Title: THERAPEUTIC APPLICATIONS OF LNFP III

Abstract: The present invention demonstrates that the administration of an immunomodulatory glycan or a glycoconjugate thereof, including, but not limited to, a glycoconjugate of lacto-N-fucopentaose III (LNFP III), during pregnancy and/or lactation is therapeutic and/or prophylactic to restore or normalize the physiologic, neurologic, metabolic, and immune functions adversely influenced in neonates and children born of obese, overweight mothers.
THERAPEUTIC APPLICATIONS OF LNFPIII

CONTINUING APPLICATION DATA

This application claims the benefit of U.S. Provisional Application Serial No. 62/031,359, filed July 31, 2014, which is incorporated by reference herein in its entirety.

GOVERNMENT FUNDING

This invention was made with government support under Grant No. R01AI056484, awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

Obesity induced inflammation leading to insulin resistance, hepatosteatosis, and cardiovascular disease is epidemic in the United States. In 2005-2006 more than 34% of US adults were obese with an additional 33% overweight ("Prevalence of overweight, obesity and extreme obesity among adults: United States, trends 1960-62 through 2005-2006" (available on the worldwide web at cdc.gov/nchs/data/hestat/overweight/overweight_adult.htm; released 2009); Ogden et al, 2006, JAMA; 295:1549-1555; and Flegal et al, 2002, JAMA; 288:1723-1727). Importantly, childhood obesity and diabetes have also increased dramatically (Ludwig et al, 2001, Lancet; 357:505-508). A recent cross-sectional study of 12-19 year olds in the US showed the prevalence of non-alcoholic fatty liver disease (NAFLD) in this age group increased from 3.9% from 1988-1994 to 10.7% in 2007-2010 (Welsh et al, 2013, J Pediatr; 162:496-500 e491). Currently there are no FDA approved drugs to treat NAFLD and the need to identify protective or therapeutic pathways to alleviate the pathologies of obesity, including childhood obesity and diabetes, is urgent.
SUMMARY OF THE INVENTION

The present invention includes a method of reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject's mother, the method including maternal administration of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, a mimetic thereof, or a conjugate thereof, to the subject's mother.

In some aspects, administration includes administration to the subject's mother prior to pregnancy, prior to lactation, during pregnancy, and/or during lactation. In some aspects, administration includes administration to the subject's mother prior to gestation of the subject. In some aspects, administration includes administration to the subject's mother during gestation of the subject. In some aspects, administration includes administration to the subject's mother prior to the initiation of breast feeding of the subject by the mother. In some aspects, administration includes administration to the subject's mother during breast feeding of the subject by the mother. In some aspects, maternal administration restores gut, metabolic, brain, neurologic and/or immune functions to normal in the offspring.

In some aspects, administration includes by injection, orally, transdermally, topically, or intranasally. In some aspects, administration by injection includes intramuscular, intradermal, or subcutaneous injection. In some aspects, administration by injection includes formulation as a slow release biodegradable complex, nanoparticles, and/or a hydrogel. In some aspects, oral administration includes administration as a liquid suspension, tablet, a capsule, or a gel. In some aspects, oral administration includes formulation as a food item, nutraceutical, or a dietary supplement. In some aspects, topical administration includes administration as an emulsion, cream, or ointment. In some aspects, transdermal administration includes administration by a transdermal skin patch or a microneedle patch.

In some aspects, a LNFPIII glycan conjugate thereof includes BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer. In some aspects, a LNFPIII glycan conjugate includes about 8 to about 10 LNFPIII per dextran.

The present invention includes a method of reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject's mother, the method including the maternal administration of an immunomodulatory glycan or a glycoconjugate
thereof to the subject's mother. In some aspects, an immunomodulatory glycan includes lacto-N-fucopentaose III (LNFPIII), LacdiNAc, fucosylated LacdiNac, or omega-1.

