Effect of Insulin Glargine and n-3FA on Carotid Intima-Media Thickness in People With Dysglycemia at High Risk for Cardiovascular Events

The Glucose Reduction and Atherosclerosis Continuing Evaluation Study (ORIGIN-GRACE)

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OBJECTIVE—To evaluate the effects of insulin glargine and n-3 polyunsaturated fatty acid (n-3FA) supplements on carotid intima-media thickness (CIMT).

RESEARCH DESIGN AND METHODS—We enrolled 1,184 people with cardiovascular (CV) disease and/or CV risk factors plus impaired fasting glucose, impaired glucose tolerance, or early type 2 diabetes in a randomized multicenter 2 × 2 factorial design trial. Participants received open-label insulin glargine (targeting fasting glucose levels ≤5.3 mmol/L [95 mg/dL]) or standard glycemic care and double-blind therapy with a 1-g capsule of n-3FA or placebo. The primary trial outcome was the annualized rate of change in maximum CIMT for the common carotid, bifurcation, and internal carotid artery segments. Secondary outcomes were the annualized rates of change in maximum CIMT for the common carotid and the common carotid plus bifurcation, respectively. Baseline followed by annual ultrasounds were obtained during a median follow-up of 4.9 years.

RESULTS—Compared with standard care, insulin glargine reduced the primary CIMT outcome, but the difference was not statistically significant (difference = 0.0030 ± 0.0021 mm/year; $P = 0.145$) and significantly reduced the secondary CIMT outcomes (differences of 0.0033 ± 0.0017 mm/year [$P = 0.049$] and 0.0045 ± 0.0021 mm/year [$P = 0.032$], respectively). There were no differences in the primary and secondary outcomes between the n-3FA supplement and placebo groups.

CONCLUSIONS—In people with CV disease and/or CV risk factors and dysglycemia, insulin glargine used to target normoglycemia modestly reduced CIMT progression, whereas daily supplementation with n-3FA had no effect on CIMT progression.

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Atherosclerosis is the major cause of death and disability in people with type 2 diabetes and lesser degrees of dysglycemia (1,2). Large epidemiological studies show consistent independent associations between glycemia and cardiovascular (CV) risk (1–4), and the metabolic abnormalities associated with dysglycemia promote atherosclerosis (5). Exogenous insulin can provide effective glycemic control, but its effects on atherosclerosis are unknown. Moreover, some studies suggest possible proatherogenic effects (6,7).

Essential long-chain n-3 polyunsaturated fatty acids (n-3FA) may have beneficial effects on atherosclerosis (8). Higher intake of fish or n-3FA supplements is associated with lower rates of coronary heart disease and death (9,10) and lower atherosclerotic burden (11,12), and some, but not all, previous trials reported reduced CV events in patients receiving n-3FA supplements (13–16). The effects of these supplements on human atherosclerosis progression were evaluated in a few small studies, which were inconclusive (17–21).

Therefore, we evaluated the effects of insulin glargine and n-3FA supplements on carotid intima-media thickness (CIMT) in people with dysglycemia and additional risk factors for atherosclerosis progression in a substudy of the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial (22–24).

RESEARCH DESIGN AND METHODS

Study design and study population

The Glucose Reduction and Atherosclerosis Continuing Evaluation substudy of ORIGIN (ORIGIN-GRACE) is an investigator-initiated, randomized, controlled, parallel-group study with a 2 × 2 factorial design. Clinical eligibility criteria, study interventions,

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and follow-up procedures are those described in detail previously (22–24), with the addition of serial carotid ultrasound (CUS) examinations. The study was conducted at 32 ORIGIN centers in seven countries, selected based on interest and availability of adequate ultrasound equipment, which met preset technical specifications, and expert sonographers, who met predefined performance criteria. Funding and regulatory support were provided by Sanofi, and capsules containing n-3FA and placebo were provided by Pronova BioPharma, Norway. Project coordination, data management, and statistical analyses were independently provided by the Population Health Research Institute in Hamilton, Canada, which was also the site for the Core CUS Laboratory. The study was approved by the ethics review boards of all participating institutions, and all participants provided written informed consent.

Between 5 February 2004 and 27 December 2005, we enrolled people ≥50 years of age with dysglycemia, defined as early diabetes on no more than one oral glucose-lowering drug, impaired glucose tolerance (IGT), or impaired fasting glucose (IFG) and with known CV disease and/or CV risk factors (detailed clinical eligibility criteria are published [22–24] and are summarized in Supplementary Appendix 2). In addition, patients were required to have an adequate baseline CUS examination, defined as a scan allowing reliable measurements from a minimum of four predefined carotid arterial segments, as per the Core Ultrasound Laboratory's review.

