Supplemental Data

GLP-1 receptor blockade reduces stimulated insulin secretion in fasted subjects with low circulating GLP-1. Sarah M. Gray[1,2,3], Andrew L. Hoselton[2,3], Radha Krishna[1,2,3], Cris A. Slentz[2,3], David A. D'Alessio[1,2,3]

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Supplemental Table 1: Characteristics of glucagon assay

Dilution Linearity				Spike Recovery			
Sample	**Dilution**	**Gcg pmol/L**	**% Recovery**	**Sample**	**Dilution**	**Gcg pmol/L**	**% Recovery**
1	Direct	38.22		Pool 1	Expected	46.71	
	1/2dil.	19.89	104%		Measured	44.17	95%
	1/4 dil.	11.46	119%				
2	Direct	53.99		Pool 2	Expected	51.80	
	1/2dil.	19.73	93%		Measured	50.45	97%
	1/4 dil.	13.53	100%				
3	Direct	39.11		Pool 3	Expected	51.45	
	1/2dil.	21.02	107%		Measured	44.93	87%
	1/4 dil.	11.13	113%				
4	Direct	33.85		Pool 4	Expected	50.02	
	1/2dil.	23.70	120%		Measured	49.31	99%
	1/4 dil.	14.11	115%				
				Pool 5	Expected	50.02	
					Measured	54.63	109%

Cross Reactivity					
Cross-Reactant	**Conc.**	**% Cross Reactivity**	**Cross-Reactant**	**Conc.**	**% Cross Reactivity**
GRPP	5 ng/ml	nd	GLP-2	5 ng/ml	nd
A5: OXM (1-17) / (Glucagon 1-17)	5 ng/ml	nd	MPGF-1	5 ng/ml	nd
A7: OXM (19-29) / (Glucagon 19-29)	5 ng/ml	nd	Insulin	5 ng/ml	nd
A9: OXM (23-37)	5 ng/ml	nd	C-peptide	5 ng/ml	nd
A1: OXM (1-37), Ansh stk sol.	5 ng/ml	nd			

Glucagon (19-29) Bachem	5 ng/ml	nd	ProG-null mouse plasma, sample 1	0 ng/ml	nd
Glicentin	5 ng/ml	nd	ProG-null mouse plasma, sample 2	0 ng/ml	nd
B3: GLP-1 (9-36-NH2)	5 ng/ml	nd			
GLP-1 (1-36)	5 ng/ml	nd			

Supplemental Table 2: Characteristics of GLP-1 assay

Dilution Linearity

Sample	Dilution	GLP-1 pmol/L	% Recovery	Sample	Dilution	GLP-1 pmol/L	% Recovery
1	Direct	18.42		6	Direct	22.52	
	1/2dil.	7.36	80%		1/2dil.	10.06	89%
	1/4 dil.	4.01	109%		1/4 dil.	4.84	96%
	1/8 dil.	1.33	66%		1/8 dil.	2.00	83%
2	Direct	35.96		7	Direct	20.63	
	1/2dil.	16.08	89%		1/2dil.	8.99	87%
	1/4 dil.	7.37	92%		1/4 dil.	4.17	93%
	1/8 dil.	3.68	100%		1/8 dil.	1.57	75%
3	Direct	36.23		8	Direct	19.71	
	1/2dil.	15.27	84%		1/2dil.	8.68	88%
	1/4 dil.	7.37	97%		1/4 dil.	3.89	90%
	1/8 dil.	3.65	99%		1/8 dil.	1.08	56%
4	Direct	18.88		9	Direct	33.19	
	1/2dil.	8.35	88%		1/2dil.	14.51	87%
	1/4 dil.	4.37	105%		1/4 dil.	6.97	96%
	1/8 dil.	1.31	60%		1/8 dil.	3.33	95%
5	Direct	25.16		10	Direct	19.21	
	1/2dil.	11.28	90%		1/2dil.	8.72	91%
	1/4 dil.	5.85	103%		1/4 dil.	3.80	87%
	1/8 dil.	2.61	89%		1/8 dil.	0.72	38%

Spike Recovery

Sample		GLP-1 pmol/L	% Recovery
Pool 1	Expected	22.73	
	Measured	20.88	92%
Pool 2	Expected	29.55	
	Measured	27.01	91%
Pool 3	Expected	25.82	
	Measured	24.20	94%

