

induced obesity (DIO), have improved hepatic insulin sensitivity, and increased hepatocyte oxygen consumption rate. Lin *et al.*, *iScience*, 2020.

SUMMARY

[0004] The present application relates, in part, to the design and generation of RNAi constructs that target the *FAM13A* gene and reduce its expression. The sequence-specific inhibition of *FAM13A* gene expression is useful for reducing abdominal adiposity, reducing body weight, reducing fat mass, improving metabolic parameters including insulin resistance and non-alcoholic steatohepatitis (NASH), and reducing risk of myocardial infarction. Accordingly, in one example, the present application provides an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region comprising a sequence that is substantially complementary to a *FAM13A* mRNA sequence. In one aspect, the present disclosure refers to an RNAi construct comprising a sense strand and an antisense strand, wherein the sense strand comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region, and wherein the antisense strand comprises: {a) a region that has substantial complementarity to at least 15 contiguous nucleotides within nucleotides 4900-5300 or 1300-1375 of the *FAM13A* mRNA sequence set forth in SEQ ID NO: 1, such that there are no more than 2 mismatches between the antisense strand's region of substantial complementarity and the contiguous nucleotides; or (b) a region that has substantial identity to at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2, such that there are no more than 2 mismatches between the antisense strand's region of substantial identity and the contiguous nucleotides. In some examples, the RNAi construct is targeted only to the liver. In some examples, the antisense strand comprises a sequence that is substantially complementary to the sequence of at least 15 contiguous nucleotides of a region of the human *FAM13A* mRNA sequence (SEQ ID NO: 1) with no more than 1, 2, or 3 mismatches. In some examples, the antisense strand comprises a region comprising a sequence that is substantially complementary to at least 15 contiguous nucleotides within particular regions of the *FAM13A* mRNA sequence set forth in SEQ ID NO: 1, such as within nucleotides 1300-1375 or 4900-5300 of SEQ ID NO: 1. In certain examples, the antisense strand comprises a region comprising at least 15 contiguous nucleotides from an antisense sequence listed in **Table 1** or **Table 2**.

[0005] In some examples, the sense strand of the RNAi constructs described herein comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length, about 17 to about 24 base pairs in length, or about 19 to about 21 base pairs in length. In some examples, the sense and antisense strands are each independently about 19 to about 30 nucleotides in length, or about 19 to about 23 nucleotides in length. In some examples, the RNAi constructs comprise one or two blunt ends. In other examples, the RNAi constructs comprise one or two nucleotide overhangs. Such nucleotide overhangs may comprise 1 to 6 unpaired nucleotides and can be located at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense and antisense strand. In certain examples, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the sense strand and the 3' end of the

antisense strand. In other examples, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the antisense strand and a blunt end at the 3' end of the sense strand/5' end of the antisense strand.

[0006] The disclosed RNAi constructs may comprise one or more modified nucleotides, including nucleotides having modifications to the ribose ring, nucleobase, or phosphodiester backbone. In some examples, the RNAi constructs comprise one or more 2'-modified nucleotides. Such 2'-modified nucleotides can include 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-alkyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNA), deoxyribonucleotides, or combinations thereof. In one particular example, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof. In some examples, all of the nucleotides in the sense and antisense strand of the RNAi construct are modified nucleotides. Abasic nucleotides may be incorporated into the disclosed RNAi constructs, for example, as the terminal nucleotide at the 3' end, the 5' end, or both the 3' end and the 5' end of the sense strand. In such examples, the abasic nucleotide may be inverted, *e.g.*, linked to the adjacent nucleotide through a 3'-3' internucleotide linkage or a 5'-5' internucleotide linkage.

