CB4211 is a Potential Treatment for Metabolic Diseases with a Novel Mechanism of Action: Sensitization of the Insulin Receptor

KENT GRINDSTAFF[1], RÉMI MAGNAN[1], ROBIN SHANG[1], EMILY STENGER[1], JENNA S. HOLLAND[2], DIEGO PEREZ-TILVE[2], KENNETH C. CUNDY[1]
[1]CohBar, Inc., Menlo Park, CA; [2]University of Cincinnati, Cincinnati, OH

Abstract

Metabolic dysfunction and insulin resistance are common underlying factors in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and many age-related diseases, including obesity and type 2 diabetes. CB4211 is a novel peptide analog of MOTS-c, a mitochondrially encoded peptide with a potential role in metabolic homeostasis. CB4211 reduces free fatty acid release from cultured adipocytes, improves NAFLD activity score (NAS) in STAM® mice, and selectively decreases fat mass in DIO mice. We investigated the mechanism of action (MOA) of CB4211 in regulating fatty acid metabolism, glucose homeostasis, and insulin sensitivity. CB4211 potentiated insulin mediated inhibition of lipolysis in isoproterenol stimulated adipocyte cultures without changing maximal response, while CB4211 alone had no effect. Inhibitors of IR auto-phosphorylation (GSK183705A) or downstream PI3K/Akt signaling pathway components (wortmannin, Akti-1/2) abolished the antilipolytic effects of insulin alone and in combination with CB4211. Further supporting sensitization of insulin signaling, CB4211 enhanced insulin mediated phosphorylation of IR, IRS-1, and Akt, without affecting IGF mediated phosphorylation of IGF-1R. Consistent with activity through IR, CB4211 potentiated insulin induced reduction in glucose production in H4-IIE cells. The acute *in vivo* effect of CB4211 on insulin tolerance was determined in fasted DIO mice. Administration of CB4211 with insulin enhanced insulin sensitivity, prolonging the reduction in blood glucose levels compared to insulin alone. In conclusion, CB4211 potentiates insulin effects on fatty acid metabolism and glucose homeostasis by acting at the level of IR. The observed MOA of CB4211 therefore supports its potential utility for treatment of NASH, obesity, type 2 diabetes, and other metabolic disorders.

Methods

Cell Culture: Cell lines were cultured according to supplier recommendations. 12-13 day differentiated 3T3-L1 (ZenBio) adipocyte cultures were placed in differentiation medium without supplemental insulin for 24-48 hours prior to addition of compounds. 5 day differentiated C2C12 (Sigma-Millipore) myotube cultures were placed in DMEM containing 1 g/L glucose for 5 hours prior to addition of compounds for 22 hours. Established H4-IIE (ATCC) cultures were placed in glucose production medium (glucose free DMEM containing lactate and pyruvate) for 24 hours prior to addition of compounds. Established MCF-7 (ATCC) cultures were serum starved for 19 hours prior to addition of compounds.

