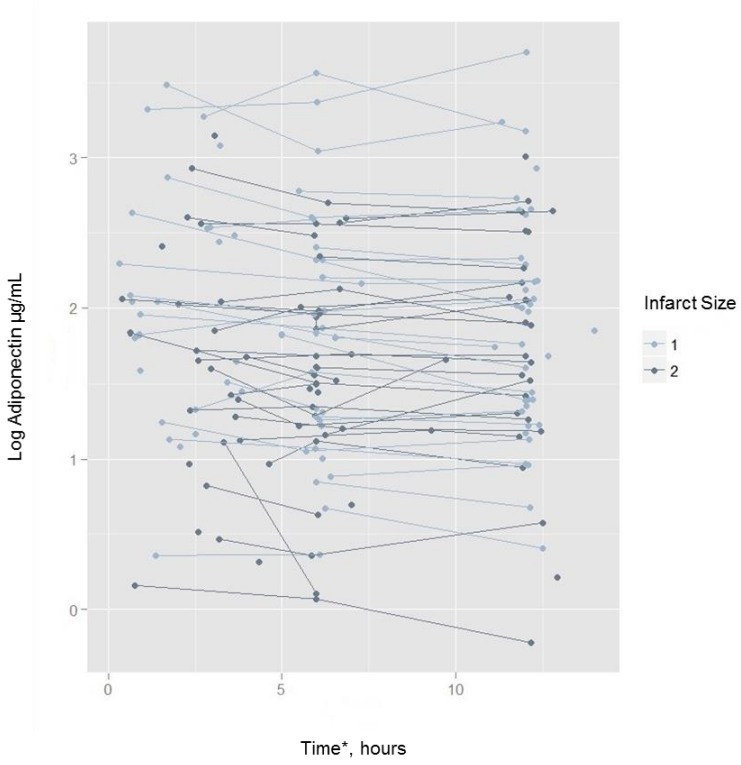
* Time from symptom onset to adiponectin measurement.

† Data analyzed using logarithmically transformed adiponectin values.

Data points without lines represent those participants with only one adiponectin measure available.

0 indicates no outcome, and 1 indicates presence of the composite outcome of one-year all-cause mortality or hospitalization for heart failure.

Figure 3.4. Individual Changes in Adiponectin Levels in Subjects with Small and Large Infarct Sizes†



* Time from symptom onset to adiponectin measurement.

† Data analyzed using logarithmically transformed adiponectin values.

Data points without lines represent those participants with only one adiponectin measure available.

Infarct size was divided at the median, with 0 indicating infarct size measured less than the median, and 1 indicates infarct size measured equal or more than the median.

Using linear mixed models, adiponectin levels decreased by -0.005 µg/mL per hour, (p= 0.035, Table 3.4). The estimated mean adiponectin levels at four, eight, and 14 hours after symptom onset, after log transformation, were 1.74 µg/mL, 1.72 µg/mL, and 1.69 µg/mL respectively (Table 3.4). After stratifying the analysis by sex, men failed to have a significant decrease in adiponectin levels over time; on the other hand, adiponectin levels decreased by -0.010 µg/mL per hour in women (p=0.02, Table 3.4).

Table 3.4. Results of Linear Mixed Model Analysis of Change in Adiponectin Levels*

Model Results[†, ‡]	**All participants (N=120)**			**Men (N=84)**			**Women (N=36)**		
Fixed Effects	**β**	**SE**	**p-value**	**β**	**SE**	**p-value**	**β**	**SE**	**p-value**
Intercept	1.76	0.075	<0.001	1.76	0.089	<0.001	1.76	0.140	<0.001
Time, hours[§]	-0.005	0.003	0.033	-0.003	0.003	0.330	-0.010	0.005	0.015
Adiponecti-n, µg/mL	**Estimated mean** [**]			**Estimated mean** [**]			**Estimated mean** [**]		
Four hours[§]	1.74			1.75			1.72		
Eight hours[§]	1.72			1.74			1.68		
Fourteen hours[§]	1.69			1.72			1.62		

Abbreviations: β, regression coefficient; SE, standard error.
[*] Data analyzed using logarithmically transformed adiponectin values
[†] Results of multivariable adjusted linear mixed models. Covariates adjusted for in the model included age.
[‡] Model included terms for random slope and random intercept, together with fixed effect terms.
[§] Time from symptom onset to adiponectin measurement.
[**] Estimated from linear mixed models.

Adiponectin and Clinical Characteristics

Adiponectin levels decreased by increasing age (β coefficient= -0.01, p= 0.02), but no other patient characteristics were found to have significant association with plasma adiponectin (Table 3.5). In addition, there was no association of baseline hs-CRP, and 30-day BNP and HbA1C levels with the plasma adiponectin levels. We next looked for associations of medical history and clinical variables with adiponectin levels stratified by sex. We also ran the analysis in the whole cohort using interaction terms with sex, however, the results were non-significant.

Table 3.5. Association between Baseline Clinical Characteristics and Adiponectin Levels*

	All participants (N=120)		**Men (N=84)**		**Women (N=36)**	
Variable	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Age, years	**-0.01**	**0.02**	**-0.01**	**0.05**	-0.01	0.25
Men	0.07	0.63	--	--	--	--
BMI, kg/m^2	0.001	0.90	<0.001	0.93	0.002	0.92
History of diabetes mellitus	-0.001	0.10	0.25	0.24	-0.35	0.15
History of MI	-0.06	0.66	-0.03	0.85	-0.04	0.83
History of heart failure	-0.26	0.26	0.03	0.93	-0.56	0.08
HbA1C, %	0.02	0.69	0.06	0.41	-0.09	0.46
Log hs-CRP, mg/L	0.08	0.47	0.14	0.34	-0.03	0.92
BNP, pg/mL	-0.0003	0.80	-0.001	0.98	-0.0001	0.58

Abbreviations: β, regression coefficient; BMI, body mass index; BNP, B-type natriuretic peptide, hs-CRP, high sensitivity c-reactive protein; HbA1C, glycosylated hemoglobin; MI, myocardial infarction.

* Data analyzed using logarithmically transformed adiponectin values. First available adiponectin measurement for each participant was used in this analysis.

Adiponectin and 30-day Infarct Size

The association between infarct size and baseline clinical characteristics can be found in Table 3.6. There was no significant correlation between serum levels of adiponectin and 30-day infarct size (r =-0.02, p=0.86). Unadjusted and multivariable adjusted linear regression models, adjusting for age, sex, and randomization to GIK, showed no significant association between 30-day infarct size and adiponectin levels (Table 3.6). In addition, stratifying the analysis by sex, adiponectin levels were not associated with infarct size for either sexes (Table 3.6). Examination of the quartile analysis of adiponectin, similarly showed no relationship between adiponectin and infarct size.

Table 3.6. Association between Adiponectin and 30-Day Infarct Size*

	All participants (N=90)		**Men (N=64)**		**Women (N=26)**	
Variables	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Univariable Models						
Age, years	0.19	0.21	0.09	0.62	0.46	0.14
Men	-0.07	0.99	-	-	-	-
BMI, kg/m^2	0.11	0.62	0.18	0.43	-0.15	0.78
History of diabetes mellitus	-1.38	0.74	-1.92	0.71	-0.62	0.94
History of MI	-4.52	0.27	-3.79	0.40	-7.64	0.45
History of heart failure	-2.35	0.77	-13.52	0.16	15.21	0.26
Log hs-CRP, mg/L	2.56	0.45	2.72	0.47	2.73	0.75
BNP, pg/mL	0.004	0.20	0.004	0.19	0.003	0.70

GIK treatment	-9.60	0.006	-10.32	0.01	-7.81	0.29
Log adiponectin, µg/mL	-2.39	0.32	0.08	0.98	-8.92	0.07
Adiponectin quartiles	**β (95% CI)**	**p-value†**	**β (95% CI)**	**p-value†**	**B (95% CI)**	**p-value†**
Quartile 1	ref	0.52	ref	0.96	ref	0.60
Quartile 2	-5.81 (-16.18-4.55)		2.85 (-9.63-15.32)		1.98 (-19.46-23.41)	
Quartile 3	-0.39 (-10.21-9.42)		2.90 (-8.48-14.27)		-1.86 (-23.29-19.58)	
Quartile 4	-5.27 (-15.17-5.66)		1.71 (-9.96-13.38)		-10.90 (-31.45-9.74)	
Multivariable Models‡						
Log adiponectin, µg/mL	-1.10	0.64	0.52	0.85	-5.30	0.32
Adiponectin quartiles	**β (95% CI)**	**p-value†**	**β (95% CI)**	**p-value†**	**β (95% CI)**	**p-value†**
Quartile 1	ref	0.10	ref	0.25	ref	0.32
Quartile 2	-4.65 (-14.85-5.55)		0.49 (-11.69-12.67)		7.58 (-14.01-29.18)	
Quartile 3	-0.06 (-9.66-9.54)		1.01 (-10.10-12.11)		8.48 (-14.62-31.58)	
Quartile 4	-2.81 (-12.73-7.11)		1.34 (-10.03-12.71)		-0.85 (-23.19-21.49)	

Abbreviations: β, regression coefficient; BMI, body mass index; BNP, B-type natriuretic peptide; GIK, glucose insulin potassium; HbA1C, glycosylated hemoglobin; hs-CRP, high sensitivity c-reactive protein; MI, myocardial infarction.

* First available adiponectin measurement for each participant was used in this analysis.

† Overall p-value.

‡ Adjusted for age, sex (in the total cohort), and GIK treatment.

Excluding patients without and infarct yielded similar results, for both continuous and quantiles of adiponectin (Table 3.7).

Table 3.7. Association between Adiponectin and 30-Day Infarct Size Excluding Subjects with no Infarct*

	All participants (N=59)	
Unadjusted models	**β**	**p-value**
Log adiponectin, µg/mL	-0.92	0.77
Adiponectin quartiles	**β (95% CI)**	**p-value†**
Quartile 1	ref	0.97
Quartile 2	-1.93 (-14.66-10.79)	
Quartile 3	0.87 (-12.01-13.82)	
Quartile 4	-0.89 (-13.59-11.86)	
Adjusted model‡	**β**	**p-value**
Log adiponectin, µg/mL	0.82	0.72
Adiponectin quartiles	**β (95% CI)**	**p-value†**
Quartile 1	ref	0.44
Quartile 2	0.87 (-12.22-13.96)	
Quartile 3	1.59 (-11.19-14.37)	
Quartile 4	1.16 (-11.61-13.92)	

Abbreviations: β, regression coefficient.
* First available adiponectin measurement for each participant was used in this analysis.
† Overall p-value.
‡ Adjusted for age, sex, and GIK treatment.
Analysis could not be stratified by sex due to small number of subjects.

Adiponectin and One-Year Composite Outcome

There were 14 participants in the adiponectin cohort who either died or were hospitalized for heart failure during the one-year follow-up period. Nine participants were hospitalized for heart failure, and 10 participants died during the one-year follow-up period. In the unadjusted Cox-proportional models, adiponectin levels, and quartiles of adiponectin

were not associated with the one-year composite outcome (Table 3.8). Due to the low number of outcomes in this cohort, we did not run multivariable adjusted models, and we were unable to stratify the composite outcome by sex.

Table 3.8. Association between Adiponectin and One-Year Composite Outcomes of All-Cause Mortality, or Hospitalization for Heart Failure (N=120)[*]

	All-cause mortality (No. of outcomes=10)		**Hospitalization for heart failure (No. of outcomes=9)**		**Composite of all-cause mortality and hospitalization for heart failure (No. of outcomes=14)**	
Variables	**HR**	**p-value**	**HR**	**p-value**	**HR**	**p-value**
Univariable Models						
Age, years	1.11	<0.001	1.08	0.011	1.09	<0.001
Men	0.28	0.046	0.19	0.021	0.30	0.025
BMI, kg/m^2	0.81	0.043	0.76	0.016	0.82	0.021
History of diabetes mellitus	1.44	0.60	1.64	0.48	1.32	0.64
History of MI	5.68	0.012	2.94	0.10	3.12	0.035
History of heart failure	9.74	<0.001	18.0	<0.001	10.3	<0.001
Log hs-CRP, µg/mL	3.25	0.024	3.91	0.034	3.20	0.014
BNP, pg/mL	1.00	0.16	1.00	0.29	1.00	0.13
GIK treatment	0.29	0.12	0.14	0.07	0.31	0.07
Log adiponectin, µg/mL	0.54	0.20	0.43	0.10	0.51	0.09
Adiponectin quartiles	**HR (95% CI)**	**p-value[†]**	**HR (95% CI)**	**p-value[†]**	**HR (95% CI)**	**p-value[†]**
Quartile 1	ref	0.21	ref	0.56	ref	0.23

Quartile 2	0.20 (0.02-1.69)		0.50 (0.09-2.73)		0.50 (0.13-1.99)	
Quartile 3	0.69 (0.16-2.87)		0.56 (0.10-3.04)		0.74 (0.21-2.61)	
Quartile 4	0.20 (0.02-1.75)		0.25 (0.03-3.04)		0.17 (0.02-1.37)	

Abbreviations: BMI, body mass index; BNP, B-type natriuretic peptide; GIK, glucose insulin potassium; HbA1C, glycosylated hemoglobin; HR, hazard ratio; hs-CRP, high sensitivity c-reactive protein; MI, myocardial infarction.

* First available adiponectin measurement for each participant was used in this analysis.

† Overall p-value.

Strengths and Weaknesses

This study has several limitations. Our sample size is small, especially for the stratified and sensitivity analyses, which might explain the non-significant p-values. Also, the lack of association between adiponectin and several clinical characteristics, may be attributed to the number of subjects included. However, it represents the first study on serial measures of adiponectin assessed early in the course of ACS, and it's relation with infarct size. In addition, the number of clinical outcomes was too small to perform adjusted analysis, limiting conclusions about the effect of adiponectin on all-cause mortality or hospitalization for heart failure. Not all participants in the biocohort had all three blood measurements available for analysis. Nevertheless, we used a linear mixed model analysis, which has the ability to accommodate missing data points often encountered in longitudinal datasets (79). We used frozen samples for adiponectin measurements. However adiponectin is considered to be a relatively stable adipokine with stable concentrations even after long periods of freezing, therefore this would unlikely affect our results (80). Due to the nature of the design of this study, we do not have plasma adiponectin levels before the development of ischemia, and at 30-days follow-up when infarct size was measured. Finally, in this study, we only measured total adiponectin levels; however, some evidence suggest that high-molecular weight

adiponectin to be more biologically active (81). Therefore, measuring specific adiponectin isoforms may add additional prognostic information than that seen with total adiponectin.

The strength of this study is a unique data set that examines changes in plasma adiponectin levels in patients with ACS shortly after the onset of symptoms of ischemia, and the association of those levels with 30-day infarct size. We utilized data collected from a randomized controlled trial, and had serial measurements of adiponectin within the first 24 hours of ischemic symptoms. There is an interest in the potential clinical use of adiponectin, and several pre-clinical and clinical trials are presently being conducted on drugs that can increase adiponectin levels, including intracoronary injections during ischemia (71; 82). However, the effect of plasma adiponectin levels in the setting of ACS remains unclear.

Chapter 4: **Methods and Results- Association of Adiponectin and Risk of Primary and Secondary Coronary Artery Disease: A Systematic Review and Meta-Analysis**

Methods

Search Strategy

For this systematic review we searched MEDLINE, EMBASE, Web of Science, Cochrane, and Google Scholar, from inception to February 18, 2015 (Table 4.1). The search was not restricted by language, publication type, date, or status. We subsequently searched through reference lists of eligible articles and related existing systematic reviews. We attempted to identify unpublished data by searching conference proceedings of the American Heart Association/American Stroke Association, American Diabetes Association, European Society of Cardiology Congress, International Congress on Nutrition and Metabolism in Renal Disease, and World Congress of Cardiology. Authors of abstracts with incomplete data were contacted when possible. This review was conducted in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (83).

Table 4.1. Search Strategies

MEDLINE, search through February 18, 2015
1. exp adiponectin/
2. APN.af.
3. ADN.af.
4. adiponectin.af.
5. adipokines.af.
6. adipocytokines.af.
7. or/1-6
8. cardiovascular diseases.af.
9. exp cardiovascular diseases/
10. coronary diseases.af.
11. exp coronary diseases/
12. coronary artery disease.af.

13. exp coronary artery disease/
14. coronary heart disease.af.
15. exp coronary heart disease/
16. coronary ischemia.af.
17. coronary event.af.
18. myocardial infarction.af.
19. exp myocardial infarction/
20. angina.af.
21. ischemic heart disease.af.
22. exp Ischemic heart disease/
23. cardiovascular mortality.af.
24. mortality.af.
25. exp mortality/
26. or/8-25
27. 7 and 26
28. remove duplicates from 27

EMBASE, search through February 18, 2015

1. 'adiponectin'/exp OR adiponectin
2. APN
3. ADN
4. 'adipokines'/exp OR adipokines
5. adipocytokines/exp OR adipocytokines
6. or/1-5
7. cardiovascular AND 'diseases'/exp OR cardiovascular AND diseases
8. coronary AND 'diseases'/exp OR coronary AND diseases
9. coronary AND artery AND 'disease'/exp OR coronary AND artery AND disease
10. coronary AND heart AND 'disease'/exp OR coronary AND heart AND disease
11. coronary AND 'ischemia'/exp OR coronary AND ischemia
12. coronary AND event OR coronary AND event
13. myocardial AND 'infarction'/exp OR myocardial AND infarction
14. 'angina'/exp OR angina
15. ischemic AND heart AND 'disease'/exp OR ischemic AND heart AND disease
16. cardiovascular AND 'mortality'/exp OR cardiovascular AND mortality
17. 'mortality'/exp OR mortality

18. or/7-17
19. 6 and 18

Eligibility Criteria

All identified citations and abstracts yielded by the searches were screened by two investigators for eligibility (Table 4.2). We included epidemiological studies of plasma adiponectin concentrations and coronary artery disease (CAD) that fulfilled the following criteria: adult subjects followed-up for the development of either primary or secondary CAD events; adiponectin levels measured before the onset of CAD events (i.e., prior to primary or secondary CAD events); and included the outcome of CAD events, either as a composite or individual outcomes. We did not limit our analysis by follow-up duration. We excluded narrative reviews, cross-sectional studies, studies on animals or cell lines, and studies of genetic variation in adiponectin-related genes. If there were multiple reports on the same cohort, the effect estimates from the first study was generally included.

Table 4.2. Systematic Review Eligibility Criteria

	Description	**Comments**
Population	Adults being followed for a CAD event, regardless of pre-existing CAD status	Studies were divided into: 1) participants free of CAD at baseline (i.e., primary events); or 2) participants with pre-existing CAD (i.e., secondary events)
Exposure	Adiponectin level in blood	Studies with adiponectin levels measured in blood collected at baseline (before primary, or secondary CAD event)

Comparator	Not applicable	Studies compare adiponectin levels
Outcomes	Composite of CAD events, including fatal/nonfatal MI, CVD mortality, new onset angina, unstable angina, coronary revascularization, stroke, heart failure, and all-cause mortality	The outcomes must be identified through review of medical records and/or death certificates CVD mortality was defined as death due to myocardial infarction, stroke, or sudden cardiac death, or other causes of death due to CVD
Study **D**esign	Longitudinal, observational studies (including case control studies), prospective or retrospective as long as adiponectin was measured before outcome	Any sample size, setting and duration

Abbreviations: CAD, coronary artery disease; CVD, cardiovascular disease; MI, myocardial infarction.

Data Extraction and End Points

Three investigators extracted data independently, and to resolve discrepancies, a fourth investigator was consulted. We extracted patient characteristics, including their CAD history; follow-up duration; serum assay methods; regression methods and variables included in the models; and all reported model results. We also collected data on study quality. We categorized studies as: 1) primary CAD (i.e., carried out in subjects free of CAD at baseline) or 2) secondary CAD (i.e., carried out in subjects with pre-existing CAD, either acute or chronic). If studies included subjects with other cardiovascular disease (CVD) and not specifically CAD, they were not included in the main analysis. In addition, when CAD status at baseline was unclear or when subjects with and without CAD were analyzed together, these studies were referred to as “mixed populations” and were not included in the main analysis.

The primary end point was a composite of CAD events, as defined by study authors, encompassing either fatal or nonfatal myocardial infarction (MI), new onset angina, unstable angina, coronary revascularization, CVD mortality, or all-cause mortality. Some studies included stroke as part of the composite outcome, these studies were still included in our analyses. For secondary CAD studies, we also allowed inclusion of hospitalization for heart failure in the composite outcome. We used composite CAD as our primary end point since the majority of studies reported results for a composite outcome but not for individual components of the outcome. If a study reported only the components of the composite outcome, we selected the analysis of MI, CVD mortality, angina, revascularization, stroke, or all-cause mortality, in this order.

Quality of Studies

Quality assessment was performed according to the Newcastle-Ottawa quality assessment scale (84), which is a validated scale for non-randomized studies. This scale awards a maximum of nine points to each study: four for selection of participants and measurement of exposure, two for comparability of cohorts on the basis of the design or analysis, and three for assessment of outcomes and adequacy of follow-up. Studies assigned zero points for at least four elements in the scale were considered to be of low quality. In addition to this scale, we developed two questions to evaluate quality of studies. The first question was based on the description of statistical model development. Each study was rated as having either a clear or no/unclear description. For a study to be given a “clear” status, all of the following components must be present in the analysis section: 1) description of the statistical method used to examine the association between adiponectin and CAD; 2) description of how the effect estimates were calculated (e.g., per 1-standard deviation [SD] increase); and 3) listing variables in the multivariable analysis. If any of these factors were not present, a study was given an

"unclear" description status. The second question aimed to assess the definition of the study outcomes. A study was given a "clear" status depending on whether they provided a clear definition of their outcomes, including description of the individual components of the composite outcome when applicable.