[0007] In some examples, the RNAi constructs comprise at least one backbone modification, such as a modified internucleotide or internucleoside linkage. In certain examples, the RNAi constructs described herein comprise at least one phosphorothioate internucleotide linkage. In particular examples, the phosphorothioate internucleotide linkages may be positioned at the 3' or 5' ends of the sense and/or antisense strands. For instance, in some examples, the antisense strand comprises two consecutive phosphorothioate internucleotide linkages between the terminal nucleotides at both the 3' and 5' ends. In some such examples, the sense strand comprises one or two phosphorothioate internucleotide linkages between the terminal nucleotides at its 3' end.

[0008] In some examples, the RNAi constructs of this application may target a particular region of the human *FAM13A* mRNA transcript set forth in SEQ ID NO: 1. In some examples, the sequence of the antisense strand may be fully complementary to the sequence of at least 15 contiguous nucleotides of the specific regions of the human *FAM13A* transcript (SEQ ID NO: 1). In some examples, the sequence of the antisense strand may be substantially complementary to the sequence of at least 15 contiguous nucleotides of the specific

regions of the human *FAM13A* transcript (SEQ ID NO: 1) with no more than 1, 2, or 3 mismatches between the sequence of the antisense strand and the sequence of the specific regions of the human *FAM13A* transcript. In certain examples, the antisense strand and/or the sense strand of the RNAi constructs may comprise or consist of a sequence from the antisense and sense sequences listed in **Table 1**. In some examples, the sense and antisense strands, respectively, comprise or consist of SEQ ID NOs: 15 and 559, SEQ ID NOs: 24 and 568, SEQ ID NOs: 125 and 669, SEQ ID NOs: 127 and 671, SEQ ID NOs: 222 and 766, SEQ ID NOs: 406 and 950, SEQ ID NOs: 448 and 992, SEQ ID NOs: 498 and 1042, SEQ ID NOs: 502 and 1046, SEQ ID NOs: 503 and 1047, SEQ ID NOs: 504 and 1048, SEQ ID NOs: 513 and 1057, SEQ ID NOs: 526 and 1070, SEQ ID NOs: 527 and 1071, SEQ ID NOs: 533 and 1077, or SEQ ID NOs: 534 and 1078.

[0009] In some examples, the RNAi construct comprises particular sequences with particular modification patterns, which are referred to as duplexes herein. In certain examples, the antisense strand and/or the sense strand of the RNAi constructs, with particular modification patterns, may comprise or consist of antisense and sense sequences listed in **Table 2** as particular duplexes. In some examples, the RNAi construct is a duplex called D-1557, D-1597, D-1612, D-1614, D-1623, D-1650, D-1667, D-1680, D-1682, D-1685, D-1686, D-1690, D-1697, D-1698, D-1699, D-1702, D-1704, D-1705, D-1709, D-1768, D-1846, D-1849, D-1853, D-1856, D-1858, D-1861, D-1862, D-1863, D-1864, D-1865, D-1866, D-1868, D-1869, D-1870, D-1871, D-1873, D-1875, D-1876, D-1877, D-1878, D-1879, D-1880, D-1881, D-1883, D-1884, D-1885, D-1886, D-1887, D-1888, D-1899, D-1896, D-1955, D-1970, D-1972, D-1975, D-1976, D-1977, D-1979, D-1980, D-1981, D-1982, D-1983, D-1984, D-1985, D-1987, D-1988, D-1989, D-1990, D-1991, D-1992, D-1993, D-1994, D-1995, D-1996, D-1997, D-1998, D-2000, D-2001, D-2002, D-2003, D-2004, D-2005, D-2012, D-2013, D-2014, D-2017, D-2021, D-2022, D-2023, D-2040, D-2044, D-2045, D-2047, D-2049, D-2051, D-2052, D-2053, D-2054, D-2058, D-2061, D-2075, D-2077, D-2079, D-2080, D-2081, D-2083, D-2090, D-2091, or D-2093. In some examples, the RNAi construct is a duplex observed to knock down FAM13A expression by greater than 80%.

