Xenin-25 Amplifies GIP-Mediated Insulin Secretion in Humans With Normal and Impaired Glucose Tolerance but Not Type 2 Diabetes

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Glucose-dependent insulinotropic polypeptide (GIP) potentiates glucose-stimulated insulin secretion (GSIS). This response is blunted in type 2 diabetes (T2DM). Xenin-25 is a 25–amino acid neurotensin-related peptide that amplifies GIP-mediated GSIS in hyperglycemic mice. This study determines if xenin-25 amplifies GIP-mediated GSIS in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or T2DM. Each fasting subject received graded glucose infusions to progressively raise plasma glucose concentrations, along with vehicle alone, GIP, xenin-25, or GIP plus xenin-25. Plasma glucose, insulin, C-peptide, glucose-stimulated insulin secretion (GSIS), and glucagon levels and insulin secretion rates (ISRs) were determined. GIP amplified GSIS in all groups. Initially, this response was rapid, profound, transient, and essentially glucagon independent. Thereafter, ISRs increased as a function of plasma glucose. Although magnitudes of insulin secretory responses to GIP were similar in all groups, ISRs were not restored to normal in subjects with IGT and T2DM. Xenin-25 alone had no effect on ISRs or plasma glucagon levels, but the combination of GIP plus xenin-25 transiently increased ISR and plasma glucagon levels in subjects with NGT and IGT but not T2DM. Since xenin-25 signaling to islets is mediated by a cholinergic relay, impaired islet responses in T2DM may reflect defective neuronal, rather than GIP, signaling. Diabetes 61:1793–1800, 2012

Peptides secreted from the gastrointestinal tract play an important role in regulating insulin secretion (1,2). To date, attention has focused on two intestinal peptides: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 is produced predominantly by L cells in the distal bowel, whereas GIP is produced mainly by K cells in the proximal intestine. Both peptides are released into the blood immediately after eating and potentiate glucose-stimulated insulin secretion (GSIS) (3–5). Unlike GLP-1, which simulates insulin secretion in type 2 diabetes (T2DM), persons with T2DM are thought to be resistant to the actions of GIP (6–8), and increasing GIP signaling has not been pursued as a therapeutic target for T2DM.

To better understand how GIP regulates the incretin response, we generated and characterized transgenic mice that lack GIP-producing cells by driving expression of an attenuated diphtheria toxin transgene with regulatory elements from the GIP gene (9). These GIP/DT mice demonstrated markedly attenuated insulin secretory responses to oral glucose even though GLP-1 release was normal (9). Moreover, the GIP/DT mice exhibited a blunted insulin secretory response to exogenously administered GIP but not GLP-1 (10). Thus, GIP-producing cells may secrete a hormone(s) in addition to GIP that plays a critical role in the incretin response.

Xenin-25 is a 25–amino acid neurotensin-related peptide reportedly produced by a subset of K cells (11,12) and would also be reduced in the GIP/DT mice. In vivo experiments demonstrated that xenin-25 potentiated the insulin secretory response to GIP but had little effect alone (10). Similar results were observed in hyperglycemic NONcNZO10/Ltj mice (10)—a polygenic model of human T2DM in which diabetes spontaneously develops with age (13,14). These studies raise the possibility that xenin-25 could increase the insulin secretory response to GIP in humans. The purpose of the current study was to determine if xenin-25, either alone or with GIP, could amplify the insulin secretory response to glucose in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and/or T2DM.

RESEARCH DESIGN AND METHODS

Studies in human subjects. All protocols were approved by Washington University’s Human Research Protection Office and the Food and Drug Administration (IND 103,374) and are registered with ClinicalTrials.gov (NCT00798915). Studies were performed in the Clinical Research Unit of the Institute of Clinical and Translational Sciences of Washington University after obtaining written informed consent. Male and female subjects with NGT, IGT, and mild T2DM were studied (Table 1). Glucose tolerance was defined by the 2-h plasma glucose level during a 75-g oral glucose tolerance test using diagnostic criteria of the American Diabetes Association (15). Baseline characteristics were determined during a screening visit after a 10-h fast. HbA1c levels were required to be ≤9.0 in all subjects. Subjects treated with insulin were excluded. Subjects treated with oral antidiabetic medications were enrolled if the agent(s) could be safely discontinued for 48 h preceding each study visit. These selection criteria were designed to exclude T2DM subjects with advanced β-cell failure and to identify participants with residual insulin secretion who have the potential to respond to incretin peptides. Women of childbearing potential were required to use birth control. Subjects were excluded if they 1) had a history of chronic pancreatitis and/or risk factors for chronic pancreatitis, 2) had a history of gastrointestinal disorders, 3) were taking nondiabetes medications known to
Affect glucose homeostasis, and 4) had significant systemic illness including heart, kidney, liver, inflammatory, or malignant disease.