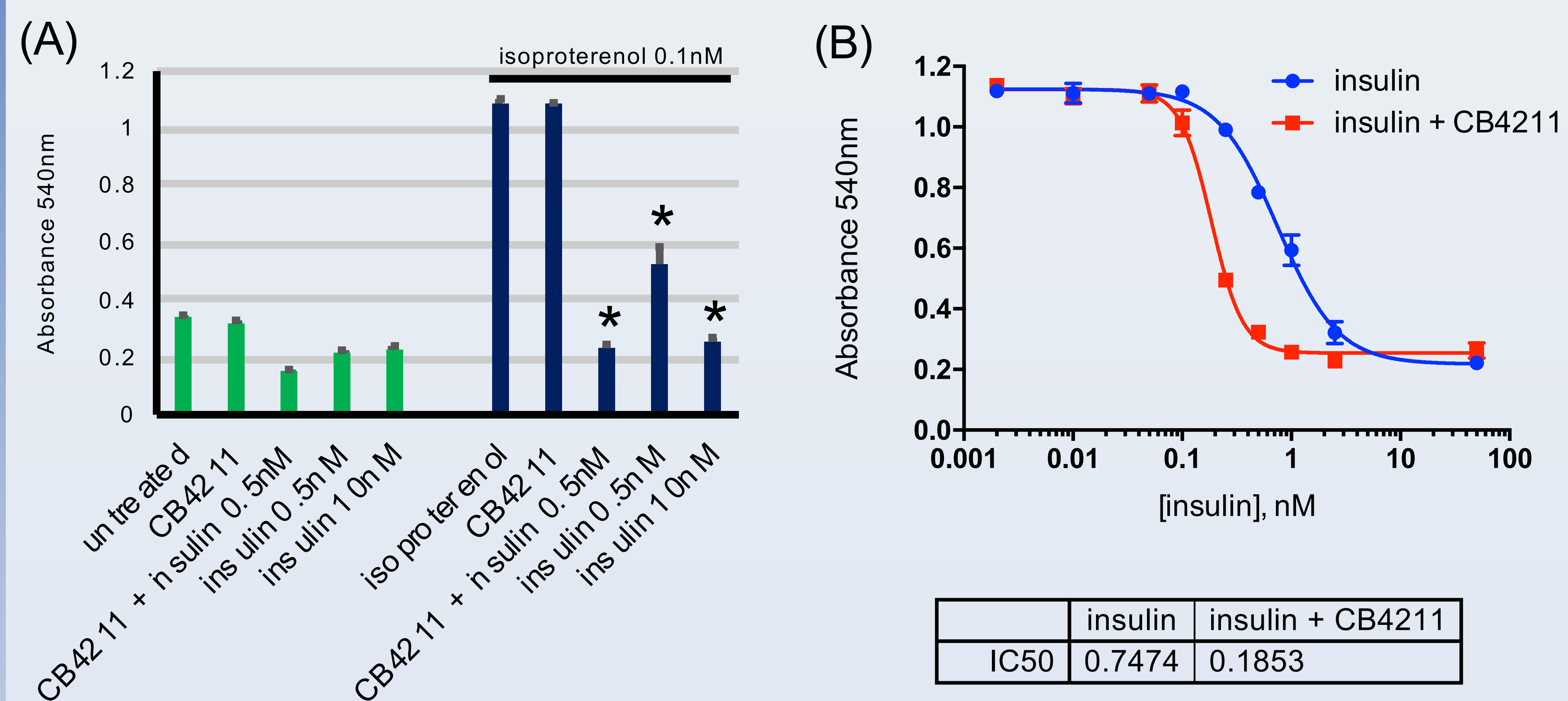
In Vitro Lipolysis: Free fatty acid release into the medium (lipolysis) was measured following a 3 hour incubation using a commercial kit according to the manufacture's instructions (ZenBio).

Insulin Signaling: Phosphorylation of IR, IRS-1, and Akt in extracts prepared following the specified incubations was determined by ELISA kits according to manufacture's instructions (Cell Signaling Technology).

Glucose Assay: Glucose content in extracellular medium was measured using a kit according to manufacture's instructions (Abcam). For C2C12 myotube cultures, compounds were added for 22 hours in DMEM containing 1 g/L glucose. For H4-IIE cultures, compounds were added for 24 hours in glucose production medium.

Acute ITT: Blood glucose levels were monitored in overnight fasted DIO mice during acute single-dose treatment with insulin alone or insulin + CB4211.

Results



	insulin	insulin + CB4211
IC50	0.7474	0.1853

Figure 1: Measurement of lipolysis in 14 day differentiated 3T3-L1 adipocyte cultures following 3 hour incubation. (A) Effect of CB4211 (50 μM) and insulin (0.5 nM and 10 nM) on basal and isoproterenol-stimulated lipolysis. (B) Insulin titration (0.005-50 nM) without and with CB4211 (25 μM). n=3 for all data points. Data are mean (SD). *p <0.001 compared to isoproterenol alone.

