Targeting Adipose Lipolysis with *INHBE* Silencing Promotes A Healthy Weight Loss Profile in Mice

Hsiu-Chiung Yang, Wei Liu, Michael Byrne, Pachamuthu Kandasamy, Keith Bowman, Stearne Briem, Cynthia Caracta, Jigar Desai, Anamitra Ghosh, Naoki Iwamoto, Tomomi Kawamoto, Anthony Lamattina, Siqi Liu, Ken Longo, Khoa Luu, Subramanian Marappan, Padma Narayanan, Lola Owen, Priyanka Prakasha, Jeanette Rheinhardt, Denise Schwahn, Himali Shah, Kuldeep Singh, Stephany Standley, Kris Taborn, Hailin Yang, Chandra Vargeese, Erik Ingelsson

Wave Life Sciences, Cambridge, MA, USA

SUMMARY

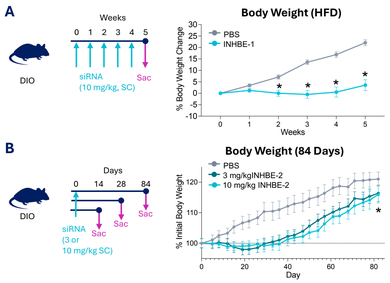
- Activin E is a hepatokine encoded by *INHBE* (Inhibin βE), and human genetic studies have suggested that it is a therapeutic target for obesity.[1-5]
- We investigated the impact of small interfering RNA (siRNA) designed to lower expression of *Inhbe* mRNA on the regulation of body weight and composition in a diet induced-obesity (DIO) model in mice.
- In mice fed a 60% high fat diet (HFD), five weekly doses of INHBE-1, an *N*-Acetylgalactosamine (GalNAc)-conjugated siRNA[6], suppressed weight gain by 18.5%. A single dose of INHBE-2, another *Inhbe* GalNAc-siRNA, significantly reduced weight gain relative to PBS, that was sustained up to 84 days of study duration.
- INHBE-3 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-3 treatment led to decreased infiltration of total and pro-inflammatory macrophages by up to 41% and 80%, respectively, and reduced fibrosis by 58% in visceral adipose tissues of DIO mice.
- INHBE-3 alters expression of specific mRNAs and pathways in inguinal and mesenteric fat, with gene expression profiles that suggest improvement of glucose utilization and insulin sensitivity and attenuation of inflammatory and fibrotic processes driven by increased adiposity.
- 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.
- INHBE-3 GalNAc-siRNA lowered levels of *Inhbe* mRNA and Activin E protein. These were only minimally affected by semaglutide, suggesting *Inhbe* GalNAc-siRNAs work through a unique mechanism of action.
- Wave Life Sciences expects to deliver clinical data in the second half of 2025 from INLIGHT, a Phase 1 clinical trial of WVE-007, an investigational *INHBE* GalNAc-siRNA, for the treatment of people living with overweight or obesity.

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 and weight loss, without loss of muscle mass.

RESULTS

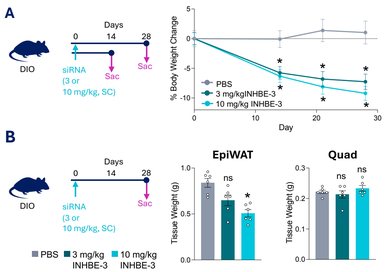
Figure 1. *Inhbe* GalNAc-siRNAs INHBE-1 and INHBE-2 durably suppress weight gain in DIO mice



Mice were started on a high fat diet or regular chow at 6 weeks old. (A) C57Bl6 mice were placed on a HFD (diet induced obesity, DIO) at 6 weeks old. At 14 weeks of age, DIO or lean mice received 5 repeated weekly subcutaneous (SC) injections of 10mg/kg INHBE-1 or PBS. Mice were sacrificed (sac) one-week-post last dose. Weight is shown as % weight change 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. (B) C57Bl6 DIO mice received a single SC injection of 3 or 10 mg/kg INHBE-2 or PBS (control). 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.