**Study design.** Studies were performed after a 10-h overnight fast. One intravenous catheter was placed into a hand vein. This hand was kept in a thermostatically controlled box (50–55°C) to facilitate venous sampling and to provide arterialized venous blood (16,17). A second intravenous line was inserted for administration of glucose/peptides. Subjects with a fasting blood glucose ≥120 mg/dL were given boluses of intravenous human insulin (2.0 units/kg) at 30-min intervals as needed to decrease the blood glucose level to 100–120 mg/dL to limit variability of initial glucose levels. Blood glucose was stable for 20 min before starting the graded glucose infusion (GGI).

All subjects were administered four separate GGIs, each separated by at least 2 weeks. During the respective GGIs, along with glucose, subjects also received an intravenous infusion with GIP plus xenin-25, GIP alone, albumin alone (no peptide), or xenin-25 alone (subjects were blinded to treatment). From 0 to 40, 40 to 80, 80 to 120, 120 to 200, and 200 to 240 min, the glucose infusion rates were 1, 2, 3, 4, 6, and 8 mg kg\(^{-1}\) min\(^{-1}\), respectively. Peptides were administered by primed-continuous intravenous infusion. Infusions rates from 0 to 3, 3 to 7, 7 to 10, and 10 to 240 min were 10.8, 7.7, 5.6, and 4.0 pmol kg\(^{-1}\) min\(^{-1}\), respectively.

**Peptides.** GIP and xenin-25 were custom synthesized under good manufacturing practice conditions (Bachem, Torrance, CA), dissolved in water (United States Pharmacopeial Convention, injectable), filter sterilized, aliquoted into cryovials as single doses, and stored at –80°C. Representative vials were analyzed for identity, purity, concentration, sterility, and endotoxins. Peptides were compounded in normal saline containing 1% Pluronic (Baxter Healthcare Corp., Westlake Village, CA) immediately before infusions.

**Peptide dosing.** GIP administered by an intravenous infusion at a dose of 4 pmol kg\(^{-1}\) min\(^{-1}\) increases insulin release during a hyperglycemic clamp in healthy individuals approximately fourfold (7,8) but is ineffective in persons with T2DM (7). Similar results were obtained using GIP at a dose of 2.4 pmol kg\(^{-1}\) min\(^{-1}\) (6). Only one human clinical trial with xenin-25 has been published (18). The authors assessed the effects of xenin-25 infused at a dose of 4 pmol kg\(^{-1}\) min\(^{-1}\) on intestinal motility. This dose increased plasma xenin-25 levels and gut motility (18). Effects on insulin secretion and glucose concentrations were not reported. On the basis of these studies, GIP and xenin-25 were each infused at a dose of 4 pmol kg\(^{-1}\) min\(^{-1}\).

**Assays.** Plasma glucose was measured bedside by the glucose oxidase technique using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin, C-peptide, glucagon, complete metabolic profiles, and Hb\(_A1C\) levels were determined by Washington University’s Core Laboratory for Clinical Studies. Insulin and C-peptide were measured using a solid-phase, two-site chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Glucagon was determined by radioimmunoassay (Millipore Corporation, St. Charles, MO). Immuno reactive (IR)-GIP and IR-xenin levels were determined using an enzyme-linked immunosorbent assay (ELISA) for total GIP (Millipore Corporation) and a custom ELISA developed in our laboratory for IR-xenin (10). Lipase and total amylase were determined by the Barnes Hospital Laboratories.