Randomization, study interventions, allocation concealment, and follow-up

Eligible participants were randomized by an automated telephone randomization system (using randomly varying block sizes, stratified by center) according to a 2 × 2 factorial design to *1*) either insulin glargine (Lantus; Sanofi) or standard approaches to glycemic control and *2*) either n-3FA (Omacor 1 g; Pronova BioPharma AS, Lysaker, Norway) containing eicosapentaenoic acid (EPA) 465 mg and docosahexaenoic acid (DHA) 375 mg or matching placebo containing ~1 g olive oil. The randomization sequence was concealed, and all study personnel (except one unblinded statistician at the project office) were unaware of the randomization procedure. The insulin glargine arm of the study used a prospective, randomized, open, blinded end point design (PROBE), so that study participants and site investigators were not blinded but all personnel at the Core CUS Laboratory and all other study personnel and investigators involved in event adjudication and data analysis were blinded to treatment assignment. Participants assigned to insulin glargine added one evening injection to their glycemic-control regimen and increased the dose at least once weekly, targeting a self-measured fasting plasma glucose (FPG) level of 5.3 mmol/L (95 mg/dL) or less. Participants assigned to standard care were treated on the basis of the investigator's best judgment and local guidelines. The n-3FA arm of the trial was blinded to study participants, site investigators, and all local and central trial personnel.

Study visits occurred at 0.5, 1, 2, and 4 months after randomization and every 4 months thereafter. FPG and glycated hemoglobin (HbA_{1c}) levels were measured at 4 months, 8 months, and annually, and fasting lipid levels were measured at baseline, 2 years, and study end in all participants. A food frequency questionnaire was administered at randomization, at 2 years, and at the end of the study, and the dietary intake of EPA and DHA was calculated using the Department of Agriculture National Nutrient Database for Standard Reference, release 23 (USDA Food Search for Windows, version 1.0).

Quantitative carotid ultrasonography

CUS examinations were performed at baseline and yearly thereafter until 1–1.3 years prior to the final ORIGIN study visit (average six scans per participant). The ultrasound methods have been reviewed in detail previously (25). Sonographer training, quality control, and CIMT measurements (readings) were performed by the Core Laboratory. Standardized and validated scanning and measurement protocols were used. All CUS scans were performed by trained and certified sonographers using high-resolution imaging systems with linear array transducers operating at a fundamental frequency of at least 7.5 MHz (for each subject, the same ultrasound imaging system and transducer were used throughout the study). A transverse B-mode scan was followed by a circumferential longitudinal scan, aimed at recording the maximum CIMT in each of 12 carotid artery segments (1-cm long), which were defined relative to the carotid flow divider as the near and far walls of the internal, bifurcation, and common left and right carotid arteries. Three trained and certified readers unaware of treatment assignment performed all measurements using the Image-ProPlus software (Media Cybernetics, Silver Spring, MD). For each carotid arterial segment, the reader selected a minimum of three frames showing the thickest CIMT. The leading edge (far wall) and the trailing edge (near wall) of the boundaries between the lumen and media and the media and adventitia were traced, obtaining measurements of segment maximum and mean CIMT. Scans were read in batch fashion and in random order for each individual in order to exclude potential reader drift in measurements and to ensure use of similar anatomical landmarks. Batches were read by a single reader to avoid interreader variability. Intraclass correlation coefficients for 250 paired baseline CUS examinations performed maximum 10 days apart were 0.98 for the average maximum CIMT from 12 carotid artery segments and ranged from 0.93 to 0.98 for segment maximum and mean CIMT measurements. At study end, intraclass correlation coefficients evaluated on 26 paired CUS examinations were 0.95 for the average maximum CIMT from 12 carotid artery segments and ranged from 0.87 to 0.98 for segment maximum and mean CIMT measurements. Completeness of data by carotid arterial segment was as follows: 99% for the common carotid far wall, 96% for the common carotid near wall, 94% for the bifurcation far wall, 91% for the bifurcation near wall, 71% for the internal far wall, and 55% for the internal near wall.

Study outcomes

The primary outcome was the annualized change in the maximum CIMT for the near and far walls of the right and left common carotid, bifurcation, and internal carotid artery segments (12 carotid artery segments) based on all scans performed during the study. The secondary outcomes were the annualized change in the maximum common carotid CIMT (four segments, the near and far walls of the right and left common carotid artery segments) and the annualized change in the maximum CIMT for the common carotid and bifurcation (eight segments, the near and far walls of the right and left common carotid and bifurcation). An additional CUS outcome was the annualized change in the maximum far wall CIMT (six segments, the right and left far walls of the common carotid, bifurcation, and internal carotid artery segments). CV

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outcome events were collected and adjudicated as part of the parent ORIGIN trial.

Statistical analysis

Sample size calculations showed that 800 participants would provide 80% power to detect a 25% treatment effect at the margins of the factorial, based on a repeated-measures analysis, assuming a control progression rate of 0.017 mm/year for the primary outcome, five CUS measurements per study participant, baseline average maximum CIMT of 1.15 in the treatment and control groups, a correlation between repeated measurements of 0.84 (between variance = 0.09613 and total variance = 0.11487, as estimated from another CIMT trial performed by our group in a high-risk population, the Study to Evaluate Carotid Ultrasound with Ramipril and vitamin E [SECURE]) (26), and no significant interaction between the treatments. In light of possible lower CIMT progression rates, and allowing for a 5% attrition rate, we increased the sample size, which was set a priori to 1,100 participants.

