ENT-03, a Centrally Acting Endogenous Spermine Bile Acid with PTP1B Inhibitory Activity has Potent Effects on Metabolism and Weight in a Mouse Model of Diet Induced Obesity (DIO)

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Background: Efforts to identify new targets to treat obesity and T2D have focused on inhibition of PTP1B based on compelling data in neuronal PTP1B KO mice. Synthetic PTP1B inhibitors, however, have had limited success largely due to poor pharmacodynamic characteristics. We describe here ENT-03, a spermine bile acid newly discovered in mammalian brain that acts centrally, has PTP1B inhibitory activity, rapidly and dramatically lowers glucose out of proportion to weight loss and progressively reduces body weight.

Methods: To determine the effect of ENT-03 on nonfasting blood glucose and body weight in DIO mice fed a high fat diet, ENT-03 (25mg/kg), semaglutide (0.04mg/kg) or vehicle were administered subcutaneously twice weekly for 10 weeks (n=5/group) (Experiment 1). In a separate experiment designed to compare persistence of effect beyond the treatment period (Experiment 2) and compared to a higher dose of semaglutide, ENT-03 (25mg/kg) or vehicle was administered s.c. twice weekly, and semaglutide (0.12mg/kg) was administered s.c. daily for 10 weeks (n=8/group) and glucose, weight and body fat were followed during treatment and for 5 weeks beyond the treatment period. Mice continued on a high fat diet after discontinuation. To determine whether ENT-03 acts centrally, a single dose of ENT-03 (60µg or 120µg) or vehicle was administered intracerebroventricularly (i.c.v.) to SD rats (n=3-4/group).

Results: In ENT-03-treated DIO mice, fed glucose fell rapidly and substantially prior to any significant weight loss ($p=2x10^{-5}$) and remained at a new steady-state thereafter. In contrast, in semaglutide treated mice, glucose fell in proportion to weight loss (p=ns) (Figure 1). In a separate experiment and at a higher dose of semaglutide the pattern was the same during the active treatment period (Figure 2). Following cessation of treatment, semaglutide-treated mice exhibited rebound weight gain and food intake. In contrast, ENT-03 treated animals defended the lower weight and the reduced food intake reached at drug discontinuation for several weeks (Figure 2). Body fat decreased significantly in both groups during treatment but increased rapidly in the semaglutide group upon discontinuation compared to ENT-03 (p=5x10-3) (Figure 3). Indirect calorimetry 3 weeks into treatment demonstrated that each dose of ENT-03 was followed by a decrease in food intake, RER, movement and energy expenditure lasting 24-48 hours (Figure 4). Following a single injection of ENT-03 to SD rats i.c.v. (60µg or 120µg), body weight fell significantly (p<0.05 and p<0.01 respectively) and remained below vehicle for an extended period (Figure 5).

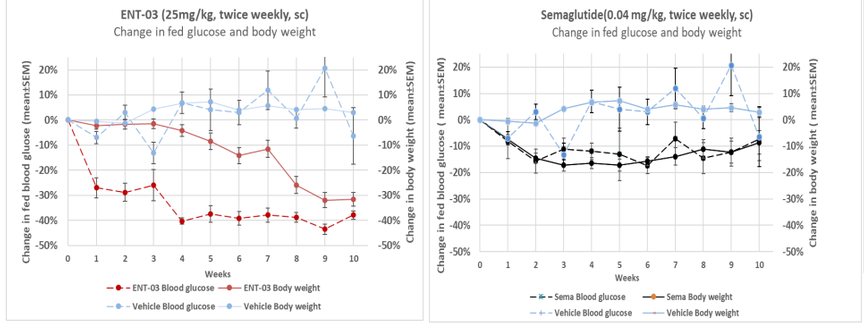

Figure 1: **The effect of ENT-03 or semaglutide on fed blood glucose and body weight in DIO mice**. ENT-03 (25mg/kg) (n=5), semaglutide (0.04mg/kg) (n=5) or vehicle were administered s.c. twice weekly for 10 weeks to high fat diet fed DIO mice.

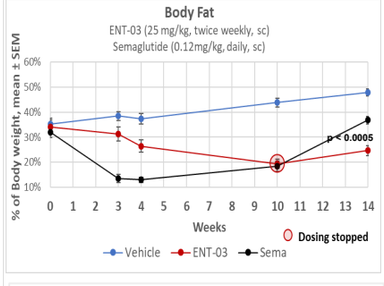

Figure 3: The effect of ENT-03 and semaglutide on body fat and lean weight (Echo/MRI). ENT-03 (25mg/kg) or vehicle were administered s.c. twice weekly, and semaglutide (0.12mg/kg) was administered s.c. daily for 10 weeks to high fat fed DIO mice (n=8/group).

