Adiponectin, free fatty acids, and cardiovascular outcomes in patients with type 2 diabetes and acute coronary syndrome

Running title: Adiponectin, FFA and cardiovascular outcomes

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ABSTRACT

Objective: In observational cohorts, adiponectin is inversely and free fatty acids (FFA) directly associated with incident coronary heart disease (CHD). Adiponectin tends to be reduced and FFA elevated in type 2 diabetes. We investigated relationships of adiponectin and FFA to major adverse cardiovascular events (MACE) and death in patients with acute coronary syndrome (ACS) and type 2 diabetes using data from the AleCardio trial that compared the PPAR-α/γ agonist aleglitazar with placebo.

Research Design: Using Cox regression adjusted for demographic, laboratory, and treatment variables, we determined associations of baseline adiponectin and FFA, or the change in adiponectin and FFA from baseline with MACE (cardiovascular death, myocardial infarction, or stroke) and death.

Results: A twofold higher baseline adiponectin (n=6998) was directly associated with risk of MACE (HR 1.17; 95% CI 1.08-1.27) and death (HR 1.53; 95% CI 1.35-1.73). A doubling of adiponectin from baseline to month 3 (n=6325) was also associated with risk of death (HR 1.20, 95% CI 1.03-1.41). Baseline FFA (n=7038), but not change in FFA from baseline (n=6365), was directly associated with greater risk of MACE and death. There were no interactions with study treatment.

Conclusions: In contrast to prior observational data for incident CHD, adiponectin is prospectively associated with MACE and death in patients with type 2 diabetes and ACS, and an increase in adiponectin from baseline is directly related to death. These findings raise the possibility that adiponectin has different effects in patients with type 2 diabetes and ACS than in

populations without prevalent cardiovascular disease. Consistent with prior data, FFA is directly associated with adverse outcomes.

Adiponectin and free fatty acids (FFA) are markers of adipocyte function. Adiponectin is a hormone secreted by adipocytes and signals through specific receptors in target tissues including myocardium and arterial wall. Adiponectin may modulate insulin action and sensitivity, and has putative anti-atherogenic and anti-inflammatory effects (1). Infusion of adiponectin in experimental animals may mitigate myocardial ischemia-reperfusion injury (2). Observational analyses show a strong and consistent association of higher adiponectin levels with lower risk of incident coronary heart disease (3,4), but Mendelian randomization analysis does not support such an association (5). Studies in patients with established coronary heart disease have also yielded conflicting evidence, with some indicating favorable and others adverse associations of adiponectin with risk of future cardiovascular events (6-9). Free fatty acids are an important energy substrate, although at elevated concentrations, FFA may exert pro-inflammatory, pro-apoptotic, or pro-arrhythmic effects, and impair endothelial function (10-12).

Adipocyte dysfunction is a hallmark of insulin-resistant states and is manifested by reduced adiponectin and elevated FFA levels. In fact, it has been postulated that low adiponectin and high FFA may contribute to increased cardiovascular risk in type 2 diabetes (3,13). Agonists of the peroxisome proliferator-activated receptor gamma (PPAR-γ) are among the most effective agents to raise adiponectin and lower FFA concentrations in circulation (14). The AleCardio (Effect of Aleglitazar on Cardiovascular Outcomes after Acute Coronary Syndrome in Patients with Type 2 Diabetes Mellitus) trial compared the effects of the dual PPAR α/γ agonist aleglitazar with placebo on cardiovascular morbidity and mortality among patients with type 2 diabetes and acute coronary syndrome (ACS) (www.ClinicalTrials.gov: NCT01042769). The AleCardio trial showed no effect of aleglitazar on cardiovascular outcomes. Using data from that

trial, we evaluated the association of adiponectin and FFA at baseline and on assigned study treatment with major adverse cardiovascular events (MACE) and death.

RESEARCH DESIGN AND METHODS

Study design and patients

This study is a pre-specified post-hoc analysis of the AleCardio Trial data. Study data and study materials are not publicly available for other researchers, but analytic methods can be requested from the corresponding author. The rationale, design, and primary results of the AleCardio trial have been published previously (15,16). The protocol was approved by institutional review boards and written informed consent was obtained from all participants. At 720 participating centers in 26 countries, 7226 patients with established or newly diagnosed type 2 diabetes and recent ACS were randomly assigned to treatment with aleglitazar 150 µg daily or placebo, added to standard of care. Randomization occurred during the interval spanning hospital discharge to 12 weeks after the index ACS event. The primary outcome measure was time to first occurrence of cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke (defined as MACE for this analysis). All-cause and cardiovascular deaths were secondary efficacy measures. After a median follow-up of 2 years, the trial was ended prematurely due to futility for efficacy and in response to a higher incidence of safety endpoints in the aleglitazar group.