Data Synthesis and Analysis

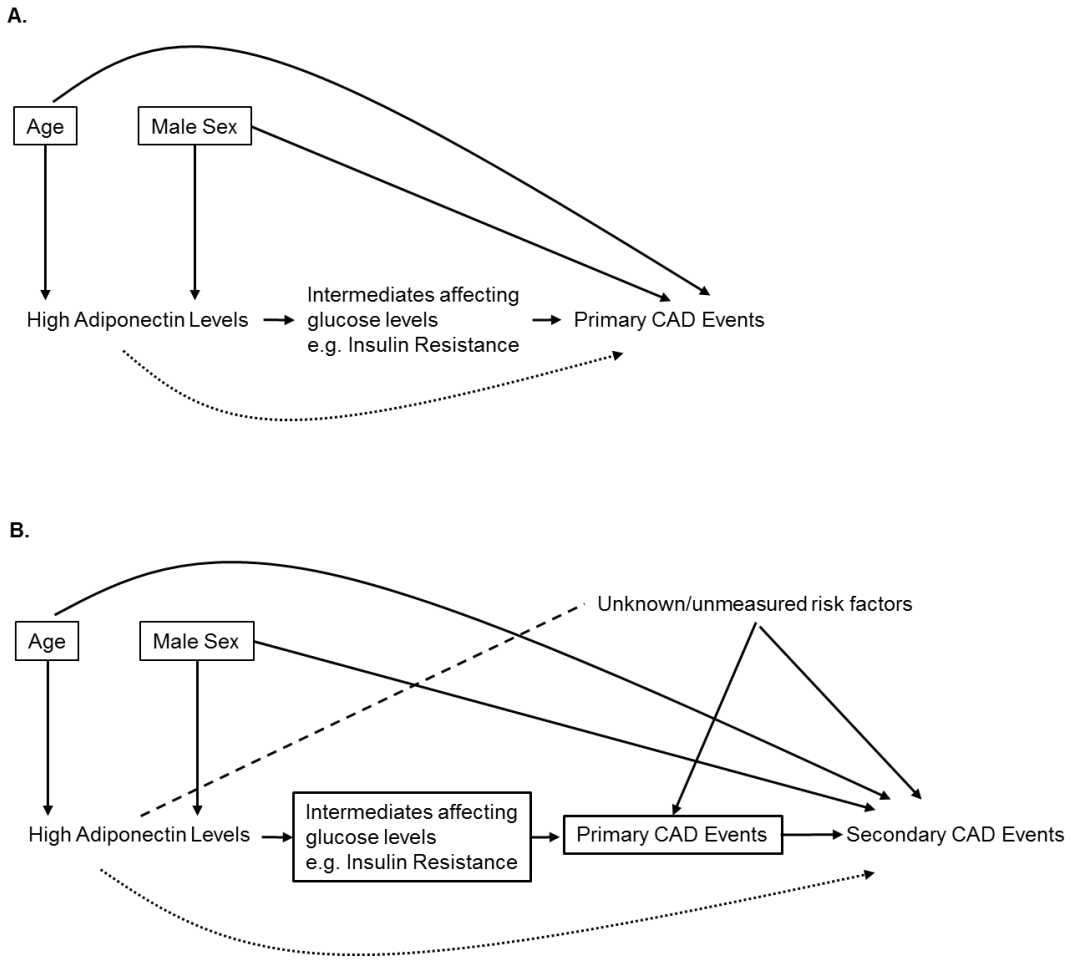
We separately analyzed and compared studies of primary and secondary CAD. From each study, models with and without intermediate variables were captured and analyzed when possible. Models without intermediate variables referred to analyses with the highest number of included possible confounders that did not include any potential intermediate variables. In this review we refer to those models as "models without intermediate variables". Models with intermediate variables, referred to the maximum adjusted models in each study, whether or not it contained potential intermediate variables. Throughout this review we refer to models with intermediate variables as "most-adjusted models". If the maximum adjusted model in a study did not contain any intermediate variable, then the effect estimate from that model was assigned for both model analyses. If a study only reports models that contain intermediate variables, then the maximum adjusted model would only be assigned as most-adjusted (i.e., models with intermediate variables) in our analysis, and that study would be missing a model without intermediate variables. Unadjusted models were used and labeled as models without intermediate variables if all of the adjusted model in a study contained intermediate variables. In case of studies only reporting unadjusted analysis, they were only included in a sensitivity analysis.

Intermediate variables include those related to glucose measure (e.g., glucose levels, glycosylated hemoglobin [HbA1C], history of diabetes), high density lipoprotein

cholesterol (HDL-c) levels, and inflammation (C reactive protein, [CRP]), which are believed to be on the causal pathway between adiponectin and CAD. These intermediate variables are likely to play a causal role between adiponectin and primary CAD events; although their role in secondary events is less-well established. Nevertheless, here we present the analysis on models without intermediate variables and most-adjusted models for both primary and secondary CAD studies. If a study reported only models which incorporated intermediate variables, or only unadjusted result, it was not included in the main analysis. If the maximally adjusted model in a study did not include any intermediate variables, then the effect estimate from that model was used in the analysis for both models without intermediate variables and most-adjusted models. Unpublished reports were summarized in narrative forms but were not included in meta-analyses.

To illustrate the effect of adjusting for intermediate variables, and conditioning study inclusion on prior CAD events we drew a simplified directed acyclic graph of the association between adiponectin and CAD events (Figure 4.1). By adjusting for intermediate variables some of the effects of adiponectin on CAD events are masked, biasing the results toward the null (Figure 4.1.A) (85). When conditioning on pre-existing CAD, this can open a path between adiponectin and CAD recurrence through unmeasured confounders that were initially not associated with adiponectin. This artificially generated confounding can bias the association between adiponectin and secondary CAD events (Figure 4.1.B).

Figure 4.1. Directed Acyclic Graph (DAG) On the Association between Adiponectin Levels and CAD Events



Abbreviations: CAD, coronary artery disease; DAG, directed acyclic graph

A. Simplified Directed Acyclic Graph (DAG) on the Association between Adiponectin Levels and Primary CAD Events

This DAG demonstrates the research question of the relationship of high adiponectin levels and primary CAD events. Both age, and male sex act as confounders of the adiponectin-primary CAD association because they predicts both variables, but not in the causal pathway between them. Therefore, they need to be adjusted for in the analysis, hence the use of the black box. However intermediate variables such as those affecting glucose levels (e.g. insulin resistance) are potentially in the causal pathway in the adiponectin-primary CAD association. If we condition on intermediate variables, this will bias the results towards null, because adjustment of variables in the causal pathway can remove some of the effects of adiponectin levels on primary CAD events.

The dashed curved line represent a direct effect between adiponectin and primary CAD events.

B. Simplified Directed Acyclic Graph (DAG) on the Association between Adiponectin Levels and Secondary CAD Events

This DAG demonstrates the research question of the relationship of high adiponectin levels and secondary CAD events. Both age, and male sex act as confounders of the adiponectin-secondary CAD association because they predicts both variables, but not in the causal pathway between them. Therefore they need to be adjusted for in the analysis, hence the use of the black box. Although intermediate variables may fall in the causal pathway between adiponectin and primary CAD, it is unknown whether they are also in the causal relationship between adiponectin and secondary CAD. Therefore, in case of secondary events it might be appropriate to adjust for variables that were previously defined as intermediates in the relationship between adiponectin and primary CAD.

Unknown, or unmeasured risk factors in this figure refer to those unmeasured variables that are associated with both primary and secondary CAD, however there is no relation between adiponectin and those unmeasured risk factors. Conditioning on (or restricting on) pre-existing CAD (primary CAD) results in a relationship between adiponectin and the unmeasured risk factors, as indicated by the straight dotted line between them, even though these two factors were not associated before conditioning on CAD incidence. This artificially generated confounding results in a biased association between adiponectin and secondary CAD events (represented by adiponectin—unmeasured risk factors→secondary CAD events). This can either bias the results towards the null, or reverse the relationship between adiponectin and secondary CAD events.

The dashed curved line represent a direct effect between adiponectin and secondary CAD events.

The association of adiponectin and CAD was often reported differently by each study (e.g., per 1-SD, per unit increase, or comparing quintiles). To allow a consistent metric for meta-analysis, the estimates were transformed to correspond to the comparison of the top versus bottom third of the adiponectin distribution in each study, using a previously described method (86). This method assumed a normally distributed exposure (adiponectin) with a log-linear association with CAD risk (i.e., linear relationship between log relative risk [RR] estimates and levels of adiponectin). Conversion factors, to convert log RRs from reported scale comparison to top versus bottom third comparison, were derived based on the ratio of expected differences in mean levels of the standardized exposure (i.e., SD scale). This method have been used in previous meta-analyses (67; 87; 88). The hazard ratios (HR) and odds ratios (OR), were assumed to approximate the same measure of RR. Below is the list of the conversion factors used in eligible studies:

1. For studies reporting effect estimates for the top versus bottom third of adiponectin (i.e., tertiles): no conversion factor required.
2. For studies reporting the effect estimate for the top versus bottom fourth of adiponectin (i.e., quartiles): multiply log relative risk (of top versus bottom quartiles) and standard error by 2.18/2.54. Where 2.18 SD units is the difference between the means of the top and bottom third of the distribution. While 2.54 is the difference, in SD units, between the means of the top and bottom quarters of distribution.
3. For studies reporting the effect estimate for the top versus bottom fifth of adiponectin (i.e., quintiles): multiply log relative risk (of top versus bottom quintiles) and standard error by 2.18/2.80. Where 2.80 is the difference, in SD units, between the means of the top and bottom quantiles of distribution.

4. For studies reporting the effect estimate for two equal groups: multiply log relative risk (of upper versus lower half) and standard error by 2.18/1.59. Where 1.59 is the difference, in SD units, between the means of the top and bottom halves of the distribution.
5. For studies reporting the effect estimate for two unequal groups: multiply log relative risk and standard error by study-specific scaling factors (2.18/x), where x is the difference in means between the unequal groups. The differences were found by simulating one million observations from the distribution used to report the comparison (i.e., normal or log normal). For example, if the effect estimate was reported for the top three quartiles versus the bottom quartile, then multiply by 2.18/1.7.
6. For studies reporting the effect estimate for 1-unit increase in adiponectin: multiply log relative risk and standard error by 2.18 times the SD, using study specific SD, and if it was not reported in published report, then it was estimated from some of the larger studies used.
7. For studies reporting the effect estimate for 1-SD increase in adiponectin: multiply log relative risk and standard error by 2.18.
8. For studies reporting the effect estimate per tertile increase in adiponectin: multiple log relative risk and standard error by 2.

We conducted random-effects model meta-analyses to calculate summary RRs (89). The Cochran Q test and the I^2 statistic were used to evaluate heterogeneity between studies (90). We considered that I^2 values of 25%, 50%, and 75% to represent low, moderate, and high heterogeneity, respectively. We also performed meta-regression analyses as described in sensitivity analyses below. All analyses were conducted using R version 3.0.1, with the metafor, and ggplots2 packages (73; 78; 91).

Subgroup and Sensitivity Analyses

We performed several sensitivity analyses on the primary outcome of CAD events to explore potential sources of heterogeneity. Random effects model meta-regression was performed to assess the effect of pre-specified study level characteristics and study quality variables including: 1) race/ethnicity (studies on predominantly Caucasian populations versus non-Caucasians); 2) sex (studies on male versus female); 3) population source (population based versus not), 4) type of study (cohort versus case-control); 5) number of subjects included (<500 versus ≥500 participants); 6) follow-up duration, using the median follow-up time across all studies (≥9 years versus <9 years for studies of subjects with primary CAD, and ≥4 years versus <4 years for studies of subjects with secondary CAD); 7) adiponectin analysis (radioimmunoassay [RIA] versus enzyme linked immunosorbent assay [ELISA]); 8) whether the composite outcome contained stroke or not; 9) acute pre-existing CAD or chronic disease (only on studies of secondary prevention); 10) description of method build-up (clear versus unclear); and 11) description of study outcomes (clear versus unclear). To evaluate the effect of studies quality, an additional analysis was done by excluding those with unclear method description, unclear outcome definition, and those with zero points in at least four elements in the Newcastle-Ottawa quality assessment scale. We also meta-analyzed all eligible studies regardless of whether they only reported models that incorporated intermediate variables or only unadjusted models. To assess whether the results obtained were affected by the conversion method used, we analyzed only the studies reporting the outcome per unit or per 1-SD increase in adiponectin, by standardizing them to per unit without further transformation.

In studies of subjects with primary CAD events, we ran a sensitivity analysis by excluding studies that recruited participants based on a history of diabetes. This was

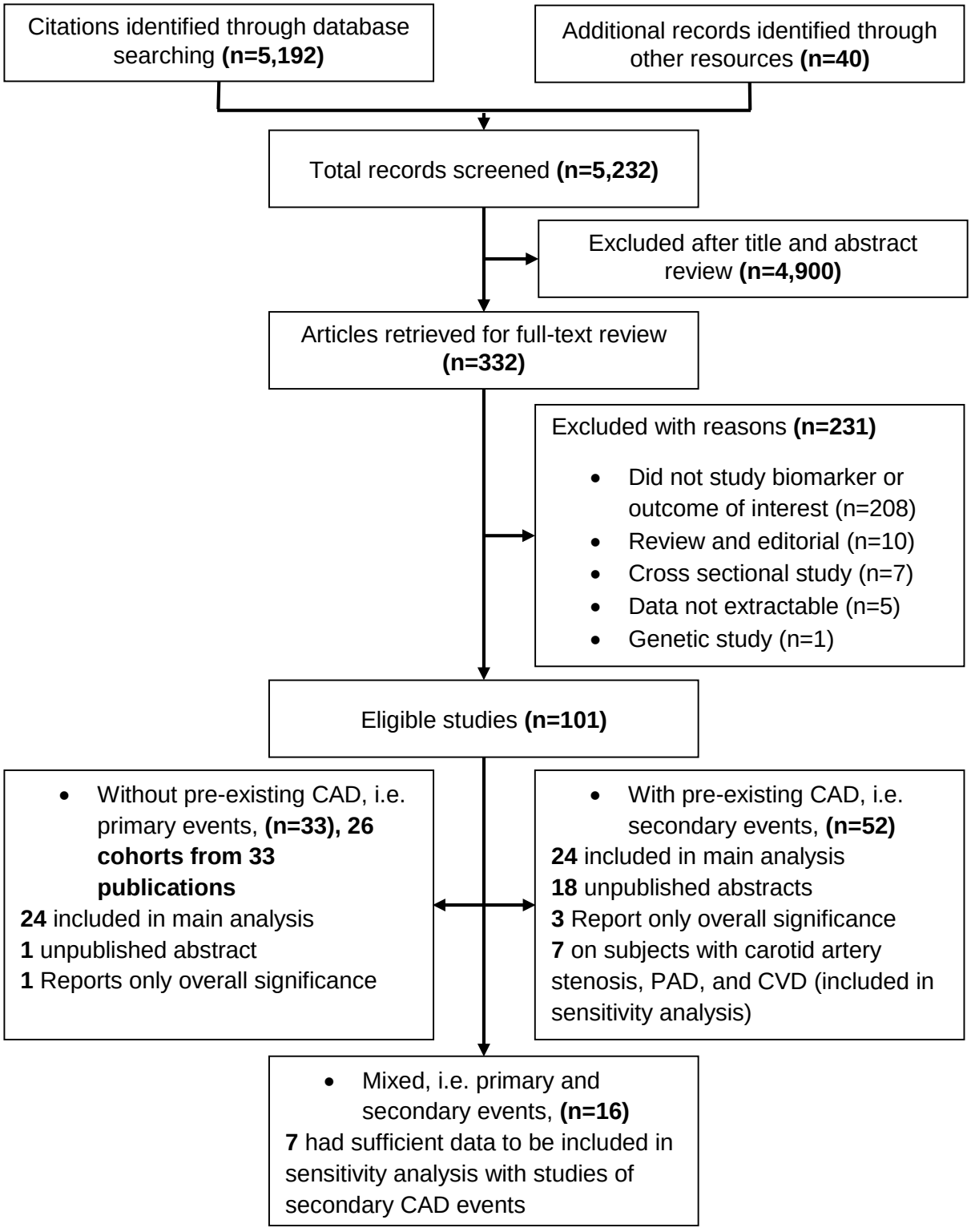
done to address the possibility of index event bias, caused by conditioning study entry to a pre-existing diabetes diagnosis. In studies of secondary CAD events, we included reports of subjects with carotid artery stenosis or peripheral arterial disease (PAD), and subjects with CVD but not CAD, in a sensitivity analysis. Studies with mixed populations of subjects with end stage renal disease, or undergoing cardiac surgery, were included in an additional sensitivity analysis with those of pre-existing CAD, if they included extractable data on models without intermediate variables and most-adjusted models. We performed post-hoc sensitivity analysis by excluding outlier studies from the main analysis. Finally, sensitivity analyses were done using the “leave-one-out” method by iteratively omitting each study and calculating the resulting effect sizes.

Results

Search Results

Of the 5,232 citations identified in the literature search, 101 studies met basic inclusion criteria (Figure 4.2). Thirty-three studies were following subjects for primary CAD events, one from an unpublished abstract, one did not report the effect estimates but only the overall significance, and seven used duplicate cohorts, leaving 24 studies eligible for analysis. Fifty-two studies were of subjects with pre-existing CAD being followed for secondary CAD events. However,18 were from unpublished reports, three reported overall significance without effect estimates, seven included subjects with other CVD (i.e. carotid artery stenosis, PAD, and subjects with CVD but not heart failure), and this left 24 studies eligible for analysis. Sixteen studies had a mixed population of subjects with and without pre-existing CAD and were not included in meta-analyses.

Figure 4.2. Flow of Studies in Review



Abbreviations: CAD, coronary artery disease; CVD, cardiovascular disease; PAD, peripheral artery disease.

Study Characteristics

In aggregate, the studies included 61,097 individuals, including 43,086 subjects in studies of primary CAD events and 19,011 subjects in studies of secondary events. The included studies were published between 2001 and 2015. Of primary CAD studies, 14 were cohorts (33,498 participants) and 10 were case-control studies (9,588 participants). Of secondary CAD studies, 21 were cohorts (15,640 participants), and three were case-control studies (3,371 participants). The sample sizes across all eligible studies ranged from 77 to 4,589 patients and the mean/median follow-up durations ranged from 10 months to 27 years. Details on eligible studies are presented in Tables 4.3 and 4.4.

Table 4.3. Characteristics of Included Studies of Subjects with Primary CAD Events

Source	Study Design	Study Name	Patient Population	Follow-Up, years	Group		No.	Age, years Mean (SD)	Adiponectin, mg/L Mean (SD)
Ai et al, 2011(92)	Prospective cohort	Framingham Offspring Study	Community residents	7.5	Men		1335	58.0 (9.7)	8.9 (6.7-11.9)*
					Women		1606	58.1 (9.6)	14.5 (10.4-19.9)*
Bidulescu et al, 2013(93)	Prospective cohort	Jackson Heart Study	African American subjects	6.3	CAD	Men	38	54 (13)	5.3 (4.3)
						Women	60		7.8 (6.1)
					No CAD	Men	1602		4.0 (3.1)
						Women	2889		6.0 (4.3)
Costacou et al, 2005(94)	Nested case-control	Pittsburgh EDC Study	Subjects with type one diabetes	10.0	Cases		28	34.5 (7.6)	16.4 (4.5)
					Controls		34	34.0 (6.4)	19.5 (7.3)
Cote et al, 2011(95)	Nested case-control	EPIC-Norfolk cohort	Healthy subjects	7.7	Cases	Men	660	65.0 (8.0)	8.7 (4.5)
						Women	375	66.0 (7.0)	12.6 (5.9)
					Control-s	Men	1209	65.0 (8.0)	9.1 (4.3)
						Women	711	66.0 (7.0)	13.4 (6.1)

Dekker et al, 2008[a(96)]	Prospective cohort	Hoorn Study	Subjects aged 50-75 years	15.0	Men	Quartile 1	267	58.3 (6.3)	5.5 (1.0)
						Quartile 2	261	60.5 (6.8)	7.9 (0.6)
						Quartile 3	277	61.7 (7.4)	10.3 (0.8)
						Quartile 4	272	63.7 (7.2)	15.6 (3.7)
					Women	Quartile 1	310	61.2 (7.3)	8.1 (1.6)
						Quartile 2	314	60.9 (7.4)	12.1 (1.1)
						Quartile 3	311	61.7 (7.3)	16.0 (1.3)
						Quartile 4	313	63.6 (7.5)	24.6 (5.9)
Frystyk et al, 2007(97)	Prospective cohort	ULSAM study	Healthy men ≥70 years	7.9	All		832	71.0 (0.6)	10.3 (4.2)
Gardener et al, 2013(98)	Prospective cohort	NOMAS study	Multi-ethnic population	10.0	All		2900	69.0 (10.0)	11.4 (6.2)**
Hatano et al, 2011(99)	Nested case-control	JMS cohort	Community residents	9.4	Cases		38	64.8 (8.4)	7.6 (5.0-12.2)†
					Controls		89	64.9 (8.4)	7.4 (5.4-11.1)†
Kanaya et al, 2006(100)	Prospective cohort	Health ABC study	Subjects 70-79 years	6.1	African Americ-ans	CAD	96	73.3 (3.0)	8.0 (7.0)*
						No CAD	948	73.3 (2.8)	8.0 (7.0)*

					Whites	CAD	166	73.8 (3.0)	10.0 (8.0)*
						No CAD	1263	73.7 (2.8)	12.0 (9.0)*
Kappelle et al, 2012(101)	Nested case-control	PREVEND cohort	Caucasian men	3.0	Cases		103	58.0 (10.0)	15.6 (9.5)†
					Controls		106	47.0 (12.0)	19.9 (11.5)†
Khalili et al, 2010(102)	Prospective cohort	Malmö preventive project	Middle aged men	27.0	Quintile 1		781	47.2 (3.0)	(0.9-3.9)*
					Quintile 2		780	47.3 (2.7)	(3.9-5.1)*
					Quintile 3		772	47.3 (2.9)	(5.1-6.4)*
					Quintile 4		776	47.6 (2.3)	(6.4-8.2)*
					Quintile 5		776	47.7 (2.1)	(8.2-24.9)*
Kizer et al, 2008(103)	Nested case-control	CHS	Subjects aged ≥65 years	7.4	Cases	Men	282	75.5 (5.4)	12.6 (11.5-13.8)†
						Women	322	75.3 (5.0)	18.5 (17-20.1)†
					Controls	Men	366	75.4 (5.4)	12.4 (11.4-13.5)†
						Women	416	75.4 (5.1)	20.3 (18.9-21.7)†

Koenig et al, 2006(104)	Prospective cohort	MONICA/ KORA	Healthy middle aged men	18.0	All		937	54.1 (5.8)	6.2 (4.4-8.9)*
Laughlin et al, 2007(105)	Prospective cohort	Rancho Bernardo Study	Community-subjects 50-91 years old	20.0	CAD		252	75.4 (74.2-76.5)*	11.2 (10.5-11.9)†
					No CAD		1100	71.2 (70.7-71.8)*	12.1 (11.8-12.6)†
Lawlor et al, 2005(55)	Prospective cohort	BWHHS	Women	4.0	Cases		165	70.3 (5.5)	14.5 (13.5-15.7)§
					Controls		334	70.1 (5.3)	15.1 (14.3-16.0)§
Lindberg et al, 2013(106)	Prospective cohort	CCHS	Community subjects	7.8	All		5624	20-94	9.8 (3.4-27.7)
Lindsay et al, 2005(54)	Case-Control	SHS	American Indian subjects	NR	Cases		251	61.7 (8.4)	9.6 (6.8 to 13.3)*
					Controls		251	60.5 (7.6)	9.4 (6.0 to 15.0)*
Luc et al, 2009(107)	Nested case-control	PRIME cohort	Healthy middle aged men	10.0	Cases		617	(50-59)*	10.6 (5.4-20.6)†
					Controls		1215	(50-59)*	11.0 (5.5-22.0)†
Onat et al, 2013(108)	Prospective cohort	TARF study	Middle aged subjects	3.8	Men	Tertile 1	561	52.2 (10.1)	4.9†
						Tertile 2		53.8 (11.0)	8.5†
						Tertile 3		58.1 (11.9)	15.4†
					Women	Tertile 1	663	52.8 (10.3)	6.1†

						Tertile 2		53.5 (11.9)	10.9†
						Tertile 3		57.1 (12.0)	19.7†
Pischon et al, 2011(69)	Nested case-control	Nurses' Health Study	Women	14.0	Cases		455	60.0 (6.5)	8.2 (4.1)
					Controls		911	59.9 (6.5)	9.3 (4.0)
Pischon et al, 2004(53)	Nested case-control	HPFS	Men aged 40-75 years	6.0	Cases		266	65.2 (8.3)	15.6 (8.5)
					Controls		532	65.2 (8.3)	17.9 (8.8)
Saito et al, 2013(109)	Nested case-control	The CIRCS and Ozu study	Subjects with high blood glucose levels	12.3	Cases		117	68.2	5.5*
					Controls		234	67.7	5.5*
Schottker et al, 2013(110)	Prospective cohort	ESTHER study	Subjects with diabetes with and without renal dysfunction	8.0	With events		161	66.0 (61-70)*	8.0 (4.5-11.7)*
					Without events		877	64.0 (60-69)*	7.6 (4.7-10.6)*
Wannamethee et al, 2011(111)	Prospective cohort	BRHS	Men aged 60-79 years	9.0	All		2879	(40-59)*	6.8 (4.4-10.9)

Abbreviations: BRHS, British Regional Heart Study; BWHHS, British Women's Heart and Health Study; CCHS, Copenhagen City Heart Study**;** CAD, coronary artery disease**;** CHS, Cardiovascular Health Stud ; CIRCS, Circulatory Risk in Communities Stud; EDC, Epidemiology of Diabetes Complications; EPIC, European Prospective Investigation into Cancer and Nutrition; Health ABC, Health, Aging, and Body Composition; HPFS, Health Professionals Follow-up Study; JMS, Jichi Medical School Cohort Study; KORA, Cooperative Health Research in the Region of Augsburg; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; NORMAS, Northern Manhattan Study; NR, not reported; PREVEND, Prevention of Renal and Vascular End-stage Disease; PRIME, Prospective Epidemiological Study of Myocardial Infarction; SHS, Strong Heart Study; TARF, Turkish Adult Risk Factor Study; ULSAM, Uppsala Longitudinal Study of Adult Men.