In some aspects, administration includes administration to the subject's mother prior to pregnancy, prior to lactation, during pregnancy, and/or during lactation. In some aspects, administration includes administration to the subject's mother prior to gestation of the subject. In some aspects, administration includes administration to the subject's mother during gestation of the subject. In some aspects, administration includes administration to the subject's mother prior to the initiation of breast feeding of the subject by the mother. In some aspects, administration includes administration to the subject's mother during breast feeding of the subject by the mother. In some aspects, maternal administration restores gut, metabolic, brain, neurologic and/or immune functions to normal in the offspring.

In some aspects, administration includes by injection, orally, transdermally, topically, or intranasally. In some aspects, administration by injection includes intramuscular, intradermal, or subcutaneous injection. In some aspects, administration by injection includes formulation as a slow release biodegradable complex, nanoparticles, and/or a hydrogel. In some aspects, oral administration includes administration as a liquid suspension, tablet, a capsule, or a gel. In some aspects, oral administration includes formulation as a food item, nutraceutical, or a dietary supplement. In some aspects, topical administration includes administration as an emulsion, cream, or ointment. In some aspects, transdermal administration includes administration by a transdermal skin patch or a microneedle patch.

In some aspects, a glycoconjugate thereof includes BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer.

The present invention includes the use of an immunomodulatory glycan or a glycoconjugate thereof as a medicament for maternal administration for reducing one or more of the effects of maternal high fat diet (HFD) obesity in the offspring. In some aspects, an immunomodulatory glycan includes lacto-N-fucopentaose III (LNFPIII), LacdiNAc, fucosylated LacdiNac, or omega-1.

The present invention includes the use of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof, as a medicament for maternal administration for reducing one or more of the effects of maternal high fat diet (HFD) obesity in the offspring.
In some aspects, a LNFPIII glycan conjugate includes BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer. In some aspects, a LNFPIII glycan conjugate includes about 8 to about 10 LNFPIII per dextran.

The present invention includes the use of an immunomodulatory glycan or a glycoconjugate thereof for reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject’s mother. In some aspects, an immunomodulatory glycan includes lacto-N-fucopentaose III (LNFPII), LacdiNAc, fucosylated LacdiNac, or omega-1.

The present invention includes the use of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof, for reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject’s mother. In some aspects, a LNFPIII glycan conjugate includes BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer. In some aspects, a LNFPIII glycan conjugate includes about 8 to about 10 LNFPIII per dextran.

The present invention includes the use of an immunomodulatory glycan or a glycoconjugate for the preparation of a medicament for reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject’s mother. In some aspects, an immunomodulatory glycan includes lacto-N-fucopentaose III (LNFPIII), LacdiNAc, fucosylated LacdiNac, or omega-1.

The present invention includes the use of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof, in the preparation of a medicament for reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject’s mother. In some aspects, a LNFPIII glycan conjugate includes BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer. In some aspects, a LNFPIII glycan conjugate includes a LNPIII dextran conjugate including about 8 to about 10 LNFPIII per dextran.

In some aspects of the uses of the present invention, maternal administration includes prior to pregnancy, prior to lactating, during pregnancy, and/or during lactating.

The present invention includes a nutraceutical or dietary supplement for consumption by a pregnant and/or lactation woman, the nutraceutical or dietary supplement comprising an immunomodulatory glycan or a glycoconjugate thereof. In some aspects, an immunomodulatory
glycan includes lacto-N-fucopentaose III (LNFPIII), LacdiNAc, fucosylated LacdiNac, or omega-1.

The present invention includes a nutraceutical or dietary supplement for consumption by a pregnant and/or lactation women, the nutraceutical or dietary supplement comprising a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof. In some aspects, a LNFPIII glycan conjugate includes BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer. In some aspects, a LNFPII glycan conjugate includes a LNPIII dextran conjugate including about 8 to about 10 LNFPIII per dextran.

The term "and/or" means one or all of the listed elements or a combination of any two or more of the listed elements.

The words "preferred" and "preferably" refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the invention.

The terms "comprises" and variations thereof do not have a limiting meaning where these terms appear in the description and claims.

Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims,
each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Panel (A): LNFP III glycan therapy of HF obese dams (HF-Glycan) normalized pup gut permeability. Gut permeability was determined in post-natal day (PND) 21 pups by quantifying plasma concentration of fluorescent dextran one hour following oral administration. Maternal obesity (HF) led to a significant increase in GI permeability in pups. One way anova, LF vs. HF p<0.01, HF-G vs. HF, p<0.05. Panel (B): LNFP III therapy of obese dams normalizes IAP activity at PND 21 and PND 90 pups. Panel (C): Pups born of HF obese dams have increased gut microflora composition. Pups born of LNFP III Glycan treated HF obese dams have normalized gut microflora composition. Significant differences between LFD and HFD were for Porphyromonadaceae (59% vs. 24.5%, p < 0.006), Lachnospiraceae (9.8% vs. 34.3%, p < 0.0006), Ruminococcaceae (6.7% vs. 15.7%, p < 0.032), and Verrucomicrobiaceae (0.3% vs. 0.1%, p < 0.016). Additionally, no Desulfovibrioaceae were present in feces from pups from dams fed LFD and the presence of small fractional contributions of this family (0.104%) in feces of pups from dams fed HFD. Two of five pups in the HFD + G groups also had low levels of this family.

Figure 2. Adult female pups (PND90) from HFD dams have decreased hippocampal LTP (long-term potentiation, a measure of synaptic plasticity that is associated with learning and
memory). The LTP deficit is restored in pups born of LNFPIII glycan treated HFD dams. Of note, after weaning (PND21), all offspring were raised on regular chow.

Figure 3. Total RNA was isolated from livers of PND21 pups from LF, HF or HF-glycan adults. qPvT-PCR was performed using specific Mir-155 primer. Gene expression was normalized with 18S endogenous control expression and fold expression was determined over LF control. Statistical significance calculated using Graphpad prism student’s t test. *, P < 0.05.

Figure 4. Impact of glycan LNFPIII on hepatic steatosis. Hepatic tissue of 2 l-day old offspring from dams fed either HFD diet or HFD diet was isolated. A subset of dams from the HFD group were given a biweekly injection of the immunomodulatory glycan LNPfIII. Panel (A) represents LFD versus HFD. Panel (B) represents HFD versus glycan. Isolated lipid from hepatic tissue was resuspended in Triton-X and assayed using a colorimetric assay for triglycerides. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter are statistically significant at p<0.05. Abbreviations: low-fat diet (LF) and high-fat diet (HF).

Figure 5. Lipogenic Gene Expression. Panel (A) presents ACACA gene expression. Panel (B) presents FASN gene expression. Panel (C) presents PPARy gene expression. Panel (D) presents SREBF1 gene expression.

Figure 6. Impact of glycan LNFPIII on insulin signaling. Hepatic mRNA was isolated from post-natal day 2 l offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). Panel (A) represents SLC2A4 gene expression for LFD versus HFD. Panel (B) represents SLC2A4 gene expression for HFD versus glycan. Panel (C) represents IRS1 gene expression for LFD versus HFD. Panel (D) represents IRS1 gene expression for HFD versus glycan. Panel (E) represents INSR gene expression for LFD versus HFD. Panel (F) represents INSR gene expression for HFD versus glycan. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05. Abbreviations: solute carrier family 2 (facilitated glucose transporter) member 4 (SLC2A4), insulin receptor substrate 1 (IRS1), insulin receptor (INSR), low-fat diet (LFD), high-fat diet (HFD).

Figure 7. Impact of glycan LNPfIII on endoplasmic reticulum stress gene expression. Hepatic mRNA was isolated from post-natal day 2 l offspring from dams fed either HFD or LFD.
A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). Panel (A) represents DDIT3 gene expression for LFD versus HFD. Panel (B) represents DDIT3 gene expression for HFD versus glycan. Panel (C) represents XBP1 spliced mRNA for LFD versus HFD. Panel (D) represents XBP1 spliced mRNA for HFD versus glycan. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05. Abbreviations: DNA damage-inducible transcript 3 (DDIT3), X-box binding protein 1- spliced variant (XBPls), low-fat diet (LFD), high-fat diet (HFD).

Figure 8. Impact of glycan LNFPIII on the expression of epigenetic regulator DNA methyltransferases 1. Hepatic mRNA was isolated from post-natal day 21 offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). Panel (A) represents DNMT1 gene expression for LFD versus HFD. Panel (B) represents DNMT1 gene expression for HFD versus glycan. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05. Abbreviations: DNA-inducible methyl transferase 1 (DNMT1), low-fat diet (LFD), high-fat diet (HFD).

