

## ORIGINAL ARTICLE

# Pharmacokinetics and pharmacodynamics of single and multiple doses of the glucagon receptor antagonist LGD-6972 in healthy subjects and subjects with type 2 diabetes mellitus

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**Aim:** To evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of single and multiple doses of a novel, oral glucagon receptor antagonist, LGD-6972, in healthy subjects and subjects with type 2 diabetes (T2DM).

**Methods:** In the single ascending dose study, LGD-6972 (2-480 mg) was administered to healthy subjects (n = 48) and T2DM subjects (n = 8). In the multiple ascending dose study, healthy subjects (n = 12) received a dose of 15 mg LGD-6972 and T2DM subjects (n = 36) received doses of 5, 10 or 15 mg of LGD-6972 daily for 14 days.

**Results:** LGD-6972 had linear plasma pharmacokinetics consistent with once-daily dosing that was comparable in healthy and T2DM subjects. Dose-dependent decreases in fasting plasma glucose were observed in all groups with a maximum of 3.15 mmol/L (56.8 mg/dL) on day 14 in T2DM subjects. LGD-6972 also reduced plasma glucose in the postprandial state. Dose-dependent increases in fasting plasma glucagon were observed, but glucagon levels decreased and insulin levels increased after an oral glucose load in T2DM subjects. LGD-6972 was well tolerated at the doses tested without dose-related or clinically meaningful changes in clinical laboratory parameters. No subject experienced hypoglycaemia.

**Conclusion:** Inhibition of glucagon action by LGD-6972 was associated with decreases in glucose in both healthy and T2DM subjects, the magnitude of which was sufficient to predict improvement in glycaemic control with longer treatment duration in T2DM patients. The safety and pharmacological profile of LGD-6972 after 14 days of dosing supports continued clinical development.

## KEYWORDS

antagonist, diabetes, glucagon receptor, pharmacodynamics, pharmacokinetics

## 1 | INTRODUCTION

Elevated glucagon levels observed in T2DM exacerbate the hyperglycaemic state and its associated complications by increasing hepatic glucose production.<sup>1</sup> Animal models of T2DM have demonstrated the utility of inhibiting glucagon action for treating T2DM.<sup>2-4</sup> Glucagon receptor (GCGR) neutralizing antibodies, antisense oligonucleotides and/or peptide and small molecule glucagon

receptor antagonists (GRAs) have been shown to significantly reduce blood glucose levels and improve glucose tolerance in various rodent obesity and/or diabetes models.<sup>5-16</sup> In T2DM patients, small molecule GRAs suppress fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) levels.<sup>17-22</sup> Thus, GCGR antagonism is a validated mechanism to control hyperglycaemia and is a logical target for the treatment of T2DM and other hyperglycaemic conditions.<sup>23-25</sup>

LGD-6972 is a novel, orally bioavailable small molecule GRA being developed as an adjunct to diet and exercise to improve glycaemic control in adults with T2DM. *In vitro*, LGD-6972 binds competitively to GCGR with high affinity and selectivity, suppressing both cAMP and glucose production.<sup>26</sup> *In vivo*, LGD-6972 reduced acute glucagon-stimulated hyperglycaemia as well as the hyperglycaemia observed in diabetic mouse models.<sup>26</sup> The pharmacological activity of LGD-6972 appears to be mediated primarily by inhibiting GCGR signaling. Herein, we describe the results of clinical studies evaluating single and multiple doses of LGD-6972 in healthy subjects and subjects with T2DM.

## 2 | MATERIALS AND METHODS

### 2.1 | Study designs

Studies L6972-01 (NCT01919684) and L6972-02 (NCT02250222) were conducted in accordance with Good Clinical Practice (GCP) guidelines. An Institutional Review Board (IRB) reviewed and approved the protocols prior to initiating the studies. All subjects provided written informed consent to participate. The primary objective of both studies was to evaluate the safety and tolerability of oral doses of LGD-6972. Secondary objectives were to characterize the pharmacokinetic (PK) and pharmacodynamic (PD) profile of LGD-6972.

Study L6972-01 was a single centre, randomized, double-blind, placebo-controlled single ascending dose (SAD) study conducted in two parts. Part 1 evaluated LGD-6972 in six groups of normal healthy subjects (eight/group) and Part 2 evaluated LGD-6972 in a single group of eight subjects with T2DM. In Part 1, healthy subjects were randomly assigned in a 3:1 ratio to receive either a single oral dose of 2, 10, 40, 120, 240 or 480 mg of LGD-6972 or placebo administered in a fasted state. Dose escalation occurred after review of safety, tolerability and preliminary PK data from previous dose levels. Following a 21-day washout period, subjects who received the 40 mg dose in a fasted state received a second 40 mg dose after a high-fat breakfast to explore food effects on pharmacokinetics of LGD-6972. In Part 2, T2DM subjects received a single dose of 40 mg LGD-6972 in a fasted state after the equivalent dose had been administered to healthy subjects and safety data had been reviewed. All subjects were confined at the site for 48 hours after dosing, and returned to the site 5, 7 and 14 days after dosing for follow-up visits.