[a] Study characteristics for participants with and without CAD at baseline. However effect estimates were reported separately for subjects with primary and secondary CAD events.
[b] Outcome data was stratified based on smoking status, however only data on smokers were reported.
* Median (IQR)
** Mean (Range)
† Geometric mean, log10 (IQR)
‡ Weighted geometric means (antilog of standard errors of log means)
§ Geometric mean (95% CI of geometric mean)

Table 4.4. Characteristics of Included Studies of Subjects with Secondary CAD Events

Source	Study Design	Study Name	Patient Population	Follow-Up, years	Group	No.	Age, years Mean (SD)	Adiponectin, mg/L Mean (SD)
Alkofide et al, 2015(112)	Prospective cohort	IMMEDIATE Trial	Subjects with ACS	1.0	All	120	65.0 (12.4)	7.1 (5.9)
Ang et a, 2009(113)	Prospective cohort	NR	Subjects with ACS	0.8	Tertile 1	147	60.0 (12.0)	(0.5-4.9)*
					Tertile 2	148	66.0 (11.0)	(4.9-8.9)*
					Tertile 3	147	69.0 (11.0)	(8.9-35.4)*
Arsenault et al, 2014(114)	Case-control	TNT	Subjects with stable CAD	4.9	With events	157	63.0 (8.4)	6.6 (4.7, 8.9)
					Without events	1349	61.4 (8.8)	6.6 (4.8, 9.3)
Beatty et al, 2012(115)	Prospective cohort	Heart and Soul Study	Subjects with stable CAD	7.1	Quartile 1	246	64.0 (10.5)	(1.2-12.6)*
					Quartile 2	245	64.9 (10.2)	(12.6-21.3)*
					Quartile 3	245	67.6 (10.9)	(21.3-35.6)*
					Quartile 4	245	70.5 (11.2)	(35.6-121)*
Cavusoglu et al, 2006(57)	Prospective cohort	NR	Subjects with angina, unstable angina and NSTEMI	2.0	Tertile 1	104	63.4 (9.4)	<=4.4*
					Tertile 2	104	65.2 (9.1)	>4.4-<=8.0*

					Tertile 3		104	68.1 (9.9)	>8.0*
Hascoet et al, 2013(116)	Case-control	GENES study	Men with stable CAD	8.1	Cases	Quartiles 1-3	542	59.3 (7.8)	<7.3*
						Quartile 4	173	63.0 (7.9)	>=7.3*
					Controls	Quartiles 1-3	588	58.5 (8.1)	<9.1*
						Quartile 4	194	60.8 (8.7)	>=9.1*
Huang et al, 2010(117)	Prospective cohort	NR	Subjects with ACS	3.6	MACE		30	63.0 (11.0)	4.0 (2.4)
					No MACE		72	62.0 (11.0)	5.8 (4.6)
Hung et al, 2010(118)	Prospective cohort	NR	Subjects with type two diabetes, CAD	1.3	All		193	64.6 (12.2)	3.7 (3.1)
Kojima et al, 2007(119)	Prospective cohort	NR	Subjects with acute MI	1.0	Men		114	61.0 (12.0)	4.7 (3.5-7.3)**
					Women		42	72.0 (10.0)	8.7 (6.6-14.1)**
Lee et al, 2009(120)	Prospective cohort	Infarction prognosis study registry	Subjects with acute MI	1.0	Tertile 1		132	57.0 (12.0)	<5.5*
					Tertile 2		132	62.0 (11.0)	5.5-10.3*
					Tertile 3		133	68.0 (11.0)	>10.3*

Li et al, 2012(121)	Prospective cohort	NR	Han Chinese with CAD	1.6	Low	276	64.5 (10.9)	<5*
					High	173	65.5 (10.8)	>5*
Lindberg et al, 2012(59)	Prospective cohort	NR	Subjects with acute STEMI	2.3	Quartiles 1-3	551	61.0 (12.0)	6.9 (5.0-10.2)*
					Quartile 4	184	69.9 (12.0)	
Maiolino et al, 2008(122)	Prospective cohort	GENICA	Subjects with chest pain/ suspected CAD going to angiography	3.8	Low adiponectin	356	60.0 (10.3)	3.9 (1.7)
					High adiponectin	356	65.0 (9.2)	11.4 (4.5)
Menzaghi et al, 2014(123)	Prospective cohort	GHS	Subjects with type two diabetes, CAD	5.4	Men	242	63.6 (8.3)	4.2 (0.97-21.8)**
					Women	117	66.1 (7.5)	5.0 (0.67-19.2)**
Morita et al, 2013(124)	Prospective cohort	NAMIS	Subjects with acute MI	3.0	All	724	64.0 (56.0-70.0)*	5.0 (3.4-7.4)*
Ohashi et al, 2010(125)	Prospective cohort	JAPAN-ACS	Subjects with ACS	1.0	All	238	62.5 (11.3)	7.8 (4.6)
Oliveira et al, 2013(126)	Prospective cohort	NR	Subjects with ACS	1.1	All	114	62.0 (10.5)	9.8 (6.1-13.9)*
Piestrzeniewicz et al, 2008(127)	Prospective cohort	NR	Men with STEMI going to PCI	1.0	MACE	9	57.0 (6.7)	4.0 (0.90)
					No MACE	68	54.0 (6.8)	9.9 (6.4)

Pilz et al, 2006(58)	Prospective cohort	LURIC study	Subjects with angiographic CAD	5.5	All	2473	64.0 (10.0)	NR
Schnabel et al, 2008(61)	Prospective cohort	AtheroGene study	Subjects with stable CAD	2.5	All	1890	63.0 (54.0-69.0)*	9.1 (6.7-13.3)*
Shioji et al, 2007(128)	Prospective cohort	NR	Subjects with angina and acute MI going to PCI	2.3	Quartile 1	46	60.1 (1.6)	<=4.5*
					Quartiles 2-4	138	68.2 (0.9)	>4.5*
Soderberg et al, 2009(129)	Nested case-control	LIPID trial cohort	Men with Stable CAD	4.4	Cases	184	64.0 (59.0-68.0)**	8.3 (5.3-11.7)**
					Controls	184	61.0 (53.0-68.0)**	7.1 (4.9-11.3)**
von Eynatten et al, 2008(130)	Prospective cohort	NR	Subjects with Stable CAD	4.7	All	1051	59.0 (8.0)	4.3 (3.3-5.5)*
Wilson et al, 2011(60)	Prospective cohort	PROVE IT–TIMI 22	Subjects with ACS	2.0	< median	1965	55.0 (48.0-63.0)**	2.8 (2.1-3.6)**
					> median	1966	61.0 (53.0-70.0)**	7.2 (5.6-9.9)**

Abbreviations: ACS, acute coronary syndrome; BiKE, Biobank of Karolinska carotid Endarterectomies; BRHS, British Regional Heart Study; CAD, coronary artery disease; CHS, Cardiovascular Health Study; CVD, cardiovascular disease; GENES, Genetique et ENvironnement en Europe du Sud; GENICA Genetic and ENvironmental factors in Coronary Atherosclerosis; GHS, Gargano Heart Study; JAPAN-ACS, Japan assessment of pitavastatin and atorvastatin in acute coronary syndrome; LIPID, Long-Term Intervention with Pravastatin in Ischaemic Disease; LURIC LUdwigshafen RIsk and Cardiovascular Health; MACE, major adverse cardiovascular events; MI, myocardial infarction; NAMIS; Nagoya Acute Myocardial Infarction Study; NR, not reported; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous coronary intervention; PROVE IT–TIMI 22, Pravastatin Or atorVastatin Evaluation and Infection Trial–Thrombolysis in Myocardial Infarction 22; SMART, Second Manifestations of ARTerial disease; STEMI, ST elevation myocardial infarction; TNT, Treating to New Targets.

* Median (IQR)

** Mean (Range)

The most common outcome reported was a composite of CAD events, followed by CVD mortality, all-cause mortality, MI, stroke, heart failure, unstable angina, and need for coronary revascularization. The definition of the composite outcome of CAD events varied in each study, with most primary event studies reporting a composite of fatal and non-fatal CAD events. Secondary CAD studies frequently included a broader outcome definition, containing all-cause mortality and heart failure (Tables 4.5, and 4.6).

Table 4.5. Outcomes in Studies of Primary CAD Events

Source	Composite outcome	Definition	MI	CVD mortality	Angina	Stroke	Revascularization	All-cause mortality	Heart Failure
Ai et al, 2011(92)	Yes	CAD events (MI, CABG, PCI, or documented coronary disease on angiography)	No	No	No	No	No	No	No
Bidulescu et al, 2013(93)	Yes	CAD events (CAD deaths, hospitalized MI)	No	No	No	Yes	No	No	No
Costacou et al, 2005(94)	Yes	CAD events (CAD determined by angina diagnosed by an ED physician or MI, or angiographic stenosis ≥50%, CABG, angioplasty or ischemic ECG changes)	No	No	No	No	No	No	No
Cote et al, 2011(95)	Yes	CAD events (unstable angina, stable angina, and MI [fatal or not])	No	No	No	No	No	No	No
Dekker et al, 2008[96]	Yes	Non-fatal CVD (documented angina pectoris, MI, heart failure, stroke or transient ischemic attack, or peripheral disease)	No	Yes	No	No	No	Yes	No

Frystyk et al, 2007(97)	Yes	CAD events (dying from CAD or first-time hospitalization due to CAD)	No	No	No	No	No	No	No
Gardener et al, 2013(98)	Yes	Vascular events (incident stroke, MI, or vascular death)	Yes	Yes (Vascular death)	No	Yes	No	No	No
Hatano et al, 2011(99)	No	NA	Yes	No	No	No	No	No	No
Kanaya et al, 2006(100)	Yes	CAD events (overnight hospitalization for MI or coronary death)	No	No	No	No	No	No	No
Kappelle et al, 2012(101)	Yes	CAD events (CVD mortality, hospitalization for MI, PCI or CABG)	No	No	No	No	No	No	No
Khalili et al, 2010(102)	Yes	CAD events (fatal and non-fatal coronary events)	No	No	No	Yes	No	No	No
Kizer et al, 2008(103)	Yes	CAD events (non-fatal events [MI, angina pectoris, CABG, or PCI] and fatal events [MI, sudden cardiac death, and procedure related death]	Yes	Yes	No	No	No	Yes	No
Koenig et al, 2006(104)	Yes	CAD events (fatal or non-fatal acute MI and sudden cardiac death)	No	No	No	No	No	No	No

Laughlin et al, 2007[a](105)	Yes	CAD events (non-fatal MI or fatal CAD)	Yes	Yes	No	No	No	No	No
Lawlor et al, 2005(55)	Yes	CAD events (CAD death, MI, first diagnosis of angina, or CABG)	No	No	No	No	No	No	No
Lindberg et al, 2013(106)	Yes	MACE (CVD mortality, hospitalization for MI, or ischemic stroke)	No	No	No	No	No	Yes	No
Lindsay et al, 2005(54)	Yes	CAD events (fatal and non-fatal CAD)	No	No	No	No	No	No	No
Luc et al, 2009(107)	Yes	CAD events (angina, unstable angina, MI and CAD death)	No	No	No	No	No	No	No
Onat et al, 2013(108)	Yes	CAD events (CAD death, and non-fatal CAD [angina pectoris, MI, or myocardial revascularization])	No	No	No	No	No	No	No
Pischon et al, 2011(69)	Yes	CAD events (non-fatal MI or fatal CAD)	No	No	No	No	No	No	No
Pischon et al, 2004(53)	Yes	CAD events (non-fatal MI or fatal CAD)	No	No	No	No	No	No	No
Saito et al, 2013(109)	Yes	CAD events (strokes and MI)	No	No	No	No	No	No	No

Schottker et al, 2013(110)	Yes	CAD events (MI, stroke and fatal CVD events)	No	No	No	No	No	No	No
Wannamethee et al, 2011(111)	Yes	CAD (fatal or non-fatal MI)	No	Yes	No	No	No	Yes	No

Abbreviations: ACS, acute coronary syndrome; CABG, coronary artery bypass graft; CAD, coronary artery disease; CV, cardiovascular; CVD, cardiovascular disease; ECG, electrocardiogram; ED, emergency department; MACE, major adverse cardiovascular events; MI, myocardial infarction; NA, not applicable; PCI, percutaneous coronary intervention.

[a] MI and CVD mortality outcomes were only reported in men, composite outcome was reported in all.

Table 4.6. Outcomes in Studies of Secondary CAD Events

Source	Composite outcome	Definition	MI	CVD mortality	Angina	Stroke	Revascularization	All-cause mortality	Heart Failure
Alkofide et al, 2015(112)	Yes	Composite (all-cause mortality, and hospitalization for heart failure)	No	No	No	No	No	Yes	Yes
Ang et a, 2009(113)	Yes	Composite (all-cause mortality, readmission with ACS [STEMI, NSTEMI, unstable angina] or admission with heart failure)	No	No	No	No	No	No	No
Arsenault et al, 2014(114)	Yes	MACE (CAD death, non-fatal non-procedure-related MI, resuscitated cardiac arrest, and fatal or non-fatal stroke)	No	No	No	No	No	No	No
Beatty et al, 2012(115)	Yes	Composite (MI, heart failure, or all-cause mortality)	Yes	No	No	No	No	Yes	Yes
Cavusoglu et al, 2006(57)	No	NA	Yes	Yes	No	No	No	Yes	No

Hascoet et al, 2013(116)	No	NA	No	No	No	No	No	No	No
Huang et al, 2010(117)	Yes	MACE (re-hospitalization due to unstable angina, non-fatal MI, PCI, CABG, ischemic stroke, CVD mortality)	No	No	No	No	No	No	No
Hung et al, 2010(118)	Yes	MACE (MI, PCI, CABG, stroke, carotid revascularization, all-cause mortality)	No	No	No	No	No	No	No
Kojima et al, 2007(119)	Yes	MACE (CVD mortality, recurrent MI, unstable angina, and heart failure requiring emergency re-hospitalization)	No	No	No	No	No	No	No
Lee et al, 2009(120)	No	NA	No	Yes	No	No	No	Yes	No
Li et al, 2012(121)	Yes	MACE (death, targeted vascular revascularization, ACS, heart failure, and transient ischemic attack/ stroke)	No	No	No	No	No	No	No
Lindberg et al, 2012(59)	No	NA	Yes	Yes	No	No	No	Yes	Yes

Maiolino et al, 2008(122)	Yes	CVD events (sudden death and death due to heart failure, ACS or stroke)	No	Yes	No	No	No	No	No
Menzaghi et al, 2014(123)	No	NA	No	Yes	No	No	No	No	No
Morita et al, 2013(124)	Yes	Composite (CVD mortality, ACS requiring revascularization, heart failure requiring re-hospitalization, stroke)	No	Yes	No	Yes	No	No	Yes
Ohashi et al, 2010(125)	Yes	MACE (CVD mortality, ACS, coronary revascularization [target lesion or target vessel revascularization or CABG])	No	No	No	No	No	No	No
Oliveira et al, 2013(126)	Yes	Composite (CVD mortality, non-fatal acute MI or re-infarction, non-fatal stroke, re-hospitalization due to recurring ischemia or revascularization)	No	No	No	No	No	No	No
Piestrzeniewicz et al, 2008(127)	Yes	Composite (CVD mortality, non-fatal MI, hospitalization for angina or heart failure)	No	No	No	No	No	No	No

Pilz et al, 2006(58)	No	NA	No	Yes	No	No	No	Yes	No
Schnabel et al, 2008(61)	Yes	CVD events (CVD mortality, non-fatal MI)	No	No	No	No	No	No	No
Shioji et al, 2007(128)	Yes	MACCE (all-cause mortality, re-infarction, repeat coronary revascularization, hospitalization for heart failure, and cerebral infarction)	No	No	No	No	No	No	No
Soderberg et al, 2009(129)	Yes	CVD events (CVD mortality, non-fatal MI, stroke)	No	No	No	No	No	No	No
von Eynatten et al, 2008(130)	Yes	CVD events (CVD mortality, non-fatal MI, or ischemic cerebrovascular event [stroke])	No	No	No	No	No	No	No
Wilson et al, 2011(60)	Yes	CVD events (all-cause mortality, MI, documented unstable angina requiring re-hospitalization, revascularization, and stroke)	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations: ACS, acute coronary syndrome; CABG, coronary artery bypass graft; CAD, coronary artery disease; CV, cardiovascular; CVD, cardiovascular disease; MACE, major adverse cardiovascular events; MACCE, major adverse cardiovascular and cerebrovascular events; MI, myocardial infarction; NA, not applicable; PCI, percutaneous coronary intervention.

In primary CAD studies, almost all models without intermediate variables contained only age and sex, and most-adjusted models included either variables affecting glucose levels, HDL-c, or CRP. In contrast, frequently in secondary CAD studies any adjusted analyses contained at least one of the intermediate variables defined earlier; therefore, the models without intermediate variables were commonly unadjusted (Tables 4.7, and 4.8).

Table 4.7. Covariates in Models in Studies of Subjects with Primary CAD Events

Source	Variables in Models Without Intermediate Variables	Variables in Most-Adjusted Models
Ai et al, 2011[a](92)	Age, sex, smoking status	Age, sex, BMI, smoking status, SBP, hypertensive treatment, cholesterol lowering medication, total cholesterol, **HDL-c, diabetes status**
Bidulescu et al, 2013[a](93)	Did not report a model without intermediate variables	Age, sex, BMI, smoking, physical activity, SBP, blood pressure medication, triglycerides, **HDL-c, CRP, insulin resistance by HOMA-IR**
Costacou et al, 2005(94)	Did not report a model without intermediate variables	Age, sex, albumin excretion rate, homocysteine, non-HDL-c, **HDL-c**
Cote et al, 2011(95)	Age, sex	Age, sex, BMI, waist circumference, smoking status, blood pressure, total cholesterol, LDL-c, **HDL-c, type 2 diabetes**
Dekker et al, 2008[a,b(96)]	Age, sex	Age, sex
Frystyk et al, 2007[a](97)	Age, sex	Age, sex BMI, smoking status, SBP, total cholesterol, **HDL-c, insulin sensitivity, intact proinsulin**
Gardener et al, 2013(98)	Age, sex, race/ethnicity	Age, sex, race/ethnicity, waist circumference, smoking status, alcohol use, physical activity, previous cardiac disease history, hypertension, LDL-c, non-HDL-c, triglycerides, **HDL-c, diabetes, hsCRP**
Hatano et al, 2011(99)	Age, sex	Age, sex, BMI, smoking status, SBP, triglyceride, **HDL-c, diabetes, hsCRP**
Kanaya et al, 2006[c](100)	Age, sex, race, study site, BMI, abdominal visceral fat, smoking status, hypertension, aspirin use, LDL-c	Age, sex, race, study site, BMI, abdominal visceral fat, smoking status, hypertension, aspirin use, LDL-c**, HDL-c, diabetes, CRP, insulin**

Kappelle et al, 2012[a](101)	Age, sex	Age, sex, waist circumference, smoking status, hypertension, microalbuminuria, triglycerides, **total cholesterol/HDL-c ratio, hsCRP, insulin sensitivity by HOMA-IR**
Khalili et al, 2010[a](102)	Age, sex	Age, sex, BMI, SBP, cholesterol, triglycerides, **glucose**
Kizer et al, 2008(103)	Age, sex, race, subclinical disease, clinic, waist to hip ratio, smoking status, alcohol consumption, SBP, serum creatinine, leptin, health status and measured weight loss or gain more than 10 poinds in past 3 years, LDL-C	Age, sex, race, subclinical disease, clinic, waist to hip ratio, smoking status, alcohol consumption, SBP, serum creatinine, leptin, health status and measured weight loss or gain more than 10 lb in past 3 years, fibrinogen, triglycerides, LDL-c, **HDL-c, diabetes, CRP**
Koenig et al, 2006[a](104)	Age, sex	Age, sex, BMI, smoking status, physical activity, alcohol consumption, actual hypertension, total cholesterol, **HDL-c, diabetes**
Laughlin et al, 2007(105)	Age, sex, weight girth	Age, sex, waist girth, triglycerides, **HDL-c, fasting plasma glucose**
Lawlor et al, 2005[a](55)	Age, sex	Age, sex, smoking status, physical activity, alcohol consumption, hypertension status, fasting triglyceride, **HDL-C, glucose, insulin, and CRP**
Lindberg et al, 2013(106)	Age, sex	Age, sex, smoking status, hypertension, DPB, SBP, GFR, hypercholesterolemia, cholesterol, LDL-C, triglycerides, **HDL-c, hsCRP, HbA1C, glucose**
Lindsay et al, 2005[b](54)	Age, sex, waist, % fat, smoking status, SBP, albumin:creatinine ratio	Age, sex, waist, % fat, smoking status, SBP, albumin:creatinine ratio
Luc et al, 2009[a](107)	Did not report a model without intermediate variables	Sex, smoking status, hypertension, total cholesterol, triglycerides, **HDL-c, diabetes**
Onat et al, 2013[a](108)	Did not report a model without intermediate variables	Age, sex, waist circumference, creatinine, non-HDL, **CRP**

Pischon et al, 2011[a](69)	Age, sex, BMI, date of blood draw, reported problems with blood draw, fasting status, smoking status, physical activity, alcohol consumption, hypertension, parental history of MI, hormone replacement therapy use, LDL-c	Age, sex BMI, date of blood draw, reported problems with blood draw, fasting status, smoking status, physical activity, alcohol consumption, hypertension, parental history of MI, hormone replacement therapy use, LDL-c, **HDL-c, diabetes, HbA1c, CRP**
Pischon et al, 2004[a](53)	Age, sex, smoking status, month of blood draw	Age, sex, month of blood draw, BMI, smoking status, physical activity, alcohol intake, hypertension, family history of MI before age 60 years, LDL-c, **HDL-c, diabetes**
Saito et al, 2013(109)	Age, sex, community	Age, sex, community, BMI, smoking status, alcohol intake, hypertension, hyperlipidemia, **type 2 diabetes**
Schottker et al, 2013(110)	Age, sex	Age, sex, smoking status, physical activity, SBP, GFR, NSAID, non-HDL-c, **HbA1C**
Wannamethee et al, 2011[a](111)	Age, sex	Age, sex social class, BMI, smoking status, physical activity, alcohol intake, treated hypertension, SBP, b-blockers, statins, forced expiratory volume in 1 sec, albumin, muscle mass, GFR, BNP, LDL-c, **HDL-c, diabetes, insulin resistance by HOMA-IR, CRP**

Abbreviations: BMI, body mass index, BNP, brain natriuretic peptide; CAD, coronary artery disease; CRP, C-reactive protein; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL-c, high density lipoprotein cholesterol, HOMA-IR, homeostatic model assessment- insulin resistance; hsCRP, high sensitivity C-reactive protein; LDL-c, low density lipoprotein cholesterol; SBP, systolic blood pressure.

