Native Oxyntomodulin Has Significant Glucoregulatory Effects Independent of Weight loss in Obese Humans With and Without Type 2 Diabetes

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ABSTRACT

Oxyntomodulin (OXM), an enteroendocrine hormone, causes appetite suppression, increased energy expenditure, and weight loss in obese humans via activation of GLP-1 and glucagon receptors. However, the effects of OXM on glucose homeostasis remain ill-defined. To address this gap, we evaluated the effects of an intravenous (IV) infusion of native OXM on insulin secretory rates (ISR) and glycemic excursion in a graded glucose infusion (GGI) procedure in two separate randomized, placebo-controlled, single dose crossover trials in 12 overweight and obese subjects without diabetes, and in 12 obese subjects with Type 2 diabetes (T2DM) respectively, using the GLP-1 analog, liraglutide as a comparator in the T2DM. In both groups, in the GGI, 3.0 pmol/kg/min of OXM significantly increased ISR and blunted glycemic excursion relative to placebo. In the T2DM, the effects of OXM were comparable to those of liraglutide, including restoration of beta cell glucose responsiveness to that of non-obese subjects without diabetes. Our findings indicate that native OXM significantly augments glucose-dependent insulin secretion acutely in obese subjects with and without diabetes, with effects comparable to pharmacologic GLP-1R activation and independent of weight loss. Native OXM has potential to improve hyperglycemia via complementary and independent induction of insulin secretion and weight loss.

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Oxyntomodulin (OXM), like GLP-1 and glucagon, is a peptide product derived from posttranslational modification of preproglucagon (1,2,3). It is secreted by the entero-endocrine cells of the small intestine in response to nutrient ingestion and has been shown *in vitro* to be a natural chimera, binding and activating both the GLP-1 receptor (GLP-1R) and the glucagon receptor (GCGR) (4). In vitro characterization of OXM indicates approximately equal potency at GLP-1R and GCGR (4). However, the physiologic and pharmacologic effects of OXM in humans are less well characterized than those of GLP-1. Bloom and colleagues have previously shown that at exposures that are well-tolerated, native OXM administered subcutaneously thrice a day for 4 weeks induces meaningful weight loss in obese subjects without diabetes (OB) (5). In similar elegantly designed and executed mechanistic studies in the same series, Bloom et al demonstrated that OXM can suppress appetite but unlike GLP-1, increase energy expenditure (6,7) as well, findings that suggest contributions from both GLP-1R and GCGR activation. Native OXM, much like GLP-1, is degraded within minutes of release, making it an impractical therapeutic candidate (1). There have been efforts to design longer half-life analogs of OXM and to manipulate the balance of GLP-1R and GCGR agonism (8,9). In rodent studies, an OXM-like analog, with approximately equal potency at GLP-1R and GCGR, was more efficacious for weight loss than an analog highly selective for GLP-1R (10,11). Furthermore, in these studies, at doses of the respective analogs chosen to closely match target engagement at the GLP-1R, equivalent suppression of food intake was observed yet weight loss was greater with the OXM-like analog, findings that appear to denote efficacy attributable to GCGR engagement (11,12,13). However, when peptide analogs

were created that were preferentially selective for GCGR over GLP-1R, glucose tolerance in the treated rodents was observed to deteriorate, despite impressive weight loss (11).

There are limited clinical data on the effect of OXM on glucose homeostasis. In the previously cited weight loss study in OB, 4 weeks of OXM administration did not cause changes in fasting glucose but the response to a glucose challenge was not examined (5). The two studies presented in the current report were undertaken to examine the acute glucoregulatory effect of native OXM in the setting of a glucose challenge, in two clinical populations who would be potentially vulnerable to adverse consequences of GCGR activation, namely, obese with and without type 2 diabetes mellitus. A graded glucose infusion (GGI) method was used to provide the glucose challenge for these studies. The GGI is a platform that has been effectively employed to characterize glucoregulatory effects of GLP-1R analogs (14). Briefly, using stepwise incrementally rising rates of glucose infusion, a GGI generates a graded hyperglycemic stimulus that not only enables the measurement of insulin secretion and its glucose-responsiveness, but also provides an integrated measure of concurrent changes in plasma glucose in response to the insulin secretion (14). Data from the current experimental series demonstrate that in OB and T2DM, native OXM has beneficial effects on glucoregulatory homeostasis through direct augmentation of insulin secretory response, and independent of its weight loss effects.

