GROWTH DIFFERENTIATION FACTOR 15 (GDF-15) POLYPEPTIDES

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 28, 2013, is named A1682WOP.txt and is 253,155 bytes in size.

FIELD OF THE INVENTION

The instant disclosure relates to GDF15 polypeptides, polypeptides comprising GDF15, and the generation and use thereof.

BACKGROUND OF THE INVENTION

Growth differentiation factor 15 (GDF15) is a divergent member of the TGFβ superfamily. It is also called macrophage inhibitory cytokine 1 (MIC1) (Bootcov MR, 1997, *Proc Natl Acad Sci* 94:11514-9.), placental bone morphogenetic factor (PLAB) (Hromas R 1997, *Biochim Biophys Acta.* 1354:40-4), placental transforming growth factor beta (PTGFB) (Lawton LN 1997, *Gene.* 203:17-26), prostate derived factor (PDF) (Paralkar VM 1998, *J Biol Chem.* 273:13760-7), and nonsteroidal anti-inflammatory drug-activated gene (NAG-1) (Baek SJ 2001, *J Biol Chem.* 276: 33384-92).

Human GDF15 gene is located on chromosome 19p13.2-13.1; rat GDF15 gene is located on chromosome 16; and mouse GDF15 gene is located on chromosome 8. The GDF15 open reading frames span two exons (Bottner M 1999, *Gene.* 237:105-11 and NCBI). The mature GDF15 peptide shares low homology with other family members (Katoh M 2006, *Int J Mol Med.* 17:951-5.).

GDF15 is synthesized as a large precursor protein that is cleaved at the dibasic cleavage site to release the carboxyterminal mature peptide. The mouse and rat GDF15 prepro-peptides both contain 303 amino acids. Human full-length precursor contains 308 amino acids. The rodent mature peptides contain 115 amino acids after processing at the RGRR (SEQ ID NO:1) cleavage site. The human mature peptide contains 112 amino acids after processing at the RGRRRAR (SEQ ID NO:2) cleavage site. Human mature GDF15 peptide shares 66.1% and 68.1% sequence similarity with rat and mouse mature GDF15 peptides (Bottner M 1999, *Gene.*

237:105-11; Bauskin AR 2000, *EMBO J.* 19:2212-20; NCBI). There is no glycosylation site in the mature GDF15 peptide.

The mature GDF15 peptide contains the seven conserved cysteine residues required for the formation of the cysteine knot motif (having three intrachain disulfide bonds) and the single interchain disulfide bond that are typical for TGFβ superfamily members. The mature GDF15 peptide further contains two additional cysteine residues that form a fourth intrachain disulfide bond. Biologically active GDF15 is a 25KD homodimer of the mature peptide covalently linked by one interchain disulfide bond.

GDF15 circulating levels have been reported to be elevated in multiple pathological and physiological conditions, most notably pregnancy (Moore AG 2000. *J Clin Endocrinol Metab* 85: 4781-4788), β-thalassemia (Tanno T 2007, Nat Med 13:1096-101) (Zimmermann MB, 2008 *Am J Clin Nutr* 88:1026-31), and congenital dyserythropoietic anemia (Tamary H 2008, *Blood.* 112:5241-4). GDF15 has also been linked to multiple biological activities in literature reports. Studies of GDF15 knockout and transgenic mice suggested that GDF15 may be protective against ischemic/reperfusion- or overload-induced heart injury (Kempf T, 2006, *Circ Res.*98:351-60) (Xu J, 2006, *Circ Res.* 98:342-50), protective against aging-associated motor neuron and sensory neuron loss (Strelau J, 2009, *J Neurosci.* 29 :13640-8), mildly protective against metabolic acidosis in kidney, and may cause cachexia in cancer patients (Johnen H 2007 *Nat Med.* 11:1333-40). Many groups also studied the role of GDF15 in cell apoptosis and proliferation and reported controversial results using different cell culture and xenograft models. Studies on transgenic mice showed that GDF15 is protective against carcinogen or Apc mutation induced neoplasia in intestine and lung (Baek SJ 2006, *Gastroenterology.* 131:1553-60; Cekanova M 2009, *Cancer Prev Res* 2:450-8).

SUMMARY OF THE INVENTION

Provided herein is a construct comprising a GDF15 polypeptide and one or more Fc sequences. In one embodiment, the construct comprises two or more Fc sequences. In a further embodiment, the one or more of the Fc sequences independently comprise a sequence selected from the group consisting of SEQ ID NOs:18, 19, 85, 86, 89, 90, 91, 99, 100, 108, 109 and 111. In another embodiment, two or more of the Fc sequences are associated. In still a further embodiment, the

GDF15 polypeptide and one or more of the Fc sequences form a contiguous sequence. In yet another embodiment, the GDF15 polypeptide and an Fc sequence are joined by a linker. In another embodiment, the construct comprises a sequence selected from the group consisting of SEQ ID NOs:44, 50, 57, 61, 65, 69, 74, 79, 84, 95 and 104. In yet a further embodiment, the construct further comprises a sequence selected from the group consisting of SEQ ID NOs:18, 19, 85, 86, 89, 90, 91, 99, 100, 108, 109 and 110. In another embodiment, a dimer comprising the constructs described herein is provided. In a particular embodiment, the construct comprises: (a) a sequence comprising SEQ ID NO:18 and a sequence comprising SEQ ID NO:44; (b) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:50; (c) a sequence comprising SEQ ID NO:18 and a sequence comprising SEQ ID NO: 57; (d) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:61; (e) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:65; (f) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:69; (g) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:74; (h) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:79; (i) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:84; (j) a sequence comprising SEQ ID NO:91 and a sequence comprising SEQ ID NO:95; or (k) a sequence comprising SEQ ID NO:100 and a sequence comprising SEQ ID NO:104, as well as a dimer comprising the construct.

Also provided herein is a construct comprising a GDF15 polypeptide and two or more Fc sequences, wherein one or more Fc sequences independently comprise a sequence selected from the group consisting of SEQ ID NOs:23, 110, 114 and 115. In one embodiment, two or more Fc sequences are associated. In a further embodiment, the GDF15 polypeptide and one or more of the Fc sequences form a contiguous sequence. In yet another embodiment, the GDF15 polypeptide and an Fc sequence are joined by a linker. In still a further embodiment, the construct comprises SEQ ID NO:113. In another embodiment a dimer comprising the constructs described herein is provided. In a particular embodiment, the construct comprises: a sequence comprising SEQ ID NO:110 and a sequence comprising SEQ ID NO:113, as well as a dimer comprising the construct.

Also provided herein is a construct comprising a GDF15 polypeptide and two or more Fc sequences, wherein the Fc sequences comprise SEQ ID NO:28 and SEQ ID NO:30. In one embodiment the two Fc sequences are joined by a linker. In

another embodiment the linker comprises a sequence selected from the group consisting of SEQ ID NOs: 87, 88 and 131. In still another embodiment, the C-terminus of a first Fc sequence is joined to the N-terminus of a second Fc sequence. In yet another embodiment, the GDF15 polypeptide and one or more of the Fc sequences form a contiguous sequence. In still a further embodiment, the GDF15 polypeptide and an Fc sequence are joined by a linker. In another embodiment, the linker comprises SEQ ID NO:31. In still a further embodiment, a construct comprising a dimer of the constructs described herein is provided. In a particular embodiment, the construct comprises a sequence selected from the group consisting of SEQ ID NOs:33, 123 and 128, as well as a dimer comprising the construct.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a series of graphics depicting the arrangement of the charged pair (delHinge) construct DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) (Figure 1A); the charged pair construct CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) (Figure 1B); and the hemi construct Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 (Figure 1C). Figure 1 discloses "$(G_4S)_4$" (SEQ ID NO: 20), "GS$(G_4S)_4$" (SEQ ID NO:31) and "$(G_4S)_8$" (SEQ ID NO: 34).

Figure 2 is a table showing the results of a food intake assay in hyperphagic ob/ob mice in which dimers of the following GDF15 constructs were studied: DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+), DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-), DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+), DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-), DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-), DhCpmFc(+)-GDF15:DhCpmFc(-), DhCpmFc(+)-G_4-GDF15:DhCpmFc(-),DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-), DhCpmFc(+)-$(G_4Q)_4$-GDF15:DhCpmFc(-),DhCpmFc(+)-(1K)-GDF15:DhCpmFc(-), DhCpmFc(+)(L351C)-G_4-GDF15:DhCpmFc(-)(L351C), DhCpmFc(+)(S354C)-G_4-GDF15:DhCpmFc(-)(Y349C) CpmFc(+)-(G4S)4-GDF15:CpmFc(-);Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15, Fc-$(G_4S)_3$-Fc-GS$(G_4S)_4$-GDF15, Fc-$(G_4S)_5$-Fc-GS$(G_4S)_4$-GDF15, GDF15 and GDF15 (H6D) variant.

Figure 3 is a plot showing the effect on food intake (g food/g body weight (BW) of ob/ob mice as a function of dose (log [g protein/kg BW]) using a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct.

Figure 4 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-) construct.

Figure 5 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct.

Figure 6 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-) construct.

Figure 7 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-) construct.

Figure 8 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-GDF15:DhCpmFc(-) construct.

Figure 9 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-G_4-GDF15:DhCpmFc(-) construct.

Figure 10 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-) construct.

Figure 11 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-$(G_4Q)_4$-GDF15:DhCpmFc(-) construct.

Figure 12 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)-(1K)-GDF15:DhCpmFc(-) construct.

Figure 13 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)(L351C)-G_4-GDF15:DhCpmFc(-)(L351C) construct.

Figure 14 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the DhCpmFc(+)(S354C)-G_4-GDF15:DhCpmFc(-)(Y349C) construct.

Figure 15 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the CpmFc(+)-$(G_4S)_4$-GDF15:CpmFc(-) construct.

Figure 16 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the Fc-$G(G_4S)_8$-Fc-$GS(G_4S)_4$-GDF15 construct.

Figure 17 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the Fc-$G(G_4S)_3$-Fc-$GS(G_4S)_4$-GDF15 construct.

Figure 18 is a plot showing the effect on food intake of ob/ob mice as a function of dose using a dimer of the Fc-G(G_4S)$_5$-Fc-GS(G_4S)$_4$-GDF15 construct.

Figure 19 is a plot showing the effect on food intake of ob/ob mice as a function of dose using native mature GDF15 dimer.

Figure 20 is a plot showing the effect on food intake of ob/ob mice as a function of dose using native mature hGDF15 dimer.

Figure 21 is a plot showing the effect on food intake of ob/ob mice as a function of dose using mature hGDF15(H6D) variant dimer

Figure 22 is a bar graph of the results of a lipid tolerance assay showing the effect on triglyceride (mg/dL) for native mature GDF15 dimer (1 mg/kg, i.v.), a dimer of the Fc-(G_4S)$_8$-Fc-GS(G_4S)$_4$-GDF15 construct (1 mg/kg, i.v.) and control.

Figure 23 is a plot of the results of a lipid tolerance assay using a dimer of the DhCpmFc(-)-(G_4S)$_4$-GDF15:DhCpmFc(+) construct, showing effect on triglyceride (mg/dL) as a function of dose (log[g construct/kg BW]).

Figure 24 is a plot showing the results of a lipid tolerance assay using a dimer of the native mature hGDF15.

Figure 25 is a plot showing the results of a lipid tolerance assay using a dimer of the mature hGDF15(H6D) variant.

Figure 26 is plot showing the results of a two week OGTT performed using a dimer of the DhCpmFc(-)-(G_4S)$_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-(G_4S)$_8$-Fc-GS(G_4S)$_4$-GDF15 construct and rosiglitizone (Rosi) in DIO (diet-induced obese) mice.

Figure 27 is bar graph summarizing the data of Figure 26 in the form of AUC data.

Figure 28 is a plot showing the results of a five week OGTT performed using a dimer of the DhCpmFc(-)-(G_4S)$_4$-GDF15:DhCpmFc(+) construct, a dimer of Fc-(G_4S)$_8$-Fc-GS(G_4S)$_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 29 is a bar graph summarizing the data of Figure 28 in the form of AUC data.

Figure 30 is a plot showing the effect on the body weight (g) of DIO mice of a dimer of the DhCpmFc(-)-(G_4S)$_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-(G_4S)$_8$-Fc-GS(G_4S)$_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 31 is a plot showing the effect on the food intake (g food/animal/day) of DIO mice of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 32 is a bar graph showing the effect on the glucose levels (mg/dL) of DIO mice after 4 hour fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 33 is a plot showing the effect on insulin levels (ng/mL) of DIO mice after 4 hour fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 34 is a bar graph showing the effect on insulin levels (ng/mL) of DIO mice after overnight fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 and rosiglitizone (Rosi).

Figure 35 is a plot showing the effect on the triglyceride levels (mg/dL) of DIO mice after 4 hour fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 36 is a bar graph showing the effect on the triglyceride levels (mg/dL) of DIO mice after overnight fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 37 is a plot showing the effect on the total cholesterol levels (mg/dL) of DIO mice after 4 hour fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 38 is a bar graph showing the effect on the total cholesterol levels (mg/dL) of DIO mice after overnight fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and rosiglitizone (Rosi).

Figure 39 is a plot showing the results of a two week OGTT after 4 hour fast performed on DIO mice dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-

GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 40 is a bar graph summarizing the data of Figure 39 in the form of AUC data.

Figure 41 is a plot showing the results of a five week OGTT after an overnight fast performed on DIO mice dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 42 is a bar graph summarizing the data of Figure 41 in the form of AUC data.

Figure 43 is a plot showing the effect on the body weight of DIO mice dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 44 is a plot showing the effect on food intake of DIO mice dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 45 is a bar graph showing the effect on glucose levels (mg/dL) of DIO mice after 4 hour fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 46 is a plot showing the effect on the insulin levels (ng/mL) of DIO mice after 4 hour fast dosed with a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 47 is a bar graph showing the effect on the insulin levels of DIO mice fed *ad libitum* using a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 48 is a bar graph showing the effect on the insulin levels (ng/ml) of DIO mice after overnight fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 49 is a bar graph showing the effect on the triglyceride levels (mg/dL) of DIO mice after 4 hour fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-

GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 50 is a bar graph showing the effect on the triglyceride levels (mg/dL) of DIO mice fed *ad libitum* of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 51 is a bar graph showing the effect on the triglyceride levels (mg/dL) of DIO mice after overnight fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 52 is a bar graph showing the effect on the total cholesterol levels (mg/dL) of DIO mice after 4 hour fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 53 is a plot showing the effect on the total cholesterol levels (mg/dL) of DIO mice fed *ad libitum* of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 54 is a bar graph showing the effect on the total cholesterol levels (mg/dL) of DIO mice after overnight fast of a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct and rosiglitizone (Rosi).

Figure 55 is a diagram graphically depicting the study design for a five-week treatment performed in obese *cynomolgous* monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct.

Figure 56 is a plot showing the serum levels (ng/mL) of a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct and a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct in the *cynomolgous* monkeys studied.

Figure 57 is a bar graph showing the results of an acclimation OGTT following an overnight fast performed on cynomolgous monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle, in the form of glucose AUC data.

Figure 58 is a bar graph showing the results of OGTT following an overnight fast performed on cynomolgous monkeys after 2 week administration of a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle, in the form of glucose AUC data.

Figure 59 is a bar graph showing the results of a OGTT following an overnight fast performed on cynomolgous monkeys after 5 week administration of a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle, in the form of glucose AUC data.

Figure 60 is a bar graph showing the results of an OGTT following an overnight fast performed on cynomolgous monkeys after 4 week of wash out using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle in the form of glucose AUC data.

Figure 61 is a bar graph showing the results of an acclimation OGTT following an overnight fast performed on cynomolgous monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle, in the form of insulin AUC data.

Figure 62 is a bar graph showing the results of OGTT following an overnight fast performed on cynomolgous monkeys after 2 week administration of a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle, in the form of insulin AUC data.

Figure 63 is a bar graph showing the results of a OGTT following an overnight fast performed on cynomolgous monkeys after 5 week administration of a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle, in the form of insulin AUC data.

Figure 64 is a bar graph showing the results of a OGTT following an overnight fast performed on cynomolgous monkeys 4 week of wash out of a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhFc(+) construct and vehicle in the form of insulin AUC data.

Figure 65 is a plot of body weight (kg) as a function of time (days) collected in the month preceding, during a 30 day treatment and subsequent washout period performed on cynomolgous monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle.

Figure 66 is a plot of daily food intake (g) as a function of time (days) collected in the week preceding, during a 5 week treatment and subsequent 4 week washout period performed on cynomolgous monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle.

Figure 67 is a plot of fasting triglyceride (mg/dL) as a function of time (days) collected in the week preceding, during a 5 week treatment and subsequent 4 week washout period performed on cynomolgous monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle.

Figure 68 is a plot of fasting insulin (ng/dL) as a function of time (days) collected in the week preceding, during a 5 week treatment and subsequent 4 week washout period performed on cynomolgous monkeys using a dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and vehicle.

DETAILED DESCRIPTION OF THE INVENTION

The instant disclosure provides GDF15 polypeptides and constructs comprising GDF15 polypeptides. Also provided is the generation and uses of the disclosed molecules, for example in treating a metabolic disorder, such as Type 2 diabetes, elevated glucose levels, elevated insulin levels, dyslipidemia or obesity.

Recombinant polypeptide and nucleic acid methods used herein, including in the Examples, are generally those set forth in Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989) or Current Protocols in Molecular Biology (Ausubel et al., eds., Green Publishers Inc. and Wiley and Sons 1994), both of which are incorporated herein by reference for any purpose.

I. General Definitions

Following convention, as used herein "a" and "an" mean "one or more" unless specifically indicated otherwise.

As used herein, the terms "amino acid" and "residue" are interchangeable and, when used in the context of a peptide or polypeptide, refer to both naturally occurring and synthetic amino acids, as well as amino acid analogs, amino acid mimetics and

non-naturally occurring amino acids that are chemically similar to the naturally occurring amino acids.

The terms "naturally occurring amino acid" and "naturally encoded amino acid" are used interchangeably and refer to an amino acid that is encoded by the genetic code, as well as those amino acids that are encoded by the genetic code that are modified after synthesis, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine.

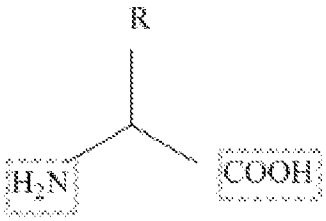
An "amino acid analog" is a compound that has the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs can have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but will retain the same basic chemical structure as a naturally occurring amino acid.

An "amino acid mimetic" is a chemical compound that has a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Examples include a methacryloyl or acryloyl derivative of an amide, β-, γ-, δ-imino acids (such as piperidine-4-carboxylic acid) and the like.

The terms "non-naturally occurring amino acid" and "non-naturally encoded amino acid" are used interchangeably and refer to a compound that has the same basic chemical structure as a naturally occurring amino acid, but is not incorporated into a growing polypeptide chain by the translation complex. "Non-naturally occurring amino acid" also includes, but is not limited to, amino acids that occur by modification (*e.g.*, posttranslational modifications) of a naturally encoded amino acid (including but not limited to, the 20 common amino acids) but are not themselves naturally incorporated into a growing polypeptide chain by the translation complex. A non-limiting lists of examples of non-naturally occurring amino acids that can be inserted into a polypeptide sequence or substituted for a wild-type residue in polypeptide sequence include β-amino acids, homoamino acids, cyclic amino acids and amino acids with derivatized side chains. Examples include (in the L-form or D-form; abbreviated as in parentheses): citrulline (Cit), homocitrulline (hCit), Nα-methylcitrulline (NMeCit), Nα-methylhomocitrulline (Nα-MeHoCit), ornithine (Orn), Nα-Methylornithine (Nα-MeOrn or NMeOrn), sarcosine (Sar), homolysine (hLys or hK), homoarginine (hArg or hR), homoglutamine (hQ), Nα-methylarginine (NMeR),

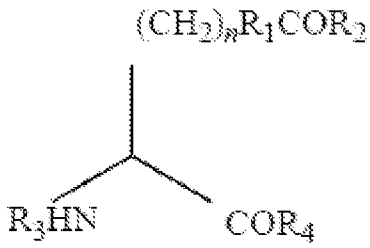
Nα-methylleucine (Nα-MeL or NMeL), N-methylhomolysine (NMeHoK), Nα-methylglutamine (NMeQ), norleucine (Nle), norvaline (Nva), 1,2,3,4-tetrahydroisoquinoline (Tic), Octahydroindole-2-carboxylic acid (Oic), 3-(1-naphthyl)alanine (1-Nal), 3-(2-naphthyl)alanine (2-Nal), 1,2,3,4-tetrahydroisoquinoline (Tic), 2-indanylglycine (IgI), para-iodophenylalanine (pI-Phe), para-aminophenylalanine (4AmP or 4-Amino-Phe), 4-guanidino phenylalanine (Guf), glycyllysine (abbreviated "K(Nε-glycyl)" or "K(glycyl)" or "K(gly)"), nitrophenylalanine (nitrophe), aminophenylalanine (aminophe or Amino-Phe), benzylphenylalanine (benzylphe), γ-carboxyglutamic acid (γ-carboxyglu), hydroxyproline (hydroxypro), p-carboxyl-phenylalanine (Cpa), α-aminoadipic acid (Aad), Nα-methyl valine (NMeVal), N-α-methyl leucine (NMeLeu), Nα-methylnorleucine (NMeNle), cyclopentylglycine (Cpg), cyclohexylglycine (Chg), acetylarginine (acetylarg), α, β-diaminopropionoic acid (Dpr), α, γ-diaminobutyric acid (Dab), diaminopropionic acid (Dap), cyclohexylalanine (Cha), 4-methyl-phenylalanine (MePhe), β, β-diphenyl-alanine (BiPhA), aminobutyric acid (Abu), 4-phenyl-phenylalanine (or biphenylalanine; 4Bip), α-amino-isobutyric acid (Aib), beta-alanine, beta-aminopropionic acid, piperidinic acid, aminocaprioic acid, aminoheptanoic acid, aminopimelic acid, desmosine, diaminopimelic acid, N-ethylglycine, N-ethylaspargine, hydroxylysine, allo-hydroxylysine, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, 4-hydroxyproline (Hyp), γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, ω-methylarginine, 4-Amino-O-Phthalic Acid (4APA), N-acetylglucosaminyl-L-serine, N-acetylglucosylaminyl-L-threonine, O-phosphotyrosine and other similar amino acids, and derivatized forms of any of those specifically listed.

