ORIGINAL RESEARCH

Randomized Trial of the Effects of Insulin and Metformin on Myocardial Injury and Stress in Diabetes Mellitus: A Post Hoc Exploratory Analysis

Pratyaksh K. Srivastava, MD; Aruna D. Pradhan, MD, MPH; Nancy R. Cook, ScD; Paul M Ridker, MD, MPH; Brendan M. Everett, MD, MPH

***Background*—**Subclinical myocardial injury, as measured by high-sensitivity cardiac troponin T (hsTnT), and myocardial stress, as measured by N-terminal pro-B-type natriuretic peptide (NT-proBNP), are related to glycemic control in patients with type 2 diabetes mellitus, and are strong predictors of adverse cardiovascular outcomes. We sought to determine whether antihyperglycemic therapy improves measures of myocardial injury and myocardial stress in patients with type 2 diabetes mellitus.

***Methods and Results*—**We randomized, in a 2×2 factorial fashion, 438 patients with type 2 diabetes mellitus to insulin glargine, metformin, the combination, or placebo and measured changes in NT-proBNP and hsTnT after 12 weeks of therapy. At baseline, the median (Q1–Q3) plasma concentration was 35.4 (15.7–86.3) ng/L for NT-proBNP and 6.7 (4.6–10.1) ng/L for hsTnT. The adjusted (95% confidence interval) change in NT-proBNP concentration was 20.7% (7.9–35.0) in the insulin arm compared with 0.13% (−10.8 to 12.5) in the no-insulin arm (P=0.03 for comparison). These changes were not related to changes in fasting or postprandial glucose, glycated hemoglobin, weight, blood pressure, or inflammation. In the metformin arm, the adjusted change in NT-proBNP was 7.8% (−3.7 to 20.7) compared with 13.0% (0.72–26.8) in the no-metformin arm (P=0.58). No significant changes in hsTnT concentrations were observed for any of the treatment arms.

***Conclusions*—**Insulin glargine was associated with a significant 20.7% increase in NT-proBNP, a marker of myocardial stress, after 12 weeks of therapy. No change in hsTnT, a marker of myocardial injury, was observed. The changes were independent of substantial improvements in glucose control.

***Clinical Trial Registration*—**URL: http://www.clinicaltrials.gov. Unique identifier: NCT00366301. **(*J Am Heart Assoc*. 2017;6: e007268. DOI: 10.1161/JAHA.117.007268.)**

Key Words: cardiac biomarkers • cardiovascular disease • diabetes mellitus • natriuretic peptide • troponin

Patients with type 2 diabetes mellitus (T2DM) have high rates of cardiovascular disease, and cardiovascular disease remains the most common cause of death in the T2DM population.[1–4] Although a reduction in cardiovascular morbidity and mortality has been a major goal of therapy in patients with T2DM, therapeutic strategies focused on intensive glycemic control in these patients have largely failed to show a substantial impact on cardiovascular morbidity and mortality, at least in the short term.[5–8] Circulating concentrations of cardiac troponin, a marker of myocardial injury, and natriuretic peptides (NPs), markers of myocardial stress, have emerged as powerful predictors of cardiovascular risk in stable patients with and without T2DM.[9–13] Both markers have also been associated with abnormalities in glucose metabolism. For example, minor elevations in glycated hemoglobin (HbA1c) in the prediabetic range are associated with elevations in cardiac troponin.[9,14] In contrast, observational and Mendelian randomization studies suggest modest elevations in NPs protect against the development of T2DM.[15,16] In addition to their role in promoting natriuresis, diuresis, and blood pressure, NPs have a number of favorable metabolic effects, including increased lipolysis and activation of brown fat.[17–19] Because of the associations between these cardiac biomarkers, glucose metabolism, and cardiovascular risk in patients with T2DM, we hypothesized that different strategies for glucose control

From the Division of General Internal Medicine, University of California Los Angeles, Los Angeles, CA (P.K.S.); Divisions of Preventive Medicine (A.D.P., N.R.C., P.M.R., B.M.E.) and Cardiovascular Medicine (P.M.R., B.M.E.), Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Division of Cardiovascular Medicine, Veterans Affairs Boston Medical Center, West Roxbury, MA (A.D.P.).

Correspondence to: Brendan M. Everett, MD, MPH, Brigham and Women's Hospital, 900 Commonwealth Ave, Boston, MA 02115. E-mail: beverett@partners.org

Received August 2, 2017; accepted October 30, 2017.

Clinical Perspective

What Is New?

- Among patients with type 2 diabetes mellitus, randomly allocated therapy with insulin glargine for 12 weeks increases concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) by ≈20%, but does not appear to alter concentrations of high-sensitivity cardiac troponin T.
- Randomly allocated metformin did not alter concentrations of either NT-proBNP or high-sensitivity cardiac troponin T.
- The increases in NT-proBNP were independent of improvements in glycemic control, weight, blood pressure, or inflammation.

What Are the Clinical Implications?

- The etiology of the changes in NT-proBNP with insulin glargine therapy are unknown, but could reflect changes in sodium or fluid retention, or alterations in glucose metabolism.
- Whether the observed changes in NT-proBNP associate with an increased risk of congestive heart failure or other major cardiovascular events remains unknown.
- These results suggest that different antihyperglycemic agents have measurable impacts on myocardial biology beyond their effects on glucose, and raise the hypothesis that markers of myocardial stress and/or myocardial injury may have a role in guiding therapy in patients with type 2 diabetes mellitus.

might lead to clinically relevant changes in these markers of cardiac injury and cardiac stress. If a particular glucose control strategy could be identified that modified these markers of important myocardial pathophysiological processes, that same strategy might be one that could be considered as a means to reduce major cardiovascular event rates in these high-risk patients.

