siRNA-INHBE Silencing in Mice Confirms Human Genetic Data and Supports a New Approach for Obesity

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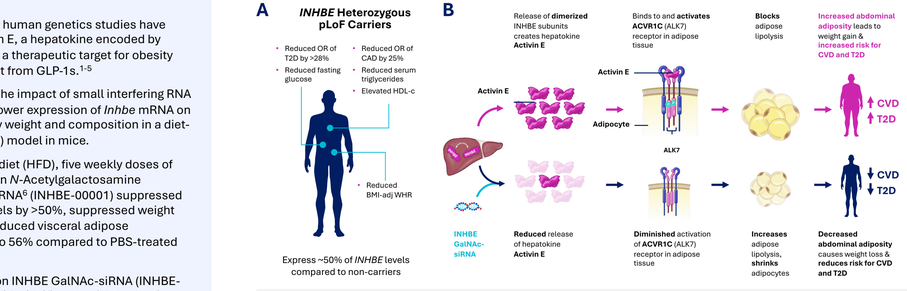
SUMMARY

- Strong evidence from human genetics studies have suggested that Activin E, a hepatokine encoded by *INHBE* (Inhibin βE), is a therapeutic target for obesity with an action distinct from GLP-1s.[1-5]
- Here, we investigate the impact of small interfering RNA (siRNA) designed to lower expression of *Inhbe* mRNA on the regulation of body weight and composition in a diet-induced-obesity (DIO) model in mice.
- In mice fed a high fat diet (HFD), five weekly doses of Wave's first generation *N*-Acetylgalactosamine (GalNAc)-modified siRNA[6] (INHBE-00001) suppressed liver *Inhbe* mRNA levels by >50%, suppressed weight gain by 18.5%, and reduced visceral adipose accumulation by up to 56% compared to PBS-treated mice.
- Wave's next generation INHBE GalNAc-siRNA (INHBE-00002) supports a rapid, significant weight reduction relative to PBS that is sustained up to 84 days after a single dose.
- Another next generation INHBE GalNAc-siRNA (INHBE-00003) supports up to a 9% weight reduction compared to PBS treatment in DIO mice 28 days after a single dose.
  - Visceral adipose tissue mass was reduced up to 40% compared to PBS-treated mice.
  - There was no loss of skeletal muscle mass compared to PBS treatment.
- When added to semaglutide (a GLP-1) treatment, INHBE-00003 doubled weight loss and reduced weight regain upon cessation of semaglutide in DIO mice, suggesting that *Inhbe* mRNA knockdown could complement GLP-1 therapies.
- Wave expects to initiate a clinical trial for its INHBE GalNAc-siRNA candidate, WVE-007, in 1Q 2025.

INTRODUCTION

- Current weight loss agents, including GLP-1s, have several drawbacks, including muscle loss, severe gastrointestinal intolerance, frequent dosing schedule, and rapid weight regain upon cessation of therapy.[7]
- Human genetics studies suggest that *INHBE* is a therapeutic target for obesity treatment (**Figure 1A**).[1-3]
- Activin E, the gene product of *INHBE*, is a hepatokine that regulates adiposity through a pathway distinct from GLP-1s (**Figure 1B**).[4-5]
- Elevated Activin E levels lead to suppression of lipolysis in adipocytes and increased abdominal adiposity, which is an independent risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD) (**Figure 1B**).[8-9]
- Small interfering RNA (siRNA) is a clinically validated therapeutic approach to reduce target gene expression. Conjugating *N*-Acetylgalactosamine (GalNAc) to siRNA enables efficient delivery to hepatocytes.[10]
- Silencing *INHBE* gene expression by ≥50% is expected to recapitulate the healthy metabolic profile of *INHBE* loss of function carriers, including reduced visceral adipose and weight loss, without loss of muscle mass (**Figure 1B**).

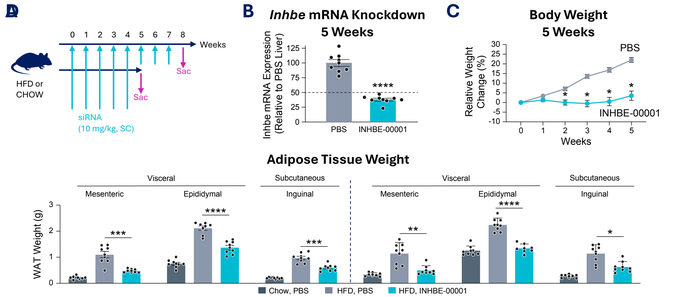
Figure 1. *INHBE* is a therapeutic target for obesity



(A) Metabolic phenotypes of human heterozygous carriers of *INHBE* predicted loss of function (pLoF) alleles. (B) Model of how Activin E contributes to obesity and metabolic disease and hypothesized impact of siRNA-mediated knockdown of *INHBE* expression. OR: odds ratio; CAD: coronary artery disease; BMI-adj WHR: body mass index-adjusted waist to hip ratio; HDL-c: high-desnsity lipoprotein cholesterol.

