

# Supplementary Information

## **Establishing a relationship between in vitro potency in cell-based assays and clinical efficacious concentrations for approved GLP-1 receptor agonists**

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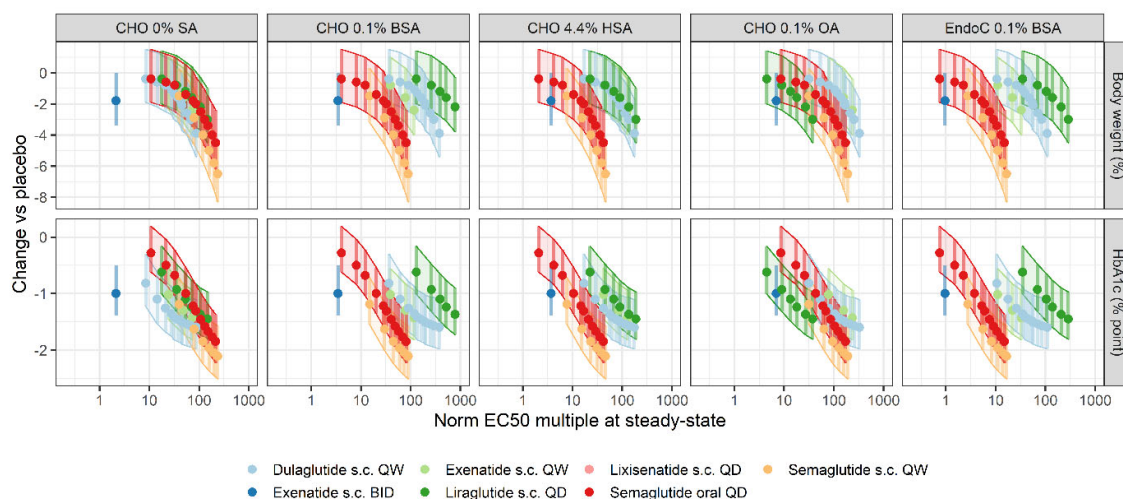
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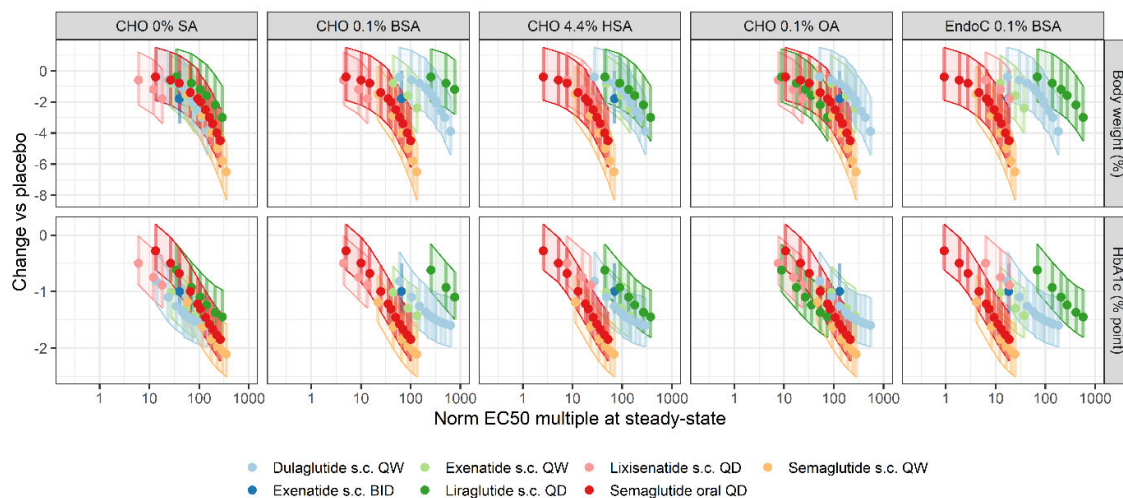
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# Figures



**Figure S1 PK/PD based on trough plasma exposure.** Simulated bodyweight (% top) and HbA1c (% point, bottom) change from placebo (comparediabetesdrugs.com; 100 x 100 trials over 52 weeks; median with 90% credible range indicated) vs fold GLP-1R  $EC_{50}$  at trough (Methods). Panels left to right shows multiples derived using the evaluated cell systems (CHO, EndoC) serum albumin conditions (0%, 0.1% BSA, 0.1% OA, 4.4% HSA).



