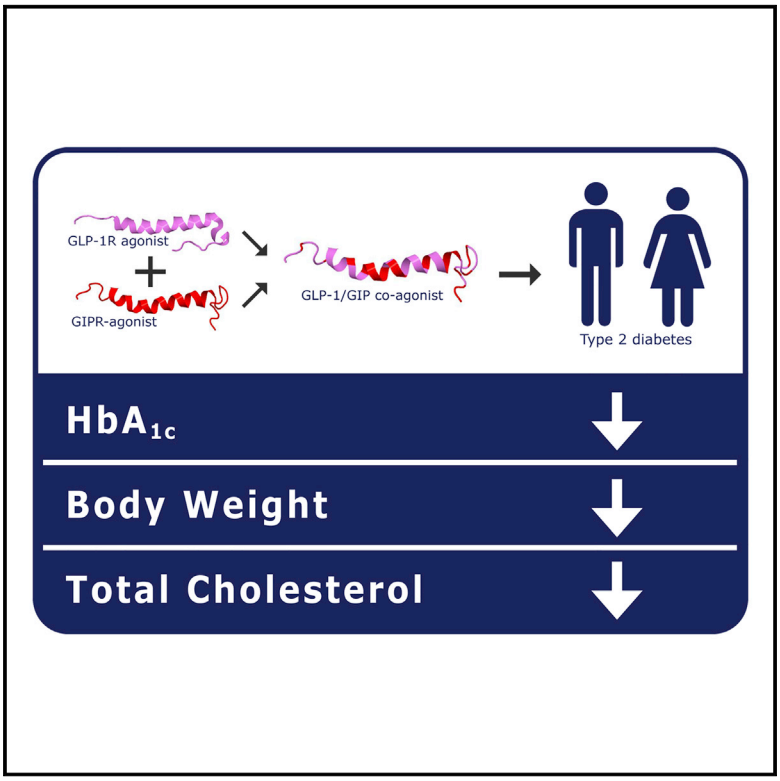
Clinical and Translational Report

Cell Metabolism

The Sustained Effects of a Dual GIP/GLP-1 Receptor Agonist, NNC0090-2746, in Patients with Type 2 Diabetes

Graphical Abstract



Authors

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In Brief

Frias et al. assessed the effects of NNC0090-2746, a unimolecular dual agonist derived from hybridized GLP-1 and GIP, in patients with T2DM. NNC0090-2746 was well tolerated and significantly improved glycemic control and reduced body weight compared with placebo. Maximum benefit was obtained by patients with the best baseline glycemic control.

Highlights

- NNC0090-2746 is a fatty-acylated GIP/GLP-1 dual agonist
- NNC0090-2746 improved glucose control in type 2 diabetic patients
- NNC0090-2746 also decreased body weight and total cholesterol
- The effect of NNC0090-2746 was dependent on initial level of glucose control

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The Sustained Effects of a Dual GIP/GLP-1 Receptor Agonist, NNC0090-2746, in Patients with Type 2 Diabetes

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SUMMARY

Unimolecular dual incretins derived from hybridized glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) sequences have demonstrated synergistic reduction of adiposity in animal models and reductions of hyperglycemia in short-duration human trials. Here, we extend the characterization of NNC0090-2746 (also known as RG7697), a fatty-acylated dual agonist possessing *in vitro* balanced GIPR and GLP-1R agonism. In this 12-week, randomized, placebo-controlled, double-blind phase 2a trial, patients with type 2 diabetes inadequately controlled with metformin received 1.8 mg of NNC0090-2746 or placebo subcutaneously once daily. Liraglutide 1.8 mg (Victoza), starting with 2-week dose escalation, was administered subcutaneously once daily as an open-label reference arm. Measurements were collected at regular intervals after randomization. NNC0090-2746 significantly improved glycemic control and reduced body weight compared with placebo. Total cholesterol, alone among a range of lipid parameters, and leptin were both significantly reduced compared with placebo. Treatment with NNC0090-2746 was generally safe and well tolerated.

INTRODUCTION

The global prevalence of both type 2 diabetes (T2D) and obesity has steadily increased over the last three decades (Ng et al., 2014; World Health Organization, 2016). Successful management of T2D frequently requires that hyperglycemia and excess body weight are simultaneously addressed. The glucagon-like peptide 1 receptor agonists (GLP-1RAs) are a class of pharmacological agents that address both conditions. GLP-1RAs are therapeutically effective and achieve significant decreases in hyperglycemia and body weight, though the magnitude of body weight reduction is limited and tends to plateau over time (Klonoff et al., 2008; le Roux et al., 2017; Lorenz et al., 2013; Neff and Kushner, 2010). Novel treatments that recruit additional mechanisms of biological action constitute one of the more promising early-phase approaches to safely enhance efficacy when current therapy no longer delivers progressive improvements in disease management (Tschöp et al., 2016).

GLP-1 and glucose-dependent insulinotropic peptide (GIP) are gut hormones secreted from intestinal L cells and K cells, respectively. Together, these hormones account for the vast majority of the incretin effect, the enhanced postprandial insulin secretion observed in healthy adults (Dupre et al., 1973; Kreymann et al., 1987; McIntyre et al., 1964; Nauck et al., 1986). Though inhibition of dipeptidyl peptidase 4 (DPP-4) increases levels of both active GLP-1 and GIP, suggesting a common pharmacological end goal, the majority of clinical research pertaining to the therapeutic use of incretins has thus far focused on GLP-1 with much less attention given to GIP (Baggio and Drucker, 2007).

The historical emphasis on GLP-1 therapy arose partly due to the early positive clinical results achieved with GLP-1RAs (Gutniak et al., 1992; Nauck et al., 1996) and partly due to reports in animals that GIP promoted obesity and impaired lipid metabolism (Gault et al., 2002, 2005; Irwin et al., 2004; McClean et al., 2007; Miyawaki et al., 2002). Additionally, single-dose trials in patients with T2D reported that GIP worsened postprandial hyperglycemia and deepened the conviction that GIP agonism may not have therapeutic benefit (Chia et al., 2009; Mentis et al., 2011; Nauck et al., 1993; Vilsbøll et al., 2002).

The diabetic state is characterized by a complex array of progressive changes, to both the diabetic pancreas and,

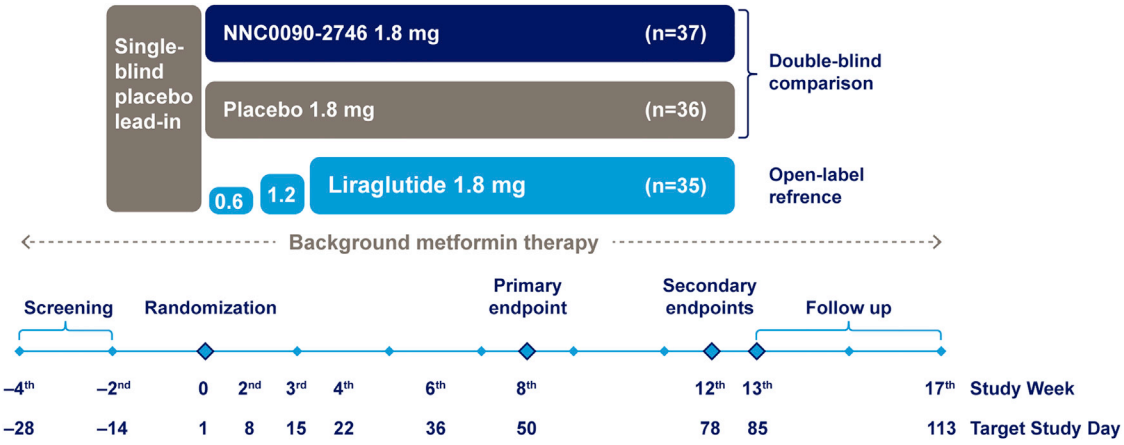

Figure 1. Trial Design
Overview of trial design showing the three trial arms and the different trial periods.

consequently, the entero-insular axis, notably manifesting in hyperglycemia, reduced β cell mass, and diminished incretin effect (Campbell and Drucker, 2013; Nauck and Meier, 2016). The incretin effect of GIP in patients with T2D is blunted primarily as a consequence of hyperglycemia rather than being a causal defect (Knop et al., 2007a, 2007b). This can be seen experimentally in patients with T2D who regain some GIP sensitivity after approaching normoglycemia with insulin therapy or treatment with DPP-4 inhibitors (Aaboe et al., 2015; Højberg et al., 2009). This requirement for glycemic control affects the interpretation of previous trials assessing acute GIP administration, suggesting that chronic therapy in well-controlled patients could result in different outcomes. Recent data suggest that GIP may regulate β cell survival through signaling pathways independent of GLP-1, supporting the hypothesis that the two incretins are not redundant and may complement one another (Campbell et al., 2016). The pharmacological integration of the activities of both incretins could conceivably function in two stages where GLP-1RA establishes glycemic control, reduces body weight, and sets the foundation upon which glucose-dependent insulinotropic peptide receptor agonists (GIP-RA) can further improve metabolism and body weight. The selectively engineered peptides intermixing balanced, potent GLP-1 and GIP agonism have demonstrated, in sustained administration in rodents and non-human primates, their ability to further decrease blood glucose and body weight when compared with peptides functioning by just one of the two mechanisms (Finan et al., 2013).