RESULTS

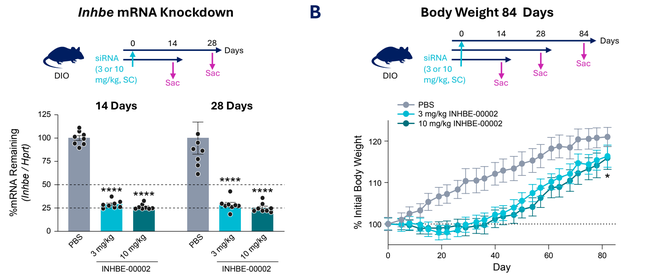
Figure 2. INHBE GalNAc-siRNA INHBE-00001 suppresses *Inhbe* expression beyond anticipated therapeutic threshold and improves metabolic phenotypes in DIO mice



(A) C57Bl6 mice were placed on standard chow (lean) or HFD (DIO) at 6 weeks old. At 14 weeks old (Week 0, W0), mice received either 5 or 8 weekly subcutaneous (SC) injections of 10mg/kg INHBE-00001 or PBS. Mice were sacrificed (sac) one-week-post last dose. (B) *Inhbe* expression in liver at W5 was evaluated by RT-qPCR. Stats: Data shown as mean ± SEM, Welch's T test **** $p < 0.0001$. (C) Weight is shown as % weight gain relative to weight at W0. Stats: Data shown as mean ± SEM (n=8), Linear Mixed Effects ANOVA with comparisons of marginal treatment effects versus PBS per timepoint * $p < 0.05$. (D) Weights of visceral fat pads evaluated one-week-post last dose from Chow, PBS; HFD, PBS; and HFD, INHBE-00001 treated mice. Stats: Data shown as mean ± SEM (n=8); white-adjusted Two-way ANOVA with Bonferroni-adjusted post hoc comparisons per tissue type allowing heteroscedasticity (comparing HFD, INHBE-00001 vs. HFD, PBS shown) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

- INHBE-00001 supported 62% knockdown of *Inhbe* mRNA in HFD mice, which is greater than the expected therapeutic threshold of 50% knockdown (**Figure 2B**).
- An 18.5% weight reduction, compared to PBS control, was observed after treatment with INHBE-00001 for 5 weeks (**Figure 2C**).
- After 5 weeks treatment INHBE-00001, mesenteric, epididymal, and inguinal fat pad weights were reduced by 56%, 35%, and 39%, respectively, compared to HFD PBS control. After 8 weeks treatment with INHBE-00001, mesenteric, epididymal, and inguinal fat pad weights were reduced by 56%, 40%, and 45%, respectively, compared to HFD PBS control (**Figure 2D**).

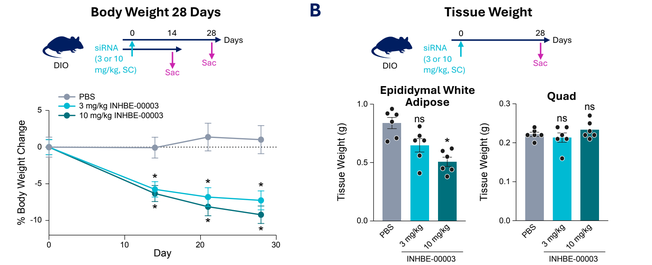
Figure 3. A single dose of INHBE-00002 supports durable weight loss in DIO mice



At ~14 weeks old (Day 0, D0), C57Bl6 DIO mice (as described in Figure 2) received a single SC injection of 3 or 10 mg/kg INHBE-00002 or PBS (control). (A) *Inhbe* expression in liver was evaluated by RT-qPCR on D14 or D28. Stats: mean ±SEM,% mRNA remaining relative to mean of PBS at D0; Two-way ANOVA with Bonferroni-adjusted post hoc tests vs. PBS per time point; **** $p < 0.0001$. (B) Mice were weighed twice weekly and sacrificed at D14, D28, or D84. Stats: Mean body weight change (%) relative to D0 ±SEM (n=8-24); Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects versus PBS per time point; * $p < 0.05$ compared to PBS; 3 mg/kg group and 10 mg/kg group were significantly different from PBS between D12-82 and D8-82, respectively.

- In DIO mice, a single 3 mg/kg SC injection of INHBE-00002 supported >50% *Inhbe* mRNA knockdown compared to PBS treatment at both 14 days and 28 days after dosing (**Figure 3A**).
- The weight of INHBE-00002-treated DIO mice remained significantly lower than PBS-treated mice up to 84 days post single injection ($p < 0.05$) (**Figure 3B**).