**Figure S2 PK/PD based on maximum plasma exposure.** Simulated bodyweight (% top) and HbA1c (% point, bottom) change from placebo (comparediabetesdrugs.com; 100 x 100 trials over 52 weeks; median with 90% credible range indicated) vs fold GLP-1R  $EC_{50}$  at  $T_{max}$  (Methods). Panels left to right shows multiples derived using the evaluated cell systems (CHO, EndoC) serum albumin conditions (0%, 0.1% BSA, 0.1% OA, 4.4% HSA).

## Tables

**Table S1.** *In vitro* potency ( $EC_{50}$ ) estimated from concentration-response (cAMP) data using a stable CHO cell line and incubated at 0.1, 0.3, 1 and 3% NSB where NSB = BSA (A) or OA (B).

A. NSB = BSA

Compound	$EC_{50}$ (pM) 0.1%	$EC_{50}$ (pM) 0.3%	$EC_{50}$ (pM) 1%	$EC_{50}$ (pM) 3%
Dulaglutide	9.07	10.8	12.1	9.7
Semaglutide	35	75.1	181	286
Liraglutide	3.9	7.59	18.2	58.1
Exenatide	2.7	1.68	1.5	1.58
Lixisenatide	2.77	2.72	4.25	2.25
GLP1 (7-36 amide)	2.14	2.03	3.91	5.11

B. NSB = OA

Compound	$EC_{50}$ (pM) 0.1%	$EC_{50}$ (pM) 0.3%	$EC_{50}$ (pM) 1%	$EC_{50}$ (pM) 3%
Dulaglutide	46.4	34.1	30.7	26
Semaglutide	3.58	19.2	9.43	16.4
Liraglutide	26.9	33.8	136	285
Exenatide	3.19	2.62	3.75	2.33
Lixisenatide	6.25	2.69	3.44	3.46
GLP1 (7-36 amide)	5.44	4.05	8.34	10

**Table S2.** Estimated fraction unbound in human plasma  $f_{unb,p}$  and 0.1% serum albumin  $f_{unb}$  (0.1% SA).

Compound	$f_{unb,p}$ (%)	$f_{unb}$ (0.1% SA) <sup>1</sup>	Reference
Exenatide	100	100	Based on serum shift assay data in Table S1
Dulaglutide	100	100	Based on serum shift assay data in Table S1
Lixisenatide	45	97	[1]
Liraglutide	0.51	18	[2]
Semaglutide	0.36	14	[3]

<sup>1</sup>Estimated from  $f_{unb,p}$  (Methods).

**Table S3.** Pharmacokinetic parameters representing rate of absorption ( $K_a$ ), central clearance ( $CL$ ), central volume ( $V_c$ ), peripheral volume ( $V_p$ ), distribution clearance ( $CL_d$ ), bioavailability ( $F$ ) and lag-time ( $T_{lag}$ ).

