

ORIGINAL ARTICLE

WILEY

Abolishing β -arrestin recruitment is necessary for the full metabolic benefits of G protein-biased glucagon-like peptide-1 receptor agonists

Charlotte E. Hinds PhD¹  | Ellie Peace BSc¹ | Shiqian Chen MRes¹ |
Iona Davies MRes¹ | Liliane El Eid MRes² | Alejandra Tomas PhD²  |
Tricia Tan MD¹  | James Minnion PhD¹  | Ben Jones MD¹  |
Stephen R. Bloom MD¹

¹Section of Endocrinology and Investigative Medicine, Imperial College London, London, UK

²Section of Cell Biology, Imperial College London, London, UK

Correspondence

Ben Jones and Stephen R. Bloom, Section of Endocrinology and Investigative Medicine, Imperial College London, W12 0NN, UK.
Email: ben.jones@imperial.ac.uk and s.bloom@imperial.ac.uk

Funding information

Diabetes UK, Grant/Award Numbers: 19/0006094, 20/0006307; Medical Research Council, Grant/Award Number: MR/R010676/1; NIHR Imperial Biomedical Research Centre; North West London Pathology

Abstract

Aim: Earlier studies have shown that peptide glucagon-like peptide-1 receptor (GLP-1R) agonists with reduced β -arrestin recruitment show enhanced anti-hyperglycaemic efficacy through avoidance of GLP-1R desensitization. However, the ligand modifications needed to decrease β -arrestin recruitment usually also reduces GLP-1R affinity, therefore higher doses are needed. Here we aimed to develop new, long-acting, G protein-biased GLP-1R agonists with acute signalling potency comparable with semaglutide, to provide insights into specific experimental and therapeutic scenarios.

Materials and Methods: New GLP-1R agonist peptides were assessed using a variety of in vitro and in vivo assays.

Results: First, we show that very substantial reductions in β -arrestin recruitment efficacy are required to realize fully the benefits of GLP-1R agonism on blood glucose lowering in mice, with more moderate reductions being less effective. Secondly, our lead compound (SRB107) performs substantially better than semaglutide for effects on blood glucose and weight loss, which may be jointly attributable to its biased agonist action and protracted pharmacokinetics. Thirdly, we show that biased agonist-specific GLP-1R internalization profiles occur at clinically relevant pharmacological concentrations. Finally, we show that SRB107 cAMP signalling is differentially modulated by single and double *GLP1R* coding variants seen in human populations, with implications for GLP-1R agonist pharmacogenomics.

Conclusions: Completely abolishing β -arrestin recruitment improves the anti-hyperglycaemic effects of GLP-1R agonists in mice.

KEYWORDS

antidiabetic drug, antiobesity drug, GLP-1 analogue, pharmacology

1 | INTRODUCTION

Glucagon-like peptide-1 receptor (GLP-1R) agonists are mainstay treatments for type 2 diabetes (T2D) and obesity, improving blood glucose levels through effects on β -cell function and insulin sensitivity via weight loss. The GLP-1R is a class B G protein-coupled receptor (GPCR) highly expressed in metabolic tissues including pancreatic β -cells and other islet cell types, anorectic neurons in the peripheral and central nervous system, and at lower levels in myriad other tissues including kidney, myocardium, blood vessels and immune cells.¹ All currently approved GLP-1R agonists are synthetic analogues of the endogenous agonist GLP-1(7-36)NH₂ or its paralogue exendin-4, with a variety of modifications that extend pharmacokinetics through proteolytic stabilization and/or avoidance of renal clearance.²

Recent work has revealed that the intracellular signalling profile of orthosteric GLP-1R peptide agonists can be profoundly altered by changing their amino acid sequence.³⁻⁸ In many cases, this results in diminished coupling to intracellular effectors, with recruitment of β -arrestins affected more so than G protein-dependent cyclic adenosine monophosphate (cAMP) signalling. This imbalance is often classified as 'bias' in favour of G protein-dependent signalling. A consistent effect of G protein-biased GLP-1R agonists is their prolonged GLP-1R signalling ability, circumventing natural mechanisms that prevent receptor overstimulation such as steric hinderance by β -arrestins and receptor internalization.^{4,5,9}

The probable importance of biased agonism has been highlighted by the realization that the GLP-1R/GIPR co-agonist tirzepatide, recently approved for the treatment of T2D, shows pronounced G protein bias at the GLP-1R.¹⁰ However, the acute cAMP signalling potency of tirzepatide at the GLP-1R is reduced substantially compared with semaglutide,¹¹ potentially accounting for the higher doses required clinically (up to 15 mg vs. up to 2.4 mg, respectively). An earlier long-acting, acylated G protein-biased GLP-1R agonist developed by our group also showed reduced acute signalling potency compared with the non-biased comparator, although it still showed better anti-diabetic effects in mice.⁹ Feasibly, restoring acute cAMP signalling potency may further add to the benefits of G-protein bias. In addition, it is unclear whether total abolition of β -arrestin recruitment (for maximum avoidance of desensitization) is preferable to a partial reduction, as β -arrestin recruitment is paradoxically linked to positive signalling effects including GLP-1R-stimulated insulin release.¹²

Like all proteins, the *GLP1R* gene sequence is subject to natural coding variation. Common and low frequency *GLP1R* variants have been associated with metabolic effects in large-scale genomic and experimental analyses.¹³⁻¹⁶ The importance of target coding variation on therapeutic and side effect profiles for GPCR-targeting drugs has recently been proposed on theoretical grounds.¹⁷ The effects of *GLP1R* missense variants on signalling characteristics of GLP-1, oxyntomodulin and exendin-4 were previously tested.^{18,19} However, the impact of coding variation on G protein-biased GLP-1R mono-agonist signalling is not yet known, and, because of larger differences in ligand signalling properties, may be more consequential. Understanding how

target variation influences responses to GLP-1R agonists will be necessary for personalized treatment selection in T2D.²⁰

In this work we integrate our previous knowledge of ligand factors responsible for G protein-biased agonism to develop new, pharmacokinetically optimized GLP-1R agonists with reduced coupling efficiency to β -arrestin recruitment but retained cAMP signalling potency. We then use these ligands to address questions pertinent to the biology of biased GLP-1R agonism, namely: (a) is partial or complete loss of β -arrestin recruitment therapeutically preferable; (b) are GLP-1R internalization effects important at clinically relevant pharmacological concentrations; and (c) are biased agonist responses differentially modulated by naturally occurring *GLP1R* single residue missense *GLP1R* variants present in human populations.

2 | MATERIALS AND METHODS

New GLP-1R agonist peptides were assessed using a variety of in vitro and in vivo assays (full methodological details are provided in Data S1).

3 | RESULTS

3.1 | Development of equipotent $G\alpha_s$ -favouring glucagon-like peptide-1 receptor agonists

Our previous development of biased GLP-1R agonists showed that substituting phenylalanine for histidine at the N-terminus of exendin-4 markedly reduced recruitment of β arrestins, while moderately reducing acute signalling potency for cAMP.⁴ G protein-biased signalling properties were maintained with a C16 fatty diacid chain installed at the peptide C-terminus for half-life protraction, but overall binding affinity was further reduced.^{9,21} Therefore, here we introduced further targeted amino acid sequence substitutions to improve peptide signalling potency and stability, in addition to optimizing the acyl chain and linker. After iterative improvements we arrived at two peptides, SRB106 and SRB107, which share a C20 fatty diacid chain at the C-terminal lysine residue and differ only at their N-termini (Figure 1A; Figure S1A). Various linkers and acyl chains were tested, and the ϵ LysGlu motif was identified as providing an optimal balance of half-life protraction and potency. Like semaglutide, SRB106 and SRB107 were GLP-1R mono-agonists for both human and mouse receptor cAMP responses in transiently transfected HEK293-derived T-REx 293 cells, with no meaningful activation of the glucagon receptor (GCGR), glucose-dependent insulinotropic polypeptide receptor (GIPR) or glucagon-like peptide-2 receptor in either species (Figure S1B, C, Table 1).