NNC0090-2746 (previously developed as RG7697) is a fatty-acylated GIP/GLP-1 dual agonist in clinical development for the treatment of T2D. It exhibits balanced activity for the human GLP-1 (EC_{50} = 5 pM) and GIP (EC_{50} = 3 pM) receptors, with virtually no meaningful agonism at any other related receptor (EC_{50} at glucagon receptor of >1 μM) (Finan et al., 2013). Recent phase 1 trials of NNC0090-2746 have shown that steady-state concentration of the peptide is achieved within 1 week by daily dosing of patients with T2D (Schmitt et al., 2017). Once-daily doses of up to 2 mg were well tolerated, with gastrointestinal (GI) adverse effects of the type typically observed with GLP-1RAs increasingly evident at higher doses (Portron et al., 2017). Reductions in fasting, postprandial, and 24-hr profile plasma glucose were observed after 2 weeks of treatment at doses ≥0.75 mg and were associated with decreases in HbA_{1c} (up to 0.67% [absolute change]) (Schmitt et al., 2017). Building upon these clinical observations, we present here a phase 2a clinical trial of NNC0090-2746 in patients with T2D inadequately controlled with metformin.

RESULTS AND DISCUSSION

Clinical Trial Design

This was a multicenter, randomized, double-blind, parallel-group, placebo-controlled trial with open-label comparison (registered on http://clinicaltrials.gov as NCT02205528). The trial was conducted at 19 investigational sites in the US and approved by an institutional review board (IRB). The trial was conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. The trial complied with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Patients who were able to perform the self-injections satisfactorily were randomized into the 12-week double-blind treatment period in a 1:1:1 manner to NNC0090-2746 (blinded, n = 37), placebo (blinded, n = 36), or liraglutide 1.8 mg with 2-week dose escalation (open label, n = 35) (Figure 1). The purpose of the trial was to evaluate the effect of NNC0090-2746 on glycemic control, body weight, and other metabolic parameters in patients with T2D inadequately controlled by metformin (HbA_{1c} ≥ 7.2% and ≤ 10.5%) as well as the safety and tolerability. The open-label liraglutide arm was included to provide a qualitative reference to a currently available GLP-1RA. The efficacy and safety results from the liraglutide arm are presented in the STAR Methods. The primary endpoint, HbA_{1c} change, was evaluated from baseline (Day 1) to Week 8 visit (W8, Trial Day 50). Secondary and exploratory endpoints were evaluated to either Week 12 visit (W12, Trial Day 78) or Week 13 visit (W13, Trial Day 85).

Patients randomized to NNC0090-2746 achieved significant reductions in HbA_{1c}, body weight, and total cholesterol (TC) compared to baseline and versus placebo.

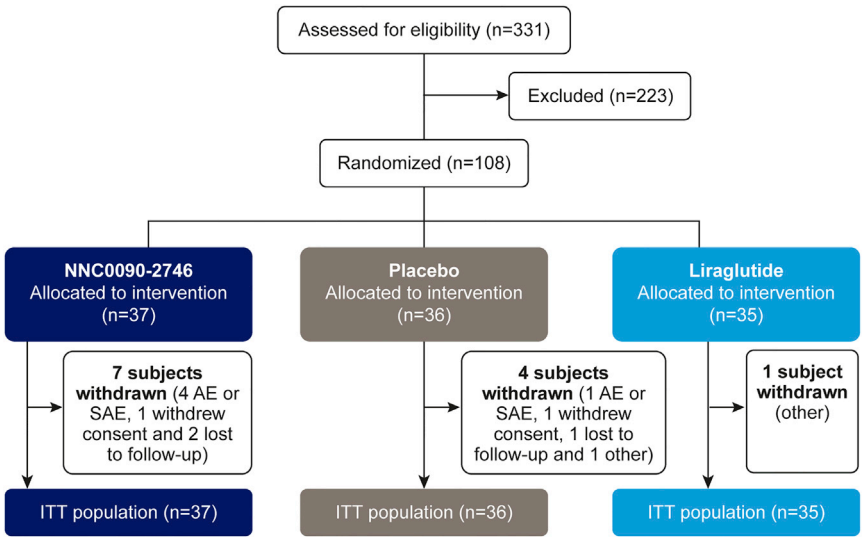

Figure 2. Participant Flow Diagram

Numbers of patients initially screened, randomized, withdrawn, and included in the ITT analysis set for the three trial arms are depicted. The ITT analysis set is defined as all patients who were randomized and had evaluable measurement of the parameter of interest at baseline and at least one post-baseline visit. AE, adverse event; ITT, intention to treat; SAE, serious adverse event.

Patient Characteristics

Of the 331 screened patients, 108 met the inclusion criteria and were randomized to NNC0090-2746, placebo, or liraglutide. Twelve patients (seven from NNC0090-2746, four from placebo, and one from liraglutide) withdrew prematurely from the trial, which led to 96 completing patients (Figure 2). Demographic baseline characteristics of the 108 patients are shown in Table 1. Patients had a mean age of 54.8 years, 54.6% were female, and the mean duration of diabetes was 8.0 years. The mean HbA_{1c} was 8.3%, and the mean fasting plasma glucose (FPG) was 166.3 mg/dL. The mean body weight was 90.9 kg, and the mean body mass index (BMI) was 33.0 kg/m^2. The baseline characteristics were similar across treatment groups.

Effects on Glycemic Control and Body Weight

Change from baseline in HbA_{1c} was statistically significant when comparing NNC0090-2746 treatment with placebo to both W8 and W12 with estimated treatment differences (ETDs) of −0.63% (−0.93; −0.33)$_{95\%\ CI}$ and −0.96% (−1.36; −0.56)$_{95\%\ CI}$, respectively, (Figure 3A; Table 2). Post hoc assessment of change in HbA_{1c} to the W13 follow-up visit, where a similar proportion of patients as to W12 were on treatment, was of comparable magnitude to prior measurements with an ETD of −1.04% (−1.45; −0.62)$_{95\%\ CI}$ (Figure S1A). Consistent with this decrease, both the mean seven-point self-measured plasma glucose (SMPG) and FPG values were significantly reduced by NNC0090-2746 treatment compared to placebo (Figure S2; Table 2). The change from baseline in mean SMPG was statistically significant from baseline to both W8 and W12 with ETDs of −27.7 mg/dL (−44.7; −10.7)$_{95\%\ CI}$ and −31.7 mg/dL (−47.0; −16.5)$_{95\%\ CI}$, respectively (Table 2). The change from baseline to W12 in FPG was statistically significant with an ETD of −38.2 mg/dL (−57.0; −19.4)$_{95\%\ CI}$ but not measured at W8 (Table 2). Decreases in FPG for both the NNC0090-2746 and placebo groups were seen at W4 and W12 (Figure S2), coincident with performance of a meal tolerance test (MTT). NNC0090-2746 improved insulin secretion as fasting C-peptide was significantly increased from baseline to W12 with NNC0090-2746 compared to placebo with an estimated treatment ratio (ETR) of 1.29 (1.13; 1.48)$_{95\%\ CI}$ (Table 2). The fasting insulin concentration was somewhat higher for the NNC0090-2746 group than the placebo group although not significant (Table 2).

Treatment with NNC0090-2746 significantly improved measures of glycemic control compared to placebo. Short-term measures of blood glucose, SMPG and FPG, were significantly reduced (Table 2). Even though FPG may be partially influenced by a stricter fasting state at W4 and W12 due to administration of the MTT, FPG was significantly lower in the NNC0090-2746 group as compared to placebo (Figure S2), and the longer-term consequences of these effects were reflected by the significant reduction in HbA_{1c} (Table 2).