Methods

The data, analytical methods, and study materials will not be made available to other researchers for purposes of reproducing the results because of legal agreements with the sponsors. Individuals included in this study were enrolled in the LANCET (Lantus for C-reactive Protein Reduction in Early Treatment of Type 2 Diabetes) trial, a 2×2 factorial trial evaluating the impact of open-label insulin glargine and placebo-controlled metformin on inflammatory biomarkers in patients with recently diagnosed T2DM. LANCET was approved by the Brigham and Women's Hospital Institutional Review Board. Informed consent was obtained from all participants. The full trial design for LANCET has been described previously.[20] Briefly, LANCET enrolled 500 adults with T2DM, suboptimal glycemic control (baseline HbA1c 7–10%), and elevated high sensitivity C-reactive protein (hsCRP; ≥2.0 mg/L). Eligible participants were undergoing either nonpharmacological treatment or monotherapy with a sulfonylurea or thiazolidinedione at time of enrollment. Participants were randomized to placebo alone, placebo plus insulin glargine, metformin alone, or metformin plus insulin glargine and followed for 12 weeks. The primary end point of the trial was reduction in hsCRP, whereas the primary end point of this LANCET post hoc exploratory analysis was the change in cardiac troponin T and the change in the N-terminal fragment of pro-B-type natriuretic peptide (NT-proBNP). This study includes 438 patients from LANCET who had adequate fasting blood sample volume for measurement of cardiac troponin T and NT-proBNP at baseline (randomization, week 2) and at trial completion (week 14).

Cardiac troponin T was measured using a high-sensitivity electrochemiluminescent immunoassay (hsTnT) with a limit of blank of 3 ng/L, a limit of detection of 5 ng/L, and an established 99th percentile of the upper reference limit in a healthy population of 14 ng/L (Roche Diagnostics, Indianapolis, IN). The 10% coefficient of variation is less than this value.[21,22] In total, 93.2% of participants had hsTnT ≥3 ng/L. The 6.8% of participants with hsTnT values <3 ng/L were assigned a value of 2.9 ng/L before natural log transformation. The assay for NT-proBNP has day-to-day variability of 3.2%, 2.4%, and 2.2% at concentrations of 175, 434, and 6781 ng/L. HbA1c was estimated by turbidimetric immunoinhibition on packed red blood cells using the Hitachi 917 analyzer (Roche Diagnostics). The assay has day-to-day variability of 1.9% and 3.0% at values of 5.5% and 9.9%, respectively. Blood glucose measurements were made with glucometers (Accu-Chek Advantage; Roche Diagnostics) on capillary blood.

Statistical Analysis

Baseline characteristics of the cohort were compared across a individual randomized treatment arm using Kruskal–Wallis and chi-square tests for continuous and categorical variables, respectively. Baseline and end-of-study hsTnT and NT-proBNP values were compared using Wilcoxon signed-rank tests. The primary efficacy end point of this LANCET substudy was the percent change in hsTnT and NT-proBNP from baseline (2 weeks) to final study visit (14 weeks). Because of skewed distributions, natural log transformations were used for fasting glucose, postprandial glucose, glycated hemoglobin, hsTnT, and NT-proBNP. Changes in logs were converted to percent change for presentation. We utilized the primary comparison of the parent LANCET trial (random allocation to insulin versus no insulin; random allocation to metformin versus no metformin) as well as the secondary comparison of

each individual treatment arm to placebo (insulin glargine versus placebo, metformin versus placebo, and metformin plus insulin glargine versus placebo). Linear regression models of the log-transformed variables were used to evaluate the impact of treatment arm on percent change in fasting glucose, postprandial glucose, HbA1c, hsTnT, and NT-proBNP between randomization (week 2) and study conclusion (week 14). We also tested for the presence of an interaction between treatment groups by including a multiplicative interaction term in the model. Model 1 was adjusted for baseline biomarker level (hsTnT or NT-proBNP) and for baseline treatment stratum (baseline use of sulfonylurea or thiazolidinedione). Model 2 adjusted for the covariables in model 1 plus baseline age, sex, race, weight, body mass index, hypertension, cholesterol, history of myocardial infarction, history of heart failure, statin use, and aspirin use. Because the parent trial demonstrated significant differences in weight change during the course of the trial,[20] model 3 was adjusted for natural log-transformed change in weight from baseline in addition to the variables in model 2. Partial correlations between change in hsTnT or NT-proBNP and change in fasting glucose, postprandial glucose, HbA1c, weight, systolic blood pressure, or hsCRP over the study's duration were evaluated with partial Spearman correlation. Each correlation adjusted for baseline hsTnT or NT-proBNP and the baseline values of the correlates of interest, as well as for randomized treatment allocation.

All statistical analyses were performed on SAS (software 9.2; SAS Institute Inc, Cary, NC). A level of significance of <0.05 was used for all hypothesis testing. Because of the post hoc, hypothesis-generating nature of the study, *P* values were not adjusted for multiplicity.

Results

Median age (Q1–Q3) and duration of diagnosed diabetes mellitus (Q1–Q3) at enrollment was 54.0 (47.0–62.0) and 2.0 (0.3–5.6) years, respectively. Median (Q1–Q3) hsTnT at baseline was 6.7 (4.6–10.1) ng/L, and 93.2% of the cohort had a hsTnT above the limit of blank (≥3 ng/L). At baseline, 13.5% of the cohort had an abnormal hsTnT (≥14 ng/L). Median (Q1–Q3) baseline NT-proBNP of the cohort was 35.4 (15.7–86.3) ng/L. Baseline characteristics of the cohort, stratified by treatment arm, are shown in Table 1. No significant differences in baseline characteristics were observed across treatment arms.

We observed no significant differences in percent change in hsTnT at 14 weeks when patients randomly allocated to insulin glargine were compared with those randomly allocated to receive no insulin, or when those randomly allocated to receive metformin were compared with those randomly allocated to no metformin (Table 2). When each of the individual treatment arms was compared with placebo, we again observed no significant differences in the percent change in hsTnT concentrations after 12 weeks of therapy (Table 2). We observed no statistically significant evidence of interaction between insulin glargine and metformin assignment and change in hsTnT.

In contrast, in adjusted models, percent change from baseline in NT-proBNP was larger (20.7% [95% confidence interval [CI], 7.9, 35.0; model 3]) in patients who were randomly allocated to receive insulin than the change observed in those allocated to no insulin (0.13% [95% CI, −10.8, 12.5; model 3]; $P=0.03$ for insulin versus no insulin comparison; Table 3). When each treatment group was analyzed individually, those groups randomly allocated to receive insulin had an increase in NT-proBNP after 12 weeks of therapy that was similar in magnitude to that observed in the insulin versus no insulin analysis. Specifically, in patients assigned to placebo plus insulin glargine, we observed a 21.4% (95% CI, 3.6, 42.3) increase in NT-proBNP, and in patients assigned to metformin plus insulin glargine, we observed a 20.1% (95% CI, 2.5, 40.7) increase in NT-proBNP. However, these changes were not significantly different from the percent change observed in the placebo group (4.7% [95% CI, −11.4, 23.9]). We observed no statistically significant evidence of interaction between insulin glargine and metformin assignment and change in NT-proBNP.