To seek evidence of biased agonism, we used the NanoBiT system to evaluate ligand-induced coupling to mini- $G\alpha_s$ (a conformational sensor for G protein-favouring active GPCR states²²) and β -arrestin-2 recruitment. Assays were performed in parallel using T-REx 293 cells stably transfected with SNAP_F-tagged human GLP-1R with a C-terminal

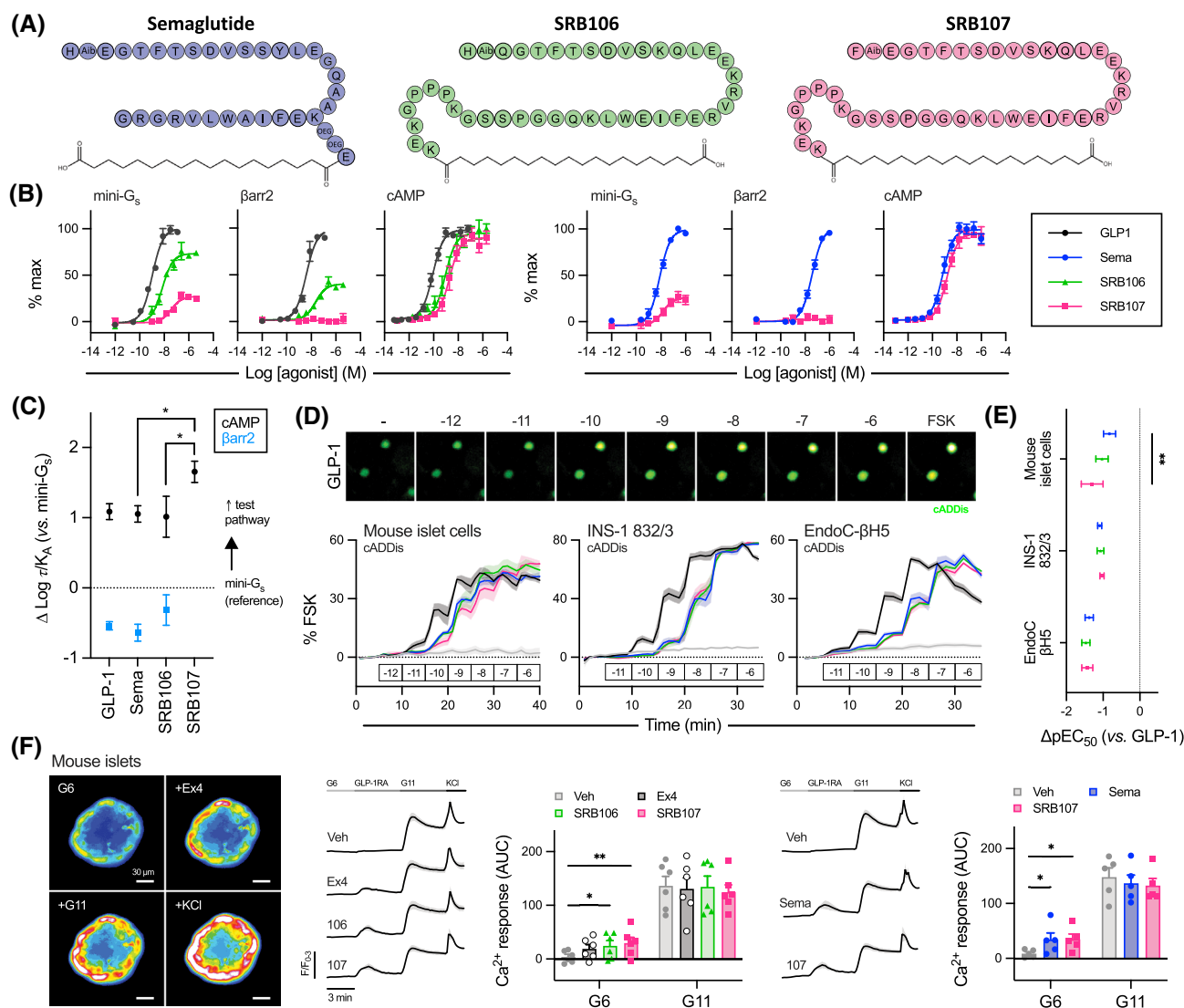


FIGURE 1 Pharmacological characterization of novel glucagon-like peptide-1 receptor (GLP-1R) agonists. (A) Ligand amino acid sequences. (B) Mini-G_s and β-arrestin-2 (βarr2) NanoBiT responses, and cyclic AMP (cAMP) in Flp-In T-REx-293-SNAP₁-GLP-1R-SmBiT cells, $n = 5$, 30 min stimulation with GLP-1, semaglutide (Sema), SRB106 or SRB107, with three-parameter fits shown. (C) Relative signal transduction ratios ($\Delta \text{Log } \tau/K_d$) calculated from (B) relative to the mini-G_s response for each ligand; statistical comparisons using mixed effects model with Tukey's test. (D) cADDIS cAMP sensor signal in response to stepwise addition of increasing concentrations of each agonist in dispersed primary mouse islet cells ($n = 5$), INS-1832/3 cells ($n = 6$) or EndoC-βH5 cells ($n = 5$). Images show intensity changes in representative mouse islet cells at different agonist concentrations. (E) Relative signalling potency (ΔpEC_{50}) for each ligand, derived from data in (D) by determining concentration responses from area under the curve (AUC) during each time window and normalizing to GLP-1 for each system; statistical comparisons by two-way repeated measures ANOVA with Tukey's test. (F) Intracellular calcium responses in wild-type mouse islets treated with 100 nM agonist (GLP1RA) or vehicle (Veh) at 6 mM or 11 mM glucose (G6, G11), $n = 5$ –6; statistical comparisons of AUC from indicated time window by two-way repeated measures ANOVA with Tukey's test. All data represented as mean $\pm$ SEM. * $p < .05$, ** $p < .01$ by indicated test.

SmBiT tag (referred to hereafter as SNAP-GLP-1R). SRB106 and SRB107 both showed reduced maximum engagement with mini-G_s and β-arrestin-2 compared with GLP-1(7-36)NH₂ and semaglutide, with SRB107 most affected (Figure 1B). Notably, the β-arrestin-2 recruitment response for SRB107 was barely detectable. To compare formally the relative coupling of each ligand to these responses we used the modified operational model,²³ setting mini-G_s as the reference pathway. This indicated enhanced cAMP signalling for SRB107 compared with semaglutide

and SRB106 (Figure 1C). Of note, pronounced loss of the β-arrestin-2 response with SRB107 was also seen in cells transiently expressing mouse or human untagged GLP-1R (Figure S1D), and both β-arrestin-1 and β-arrestin-2 recruitment were comparably reduced when tested using the PathHunter GLP-1R system (Figure S1E).

To assess the signalling performance of each agonist at natively expressed GLP-1R, we also used the sensitive, fluorescent cAMP biosensor cADDIS,²⁴ transduced into dispersed primary mouse islet cells,

TABLE 1 cAMP signalling potency estimates.

	GLP-1	Semaglutide	SRB106	SRB107
hGLP-1R	9.96 ± 0.06	8.84 ± 0.15	9.14 ± 0.12	8.44 ± 0.12
hGCGR	NC	NC	NC	NC
hGIPR	NC	NC	NC	NC
hGLP-2R	NC	NC	NC	NC
mGLP-1R	9.87 ± 0.10	8.94 ± 0.08	9.25 ± 0.20	9.02 ± 0.06
mGCGR	NC	NC	NC	NC
mGIPR	NC	NC	NC	NC
hGLP-2R	NC	NC	NC	NC
Dispersed mouse islets	10.07 ± 0.12	9.24 ± 0.08	9.04 ± 0.15	8.77 ± 0.31
INS-1832/3	9.10 ± 0.13	8.01 ± 0.13	8.03 ± 0.12	8.08 ± 0.13
EndoC-βH5	9.51 ± 0.11	8.13 ± 0.09	8.04 ± 0.05	8.10 ± 0.10

Note: pEC₅₀ ± SEM, i.e. negative logarithm of EC₅₀ in M, for data presented in Figure 1 and Figure S1.

Abbreviations: GLP-2R, glucagon-like peptide-2 receptor; h, as prefix for human; m, as prefix for mouse; NC, not calculated.

rat-derived INS-1832/3 insulinoma cells²⁵ and the recently developed human β-cell model EndoC-βH5.²⁶ Stepwise addition of increasing concentrations of agonist was used to assess cAMP responses across a wide concentration range by fluorescence imaging (Figure 1D). These experiments indicated that semaglutide, SRB106 and SRB107 show similar maximal responses and potency at physiological GLP-1R expression levels, except for a modestly reduced potency for SRB107 in mouse islet cells (Figure 1E, Figure S1F, Table 1). We also found that 100 nM of each test agonist led to a similar increase in intracellular calcium concentrations in intact mouse islets at 6 mM glucose (Figure 1F).

Overall, these data indicate that semaglutide, SRB106 and SRB107 are approximately equal potency GLP-1R mono-agonists for cAMP, but with the latter two showing partial agonism for β-arrestin recruitment. The partial versus complete loss of β-arrestin recruitment with SRB106 and SRB107, respectively, along with relatively preserved cAMP signalling potency, provides an opportunity to examine the optimum degree of β-arrestin response attenuation for maximum metabolic benefits.

3.2 | Glucagon-like peptide-1 receptor internalization is slower with SRB107

G protein-biased GLP-1R agonists with reduced β-arrestin-2 recruitment typically show reduced GLP-1R internalization.^{3–8} When expressed T-REx 293 cells, INS-1832/3 cells or EndoC-βH3²⁷ β-cells, the SNAP-tagged GLP-1R appeared more membrane-resident after treatment with SRB107 than other ligands applied at 100 nM (Figure 2A). A real-time assay²¹ also showed notably slower clustering of SNAP-GLP-1R into endosomal puncta with 100 nM SRB107 compared with other ligands (Figure 2B). Moreover, a reversible labelling assay, in which residual surface SNAP-GLP-1R labelling is stripped using the membrane-impermeant reducing agent Mesna, confirmed that less GLP-1R was internalized after 100 nM SRB107 than other

ligands (Figure 2C). Nevertheless, at very high agonist concentrations, more extensive GLP-1R internalization with SRB107 could be detected (Figure 2D).