Study L6972-02 was a randomized, double-blind, placebo-controlled, sequential, multiple ascending dose (MAD) study conducted at three sites in normoglycaemic healthy subjects ( $n = 12$ ) and subjects with T2DM who were inadequately controlled with stable metformin monotherapy ( $n = 36$ ). Twelve healthy subjects were randomized (3:1) to oral doses of 15 mg LGD-6972 or placebo once daily in a fasted state for 14 days. T2DM subjects (12 subjects/dose group) were randomized (3:1) to 5, 10 or 15 mg LGD 6972 or placebo once daily in the fasted state for 14 days. Subjects were confined at the site for the entire 14-day treatment period, and returned to the site for up to three weekly follow-up visits. Initiation of dosing

and dose escalation occurred in the T2DM subjects after review of safety, tolerability and preliminary PK data from previous dose levels.

In both studies, subjects received once-daily placebo or LGD-6972 as an aqueous solution formulated with CAPTISOL<sup>®</sup> (betadex [ $\beta$ -cyclodextrin] sulfobutylether sodium). Subjects received standardized meals during confinement in the clinical pharmacology unit. Safety and tolerability were assessed during periodic physical examinations and measurement of vital signs, clinical laboratory tests, 12-lead electrocardiograms (ECGs) and continual adverse event observation.

### 2.2 | Subjects

In the SAD study, subjects were healthy men and women, 21–65 years of age. Eligibility criteria for T2DM subjects included HbA1c  $\geq 6.5\%$  and  $\leq 10\%$ , FPG  $< 12.21$  mmol/L, and BMI of 18.5–38.0 kg/m<sup>2</sup>. T2DM subjects were required to discontinue any antidiabetic medication 2 weeks prior to admission until after the last follow-up visit. In the MAD study, subjects were men and women, 21–65 years of age. T2DM subjects were required to be on a stable dose of metformin for  $\geq 12$  weeks without use of other antidiabetic medications for  $> 3$  weeks, and have HbA1c  $\geq 6.5\%$  and  $\leq 10.5\%$ , FPG  $\geq 6.94$  mmol/L and  $\leq 14.43$  mmol/dL, and BMI of 20 and 45 kg/m<sup>2</sup>.

Key exclusion criteria for both studies included: significant illness such as cardiovascular, haematologic, respiratory, renal or gastrointestinal disease; history of uncontrolled blood pressure; liver transaminase levels (AST, alanine aminotransferase or ALT, aspartate aminotransferase)  $> 10\% \times$  ULN; creatine kinase (CK) levels  $> 2 \times$  ULN; serum triglyceride level  $> 4.52$  mmol/L. To be eligible, women had to be either postmenopausal, surgically sterile or practicing an effective method of birth control. Male subjects must either have had a vasectomy or agreed that they and any female partners would use two acceptable forms of contraception.

### 2.3 | Measurements

#### 2.3.1 | Pharmacokinetics

Plasma concentrations of LGD-6972 were measured by a validated LC-MS/MS method. A time-exposure profile was measured throughout a 24-hour period on day 1 in the SAD study, and on day 1 and day 14 in the MAD study. Additional trough concentrations were measured at several time points in the MAD study to investigate steady state pharmacokinetics and clearance rates.

#### 2.3.2 | Pharmacodynamics

In the SAD study, FPG, fasting plasma glucagon, insulin and glucagon-like peptide-1 (GLP-1) were evaluated in healthy and T2DM subjects. In the MAD study, PD variables in both healthy and T2DM subjects included FPG, fasting glucagon, total and active glucagon-like peptide-1, and insulin measured at baseline and throughout the 14-day treatment. Seven-point plasma glucose measurements were performed at baseline (day  $-1$ ) and at day 14 in all T2DM subjects. A 4-hour oral glucose tolerance test (OGTT) was performed in T2DM subjects receiving 10-mg LGD-6972 on day  $-1$  and day 14 for measurement of

within-subject change from baseline for glucose, glucagon, insulin and active and total GLP-1.

A direct  $E_{\max}$  model was developed to evaluate the relationship between plasma LGD-6972 concentration and change from baseline fasting plasma glucose. The model estimated the maximum glucose lowering effect ( $E_{\max}$ ) and plasma LGD-6972 concentration required to attain 50% of the maximum glucose effect ( $EC_{50}$ ).

Descriptions of the analytical and statistical methods are available online (Appendix S1, Supporting Information).

### 3 | RESULTS

#### 3.1 | Subjects and demographics

Baseline characteristics and demographics for the SAD and the MAD studies are shown in Table S1, Supporting Information. In both studies, all subjects completed the study as planned and no subjects discontinued the study. No meaningful differences in demographic or baseline characteristics were noted across treatment groups for healthy subjects or T2DM subjects in either study. Mean baseline HbA1c for subjects with T2DM was 7.3% in the SAD study and 8.3% in the MAD study, with no differences between treatment groups.