The observed changes in fasting plasma glucose, postprandial glucose, and HbA1c according to randomized treatment group were similar to those observed in the parent trial (Tables 4 and 5; Figures 1 and 2).[20] We observed significant reductions in fasting plasma glucose, postprandial glucose, and HbA1c in the insulin glargine (versus no insulin glargine) and metformin (versus no metformin) treatment arms, even after adjustment for a number of possible baseline confounders, and for change in weight during the trial (Tables 4 and 5; Figure 1). The increase in NT-proBNP in the insulin glargine arm, and the lack of change in hsTnT in both arms, can also be observed (Figure 1). In sensitivity analysis stratified by fasting insulin concentration and body mass index at baseline, we observed no evidence for heterogeneity of the effect of insulin glargine on NT-proBNP, and no evidence for a significant effect on hsTnT. Each of the 4 treatment groups is displayed individually in Figure 2. The observed changes in NT-proBNP and hsTnT were not significantly correlated with changes in fasting glucose, postprandial glucose, HbA1c, weight, systolic blood pressure, or hsCRP over the duration of the study (Table 6).

Discussion

In this post hoc exploratory analysis of a randomized, placebo-controlled trial of insulin glargine, metformin, or the combination in patients with T2DM, we report that therapy with

Table 1. Baseline Characteristics of the Cohort Stratified by Randomized Treatment Arm

Variable*	Randomized Treatment Arm				Total Cohort (n=438)
	Placebo Alone (n=102)	Placebo and Insulin Glargine (n=112)	Metformin Alone (n=107)	Metformin and Insulin Glargine (n=117)	
Age, y	55.0 (46.0–62.0)	53.0 (46.0–63.0)	55.0 (47.0–64.0)	55.0 (48.0–62.0)	54.0 (47.0–62.0)
Duration of diabetes mellitus, years (interquartile range)	1.8 (0.2–5.4)	2.7 (0.4–5.9)	1.0 (0.2–6.0)	2.0 (0.3–5.2)	2.0 (0.27–5.6)
Women, N (%)	54 (53)	73 (65)	55 (51)	62 (53)	244 (55.7)
White, N (%)	77 (75)	86 (77)	77 (72)	85 (73)	325 (74.2)
Black, N (%)	19 (19)	22 (20)	27 (25)	23 (20)	91 (20.8)
Other race, N (%)	6 (6)	4 (4)	3 (3)	9 (8)	22 (5.0)
Body mass index, kg/m^2	36.4 (32.3–41.9)	35.7 (31.8–40.8)	34.6 (30.1–40.5)	34.8 (30.2–39.1)	35.3 (31.1–40.7)
Hypertension, N (%)	68 (67)	75 (67)	76 (71)	85 (73)	304 (69.4)
Myocardial infarction, N (%)	7 (7)	10 (9)	9 (8)	7 (6)	33 (7.5)
History of heart failure, N (%)	1 (1)	1 (1)	0 (0)	0 (0)	2 (0.5)
Sulfonylurea use, N (%)	32 (31)	35 (31)	35 (33)	37 (32)	139 (31.7)
Thiazolidinedione use, N (%)	13 (13)	16 (14)	14 (13)	15 (13)	58 (13.2)
Total cholesterol, mg/dL	168 (156–210)	176 (154–204)	177 (151–199)	174 (152–197)	174 (154–199)
hsCRP, mg/L	5.1 (2.5–12.3)	4.0 (2.3–6.9)	4.4 (1.9–10.4)	4.8 (2.9–7.2)	4.6 (2.4–8.1)
Glycated hemoglobin, %	6.9 (6.4–7.8)	6.9 (6.3–7.5)	6.7 (6.3–7.5)	7.1 (6.4–7.8)	6.9 (6.3–7.7)
Fasting glucose, mg/dL	147 (125–187)	148 (128–181)	147 (130–172)	144 (125–181)	147 (127–179)
2 hour postprandial glucose, mg/dL	189 (163–230)	192 (167–216)	197 (168–218)	195 (162–232)	193 (165–225)
Fasting insulin, mU/L	20.0 (13.1–27.8)	16.8 (11.8–26.1)	17.4 (10.2–29.0)	17.0 (11.0–26.2)	17.6 (11.4–27.8)
hsTnT, ng/L	6.9 (4.6–12.0)	7.7 (4.8–10.8)	6.5 (4.5–9.0)	6.5 (4.5–8.9)	6.7 (4.6–10.1)
NT-proBNP, ng/L	28.4 (11.2–86.3)	38.1 (16.3–87.0)	31.9 (15.3–82.4)	41.7 (18.5–87.2)	35.4 (15.7–86.3)

hsCRP indicates high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.
*Continuous variables presented as median (25th–75th percentile) and categorical variables presented as N (%). Continuous and categorical variables were compared across treatment arm using Kruskal–Wallis and chi-square tests, respectively, with no significant differences.

insulin glargine increases NT-proBNP by ≈20% during the course of 12 weeks of therapy. This change was not related to the substantial improvements in glucose control observed during the trial, nor was it accounted for by changes in weight, blood pressure, or hsCRP. By contrast, concentrations of hsTnT did not change significantly in any of the treatment arms, in spite of significant improvements in fasting and postprandial glucose and HbA1c.

These findings represent an important evaluation of the hypothesis that improved glucose control can reduce subclinical myocardial injury in patients with T2DM. Ambulatory patients with T2DM have circulating concentrations of cardiac troponin T that frequently exceed the 99th percentile of the upper reference limit. In this population of patients with relatively recent-onset T2DM, that proportion was 13.5%. Higher proportions have been reported in other cohorts (eg, 39% in the BARI 2D [Bypass Angioplasty Revascularization Investigation 2 Diabetes] trial).[10] In patients with diabetes mellitus with and without established cardiovascular disease, cardiac troponin concentrations are a significant predictor of myocardial infarction, stroke, heart failure, and cardiovascular death.[9,10,23,24] As a result, there is considerable interest in identifying treatment strategies that might modify troponin concentrations and thus confer cardiovascular benefit. Our data suggest that glucose control with either insulin glargine, metformin, or the combination, at least over the relatively short-term follow-up of this trial, might be added to the list of interventions (including coronary revascularization and statin therapy) that do not appear to lead to clinically meaningful reductions in troponin concentrations.[10,25]