To extend our analysis to untagged GLP-1R, we designed fluorescent conjugates of GLP-1, semaglutide, SRB106 and SRB107. For GLP-1 and semaglutide, tetramethylrhodamine (TMR) was appended at the C-terminus after a GK linker; for SRB106 and SRB107, TMR was inserted at position K12 (Figure S2A). cAMP signalling properties of each ligand in T-REx 293 cells stably expressing untagged human GLP-1R were preserved, except for a moderate reduction in potency of SRB107-TMR (Figure S2B). The expected pattern of ligand uptake was observed with both untagged or SNAP-tagged GLP-1R, with the latter showing strong colocalization of agonist and receptor (Figure S2C, D). Reduced uptake of 100 nM SRB107-TMR was confirmed quantitatively using an acid wash protocol to remove residual surface-bound ligand (Figure S2E). We noted in the latter study that semaglutide-TMR showed unexpectedly reduced uptake, despite its evident endosomal distribution when imaged at high resolution; this will require further studies to resolve. In addition, after pre-treatment with 100 nM unmodified SRB107, there was more GLP-1R available at the cell surface for subsequent binding of fluorescent exendin-4-Cy5,⁶ suggesting less receptor had been internalized (Figure S2F).

To identify any potential species-specific agonist response differences, we repeated these experiments in T-REx 293 cells transiently transfected with human or mouse GLP-1R (Figure S2G–J). These experiments all pointed to reduced GLP-1R internalization with SRB107, although we note this effect appeared less marked at the mouse compared with human GLP-1R. Finally, to assess internalization effects with endogenously expressed GLP-1R, we imaged primary mouse pancreatic islets after TMR agonist treatment, which again showed predominant membrane retention of SRB107-TMR (Figure 2E).

These experiments confirm a specific reduction in the ability of SRB107 to promote GLP-1R internalization.

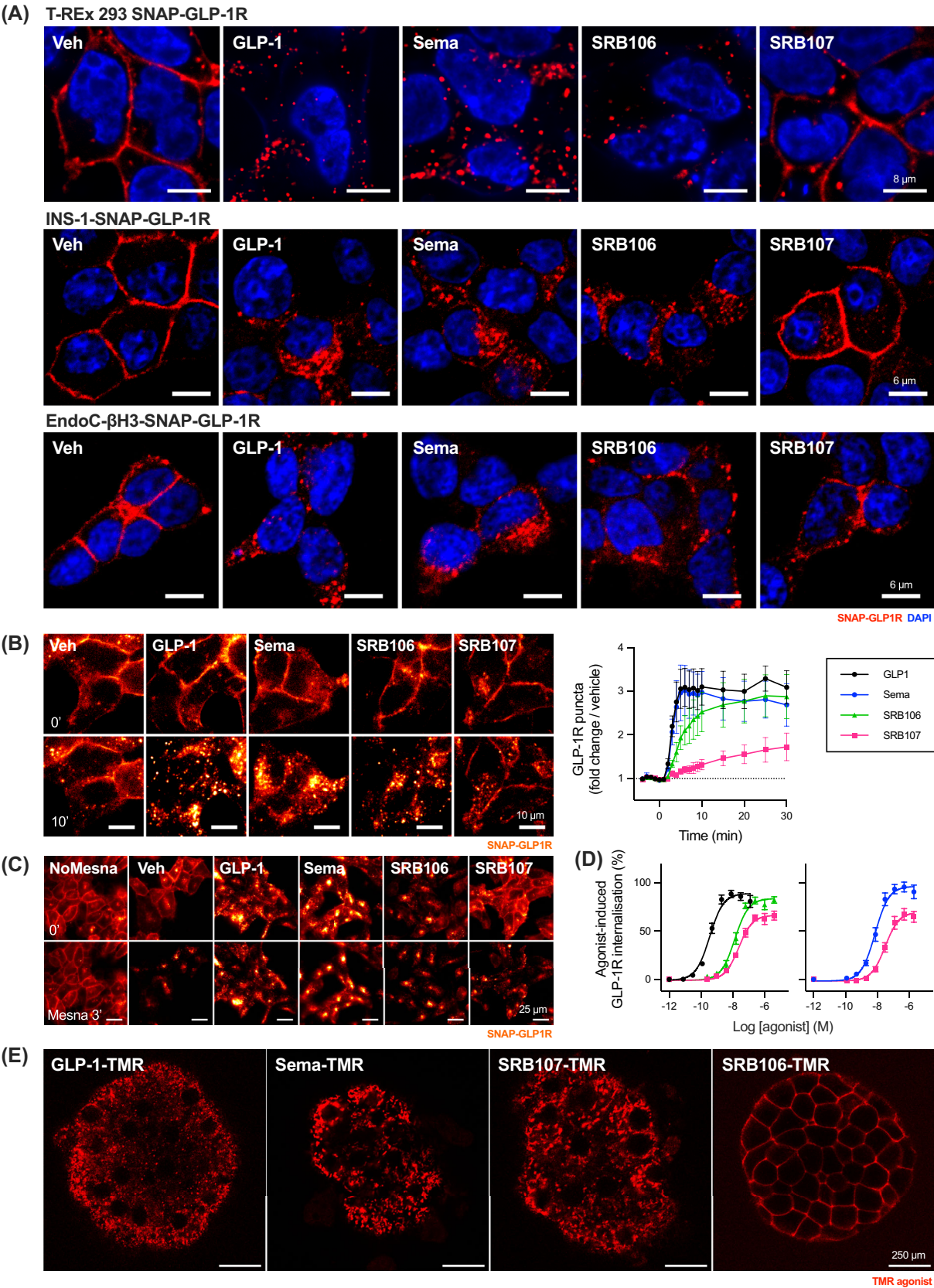


FIGURE 2 Legend on next page.

3.3 | Sustained stimulation effects in the presence of physiological albumin concentration

Acylated GLP-1R agonists are extensively bound to albumin, reducing free drug concentration in plasma. Indeed, compared with experiments conducted at 0.1% bovine serum albumin (Figure S2B), the presence of a physiological concentration of human serum albumin (HSA; 4%) led to a right shift in cAMP signalling potency for semaglutide, SRB106 and SRB107 in the same cell type (Figure 3A), with EC₅₀ estimates of 0.4, 0.5 and 0.6 nM (Figure S2B) shifting to 16.2, 6.9 and 14.0 nM (Figure 2A), respectively. We note that this right shift is less marked than the HSA-induced shift in binding affinity noted during the development of semaglutide,²⁸ which is not surprising as the cAMP pathway is highly amplified.

To probe whether GLP-1R internalization effects would probably be operative under conditions mimicking in vivo drug exposure, we measured the effect of sustained agonist stimulation on the net loss of surface SNAP-GLP-1R in T-REx 293 cells in media supplemented with 4% HSA. Here, at the highest concentration tested (1000 nM), 12 h stimulation with semaglutide or SRB106, but not SRB107, led to marked SNAP-GLP-1R downregulation (Figure 3B). Importantly, differences in the extent of surface GLP-1R loss with each agonist were observed within the reported range of steady state plasma semaglutide concentrations achieved with 2.4 mg weekly dosing in humans,²⁹ and for mice after a single 10 nmol/kg dose as in our study.³⁰ We also assessed the same phenomenon in INS-1832/3 cells and intact mouse pancreatic islets using post-labelling with exendin-4-Cy5⁶; here, with 4% supplemental HSA treatment, 100 nM SRB107 again led to moderately preserved surface GLP-1R compared with SRB106 (Figure 3C, D). We note that this difference was less marked than in SNAP-GLP-1R cells, which could reflect receptor species-related differences (as in Figure S2), other cell-specific differences in endocytosis machinery, or assay considerations: residual binding of SRB107 at the cell surface may have reduced exendin-4-Cy5 binding during the labelling phase as both compete for the GLP-1R orthosteric site. As an additional corroboration, we measured TMR agonist uptake in INS-1832/3 cells after 12 h stimulation at 100 nM in the presence of 4% HSA, also showing reduced uptake of SRB107-TMR compared with SRB106-TMR (Figure S3A).

Agonist-specific reductions in β -arrestin recruitment and GLP-1R internalization are expected to facilitate extended GLP-1R signalling, e.g. via avoidance of desensitization. To assess this in cells with

endogenous GLP-1R expression, we measured the effect of 12 h of treatment with 100 nM of each agonist in the presence of 4% HSA on steady-state cAMP levels in both INS-1832/3 cells and intact mouse islets from CAMPER mice³¹ globally expressing the cAMP FRET sensor ^TEpac^{VV}. In both cases, cAMP levels at steady state were increased with SRB107 compared with SRB106 and semaglutide (Figure 3E, F).