All analyses are by intention to treat and were performed in SAS version 9.1 for Solaris. The primary analyses compared the primary, secondary, and additional CUS outcomes between the insulin glargine versus standard glycemic care and between the n-3FA versus the placebo groups, after confirming that there was no significant interaction between the study treatments for the primary, secondary, or additional CUS outcomes (P = 0.496, 0.749, 0.789, and 0.353, respectively, for interaction terms in the regression models). The main efficacy analysis included all participants with at least one adequate CUS examination after the baseline scan. As previously described (25,27–29), a repeated-measures linear mixed-effects model was used to analyze the annualized rate of change in maximum CIMT, including all segment maximum measurements for each patient as the dependent variables, with random intercepts and slopes as a function of time and fixed effects for geographic region, age, sex, treatment assignment for the other arm of the factorial design, carotid segment, treatment, time, and interaction between time and treatment. Testing was two sided and conducted with a 5% type I error rate. Similar analyses were used for the secondary and additional CIMT outcomes. Additional models were computed with the addition of fixed effects for baseline and average on treatment HbA_{1c}, FPG, triglyceride, LDL cholesterol, HDL cholesterol, and blood pressure (BP) levels (entered individually and sequentially). Prespecified subgroup analyses were performed for age (above and below 65 years), sex, baseline glycemic status (diabetes or no diabetes), CV history (previous CV event or no previous CV event), baseline CIMT, HbA_{1c}, FPG, and triglyceride levels (above and below median), and baseline treatment with statins and ACE inhibitors or angiotensin receptor blockers (ARBs). Laboratory measurements were analyzed by ANCOVA, using terms for treatment assignment to the other arm of the factorial, baseline metabolic status (known diabetes, new diabetes, or IFG/IGT), status with respect to a history of CV disease, and the baseline laboratory measurement as covariate. BP and heart rate changes were analyzed by repeated-measures analyses, and clinical outcomes were analyzed as part of the main ORIGIN trial, as previously described (22–24).

RESULTS

Study population, adherence, and safety

A total of 1,184 participants at 32 centers met clinical and CUS eligibility criteria; 580 were randomly assigned to insulin glargine, 604 to standard glycemic care, 585 to n-3FA, and 599 to placebo. Of these, 25 died before the scheduled first postrandomization CUS at the 1 year visit and 68 did not have any adequate follow-up CUS examination. In total, 1,091 patients (92.2%) had at least one adequate follow-up CUS examination and are evaluated in the primary efficacy analysis (533 allocated to insulin glargine and 558 to standard glycemic care; 539 to n-3FA and 552 to placebo). All 1,184 participants were followed for safety and clinical outcomes for a median of 6.2 years (interquartile range [IQR] 5.8–6.5). The median time from the baseline to the study end CUS was 4.9 years (IQR 3.0–5.0). At study end, vital status was unknown in two participants (Supplementary Fig. 1).

Baseline characteristics of the 1,184 participants randomized in the GRACE study were well balanced between the treatment groups, were generally similar to those of the entire ORIGIN study population (except for the geographic distribution, with proportionally more participants from South America and fewer from Europe in GRACE compared with ORIGIN), and confirm participants' high risk. Baseline CIMT did not differ significantly between the treatment groups (Table 1).

Adherence to insulin glargine at 1, 2, 3, 4, and 5 years and at study end was 94.0, 93.0, 91.0, 90.1, 89.3, and 86.3%, respectively. Nonstudy insulin was used at study end in 3.1% of patients in the insulin glargine and 10.4% in the standard care group. For the n-3FA supplement arm of the trial, adherence rates were 97.2% for active n-3FA and 97.3% for placebo at 1 year, 96.6 and 95.8% at 2 years, 95.5 and 94.6% at 3 years, 94.5 and 94.5% at 4 years, 93.8 and 94.3% at 5 years, and 91.4 and 92.6%, respectively, at study end. A total of 91 participants (15.7%) permanently discontinued insulin glargine, most frequently due to patient preference (76 patients) and hypoglycemia (9 patients). Sixty-six (11.3%) participants in the n-3FA group and 64 (10.7%) in the placebo group permanently discontinued the study drug, most frequently due to patient preference (45 and 43 patients, respectively), abdominal discomfort (4 and 2 patients, respectively), and lower gastrointestinal problems (2 and 4 patients, respectively). Intracranial bleeding occurred in four patients receiving n-3FA and four patients in the placebo group. Baseline characteristics, adherence rates, and side effects of the 1,091 patients included in the primary efficacy analysis were similar to those of the entire GRACE study population (Supplementary Tables 1 and 2).

Changes in CV risk factor levels

Compared with the standard care group, FPG, HbA_{1c}, and triglyceride levels were lower in the insulin glargine group at 2 years and at study end (Fig. 1). There were no significant differences in BP, heart rate, and in total, LDL, and HDL cholesterol levels (Supplementary Fig. 2). There were no significant differences in BP, heart rate, lipid, and glycemia measures between the n-3FA and the placebo groups (Supplementary Fig. 3). Dietary n-3FA consumption remained similar in the n-3FA and placebo groups at 2 years (median 58.8 mg/day [IQR 0.7–230] and 60.3 mg/day [0.3–230.2]) and at study end (median 95.3 mg/day [0.7–287.1] and 93.1 mg/day [1.5–268.4]).