[a] These studies did not include sex as a covariate but their analysis was either stratified by sex or the study was done only in men or women, therefore they were considered to be sex-adjusted.

[b] This study did not adjust for race, however their analysis was stratified by African Americans versus Whites, therefore it was considered to be race adjusted.

[c] There were no models that included intermediate variables in these studies, therefore the same multivariable model was used in models without intermediate variables and most-adjusted analyses.

Notes:

1- Bold covariates indicates intermediate variables.

2- The studies could report models more than those listed here, however for this analysis, we only included two models. One not adjusting for any intermediate variables (also known as models without intermediate variables), and one that includes the maximum number of covariates in a study, which can contain intermediate variables (also known as most-adjusted models).

Table 4.8. Covariates in Models in Studies of Subjects with Secondary CAD Events

Source	Variables in Models Without Intermediate Variables	Variables in Most-Adjusted Models
Alkofide et al, 2015(112)	None	Did not report an adjusted model
Ang et a, 2009(113)	None	Age, sex, BMI, smoking status, hypertension, Killip class II, III or IV, ST deviation, left bundle branch block, troponin T on admission, chronic kidney disease stages 3, 4 or 5, LV systolic dysfunction, hemoglobin, total cholesterol, **HDL-c, diabetes**
Arsenault et al, 2014[a](114)	Age, sex, and treatment effect (randomization to atorvastatin dose)	Age, sex, and treatment effect (randomization to atorvastatin dose)
Beatty et al, 2012(115)	Age, sex, race	Age, sex, race, BMI, GFR, b-blocker, aspirin, statin, LV ejection fraction, diastolic dysfunction, inducible ischemia, NT-proBNP, triglycerides, non-HDL-c, **HDL-c, diabetes, HgA1c, insulin, glucose, CRP**
Cavusoglu et al, 2006(57)	Did not report a model without intermediate variables	Age, MI on presentation, b-blocker use, number of diseased coronary arteries, SrCr, TIMP-1, **diabetes**
Hascoet et al, 2013[a](116)	Sex, case-control design	Age, sex, BMI, waist, smoking status, gamma-glutamyl transferase, apolipoprotein A1, resting heart rate, GFR or history of kidney failure, ankle–arm index, case–control design, **treatment for diabetes, fasting glucose, hsCRP**
Huang et al, 2010(117)	Did not report a model without intermediate variables	Age, BMI, smoking status, SBP, ACEI/ARBS, LDL-c, **HDL-c, fasting glucose**
Hung et al, 2010(118)	Age, sex	Age, sex, waist circumference, smoking status, MBP, antihypertensive therapy (ARB/ACEI), statins, aspirin, **metabolic syndrome, lipid profile, fasting sugar**

Kojima et al, 2007[a](119)	Sex	Age, sex, BMI, smoking status, hypertension, time to admission to hospital, Killip class, culprit coronary artery, multivessel involvement, total cholesterol, triglyceride, **HDL-c, diabetes**
Lee et al, 2009[d](120)	None	Age, hypertension, renal insufficiency, resistin, LV systolic dysfunction, early invasive therapy, b-blocker use, statin use, **fasting glucose, hs-CRP**
Li et al, 2012(121)	Did not report a model without intermediate variables	Age, sex, weight, smoking status, hypertension, LVEF, CrCl, vascular score, stenosis score, extent score, dyslipidemia, total cholesterol, triglycerides, LDL-c, **HDL-c, glucose, diabetes, CRP**
Lindberg et al, 2012[c](59)	None	Smoking status, hypertension, multivessel disease, **hypercholesterolemia**
Maiolino et al, 2008[b](122)	Age, LV ejection fraction, modified Duke coronary artery disease score, calcium-channel blocker treatment	Age, LV ejection fraction, modified Duke coronary artery disease score, calcium-channel blocker treatment
Menzaghi et al, 2014(123)	None	Age, sex, BMI, smoking status, hypertension, total cholesterol, triglycerides, **HDL-c, HbA1c, anti-diabetic therapy, hsCRP**
Morita et al, 2013(124)	Age, sex, BMI, smoking status	Age, sex, BMI, smoking status, previous MI, BNP level, **diabetes**
Ohashi et al, 2010(125)	Did not report a model without intermediate variables	Sex, history of prior PCI, total bilirubin, creatinine, **diabetes**
Oliveira et al, 2013(126)	Did not report a model without intermediate variables	Previous angina, arterial hypertension, Killip classification, leptin, creatinine, CKMB activity, CKMB mass, troponin, BNP, urea, **fasting glucose**
Piestrzeniewicz et al, 2008(127)	None	Ejection fraction, **diabetes**
Pilz et al, 2006(58)	Age, sex	Age, sex, BMI, smoking, hypertension, CAD, fibrinogen, GFR, homocysteine, NT-pro-BNP, triglycerides, LDL-c, **HDL-c, metabolic syndrome/type 2 diabetes, CRP**

Schnabel et al, 2008(61)	None	Age, sex, BMI, smoking status, hypertension, family history, statins, b-blocker, BNP, **diabetes, CRP**
Shioji et al, 2007(128)	Did not report a model without intermediate variables	Age, sex, BMI, final diameter stenosis, final reference diameter, statins, **hypoglycemic drugs, insulin, diabetes, fasting blood glucose, HbA1C**
Soderberg et al, 2009[a](129)	Sex	Age, sex, randomization to pravastatin, BMI, smoking status, hypertension, nature of prior ACS, revascularization, stroke, total cholesterol, **HDL-c, diabetes**
von Eynatten et al, 2008(130)	Age, sex	Age, sex, smoking status, family status, severity of CAD, intake of lipid-lowering drugs, ACEI, initial management of CAD (three categories: conservative, PCI, CABG), NT-proBNP, CrCl, LDL-C, triglyceride, **HDL-c, diabetes, hsCRP**
Wilson et al, 2011(60)	None	Age, sex, race, BMI, smoking status, SBP, qualifying event, GFR, treatment group (atorvastatin), BNP, triglycerides, **diabetes, CRP**

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors, ACS, acute coronary syndrome; ARB, angiotensin receptor blockers; BMI, body mass index, BNP, brain natriuretic peptide; CAD, coronary artery disease; CKMB, creatine kinase-MB; CrCl, creatinine clearance; CRP, C-reactive protein; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL-c, high density lipoprotein cholesterol, HOMA-IR, homeostatic model assessment- insulin resistance; hsCRP, high sensitivity C-reactive protein; IL, interleukin; LDL-c, low density lipoprotein cholesterol; LV, left ventricular dysfunction; NT-proBNP, N-terminal pro-brain natriuretic peptide; NSTEMI, non-ST elevation myocardial infarction; SBP, systolic blood pressure; SrCr, serum creatinine; STEMI, ST-elevation myocardial infarction.

[a] These studies did not include sex as a covariate but their analysis was either stratified by sex or the study was done only in men or women, therefore they were considered to be sex-adjusted.

[b] There were no models that included intermediate variables in these studies, therefore the same multivariable model was used in models without intermediate variables and most-adjusted analyses.

[c] The only model available in this study includes hypercholesterolemia, which may or may not indicate adjustment for HDL-c. This was included as a “most-adjusted model”. However, we ran a sensitivity analysis considering this model as a “model without intermediate variables” and the results did not differ.

[d] Adjusted models were only used for the outcome of all-cause mortality, not cardiovascular disease mortality.

Notes:

1- Bold covariates indicates intermediate variables.

2- The studies could report models more than those listed here, however for this analysis, we only included two models. One not adjusting for any intermediate variables (also known as models without intermediate variables), and one that includes the maximum number of covariates in a study, which can contain intermediate variables (also known as most-adjusted models).
3- None, indicates no variables were included in those models (i.e. they were unadjusted).

Using the Newcastle-Ottawa quality assessment tool, most studies scored well in exposure ascertainment, and demonstrated that the outcome of interest was not present at the start of the study. Among eligible studies, seven earned zero points in at least four of the scale elements; one of primary CAD and five of secondary CAD (Tables 4.9, and 4.10,). In the 24 primary CAD studies, eight had unclear descriptions, six of model development and two of outcome definition. In 24 secondary CAD studies, 14 had an unclear description of model development and three did not adequately define study outcomes.

Table 4.9. Quality Assessment in Studies of Subjects with Primary CAD Events

a. Cohort Studies								
Section	**Selection**				**Comparability**	**Outcomes**		
Source/Element	**Representativeness of the exposed cohort**	**Selection of the non-exposed cohort**	**Ascertainment of exposure**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of cohorts on the basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur**	**Adequacy of follow up of cohorts**
Ai et al, 2011(92)	1	1	1	1	2	0	1	0
Bidulescu et al, 2013(93)	1	1	1	1	2	1	1	0
Dekker et al, 2008[96]	1	1	1	1	1	1	1	0
Frystyk et al, 2007(97)	1	1	1	1	2	1	1	1
Gardener et al, 2013(98)	1	1	1	1	2	1	1	0
Kanaya et al, 2006(100)	1	1	1	1	2	1	1	0
Khalili et al, 2010(102)	0	1	1	0	2	1	1	1

Koenig et al, 2006(104)	1	1	1	1	2	1	1	0
Laughlin et al, 2007(105)	1	1	1	1	2	1	1	1
Lawlor et al, 2005(55)	1	1	1	1	2	1	1	0
Lindberg et al, 2013(106)	0	1	1	1	2	1	1	1
Onat et al, 2013(108)	1	1	1	1	2	0	1	0
Schottker et al, 2013(110)	0	1	1	1	2	1	1	1
Wannamethee et al, 2011(111)	1	1	1	1	2	1	1	1
b. Case-Control Studies								
Costacou et al, 2005(94)	0	1	1	1	2	1	1	0
Cote et al, 2011(95)	1	1	1	1	2	1	1	1
Hatano et al, 2011(99)	1	1	1	0	2	1	1	1
Kappelle et al, 2012(101)	1	1	1	1	2	1	1	0
Kizer et al, 2008(103)	1	1	1	1	2	1	1	0
Lindsay et al, 2005(54)	0	0	1	0	2	1	1	0

Luc et al, 2009(107)	1	1	1	1	2	1	1	0
Pischon et al, 2011(69)	1	1	1	1	2	1	1	0
Pischon et al, 2004(53)	1	1	1	1	2	1	1	1
Saito et al, 2013(109)	0	0	1	1	2	1	1	0

Abbreviations: CAD, coronary artery disease.

Table 4.10. Quality Assessment in Studies of Subjects with Secondary CAD Events

a. Cohort Studies								
Section	**Selection**				**Comparability**	**Outcomes**		
Source/Element	**Representativeness of the exposed cohort**	**Selection of the non-exposed cohort**	**Ascertainment of exposure**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of cohorts on the basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur**	**Adequacy of follow up of cohorts**
Alkofide et al, 2015(112)	1	1	1	1	0	1	0	1
Ang et al, 2009(113)	1	1	1	1	2	1	0	0
Beatty et al, 2012(115)	1	1	1	1	2	1	1	1
Cavusoglu et al, 2006(57)	0	1	1	1	1	1	0	0
Huang et al, 2010(117)	0	1	1	1	1	1	1	1
Hung et al, 2010(118)	1	1	1	1	2	0	0	0
Kojima et al, 2007(119)	0	1	1	1	0	0	0	1

Lee et al, 2009(120)	0	1	1	1	1	1	0	1
Li et al, 2012(121)	1	1	1	1	2	1	0	1
Lindberg et al, 2012(59)	1	1	1	1	2	1	1	1
Maiolino et al, 2008(122)	0	1	1	1	0	1	1	1
Menzaghi et al, 2014(123)	0	1	1	1	2	1	1	0
Morita et al, 2013(124)	1	1	1	1	2	1	1	0
Ohashi et al, 2010(125)	0	1	1	1	1	1	0	0
Oliveira et al, 2013(126)	0	1	1	1	1	0	0	1
Piestrzeniewicz et al, 2008(127)	0	1	1	1	0	0	0	0
Pilz et al, 2006(58)	0	1	1	1	2	0	1	1
Schnabel et al, 2008(61)	0	1	1	1	2	0	1	1
Shioji et al, 2007(128)	0	1	1	1	1	0	1	1
von Eynatten et al, 2008(130)	0	1	1	1	2	0	1	1
Wilson et al, 2011(60)	0	1	1	1	2	1	1	0

b. Case-Control Studies								
Arsenault et al, 2014(114)	1	1	0	0	1	1	1	0
Hascoet et al, 2013(116)	0	1	1	1	2	1	1	0
Soderberg et al, 2009(129)	0	1	0	0	2	1	1	0

Abbreviations: CAD, coronary artery disease.

About half of the studies used a continuous measure of adiponectin in their analysis (e.g. per unit, or per SD). Other studies used categorical adiponectin for analysis (e.g. comparing quantiles). Few studies used the bottom third versus the top third of adiponectin distribution. Tables 4.11, and 4.12 list the details of the conversion method applied in each study.

Table 4.11. Type of Analysis and Conversion Method in Studies of Subjects with Primary CAD

Source	Type of Analysis	SD in Study *	Estimated SD*	Conversion Method	x (for unequal group comparisons) **	Final Conversion factor
Ai et al, 2011(92)	Two unequal groups			2.18/1.70	1.7	1.28
Bidulescu et al, 2013(93)	Per SD log			2.18		2.18
Costacou et al, 2005(94)	Per SD log			2.18		2.18
Cote et al, 2011(95)	Per Unit	5.93		2.18*5.93		12.93
Dekker et al, 2008(96)	Per SD			2.18		2.18
Frystyk et al, 2007(97)	Per SD			2.18		2.18
Gardener et al, 2013(98)	Per Unit	6.20		2.18*6.20		13.52
Hatano et al, 2011(99)	Tertile (tertile 3 reference)			1.00		1.00
Kanaya et al, 2006(100)	Doubling (log2)†		0.70	2.18*4.80		1.53

Kappelle et al, 2012(101)	Per SD log			2.18		2.18
Khalili et al, 2010(102)	Quintile (Quintile 5 reference)			2.18/2.80		0.78
Kizer et al, 2008(103)	Quintile			2.18/2.80		0.78
Koenig et al, 2006(104)	Tertile			1.00		1.00
Laughlin et al, 2007(105)	Per SD log			2.18		2.18
Lawlor et al, 2005(55)	Quartile			2.18/2.54		0.86
Lindberg et al, 2013(106)	5 unit increase‡		4.00	(2.18*4.00)/ 5.00		1.74
Lindsay et al, 2005(54)	Per SD log			2.18		2.18
Luc et al, 2009(107)	Per SD log			2.18		2.18
Onat et al, 2013(108)	Tertile			1.00		1.00
Pischon et al, 2011(69)	Quintile			2.18/2.8.00		0.78
Pischon et al, 2004(53)	Quintile			2.18/2.8.00		0.78
Saito et al, 2013(109)	Quartile			2.18/2.54		0.86
Schottker et al, 2013(110)	Tertile			1.00		1.00
Wanname thee et al, 2011(111)	Tertile			1.00		1.00

Abbreviations: CAD coronary artery disease; SD, standard deviation.

* SD was only reported when analysis required using SD for transformation. If not available from a study it was estimated from larger studies

** x is a factor used when studies report two unequal groups, and it was derived from difference in means between the unequal groups. The differences were found by simulating one million observations from the distribution used to report the comparison (i.e., normal or log normal). For example, if the effect estimate was reported for the top three quartiles vs the bottom quartile, then multiply by 2.18/1.7
† Doubling of adiponectin means increase in log2 units of adiponectin. This was treated as unit increase, were the log effect estimate was multiplied by SD. The SD used here was derived from log adiponectin, which was 0.7 as estimated from larger studies that reported log adiponectin SD
‡ This was transformed by dividing log effect estimate by 5, to convert to per unit increase, and multiplying by SD*2.18 to convert to the top versus bottom thirds of serum adiponectin distribution
For all groups reference group was the lower category unless otherwise stated.

Table 4.12. Type of Analysis and Conversion Method in Studies of Subjects with Secondary CAD

Source	Type of Analysis	SD in Study *	Estimated SD*	Conversion Method	x (for unequal group comparisons) **	Final Conversion factor
Alkofide et al, 2015(112)	Tertile			1.00		1.00
Ang et a, 2009(113)	Per unit log	NA	0.70	2.18*SD		1.53
Arsenault et al, 2014(114)	Doubling (log 2)†	NA	0.70	2.18*SD		1.53
Beatty et al, 2012(115)	Quartile and Per SD log for composite outcome			2.18/2.54		0.86
Cavusoglu et al, 2006(57)	Per SD log	0.65		2.18		2.18
Hascoet et al, 2013(116)	Two unequal groups			2.18/x	1.70	1.28
Huang et al, 2010(117)	Two unequal groups			2.18/x	1.64	1.33
Hung et al, 2010(118)	Two equal groups			2.18/x	1.59	1.37

Kojima et al, 2007(119)	Per unit log	NA	0.70	2.18*SD		1.53
Lee et al, 2009(120)	Per tertile			2.00		2.00
Li et al, 2012(121)	Two unequal groups (higher group reference)			2.18/x	1.64	1.33
Lindberg et al, 2012(59)	Two unequal groups			2.18/x	1.70	1.28
Maiolino et al, 2008(122)	Two equal groups			2.18/x	1.59	1.37
Menzaghi et al, 2014(123)	Per SD			2.18		2.18
Morita et al, 2013(124)	Per unit log	NA	0.70	2.18*SD		1.53
Ohashi et al, 2010(125)	Per unit	4.6		2.18*SD		10.00
Oliveira et al, 2013(126)	Per unit	NA	4.00	2.18*SD		8.72
Piestrzeniewicz et al, 2008(127)	Per unit	NA	4.00	2.18*SD		8.72
Pilz et al, 2006(58)	Per SD			2.18		2.18
Schnabel et al, 2008(61)	Per unit	NA	4.00	2.18*SD		8.72
Shioji et al, 2007(128)	Two unequal groups (higher group reference)			2.18/x	1.70	1.28

Soderberg et al, 2009(129)	Quartile (quartile 4 reference)			2.18/2.54		0.86
von Eynatten et al, 2008(130)	Per unit	NA	4.00	2.18*SD		8.72
Wilson et al, 2011(60)	Two equal groups			2.18/x	1.59	1.37

Abbreviations: CAD, coronary artery disease; SD, standard deviation.

* SD was only reported when analysis required using SD for transformation. If not available from a study it was estimated from larger studies

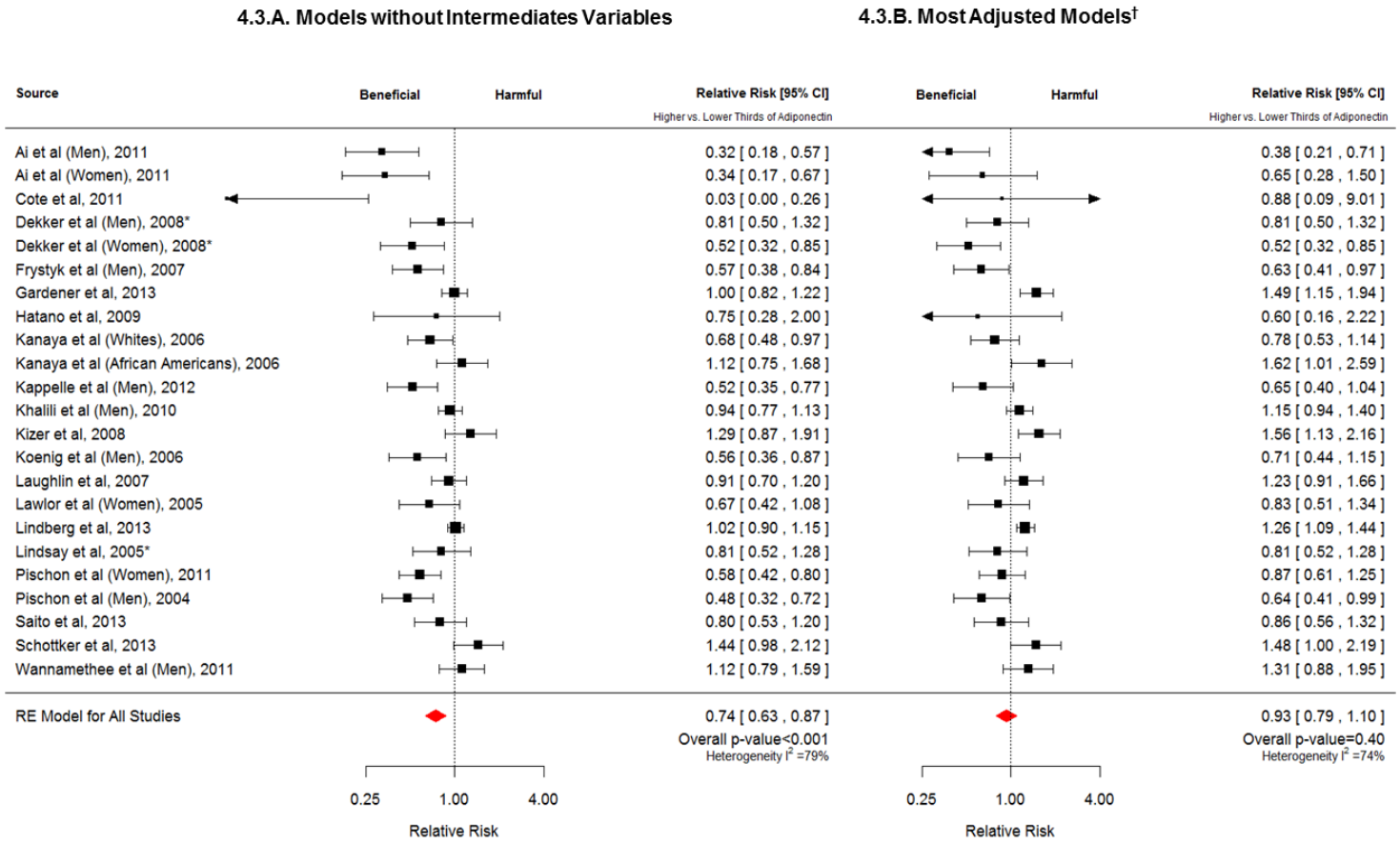
** x is a factor used when studies report two unequal groups, and it was derived from difference in means between the unequal groups. The differences were found by simulating one million observations from the distribution used to report the comparison (i.e., normal or log normal). For example, if the effect estimate was reported for the top three quartiles vs the bottom quartile, then multiply by 2.18/1.7

† Doubling of adiponectin means increase in log2 units of adiponectin. This was treated as unit increase, were the log effect estimate was multiplied by SD. The SD used here was derived from log adiponectin, which was 0.7 as estimated from larger studies that reported log adiponectin SD For all groups reference group was the lower category unless otherwise stated.

