# Silencing Hepatokine Activin E Promotes a Healthy Body Composition and Metabolic Profile in Mice

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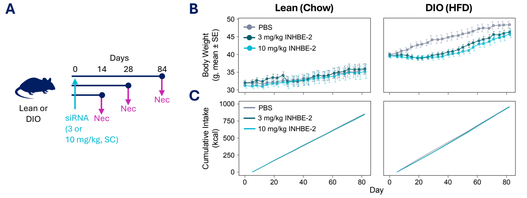
## SUMMARY

- Activin E is a hepatokine encoded by *INHBE* (Inhibin βE), and human genetic studies have suggested that it is a therapeutic target for obesity.[1-5]
- We investigated the impact of *N*-Acetylgalactosamine (GalNAc)-conjugated stereopure small interfering RNAs (SpiNA-GalNAc siRNAs)[6] designed to lower expression of *Inhbe* mRNA on the regulation of body weight and composition in a diet induced-obesity (DIO) model in mice.
  - INHBE-2 durably suppressed weight gain in DIO mice, but not lean mice. This suppression of weight gain was not associated with reduced food intake, suggesting a distinct mechanism of action from GLP-1 therapies.
  - INHBE-3, another *Inhbe* SpiNA-GalNAc siRNA, supported statistically significant weight reduction in mice, compared to PBS treatment, observed over 28 days of study duration after a single dose.
  - Visceral adipose tissue mass and adipocyte size were reduced in INHBE-3 treated mice compared to controls, without loss of skeletal mass, suggesting *Inhbe* mRNA knockdown induces healthy weight loss.
  - *Inhbe*-silencing leads to adipose tissue remodeling compared to PBS controls. Robust changes in gene expression profiles, total and pro-inflammatory macrophage infiltration, and fibrosis were observed in the adipose of INHBE-3-treated DIO mice compared to PBS controls.
- When added to semaglutide (a GLP-1) treatment, INHBE-3 doubled weight loss in mice. INHBE-3 reduced weight regain upon cessation of semaglutide, suggesting that *Inhbe* mRNA knockdown could complement GLP-1 therapies.
- The results support multiple treatment options with *INHBE*-lowering siRNA, including as 1) monotherapy, 2) an add-on to incretins to potentiate weight loss, and 3) to curtail weight regain upon cessation of treatment wih incretins. Wave Life Sciences is evaluating investigational WVE-007, an *INHBE*-GalNAc-siRNA, for the treatment of overweight and obesity, in the INLIGHT™ clinical trial.

## INTRODUCTION

- Current weight loss agents, including GLP-1s, have several limitations, 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.[1-3]
- Activin E, the gene product of *INHBE*, is a hepatokine that regulates adiposity through a pathway distinct from GLP-1s.[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).[8-9]
- siRNA is a clinically validated therapeutic approach to reduce target gene expression. Conjugating GalNAc to siRNA enables efficient delivery to hepatocytes.[10]
- Silencing *INHBE* gene expression by ≥50% is expected to recapitulate the healthy metabolic profile of heterozygous *INHBE* loss of function carriers, including reduced visceral adipose, without loss of muscle mass, and favorable lipid and glucose profiles.

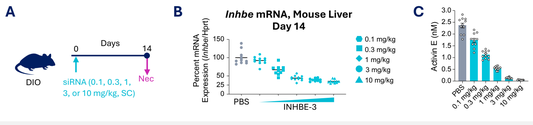
## Figure 1. *Inhbe*-silencing durably suppresses weight gain in DIO mice, but not lean mice, without impacting food intake




(A) C57Bl6 mice were started on a regular chow diet (lean) or a high fat diet (HFD) to promote diet-induced obesity (DIO) at 6 weeks old. Mice received a single SC injection of 3 or 10 mg/kg INHBE-2 or or PBS (phosphate buffered saline, control). Mice were weighed twice weekly and sacrificed at D14, D28, or D84. (B) Body weight ± 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. (C) Food intake was measured each time HFD was changed, as weight of current food in the hopper versus previous weight.