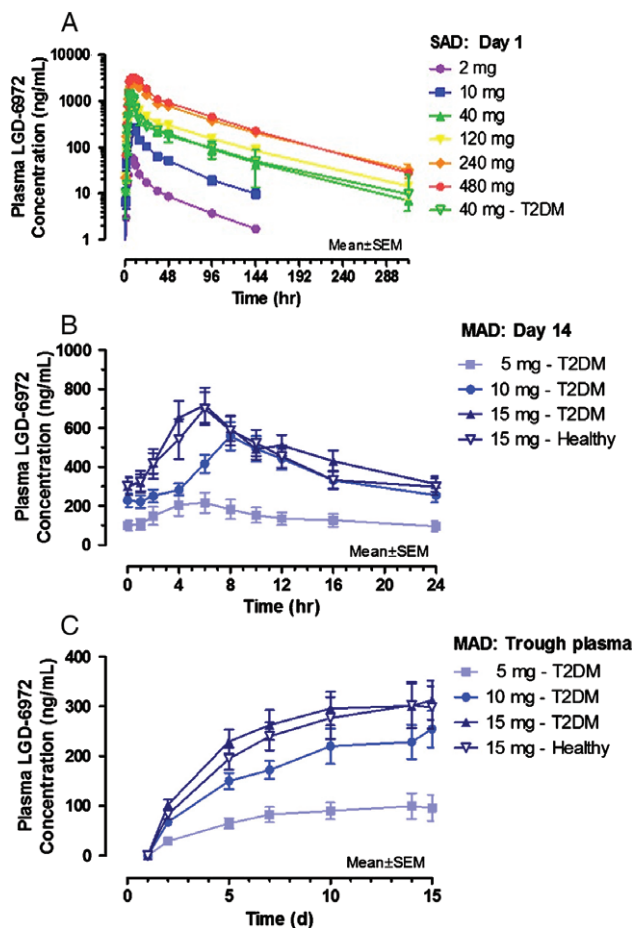
#### 3.2 | Pharmacokinetics

##### 3.2.1 | SAD study

LGD-6972 was well absorbed after single oral doses ranging from 2 to 480 mg. Figure 1A displays the mean LGD-6972 plasma concentrations by dose for healthy and T2DM subjects following fasted administration. Time to maximum concentration ( $T_{\max}$ ) was achieved for most doses approximately 6–8 hours postdose (Table S2, Supporting Information). The maximum concentration ( $C_{\max}$ ) and overall exposure [area under the curve (AUC)] increased with increasing doses of LGD-6972 in healthy subjects. The elimination half-life across all dose groups ranged from 39.2 to 58.5 hours. LGD-6972 was not detected in urine (data not shown). The  $C_{\max}$  and AUCs were 22.5% higher in fasted condition than in fed condition in healthy subjects after administration of 40 mg of LGD-6972 (Table S2, Supporting Information). The  $C_{\max}$  was higher in T2DM subjects than in healthy subjects, but overall exposure (AUC) was similar between T2DM and healthy subjects (Table S2, Supporting Information).

##### 3.2.2 | MAD study

The plasma PK of LGD-6972 following repeat dosing was comparable and predictable from what was observed in the SAD study. The mean plasma LGD-6972 concentrations over time on day 14, as well as mean plasma LGD-6972 trough concentrations, are shown in Figure 1B and C. Group mean plasma LGD-6972 PK parameters are presented in Table S3, Supporting Information. The  $C_{\max}$  and exposures increased dose-proportionately. The PK profiles were similar between healthy and T2DM subjects following 14 days of treatment with 15 mg LGD-6972 (Figure 1B). LGD-6972, as in the SAD study,



**FIGURE 1** Mean ( $\pm$  SEM) plasma LGD-6972 concentration versus time profiles by dose for healthy and T2DM subjects in the SAD and the MAD studies. A, Healthy and T2DM subjects in the SAD study. B, T2DM and healthy subjects on day 14 in the MAD study. C, Trough plasma LGD-6972 concentrations versus time profiles for T2DM and healthy subjects in the MAD study. hr, hours; d, day; SEM, standard error of the mean.

exhibited a long half-life in all dose groups (ranging from 43.7 to 58.6 hours), resulting in accumulation ratios of 2.5 to 3.1 in  $AUC_{0-24hr}$  following 14 days of treatment in T2DM subjects (Table S3, Supporting Information). Steady state PK was achieved in all groups by end of treatment (Figure 1C).