Cross Reactivity

Cross-Reactant	Expected	% Cross reactivity
MPGF-2	0.194 ng/ml	nd
	0.39 ng/ml	nd
	0.781 ng/ml	nd
	1.562 ng/ml	nd
	3.125 ng/ml	nd
	6.25 ng/ml	nd
	12.5 ng/ml	8%
	25 ng/ml	34%
	50 ng/ml	159%
OXM	10 ng/ml	nd
Glucagon	10 ng/ml	nd
Glicentin	10 ng/ml	nd
GLP-2	10 ng/ml	nd
Insulin	10 ng/ml	nd

Supplemental Table 3: Baseline values in nondiabetic subjects

	Drug		**Treatment**		**Time**		***p* values**			
	Sitagliptin	Placebo	Ex-9	Saline	Arg1	Arg2	Drug	Trt	Time	Trt* Time
Insulin pmol/L	108.3 (95.3-123.2)	108.1 (95.1-122.9)	111.2 (97.9-126.4)	105.3 (92.6-119.7)	113.4 (99.7-128.9)	103.3 (90.9-117.4)	0.939	0.0457	0.001	0.012
C-Peptide pmol/L	456.2 (384.0-542.1)	416.3 (350.3-494.5)	448.9 (377.9-533.4)	422.9 (356.0-502.5)	450.1 (378.8-534.7)	422.0 (355.1-501.3)	0.0036	0.0533	0.0372	ns
Glucagon pmol/L	15.1 (13.1-17.4)	14.3 (12.4-16.5)	14.5 (12.5-16.7)	15.0 (13.0-17.2)	14.3 (12.4-16.5)	15.1 (13.1-17.4)	0.422	0.6117	0.4452	0.005
GLP-1 pmol/L	3.6 (2.5-5.1)	3.8 (2.6-5.4)	3.6 (2.5-5.2)	3.7 (2.6-5.4)	3.8 (2.6-5.5)	3.5 (2.5-5.1)	0.5932	0.7485	0.5386	ns

Pairwise comparisons of trt*time interaction

	Ex-9		Saline	
	Arg1	Arg2	Arg1	Arg2
Insulin pmol/L	100.3 (85.7-117.4)	123.3 (105.3-144.4)	128.2 * (109.5-150.0)	86.5 † ‡ (73.9-101.3)
Glucagon pmol/L	17.0 (13.7-21.0)	12.3 (10.1-14.9)	12.1 (10.0-14.7)	18.5 † ‡ (15.0-22.8)

Data are reported as LS mean ± 95% CI. Main effect *p* values are from type 3 tests of fixed effects. Pairwise post-hoc comparisons are reported with *p* from Tukey-Kramer poc-hoc test for multiple comparisons. *$p < 0.05$ between Ex-9/Arg1 and Saline/Arg1; † $p < 0.05$ between Ex-9/Arg2 and Saline/Arg2; ‡ $p < 0.05$ between Saline/Arg1 and Saline/Arg 2; ns, not significant.

Supplemental Table 4: Pairwise comparisons of C-P_{Arg}

C-P_{Arg} (pmol/L)	Sitagliptin		Placebo	
	Ex-9	Saline	Ex-9	Saline
Nondiabetic subjects	320.1 (263.9-388.3)	318.7 (262.7-386.6)	280.0 (230.8-339.7)	371.4 * (306.2-450.6)
Diabetic subjects	243.4 (185.4-319.4)	319.4 † (243.4-419.3)	251.2 (191.5-329.7)	249.2 ‡ (189.5-327.8)

Pairwise post-hoc comparisons are reported with *p* from Tukey-Kramer poc-hoc test for multiple comparisons. * $p = 0.0006$ between placebo/Ex-9 and placebo/saline; † $p = 0.0095$ between sitagliptin/Ex-9 and sitagliptin/saline; ‡ $p = 0.0215$ between placebo/saline and sitagliptin/saline.

Supplemental Table 5: Baseline values in diabetic subjects

	Drug		**Treatment**		**Time**		***p* values**			
	Sitagliptin	Placebo	Ex-9	Saline	Arg1	Arg2	Drug	Trt	Time	Trt* Time
Insulin pmol/L	139.4 (117.2-165.7)	135.3 (113.8-160.9)	135.6 (114.0-161.2)	139.1 (117.0-165.4)	137.9 (116.0-164.0)	136.8 (115.0-162.6)	0.3228	0.4123	0.7888	ns
C-Peptide pmol/L	661.4 (506.3-864.3)	576.2 (441.0-753.0)	598.8 (458.2-782.3)	636.6 (487.3-831.9)	617.6 (472.6-807.0)	617.3 (472.4-806.5)	<0.0001	0.0063	0.9798	ns
Glucagon pmol/L	15.8 (13.6-22.7)	17.6 (12.3-20.4)	17.3 (13.5-22.4)	16.1 (12.4-20.7)	17.0 (13.1-21.9)	16.4 (12.7021.2)	0.0991	0.2198	0.6193	ns
GLP-1 pmol/L	5.0 (3.7-6.7)	6.3 (4.7-8.4)	5.4 (4.0-7.3)	5.8 (4.3-7.7)	5.6 (4.2-7.5)	5.6 (4.2-7.5)	0.0044	0.4063	0.9127	ns