**Insulin secretion rates.** Insulin secretion rates (ISR) were derived by stochastic deconvolution of the peripheral C-peptide concentrations as previously described using population-based estimates of C-peptide clearance kinetics (19–21).

**Statistics.** Physiologic data were analyzed using mixed-effects models with subject as a random effect and peptide as a fixed effect. Pairwise comparisons were limited to evaluating the effects of 1) xenin-25 alone versus albumin, 2) GIP alone versus albumin, and 3) GIP plus xenin-25 versus GIP alone. One-tailed t tests were used when testing the hypotheses that ISRs were greater during infusion with 1) GIP plus xenin-25 compared with GIP alone and 2) GIP alone compared with albumin. Two-tailed t tests were used when testing the hypothesis that ISRs were similar during infusions with albumin alone and xenin-25 alone. All glucagon data were analyzed using two-tailed t tests.

**RESULTS**

**Subject characteristics.** Groups were well-matched and as anticipated, the 2-h and fasting plasma glucose and Hb\(_A1C\) levels progressively increased in groups with IGT and T2DM compared with NGT (Table 1). Subjects with T2DM did not have gastroparesis or clinically evident peripheral neuropathies. Five patients with T2DM were taking metformin and two were also on a sulfonylurea. No subjects were receiving incretin-based therapies. Three subjects with T2DM received insulin to lower basal glucose levels before one, one, and two of their four respective GGIs.

**Symptomatic effects of peptide infusions.** Plasma total amylase levels (salivary plus pancreatic) were unaffected by any of the infusions (data not shown). On the basis of qualitative surveys taken before, during, and after each infusion, peptides were not associated with nausea, vomiting, chest pains, dizziness, heart palpitations, shortness of breath, fever, chills, blurred vision, or changes in salivation, sweating, or frequency of urination. Mild diarrhea and/or loose stools were observed in 57, 14, 21, and 0% of subjects who received GIP plus xenin-25, GIP alone, xenin-25 alone, or albumin alone, respectively. When experienced, diarrhea occurred within several hours of the infusion and was noted in all three groups. Stools did not contain blood, and diarrhea did not require pharmacologic or medical intervention. Infusion with GIP alone reduced mean arterial blood pressure 10–15 mmHg and increased resting heart rate ~3 bpm in all three groups. In contrast, xenin-25, either alone or with GIP, had no effect on either parameter. Thus, xenin-25 does not appear to affect autonomic function.

**GIP and xenin-25 levels during GGIs.** In subjects with NGT, IGT, and T2DM (Fig. 1; some data not shown), fasting, preinfusion plasma levels of IR-GIP were <10 pmol/L, and fasting IR-xenin levels were below the limit of detection (typically <2 pmol/L). Infusion of GIP and xenin-25 increased steady-state levels of plasma IR-GIP and IR-xenin to ~400 and ~125 pmol/L, respectively. These levels were unaffected by infusion with the other peptide. Peak plasma IR-GIP levels after mixed-meal ingestion were ~80 pmol/L, and plasma IR-xenin was undetectable (<2 pmol/L). Thus, pharmacologic levels of GIP and xenin-25 were maintained throughout the GGIs. On the basis of IR-xenin levels measured after peptide infusions were terminated.
Xenin-25 amplifies the effects of GIP on ISRs in humans. Subjects were next administered GGIs with GIP or GIP plus xenin-25. GIP plus or minus xenin-25 increased insulin secretory responses that varied as functions of time, plasma glucose level, and glucose tolerance (Figs. 2 and 3). At all glucose levels studied (Fig. 3), ISRs were greater within each group during GGIs with GIP and GIP plus xenin-25 compared with albumin alone or xenin-25 alone. Since the effects of GIP plus or minus xenin-25 on ISRs were not linear as a function of plasma glucose levels, iAUCs rather than slopes were used to quantify ISRs. Compared with albumin, infusion with GIP increased the 240-min ISR iAUC/glucose iAUC (Fig. 3) 1.7-, 1.5-, and 1.9-fold in subjects with NGT, IGT, and T2DM, respectively (all P values ≤ 0.007 vs. albumin), and these ratios were further increased during infusion with GIP plus xenin-25 in subjects with IGT (1.7-fold further increase vs. GIP alone; P = 0.002). In subjects with NGT and T2DM, the differences were not significant. Since the 240-min summary measures are taken over differing ranges of glucose levels between groups, these fold increases do not directly compare peptide effects between the three groups.