[0010] The disclosed RNAi constructs may comprise a ligand to facilitate delivery or uptake of the RNAi constructs to specific tissues or cells, such as liver or adipose cells. In certain examples, the ligand targets delivery of the RNAi constructs to hepatocytes. In these and other examples, the ligand may comprise galactose, galactosamine, or N-acetyl-

galactosamine (GalNAc). In certain examples, the ligand comprises a multivalent galactose or multivalent GalNAc moiety, such as a trivalent or tetravalent galactose or GalNAc moiety. The ligand may be covalently attached to the 5' or 3' end of the sense strand of the RNAi construct, optionally through a linker. In some examples, the RNAi constructs comprise a ligand and linker comprising a structure according to any one of Formulas I to IX described herein. In certain examples, the RNAi constructs comprise a ligand and linker comprising a structure according to Formula VII. In other examples, the RNAi constructs comprise a ligand and linker comprising a structure according to Formula IV. In some examples, the ligand comprises a long-chain fatty acid such as lauric acid (C12), myristic acid (C14), palmitic acid (C16), stearic acid (C18), eicosapentaenoic acid (C20), or docosanoic acid (C22). In some examples, the ligand is attached through a phosphodiester or phosphorothioate linkage.

[0011] The present application also provides pharmaceutical compositions comprising any of the RNAi constructs described herein and a pharmaceutically acceptable carrier, excipient, or diluent. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the RNAi construct as described herein and a pharmaceutically acceptable carrier or excipient. Such pharmaceutical compositions are particularly useful for reducing expression of the *FAM13A* gene in the cells (*e.g.*, liver or adipose cells) of a patient in need thereof. Patients who may be administered a disclosed pharmaceutical composition include patients diagnosed with or at risk of obesity, including patients displaying a high WHR and patients diagnosed with abdominal obesity. Patients who may be administered a disclosed pharmaceutical composition also can include patients diagnosed with or at risk of metabolic conditions such as fatty liver disease (*e.g.*, NAFLD, NASH, alcoholic fatty liver disease, or alcoholic steatohepatitis), insulin resistance and type 2 diabetes (T2D), hypertriglyceridemia, or hypercholesterolemia. The present application also provides methods of treating patients in need of reduction of expression of the *FAM13A* gene expression in their cells, including patients diagnosed with or at risk of obesity, abdominal obesity, fatty liver disease (*e.g.*, NAFLD, NASH, alcoholic fatty liver disease, or alcoholic steatohepatitis), insulin resistance and type 2 diabetes (T2D), hypertriglyceridemia, or hypercholesterolemia. These methods comprise administering an RNAi construct or pharmaceutical composition described herein. In some examples, the RNAi construct is administered with a ligand that targets the RNAi construct to the liver or hepatocytes.

[0012] The use of *FAM13A*-targeting RNAi constructs in any of the methods described herein or for preparation of medicaments for administration according to the methods described herein is specifically contemplated. For instance, the present application includes a *FAM13A*-targeting RNAi construct for use in treating, preventing, or reducing the risk of developing

obesity, abdominal obesity, fatty liver disease (*e.g.*, NAFLD, NASH, alcoholic fatty liver disease, or alcoholic steatohepatitis), insulin resistance and type 2 diabetes (T2D), hypertriglyceridemia, or hypercholesterolemia in a patient in need thereof. In one aspect, the present disclosure refers to the use of the RNAi construct as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for reducing the expression of FAM13A protein in a patient in need thereof. In yet another aspect, the present disclosure refers to the use of the RNAi construct as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for reducing the body weight or fat mass of a patient.

BRIEF DESCRIPTION OF THE FIGURES

[0013] **Figure 1** shows the results of a genomic analysis performed to examine the association of three common *FAM13A* variants for their association with adjusted for BMI (WHRadjBMI), triglyceride levels, HDL cholesterol levels, systolic blood pressure, and *FAM13A* expression in subcutaneous adipose tissue eQTL data.