Adiponectin in Studies of Primary CAD Events

Of the 24 studies eligible for analysis of primary CAD events, 20 reported models without intermediate variables, two reported the data separately for men and women, and one stratified the analysis for African American versus white participants. The pooled RR for primary CAD events, using models without intermediate variables, was 0.73 (95% confidence interval [CI] 0.63–0.87; p<0.001) for the top versus bottom thirds of the serum adiponectin distribution (Figure 4.3.A), indicating a protective association between plasma adiponectin levels and primary CAD events. The I^2 was 79%, reflecting substantial between-study heterogeneity. When analyzing the most-adjusted models, the association between higher adiponectin levels and fewer primary CAD events was null with a pooled RR of 0.93 (95% CI 0.79–1.10; P=0.40; I^2=74%) for the top versus bottom thirds of the adiponectin distribution (Figure 4.3.B).

Figure 4.3. Association of Adiponectin Levels with Primary CAD Events



Abbreviations: CAD, coronary artery disease; CI, confidence interval; RE, random effect.

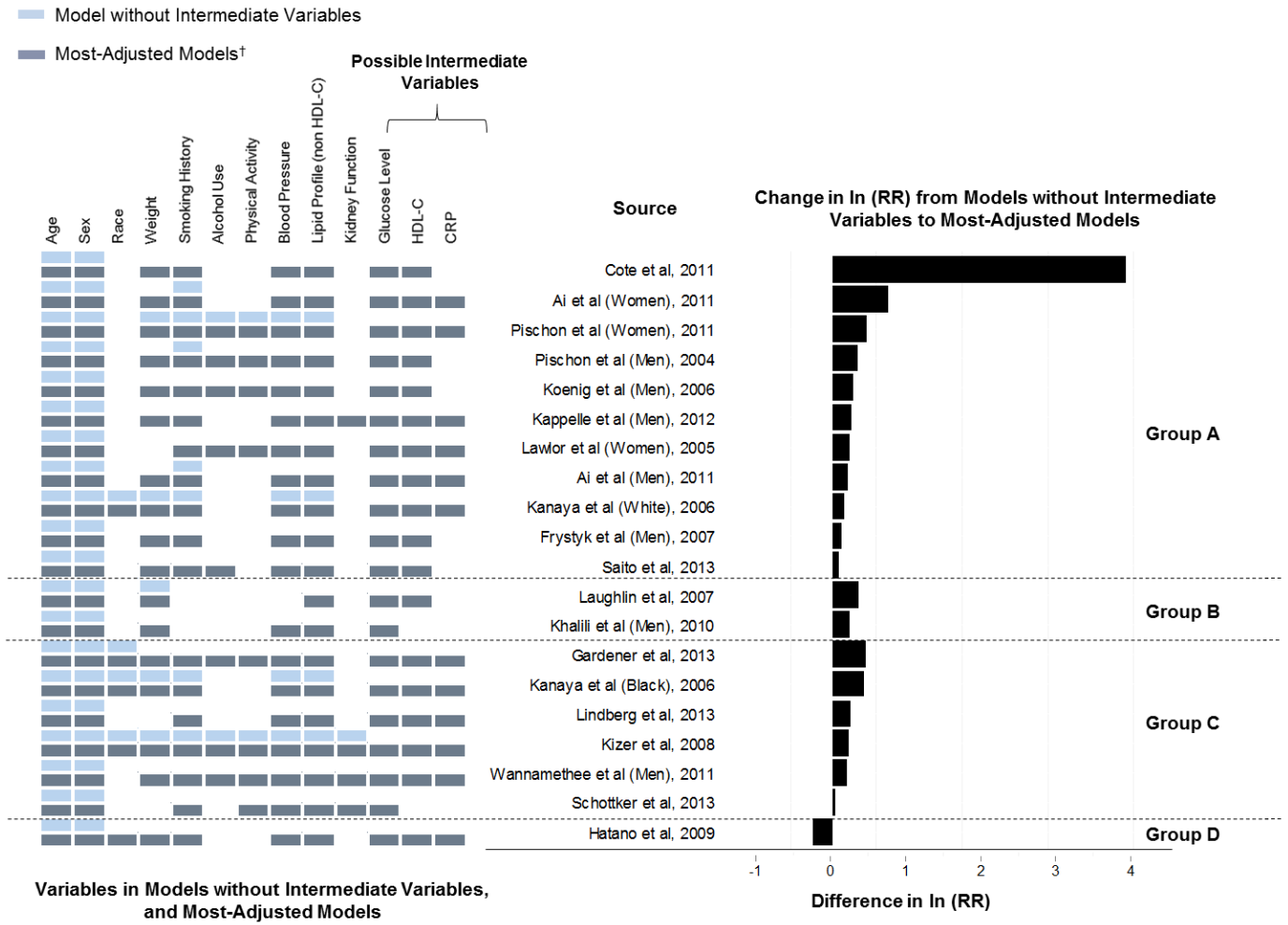
* Effect estimates were the same for models without intermediate variables and most-adjusted models.

† Most-adjusted models refers to models with intermediate variables.

Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in each meta-analysis.

The models without intermediate variables, all included age and sex as covariates, while nine studies adjusted for additional covariates. In all but one study, RRs were closer to one in most-adjusted models compared to models without intermediate variables. The difference in RR between models and the corresponding covariates in these models are presented in Figure 4.4.

Figure 4.4. Covariates in Models with and without Intermediate Variables of Studies with Primary CAD Events and the Corresponding Difference in RR



Difference in ln (RRs) in models without intermediate variables and most-adjusted models in each study, sorted from largest to smallest difference, and grouped depending on the shift in RR from models without intermediate variables to most-adjusted models, where A, B, C, D, and E corresponds to:

	RR in models without intermediate variables	RR in most-adjusted model
Group A	<1	increased and remained <1
Group B	<1	increased and shifted to >1
Group C	>1	increased and remained >1
Group D	<1	decreased and remained <1
Group E	>1	decreased and shifted to <1

Light boxes correspond to models without intermediate variables, while dark boxes correspond to most-adjusted models.
Covariates related to glucose level, HDL-c, or CRP were considered intermediate variables.
Abbreviations: HDL-c, high density lipoprotein cholesterol; CRP, C-reactive protein; RR, relative risk.
† Most-adjusted models refers to models with intermediate variables.

Notes:
A study was considered to be sex adjusted if sex was included in the model, stratified analysis by sex, or study was done in a sex-specific population.
Studies were the same effect estimate was used for models without intermediate variables and most-adjusted models were not included in this figure.
Adjustment for weight included any of the following: body mass index, visceral fat, actual body weight, abdominal girth.
Adjustment for blood pressure included any of the following: systolic blood pressure, diastolic blood pressure, history of hypertension, and the use of antihypertensive medications.
Adjustment for kidney function included any parameter that would reflect kidney disease (e.g. serum creatinine).
Adjustment for glucose level included any covariates such as history of diabetes, insulin resistance, and diabetes medication, which might influence glucose level.

For both models with and without intermediate variables, the pooled RR estimates for CAD events did not differ significantly by any of the study level characteristics tested including race/ethnicity, sex, population source, study type, number of subjects included, follow-up duration, type of assay used, whether the composite outcome contained stroke, and quality indicators (Table 4.13).

Table 4.13. Stratified Analysis on the Association of Adiponectin and Primary CAD Events

Factors Related to Study Characteristics					
		Models without Intermediate Variables		**Most-Adjusted Models**	
Variable	**N**	**RR (95% CI)**	**p-value (interaction)**	**RR (95% CI)**	**p-value (interaction)**
Sex*					
Men	10	1.05 (0.67-1.62)	0.84	0.91 (0.59-1.40)	0.66
Women	6	0.95 (0.62-1.48)		1.10 (0.71-1.70)	
Race*					
White	11	1.30 (0.88-1.93)	0.19	1.03 (0.68-1.54)	0.89
Non-White	4	0.77 (0.52-1.14)		0.97 (0.65-1.46)	
Follow-up					
< Median (9 years)	12	0.87 (0.63-1.21)	0.42	0.96 (0.69-1.33)	0.27
≥ Median (9 years)	11	1.15 (0.82-1.59)		1.04 (0.75-1.45)	
Study Type					
Prospective Cohort	15	1.17 (0.82-1.66)	0.38	1.12 (0.79-1.60)	0.64
Case-Control	8	0.85 (0.60-1.22)		0.89 (0.62-1.27)	
Population Based					
Yes	21	0.67 (0.39-1.15)	0.15	0.80 (0.46-1.40)	0.44
No	2	1.50 (0.87-2.60)		1.25 (0.71-2.18)	

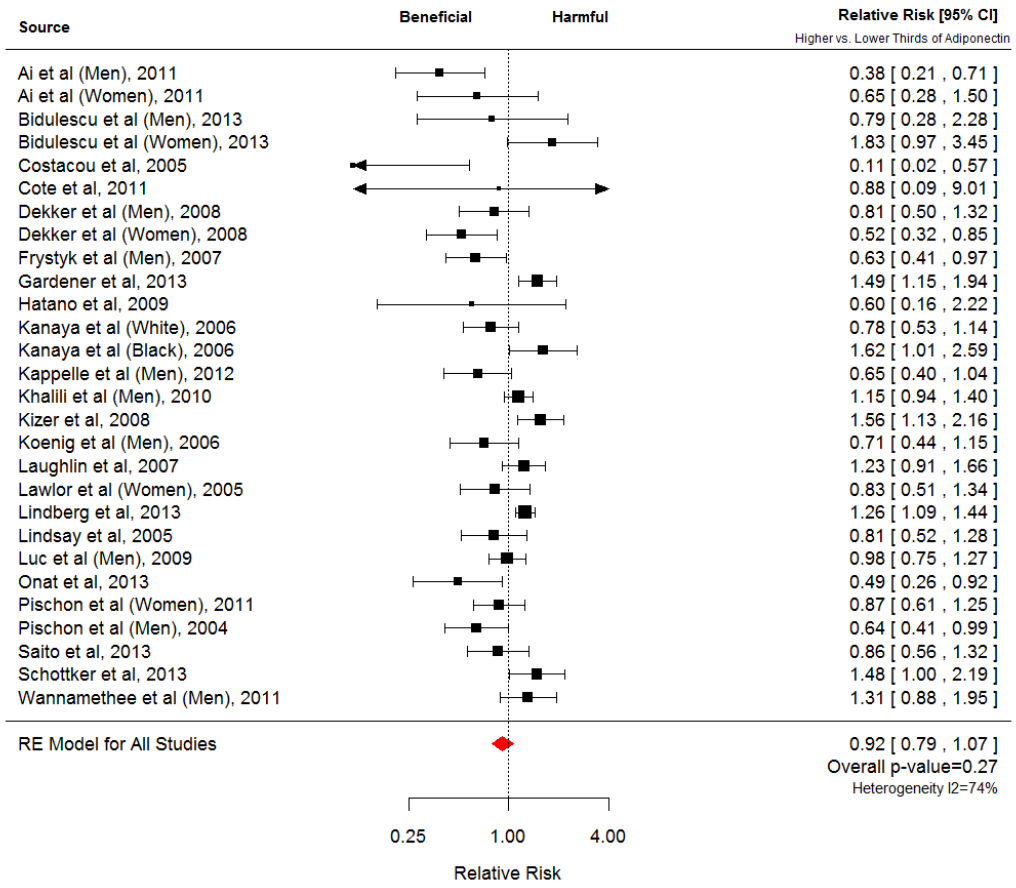
Type of Assay Used					
RIA	12	0.87 (0.63-1.21)	0.84	0.82 (0.60-1.14)	0.24
ELISA	11	1.15 (0.83-1.60)		1.21 (0.88-1.67)	
Number of Patients					
< 500	4	0.87 (0.56-1.37)	0.56	0.78 (0.50-1.46)	0.29
≥ 500	19	1.14 (0.73-1.80)		1.23 (0.81-2.01)	
Stroke in Composite Outcome					
Yes	6	1.33 (0.95-1.87)	0.09	1.18 (0.83-1.68)	0.35
No	17	0.75 (0.53-1.05)		0.85 (0.60-1.20)	
Definition of Outcome					
Clear	14	0.95 (0.68-1.33)	0.28	0.95 (0.68-1.32)	0.74
No/Unclear	9	1.05 (0.75-1.47)		1.06 (0.76-1.47)	
Model Build-up					
Clear	18	1.29 (0.87-1.91)	0.20	1.30 (0.88-1.92)	0.19
No/Unclear	5	0.78 (0.52-1.15)		0.77 (0.52-1.14)	

Abbreviations: CAD, coronary artery disease events; CI, confidence interval; ELISA, Enzyme Linked Immunosorbent Assay; RIA, Radioimmunoassay; RR, relative risk.
*Done on studies that either provided stratified analysis based on sex or race, or sex/ race specific studies.

Sensitivity Analyses

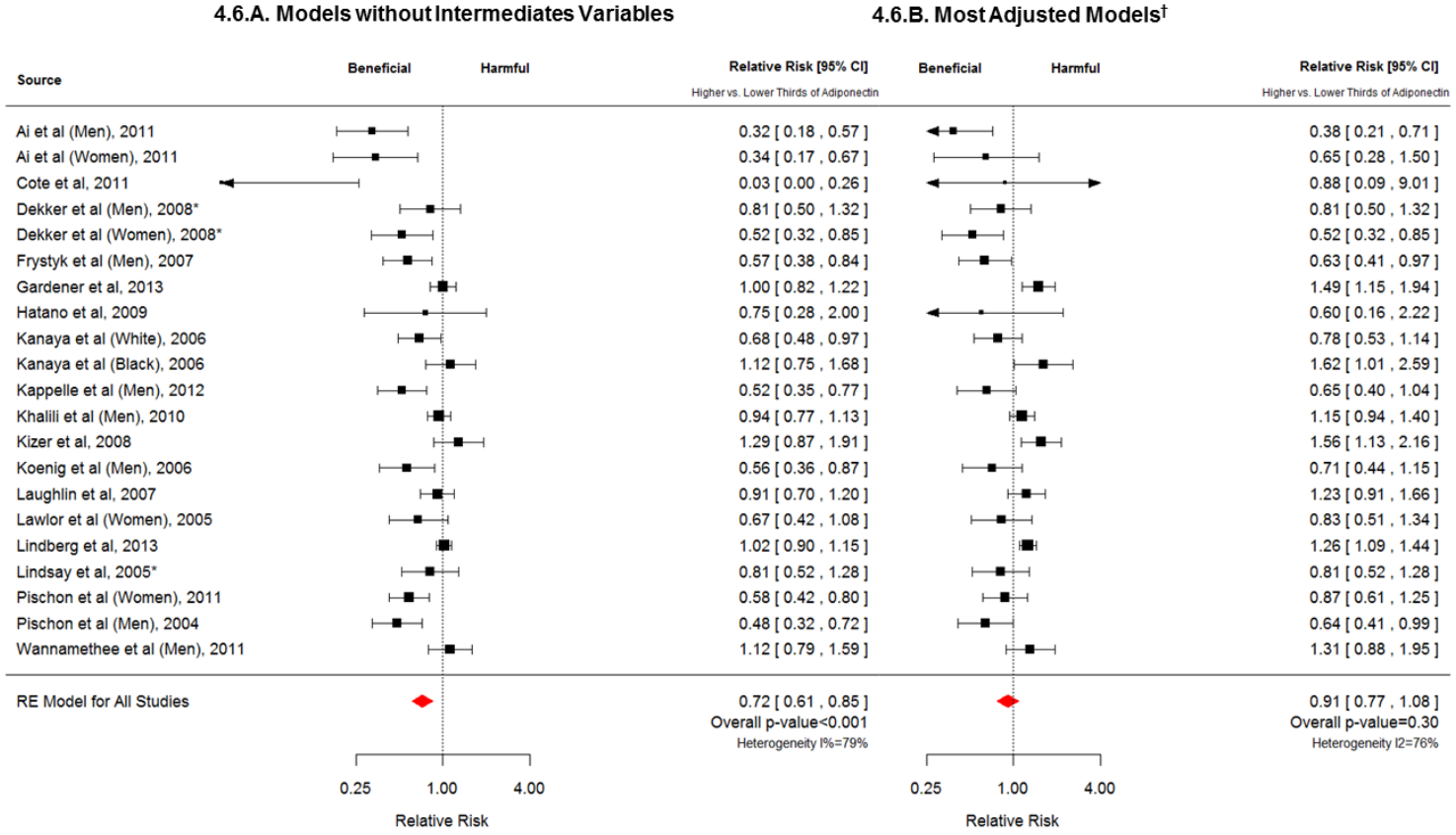
After including all eligible studies (i.e., including those that did not report models without intermediate variables), N=24, the pooled RR for primary CAD events was 0.92 (95% CI 0.79–1.07; P=0.27; I^2=74%) using the most-adjusted models in each study (Figure 4.5). When excluding studies that recruited subjects based on a history of diabetes (N=2), the results did not differ substantially for models without intermediate variables (RR 0.72; 95% CI 0.61-0.85; p<0.001; I^2=79%) versus most-adjusted models (RR 0.91; 95% CI 0.77-1.08; P=0.30, I^2=76%) (Figure 4.6).

Figure 4.5. Association of Adiponectin Levels with Primary CAD Events using Most-Adjusted Models in All Studies Including Studies Only Reporting Models Incorporating Intermediate Variables



Abbreviations: CAD, coronary artery disease; CI, confidence interval; RE, random effect.
Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in the overall meta-analysis.

Figure 4.6. Effect of Adiponectin Levels on CAD Incidence in Studies of Subjects with Primary CAD Events, Excluding Studies on Exclusively Diabetic Population



Abbreviations: CAD, coronary artery disease; CI, confidence interval; RE, random effect.

* Effect estimates were the same for models without intermediate variables and most-adjusted models.

† Most-adjusted models refers to models with intermediate variables.

Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in each meta-analysis.

Excluding studies that were of low quality on the Newcastle-Ottawa scale, and those labeled with an “unclear” definition for the primary outcome or model development (N=12), yielded similar results. When we included only studies reporting effect estimates per unit/SD increase in adiponectin (N=10), the results did not change appreciably (Table 4.14). Further sensitivity analysis using the “leave-one-out” method did not indicate that any individual study had a major impact on the pooled RR in either model.

Table 4.14. Sensitivity Analyses on the Association of Adiponectin and Primary CAD Events

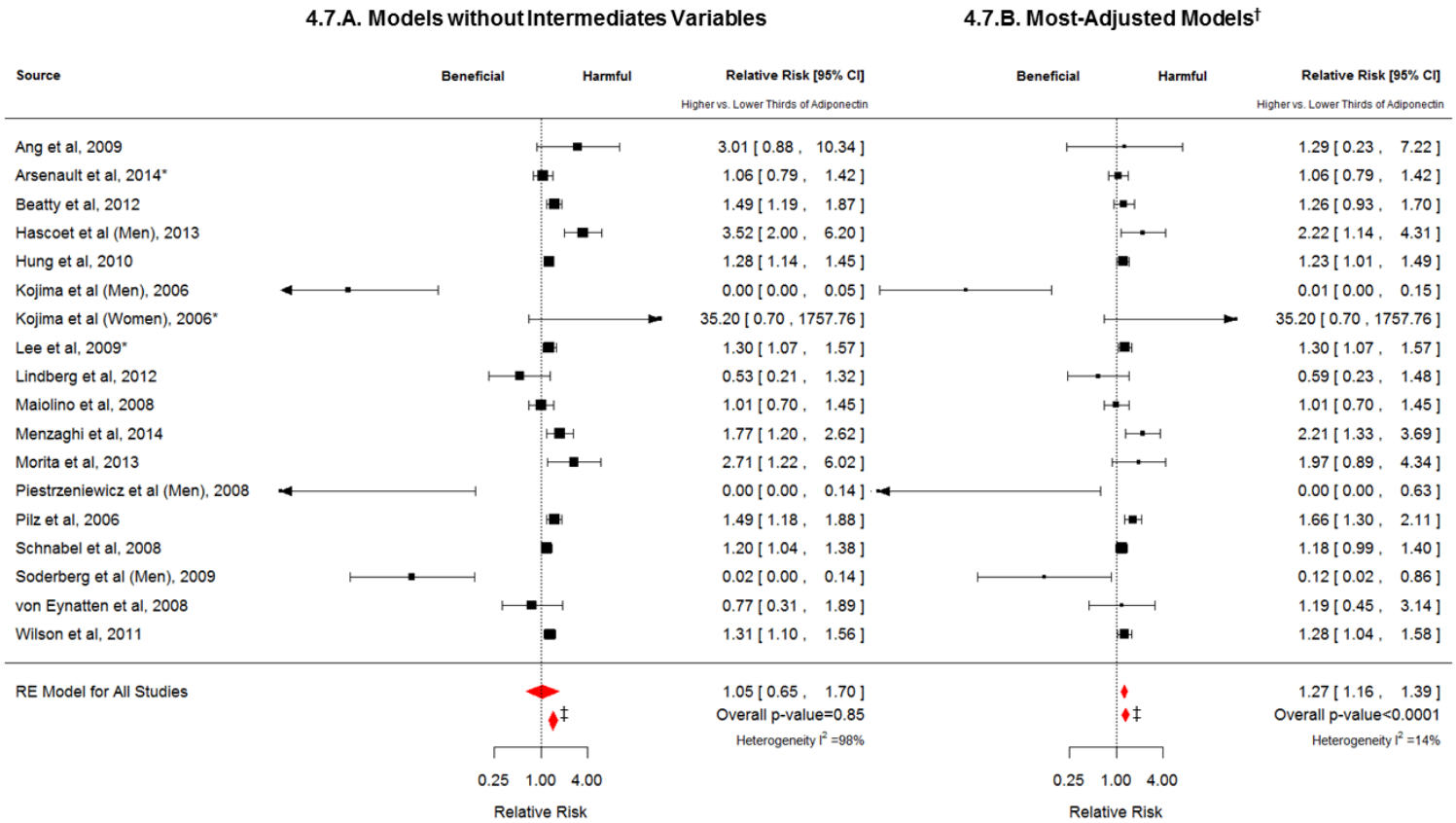
		Models without Intermediate Variables			Most-Adjusted Models		
Sensitivity Analyses	**No.**	**RR (95% CI)**	**p-value**	**I^2%**	**RR (95% CI)**	**p-value**	**I^2%**
Methodological Quality							
Clear Definition of Outcome	13	0.73 (0.58-0.91)	0.005	75	0.91 (0.73-1.13)	0.39	70
Clear Description of Model build-up	16	0.79 (0.67-0.93)	0.006	71	0.99 (0.84-1.17)	0.91	68
0 points in ≤3 elements in quality scale	19	0.74 (0.62-0.88)	<0.001	81	0.94 (0.79-1.11)	0.45	76
All of the above	12	0.74 (0.58-0.94)	0.01	76	0.99 (0.84-1.17)	0.91	68
Type of Analysis							
Per Unit/Per SD Increase*	10	0.89 (0.82-0.97)	0.008	70	0.97 (0.87-1.09)	0.63	78

Abbreviations: CAD, coronary artery disease; SD, standard deviation
*Per unit effect estimates were converted to per SD by multiplying with the SD in the reported studies, or estimated from larger studies.