Primary efficacy analysis: treatment effects on CIMT

For the insulin glargine arm of the study, we observed a statistically nonsignificant reduction in CIMT progression for the

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Table 1—*Baseline characteristics by treatment group*

	Insulin glargine (n = 580)	Standard care (n = 604)	n-3FAs (n = 585)	Placebo (n = 559)
Demographic characteristics				
Mean age (years)	63.0 ± 7.9	63.2 ± 7.8	63.0 ± 7.8	63.2 ± 7.9
Women, *n* (%)	206 (35.5)	223 (36.9)	224 (38.3)	205 (34.2)
Geographic distribution, *n* (%)				
North America	80 (13.8)	86 (14.2)	81 (13.8)	85 (14.2)
South America	405 (69.8)	419 (69.4)	407 (69.6)	417 (69.6)
Europe	6 (1.0)	8 (1.3)	7 (1.1)	7 (1.1)
India	87 (15.0)	86 (14.2)	86 (14.7)	87 (14.5)
Australia	2 (0.3)	5 (0.8)	4 (0.7)	3 (0.5)
History of CV disease and of CV risk factors, *n* (%)				
Prior CV event	303 (52.2)	280 (46.4)	287 (49.1)	296 (49.4)
Prior or new diabetes	524 (90.3)	547 (90.6)	536 (91.6)	535 (89.3)
IGT or IFG	56 (9.7)	57 (9.4)	49 (8.4)	64 (10.7)
Hypertension	466 (80.3)	485 (80.3)	466 (79.7)	485 (81.0)
Hyperlipidemia	344 (59.3)	363 (60.1)	344 (58.8)	363 (60.6)
Current smoking	55 (9.5)	67 (11.1)	59 (10.1)	63 (10.5)
Microalbuminuria or macroalbuminuria	107 (18.4)	96 (15.9)	97 (16.6)	106 (17.7)
Dietary EPA-DHA intake (mg/day)*	47.3 (0.3–230.2)	53.3 (0.3–230.2)	53.3 (0.7–230.0)	53.4 (0.3–230.2)
Physical examination				
BMI (kg/m^2)	29.5 ± 5.4	30.1 ± 5.9	29.8 ± 5.6	30.1 ± 5.8
Heart rate (bpm)	71.1 ± 13.0	70.6 ± 12.6	70.4 ± 12.6	70.6 ± 12.9
Systolic BP (mmHg)	146.3 ± 23.1	146.2 ± 22.3	145.7 ± 21.8	147.8 ± 23.6
Diastolic BP (mmHg)	84.0 ± 12.4	84.0 ± 12.4	83.6 ± 12.0	84.8 ± 12.9
Ankle/brachial index	1.16 ± 0.2	1.16 ± 0.2	1.16 ± 0.2	1.16 ± 0.2
Laboratory investigations				
FPG (mmol/L)	7.3 ± 2.0	7.2 ± 2.1	7.3 ± 2.0	7.2 ± 2.0
Glycated hemoglobin (%)	6.8 ± 1.0	6.7 ± 1.0	6.8 ± 1.0	6.7 ± 1.0
Total cholesterol (mmol/L)	4.9 ± 1.1	4.9 ± 1.1	4.9 ± 1.1	5.0 ± 1.1
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
LDL cholesterol (mmol/)	3.0 ± 1.0	2.9 ± 1.0	2.9 ± 1.0	3.0 ± 1.0
Triglycerides (mmol/L)	1.9 ± 1.2	1.9 ± 1.1	1.8 ± 1.0	1.9 ± 1.3
Serum creatinine (μmol/)	91.3 ± 21.2	89.8 ± 22.2	89.9 ± 21.8	91.3 ± 21.2
Estimated glomerular filtration rate (mL/min/1.73 m^2)	73.9 ± 17.8	76.0 ± 23.3	75.1 ± 19.5	74.3 ± 18.3
Urinary albumin-to-creatinine ratio*	0.7 (0.3–3.0)	0.6 (0.3–2.1)	0.7 (0.3–2.3)	0.6 (0.3–3.0)
Cardiovascular and oral hypoglycemic drugs, *n* (%)†				
Aspirin or antiplatelet agent	365 (62.9)	384 (63.6)	370 (63.2)	379 (63.3)
Statin	235 (40.5)	250 (41.4)	231 (39.5)	254 (42.4)
ACE inhibitor or ARB	389 (67.1)	416 (68.9)	406 (69.4)	399 (66.6)
Thiazide diuretic	76 (13.1)	79 (13.1)	76 (13.0)	79 (13.2)
Anticoagulant	29 (5.0)	33 (5.5)	37 (6.3)	25 (4.2)
β-Blocker	273 (51.2)	286 (51.3)	298 (50.9)	295 (49.2)
Calcium channel blocker	131 (22.6)	140 (23.2)	130 (22.2)	141 (23.5)
Metformin	143 (24.7)	159 (26.3)	145 (24.8)	157 (26.2)
Sulfonylurea	244 (42.1)	233 (38.6)	250 (42.7)	227 (37.9)
Carotid ultrasound				
Average maximum CIMT (mm)	1.08 ± 0.34	1.09 ± 0.34	1.08 ± 0.33	1.10 ± 0.35
Average of maximum common carotid CIMT (mm)	0.88 ± 0.25	0.89 ± 0.25	0.87 ± 0.24	0.89 ± 0.26
Average maximum common and bifurcation CIMT (mm)	1.10 ± 0.33	1.11 ± 0.33	1.09 ± 0.31	1.12 ± 0.34
Average maximum far wall CIMT (mm)	1.08 ± 0.38	1.09 ± 0.34	1.07 ± 0.35	1.10 ± 0.37

Plus/minus values are means ± SD. Prior vascular event refers to history of myocardial infarction, stroke, or revascularization. *Values are medians and IQRs. †At study end, 51% were taking statins, 75% ACE inhibitors or ARBs, 70% aspirin, 55% β-blockers, 28% calcium channel blockers, and 18% thiazide diuretics (similar in the treatment and control groups). At study end, metformin and sulfonylurea use were 56 and 25% in the insulin glargine and 61 and 53% in the standard care groups. Study-end use of oral hypoglycemic drugs remained well balanced between the n-3FA and placebo groups.