By contrast, we observed a 20.7% increase in NT-proBNP among patients randomized to insulin glargine. This change was independent of changes in glucose control, body weight, and systolic blood pressure. Patients with T2DM tend to be obese (the median body mass index in LANCET was

Table 2. Effect of Randomized Treatment Arm on Change in High-Sensitivity Cardiac Troponin T

Treatment Arm	N	Model 1: % Change From Baseline (95% CI)*	Model 1 *P* Value†	Model 2: % Change From Baseline (95% CI)‡	Model 2 *P* Value†	Model 3: % Change From Baseline (95% CI)§	Model 3 *P* Value†
Main group effect							
Insulin glargine	229	3.4 (−1.1 to 8.2)	0.74	2.9 (−1.7 to 7.8)	0.89	3.2 (−1.6 to 8.1)	0.96
No insulin glargine	209	2.3 (−2.4 to 7.2)		2.4 (−2.4 to 7.5)		3.0 (−1.9 to 8.2)	
Metformin	224	1.6 (−3.0 to 6.3)	0.43	1.4 (−3.3 to 6.2)	0.44	1.1 (−3.7 to 6.1)	0.27
No metformin	214	4.3 (−0.47 to 9.3)		4.1 (−0.76 to 9.2)		5.2 (0.1–10.4)	
Individual group effect							
Placebo alone	102	4.6 (−2.2 to 11.9)	…	4.8 (−4.1 to 9.3)	…	6.7 (−0.6 to 14.6)	…
Placebo+insulin glargine	112	4.0 (−2.5 to 10.9)	0.91	3.6 (−3.1 to 10.7)	0.81	3.9 (−2.9 to 11.1)	0.59
Metformin alone	107	0.13 (−6.3 to 7.0)	0.37	0.29 (−6.3 to 7.3)	0.39	−0.42 (−7.1 to 6.7)	0.18
Metformin+insulin glargine	117	2.9 (−3.4 to 9.6)	0.73	2.4 (−4.1 to 9.3)	0.64	2.5 (−4.1 to 9.7)	0.43

CI indicates confidence interval.
*Model 1: adjusted for baseline sulfonylurea or thiazolidinedione use and for baseline high-sensitivity cardiac troponin T.
†*P* value for comparison with no insulin or no metformin for main group effect; comparison with placebo for individual group effect.
‡Model 2: adjusted for variables in model 1 plus for baseline age, sex, race, weight, body mass index, hypertension, cholesterol, myocardial infarction history, heart failure history, statin use, and aspirin use.
§Model 3: adjusted for variables in model 2 plus for weight change from baseline.

35 kg/m^2), and obese patients have NT-proBNP concentrations that are 10% to 30% lower than the nonobese, perhaps because of the hyperinsulinemia and insulin resistance commonly observed in obese patients.[26,27] Some have postulated that this natriuretic "handicap" might partially explain the susceptibility of obese and overweight individuals to salt retention, hypertension, and heart failure.[27,28] We and others have published evidence that elevations in NPs are associated with decreased incidence of diabetes mellitus,[15,29] and Mendelian randomization studies have suggested that these associations may be causal in nature.[16] Thus, the increases in NT-proBNP we observed with insulin therapy may represent a return of NP concentrations toward normal concentrations observed in nonobese patients.[26] However, this explanation would not account for the differences in effects of insulin glargine and metformin, or for the fact that the observed changes were independent of improvements in glucose control.

Table 3. Effect of Randomized Treatment Arm on Change in N-Terminal Pro-B-Type Natriuretic Peptide

Treatment Arm	N	Model 1: % Change From Baseline (95% CI)*	Model 1 *P* Value†	Model 2: % Change From Baseline (95% CI)‡	Model 2 *P* Value†	Model 3: % Change From Baseline (95% CI)§	Model 3 *P* Value†
Main group effect							
Insulin glargine	229	18.7 (6.8–32.0)	0.02	19.5 (7.2–33.0)	0.02	20.7 (7.9–35.0)	0.03
No insulin glargine	209	−0.5 (−11.0 to 11.2)		−0.9 (−11.4 to 10.8)		0.13 (−10.8 to 12.5)	
Metformin	224	7.9 (−3.1 to 20.1)	0.77	7.1 (−4.0 to 19.4)	0.62	7.8 (−3.7 to 20.7)	0.58
No metformin	214	10.4 (−1.1 to 23.2)		11.4 (−0.3 to 24.5)		13.0 (0.72 to 26.8)	
Individual group effect							
Placebo alone	102	2.2 (−12.9 to 19.8)	…	4.0 (−11.5 to 22.3)	…	4.7 (−11.4 to 23.9)	…
Placebo+insulin glargine	112	18.5 (1.8–37.9)	0.19	18.6 (1.7–38.4)	0.25	21.4 (3.6–42.3)	0.21
Metformin alone	107	−3.0 (−17.0 to 13.3)	0.64	−5.5 (−19.2 to 10.6)	0.40	−3.9 (−18.3 to 13.0)	0.47
Metformin+insulin glargine	117	19.0 (2.5–38.0)	0.17	20.2 (3.2–39.8)	0.21	20.1 (2.5–40.7)	0.24

CI indicates confidence interval.
*Model 1: adjusted for baseline sulfonylurea or thiazolidinedione use and for baseline N-terminal pro-B-type natriuretic peptide.
†*P* value for comparison with no insulin or no metformin for main group effect; comparison with placebo for individual group effect.
‡Model 2: adjusted for variables in model 1 plus for baseline age, sex, race, weight, body mass index, hypertension, cholesterol, myocardial infarction history, heart failure history, statin use, and aspirin use.
§Model 3: adjusted for variables in model 2 plus for weight change from baseline.