Also included in the definition of "non-naturally occurring amino acid" is any amino acid comprising the structure



wherein the R group is any substituent other than the one used in the twenty natural amino acids.

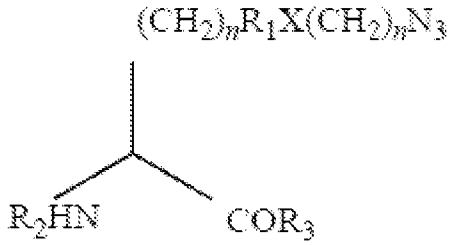
In some embodiments, the non-naturally encoded amino acid comprises a carbonyl group. In some embodiments, the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R_1 is an alkyl, aryl, substituted alkyl, or substituted aryl; R_2 is H, an alkyl, aryl, substituted alkyl, and substituted aryl; and R_3 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R_4 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

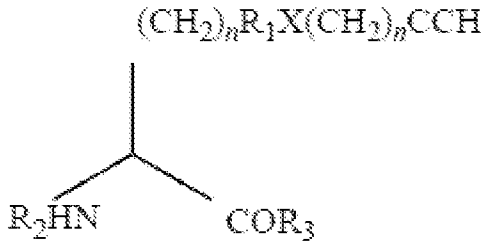
In some embodiments, the non-naturally encoded amino acid comprises an aminooxy group. In some embodiments, the non-naturally encoded amino acid comprises a hydrazide group. In some embodiments, the non-naturally encoded amino acid comprises a hydrazine group. In some embodiments, the non-naturally encoded amino acid residue comprises a semicarbazide group.

In some embodiments, the non-naturally encoded amino acid residue comprises an azide group. In some embodiments, the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R_1 is an alkyl, aryl, substituted alkyl, substituted aryl or not present; X is O, N, S or not present; m is 0-10; R_2 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R_3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

In some embodiments, the non-naturally encoded amino acid comprises an alkyne group. In some embodiments, the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R_1 is an alkyl, aryl, substituted alkyl, or substituted aryl; X is O, N, S or not present; m is 0-10, R_2 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R_3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

The term "substituted" means that a hydrogen atom on a molecule or group is replaced with a group or atom, which is referred to as a substituent. Typical substitutents include: halogen, C_{1-8}alkyl, hydroxyl, C_{1-8}alkoxy, $-NR^xR^x$, nitro, cyano, halo or perhaloC_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, $-SR^x$, $-S(=O)_2R^x$, $-C(=O)OR^x$, $-C(=O)R^x$, wherein each R^x is independently hydrogen or C_1-C_8 alkyl. It is noted that when the substituent is $-NR^xR^x$, the R^x groups may be joined together with the nitrogen atom to form a ring.

The term "alkyl" means a straight or branched chain hydrocarbon. Representative examples of alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, sec-butyl, pentyl and hexyl. Typical alkyl groups are alkyl groups having from 1 to 8 carbon atoms, which groups are commonly represented as C_{1-8}alkyl.

The term "alkoxy" means an alkyl group bonded to an oxygen atom. Representative examples of alkoxy groups include methoxy, ethoxy, tert-butoxy, propoxy and isobutoxy. Common alkoxy groups are C_{1-8}alkoxy.

The term "halogen" or "halo" means chlorine, fluorine, bromine or iodine.

The term "alkenyl" means a branched or straight chain hydrocarbon having one or more carbon-carbon double bonds. Representative examples alkenyl groups include ethenyl, propenyl, allyl, butenyl and 4-methylbutenyl. Common alkenyl groups are C_{2-8}alkenyl.

The term "alkynyl" means a branched or straight chain hydrocarbon having one or more carbon-carbon triple bonds. Representative examples of alkynyl groups include ethynyl, propynyl (propargyl) and butynyl. Common alkynyl groups are C_{2-8} alkynyl.

The term "cycloalkyl" means a cyclic, nonaromatic hydrocarbon. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl. A cycloalkyl group can contain one or more double bond. Examples of cycloalkyl groups that contain double bonds include cyclopentenyl, cyclohexenyl, cyclohexadienyl and cyclobutadienyl. Common cycloalkyl groups are C_{3-8} cycloalkyl groups.

The term "perfluoroalkyl" means an alkyl group in which all of the hydrogen atoms have been replaced with fluorine atoms. Common perfluoroalkyl groups are C_{1-8}perfluoroalkyl. An example of a common perfluoroalkyl group is $-CF_3$.

The term "acyl" means a group derived from an organic acid by removal of the hydroxy group (-OH). For example, the acyl group $CH_3C(=O)-$ is formed by the removal of the hydroxy group from $CH_3C(=O)OH$.

The term "aryl" means a cyclic, aromatic hydrocarbon. Examples of aryl groups include phenyl and naphthyl. Common aryl groups are six to thirteen membered rings.

The term "heteroatom" as used herein means an oxygen, nitrogen or sulfur atom.

The term "heteroaryl" means a cyclic, aromatic hydrocarbon in which one or more carbon atoms of an aryl group have been replaced with a heteroatom. If the heteroaryl group contains more than one heteroatom, the heteroatoms may be the same or different. Examples of heteroaryl groups include pyridyl, pyrimidinyl, imidazolyl, thienyl, furyl, pyrazinyl, pyrrolyl, indolyl, triazolyl, pyridazinyl, indazolyl, purinyl, quinolizinyl, isoquinolyl, quinolyl, naphthyridinyl, quinoxalinyl, isothiazolyl and benzo[b]thienyl. Common heteroaryl groups are five to thirteen membered rings that contain from 1 to 4 heteroatoms. Heteroaryl groups that are five and six membered rings that contain 1 to 3 heterotaoms are particularly common.

The term "heterocycloalkyl" means a cycloalkyl group in which one or more of the carbon atoms has been replaced with a heteroatom. If the heterocycloalkyl group contains more than one heteroatom, the heteroatoms may be the same or different. Examples of heterocycloalkyl groups include tetrahydrofuryl, morpholinyl, piperazinyl, piperidinyl and pyrrolidinyl. It is also possible for the heterocycloalkyl group to have one or more double bonds, but is not aromatic. Examples of heterocycloalkyl groups containing double bonds include dihydrofuran. Common heterocycloalkyl groups are three to ten membered rings containing from 1 to 4 heteroatoms. Heterocycloalkyl groups that are five and six membered rings that contain 1 to 2 heterotaoms are particularly common.

It is also noted that the cyclic ring groups, i.e., aryl, heteroaryl, cycloalkyl, and heterocycloalkyl, can comprise more than one ring. For example, the naphthyl group is a fused bicyclic ring system. It is also intended that the present invention include ring groups that have bridging atoms, or ring groups that have a spiro orientation.

Representative examples of five to six membered aromatic rings, optionally having one or two heteroatoms, are phenyl, furyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, pyridinyl, pyridiazinyl, pyrimidinyl, and pyrazinyl.

Representative examples of partially saturated, fully saturated or fully unsaturated five to eight membered rings, optionally having one to three heteroatoms, are cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and phenyl. Further exemplary five membered rings are furyl, thienyl, pyrrolyl, 2-pyrrolinyl, 3-pyrrolinyl, pyrrolidinyl, 1,3-dioxolanyl, oxazolyl, thiazolyl, imidazolyl, 2H-imidazolyl, 2-imidazolinyl, imidazolidinyl, pyrazolyl, 2-pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, 1,2-dithiolyl, 1,3-dithiolyl, 3H-1,2-oxathiolyl, 1,2,3-oxadizaolyl, 1,2,4-

oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4oxadiazolyl, 1,2,3-triazolyl, 1,2,4-trizaolyl, 1,3,4-thiadiazolyl, 3H-1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, 1,3,4-dioxazolyl, 5H-1,2,5-oxathiazolyl, and 1,3-oxathiolyl.

Further exemplary six membered rings are 2H-pyranyl, 4H-pyranyl, pyridinyl, piperidinyl, 1,2-dioxinyl, 1,3-dioxinyl, 1,4-dioxanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, pyndazinyl, pyrimidinyl, pyrazinyl, piperazinyl, 1,3,5-triazinyl, 1,2,4-triazinyl, 1,2,3-triazinyl, 1,3,5-trithianyl, 4H-1,2-oxazinyl, 2H-1,3-oxazinyl, 6H-1,3-oxazinyl, 6H-1,2-oxazinyl, 1,4-oxazinyl, 2H-1,2-oxazinyl, 4H-1,4-oxazinyl, 1,2,5-oxathiazinyl, 1,4-oxazinyl, o-isoxazinyl, p-isoxazinyl, 1,2,5-oxathiazinyl, 1,2,6-(3 oxathiazinyl, and 1,4,2-oxadiazinyl.

Further exemplary seven membered rings are azepinyl, oxepinyl, thiepinyl and 1,2,4-triazepinyl.

Further exemplary eight membered rings are cyclooctyl, cyclooctenyl and cyclooctadienyl.

Exemplary bicyclic rings consisting of two fused partially saturated, fully saturated or fully unsaturated five and/or six membered rings, optionally having one to four heteroatoms, are indolizinyl, indolyl, isoindolyl, indolinyl, cyclopenta(b)pyridinyl, pyrano(3,4-b)pyrrolyl, benzofuryl, isobenzofuryl, benzo(b)thienyl, benzo(c)thienyl, 1H-indazolyl, indoxazinyl, benzoxazolyl, anthranilyl, benzimidazolyl, benzthiazolyl, purinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, indenyl, isoindenyl, naphthyl, tetralinyl, decalinyl, 2H-1-benzopyranyl, pyrido(3,4-b)pyridinyl, pyrido(3,2-b)pyridinyl, pyrido(4,3-b)-pyridinyl, 2H-1,3-benzoxazinyl, 2H-1,4-benzoxazinyl, 1H-2,3-benzoxazinyl, 4H-3,1-benzoxazinyl, 2H-1,2-benzoxazinyl and 4H-1,4-benzoxazinyl.

A cyclic ring group may be bonded to another group in more than one way. If no particular bonding arrangement is specified, then all possible arrangements are intended. For example, the term "pyridyl" includes 2-, 3-, or 4-pyridyl, and the term "thienyl" includes 2-, or 3-thienyl.

The term “isolated nucleic acid molecule” refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5’ to the 3’ end (*e.g.*, a GDF15 nucleic acid sequence provided herein), or an analog thereof, that has been separated from at least about 50 percent of polypeptides, peptides, lipids, carbohydrates, polynucleotides or other materials with which the nucleic acid is

naturally found when total nucleic acid is isolated from the source cells. Preferably, an isolated nucleic acid molecule is substantially free from any other contaminating nucleic acid molecules or other molecules that are found in the natural environment of the nucleic acid that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "isolated polypeptide" refers to a polypeptide (*e.g.*, a GDF15 polypeptide sequence provided herein) that has been separated from at least about 50 percent of polypeptides, peptides, lipids, carbohydrates, polynucleotides, or other materials with which the polypeptide is naturally found when isolated from a source cell. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "encoding" refers to a polynucleotide sequence encoding one or more amino acids. The term does not require a start or stop codon. An amino acid sequence can be encoded in any one of the different reading frames provided by a polynucleotide sequence.

The terms "identical" and percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. "Percent identity" means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) can be addressed by a particular mathematical model or computer program (*i.e.*, an "algorithm"). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in *Computational Molecular Biology*, (Lesk, A. M., ed.), (1988) New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., (1987) Sequence Analysis in Molecular Biology, New York: Academic Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo *et al.*, (1988) *SIAM J. Applied Math.* 48:1073.

In calculating percent identity, the sequences being compared are aligned in a way that gives the largest match between the sequences. The computer program used to determine percent identity is the GCG program package, which includes GAP (Devereux *et al.*, (1984) *Nucl. Acid Res.* 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI). The computer algorithm GAP is used to align the two polypeptides or polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3x the average diagonal, wherein the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. In certain embodiments, a standard comparison matrix (*see*, Dayhoff *et al.*, (1978) *Atlas of Protein Sequence and Structure* 5:345-352 for the PAM 250 comparison matrix; Henikoff *et al.*, (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89:10915-10919 for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Recommended parameters for determining percent identity for polypeptides or nucleotide sequences using the GAP program are the following:

Algorithm: Needleman *et al.*, 1970, *J. Mol. Biol.* 48:443-453;

Comparison matrix: BLOSUM 62 from Henikoff *et al.*, 1992, *supra*;

Gap Penalty: 12 (but with no penalty for end gaps)

Gap Length Penalty: 4

Threshold of Similarity: 0

Certain alignment schemes for aligning two amino acid sequences can result in matching of only a short region of the two sequences, and this small aligned region can have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, the selected alignment method (*e.g.*, the GAP program) can be adjusted if so desired to result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

The terms "GDF15 polypeptide" and "GDF15 protein" are used interchangeably and mean a naturally-occurring wild-type polypeptide expressed in a mammal, such as a human or a mouse. For purposes of this disclosure, the term

"GDF15 polypeptide" can be used interchangeably to refer to any full-length GDF15 polypeptide, *e.g.*, SEQ ID NO:4, which consists of 308 amino acid residues and which is encoded by the nucleotide sequence SEQ ID NO:3; any form comprising the active and prodomains of the polypeptide, *e.g.*, SEQ ID NO:8, which consists of 279 amino acid residues and which is encoded by the nucleotide sequence SEQ ID NO:7, and in which the 29 amino acid residues at the amino-terminal end of the full-length GDF15 polypeptide (*i.e.*, which constitute the signal peptide) have been removed; and any form of the polypeptide comprising the active domain from which the prodomain and signal sequence have been removed, *e.g.*, SEQ ID NO:12, which consists of 112 amino acid residues and which is encoded by the nucleotide sequence SEQ ID NO:11, in which the signal sequence and the prodomain have been removed. GDF15 polypeptides can but need not comprise an amino-terminal methionine, which may be introduced by engineering or as a result of a bacterial expression process.

The term "GDF15 mutant polypeptide" encompasses a GDF15 polypeptide in which a naturally occurring GDF15 polypeptide sequence (*e.g.*, SEQ ID NOs:4, 8 or 12) has been modified. Such modifications include, but are not limited to, one or more amino acid substitutions, including substitutions with non-naturally occurring amino acids non-naturally-occurring amino acid analogs and amino acid mimetics.

In one aspect, the term "GDF15 mutant polypeptide" refers to a GDF15 polypeptide sequence (e.g., SEQ ID NOs:4, 8 or 12) in which at least one residue normally found at a given position of a native GDF15 polypeptide is deleted or is replaced by a residue not normally found at that position in the native GDF15 sequence. In some cases it will be desirable to replace a single residue normally found at a given position of a native GDF15 polypeptide with more than one residue that is not normally found at the position; in still other cases it may be desirable to maintain the native GDF15 polypeptide sequence and insert one or more residues at a given position in the protein; in still other cases it may be desirable to delete a given residue entirely; all of these constructs are encompassed by the term "GDF15 mutant polypeptide."

In various embodiments, a GDF15 mutant polypeptide comprises an amino acid sequence that is at least about 85 percent identical to a naturally-occurring GDF15 polypeptide (*e.g.*, SEQ ID NOs:4, 8 or 12). In other embodiments, a GDF15 polypeptide comprises an amino acid sequence that is at least about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to a naturally-occurring GDF15

polypeptide amino acid sequence (*e.g.*, SEQ ID NOs:4, 8 or 12). Such GDF15 mutant polypeptides preferably, but need not, possess at least one activity of a wild-type GDF15 mutant polypeptide, such as the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; the ability to reduce body weight; or the ability to improve glucose tolerance, energy expenditure, or insulin sensitivity. The present invention also encompasses nucleic acid molecules encoding such GDF15 mutant polypeptide sequences.

As stated herein, a GDF15 mutant polypeptide can comprise a signal sequence (residues 1-29 of SEQ ID NO:4) or it can have the signal sequence removed (providing SEQ ID NO:8). In other embodiments, a GDF15 mutant polypeptide can have the signal sequence removed and can also be cleaved at residue 196, separating the primary sequence of the prodomain (residues 30-196 of SEQ ID NO:4) from the primary sequence of the active domain. The naturally-occurring biologically active form of a GDF15 mutant polypeptide is a homodimer comprising the processed mature peptide (SEQ ID NO:12; residues 197-308 of SEQ ID NO:4). Although the GDF15 polypeptides and GDF15 mutant polypeptides, and the constructs comprising such polypeptides are primarily disclosed in terms of human GDF15, the invention is not so limited and extends to GDF15 polypeptides and GDF15 mutant polypeptides and the constructs comprising such polypeptides where the GDF15 polypeptides and GDF15 mutant polypeptides are derived from other species (*e.g.*, cynomolgous monkeys, mice and rats). In some instances, a GDF15 polypeptide or a GDF15 mutant polypeptide can be used to treat or ameliorate a metabolic disorder in a subject is a mature form of a GDF15 mutant polypeptide that is derived from the same species as the subject.

A GDF15 mutant polypeptide is preferably biologically active. In various respective embodiments, a GDF15 polypeptide or a GDF15 mutant polypeptide has a biological activity that is equivalent to, greater to or less than that of the naturally occurring form of the mature GDF15 protein or GDF15 mutant polypeptide from which the signal peptide has been removed from the N-terminus of a full length GDF15 mutant polypeptide sequence and in which the prodomain has been cleaved (but not necessarily removed from) the active domain. Examples of biological activities include the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; the ability to reduce body weight; or the ability to improve glucose

tolerance, lipid tolerance, or insulin sensitivity; the ability to lower urine glucose and protein excretion.

The terms "therapeutically effective dose" and "therapeutically effective amount," as used herein, means an amount of GDF15 or GDF15 mutant polypeptide that elicits a biological or medicinal response in a tissue system, animal, or human being sought by a researcher, physician, or other clinician, which includes alleviation or amelioration of the symptoms of the disease or disorder being treated, *i.e.*, an amount of GDF15 or GDF15 mutant polypeptide that supports an observable level of one or more desired biological or medicinal response, for example lowering blood glucose, insulin, triglyceride, or cholesterol levels; reducing body weight; or improving glucose tolerance, energy expenditure, or insulin sensitivity.

II. GDF15 Polypeptides and Constructs Comprising GDF15, Including Mutant Forms Thereof, and Polynucleotides

A range of GDF15 polypeptides and constructs comprising a GDF15 polypeptide are provided herein. Some of these molecules were studied in a variety of assays, as described in the Examples presented herein below. Some of the GDF15 polypeptides and constructs comprising a GDF15 polypeptide provided herein include those described below.