Compound	$K_a$ (h <sup>-1</sup> )	$CL$ (L/h)	$V_c$ (L)	$V_p$ (L)	$CL_d$ (L/h)	$F$	$T_{lag}$ (h)	Population	Reference
Dulaglutide	0.00769	0.0593	2.25	3.75	0.0201	0.47 at 1.5 mg	-	T2D	[4]
Semaglutide oral	2.09	0.039	3.7	4.2	0.31	0.0069	-	T2D,obesity	[5]
Semaglutide sc	0.0253	0.039	3.7	4.2	0.31	0.847	-	T2D	[6]
Liraglutide	0.154	0.91	11.2	-	-	0.51	6	T2D	[7]
Lixisenatide	0.317	38.6*	41.2*					T2D	[8]
Exenatide BID	0.734	8.829*	26.2*					T2D	[7]
Exenatide QW	Variable	9.1*	28.2*					T2D	[9]

\*CL/F and V/F

**Table S4.** Average ( $C_{avg}$ ), maximum ( $C_{max}$ ) and trough ( $C_{min}$ ) exposure at steady-state.

Compound	Exposure at steady-state		
	$C_{max}$ (nM)	$C_{avg}$ (nM)	$C_{min}$ (nM)
Dulaglutide 1.5 mg s.c. QW	1.4	1.2	0.83
Exenatide 10 ug s.c. BID	0.024	0.011	0.0015
Exenatide 2 mg s.c. QW	0.07	0.068	0.066
Liraglutide 3 mg s.c. QD	19	15	9.5
Lixisenatide 20 ug s.c. QD	0.02	0.0045	0.000026
Semaglutide 14 mg oral QD	14	12	11
Semaglutide 2.4 mg s.c. QW	86	75	57

**Table S5.** Parameters associated with non-parametric correlation analysis (Spearman) for each endpoint and assay potency. <sup>1</sup>A correlation coefficient rho of +/- 1 indicate perfect correlation and <sup>2</sup>the p-value reflects the probability that the true rho = 0.

PK metric	C <sub>avg</sub>		C <sub>max</sub>		C <sub>min</sub>	
	rho <sup>1</sup>	p value <sup>2</sup>	rho <sup>1</sup>	p value <sup>2</sup>	rho <sup>1</sup>	p value <sup>2</sup>
<b>CHO 0% SA</b>						
HbA1c (% point)	-0.85	$1.5 \times 10^{-12}$	-0.85	$1.7 \times 10^{-12}$	-0.85	$3.7 \times 10^{-12}$
Body weight (%)	-0.87	$1.0 \times 10^{-13}$	-0.89	$6.3 \times 10^{-15}$	-0.88	$6.5 \times 10^{-14}$
<b>CHO 0.1% BSA</b>						
HbA1c (% point)	-0.42	$6.7 \times 10^{-3}$	-0.40	$9.3 \times 10^{-3}$	-0.42	$6.0 \times 10^{-3}$
Body weight (%)	-0.27	$9.2 \times 10^{-2}$	-0.27	$9.3 \times 10^{-2}$	-0.27	$8.8 \times 10^{-2}$
<b>CHO 0.1% OA</b>						
HbA1c (% point)	-0.81	$1.1 \times 10^{-10}$	-0.81	$8.8 \times 10^{-11}$	-0.82	$7.7 \times 10^{-11}$
Body weight (%)	-0.66	$2.8 \times 10^{-6}$	-0.68	$1.0 \times 10^{-6}$	-0.66	$2.2 \times 10^{-6}$
<b>EndoC 0.1% BSA</b>						
HbA1c (% point)	-0.35	$2.5 \times 10^{-2}$	-0.26	$1.0 \times 10^{-1}$	-0.38	$1.5 \times 10^{-2}$
Body weight (%)	-0.20	$2.0 \times 10^{-1}$	-0.17	$3.0 \times 10^{-1}$	-0.21	$1.8 \times 10^{-1}$
<b>CHO 4.4% HSA</b>						
HbA1c (% point)	-0.47	$2.1 \times 10^{-3}$	-0.42	$5.7 \times 10^{-3}$	-0.51	$6.1 \times 10^{-4}$
Body weight (%)	-0.34	$2.8 \times 10^{-2}$	-0.33	$3.7 \times 10^{-2}$	-0.37	$1.7 \times 10^{-2}$

**Table S6.** Parameter point estimates with 90% confidence interval resulting from regression using a linear, power and sigmoid drug effect model to describe relation to weight and HbA1c effect. Linear model:  $y = a \cdot x$ ; Power model  $y = a \cdot xb$ ; Sigmoid model  $y = a + (b - a) \cdot xc/(xc + dc)$ . AICc = corrected Akaike information criterion, rmse = root mean square error. NaN = not a number, indicating ambiguous fit obtained using specified model.