In addition to improvements in glycemic control, patients also experienced reductions in body weight. A continued decrease in body weight from baseline to W12 was observed for the NNC0090-2746 group (Figure 3B). Percent change in body weight with NNC0090-2746 treatment from baseline was significant to W8, though not to W12, with ETDs of −1.80% (−3.24; −0.37)$_{95\%\ CI}$ and −1.67% (−3.43; 0.09)$_{95\%\ CI}$, respectively, compared to placebo (Table 2). Post hoc assessment of change in body weight to W13, where a similar proportion of patients as to W12 were on treatment, was of comparable magnitude to prior measurements with an ETD of −1.87% (−3.66; −0.08)$_{95\%\ CI}$ (Figure S1B).

Results from the Meal Tolerance Test

Placebo and NNC0090-2746 patients also underwent an MTT. NNC0090-2746 significantly reduced the 2-hr postprandial concentration (C_{2hr}) of glucose (ETD: −74.6 mg/dL [100.2; −48.9]$_{95\%\ CI}$), as well as the area under the curve ($AUC_{0–3hr}$) of glucose (ETD: −181.3 mg × hr/dL [−252.4; −110.2]$_{95\%\ CI}$), from baseline to W12 compared with placebo (Figures 3C and 3D; Table 2). Furthermore, NNC0090-2746 significantly reduced $AUC_{0–3hr}$ of insulin (ETR: 0.70 ng × hr/mL [0.52; 0.95]$_{95\%\ CI}$), but not the C_{2hr} of insulin, from baseline to W12 compared to placebo (Table 2). No significant change in C_{2hr} and $AUC_{0–3hr}$ of C-peptide during the MTT from baseline to W12 compared to placebo was observed (Figures 3E and 3F; Table 2). A decrease in glucose $AUC_{0–3hr}$ was found during the MTT from baseline to W12. However, the effect of NNC0090-2746 on insulin production is unclear since the $AUC_{0–3hr}$ of insulin was reduced, but no difference was found in $AUC_{0–3hr}$ C-peptide (Table 2). Hence, the effect of NNC0090-2746 on insulin sensitivity cannot be concluded. A more rigorous focus on these parameters in future

Table 1. Baseline Characteristics for All Treatment Groups

Characteristic	NNC0090-2746 (n = 37)	Placebo (n = 36)	Liraglutide (n = 35)
Age at randomization (years)	54.7 (8.55)	54.6 (7.91)	55.2 (7.87)
Female (%)	51.4	61.1	51.4
Duration of diabetes (years)	8.5 (5.54)	7.6 (5.71)	7.8 (5.24)
Race (%)			
White	81.1	66.7	71.4
Black or African-American	18.9	30.6	25.7
Asian	0.0	2.8	2.9
Hispanic or Latino	45.9	55.6	34.3
Weight (kg)	91.8 (18.87)	89.9 (21.05)	90.9 (17.98)
BMI (kg/m^2)	33.45 (4.860)	33.29 (5.018)	32.35 (4.308)
HbA_{1c} (%)	8.37 (0.907)	8.22 (0.871)	8.36 (0.955)
$HbA_{1c} \geq 8.5\%$ (%)	51.4	38.9	37.1
FPG (mg/dl)	164.8 (39.23)	167.2 (40.91)	167.1 (36.40)
Fasting insulin (μIU/mL)	17.77 (14.006)	28.48 (55.707)	16.19 (9.755)
Fasting C-peptide (ng/mL)	3.624 (1.6971)	3.877 (1.7764)	3.456 (1.3102)
Total cholesterol (mg/dL)	196.0 (40.54)	193.8 (48.40)	181.9 (36.48)
Triglycerides (mg/dL)	210.5 (149.02)	170.2 (110.44)	145.1 (93.52)

Demographic and clinical characteristics of trial patients at time of randomization by treatment group. All values are mean (SD). BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; n, number of patients; SD, standard deviation.

studies would provide a clearer picture of effect of NNC0090-2746 on insulin secretion, clearance, and sensitivity.

Effects on Lipid Profile and Adipose Markers

The effects of NNC0090-2746 on fasting lipid parameters and adipose biomarkers were evaluated. A decrease in TC was seen for the NNC0090-2746 group with an estimated mean value at W13 of 169.7 mg/dL compared to 184.7 mg/dL for the placebo group. The change from baseline was significant and equaled a decrease of 8% to W13 with NNC0090-2746, relative to placebo (ETR: 0.92 $[0.85; 0.99]_{95\%\ CI}$) (Figure 4A). A trend for lowering lipids in general (low-density lipoprotein [LDL], triglycerides, free fatty acids, and apolipoprotein B) was observed (Figure 4A). Change in plasma leptin was significant, and a reduction by 22% (ETR: 0.78 $[0.63; 0.96]_{95\%\ CI}$) with NNC0090-2746 relative to placebo was found from baseline to W12 (Figure 4B). Among biomarkers, no significant changes in adiponectin and resistin were seen.

Leptin levels were significantly reduced compared to placebo, and though assessed in this trial as an adipose marker, the magnitude and timing of the reduction in leptin are greater than that expected by the change in body weight over the same period (Maffei et al., 1995; Rosenbaum et al., 1997). The reduction in plasma leptin may potentially reflect a restoration of leptin sensitivity, though this cannot be definitively concluded from the design of the current trial.

TC was significantly lowered by 8% compared to placebo from baseline to W13. Lowering in fasting lipids is not consistently seen for GLP-1RAs, with the effect varying by trial length and size (Davies et al., 2015; Hermansen et al., 2013; Nauck et al., 2013; Plutzky et al., 2009). The open-label liraglutide arm of this trial did not exhibit any appreciable change in total or LDL cholesterol (Figure S3). The reduction in TC observed with NNC0090-2746 is driven by a reduction in LDL cholesterol, though not statistically significant in this trial. Reduction in TC is consistent with the phase 1 MAD trial of the same compound (Schmitt et al., 2017). These data align with associations emerging from a retrospective analysis of the ADDITION-PRO trial in patients at risk of developing T2D whereby increased GIP correlated with lower fasting LDL (Møller et al., 2016). The mechanistic basis for the relative difference in how GIP and GLP impact cholesterol is an important observation that warrants additional study. It is consistent with the physiological need for two incretins of overlapping biological function but with unique beneficial virtues.

Safety

Overall in the NNC0090-2746 and placebo groups, 135 adverse events (AEs) were reported, and of these, 112 were treatment-emergent AEs (TEAEs) (Table 3). 91 AEs were reported in the NNC0090-2746 group and 44 in the placebo group. The majority of the TEAEs in the two treatment groups were mild (63%) or moderate (32%) in severity and chiefly comprised "gastrointestinal disorders" with nausea, vomiting, and diarrhea being the most frequently reported terms (Table 3). The remaining 5% were severe TEAEs and included hepatic enzyme increased, insomnia, lipase increased, alanine aminotransferase increased, and seasonal allergy (coded by MedDRA preferred terms). None of the severe TEAEs in the NNC0090-2746 and placebo groups were serious adverse events (SAEs) and all recovered.

Five patients (four from the NNC0090-2746 group; one from the placebo group) discontinued prematurely from the trial due to one SAE and eight AEs that were considered related to treatment (coded by MedDRA preferred terms): abdominal distension, diarrhea, flatulence, nausea, alanine aminotransferase increased, lipase increased, appetite decreased, and hepatic