[0014] **Figures 2A and 2B** show the results of an *in vitro* dose-response study of *Fam13a* siRNA's effects in Renca cells and primary adipocytes.

[0015] **Figures 3A-3D** show the results of an *in vivo* study of *Fam13a* siRNA's ability to knock down murine *Fam13a* mRNA expression levels in the liver and white adipose tissue of mice.

[0016] **Figures 4A-4C** show the results of an *in vivo* study of *Fam13a* siRNA's effects on body weight and fat mass of mice.

[0017] **Figure 5** is a table showing the effects of C16- and GalNAc-linked *Fam13a* siRNA in obese mice after 60 days of treatment. *Fam13a* siRNAs had significant effects on body weight, fat mass, cumulative food intake, liver weight, insulin levels, total cholesterol, LDL cholesterol, and ALT levels.

[0018] **Figure 6** is a diagram compiling the locations which of a range of human *FAM13A* siRNA triggers target on the human *FAM13A* mRNA transcript. The depicted triggers were all efficacious in reducing *FAM13A* mRNA levels and are divided in this diagram according to whether the maximum observed knockdown for that trigger fell within the range of 40-60% knockdown, 60-80% knockdown, or greater than 80% knockdown.

[0019] **Figures 7A-7R** are depictions of different modification patterns that may be applied to siRNA trigger sequences, with each figure showing a hybridized sense (top) and antisense (bottom) strand. In these figures, the solid circles correspond to 2'-O-methyl ribonucleotides, the open circles correspond to 2'-deoxy-2'-fluoro ("2'-fluoro") ribonucleotides, and the hatched circles correspond to inverted abasic deoxynucleotides. Bold lines indicate where a phosphorothioate bond is used in place of the standard phosphodiester bond between

CLAIMS

What is claimed:

1. An RNAi construct comprising a sense strand and an antisense strand, wherein the sense strand comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region, and

wherein the antisense strand comprises:
 - (a) a region that has substantial complementarity to at least 15 contiguous nucleotides within nucleotides 4900-5300 or 1300-1375 of the *FAM13A* mRNA sequence set forth in SEQ ID NO: 1, such that there are no more than 2 mismatches between the antisense strand's region of substantial complementarity and the contiguous nucleotides;

or
 - (b) a region that has substantial identity to at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2, such that there are no more than 2 mismatches between the antisense strand's region of substantial identity and the contiguous nucleotides.
2. The RNAi construct of claim 1, wherein the antisense strand comprises (a) a region that consists of at least 15 contiguous nucleotides within nucleotides 4900-5300 or 1300-1375 of the *FAM13A* mRNA sequence set forth in SEQ ID NO: 1, or (b) a region that consists of at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2.
3. The RNAi construct of claim 1 or claim 2, wherein the sense strand and antisense strand form a duplex region of 19 to 23 base pairs in length.
4. The RNAi construct of any one of claims 1 to 3, wherein the RNAi construct comprises one or two nucleotide overhangs of 1 to 4 unpaired nucleotides.
5. The RNAi construct of any one of claims 1 to 4, wherein the RNAi construct comprises one or more modified nucleotides.