Adiponectin in Studies of Secondary CAD Events

Of the 24 studies eligible for analysis of secondary CAD events, 17 reported both models without intermediate variables and most-adjusted models, with one reporting the analysis stratified by sex. The pooled RR for secondary CAD events was 1.05 (95% CI, 0.65–1.70; P=0.85) using models without intermediate variables for the top versus bottom thirds of adiponectin distribution (Figure 4.7.A), indicating no association between serum adiponectin and secondary CAD events. The I^2 was 98%, indicating high between study heterogeneity. For the most-adjusted models, the pooled RR for secondary CAD events was 1.27 (95% CI 1.16–1.39; p<0.001) for the top versus bottom thirds of the serum adiponectin distribution, with low between study heterogeneity (I^2=14%) (Figure 4.7.B), indicating a harmful association.

Figure 4.7. Association of Adiponectin Levels with Secondary CAD Events



Abbreviations: CAD, coronary artery disease; CI, confidence interval; RE, random effect.

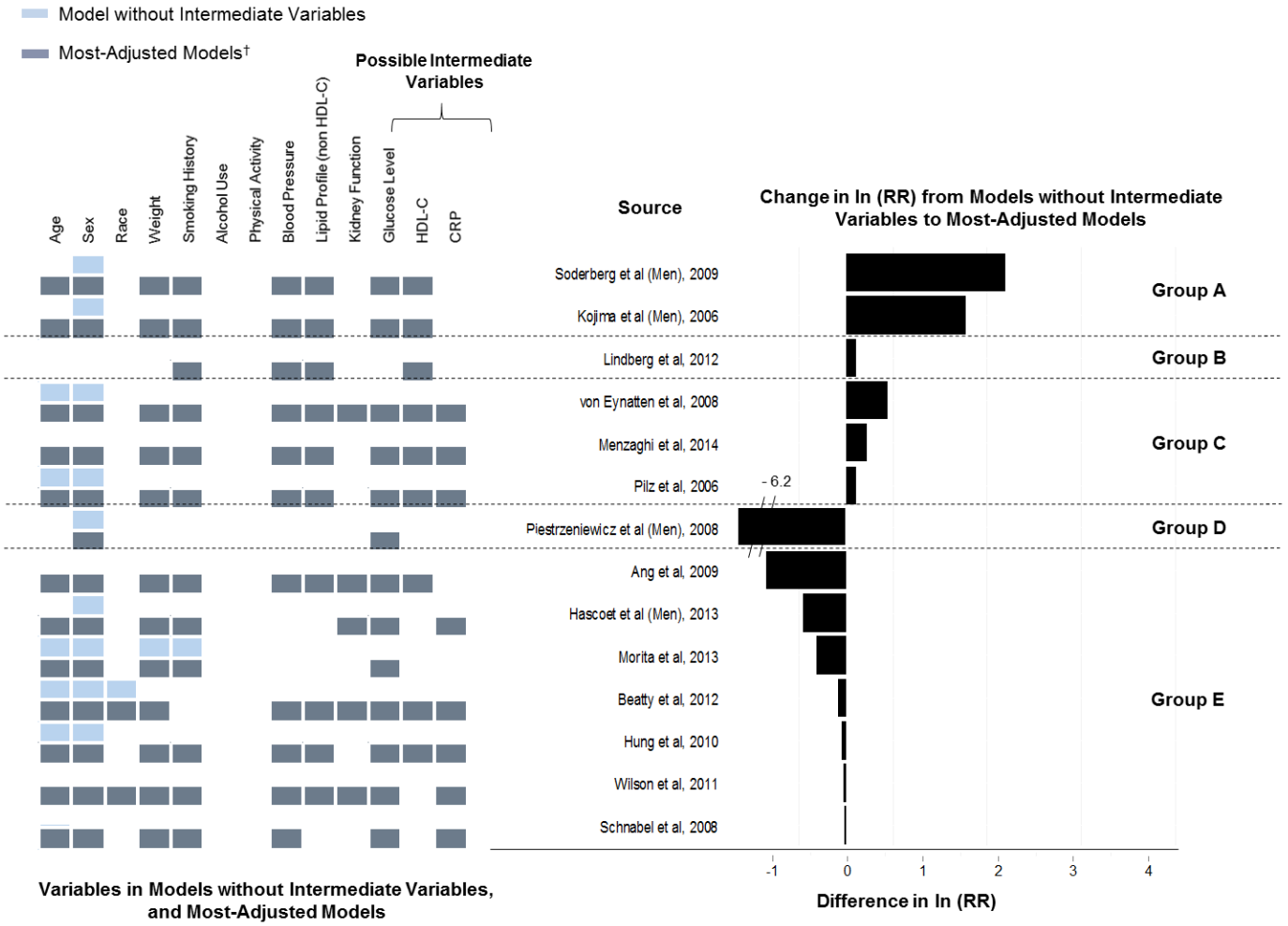
* Effect estimates were the same for models without intermediate variables and most-adjusted models.

† Most-adjusted models refers to models with intermediate variables.

‡ Results of post-hoc analysis after removing three outlier studies from the analysis. RR for models without intermediate variables=1.33 (95% CI 1.22-1.45) and I^2=29%. RR for most-adjusted models=1.28 (95% CI 1.17-1.41) and I^2=16%. For both models, p-value<0.001. Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in each meta-analysis.

Some of the models without intermediate variables in the included studies did not adjust for any confounders (i.e. they were unadjusted). However, we included them in our analysis, to allow for comparison with most-adjusted models from the same studies. Figure 4.8 shows the difference between the RR based on the inclusion of intermediate variables. In six studies, the RR increased in models without intermediate variables versus models with them, while it decreased in other studies. None of the studies adjusted for alcohol use or physical activity.

Figure 4.8. Covariates in Models with and without Intermediate Variables of Studies with Secondary CAD Events and the Corresponding Difference in RR



Difference in ln (RRs) in models without intermediate variables and most-adjusted models in each study, sorted from largest to smallest difference, and grouped depending on the shift in RR from models without intermediate variables to most-adjusted models, where A, B, C, D, and E corresponds to:

	RR in models without intermediate variables	RR in most-adjusted model
Group A	<1	increased and remained <1
Group B	<1	increased and shifted to >1
Group C	>1	increased and remained >1
Group D	<1	decreased and remained <1
Group E	>1	decreased and shifted to <1

Light boxes correspond to models without intermediate variables, while dark boxes correspond to most-adjusted models.
Covariates related to glucose level, HDL-c, or CRP were considered intermediate variables.
Abbreviations: HDL-c, high density lipoprotein cholesterol; CRP, C-reactive protein; RR, relative risk

Notes:
A study was considered to be sex adjusted if sex was included in the model, stratified analysis by sex, or study was done in a sex-specific population.
Studies were the same effect estimate was used for models without intermediate variables and most-adjusted models were not included in this figure.
Adjustment for weight included any of the following: body mass index, visceral fat, actual body weight, abdominal girth.
Adjustment for blood pressure included any of the following: systolic blood pressure, diastolic blood pressure, history of hypertension, and the use of antihypertensive medications.
Adjustment for kidney function included any parameter that would reflect kidney disease (e.g. serum creatinine).
Adjustment for glucose level included any covariates such as history of diabetes, insulin resistance, and diabetes medication, which might influence glucose level.

For both models, pooled RR estimates for CAD events did not differ significantly by any of the study level characteristics tested including race/ethnicity, sex, population source, study type, number of subjects included, follow-up duration, whether the composite outcome contained stroke, and quality indicators (Table 4.15).

Table 4.15. Stratified Analysis on the Association of Adiponectin and Secondary CAD Events

Factors Related to Study Characteristics					
		Models without Intermediate Variables		**Most-Adjusted Models**	
Variable	**N**	**RR (95% CI)**	**p-value (interaction)**	**RR (95% CI)**	**p-value (interaction)**
Sex*					
Men	5	0.02 (5.05-6.08)	0.18	0.47 (0.04-5.51)	0.24
Women	2	57.04 (0.16-19799.26)		16.40 (0.16-1735.93)	
Race*					
White	12	4.31 (0.07-254)	0.48	5.00 (0.32-78.54)	0.25
Non-White	4	0.23 (0.004-13.67)		0.20 (0.01-3.15)	
Follow-up					
< Median (4 years)	13	0.24 (0.002-27.39)	0.55	1.08 (0.31-3.74)	0.90
≥ Median (4 years)	11	4.22 (0.04-488.33)		0.93 (0.28-3.22)	
Study Type					
Prospective Cohort	14	1.29 (0.03-51.11)	0.89	1.11 (0.44-2.78)	0.82
Case-Control	3	0.78 (0.02-30.94)		0.90 (0.36-2.26)	
Type of Assay Used					
RIA	3	0.39 (0.01-10.99)	0.58	0.57 (0.25-1.35)	0.20
ELISA	14	2.53 (0.09-70.64)		1.74 (0.74-4.09)	
Number of Patients					
< 500	10	0.16 (0.005-4.92)	0.29	0.45 (0.16-1.25)	0.12

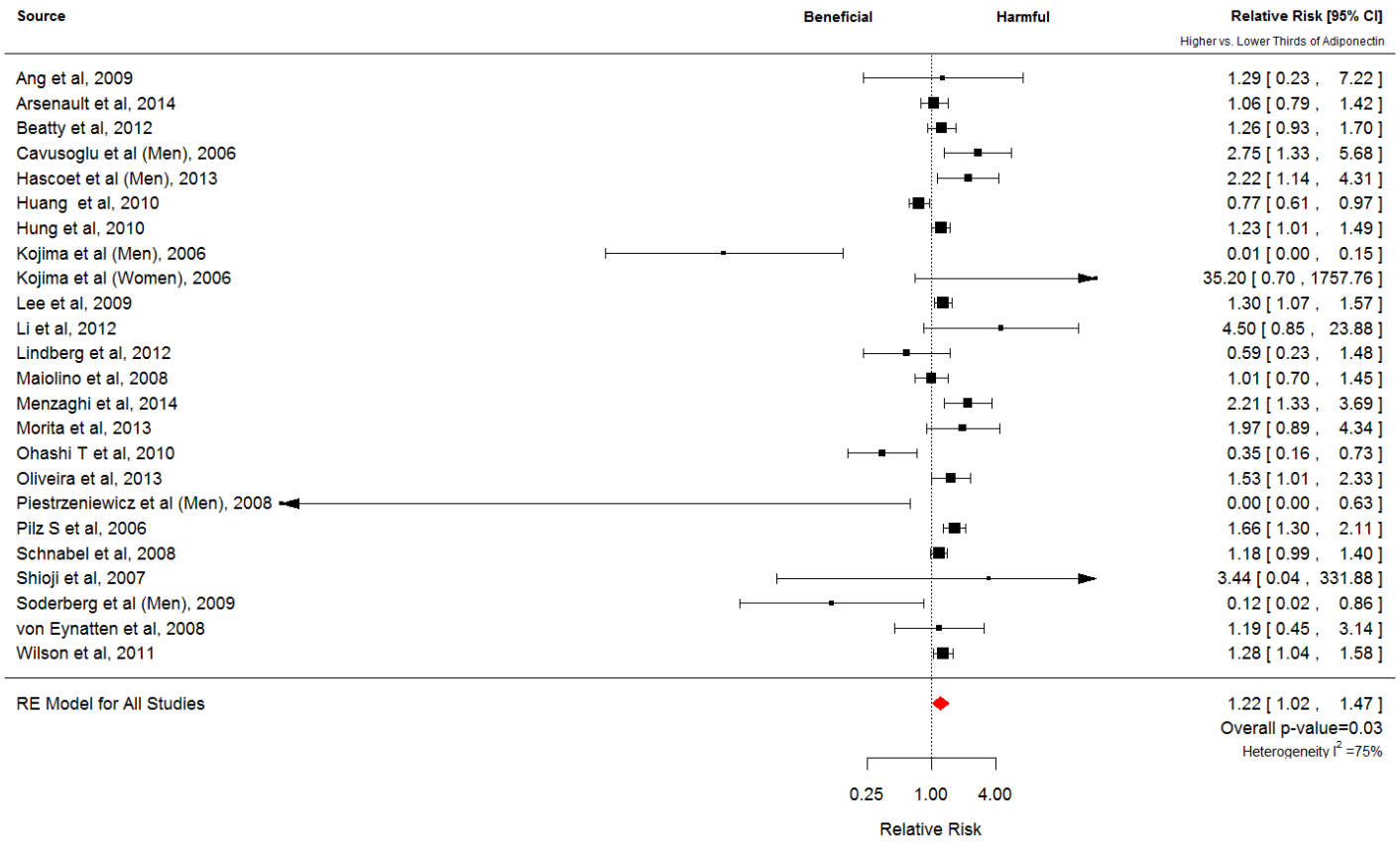
≥ 500	7	6.45 (0.20-205.22)		2.24 (0.80-6.24)	
Stroke in Composite Outcome					
Yes	7	0.67 (0.07-6.89)	0.74	0.66 (0.35-1.27)	0.22
No	10	1.48 (0.15-15.18)		1.51 (0.79-2.88)	
Acute versus Chronic CAD					
Acute	8	2.34 (0.03-176.71)	0.70	0.54 (1.67-1.74)	0.30
Chronic	9	0.43 (0.006-32.18)		1.86 (0.58-6.01)	
Definition of Outcome					
Clear	3	0.68 (0.03-13.73)	0.79	0.72 (0.35-1.48)	0.38
No/Unclear	14	1.50 (0.07-30.85)		1.38 (0.68-2.82)	
Model Build-up					
Clear	9	0.28 (0.005-17.64)	0.55	0.51 (0.19-1.40)	0.19
No/Unclear	8	3.54 (0.06-220.72)		1.96 (0.71-5.38)	

Abbreviations: CAD, coronary artery disease events; CI, confidence interval; ELISA, Enzyme Linked Immunosorbent Assay; RIA, Radioimmunoassay; RR, relative risk.
* Done on studies that either provided stratified analysis based on sex or race, or sex/ race specific studies.
All studies were non-population based, therefore no stratified analysis was done by type of population.

Sensitivity Analyses

We performed a post-hoc sensitivity analysis by removing three outlier studies from the association between adiponectin and secondary CAD events. These three studies were also deemed to be of low quality. Results of models without intermediate variables, and most-adjusted models yielded RR=1.31 (95% CI 1.22-1.45), and 1.28 (95% CI 1.17-1.41) respectively. The heterogeneity was low for both models when these three studies were excluded. When including all studies (N=23), even those only reporting models which incorporating intermediate variables, the pooled RR was 1.22 (95% CI 1.02–1.47; P=0.03, I^2=75%) (Figure 4.9). Following the addition of studies that did not report any adjusted analysis (N=1), the results remained the same (Figure 4.10).

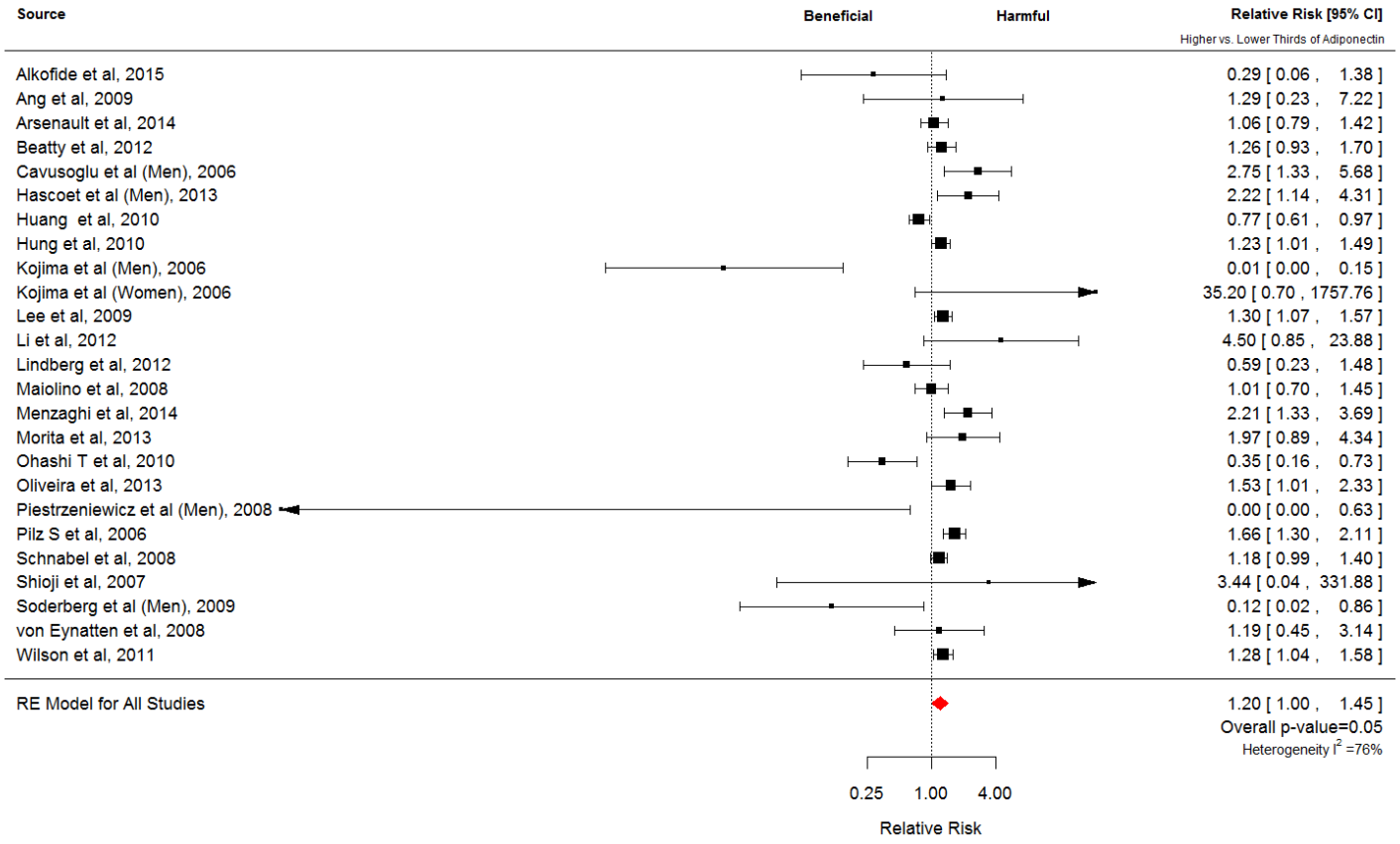
Figure 4.9. Association of Adiponectin levels with Secondary CAD Events using Most-Adjusted Models in All Studies, Including Studies Only Reporting Models Incorporating Intermediate Variables



Abbreviations: CAD, coronary artery disease; CI, confidence interval; RE, random effect.

Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in the overall meta-analysis.

Figure 4.10. Associations of Adiponectin Levels with Secondary CAD Events using Most-Adjusted Models in All Studies, Including Studies Only Reporting Models Incorporating Intermediate Variables and Unadjusted Models



Abbreviations: CAD, coronary artery disease; CI, confidence interval; RE, random effect.
Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in the overall meta-analysis.

After including high quality studies on Newcastle-Ottawa Scale (N=13), the RR was 1.35 (95% CI 1.23-1.48) for models without intermediate variables, and 1.35 (95% CI 1.23-1.48) for most-adjusted models. Limiting the analysis to studies reporting effect estimates per unit/SD increase in adiponectin (N=10) yielded similar results (Table 4.16).

Table 4.16. Sensitivity Analyses on the Association of Adiponectin and Secondary CAD Events

		Models without Intermediate Variables			Most-Adjusted Models		
Sensitivity Analyses	**No.**	**RR (95% CI)**	**p-value**	**I^2%**	**RR (95% CI)**	**p-value**	**I^2%**
Methodological Quality							
Clear Definition of Outcome	14	0.81 (0.35-1.89)	0.63	99	**1.25 (1.10-1.43)**	**<0.001**	**25**
Clear Description of Model build-up	9	1.01 (0.56-1.81)	0.98	97	**1.27 (1.10-1.46)**	**0.001**	**34**
"0" points in ≤3 elements in quality scale	13	**1.35 (1.23-1.48)**	**<0.0001**	**30**	**1.31 (1.19-1.43)**	**<0.0001**	**16**
All of the above	6	1.38 (0.91-2.08)	0.13	91	**1.36 (1.12-1.65)**	**0.002**	**36**
Type of Analysis							
Per Unit/Per SD Increase*	10	**1.15 (1.07-1.24)**	**<0.001**	**40**	**1.14 (1.05-1.25)**	**0.004**	**44**

Abbreviations: CAD, coronary artery disease; SD, standard deviation
*Per unit effect estimates were converted to per SD by multiplying with the SD in the reported studies, or estimated from larger studies.

Adding studies of subjects with carotid artery stenosis, PAD, other CVD, and mixed populations (N=14), results did not change results substantially (Tables 4.17, 4.18, and Figures 4.11, 4.12); however, the pooled RR for most-adjusted models lost statistical significance. Further sensitivity analysis using the "leave-one-out" method did not indicate that any particular study had a major impact on the pooled RR, for models without intermediate variables and most-adjusted models.