#### 3.3 | Safety and tolerability

##### 3.3.1 | SAD study

LGD-6972 was well tolerated up to the highest dose tested (480 mg). No healthy subjects or T2DM subjects had a serious adverse event (SAE) or were discontinued from the study because of an AE. There were no clinically significant or dose-dependent changes in haematology, clinical chemistry, urinalysis, ECG or vital signs, and there were no reports of hypoglycaemia. Study drug-related treatment emergent adverse events (TEAE) were observed in healthy subjects but not in any T2DM subjects. Table 1 provides an overview of AEs by treatment for healthy subjects. The most common TEAEs were headache ( $n = 5$ ) and gastrointestinal disorders ( $n = 4$ ). Most

**TABLE 1** Treatment-emergent adverse events by treatment for healthy subjects in the SAD study

Volunteers	LGD-6972 dose level						
	PBO	2 mg	10 mg	40 mg	120 mg	240 mg	480 mg
Number of subjects	12	6	6	6	6	6	6
Any TEAE (%)	3 (25.0)	1 (16.7)	1 (16.7)	1 (16.7)	4 (66.7)	4 (66.7)	3 (50.0)
Any study drug-related TEAE (%)	1 (8.3)	0 (0.0)	1 (16.7)	0 (0.0)	2 (33.3)	2 (33.3)	2 (33.3)
<b>Detail of study drug-related TEAE</b>							
Gastrointestinal disorders (%)	Nausea	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
	Diarrhea	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
	Vomiting	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
Nervous system disorders (%)	Headache	1 (8.3)	0 (0.0)	1 (16.7)	0 (0.0)	2 (33.3)	1 (16.7)

Note: Treatment-emergent adverse events were defined as adverse events that occurred for the first time on or after the first dosing date/time of study drug, or existed prior to dosing but worsened in severity or frequency during the treatment period. Percentages (%) were calculated using the number of subjects as the denominator.

TEAE, treatment-emergent adverse event; PBO, placebo, SAD, single ascending dose.

TEAEs were mild or moderate in severity; however, one healthy subject who received a 480-mg dose experienced two severe TEAEs (headache and nausea).

### 3.3.2 | MAD study

LGD-6972 was well tolerated in healthy and T2DM subjects following 14 days of dosing, with no clinically significant or dose-dependent changes in haematology, clinical chemistry, urinalysis, ECG or vital signs. There were no serious adverse events and no study discontinuations. Table 2 provides an overview of AEs for healthy and T2DM subjects. Two healthy subjects who received 15 mg LGD-6972 experienced study drug-related TEAEs (headache). For T2DM subjects, the most common study drug-related TEAEs were headache (n = 4) and gastrointestinal disorders (n = 4). No other specific TEAEs were

experienced by more than one subject. Most TEAEs were of mild or moderate severity (grade 1 or 2); however, one T2DM subject who received 15 mg LGD-6972 had TEAEs with a maximum severity of grade 3 (abdominal discomfort, abdominal pain, headache and nausea). One T2DM subject who received 5 mg LGD-6972 developed increased ALT (>3X ULN), AST and gamma-glutamyl-transferase (GGT), along with elevated per cent neutrophil and white blood cells and haematuria during the follow-up period (14 days after dosing ended). Bilirubin was not elevated (no Hy's Law violation). All findings were mild in severity and resolved by the end of study participation. There were no cases of symptomatic hypoglycaemia. Small increases in ALT from baseline were observed by day 14 in the T2DM subjects given 5, 10 or 15 mg LGD-6972 (15.6, 2.6 and 5.6 U/L, respectively). However, these increases were not dose dependent and group means

**TABLE 2** Treatment-emergent adverse events by treatment for healthy and T2DM subjects in the MAD study who received PBO or 5, 10 or 15 mg LGD-6972

Volunteers	Healthy		T2DM			
	PBO	15 mg	PBO	5 mg	10 mg	15 mg
Number of subjects	3	9	9	9	9	9
Any TEAE (%)	1 (33.3)	4 (44.4)	4 (44.4)	2 (22.2)	6 (66.7)	4 (44.4)
Any study drug-related TEAE (%)		2 (22.2)	1 (11.1)	1 (11.1)	5 (55.6)	2 (22.2)
<b>Detail of study drug-related TEAE</b>						
Gastrointestinal disorders (%)			1 (11.1)	1 (11.1)	1 (11.1)	1 (11.1)
Investigations (%)				1 (11.1) <sup>1</sup>		
				1 (11.1) <sup>1</sup>		
				1 (11.1) <sup>1</sup>		
				1 (11.1) <sup>1</sup>		
				1 (11.1) <sup>1</sup>		
				1 (11.1) <sup>1</sup>		
				1 (11.1) <sup>1</sup>		
Nervous system disorders	Headache (%)	2 (22.2)			3 (33.3)	1 (11.1)
Respiratory disorder (%)	Oropharyngeal pain				1 (11.1)	

Note: Treatment-emergent adverse events were defined as adverse events that occurred for the first time on or after the first dosing date/time of study drug, or existed prior to dosing but worsened in severity or frequency during the treatment period. Percentages (%) were calculated using the number of subjects as the denominator.

TEAE, treatment-emergent adverse event; PBO, placebo; T2DM, type 2 diabetes mellitus; MAD, multiple ascending dose; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

<sup>1</sup>Abnormal laboratory values occurred in same T2DM subject 14 days after dosing had ended.

remained within the normal range (Figure S1, Supporting Information). Changes in AST were generally smaller than those in ALT. No clinically meaningful or dose-dependent changes in total, LDL or HDL cholesterol or triglycerides were observed (Tables S4 and S5, Supporting Information).