Laboratory assessments

All laboratory analyses were conducted by a central laboratory. Blood samples were collected after an overnight fast of at least 8 hours at randomization and after 3 months of assigned study treatment. Plasma adiponectin was measured by a quantitative sandwich ELISA

(Quantikine ™ Adiponectin/Acrp30 Immunoassay, R&D Systems, Minneapolis, MN), with an intra-assay coefficient of variation of 2.5 – 4.7% and an inter-assay coefficient of variation of 5.8 – 6.9%. FFA were measured by enzymatic colorimetry (NEFA HR2, WAKO Chemicals, Richmond, VA) with an intra-assay coefficient of variation of 0.61 – 0.75% and an inter-assay coefficient of variation of 0.03 – 0.37%. Results were reported with a precision of 0.1 mmol/L. Testing was performed on Roche Modular Analyzers.

Statistical methods

We compared baseline characteristics among quartiles of adiponectin and 4 categories of FFA concentrations using ANOVA or Kruskal-Wallis test for continuous variables, depending on the distribution, and Chi-square for categorical variables. Categories of FFA were used instead of exact quartiles because the precision of measurement of FFA concentrations was 0.1 mmol/L, resulting in an unequal distribution of participants across quartiles. The distributions of adiponectin and FFA concentrations were checked and log transformation was conducted if required for further analysis.

Correlation between 2 variables was specified by the Pearson's or the Spearman's correlation coefficient, as appropriate. Cox proportional hazard regression models were used to analyze the association between baseline adiponectin or FFA levels and time to event for MACE, all-cause death, and cardiovascular death. Additionally, we modeled the association between change in adiponectin or change in FFA from baseline to Month 3 of assigned study treatment with each of the 3 endpoints (occurring after Month 3). We adjusted all models for covariates and treatment and we stratified for the type of index ACS event (unstable angina, non-ST-segment elevation myocardial infarction, or ST-segment elevation myocardial infarction) and the

need for reperfusion therapy for the index ACS event. We checked for a potential interaction with treatment and sex and stratified if necessary. The proportional-hazards assumption and functional form of the covariates was evaluated by using the ASSESS statement in SAS. Visual inspection of the cumulative Martingale residuals and the formal hypothesis based on simulation were assessed.

We included the following covariates in our model: age, sex, race, geographical region, prior history of myocardial infarction, stroke or transient ischemic attack, heart failure, hypertension, duration of diabetes, smoking history, body mass index, time from ACS to randomization, systolic and diastolic blood pressure, use of anti-hyperglycemic agents (insulin, sulfonylureas, biguanides), HbA_{1c}, fasting plasma glucose, LDL cholesterol, HDL cholesterol, triglycerides, high sensitivity C-reactive protein (hs-CRP), and estimated glomerular filtration rate (eGFR). Adiponectin or FFA concentration was added as a covariate, depending upon which of those was the variable of interest in the analysis. All covariates were selected *a priori* based on their relation with adiponectin, FFA or cardiovascular outcomes as described in prior literature.

In the analysis on change in adiponectin or FFA from baseline to Month 3, additional adjustment was performed for concurrent changes in systolic and diastolic blood pressure, glucose, insulin, HbA_{1c}, LDL cholesterol, HDL cholesterol, triglycerides, eGFR, adiponectin (for analysis on change in FFA), and FFA (for analysis on change in adiponectin).

Two sensitivity analyses were performed. In the first, patients treated with exogenous insulin were excluded. In the second, N-terminal pro-B-type natriuretic peptide (NT-proBNP) was added as a covariate to the multivariable model, because prior studies showed that NT-proBNP may be related to adiponectin concentrations (17,18).

Missing covariate data were replaced with use of multiple imputation (Markov chain Monte Carlo method). Results were considered significantly different at a P-value of <0.05. Statistical analyses were performed using SAS statistical software version 9.2 (SAS Institute, Cary, NC, USA). The first author (ICS) had full access to all the data in the study and takes responsibility for its integrity and the data analysis.

RESULTS

Baseline characteristics

Baseline adiponectin and FFA were available for 6998 and 7038 patients, respectively. The distribution of baseline levels is tabulated in **Supplemental Table S1** and illustrated in **Supplemental Figure S1**. Adiponectin and FFA values both have a skewed distribution. Median (IQR [interquartile range]) of adiponectin was 4.0 (2.7-6.1) µg/mL. The four categories of FFA were 0.1-0.3 mmol/L (n=1878), 0.4-0.5 mmol/L (n=2308), 0.6-0.7 mmol/L (n=1683), and 0.8-3.3 mmol/L (n=1169). As expected, baseline adiponectin and FFA concentrations did not differ between the aleglitazar and placebo groups. Paired baseline and Month 3 measurements of adiponectin and FFA were available for 6325 and 6365 patients, respectively. **Table 1 and 2** show baseline characteristics stratified according to adiponectin quartiles and FFA categories. Patients with higher adiponectin levels were older, more frequently women, and had a longer duration of type 2 diabetes. They had lower insulin, triglyceride, and eGFR, but higher NT-proBNP, HDL cholesterol, and LDL cholesterol levels. Further, they were more likely to be treated with exogenous insulin, but less likely to be treated with biguanides and diuretics.