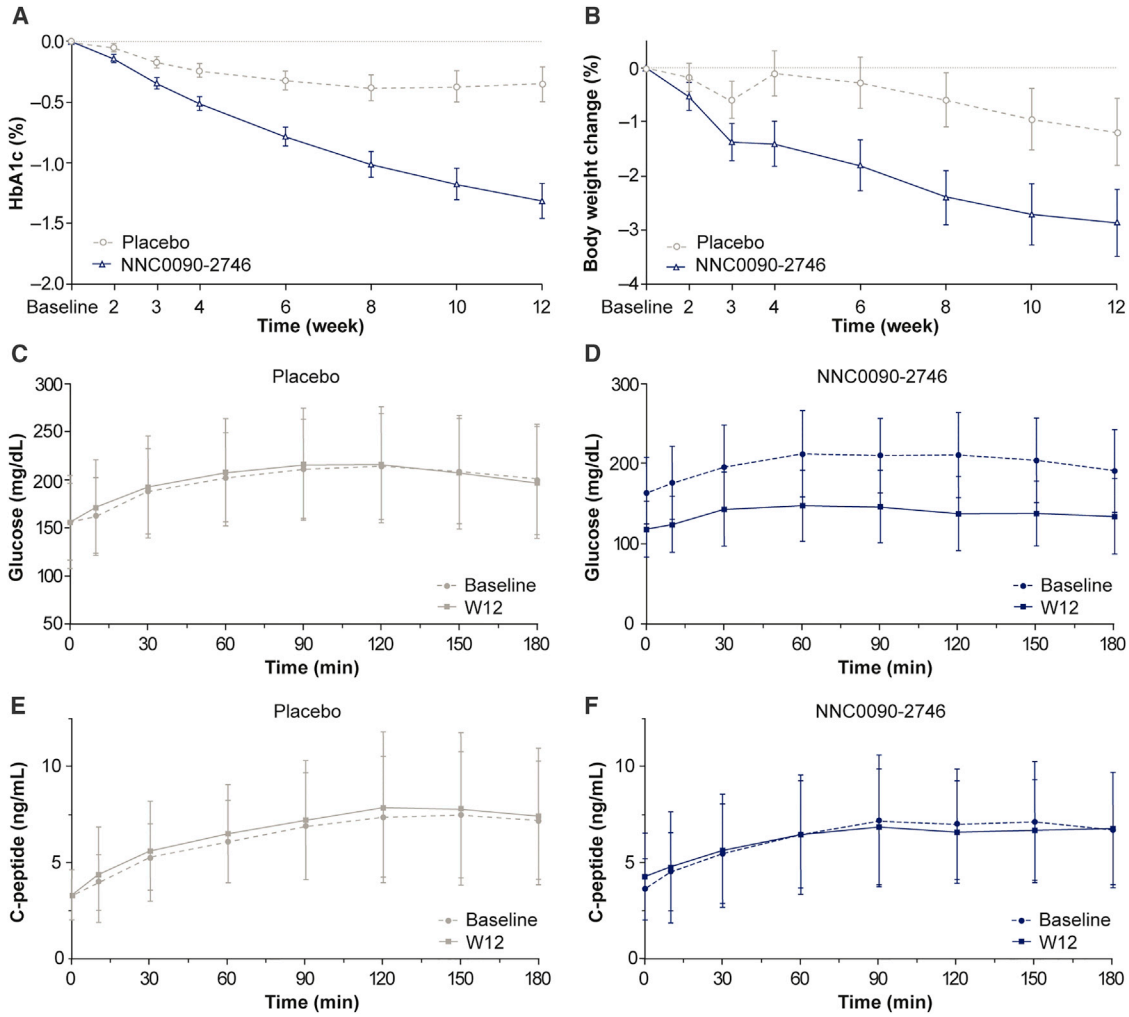

Figure 3. Effect of NNC0090-2746 on HbA_{1c}, Body Weight, and MTT Parameters

Time course of mean (±SEM) change from baseline in HbA_{1c} (A) and body weight (B) as well as plasma glucose (C and D) and C-peptide (E and F) responses during a meal tolerance test at baseline (Week 1) and Week 12. MTT, meal tolerance test. See also Figure S1.

enzyme increased (placebo patient). The SAE occurred on Trial Day 64 when a patient from the NNC0090-2746 group experienced an event of atrial fibrillation, which was moderate in intensity and resolved 4 days after the onset (patient narrative can be found in Table S1). The SAE was not considered to be related to the trial product according to the investigator's assessment. No deaths were reported during this trial.

Therapeutic administration of GLP-1RAs is commonly associated with GI adverse events, especially during treatment initiation and dose escalation. NNC0090-2746 has previously been shown to have a broadly equivalent *in vitro* affinity for both glucose-dependent insulinotropic peptide receptor (GIPR) and GLP-1R, and the unimolecular dual agonist molecule is expected to bind to either the GIP or the GLP-1 receptor, not both simultaneously (Finan et al., 2013). Proportionally reduced binding to the GLP-1 receptor would be expected to proportionally reduce GI adverse reactions associated with GLP-1RAs. In the NNC0090-2746 group, 35.1% of patients reported at least one GI-related AE, which is a comparable proportion to that observed previously in the LEAD-2 trial (44%), although that was a 26-week trial (Nauck et al., 2009), and to the open-label liraglutide 1.8 mg arm in the current trial (31.4%, Table S4). The previously mentioned lack of dose escalation for NNC0090-2746 could imply a more benign GI profile of NNC0090-2746 compared to liraglutide as similar proportions of AEs were observed for these two differently applied compounds; however, this cannot be confirmed from the current trial. More work remains to determine equivalent exposure for liraglutide and NNC0090-2746 before a direct comparison can be made. No additional safety concerns with NNC0090-2746 relative to liraglutide were identified (Table 3; Table S4).

During the clinical laboratory evaluation, statistically significant amylase increases for NNC0090-2746-treated patients compared to placebo were observed from baseline to W6 (ETR: 1.13 $[1.01; 1.26]_{95\%\ CI}$). The values then decreased although did not reach baseline. No amylase-related clinical laboratory AEs (CLAEs) were reported, and none of the increases were symptomatic. Mean lipase level was statistically

Table 2. Summary of Change in HbA_{1c}, Body Weight, and Glycemic Control Parameters

Parameter	NNC0090-2746	Placebo	NNC0090-2746 versus placebo ETD [95% CI]	p value
HbA_{1c} (%)	n = 36	n = 36		
Week 8	7.33	7.96	−0.63 [−0.93; −0.33]	<0.0001
Week 12	7.02	7.99	−0.96 [−1.36; −0.56]	<0.0001
Mean of 7-point SMPG (mg/dL)	n = 31	n = 30		
Week 8	145.4	173.1	−27.7 [−44.7; −10.7]	0.0017
Week 12	136.0	167.7	−31.7 [−47.0; −16.5]	<0.0001
FPG (mg/dL)	n = 36	n = 36		
Week 12	116.5	154.7	−38.2 [−57.0; −19.4]	0.0001
MTT glucose $AUC_{0–3hr}$ (mg × h/dL)	n = 30	n = 31		
Week 12	419.9	601.2	−181.3 [−252.4; −110.2]	<0.0001
MTT glucose C_{2hr} (mg/dL)	n = 32	n = 31		
Week 12	139.2	213.7	−74.6 [100.2; −48.9]	<0.0001
Body weight (%)	n = 36	n = 36		
Week 8	−2.40	−0.59	−1.80% [−3.24; −0.37]	0.0141
Week 12	−2.86	−1.19	−1.67% [−3.43; 0.09]	0.0621
Parameter	NNC0090-2746	Placebo	NNC0090-2746 versus placebo ETR [95% CI]	p value
MTT insulin $AUC_{0–3hr}$ (ng × h/mL)	n = 26	n = 30		
Week 12	94.58	132.20	0.70 [0.52; 0.95]	0.0238
MTT insulin C_{2hr} (ng/mL)	n = 31	n = 30		
Week 12	35.06	48.18	0.73 [0.47; 1.12]	0.1443
MTT C-peptide $AUC_{0–3hr}$ (ng × h/mL)	n = 28	n = 30		
Week 12	17.052	18.274	0.93 [0.80; 1.09]	0.3710
MTT C-peptide C_{2hr} (ng/mL)	n = 32	n = 30		
Week 12	6.197	6.851	0.90 [0.76; 1.07]	0.2425
Fasting insulin (μIU/mL)	n = 36	n = 36		
Week 12	14.41	11.88	1.21 [0.95; 1.55]	0.1212
Fasting C-peptide (ng/mL)	n = 36	n = 36		
Week 12	3.787	2.929	1.29 [1.13; 1.48]	0.0002

Estimated mean values for several metabolic parameters for the NNC0090-2746 and placebo groups as well as the estimated treatment difference (ETD) or estimated treatment ratio (ETR) with 95% confidence interval (CI) and p value for NNC0090-2746 versus placebo. AUC, area under the curve; C_{2hr}, 2-hr postprandial concentration; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; MTT, meal tolerance test; n, number of patients included in the model; SMPG, self-measured plasma glucose. See also Figure S2 and Tables S2 and S3.

significantly higher in the NNC0090-2746 group compared to the placebo group at all assessed time points during the trial, with highest ETRs at W6 (1.68 $[1.29; 2.18]_{95\%\ CI}$) and W13 (1.63 $[1.29; 2.07]_{95\%\ CI}$). However, only two patients treated with NNC0090-2746 met the >3× upper limit of normal (ULN) threshold at single time points during the trial (at W3 and at an unscheduled visit after W3) and were reported as CLAEs. There were no clinical findings indicative of pancreatitis in association with the increased lipase. A total of 15 other reported non-lipase CLAEs were sporadically distributed between treatment groups. No other clinically significant changes in laboratory assessments of hematological or biochemical parameters were observed.