Xenin-25 amplifies a rapid and transient increase in GIP-mediated ISR in humans with NGT and IGT but not T2DM. Infusion with GIP alone at the start of the GGI caused rapid and transient increases in insulin, C-peptide, and ISRs in all groups (Figs. 2 and 3). This initial response occurred with little change in plasma glucose levels and was absent during infusions with albumin alone. Since this transient response was not anticipated, a post hoc analysis of the first 40 min was conducted (Fig. 4). In subjects with NGT, this early insulin secretory response to GIP peaked within the first 10 min of the GGI. ISRs then declined until −40 min but remained greater than levels during infusion with albumin or xenin-25 alone. A similar response to GIP was noted in subjects with IGT and T2DM, except peak ISRs were not reached until 20 min into the infusions and then decreased until −60–70 min into the GGI. Within each group, the iAUCs for the ISR during the first 40 min of the GGIs (pmol insulin secreted/40 min) were greater during infusion with GIP alone compared with albumin (iAUCs increased 4.6-, 6.1-, and 8.7-fold in subjects with NGT, IGT, and T2DM, respectively; all P values ≤ 0.0005). Moreover, the incremental insulin secretory responses to GIP during the first 40 min of the GGI were remarkably similar in humans with NGT (7,998 ± 1,263), IGT (10,016 ± 1,524), and T2DM (10,062 ± 2,290).

Compared with albumin, xenin-25 did not significantly affect plasma insulin, C-peptide, or glucose levels or ISRs in any group (Fig. 2). Small and transient, but nonsignificant, increases in insulin, C-peptide, and ISR were noted at the 10- and 20-min time points during infusion of xenin-25 alone (see below). Plots of ISRs versus plasma glucose levels in the presence and absence of xenin-25 were also nearly identical within each group (Fig. 3). Incremental areas under the curve (iAUCs) were calculated for ISR and plasma glucose for each individual during the 240-min GGI with and without infusion of the xenin-25. The ratio of these iAUCs represents a summary measure of β-cell sensitivity (ISR per glucose concentration) over the entire range of glucose levels. As shown in Fig. 3, the means of the ratios were not different within each group during infusions with albumin versus xenin-25 alone in subjects with NGT (8.7 ± 1.4 vs. 9.5 ± 1.4), IGT (6.2 ± 0.8 vs. 6.9 ± 0.8), and T2DM (3.6 ± 1.8 vs. 3.4 ± 1.8). Thus, infusion of xenin-25 alone does not significantly affect ISR over a broad range of plasma glucose levels in humans with NGT, IGT, or T2DM.

(n = 6), the circulating half-life of IR-xenin is 2.5 ± 0.3 min (data not shown).

Xenin-25 alone does not affect ISR or glucose levels in humans. During infusion with albumin alone, plasma insulin, C-peptide, and glucose levels, as well as ISRs (Fig. 2), progressively increased in response to the stepwise increases in glucose infusion rates. Maximal plasma glucose levels were higher in the group with T2DM (313 ± 13 mg/dL) versus IGT (240 ± 12 mg/dL) and NGT (186 ± 11 mg/dL) (P < 0.003 for IGT vs. NGT, P < 0.0001 for T2DM vs. NGT). In spite of the progressively higher plasma glucose levels, plasma insulin and C-peptide levels, as well as ISRs, tended to reach lower maximal levels (in pmol/min) in subjects with T2DM (838 ± 224) versus IGT (1,003 ± 118) and NGT (1,037 ± 141). As shown in Fig. 3, ISRs increased linearly as a function of plasma glucose level during infusion with albumin alone. However, the slope of the line relating ISR to glucose declined in the progression from NGT to IGT to T2DM (slopes in pmol/min per mg/dL are 9.0, 6.1, and 3.1, respectively), reflecting worsening β-cell insulin secretory responses to glucose with IGT and T2DM.