### 3.4 | Pharmacodynamics

#### 3.4.1 | SAD study

LGD-6972 decreased FPG 24 hours after dosing in healthy subjects, approximating dose-dependency (Figure 2A). In T2DM subjects (Figure 2B), a single dose of 40 mg LGD-6972 decreased FPG by 2.78 mmol/L. The maximum effect was observed at 24 hours post-dose but a reduction in FPG compared to baseline persisted throughout the 144 hours of measurement.

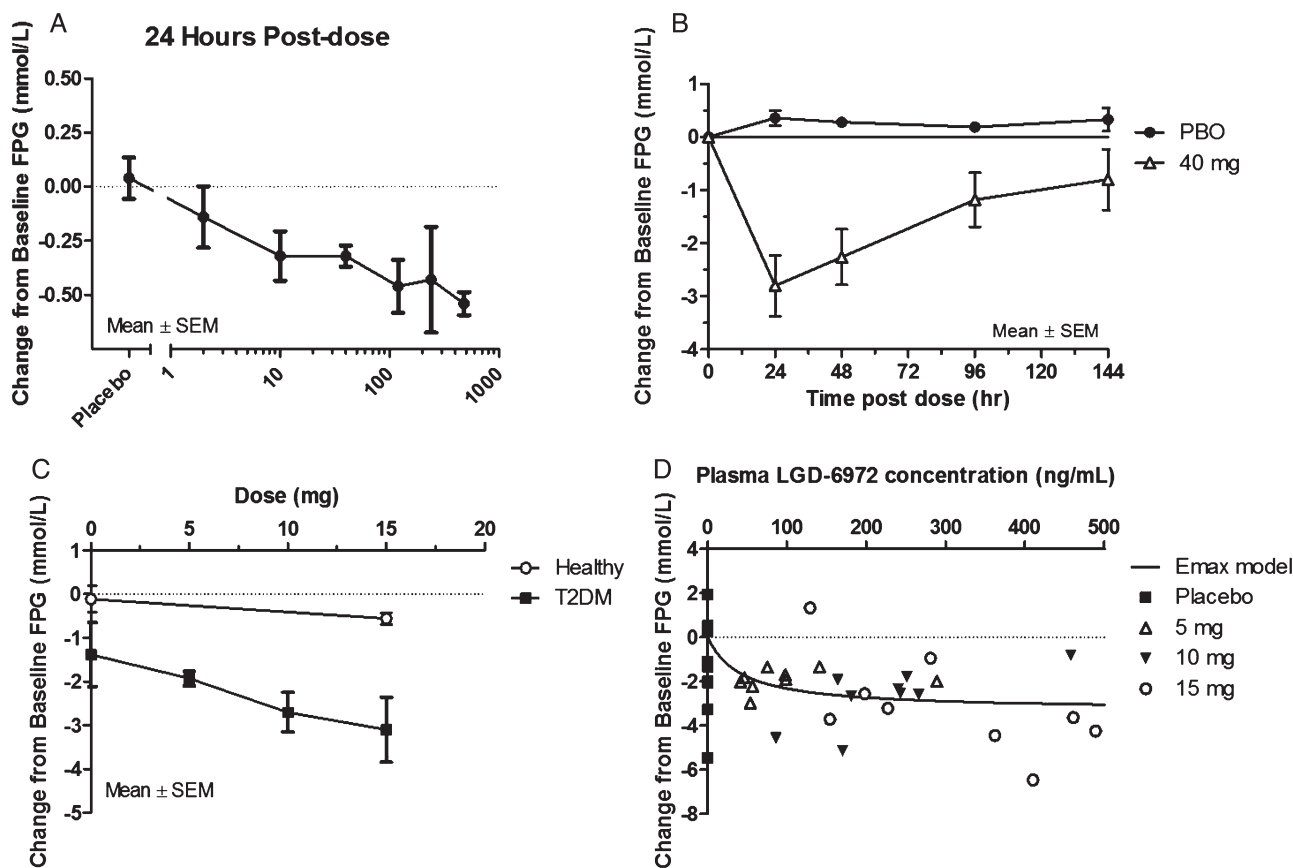
LGD-6972 increased fasting plasma glucagon in healthy subjects in a dose-dependent manner and in T2DM subjects given a 40 mg dose (Figure S2, Supporting Information). LGD-6972 increased fasting plasma total GLP-1 in healthy and T2DM subjects; however, active GLP-1 and insulin were not consistently altered following LGD-6972 administration (data not shown).

#### 3.4.2 | MAD study

Treatment with 15 mg LGD-6972 once daily for 14 days resulted in a slight decrease in FPG from baseline in healthy subjects and a larger dose-dependent decrease in T2DM subjects (Figure 2C). PK/PD modeling with an  $E_{\max}$  model predicted a maximal decrease of  $-3.31$  mmol/L ( $-59.6$  mg/dL) with an  $EC_{50}$  of 42.2 ng/dL (Figure 2D). LGD-6972 effects were reversible during the follow-up period.