Table 4. Effect of Randomized Treatment Arm on Change in Fasting Glucose

Treatment Arm	N	Model 1: % Change From Baseline (95% CI)*	Model 1 *P* Value†	Model 2: % Change From Baseline (95% CI)‡	Model 2 *P* Value†	Model 3: % Change From Baseline (95% CI)§	Model 3 *P* Value†
Main group effect							
Insulin glargine	216	−32.8 (−35.1 to −30.5)	<0.0001	−33.0 (−35.2 to −30.6)	<0.0001	−33.0 (−35.3 to −30.7)	<0.0001
No insulin glargine	198	−9.4 (−12.6 to −6.1)		−9.8 (−13.0 to −6.5)		−9.5 (−12.6 to −6.1)	
Metformin	211	−27.6 (−30.0 to −25.0)	<0.0001	−28.0 (−30.4 to −25.4)	<0.0001	−27.6 (−30.1 to −25.1)	<0.0001
No metformin	203	−16.9 (−19.8 to −13.9)		−16.8 (−19.6 to −13.7)		−16.9 (−19.8 to −13.9)	
Individual group effect							
Placebo alone	95	0.27 (−4.7 to 5.5)	…	0.36 (−4.7 to 5.6)	…	0.65 (−4.4 to 6.0)	…
Placebo+insulin glargine	108	−30.0 (−33.3 to −26.5)	<0.0001	−29.9 (−33.2 to −26.4)	<0.0001	−30.3 (−33.6 to −26.8)	<0.0001
Metformin alone	103	−17.8 (−21.7 to −13.6)	<0.0001	−18.5 (−22.4 to −14.3)	<0.0001	−18.1 (−22.0 to −13.9)	<0.0001
Metformin+insulin glargine	108	−35.4 (−38.5 to −32.3)	<0.0001	−35.8 (−38.8 to −32.6)	<0.0001	−35.5 (−38.5 to −32.3)	<0.0001

CI indicates confidence interval.
*Model 1: adjusted for baseline sulfonylurea or thiazolidinedione use and for baseline fasting glucose.
†*P*-value for comparison with no insulin or no metformin for main group effect; comparison with placebo for individual group effect.
‡Model 2: adjusted for variables in model 1 plus for baseline age, sex, race, weight, body mass index, hypertension, cholesterol, myocardial infarction history, heart failure history, statin use, and aspirin use.
§Model 3: adjusted for variables in model 2 plus for weight change from baseline.

Alternatively, insulin therapy is known to lead to sodium and fluid retention, thus leading to increases in myocardial wall stress and NP release.[30] If so, our observations may represent an early sign of cardiovascular hazard, particularly of heart failure, in susceptible patients. Whereas observational studies have reported an increased risk of heart failure among patients with history of heart failure and T2DM receiving insulin,[31] the ORIGIN (Outcome Reduction with an Initial Glargine Intervention) randomized trial of insulin glargine versus usual care reported a nonsignificant reduction in heart failure hospitalization for patients randomized to insulin (hazard ratio, 0.90; 95% CI, 0.77–1.05).[32] The absence of

Table 5. Effect of Randomized Treatment Arm on Change in Postprandial Glucose

Treatment Arm	N	Model 1: % Change From Baseline (95% CI)*	Model 1 *P* Value†	Model 2: % Change From Baseline (95% CI)‡	Model 2 *P* Value†	Model 3: % Change From Baseline (95% CI)§	Model 3 *P* Value†
Main group effect							
Insulin glargine	209	−22.6 (−24.8 to −20.3)	<0.0001	−22.9 (−25.1 to −20.6)	<0.0001	−23.3 (−25.5 to −21.0)	<0.0001
No insulin glargine	192	−13.5 (−16.1 to −10.8)		−13.3 (−15.9 to −10.7)		−12.9 (−15.5 to −10.1)	
Metformin	204	−23.0 (−25.3 to −20.7)	<0.0001	−23.1 (−25.3 to −20.7)	<0.0001	−22.8 (−25.0 to −20.5)	<0.0001
No metformin	197	−13.2 (−15.8 to −10.5)		−13.4 (−15.9 to −10.7)		−13.6 (−16.2 to −11.0)	
Individual group effect							
Placebo alone	92	−8.0 (−12.0 to −3.8)	…	−7.9 (−11.9 to −3.7)	…	−7.6 (−11.6 to −3.4)	…
Placebo+insulin glargine	105	−17.8 (−21.1 to −14.3)	0.0003	−18.2 (−21.5 to 14.7)	<0.0002	−18.9 (−22.2 to −15.4)	<0.0001
Metformin alone	100	−18.5 (−21.9 to −14.9)	0.0001	−18.3 (−21.7 to 14.8)	<0.0002	−17.6 (−21.1 to −14.0)	0.0003
Metformin+insulin glargine	104	−26.9 (−29.9 to −23.8)	<0.0001	−27.2 (−30.2 to −24.1)	<0.0001	−27.3 (−30.2 to −24.2)	<0.0001

CI indicates confidence interval.
*Model 1: adjusted for baseline sulfonylurea or thiazolidinedione use and for baseline postprandial glucose.
†*P*-value for comparison with no insulin or no metformin for main group effect; comparison with placebo for individual group effect.
‡Model 2: adjusted for variables in model 1 plus for baseline age, sex, race, weight, body mass index, hypertension, cholesterol, myocardial infarction history, heart failure history, statin use, and aspirin use.
§Model 3: adjusted for variables in model 2 plus for weight change from baseline.