Compared with albumin, xenin-25 did not significantly affect plasma insulin, C-peptide, or glucose levels or ISRs in any group (Fig. 2). Small and transient, but nonsignificant, increases in insulin, C-peptide, and ISR were noted at the 10- and 20-min time points during infusion of xenin-25 alone (see below). Plots of ISRs versus plasma glucose levels in the presence and absence of xenin-25 were also nearly identical within each group (Fig. 3). Incremental areas under the curve (iAUCs) were calculated for ISR and plasma glucose for each individual during the 240-min GGI with and without infusion of the xenin-25. The ratio of these iAUCs represents a summary measure of β-cell sensitivity (ISR per glucose concentration) over the entire range of glucose levels. As shown in Fig. 3, the means of the ratios were not different within each group during infusions with albumin versus xenin-25 alone in subjects with NGT (8.7 ± 1.4 vs. 9.5 ± 1.4), IGT (6.2 ± 0.8 vs. 6.9 ± 0.8), and T2DM (3.6 ± 1.8 vs. 3.4 ± 1.8). Thus, infusion of xenin-25 alone does not significantly affect ISR over a broad range of plasma glucose levels in humans with NGT, IGT, or T2DM.

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Xenin-25 amplifies the effects of GIP on glucagon levels in humans with NGT and IGT. Glucagon plays a central role in the regulation of insulin secretion and glucose metabolism. Fasting, basal plasma glucagon levels were similar in all three groups and progressively decreased in humans with NGT, IGT, and T2DM during the GGI when albumin alone was infused (Fig. 5). As expected, the ability of plasma glucose to suppress plasma glucagon levels was attenuated in subjects with T2DM since glucagon levels at the end of the GGI declined by 31, 34, and 21 pg/mL in subjects with NGT, IGT, and T2DM, respectively. Compared with albumin, infusion with xenin-25 alone had no statistically significant effect on glucagon levels in any group. However, compared with GIP alone, infusion with GIP plus xenin-25 increased plasma glucagon levels in subjects with NGT and IGT during the first 40 min and during the entire 240 min of the GGI. In contrast, xenin-25 failed to amplify the effects of GIP on plasma glucagon levels in subjects with T2DM during either time period. Thus, xenin-25 amplified the glucagon response to GIP in the subjects with NGT and IGT but not T2DM.

DISCUSSION
GIP is an incretin hormone that in people with NGT, significantly increases the insulin secretory response to glucose. However, since GIP-stimulated insulin release is reportedly blunted in humans with T2DM, this peptide has not been pursued as a therapy for T2DM. The current study was undertaken to further our understanding of attenuated β-cell responsiveness to GIP in T2DM and to elucidate the mechanisms involved in GIP signaling in the β-cell.

Previous studies from our laboratory show that xenin-25 potentiates GIP-mediated insulin release in two mouse...
models that exhibit blunted insulin secretory responses to GIP (10). In contrast, xenin-25 alone had no effect on insulin secretion in either mouse model. Moreover, xenin-25 did not act directly on islet β-cells. Rather, xenin-25 activated a cholinergic relay in the periphery that did not require neurons in the central nervous system. The results presented in this study extend these observations and show that during GGI at the peptide and glucose levels studied, xenin-25 alone does not affect ISR in humans but does potentiate GIP-mediated insulin secretion in humans with NGT and IGT but not T2DM. Albumin alone, Alb; GIP alone, GIP; xenin-25 alone, Xen; combination of GIP plus xenin-25, G+X.

FIG. 3. A–F: Xenin-25 amplifies the insulin secretory response to GIP in humans with NGT and IGT but not T2DM. Data are shown for the first 40 min of each GGI. A–C: ISR IAUCs. D–F: ISR total AUC (tAUC) divided by the glucose tAUC. Albumin alone, Alb; GIP alone, GIP; xenin-25 alone, Xen; combination of GIP plus xenin-25, G+X.

FIG. 4. Xenin-25 rapidly and transiently amplifies GIP-mediated ISR in humans with NGT and IGT but not T2DM. Data are shown for the first 40 min of each GGI. A–C: ISR IAUCs. D–F: ISR total AUC (tAUC) divided by the glucose tAUC. Albumin alone, Alb; GIP alone, GIP; xenin-25 alone, Xen; combination of GIP plus xenin-25, G+X.
consistent with the idea proposed by Meier and Nauck (31) that humans with T2DM may retain the ability to respond to GIP, but reduced GIP responses in these subjects may reflect worsening insulin secretory responses to glucose rather than defective GIP signaling. This is further supported by the fact that the effects of GIP on blood pressure and heart rate were not blunted in humans with T2DM. Moreover, the effects of GIP on ISRs remained elevated even after this initial transient response, suggesting that like the cephalic response (32), this early response to GIP may play an important role in amplifying subsequent insulin secretion.