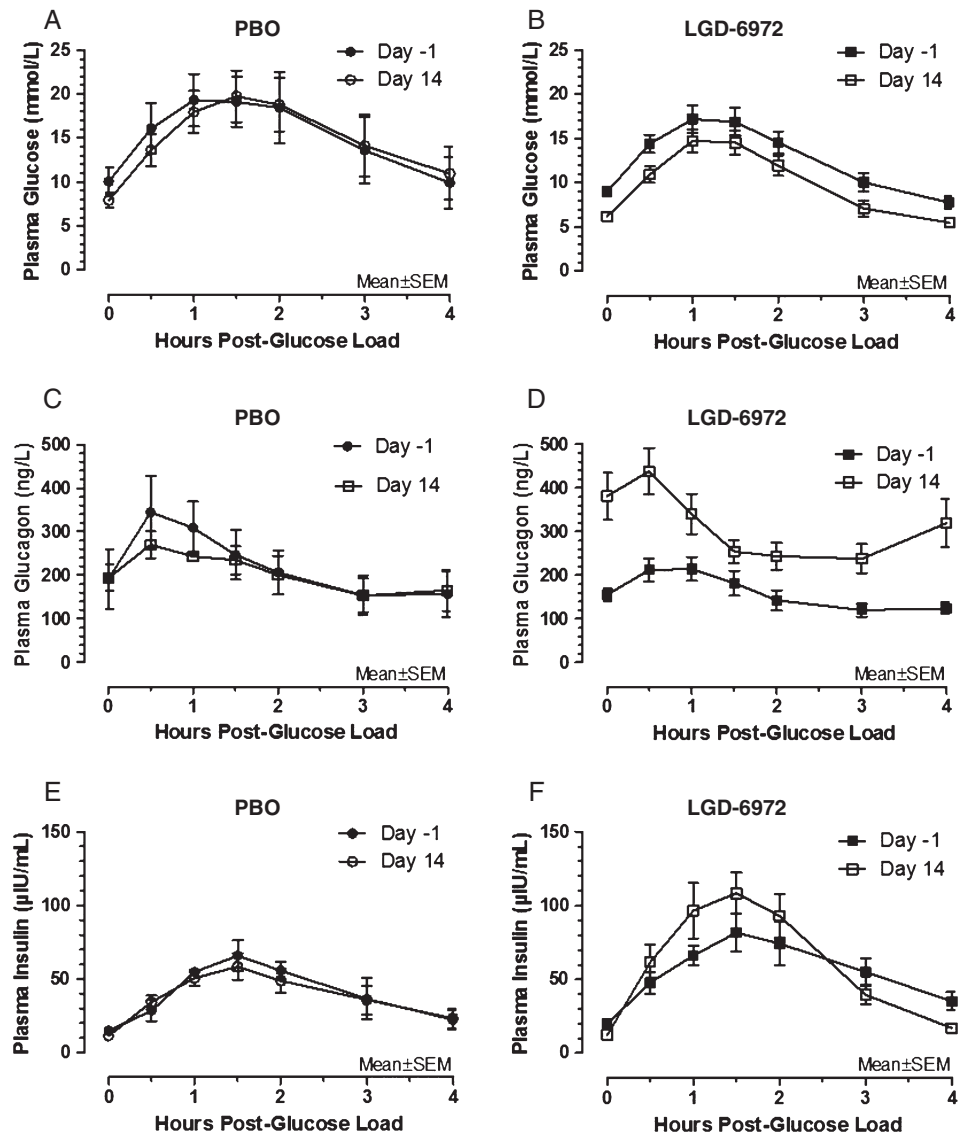
Mean weighted average 7-point glucose was lower on day 14 relative to day -1 for T2DM subjects who received 5 mg ( $-2.28$  mmol/L), 10 mg ( $-2.36$  mmol/L) or 15 mg LGD-6972 ( $-2.72$  mmol/L). No change from day -1 was observed for subjects who received placebo (Figure S3, Supporting Information). Day 14 mean glucose was decreased at all seven time points pre- and postmeal across all LGD-6972 treatments versus the placebo group.

Effects on glycaemia were accompanied by increases from baseline in fasting glucagon (305.0 ng/L) and total GLP-1 (2.1 pmol/L) following 14 days of treatment in healthy subjects who received 15 mg LGD-6972 (Table S6, Supporting Information). Similarly, in T2DM subjects who received 5, 10 or 15 mg LGD-6972, dose-related increases from baseline in fasting glucagon (168.9, 211.9 or 323.8 ng/L, respectively), total GLP-1 ( $-0.3$ , 2.0 or 2.1 pmol/L, respectively) and active GLP-1 ( $-0.52$ , 0.29 or 0.376 pmol/L,



**FIGURE 2** Mean ( $\pm$  SEM) change from baseline fasting plasma glucose (FPG) versus time profiles for healthy and T2DM subjects in the SAD and the MAD studies who received PBO or LGD-6972, and a direct  $E_{\max}$  model of the relationship between plasma LGD-6972 concentration and change from baseline FPG in T2DM subjects in the MAD study. A, Healthy subjects in the SAD study. B, T2DM subjects in the SAD study. C, Healthy subjects and T2DM subjects in the MAD study. D,  $E_{\max}$  model in T2DM subjects in the MAD study. PBO, placebo; SEM, standard error of the mean; hr, hours; SD, standard deviation; d, day.