METHODS

Study Subjects: Two separate trials with an IV infusion of native OXM or placebo (PBO) in the setting of a graded glucose infusion procedure were conducted to investigate the

effect of OXM on insulin secretion and glucose homeostasis. The first trial enrolled OB and the second trial enrolled T2DM. Both trials were randomized, double-blind, single dose and PBO-controlled, with a crossover design such that all subjects received all treatments in random sequence, and underwent a GGI procedure during each treatment period. The studies were conducted at PRA International in Zuidlaren, Netherlands, after receiving approval from the Independent Ethics Committee of the Stichting Beoordeling Ethiek Bio-Medisch Onderzoek in the Netherlands. Subjects were enrolled in the trial after providing written informed consent and meeting study entry criteria. All subjects had routine physical examinations and safety laboratory measurements done at baseline and end of study. Subjects were instructed to maintain their usual dietary and physical activity habits throughout the study, and were required to refrain from alcohol and vigorous exercise for 24 hours and from tobacco for 12 hours prior to study procedures. Following randomization, for each treatment assessment, subjects were admitted to the clinical research unit the day prior to the GGI procedure and fed a standardized meal. All metabolic measurements and GGI procedures were performed following an overnight fast and after administration of study drug or PBO. On completion of the first study period, and following a 7-day washout, subjects were crossed over to a second treatment, and subsequently to a third treatment following another 7-day washout, and in the T2DM, a fourth treatment following a 7-day washout.

OB: A group of 12 overweight and obese otherwise healthy male volunteers with a body mass index >27 kg/m^2 and ≤35 kg/m^2 and not routinely on any medications were recruited. During each study period, each subject underwent a 160-minute GGI procedure while receiving a continuous IV infusion of OXM or PBO that was maintained

throughout the procedure. The GGI procedure was conducted as previously described in detail (14). Briefly, glucose (20% Dextrose in water) was infused at pre-defined glucose infusion rates (GIR) in ascending order (2, 4, 6 and 12 mg/kg/min), each for 40 minutes. In each study period, subjects received one of the following three treatments in random order: 3.0 pmol/kg/min OXM (OXM-HI), 0.6 pmol/kg/min OXM (OXM-LO), or PBO, with all subjects having received all three treatments. Sampling for bedside and central laboratory glucose, insulin, C-peptide and other analytes was performed at regular intervals throughout the GGI procedure, as previously described (14).

T2DM: A group of 12 male volunteers with physician-diagnosed T2DM per FPG or HbA1c, and naïve to GLP-1 analogs and DPP4-inhibitor therapies, were recruited. Three subjects were on lifestyle measures only, 9 subjects were on metformin, and of these, 4 were on metformin and sulfonylureas, and these background antihyperglycemic agents were held for 24 hours prior to the GGI. Each subject underwent four GGI procedures a week apart, during each of 4 treatment arms. Treatments were administered in random order in a crossover design: OXM-HI plus PBO for liraglutide (OXM); LIRA 0.6 mg plus PBO for OXM (LIRA0.6), LIRA 1.2 mg plus PBO for OXM (LIRA1.2); and PBO for both LIRA and OXM (PBO). To maintain blinding, during each study period, each subject received one subcutaneous injection (a single dose of LIRA or PBO) and one administration of a continuous IV infusion (of OXM or PBO). All 12 subjects completed a GGI with PBO, OXM and LIRA0.6; six of these 12 completed the fourth GGI after receiving LIRA1.2; and the remaining 6 completed the fourth GGI with PBO to assess reproducibility. LIRA/PBO was administered as a single subcutaneous injection approximately 9 hours preceding the start of the GGI, to achieve C_{max} during the GGI.

OXM was administered as a continuous IV infusion, starting approximately 15 minutes prior to initiation of the GGI, and maintained throughout the 160 minutes of the GGI. The GGI procedure itself comprised of four 40-minute periods of glucose infusion administered in ascending steps, as described above. In this cohort of T2DM, the highest GIR was limited to 10 mg/kg/min, to avoid risk of excessive hyperglycemia. Also, T2DM received a low-dose infusion of insulin overnight, to achieve similar baseline plasma glucose across treatment arms prior to starting the GGI. The insulin infusion was turned off for approximately 30 minutes prior to initiating the GGI to avoid suppression of endogenous insulin secretion. Sampling for bedside and central laboratory glucose, insulin, C-peptide and other analytes was performed at regular intervals throughout the procedure.

Analysis of Blood Samples

Central laboratory glucose was assayed using the Roche Modular platform using Roche reagents (inter assay CV: 2.3-3.8%, intra assay CV: 2.6-3.0%). Insulin and C-peptide assays were performed using the Immulite 2000 system using reagents from Siemens (Insulin has an inter-assay CV of 1.25-1.59% and an intra-assay CV of 1.93-3.41%; C-peptide has an inter-assay CV of 1.40-2.70% and an intra-assay CV of 1.30-2.70%). Bedside glucose was assayed using YSI (Yellowspring Instruments) apparatus, by the glucokinase method. Plasma total, LDL and HDL cholesterol, as well as triglyceride levels were measured at baseline. OXM levels were measured by LC/MS/MS.