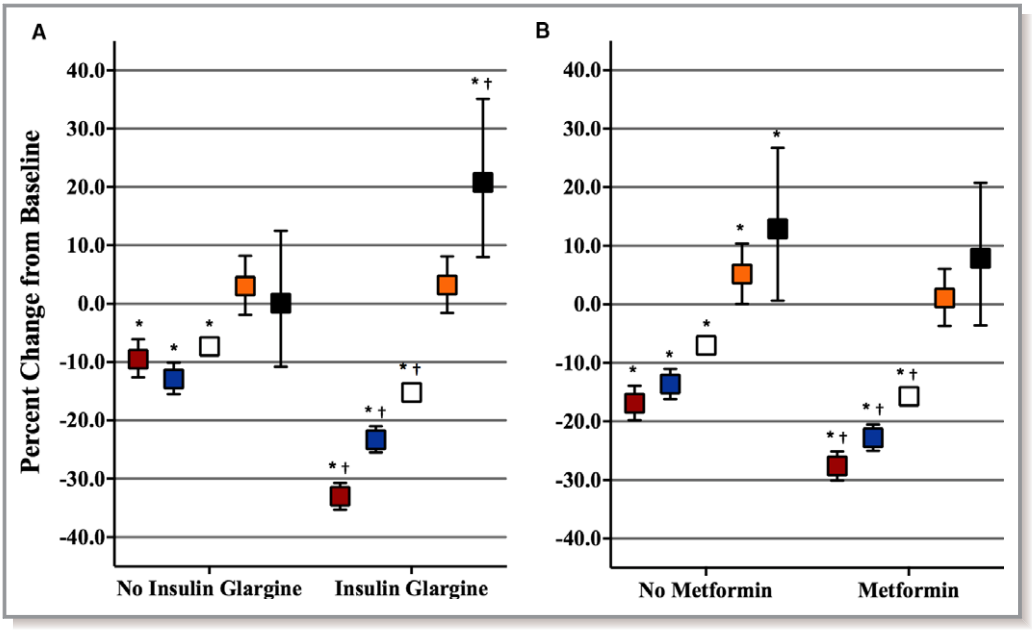

Figure 1. A and B, Impact of randomly allocated antihyperglycemic therapy on fasting glucose (red box), postprandial glucose (blue box), glycated hemoglobin (white box), high-sensitivity cardiac troponin T (orange box), and N-terminal pro-B-type natriuretic peptide (black box) by main treatment (A—insulin glargine vs no insulin glargine; B—metformin vs no metformin) group. Models adjusted for baseline biomarker, treatment stratum, age, sex, race, weight, body mass index, hypertension, cholesterol, history of myocardial infarction, history of heart failure, statin use, aspirin use, and change in weight from baseline. 95% confidence intervals for glycated hemoglobin are narrower than box presented, and so are not displayed. *Significant percent change from baseline. †Significant percent change compared with no insulin group (A) or no metformin group (B).

an effect of metformin on NT-proBNP in our study is notable, given its status as a first-line therapy for patients with glucose intolerance and newly diagnosed diabetes mellitus.

Whether the relatively substantial increase in NT-proBNP we observed in our study represents cardiovascular benefit or hazard remains unclear. In patients with T2DM in the UKPDS (United Kingdom Prospective Diabetes Study), glucose control with the combination of a sulfonylurea and insulin offered a cardiovascular benefit when compared with diet alone in long-term follow-up.[33] However, in patients with impaired fasting glucose, insulin glargine was not associated with any changes in cardiovascular event rates when compared with placebo in the ORIGIN trial.[32]

Concerns about the effects of exogenous insulin on weight gain, hypoglycemic episodes, and cardiovascular risk remain,[34–36] and the effects of novel antihyperglycemic therapies on cardiovascular events is an important regulatory concern.[37] Recently, a number of novel antihyperglycemic therapies, such as the sodium-glucose cotransporter 2 inhibitor, empagliflozin, and the glucagon-like peptide 1 analogue, liraglutide, have shown significant and clinically meaningful reductions in the occurrence of major cardiovascular events.[38,39] The effects of these agents on surrogate markers of myocardial stress (NT-proBNP) or injury (cardiac troponin) have not been well studied. One study has reported evidence that liraglutide leads to natriuresis without altering NP concentrations, and another has reported nonsignificant reductions in NT-proBNP concentrations in patients randomly allocated to liraglutide therapy.[40,41] Data on the effects of these agents on cardiac troponin are scarce. Overall, markers of cardiovascular risk—such as NPs and cardiac troponins—have not been well evaluated as tools to help guide therapies, including glycemic control strategies, for patients with T2DM.[42]

Our study has a number of strengths, including the random allocation of different antihyperglycemic therapies and the ability to compare insulin glargine to no insulin glargine and metformin to no metformin. Furthermore, the ability to compare each of the 3 active treatment arms (insulin glargine, metformin, or the combination) with a placebo arm is an important strength, but our statistical power for those comparisons was limited. Our conclusions on the effects of glucose control on circulating concentrations of hsTnT and NT-proBNP are limited by the relatively short duration of the trial. A more-sustained effort at glucose control might lead to improvements in hsTnT that were not observed within 12 weeks, although no changes were noted in the BARI 2D or LIPID (Long-Term Intervention with Pravastatin in Ischaemic

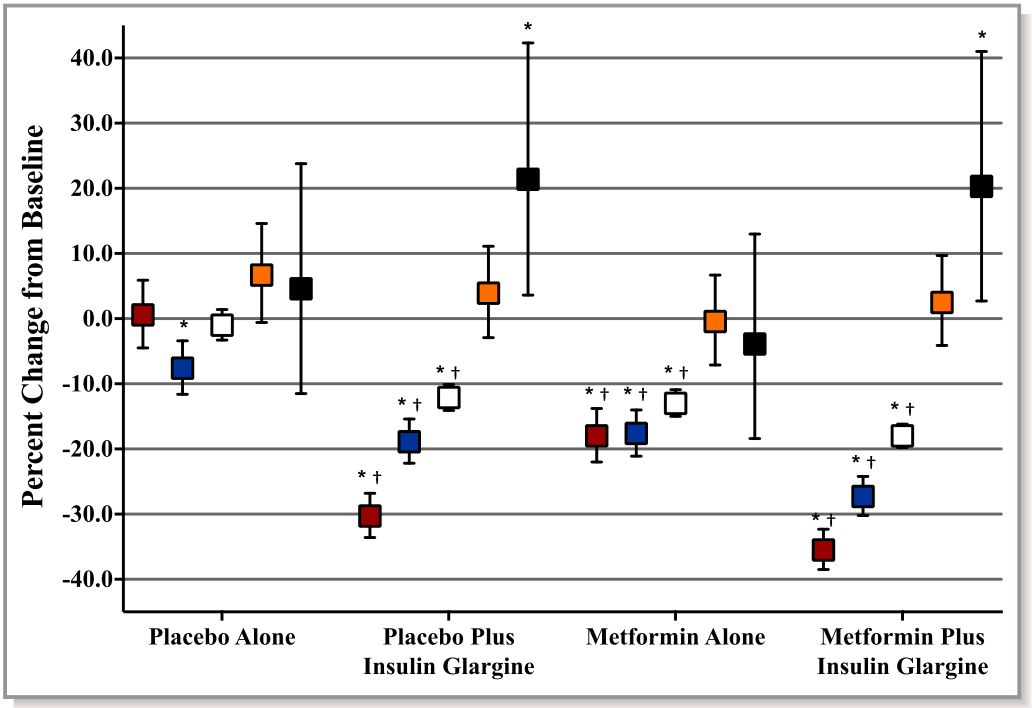

Figure 2. Impact of randomly allocated antihyperglycemic therapy on fasting glucose (red box), postprandial glucose (blue box), glycated hemoglobin (white box), high-sensitivity cardiac troponin T (orange box), and N-terminal pro-B-type natriuretic peptide (black box) by individual treatment group. Models adjusted for baseline biomarker, treatment stratum, age, sex, race, weight, body mass index, hypertension, cholesterol, history of myocardial infarction, history of heart failure, statin use, aspirin use, and change in weight from baseline. *Significant percent change from baseline. †Significant percent change compared with placebo alone group.