II.A. Native Mature GDF15 and Variants

II.A.1 Native Mature GDF15

Bearing in mind that the biologically active form of GDF15 comprises a homodimer, the construct designated "native mature GDF15 dimer" in the instant disclosure refers to a homodimer comprising two mature GDF15 monomers, each of which comprises SEQ ID NO:12. The monomer that homodimerizes to form the native mature GDF15 dimer is encoded by the nucleic acid sequence:

```
gcgcgcaacggggaccactgtccgctcgggcccgggcgttgct
gccgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgat
tgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtg
cccgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcc
tgcaccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgcc
agctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgct
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ccagacctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID NO:11)

and comprises the amino acid sequence:

ARNGDHCPLGPGRCCRLHTVRASLEDLGWAD
WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPA
SYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:12).

Thus, the "native mature GDF15 dimer" comprises two covalently associated monomers comprising SEQ ID NO:12.

In some embodiments, a leader, or signal, sequence may be used to direct secretion of a polypeptide. A signal sequence may be positioned within or directly at the 5' end of a polypeptide coding region. Many signal sequences have been identified and may be selected based upon the host cell used for expression, e.g., the cleaved VH21 signal sequence (*see* EP2330197 for discussion of the VH21 signal sequence).

In an embodiment employing the VH21 signal sequence, the monomer that homodimerizes to form the native mature GDF15 dimer is encoded by the nucleic acid sequence (VH21 signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgaccggtgt
ccactccgcgcgcaacggggaccactgtccgctcgggcccgggcgttgct
gccgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgat
tgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtg
cccgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcc
tgcaccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgcc
agctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgct
ccagacctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID NO:15)

and comprises the amino acid sequence (cleaved VH21 signal sequence underlined):

MEWSWVFLFFLSVTTGVHSARNGDHCPLGPGRCCRLHTVRASLEDLGWAD
WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPA
SYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:16)

II.A.2 Mature GDF15(H6D) Variant

The GDF15(H6D) variant is a naturally occurring human GDF15 variant due to a C → G polymorphism, resulting in the His to Asp change at residue 202 in full-length peptide (SEQ ID NO:4); residue 6 in mature peptide (SEQ ID NO:12).

The construct designated "mature GDF15(H6D) dimer" in the instant disclosure refers to a homodimer comprising two mature GDF15(H6D) monomers, each of which comprises SEQ ID NO:38. The monomer that homodimerizes to form the mature GDF15(H6D) dimer is encoded by the nucleic acid sequence:

gcgcgcaacggggacgattgtccgctcgggcccgggcgttgct
gccgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgat
tgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtg
cccgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcc
tgcaccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgcc
agctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgct
ccagacctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID NO:37)

and comprises the amino acid sequence:

ARNGDDCPLGPGRCCRLHTVRASLEDLGWAD
WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPA
SYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:38)

Thus, the "mature GDF15(H6D) dimer" comprises two covalently associated monomers comprising SEQ ID NO:38.

In an embodiment employing the VH21 signal sequence, the monomer that homodimerizes to form the mature GDF15(H6D) dimer is encoded by the nucleic acid sequence (signal sequence underlined):

<u>atggaatggagctgggtctttctcttcttcctgtcagtaacgaccggtgt
ccactcc</u>gcgcgcaacggggacgattgtccgctcgggcccgggcgttgct
gccgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgat
tgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtg
cccgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcc
tgcaccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgcc
agctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgct

ccagacctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID NO:35)

and comprises the amino acid sequence (signal sequence underlined):

MEWSWVFLFFLSVTTGVHSARNGDDCPLGPGRCCRLHTVRASLEDLGWAD
WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPA
SYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:36)

II.A.3 Mature GDF15(N3Q) Variant

The GDF15(N3Q) variant is a human GDF15 mutant with Asn at residue 3 of the mature peptide (SEQ ID NO:12) replaced by Gln to avoid potential N deamidation.

The construct designated "mature GDF15(N3Q) dimer" in the instant disclosure refers to a homodimer comprising two mature GDF15(N3Q) monomers, each of which comprises SEQ ID NO:42. The monomer that homodimerizes to form the mature GDF15(N3Q) dimer is encoded by the nucleic acid sequence:

gcgcgccagggagaccactgtccgctcgggcccgggcgttgct
gccgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgat
tgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtg
cccgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcc
tgcaccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgcc
agctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgct
ccagacctatgatgacttgttagccaaagactgccactgcata (SEQ ID NO:41)

and comprises the amino acid sequence:

ARQGDHCPLGPGRCCRLHTVRASLEDLGWAD
WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPA
SYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI SEQ ID NO:42)

In an embodiment employing the VH21 signal sequence, the monomer that homodimerizes to form the native mature GDF15 dimer is encoded by the nucleic acid sequence (including the cleaved VH21 signal sequence, which is underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgaccggtgt
ccactccgcgcgccagggagaccactgtccgctcgggcccgggcgttgct
gccgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgat
tgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtg
cccgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcc
tgcaccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgcc
agctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgct
ccagacctatgatgacttgttagccaaagactgccactgcata (SEQ ID
NO:39)

and comprises the amino acid sequence (cleaved VH21 signal sequence underlined):

MEWSWVFLFFLSVTTGVHSARQGDHCPLGPGRCCRLHTVRASLEDLGWAD
WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPA
SYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI SEQ ID NO:40).

Thus, the "mature GDF15(N3Q) dimer" comprises two covalently associated monomers comprising SEQ ID NO:42.

II.B. Charged pair (delHinge) Constructs

Constructs designated "charged pair (delHinge)" or "charged pair (delHinge) Fc" in the instant disclosure refer to a construct comprising (i) a "negatively charged" Fc sequence lacking the hinge region and comprising a charged pair mutation and (ii) a "positively charged" Fc sequence lacking the hinge region and comprising a charged pair mutation. Note that use of the terms "positively charged" and "negatively charged" is for ease of reference (*i.e.*, to describe the nature of the charge pair mutations in the Fc sequences) and not to indicate that the overall sequence or construct necessary has a positive or negative charge.

The introduction of an aspartatic acid-to-lysine mutation (E356K) and a glutamic acid-to-lysine mutation (D399K) into the unmodified Fc sequence lacking the hinge region provides the positively charged Fc sequence lacking the hinge region (referred to herein as "DhCpmFc(+)"). The introduction of two lysine-to-aspartate mutations (K392D, K409D) into the unmodified Fc sequence lacking the hinge region provides the negatively charged Fc sequence lacking the hinge region (referred to herein as "DhCpmFc(-)"). The C-terminal lysine (K477) optionally also may be

deleted in the negatively charged DhCpmFc(-) sequence, the positively charged DhCpmFc(+) sequence, or both. *See, e.g.,* SEQ ID NOs: 18, 19, 85, 86, 89, 90, 91, 99, 100, 108, 109 and 111 (DhCpmFc sequences).

When incubated together, the aspartate residues associate with the lysine residues through electrostatic force, facilitating formation of Fc heterodimers between the DhCpmFc(+) and DhCpmFc(-) sequences, and reducing or preventing formation of Fc homodimers between the DhCpmFc(+) sequences or between DhCpmFc(-) sequences.

In some embodiments a heterodimer comprises (i) a mature GDF15 sequence linked directly or via a linker to the C-terminus of a DhCpmFc(-) and (ii) a DhCpmFc(+). In other embodiments the heterodimer comprises (i) a mature GDF15 sequence linked directly or via a linker to the C-terminus of a DhCpmFc(+) and (ii) a DhCpmFc(-). In either event, two such heterodimers associate to form tetramer in which the heterodimers are linked via an interchain disulfide bond between the two GDF15 sequences. *See* Figure 1 for a graphic depiction of a tetramer comprising two heterodimers linked via an interchain disulfide bond between the two human GDF15 sequences, in which each heterodimer is the charged pair (delHinge) construct designated "DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(-)" in the instant disclosure (*i.e.*, where each heterodimer comprises (i) a first monomer comprising a mature GDF15 polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a DhCpmFc(-) sequence and (ii) a second monomer comprising a DhCpmFc(+) sequence).

II.B.1 DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+)

The charged pair (delHinge) construct designated "DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a DhCpmFc(-) sequence and (ii) a second monomer comprising a DhCpmFc(+) sequence. The negatively charged DhCpmFc(-)-$(G_4S)_4$-hGDF15 chain associates with the negatively charged DhCpmFc(-) chain to form the heterodimer. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising the sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK (SEQ ID NO:18),

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising the sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG (SEQ ID NO:19),

and

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising the sequence:

GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:20)

at the N-terminus of the GDF15 polypeptide via peptide bond to the negatively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct

cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagaccactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:43)

and comprises the amino acid sequence (linker double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:44).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta

cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagaccactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:21)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:22).

The second monomer comprising the heterodimer is encoded by the nucleic acid sequence:

gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:46)

and comprises the amino acid sequence of SEQ ID NO:18.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

<u>atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt</u>
<u>ccactcc</u>gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:17)

and comprises the amino acid sequence (signal sequence underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:45).

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct) comprises two monomers comprising SEQ ID NO:18 and two monomers comprising SEQ ID NO:44.

II.B.2 DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a DhCpmFc(+) sequence and (ii) a second monomer comprising a DhCpmFc(-) sequence. The positively charged DhCpmFc(+)-$(G_4S)_4$-hGDF15 chain associates with the negatively charged DhCpmFc(-) chain to form the heterodimer. Two such heterodimers associate to form tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising the sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG (SEQ ID NO:85)

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising the sequence

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK (SEQ ID NO:86),

and

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising SEQ ID NO:20 at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagaccactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa

gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:49)

and comprises the amino acid sequence (linker double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI(SEQ ID NO:50).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagaccactgtccgctcgggcccgg

gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:47)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI(SEQ ID NO:48).

The second monomer comprising the heterodimer is encoded by the nucleic acid sequence:

gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat

gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:53)

and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:51)

and comprises the amino acid sequence (signal sequence underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:52).

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:86 and two monomers comprising SEQ ID NO:50.

II.B.3. DhCpmFc(-)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(+)

The charged pair (delHinge) construct designated "DhCpmFc(-)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(+)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15(H6D) polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a DhCpmFc(-) sequence and (ii) a second monomer comprising a DhCpmFc(+) sequence. The negatively charged DhCpmFc(-)-$(G_4S)_4$-GDF15(H6D) chain associates with the positively charged DhCpmFc(+) chain. Two such heterodimers associate to form a tetramer in which the heterodimers linked via an interchain disulfide bond between the two human GDF15(H6D) sequences.

More particularly, in a specific embodiment, the DhCpmFc(-)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(+) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:18,

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:19, and

(c) two chains (one each heterodimer) of a mature human GDF15(H6D) polypeptide comprising SEQ ID NO:38.

The GDF15(H6D) polypeptide is fused via a linker comprising SEQ ID NO:20 at the N-terminus of the GDF15(H6D) polypeptide via peptide bond to the negatively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

```
gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
```

cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagacgactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:56)

and comprises the amino acid sequence (linker double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDDCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:57)

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct

cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagacgactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:54)

and comprises the amino acid sequence (signal sequence underlined, linker double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDDCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:55)

The second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:46 and comprises the amino acid sequence of SEQ ID NO:18.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:17 and comprises the amino acid sequence of SEQ ID NO: 45.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(+) construct) comprises two monomers comprising SEQ ID NO:18 and two monomers comprising SEQ ID NO:57.

II.B.4. DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15(H6D) polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a DhCpmFc(+) sequence and (ii) a second monomer comprising a DhCpmFc(-) sequence. The positively charged DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D) chain associates with the negatively charged DhCpmFc(-) chain. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15(H6D) sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:85,

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:86, and

(c) two chains (one each heterodimer) of a mature human GDF15(H6D) polypeptide comprising SEQ ID NO:38.

The GDF15(H6D) polypeptide is fused via a linker comprising SEQ ID NO:20 at the N-terminus of the GDF15(H6D) polypeptide via peptide bond to the positively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