Table 4.17. Characteristics of Studies of Subjects with Carotid Artery Stenosis, PAD, and CVD

Source	Study Design	Patient Population	No.	Age, years Mean (SD)	Follow-Up, years	Composite Outcome, Models without Intermediate Variables	Composite Outcome, Most-Adjusted Models	Type of Analysis
Dekker et al, 2008[a(96)]	Prospective cohort	Subjects aged 50-75 years with history of CVD	417	50–75 (range)	15.0	No composite outcome. CVD mortality HR 1.27 95% CI (0.98 – 1.63); all-cause mortality HR 1.29 95% CI (1.07–1.55); both adjusted for age and sex	Did not provide most-adjusted, therefore models without intermediate variables were used in analysis	Per SD change
Dieplinger et al, 2010(131)	Prospective cohort	Subjects with shortness of breath	251	Survivors 71 (61–79), decedents 78 (72–83); [median (range)];	1.0	OR 1.9 95% CI (1.5–2.5); unadjusted	Did not provide most-adjusted, therefore models without intermediate variables were used in analysis	Per SD change
Hajer et al, 2007(132)	Nested case-control	Subjects with vascular disease	431	Cases 65 (10), controls 59 (10)	2.3	HR 0.50 95% CI (0.25-0.99); adjusted for age, sex, and renal function	HR 0.48 95% CI (0.23-1.00); adjusted for age, sex, renal function, BMI, ACEIs, and hsCRP	Quartiles (Quartile 4 reference)

Kizer et al, 2012(133)	Prospective cohort	Subjects with CVD, no heart failure	1030	75.6 (5.3)	11.8	No composite outcome. CVD mortality HR 1.00 95% CI (0.88–1.14); all-cause mortality HR 1.03 95% CI (0.95–1.13); both adjusted for age, sex, race, center, BMI, smoking, alcohol, SBP, B-blockers, ACEIs, self-reported health status, and GFR	No composite outcome. CVD mortality HR 1.12 95% CI (0.95–1.32); all-cause mortality HR 1.08 95% CI (0.97–1.21); both adjusted for age, sex, race, BMI, smoking, alcohol, SBP, B-blockers, ACEI, self-reported health status, GFR, LDL-c, HDL-c, CRP	Per SD change
Persson et al, 2013(134)	Prospective cohort	Subjects with carotid stenosis undergoing carotid endarterectomy	292	Men 69.9 (8.6), women 69.3 (9.0)	5.2	HR 0.89 95% CI (0.68-1.18); adjusted for age	HR 1.00 95% CI (0.73-1.36); adjusted for age, BMI, IL-6, diabetes, and CRP	Per SD change
Urbonaviciene et al, 2010(135)	Prospective cohort	Subjects with intermittent claudication or chronic critical lower-extremity ischemia	468	65.7 (9.6)	3.5	HR 0.83 95% CI (0.65-1.07); unadjusted	HR 0.73, 95% CI 0.54–0.98); adjusted for age, sex, BMI, systemic hypertension, smoking, previous MI, ABI, leg ischemia, total cholesterol, B-blockers, ACEIs, and diabetes	Quartiles
Wannamethee et al, 2007(136)	Prospective cohort	Subjects with CVD no heart failure	830	Tertile (1) 68.8, Tertile (2) 70.1, Tertile (3) 71.0	6	No composite outcome. CVD mortality HR 1.26 95% CI (0.81-1.96); all-cause mortality HR 1.12 95% CI (0.81-1.55); both adjusted for age and sex	No composite outcome. CVD mortality HR 1.49 95% CI (0.90-2.47); all-cause mortality HR 1.19 95% CI (0.82-1.73); both adjusted for age, sex, BMI, social	Tertiles

							class, smoking, physical activity, alcohol, LVH, hypertension, b-blockers, statins, FEV1, albumin, weight loss, GFR, HDL-c, diabetes, HOMA-IR, CRP	

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors, ACS, acute coronary syndrome; ARB, angiotensin receptor blockers; BMI, body mass index, BNP, brain natriuretic peptide; CAD, coronary artery disease; CKD, chronic kidney disease; CI, confidence interval; CRCL, creatinine clearance; CRP, C-reactive protein; CVD, cardiovascular disease; GFR, glomerular filtration rate; ESRD, end stage renal disease; FEV1, forces expiratory volume in one second; HbA1c, glycosylated hemoglobin; HD, hemodialysis; HDL-c, high density lipoprotein cholesterol, HOMA-IR, homeostatic model assessment- insulin resistance; HR, hazard ratio; hs-CRP, high sensitivity C-reactive protein; IL, interleukin; LDL-C, low density lipoprotein cholesterol; LVH, left ventricular hypertrophy; NR, not reported; NT-proBNP, N-terminal pro-brain natriuretic peptide; NSTEMI, non-ST elevation myocardial infarction; SD, standard deviation; SBP, systolic blood pressure; SrCr, serum creatinine; STEMI, ST-elevation myocardial infarction.

Table 4.18. Characteristics of Studies with Mixed Population

Source	Study Design	Patient Population	No.	Age, years Mean (SD)	Composite Outcome, Models without Intermediate Variables	Composite Outcome, Most-Adjusted Models	Type of Analysis	Comments
Abdallah et al, 2012(137)	Prospective cohort	Subjects with ESRD on HD	133	54.6 (17.3)	HR 0.98 95% CI (0.95-0.99)	HR 1.23 95% CI (1.11-1.3); adjusted for age, sex, smoking, LV function, previous CVD, duration of HD, CRP	Not clear	Not included in sensitivity analysis/ unclear analysis
Diez et al, 2009(138)	Retrospective cohort	Uremic subjects undergoing dialysis therapy	184	PD 52.1 (16.9); HD 67.8 (11.7)	NR	HR 0.75 95% CI (0.55-1.02); adjusted for sex, age, dialysis, smoking, CVD, blood pressure, statins, renal function (PD patients), lipids, and diabetes	Per SD change	Included in sensitivity analysis in studies of subjects with secondary CAD events
Drechsler et l, 2009(139)	Prospective cohort	Type 2 diabetic HD subjects	1255	65.7 (8.3)	HR 1.26 95% CI (1.05-1.51)	HR 1.03 95% CI (0.85-1.25), adjusted for age, sex, BMI, smoking, atorvastatin, CAD, heart failure, SBP, NT-pro-BNP, diabetes duration	Per unit increase in log	Included in sensitivity analysis in studies of subjects with secondary CAD events
Forsblom et al, 2011(140)	Prospective cohort	Subjects with type 1 diabetes	2034	39 (median)	NR	No composite. CVD mortality, HR 1.02 95% CI (1.00–1.04); adjusted for age, CKD, CVD, triglycerides, diabetes, and glucose	Not clear	Not included in sensitivity analysis/ unclear analysis
Iwashima et al, 2006(141)	Prospective cohort	Severe chronic kidney disease subjects	150	67.7 (0.80)	HR 0.84 95% CI (0.72–0.94), adjusted for CKD stage	HR 0.86 95% CI (0.75–0.96), adjusted for CAD, smoking, and CKD stages	Per unit increase	Included in sensitivity analysis in studies of

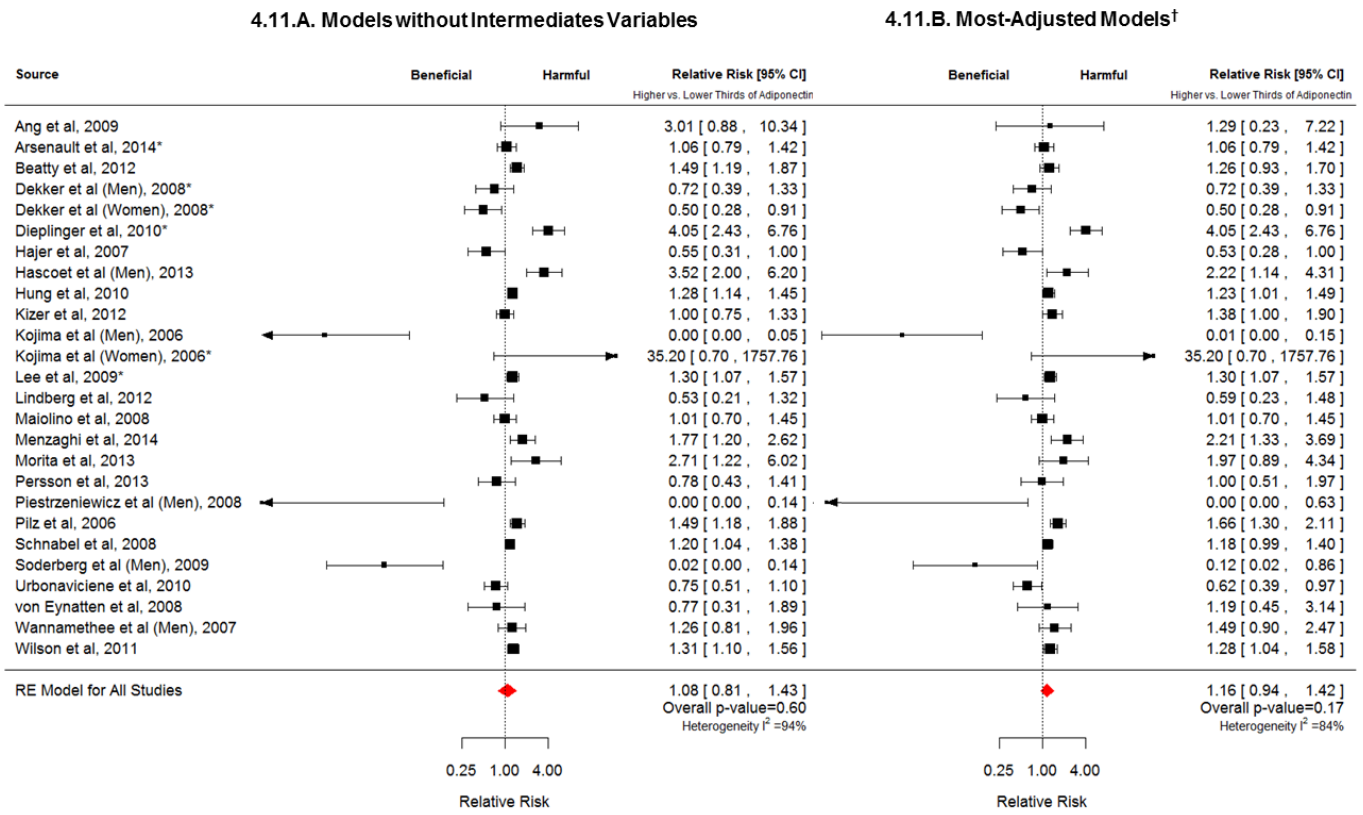
								subjects with secondary CAD events
Lim et al, 2008(142)	Prospective cohort	Subjects with type 2 diabetes	343	65.0 (9.2)	NR	HR 3.03 95% CI (1.09-8.41), adjusted for age, sex, BMI, smoking, SrCr, ECG abnormality, CVD, blood pressure, lipids, diabetes, and HbA1C	Quartiles	Not included in sensitivity analysis/ no data on models without intermediate variables
Mikkelse-n et al, 2010(143)	Prospective cohort	Subjects undergoing elective cardiac surgery	836	68 (59-750, median (IQR)	OR 2.00 95% CI (1.20-3.50)	OR 1.70 95% CI (0.90-3.10), adjusted for logistic EuroSCORE, Charlson Comorbidity Index and type of surgery	Two unequal groups (quartile)	Included in sensitivity analysis in studies of subjects with secondary CAD events
Nishida et al, 2007(144)	Prospective cohort	Subjects with multiple CVD risk factors or disease	121	67.6 (9.5)	HR 1.08 95% CI (0.99-1.16) unadjusted	NR	Per unit increase	Included in sensitivity analysis in studies of subjects with secondary CAD events
Poehls et al, 2009(145)	Prospective cohort	Older adults	3075	73.6 (2.9)	No composite outcome. CVD mortality HR 1.14 95% CI (0.99–1.31) unadjusted	No composite outcome. CVD mortality HR 1.36 95% CI (1.14-1.61) adjusted for age, sex, race, BMI, smoking, blood pressure, CAD, lipids, cystatin-C, estrogen, abdominal fat, HDL-c, diabetes duration, and insulin	Per SD change	Not included in sensitivity analysis/ data from same cohort was analyzed in another study excluding subjects with established CAD

Rao et al, 2008(146)	Prospective cohort	HD subjects	182	62.2 (12.3)	HR 1.01 95% CI (0.83-1.24) unadjusted	HR 0.48 95% CI (0.29-0.79) adjusted for age, race, smoking, duration of HD, and serum IL6	Per SD change	Included in sensitivity analysis in studies of subjects with secondary CAD events
Sattar et al, 2006(56)	Prospective cohort	Subjects from general population but some had evidence of CAD at baseline	1820	Cases 52.6 (5.2); Controls 52.5 (5.3)	HR 0.79 95% CI (0.58-1.06) adjusted for age and town	HR 0.88 95% CI (0.63-1.24) adjusted for adjusted for age, town, BMI, total cholesterol, HDL-c, triglycerides, smoking, alcohol, physical activity, social class, and SBP	Tertiles	Not included in sensitivity analysis/ data from same cohort was analyzed in another study excluding subjects with established CAD
Singer et al, 2012(147)	Prospective cohort	Elderly subjects with type two diabetes	609	72.0 (6.3)	No composite outcome. All-cause mortality HR 2.7 95% CI (1.4-5.0) adjusted for age and race	No composite outcome. All-cause mortality HR 4.0 95% CI (1.8-9.0) adjusted for age, race, SPB, diabetes, prior MI, insulin use, metformin, thiazolidinedione, LDL-c, HDL-c, CRP	Quartiles	Included in sensitivity analysis in studies of subjects with secondary CAD events
Takemoto et al, 2009(148)	Prospective cohort	HD subjects	68	Men 58.8 (13.6); Women 61.0 (8.2)	Men HR 0.74 95% CI (0.57–0.97); Women 0.79 95% CI (0.67–0.94); both unadjusted	Women HR 0.79, P=0.009, adjustment variables not clear	Not clear	Not included in sensitivity analysis/ unclear analysis
Watt et al, 2014(149)	Nested case-control	Subjects with liver transplant	154	Cases 55.0 (9.6); Controls 55.0 (8.8)	HR 0.84 95% CI (0.72-0.99), unadjusted	HR 0.85 95% CI (0.70-1.02), adjusted, variables used unclear	Per unit increase	Not included in sensitivity analysis/ unclear if any of the subjects

								had previous CVD or not
Yu et al, 2008(150)	Prospective cohort	Non-diabetic subjects undergoing PD	59	49.6 (14.1)	NR	HR 0.85 95% CI (0.75-0.96) adjusted for age, BMI, dialysis duration, previous CVD, IL-6, triglyceride, and HDL-c	Per unit increase	Not included in sensitivity analysis/ no data on models without intermediate variables
Zoccali et al, 2001(151)	Prospective cohort	HD subjects	227	59.9 (15.0)	HR 0.97 95% CI (0.93-0.99) unadjusted	HR 0.97 95% CI (0.93-0.99) adjusted for traditional risk factors, end stage renal disease, and emerging risk factors	Per unit increase	Included in sensitivity analysis in studies of subjects with secondary CAD events

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors, ACS, acute coronary syndrome; ARB, angiotensin receptor blockers; BMI, body mass index, BNP, brain natriuretic peptide; CAD, coronary artery disease; CKD, chronic kidney disease; CI, confidence interval; CRCL, creatinine clearance; CRP, C-reactive protein; GFR, glomerular filtration rate; ESRD, end stage renal disease; HbA1c, glycosylated hemoglobin; HD, hemodialysis; HDL-c, high density lipoprotein cholesterol, HOMA-IR, homeostatic model assessment- insulin resistance; HR, hazard ratio; IL, interleukin; LDL-C, low density lipoprotein cholesterol; LV, left ventricular dysfunction; MI, myocardial infarction; NR, not reported; NT-proBNP, N-terminal pro-brain natriuretic peptide; NSTEMI, non-ST elevation myocardial infarction; SD, standard deviation; SBP, systolic blood pressure; SrCr, serum creatinine; STEMI, ST-elevation myocardial infarction.

Figure 4.11. Associations of Adiponectin Levels with Secondary CAD Events, Including Studies of Subjects with Carotid Artery Stenosis, PAD, and CVD



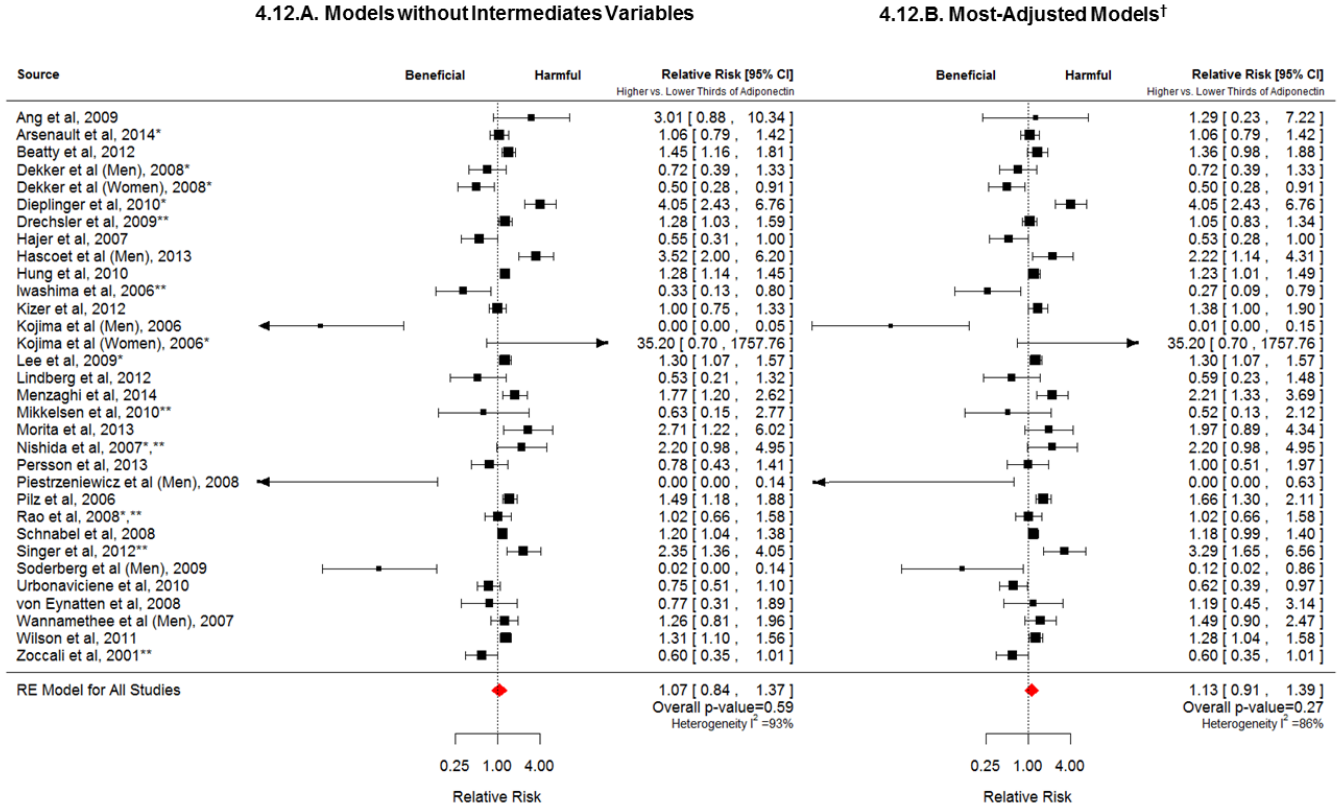
Abbreviations: CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; PAD, peripheral artery disease; RE, random effect.

* Effect estimates were the same for models without intermediate variables and most-adjusted models.

† Most-adjusted models refers to models with intermediate variables.

Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in the each meta-analysis.

Figure 4.12. Associations of Adiponectin Levels with Secondary CAD Events, Including Studies of Subjects with Carotid Artery Stenosis, PAD, CVD, and Mixed Population



Abbreviations: CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; PAD, peripheral artery disease; RE, random effect.

* Effect estimates were the same for models without intermediate variables and most-adjusted models.

† Most-adjusted models refers to models with intermediate variables.

**Studies of mixed population

Pooled RR for top third serum adiponectin levels versus bottom third. The black dotted line, is the line of no effect. The horizontal size of the diamond represents the width of the 95% CI. RRs (squares) and 95% CIs (horizontal lines) for individual studies are also shown. The size of the squares is proportional to the weight of each study in the each meta-analysis.

Components of the Composite Outcome

Results on the association between adiponectin and different components of the composite outcome are summarized in Tables 4.19.

Table 4.19. Association of Adiponectin with Components of the Composite Outcome in Studies of Primary and Secondary CAD Events

		Models without Intermediate Variables			Most-Adjusted Models		
Outcome	**No.**	**RR (95% CI)**	**p-value**	**I^2%**	**RR (95% CI)**	**p-value**	**I^2%**
Studies on Subjects with Primary CAD Events							
MI	3	0.69 (0.43-0.32)	0.12	54	0.87 (0.42-1.83)	0.72	68
CVD Mortality	6	1.14 (0.98-1.33)	0.10	0	1.49 (1.23-1.80)	<0.0001	0
All-Cause Mortality	3	1.20 (1.11-1.29)	<0.0001	0	1.39 (1.27-1.51)	<0.0001	0
Stroke*	2	[1.14 (0.87-1.49)] [1.12 (0.81-1.55)]	NA	NA	[1.49 (1.14-2.20)] [1.17 (0.83-1.64)]	NA	NA
Studies on Subjects with Secondary CAD Events							
MI	3	1.13 (0.56-2.30)	0.73	63	1.18 (0.62-2.28)	0.61	54
CVD Mortality	13	1.83 (1.41-2.38)	<0.0001	77	1.78 (1.48-2.15)	<0.0001	47
All-Cause Mortality	12	1.97 (1.50-2.60)	<0.0001	86	1.82 (1.44-2.31)	<0.0001	76
Stroke	3	1.08 (0.86-1.35)	0.63	0	1.06 (0.84-1.33)	0.64	0
Heart Failure	4	1.30 (0.79-2.15)	0.30	74	1.30 (0.98-1.72)	0.07	28
Unstable Angina*	1	[1.68 (0.77-1.78)]	NA	NA	[0.87 (0.53-1.40)]	NA	NA
Coronary Revascularization*	1	[1.14 (0.93-1.40)]	NA	NA	[1.24 (0.97-1.59)]	NA	NA

Abbreviations: CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; MI, myocardial infarction; NA, not applicable.
*Effect estimates from individual studies without performing meta-analysis.

Unpublished Reports

There was one unpublished study on studies of subjects with primary CAD events, and 18 unpublished studies on subjects of secondary CAD events. These studies did not have sufficient information to be analyzed in our main analysis, however a summary of the results of these reports is presented in table 4.20.