Quantitative and Statistical Methods

Estimation of Insulin Secretion Rates (ISR) was done by deconvolution of circulating C-peptide measurements, using the methods of Eaton (15) and Van Cauter (16). Other key endpoints for analysis included circulating glucose (G), insulin and C-peptide concentrations measured during the GGI, estimated as the time-weighted average (TWA) and maximum glycemic excursion (G_{max}). ISR and ISR/G were also computed. All parameters were estimated or computed for the entire duration of the GGI ($TWA_{0\text{-}160\ minutes}$), and during the period of highest GIR alone ($TWA_{120\text{-}160\ minutes}$). $TWA_{0\text{-}160\ minutes}$ for glucose is described as mean plasma glucose for each of the groups. The slopes of the regression for change from baseline in ISR on change from baseline in prevailing glucose (slope ΔISR vs ΔG) throughout the GGI were computed, to provide an index of beta-cell glucose sensing and responsiveness. All analyses were conducted using the individual predose values as a covariate. Prior to the analysis of covariance, the distributional assumptions were evaluated for each endpoint. For all of the concentration endpoints and ISR described above, there was no indication of departures from normality. Therefore, the data were analyzed on the raw scale. All concentration endpoints were analyzed on the log scale and back-transformed for reporting. ISR and slope of the ISR versus glucose were analyzed on the raw scale. A linear mixed effects model appropriate for a 3-period crossover design in OB and 4-period crossover design in T2DM was used to compare the effects of a single dose of OXM to PBO on glycemic and insulinotropic changes during the GGI in the subjects without diabetes and of a single dose of OXM or LIRA on glycemic changes during the GGI in subjects with diabetes. The model included fixed effects for treatment, sequence and period. An unstructured covariance structure was

assumed to account for the within-subject correlation unless convergence issues were found. In those cases, a compound symmetric covariance structure was used. No evidence of carryover was found. All comparisons were based on a one-sided t-test at $\alpha = 0.05$ level.

RESULTS

Effects of a Single Dose of OXM in OB (Table 1 and Fig.1)

Baseline characteristics of OB are shown in Table 1. The treatments and procedures were well tolerated, with no nausea or emesis. Each subject completed 3 GGI procedures, performed respectively with an infusion of PBO, OXM-LO, or OXM-HI. In response to the escalating glycemic stimulus at each of the four steps of the GGI, plasma glucose rose significantly ($p<0.001$) over baseline in all three groups (Fig. 1A) and was accompanied by significant ($p<0.001$) increases in plasma insulin, C-peptide and insulin secretion rates (ISR) with each of the above interventions (Fig. 1B, C, D). OXM at both doses led to increases in circulating insulin and C-peptide and ISR that were significantly greater than in PBO, and these responses were more robust for OXM-HI than OXM-LO ($p<0.05$) (Fig.1B, C, D). At these matched steps of GIR within the GGI, plasma glucose was highest in PBO, lowest in OXM-HI, and intermediate in OXM-LO (Fig.1A). At the high dose, OXM led to a robust and significant ($p<0.001$) decrease in mean plasma glucose by 18.4%, while with low dose OXM, a trend ($p=0.052$) for lower plasma glucose by 6.4% was observed. At the highest GIR of the GGI (12 mg/kg/min), OXM-HI was associated with a 28.8% reduction in plasma glucose compared to PBO ($p<0.001$). These between group differences in ISR and plasma glucose when expressed in the form of a ratio of

ISR/G, was significantly increased for OXM-HI and OXM-LO compared to PBO (Fig.1E). The most robust differences between groups for all parameters were observed at the highest GIR (12 mg/kg/min).

The slopes of ISR/G and ΔISR/ΔG are integrated measures of beta-cell responsiveness to glucose. Statistically significant and dose related increases were observed for OXM-HI and OXM-LO, relative to PBO (↑110%, $p<0.001$; and ↑33%, $p<0.05$), respectively, Fig. 1F.

Effects of OXM in T2DM in a GGI (Table 1 and Fig. 2 and 3)

Baseline characteristics of the research volunteers with T2DM are presented in Table 1. Each subject was studied on 4 occasions in the setting of a GGI, receiving in randomized order: PBO, OXM, LIRA0.6, or LIRA1.2, as described in the methods section. The GGI procedure and treatments were well tolerated, without nausea or emesis. During infusion of OXM, mean plasma OXM levels were 243±36 pmol/L. Plasma glucose rose significantly ($p<0.001$) during the GGI in all treatment arms, as shown in Fig. 2A. Compared to PBO, mean plasma glucose was significantly reduced by OXM, by 13% ($p=0.02$) and the maximal glucose excursion was lowered by 23% ($p<0.01$). As expected (12), LIRA lowered mean plasma glucose, with a reduction of 11% ($p<0.05$) for LIRA0.6 and 30% ($p=0.001$) for LIRA1.2. Maximal glucose excursions were significantly blunted by 23% ($p<0.01$) and 42% ($p<0.001$) for LIRA0.6 and LIRA1.2, respectively. The reductions in mean plasma glucose were comparable between OXM and LIRA0.6 ($p=0.36$). The mean plasma glucose for LIRA1.2 was significantly lower than with LIRA0.6 ($p=0.04$), and was numerically but not significantly lower than with OXM

(p=0.053). The effects of OXM and LIRA0.6 were equivalent and the effect of LIRA1.2 about 25% stronger than either; however, these differences were of borderline significance (p=0.05).