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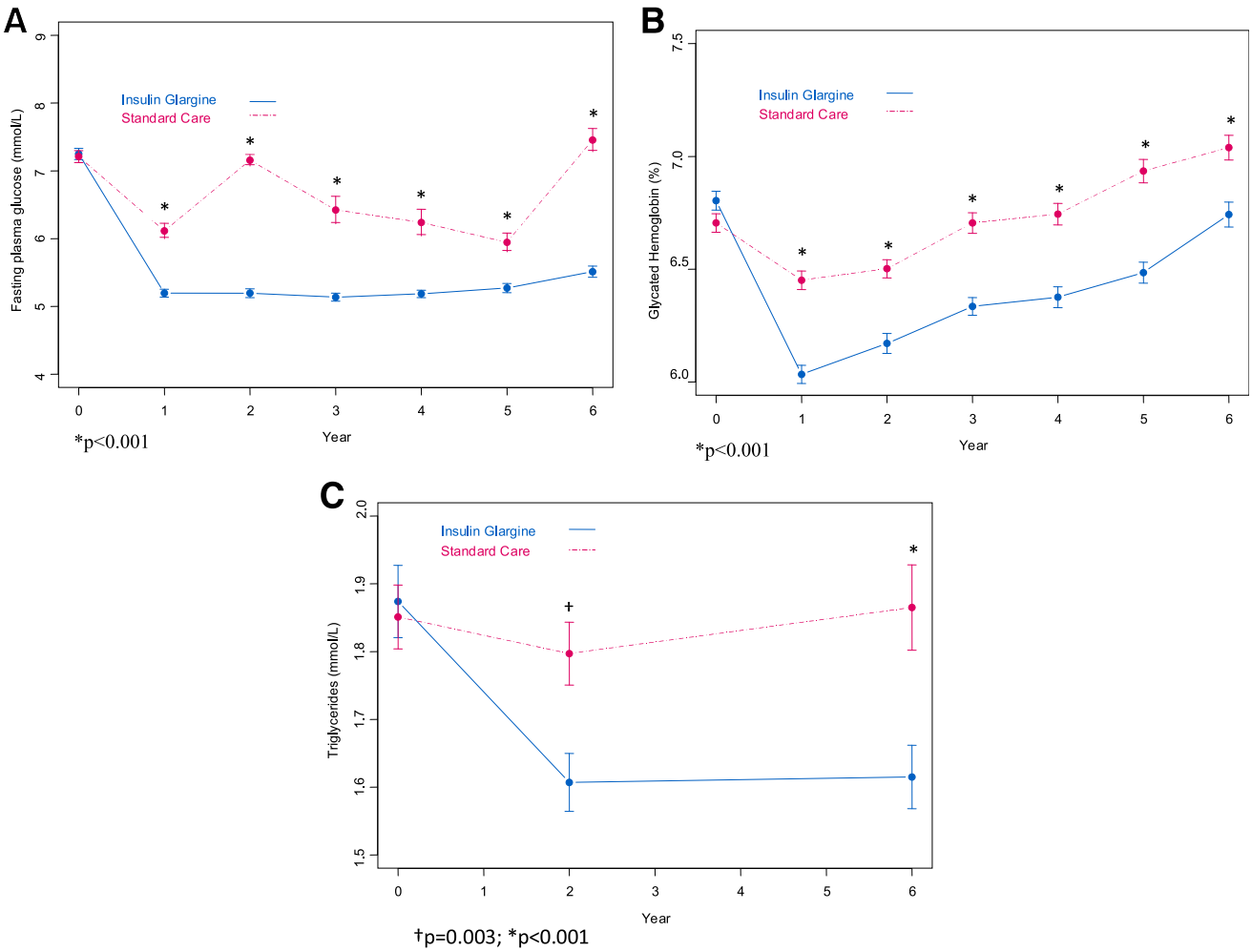

Figure 1—*Changes in levels of FPG* (A)*, HbA_{1c}* (B)*, and triglycerides* (C) *in patients receiving insulin glargine and standard care.*

primary outcome and significant differences, favoring insulin glargine therapy for the secondary CIMT outcomes. These findings did not differ significantly in models adjusting for baseline and average on treatment FPG, HbA_{1c}, lipids, and BP. There were no significant differences for the primary, secondary, and additional CIMT outcomes between the n-3FA and placebo groups (Table 2 and Fig. 2). The effect of both interventions on the primary outcome was similar across predefined subgroups (Supplementary Fig. 4) and across geographic regions.