Differences in baseline characteristics stratified according to FFA categories were less pronounced than those observed across quartiles of adiponectin. Nonetheless, patients with

higher FFA levels were older, more frequently women, less frequently past/current smokers, and had a longer duration of type 2 diabetes. They had lower insulin, higher adiponectin and higher NT-proBNP levels compared to patients with lower FFA levels.

In the aleglitazar arm, median (IQR) adiponectin concentration increased from baseline (4.0 [2.7-6.1] µg/mL) to Month 3 (11.9 [7.1-19.4] µg/mL); an increase of 7.5 (3.7, 13.7) ug/mL, p<0.001). In the placebo group, adiponectin at Month 3 was 4.1 (2.6-6.0) ug/mL, a change of 0.0 (-0.9, 0.9) ug/mL from baseline (**Supplemental Table S1, Figure S2, and Figure S3**). FFA decreased from 0.5 ± 0.3 mmol/L at baseline to 0.4 ± 0.2 mmol/L at Month 3 in the aleglitazar arm; a decrease of -0.1 ± 0.3 mmol/L, P<0.001). In the placebo group, FFA at Month 3 was 0.5 ± 0.3 mmol/L, a change of 0.0 ± 0.3 mmol/L from baseline.

Association between baseline adiponectin and FFA levels and outcomes

A total of 684 (10%) and 688 (10%) MACE, 276 (4%) and 281 (4%) all-cause deaths, and 202 (3%) and 206 (3%) cardiovascular deaths occurred in patients with baseline adiponectin and FFA data available, respectively. Median (interquartile range [IQR]) follow-up time was 1.98 (1.55-2.46) years. **Figure 1** shows the Kaplan-Meier estimates of survival free of MACE, all-cause death, and cardiovascular death according to adiponectin quartiles and FFA categories. The risk for each endpoint increased across quartiles of adiponectin (P<0.001) and categories of FFA (P<0.05).

Table 3 shows the crude and adjusted risk for MACE, all-cause death, and cardiovascular death according to baseline adiponectin and FFA concentrations. Baseline adiponectin that was 2 times higher was associated with higher risk for MACE (HR 1.28 [1.18-1.38]; adjusted HR 1.17 [1.08-1.27], P<0.001), all-cause death (HR 1.75 [1.56-1.98]; adjusted

HR 1.53 [1.35-1.73], P<0.001), and cardiovascular death (HR 1.67 [1.45-1.92]; adjusted HR 1.51 [1.30-1.76], P<0.001). No interaction existed between randomized treatment assignment and baseline adiponectin concentrations on the risk for any endpoint event (**Table 3**). Furthermore, no interaction was found for sex.

A baseline FFA level that was 2 times higher was associated with a higher risk for MACE (HR 1.15 [1.04-1.27]; adjusted HR 1.12 [1.02-1.24], P=0.019), all-cause death (HR 1.31 [1.11-1.54]; adjusted HR 1.20 [1.03-1.40], P=0.018), and cardiovascular death (HR 1.28 [1.06-1.54], adjusted HR 1.19 [0.99-1.42], P=0.062). As with adiponectin, the interaction between treatment and baseline FFA concentrations on endpoint events was not significant.

In the first sensitivity analysis, patients treated with exogenous insulin were excluded. The associations of baseline adiponectin with clinical outcomes remained significant with minimal effect on the point estimates of hazard ratios (data not shown); however, the association of baseline FFA with adverse outcomes was no longer significant. Adiponectin and NT-proBNP were weakly correlated with r^2=0.09 (P<0.001). In the second sensitivity analysis with NT-proBNP added as a covariate to the regression model a baseline adiponectin level that was 2 times higher remained significantly associated with MACE (adjusted HR 1.14 [1.04-1.26], P=0.008), all-cause death (adjusted HR 1.19 [1.02-1.39], P=0.025), and cardiovascular death (adjusted HR 1.21 [1.01-1.44], P=0.040), although the associations were attenuated (**Table 3**). With the addition of NT-proBNP as a covariate, the associations of baseline FFA with death and cardiovascular death remained significant, but the association with MACE was attenuated. A similar effect was seen for the separate endpoint non-fatal MI (**Supplemental Table S2**).

Association between change in adiponectin or FFA and outcomes

The associations between changes in adiponectin or FFA from baseline to Month 3 and outcomes are shown in **Table 3**. A doubling in adiponectin from baseline to Month 3 was associated with a higher risk for all-cause death (HR 1.20 [1.03-1.41], P=0.022), and cardiovascular death (HR 1.22 [1.02-1.46], P=0.029), but not with MACE (HR 1.03 [0.93-1.15], P=0.540) after multivariable adjustment. Since a change in adiponectin over time was identified only in the aleglitazar group **(Supplemental Table S1,** P<0.001) we investigated interaction effects by treatment and conducted stratified analysis by treatment. No significant interaction effects were observed. In addition, interaction with sex was investigated and no interaction was found, with the exception for the association between change in adiponectin and MACE (P=0.031). Stratified analysis showed no association between change in adiponectin and MACE in men (HR 0.99 [0.87-1.12], P=0.82), while a borderline significant association for women existed (HR 1.19 [1.00-1.42], P=0.052). The change in FFA from baseline to Month 3 was not associated with outcomes in crude or adjusted models.