The above glycemic changes in the GGI were accompanied by robust and significant increases in insulin, C-peptide and ISR across the three treatment arms compared to PBO (all p<0.001) (Fig. 2B, C, and D). OXM increased ISR robustly and significantly by 67% relative to PBO (p<0.001). The increases observed with LIRA0.6 and LIRA1.2 were somewhat higher, at 113% and 139%, both strongly significant compared to PBO (p<0.001), and both significantly greater than OXM (p<0.01). Mean ISR during the GGI was similar for LIRA0.6 versus LIRA1.2 (p=0.14). Similar findings were observed for comparisons across treatments for the ratio of ISR/G at the maximal glucose excursion (Fig. 2E). The slope of ISR vs G provides an integrated parameter of beta-cell glucose sensing and response, and the plots are shown in Fig. 3. A significant (p<0.001) increase in the sensitivity and responsiveness of the beta cell to glucose, estimated as the slope of ISR/G and slope of Δ ISR/Δ G, over the duration of the GGI was detected with OXM and LIRA (Fig. 3A). Relative to PBO, the infusion of OXM increased the slope by approximately 2-fold (p<0.001), while LIRA0.6 and LIRA1.2 increased the slope by 3.5-fold and 5-fold, respectively. The difference in beta cell glucose responsiveness between the two doses of LIRA was not significant (p=0.07), but was significantly greater than with OXM (p<0.02 vs LIRA0.6, and p<0.01 vs LIRA1.2). Notably, the slope of ISR/G in the T2DM following OXM was comparable (p=NS) to that in non-obese subjects without diabetes, treated with PBO (Fig. 3B).

Six of the T2DM underwent two PBO based GGI, enabling an assessment of the reproducibility of response. The ratio of ISR/G at maximal glucose excursion, and the slope of ISR vs G on the two occasions was strongly reproducible, with an Intraclass Correlation Coefficient (ICC) of 0.92 (90% CI of 0.72, 0.98) and a within subject CV of 4.8%.

DISCUSSION

The two clinical trials in the current report were undertaken to address the acute effects of exogenous native OXM on glucose homeostasis in obese subjects with and without T2DM. The key finding from our work was that OXM acutely improved glucose homeostasis in obese without T2DM, and more importantly, in the obese T2DM as well, as measured in a graded-glucose infusion. The principal mechanism for this salutary effect on glucoregulation was stimulation of insulin secretion, enhancing glucose-responsiveness of insulin secretion relative to PBO and evident in a dose-dependent manner in the obese subjects without T2DM. In the T2DM, the effect of OXM to stimulate insulin secretion and enhance glucose-responsiveness of insulin secretion was clearly evident and statistically robustly significant relative to PBO, with the latter essentially restored to that of non-obese subjects without T2DM. Notably, the glycemic excursion in the T2DM treated with OXM was comparable to that after a single dose of comparator, LIRA, in the same group of patients.

The current findings mitigate legitimate concerns that dual-agonism of both GLP-1R and GCGR will lead to deterioration of glucose tolerance in those at risk for T2DM or deterioration of glycemic control in those with T2DM. In the first of the two studies, research subjects were overweight and obese volunteers without diabetes, and an OXM-HI infusion yielded favorable effects on the glycemic response during a GGI, on insulin secretion and on beta cell glucose responsiveness, whereas an OXM-LO infusion had nominal effect. The slope of insulin secretion rate vs glucose captures glucose-dependency of the stimulation of insulin secretion and again, a positive effect was achieved by OXM. It is noteworthy that plasma exposure attained during the OXM-HI

infusion was in the same range observed in the prior successful weight loss study that was pivotal in generating interest in the potential of this gut hormone as a weight loss therapeutic (3). These observations in obese subjects without diabetes are important because these support the notion that in the face of engagement of GCGR, co-agonism of GLP-1R appears to offset an adverse effect of GCGR engagement upon glucose homeostasis, and elicits a salutary effect instead. To our knowledge, there were limited prior data that addressed this issue.