Data are reported as LS mean ± 95% CI. Main effect *p* values are from type 3 tests of fixed effects. ns, not significant.

Supplemental Table 6: Secondary analysis of baseline characteristics, comparing nondiabetic to diabetic subjects.

	Group		**Drug**		**Treatment**		**Time**		***p* values**				
	ND	T2DM	Sita	Placebo	Ex-9	Saline	Arg1	Arg2	Group	Drug	Trt	Time	Trt* Time
Insulin pmol/L	108.2 (94.6-123.8)	140.8 (119.6-165.7)	124.4 (111.8-138.5)	122.5 (109.9-136.4)	124.6 (111.9-138.8)	122.3 (109.8-136.1)	126.8 (113.9-141.3)	120.1 (107.9-133.8)	0.015	0.454	0.366	0.012	0.009
C-peptide pmol/L	435.8 (360.8-526.2)	617.4 (491.5-775.7)	548.1 (472.0-636.6)	490.8 (422.7-569.9)	520.7 (448.3-604.6)	516.7 (445.0-600.0)	527.1 (453.9-612.0)	510.5 (439.6-592.8)	0.022	<0.0001	0.722	0.132	ns
Glucagon pmol/L	49.9 (40.9-60.7)	57.8 (47.0-71.1)	53.2 (45.7-61.8)	54.2 (46.6-63.0)	54.2 (46.6-62.9)	53.2 (45.8-61.8)	53.3 (45.9-62.0)	54.0 (46.4-62.8)	0.3070	0.6831	0.7020	0.7850	ns
GLP-1 pmol/L	12.1 (8.8-16.4)	18.6 (13.4-25.7)	14.0 (11.1-17.7)	16.0 (12.7-20.2)	14.6 (11.6-18.4)	15.3 (12.1-19.3)	15.2 (12.1-19.2)	14.6 (11.6-18.5)	0.0591	0.412	0.4686	0.56	ns

Pairwise comparisons of trt*time interaction

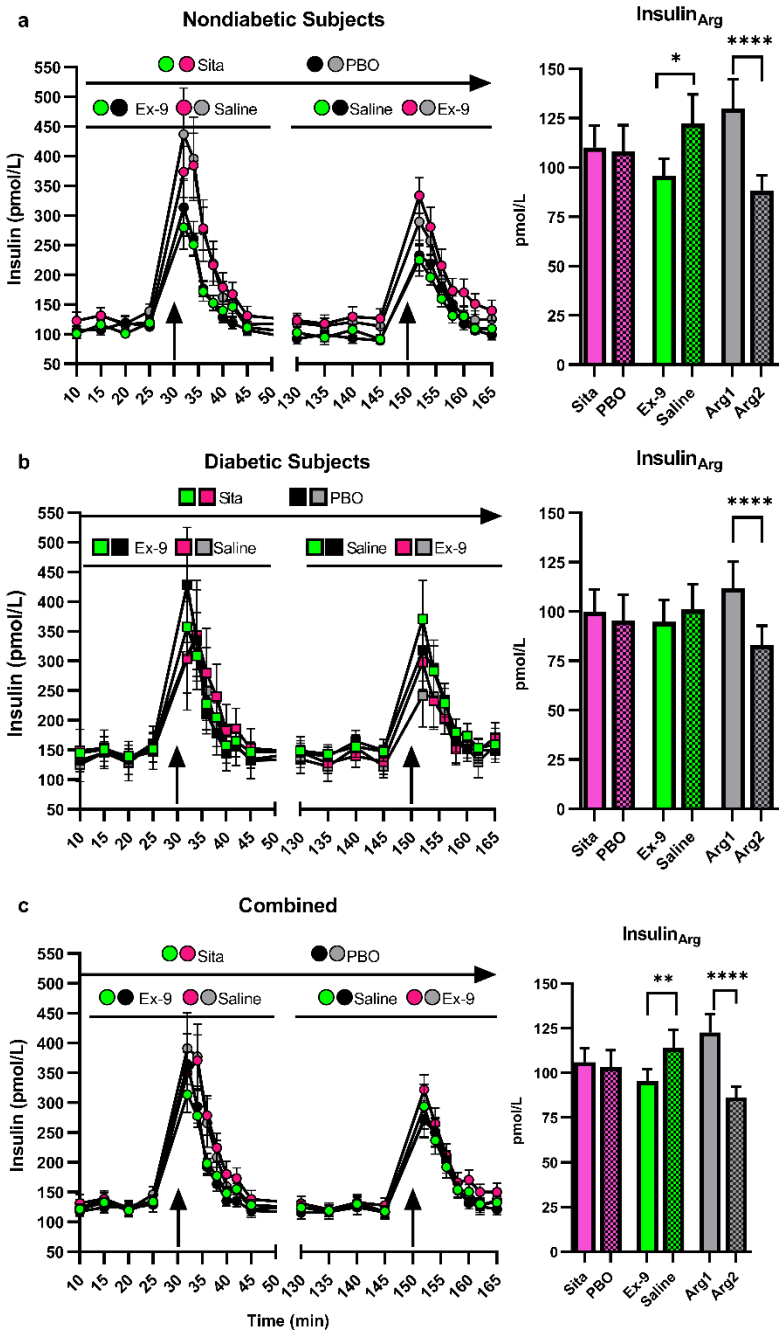
	Ex-9		Saline	
	Arg1	Arg2	Arg1	Arg2
Insulin pmol/L	115.0 (101.0-131.0)	135.0 (117.3-155.5)	139.9 (121.5-161.1)	106.9 * † (93.8-121.6)