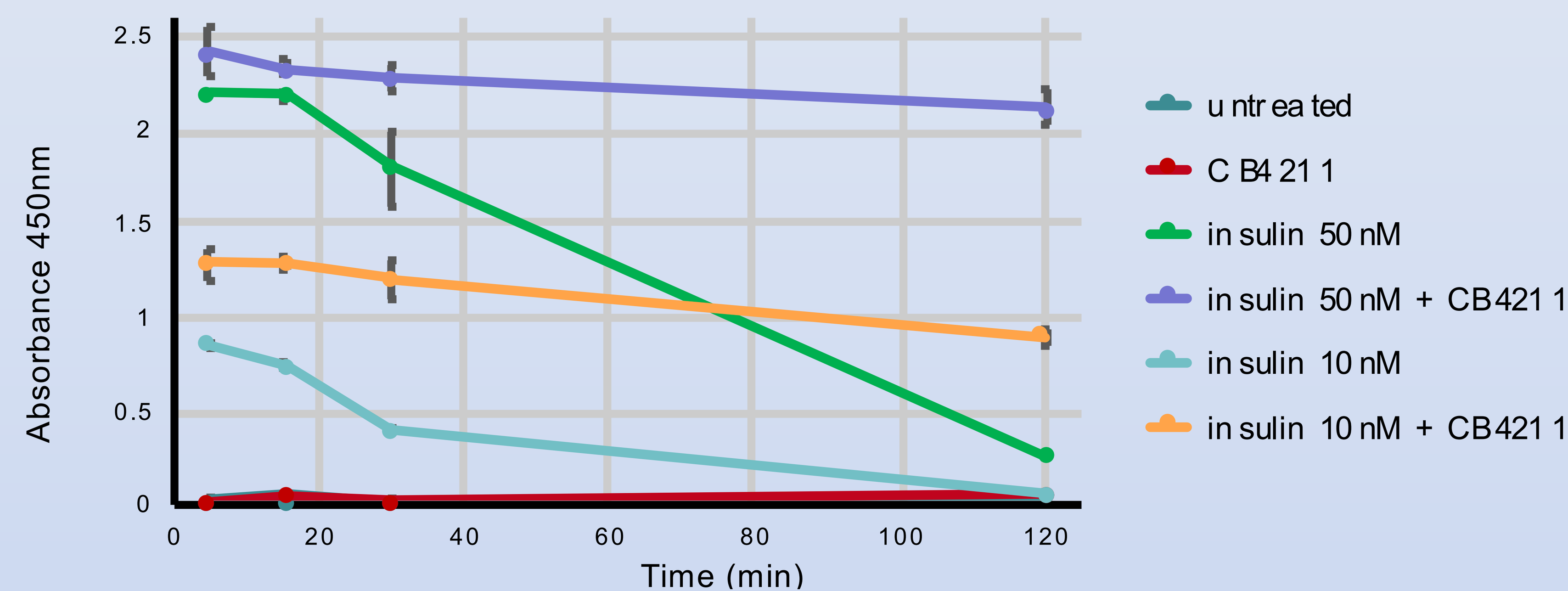


Figure 2: Effect of CB4211 (50 μM) and insulin (10 nM and 50 nM) on time course of IR auto-phosphorylation. IR phospho-Y1150/1151 ELISA of 14 day differentiated 3T3-L1 adipocyte cultures. n=3 for all data points. Data are mean (SD). Note: similar profiles were obtained with IR phospho-pan-Y, IR phospho-Y1146, IRS-1 phospho-pan-Y, and Akt phospho-S473 ELISAs.