We conclude from these experiments that differences in GLP-1R endocytosis and sustained cAMP signalling with G protein-biased agonists can occur at pharmacologically relevant concentrations.

3.4 | Complete attenuation of β -arrestin-2 recruitment is required for maximum anti-hyperglycaemic effects of biased glucagon-like peptide-1 receptor agonists

The pharmacokinetics of semaglutide, SRB106 and SRB107 were compared in pigs, showing that both SRB peptides have similar half-life protraction, at approximately twice that of semaglutide (Figure 4A). This allowed us to test directly the effects of the different receptor pharmacology of SRB106 and SRB107 on physiological responses, with semaglutide also included as a therapeutically relevant comparator.

We first identified doses of each ligand with similar effects on appetite suppression: when assessed 72 h after a single dose, SRB106 and SRB107 at 3 nmol/kg were similarly anorectic to each other, and to 10 nmol/kg semaglutide (Figure 4B, C). Of note, at these doses, semaglutide led to markedly greater malaise acutely, as assessed by treatment-blinded behavioural observations and potentially indicative of nausea propensity (Figure 4D). We then tested for glycaemic effects via intraperitoneal (IP) glucose tolerance tests performed immediately at the time of dosing (d0) and after a 72-h delay (d3) (Figure 4E). Here, 10 nmol/kg semaglutide was highly effective acutely, but its anti-hyperglycaemic effect was lost by 72 h. At the sub-anorectic 1 nmol/kg dose, both SRB peptides showed little effect until the final time point of the acute IP glucose tolerance test, but interestingly, SRB107 was superior to SRB106 when assessed at 72 h. At 3 nmol/kg however, SRB106 and SRB107 were similar acutely, but only SRB107 was able to improve glucose tolerance at 72 h. SRB107 resulted in a greater plasma insulin response to IP glucose administration at the 72-h time point (Figure 4F), supporting a β -cell-mediated mechanism. There was no evidence of altered insulin sensitivity under the same

FIGURE 2 Glucagon-like peptide-1 receptor (GLP-1R) trafficking responses. (A) Representative images ($n = 2$) of Flp-In T-REx-293-SNAP_{GLP-1R}-SmBiT cells, INS-1-SNAP-GLP-1R cells, and EndoC- β H3 cells transduced with SNAP-GLP-1R adenovirus, labelled with SNAP-Surface probe and treated with 100 nM GLP-1, semaglutide (Sema), SRB106, SRB107 or vehicle (Veh) for 10 min. Scale bars are 6 or 8 μ m, as indicated. (B) Representative images and quantification of GLP-1R endosomal puncta formation in Flp-In T-REx-293-SNAP_{GLP-1R}-SmBiT cells treated with 100 nM agonist, $n = 5$, scale bar: 10 μ m. (C) Representative images ($n = 2$) of Flp-In T-REx-293-SNAP_{GLP-1R}-SmBiT cells surface labelled with BG-SS-649, treated with agonist for 30 min, and imaged pre- and 3 min post-Mesna treatment; scale bar: 25 μ m. (D) Agonist-induced GLP-1R internalization in Flp-In T-REx-293-SNAP_{GLP-1R}-SmBiT cells assessed using Mesna assay, $n = 5$, 30 min stimulation, three-parameter fits shown. (E) Tetramethylrhodamine (TMR) agonist distribution in wild-type mouse islets treated for 20 min with 100 nM agonist, $n = 2$. Numerical data represented as mean $\pm$ SEM.

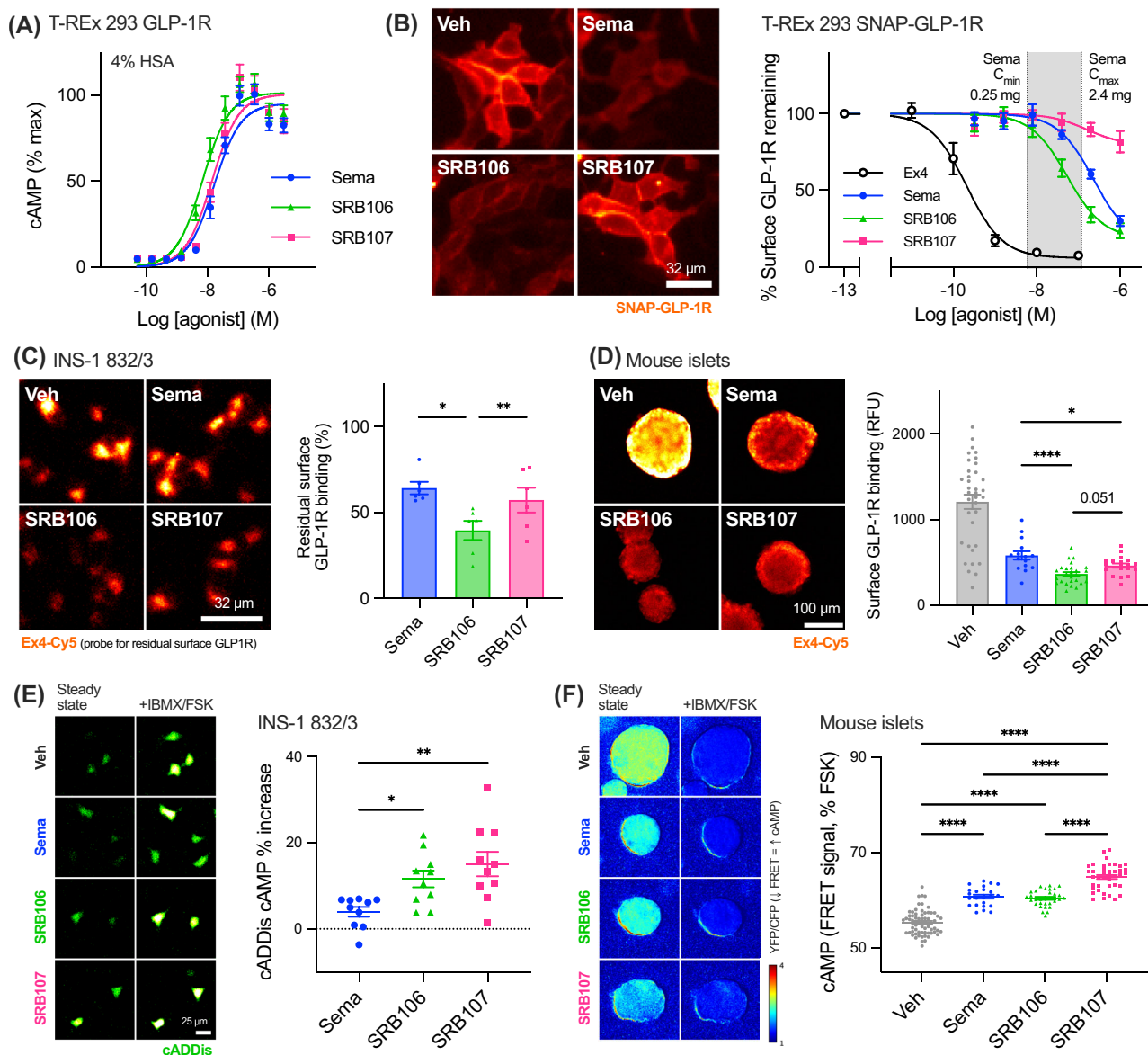


FIGURE 3 Responses in the presence of physiological albumin levels. (A) cAMP responses in Flp-In T-REx-293-GLP-1R (untagged) cells, 30 min stimulation with semaglutide (Sema), SRB106 or SRB107 in the presence of 4% human serum albumin (HSA), $n = 8$. (B) Effect of 12-h agonist treatment on surface GLP-1R in Flp-In T-REx-293-SNAP_{GLP-1R}-SmBiT cells, experiment conducted in normal growth media (10% fetal bovine serum) supplemented with 4% HSA, $n = 6$. Shaded area represents the C_{min} for low dose (0.25 mg) to the C_{max} for high dose (2.4 mg) semaglutide from phase 3 data. Representative images are shown (scale bar: 32 μ m). (C) Residual surface GLP-1R in INS-1832/3 cells after 12-h 100 nM agonist or vehicle (Veh) treatment in 10% fetal bovine serum $\pm$ 4% HSA, $n = 6$. Representative images are shown (scale bar: 32 μ m). Statistical comparisons by one-way repeated measures ANOVA with Tukey's test. (D) As for (C) but using intact wild-type mouse islets, with statistical comparisons by one-way ANOVA with Tukey's test for agonist conditions only. Scale bar: 100 μ m. (E) Representative images and quantification of steady-state cyclic AMP (cAMP) levels in INS-1832/3 cells transduced with cADDIS and treated for 12 h with 100 nM agonist in the presence of 4% HSA, $n = 10$. Statistical comparisons by one-way repeated measures ANOVA with Tukey's test. (F) As for (E) but in intact islets from CAMPER mice. All data represented as mean $\pm$ SEM. * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$ by indicated test.

conditions (Figure 4G). In diet-induced obese (DIO) mice, the same pharmacodynamic pattern was recapitulated, with 1.5 nmol/kg SRB107 but not SRB106 leading to improved glucose tolerance 72 h after dosing, and no clear difference in food intake or body weight effects between agonists at these doses (Figure 4H–J).