In the second of the two studies, research subjects had previously diagnosed T2DM controlled by diet or oral medication and the findings were consonant with those obtained in obese nondiabetic subjects. A 3 pmol/kg/min infusion rate of OXM given during the GGI procedure blunted the rise of plasma glucose (compared to response during a GGI conducted with PBO), and was associated with a highly significant effect to increase rates of insulin secretion and improved beta cell glucose responsiveness. As a positive comparator in the GGI study in subjects with T2DM, the selective GLP-1 agonist LIRA was used. Our presumption was that a pure GLP-1 agonist would likely be better than OXM on the glycemic response to the GGI challenge and to the insulin secretory response. Our findings support this notion, but arguably, the differences in response to OXM and the LIRA0.6 were modest. The glycemic response to OXM was identical to that of LIRA0.6; LIRA1.2 lowered plasma glucose response significantly more than OXM. LIRA did elicit a stronger insulin secretory response and this finding in the face of similar effect to lower the hyperglycemic response is further discussed below.

Taken together, our findings indicate that at least with a single exposure in the setting of an acute GGI challenge, the net effect of native OXM on glucose homeostasis is salutary,

both in subjects at risk for potential developing T2DM (viz., obese subjects without diabetes) and in those with established T2DM. Overall the glycemic responses during the GGI challenge in T2DM were not dissimilar to a clinically relevant dose of LIRA, a pure GLP-1 agonist, and an agent known to achieve clinical translation for positive efficacy in long-term control of glycemia (17). The current studies do not address the long-term effect of OXM, or of an OXM-like analog, on chronic glycemic control, but we would speculate that if the acute favorable effect is sustained, and weight loss were achieved with OXM, thereby lessening obesity-related insulin resistance, long-term favorable effects on glucose homeostasis would likely be observed. This is a testable hypothesis and the novel findings from the current study that revealed acute favorable effect on glucose homeostasis add impetus to carry out such an investigation.

In the study in T2DM, the acute effect of OXM was not identical to that of LIRA with respect to stimulation of insulin secretion. OXM significantly increased rates of insulin secretion and the ratio of ISR to plasma glucose, relative to observations during the GGI conducted with PBO. Notably, the effect of OXM on ISR during the GGI in subjects with T2DM essentially restored this response to what we have previously reported in PBO-treated non-obese subjects without diabetes (14). However, the insulin secretory response in T2DM was somewhat greater with LIRA than OXM and in a dose-responsive manner, numerically greater with the LIRA1.2 than LIRA0.6. The positive effects of LIRA are entirely consistent with previously reported findings (21,22). Yet despite these differences in insulin secretory response, there is the intriguing observation that the effect to blunt the rise of plasma glucose response during the GGI challenge was highly similar between OXM and LIRA0.6, and not much different from that achieved with LIRA1.2.

One possible explanation is that the insulin secretory response to OXM may already lead to near maximal insulin action and glucose disposal, and the additional increase in insulin secretion by LIRA may not necessarily lead to any further augmentation in insulin action. Alternatively, OXM may have metabolic or glucoregulatory effects besides enhancement of insulin secretion, perhaps on glucose disposal. Finally, it is possible that OXM may act on receptors besides the GLP-1R and GCGR. While it is possible that comparison of effects at steady-state conditions for both compounds may have yielded different outcomes, it should be noted that care was taken to evaluate effects at C_{max} of liraglutide. These various considerations need further experimental exploration beyond the scope of the current trial. The effects of OXM in this trial were observed at exposures that were in the range reported previously by Bloom et al (6,7), and that approximate ~EC_{25} at the respective receptors. This is particularly relevant since there were no adverse effects observed at these exposures. Taken together, the findings from this experimental series of beneficial glucoregulatory effects, the absence of adverse effects and exposures suggesting room for further dose enhancement, all augur well for the potential for OXM-like balanced analogs towards achieving maximizing weight loss, while retaining clinically meaningful direct glucoregulatory effects.

Another important point to note is the clinical relevance of the effects observed. The 3 pmol/kg/min dose of OXM at which we observed significant glucoregulatory effects in the obese with and without T2DM was the dose of OXM at which Bloom et al showed significant appetite suppression. More importantly, at exposures of OXM that matched those at the 3 pmol/kg/min dose, Bloom et al have reported increased energy expenditure after 4 days of treatment, and significant weight loss after 4 weeks of

treatment (5,7). Taken together, it is reasonable to conclude that the glucoregulatory effects of OXM observed in the current series are truly representative of a clinically relevant pharmacologic exposure of OXM, and therefore of co-activation of the GLP-1R and GCGR.