Anti-drug antibodies developed in 16 patients (43%) exposed to NNC0090-2746 from W6 and onward. Five of these patients had high titers of antibodies (i.e., ≥625). Two patients had cross-reactivity against native GIP and/or GLP-1. The *in vitro* neutralizing effect of the antibodies was not measured. The development of antibodies against NNC0090-2746 is probably a consequence of the molecule's exenatide-like sequence homology, which has been previously associated with an immunogenic response. A recent trial reported development of anti-drug antibodies in 44.6% of patients receiving exenatide alone (Milicevic et al., 2016), a comparable proportion to the NNC0090-2746 group.

Heart rate was significantly increased from baseline to W12 with NNC0090-2746 compared to placebo (ETD: 5.6 beats/minute $[1.5; 9.7]_{95\%\ CI}$). Of patients reaching the >100 beats/minute threshold, two were in the placebo group and 14 in the NNC0090-2746 group during the whole trial. At W17, heart rate appeared to return to pre-trial rates. In summary, more TEAEs were reported, and more of those led to discontinuation in the NNC0090-2746 group compared with placebo. No other clinically relevant changes except increases in lipase and heart rate were found for NNC0090-2746 compared with placebo. Treatment with NNC0090-2746 was found to be safe and well tolerated in the investigated trial population.

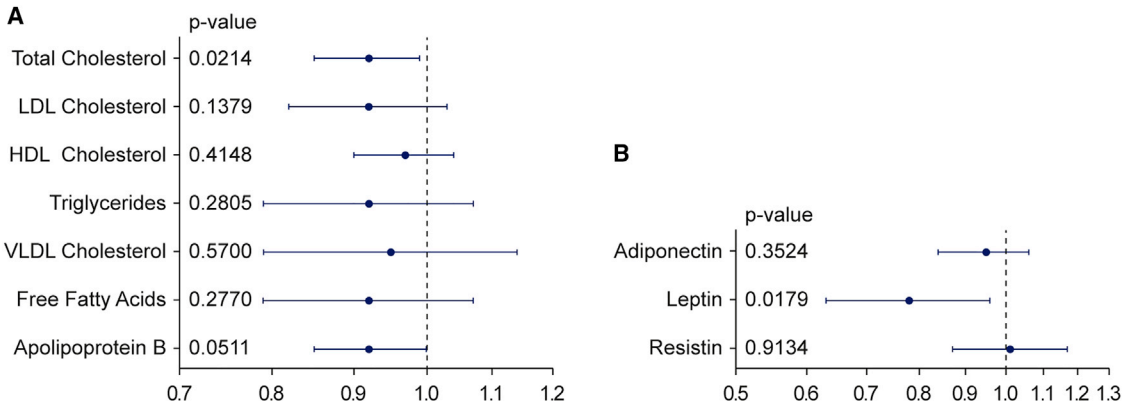

Figure 4. Effect of NNC0090-2746 on Adipose Biomarkers and Lipid Parameters

Treatment ratios with 95% CI for fasting lipids from baseline to W13 (A) and for adipose biomarkers from baseline to W12 (B) with NNC0090-2746 compared to placebo. HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein. See also Figure S3.

Post Hoc Analyses

Differences in treatment effects on HbA_{1c} and body weight between patients with baseline $HbA_{1c} < 8.5\%$ and $\geq 8.5\%$ were examined in post hoc analyses. Baseline HbA_{1c} affected body weight outcome, shown by a significant difference in the effect on body weight (NNC0090-2746 versus placebo) to W12 for patients with a baseline HbA_{1c} of <8.5% compared to those ≥8.5% (interaction p value = 0.0409) (Table S2). These results showed that patients with starting HbA_{1c} levels < 8.5% lost significantly more weight (ETD: −3.38% $[-5.76; -1.00]_{95\%\ CI}$) than those with $HbA_{1c} \geq 8.5\%$ (ETD: 0.27% $[-2.29; 2.83]_{95\%\ CI}$). No significant difference in the effect on HbA_{1c} (NNC0090-2746 versus placebo) was found to W12 between the subgroups with a baseline HbA_{1c} of <8.5% compared to those ≥8.5% (interaction p value = 0.0596), although the results suggested that the difference in baseline HbA_{1c} may have an effect on HbA_{1c} outcome. Notably, patients with a baseline $HbA_{1c} < 8.5\%$ had an ETD of −1.34% $(-1.86; -0.81)_{95\%\ CI}$ compared to −0.60% $(-1.16; -0.05)_{95\%\ CI}$ for those with a baseline $HbA_{1c} \geq 8.5\%$ relative to placebo (Table S2).

Evidence from rodent models and clinical trials suggests that GIP has more effect when glucose concentration is approaching target (Knop et al., 2007a; Xu et al., 2007). This is supported by post hoc analyses in which the estimated treatment effect of NNC0090-2746 on change in body weight (%) was significantly different between baseline HbA_{1c} subgroups (interaction p value = 0.0409). The effect of NNC0090-2746 on body weight in the full trial population was exclusively driven by the effect in the subgroup with baseline $HbA_{1c} < 8.5\%$ as the estimated treatment effect of NNC0090-2746 on change in body weight (%) was essentially zero, and even slightly positive, in the subgroup with baseline $HbA_{1c} \geq 8.5\%$. A similar tendency was seen on change in HbA_{1c} (%), where the estimated treatment effect in the subgroup with baseline $HbA_{1c} < 8.5\%$ was roughly twice as large as in the subgroup with baseline $HbA_{1c} \geq 8.5\%$. The difference between subgroups was, however, not significant (interaction p value = 0.0596) (Table S2), yet baseline HbA_{1c} may have a sizable effect on HbA_{1c} outcome. A greater reduction in HbA_{1c} with lower baseline HbA_{1c} is contrary to what is commonly seen with other glucose-lowering agents and supports the hypothesis that the effects of GIP are more prominent as patients are approaching improved glycemic control. It will be valuable to determine the molecular basis for this restoration in GIP function at moderate hyperglycemia. Specifically, it is plausible that such improvement is mechanistically linked with the recently reported GIP-responsive islet transcription factor TCF1 (Campbell et al., 2016).

Differences in the treatment effects on lipid parameters between patients treated with statins (n = 33) or without statins (n = 34) were evaluated in a post hoc analysis. No significant differences in the treatment effect of change in lipid parameters were found from baseline to W13 between statin-treated and non-statin-treated patients (data not shown).

Lastly, differences in the treatment effects on HbA_{1c} and body weight between patients who did and did not develop positive NNC0090-2746 antibodies during the trial were evaluated in post hoc analyses. No significant difference in the effect on change in HbA_{1c} was found between NNC0090-2746 antibody-positive (ETD: −1.23% $[-1.74; -0.72]_{95\%\ CI}$) and -negative patients (ETD: −0.75% $[-1.22; -0.28]_{95\%\ CI}$). A statistically significant difference in the treatment effect on body weight (kg) was found from baseline to W8 with greater effect in the positive antibody (interaction p value = 0.0143). No significant difference in the treatment effect of change in body weight (kg) was found from baseline to W12 or W13 (data not shown). No signs of reduced effect were observed in patients who developed anti-drug antibodies. A review of trials in which patients who received exenatide reached a similar conclusion; while at the extreme upper range of anti-exenatide titers there was a trend toward a smaller reduction in HbA_{1c}, subjects with exenatide anti-drug antibodies showed a reduction in HbA_{1c} consistent with those without anti-drug antibodies (Fineman et al., 2012). Patients with anti-NNC0090-2746 antibodies did lose significantly more weight from baseline to W8, though not to W12, than those without anti-NNC0090-2746 antibodies; however, the explanation for this would need further investigation.