When sensitivity analysis was performed by adding NT-proBNP as covariate to the model, associations between change in adiponectin (baseline to Month 3) and outcomes were no longer significant (**Table 3**).

CONCLUSIONS

This study shows that both FFA and adiponectin levels are directly associated with the risk of major adverse cardiovascular events and death in patients with type 2 diabetes and recent ACS. These findings extend the previously reported data on the relation between FFA levels and cardiovascular outcomes, although the observed relationships for adiponectin are opposite to conclusions from prior observational data in patients initially free of cardiovascular events.

In prior cohort studies without prevalent cardiovascular disease, higher adiponectin concentrations were related to a lower risk of incident cardiovascular disease and mortality (3,4). However, the current data are aligned with findings in patients with heart failure (19) or coronary artery disease (20), and in elderly people (21,22) that associated higher concentrations of adiponectin with greater risk of cardiovascular and all-cause death. Furthermore, an analysis of the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care in Patients with Type 2 Diabetes Mellitus and Acute Coronary Syndrome trial (EXAMINE) showed a positive association of adiponectin with cardiovascular and all-cause death (23). A post hoc analysis of the Pravastatin or Atorvastatin Evaluation and Infection Trial (PROVE IT) showed an adverse association of adiponectin with 2-year MACE outcomes but not death in 3933 patients with recent ACS (24). Additionally, in patients without diabetes and a recent acute myocardial infarction, higher adiponectin levels have been associated with higher mortality but not cardiovascular mortality (25). The current data extend these findings by demonstrating associations between baseline adiponectin and death, as well as composite MACE outcomes. Furthermore, the current analysis indicates that a rise in adiponectin during the early period after ACS is associated with a greater risk of death, independent of treatment assignment in the clinical trial that provided the source data.

The underlying mechanisms for the adverse associations of adiponectin and FFA with death and MACE are unclear. Although most evidence supports the view of adiponectin as an anti-inflammatory mediator (26), a growing body of *in vitro* data indicates that adiponectin also has the potential to induce pro-inflammatory effects. Studies in a variety of cell types, including astrocytes (27), renal tubular cells (28), synovial cells (29), macrophages and T-lymphocytes (30), demonstrate stimulation of inflammatory signalling pathways by adiponectin. In clinical

studies of patients with inflammatory or vascular disease, higher adiponectin levels correlated with greater severity of rheumatoid arthritis (31), higher likelihood of proliferative diabetic retinopathy (32), and greater aortic stiffness in patients with acute myocardial infarction (33). Alternatively, higher circulating concentrations of adiponectin may reflect "adiponectin resistance" due to a decrease in adiponectin receptor expression or responsiveness in target tissues (34). Under this concept, an adverse association of adiponectin with outcomes might not reflect adverse actions of the adipokine, but rather conditions that impair signaling its favorable effects.

Prior studies have shown a consistent positive association between the circulating concentrations of adiponectin and natriuretic peptides (17,19,35), with evidence that natriuretic peptides stimulate the synthesis and release of adiponectin (18,36). Natriuretic peptides are increased in heart failure and predict poor outcomes. Therefore, we investigated the possibility that the adverse association of adiponectin with outcomes after ACS reflect an underlying adverse association of heart failure and elevated natriuretic peptides. In sensitivity analyses incorporating levels of NT-proBNP as a covariate in Cox regression models, significant adverse associations persisted between baseline adiponectin and death and MACE outcomes, although the hazard ratios were somewhat attenuated. Thus, higher levels of natriuretic peptides may explain part, but not all, of the paradoxical association of adiponectin with adverse outcomes in this study.

Elevated FFA levels in our study population are comparable to levels seen in obese and type 2 diabetes patients (37,38). Elevated FFA has been postulated to be a risk factor for arrhythmic and atherothrombotic events (9). In prior studies, higher FFA concentrations have been associated with greater risk of incident coronary heart disease (39), MACE in patients with

coronary heart disease (40), and sudden death (41). The present study extends those findings by demonstrating a strong association of FFA with MACE and death in patients with type 2 diabetes and ACS.