CV events

Major CV events (defined as CV death, nonfatal myocardial infarction, nonfatal stroke, or hospitalized heart failure) occurred in 29.5% of participants in the insulin glargine and 29.0% in the standard care groups and in 28.4% in the n-3FA and 29.9% in the placebo groups, respectively. There were also no differences in all-cause and CV death, myocardial infarction, stroke, revascularization, and angina rates. More robust data on the effect of the study drugs on clinical events are provided by the larger, parent ORIGIN trial (23,24).

CONCLUSIONS—ORIGIN-GRACE is the largest reported clinical trial that evaluated the effects of insulin and n-3FA supplements on atherosclerosis progression. Insulin glargine, a basal insulin, titrated to achieve normoglycemia, significantly lowered FPG, HbA_{1c}, and triglyceride levels and had consistent favorable effects on CIMT progression, whereas n-3FA supplements had no significant effect on glycemia, lipids, and CIMT.

Once-daily subcutaneous injections with insulin glargine were acceptable to patients, as evidenced by the high adherence rates, were generally safe, and resulted in excellent glycemic control, with mean FPG and HbA_{1c} levels of 5.2 mmol/L and 6.0% at 1 year and 5.3 mmol/L and 6.5% at 5 years, respectively. They modestly lowered triglyceride levels and had no significant effects on BP, heart rate, and cholesterol levels. There was a statistically nonsignificant 11% reduction in the slope of CIMT progression for the primary outcome and significant 20 and 18%, respectively, for the secondary outcomes, with similar trends, 15% reduction, for the additional CIMT end point. These differences in CIMT progression could not be explained by differences in FPG and HbA_{1c}, suggesting that these may be independent of glucose lowering.

We selected a priori as our primary study outcome the annualized change in the maximum CIMT from all 12 carotid arterial segments and found a statistically nonsignificant trend toward a lower progression rate with insulin glargine. However, the optimal CIMT outcome in clinical trials remains controversial (30,31). Whereas some groups favor the

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Table 2—*Main efficacy analysis: annualized changes (slopes) for the primary, secondary, and additional efficacy outcomes

	Insulin glargine arm of the trial			
	Insulin glargine (n = 533) slope LSM ± SE (mm/year)	Standard care (n = 558) slope LSM ± SE (mm/year)	Difference (glargine, standard care) LSM ± SE (mm/year)	P value
Primary outcome				
Maximum CIMT for 12 carotid artery segments	0.0234 ± 0.0015	0.0264 ± 0.0015	−0.0030 ± 0.0021	0.145
Secondary outcomes				
Maximum CIMT for the four common carotid artery segments	0.0126 ± 0.0012	0.0158 ± 0.0012	−0.0033 ± 0.0017	0.049
Maximum CIMT for the eight common carotid and bifurcation segments	0.0209 ± 0.0015	0.0254 ± 0.0015	−0.0045 ± 0.0021	0.032
Additional outcome				
Maximum far wall CIMT for six carotid artery segments	0.0241 ± 0.0015	0.0285 ± 0.0015	−0.0044 ± 0.0023	0.061
	n-3FA arm of the trial			
	n-3FA (n = 539)	Placebo (n = 552)	Difference (active placebo)	P value
Primary outcome				
Maximum CIMT for 12 carotid artery segments	0.0254 ± 0.0015	0.0244 ± 0.0015	0.0009 ± 0.0021	0.650
Secondary outcome				
Maximum CIMT for the four common carotid artery segments	0.0140 ± 0.0012	0.0144 ± 0.0012	−0.0004 ± 0.0017	0.812
Maximum CIMT for the eight common carotid and bifurcation segments	0.0243 ± 0.0015	0.0221 ± 0.0015	0.0022 ± 0.0021	0.288
Additional outcome				
Maximum far wall CIMT for six carotid artery segments	0.0280 ± 0.0017	0.0247 ± 0.0016	0.0033 ± 0.0023	0.152

Differences in CIMT outcomes between the insulin glargine and standard care groups in additional models were as follows: Model 1 was adjusted for baseline and average on treatment HbA_{1c}: −0.0030 ± 0.0021 (P = 0.145) for the primary outcome, −0.0033 ± 0.0017 (P = 0.051) and −0.0044 ± 0.0021 (P = 0.036) for the secondary outcomes, and −0.0043 ± 0.0024 (P = 0.070) for the additional CIMT outcome. Model 2 was adjusted for baseline and average on treatment FPG levels: −0.0030 ± 0.0021 (P = 0.145) for the primary outcome, −0.0033 ± 0.0017 (P = 0.049) and −0.0045 ± 0.0021 (P = 0.032) for the secondary outcomes, and −0.0044 ± 0.0023 (P = 0.061) for the additional CIMT outcome. Model 3 was adjusted for baseline and average on treatment HbA_{1c} and FPG levels: −0.0031 ± 0.0021 (P = 0.146) for the primary outcome, −0.0034 ± 0.0017 (P = 0.047) and −0.0046 ± 0.0021 (P = 0.032) for the secondary outcomes, and −0.0044 ± 0.0024 (P = 0.065) for the additional CIMT outcome. There were no differences in the primary, secondary, and additional CIMT outcomes between the n-3FA and placebo groups in any models adjusted for risk factor levels. Model 4 was adjusted for baseline and average on treatment triglyceride levels: −0.0032 ± 0.0021 (P = 0.1305) for the primary outcome, −0.0034 ± 0.0017 (P = 0.043) and −0.0046 ± 0.0021 (P = 0.028) for the secondary outcomes, and −0.0045 ± 0.0023 (P = 0.056) for the additional CIMT outcome. Model 5 was adjusted for baseline and average on treatment HbA_{1c}, FPG, triglycerides, systolic BP, diastolic BP, and LDL and HDL cholesterol: −0.0030 ± 0.0021 (P = 0.153) for the primary outcome, −0.0035 ± 0.0017 (P = 0.041) and −0.0045 ± 0.0021 (P = 0.034) for the secondary outcomes, and −0.0044 ± 0.0024 (P = 0.066) for the additional CIMT outcome. *Insulin glargine reduced FPG, HbA_{1c}, and triglyceride levels and had no effect on BP, heart rate, and LDL and HDL cholesterol. Additional models adjusted for baseline and average on treatment levels of these variables did not significantly alter the differences in the primary, secondary, or additional outcomes between the insulin and standard care groups. LSM, least squares mean.