Figure 9. Impact of glycan LNFPIII on hepatic function. Panels (A) and (C) represent ACTA2 gene expression for LFD versus HFD. Panels (B) and (D) represents ACTA2 gene expression for HFD versus glycan. In Panels (A)-(D), hepatic mRNA was isolated from post-natal day 21 offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). In Panels (E) and (F) total protein was isolated from frozen livers. Total protein concentration and ALT concentration was performed using manufacturer's protocols. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05. Abbreviations: alanine aminotransferase (ALT), actin alpha 2 (ACTA2), glutamic pyruvate transaminase 2 (GPT2), low-fat diet (LFD), high-fat diet (HFD).

Figure 10. Direct impact of glycan LNFPIII on microbiota (Phyla) composition of dams.

Figure 11. Transgenerational impact of glycan LNFPIII on microbiota (Phyla) composition at postnatal day 21 (PND 21).
Figure 12. Transgenerational impact of glycan LNFPIII on microbiota (Phyla) composition at postnatal day 35 (PND 35).

Figure 13. Transgenerational impact of glycan LNFPIII on microbiota (Class) composition at postnatal day 21 (PND 21).

Figure 14. Transgenerational impact of glycan LNFPIII on microbiota (PCA) composition at postnatal day 21 (PND 21).

Figure 15. Transgenerational impact of glycan LNFPIII on microbiota (Phyla) composition at postnatal day 90 (PND 90).

Figure 16. Transgenerational impact of glycan LNFPIII on microbiota (Class) composition at postnatal day 90 (PND 90).

Figure 17. The effects of maternal high-fat diet (HFD) consumption and maternal administration of the LNFPIII glycan on dopamine (DA) homeostasis in the dorsal (dHIPP) and ventral (vHIPP) hippocampus. Panel (A) represents dorsal (dHIPP). Panel (B) represents ventral (vHIPP) hippocampus.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Maternal high fat diets have been shown to influence the risk of obesity and metabolic diseases, specifically non-alcoholic fatty liver disease (NALFD), in the offspring of various species (Tamashiro and Moran, 2010, *Physiology and Behavior;* 100:560-566; Bruce et al, 2009, *Hepatology;* 50:1796-1808; and Oben et al, 2010, *Journal of Hepatology;* 52:913-920). NALFD is the third most prevalent obesity co-morbidity affecting 20-30% of adults in Westernized societies (Preiss and Sattar, 2008, *Clinical Science;* 115:141-150). What is more concerning is the increasing trend of NALFD in children. Autopsy reports suggest that up to 9% of US children may have NALFD, with 27% of these children experiencing non-alcoholic steatohepatitis and 9% experiencing fibrosis (Schwimmer et al, 2006, *Pediatrics;* 118:1388-1393). Obese children are more likely to become obese adults; therefore children diagnosed with NALFD will most likely have worsening liver conditions throughout their lifespan.

The prevalence of childhood obesity and diabetes has increased significantly in the United States and there is evidence that part of this increased prevalence of childhood obesity is in part, due to gestational exposure to a high fat environment. In the US, approximately 30% of

Taken together, maternal obesity during gestation increases the risk of children being born with, or developing metabolic disease(s) and/or negative neural physiology. Thus, children born of obese mothers will have increased prevalence of Autism Spectral Disease, fatty liver disease and/or diabetes. Importantly for diabetes and fatty liver disease, children born of obese mothers may develop these diseases in the first two decades of life rather than in their 30s and 40s, significantly increasing the public health burden and health care costs due to obesity.

With the present invention it has been shown that the maternal administration of an immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate thereof during pregnancy and/or lactation prevents or reduces the severity of the adverse health effects in the offspring born to a mother with high fat diet (HFD) obesity, including, but not limited to,
preventing or reducing adverse effects on physiologic, neurologic metabolic, and/or immune functions.