As with ISR, xenin-25 also amplified the early and transient GIP-mediated increase in glucagon levels in humans with NGT and IGT but not T2DM. GIP rapidly and transiently increased plasma glucagon levels in healthy humans when administered during euglycemic conditions (33) but not during hyperglycemic clamps (34). The very early postprandial glucagon response is thought to prevent hypoglycemia as cells recruit Glut4 and amino acid transporters to the cell surface in anticipation of increased levels of nutrients in the blood (35). Thus, the rapid and transient amplification of GIP-mediated glucagon release by xenin-25 may reflect a normal islet secretory response to nutrients after an overnight fast. These results also suggest that in humans with T2DM, islets exhibit a blunted response to xenin-25 signaling.

Mouse studies indicate that pharmacologic doses of xenin-25 increase GIP-mediated insulin release via a cholinergic relay requiring muscarinic receptors in the periphery (10), and M3 muscarinic receptors in islets are required for both insulin and glucagon release (36). Results in this study suggest that at the dose required to amplify GIP-mediated insulin secretion in humans, xenin-25 does not increase parasympathetic activity since it had no effect on heart rate, blood pressure, salivation, or need to urinate. Thus, an analogous xenin-25–mediated cholinergic relay is the most likely mechanism for increasing insulin and glucagon release in humans. It is worth noting that in contrast to a previous report (37), we have been unable to detect endogenous IR-xenin in either mouse or human plasma prepared under fasting, fed, or postprandial conditions using an ELISA that can detect 2 pmol/L peptide, raising the possibility that xenin-25 is not a physiologic peptide. Moreover, we have not seen other reports where endogenous xenin has been measured in plasma or purified K cells. Alternatively, endogenous xenin-25 may be modified such that it is not detected by our ELISA. Characterization of xenin-25 purified from plasma or isolated K cells would be required to address this issue. If xenin-25 is a physiologic peptide, it is unclear if local levels (e.g., at nerve endings near the K cell) would be high enough to modify islet function in vivo. However, our results with exogenously administered xenin-25 provide important insights into β-cell function during the progression from NGT to IGT to T2DM even if xenin-25 is not a physiologic peptide or if physiologic levels do not modify islet function. These results also suggest that defects in cholinergic or neuronal signaling in the periphery may contribute to defective insulin secretion in humans with T2DM. For example, xenin-25–responsive cells involved in this relay may

FIG. 5. Xenin-25 amplifies the effects of GIP on plasma glucagon levels in humans with NGT and IGT but not T2DM. A, D, and G: Group average changes in plasma glucagon ± SEM. Changes in plasma glucagon levels during the first 40 min (B, E, and H) or the entire 240 min (C, F, and I). Alb; Albumin alone; GIP; GIP alone; Xen; xenin-25 alone; G+X; combination of GIP plus xenin-25.
produce and/or release reduced amounts of acetylcholine, or islet endocrine cells may exhibit reduced sensitivity to acetylcholine. Alternatively, transmitters produced and released by xenin-25–responsive cells in humans with T2DM may be different from those in humans with NGT and IGT. Studies are underway to distinguish between these different mechanisms.

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Washington University is pursuing a patent related to the use of xenin-25 to treat T2DM. In the future, this could lead to personal financial benefit to B.M.W., K.S.P., and Washington University. No other potential conflicts of interest relevant to this article were reported.

B.M.W., D.N.R., B.W.P., and K.S.P. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript. H.D.T. and M.J.W. researched data and reviewed and edited the manuscript. D.L.C., J.H.L., and D.T.V. researched data, contributed to discussion, and reviewed and edited the manuscript. J.D. contributed to discussion and reviewed and edited the manuscript. B.M.W. is the guarantor of this work and, as such, had full access to all of 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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