```
gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
```

agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagacgactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:60)

and comprises the amino acid sequence (linker double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDDCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:61).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc

caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgcaacggagacgactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:58)

and comprises the amino acid sequence (signal sequence single underlined, linker double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARNGDDCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:59).

The second monomer comprising the heterodimer is encoded by the nucleic acid of SEQ ID NO:53 and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:51 and comprises the amino acid sequence of SEQ ID NO:52.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:86 and two monomers comprising SEQ ID NO:61.

II.B.5. DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15(N3Q) polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a DhCpmFc(+)") sequence and (ii) a second monomer comprising a DhCpmFc(-) sequence. The positively charged DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q) chain then associates with the negatively charged DhCpmFc(-) chain. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15(N3Q) sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:85

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:86, and

(c) two chains (one each heterodimer) of a mature human GDF15(N3Q) polypeptide comprising SEQ ID NO:42.

The GDF15(N3Q) polypeptide is fused via a linker comprising SEQ ID NO:20 at the N-terminus of the GDF15(N3Q) polypeptide via peptide bond to the positively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgccgccagggagaccactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:64)

and comprises the amino acid sequence (linker sequence double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS
GGGGSARQGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:65)

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccggaaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctgaagtccgacggctccttcttcctctatagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtggaggtggtggatccggaggcggtggaagcggaggtggtggatct
ggaggcggtggaagcgcgcgccagggagaccactgtccgctcgggcccgg
gcgttgctgccgtctgcacacggtccgcgcgtcgctggaagacctgggct
gggccgattgggtgctgtcgccacgggaggtgcaagtgaccatgtgcatc
ggcgcgtgcccgagccagttccgggcggcaaacatgcacgcgcagatcaa
gacgagcctgcaccgcctgaagcccgacacggtgccagcgccctgctgcg
tgcccgccagctacaatcccatggtgctcattcaaaagaccgacaccggg
gtgtcgctccagacctatgatgacttgttagccaaagactgccactgcat
atga (SEQ ID NO:62)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS

GGGGSARQGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:63)

The second monomer comprising the heterodimer is encoded by the nucleic acid of SEQ ID NO: 53 and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:51 and comprises the amino acid sequence of SEQ ID NO:52.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:86 and two monomers comprising SEQ ID NO:65.

II.B.6. DhCpmFc(+)-GDF15:DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-GDF15:DhCpmFc(-)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a one mature human GDF15 polypeptide linked to the C-terminus of a DhCpmFc(+) sequence and (ii) a second monomer comprising a DhCpmFc(-) sequence. The positively charged DhCpmFc(+)-GDF15 chain associates with the negatively charged DhCpmFc(-) chain to form the heterodimer. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-GDF15:DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:85

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:86, and

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggagcacggaacggggaccattgtcccctgggacctggtcggtgctgc
cggcttcacaccgtcagagcctctctggaggaccttggatgggctgattg
ggtgctgagccctcgggaggtgcaagtcaccatgtgcatcggggcctgcc
ctagccagttccgcgcagccaacatgcacgctcagatcaaaacctctctt
cacagactgaagcccgacaccgtgccagcaccttgctgtgtgccggcctc
ttataaccccatggtcctcattcagaaaaccgacaccggagtgtcacttc
agacttacgatgacctcctggccaaggactgccactgtatttga (SEQ ID
NO:68)

and comprises the amino acid sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGARNGDHCPLGPGRCC
RLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSL

HRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:69).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggagtggtcttgggtctttctgttcttcctctccgtcaccaccggtgt
gcattctgccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggagcacggaacggggaccattgtcccctgggacctggtcggtgctgc
cggcttcacaccgtcagagcctctctggaggaccttggatgggctgattg
ggtgctgagccctcgggaggtgcaagtcaccatgtgcatcggggcctgcc
ctagccagttccgcgcagccaacatgcacgctcagatcaaaacctctctt
cacagactgaagcccgacaccgtgccagcaccttgctgtgtgccggcctc
ttataaccccatggtcctcattcagaaaaccgacaccggagtgtcacttc
agacttacgatgacctcctggccaaggactgccactgtatttga (SEQ ID NO:66)

and comprises the amino acid sequence (signal sequence underlined,):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT

VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGARNGDHCPLGPGRCC
RLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSL
HRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:67).

The second monomer comprising the heterodimer is encoded by the nucleic acid of SEQ ID NO:53 and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:51 and comprises the amino acid sequence of SEQ ID NO:52.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-GDF15:DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:19 and two monomers comprising SEQ ID NO:69.

II.B.7. DhCpmFc(+)-G_4-GDF15:DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-G_4-GDF15:DhCpmFc(-)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via G_4 (SEQ ID NO:70) linker to the C-terminus of a DhCpmFc(+) sequence and (ii) a second monomer comprising aDhCpmFc(-) sequence. The positively charged DhCpmFc(+)-G_4-GDF15 chain associates with the negatively charged DhCpmFc(-) chain. Two such heterodimers associate to form a tetramer in which the heterodimers linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-G_4-GDF15:DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:85,

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:86, and

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising the sequence

GGGG (SEQ ID NO:70)

at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggcggaggtggaggagcacggaacggggaccattgtcccctgggacct
ggtcggtgctgccggcttcacaccgtcagagcctctctggaggaccttgg
atgggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgca
tcggggcctgccctagccagttccgcgcagccaacatgcacgctcagatc
aaaacctctcttcacagactgaagcccgacaccgtgccagcaccttgctg
tgtgccggcctcttataaccccatggtcctcattcagaaaaccgacaccg
gagtgtcacttcagacttacgatgacctcctggccaaggactgccactgt
atttga (SEQ ID NO:73)

and comprises the amino acid sequence (linker sequence double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT

VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGARNGDHCPLGP
GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI
KTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHC
I (SEQ ID NO:74).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (cleaved signal sequence underlined):

atggagtggtcttgggtctttctgttcttcctctccgtcaccaccggtgt
gcattctgccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggcggaggtggaggagcacggaacggggaccattgtcccctgggacct
ggtcggtgctgccggcttcacaccgtcagagcctctctggaggaccttgg
atgggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgca
tcggggcctgccctagccagttccgcgcagccaacatgcacgctcagatc
aaaacctctcttcacagactgaagcccgacaccgtgccagcaccttgctg
tgtgccggcctcttataaccccatggtcctcattcagaaaaccgacaccg
gagtgtcacttcagacttacgatgacctcctggccaaggactgccactgt
atttga (SEQ ID NO:71)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGARNGDHCPLGP
GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI
KTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHC
I (SEQ ID NO:72).

The second monomer comprising the heterodimer is encoded by the nucleic acid of SEQ ID NO:53 and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:51 and comprises the amino acid sequence of SEQ ID NO:52.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-G_4-GDF15:DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:86 and two monomers comprising SEQ ID NO:74.

II.B.8. DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-)" in the instant disclosure refers to a construct comprising heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a $(G_4S)_2$ (SEQ ID NO:75) linker to the C-terminus of a DhCpmFc(+) sequence and (ii) a second monomer comprising aDhCpmFc(-) sequence. The positively charged DhCpmFc(+)-$(G_4S)_2$-GDF15 chain associates with the negatively charged DhCpmFc(-) chain. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:85,

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:86 and

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising the sequence

GGGGSGGGGS (SEQ ID NO:75)

at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gcgccggaactgctgggcggcccgagcgtgtttctgtttccgc
cgaaaccgaaagataccctgatgattagccgcaccccggaagtgacctgc
gtggtggtggatgtgagccatgaagatccggaagtgaaatttaactggta
tgtggatggcgtggaagtgcataacgcgaaaaccaaaccgcgcgaagaac
agtataacagcacctatcgcgtggtgagcgtgctgaccgtgctgcatcag
gattggctgaacggcaaagaatataaatgcaaagtgagcaacaaagcgct
gccggcgccgattgaaaaaaccattagcaaagcgaaaggccagccgcgcg
aaccgcaggtgtataccctgccgccgagccgcaaagaaatgaccaaaaac
caggtgagcctgacctgcctggtgaaaggcttttatccgagcgatattgc
ggtggaatgggaaagcaacggccagccggaaaacaactataaaaccaccc
cgccggtgctgaaaagcgatggcagcttttttctgtatagcaaactgacc
gtggataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgat
gcatgaagcgctgcataaccattatacccagaaaagcctgagcctgagcc
cgggcggcggcggcggcagcggcggcggcggcagcgcgcgcaacggcgat
cattgcccgctgggcccgggccgctgctgccgcctgcataccgtgcgcgc
gagcctggaagatctgggctgggcggattgggtgctgagcccgcgcgaag
tgcaggtgaccatgtgcattggcgcgtgcccgagccagtttcgcgcggcg
aacatgcatgcgcagattaaaaccagcctgcatcgcctgaaaccggatac
cgtgccggcgccgtgctgcgtgccggcgagctataacccgatggtgctga
ttcagaaaaccgataccggcgtgagcctgcagacctatgatgatctgctg
gcgaaagattgccattgcatttga (SEQ ID NO:78)

and comprises the amino acid sequence (linker sequence double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSARNGD
HCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAA
NMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLL
AKDCHCI (SEQ ID NO:79).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcgccggaactgctgggcggcccgagcgtgtttctgtttccgc
cgaaaccgaaagataccctgatgattagccgcaccccggaagtgacctgc
gtggtggtggatgtgagccatgaagatccggaagtgaaatttaactggta
tgtggatggcgtggaagtgcataacgcgaaaaccaaaccgcgcgaagaac
agtataacagcacctatcgcgtggtgagcgtgctgaccgtgctgcatcag
gattggctgaacggcaaagaatataaatgcaaagtgagcaacaaagcgct
gccggcgccgattgaaaaaaccattagcaaagcgaaaggccagccgcgcg
aaccgcaggtgtataccctgccgccgagccgcaaagaaatgaccaaaaac
caggtgagcctgacctgcctggtgaaaggcttttatccgagcgatattgc
ggtggaatgggaaagcaacggccagccggaaaacaactataaaaccaccc
cgccggtgctgaaaagcgatggcagctttttctgtatagcaaactgacc
gtggataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgat
gcatgaagcgctgcataaccattatacccagaaaagcctgagcctgagcc
cgggcggcggcggcggcagcggcggcggcggcagcgcgcgcaacggcgat
cattgcccgctgggcccgggccgctgctgccgcctgcataccgtgcgcgc
gagcctggaagatctgggctgggcggattgggtgctgagcccgcgcgaag
tgcaggtgaccatgtgcattggcgcgtgcccgagccagtttcgcgcggcg
aacatgcatgcgcagattaaaaccagcctgcatcgcctgaaaccggatac
cgtgccggcgccgtgctgcgtgccggcgagctataacccgatggtgctga
ttcagaaaaccgataccggcgtgagcctgcagacctatgatgatctgctg
gcgaaagattgccattgcatttga (SEQ ID NO:76)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSARNGD
HCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAA
NMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLL
AKDCHCI (SEQ ID NO:77).

The second monomer comprising the heterodimer is encoded by the nucleic acid of SEQ ID NO:53 and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:51 and comprises the amino acid sequence of SEQ ID NO:52.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:86 and two monomers comprising SEQ ID NO:79.

II.B.9. DhCpmFc(+)-$(G_4Q)_2$-GDF15:DhCpmFc(-)

The charged pair (delHinge) construct designated "DhCpmFc(+)-$(G_4Q)_4$-GDF15:DhCpmFc(-)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a $(G_4Q)_4$ (SEQ ID NO:80) linker to the C-terminus of a DhCpmFc(+) sequence and (ii) a second monomer comprising a DhCpmFc(-) sequence. The positively charged DhCpmFc(+)-$(G_4Q)_4$-GDF15chain associates with the negatively charged DhCpmFc(-) chain. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)-$(G_4Q)_4$-GDF15:DhCpmFc(-) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc sequence comprising SEQ ID NO:85,

(b) two chains (one each heterodimer) of an engineered negatively charged Fc sequence comprising SEQ ID NO:86, and

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising the sequence

GGGGQGGGGQGGGGQGGGGQ (SEQ ID NO:80)

at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer is encoded by the nucleic acid sequence:

gccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggaggtggcggagggcagggtggtggaggtcagggaggcggaggacag
ggaggaggtggacaagcacggaacggggaccattgtcccctgggacctgg
tcggtgctgccggcttcacaccgtcagagcctctctggaggaccttggat
gggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgcatc
ggggcctgccctagccagttccgcgcagccaacatgcacgctcagatcaa
aacctctcttcacagactgaagcccgacaccgtgccagcaccttgctgtg
tgccggcctcttataaccccatggtcctcattcagaaaaccgacaccgga

gtgtcacttcagacttacgatgacctcctggccaaggactgccactgtat
ttga (SEQ ID NO:83)

and comprises the amino acid sequence (linker sequence double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGQGGGGQGGGGQ
GGGGQARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:84).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggagtggtcttgggtctttctgttcttcctctccgtcaccaccggtgt
gcattctgccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggaggtggcggagggcagggtggtggaggtcagggaggcggaggacag
ggaggaggtggacaagcacggaacggggaccattgtcccctgggacctgg
tcggtgctgccggcttcacaccgtcagagcctctctggaggaccttggat
gggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgcatc

ggggcctgccctagccagttccgcgcagccaacatgcacgctcagatcaa
aacctctcttcacagactgaagcccgacaccgtgccagcaccttgctgtg
tgccggcctcttataaccccatggtcctcattcagaaaaccgacaccgga
gtgtcacttcagacttacgatgacctcctggccaaggactgccactgtat
ttga (SEQ ID NO:81)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGQGGGGQGGGGQ
GGGGQARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCI
GACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTG
VSLQTYDDLLAKDCHCI (SEQ ID NO:82).

The second monomer comprising the heterodimer is encoded by the nucleic acid of SEQ ID NO:53 and comprises the amino acid sequence of SEQ ID NO:86.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence of SEQ ID NO:51 and comprises the amino acid sequence of SEQ ID NO:52.

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)-$(G_4Q)_4$-GDF15:DhCpmFc(-) construct) comprises two monomers comprising SEQ ID NO:86 and two monomers comprising SEQ ID NO:84.

II.B.10. DhCpmFc(+)(L351C)-G_4-GDF15:DhCpmFc(-)(L351C)

The charged pair (delHinge) construct designated "DhCpmFc(+)(L351C)-G_4-GDF15:DhCpmFc(-)(L351C)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a G_4 (SEQ ID ID:70) linker to the C-terminus of a positively charged Fc monomer lacking the hinge region (referred to herein as

"DhCpmFc(+)(L351C)") and one negatively charged Fc monomer lacking the hinge region (referred to herein as "DhCpmFc(-)(L351C)").

As discussed above, the introduction of an aspartatic acid-to-lysine mutation (E356K) and a glutamic acid-to-lysine mutation (D399K) in the unmodified Fc sequence lacking the hinge region provides the positively charged DhCpmFc(+) sequence. The introduction of two lysine-to-aspartate mutations (K392D, K409D) provides the negatively charged DhCpmFc(-) sequence. The C-terminal lysine (K477) optionally may also deleted in the negatively charged DhCpmFc(-) sequence, the positively charged DhCpmFc(+) sequence, or both. When incubated together, the aspartate residues associate with the lysine residues through electrostatic force, facilitating formation of Fc heterodimers between the DhCpmFc(+) and DhCpmFc(-) sequences, and reducing or preventing formation of Fc homodimers between DhCpmFc(+) sequences or between DhCpmFc(-) sequences.

Introduction of a leucine-to-cysteine mutation (L351C) in the positively charged DhCpmFc(+) sequence (referred to herein as "DhCpmFc(+)(L351C)") and a leucine-to-cysteine mutation (L351C) in the negatively charged DhCpmFc(-) sequence (referred to herein as "DhCpmFc(-)(L351C)") further enhances the formation of Fc heterodimers between the DhCpmFc(+)(L351C) and DhCpmFc(-)(L351C) sequences by the formation of a disulfide bond (or "cysteine clamp") between them. The positively charged DhCpmFc(+)(L351C)-G_4-GDF15chain associates with the negatively charged DhCpmFc(-)(L351C) chain. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)(L351C)-G_4-GDF15:DhCpmFc(-)(L351C) tetramer comprises:

(a) two chains (one each heterodimer) of an engineered positively charged Fc(L351) sequence comprising:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTCPPSRKEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG (SEQ ID NO:90

(b) two chains (one each heterodimer) of an engineered negatively charged Fc(L351) sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTCPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK (SEQ ID NO:91)

and.

(c) two chains (one each heterodimer) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising SEQ ID NO:70 at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc(L351C) sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer is encoded by the nucleic acid sequence:

gccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacacctgtcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggcggaggtggaggagcacggaacggggaccattgtcccctgggacct
ggtcggtgctgccggcttcacaccgtcagagcctctctggaggaccttgg
atgggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgca
tcggggcctgccctagccagttccgcgcagccaacatgcacgctcagatc
aaaacctctcttcacagactgaagcccgacaccgtgccagcaccttgctg

tgtgccggcctcttataaccccatggtcctcattcagaaaaccgacaccg
gagtgtcacttcagacttacgatgacctcctggccaaggactgccactgc
atatga (SEQ ID NO:94)

and comprises the amino acid sequence (linker sequence double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTCPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGARNGDHCPLGP
GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI
KTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHC
I (SEQ ID NO:95).

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctgttcttcctctccgtcaccaccggtgt
gcattctgccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacacctgtcctccgagcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggcggaggtggaggagcacggaacggggaccattgtcccctgggacct
ggtcggtgctgccggcttcacaccgtcagagcctctctggaggaccttgg
atgggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgca

tcggggcctgccctagccagttccgcgcagccaacatgcacgctcagatc
aaaacctctcttcacagactgaagcccgacaccgtgccagcaccttgctg
tgtgccggcctcttataaccccatggtcctcattcagaaaaccgacaccg
gagtgtcacttcagacttacgatgacctcctggccaaggactgccactgc
atatga (SEQ ID NO:92)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTCPPSRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGARNGDHCPLGP
GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI
KTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHC
I (SEQ ID NO:93).

The second monomer comprising the heterodimer is encoded by the nucleic acid sequence:

gcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacacctgtcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:98)

and comprises the amino acid sequence of SEQ ID NO:91.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacacctgtcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:96)

and comprises the amino acid sequence (signal sequence underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTCPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:97)

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the DhCpmFc(+)(L351C)-G_4-GDF15:DhCpmFc(-)(L351C) construct comprises two monomers comprising SEQ ID NO:91 and two monomers comprising SEQ ID NO:95.

II.B.11. DhCpmFc(+)(S354C)-G_4-GDF15:DhCpmFc(-)(Y349C)

The charged pair (delHinge) construct designated "DhCpmFc(+)(S354C)-G_4-GDF15:DhCpmFc(-)(Y349C)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a G_4 (SEQ ID NO:70) linker to the C-terminus of a positively charged Fc monomer lacking the hinge region (referred to herein as "DhCpmFc(+)(S354C)") and one negatively charged Fc monomer lacking the hinge region (referred to herein as "DhCpmFc(-)(Y349C)").

As discussed above, the introduction of an aspartatic acid-to-lysine mutation (E356K) and a glutamic acid-to-lysine mutation (D399K) in the unmodified Fc sequence lacking the hinge region provides the positively charged DhCpmFc(+) sequence. The introduction of two lysine-to-aspartate mutations (K392D, K409D) provides the negatively charged DhCpmFc(-) sequence. . The C-terminal lysine (K477) optionally may also deleted in the negatively charged DhCpmFc(-) sequence, the positively charged DhCpmFc(+) sequence, or both. When incubated together, the aspartate residues associate with the lysine residues through electrostatic force, facilitating formation of Fc heterodimers between the DhCpmFc(+) and DhCpmFc(-) sequences, and reducing or preventing formation of Fc homodimers between DhCpmFc(+) sequences or between DhCpmFc(-) sequences.

Introduction of a leucine-to-cysteine mutation (S354C) in the positively charged DhCpmFc(+) sequence (referred to herein as "DhCpmFc(+)(S354C)") and a leucine-to-cysteine mutation (Y349C) in the negatively charged DhCpmFc(-) sequence (referred to herein as "DhCpmFc(-)(L351C)") further enhances the formation of Fc heterodimers between the two DhCpmFc(+)(S354C) and DhCpmFc(-)(Y349C) sequences by the formation of a disulfide bond (or "cysteine clamp") between them. The positively charged DhCpmFc(+)(S354C)-G_4-GDF15 chain associates with the negatively charged DhCpmFc(-)(Y349C) chain. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the DhCpmFc(+)(S354C)-G_4-GDF15:DhCpmFc(-)(Y349C) tetramer comprises

(a) two chains (one each heterodimer) of an engineered positively charged Fc(S354C) comprising the sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPCRKEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG (SEQ ID NO:99),

(b) two chains (one each heterodimer) of an engineered negatively charged Fc(Y349C) comprising the sequence:

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK (SEQ ID NO:100

and

(c) two chains (one each) of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising SEQ ID NO:70 at the N-terminus of the GDF15 polypeptide via peptide bond to the positively charged Fc(S354C) sequence.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer is encoded by the nucleic acid sequence:

gccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgtgcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc
cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat

gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggcggaggtggaggagcacggaacggggaccattgtcccctgggacct
ggtcggtgctgccggcttcacaccgtcagagcctctctggaggaccttgg
atgggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgca
tcggggcctgccctagccagttccgcgcagccaacatgcacgctcagatc
aaaacctctcttcacagactgaagcccgacaccgtgccagcaccttgctg
tgtgccggcctcttataaccccatggtcctcattcagaaaaccgacaccg
gagtgtcacttcagacttacgatgacctcctggccaaggactgccactgc
atatga (SEQ ID NO:103)

and comprises the amino acid sequence (linker sequence double underlined):

APELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGARNGDHCPLGP
GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI
KTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHC
I (SEQ ID NO:104)

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctgttcttcctctccgtcaccaccggtgt
gcattctgccccagagctgcttggtggaccatccgtgttcctgtttcctc
caaagccgaaggacaccctgatgatctcaagaactccggaagtgacttgc
gtcgtcgtggacgtgtcacatgaggatccagaggtcaagttcaattggta
tgtggacggagtggaagtgcataacgccaagaccaaaccccgcgaagaac
agtacaatagcacctaccgcgtggtgagcgtccttactgtgctccaccag
gactggcttaatgggaaggaatacaagtgtaaggtgtccaacaaggccct
ccccgctcccatcgaaaagaccatctcaaaggcaaaggggcaaccaaggg
aacctcaagtgtacaccctgcctccgtgcaggaaggagatgaccaagaac
caggtcagcctgacttgtctcgtgaagggcttctatcccagcgatattgc
tgtggaatgggagtcaaatggccagcccgagaataactacaaaactaccc

cacccgtgctgaaatctgatgggtccttcttcctttactccaagctgacc
gtggacaagagccgctggcaacaaggcaatgtctttagctgctcagtgat
gcatgaggctctccataatcactacactcagaagtcactgtccctgtcac
ctggcggaggtggaggagcacggaacggggaccattgtcccctgggacct
ggtcggtgctgccggcttcacaccgtcagagcctctctggaggaccttgg
atgggctgattgggtgctgagccctcgggaggtgcaagtcaccatgtgca
tcggggcctgccctagccagttccgcgcagccaacatgcacgctcagatc
aaaacctctcttcacagactgaagcccgacaccgtgccagcaccttgctg
tgtgccggcctcttataaccccatggtcctcattcagaaaaccgacaccg
gagtgtcacttcagacttacgatgacctcctggccaaggactgccactgc
atatga (SEQ ID NO:101)

and comprises the amino acid sequence (signal sequence single underlined, linker sequence double underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRKEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGARNGDHCPLGP
GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI
KTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHC
I (SEQ ID NO:102)

The second monomer comprising the heterodimer is encoded by the nucleic acid sequence:

gcacctgaactcctgggggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtgcaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc

cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:107)

and comprises the amino acid sequence of SEQ ID NO:100.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgcacctgaactcctgggggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtgcaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacgacaccacgc
ctcccgtgctggactccgacggctccttcttcctctatagcgacctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctc
cgggtaaatga (SEQ ID NO:105)

and comprises the amino acid sequence (signal sequence underlined):

MEWSWVFLFFLSVTTGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:106)

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer

(a dimer of the DhCpmFc(+)(S354C)-G_4-GDF15:DhCpmFc(-)(Y349C) construct) comprises two monomers comprising SEQ ID NO:100 and two monomers comprising SEQ ID NO:104.

II.C. Charged pair Construct

Constructs designated "charged pair" or "charged pair Fc" in the instant disclosure refer to a construct comprising a "negatively charged" Fc sequence comprising a charge pair mutation and (ii) a positively charged Fc sequence comprising a charged pair mutation. Note that use of the terms "positively charged" and "negatively charged" is for ease of reference (*i.e.*, to describe the nature of the charge pair mutations in the Fc sequences) and not to indicate that the overall sequence or construct necessarily has a positive or negative charge.

The introduction of an aspartatic acid-to-lysine mutation (E356K) and a glutamic acid-to-lysine mutation (D399K) in the unmodified Fc sequence provides the positively charged Fc sequence (referred to herein as "CpmFc(+)"). The introduction of two lysine-to-aspartate mutations (K392D, K409D) into the unmodified Fc sequence provides the negatively charged Fc sequence (referred to herein as "CpmFc(-)"). The C-terminal lysine (K477) optionally also may be deleted in the negatively charged CpmFc(-) sequence, the positively charged CpmFc(+) sequence, or both. *See, e.g.,* SEQ ID NOs: 23, 110, 114 and 115 (CpmFc sequences).When incubated together, the aspartate residues associate with the lysine residues, facilitating forming of Fc heterodimers between the CpmFc(+) and CpmFc(-) sequences, and reducing or preventing forming of Fc homodimers between the CpmFc(+) and CpmFc(-) sequences.

In some embodiments a heterodimer comprises (i) a mature GDF15 sequence linked directly or via a linker to the C-terminus of a CpmFc(-) and (ii) a CpmFc(+). In other embodiments the heterodimer comprises (i) a mature GDF15 sequence linked directly or via a linker to the C-terminus of a CpmFc(+) and (ii) a CpmFc(-). In either event, two such heterodimers associate to form tetramer in which the heterodimers are linked via an interchain disulfide bond between the two GDF15 sequences. *See* Figure 1 for a graphic depiction of a tetramer comprising two heterodimers linked via an interchain disulfide bond between two human GDF15 sequences, in which each heterodimer is the charged pair construct designated "CpmFc(-)-$(G_4S)_4$-

GDF15CpmFc(-)" in the instant disclosure (*i.e.*, where each heterodimer comprises (i) a first monomer comprising a mature GDF15 polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a CpmFc(-) sequence and (ii) a second monomer comprising a CpmFc(+) sequence).

CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+)

The charged pair construct designated "CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+)" in the instant disclosure refers to a construct comprising a heterodimer, which comprises (i) a first monomer comprising a mature human GDF15 polypeptide linked via a $(G_4S)_4$ (SEQ ID NO:20) linker to the C-terminus of a CpmFc(-) sequence and (ii) a second monomer comprising a CpmFc(+) sequence. Although the hinge region is present, the negatively charged CpmFc(-)-$(G_4S)_4$-hGDF15 chain associates with the negatively charged CpmFc(-) chain to form the heterodimer. Two such heterodimers associate to form a tetramer in which the heterodimers are linked via an interchain disulfide bond between the two human GDF15 sequences.

More particularly, in a specific embodiment, the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct comprises:

(a) two chains (one each heterodimer) of an engineered positively charge Fc sequence comprising the sequence (with the hinge region indicated by parentheses):

(DKTHTCPPCP)APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:110),

(b) two chains (one each) of an engineered negatively charged Fc sequence comprising the sequence (with the hinge region indicated by parentheses):

(DKTHTCPPCP)APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:23,

and

(c) two chains of a native mature human GDF15 polypeptide comprising SEQ ID NO:12.

The GDF15 polypeptide is fused via a linker comprising SEQ ID NO:20 at the N-terminus of the GDF15 polypeptide via peptide bond to the negatively charged Fc monomer.

In its final form, the first monomer comprising the heterodimer, that with another such heterodimer forms the tetramer, is encoded by the nucleic acid sequence:

gacaaaactcacacatgcccaccgtgcccagcacctgaactcc
tgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctc
atgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagcca
cgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgc
ataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaa
ccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
cccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
ggcagccggagaacaactacgacaccacgcctcccgtgctggactccgac
ggctccttcttcctctatagcgacctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
actacacgcagaagagcctctccctgtctccgggtggaggtggtggatcc
ggaggcggtggaagcggaggtggtggatctggaggcggtggaagcgcgcg
caacggagaccactgtccgctcgggcccgggcgttgctgccgtctgcaca
cggtccgcgcgtcgctggaagacctgggctgggccgattgggtgctgtcg
ccacgggaggtgcaagtgaccatgtgcatcggcgcgtgcccgagccagtt
ccgggcggcaaacatgcacgcgcagatcaagacgagcctgcaccgcctga
agcccgacacggtgccagcgccctgctgcgtgcccgccagctacaatccc
atggtgctcattcaaaagaccgacaccggggtgtcgctccagacctatga
tgacttgttagccaaagactgccactgcatatga(SEQ ID NO:112)

and comprises the amino acid sequence (hinge region indicated by parentheses and linker sequence double underlined):

(DKTHTCPPCP)APELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST

YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLD
SDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGG
GSGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWV
LSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASY
NPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:113)

In an embodiment employing the VH21 signal sequence, in its final form, the first monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgacaaaactcacacatgcccaccgtgcccagcacctgaactcc
tgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctc
atgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagcca
cgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgc
ataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaa
ccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
cccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
ggcagccggagaacaactacgacaccacgcctcccgtgctggactccgac
ggctccttcttcctctatagcgacctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
actacacgcagaagagcctctccctgtctccgggtggaggtggtggatcc
ggaggcggtggaagcggaggtggtggatctggaggcggtggaagcgcgcg
caacggagaccactgtccgctcgggcccgggcgttgctgccgtctgcaca
cggtccgcgcgtcgctggaagacctgggctgggccgattgggtgctgtcg
ccacgggaggtgcaagtgaccatgtgcatcggcgcgtgcccgagccagtt
ccgggcggcaaacatgcacgcgcagatcaagacgagcctgcaccgcctga
agcccgacacggtgccagcgccctgctgcgtgcccgccagctacaatccc
atggtgctcattcaaaagaccgacaccggggtgtcgctccagacctatga
tgacttgttagccaaagactgccactgcatatga(SEQ ID NO:24)

and comprises the amino acid sequence (signal sequence single underlined, hinge region in parentheses and linker sequence double underlined):

MEWSWVFLFFLSVTTGVHS(DKTHTCPPCP)APELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLD
SDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGG
GSGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWV
LSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASY
NPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:25).

The second monomer comprising the heterodimer is encoded by the nucleic acid sequence:

gacaaaactcacacatgcccaccgtgcccagcacctgaactcc
tgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctc
atgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagcca
cgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgc
ataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaa
ccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
cccccatcccggaaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
ggcagccggagaacaactacaagaccacgcctcccgtgctgaagtccgac
ggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
actacacgcagaagagcctctccctgtctccgggtaaatga (SEQ ID NO:116)

and comprises the amino acid sequence of SEQ ID NO:110.

In an embodiment employing the VH21 signal sequence, the second monomer comprising the heterodimer is encoded by the nucleic acid sequence (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactccgacaaaactcacacatgcccaccgtgcccagcacctgaactcc

tgggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctc
atgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagcca
cgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgc
ataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaa
ccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
cccccatcccggaaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
ggcagccggagaacaactacaagaccacgcctcccgtgctgaagtccgac
ggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
actacacgcagaagagcctctccctgtctccgggtaaatga (SEQ ID NO:26)

and comprises the amino acid sequence (signal sequence underlined, hinge region in parentheses):

<u>MEWSWVFLFFLSVTTGVHS</u>(DKTHTCPPCP)APELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLK
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:27).

The first and second monomers associate to form the heterodimer. Two such heterodimers associate to form the final tetramer. Accordingly, the resulting tetramer (a dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct) comprises two monomers comprising SEQ ID NO:110 and two monomers comprising SEQ ID NO:113.

<u>II.D. HemiFc Constructs</u>

Constructs designated "hemi" or "hemiFc" in the instant disclosure refer to a construct comprising two Fc sequences joined in tandem by a linker that connects the N-terminus of a first Fc sequence (e.g., SEQ ID NOs: 28 or 30) to the C-terminus of a second Fc sequence (e.g., SEQ ID NOs: 28 or 30). In some embodiments, a monomer comprises a mature GDF15 sequence linked to the first Fc sequence by a first linker

that connects the N-terminus of the GDF15 sequence to the C-terminus of the first Fc sequence, wherein the first Fc sequence is linked to the second Fc sequence by a second linker that connects the N-terminus of the first Fc sequence to the C-terminus of the second Fc sequence. The first and second Fc sequences also are associated by the Fc hinge regions. Two such monomers associate to form a dimer in which the monomers are linked via an interchain disulfide bond between the two GDF15 sequences. *See* Figure 1 for a graphic depiction of a hemi construct "Fc-$(G_4S)_8$-Fc-$GS(G_4S)_4$-GDF15" (*i.e.*, two monomers, each comprising a mature GDF15 polypeptide linked via a $GS(G_4S)_4$ linker to the C-terminus of a first Fc sequence, the N-terminus of which is linked via a $(G_4S)_8$ linker to the C-terminus of a second Fc sequence; and where the two mature GDF15 sequences are linked via an interchain disulfide bond).

II.D.1 Fc-$(G_4S)_8$-Fc-$GS(G_4S)_4$-GDF15

The hemiFc construct designated "Fc-$(G_4S)_8$-Fc-$GS(G_4S)_4$-GDF15" in the instant disclosure refers to a construct comprising a monomer, which comprises a mature human GDF15 sequence linked to a first Fc sequence via a first linker comprising SEQ ID NO:31 that connects the N-terminus of the human GDF15 sequence to the C-terminus of the first Fc sequence and a second linker comprising the amino acid sequence:

GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:34).

that connects the N-terminus of the first Fc sequence to the C-terminus of the second Fc sequence. The first and second Fc sequences also are associated by the Fc hinge regions. Two such monomers associate to form a homodimer in which the monomers are linked via an interchain disulfide bond between the two GDF15 sequences.

More particularly, in a specific embodiment, the hemi construct Fc-$(G_4S)_8$-Fc-$GS(G_4S)_4$-GDF15 comprises a monomer comprising a second Fc chain comprising the sequence (hinge region in parentheses):

GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSP (SEQ ID NO:28)

Joined by the linker:

GGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:29)

to a first Fc chain comprising the sequence (hinge region in parentheses):

(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY
KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSP (SEQ ID NO:30)

to the C-terminus of which, the GDF15 polypeptide comprising SEQ ID NO:12 is joined by a linker having the sequence:

GSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:31)

In its final form, the monomer is encoded by the nucleic acid sequence:

ggaggtggagagcgcaaatcttctgtcgagtgcccaccgtgcccagcacc
acctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggaca
ccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtg
agccacgaagaccccgaggtccagttcaactggtacgtggacggcgtgga
ggtgcataatgccaagacaaaaccacgggaggagcagttcaacagcacgt
tccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggc
aaggagtacaagtgcaaggtctccaacaaaggcctcccagcccccatcga
gaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtaca
ccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacc
tgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagag
caatgggcagccggagaacaactacaagaccacacctcccatgctggact
ccgacggctccttcttcctctacagcaagctcaccgtggacaagagcagg
tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgca
caaccactacacgcagaagagcctctccctgtctccgggtggaggtggcg
gtagcggtggcggaggttcaggtggtggcggttctggcggaggtggcagt
ggcggtggcggatcaggtggcggtggcagcggtggcggcggaagcggtgg
aggaggttcagagcggaaatccagcgttgaatgtcctccgtgccctgctc
cacccgtcgcggggcctagtgtcttcctttccctccaaaaccaaaggat
acactgatgatcagccggacccccgaggttacgtgcgtcgtcgtcgatgt

ctcccacgaggatccagaggtccaattcaactggtacgtggacggggtcg
aggtgcataatgcaaagacaaagccacgggaagagcagtttaactctact
ttccgcgtggtttctgtgctgaccgtggtgcaccaagattggctcaacgg
caaggagtacaagtgcaaggtaagcaataaggggctccctgcccccattg
agaagactatctccaagacaaagggacagccacgcgagccacaagtctat
acactcccccttcccgcgaagaaatgaccaagaatcaggttagcctgac
atgcttggttaagggtttctacccctctgacatagccgtggagtgggaga
gcaatggacaaccagagaacaactacaagaccaccccacccatgctggat
agcgacggttcattctttctgtatagtaagcttaccgtggacaagtcccg
gtggcaacaaggaaatgtcttttcatgctctgtgatgcacgaggccttgc
ataatcactatactcagaagagcttgagcctcagccccggatctggaggt
ggcggatccgggggcggtggaagcggaggtggtggatcgggaggcggtgg
aagcgcgcgcaacggcgaccactgtccgctcgggcccggacgttgctgcc
gtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgattgg
gtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtgccc
gagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcctgc
accgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgccagc
tacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgctcca
gacctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID
NO:32)

and comprises the amino acid sequence (hinge regions in parentheses, linkers sequences double underlined):

GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS
GGGGSGGGGSGGGGSGGGGS(ERKSSVECPPCP)APPVAGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGG
GGSGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADW

VLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPAS
YNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:33)

In an embodiment employing the VH21 signal sequence, in its final form, the monomer is encoded by the nucleic acid (signal sequence underlined):

atggaatggagctgggtctttctcttcttcctgtcagtaacgactggtgt
ccactcc
ggaggtggagagcgcaaatcttctgtcgagtgcccaccgtgcccagcacc
acctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggaca
ccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtg
agccacgaagaccccgaggtccagttcaactggtacgtggacggcgtgga
ggtgcataatgccaagacaaaaccacgggaggagcagttcaacagcacgt
tccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggc
aaggagtacaagtgcaaggtctccaacaaaggcctcccagcccccatcga
gaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtaca
ccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacc
tgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagag
caatgggcagccggagaacaactacaagaccacacctcccatgctggact
ccgacggctccttcttcctctacagcaagctcaccgtggacaagagcagg
tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgca
caaccactacacgcagaagagcctctccctgtctccgggtggaggtggcg
gtagcggtggcggaggttcaggtggtggcggttctggcggaggtggcagt
ggcggtggcggatcaggtggcggtggcagcggtggcggcggaagcggtgg
aggaggttcagagcggaaatccagcgttgaatgtcctccgtgccctgctc
cacccgtcgcggggcctagtgtcttcctttcccctccaaaaccaaaggat
acactgatgatcagccggacccccgaggttacgtgcgtcgtcgtcgatgt
ctcccacgaggatccagaggtccaattcaactggtacgtggacggggtcg
aggtgcataatgcaaagacaaagccacgggaagagcagtttaactctact
ttccgcgtggtttctgtgctgaccgtggtgcaccaagattggctcaacgg
caaggagtacaagtgcaaggtaagcaataaggggctccctgcccccattg
agaagactatctccaagacaaagggacagccacgcgagccacaagtctat
acactccccccttcccgcgaagaaatgaccaagaatcaggttagcctgac
atgcttggttaagggtttctacccctctgacatagccgtggagtgggaga
gcaatggacaaccagagaacaactacaagaccaccccacccatgctggat

agcgacggttcattctttctgtatagtaagcttaccgtggacaagtcccg
gtggcaacaaggaaatgtcttttcatgctctgtgatgcacgaggccttgc
ataatcactatactcagaagagcttgagcctcagccccggatctggaggt
ggcggatccgggggcggtggaagcggaggtggtggatcgggaggcggtgg
aagcgcgcgcaacggcgaccactgtccgctcgggcccggacgttgctgcc
gtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgattgg
gtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtgccc
gagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcctgc
accgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgccagc
tacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgctcca
gacctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID NO:117)

and comprises the amino acid sequence (signal sequence underlined, hinge region in parentheses and linker sequences double underlined):

MEWSWVFLFFLSVTTGVHS
GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS
GGGGSGGGGSGGGGSGGGGS(ERKSSVECPPCP)APPVAGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGG
GGSGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADW
VLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPAS
YNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:118)

Two such monomers associate to form the dimer in which the two hGDF15 polypeptides are linked via a disulfide bond between two naturally occurring (*i.e.*, not engineered) cysteine residues. Accordingly, the specific hemiFc dimeric construct Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 comprises two monomers comprising SEQ ID NO:33.

II.D.2 Fc-$(G_4S)_3$-Fc-GS$(G_4S)_4$-GDF15

The hemiFc construct designated "Fc$(G_4S)_3$-Fc-GS$(G_4S)_4$-GDF15" in the instant disclosure refers to a construct comprising a monomer, which comprises a mature human GDF15 sequence linked to a first Fc sequence via a first linker comprising SEQ ID NO:31 that connects the N-terminus of the human GDF15 sequence to the C-terminus of the first Fc sequence and a second linker comprising the amino acid sequence:

GGGGSGGGGSGGGGS (SEQ ID NO:87).

that connects the N-terminus of the first Fc sequence to the C-terminus of the second Fc sequence. The first and second Fc sequences are also associated by the Fc hinge regions. Two such monomers associate to form a homodimer in which the monomers are linked via an interchain disulfide bond between the two GDF15 sequences.

More particularly, in a specific embodiment, the hemiFc construct Fc$(G_4S)_3$-Fc-GS$(G_4S)_4$-GDF15 comprises a monomer comprising a second Fc chain comprising the sequence of SEQ ID NO:28 joined by the linker:

GGGGGSGGGGSGGGGS (SEQ ID NO:119)

to a first Fc chain comprising the sequence of SEQ ID NO:30 to the C-terminus of which, the GDF15 polypeptide comprising SEQ ID NO:12 is joined by a linker having the sequence of SEQ ID NO:31.

In its final form, the monomer is encoded by the nucleic acid sequence:

ggaggaggcgagaggaagagctccgtggagtgtccaccctgcc
ctgctccgcctgtggctggaccctctgtgttcctgtttccgccgaagccg
aaagacaccctcatgatcagcaggactcccgaggtcacttgtgtggtcgt
ggatgtgagccatgaggacccagaggtgcagttcaactggtacgtggacg
gcgtggaagtccacaacgccaagaccaagccacgcgaggaacagttcaat
agcaccttccgcgtggtcagcgtcctcaccgtggtccaccaggattggct
taacggaaaggaatacaaatgcaaggtgtccaacaaggggcttcctgccc
cgattgaaaagaccatctccaagaccaagggacagccaagggagccccaa
gtgtacactctgccacccagccgcgaagaaatgactaagaatcaagtgtc
tctgacctgtcttgtcaaaggcttctaccccagcgacatcgctgtcgagt
gggaatcaaacgggcagcccgagaacaactacaagaccactcctccaatg

ctcgactcagatggcagcttttcctttactccaagctgaccgtggacaa
gtcaagatggcaacagggtaacgtgttctcatgctccgtgatgcacgaag
ccctccataatcactatacccagaaatctctgtctctttccccgggagga
ggaggggatctggtggaggaggctctggtggtggaggtagcgaacggaa
atcctcagtggagtgcccaccatgcccggctcctccagtggctggtccat
ctgtctttcttttttcctccgaaacccaaggacacccttatgatctctcgc
acccctgaagtgacttgcgtggtcgtcgatgtgtcacatgaagaccctga
ggtccagttcaattggtatgtggacggagtcgaggtgcataacgccaaaa
ccaaacctcgcgaagaacaattcaactctaccttccgggtggtgtctgtg
ctcactgtcgtccatcaggactggctgaacgggaaggagtacaagtgtaa
ggtgtctaacaaaggcctgccggctcccatcgaaaagactatcagcaaga
ctaaggggcaacccagagaaccccaagtctacaccctgcctccgtcacgg
gaggagatgaccaagaatcaggtgtccctcacctgtctggtcaagggttt
ctaccctagcgacattgctgtggagtgggagagcaatggacagcccgaaa
acaattacaagactaccccacccatgctggactcagacggatcattttc
ctctactctaagctcactgtggacaagagccggtggcagcaagggaatgt
gttcagctgttcagtgatgcatgaggccctgcataaccactacacccaga
agagcctttcactgtcacccgggtctggtggcggtgggtcaggtggcgga
ggatcaggaggaggtggaagcggcggaggaggatctgccaggaacggtga
tcactgccctctgggccctggtcgctgctgtaggcttcacactgtgcggg
cttccctcgaagatctgggatgggccgactgggtgctgagcccaagagag
gtgcaagtgaccatgtgcatcggggcatgtccctcccaattccgcgctgc
aaacatgcatgctcagattaagacttcactgcatagactgaagccagata
ccgtcccagcaccctgttgtgtgcccgcttcatacaaccccatggtcctg
attcaaaagaccgacaccggggtgtctctccagacctatgatgatcttct
tgcaaaggactgccactgcatctga (SEQ ID NO:122)

and comprises the amino acid sequence (hinge regions in parentheses, linker sequences double underlined):

GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS(ERKSS

VECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSGGGGSARNGDHC
PLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANM
HAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAK
DCHCI (SEQ ID NO:123).

In an embodiment employing the VH21 signal sequence, in its final form, the monomer is encoded by the nucleic acid (signal sequence underlined):

atggaatggtcatgggtgttccttttctttctctccgtcactaccggtgt
gcactccggaggaggcgagaggaagagctccgtggagtgtccaccctgcc
ctgctccgcctgtggctggaccctctgtgttcctgtttccgccgaagccg
aaagacaccctcatgatcagcaggactcccgaggtcacttgtgtggtcgt
ggatgtgagccatgaggacccagaggtgcagttcaactggtacgtggacg
gcgtggaagtccacaacgccaagaccaagccacgcgaggaacagttcaat
agcaccttccgcgtggtcagcgtcctcaccgtggtccaccaggattggct
taacggaaaggaatacaaatgcaaggtgtccaacaaggggcttcctgccc
cgattgaaaagaccatctccaagaccaagggacagccaagggagccccaa
gtgtacactctgccacccagccgcgaagaaatgactaagaatcaagtgtc
tctgacctgtcttgtcaaaggcttctaccccagcgacatcgctgtcgagt
gggaatcaaacgggcagcccgagaacaactacaagaccactcctccaatg
ctcgactcagatggcagcttttcctttactccaagctgaccgtggacaa
gtcaagatggcaacagggtaacgtgttctcatgctccgtgatgcacgaag
ccctccataatcactatacccagaaatctctgtctctttccccgggagga
ggaggggatctggtggaggaggctctggtggtggaggtagcgaacggaa
atcctcagtggagtgcccaccatgcccggctcctccagtggctggtccat
ctgtctttcttttttcctccgaaacccaaggacacccttatgatctctcgc
acccctgaagtgacttgcgtggtcgtcgatgtgtcacatgaagaccctga
ggtccagttcaattggtatgtggacggagtcgaggtgcataacgccaaaa
ccaaacctcgcgaagaacaattcaactctaccttccgggtggtgtctgtg
ctcactgtcgtccatcaggactggctgaacgggaaggagtacaagtgtaa
ggtgtctaacaaaggcctgccggctcccatcgaaaagactatcagcaaga

ctaaggggcaacccagagaaccccaagtctacaccctgcctccgtcacgg
gaggagatgaccaagaatcaggtgtccctcacctgtctggtcaagggttt
ctaccctagcgacattgctgtggagtgggagagcaatggacagcccgaaa
acaattacaagactaccccacccatgctggactcagacggatcatttttc
ctctactctaagctcactgtggacaagagccggtggcagcaagggaatgt
gttcagctgttcagtgatgcatgaggccctgcataaccactacacccaga
agagcctttcactgtcacccgggtctggtggcggtgggtcaggtggcgga
ggatcaggaggaggtggaagcggcggaggaggatctgccaggaacggtga
tcactgccctctgggccctggtcgctgctgtaggcttcacactgtgcggg
cttccctcgaagatctgggatgggccgactgggtgctgagcccaagagag
gtgcaagtgaccatgtgcatcggggcatgtccctcccaattccgcgctgc
aaacatgcatgctcagattaagacttcactgcatagactgaagccagata
ccgtcccagcaccctgttgtgtgcccgcttcatacaaccccatggtcctg
attcaaaagaccgacaccggggtgtctctccagacctatgatgatcttct
tgcaaaggactgccactgcatctga (SEQ ID NO:120)

and comprises the amino acid sequence (signal sequence single underlined, hinge regions in parentheses and linker sequences double underlined):

MEWSWVFLFFLSVTTGVHS
GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS(ERKSS
VECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSGGGGSARNGDHC
PLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANM
HAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAK
DCHCI (SEQ ID NO:121).

Two such monomers associate to form the dimer in which the two hGDF15 polypeptides are linked via a disulfide bond between two naturally occurring (*i.e.*, not

engineered) cysteine residues. Accordingly, the specific hemiFc dimeric construct Fc-$(G_4S)_3$-Fc-GS$(G_4S)_4$-GDF15 comprises two monomers comprising SEQ ID NO:123.

II.D.3 Fc-$(G_4S)_5$-Fc-GS$(G_4S)_4$-GDF15

The hemiFc construct designated "Fc-$(G_4S)_5$-Fc-GS$(G_4S)_4$-GDF15" in the instant disclosure refers to a construct comprising a monomer, which comprises a mature human GDF15 sequence linked to a first Fc sequence via a first linker comprising SEQ ID NO:31 that connects the N-terminus of the human GDF15 sequence to the C-terminus of the first Fc sequence and a second linker comprising the amino acid sequence:

GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:88).