Limitations of this trial include a trial design where patients randomized to liraglutide had 2 weeks to dose-escalate within the 12-week treatment period, whereas patients receiving NNC0090-2746 started immediately on a dose of 1.8 mg. The

Table 3. Adverse Events and Treatment-Emergent Adverse Events for Placebo and NNC0090-2746 Groups

Category	Placebo (N = 36)			NNC0090-2746 (N = 37)		
AEs	n = 15	41.7%	E = 44	n = 24	64.9%	E = 91
TEAEs	n = 15	41.7%	E = 34	n = 24	64.9%	E = 78
AEs leading to death	n = 0	0%	E = 0	n = 0	0%	E = 0
AEs leading to discontinuation	n = 1	2.8%	E = 1	n = 4	10.8%	E = 8
TEAEs leading to discontinuation	n = 1	2.8%	E = 1	n = 4	10.8%	E = 8
AE related to trial drug	n = 8	22.2%	E = 11	n = 15	40.5%	E = 42
TEAE related to trial drug	n = 7	19.4%	E = 9	n = 15	40.5%	E = 40
SAE	n = 0	0%	E = 0	n = 1	2.7%	E = 1
TEAEs Reported by ≥5% of Patients in Any Treatment Group by SOC						
Gastrointestinal disorders	n = 6	16.7%	E = 13	n = 13	35.1%	E = 30
Muscoloskeletal and connective tissue disorders	n = 1	2.8%	E = 1	n = 6	16.2%	E = 10
Investigations	n = 5	13.9%	E = 8	n = 4	10.8%	E = 5
Infections and infestations	n = 4	11.1%	E = 4	n = 4	10.8%	E = 5
Metabolism and nutrition disorders	n = 2	5.6%	E = 2	n = 6	16.2%	E = 7
General disorders and administration site conditions	n = 0	0.0%	E = 0	n = 4	10.8%	E = 5
Nervous system disorders	n = 1	2.8%	E = 2	n = 3	8.1%	E = 3
Injury, poisoning, and procedural complications	n = 0	0.0%	E = 0	n = 4	10.8%	E = 4
Respiratory, thoracic, and mediastinal disorders	n = 0	0.0%	E = 0	n = 3	8.1%	E = 3

Adverse events (AEs), treatment-emergent AEs (TEAEs), and serious AEs (SAEs) for patients on NNC0090-2746 and placebo in the safety population. AEs are coded by MedDRA. E, the number of events in each category; N, total number of patients; n, number of patients in each category. See also Tables S1 and S4.

dose titration of liraglutide has been refined through a decade of clinical optimization to minimize adverse gut effects and achieve a maximally efficacious therapeutic dose. Furthermore, liraglutide was included as an open-label comparison, as its primary purpose was to provide benchmark to historical clinical outcomes. While direct comparisons of the blinded NNC0090-2746 treatment with the open-label liraglutide arm are tempting to make, it needs to be done cautiously in recognition of the inherent differences in trial design. However, the reductions in HbA_{1c} seen for NNC0090-2746 in this trial are consistent with those previously reported in the literature for other GLP-1RAs in combination with metformin and supported by the open-label trial arm, where reductions in HbA_{1c} were similar (Figure S1A; Table S3) (Dungan et al., 2014; Nauck et al., 2009; Reusch et al., 2014; Wysham et al., 2014). While the reductions in body weight for NNC0090-2746 and liraglutide in this trial were similar, the reduction with NNC0090-2746 was approximately 0.5% greater from baseline to W12 (Figure S1B; Table S3; Table 2). Refinement of dose and application of a dose escalation similar to what is used with liraglutide could be of benefit in future trials with NNC0090-2746. Based upon the post hoc observations and the established difference in GIP responsiveness to baseline HbA_{1c} levels, initiating therapy at a point of improved glycemic control or continuing the trial for a more extended period to allow sufficient time for GIP agonism to make its full contribution might be considered.

In summary, this proof of concept trial shows that the unimolecular dual GIP/GLP-1 receptor agonist NNC0090-2746 improves glycemic control, reduces body weight, and lowers circulating cholesterol. In addition, a post hoc analysis of weight loss by baseline HbA_{1c} suggests a striking relationship between glycemic control at baseline and ultimate efficacy of NNC0090-2746—namely that maximum benefit is obtained by patients with better glycemic control. This relationship confirms the correlation suggested by retrospective analyses of trial populations and animal models. Though more work is clearly needed, notably a dose-finding trial, a refined escalation schedule, and longer trial duration, these data suggest that integrated GIP agonism may offer additional benefits on weight loss and total cholesterol as patients approach normoglycemia.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2017.07.011.

AUTHOR CONTRIBUTIONS

Conceptualization, C.S. and R.D.D.; Formal Analysis, R.H.C., R.D.D., and E.J.B.; Investigation, J.P.F. and E.J.B.; Data Curation: E.J.B.; Writing – Review & Editing, J.P.F., E.J.B., L.V., M.H.T., C.S., K.O., R.H.C., and R.D.D.

CONFLICTS OF INTEREST

J.P.F. is a consultant for AstraZeneca, BMS, Johnson and Johnson, Novo Nordisk, and Sanofi, a member of the advisory panel for AstraZeneca and Sanofi, a member of the speaker's bureau for Sanofi and Novo Nordisk, and has received research support from AbbVie, AstraZeneca, Boehringer Ingelheim, BMS, Elcelyx, Eli Lilly & Co., Ionis, Janssen, Johnson and Johnson, Ligand, Merck, Mylan, Novartis, Novo Nordisk, Pfizer, Sanofi, Theracos, and vTv. E.J.B. was Vice President Medical for MB2 LLC; Principal, Diabetes Drug Development Associates LLC; Scientific Consultant to Viacyte, Inc.; and Associate Professor at Indiana University School of Medicine. L.V. was a consultant for MB2 LLC. M.H.T. is a consultant for Bionorica SE, an advisor for Novo Nordisk, and a member of the scientific advisory board of ERX Pharmaceuticals, Inc.; he also receives research funds from Novo Nordisk. C.S. is an employee of F. Hoffman-La Roche AG. K.O. and R.D.D. are employees and K.O. is shareholder of Novo Nordisk. R.H.C. was employed at Novo Nordisk during preparation of the article. Furthermore, R.D.D. is co-inventor of the peptide IP.

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REFERENCES

Aaboe, K., Akram, S., Deacon, C.F., Holst, J.J., Madsbad, S., and Krarup, T. (2015). Restoration of the insulinotropic effect of glucose-dependent insulinotropic polypeptide contributes to the antidiabetic effect of dipeptidyl peptidase-4 inhibitors. Diabetes Obes. Metab. *17*, 74–81.

Baggio, L.L., and Drucker, D.J. (2007). Biology of incretins: GLP-1 and GIP. Gastroenterology *132*, 2131–2157.

Campbell, J.E., and Drucker, D.J. (2013). Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab. *17*, 819–837.

Campbell, J.E., Ussher, J.R., Mulvihill, E.E., Kolic, J., Baggio, L.L., Cao, X., Liu, Y., Lamont, B.J., Morii, T., Streutker, C.J., et al. (2016). TCF1 links GIPR signaling to the control of beta cell function and survival. Nat. Med. *22*, 84–90.

Chia, C.W., Carlson, O.D., Kim, W., Shin, Y.K., Charles, C.P., Kim, H.S., Melvin, D.L., and Egan, J.M. (2009). Exogenous glucose-dependent insulinotropic polypeptide worsens post prandial hyperglycemia in type 2 diabetes. Diabetes *58*, 1342–1349.

Davies, M.J., Bergenstal, R., Bode, B., Kushner, R.F., Lewin, A., Skjøth, T.V., Andreasen, A.H., Jensen, C.B., and DeFronzo, R.A.; NN8022-1922 Study Group (2015). Efficacy of liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial. JAMA *314*, 687–699.

Dungan, K.M., Povedano, S.T., Forst, T., González, J.G., Atisso, C., Sealls, W., and Fahrbach, J.L. (2014). Once-weekly dulaglutide versus once-daily liraglutide in metformin-treated patients with type 2 diabetes (AWARD-6): a randomised, open-label, phase 3, non-inferiority trial. Lancet *384*, 1349–1357.

Dupre, J., Ross, S.A., Watson, D., and Brown, J.C. (1973). Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab. *37*, 826–828.

Finan, B., Ma, T., Ottaway, N., Müller, T.D., Habegger, K.M., Heppner, K.M., Kirchner, H., Holland, J., Hembree, J., Raver, C., et al. (2013). Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. Sci. Transl. Med. *5*, 209ra151.