The present invention includes the maternal administration of an immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate thereof. Such administration may include administration to a female individual during pregnancy and/or lactation. Such administration may be during any stage of pregnancy and for any length of time during a pregnancy. For example, administration may be for the entire pregnancy. For example, administration may be during the first, second, and/or third trimesters. In some embodiments, the pregnant woman is obese. In some embodiments, administration may decrease the risk of preeclampsia.

In some embodiments, administration may begin with the diagnosis of one of more of the symptoms of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, metabolic disease, and/or non-alcoholic fatty liver disease (NAFLD) in a pregnant woman. Non-alcoholic fatty liver disease includes the spectrum of disorders resulting from an accumulation of fat in liver cells in individuals with no history of excessive alcohol consumption. In the mildest form, NAFLD refers to hepatic steatosis. The term NAFLD may also include the more severe and advanced form non-alcoholic steatohepatitis (NASH). Metabolic syndrome is a collection of risk factors estimated to affect over 50 million Americans. Criteria for diagnosing metabolic syndrome include, for example, abdominal obesity, atherogenic dislipidemia, elevated blood pressure, insulin resistance or glucose intolerance, prothrombotic state, and a proinflammatory state. Risk factors for metabolic syndrome (MetS) may include a high level of triglycerides (>150 mg/dL), a low level of high density lipoprotein (HDL) cholesterol (<50 mg/dL for women), high blood pressure (>130/85 mmHg), high levels of fasting blood glucose (>100 mg/dL), and a large waist circumference (>35 inches for women) (see, for example, Gupta at al, 2010, *Bioscience Trends*; 4:204-1).

In some embodiments, administration may be to a woman prior to pregnancy, in preparation for a pregnancy. In some embodiments, the woman is obese. In some embodiments, the women has been diagnosed with one of more of the symptoms of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, metabolic disease, and/or non-alcoholic fatty liver disease (NAFLD).
Administration may include administration to a nursing woman during lactation. Such administration may be during any stage of lactation and for any length of time during lactation. For example, administration may be for the entire period a mother chooses to breast feed her infant. Administration may be for the period of colostrum production. Administration may be for one week, two weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, one year, or more. In some embodiments, administration may be to a woman prior to lactation, in preparation for lactation.

Maternal obesity and over nutrition can imprint an increased risk of metabolic diseases, including non-alcoholic fatty liver disease, in the offspring. With the methods of the present invention, maternal administration reduces one or more of the adverse effects of a high fat diet or metabolic disease in the offspring of such mothers. Administration may reduce or normalize the physiologic, neurologic, metabolic and immune functions adversely influenced in neonates and children born of obese, overweight mothers. Such maternal administration may result in one or more of the following in the offspring: restored gut-barrier function and gut permeability, restored brain function, restored learning and memory, normalized inflammatory mediators, normalized metabolic indicators, reduced adiposity, reduced incidence of negative behavioral and/or neurologic pathology, restoration of metabolic measurements to normal, restore impaired glucose tolerance, and/or restore insulin sensitivity.

For the methods described herein, offspring may exhibit a reduction in one or more of the symptom(s) of the effects of a maternal high fat diet (HFD). Offspring may exhibit a reduction in one or more symptom(s) or biomarker levels caused by, or associated with NAFLD or metabolic syndrome. Offspring may exhibit a reduction in one or more symptom(s) or biomarker levels described in the examples section included herewith. Therapeutic effectiveness of the treatments may be demonstrated, for example, by improvements in liver histology (e.g., by liver biopsy), levels of fatty acids (e.g., triglycerides), levels of biomarkers (including, but not limited to any of those described in the examples section included herewith. In some embodiments, administration may reduce the risk of autism in offspring.

For the methods described herein, offspring may exhibit a 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater reduction, up to a 75-90%, or 95% or greater, reduction in one or more symptom(s) or biomarker levels caused by, or associated with NAFLD
or metabolic syndrome. In a specific embodiment, the level of one or more liver enzymes, such as for example, ALT or AST, is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% in a subject administered the treatments of the invention. In another embodiment, the level of one or more fatty acids, preferably triglycerides, in hepatocytes or liver tissue are reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%.