That connects the N-terminus of the first Fc sequence to the C-terminus of the second Fc sequence. The first and second Fc sequences are also associated by the Fc hinge regions. Two such monomers associate to form a homodimer in which the monomers are linked via an interchain disulfide bond between the two GDF15 sequences.

More particularly, in a specific embodiment, the hemiFc construct Fc-$(G_4S)_5$-Fc-GS$(G_4S)_4$-GDF15 comprises a monomer comprising a second Fc chain comprising the sequence of SEQ ID NO:28 joined by the linker:

GGGGGSGGGGSGGGGSGGGGGSGGGGGS (SEQ ID NO:124)

to a first Fc chain comprising the sequence of SEQ ID NO:30 to the C-terminus of which, the GDF15 polypeptide comprising SEQ ID NO:12 is joined by a linker having the sequence of SEQ ID NO:31.

In its final form, the monomer is encoded by the nucleic acid sequence:

ggcggtggagagcgcaagtcatctgtcgagtgtccgccctgcc
ccgctccgccggtggctggaccctcagtgttcctctttccaccgaagccg
aaggacacccttatgattagccggaccccagaggtcacttgcgtcgtcgt
ggacgtgtcccatgaggatcccgaagtgcagtttaactggtatgtggacg
gagtggaggtccataacgccaagaccaagccaagggaagaacagttcaat
agcaccttccgggtggtgtccgtgctcaccgtggtgcatcaagactggct
gaatggcaaagagtacaaatgtaaggtgtcaaacaaggggctcccagccc
ctattgaaaagaccatctcaaagactaagggacagccacgcgaacctcaa

gtgtataccctcccgccttcacgcgaagaaatgactaagaatcaggtcag
ccttacttgtctggtcaagggcttctacccgagcgacattgcagtcgaat
gggagagcaatggtcagccagagaataactacaagaccactcctcccatg
cttgatagcgatggaagcttttttcctttacagcaagcttactgtggataa
gtctcgctggcaacagggaaatgtgttcagctgttcagtgatgcatgaag
cactccacaatcattacacccagaagtcactcagcctctcacccggagga
ggaggcggttctggtggaggagggtctggaggtggagggagcggcggagg
cgggtctggcggtggtgggtctgagaggaagtcatcagtggaatgcccac
catgccctgctcctcccgtggccggtccgagcgtgtttctcttcccacct
aagcccaaggacactctgatgatctcacggactccggaagtgacttgtgt
ggtggtggacgtgtctcatgaggaccctgaagtgcagttcaactggtacg
tggacggcgtggaggtgcacaatgctaagaccaagcctagagaggaacag
ttcaattccacctttcgcgtggtgagcgtcctgaccgtcgtgcaccagga
ctggcttaacggaaaggaatacaagtgcaaggtgtccaacaaaggccttc
cagctcccattgagaaaaccatctctaaaactaagggtcaaccaagggaa
ccccaagtctacaccctccctccgtctagagaagagatgaccaaaaacca
ggtgtccctgacctgtctggtgaagggattttacccctcagacatcgccg
tggagtgggaaagcaacggacagcccgaaaacaactataagactacccct
cctatgctggactcagacggatctttcttcctctatagcaagctcactgt
ggacaaatccagatggcaacaagggaatgtgttctcatgcagcgtgatgc
acgaggctcttcacaaccactatacccagaagagcctgtctctttcacct
ggttccggaggtggtgggagcggaggggtggatcaggtggtggagggtc
cggaggcggaggatccgcacggaatggcgaccactgtccactgggacccg
gaagatgttgtcgcctccacaccgtgagggcctctctggaggaccttggc
tgggccgactgggtcctgtcacctcgggaggtccaagtcaccatgtgtat
cggagcctgccccagccaattcagagcagcaaatatgcacgcacagatta
agaccagcctgcatcggcttaaacctgatactgtgccggctccttgttgc
gtgccagcatcttacaacccgatggtgctgatccagaaaaccgataccgg
tgtctccctccagacttacgacgacctccttgcaaaggactgccattgca
tctga (SEQ ID NO:127)

and comprises the amino acid sequence (hinge regions in parentheses, linker sequences double underlined):

GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS
GGGGS(ERKSSVECPP)CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWL
NGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSGG
GGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGA
CPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVS
LQTYDDLLAKDCHCI (SEQ ID NO:128)

In an embodiment employing the VH21 signal sequence, in its final form, the monomer is encoded by the nucleic acid (signal sequence underlined):

atggagtggagctgggtctttcttttctttctgtctgtgactaccggagt
ccattcaggcggtggagagcgcaagtcatctgtcgagtgtccgccctgcc
ccgctccgccggtggctggaccctcagtgttcctctttccaccgaagccg
aaggacacccttatgattagccggaccccagaggtcacttgcgtcgtcgt
ggacgtgtcccatgaggatcccgaagtgcagtttaactggtatgtggacg
gagtggaggtccataacgccaagaccaagccaagggaagaacagttcaat
agcaccttccgggtggtgtccgtgctcaccgtggtgcatcaagactggct
gaatggcaaagagtacaaatgtaaggtgtcaaacaaggggctcccagccc
ctattgaaaagaccatctcaaagactaagggacagccacgcgaacctcaa
gtgtataccctcccgccttcacgcgaagaaatgactaagaatcaggtcag
ccttacttgtctggtcaagggcttctacccgagcgacattgcagtcgaat
gggagagcaatggtcagccagagaataactacaagaccactcctcccatg
cttgatagcgatggaagcttttttcctttacagcaagcttactgtggataa
gtctcgctggcaacagggaaatgtgttcagctgttcagtgatgcatgaag
cactccacaatcattacacccagaagtcactcagcctctcacccggagga
ggaggcggttctggtggaggagggtctggaggtggagggagcggcggagg
cgggtctggcggtggtgggtctgagaggaagtcatcagtggaatgcccac
catgccctgctcctcccgtggccggtccgagcgtgtttctcttcccacct

aagcccaaggacactctgatgatctcacggactccggaagtgacttgtgt
ggtggtggacgtgtctcatgaggaccctgaagtgcagttcaactggtacg
tggacggcgtggaggtgcacaatgctaagaccaagcctagagaggaacag
ttcaattccacctttcgcgtggtgagcgtcctgaccgtcgtgcaccagga
ctggcttaacggaaaggaatacaagtgcaaggtgtccaacaaaggccttc
cagctcccattgagaaaaccatctctaaaactaagggtcaaccaagggaa
ccccaagtctacaccctccctccgtctagagaagagatgaccaaaaacca
ggtgtccctgacctgtctggtgaagggattttacccctcagacatcgccg
tggagtgggaaagcaacggacagcccgaaaacaactataagactacccct
cctatgctggactcagacggatctttcttcctctatagcaagctcactgt
ggacaaatccagatggcaacaagggaatgtgttctcatgcagcgtgatgc
acgaggctcttcacaaccactatacccagaagagcctgtctctttcacct
ggttccggaggtggtgggagcggaggggtggatcaggtggtggagggtc
cggaggcggaggatccgcacggaatggcgaccactgtccactgggacccg
gaagatgttgtcgcctccacaccgtgagggcctctctggaggaccttggc
tgggccgactgggtcctgtcacctcgggaggtccaagtcaccatgtgtat
cggagcctgccccagccaattcagagcagcaaatatgcacgcacagatta
agaccagcctgcatcggcttaaacctgatactgtgccggctccttgttgc
gtgccagcatcttacaacccgatggtgctgatccagaaaaccgataccgg
tgtctccctccagacttacgacgacctccttgcaaaggactgccattgca
tctga (SEQ ID NO:125)

and comprises the amino acid sequence (signal sequence single underlined, hinge regions in parentheses and linker sequences double underlined):

MEWSWVFLFFLSVTTGVHS
GGG(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS
GGGGS(ERKSSVECPPCP)APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWL
NGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSGG
GGSARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGA
CPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVS
LQTYDDLLAKDCHCI (SEQ ID NO:126)

Two such monomers associate to form the dimer in which the two hGDF15 polypeptides are linked via a disulfide bond between two naturally occurring (*i.e.*, not engineered) cysteine residues. Accordingly, the specific hemiFc dimeric construct Fc-$(G_4S)_5$-Fc-GS$(G_4S)_4$-GDF15 comprises two monomers comprising SEQ ID NO:128.

III. GDF15 Polypeptides and Constructs Comprising GDF15, Including Mutant Forms Thereof

As disclosed herein, the GDF15 polypeptides (including the full length and mature forms of human GDF15) and the constructs comprising GDF15 described in the instant disclosure can be engineered and/or produced using standard molecular biology methodology to form a mutant form of the GDF15 polypeptides and constructs provided herein. In various examples, a nucleic acid sequence encoding a mutant form of the GDF15 polypeptides and constructs provided herein, which can comprise all or a portion of SEQ ID NOs:4, 8 or 12 can be isolated and/or amplified from genomic DNA, or cDNA using appropriate oligonucleotide primers. Primers can be designed based on the nucleic and amino acid sequences provided herein according to standard (RT)-PCR amplification techniques. The amplified GDF15 mutant polypeptide nucleic acid can then be cloned into a suitable vector and characterized by DNA sequence analysis.

Oligonucleotides for use as probes in isolating or amplifying all or a portion of a mutant form of the GDF15 polypeptides and constructs provided herein can be designed and generated using standard synthetic techniques, *e.g.*, automated DNA synthesis apparatus, or can be isolated from a longer sequence of DNA.

III.A. GDF15 Polypeptide and Polynucleotide Sequences

In vivo, GDF15 is expressed as a contiguous amino acid sequence comprising a signal sequence, a pro domain and an active domain.

The 308 amino acid sequence of full length human GDF15 is:

MPGQELRTVNGSQMLLVLLVLSWLPHGGALSLAEASRASFPGPSELHSED
SRFRELRKRYEDLLTRLRANQSWEDSNTDLVPAPAVRILTPEVRLGSGGH
LHLRISRAALPEGLPEASRLHRALFRLSPTASRSWDVTRPLRRQLSLARP
QAPALHLRLSPPPSQSDQLLAESSSARPQLELHLRPQAARGRRRARARNG
DHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRA
ANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDL
LAKDCHCI (SEQ ID NO:4)

and is encoded by the DNA sequence:

atgcccgggcaagaactcaggacggtgaatggctctcagatgctcctggt
gttgctggtgctctcgtggctgccgcatgggggcgccctgtctctggccg
aggcgagccgcgcaagtttcccgggaccctcagagttgcactccgaagac
tccagattccgagagttgcggaaacgctacgaggacctgctaaccaggct
gcgggccaaccagagctgggaagattcgaacaccgacctcgtcccggccc
ctgcagtccggatactcacgccagaagtgcggctgggatccggcggccac
ctgcacctgcgtatctctcgggccgcccttcccgaggggctccccgaggc
ctcccgccttcaccgggctctgttccggctgtccccgacggcgtcaaggt
cgtgggacgtgacacgaccgctgcggcgtcagctcagccttgcaagaccc
caggcgcccgcgctgcacctgcgactgtcgccgccgccgtcgcagtcgga
ccaactgctggcagaatcttcgtccgcacggccccagctggagttgcact
tgcggccgcaagccgccaggggcgccgcagagcgcgtgcgcgcaacggg
gaccactgtccgctcgggcccgggcgttgctgccgtctgcacacggtccg
cgcgtcgctggaagacctgggctgggccgattgggtgctgtcgccacggg
aggtgcaagtgaccatgtgcatcggcgcgtgcccgagccagttccgggcg
gcaaacatgcacgcgcagatcaagacgagcctgcaccgcctgaagcccga
cacggtgccagcgccctgctgcgtgcccgccagctacaatcccatggtgc
tcattcaaaagaccgacaccggggtgtcgctccagacctatgatgacttg
ttagccaaagactgccactgcatatga (SEQ ID NO:3).

The 303 amino acid sequence of full length murine GDF15 is:

MAPPALQAQPPGGSQLRFLLFLLLLLLLLSWPSQGDALAMPEQRPSGPES
QLNADELRGRFQDLLSRLHANQSREDSNSEPSPDPAVRILSPEVRLGSHG
QLLLRVNRASLSQGLPEAYRVHRALLLLTPTARPWDITRPLKRALSLRGP

RAPALRLRLTPPPDLAMLPSGGTQLELRLRVAAGRGRRSAHAHPRDSCPL
GPGRCCHLETVQATLEDLGWSDWVLSPRQLQLSMCVGECPHLYRSANTHA
QIKARLHGLQPDKVPAPCCVPSSYTPVVLMHRTDSGVSLQTYDDLVARGC
HCA (SEQ ID NO:6)

and is encoded by the DNA sequence:

atggccccgcccgcgctccaggcccagcctccaggcggctctcaactgag
gttcctgctgttcctgctgctgttgctgctgctgctgtcatggccatcgc
aggggacgccctggcaatgcctgaacagcgaccctccggccctgagtcc
caactcaacgccgacgagctacggggtcgcttccaggacctgctgagccg
gctgcatgccaaccagagccgagaggactcgaactcagaaccaagtcctg
acccagctgtccggatactcagtccagaggtgagattggggtcccacggc
cagctgctactccgcgtcaaccgggcgtcgctgagtcagggtctccccga
agcctaccgcgtgcaccgagcgctgctcctgctgacgccgacggcccgcc
cctgggacatcactaggcccctgaagcgtgcgctcagcctccggggaccc
cgtgctcccgcattacgcctgcgcctgacgccgcctccggacctggctat
gctgccctctggcggcacgcagctggaactgcgcttacgggtagccgccg
gcaggggcgccgaagcgcgcatgcgcacccaagagactcgtgcccactg
ggtccggggcgctgctgtcacttggagactgtgcaggcaactcttgaaga
cttgggctggagcgactgggtgctgtccccgcgccagctgcagctgagca
tgtgcgtgggcgagtgtccccacctgtatcgctccgcgaacacgcatgcg
cagatcaaagcacgcctgcatggcctgcagcctgacaaggtgcctgcccc
gtgctgtgtcccctccagctacaccccggtggttcttatgcacaggacag
acagtggtgtgtcactgcagacttatgatgacctggtggcccggggctgc
cactgcgcttga (SEQ ID NO:5).

The amino acid sequence of human GDF15 following cleavage of the 29 residue signal sequence is:

LSLAEASRASFPGPSELHSEDSRFRELRKRYEDLLTRLRANQSWEDSNTD
LVPAPAVRILTPEVRLGSGGHLHLRISRAALPEGLPEASRLHRALFRLSP
TASRSWDVTRPLRRQLSLARPQAPALHLRLSPPPSQSDQLLAESSSARPQ
LELHLRPQAARGRRRARARNGDHCPLGPGRCCRLHTVRASLEDLGWADWV
LSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASY
NPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:8)

and is encoded by the DNA sequence:

ctgtctctggccgaggcgagccgcgcaagtttcccgggaccctcagagtt
gcactccgaagactccagattccgagagttgcggaaacgctacgaggacc
tgctaaccaggctgcgggccaaccagagctgggaagattcgaacaccgac
ctcgtcccggcccctgcagtccggatactcacgccagaagtgcggctggg
atccggcggccacctgcacctgcgtatctctcgggccgcccttcccgagg
ggctccccgaggcctcccgccttcaccgggctctgttccggctgtccccg
acggcgtcaaggtcgtgggacgtgacacgaccgctgcggcgtcagctcag
ccttgcaagaccccaggcgcccgcgctgcacctgcgactgtcgccgccgc
cgtcgcagtcggaccaactgctggcagaatcttcgtccgcacggccccag
ctggagttgcacttgcggccgcaagccgccaggggcgccgcagagcgcg
tgcgcgcaacggggaccactgtccgctcgggcccgggcgttgctgccgtc
tgcacacggtccgcgcgtcgctggaagacctgggctgggccgattgggtg
ctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtgcccgag
ccagttccgggcggcaaacatgcacgcgcagatcaagacgagcctgcacc
gcctgaagcccgacacggtgccagcgccctgctgcgtgcccgccagctac
aatcccatggtgctcattcaaaagaccgacaccggggtgtcgctccagac
ctatgatgacttgttagccaaagactgccactgcatatga (SEQ ID NO:7)

The amino acid sequence of murine GDF 15 following cleavage of the 32 residue signal sequence is:

SQGDALAMPEQRPSGPESQLNADELRGRFQDLLSRLHANQSREDSNSEPS
PDPAVRILSPEVRLGSHGQLLLRVNRASLSQGLPEAYRVHRALLLLTPTA
RPWDITRPLKRALSLRGPRAPALRLRLTPPPDLAMLPSGGTQLELRLRVA
AGRGRRSAHAHPRDSCPLGPGRCCHLETVQATLEDLGWSDWVLSPRQLQL
SMCVGECPHLYRSANTHAQIKARLHGLQPDKVPAPCCVPSSYTPVVLMHR
TDSGVSLQTYDDLVARGCHCA (SEQ ID NO:10)

and is encoded by the DNA sequence:

tcgcaggggacgccctggcaatgcctgaacagcgaccctccggccctga
gtcccaactcaacgccgacgagctacggggtcgcttccaggacctgctga
gccggctgcatgccaaccagagccgagaggactcgaactcagaaccaagt
cctgacccagctgtccggatactcagtccagaggtgagattggggtccca
cggccagctgctactccgcgtcaaccgggcgtcgctgagtcagggtctcc

ccgaagcctaccgcgtgcaccgagcgctgctcctgctgacgccgacggcc
cgcccctgggacatcactaggcccctgaagcgtgcgctcagcctccgggg
accccgtgctcccgcattacgcctgcgcctgacgccgcctccggacctgg
ctatgctgccctctggcggcacgcagctggaactgcgcttacgggtagcc
gccggcaggggcgccgaagcgcgcatgcgcacccaagagactcgtgccc
actgggtccggggcgctgctgtcacttggagactgtgcaggcaactcttg
aagacttgggctggagcgactgggtgctgtccccgcgccagctgcagctg
agcatgtgcgtgggcgagtgtccccacctgtatcgctccgcgaacacgca
tgcgcagatcaaagcacgcctgcatggcctgcagcctgacaaggtgcctg
ccccgtgctgtgtcccctccagctacaccccggtggttcttatgcacagg
acagacagtggtgtgtcactgcagacttatgatgacctggtggcccgggg
ctgccactgcgcttga (SEQ ID NO:9)

The amino acid sequence of the recombinant active form of the human GDF15, which comprises a homodimer comprising nine cysteines in each monomer to form one interchain disulfide bond and four intrachain disulfide bonds (shown with an optional N-terminal methionine residue in parentheses), is:

(M)ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGA
CPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVS
LQTYDDLLAKDCHCI (SEQ ID NO:129)

and is encoded by the DNA sequence (shown with an optional N-terminal methionine codon in parentheses):

(atg)gcgcgcaacggggaccactgtccgctcgggcccgggcgttgctgc
cgtctgcacacggtccgcgcgtcgctggaagacctgggctgggccgattg
ggtgctgtcgccacgggaggtgcaagtgaccatgtgcatcggcgcgtgcc
cgagccagttccgggcggcaaacatgcacgcgcagatcaagacgagcctg
caccgcctgaagcccgacacggtgccagcgccctgctgcgtgcccgccag
ctacaatcccatggtgctcattcaaaagaccgacaccggggtgtcgctcc
agacctatgatgacttgttagccaaagactgccactgcatataa (SEQ ID
NO:130).

The amino acid sequence of the recombinant active form of the murine GDF15, which comprises a homodimer comprising nine cysteines in each

monomer to form one interchain disulfide bond and four intrachain disulfide bonds, is:

(M)SAHAHPRDSCPLGPGRCCHLETVQATLEDLGWSDWVLSPRQLQLSMC
VGECPHLYRSANTHAQIKARLHGLQPDKVPAPCCVPSSYTPVVLMHRTDS
GVSLQTYDDLVARGCHCA (SEQ ID NO:14)

and is encoded by the DNA sequence:

(atg)agcgcgcatgcgcacccaagagactcgtgcccactgggtccgggg
cgctgctgtcacctggagactgtgcaggcaactcttgaagacttgggctg
gagcgactgggtgttgtccccgcgccagctgcagctgagcatgtgcgtgg
gcgagtgtccccacctgtatcgctccgcgaacacgcatgcgcagatcaaa
gcacgcctgcatggcctgcagcctgacaaggtgcctgccccgtgctgtgt
cccctccagctacaccccggtggttcttatgcacaggacagacagtggtg
tgtcactgcagacttatgatgacctggtggcccggggctgccactgcgct
tga (SEQ ID NO:13).

As stated herein, the term "GDF15 polypeptide" refers to a GDF polypeptide comprising the human amino acid sequences SEQ ID NOs:4, 8 and 12. The term "GDF15 mutant polypeptide," however, encompasses polypeptides comprising an amino acid sequence that differs from the amino acid sequence of a naturally occurring GDF polypeptide sequence, *e.g.*, SEQ ID NOs: 4, 8 and 12, by one or more amino acids, such that the sequence is at least 85% identical to SEQ ID NOs: 4, 8 and 12. GDF15 polypeptides can be generated by introducing one or more amino acid substitutions, either conservative or non-conservative and using naturally or non-naturally occurring amino acids, at particular positions of the GDF15 polypeptide, or by deleting particular residues or stretches of residues.

A "conservative amino acid substitution" can involve a substitution of a native amino acid residue (*i.e.*, a residue found in a given position of the wild-type GDF15 polypeptide sequence) with a non-native residue (*i.e.*, a residue that is not found in a given position of the wild-type GDF15 polypeptide sequence) such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues (as defined herein) that are typically incorporated by chemical peptide

synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues can be divided into classes based on common side chain properties:

(1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr;

(3) acidic: Asp, Glu;

(4) basic: Asn, Gln, His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro; and

(6) aromatic: Trp, Tyr, Phe.

Additional groups of amino acids can also be formulated using the principles described in, *e.g.*, Creighton (1984) PROTEINS: STRUCTURE AND MOLECULAR PROPERTIES (2d Ed. 1993), W.H. Freeman and Company. In some instances it can be useful to further characterize substitutions based on two or more of such features (*e.g.*, substitution with a "small polar" residue, such as a Thr residue, can represent a highly conservative substitution in an appropriate context).

Conservative substitutions can involve the exchange of a member of one of these classes for another member of the same class. Non-conservative substitutions can involve the exchange of a member of one of these classes for a member from another class.

Synthetic, rare, or modified amino acid residues having known similar physiochemical properties to those of an above-described grouping can be used as a "conservative" substitute for a particular amino acid residue in a sequence. For example, a D-Arg residue may serve as a substitute for a typical L-Arg residue. It also can be the case that a particular substitution can be described in terms of two or more of the above described classes (*e.g.*, a substitution with a small and hydrophobic residue means substituting one amino acid with a residue(s) that is found in both of the above-described classes or other synthetic, rare, or modified residues that are known in the art to have similar physiochemical properties to such residues meeting both definitions).

Nucleic acid sequences encoding a GDF15 mutant polypeptide provided herein, including those degenerate to SEQ ID NOs: 3, 7, 11 and 15, and those encoding polypeptide variants of SEQ ID NOs:4, 8 and 12, form other aspects of the instant disclosure.

III.B. Vectors Useful for Expressing GDF15 Polypeptides and Constructs Comprising GDF15, Including Mutant Forms Thereof

In order to express the nucleic acid sequences encoding the GDF15 polypeptides and construct comprising GDF15 provided herein, the appropriate coding sequences, *e.g.*, SEQ ID NOs:3, 7, 11, 15, 17, 21, 24, 26 and 32, can be cloned into a suitable vector and after introduction in a suitable host, the sequence can be expressed to produce the encoded polypeptide according to standard cloning and expression techniques, which are known in the art (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989). The invention also relates to such vectors comprising a nucleic acid sequence according to the invention.

A "vector" refers to a delivery vehicle that (a) promotes the expression of a polypeptide-encoding nucleic acid sequence; (b) promotes the production of the polypeptide therefrom; (c) promotes the transfection/transformation of target cells therewith; (d) promotes the replication of the nucleic acid sequence; (e) promotes stability of the nucleic acid; (f) promotes detection of the nucleic acid and/or transformed/transfected cells; and/or (g) otherwise imparts advantageous biological and/or physiochemical function to the polypeptide-encoding nucleic acid. A vector can be any suitable vector, including chromosomal, non-chromosomal, and synthetic nucleic acid vectors (a nucleic acid sequence comprising a suitable set of expression control elements). Examples of such vectors include derivatives of SV40, bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral nucleic acid (RNA or DNA) vectors.

A recombinant expression vector can be designed for expression of a GDF15 mutant polypeptide in prokaryotic (*e.g.*, *E. coli*) or eukaryotic cells (*e.g.*, insect cells, using baculovirus expression vectors, yeast cells, or mammalian cells). Representative host cells include those hosts typically used for cloning and expression, including *Escherichia coli* strains TOP10F′, TOP10, DH10B, DH5a, HB101, W3110, BL21(DE3) and BL21 (DE3)pLysS, BLUESCRIPT (Stratagene), mammalian cell lines CHO, CHO-K1, HEK293, 293-EBNA pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264: 5503-5509 (1989); pET vectors (Novagen, Madison

Wis.). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase and an in vitro translation system. Preferably, the vector contains a promoter upstream of the cloning site containing the nucleic acid sequence encoding the polypeptide. Examples of promoters, which can be switched on and off, include the lac promoter, the T7 promoter, the trc promoter, the tac promoter and the trp promoter.

Thus, provided herein are vectors comprising a nucleic acid sequence encoding a GDF15 polypeptide or construct comprising a GDF15 polypeptide, including mutant forms thereor, that facilitate the expression of the polypeptide or construct of interest. In various embodiments, the vectors comprise an operably linked nucleotide sequence which regulates the expression of a GDF15 polypeptide construct comprising a GDF15 polypeptide or a mutant form thereof. A vector can comprise or be associated with any suitable promoter, enhancer, and other expression-facilitating elements. Examples of such elements include strong expression promoters (*e.g.*, a human CMV IE promoter/enhancer, an RSV promoter, SV40 promoter, SL3-3 promoter, MMTV promoter, or HIV LTR promoter, EF1alpha promoter, CAG promoter), effective poly (A) termination sequences, an origin of replication for plasmid product in *E. coli*, an antibiotic resistance gene as a selectable marker, and/or a convenient cloning site (*e.g.*, a polylinker). Vectors also can comprise an inducible promoter as opposed to a constitutive promoter such as CMV IE. In one aspect, a nucleic acid comprising a sequence encoding a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, which is operatively linked to a tissue specific promoter which promotes expression of the sequence in a metabolically-relevant tissue, such as liver or pancreatic tissue is provided.

III.C. Host Cells

In another aspect of the instant disclosure, host cells comprising the nucleic acids and vectors disclosed herein are provided. In various embodiments, the vector or nucleic acid is integrated into the host cell genome, which in other embodiments the vector or nucleic acid is extra-chromosomal.

Recombinant cells, such as yeast, bacterial (*e.g.*, *E. coli*), and mammalian cells (*e.g.*, immortalized mammalian cells) comprising such a nucleic acid, vector, or combinations of either or both thereof are provided. In various embodiments, cells

comprising a non-integrated nucleic acid, such as a plasmid, cosmid, phagemid, or linear expression element, which comprises a sequence coding for expression of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, are provided.

A vector comprising a nucleic acid sequence encoding a GDF15 mutant polypeptide provided herein can be introduced into a host cell by transformation or by transfection. Methods of transforming a cell with an expression vector are well known.

A nucleic acid encoding a GDF15 polypeptide-, construct comprising a GDF15 polypeptide or a mutant form thereof can be positioned in and/or delivered to a host cell or host animal via a viral vector. Any suitable viral vector can be used in this capacity. A viral vector can comprise any number of viral polynucleotides, alone or in combination with one or more viral proteins, which facilitate delivery, replication, and/or expression of the nucleic acid of the invention in a desired host cell. The viral vector can be a polynucleotide comprising all or part of a viral genome, a viral protein/nucleic acid conjugate, a virus-like particle (VLP), or an intact virus particle comprising viral nucleic acids and a GDF15 mutant polypeptide-encoding nucleic acid. A viral particle viral vector can comprise a wild-type viral particle or a modified viral particle. The viral vector can be a vector which requires the presence of another vector or wild-type virus for replication and/or expression (*e.g.*, a viral vector can be a helper-dependent virus), such as an adenoviral vector amplicon. Typically, such viral vectors consist of a wild-type viral particle, or a viral particle modified in its protein and/or nucleic acid content to increase transgene capacity or aid in transfection and/or expression of the nucleic acid (examples of such vectors include the herpes virus/AAV amplicons). Typically, a viral vector is similar to and/or derived from a virus that normally infects humans. Suitable viral vector particles in this respect, include, for example, adenoviral vector particles (including any virus of or derived from a virus of the adenoviridae), adeno-associated viral vector particles (AAV vector particles) or other parvoviruses and parvoviral vector particles, papillomaviral vector particles, flaviviral vectors, alphaviral vectors, herpes viral vectors, pox virus vectors, retroviral vectors, including lentiviral vectors.

III.D. Isolation of a GDF15 Polypeptide, Construct Comprising a GDF15 Polypeptide or a Mutant Form Thereof

A GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof expressed as described herein can be isolated using standard protein purification methods. A GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof can be isolated from a cell that has been engineered to express a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, for example a cell that does not naturally express native GDF15.