Finally, we performed a 28-day chronic administration study of SRB107 versus semaglutide in DIO mice, with both ligands

dose-escalated from 1 to 5 nmol/kg over 19 days via daily subcutaneous injection. Notably, anorectic and weight loss effects of each agent were similar for the first 96 h but diverged thereafter, with SRB107 leading to greater food intake inhibition over time and an additional 10% body weight loss compared with semaglutide (Figure 4K, L).

These findings highlight how the anti-hyperglycaemic effects of SRB107 are longer lasting than both semaglutide and

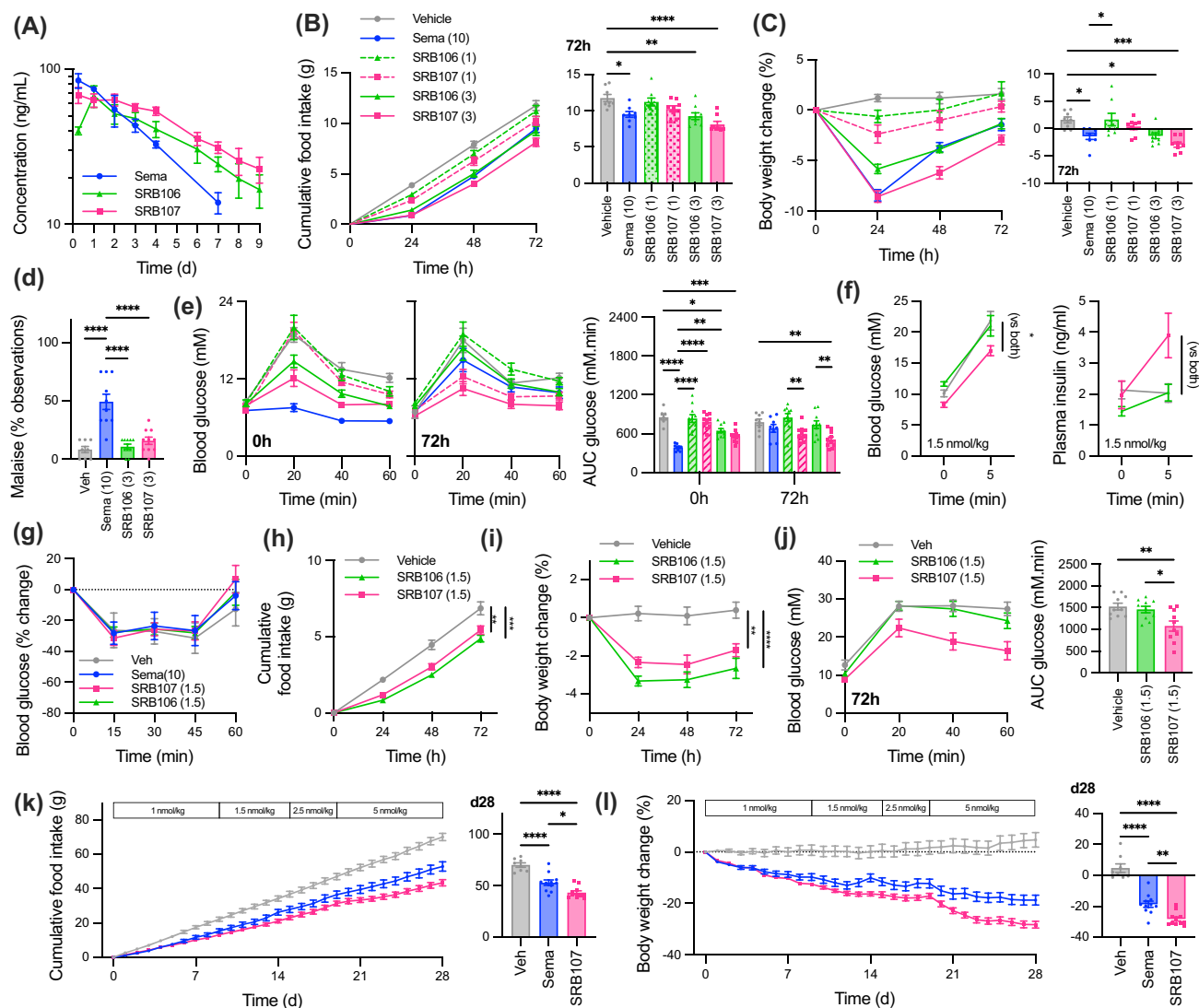


FIGURE 4 SRB107 has long-lasting anti-hyperglycaemic effects in vivo. (A) Pharmacokinetics of semaglutide (Sema; $n = 2$), SRB106 ($n = 3$) and SRB107 ($n = 3$) in pigs. (B) Food intake effects of vehicle (Veh; $n = 8$), 10 nmol/kg semaglutide ($n = 8$), and 1 and 3 nmol/kg SRB106 ($n = 8$ and 9) and SRB107 ($n = 8$ and 8), following a single intraperitoneal (IP) injection in lean mice. (C) Corresponding change in body weight 1 h after IP administration of vehicle ($n = 8$), 10 nmol/kg semaglutide ($n = 10$), and 3 nmol/kg of SRB106 ($n = 10$) and SRB107 ($n = 10$) in lean mice. (D) Acute anti-hyperglycaemic effects of vehicle ($n = 8$), 10 nmol/kg semaglutide ($n = 9$) and 1 and 3 nmol/kg SRB106 ($n = 10$ and 10) and SRB107 ($n = 10$ and 10) during intraperitoneal glucose tolerance tests in lean mice performed 0 (0 h) and 72 h (72 h) after dosing. (E) Glucose-stimulated insulin secretion in lean mice 72 h after IP administration of vehicle ($n = 6$) or 1.5 nmol/kg SRB106 ($n = 7$) or SRB107 ($n = 7$). (F) Insulin tolerance test performed in lean mice 72 h after IP administration of vehicle ($n = 5$), 10 nmol/kg semaglutide ($n = 5$) or 1.5 nmol/kg SRB106 ($n = 7$) or SRB107 ($n = 7$). (G) Food intake effects of vehicle, 1.5 nmol/kg SRB106 and SRB107 following a single IP injection in diet-induced obese (DIO) mice ($n = 15$ /group). (H) Corresponding change in body weight from baseline (%) to graph (H). (I) Acute anti-hyperglycaemic effects of vehicle and 1.5 nmol/kg SRB106 and SRB107 72 h after IP peptide administration in DIO mice ($n = 10$ /group). (J) Chronic food intake effects of vehicle ($n = 8$), SRB107 ($n = 11$) and semaglutide ($n = 11$) following daily SC injection in DIO mice. Peptide dose escalation as indicated. (L) Corresponding change in body weight from baseline (%) to graph (K). All data represented as mean $\pm$ SEM. * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$. For clarity, in some cases not every statistically significant comparison is marked; details and full results of statistical testing are reported in Table S1.

SRB106. While the former could be explained by a longer plasma half-life, this does not apply when comparing SRB106 and SRB107. We conclude therefore that the complete abolition of β -arrestin-2 recruitment with SRB107 probably allows it to retain signalling capacity and metabolic effectiveness over an extended period.

3.5 | Biased glucagon-like peptide-1 receptor agonist signalling is differentially affected by glucagon-like peptide-1 receptor coding variants

Finally, we investigated the potential for *GLP1R* coding variation to influence signalling properties of semaglutide and SRB107. Using

exome sequencing data from the gnomAD dataset³² we identified single nucleotide coding *GLP1R* variants with an allele frequency of at least 1×10^{-3} in one or more of the genetic ancestral groups described (Figure 5A, B) and generated isogenic stable cell lines expressing each missense variant using the Flp-In system.³³ Mutations from the signal peptide region (residues 1-23) were not included as the GLP-1R signal peptide in this system has been replaced with an N-terminal SNAP-tag.

