

WNT7A mRNA Lipid Nanoparticles Promote Muscle Hypertrophy and Reduce Fatty Infiltration

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Introduction: Myosteatosis, the infiltration of fat into skeletal muscle, and muscle atrophy are key pathologic features of muscle degeneration in chronic injuries, degenerative myopathies, and aging, correlating with increased disease severity, diminished function, and poor surgical repair outcomes.¹⁻³ Recombinant, secretory protein wingless-type MMTV integration site family 7a (WNT7A) has shown promise in stimulating muscle hypertrophy and reducing fatty infiltration without fibrosis, making it a promising therapeutic candidate for muscle regeneration.^{4,5} However, recombinant protein therapies face challenges related to scalability, delivery, and cost.^{6,7} Lipid nanoparticle (LNP) delivery of mRNA offers a promising alternative with scalable production and effective therapeutic delivery.^{8,9} Here, we assess the feasibility of LNP-mediated mRNA delivery of WNT7A (W7a-LNP) as a strategy for mitigating muscle degeneration by reducing fatty infiltration and promoting myofiber hypertrophy.

Methods: WNT7A mRNA-LNP synthesis. Using the human WNT7A sequence as a template, modified RNAs were *in vitro* transcribed using HiScribe® T7 High Yield RNA Synthesis Kit and fully substituted with N¹-methylpseudouridine-5'-triphosphate (m¹Ψ). LNPs were formulated via microfluidic mixing of lipid components—SM-102, DOPE, cholesterol, and DMG-PEG₂₀₀₀, at a molar ratio of 50:10:38.5:1.5—and mRNA. ***In vivo proof-of-concept.*** All animal studies were performed following the approved IACUC protocol. Cre mRNA-LNPs (15 μg/ 30 μL) were injected in the tibialis anterior (TA) muscle of Ai14-tdTomato mice. TAs were harvested 12-13 days post-injection and cryosectioned. LNP uptake and mRNA expression in skeletal muscle was assessed by visualization of fluorescent tdTomato expression in myofibers. ***In vitro dose-response.*** Primary fibro-adipogenic progenitors (FAPs), isolated from C57BL/6 mice, and C2C12 myoblasts were cultured in growth media and differentiation media, with/without recombinant WNT7A (rWNT7A; 200 ng/mL) or W7a-LNPs (1, 10, 100 ng/mL), assessing for adipogenic inhibition and increased myotube size, respectively. ***In vivo single and multiple W7a-LNP administration.*** Vehicle (30 μL) and W7a-LNP (10 μg/ 30 μL) were injected into contralateral uninjured supraspinatus (SS) muscles of C56BL/6 mice. For multi-dose regimen, injections were repeated every two weeks for a total of three doses. SS muscles were collected 4 weeks after the final treatment. Myofiber hypertrophy was assessed by

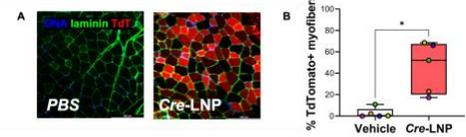


Fig. 1 Proof-of-concept mRNA-LNP delivery in skeletal muscle. (A) Representative TA muscles of Ai14-tdTomato mice injected with Cre-LNP (15 μg/35 μL) or PBS (35 μL). Scale bar: 100 μm. (B) Percentage of tdT+ myofibers. n=5 mice. *p<0.05.

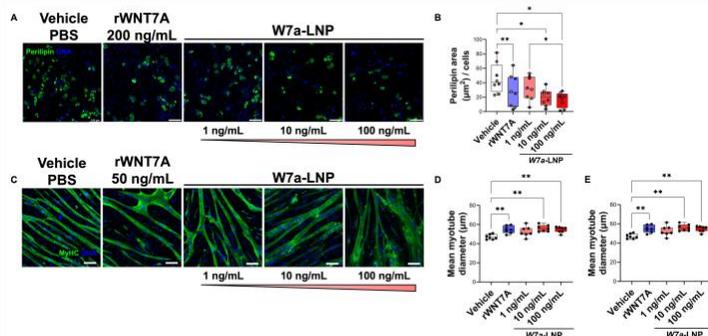


Fig. 2 W7a-LNPs reduce FAP adipogenesis and increase myotube size. (A) Representative immunofluorescent images of perilipin-labeled FAPs cultured in adipogenic differentiation media ± rWNT7A (200 ng/mL)/W7a-LNP (1, 10, 100 ng/mL). Scale bar: 100 μm. (B) Perilipin area normalized by cell quantity. n=8 mice/group (4 female/male). *p<0.05; **p<0.01. (C) Representative immunofluorescent images of myosin heavy chain-labeled myotubes cultured in differentiation media ± rWNT7A (200 ng/mL)/W7a-LNP (1, 10, 100 ng/mL). Scale bar: 100 μm. (D) Mean myotube diameter and (E) fusion index (%). n=7 technical replicates/group. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

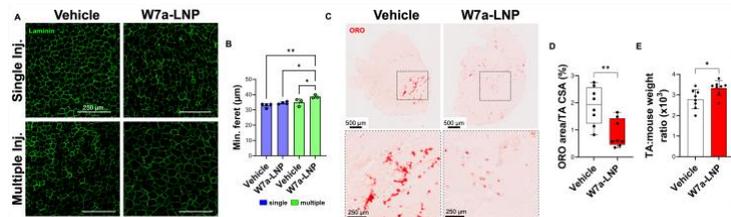


Fig. 3 W7a-LNPs promote myofiber hypertrophy and reduce IMAT. (A) Representative images of laminin-labeled, uninjured SS muscle following single or repeated W7a-LNP injections (10 μg/35 μL). Scale bar: 250 μm. (B) Minimum Feret's diameter of myofibers. n=3-4 mice/group (2 females, 1-2 males). *p<0.05; **p<0.01. (C) Representative ORO-stained sections of glycerol-injured TA muscle following vehicle (35 μL) or W7a-LNP (10 μg/35 μL) treatment. Scale bar: 500 μm; inset scale bar: 250 μm. (D) ORO area normalized by TA cross-sectional area and (E) TA wet weight normalized by body weight. n=8 mice/group (all female). *p<0.05; **p<0.01.

Discussion: We show that LNPs effectively deliver mRNA payloads to muscle cells, producing and secreting WNT7A, reducing FAP adipogenesis and increasing myotube fusion and size *in vitro*. Our *in vivo* models show that W7a-LNPs can promote myofiber hypertrophy and reduce fatty infiltration of skeletal muscle after glycerol injury.

Significance: Our findings establish W7a-LNPs as more scalable and an effective strategy to reduce adipogenesis, prevent IMAT accumulation, and enhance myofiber hypertrophy, potentially at lower doses than recombinant WNT7A. This approach addresses challenges of dose toxicity, scalability, and production costs, and suggests broader applications of mRNA-LNPs as a therapeutic platform for chronic muscle injuries, myopathies, and other degenerative conditions.

References: [1] Li+ *Neuromuscul Disord.* 2015; [2] Gladstone+ *Am J Sports Med.* 2007; [3] Norris+ *Cell Rep.* 2025; [4] Le Grand+ *Cell Stem Cell.* 2009; [5] Fu+ *Stem Cell Reports.* 2023; [6] Niazi+ *Biologics.* 2023; [7] Vavilis+ *Pharmaceutics.* 2023; [8] Hou+ *Nat Rev Mater.* 2021; [9] Kenjo+ *Nat Commun.* 2021; [10] Norris+ *Skelet Muscle.* 2024

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