Protein purification methods that can be employed to isolate a GDF15 mutant polypeptide, as well as associated materials and reagents, are known in the art. Exemplary methods of purifying a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof are provided in the Examples herein below. Additional purification methods that may be useful for isolating a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof can be found in references such as Bootcov MR, 1997, *Proc. Natl. Acad. Sci. USA 94:11514-9*, Fairlie WD, 2000, *Gene 254: 67-76.*

IV. Pharmaceutical Compositions Comprising a GDF15 Polypeptide, Construct Comprising a GDF15 Polypeptide or a Mutant Form Thereof

Pharmaceutical compositions comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof are provided. Such polypeptide pharmaceutical compositions can comprise a therapeutically effective amount of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation agents suitable for accomplishing or enhancing the delivery of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof into the body of a human or non-human subject. The term includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In some cases it will be preferable to include isotonic agents, for example, sugars,

polyalcohols such as mannitol, sorbitol, or sodium chloride in a pharmaceutical composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof polypeptide can also act as, or form a component of, a carrier. Acceptable pharmaceutically acceptable carriers are preferably nontoxic to recipients at the dosages and concentrations employed.

A pharmaceutical composition can contain formulation agent(s) for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation agents include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as free serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as Polysorbate 20 or Polysorbate 80; Triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides – preferably sodium or potassium chloride – or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants (see, *e.g.*, REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 19th edition, (1995); Berge et al., J. Pharm. Sci., 6661), 1-19 (1977). Additional relevant

principles, methods, and agents are described in, *e.g.*, Lieberman et al., PHARMACEUTICAL DOSAGE FORMS-DISPERSE SYSTEMS (2nd ed., vol. 3, 1998); Ansel et al., PHARMACEUTICAL DOSAGE FORMS & DRUG DELIVERY SYSTEMS (7th ed. 2000); Martindale, THE EXTRA PHARMACOPEIA (31st edition), Remington's PHARMACEUTICAL SCIENCES (16th-20th and subsequent editions); The Pharmacological Basis Of Therapeutics, Goodman and Gilman, Eds. (9th ed.--1996); Wilson and Gisvolds' TEXTBOOK OF ORGANIC MEDICINAL AND PHARMACEUTICAL CHEMISTRY, Delgado and Remers, Eds. (10th ed., 1998). Principles of formulating pharmaceutically acceptable compositions also are described in, e.g., Aulton, PHARMACEUTICS: THE SCIENCE OF DOSAGE FORM DESIGN, Churchill Livingstone (New York) (1988), EXTEMPORANEOUS ORAL LIQUID DOSAGE PREPARATIONS, CSHP (1998), incorporated herein by reference for any purpose).

The optimal pharmaceutical composition will be determined by a skilled artisan depending upon, for example, the intended route of administration, delivery format, and desired dosage (see, *e.g.*, Remington's PHARMACEUTICAL SCIENCES, *supra*). Such compositions can influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof.

The primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with free serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute. In one embodiment of the present invention, compositions comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's PHARMACEUTICAL SCIENCES, *supra*) in the form of a lyophilized cake or an aqueous solution. Furthermore, a product comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form

thereof can be formulated as a lyophilizate using appropriate excipients such as sucrose.

The polypeptide pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions can be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention can be in the form of a pyrogen-free, parenterally acceptable, aqueous solution comprising a desired GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which can then be delivered via a depot injection. Hyaluronic acid can also be used, and this can have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In one embodiment, a pharmaceutical composition can be formulated for inhalation. For example, a GDF15 mutant polypeptide can be formulated as a dry powder for inhalation. GDF15 polypeptide inhalation solutions can also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions can be nebulized. Pulmonary administration is further described in International Publication No. WO 94/20069, which describes the pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations can be administered orally. In one embodiment of the present invention, a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof that is administered in this fashion can

be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule can be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders can also be employed.

Another pharmaceutical composition can involve an effective quantity of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof will be evident to those skilled in the art, including formulations comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art (see, *e.g.*, International Publication No. WO 93/15722, which describes the controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions, and Wischke & Schwendeman, 2008, Int. J. Pharm. 364: 298-327, and Freiberg & Zhu, 2004, Int. J. Pharm. 282: 1-18, which discuss microsphere/microparticle preparation and use). As described herein, a hydrogel is an example of a sustained- or controlled-delivery formulation.

Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices can include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919 and European Patent No. 0 058 481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, Biopolymers 22: 547-56), poly(2-

hydroxyethyl-methacrylate) (Langer et al., 1981, J. Biomed. Mater. Res. 15: 167-277 and Langer, 1982, Chem. Tech. 12: 98-105), ethylene vinyl acetate (Langer et al., supra) or poly-D(-)-3-hydroxybutyric acid (European Patent No. 0 133 988). Sustained-release compositions can also include liposomes, which can be prepared by any of several methods known in the art. See, *e.g.*, Epstein et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82: 3688-92; and European Patent Nos. 0 036 676, 0 088 046, and 0 143 949.

A pharmaceutical composition comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, which is to be used for *in vivo* administration typically should be sterile. This can be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration can be stored in lyophilized form or in a solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations can be stored either in a ready-to-use form or in a form (*e.g.*, lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits can each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (*e.g.*, liquid syringes and lyosyringes).

The effective amount of pharmaceutical composition comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, which is to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, is being used, the route of administration, and the size (body weight, body surface, or organ size) and condition

(the age and general health) of the patient. Accordingly, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage can range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage can range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg; or 5 μg/kg, 10 μg/kg, 15 μg/kg, 20 μg/kg, 25 μg/kg, 30 μg/kg, 35 μg/kg, 40 μg/kg, 45 μg/kg, 50 μg/kg, 55 μg/kg, 60 μg/kg, 65 μg/kg, 70 μg/kg, 75 μg/kg, 100 μg/kg, 200 μg/kg or up to about 10 mg/kg.

The frequency of dosing will depend upon the pharmacokinetic parameters of the GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, in the formulation being used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition can therefore be administered as a single dose, as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages can be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, *e.g.*, orally; through injection by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes; by sustained release systems (which may also be injected); or by implantation devices. Where desired, the compositions can be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition can be administered locally via implantation of a membrane, sponge, or other appropriate material onto which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device can be implanted into any suitable tissue or organ, and delivery of the desired molecule can be via diffusion, timed-release bolus, or continuous administration.

In order to deliver drug, *e.g.*, a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, at a predetermined rate such that the

drug concentration can be maintained at a desired therapeutically effective level over an extended period, a variety of different approaches can be employed. In one example, a hydrogel comprising a polymer such as a gelatin (*e.g.*, bovine gelatin, human gelatin, or gelatin from another source) or a naturally-occurring or a synthetically generated polymer can be employed. Any percentage of polymer (*e.g.*, gelatin) can be employed in a hydrogel, such as 5, 10, 15 or 20%. The selection of an appropriate concentration can depend on a variety of factors, such as the therapeutic profile desired and the pharmacokinetic profile of the therapeutic molecule.

Examples of polymers that can be incorporated into a hydrogel include polyethylene glycol ("PEG"), polyethylene oxide, polyethylene oxide-co-polypropylene oxide, co-polyethylene oxide block or random copolymers, polyvinyl alcohol, poly(vinyl pyrrolidinone), poly(amino acids), dextran, heparin, polysaccharides, polyethers and the like.

Another factor that can be considered when generating a hydrogel formulation is the degree of crosslinking in the hydrogel and the crosslinking agent. In one embodiment, cross-linking can be achieved via a methacrylation reaction involving methacrylic anhydride. In some situations, a high degree of cross-linking may be desirable while in other situations a lower degree of crosslinking is preferred. In some cases a higher degree of crosslinking provides a longer sustained release. A higher degree of crosslinking may provide a firmer hydrogel and a longer period over which drug is delivered.

Any ratio of polymer to crosslinking agent (*e.g.*, methacrylic anhydride) can be employed to generate a hydrogel with desired properties. For example, the ratio of polymer to crosslinker can be, *e.g.*, 8:1, 16:1, 24:1, or 32:1. For example, when the hydrogel polymer is gelatin and the crosslinker is methacrylate, ratios of 8:1, 16:1, 24:1, or 32:1 methyacrylic anhydride:gelatin can be employed.

V. Therapeutic Uses of a GDF15 Polypeptide, Construct Comprising a GDF15 Polypeptide or a Mutant Form Thereof

A GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, can be used to treat, diagnose or ameliorate, a metabolic condition or disorder. In one embodiment, the metabolic disorder to be treated is diabetes, *e.g.*, type 2 diabetes. In another embodiment, the metabolic condition or disorder is obesity. In other embodiments the metabolic condition or disorder is dyslipidemia,

elevated glucose levels, elevated insulin levels or diabetic nephropathy. For example, a metabolic condition or disorder that can be treated or ameliorated using a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, includes a state in which a human subject has a fasting blood glucose level of 125 mg/dL or greater, for example 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 , 200 or greater than 200 mg/dL. Blood glucose levels can be determined in the fed or fasted state, or at random. The metabolic condition or disorder can also comprise a condition in which a subject is at increased risk of developing a metabolic condition. For a human subject, such conditions include a fasting blood glucose level of 100 mg/dL. Conditions that can be treated using a pharmaceutical composition comprising a GDF15 mutant polypeptide can also be found in the American Diabetes Association Standards of Medical Care in Diabetes Care-2011, American Diabetes Association, Diabetes Care Vol. 34, No. Supplement 1, S11-S61, 2010, incorporated herein by reference.

In application, a metabolic disorder or condition, such as Type 2 diabetes, elevated glucose levels, elevated insulin levels, dyslipidemia, obesity or diabetic nephropathy, can be treated by administering a therapeutically effective dose of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, to a patient in need thereof. The administration can be performed as described herein, such as by IV injection, intraperitoneal (IP) injection, subcutaneous injection, intramuscular injection, or orally in the form of a tablet or liquid formation. In some situations, a therapeutically effective or preferred dose of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, can be determined by a clinician. A therapeutically effective dose of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, will depend, *inter alia*, upon the administration schedule, the unit dose of agent administered, whether the GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof is administered in combination with other therapeutic agents, the immune status and the health of the recipient. The term "therapeutically effective dose," as used herein, means an amount of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, that elicits a biological or medicinal response in a tissue system, animal, or human being sought by a researcher, medical doctor, or other clinician, which includes alleviation or amelioration of the symptoms of the disease or disorder being treated,

i.e., an amount of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, that supports an observable level of one or more desired biological or medicinal response, for example lowering blood glucose, insulin, triglyceride, or cholesterol levels; reducing body weight; or improving glucose tolerance, energy expenditure, or insulin sensitivity.

It is noted that a therapeutically effective dose of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, can also vary with the desired result. Thus, for example, in situations in which a lower level of blood glucose is indicated a dose of a GDF15 mutant polypeptide will be correspondingly higher than a dose in which a comparatively lower level of blood glucose is desired. Conversely, in situations in which a higher level of blood glucose is indicated at a dose of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, will be correspondingly lower than a dose in which a comparatively higher level of blood glucose is desired.

In various embodiments, a subject is a human having a blood glucose level of 100 mg/dL or greater can be treated with a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof.

In one embodiment, a method of the instant disclosure comprises first measuring a baseline level of one or more metabolically-relevant compounds such as glucose, insulin, cholesterol, lipid in a subject. A pharmaceutical composition comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, is then administered to the subject. After a desired period of time, the level of the one or more metabolically-relevant compounds (*e.g.*, blood glucose, insulin, cholesterol, lipid) in the subject is again measured. The two levels can then be compared in order to determine the relative change in the metabolically-relevant compound in the subject. Depending on the outcome of that comparison another dose of the pharmaceutical composition comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, can be administered to achieve a desired level of one or more metabolically-relevant compound.

It is noted that a pharmaceutical composition comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, can be co-administered with another compound. The identity and properties of compound co-administered with the GDF15 mutant polypeptide will depend on the nature of the

condition to be treated or ameliorated. A non-limiting list of examples of compounds that can be administered in combination with a pharmaceutical compostion comprising a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof, include rosiglitizone, pioglitizone, repaglinide, nateglitinide, metformin, exenatide, stiagliptin, pramlintide, glipizide, glimepriride acarbose, and miglitol.

VI. Kits

Also provided are kits for practicing the disclosed methods. Such kits can comprise a pharmaceutical composition such as those described herein, including nucleic acids encoding the peptides or proteins provided herein, vectors and cells comprising such nucleic acids, and pharmaceutical compositions comprising such nucleic acid-containing compounds, which can be provided in a sterile container. Optionally, instructions on how to employ the provided pharmaceutical composition in the treatment of a metabolic disorder can also be included or be made available to a patient or a medical service provider.

In one aspect, a kit comprises (a) a pharmaceutical composition comprising a therapeutically effective amount of a GDF15 polypeptide, construct comprising a GDF15 polypeptide or a mutant form thereof; and (b) one or more containers for the pharmaceutical composition. Such a kit can also comprise instructions for the use thereof; the instructions can be tailored to the precise metabolic disorder being treated. The instructions can describe the use and nature of the materials provided in the kit. In certain embodiments, kits include instructions for a patient to carry out administration to treat a metabolic disorder, such as elevated glucose levels, elevated insulin levels, obesity, type 2 diabetes, dyslipidemia or diabetic nephropathy.

Instructions can be printed on a substrate, such as paper or plastic, etc, and can be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (*e.g.*, associated with the packaging), etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, such as over the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded.

Often it will be desirable that some or all components of a kit are packaged in suitable packaging to maintain sterility. The components of a kit can be packaged in a kit containment element to make a single, easily handled unit, where the kit containment element, *e.g.*, box or analogous structure, may or may not be an airtight container, *e.g.*, to further preserve the sterility of some or all of the components of the kit.

EXAMPLES

The following examples, including the experiments conducted and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention.

EXAMPLE 1

Preparation of Native GDF15 Polypeptides

CHO-S stable cell line growth and transfection was carried out using six-well plates containing a transfection medium (CD-CHO, 4x L-Glutamine, 1x HT, 1x P/S/G). The day before transfection, cultured cells were split to a concentration of $5e^5$ viable cells (vc)/ml. A transfection complex formation was made using 4 µg Linear DNA/500 Optimem, 10 µl LF-LTX/500 µl Optimem, and a 20 minute incubation period. Cells were then prepared by centrifugation at $1e^6$ vc/well, washed in DPBS, and resuspend in 1 ml Optimem. Next, 1 ml of transfection complex was added to $1e^6$ cells in the 6-well plates and incubated for 6 hours with shaking followed by addition 2 ml of MIX-6 Media (w/o antibiotics). Selection was started on the second day using MIX-6 Media with 6 µg/ml Puromycin and replacing media every 2-3 days for 10-12 days by centrifugation of the cells and re-suspension in media.

Cells that were transformed with a GDF15 expression vector constructed with an affinity tag were grown to an optical density of 9 at 600 nm and then induced and harvested at an optical density of 63 by centrifugation 6 hours later. Frozen cell paste was thawed and re-suspended into buffer at 15 % (wt./vol.) with a low shear homogenizer until the slurry was homogeneous. The cells were then subjected to high shear homogenization to break open and release product-containing inclusion bodies. The resulting homogenate was then centrifuged at 5,000 x g for an hour at 5 C to harvest the inclusion bodies as a pellet, leaving the cytoplasmic contaminants in the discarded supernatant. The residual cytoplasm is washed from the inclusion bodies by homogeneously re-suspending the pellet to the original homogenate volume using

chilled water and a low shear homogenizer followed by centrifugation as before. The resulting pellet, washed inclusion bodies (WIBS), is then frozen at -80 C.

A sufficient amount of WIBS and guanidine hydrochloride (GnHCl) was used at pH 8.5 in a reducing-solubilization to result in approximately 25 mg/ml reduced product and 6 M GnHCl final concentrations. The solubilization was then rapidly diluted 25-fold with stirring into a refolding buffer containing redox reagents, chaotrope and co-solvents at alkaline pH. The refold solution was allowed to gently stir and air oxidize at 6 C for 72 hours or until the solution was negative to Ellman's reagent. The refold solution at 5 C was then clarified by depth filtration to allow for a 10-fold ultra-filtration concentration and subsequent diafiltration into a buffer containing 50 mM sodium phosphate and low chaotrope concentration at pH 8.5. The subsequent retentate was warmed to 25 C and then the pH lowered into the acidic range to cause precipitation of contaminants. The precipitate was removed by centrifugation at 5,000 x g for 30 min at 25 C and the resulting supernatant further clarified by 0.45 um filtration. The filtrate (AP) was then adjusted to pH 8.5, and low salt concentration to permit the first step of purification involving immobilized metal affinity chromatography (IMAC).

Following protein folding and AP, the GDF15 was purified using a two-step chromatography train. The adjusted AP was applied to an IMAC column that is equilibrated with buffered chaotrope containing a low salt concentration at pH 8.5. The column was next washed with equilibration buffer until a baseline ultraviolet (UV) level is obtained. Product and contaminants are eluted by step-wise increases in displacer concentration and the elutions were collected and subsequently assayed by Coomasie-stained SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) to identify which eluate fractions contained a polypeptide that migrates at the predicted molecular weight of GDF15. After the IMAC was completed, the pooled fraction containing product is adjusted to pH 7.2 and 5 mM EDTA at 25 C. The product was converted into the mature length GDF15 by adding a low concentration of an enzyme to cleave off the affinity tag at 25 C for several hours. The cleavage reaction mixture was adjusted with an organic modifier and acidic pH by the addition of acetic acid and organic solvent. This allowed for the final chromatography step consisting of a linear gradient elution of product from a reverse phase column conducted at 25 C. The elution from the chromatography was collected as fractions and then assayed by SDS-PAGE to determine the appropriate

fractions to pool for homogeneous product. The resulting pool was buffer exchanged by diafiltration into a weakly acidic buffer, concentrated by ultra-filtration, sterile filtered, and finally stored at 5 C or frozen.

EXAMPLE 2

Suppression of Food Intake in Hyperphagic ob/ob Mice by Fc Fusion GDF15 Proteins

GDF15 reduces food intake in hyperphagic ob/ob mice, and a food intake assay was used to evaluate efficacy of different forms of GDF15 analogs. As the half-life of human GDF15 polypeptide in mouse was observed to be approximately 3 hours, an Fc fusion strategy was used to extend protein half-life. Different Fc fusion GDF15 polypeptides were generated and analyzed for *in vivo* GDF15 activity, by introducing the Fc fusion GDF15 and wild type GDF15 polypeptides into hyperphagic leptin-deficient ob/ob mice, and measuring the ability of a particular Fc fusion GDF15 polypeptide to suppress food intake in these animals. The Fc fusion GDF15 polypeptide to be tested was injected subcutaneously into a 7-8 week old ob/ob mouse (Jackson Laboratory) between 4-5pm on day 0. Animals were transferred after injection to cages where food had been premeasured, and food intake was measured between 9-10 AM the next day.

The results of representative experiments for wild type GDF15 and each Fc fusion GDF15 polypeptide are provided in Figures 3-21, which show dose response curves in the food intake assay for the dimers of the charged pair (delHinge) constructs: DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+); DhCpmFc(+)-$(G_4S)_4$-GDF15:DhCpmFc(-); DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+); DhCpmFc(+)-$(G_4S)_4$-GDF15(H6D):DhCpmFc(-); DhCpmFc(+)-$(G_4S)_4$-GDF15(N3Q):DhCpmFc(-); DhCpmFc(+)-GDF15:DhCpmFc(-); DhCpmFc(+)-G_4-GDF15:DhCpmFc(-); DhCpmFc(+)-$(G_4S)_2$-GDF15:DhCpmFc(-); DhCpmFc(+)-$(G_4Q)_4$-GDF15:DhCpmFc(-); DhCpmFc(+)-(1K)-GDF15:DhCpmFc(-); DhCpmFc(+)(L351C)-$(G_4S)_4$-GDF15:DhCpmFc(-)(L351C); and DhCpmFc(+)(S354C)-$(G_4S)_4$-GDF15:DhCpmFc(-)(Y349C); dimers of the charged pair construct DhCpmFc(+)(S354C)-$(G_4S)_4$-GDF15:DhCpmFc(-)(Y349C); dimers of the hemiFc constructs Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15; Fc-$(G_4S)_3$-Fc-GS$(G_4S)_4$-GDF15; and Fc-$(G_4S)_5$-Fc-GS$(G_4S)_4$-GDF15; native mature hGDF15 homodimer; and mature hGDF15(H6D) variant homodimer.