There is contemporary interest in developing OXM-like analogs that can act as dual agonists engaging both GLP-1R and GCGR but with longer half-life than the short-lived native hormone (8,9). Rodent studies employing such analogs support the concept that dual agonists can achieve greater weight loss than a pure GLP-1R analog, even when care is taken to match for equivalent GLP-1R engagement (11,12). While OXM is regarded as evenly balanced between GLP-1R and GCGR potency, a broader range of respective balances is being explored to identify the most desirable ratio of receptor activation to achieve and maintain weight loss while maintaining desirable glycemic efficacy. While the current findings of a salutary effect of OXM on glucose and insulin homeostasis cannot be extrapolated to potential analogs designed to bias toward increased GCGR potency relative to that for GLP-1R, they do reinforce that as a platform for rigorous and efficient inquiry, the GGI has value in screening such therapeutic candidates and that it can be used in populations of clinical interest (14).

The proposed overall metabolic effect of OXM invokes a dual component path to glucose lowering, a direct weight-loss independent component via enhancement of GDIS that has an acute onset, and an indirect component via weight loss that has a more gradual onset and protracted time course profile. The results from the current series of experiments demonstrate for the first time, the significant direct, weight-loss independent glucoregulatory effects of OXM in overweight and obese humans with and without

T2DM. Knowledge of the magnitude and proportion of these direct acute effects of OXM in man should enable more accurate prediction of the overall glucose-lowering potential of OXM itself, and of other similar peptides. Further work to characterize the mechanisms and magnitude of weight loss associated with OXM and similar peptides in longer-term trials should add considerably to our understanding of the native peptide as well as the potential therapeutic value of this class of peptides.

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Table 1. Baseline characteristics of subjects

	Overweight and Obese without Diabetes		Type 2 diabetes	
Parameter	**Mean**	**SD**	**Mean**	**SD**
BMI (kg/m^2)	30.9	1.9	28.0	3.1
Age (years)	30.1	8.2	59.3	3.7
Fasting Plasma Glucose (mg/dL)	99.6	7.5	133.0	22.9
Fasting Insulin (μIU/mL)	14.8	6.9	6.8	2.8
Fasting C-peptide (ng/mL)	2.3	0.4	1.3	0.4
Insulin Secretion Rate (ng/min)	0.49	0.15	0.28	0.12
ISR / Glucose (ng/min)/(mg/dL)	0.0053	0.0019	0.0039	0.0015
HbA1c (%)	N.M.	N/A	6.9	0.7
Duration of Type 2 diabetes in years	N/A	N/A	4.6	4.6

Figure Legends.

Figure 1. In obese non-diabetic subjects, during a GGI challenge, the plasma glucose response (Panel A), C-peptide concentrations (Panel B), insulin concentrations (Panel C), insulin secretion rates (Panel D), and ratio of ISR/glucose (Panel E) are shown under conditions of PBO administration (filled circles), infusion of OXM at 0.6 pmol/kg-min (open triangles), and infusion of OXM at 3.0 pmol/kg-min (filled triangles). Panel F shows beta cell sensitivity and responsiveness to glucose, represented by the slope of insulin secretion rates (ISR) vs glucose (G) across the GGI. * = $p<0.001$ versus PBO, † = $p<0.05$ versus PBO, and ‡ = $p<0.001$ versus OXM 0.6 mg.

Figure 2. In T2DM subjects, during a GGI challenge, the plasma glucose responses (Panel A), C-peptide concentrations (Panel B), insulin concentrations (Panel C), insulin secretion rates (Panel D), and ratio of ISR/glucose (Panel E) are shown under conditions of PBO administration (filled circles), infusion of OXM at 3.0 pmol/kg-min (open triangles), post-injection of LIRA 0.6 mg (open diamonds) and post-injection of LIRA 1.2 mg (open triangles). * = $p<0.001$ versus PBO, † = $p<0.01$ versus PBO, and ‡ = $p<0.01$ versus LIRA 0.6.

Figure 3. Beta Cell Sensitivity and Responsiveness to Glucose, represented by the slope of insulin secretion rates (ISR) vs glucose (G) across the GGI in in T2DM subjects (Panel

A) treated with OXM or LIRA or PBO, and Panel B – a single dose of OXM restores beta cell responsiveness in T2DM to that of PBO-treated non-obese subjects without T2DM (14). * = $p<0.001$ versus PBO-T2DM, and ‡ = $p<0.05$ versus LIRA 0.6. [Panel B: OXM vs PBO (non-obese) = NS]

DISCLOSURES

Guarantor

Sudha S. Shankar and Lori A Mixson are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Compliance with Ethics Guidelines

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all patients included in the study.

Duality of interest

R. Ravi Shankar, Lori A Mixson, Deborah L Miller, Donna M Williams and S. Aubrey Stoch are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. Kenilworth, NJ, USA and own stock and/or hold stock options in the company.

Sudha S Shankar, Barnali Pramanik, Amy K. O'Dowd, Clay B. Frederick, Chan R Beals, Helmut O Steinberg, and David E Kelley are former employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and may own stock and/or hold stock options in the company.