- Weight gain over 5 weeks was reduced from 22% of body weight (PBS) to 3.5% of body weight by treatment with INHBE-1 (**Figure 1A**).
- The weight of INHBE-2-treated DIO mice remained significantly lower than PBS-treated mice for the entire duration of the study (**Figure 1B**).

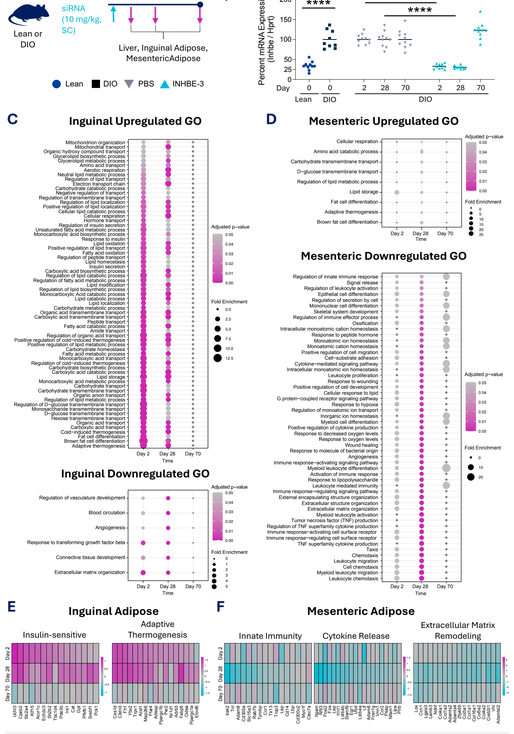
Figure 2. A single dose of INHBE-3 supports statistically significant weight loss after 28 days without loss of muscle mass



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 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 2A**).
- 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 2B**).
- Under the same conditions, quadricep muscle mass was not impacted by INHBE-3 at either dose relative to PBS treatment (**Figure 2B**).

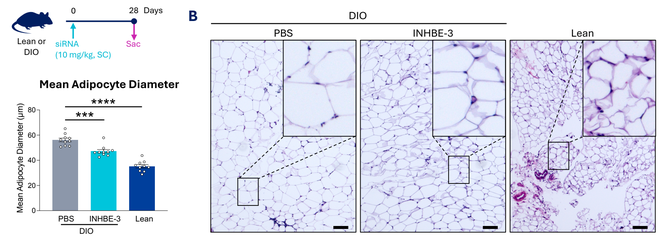
Figure 3. INHBE-3 increases expression of metabolic genes and decreases expression of inflammatory and fibrotic genes in fat



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, D, E, F: Raw mRNA sequencing reads were aligned to the mm11. 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 for each day. Chow-fed mice data is normalized to day 0 lean PBS. **** p <0.0001.

- *Inhbe* mRNA levels in the liver were reduced by 67% on Day 2, and persisted to Day 28 (70% reduction), in mice treated with INHBE-3 relative to PBS-treated controls. By Day 70, *Inhbe* mRNA levels were comparable across treatment groups (**Figure 3B**).
- Robust changes in gene expression were observed in the inguinal (**Figure 3C**) and mesenteric fat (**Figure 3D**) following treatment with INHBE-3. These gene expression changes occurred early after treatment, starting on Day 2, in the inguinal fat (**Figure 3C**). Kinetics were delayed in the mesenteric fat, with gene expression changes occurring by Day 28 (**Figure 3D**).
- 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 3E**).
- 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 3F**).