Study limitations

The present study is a post-hoc, observational analysis of a randomized clinical trial. As such, it cannot determine the biological mechanisms responsible for the adverse association of adiponectin or FFA with outcomes. Second, unaccounted factors associated with adiponectin or FFA may introduce an unknown degree of residual confounding. For example, we did not measure, and therefore cannot account for relationships with other adipokines (e.g. leptin, ghrelin). Third, because of missing data in baseline or change in adiponectin and FFA concentrations, we had to exclude approximately 3% and 12% of the patients from our analyses, respectively. Fourth, analyses relating the change in adiponectin or FFA from baseline to Month 3 to outcomes have substantially less power than those relating baseline concentrations to outcomes. This is because there were fewer patients with data from both time points, and because the analyses of the changes in biomarkers over time only consider events occurring after Month 3. Moreover, the median change from baseline in FFA was modest, further reducing power in that analysis. Fifth, the relationship of adiponectin and FFA concentrations with the qualifying (index) ACS event for the AleCardio study is unknown. Therefore, we cannot exclude index event bias as an explanation for the current findings (42). Sixth, adiponectin and FFA were measured only once at each time point. Intra-individual variability in these measures may have weakened the apparent associations with outcomes. By analogy, intra-individual variability in NT-proBNP may have weakened the effects of adjustment for that variable. Furthermore, a total

of 452 patients in AleCardio either withdrew consent or were lost to follow-up prior to the common study end date. We cannot exclude the possibility of resulting bias in our reported results. Finally, the reported adiponectin concentrations represent total adiponectin levels, without discriminating between the low- and more metabolically active high-molecular weight fractions. However, Kizer et al. found a similar direct positive relation for total and high-molecular weight adiponectin with cardiovascular and all-cause mortality in older people from the Cardiovascular Health Study (43).

Conclusions

In patients with type 2 diabetes and recent ACS, both baseline adiponectin and FFA levels are directly associated with the risk of MACE and death. These relationships persist after multivariable adjustment. Additional adjustment for NT-proBNP attenuates, but does not abrogate these associations. Moreover, an increase in adiponectin during the 3 months following the ACS event is associated with higher risk for all-cause and cardiovascular death after multivariable adjustment. The neutral results of the AleCardio trial may reflect a balance between beneficial and adverse effects of aleglitazar. Beneficial effects may include reduced FFA, as well as reduced glycemic indices and triglycerides and increased HDL cholesterol. Adverse effects of aleglitazar may include increased adiponectin, as well as increased LDL cholesterol and creatinine levels, as previously described (16). The present results suggest that interventions the are specifically intended to increase adiponectin are unlikely to be useful in patients with coronary heart disease.

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FIGURE LEGENDS

Figure 1. Kaplan-Meier curves of survival free of MACE, all-cause death and cardiovascular death by (A) baseline adiponectin quartiles and (B) FFA categories.

TABLES

Table 1. Baseline characteristics of AleCardio participants by adiponectin quartiles and FFA categories

		Adiponectin	Adiponectin	Adiponectin	Adiponectin		FFA	FFA	FFA	FFA	
	All	**quartile 1**	**quartile 2**	**quartile 3**	**quartile 4**		**category 1**	**category 2**	**category 3**	**category 4**	
	n=7060	n=1749	n=1750	n=1750	n=1749		n=1878	n=2308	n=1683	n=1169	
Variable [a]		0.4-2.7	2.7-4.0	4.0-6.1	6.1-50.0	**P-value***	0.1-0.3	0.4-0.5	0.6-0.7	0.8-3.3	**P-value***
Demographics											
Age, years (mean ± SD)	60.8 ± 9.9	57.2 ± 9.4	59.6 ± 9.7	61.9 ± 9.5	64.5 ± 9.6	<0.001	59.8 ± 9.7	60.3 ± 9.8	61.2 ± 10.1	62.7 ± 9.9	<0.001
Female sex [n (%)]	1909 (27)	239 (14)	372 (21)	532 (30)	754 (43)	<0.001	364 (19)	510 (22)	526 (31)	499 (43)	<0.001
Race [n (%)]						<0.001					<0.001
White	4707 (67)	1047 (60)	1188 (68)	1207 (69)	1222 (70)		1312 (70)	1511 (66)	1117 (66)	749 (64)	
Asian	1846 (26)	567 (32)	436 (25)	432 (25)	398 (23)		413 (22)	613 (27)	463 (28)	357 (31)	
Black	215 (3)	59 (3)	47 (3)	48 (3)	57 (3)		84 (4)	70 (3)	39 (2)	22 (2)	
Other	290 (4)	76 (4)	78 (4)	63 (4)	71 (4)		69 (4)	113 (5)	63 (4)	41 (4)	
Region [n (%)]						<0.001					<0.001
Europe	2485 (35)	592 (34)	641 (37)	643 (37)	584 (33)		676 (36)	773 (34)	570 (34)	454 (39)	
Asia/Pacific	1934 (27)	577 (33)	466 (27)	457 (26)	421 (24)		450 (24)	620 (27)	494 (29)	368 (31)	
North America	1954 (28)	419 (24)	483 (28)	470 (27)	564 (32)		569 (30)	655 (28)	462 (27)	263 (23)	
South America	679 (10)	157 (9)	158 (9)	178 (10)	180 (10)		178 (10)	258 (11)	156 (9)	84 (7)	
Medical history											
Prior myocardial infarction [n (%)]	1613 (23)	374 (21)	387 (22)	403 (23)	439 (25)	0.053	429 (23)	515 (22)	396 (24)	268 (23)	0.844
Prior stroke or TIA [n (%)]	551 (8)	110 (6)	128 (7)	140 (8)	168 (10)	0.003	144 (8)	172 (7)	135 (8)	98 (8)	0.777
History of heart failure [n (%)]	746 (11)	155 (9)	167 (10)	176 (10)	239 (14)	<0.001	220 (12)	211 (9)	180 (11)	133 (11)	0.038