Author Contribution

All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published. Sudha S. Shankar, R. Ravi Shankar, Lori A. Mixson, Deborah L. Miller, Barnali Pramanik, Amy K. O'Dowd, Donna M. Williams, Clay B. Frederick, Chan R. Beals, S. Aubrey Stoch, Helmut O. Steinberg, and David E. Kelley agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Sudha S. Shankar, R. Ravi Shankar, Lori A. Mixson, Deborah L. Miller, Barnali Pramanik, Amy K. O'Dowd, Donna M. Williams, Clay B. Frederick, Chan R. Beals, S. Aubrey Stoch, Helmut O. Steinberg, and David E. Kelley conceived, designed, and/or planned the study. Sudha S. Shankar, and Deborah L. Miller acquired the data. Sudha S. Shankar, Lori A. Mixson, Barnali Pramanik, Chan R. Beals, Helmut O. Steinberg, and David E. Kelley analyzed the data. Sudha S. Shankar, R. Ravi Shankar, S. Aubrey Stoch, Helmut O. Steinberg, and David E. Kelley interpreted the results. Sudha S. Shankar, R. Ravi Shankar, Lori A. Mixson, and David E. Kelley drafted the manuscript. Sudha S. Shankar, R. Ravi Shankar, Lori A. Mixson, Deborah L. Miller, Barnali Pramanik, Amy

K. O'Dowd, Donna M. Williams, Clay B. Frederick, Chan R. Beals, S. Aubrey Stoch, Helmut O. Steinberg, and David E. Kelley critically reviewed and/or revised the manuscript for important intellectual content.

Data Availability

Merck & Co., Inc.'s data sharing policy, including restrictions, is available at http://engagezone.merck.com/ds_documentation.php. Requests for access to the study data can be submitted through the EngageZone site or via email to dataaccess@merck.com.

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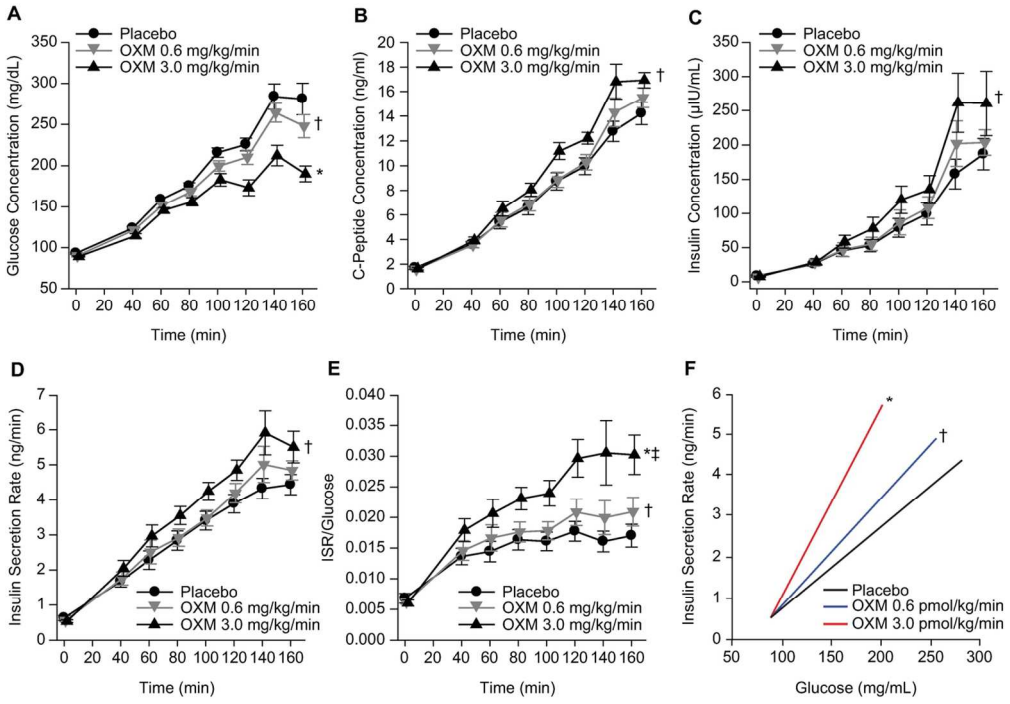