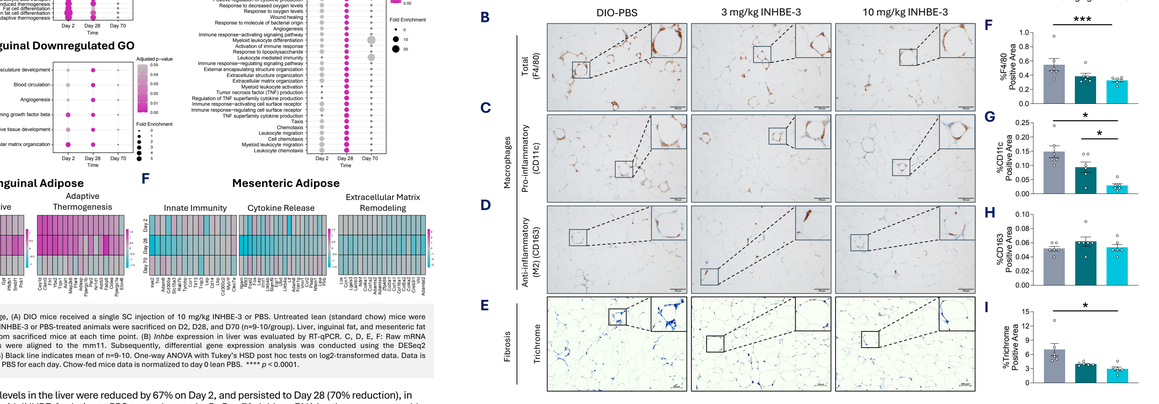
Figure 4. INHBE-3 decreases adipocyte size in mesenteric white adipose tissue (MesWAT) of DIO mice



(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 4B, C**).
- Treatment with INHBE-3 for 4 weeks suppressed the high fat diet-induced adipocyte size increase by 43% (p <0.001) (**Figure 4B, C**).

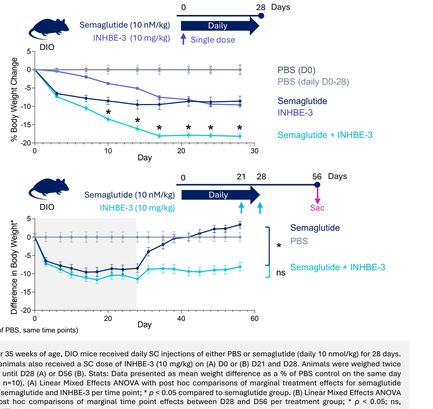
Figure 5. INHBE-3 decreases total and pro-inflammatory macrophage recruitment in the epididymal white adipose tissue (epiWAT) of DIO mice



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-D) 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 5B, F**) in the epiWAT. Infiltration of CD68+ activated macrophages was reduced in INHBE-3 treated mice compared to PBS-treated controls.
- Pro-inflammatory macrophages (CD11c+, **Figure 5C, 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 5D, H**) in the epiWAT were comparable between PBS- and INHBE-3 treated mice.
- Fibrosis (trichrome+ area, **Figure 5E, I**) was reduced by 58% in the epiWAT of mice treated with 10mpk INHBE-3 compared to PBS-treated mice.

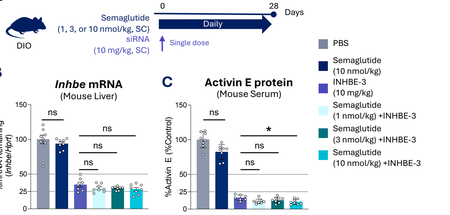
Figure 6. INHBE-3 augments semaglutide-induced weight management in DIO mice



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 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-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 6A**).
- 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 6B**).

Figure 7. Activin E protein is lowered by *Inhbe* GalNAc-siRNA but minimally affected by semaglutide in mice



At 29 weeks (D0), DIO mice received daily SC injections of PBS (for 28 days), as single dose of INHBE-3 (10 mpk), daily semaglutide (for 28 days), or a single dose of INHBE-3 + daily semaglutide (for 28 days). Mice sacrificed on D28. *Inhbe* expression in liver was evaluated by RT-qPCR. Stats: Left, middle: Data analyzed by 1-way ANOVA followed by Tukey's HSD post-hoc comparisons on log2-transformed data; * p <0.05. n=7 or 8/group.

- INHBE-3 GalNAc-siRNA lowered *Inhbe* mRNA (**Figure 7B**) and Activin E protein (**Figure 7B**).
- Semaglutide treatment alone or in combination with INHBE GalNAc-siRNA minimally impacted *Inhbe* mRNA and Activin E protein levels (**Figure 7B, C**).

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. **Acknowledgments:** The authors are grateful to Darienne Myerbeck and Nicole Neuman (Wave Life Sciences) for editorial support and to Eric Smith for graphical support. This work was funded by Wave Life Sciences.