We used the ' $\Delta\Delta\log(\max/EC_{50})$ ' approach to define the impact of each variant on acute cAMP signalling responses to semaglutide and SRB107, with the high affinity full agonist exendin-4 used as a reference ligand to reveal variant-specific differences in signal transduction.³⁴ Note

that this approach, rather than ascribing overall gain- or loss-of-function to each variant, highlights differences in their ligand 'preference'. For example, compared with exendin-4, SRB107 is a low potency ligand at wild-type GLP-1R, but this loss of potency is somewhat mitigated by the D344E variant (Figure 5C). Across our panel of variants, we observed both disproportionate enhancement and disproportionate loss of test ligand responses; for example, the common G168S variant showed relatively enhanced responses to semaglutide and SRB107 compared with exendin-4, as did the low frequency A316T and D344E variants (Figure 5D). Note that in this analysis, statistical significance is inferred when the 95% confidence intervals shown do not cross zero. Re-analysis of the same data suggests that

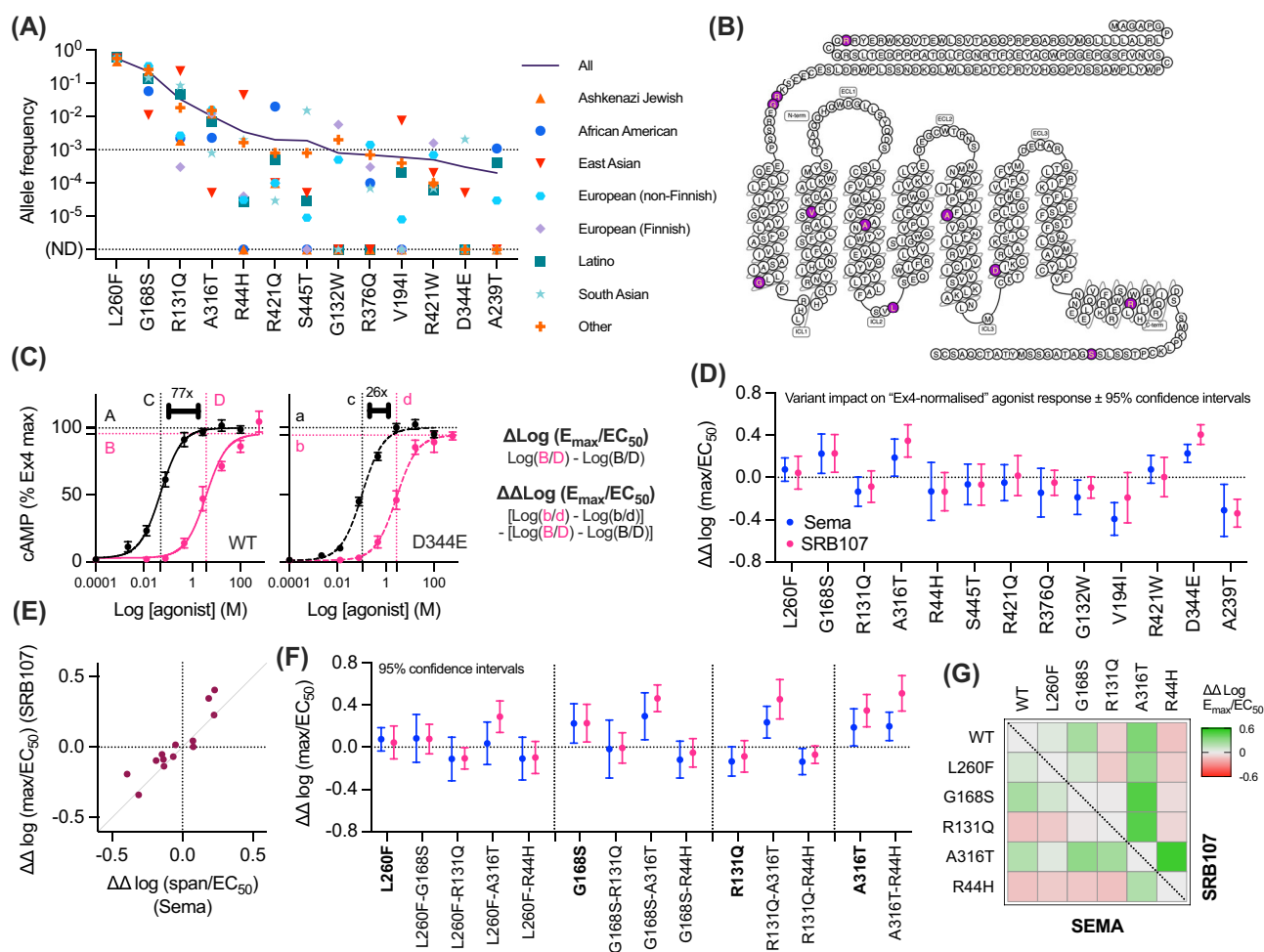


FIGURE 5 Effect of *GLP1R* missense coding variants on signalling responses. (A) Population prevalence of indicated *GLP1R* missense variants in the gnomAD dataset, with breakdown according to recorded genetic ancestry. (B) Snake plot from *gpcrdb* highlighting the position of evaluated *GLP1R* variants. (C) Pooled cAMP response to exendin-4 (Ex4) and SRB107 in wild-type and D344E Flp-In T-REx-293-SNAP-GLP-1R-SmBiT cells, $n = 8$, with mean $\pm$ SEM shown. Variant-specific potency ratios for SRB107 relative to Ex4 are shown on each graph, and the determination of $\Delta\Delta\log(E_{\max}/EC_{50})$ is explained. (D) Semaglutide (Sema/SEMA) and SRB107 responses for single missense *GLP1R* variants, after normalization to exendin-4 for each variant using the $\Delta\Delta\log(E_{\max}/EC_{50})$ approach, $n = 8$ for each variant. Data are represented as mean $\pm$ 95% confidence intervals, meaning that statistically significant effects are inferred when the confidence intervals do not cross zero. (E) Relationship between $\Delta\Delta\log(E_{\max}/EC_{50})$ estimates for SRB107 and semaglutide for variants shown in (D). (F) As for (D) but for double missense *GLP1R* variants; the relevant single missense variant data are the same as in (D). (G) Heatmap representation of data from (F), showing how double missense variant responses tend to mimic those of the individual missense variants included. Data for semaglutide are on the bottom left, and data for SRB107 are at the top right.

for A316T and D344E, this shift in ligand preference represents 'rescue' by semaglutide and SRB107 of a diminished exendin-4 response (Figure S4A). Contrasting with this, semaglutide and SRB107 responses with the V194I and A239T variants were disproportionately reduced, failing to rescue an already reduced response to exendin-4 (Figure 5D, Figure S4A). Overall, these differences were relatively modest, with the largest shifts in SRB107 preference observed with the D344E and A239T variants, amounting to a 5.5-fold selectivity difference (Figure 5D). Variant effects tended to be similar for SRB107 and semaglutide (Figure 5E).

We also performed experiments to determine the impact of more than one natural *GLP1R* variant being simultaneously present in the same receptor by generating 'double mutants' for the commonest *GLP1R* variants (Figure 5F, G, Figure S4B). Here, the effect of each additional mutation tended to imitate that of the same mutation present in isolation; for example, SRB107 signalling was enhanced by the presence of the A316T mutation irrespective of the presence of other coding variants.

These experiments show how some of the commoner *GLP1R* variants lead to ligand-specific changes in cAMP signalling.

4 | DISCUSSION

GLP-1R agonists are widely prescribed for T2D and obesity.³⁵ The current generation of GLP-1R agonists used clinically, including semaglutide, show greater therapeutic efficacy with respect to weight loss than earlier iterations such as exenatide and liraglutide.^{36,37} Nevertheless, there remains an intense interest in the development of novel GLP-1R agonists for use either as monotherapy or as a component of multi-PCR-targeting strategies.³⁸ A more effective GLP-1R agonist might have a longer half-life to allow less frequent dosing, be more potent to allow for lower doses and manufacturing costs, or be more tolerable to increase the therapeutic window and achievable therapeutic efficacy.

Biased signalling is one potential route via which the pharmacological profile of GLP-1R agonists could be improved. Promising results have been obtained in several preclinical studies,³⁻⁸ and the impressive performance in the phase 3 SURPASS-2 study of tirzepatide,³⁹ developed as a GLP-1R/GIPR co-agonist but also showing a GLP-1R signalling profile similar to several G protein-selective GLP-1R agonists,¹⁰ places the concept of biased agonism centre-stage. However, tirzepatide and other N-terminally modified G protein-biased GLP-1R agonists typically show reduced binding affinity,^{9,11} probably because of the important role of the peptide N-terminus in orthosteric binding to the receptor.⁴⁰ Here we have generated two novel ligands with similar acute cAMP signalling potency to semaglutide, but with reduced or absent recruitment of β -arrestin-2. Preserved acute signalling potency in the context of reduced desensitization tendency is predicted to be therapeutically advantageous.

β -arrestins have a canonical role in GPCR desensitization, but they may play a role in orchestrating G protein-independent

signalling events. GLP-1-regulated insulin release has been linked to β -arrestin-1 recruitment,¹² raising the possibility that abolishing this response could have undesirable consequences. However, β -cell-specific conditional knockout studies of β -arrestin-1 and -2 in mice showed no acute insulin secretory deficit in response to GLP-1R agonists,^{41,42} although we recently found that loss of β -arrestin-2 may lead to reduced GLP-1R agonist-induced insulin secretion in female mice and DIO male mice, omitted from earlier studies.⁴³ With this in mind, we aimed to test whether partial or complete loss of GLP-1R agonist-induced β -arrestin recruitment is optimal for enhancing insulin secretion using SRB106 and SRB107. It was clear that the greater avoidance of desensitization with SRB107 obviates any possible role for β -arrestin recruitment in regulating insulin secretion, both in vitro and in vivo. It seems plausible that studies in which β -arrestins are genetically depleted, even using inducible gene knockout strategies, can result in complex effects on islet function, which obfuscate clear roles in GLP-1R signal transduction. More rapid methods to inhibit or deplete β -arrestins, e.g. pharmacological inhibitors or rapid degradation approaches such as Trim-Away,⁴⁴ are needed to corroborate results from biased agonist studies to resolve important questions regarding the role of β -arrestins.