Table 1 continued	**All**	**Adiponectin quartile 1**	**Adiponectin quartile 2**	**Adiponectin quartile 3**	**Adiponectin quartile 4**		**FFA category 1**	**FFA category 2**	**FFA category 3**	**FFA category 4**	
	n=7060	n=1749	n=1750	n=1750	n=1749		n=1878	n=2308	n=1683	n=1169	
Variable		0.4-2.7	2.7-4.0	4.0-6.1	6.1-50.0	**P-value***	0.1-0.3	0.4-0.5	0.6-0.7	0.8-3.3	**P-value***
History of hypertension [n (%)]	5498 (78)	1286 (74)	1355 (77)	1380 (79)	1431 (82)	<0.001	1418 (76)	1788 (77)	1314 (78)	959 (82)	<0.001
Smoking, current or previous [n (%)]	4332 (61)	1207 (69)	1118 (64)	1014 (58)	953 (54)	<0.001	1243 (66)	1508 (65)	988 (59)	578 (50)	<0.001
Duration of diabetes, years [median (IQR)]	5.6 (1.8-11.1)	4.5 (1.1-9.8)	5.2 (1.6-10.5)	5.7 (1.8-10.9)	7.6 (2.6-14.6)	<0.001	5.8 (1.8-11.3)	5.2 (1.7-10.4)	5.5 (1.6-11.3)	6.6 (2.3-12.4)	<0.001
BMI, kg/m^2 [median (IQR)]	29 (26-32)	29 (26-32)	29 (26-33)	29 (26-32)	28 (25-32)	<0.001	28 (25-32)	28 (26-32)	29 (26-33)	28 (25-33)	0.002
SBP, mmHg [mean ± SD]	128 ± 18	126 ± 16	128 ± 17	129 ± 18	130 ± 18	<0.001	126 ± 18	128 ± 17	129 ± 17	130 ± 18	<0.001
DBP, mmHg [mean ± SD]	76 ± 10	76 ± 9	76 ± 9	76 ± 10	76 ± 10	0.184	75 ± 10	76 ± 10	76 ± 10	77 ± 10	0.002
Index ACS event						<0.001					0.495
NSTEMI [n (%)]	2575 (36)	627 (36)	624 (36)	628 (36)	676 (39)		684 (36)	846 (37)	611 (36)	426 (36)	
STEMI [n (%)]	2775 (39)	742 (42)	713 (41)	652 (37)	643 (37)		746 (40)	932 (40)	642 (38)	446 (38)	
UA [n (%)]	1709 (24)	379 (22)	413 (24)	470 (27)	430 (25)		448 (24)	529 (23)	430 (26)	297 (25)	
Time from ACS to randomization, days (mean ± SD)	29 ± 15	29 ± 15	28 ± 15	29 ± 14	29 ± 15	0.174	28 ± 15	29 ± 15	29 ± 15	28 ± 15	0.148
Reperfusion therapy for ACS event [n (%)]	5654 (80)	1465 (84)	1423 (82)	1404 (80)	1313 (75)	<0.001	1521 (81)	1884 (82)	1306 (78)	926 (79)	0.008
Assignment to aleglitazar [n (%)]	3536 (50)	860 (49)	919 (53)	873 (50)	853 (49)	0.114	960 (51)	1162 (50)	853 (51)	553 (47)	0.194
Medication [n (%)]											
Aspirin	6604 (94)	1637 (94)	1664 (95)	1629 (93)	1615 (92)	0.009	1749 (93)	2173 (94)	1558 (93)	1103 (94)	0.120
Other anti-platelet agents	6123 (87)	1564 (89)	1547 (88)	1505 (86)	1455 (83)	<0.001	1638 (87)	1993 (86)	1451 (86)	1020 (87)	0.720
ACE inhibitors or ARB	5772 (82)	1437 (82)	1445 (83)	1447 (83)	1395 (80)	0.083	1536 (82)	1912 (83)	1360 (81)	945 (81)	0.321