Table 4.20. Summary of Unpublished Reports

a. Studies of Primary CAD Events					
Source	**Study Design**	**Study Name**	**Patient Population**	**No.**	**Results**
Persson et al, 2014(152)	NR	NR	Subjects with high cardiovascular risk but no prevalent disease	3430	Adiponectin levels were inversely associated with cardiovascular events during 30-months follow-up (HR 0.69, p<0.001)
b. Studies of Subjects with Secondary CAD Events					
Bourron et al, 2013(153)	Prospective cohort	Fast-MI	STEMI/NSTEMI subjects	932	High adiponectin levels was an independent predictor of death or recurrent MI during 2-year follow-up (HR 1.70, 95% CI 1.24-2.32), using multivariable models
Chen et al, 2012(154)	NR	NR	STEMI	206	Higher adiponectin levels increased MACE at discharge and during 6-month follow-up (p<0.05)
De Rosa et al, 2012*(155)	NR	NR	Subjects who underwent coronary angiography or PCI for stable CAD or ACS	311	Lower adiponectin levels as an independent predictor of MACE during 18-months follow-up (Exp(B) 5.25, 95% CI 1.61-17.09), using multivariable models
De Rosa et al, 2010*(156)	NR	NR	Subjects who underwent PCI	400	Lower adiponectin level as a stronger independent predictor for MACE during 4-months follow up (Exp(B) 4.37, 95% CI 1.71-11.13)
Djaberi et al, 2010(157)	Prospective cohort	NR	Asymptomatic subjects with type 2 diabetes	97	Low plasma adiponectin is an independent predictor of obstructive CAD (HR 0.79, 95% CI 0.68-0.92)
Dvoryashina et al, 2010(158)	NR	NR	Male subjects with IHD underwent CABG	101	Low adiponectin levels a risk factor for recurrent angina during 1-year follow-up (P=0.031)

Elkalioubie et al, 2014(159)	NR	NR	Subjects with advanced CAD with at least one coronary artery occlusion	408	Adiponectin was an independent predictor of MACE during 9-year follow-up (HR=1.04, 95% CI=1.02-1.07, per unit increase in adiponectin) and CV mortality (HR 1.06, 95% CI 1.03-1.09), after adjusting for age and sex
Kumada et al, 2012(160)	NR	NR	Subjects having HD	203	High adiponectin was independent risk factors of CVD mortality during 5-year follow-up (HR 2.54, 95% CI 1.01-6.59), using multivariable models
Minami et al, 2014(161)	NR	NR	Subjects with STEMI treated with primary PCI	252	High adiponectin levels positively correlated with target vessel revascularization during 1050-days follow-up (HR 3.51, 95%CI 1.18- 11.21), and all-cause mortality (HR 5.44, 95% CI 1.68- 24.66)
Nakashima et al, 2013(162)	NR	NR	Subjects who underwent primary PCI	254	Higher adiponectin levels predicted ACS during 1096-days follow-up (HR, median, 4.46, P=0.024; highest, 6.63, P=0.005), using multivariable models
Nakashima et al, 2010(163)	NR	NR	Subjects with STEMI	212	High adiponectin level was significantly correlated with and in-hospital death (OR 10.5, P=0.044)
Ohuchi et al, 2012(164)	NR	NR	Fontan operation Subjects	166	High adiponectin levels independently predicted unexpected hospitalization during 2-years follow-up (p<0.05)
Peters et al, 2010(165)	NR	Fremantle Diabetes Study (FDS)	Subjects with type 1 diabetes	116	Females in the lowest serum adiponectin quintile had higher incidence of all-cause death during 12-years follow-up (HR 10.9, 95% CI 2.46-48.05). No significant association between quintiles of serum adiponectin and all-cause mortality in males
Sotoodeh et al, 2014**(166)	Case-control	NR	Subjects with acute MI	86	Lower adiponectin were independently associated with higher risk of acute MI (OR 8.97, 95% CI 2.30-34.50)
Wang et al, 2012(167)	NR	NR	Chronic PD subjects	238	Subjects with higher plasma adiponectin levels had a 2.47-fold increased risk of mortality and CVD death during 48-months follow-up (95% CI, 1.09-5.63; P=0.03), using multivariable models
Witzenbichler et al, 2011(168)	NR	HORIZONS-AMI trial	Subjects with STEMI treated with primary PCI	502	Adiponectin levels predicted MACE during 3-years follow-up

Xueying et al, 2010(169)	NR	NR	Subjects undergoing coronary angiography	105	Lower levels of adiponectin was independent and strong predictor for MACE during 1-year follow-up (OR 0.15, 95% CI 0.02-1.21)
Yajima et al, 2009(170)	NR	NR	Subjects who had acute MI	91	Adiponectin level >5.14 ng/dl was predictive of combined cardiac mortality and HF hospitalization after acute MI during 6-months follow-up (P=0.005)

Abbreviations: BMI, body mass index, CAD, coronary artery disease; CRP, C-reactive protein; DBP, diastolic blood pressure; Fast-MI, French registry of Acute ST elevation or non-ST-elevation Myocardial Infarction; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin; HD, hemodialysis; HDL-c, high density lipoprotein cholesterol; HORIZONS-AMI, Harmonizing Outcomes with RevasculariZatiON and Stents in Acute Myocardial Infarction; LDL-C, low density lipoprotein cholesterol; MACE, major adverse cardiovascular events; MI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous coronary intervention; PD, peritoneal dialysis; SBP, systolic blood pressure; and STEMI, ST-segment elevation myocardial infarction.

* Unclear if the two abstracts are using the same cohorts.

** Unclear whether this was through long term follow-up or cross-sectional analysis.

Strengths and Weaknesses

One of the strengths of our study is the large sample size and number of studies included, which make the findings robust to the effects of any single study. Additionally, we used a comprehensive search strategy and included publications in any language, minimizing the risk of missing eligible studies. An important strength of our analysis is stratifying studies based on CAD status at baseline, to assess the potential effect of index event bias that was observed in previous reports. Although not all studies reported the effect estimates in a consistent manner, we used a well-validated method to convert all estimates to a common scale before pooling the data. Finally, although the level of adjustment in each model in included studies varied widely, we investigated the impact that adjusting on potential intermediate variables had on effect estimates.

Our study has several limitations. It addresses only a composite outcome of CAD events, which varied across studies. Also, it reports findings related to total adiponectin, not high-, medium-, and low molecular weight oligomers of adiponectin. However, a study showed that high molecular weight and total adiponectin were highly correlated, and differences in effect estimates based on different oligomers of adiponectin were not detected (69). In general, the between-study heterogeneity was high, likely due in part to underlying clinical differences across study populations. While we examined the heterogeneity by meta-regression, the observed statistical between-study heterogeneity remained unexplained. Information on subgroups such as sex and race were limited. Finally, we cannot exclude publication bias as null or negative estimates may have been less likely to be published, and although we sought unpublished abstracts, not enough were identified to permit analysis.

Chapter 5: **Discussion and Future Directions**

This dissertation sought to broaden the understanding of the role of inflammatory and metabolic processes that can be targeted for coronary artery disease (CAD) prevention and management. This was done using data collected from a randomized controlled trial and by performing a systematic review and meta-analysis of available literature. The first study aimed to compare the effect of early glucose-insulin-potassium (GIK) administration on C-reactive protein (CRP) levels in the first 12 hours following symptoms of acute coronary syndrome (ACS). In addition, we also investigated the association between these early CRP levels and infarct size. The second study aimed to describe the changes in adiponectin levels early in the course of ACS, and how those levels are correlated with infarct size and one-year clinical outcomes. Finally, the third study aimed to assess the association between adiponectin and primary and secondary CAD events. We also assessed the effect of adjustment for intermediate variables, meaning those hypothesized to be on the causal pathway between adiponectin and CAD, on this relationship.

C-Reactive Protein Reactions to Glucose-Insulin-Potassium Infusion and Relations to Infarct Size in Patients with Acute Coronary Syndromes

We began by analyzing data collected from participants enrolled in the IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care) Trial (31). A total of 143 subjects were included in the IMMEDIATE Trial biocohort; 68 received GIK and 75 placebo. Our data confirm that plasma high sensitivity CRP (hs-CRP) concentrations are increased in participants presenting to the emergency department (ED) with ACS, presumably reflecting a state of inflammation. However, the administration of GIK early in the course of an ACS did not have a significant impact on hs-CRP levels. Although there was a modest difference between the 12-hour hs-CRP levels with GIK, the delta hs-CRP values was not different

between treatment arms. In addition, results of mixed models, adjusting for within and between subjects variability, demonstrated no significant effect of GIK on hs-CRP over the three time points. These results may suggest that the beneficial effects of GIK in ACS observed in the main study (31), at least as reflected in the absence of an effect on hs-CRP, are less likely to be through an anti-inflammatory effect, and may be more exerted through a metabolic effect.

Previous studies on the effect of GIK on CRP yielded conflicting results. In a study by Chaudhuri et al., GIK administration started in the ED in patients presenting with ST-elevation myocardial infarction (STEMI) (N=32) and lasting 48 hours showed significantly reduced hs-CRP values at 24 and 48 hours post-infusion compared to placebo (34). In contrast, Parikh et al., demonstrated in 25 patients with STEMI that a 24-hour infusion of GIK produced no statistically significant difference in 24 hour hs-CRP levels compared with placebo (35). Additionally a study by Hashemian et al., showed no effect of GIK on hs-CRP levels in 72 patients with STEMI treated within 12 hours from symptom onset (36). Although those studies added GIK to standard care, there are important differences in the use of GIK in the IMMEDIATE Trial. First, in prior clinical trials GIK was started typically an average of six hours after onset of ischemic symptoms, following documentation of acute myocardial infarction (MI) (22; 26; 30). However, in the IMMEDIATE Trial, the study drug was started prior to arrival to the ED, and upon emergency medical services (EMS) arrival in the community following a 9-1-1 call, at an average of 90 minutes after symptom onset (31). Moreover, the previous studies only included participants with STEMI. In contrast, the IMMEDIATE Trial included subjects with ACS, i.e., either unstable angina or acute MI (whether or not STEMI) (31).

Infarct size has shown to be a prognostic marker of adverse clinical outcomes after an acute coronary event (171). Baseline CRP levels in healthy individuals or in patients with stable angina are independent risk factor for cardiovascular events (172). Also the rise in CRP after acute MI or during unstable angina pectoris is related to adverse clinical outcomes (10; 173; 174). In this study on participants presenting to EMS with ACS, we document a relationship between hs-CRP level measured at 12 hours and 30-day infarct size. We also found that the magnitude of change in hs-CRP levels, between the initial and 12-hour values, was related to infarct size. Limiting the analysis to those participants with a documented infarct size measurement, and also to those in the placebo group only, showed similar results.

Studies have shown no clear relationship between CRP levels at hospital admission and infarct size in patients with presenting with acute coronary events (175); but cumulative or peak CRP levels have been correlated with infarct size (176). Peak CRP levels are reached by no earlier than 24 hours after infarction (177). In contrast, our results show that an early rise, within 12 hours of ACS symptom onset, in hs-CRP levels correlates with 30-day infarct size. This may reflect a general correlation between the magnitude of acute phase response and infarct size. One study suggests that the CRP-mediated complement activation may contribute to infarct size and outcome in subjects with an acute MI (178). This is supported by a report that shows an injection of human CRP into rats after ligation of the coronary artery leads to a significant enhancement of the size of the resulting MI (179).

Imaging studies in IMMEDIATE Trial biocohort participants at 30-days showed that GIK was associated with a reduction in infarct size, both for the entire ACS cohort (N=110) and in those presenting with STEMI (N=75) (31). Therefore the modest reduction seen in

hs-CRP levels at 12 hours between the two groups may be indirectly related to infarct size. However, given that the decline in hs-CRP levels was very small, and only observed in unadjusted analyses, we are unable to draw conclusions on relationship between GIK, infarct size, and hs-CRP levels in a cohort of subjects with ACS.

Serum Adiponectin Levels in Patients with Acute Coronary Syndromes: Serial Changes and Relation to Infarct Size

We also used data from the IMMEDIATE Trial biocohort to analyze adiponectin levels shortly after ACS symptoms. A total of 120 participants were included in this analysis. Plasma adiponectin levels decreased slightly in the first few hours after the onset of ACS symptoms. This suggests that adiponectin may respond to the acute phase of CAD. The exact mechanism of the decrease in plasma adiponectin levels shortly following the onset of ACS is not known. One possible explanation is that rupture of the coronary plaque may lead to decrease in plasma adiponectin levels. Animals and human studies show that adiponectin is found in injured vessels rather than in intact vascular walls (180). Therefore, it is possible that adiponectin targets ruptured plaques resulting in its consumption in the circulating plasma (180; 181). Previous literature have shown that adiponectin levels tend to decline after acute MI. In one report mean plasma adiponectin concentrations declined significantly 24 hours (6.2 mcg/ml) and 72 hours (5.8 mcg/ml) after acute MI compared to the concentrations on admission (8.1 mcg/ml) (182). In addition a recent study showed that plasma adiponectin levels fluctuated before and after percutaneous coronary intervention (PCI) in subjects with STEMI. Here, we observed a slight decrease in adiponectin values within the first 24 hours of symptom onset (183). Although this reduction was minimal, the results of our study, and others suggest that levels of systemic adiponectin can vary depending on the time of sampling after presentation.

When the analysis was stratified by sex, the small decrease observed in adiponectin levels, was only noticeable in women. The mechanisms behind this sex difference is unclear. One possible explanation is variations in medical therapy at the very early stages of ACS between men and women. A study by Sullivan et al, examining the duration of time for sequence of care in patients with suspected ACS, using data from the IMMEDIATE Trial, found significant delays in women compared to men (184). Therefore the differences in access to early treatments may have affected adiponectin levels shortly after symptoms of ACS. Nevertheless, biological differences between sexes could explain our findings. In the general population, women have higher plasma adiponectin levels than men (44; 185), and several reports show that sex hormones affect adiponectin, and may result in the differences seen between sexes (186). Future research is required to explore the changes in adiponectin levels early after ACS between men and women, possibly focusing on the effect of early management of ACS on adiponectin.

When studying the association between plasma adiponectin levels on admission and patient's clinical and demographic characteristics, older individuals had lower adiponectin levels. Other clinical characteristics that are known to be correlated with plasma adiponectin (187), were not associated with this biomarker in our cohort. For example, in the general population, plasma adiponectin levels are highly correlated with glycosylated hemoglobin (HbA1C), markers of inflammation, and body mass index (BMI) (53). However, few observational studies in patients with established CAD did not find an association between plasma adiponectin and the presence of diabetes mellitus, and CRP levels (113; 188). In addition, a study in subjects with CAD, found no correlation between adiponectin and BMI in those with a BMI over 30 kg/m^2 (189). The average BMI in our cohort was around 29 kg/m^2, and similarly, we did not observe an association

between BMI and adiponectin. The findings in our study suggests the possibility that the role of systemic inflammation, body weight, and glucose metabolism, as part of the relationship between adiponectin and atherosclerosis may be decreased in the course of CAD.

Adiponectin-deficient mice have been found to have a larger infarct size following myocardial ischemia and reperfusion compared with control mice (62). In addition intracoronary injections of adiponectin in animals resulted in a decrease in infarct size (82). In contrast to animal models, we could not establish such a correlation between infarct size and plasma adiponectin levels. It is important to note that the lack of association between the two could be attributed to the heterogeneous cohort in this study. We included subjects with unstable angina, who did not have an infarct, together with subjects who had a measurable infarct. This can bias the results towards the null. Nevertheless, when we ran a sensitivity analysis excluding subjects with infarct size of 0, the results remained non-significant. However the magnitude and the direction of effect sizes changes slightly, indicating the possibility that the relationship between adiponectin and infarct size could be different in subjects with unstable angina versus those with an acute MI. In addition, using only unadjusted hazard models, plasma adiponectin levels were not associated with the composite clinical outcomes or all-cause mortality or hospitalization for heart failure, during the one-year follow-up period. The findings in our study support those from a number of observational studies that have found no link between plasma adiponectin levels and clinical outcomes, including future acute coronary events in patients with various degrees of CAD (54-56).

Association of Adiponectin and Risk of Primary and Secondary Coronary Artery Disease: A Systematic Review and Meta-Analysis

In this largest-to-date and most comprehensive systematic review and meta-analysis on the association between plasma adiponectin and CAD events, we synthesized evidence from 48 studies that included 61,097 individuals—43,086 subjects in studies of primary CAD events and 19,011 subjects in those with secondary events. We found that higher adiponectin levels were associated with 27% lower risk of primary CAD when comparing the highest to lowest tertile, using models without intermediate variables that are considered biologically influenced by adiponectin. Paradoxically, we found an increased risk of secondary events in subjects with established CAD disease, using maximally adjusted models. The striking aspect of our results, which could explain the discrepancies in the literature regarding the relationship between adiponectin and CAD risk, is how the findings were heavily affected by 1) selection of the baseline population (CAD versus no CAD at baseline); and 2) the inclusion of adjustments for intermediate pathway variables (e.g., glycaemia measures) in the models.

Including studies of subjects with established CAD may alter study results by introducing index event bias, which is a consequence of selecting subjects based on having a prior CAD events (43). This can results in an inversion in the relationship between adiponectin and recurrent CAD events (43). An example of this bias is demonstrated in a study by Canto et al., showing that among people with MI, well-established cardiovascular disease (CVD) risk factors (such as hypertension, smoking, dyslipidemia, diabetes, and family history of CAD) had protective effects on the risk of hospital mortality (172). This bias is also suggested in our analysis as the relative risk (RR) for primary CAD was 0.73 and 0.93 (in models without intermediate variables and most-adjusted models, respectively), as compared to RR=1.05 and 1.27 (in models without intermediate

variables and most-adjusted models, respectively) for secondary events. In line with our findings, the meta-analysis presented by Zhang et al. showed a protective association of adiponectin in subjects without established CAD (summary RR 0.83; 95% confidence interval [CI], 0.69–0.98; P=0.031) (39). Meta-analyses that included a mix of studies of subjects with and without CAD did not find a beneficial association between adiponectin and future CAD events (41; 42). Furthermore, one meta-analysis suggests adiponectin is associated with increased risk of secondary CAD events (RR 1.12; 95% CI 1.02–1.22) (41). However only seven studies were included in this review, as compared to our analysis which included 24 studies.

Another possible explanation for the apparent paradoxical relationship between adiponectin and secondary CAD events is a compensatory mechanism, where adiponectin levels increase in subjects with vascular injuries (173). In advanced CAD, this compensation may be less adequate, leading to loss of adiponectin's protective effects (173). Also subjects with established CAD can have adiponectin resistance, a concept that is currently under investigation (174). For example, a recent study showed a decrease in adiponectin receptor gene expression, and higher circulating levels of adiponectin, in subjects with CAD compared to healthy subjects, suggesting a state of adiponectin resistance (175). Future animal and human studies are required to determine whether the observed effects of adiponectin in subjects with CAD is the result of a physiological mechanism (i.e., adiponectin resistance) or due to the selection of study population (i.e., index event bias).

Previous meta-analyses summarized available multivariable models (38-42), even when they incorporated intermediate variables, which can lead to over-adjustment bias. Genetic studies indicate that genotypes which affect adiponectin levels also influence

insulin sensitivity (34; 45). A randomized controlled trial showed that an increase in adiponectin level, after lifestyle intervention, mediated an increase in high density lipoprotein cholesterol (HDL-c) levels in obese diabetic individuals (176). Furthermore, the genetic component of the HDL-c and plasma insulin overlaps significantly with that of plasma adiponectin, as single nucleotide polymorphisms (SNPs) that contribute to the heritability of plasma adiponectin, were also associated with plasma insulin ad HDL-c levels (177). Animal models have indicated that lower adiponectin levels can exacerbate the pro-inflammatory state by inducing CRP production (178). These lines of evidence suggest that variables affecting glucose, HDL-c, and CRP levels could be on the causal pathway between adiponectin and CAD (179; 180).

Our results also support the hypothesis that these intermediate variables fall on the causal pathway between adiponectin and CAD events. This is specifically observed in studies of primary CAD events, as we saw associations were attenuated once those variables were incorporated in the analysis. By adjusting for intermediate variables (e.g., those influencing glucose levels) some of the effects of adiponectin on CAD events are masked, biasing the results toward the null (106). As noted earlier, the relationship between adiponectin and the intermediate variables were derived from reports in subjects free of CAD. Observational studies in subjects with established CAD showed no association between plasma adiponectin levels and the presence of diabetes or CRP levels, which are known to be highly correlated in studies of the general population (97; 133; 181). Therefore, in the analysis of studies with secondary CAD events it may be appropriate to use the maximally adjusted models, whether or not they included variables related to glucose, HDL-c, and CRP levels. When we limited the analysis to high quality studies, and excluded outlier studies both models, without intermediate variables and most-adjusted models, showed an increased risk of secondary events with

higher adiponectin levels. This support our results of a trend toward a harmful effect of adiponectin on disease recurrence regardless to whether or not intermediate variables were included in the models.

Future Research Recommendations

The results of this study highlights important considerations for future work. First to better understand the mechanisms by which GIK exerts its benefits in subjects with ACS, other markers of metabolic abnormalities such as those related to endothelial dysfunction, microvascular dysfunction, and coagulation, should be tested. Second, studies with larger samples of subjects are required to explore the early changes in adiponectin shortly after ACS symptoms, especially for conducting stratified analysis by sex. Third, given the paradoxical relationship between adiponectin and CAD events, we encourage researchers to exercise caution when investigating interventions that raise adiponectin levels, as evidence is still required to confirm its beneficial effect in CAD, which is especially doubtful in subjects with established disease. Finally, the observations from the meta-analysis we performed highlights the pitfalls and limitations of observational studies designed to understand the relationship between biomarkers and clinical outcomes. Researchers and clinicians should carefully consider the type of baseline population selected in a study, especially when examining risk of recurrent events. Also, there should be a cautious selection for variables in multivariable models as inappropriate adjustment can bias the results.

There is an interest in the potential clinical use of adiponectin, and several pre-clinical and clinical trials are presently being conducted on drugs that can increase adiponectin levels, including intracoronary injections during ischemia (71; 82). However, the effect of plasma adiponectin levels in the setting of ACS remains unclear. In general,

observational studies are limited in their ability in establishing causal associations between modifiable exposures and disease. Mendelian randomization studies, are becoming more widely used to establish causal relationships between biomarkers and CVD, and CVD risk factors. These studies uses genetic variants as instrumental variables to identify whether gene variants that can alter a biomarker level, are also related to disease risk (190). To our knowledge no studies have been conducted between adiponectin and CAD risk. Such approach may help in understanding whether abnormal adiponectin levels can actually lead to CAD development (i.e. establish a causal relationship), or whether the altered plasma adiponectin levels are just a consequence of disease progression (190).

In our future research we will aim to apply the meta-analysis methodology performed in this projects to other clinical scenarios including:

1) Studying the relationship between adiponectin and mortality, both all-cause and CVD mortality
2) Addressing the association between adiponectin and other CVDs, such as heart failure
3) Applying the meta-analysis methodology we performed to assess the association between other biomarkers and CVD risk
4) Exploring other methodological techniques to convert effect estimates that are not reported in similar manner before pooling them in one analysis

Conclusions

In summary, the results of this dissertation research have enriched our understanding of the complexity of several metabolic and inflammatory targets for the management and

prevention of CAD. First, in patients with an ACS, early administration of GIK appears to have no significant effect on hs-CRP levels measured in the first 12 hours of treatment infusion. This is consistent with a model that the primary immediate benefits of GIK are more directly metabolic rather than anti-inflammatory in nature. Our findings of a possible association between the early rise in hs-CRP levels and infarct size, are consistent with the role of inflammation in extent of infarction.

Second, unlike previous research we found that plasma adiponectin levels tend to slightly decrease after symptoms of ischemia in patients with ACS. This reduction, although weak, was present only in women in the stratified analysis. We also show that the relationship between adiponectin and clinical characteristics and adverse outcomes seen in the general population does not necessarily apply to those with established disease. These results suggest that adiponectin might react to the acute phase of ischemia, and this response may differ by sex. In addition, the role of adiponectin in subjects with ACS seems to be different than that observed in other populations.

Finally, the results of the large systematic review concluded that while higher adiponectin levels are associated with fewer primary CAD events, they are also linked with an increased risk of secondary events. This paradoxical finding could be attributed to index event bias in studies selecting subjects with prevalent CAD. In addition, the statistical significance of the results varied when included studies adjusted for intermediate variables. These observations represent a general problem when studying the associations between biomarkers and clinical outcomes. The type of baseline population, especially when examining risk of recurrent events, and the variables used in multivariable models can significantly bias the results of observational studies.

In conclusion, this dissertation suggests that metabolic therapy with GIK exerts its benefits in subjects with ACS probably through metabolic, rather than inflammatory pathways. This study also shows, for the first time, a modest response of adiponectin levels to the acute phase of ischemic events. We could not establish a relationship between adiponectin and infarct and clinical outcomes in subjects with CAD. However, the finding from the largest systematic review to date, which incorporated novel approaches, showed a paradoxical relationship between plasma adiponectin levels and CAD risk. While higher levels of adiponectin were associated with less risk of CAD events in subjects without history of the disease, it was associated with higher risk of CAD outcomes among subjects with pre-existing CAD. This study illustrate the complex relationship between metabolic and inflammatory biomarkers and CAD.

Chapter 6: **References**

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