Disease) studies after 1 year of follow-up.[10,25] We did not collect measures of renal function, urine microalbumin, New York Heart Association class, or a history of hypoglycemic episodes. We would anticipate that these covariates would be balanced at baseline by randomization across the 4 treatment arms of this study, as were the other baseline covariates described in Table 1. Finally, the study was not designed to collect the occurrence of major cardiovascular events, such as myocardial infarction or heart failure, so we cannot relate the observed changes in NT-proBNP to end points that have more clinical relevance to patients.

Table 6. Partial Spearman Correlations Between Change From Baseline to Follow-up in hsTnT and NT-proBNP and Change From Baseline to Follow-up in Fasting Glucose, Postprandial Glucose, Hb1AC, Weight, Systolic Blood Pressure, and hsCRP

	Spearman Partial Correlation Coefficient (rho)*	
	Δ hsTnT	Δ NT-proBNP
Δ Fasting glucose	−0.004	−0.002
Δ Postprandial glucose	0.06	0.02
Δ Hb1AC	0.03	0.01
Δ Weight	−0.07	0.01
Δ Systolic blood pressure	−0.02	−0.08
Δ hsCRP	−0.0002	0.02

Hb1AC indicates glycated hemoglobin; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide. *Partial correlations adjusted for baseline hsTnT or NT-proBNP, baseline values of correlates of interest, and for randomized treatment assignment. None of the correlations were statistically significant (each $P \geq 0.12$).

Conclusions

In this ancillary study of the LANCET randomized trial, we found that insulin glargine led to an ≈20% increase in concentrations of NT-proBNP after 12 weeks of therapy compared with little change in noninsulin groups. No changes in NT-proBNP were observed with randomly allocated metformin therapy, and no changes in hsTnT were noted in any of the treatment arms. None of the observed changes in NT-proBNP were related to improvements in glucose control, weight, systolic blood pressure, or markers of inflammation. Whether the observed changes in NT-proBNP associate with changes in cardiovascular risk, as well as the biological mechanisms underlying the changes,

remains unknown. These results suggest that different antihyperglycemic agents have measurable impacts on myocardial biology beyond their effects on glucose, and raise the hypothesis that markers of myocardial stress and/or myocardial injury may have a role in guiding therapy in patients with type 2 diabetes mellitus.

Sources of Funding

This research was supported by an investigator-initiated research grant from Roche Diagnostics (Indianapolis, Indiana) to Dr Everett. The LANCET trial was funded by Sanofi-Aventis (Bridgewater, NJ). *Role of the Sponsor:* The funder had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Disclosures

Dr Pradhan reports funding from Kowa Research Institute. Dr Ridker reports grants from the NHLBI, Novartis, Pfizer, and Kowa. Dr Everett reports investigator-initiated research awards from Roche Diagnostics and Novartis and serves as consultant/advisor for Roche Diagnostics and Abbott Diagnostics. The remaining authors have no disclosures to report.