Fineman, M.S., Mace, K.F., Diamant, M., Darsow, T., Cirincione, B.B., Booker Porter, T.K., Kinninger, L.A., and Trautmann, M.E. (2012). Clinical relevance of anti-exenatide antibodies: safety, efficacy and cross-reactivity with long-term treatment. Diabetes Obes. Metab. *14*, 546–554.

Gault, V.A., O'Harte, F.P., Harriott, P., and Flatt, P.R. (2002). Characterization of the cellular and metabolic effects of a novel enzyme-resistant antagonist of glucose-dependent insulinotropic polypeptide. Biochem. Biophys. Res. Commun. *290*, 1420–1426.

Gault, V.A., Irwin, N., Green, B.D., McCluskey, J.T., Greer, B., Bailey, C.J., Harriott, P., O'harte, F.P., and Flatt, P.R. (2005). Chemical ablation of gastric inhibitory polypeptide receptor action by daily (Pro3)GIP administration improves glucose tolerance and ameliorates insulin resistance and abnormalities of islet structure in obesity-related diabetes. Diabetes *54*, 2436–2446.

Gutniak, M., Orskov, C., Holst, J.J., Ahrén, B., and Efendic, S. (1992). Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. N. Engl. J. Med. *326*, 1316–1322.

Hermansen, K., Bækdal, T.A., Düring, M., Pietraszek, A., Mortensen, L.S., Jørgensen, H., and Flint, A. (2013). Liraglutide suppresses postprandial triglyceride and apolipoprotein B48 elevations after a fat-rich meal in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, cross-over trial. Diabetes Obes. Metab. *15*, 1040–1048.

Højberg, P.V., Vilsbøll, T., Rabøl, R., Knop, F.K., Bache, M., Krarup, T., Holst, J.J., and Madsbad, S. (2009). Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. Diabetologia *52*, 199–207.

Irwin, N., Gault, V.A., Green, B.D., Greer, B., McCluskey, J.T., Harriott, P., O'Harte, F.P., and Flatt, P.R. (2004). Effects of short-term chemical ablation of the GIP receptor on insulin secretion, islet morphology and glucose homeostasis in mice. Biol. Chem. *385*, 845–852.

Klonoff, D.C., Buse, J.B., Nielsen, L.L., Guan, X., Bowlus, C.L., Holcombe, J.H., Wintle, M.E., and Maggs, D.G. (2008). Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. Curr. Med. Res. Opin. *24*, 275–286.

Knop, F.K., Vilsbøll, T., Højberg, P.V., Larsen, S., Madsbad, S., Holst, J.J., and Krarup, T. (2007a). The insulinotropic effect of GIP is impaired in patients with chronic pancreatitis and secondary diabetes mellitus as compared to patients with chronic pancreatitis and normal glucose tolerance. Regul. Pept. *144*, 123–130.

Knop, F.K., Vilsbøll, T., Højberg, P.V., Larsen, S., Madsbad, S., Vølund, A., Holst, J.J., and Krarup, T. (2007b). Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? Diabetes *56*, 1951–1959.

Kreymann, B., Williams, G., Ghatei, M.A., and Bloom, S.R. (1987). Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet *2*, 1300–1304.

le Roux, C.W., Astrup, A., Fujioka, K., Greenway, F., Lau, D.C.W., Van Gaal, L., Ortiz, R.V., Wilding, J.P.H., Skjøth, T.V., Manning, L.S., and Pi-Sunyer, X.; SCALE Obesity Prediabetes NN8022-1839 Study Group (2017). 3 years of liraglutide versus placebo for type 2 diabetes risk reduction and weight management in individuals with prediabetes: a randomised, double-blind trial. Lancet *389*, 1399–1409.

Lorenz, M., Evers, A., and Wagner, M. (2013). Recent progress and future options in the development of GLP-1 receptor agonists for the treatment of diabesity. Bioorg. Med. Chem. Lett. *23*, 4011–4018.

Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., et al. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. *1*, 1155–1161.

McClean, P.L., Irwin, N., Cassidy, R.S., Holst, J.J., Gault, V.A., and Flatt, P.R. (2007). GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet. Am. J. Physiol. Endocrinol. Metab. *293*, E1746–E1755.

McIntyre, N., Holdsworth, C.D., and Turner, D.S. (1964). New interpretation of oral glucose tolerance. Lancet *2*, 20–21.

Mentis, N., Vardarli, I., Köthe, L.D., Holst, J.J., Deacon, C.F., Theodorakis, M., Meier, J.J., and Nauck, M.A. (2011). GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. Diabetes *60*, 1270–1276.

Milicevic, Z., Anglin, G., Harper, K., Konrad, R.J., Skrivanek, Z., Glaesner, W., Karanikas, C.A., and Mace, K. (2016). Low incidence of anti-drug antibodies in patients with type 2 diabetes treated with once-weekly glucagon-like peptide-1 receptor agonist dulaglutide. Diabetes Obes. Metab. *18*, 533–536.

Miyawaki, K., Yamada, Y., Ban, N., Ihara, Y., Tsukiyama, K., Zhou, H., Fujimoto, S., Oku, A., Tsuda, K., Toyokuni, S., et al. (2002). Inhibition of gastric inhibitory polypeptide signaling prevents obesity. Nat. Med. *8*, 738–742.

Møller, C.L., Vistisen, D., Færch, K., Johansen, N.B., Witte, D.R., Jonsson, A., Pedersen, O., Hansen, T., Lauritzen, T., Jørgensen, M.E., et al. (2016). Glucose-dependent insulinotropic polypeptide is associated with lower low-density lipoprotein but unhealthy fat distribution, independent of insulin: the ADDITION-PRO study. J. Clin. Endocrinol. Metab. *101*, 485–493.

Nauck, M.A., and Meier, J.J. (2016). The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. Lancet Diabetes Endocrinol. *4*, 525–536.

Nauck, M.A., Homberger, E., Siegel, E.G., Allen, R.C., Eaton, R.P., Ebert, R., and Creutzfeldt, W. (1986). Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J. Clin. Endocrinol. Metab. *63*, 492–498.

Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R., and Creutzfeldt, W. (1993). Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J. Clin. Invest. *91*, 301–307.

Nauck, M.A., Wollschläger, D., Werner, J., Holst, J.J., Orskov, C., Creutzfeldt, W., and Willms, B. (1996). Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. Diabetologia *39*, 1546–1553.

Nauck, M., Frid, A., Hermansen, K., Shah, N.S., Tankova, T., Mitha, I.H., Zdravkovic, M., Düring, M., and Matthews, D.R.; LEAD-2 Study Group (2009). Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. Diabetes Care *32*, 84–90.

Nauck, M., Frid, A., Hermansen, K., Thomsen, A.B., During, M., Shah, N., Tankova, T., Mitha, I., and Matthews, D.R. (2013). Long-term efficacy and safety comparison of liraglutide, glimepiride and placebo, all in combination with metformin in type 2 diabetes: 2-year results from the LEAD-2 study. Diabetes Obes. Metab. *15*, 204–212.

Neff, L.M., and Kushner, R.F. (2010). Emerging role of GLP-1 receptor agonists in the treatment of obesity. Diabetes Metab. Syndr. Obes. *3*, 263–273.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F., et al. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet *384*, 766–781.

Plutzky, J., Garber, A., Falahati, A., Toft, A.D., and Poulter, N.R. (2009). Reductions in lipids and CV risk markers in patients with type 2 diabetes treated with liraglutide: a meta-analysis. Can. J. Diabetes *33*, 209–210.

Portron, A., Jadidi, S., Sarkar, N., DiMarchi, R., and Schmitt, C. (2017). Pharmacodynamics, pharmacokinetics, safety and tolerability of the novel dual GIP/GLP-1 agonist (RG7697) after single subcutaneous administration in healthy subjects. Diabetes Obes. Metab. http://dx.doi.org/10.1111/dom.13025.

Reusch, J., Stewart, M.W., Perkins, C.M., Cirkel, D.T., Ye, J., Perry, C.R., Reinhardt, R.R., and Bode, B.W. (2014). Efficacy and safety of once-weekly glucagon-like peptide 1 receptor agonist albiglutide (HARMONY 1 trial): 52-week primary endpoint results from a randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes mellitus not controlled on pioglitazone, with or without metformin. Diabetes Obes. Metab. *16*, 1257–1264.

Rosenbaum, M., Nicolson, M., Hirsch, J., Murphy, E., Chu, F., and Leibel, R.L. (1997). Effects of weight change on plasma leptin concentrations and energy expenditure. J. Clin. Endocrinol. Metab. *82*, 3647–3654.