- INHBE-2 induced a significant reduction in weight gain relative to PBS that was durable up to 84 days (**Figure 1B, right**).
- No dose of INHBE-2 tested significantly affected the weight of lean (chow-fed) mice at any point in the study(**Figure 1B, left**).
- INHBE-2 had no significant impact on final cumulative food intake over the course of the study for either diet (**Figure 1C**).

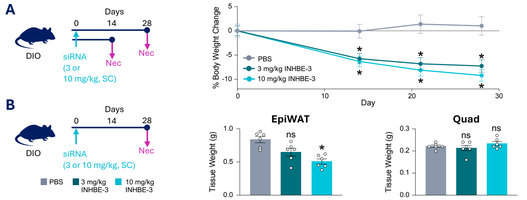
## Figure 2. Potent and dose-dependent silencing of liver *Inhbe* mRNA and serum Activin E protein in DIO mice




(A) At 24 weeks of age, DIO mice received a single SC injection of INHBE-3 (0.1, 0.3, 1, 3, or 10 mg/kg) or PBS. Mice were sacrificed on day 14, and liver was collected. (B) *Inhbe* expression in liver was evaluated by RT-qPCR. (C) Serum Activin E levels were measured.

- INHBE-3 led to dose-dependent knockdown of *Inhbe* mRNA in the liver (**Figure 2B**) and Activin E protein in the serum (**Figure 2C**) of DIO mice 14 days after a single dose. A 65% reduction in *Inhbe* mRNA levels was observed with the highest dose (10 mg/kg) of INHBE-3, compared to PBS-treated mice (**Figure 2B**).

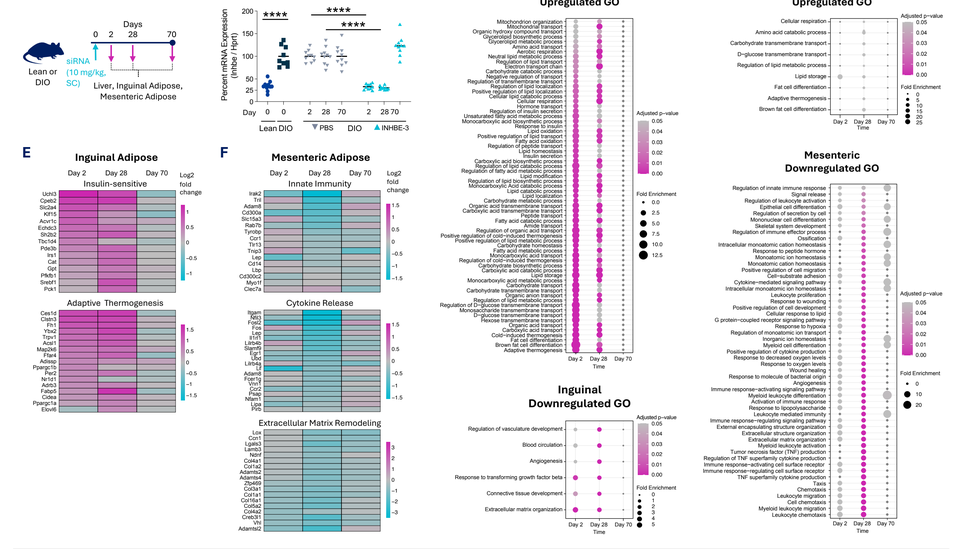
## Figure 3. A single dose of INHBE-3 supports statistically significant weight loss through 28 days without loss of muscle mass




At 25 weeks of age, DIO mice received a single SC injection of 3 or 10 mg/kg INHBE-3 or PBS. 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 white adipose tissue (EpiWAT, visceral adipose) (left) and quadriceps muscle (right) were collected and weighed on D28. Stats: Mean weight (g) ±SEM (n=6). Linear Mixed Effects ANOVA on data Z-score-standardized per tissue type 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-3, DIO mice show statistically significant weight loss compared to PBS (**Figure 3A**).
- In INHBE-3-treated mice, 28 days after a single SC 10 mg/kg dose, epididymal visceral fat mass was reduced by 40% relative to PBS ($p$ <0.05) (**Figure 3B**).
- Under the same conditions, quadriceps muscle mass was not impacted by INHBE-3 at either dose relative to PBS treatment (**Figure 3B**).