6. The RNAi construct of claim 5, wherein the one or more modified nucleotides are 2'-fluoro modified nucleotides, a 2'-O-methyl modified nucleotides, a 2'-O-methoxyethyl modified nucleotides, 2'-O-alkyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acid (BNA), deoxyribonucleotides, or combinations thereof.
7. The RNAi construct of any one of claims 1 to 6, wherein the sense strand comprises an abasic nucleotide as the terminal nucleotide at its 3' end, its 5' end, or both its 3' and 5' ends.
8. The RNAi construct of any one of claims 1 to 7, wherein the sense strand, the antisense strand, or both the sense and antisense strands comprise one or more phosphorothioate internucleotide linkages.
9. The RNAi construct of any one of claims 1 to 8, wherein the sense and antisense strands, respectively, comprise or consist of SEQ ID NOs: 15 and 559, SEQ ID NOs: 24 and 568, SEQ ID NOs: 125 and 669, SEQ ID NOs: 127 and 671, SEQ ID NOs: 222 and 766, SEQ ID NOs: 406 and 950, SEQ ID NOs: 448 and 992, SEQ ID NOs: 498 and 1042, SEQ ID NOs: 502 and 1046, SEQ ID NOs: 503 and 1047, SEQ ID NOs: 504 and 1048, SEQ ID NOs: 513 and 1057, SEQ ID NOs: 526 and 1070, SEQ ID NOs: 527 and 1071, SEQ ID NOs: 533 and 1077, or SEQ ID NOs: 534 and 1078.
10. The RNAi construct of any one of claims 1 to 8, wherein the RNAi construct is D-1557, D-1597, D-1612, D-1614, D-1623, D-1650, D-1667, D-1680, D-1682, D-1685, D-1686, D-1690, D-1697, D-1698, D-1699, D-1702, D-1704, D-1705, D-1709, D-1768, D-1846, D-1849, D-1853, D-1856, D-1858, D-1861, D-1862, D-1863, D-1864, D-1865, D-1866, D-1868, D-1869, D-1870, D-1871, D-1873, D-1875, D-1876, D-1877, D-1878, D-1879, D-1880, D-1881, D-1883, D-1884, D-1885, D-1886, D-1887, D-1888, D-1899, D-1896, D-1955, D-1970, D-1972, D-1975, D-1976, D-1977, D-1979, D-1980, D-1981, D-1982, D-1983, D-1984, D-1985, D-1987, D-1988, D-1989, D-1990, D-1991, D-1992, D-1993, D-1994, D-1995, D-1996, D-1997, D-1998, D-2000, D-2001, D-2002, D-2003, D-2004, D-2005, D-2012, D-2013, D-2014, D-2017, D-2021, D-2022, D-2023, D-2040, D-2044, D-2045, D-2047, D-2049, D-2051, D-2052, D-2053, D-2054, D-2058, D-2061, D-2075, D-2077, D-2079, D-2080, D-2081, D-2083, D-2090, D-2091, or D-2093.

11. The RNAi construct of any one of claims 1 to 10, wherein the RNAi construct further comprises a ligand.
12. The RNAi construct of claim 11, wherein the ligand comprises a multivalent galactose moiety or multivalent N-acetyl-galactosamine moiety.
13. The RNAi construct of claim 11, wherein the ligand is a long-chain fatty acid.
14. The RNAi construct of claim 13, wherein the long-chain fatty acid is C22.
15. The RNAi construct of any one of claims 11 to 14, wherein the ligand is covalently attached to the sense strand through a linker.
16. The RNAi construct of any of claims 11 to 14, wherein the ligand is attached through a phosphodiester or phosphorothioate linkage.
17. A pharmaceutical composition comprising the RNAi construct of any one of claims 1 to 16 and a pharmaceutically acceptable carrier or excipient.
18. Use of the RNAi construct of any one of claims 1 to 16 or the pharmaceutical composition of claim 17 in the manufacture of a medicament for reducing the expression of FAM13A protein in a patient in need thereof.
19. The use of claim 18, wherein the patient is diagnosed with or at risk for obesity, abdominal obesity, NASH, hepatosteatosis, insulin resistance, type 2 diabetes, hypertriglyceridemia, or hypercholesterolemia.
20. Use of the RNAi construct of any one of claims 1 to 16 or the pharmaceutical composition of claim 17 in the manufacture of a medicament for reducing the body weight or fat mass of a patient.
21. The use of any of claims 18 to 20, wherein the patient has a waist to hip ratio greater than 1.0.
22. The use of any of claims 18 to 21, wherein the RNAi construct or pharmaceutical composition is formulated for intravenous or subcutaneous administration.

23. The RNAi construct of any one of claims 1 to 16 or the pharmaceutical composition of claim 17 for use in therapy.