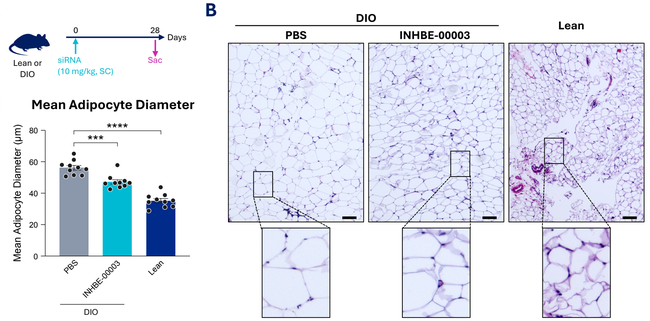
Figure 4. A single dose of INHBE-00003 supports up to 9% weight loss after 28 days without loss of muscle mass



At ~25 weeks old (Day 0, D0), C57Bl6 DIO mice (as described in Figure 2) received a single SC injection of 3 or 10 mg/kg INHBE-00003 or PBS (control). Mice were sacrificed at D14 or D28. (A) Mean body weight change (%) from D0 ±SEM (n=12-18); Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects versus PBS per timepoint; * $p < 0.05$. (B) Epididymal visceral adipose (left) and quadriceps muscle (right) were collected and weighed on D28. Stats: Mean weight (g) ±SEM (n=6). Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects versus PBS per tissue type; * $p < 0.05$; ns, nonsignificant.

- 28 days after a single SC 3 mg/kg or 10 mg/kg dose of INHBE-00003, DIO mice show 7% or 9% weight loss, respectively, compared to PBS (**Figure 4A**).
- In INHBE-00003-treated mice, 28 days after a single SC 10 mg/kg dose, epididymal visceral fat mass was reduced by 40% relative to PBS (**Figure 4B**).
- Under the same conditions, quadricep muscle mass was not impacted by INHBE-00003 at either dose relative to PBS treatment (**Figure 4B**).

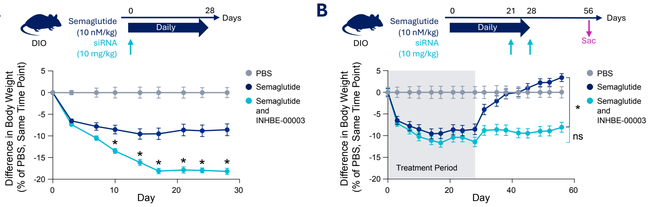
Figure 5. INHBE-00003 decreases adipocyte size in MesWAT of DIO mice



(A) At 23 weeks old (D0), DIO C57Bl6 mice (as described in Figure 2) received a single SC injection of 10mg/kg INHBE-00003 or PBS. Untreated lean mice were sacrificed on D0; INHBE-00003 or PBS treated animals were sacrificed on D28 (n=10/group). (B) Representative images of H&E stained MesWAT tissues, overlaid with segmentation by ImageJ Adiposoft plugin, used to characterize adipocyte dimensions. Scale bar: 100 µm. (C) Mean ±SEM MesWAT adipocyte diameter, calculated from H&E stained sections, using ImageJ Adiposoft plugin. One representative field of view was analyzed per animal. Stats: One-way ANOVA followed by Tukey HSD post hoc tests; not all comparisons are shown; *** $p < 0.001$, **** $p < 0.0001$.

- As expected, PBS-treated DIO mice (D28) displayed MesWAT adipocytes with significantly larger mean diameter ($p < 0.0001$) compared to age-matched lean mice (D0) (**Figure 5B,C**).
- Treatment with INHBE-00003 for 4 weeks suppressed the high fat diet-induced adipocyte size increase by 43% ($p < 0.001$) (**Figure 5B,C**).

Figure 6. INHBE-00003 augments semaglutide-induced weight management in DIO mice



At 30-35 wks old (D0), DIO C57Bl6 mice (as described in Figure 2) received daily SC injections of either PBS or semaglutide (10 nmol/kg) for 28 days. Some animals receiving semaglutide also received a SC dose of INHBE-00003(10 mg/kg) on (A) D0 or (B) D21 and D28. Animals were weighed twice weekly until D28 (A) or D56 (B). Stats: Data presented as mean weight difference as a % of PBS control on the same day (±SEM, n=10). (A) Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects for semaglutide versus semaglutide and INHBE-00003 per time point; * $p < 0.05$ compared to semaglutide group. (B) Linear Mixed Effects ANOVA with post hoc comparisons of marginal time point effects between D28 and D56 per treatment group; * $p < 0.05$; ns, nonsignificant.

- After 28 days, daily semaglutide resulted in a 8.5% reduction in baseline-adjusted weight relative to PBS in DIO mice. When added to daily semaglutide, a single dose of INHBE-00003 delivered at D0 doubled (8.5% versus 18%) the reduction in baseline-adjusted weight relative to PBS at D28 (**Figure 6A**).
- INHBE-00003 also suppressed weight regain upon discontinuation of semaglutide. DIO mice given daily semaglutide regained weight quickly (D28 versus D56, $p < 0.05$), resulting in their baseline-adjusted weight exceeding PBS-treated mice by D56. By comparison, DIO mice given both daily semaglutide and INHBE-00003 (on D21 and D28) regained weight more slowly, maintaining baseline-adjusted weight loss relative to PBS-treated mice at D56 (comparing D28 versus D56, p = ns) (**Figure 6B**).

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