The mechanism for improved metabolic effects of G protein-biased GLP-1R agonists is presumed to revolve around reduced desensitization and/or avoiding depletion of surface GLP-1R. We aimed to test whether clinically relevant pharmacological concentrations of acylated GLP-1R agonists, in the presence of physiological albumin levels, are sufficient to induce the extensive degree of GLP-1R internalization needed to diminish signalling responses purely via restricting surface receptor availability. While acknowledging that the in vitro systems used carry their own limitations, we conclude that measurable GLP-1R loss can occur at plasma concentrations that are readily achieved during at standard clinical doses, and that this is reduced with SRB107 when compared with agonists with greater inherent GLP-1R endocytic propensity. On the other hand, even with the full agonist semaglutide, GLP-1R disappearance under these conditions was far from complete, and it is unclear whether this supports a lack of agonist-mediated GLP-1R downregulation as a primary driver of the distinct biased agonist effects. If not via avoiding extensive GLP-1R downregulation, how could biased GLP-1R agonists achieve longer lasting biological effects? Another plausible explanation is that individual GLP-1Rs occupied by G protein-biased versus non-biased agonist molecules can avoid signal termination through reduced tendency to recruit β -arrestin, as suggested from differences in coupling to mini-G versus β -arrestin-2 from our initial pharmacological characterization. This advantage should apply irrespective of fractional receptor occupancy, i.e. across a full concentration range, although the concomitant reduced efficacy for G protein coupling also needs to be considered.

The rapid expansion of next-generation sequencing technology has highlighted extensive genomic coding variation in drug targets.¹⁷ Current GLP-1R agonists are effective for many patients, but a significant minority fail to achieve treatment targets.⁴⁵ Variation in the *GLP1R* coding sequence is one possible explanation, which may be

particularly relevant for biased GLP-1R agonists. Therefore, we tested for differential effects of missense coding variants on cAMP response to semaglutide and SRB107 compared with exendin-4, identifying three variants (G168S, A316T and D344E) with relatively enhanced acute responses to both ligands, and two with relatively decreased acute responses (A239T and V194I). Enhanced acute signalling responses to tirzepatide, also a biased GLP-1R agonist, with the G168S and A316T variants have been reported elsewhere.⁴⁶ These observations implicate *GLP1R* coding variation as one source of heterogeneity in GLP-1R agonist treatment responses. However, because our data showed the effects of each variant on semaglutide and SRB107 were correlated, we cannot conclude at this point that biased ligands per se will carry a specific therapeutic advantage for certain genotypes. Moreover, current clinical data relevant to these phenomena are conflicting. For example, the G168S variant is associated with a greater suppression of glucose excursion after a mixed meal but a reduced response to DPP-4 inhibitor treatment.¹⁶ Two small studies reported greater response to GLP-1R agonist treatment with the G168S variant,^{47,48} but a recent meta-analysis found G168S results in a smaller HbA1c reduction.⁴⁹ Analysis of the semaglutide phase 3 data highlighted inter-individual pharmacokinetic parameters as the key driver of treatment response variability, implying a more limited role for pharmacogenomic differences.⁵⁰ If pharmacogenomic datasets from large GLP-1R agonist trials become available, it will be informative to compare whether variant effects are modulated by the ligand tested. In addition, our analysis has focused on observing whether variant effects differ according to the agonist used; further work should explore the structural and pharmacological mechanisms underlying these differences.

Concurrent targeting of more than one receptor is now a major focus for drug development. Within this field, the GLP-1R/GIPR co-agonist tirzepatide is the most advanced, with highly promising results also seen with the GLP-1R/GIPR/GCGR triple agonist retatrutide.⁵¹ Two possible drawbacks with the unimolecular dual agonist approach are that (a) the activation profile for each receptor may be sub-optimal because of conflicting requirements for two targets, and (b) the relative contribution of each receptor component cannot be fine-tuned for individual patients. SRB107 shares a GLP-1R pharmacological profile with tirzepatide, albeit with better potency, thereby providing a platform for combined administration alongside additional mono-agonist ligands for other receptors; in this multi-molecular model, dose ratios can be adjusted to accentuate certain effects depending on the clinical need. SRB107 should also be useful for delineating the relative contributions of biased GLP-1R agonism versus GIPR co-agonism to the effects of tirzepatide, as most studies have used non-biased semaglutide as the comparator GLP-1R mono-agonist for this purpose.^{11,52}

With our results in mind, the obvious additional question is whether therapeutically beneficial effects of biased agonists will also be observed in humans. The fact that loss-of-function variants in β -arrestin-1 are associated with greater anti-hyperglycaemic responses to GLP-1R agonists in humans provide initial genetic evidence that this may be the case.⁵³

ACKNOWLEDGMENTS

The Section of Endocrinology and Investigative Medicine is funded by grants from the MRC, NIHR and is supported by the NIHR Biomedical Research Centre Funding Scheme and the NIHR/Imperial Clinical Research Facility. AT and LEE acknowledge funding by Diabetes UK (19/0006094) and the MRC (MR/R010676/1). BJ has received funding from Diabetes UK (20/0006307), the MRC (MR/R010676/1), the IPPRF scheme and North West London Pathology.

CONFLICT OF INTEREST STATEMENT

CEH, TT, JM and SRB are employees and/or shareholders in Zihipp Ltd, an Imperial College spin-out company developing analogues of gut hormones for treatment of obesity. AT and LEE have received funding from Diabetes UK (19/0006094) and the MRC (MR/R010676/1). BJ has received funding from Diabetes UK (20/0006307), the MRC (MR/R010676/1), the IPPRF scheme and North West London Pathology. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15288>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Charlotte E. Hinds  <https://orcid.org/0000-0002-7414-9495>

Alejandra Tomas  <https://orcid.org/0000-0002-2290-8453>

Tricia Tan  <https://orcid.org/0000-0001-5873-3432>

James Minnion  <https://orcid.org/0000-0002-4987-1224>

Ben Jones  <https://orcid.org/0000-0003-0461-2584>

REFERENCES

- Müller TD, Finan B, Bloom SR, et al. Glucagon-like peptide 1 (GLP-1). *Mol Metab*. 2019;30:72-130.
- Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes – state-of-the-art. *Mol Metab*. 2020; 101102. doi:10.1016/j.molmet.2020.101102
- Zhang H, Sturchler E, Zhu J, et al. Autocrine selection of a GLP-1R G-protein biased agonist with potent antidiabetic effects. *Nat Commun*. 2015;6:8918.
- Jones B, Buenaventura T, Kanda N, et al. Targeting GLP-1 receptor trafficking to improve agonist efficacy. *Nat Commun*. 2018;9:1602.
- Fremaux J, Venin C, Mauran L, et al. Ureidopeptide GLP-1 analogues with prolonged activity in vivo via signal bias and altered receptor trafficking. *Chem Sci*. 2019;10:9872-9879.
- Fang Z, Chen S, Manchanda Y, et al. Ligand-specific factors influencing GLP-1 receptor post-endocytic trafficking and degradation in pancreatic Beta cells. *Int J Mol Sci*. 2020;21:8404.
- Fang Z, Chen S, Pickford P, et al. The influence of peptide context on signaling and trafficking of glucagon-like Peptide-1 receptor biased agonists. *ACS Pharmacol Transl Sci*. 2020;3:345-360.