Potency assay	Endpoint	Model	a	b	c	d	rmse	AICc
CHO 0% SA	Body weight	linear	-0.01 (-0.012 to -0.0085)				0.28	-109
		power	-0.46 (-0.55 to -0.38)	0.25 (0.21 to 0.29)			0.086	-205
		sigmoid	0 (NaN to NaN)	-2.5 (NaN to NaN)	0.45 (0.36 to 0.54)	47 (36 to 58)	0.094	-187
	HbA1c	linear	-0.021 (-0.024 to -0.019)				0.38	-82.9
		power	-0.15 (-0.24 to -0.057)	0.62 (0.5 to 0.75)			0.33	-94.9
		sigmoid	0 (NaN to NaN)	-2.5 (NaN to NaN)	0.94 (0.15 to 1.7)	11 (-0.19 to 23)	0.51	-47.7
CHO 0.1% OA	Body weight	linear	-0.0079 (-0.0092 to -0.0066)				0.28	-106
		power	-0.48 (-0.6 to -0.36)	0.22 (0.17 to 0.27)			0.1	-190
		sigmoid	0 (NaN to NaN)	-2.5 (NaN to NaN)	0.41 (0.32 to 0.51)	64 (46 to 82)	0.11	-177
	HbA1c	linear	-0.015 (-0.018 to -0.012)				0.58	-47.7
		power	-0.42 (-0.74 to -0.087)	0.37 (0.21 to 0.53)			0.47	-66.1
		sigmoid	0 (NaN to NaN)	-2.5 (NaN to NaN)	0.93 (0.19 to 1.7)	11 (-1.2 to 24)	0.52	-46.5

## References

1. chmp, *Lyxumia*. 2012, EMA.
2. Ungewiss, J., S. Gericke, and H. Boriss, *Determination of the plasma protein binding of liraglutide using the escalate\* equilibrium shift assay*. Journal of Pharmaceutical Sciences, 2019. **108**(3): p. 1309-1314.
3. CHMP, *Ozempic Assessment report*. 2017, EMA.
4. Geiser, J.S., et al., *Clinical pharmacokinetics of dulaglutide in patients with type 2 diabetes: analyses of data from clinical trials*. Clinical pharmacokinetics, 2016. **55**: p. 625-634.
5. Overgaard, R.V., et al., *Clinical pharmacokinetics of oral semaglutide: analyses of data from clinical pharmacology trials*. Clinical Pharmacokinetics, 2021. **60**: p. 1335-1348.
6. Overgaard, R.V., et al., *Population pharmacokinetics of semaglutide for type 2 diabetes*. Diabetes Therapy, 2019. **10**: p. 649-662.
7. Watson, E., et al., *Population pharmacokinetics of liraglutide, a once-daily human glucagon-like peptide-1 analog, in healthy volunteers and subjects with type 2 diabetes, and comparison to twice-daily exenatide*. J Clin Pharmacol, 2010. **50**(8): p. 886-94.
8. Frank, T., *Population pharmacokinetics of lixisenatide, a once-daily human glucagon-like peptide-1 receptor agonist, in healthy subjects and in patients with type 2 diabetes*. J Pharm Drug Deliv Res, 2013. **2**: p. 1.
9. Trägårdh, M., et al., *Input estimation for extended-release formulations exemplified with exenatide*. Frontiers in Bioengineering and Biotechnology, 2017. **5**: p. 24.