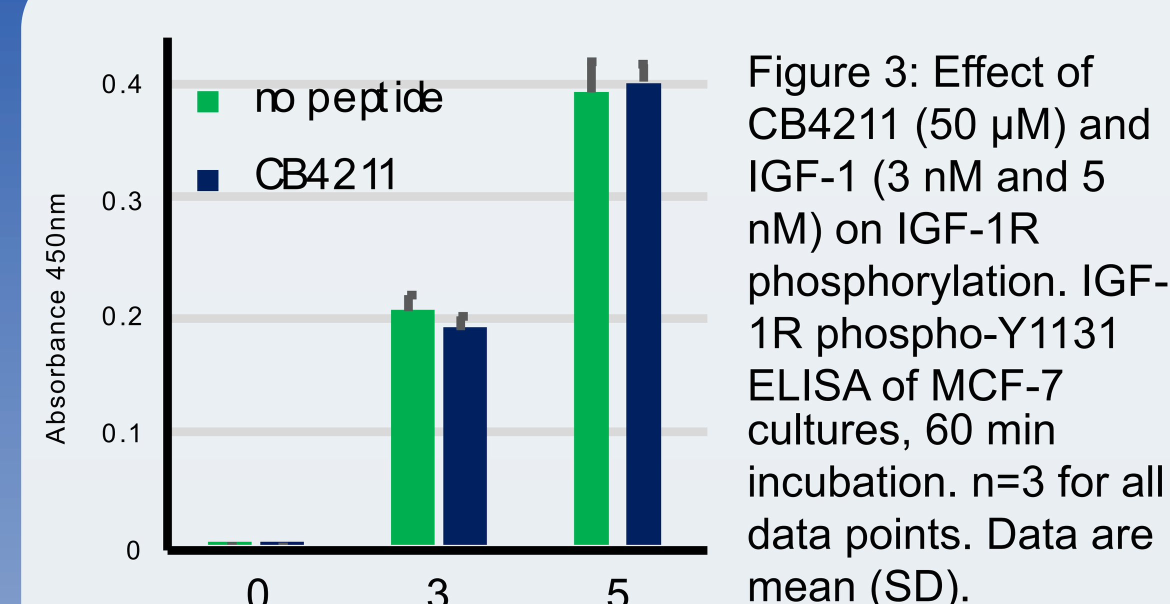
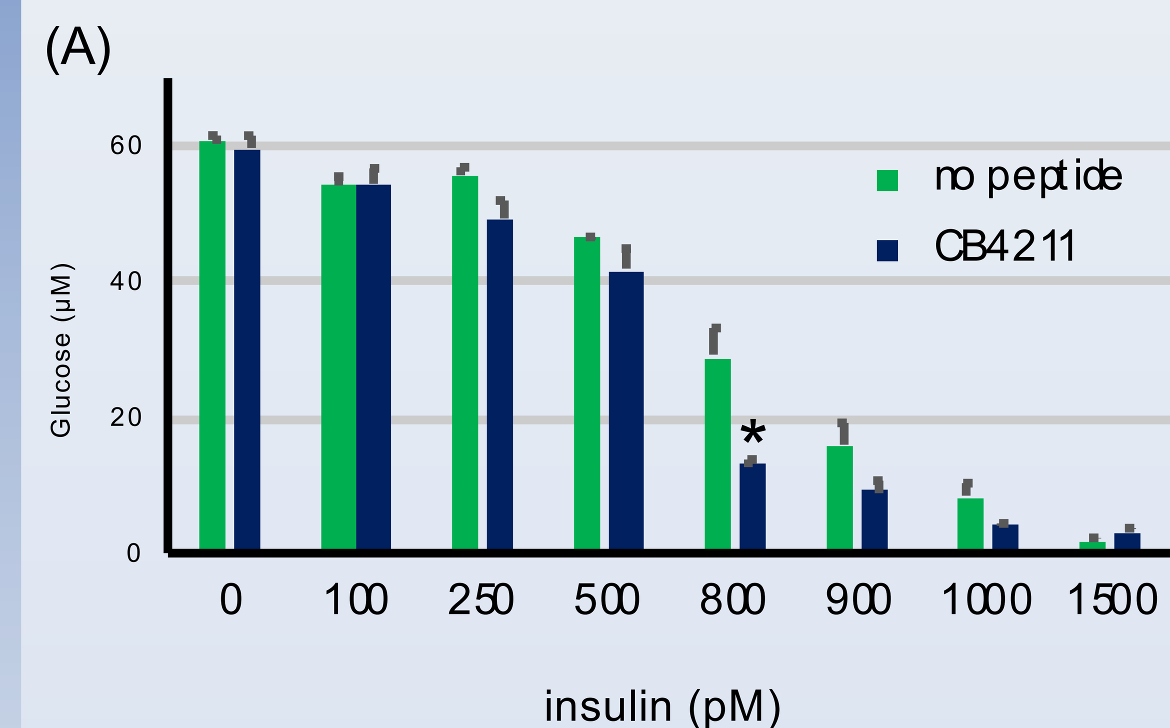


Figure 3: Effect of CB4211 (50 μM) and IGF-1 (3 nM and 5 nM) on IGF-1R phosphorylation. IGF-1R phospho-Y1131 ELISA of MCF-7 cultures, 60 min incubation. n=3 for all data points. Data are mean (SD).



