Oral Controlled Metabolic Accelerator Drives Fat-Specific Weight Loss and Augments GLP-1 Effect

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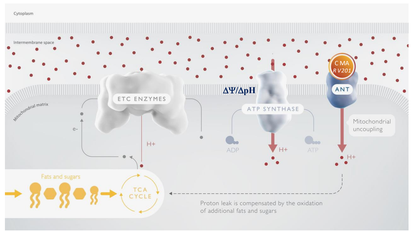
Abstract #119

Background

- Controlled Metabolic Accelerators (CMAs) are oral small molecules in development for the treatment of obesity and related cardiometabolic diseases.
- By engaging the natural metabolic process of mitochondrial uncoupling, CMAs are engineered to increase resting metabolic rate, resulting in increased energy expenditure (EE) in a manner that is precision-controlled and imperceptible to the patient.
- CMAs specifically act by activating the Adenine Nucleotide Translocase (ANT) transporter, a carrier protein in the mitochondrial inner membrane that mediates proton leak. This process significantly contributes to basal proton conductance and regulates mitochondrial energy output. The proton leak represents a "futile cycle," wherein protons re-enter the mitochondrial matrix in an ATP synthase-independent manner, thereby increasing substrate (fat) oxidation to maintain the proton motive force ($\Delta\Psi/\Delta pH$) required to produce ATP.
- By selectively enhancing carbon oxidation from fat, Rivus' CMAs are designed to induce sustained, fat-selective, muscle-preserving weight loss.
- RV201 is an oral small molecule CMA, structurally distinct from known uncouplers. Here, we assessed RV201's potency, *in vivo* target engagement, and weight loss (WL) efficacy in diet-induced obese (DIO) mice, both as monotherapy and in combination with low or high-dose Semaglutide (SEMA).



Methods

***In Vitro* Studies:**

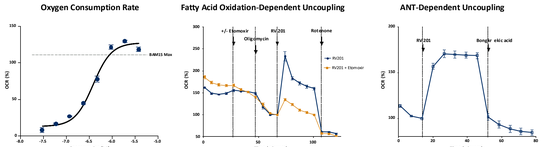
- Oxygen consumption rate (OCR) was measured in HepG2 human hepatoma cells and C2C12 mouse myoblasts cells using Seahorse XF Analyzers to assess mitochondrial uncoupling activity and potency of CMAs.
- Fat oxidation was assessed using Seahorse XF Palmitate Oxidation Stress Test Kit. OCR was measured in the presence and absence of etomoxir, an inhibitor of carnitine palmitoyltransferase-1 (CPT-1), to prevent the formation of acyl carnitines and thus, transport of long-chain fatty acids to the mitochondria. Oligomycin (ATP synthase inhibitor) was added to measure ATP-linked respiration, and Rotenone (Complex I inhibitor) was added to inhibit mitochondria-dependent oxygen consumption.
- ANT dependency was assessed by measuring OCR in the presence of ANT inhibitor bongkrekic acid.

Animal Studies:

- Target engagement studies: Male C57BL/6 lean mice were treated with vehicle (veh) or RV201 (1, 3, 10 mg/kg PO, QD) for 3 days at thermoneutrality. Oxygen consumption (VO2), carbon dioxide production (VCO2), energy expenditure (EE), and fat oxidation were assessed using the Promethion (Comprehensive, High-resolution Behavioral Analysis Systems, Sable Systems International). Body temperature was monitored twice daily.
- Efficacy studies: Male DIO mice were fed high-fat diet for at least 10 weeks and received veh, low-dose SEMA (1 nmol/kg/day SC), or high-dose SEMA (15 nmol/kg/day SC) for 8 days, with or without RV201 (10 mg/kg PO, Q3D). Treatment ceased at day 9, with a 6-day follow-up. Energy expenditure (EE) and fat oxidation were assessed using indirect calorimetry (Promethion). Food intake and body weight were measured throughout the study. Body composition (BC) was measured by Echo-MRI.
- *In vivo* studies were performed by academic collaborators with oversight from Rivus personnel.

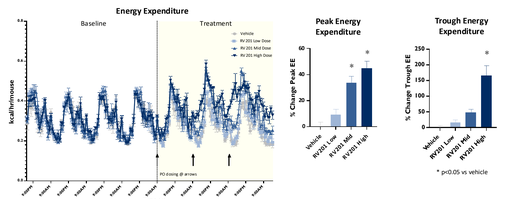
Results

1 *In Vitro* Profiling of RV201, a Potent ANT-Dependent Uncoupler



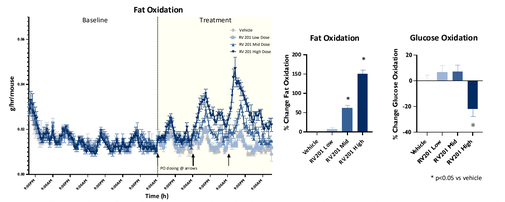
- RV201 is a potent uncoupler that is structurally dissimilar to historic uncouplers such as DNP, BAM15, or FCCP. RV201 increased OCR in HepG2 (EC_{50} = 224 ± 104 nM; E_{max} = 133 ± 16%, n=3) and mouse C2C12 cells (EC_{50} = 353, nM; E_{max}= 194%).
- RV201's OCR increase was mostly fatty acid oxidation (FAO)-dependent (% FAO = 77 ± 1, n=2).
- Inhibiting ANT with bongkrekic acid decreased RV201's uncoupling activity, suggesting it acts as an ANT activator.