Schmitt, C., Portron, A., Jadidi, S., Sarkar, N., and DiMarchi, R. (2017). Pharmacodynamics, pharmacokinetics and safety of multiple ascending doses of the novel dual GIP/GLP-1 agonist RG7697 in patients with type 2 diabetes mellitus. Diabetes Obes. Metab. http://dx.doi.org/10.1111/dom.13024.

Tschöp, M.H., Finan, B., Clemmensen, C., Gelfanov, V., Perez-Tilve, D., Müller, T.D., and DiMarchi, R.D. (2016). Unimolecular polypharmacy for treatment of diabetes and obesity. Cell Metab. *24*, 51–62.

Vilsbøll, T., Krarup, T., Madsbad, S., and Holst, J.J. (2002). Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. Diabetologia *45*, 1111–1119.

World Health Organization (2016). Global Report on Diabetes. Available at: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf. Accessed July 4, 2017.

Wysham, C., Blevins, T., Arakaki, R., Colon, G., Garcia, P., Atisso, C., Kuhstoss, D., and Lakshmanan, M. (2014). Efficacy and safety of dulaglutide added onto pioglitazone and metformin versus exenatide in type 2 diabetes in a randomized controlled trial (AWARD-1). Diabetes Care *37*, 2159–2167.

Xu, G., Kaneto, H., Laybutt, D.R., Duvivier-Kali, V.F., Trivedi, N., Suzuma, K., King, G.L., Weir, G.C., and Bonner-Weir, S. (2007). Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. Diabetes *56*, 1551–1558.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Dual GIP/GLP-1 Receptor Agonist	Roche; Finan et al., 2013	NNC0090-2746

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the communicating author, Contact, Richard DiMarchi (rdmh@novonordisk.com).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human Subjects

Trial Design

This was a multi-center, randomized, double-blind, parallel-group, placebo-controlled trial including 108 patients. It included a screening period and a single-blind placebo lead-in period both of 2 weeks' duration as well as a double-blind treatment period and a follow-up period (Figure 1). The lead-in period was to ensure that all patients were able to perform subcutaneous (s.c.) injections. Patients were randomized to NNC0090-2746, placebo, or liraglutide 1.8 mg (Victoza) in a 1:1:1 manner and their baseline characteristics can be found in Table 1. Randomization was stratified according to HbA_{1c} (< 8.5% or ≥ 8.5%) and body weight (< 85 kg or ≥ 85 kg) at screening. The open-label liraglutide 1.8 mg reference arm was included to explore the pharmacodynamic effects and tolerability profile of NNC0090-2746 compared to that of once-daily liraglutide 1.8 mg. The trial was conducted at 19 investigational sites in the US and approved by an institutional review board (IRB). The trial was conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. The trial complied with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Patient Eligibility

Eligible patients were between 18 and 70 years old (both inclusive) with HbA_{1c} levels of 7.2% to 10.5% (both inclusive) at screening and had a diagnosis of T2D for at least 3 months before screening despite being on a stable dose of metformin monotherapy for at least 8 weeks. Furthermore eligible patients had an FPG < 250 mg/dL, a fasting C-peptide > 1.5 ng/mL, and a BMI between 27 kg/m^2 and 44 kg/m^2 (both inclusive) at the time of screening. Patients were ineligible if they used any oral antidiabetic medication other than metformin, insulin, incretin, or glucagon analogs, or weight loss medication, or had a history of significant diabetic or acute metabolic complications.

METHOD DETAILS

Trial Medication and Blinding

NNC0090-2746 1.8 mg and matching placebo were administered by once-daily s.c. injection in the abdomen by the patients. All efforts were to be made to administer the products at approximately the same time every morning (±6-h window). No dose escalation was performed. Temporary down-titration to 1.5 mg was permitted in case of intolerable or unacceptable AEs. In case of down-titration, all efforts were to be made to resume the daily target dose of 1.8 mg as soon as judged appropriate by the investigator. Liraglutide 1.8 mg was administered by once-daily s.c. injection in the abdomen by the patients according to the manufacturer's recommended dose-escalation scheme. Patients were to remain at their pre-trial metformin dose and schedule throughout the trial including during the screening and follow-up periods. Adjustments of the metformin dosing regimen were not permitted during the trial unless medically indicated. Patients, investigators, individuals in direct contact with the patients at the investigative site, and sponsor and contract research organizations (CROs) were blinded to the trial product assignment of the NNC0090-2746 and placebo treatment groups.

Trial Procedures

After completing the lead-in period patients were randomized to NNC0090-2746, liraglutide, or placebo by the use of an interactive voice/web-based response system (IxRS). The primary endpoint was defined as change from baseline in HbA_{1c} after 8 weeks, interpreted as the Week 8 visit (W8) corresponding to Trial Day 50. Secondary endpoints included: change from baseline in HbA_{1c} after 12 weeks, interpreted as the Week 12 (W12) corresponding to Trial Day 78; change and percent change in body weight to W8 and W12; change in FPG to W12; change in SMPG W8 and W12; and change in glucose, insulin, and C-peptide following ingestion of an

MTT to W4 and W12. Change from baseline in fasting lipids and adipose biomarkers were exploratory secondary efficacy endpoints and were measured at W13 and W12, respectively. The W13 visit, corresponding to Trial Day 85, was scheduled as a follow-up visit with the same requirements as the previous visits, i.e., patients were fasting. A similar proportion of patients were still on treatment when they attended the W13 visit as for the W12 visit. Body weight and HbA_{1c} were measured at baseline (W1), W2, W3, W4, W6, W8, W10, W12, and W13. Fasting glucose, insulin, and C-peptide were measured at baseline (W1), W2, W4, W6, W12, and W13. Fasting lipids were measured at baseline (W1), W6, and W13. Biomarkers were measured at baseline (W1), W4, and W12. An MTT was performed at W−1 (all three groups), W4 (only NNC0090-2746 and placebo), and W12 (only NNC0090-2746 and placebo), and a 7-point SMPG profile was performed during W−1, W8, and W12. Throughout the trial patients were to maintain their existing diet and exercise habits.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Analysis

The planned sample size was determined to detect a treatment difference of 0.8% between NNC0090-2746 and placebo in change from baseline in HbA_{1c} with more than 80% power, a two-sided t test at a 5% significance level, and a standard deviation of 1.2%. The required sample size was found to be 105 patients, 35 per treatment group. This sample size did not provide sufficient statistical power for detecting or elucidating small or moderate clinical differences between NNC0090-2746 and liraglutide.

The primary analysis of the primary efficacy endpoint was based on a mixed model for repeated-measurements (MMRM) with treatment, week of visit, and strata ($HbA_{1c} < 8.5\%$ and $\geq 8.5\%$ each intersected by body weight < 85 kg and ≥ 85 kg) as fixed factors, baseline value as covariate, and the interactions of treatment, strata, and baseline with visit. An unstructured within-subject variance-covariance matrix was used. Most continuous secondary endpoints were analyzed with the same MMRM as the primary endpoint. The model was applied to the intention to treat (ITT) analysis set unless otherwise stated. For insulin, C-peptide, lipids, and adipose biomarkers the values were logarithmically transformed before analysis and back transformed to the original scale.

The ETD for NNC0090-2746 versus placebo and the corresponding two-sided p values for tests of the no-difference hypothesis and 95% confidence intervals presented are based on the MMRM model. Denominator degrees of freedom were estimated using Satterthwaite's approximation. Estimated means and estimated mean changes from baseline are adjusted to the observed baseline distribution of patients contributing to the model. These means are therefore representative of a typical patient among those contributing to the model across all three treatment groups.

Post hoc analyses included subgroup analyses of HbA_{1c} and body weight by statin subgroups (any record of statin use during trial period [yes/no]), by anti-drug antibody subgroups (development of anti-drug antibodies against NNC0090-2746 [positive/negative]), and by HbA_{1c} subgroups (HbA_{1c} strata [$< 8.5\%$, $\geq 8.5\%$]). Subgroup analyses were conducted using the same MMRM as for the prespecified continuous endpoints with the addition of the fixed effect for the interaction between given strata, treatment, and visit. Estimated means are adjusted to the observed baseline distribution of patients contributing to the model in each subgroup and are therefore representative for a typical patient across all three treatment arms within each subgroup.

ADDITIONAL RESOURCES

Clinical trial registry number NCT02205528: https://clinicaltrials.gov/ct2/show/NCT02205528.