Table 1 continued	**All**	**Adiponectin quartile 1**	**Adiponectin quartile 2**	**Adiponectin quartile 3**	**Adiponectin quartile 4**		**FFA category 1**	**FFA category 2**	**FFA category 3**	**FFA category 4**	
	n=7060	n=1749	n=1750	n=1750	n=1749		n=1878	n=2308	n=1683	n=1169	
Variable		0.4-2.7	2.7-4.0	4.0-6.1	6.1-50.0	**P-value***	0.1-0.3	0.4-0.5	0.6-0.7	0.8-3.3	**P-value***
Statins	6587 (93)	1648 (94)	1655 (95)	1638 (94)	1590 (91)	<0.001	1772 (94)	2148 (93)	1570 (93)	1075 (92)	0.076
Beta blockers	6385 (90)	1617 (92)	1601 (91)	1584 (91)	1530 (87)	<0.001	1710 (91)	2114 (92)	1493 (89)	1047 (90)	0.011
Diuretics	2228 (32)	422 (24)	506 (29)	582 (33)	692 (40)	<0.001	579 (31)	679 (29)	526 (31)	435 (37)	<0.001
Insulin	2065 (29)	481 (28)	483 (28)	521 (30)	563 (32)	0.006	663 (35)	578 (25)	462 (27)	353 (30)	<0.001
Sulfonylureas	2437 (35)	597 (34)	612 (35)	599 (34)	609 (35)	0.939	605 (32)	791 (34)	596 (35)	435 (37)	0.032
Biguanides	4720 (67)	1232 (70)	1215 (69)	1164 (67)	1069 (61)	<0.001	1189 (63)	1548 (67)	1176 (70)	794 (68)	<0.001

Values are presented as mean ± SD, median (IQR) or numbers (%).

*Two sides p-values for overall differences between adiponectin quartiles from ANOVA, Kruskal-Wallis or Chi-square tests.

ACE = angiotensin converting enzyme, ACS = acute coronary syndrome, ARB = angiotensin receptor blocker, DBP = diastolic blood pressure; NSTEMI = non-ST segment elevation myocardial infarction, SBP = systolic blood pressure, STEMI = ST segment elevation myocardial infarction, TIA = transient ischemic attack, UA = unstable angina.

Table 2. Baseline laboratory values by adiponectin quartiles and FFA categories

		adiponectin	adiponectin	adiponectin	adiponectin		FFA	FFA	FFA	FFA	
	All	**quartile 1**	**quartile 2**	**quartile 3**	**quartile 4**		**category 1**	**category 2**	**category 3**	**category 4**	
	n=7060	n=1749	n=1750	n=1750	n=1749		n=1878	n=2308	n=1683	n=1169	
Variable		0.4-2.7	2.7-4.0	4.0-6.1	6.1-50.0	**P-value***	0.1-0.3	0.4-0.5	0.6-0.7	0.8-3.3	**P-value***
FFA (mmol/L)	0.5 (0.3-0.7)	0.5 (0.3-0.6)	0.5 (0.3-0.6)	0.5 (0.3-0.7)	0.5 (0.4-0.7)	<0.001	n/a	n/a	n/a	n/a	n/a
Adiponectin (µg/mL)	4.0 (2.7-6.1)	n/a	n/a	n/a	n/a	n/a	3.9 (2.6-5.8)	3.7 (2.5-5.7)	4.1 (2.8-6.2)	4.6 (3.0-7.2)	<0.001
HbA_{1c} (%)	7.8 ± 1.6	7.9 ± 1.5	7.9 ± 1.7	7.7 ± 1.6	7.7 ± 1.8	0.003	7.9 ± 1.7	7.7 ± 1.6	7.8 ± 1.6	7.8 ± 1.6	0.053
HbA_{1c} mmol/mol)	62 ± 18	63 ± 16	63 ± 19	61 ± 18	61 ± 20	0.003	63 ± 19	61 ± 18	62 ± 18	62 ± 18	0.053
FPG (mmol/L)	8.3 ± 3.2	8.3 ± 2.9	8.4 ± 3.1	8.3 ± 3.2	8.2 ± 3.6	0.233	8.4 ± 3.6	8.1 ± 2.8	8.2 ± 3.0	8.7 ± 3.6	<0.001
Insulin (pmol/L)	69 (44-117)	80 (50-135)	75 (49-124)	66 (44-112)	56 (34-96)	<0.001	92 (51-185)	66 (42-107)	66 (42-105)	64 (41-95)	<0.001
HDL cholesterol (mg/dL)	1.08 ± 0.28	0.97 ± 0.21	1.04 ± 0.24	1.10 ± 0.25	1.22 ± 0.34	<0.001	1.06 ± 0.27	1.06 ± 0.27	1.09 ± 0.27	1.16 ± 0.31	<0.001
LDL cholesterol (mg/dL)	2.05 ± 0.80	1.96 ± 0.74	2.03 ± 0.79	2.06 ± 0.80	2.15 ± 0.86	<0.001	2.02 ± 0.76	2.05 ± 0.78	2.08 ± 0.84	2.07 ± 0.84	0.300
Triglycerides (mmol/L)	1.73 ± 1.08	1.93 ± 1.39	1.82 ± 1.17	1.66 ± 0.80	1.52 ± 0.77	<0.001	1.69 ± 0.91	1.72 ± 0.91	1.75 ± 1.09	1.80 ± 1.53	0.123
hs-CRP (nmol/L)	64 ± 134	57 ± 115	64 ± 146	68 ± 136	66 ± 137	0.115	59 ± 120	66 ± 141	65 ± 134	65 ± 137	0.045
eGFR (mL/min/1.73m^2)	78 ± 20	82 ± 19	80 ± 19	77 ± 20	73 ± 22	<0.001	78 ± 20	79 ± 20	78 ± 20	76 ± 21	0.031
NT-proBNP (pg/mL)	832 ± 1510	446 ± 609	595 ± 772	779 ± 995	1490 ± 2493	<0.001	794 ± 1222	741 ± 1271	841 ± 1634	1054 ± 2056	<0.001

Values are presented as mean ± SD or median (IQR).