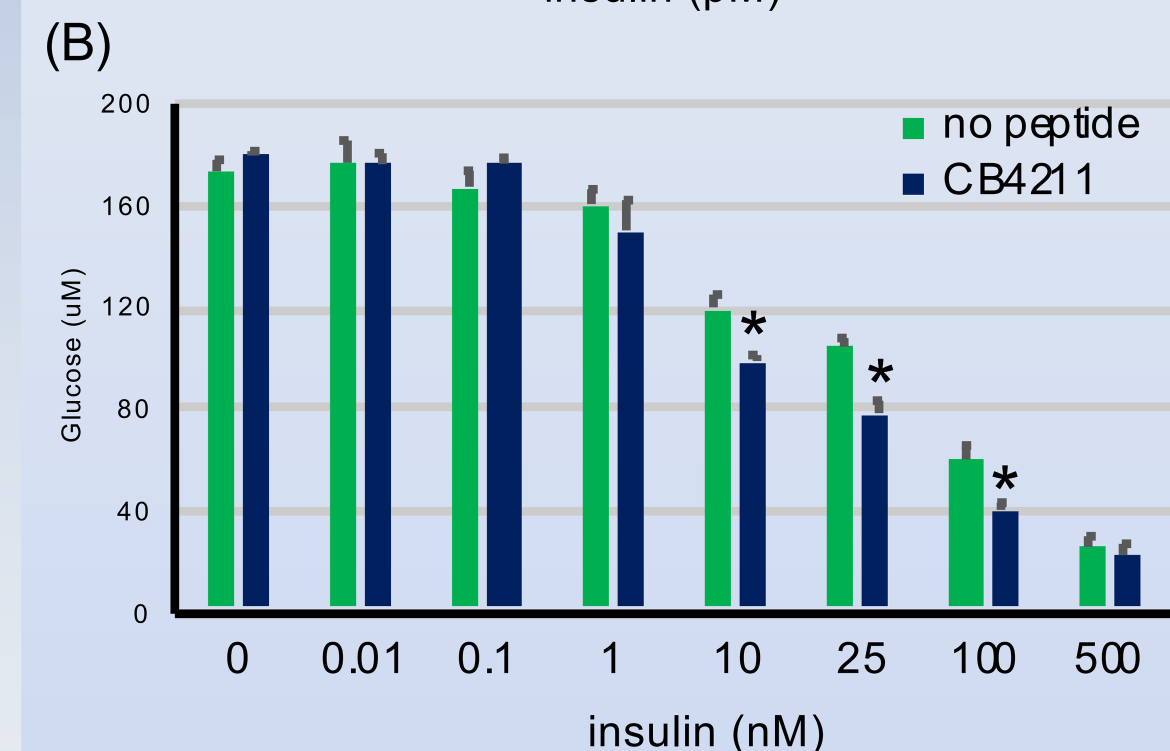


Figure 4: Effect of CB4211 (50 μM) on (A) glucose production by H4-IIE cells and (B) glucose consumption by 6 day differentiated C2C12 myotubes. Glucose concentrations determined in medium following 22-24 hour incubation. n=3 for all data points. Data are mean (SD). *p <0.05 compared to no peptide at same insulin concentration.