As used herein, terms such as "subject," "individual," "patient," "mother," "women," "female," "offspring," and "child," are intended to include human and nonhuman animals. In preferred embodiments, such terms refer to human animals. The term "mammals" includes all vertebrates, for example, humans, nonhuman primates, sheep, dogs, cats, horses, cows, rodents and transgenic non-human animals.

Immunomodulatory glycans include, but are not limited to, glycans found in breast milk, such as human breast milk, and/or glycans expressed on parasitic helminths, such as the parasite *Schistosoma mansoni*. This includes, but is not limited to, immunomodulatory glycans such as lacto-N-fucopentaose III (LNFPIII), LacdiNac, fucosylated LacdiNac, and omega-1 (see, for example, Atochina et al., 2001, J Immunol; 167:4293-4302; Atochina et al., 2008, Immunol; 125(1): 111-121; Everts et al, 2009, J Exp Med; 206:1673-1680; Steinfelder et al, 2009, J Exp Med; 206:1681-1690; and Van der Kleij et al, 2002, J Infect Dis; 185:531-539).

In some aspects of the methods described herein, an immunomodulatory glycan includes the Lewis^x^ containing immunomodulatory glycan Lacto-N-fucopentaose III (LNFPIII). LNFPIII is a fucosylated glycan/sugar found in human milk and on the helminth parasite *Schistosoma mansoni*. It has been shown to contain a Lewis(x) trisaccharide and to demonstrate immunomodulatory activities. See, for example Bhargava et al., 2012, Nat Med; 18(11): 1665-72; Srivastava et al, 2014, Infect Immun; 82(5):1891-903; Zhu et al, 2012, Clin Immunol; 142(3):351-61; and Dutta et al, Transplantation; 90(10): 1071-8, each of which is hereby incorporated by reference in its entirety. A LNFPIII glycan has the structure: \{Gal(pi-3)[Fuc(al-3)]GlcNAc(pi-3)Gal (P)Glc\} and comprises the Lewis^x^ antigen.

In some aspects of the methods described herein, an immunomodulatory glycan includes a structural derivative of LNFPIII that has a similar activity profile *in vivo*. Such derivatives thereof may include one or more of those described in more detail in U.S. Patent Application
20120202753 ("Inhibiting Inflammation with Milk Oligosaccharides"), which is hereby incorporated by reference in its entirety.

In some aspects of the methods described herein, an immunomodulatory glycan includes a mimetic of LNFPIII that mimics structural aspects of LNFPIII and mimics one or more of the biological activities of LNFPIII. Such a glycomimetic may be, for example, a small molecule, a peptide, or an aptamer.