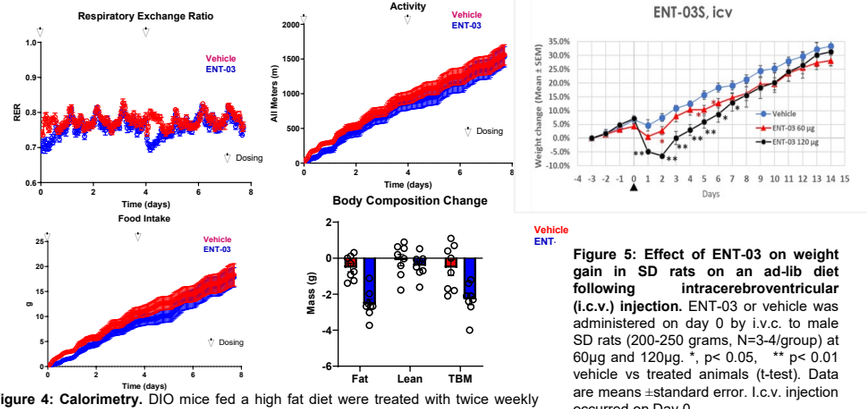

Figure 4: Calorimetry. DIO mice fed a high fat diet were treated with twice weekly s.c. ENT-03 (25mg/kg) or vehicle for 10 weeks. Calorimetry was conducted at 3-4 weeks.The first dose of ENT-03 was on Day 0 (4pm 3/27), and the second dose right before Day 4 (4pm, 3/31).

Figure 5: Effect of ENT-03 on weight gain in SD rats on an ad-lib diet following intracerebroventricular (i.c.v.) injection. ENT-03 or vehicle was administered on day 0 by i.v.c. to male SD rats (200-250 grams, N=3-4/group) at 60µg and 120µg. *, p< 0.05, ** p< 0.01 vehicle vs treated animals (t-test). Data are means ±standard error. I.c.v. injection occurred on Day 0.

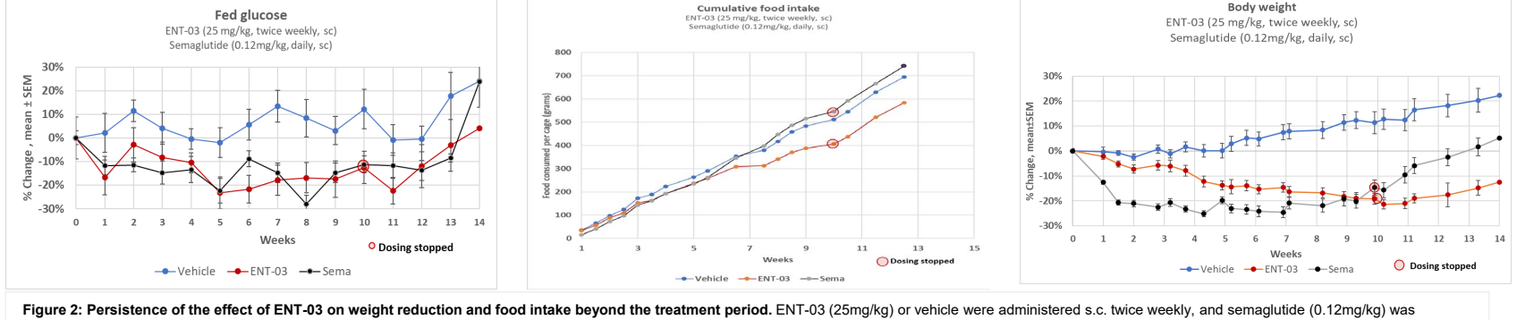

Figure 2: Persistence of the effect of ENT-03 on weight reduction and food intake beyond the treatment period. ENT-03 (25mg/kg) or vehicle were administered s.c. twice weekly, and semaglutide (0.12mg/kg) was administered s.c. daily for 10 weeks to high fat fed DIO mice (n=8/group).

Conclusion:
ENT-03 is a novel, endogenous, centrally acting mammalian aminosterol which rapidly lowers fed glucose out of proportion to body weight loss and causes progressive and sustained weight loss in DIO mice. On discontinuation of treatment, the new body weight is defended for weeks. These data support a potential role for ENT-03 in the treatment of T2D and obesity. Phase 1 studies in obese and diabetic subjects are currently in progress

Unanswered questions:

1. What are the neural and metabolic pathways responsible for the rapid fall in blood glucose?
2. What are the neural pathways involved in driving weight loss and reduction in food intake?
3. What is the target organ involved in driving the rapid fall in blood glucose? Is hepatic gluconeogenesis inhibited? Is muscle glucose uptake increased?
4. What are the mechanisms responsible for persistence of weight loss?
5. What is the role of PTP1B inhibition in ENT-03 pharmacology?

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