**FIGURE 3** Effect of LGD-6972 on mean ( $\pm$  SEM) plasma glucose, glucagon and insulin levels in response to an oral glucose load during an oral glucose tolerance test in the MAD study. A, Glucose in T2DM subjects who received PBO. B, Glucose in T2DM subjects who received 10 mg LGD-6972. C, Glucagon in T2DM subjects who received PBO. D, Glucagon in T2DM subjects who received 10 mg LGD-6972. E, Insulin in T2DM subjects who received PBO. F) Insulin in T2DM subjects who received 10 mg LGD-6972. PBO, placebo; SEM, standard error of the mean.

respectively) were observed (Table S7, Supporting Information). No dose-related effect of LGD-6972 on fasting insulin was observed for healthy or T2DM subjects.

Figure 3 displays the mean change from preglucose load on day -1 and day 14 for glucose, glucagon and insulin in T2DM subjects in the 10-mg LGD-6972 group who received an OGTT. Placebo-treated T2DM subjects had comparable OGTT results on day -1 and day 14. As expected, there was a transient increase in plasma glucagon, followed by a late rise in insulin levels.<sup>30</sup> Although fasting glucagon levels were elevated on day 14 in T2DM subjects receiving 10 mg LGD-6972, the incremental change [area under the curve from time 0 to 4 hours ( $AUC_{0-4hr}$ )] in glucagon following a glucose load generally decreased on day 14, relative to the  $AUC_{0-4hours}$  on day -1 (Figure 3C and D). In contrast, while fasting insulin levels were unchanged on day 14, there was an increase in insulin  $AUC_{0-4hours}$  when compared with day -1 (Figure 3E and F). Although baseline fasting glucose was decreased on day 14, there was no difference in the incremental change ( $AUC_{0-4hours}$ ) in glucose (Figure 3A and B) or in total or active GLP-1 (Figure S4A–D, Supporting Information) during the OGTT on day 14 relative to day -1.

## 4 | DISCUSSION

GCGR antagonism provides a novel mechanism to modulate plasma hyperglycaemia for the treatment of T2DM that would be complementary to some of the currently marketed agents.<sup>23</sup> GCGR antagonism has also been proposed as an effective treatment for stress-induced hyperglycaemia.<sup>25</sup> GRAs reduce hepatic glucose production by inhibiting the action of glucagon. Recently, it has been shown that small molecule GRAs,<sup>17–22</sup> a GCGR monoclonal antibody<sup>27</sup> and an antisense GCGR antagonist<sup>28</sup> can reduce fasting plasma glucose (FPG) and HbA1c in T2DM patients. Herein we report that treatment with the novel, potent GRA, LGD-6972, results in dose-related decreases from baseline in FPG in subjects with T2DM. Glucose reduction was observed in fasting and postprandial states. The effects on glycaemia in T2DM subjects were accompanied by dose-related increases from baseline in fasting glucagon, total GLP-1 and active GLP-1. As expected, in normal healthy subjects the reductions in FPG were more modest but nearly equivalent proportional increases in glucagon and total GLP-1 were observed. Decreases in FPG and increases in glucagon and GLP-1 were expected because similar

effects have been reported in clinical studies with other GRAs.<sup>17–22</sup> However, unlike reports concerning other small molecule GRAs, the robust glycaemic responses in T2DM subjects treated with LGD-6972 were not associated with dose-related or clinically meaningful changes in liver enzymes during the 14-day treatment or follow-up period.

In the MAD study, the decreases in FPG in healthy subjects and T2DM subjects treated with metformin monotherapy were apparent 24 hours after the first dose, reached maximal effect by day 5 and were sustained at this level through the end of dosing. Decreases from baseline in mean weighted average 7-point glucose were also observed in T2DM subjects. Indeed, mean glucose values were decreased at all seven time points on day 14 across all LGD-6972 treatments versus placebo, indicating that LGD-6972 was effective throughout the day at modulating glucagon effects on fasting, pre-meal and postprandial glucose. Based on the mean change from baseline in the weighted average 7-point glucose on day 14, the projected HbA1c reduction with longer-term chronic dosing is estimated to be as much as 1.5%.<sup>29</sup>

For T2DM subjects, no dose-related effect on fasting insulin was detected after 14 days of treatment compared to baseline. Considered in the context of decreased FPG, this result may reflect decreased insulin resistance in the fasting state.