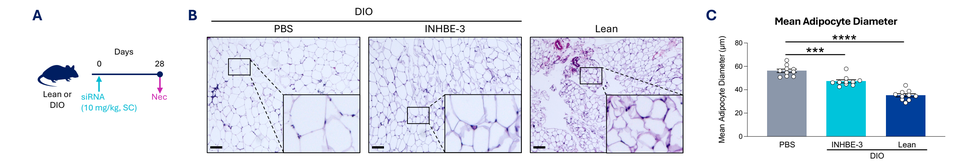
## Figure 4. INHBE-3 increases expression of metabolic genes and decreases expression of inflammatory and fibrotic genes in fat




At 23 weeks of age, (A) DIO mice received a single SC injection of 10 mg/kg INHBE-3 or PBS. Untreated lean (standard chow) mice were sacrificed on D0; INHBE-3 or PBS-treated animals were sacrificed on D2, D28, and D70 (n=9-10/group). Liver, inguinal fat, and mesenteric fat were collected from sacrificed mice at each time point. (B) *Inhbe* expression in liver was evaluated by RT-qPCR. (C-F) Raw mRNA sequencing reads were aligned to the mm10 genome. Subsequently, differential gene expression analysis was conducted using the DESeq2 package. Stats: (B) Black line indicates mean of n=9-10. One-way ANOVA with Tukey's HSD post hoc tests on log2-transformed data. Data is normalized to DIO PBS per day for days 2, 28, and 70. Day 0 mice data is normalized to DIO. **** $p <$ 0.0001.

- *Inhbe* mRNA levels in the liver were reduced by 67% on Day 2, which persisted to Day 28 (70% reduction), in mice treated with INHBE-3 relative to PBS-treated controls. By Day 70, mRNA levels were comparable across treatment groups (**Figure 4B**).
- Robust changes in gene expression were observed in the inguinal (subcutaneous) (**Figure 4C**) and mesenteric (visceral) white adipose tissue (mesWAT) (**Figure 4D**) following treatment with INHBE-3. These gene expression changes occurred early after treatment, starting on Day 2, in the inguinal fat (**Figure 4C**). Kinetics were delayed in the mesenteric fat, with gene expression changes occurring by Day 28 (**Figure 4D**).
- In the inguinal fat, INHBE-3 treatment led to an upregulation of genes associated with metabolic improvement of insulin sensitization and increased beiging of white adipose tissue, including glucose and fat utilization, insulin sensitivity and adaptive thermogenesis (**Figure 4E**).
- In the mesenteric fat, there was a downregulation of genes associated with inflammation and fibrosis, including innate immunity, cytokine release, and extracellular matrix remodeling, following treatment with INHBE-3 (**Figure 4F**).

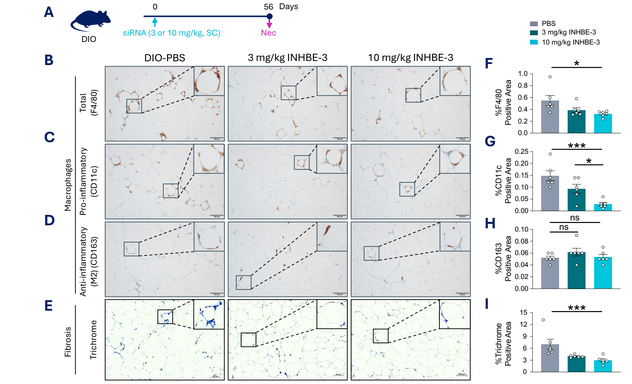
## Figure 5. INHBE-3 decreases adipocyte size in mesenteric white adipose tissue of DIO mice




(A) At 23 weeks of age, DIO mice received a single SC injection of 10 mg/kg INHBE-3 or PBS. Untreated lean (standard chow) mice were sacrificed on D0; INHBE-3 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. 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.

- 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-3 for 4 weeks suppressed the high fat diet-induced adipocyte hypertrophy by 43% ($p$ <0.001) (**Figure 5B, C**).