8. Marzook A, Chen S, Pickford P, et al. Evaluation of efficacy-versus affinity-driven agonism with biased GLP-1R ligands P5 and exendin-F1. *Biochem Pharmacol*. 2021;114:656. doi:10.1016/j.bcp.2021.114656
9. Lucey M, Pickford P, Bitsi S, et al. Disconnect between signalling potency and in vivo efficacy of pharmacokinetically optimised biased glucagon-like peptide-1 receptor agonists. *Mol Metab*. 2020;37:100991.
10. Willard FS, Douros JD, Gabe MBN, et al. Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist. *JCI Insight*. 2020;5:1202.
11. Coskun T, Sloop KW, Loghin C, et al. LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: from discovery to clinical proof of concept. *Mol Metab*. 2018;18:3-14.
12. Sonoda N, Imamura T, Yoshizaki T, et al. Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. *Proc Natl Acad Sci U S A*. 2008;105:6614-6619.
13. Wessel J, Chu AY, Willems SM, et al. Low-frequency and rare exome chip variants associate with fasting glucose and type 2 diabetes susceptibility. *Nat Commun*. 2015;6:5897.
14. Scott RA, Freitag DF, Li L, et al. A genomic approach to therapeutic target validation identifies a glucose-lowering GLP1R variant protective for coronary heart disease. *Sci Transl Med*. 2016;8:341ra76-341ra76.
15. Suzuki K, Akiyama M, Ishigaki K, et al. Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. *Nat Genet*. 2019;51:379-386.
16. Mashayekhi M, Wilson JR, Jafarian-Kerman S, et al. Association of a glucagon-like peptide-1 receptor gene variant with glucose response to a mixed meal. *Diabetes Obes Metab*. 2021;23:281-286.
17. Hauser AS, Chavali S, Masuho I, et al. Pharmacogenomics of GPCR drug targets. *Cell*. 2017;172:41-54.e19. doi:10.1016/j.cell.2017.11.033
18. Fortin J-P, Schroeder JC, Zhu Y, Beinborn M, Kopin AS. Pharmacological characterization of human incretin receptor missense variants. *J Pharmacol Exp Ther*. 2010;332:274-280.
19. Koole C, Wootten D, Simms J, et al. Polymorphism and ligand dependent changes in human glucagon-like peptide-1 receptor (GLP-1R) function: allosteric rescue of loss of function mutation. *Mol Pharmacol*. 2011;80:486-497.
20. Rathmann W, Bongaerts B. Pharmacogenetics of novel glucose-lowering drugs. *Diabetologia*. 2021;64:1201-1212.
21. Lucey M, Ashik T, Marzook A, et al. Acylation of the incretin peptide exendin-4 directly impacts GLP-1 receptor signalling and trafficking. *Mol Pharmacol*. 2021;100:319-334. doi:10.1124/molpharm.121.000270
22. Wan Q, Okashah N, Inoue A, et al. Mini G protein probes for active G protein-coupled receptors (GPCRs) in live cells. *J Biol Chem*. 2018;293:7466-7473.
23. Kenakin T, Watson C, Muniz-Medina V, Christopoulos A, Novick S. A simple method for quantifying functional selectivity and agonist bias. *ACS Chem Neurosci*. 2012;3:193-203.
24. Tewson PH, Martinka S, Shaner NC, Hughes TE, Quinn AM. New DAG and cAMP sensors optimized for live-cell assays in automated laboratories. *J Biomol Screen*. 2015;21:298-305.
25. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB. Isolation of INS-1-derived cell lines with robust ATP-sensitive K⁺ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes*. 2000;49:424-430.
26. Szczerbinska I, Tessitore A, Hansson LK, et al. Large-scale functional genomics screen to identify modulators of human β -cell insulin secretion. *Biomed*. 2022;10:103.
27. Benazra M, Lecomte MJ, Colace C, et al. A human beta cell line with drug inducible excision of immortalizing transgenes. *Mol Metab*. 2015;4:916-925.
28. Lau J, Bloch P, Schäffer L, et al. Discovery of the once-weekly glucagon-like Peptide-1 (GLP-1) analogue Semaglutide. *J Med Chem*. 2015;58:7370-7380.
29. Agency EM. Wegovy Assessment Report. https://www.ema.europa.eu/en/documents/assessment-report/wegovy-epar-public-assessment-report_en.pdf 2021.
30. Nestor JJ, Parkes D, Feigh M, Suschak JJ, Harris MS. Effects of ALT-801, a GLP-1 and glucagon receptor dual agonist, in a translational mouse model of non-alcoholic steatohepatitis. *Sci Rep-Uk*. 2022;12:6666.
31. Muntean BS, Zucca S, MacMullen CM, et al. Interrogating the spatio-temporal landscape of Neuromodulatory GPCR signaling by real-time imaging of cAMP in intact neurons and circuits. *Cell Rep*. 2018;22:255-268.
32. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434-443.
33. Ward RJ, Alvarez-Curto E, Milligan G. Receptor signal transduction protocols, Third Edition. *Methods Mol Biol*. 2011;746:21-37.
34. Kenakin T. A scale of Agonism and allosteric modulation for assessment of selectivity, bias, and receptor mutation. *Mol Pharmacol*. 2017;92:414-424.
35. Adhikari R, Jha K, Dardari Z, et al. National Trends in use of sodium-glucose Cotransporter-2 inhibitors and glucagon-like Peptide-1 receptor agonists by cardiologists and other specialties, 2015 to 2020. *J Am Hear Assoc Cardiovasc Cerebrovasc Dis*. 2021;11:e023811.
36. Ahmann AJ, Capehorn M, Charpentier G, et al. Efficacy and safety of once-weekly Semaglutide versus Exenatide ER in subjects with type 2 diabetes (SUSTAIN 3): A 56-week, open-label, randomized clinical trial. *Diabetes Care*. 2017;41:258-266.
37. Rubino DM, Greenway FL, Khalid U, et al. Effect of weekly subcutaneous Semaglutide vs daily Liraglutide on body weight in adults with overweight or obesity without diabetes. *JAMA*. 2022;327:138-150.
38. Finan B, Yang B, Ottaway N, et al. A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. *Nat Med*. 2014;21:27-36.
39. Frías JP, Davies MJ, Rosenstock J, et al. Tirzepatide versus Semaglutide once weekly in patients with type 2 diabetes. *New Engl J Med*. 2021;385:503-515. doi:10.1056/nejmoa2107519
40. Al-Sabah S, Donnelly D. A model for receptor-peptide binding at the glucagon-like peptide-1 (GLP-1) receptor through the analysis of truncated ligands and receptors. *Br J Pharmacol*. 2003;140:339-346.
41. Zhu L, Almaça J, Dadi PK, et al. β -Arrestin-2 is an essential regulator of pancreatic β -cell function under physiological and pathophysiological conditions. *Nat Commun*. 2017;8:14295.
42. Barella LF, Rossi M, Zhu L, et al. β -Cell-intrinsic β -arrestin 1 signaling enhances sulfonylurea-induced insulin secretion. *J Clin Invest*. 2019;129:3732-3737.
43. Bitsi S, Manchanda Y, ElEid L, et al. Divergent acute versus prolonged pharmacological GLP-1R responses in adult β cell-specific β -arrestin 2 knockout mice. *Sci Adv*. 2023;9(8):eadf7737.
44. Clift D, So C, McEwan WA, James LC, Schuh M. Acute and rapid degradation of endogenous proteins by trim-away. *Nat Protoc*. 2018;13:2149-2175.
45. Pratley RE, Aroda VR, Lingvay I, et al. Semaglutide versus dulaglutide once weekly in patients with type 2 diabetes (SUSTAIN 7): a randomised, open-label, phase 3b trial. *Lancet Diabetes Endocrinol*. 2018;6:275-286.
46. Lagou V, Jiang L, Ulrich A, et al. GWAS of random glucose in 476,326 individuals provide insights into diabetes pathophysiology, complications and treatment stratification. *Nat Genet*. 2023;55(9):1448-1461. doi:10.1038/s41588-023-01462-3

47. de Luis DA, Soto GD, Izaola O, Romero E. Evaluation of weight loss and metabolic changes in diabetic patients treated with liraglutide, effect of RS 6923761 gene variant of glucagon-like peptide 1 receptor. *J Diabetes Complications*. 2015;29:595-598.
48. Maselli D, Atieh J, Clark MM, et al. Effects of liraglutide on gastrointestinal functions and weight in obesity: A randomized clinical and pharmacogenomic trial. *Obesity*. 2022;30:1608-1620.
49. Dawed AY, Mari A, Brown A, et al. Pharmacogenomics of GLP-1 receptor agonists: A genome wide analysis of observational data and large randomized controlled trials. *Lancet Diabetes Endocrinol*. 2023; 11(1):33-41. doi:[10.1016/S2213-8587\(22\)00340-0](https://doi.org/10.1016/S2213-8587(22)00340-0)
50. Overgaard RV, Hertz CL, Ingwersen SH, Navarria A, Drucker DJ. Levels of circulating semaglutide determine reductions in HbA1c and body weight in people with type 2 diabetes. *Cell Rep Med*. 2021;2: 100387.
51. Jastreboff AM, Kaplan LM, Frías JP, et al. Triple-hormone-receptor agonist Retatrutide for obesity — A phase 2 trial. *N Engl J Med*. 2023; 389:514-526. doi:[10.1056/nejmoa2301972](https://doi.org/10.1056/nejmoa2301972)
52. Urva S, Coskun T, Loghin C, et al. The novel dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 (GLP-1) receptor agonist tirzepatide transiently delays gastric emptying similarly to selective long-acting GLP-1 receptor agonists. *Diabetes Obes Metab*. 2020;22:1886-1891.
53. Dawed AY, Mari A, Brown A, et al. Pharmacogenomics of GLP-1 receptor agonists: a genome-wide analysis of observational data and large randomised controlled trials. *Lancet Diabetes Endocrinol*. 2023; 11:33-41.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hinds CE, Peace E, Chen S, et al. Abolishing β -arrestin recruitment is necessary for the full metabolic benefits of G protein-biased glucagon-like peptide-1 receptor agonists. *Diabetes Obes Metab*. 2024;26(1):65-77. doi:[10.1111/dom.15288](https://doi.org/10.1111/dom.15288)