In contrast to the effects of fasting glycaemia, the pharmacodynamic response observed during the OGTT was not anticipated. As previously reported by Rizza,<sup>30</sup> glucagon levels increased “paradoxically” in placebo-treated T2DM subjects on metformin only after an oral glucose load. However, in this study, the mean change in glucagon levels from baseline and the incremental change in glucagon AUC<sub>0–4hours</sub> were decreased in LGD-6972-treated T2DM subjects versus those receiving placebo. The observed decrease in glucagon levels in response to an oral glucose load may reflect the accompanied increased peak OGTT insulin levels and incremental change in insulin AUC<sub>0–4hours</sub> observed on day 14 versus day –1. As might be expected, the increased insulin levels in subjects who received LGD-6972 were followed by a greater decrement in subsequent glucose levels when compared to the response in subjects who received placebo. This apparent improvement in insulin sensitivity and insulin secretion in response to glucose load, accompanied by increases that occurred in both active and total GLP-1, may be responsible for the unexpected decrease in glucagon levels, as glucagon secretion is normally highly sensitive to insulin and GLP-1 levels. If confirmed in subsequent studies of longer duration, the clinical significance of these findings suggests that inhibition of the glucagon response by LGD-6972 in the fasted state may abrogate the increased overnight gluconeogenesis found in early T2DM, while the potential concomitant improvements in fasting insulin sensitivity and insulin secretion in response to an oral glucose load may address the impaired insulin action in the fed state in clinically manifest T2DM.

LGD-6972 was generally well tolerated in these studies, with a safety profile supporting further development. There were no SAEs and TEAEs were generally mild to moderate and related to headaches or gastrointestinal disorders. There was a trend towards increased AEs with increased dose and larger, longer term studies

will be needed to better define the safety profile of LGD-6972. One potential concern with this mechanism of action is hypoglycaemia. In both studies, no healthy subjects or T2DM subjects experienced a hypoglycaemic event during the treatment or follow-up periods. Likewise, in studies with LY2409021 in T2DM, events of hypoglycaemia were mild and infrequent, even at high drug exposure.<sup>19,20</sup> This suggests that glucagon-independent counter-regulatory mechanisms, such as a sympathomimetic response, may be adequate in this setting to minimize hypoglycaemia in the presence of competitive GCGR blockade.

Elevations in liver enzymes, predominantly ALT and to a lesser extent AST, have been observed in the clinical trials of other GRAs, including small molecules,<sup>17–22</sup> a monoclonal antibody<sup>27</sup> and an antisense oligonucleotide,<sup>28</sup> suggesting that it may be a pharmacologic effect of GCGR blockade, although the mechanism is not known. The increases in liver enzymes were reversible, and were not reported to be associated with elevated bilirubin levels or other signs or symptoms of liver injury in any of the studies. Elevations in liver enzymes observed after treatment with LGD-6972 were not dose dependent, and were not regarded as clinically significant despite robust glycaemic efficacy. Larger clinical trials of longer duration will be required to fully examine the effects of LGD-6972 on liver metabolism.

The GRAs MK-0893, MK 3577 and LY2409021<sup>17,18,31,32</sup> were reported to have effects on plasma lipids, bodyweight and blood pressure. These effects were not observed during treatment with LGD-6972 or ISIS-GCGRX<sup>28</sup> at doses that displayed significant glucose lowering. Whether this discrepancy is explained by differences in study design, patient population or pharmacological effects among the compounds is currently unknown.

In conclusion, the results of the Phase 1 studies reported here demonstrate that oral administration of LGD-6972 once daily achieved sustained, pharmacologically relevant plasma levels of drug that were associated with glycaemic response in both normal and T2DM subjects. The reduction in glucose with LGD-6972 was observed in both fasting and postprandial states, and was accompanied by an increase in insulin and a decrease in glucagon in response to an oral glucose load. The extent and magnitude of the glycaemic response in subjects with T2DM was sufficient to predict a significant beneficial effect on glycaemic control when testing longer duration of treatment in patients with T2DM. No meaningful safety or tolerability issues were observed. These results support continuation of the development of once-daily LGD-6972 into Phase 2 in patients with T2DM.

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## Conflict of interest

Both studies were sponsored by Ligand Pharmaceuticals Incorporated. EGV, JDP, LZ and KBM are employees and/or stockholders of Ligand Pharmaceuticals Incorporated.

## Author contributions

All authors contributed to the writing and review process and approved the final manuscript. EGV, DJP, JDP, LZ and KBM contributed to the study concept and design, analysis and interpretation of data, drafting of the manuscript and critical revisions. DL was the principal investigator for the SAD study and a principal investigator in the MAD study; he was involved in the study design, analysis and interpretation of data. KL and DA were principal investigators in the MAD study and involved in the study design, analysis and interpretation of data. RZ and XW contributed to the study design, analysis and interpretation of data, and critical revisions to the manuscript. Y-XL contributed to the analysis and interpretation of data.

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## SUPPORTING INFORMATION

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