*Two sides p-values for overall differences between adiponectin quartiles from ANOVA or Kruskal-Wallis tests.

eGFR = estimated glomerular filtration rate, FFA = free fatty acid, FPG = fasting plasma glucose, hs-CRP = high sensitive C-reactive protein, NT-proBNP = N-terminal pro b-type natriuretic peptide.

Table 3. Hazard ratios and point estimates of endpoints per doubling of adiponectin and FFA concentrations at baseline and from baseline to Month 3

				Crude model	Multivariable model	Interaction of treatment assignment Point estimate	Multivariable model + NT-proBNP
		N	Events (%)	HR (95%CI)	HR (95%CI)	(95%CI)*	HR (95%CI)
Baseline adiponectin	MACE	6998	684 (10%)	1.28 (1.18-1.38) †	1.17 (1.08-1.27) †	1.12 (0.96-1.31)	1.14 (1.04-1.26) †
	All-cause death	6998	276 (4%)	1.75 (1.56-1.98) †	1.53 (1.35-1.73) †	1.07 (0.85-1.36)	1.19 (1.02-1.39) ‡
	CV death	6998	202 (3%)	1.67 (1.45-1.92) †	1.51 (1.30-1.76) †	1.02 (0.77-1.35)	1.21 (1.01-1.44) ‡
Baseline FFA	MACE	7038	688 (10%)	1.15 (1.04-1.27) †	1.12 (1.02-1.24) ‡	0.94 (0.78-1.14)	1.02 (0.93-1.11)
	All-cause death	7038	281 (4%)	1.31 (1.11-1.54) †	1.20 (1.03-1.40) ‡	1.07 (0.79-1.44)	1.22 (1.07-1.40) †
	CV death	7038	206 (3%)	1.28 (1.06-1.54) ‡	1.19 (0.99-1.42)	1.05 (0.74-1.51)	1.14 (0.97-1.33)
Change in adiponectin	MACE	6212	443 (7%)	0.92 (0.84-1.01)	1.03 (0.93-1.15)	0.95 (0.73-1.25)	1.00 (0.90-1.10)
	All-cause death	6325	188 (3%)	1.00 (0.87-1.14)	1.20 (1.03-1.41) ‡	0.84 (0.56-1.28)	1.06 (0.90-1.24)
	CV death	6325	130 (2%)	1.03 (0.87-1.21)	1.22 (1.02-1.46) ‡	0.91 (0.55-1.49)	1.06 (0.89-1.26)
Change in FFA	MACE	6253	448 (7%)	0.96 (0.87-1.06)	0.92 (0.84-1.02)	1.12 (0.92-1.36)	0.93 (0.85-1.03)

All-cause death	6365	191 (3%)	0.88 (0.73-1.06)	0.93 (0.80-1.08)	1.01 (0.75-1.35)	0.93 (0.80-1.08)
CV death	6365	131 (2%)	0.90 (0.77-1.04)	0.90 (0.75-1.08)	1.12 (0.79-1.59)	0.89 (0.74-1.07)

Multivariable model: adjusted for treatment, baseline log_2(FFA) or log_2(adiponectin), age, sex, race, region, prior history of MI, stroke or TIA, heart failure, and hypertension, duration of diabetes, smoking history, body mass index, time from ACS to randomization, systolic and diastolic blood pressure, use of anti-hyperglycemic agents (insulin, sulfonylureas, biguanides), HbA_{1c}, fasting plasma glucose, LDL, HDL, triglyceride, hs-CRP, and eGFR. Model was stratified by ACS index event and reperfusion therapy for ACS.

Interaction model: multivariable model with extra adjustment for interaction with treatment.

* Point estimate shown is the ratio by which the HR of the multivariable model changes when going from aleglitazar to placebo.

Multivariable model + NT-proBNP: multivariable model with additional adjustment for log (NT-proBNP).

Change models were additionally adjusted for change in log_2(FFA) or log_2(adiponectin), and change in systolic and diastolic blood pressure, HbA_{1c}, fasting plasma glucose, LDL, HDL, triglyceride, hs-CRP, and eGFR from baseline to Month 3.

† $P<0.01$, ‡ $P<0.05$.

CV = cardiovascular, FFA = free fatty acid; MACE = major adverse cardiovascular events (Cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke), MI = myocardial infarction, NT-proBNP = N-terminal pro b-type natriuretic peptide, TIA = transient ischemic attack