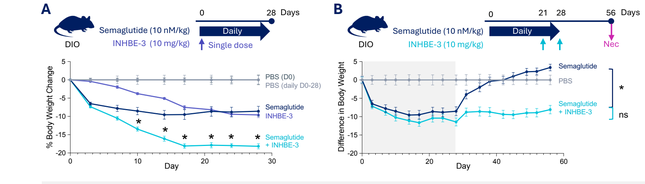
## Figure 6. INHBE-3 decreases total and pro-inflammatory macrophage recruitment in the epiWAT of DIO mice




At 25 weeks of age, DIO mice received a single SC injection of 3 or 10 mg/kg INHBE-3 or PBS. Animals were sacrificed on D56 (n=6/group). (B-E) Representative images (5X) of F4/80 (B), CD11c (C), CD163 (D), and Trichrome (E) stained epiWAT tissues with values similar to the group mean, captured using an Olympus camera (DP74) and microscope (BX53). Scale bar: 100 µm. (F-I) Mean +SEM of percentage stain-positive area, calculated from F4/80 (F), CD11c (G), and CD163 (H), Trichrome (I) stained sections, using HALO image analysis platform . Stats: (F,I) Kruskal-Wallis test with Dunn's multiple comparison test. (G, H) One-way ANOVA with Tukey multiple comparison test. *** $p <$0.001, *$p <$0.05.

- 56 days after treatment with a single dose of INHBE-3, DIO mice show a 41% reduction in total macrophages (F4/80+, **Figure 6B, F**) in the epiWAT.
- Pro-inflammatory macrophages (CD11c+, **Figure 6C, G**) were reduced in a dose-dependent manner, with decreases up to 80% in the epiWAT, compared to PBS-treated controls.
- Anti-inflammatory macrophages (CD163, **Figure 6D, H**) in the epiWAT were comparable between PBS- and INHBE-3 treated mice.
- Fibrosis (trichrome+ area, **Figure 6E, I**) was reduced by 58% in the epiWAT of mice treated with 10 mg/kg INHBE-3 compared to PBS-treated mice.

## Figure 7. INHBE-3 augments semaglutide-induced weight management in DIO mice




At 30 or 35 weeks of age, DIO mice received daily SC injections of either PBS or semaglutide (daily 10 nmol/kg) for 28 days. Some animals also received a SC dose of INHBE-3 (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 difference in baseline-adjusted weight % relative to PBS control on same day (±SEM, n=10). (A) Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects for semaglutide versus semaglutide and INHBE-3 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.

- 28 days of daily semaglutide resulted in a reduction in baseline-adjusted weight relative to PBS in DIO mice. A single dose of INHBE-3 led to a similar reduction in weight relative to PBS in DIO mice. When added to daily semaglutide, a single dose of INHBE-3 delivered at D0 doubled the reduction in baseline-adjusted weight relative to PBS at D28 ($p$ <0.05) (**Figure 7A**).
- INHBE-3 also suppressed weight regain upon discontinuation of semaglutide. DIO mice given daily semaglutide for 28 days regained weight quickly upon cessation (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 (D0-D28) and INHBE-3 (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 7B**).

**References:** 1. Akbari P et al., *Nat Commun.* 2022 Aug 23;13(1):4844; 2. Deaton AM et al., *Nat Commun.* 2022 Jul 27;13(1):4319; 3. Suglyama M et al., *PLoS ONE* 2018;13(3): e0194798; 4. Adam RC et al., *PNAS* 2023 Aug 8;120(32):e2309967120; 5. Griffin JD et al., *Mol Metab.* 2023 Dec;78:101830; 6. Liu W et al., *Nucleic Acids Res.* 2023 May 22;51(9):4126; 7. Forst T et al., *Diabetes Obes Metab.* 2024 Oct;26(10):4178; 8. Klaus VS et al., *Mol Metab.* 2021 Nov; 53:101295; 9. Emdin CA et al., *JAMA.* 2017;317(6):626; 10. Egli M & Manoharan M, *Nucl Acid Res.* 2023 Apr 11; 51(6):2529-2573.

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