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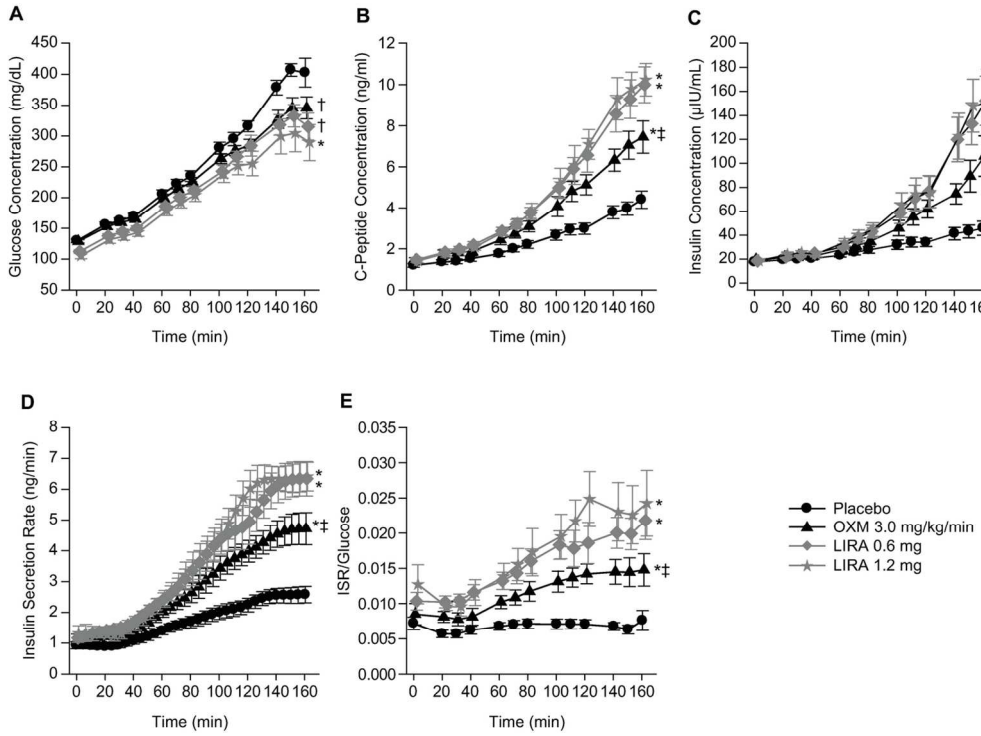

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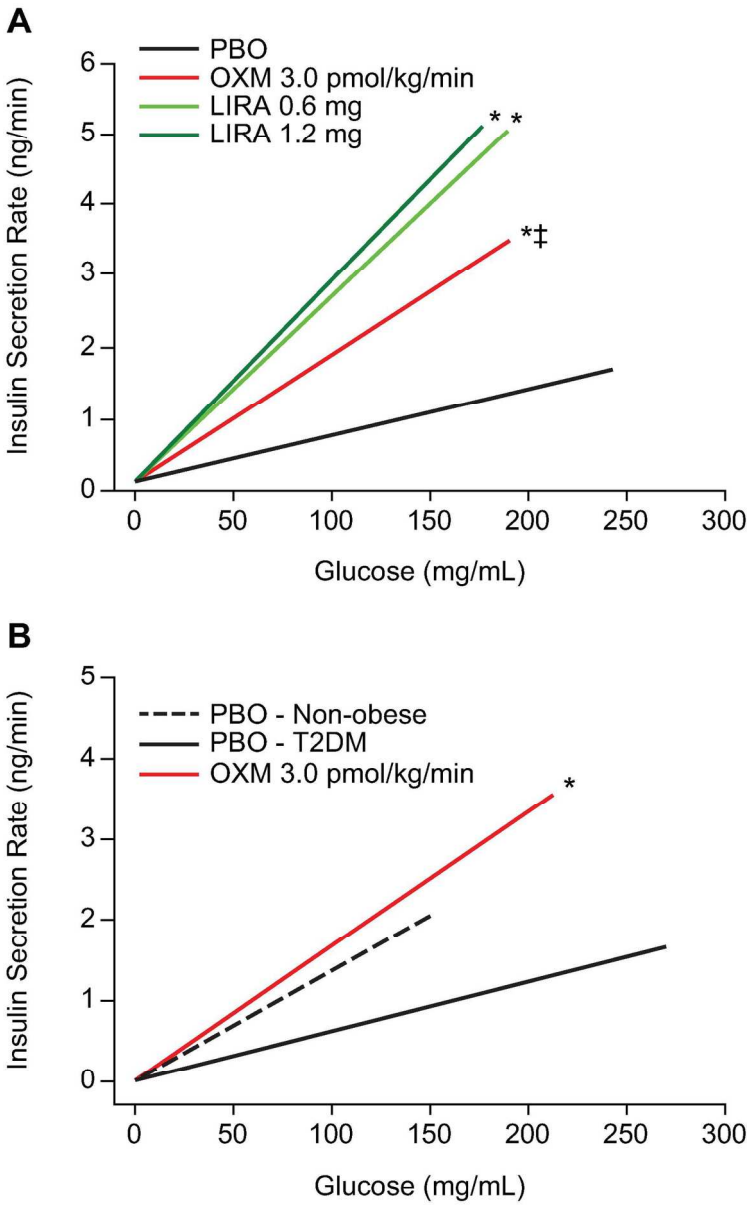

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