Data are reported as LS mean ± 95% CI. Main effect *p* values are from type 3 tests of fixed effects. Pairwise post-hoc comparisons are reported with *p* from Tukey-Kramer poc-hoc test for multiple comparisons. *$p = 0.0294$ between Ex-9/Arg2 and Saline/Arg 2; † $p = 0.009$ between saline/Arg1 and saline/Arg 2; ns, not significant.

Supplemental Table 7: Secondary analysis of arginine-stimulated responses, comparing nondiabetic to diabetic subjects.

	Group		**Drug**		**Treatment**		**Time**		***p* values**			
	ND	T2DM	Sita	Placebo	Ex-9	Saline	Arg1	Arg 2	Group	Drug	Trt	Time
$Insulin_{Arg}$ pmol/L	95.6 (72.4-123.3)	84.8 (59.5-116.4)	91.8 (73.3-113.3)	88.3 (70.3-109.3)	83.5 (66.2-103.7)	97.0 (77.7-119.2)	106.2 (85.6-129.7)	75.7 (59.5-94.7)	0.578	0.466	0.006	<0.0001
$C\text{-}P_{Arg}$ pmol/L	321.0 (266.7-386.3)	265.3 (212.1-332.0)	296.9 (255.4-345.1)	286.8 (246.7-333.4)	272.0 (234.0-316.2)	313.0 (269.2-364.0)	338.6 (291.3-393.5)	251.5 (216.3-292.4)	0.196	0.384	0.001	<0.0001
$Glucagon_{Arg}$ pmol/L	39.4 (30.3-48.6)	48.1 (38.5-57.7)	45.0 (38.1-51.9)	42.5 (35.7-49.4)	44.9 (38.0-51.8)	42.6 (35.7-49.5)	44.1 (37.2-50.9)	43.4 (36.5-50.3)	0.1970	0.1760	0.2110	0.7200
$GLP\text{-}1_{Arg}$ pmol/L	1.5 (1.1-1.9)	1.6 (1.2-2.1)	1.8 (1.4-2.3)	1.3 (1.0-1.7)	1.4 (1.1-1.8)	1.7 (1.3-2.2)	1.7 (1.4-2.2)	1.3 (1.0-1.7)	0.6800	0.0496	0.235	0.111

Supplemental Figure 1: Insulin concentrations throughout the experiment for nondiabetic (a), diabetic (b), and combined subjects (c). Arrows indicate time of arginine (5 g) infusion. Data are shown as mean +/- SEM and compared with mixed model. *$p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.



Supplemental Figure 2: C-peptide concentrations throughout the experiment for combined subjects. Arrows indicate time of arginine (5 g) infusion. Data are shown as mean +/- SEM and compared with mixed model. *** p <0.001, ****p < 0.0001.