2 RV201's Dose-Dependent Increase in Energy Expenditure in Lean Mice



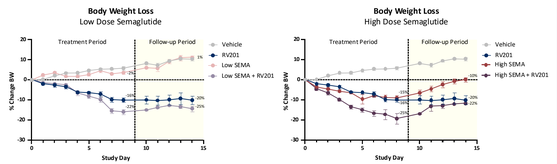
- In lean mice, RV201dose-dependently and continuously raised EE (up to 50% vs vehicle) over 3 days without increasing body temperature.
- Robust and significant dose-dependent changes in EE were observed during both active (peak) and inactive (trough) cycles.

3 RV201's Dose-Dependent Increase in Fat Oxidation in Lean Mice



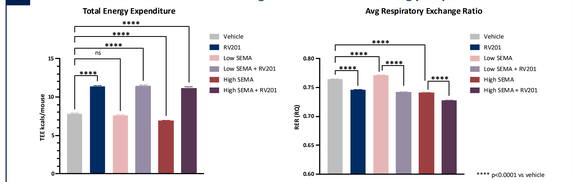
- When orally dosed, QD for 3 days, RV201 induced a dose- and time-dependent decrease in respiratory exchange ratio (RER), which translated into increased fat oxidation (up to 150% vs vehicle) and decreased glucose oxidation (-22%) at the high dose.
- These findings indicate that RV201 induces a dose-dependent shift towards lipid metabolism.

4 RV201 Augments Semaglutide Weight Loss in DIO Mice



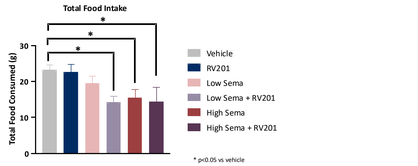
- When orally dosed (Q3D) in DIO mice, RV201 alone induced significant WL (16%) after 8 days.
- High SEMA (dosed SC, QD) caused 15% WL, while low SEMA was ineffective (2% WL). Adding RV201 enhanced SEMA efficacy at both low (22% total WL) and high doses (25% total WL).
- Notably, WL persisted in combination groups 6 days after stopping treatment.

5 RV201 Increases While Semaglutide Decreases Energy Expenditure



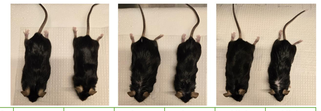
- When orally dosed (Q3D) in DIO mice, RV201 alone induced a significant increase in EE after 8 days (45% increase). In contrast, SEMA (dosed SC,QD) caused a dose-dependent decrease in EE. Adding RV201 to SEMA rescued and further increased EE.
- RV201 and High SEMA monotherapy caused a reduction in RER. Adding RV201 to SEMA further reduced RER, driving increased fat oxidation.

6 RV201 Accentuates the Semaglutide Increase in Satiety at Low Dose

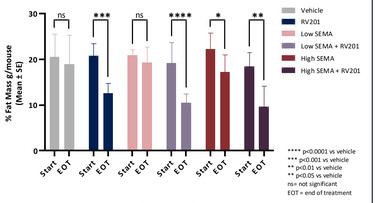


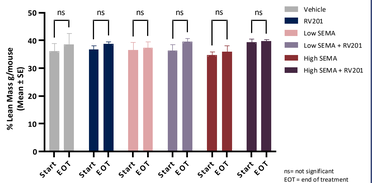
- As expected, high-dose SEMA reduced food intake as a monotherapy and in combination with RV201.
- RV201 alone did not affect food intake but amplified satiety effects with low SEMA, rendering the low dose of SEMA effective in combination with RV201.

7 RV201 Reduces Fat Mass While Preserving Lean Body Mass



SEMA	-	-	Low	Low	High	High
RV201	-	+	-	+	-	+





- Compared to baseline, RV201 drove greater fat mass loss (-46%) than low (-7%) or high SEMA (-24%) while also preserving lean mass.
- The combinations further reduced fat mass (-57% and -63% for low/high SEMA + RV201, respectively).
- RV201-treated DIO mice looked leaner and more robust at the end of the study.

Conclusions

RV201, an oral CMA, increases fat-dependent OCR in ANT.

In lean mice, RV201 induces a dose-dependent increase in EE and a shift towards fat oxidation.

Importantly, RV201 promotes fat-specific WL in DIO mice by increasing EE and fat oxidation.

Combined with Semaglutide, RV201 enhanced and sustained weight and fat loss, even at sub-efficacious Semaglutide doses, while preserving lean mass.

The combination of RV201 with low-dose incretins like Semaglutide may provide more effective, durable WL with potentially improved tolerability vs high-dose incretin monotherapy.

Combining Rivus' CMAs with low-dose incretin offers the potential for improved efficacy, better tolerability, and fat-selective, muscle-preserving weight loss compared to current standard-of-care therapies for treating obesity and related cardiometabolic disorders.

Acknowledgements

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