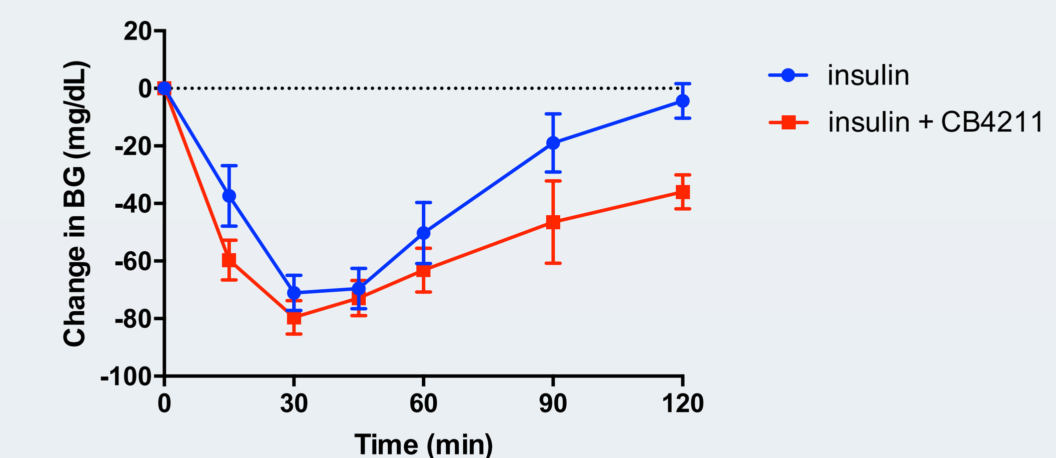


Figure 5: Effect of CB4211 on insulin tolerance test in fasted DIO mice. Blood glucose concentrations following intraperitoneal administration of insulin (1.25 U/kg) alone or 1 hour after intraperitoneal CB4211 (15 mg/kg). n=12 mice per treatment arm. Data are mean (SEM).

Compound	MOA	Lipolysis Stimulator	Treatment (with isoproterenol)		% of Stimulated Lipolysis	% CV
N/A	N/A	isoproterenol (0.1nM)	insulin (10nM)	no addition	**23.9**	10
			insulin (2.5nM)		**34.4**	11
			insulin (0.5nM)		**58.9**	8.5
			CB4211 (50μM) + insulin (0.5nM)		**24.7**	4.9
BMS754807	inhibitor of IR auto-phosphorylation	isoproterenol (0.1nM)	insulin (10nM)	+ BMS754807 (500nM)	**99.2**	1.5
			insulin (0.5nM)		**99.7**	2.7
			CB4211 (50μM) + insulin (0.5nM)		**99.2**	1.7
GSK183705A	inhibitor of IR auto-phosphorylation	isoproterenol (0.1nM)	insulin (10nM)	+ GSK183705A (1μM)	**90.1**	2.9
			insulin (0.5nM)		**95.7**	4.9
			CB4211 (50μM) + insulin (0.5nM)		**91.8**	9.3
Wortmannin	inhibitor of PI3K	isoproterenol (0.1nM)	insulin (10nM)	+ Wortmannin (100nM)	**106**	1.3
			insulin (0.5nM)		**107**	1.6
			CB4211 (50μM) + insulin (0.5nM)		**106**	1.3
GDC0032	inhibitor of PI3K	isoproterenol (0.1nM)	insulin (10nM)	+ GDC0032 (1μM)	**96.3**	1.5
			insulin (0.5nM)		**108**	2.7
			CB4211 (50μM) + insulin (0.5nM)		**107**	2.1
Akti 1/2	inhibitor of Akt	isoproterenol (0.1nM)	insulin (2.5nM)	+ Akti 1/2 (7.5μM)	**79.7**	6.4
			insulin (0.5nM)		**93.9**	7.8
			CB4211 (50μM) + insulin (0.5nM)		**93.7**	6.1

Table 1: Effect of IR pathway inhibitors on anti-lipolytic activity of insulin alone and insulin + CB4211. n=3 for all data points.

Conclusions

- CB4211 potentiates insulin activity in *in vitro* models of lipolysis, IR pathway activation, and glucose homeostasis
- CB4211 alone does not alter basal responses, activity dependent on insulin
- Activity of CB4211 observed only at intermediate insulin concentrations
- IR pathway inhibitors block anti-lipolytic effect of CB4211
- CB4211 has no effect on IGF-1 mediated phosphorylation of IGF-1R
- Acute administration of CB4211 enhances insulin sensitivity *in vivo*
- CB4211 effects on insulin signaling potentially offer a novel approach for treatment of NAFLD, NASH, and other metabolic disorders

Disclosures

Kent Grindstaff, Rémi Magnan, Robin Shang, Emily Stenger, Kenneth C. Cundy: Employees and shareholders of CohBar, Inc. Diego Perez-Tilve: Research funds from CohBar, Inc. Jenna S. Holland: None.