These experiments demonstrated that the dimers of the charged pair (delHinge), charged pair and hemiFc constructs exhibit a decrease in food intake in ob/ob mice, with greater potency than those of the native mature hGDF15 homodimer The food intake measurement was taken 17 hours after a single injection of the polypeptides, and the stronger potency and efficacy may have resulted from an extended half-life of the Fc fusion polypeptides.

EXAMPLE 3

Improvement of Lipid Tolerance by Fc fusion GDF15 Constructs

Dimers of charged pair (delHinge) and hemiFc constructs were analyzed for *in vivo* GDF15 activity, by introducing these constructs, as well as native mature hGDF15 homodimer, into obese B6D2F1 mice (obesity was induced by feeding the mice a 60% high fat diet), and measuring the ability of each particular polypeptide or construct to improve oral lipid tolerance in these animals.

Male B6D2F1 mice were fed 60% high fat diet (Research Diets D12492i) at 5 weeks old for 6-10 weeks and were stratified by body weight and 3 hour fasting serum triglyceride levels 2-4 days before study. On the day of the study, a dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct (*see* Section II.B.1), a dimer of Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct (*see* Section II.D.1), and native mature hGDF15 homodimer were injected intravenously into mice that had been fasted for 3 hours. In the dose response studies for native mature hGDF15 or or mature hGDF15(H6D) variant, proteins were injected subcutaneously. Three hours after protein injection, 20% Intralipid was orally administered at 20ml/kg. Another 90 min later, blood samples were collected through tail bleeding and serum triglyceride levels were measured.

The results of representative experiments involving the native mature hGDF15 homodimer, the mature hGDF15(H6D) variant homodimer, the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct, and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct are shown in Figures 22-25. In Figures 23-25, the native mature hGDF15 homodimer, the mature hGDF15(H6D) variant and the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct improved lipid tolerance in a dose dependent fashion. In Figure 22, the native mature hGDF15 homodimer and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct are shown to be efficacious in the same assay at 1 mg/kg IV dose.

EXAMPLE 4

Chronic Efficacy of hGDF15 and Constructs Comprising hGDF15 in DIO mice

GDF15 lowers blood glucose, insulin, triglyceride, or cholesterol levels; reduces body weight; or improves glucose tolerance and insulin sensitivity. To assess the ability of hGDF15 and various Fc fusion GDF15 constructs to improve these parameters, chronic studies were performed.

Example 4.1 Study Design

Two separate studies were conducted to evaluate the chronic efficacy of different Fc fusion GDF15 polypeptides. The first study (Study #1) included the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct (*see* Section II.B.1) and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct (*see* Section II.D.1). In this study, the dimer of the charged pair (delHinge) construct and the dimer of the hemiFc constructs were administered chronically and subcutaneously into male C57Bl/6 mice (HAR) fed 60Kcal% high fat diet (Research Diets) at 4 weeks of age for 16 weeks. Animals were acclimated to single housing conditions for 6 weeks. Weekly handling and subcutaneous injection of saline were performed for 3 weeks prior to administration of any test compound. Mice were divided into 7 groups of 12 and one group of 6 based on weekly food intake, and body weight, 4hr fasting blood glucose levels, 4hr fasting serum insulin levels, triglyceride and cholesterol levels 2 days before the first protein injections. Three different dose levels were selected: 10, 1, 0.1nmol/kg, which are equivalent to 1.29, 0.129, 0.0129 mg/kg for the dimer of the charged pair (delhinge) construct, or 1.35, 0.135, 0.0135mg/kg for the dimer of the hemiFc construct. The dosing regimen of weekly dosing was selected based on mouse PK data. One group of 12 animals was subcutaneously injected with vehicle buffer weekly as control group. One group of 6 animals was subcutaneously injected with vehicle buffer and given diet containing 0.014% rosiglitazone to target 10mg/kg oral daily dose as positive control. Studies were carried out for 5 weeks, with the last dose on day 28. The results are shown in Figures 26-38.

The second study (Study#2) included the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct (*see* Section II.B.1) and the dimer of the CpmFc(-)-$(G_4S)_4$-GDF15:CpmFc(+) construct (*see* Section II.C). In this study, the dimer of the charged pair (delHinge) construct and the dimer of the charged pair construct were

administered chronically and subcutaneously into male C57Bl/6 mice (HAR) fed 60Kcal% high fat diet (Research Diets) at 4 weeks of age for 15 weeks. Animals were acclimated to single housing conditions for 5 weeks. Weekly handling and subcutaneous injection of saline were performed for 3 weeks prior to administration of any test compound. Mice were divided into groups of 12 based on weekly food intake, and body weight, 4hr fasting blood glucose levels, 4hr fasting serum insulin, triglyceride and cholesterol levels 4 days before the first protein injection. Three different dose levels were selected at 10, 1, 0.1nmol/kg, equivalent to 1.25, 0.125, 0.0125 mg/kg for the dimer of the charged pair construct. Two different dose levels were selected at 10 and 1 nmol/kg, equivalent to 1.29 and 0.129 mg/kg, for the dimer of the charged pair (delHinge) construct. Dosing regimen of weekly dosing was selected based on mouse PK data. One group of 12 animals was subcutaneously injected with vehicle buffer weekly as control group. One group of 6 animals was subcutaneously injected with vehicle buffer and given diet containing 0.014% rosiglitazone to target 10mg/kg oral daily dose as positive control. Studies were carried for 5 weeks with the last dose on day 28. The results are shown in Figures 39-54.

Body weight and food intake were measured weekly throughout the study. One oral glucose tolerance tests (OGTT) was performed 2 weeks after the first protein injection in animals fasted for 4 hours. One oral glucose tolerance tests (OGTT) was performed 5 weeks after the first protein injection in animals fasted for 16 hours. Blood samples were collected from 4hr fasted animals at 2 and 4 weeks after first protein injection, from animals with free access to food 3 weeks after the first protein injection, and from animals fasted for 16hr 5 weeks after the first protein injection. Serum samples were used to measure insulin, triglyceride and cholesterol levels, as well as the levels of test compound.

Example 4.2 Effect of Test Compounds on Body Weight

Body weight was followed weekly throughout the study, both pre- and post-administration of test compounds. Body weight from baseline of the vehicle animals increased with time, whereas body weight of animals treated with 10 or 1 nmol/kg of the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct and the dimer of the hemiFc construct decreased over the course of the 5 week treatment period and started to come back during the compound wash-out

phase, as shown in Figures 30 and 43. The change of body weight in animals treated with 1 nmol/kg of the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct and the dimer of the hemiFc constructs did not reach statistical significance.

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Example 4.3. Effect of Test Compounds on Food Intake

Food intake was measured every week. Average daily food intake was calculated by dividing weekly food intake by 7. At 10 nmol/kg, the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct and the 10 dimer of the hemiFc construct lowered food intake, with statistical significance in most weekly measurements, as demonstrated in Figures 31 and 44. The effect at 1 nmol/kg dose was less significant, but a trend was clear. The food intake in animals treated with 1nmol/kg of each of the three constructs (*i.e.*, the dimer of the charged pair (delHinge) construce, the dimer of the charged pair construct and the dimer of the 15 hemiFc constructs) were lower compared to food intake in animals treated with vehicle buffer.

Example 4.4 Effect of Test Compounds on Glucose and OGTT

OGTTs (OGTT1 and OGTT2) were performed after treatment was initiated. 20 OGTT1 was conducted 14 days after the first protein injection, in animals fasted for 4 hr from 6am. 4hr fasting glucose levels before the oral glucose challenge were compared with the baseline 4hr fasting glucose levels measured 2 days or 4 days before protein injection (Figures 32 and 45). The glucose profile of OGTT1 is shown in Figures 26, 27, 39 and 40. Animals treated with 10 and 1 nmol/kg of the dimer of 25 the charged pair (delHinge) construct, the dimer of the charged pair Fc-hGDF15 construct or the dimer of the hemiFc constructs exhibited improved glucose levels and oral glucose tolerance when compared to vehicle treated animals, as demonstrated by glucose levels before and during OGTT and glucose AUC during the OGTT. Animals treated with 0.1 nmol/kg of the dimer of the charged pair (delHinge) 30 construct, the dimer of the charged pair construct and the dimer of the hemiFc construct exhibited improved glucose tolerance, but the improvement in glucose levels in these animals was not as significant. The improved glucose tolerance in 0.1nmol/kg dimer of the hemiFc construct treated animals 2 weeks after the first protein injection is indicative of improved glucose tolerance independently from body

weight or food intake changes, since these animals have similar body weight and food intake to vehicle treated animals.

OGTT2 was conducted 35 days after the first protein injection, 7 days after the last protein injection, in animals fasted for 16hr from 5pm the day before the oral glucose challenge. The glucose profile of OGTT2 is shown in Figures 28, 29, 41 and 42. Treatment with 10, 1, or 0.1 nmol/kg of each of the three constructs (*i.e.*, the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct and the dimer of the hemiFc construct) for 5 weeks significantly improved oral glucose tolerance, as demonstrated by glucose levels and glucose curve AUC during the OGTT.

Example 4.5. Effect of Test Compounds on Insulin

Insulin levels were measured in blood samples that had been collected from 4hr fasted animals on day 4, day 14 and day 28, from animals with free access to food on day 21, and from animals fasted for 16hr on day 35. Mice consume majority of their food during the dark cycle. The protocol of 4hr fasting from 6am was used for longitudinal comparison within the same group to remove variation induced by nibbling of food during the light cycle. Observed insulin levels are shown in Figures 33, 34 and 46-48.

The 4hr fasting insulin levels in vehicle treated animals increased over time, as insulin resistance further progresses in these animals. Treatment with 10 or 1 nmol/kg of the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct or the dimer of the hemiFc construct brought insulin levels to levels lower than where the animals started before the treatment was initiated, indicative of a reversal of high fat diet-induced insulin resistance. 0.1 nmol/kg dosage prevented the increase of insulin levels over time. .

All three Fc fusion GDF15 constructs also demonstrated similar dose-dependent improvement in insulin levels in animals fasted overnight on day 35. Data collected for Study #2 demonstrated that the dimer of the charged pair (delHinge) construct and the dimer of the charged pair construct lower insulin levels in animals with free access to food.

Example 4.6 Effect of Test Compounds on Triglyceride Levels

Triglyceride levels were measured in blood samples that had been collected from 4hr fasted animals at day -4, day 14 and day 28, from animals with free access to food on day 21, and from animals fasted for 16hr on day 35. Mice consume majority of their food during the dark cycle. The protocol of 4hr fasting from 6am was used for longitudinal comparison within the same group to remove variation induced by nibbling of food during the light cycle.

The effect of the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct and the dimer of the hemiFc constructs on triglyceride levels in animals fasted for 4 hr or 16 hr were not robust but statistically significant, as demonstrated in Figures 35, 36, 49 and 51. At 10nmol/kg, the dimer of the charged pair (delHinge) Fc- construct consistently lowered serum triglyceride levels at day 28 and day 35. Data collected from Study #2 demonstrated that in animals with free access to food, the effect of 10 and 1 nmol/kg of both the dimer of the charged pair (delHinge) construct and the dimer of the charged pair construct were statistically significant, as shown in Figure 50. We have demonstrated in earlier sections that GDF15 improves oral lipid tolerance. The observation of more potent efficacy in lowering serum triglyceride levels in animals with free access food may indicate improvement of postprandial lipid profile and may have resulted from the improvement in oral/diet lipid tolerance.

Example 4.7 Effect of Test Compounds on Cholesterol Levels

Total cholesterol levels were measured in blood samples that had been collected from 4hr fasted animals at day -4, day 14 and day 28, from animals with free access to food on day 21, and from animals fasted for 16hr on day 35. Mice consume majority of their food during the dark cycle. The protocol of 4hr fasting from 6am was used for longitudinal comparison within the same group to remove variation induced by nibbling of food during the light cycle.

All three constructs (*i.e.*, the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construce and the dimer of the hemiFc construct) dose-dependently lowered total cholesterol levels, and this effect was impacted by the feeding state of the animals, as demonstrated in Figures 37, 38 and 52-54.

Conclusions From Example 4

All three constructs, namely the dimer of the charged pair (delHinge) construct, the dimer of the charged pair construct and the dimer of the hemiFc construct, demonstrated efficacy in improving various metabolic parameters, including body weight, blood glucose levels and glucose tolerance, serum insulin levels, serum cholesterol levels, serum triglyceride levels and oral lipid tolerance. The polypeptides were injected once per week, and efficacy was dose-dependent, with 10nom/kg dosage demonstrating the most robust efficacy.

EXAMPLE 5

Chronic Efficacy of hGDF15 and Constructs Comprising hGDF15 in Obese Cynomolgous Monkey

The charged pair (delHinge) construct "DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+)" and the hemiFc construct "Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15" were analyzed for *in vivo* GDF15 activity by introducing these constructs, as well as vehicle (A4.5Su) into obese cynomolgous monkeys. .

Example 5.1 Study Design

The study was conducted in cynomolgous monkeys. The monkeys were 11-14 years old. Their body weights ranged from 7-10 kg and BMI ranged from 38-58 kg/m^2. 40 monkeys were acclimated for 6 weeks prior to the initiation of compound administration. During the acclimation period, monkeys were trained 4 times a week for 6 weeks to familiarize the procedures including chair-restrain, subcutaneous injection (PBS, 0.1 ml/kg), gavage (Water, 10 ml/kg), blood drawn for non OGTT and OGTT samples. During 6 weeks of training, baseline OGTT and plasma metabolic parameters were measured. 30 out of 40 monkeys were selected and randomized into three treatment groups to achieve similar baseline levels of body weight, OGTT AUC response, and plasma glucose, insulin and triglyceride levels.

The study was conducted in a blind fashion. Vehicle (n=10), dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct (n=10) and dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct (n=10) were labeled as compound A, B and C and administered once a week via subcutaneous injection. Compounds were given at 1.5 mg/kg/week. After 6 weeks of compound treatments, animals were monitored for additional 5 weeks for compound washout and recovery from treatments. Food intake, body weight, clinical chemistry and OGTT were monitored throughout the study.

Food intake was measured at every meal. Body weight was measured weekly. Blood samples were collected weekly 6 days post each injection to measure glucose, triglyceride. OGTTs were conducted on day 13, 34 and 55 after the initiation of treatments. The day starting the treatment is designated as 0 and the detailed study design is shown in Figure 55.

The results shown in this example are data collected at the end of 6 weeks treatment and during the washout.

Example 5.2 Compounds exposure

Compounds (dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct) exposure measurements were performed on samples collected after an overnight fast and the day after in non fasted animals. These measurements were made every week during the treatment and washout phase. The dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct showed a greater exposure than the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct. Two out of ten animals in the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct group presented antibody whereas five out of ten animals were identified in the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct group. The loss of pharmacodynamic responses in suspected animals with antibody correlates with loss of drug exposure. The exposure levels for each compound are shown in Figure 56.

Example 5.3 Effect of Test Compounds on Oral Glucose Tolerance Test (OGTT)

OGTTs were conducted before and after initiation of treatments. Figures 57-64 show pre- and post-OGTT area under the OGTT curve (AUC). Acclimation OGTT was performed before the treatment was initiated, post-dose OGTTs were performed on week 2 and week 5 and washout OGTT was performed on week 8 (3 weeks after the last dose). Glucose and insulin (AUC) were measured for glucose (Figure 57-60) and insulin (Figures 61-64). The dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct significantly improved OGTT glucose AUC on week 2 and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct on week 5. OGTT Glucose AUC was still improved on week 8 (during washout phase with the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15) construct. OGTT insulin AUC was significantly ameliorated on week 2 and 5 for the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15

construct and on week 2 only for the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct.

Example 5.4 Effect of Test Compounds on Fasting Insulin Levels

Blood was collected from overnight fasted animals. The blood drawn was conducted weekly at 6 days post each injection. Both the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct reduced fasting blood insulin levels. This effect was not seen during the washout period. Figure 68 shows the levels of fasting insulin during the course of the study.

Example 5.5 Effect of Test Compounds on Food Intake

Animals were fed twice a day and food intake was recorded at each meal. The feeding times were from 8:00 AM to 9:00 AM (±30 minutes) and then from 4:00PM to 5:00PM (±30 minutes). Apple (150 g) was supplied to each animal at 1:30-2:30 PM (±30 minutes) every day.

Compared with vehicle, both the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct reduced food intake in monkeys (Figure 66). However, the effect diminished and the food intake returned close to baseline or control levels after about 30 days of treatment.

Example 5.6 Effect of Test Compounds on Trigylceride Levels

Blood was collected from overnight fasted animals. The blood drawn was conducted weekly at 6 days post each injection. Triglyceride levels were significantly reduced in animals treated with the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct throughout the treatment phase. This effect diminished during the washout period. Figure 67 shows the levels of fasting plasma triglycerides during the course of the study.

Example 5.7 Effect of Test Compounds on Body Weight

Body weight was monitored weekly throughout the study. Over the course of the 6 week treatments, the body weight of animals treated with vehicle remained constant or slightly increased while body weight of animals treated with the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct progressively decreased as shown in Figure 65.

Conclusions from Example 5

In a study conducted in male obese *cynomolgous* monkeys, animals treated with the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct showed improved metabolic parameters. Body weight was reduced. Short-term reduction of food intake was observed and the effect diminished and the food intake recovered to baseline or control levels at the mid term of the study. Fasting triglyceride levels were also reduced by both compounds, the dimer of the DhCpmFc(-)-$(G_4S)_4$-GDF15:DhCpmFc(+) construct and the dimer of the Fc-$(G_4S)_8$-Fc-GS$(G_4S)_4$-GDF15 construct. OGTT glucose and insulin AUC were improved.

While the present invention has been described in terms of various embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed. In addition, the section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

All references cited in this application are expressly incorporated by reference herein for any purpose.

CLAIMS

What is claimed is:

1. A construct comprising a GDF15 polypeptide and one or more Fc sequences.

2. The construct of claim 1, wherein the construct comprises two or more Fc sequences.

3. The construct of claim 2, wherein one or more of the Fc sequences independently comprise a sequence selected from the group consisting of SEQ ID NOs:18, 19, 85, 86, 89, 90, 91, 99, 100, 108, 109 and 111.

4. The construct of claim 3, wherein two or more of the Fc sequences are associated.

5. The construct of claim 4, wherein the GDF15 polypeptide and one or more of the Fc sequences form a contiguous sequence.

6. The construct of claim 5, wherein the GDF15 polypeptide and an Fc sequence are joined by a linker.

7. The construct of claim 6, wherein the construct comprises a sequence selected from the group consisting of SEQ ID NOs: 44, 50, 57, 61, 65, 69, 74, 79, 84, 95 and 104.

8. The construct of claim 7, wherein the construct further comprises a sequence selected from the group consisting of SEQ ID NOs:18 19, 85, 86, 89, 90, 91, 99, 100, 108, 109 and 110.

9. A dimer comprising the construct of claim 8.

10. The construct of claim 1, wherein the construct comprises a construct selected from the group consisting of:

(a) a sequence comprising SEQ ID NO:18 and a sequence comprising SEQ ID NO:44;

(b) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:50;

(c) a sequence comprising SEQ ID NO:18 and a sequence comprising SEQ ID NO:57;

(d) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:61;

(e) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:65;

(f) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:69;

(g) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:74;

(h) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:79;

(i) a sequence comprising SEQ ID NO:86 and a sequence comprising SEQ ID NO:84;

(j) a sequence comprising SEQ ID NO:91 and a sequence comprising SEQ ID NO:95; or

(k) a sequence comprising SEQ ID NO:100 and a sequence comprising SEQ ID NO:104.

11. A construct comprising a dimer comprising the construct of claim 10.

12. The construct of claim 2, wherein one or more Fc sequences independently comprise a sequence selected from the group consisting of SEQ ID NOs:23, 110, 114 and 115.

13. The construct of claim 12, wherein two or more Fc sequences are associated.

14. The construct of claim 13, wherein the GDF15 polypeptide and one or more of the Fc sequences form a contiguous sequence.

15. The construct of claim 14, wherein the GDF15 polypeptide and an Fc sequence are joined by a linker.

16. The construct of claim 15, wherein the construct comprises SEQ ID NO:113.

17. A dimer comprising the construct of claim 16.

18. The construct of claim 12, wherein the construct comprises a sequence comprising SEQ ID NO: 110 and a sequence comprising SEQ ID NO:113.

19. A dimer comprising the construct of claim 18.

20. The construct of claim 2, wherein one or more of the Fc sequences independently comprise a sequence selected from the group consisting of SEQ ID NOs:28 and 30.

21. The construct of claim 20, wherein two or more of the Fc sequences are joined by a linker.

22. The construct of claim 21, wherein the linker comprises a sequence selected from the group consisting of SEQ ID NOs: 87, 88 and 131.

23. The construct of claim 22, wherein the C-terminus of a first Fc sequence is joined to the N-terminus of a second Fc sequence.

24. The construct of claim 23, wherein the GDF15 polypeptide and one or more of the Fc sequences form a contiguous sequence.

25. The construct of claim 24, wherein the GDF15 polypeptide and an Fc sequence are joined by a linker.

26. The construct of claim 25, wherein the linker comprises SEQ ID NO:31.

27. A dimer comprising the construct of claim 26.

28. The construct of claim 20, wherein the construct comprises a sequence selected from the group consisting of SEQ ID NOs:33, 123 and 128.

29. A dimer comprising the construct of claim 28.