change in the maximum CIMT across 12 carotid segments (as a more comprehensive approach), others favor changes in our predefined secondary outcomes (i.e., the common carotid and the common carotid plus bifurcation segments), due to fewer missing data and higher reproducibility (especially for the common carotid artery), or our additional CIMT outcome, because of higher accuracy for far wall measurements (30,31). Indeed, in our trial, missing data were highest for the internal carotid artery segments.

Our findings provide further data supporting the CV safety of insulin glargine. The parent ORIGIN trial found no increase in clinical CV events with insulin glargine after 6.2 years, and the GRACE substudy found no adverse effects on atherosclerosis after 5 years. Diabetic patients frequently require glycemic control for decades. The lack of adverse effects of basal insulin on the arterial wall over 5 years suggests that longer-term therapy is likely to remain safe with regards to CV outcomes and may result in clinical CV benefits. The U.S. Food and Drug Administration recently mandated proof of CV safety as a major requirement for the approval of new hypoglycemic drugs (32). The findings of ORIGIN and its atherosclerosis substudy, GRACE, provide a very robust body of evidence for the CV safety of insulin glargine.

In experimental studies, insulin reduced inflammatory markers (5,33) and improved endothelial function (34) and atherogenic plasma lipid patterns (35), although some studies suggested a possible proatherogenic effect of exogenous insulin in insulin-resistant states associated with compensatory hyperinsulinemia, possibly by stimulating cell proliferation through the MAPK pathway (6,7). There are surprisingly few studies on the effects of insulin on human atherosclerosis progression. The long-term follow-up of the Diabetes Control and Complications Trial

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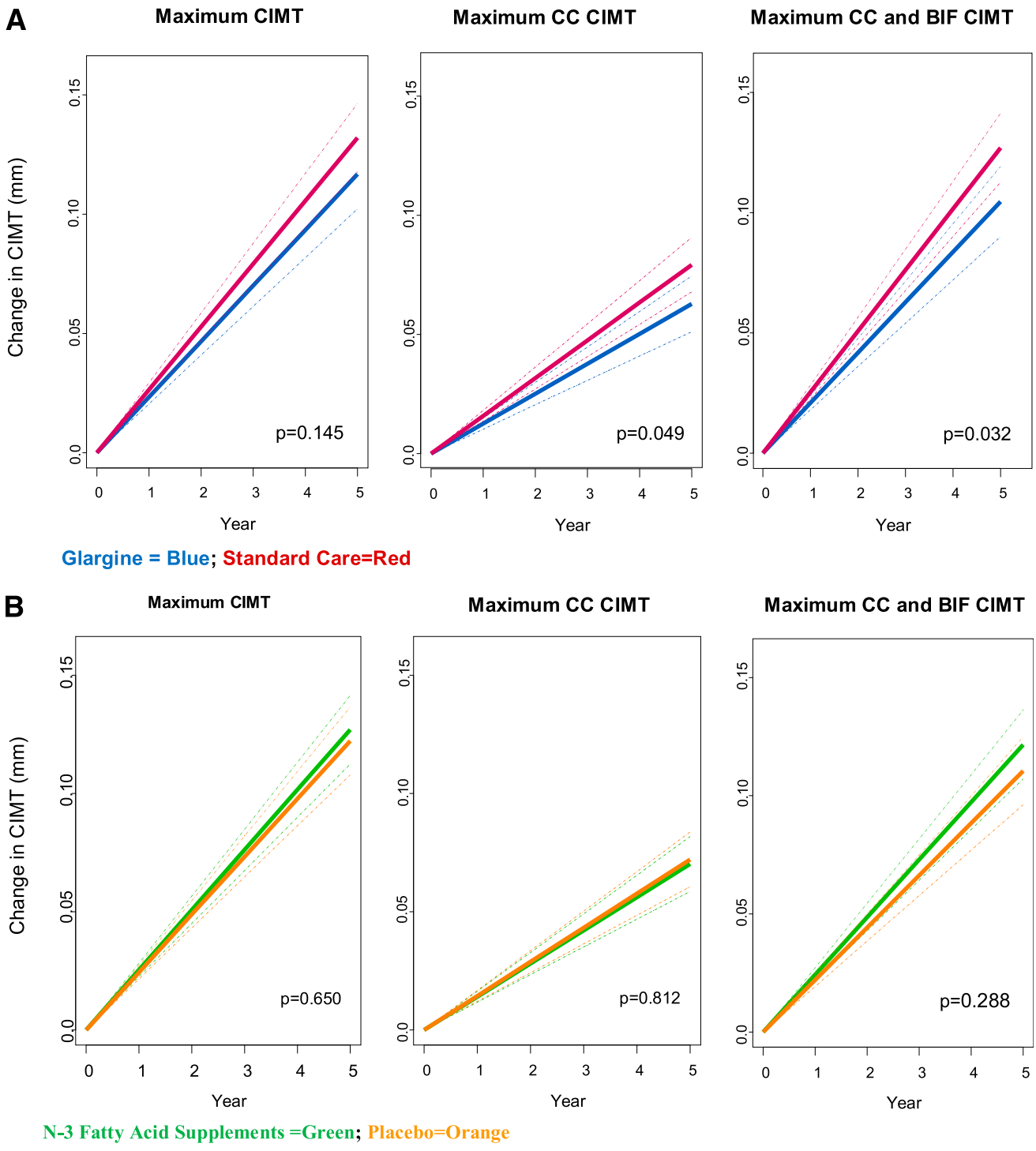