References

1. Emerging Risk Factors C, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet*. 2010;375:2215–2222.
2. Fox CS, Coady S, Sorlie PD, Levy D, Meigs JB, D'Agostino RB Sr, Wilson PW, Savage PJ. Trends in cardiovascular complications of diabetes. *JAMA*. 2004;292:2495–2499.
3. Preis SR, Hwang SJ, Coady S, Pencina MJ, D'Agostino RB Sr, Savage PJ, Levy D, Fox CS. Trends in all-cause and cardiovascular disease mortality among women and men with and without diabetes mellitus in the Framingham Heart Study, 1950 to 2005. *Circulation*. 2009;119:1728–1735.
4. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia*. 2001;44(Suppl 2):S14–S21.
5. Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med*. 2008;358:2545–2559.
6. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD; VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med*. 2009;360:129–139.
7. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2008;358:2560–2572.
8. Cushman WC, Evans GW, Byington RP, Goff DC Jr, Grimm RH Jr, Cutler JA, Simons-Morton DG, Basile JN, Corson MA, Probstfield JL, Katz L, Peterson KA, Friedewald WT, Buse JB, Bigger JT, Gerstein HC, Ismail-Beigi F. Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med*. 2010;362:1575–1585.
9. Everett BM, Cook NR, Magnone MC, Bobadilla M, Kim E, Rifai N, Ridker PM, Pradhan AD. Sensitive cardiac troponin T assay and the risk of incident cardiovascular disease in women with and without diabetes mellitus: the Women's Health Study. *Circulation*. 2011;123:2811–2818.
10. Everett BM, Brooks MM, Vlachos HE, Chaitman BR, Frye RL, Bhatt DL; BARI 2D Study Group. Troponin and cardiac events in stable ischemic heart disease and diabetes. *N Engl J Med*. 2015;373:610–620.
11. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, Vasan RS. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med*. 2004;350:655–663.
12. Di Angelantonio E, Chowdhury R, Sarwar N, Ray KK, Gobin R, Saleheen D, Thompson A, Gudnason V, Sattar N, Danesh J. B-type natriuretic peptides and cardiovascular risk: systematic review and meta-analysis of 40 prospective studies. *Circulation*. 2009;120:2177–2187.
13. Everett BM, Berger JS, Manson JE, Ridker PM, Cook NR. B-type natriuretic peptides improve cardiovascular disease risk prediction in a cohort of women. *J Am Coll Cardiol*. 2014;64:1789–1797.
14. Rubin J, Matsushita K, Ballantyne CM, Hoogeveen R, Coresh J, Selvin E. Chronic hyperglycemia and subclinical myocardial injury. *J Am Coll Cardiol*. 2012;59:484–489.
15. Everett BM, Cook N, Chasman DI, Magnone MC, Bobadilla M, Rifai N, Ridker PM, Pradhan AD. Prospective evaluation of B-type natriuretic peptide concentrations and the risk of type 2 diabetes in women. *Clin Chem*. 2013;59:557–565.
16. Pfister R, Sharp S, Luben R, Welsh P, Barroso I, Salomaa V, Meirhaeghe A, Khaw KT, Sattar N, Langenberg C, Wareham NJ. Mendelian randomization study of B-type natriuretic peptide and type 2 diabetes: evidence of causal association from population studies. *PLoS Med*. 2011;8:e1001112.
17. Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, Yamahara K, Taura D, Inuzuka M, Sonoyama T, Nakao K. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. *Diabetes*. 2009;58:2880–2892.
18. Bordicchia M, Liu D, Amri EZ, Ailhaud G, Dessi-Fulgheri P, Zhang C, Takahashi N, Sarzani R, Collins S. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*. 2012;122:1022–1036.
19. Engeli S, Birkenfeld AL, Badin PM, Bourlier V, Louche K, Viguerie N, Thalamas C, Montastier E, Larrouy D, Harant I, de Glisezinski I, Lieske S, Reinke J, Beckmann B, Langin D, Jordan J, Moro C. Natriuretic peptides enhance the oxidative capacity of human skeletal muscle. *J Clin Invest*. 2012;122:4675–4679.
20. Pradhan AD, Everett BM, Cook NR, Rifai N, Ridker PM. Effects of initiating insulin and metformin on glycemic control and inflammatory biomarkers among patients with type 2 diabetes: the LANCET randomized trial. *JAMA*. 2009;302:1186–1194.
21. Giannitsis E, Becker M, Kurz K, Hess G, Zdunek D, Katus HA. High-sensitivity cardiac troponin T for early prediction of evolving non-ST-segment elevation myocardial infarction in patients with suspected acute coronary syndrome and negative troponin results on admission. *Clin Chem*. 2010;56:642–650.
22. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem*. 2010;56:254–261.
23. de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, Hashim I, Berry JD, Das SR, Morrow DA, McGuire DK. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. *JAMA*. 2010;304:2503–2512.
24. Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, Hoogeveen RC, Liu X, Astor BC, Mosley TH, Folsom AR, Heiss G, Coresh J, Ballantyne CM. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. *Circulation*. 2011;123:1367–1376.
25. White HD, Tonkin A, Simes J, Stewart R, Mann K, Thompson P, Colquhoun D, West M, Nestel P, Sullivan D, Keech AC, Hunt D, Blankenberg S; LIPID Study Investigators. Association of contemporary sensitive troponin I levels at baseline and change at 1 year with long-term coronary events following myocardial infarction or unstable angina: results from the LIPID Study (Long-Term Intervention With Pravastatin in Ischaemic Disease). *J Am Coll Cardiol*. 2014;63:345–354.
26. Khan AM, Cheng S, Magnusson M, Larson MG, Newton-Cheh C, McCabe EL, Coviello AD, Florez JC, Fox CS, Levy D, Robins SJ, Arora P, Bhasin S, Lam CS, Vasan RS, Melander O, Wang TJ. Cardiac natriuretic peptides, obesity, and insulin resistance: evidence from two community-based studies. *J Clin Endocrinol Metab*. 2011;96:3242–3249.
27. Wang TJ, Larson MG, Keyes MJ, Levy D, Benjamin EJ, Vasan RS. Association of plasma natriuretic peptide levels with metabolic risk factors in ambulatory individuals. *Circulation*. 2007;115:1345–1353.

28. Dessi-Fulgheri P, Sarzani R, Tamburrini P, Moraca A, Espinosa E, Cola G, Giantomassi L, Rappelli A. Plasma atrial natriuretic peptide and natriuretic peptide receptor gene expression in adipose tissue of normotensive and hypertensive obese patients. *J Hypertens*. 1997;15:1695–1699.
29. Lazo M, Young JH, Brancati FL, Coresh J, Whelton S, Ndumele CE, Hoogeveen R, Ballantyne CM, Selvin E. NH2-terminal pro-brain natriuretic peptide and risk of diabetes. *Diabetes*. 2013;62:3189–3193.
30. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest*. 1975;55:845–855.
31. Smooke S, Horwich TB, Fonarow GC. Insulin-treated diabetes is associated with a marked increase in mortality in patients with advanced heart failure. *Am Heart J*. 2005;149:168–174.
32. ORIGIN Trial Investigators, Gerstein HC, Bosch J, Dagenais GR, Diaz R, Jung H, Maggioni AP, Pogue J, Probstfield J, Ramachandran A, Riddle MC, Ryden LE, Yusuf S. Basal insulin and cardiovascular and other outcomes in dysglycemia. *N Engl J Med*. 2012;367:319–328.
33. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*. 2008;359:1577–1589.
34. Holden SE, Currie CJ. Endogenous hyperinsulinaemia and exogenous insulin: a common theme between atherosclerosis, increased cancer risk and other morbidities. *Atherosclerosis*. 2012;222:26–28.
35. Pontiroli AE, Miele L, Morabito A. Increase of body weight during the first year of intensive insulin treatment in type 2 diabetes: systematic review and meta-analysis. *Diabetes Obes Metab*. 2011;13:1008–1019.
36. Rensing KL, Reuwer AQ, Arsenault BJ, von der Thusen JH, Hoekstra JB, Kastelein JJ, Twickler TB. Reducing cardiovascular disease risk in patients with type 2 diabetes and concomitant macrovascular disease: can insulin be too much of a good thing? *Diabetes Obes Metab*. 2011;13:1073–1087.
37. U.S. Food And Drug Administration. Guidance for Industry Diabetes mellitus—evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); December 2008: https://www.fda.gov/downloads/Drugs/.../Guidances/ucm071627.pdf. Accessed December 7, 2017.
38. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117–2128.
39. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2016;375:311–322.
40. Nomoto H, Miyoshi H, Furumoto T, Oba K, Tsutsui H, Miyoshi A, Kondo T, Tsuchida K, Atsumi T, Manda N, Kurihara Y, Aoki S; SAIS Study Group. A comparison of the effects of the GLP-1 analogue liraglutide and insulin glargine on endothelial function and metabolic parameters: a randomized, controlled Trial sapporo athero-incretin study 2 (SAIS2). *PLoS One*. 2015;10:e0135854.
41. Lovshin JA, Barnie A, DeAlmeida A, Logan A, Zinman B, Drucker DJ. Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. *Diabetes Care*. 2015;38:132–139.
42. American Diabetes Association. Approaches to glycemic treatment. *Diabetes Care*. 2016;39 (Supplement 1):S52–S59. DOI:10.2337/dc16-S010.