Figure 2—*Changes in the primary and secondary CIMT outcomes by treatment group for the insulin glargine* (A) *and n-3FA* (B) *arms of the trial (slopes of carotid intima-media change and 95% CIs). BIF, bifurcation; CC, common carotid.*

(DCCT) cohort, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, which compared CIMT in 1,229 patients with type 1 diabetes, 611 who had been randomly assigned to conventional diabetes treatment during the DCCT and 618 to intensive insulin treatment, found that 6 years after completion of the randomized DCCT intervention trial, CIMT was significantly lower in the group that had received intensive insulin therapy during the trial (36). Several studies confirmed the presence of increased CIMT in people with type 2 diabetes or prediabetes (37). However, there are only a few small, and therefore not reliable, intervention trials with insulin on atherosclerosis progression (38,39). Clinical CV end point trials conducted prior to ORIGIN also fail to provide clear answers regarding the impact of exogenous insulin on CV events. Extended follow-up of the UK Prospective Diabetes Study (UKPDS) trial found a

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legacy effect, with 15% reduction in myocardial infarction and 13% reduction in death among people with new-onset type 2 diabetes treated with insulin and sulfonylurea (40). Subsequent large-outcome trials of more versus less intense glucose lowering failed to demonstrate clear CV benefits, although insulin was used in both study groups in these strategy trials (41). The ORIGIN trial found a neutral effect of insulin glargine on CV events over 6.2 years (23). The GRACE substudy shows a modest decrease in carotid atherosclerosis consistent with EDIC, providing a rationale for an extended follow-up to assess whether the observed differences in atherosclerosis persist and whether these differences translate into clinical event reduction.

n-3FA were reported to have several potentially antiatherogenic effects, such as improving endothelial function, lowering BP, inhibiting platelet aggregation, reducing triglycerides, and raising HDL_2 cholesterol levels (8). Observational studies indicate associations between n-3FA intake and lower risk of CV events, and some clinical trials found clinical CV event reduction with n-3FA supplements (8–15). We found no significant effects of daily intake over 4.9 years of 1 g of n-3FA supplements on BP, lipid levels, and CIMT, and the parent ORIGIN trial found no effect on clinical CV events over 6.2 years (24). This is consistent with the results of previous smaller studies examining the effects on carotid and coronary atherosclerosis (17,20,21), as well as a recent meta-analysis of the effects of n-3FA on clinical outcomes (16). It is unclear if these findings are unique to our study population and the n-3FA dose and formulation used. Ongoing clinical end point trials will provide further insight into the role of n-3FA supplements in CV prevention (24). Moreover, our study does not address the CV effects of dietary fish consumption.

In conclusion, treatment with basal insulin glargine over 4.9 years had a modest beneficial effect, whereas 1 g of n-3FA supplements had no impact on carotid atherosclerosis. Our findings confirm the CV safety of insulin and raise the possibility that longer-term treatment might result in CV event reduction. This hypothesis is currently under evaluation in the ORIGIN passive extended follow-up, the ORIGIN and Legacy Effects (ORIGINALE) study. Our findings do not support the use of n-3FA supplements in high-risk people with dysglycemia.

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E.M.L. designed the trial, obtained funding, supervised the activities of the Core Ultrasound Laboratory, analyzed data, interpreted results, and drafted the manuscript. J.B., S.Y., and H.C.G. designed the trial, obtained funding, interpreted results, and critically reviewed the manuscript. R.D., P.L.-J., A.R., N.H., M.H., and H.K. acquired data and critically reviewed the manuscript. L.R. designed the trial and critically reviewed the manuscript. S.S. coordinated the Core Ultrasound Laboratory activities and critically reviewed the manuscript. M.J.M. directed the Core Biochemistry Laboratory and critically reviewed the manuscript. L.D. conducted the statistical analysis. E.M.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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