In some aspects of the methods described herein, an immunomodulatory glycan includes any one or more of the helminth-derived glycans and glycoconjugates as described in more detail in U.S. Patent Application 20140315781 ("Methods of Treating Fatty Liver Disease with Helminth-Derived Glycan-Containing Compounds"), which is hereby incorporated by reference in its entirety. Briefly, such a helminth-derived glycan may include, for example, a Lewis\(^x\) antigen, a non-Lewis\(^x\) antigen, derivatives thereof, or a combination thereof. In certain embodiments, a helminth-derived glycan for use in the methods described herein includes LNFPIII, LNN\(\text{T}, LDN, LDNF, SEA, or combinations thereof. As used herein, "Lewis antigen" includes carbohydrates having as a core sequence either the lacto type I structure \{Gal(pi-3)GlcNac\} or the lacto type II structure \{Gal(pi-4)GlcNac\}, substituted with one or more fucosyl residues. The Lewis antigen may include a single substituted core sequence or a repetitive series of substituted core sequences. Moreover, the core sequence may be present within a larger sugar. Accordingly, a Lewis antigen-containing oligosaccharide can be, for example, a trisaccharide, a tetrasaccharide, a pentasaccharide, and so on. Types of Lewis antigens include Lewis\(^x\), Lewis\(^y\), Lewis\(^a\), and Lewis\(^b\) oligosaccharides and derivatives thereof. Synthetic structural homologues of these carbohydrates that retain the immunomodulatory capacity are also intended to be encompassed by the term "Lewis antigen." As used herein, "Lewis\(^x\) oligosaccharide" refers to a lacto type II carbohydrate having the structure: \{Gal(pi-4)[Fuc(al-3)]GlcNac\}. As used herein, "Lewis\(^y\) oligosaccharide" refers to a lacto type II carbohydrate comprising the structure: \{Fuc(al-2)Gal(pi-4)[Fuc(al-3)]GlcNac\}. As used herein, "Lewis\(^a\) oligosaccharide" refers to a lacto type I carbohydrate having the structure: \{Gal(pi-3)[Fuc(al-4)]GlcNac\}. As used herein, "Lewis\(^b\) oligosaccharide" refers to a lacto type I carbohydrate comprising the structure: \{Fuc(al-2)Gal(pi-3)[Fuc(al-4)]GlcNac\}. As used herein, a "derivative" of a Lewis oligosaccharide includes a Lewis oligosaccharide having one or more additional substituent groups, for example, terminally sialylated forms of Lewis
oligosaccharides (such as, for example, sialyl-Lewis χ, sialyl-Lewis ω, sialyl-Lewis α, sialyl-
Lewis β), sulfated forms of Lewis oligosaccharides, and sulfo-sialylated forms of Lewis
oligosaccharides. As used herein, "SEA" refers to schistosome egg antigen or soluble egg
antigen, including Lewis x and non-Lewis x antigens. As used herein, "LN" refers to a glycan
with the structure: \{Gal(P)GlcNAc\}. As used herein, "LNnT" (lacto-N-neotetraose) refers to a
cyclodextran. Alternative to a carrier protein, immunomodulatory glycans can be conjugated to
an antigen-containing polyol, polylysine avidin, a lipid, a lipid emulsion, a liposome, a dendrimer, or a
cyclodextran. Alternative to a carrier protein, immunomodulatory glycans can be conjugated to

Glycans for use in the methods of the invention can be purchased commercially or can be
purified or synthesized by standard methods. For example, LNFPIII is available from Neose
Technologies Inc. (Horsham, PA) and Sigma-Aldrich (USA). Further, conjugates of Lewis
antigen-containing sugars and a carrier protein (e.g., HSA) are available from Accurate
Chemicals, Westbury, N.Y. Conjugates of Lewis antigen-containing sugars and polyacrylamide
are available from GlycoTech, Rockville, MD. Schistosome egg antigen (SEA) can be purified
from Schistosoma mansoni eggs as described in Ham et al., 1984, J Exp Med; 159:1371-1387.

In some embodiments, a conjugate of one or more immunomodulatory glycans, a
mimetic thereof, and/or a derivative thereof is administered. An immunomodulatory glycan,
mimetic thereof, or derivative thereof may be conjugated to any of a variety of carriers,
including, but not limited to, a protein, such as, for example, human serum albumin (HSA),
bovine serum albumin (BSA), mouse gamma globulin (MGG), human gamma globulin (HGG),
keyhole limpet hemocyanin (KLH), ovalbumin (OVA), a carbohydrate polymer, such as, for
example, dextran (Dex), a polysaccharide polymer, polyacrylamide, Examples include, but are
not limited to, a polyl, polylysine avidin, a lipid, a lipid emulsion, a liposome, a dendrimer, or a
cyclodextran. Alternative to a carrier protein, immunomodulatory glycans can be conjugated to
other carrier molecules, for example carriers that will protect the compound against rapid
elimination from the body, such as a controlled release formulation, including implants and
microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such
as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and
polylactic acid.

With respect to the density of glycans conjugated to the carrier, the glycan molecules
may comprise at least about 10% of the conjugate by weight, at least about 15% of the conjugate
by weight, at least about 20% of the conjugate by weight, at least about 25% of the conjugate by
weight, at least about 25% of the conjugate by weight, at least about 30% of the conjugate by
weight, at least about 35% of the conjugate by weight, at least about 40% of the conjugate by
weight, or at least about 45% of the conjugate by weight. In certain embodiments, the glycan
molecules comprise about 10-25% of the conjugate by weight, about 15-25% of the conjugate by
weight, about 20-25% of the conjugate by weight, about 30-35% by weight, about 35-40% by
weight, or about 40-45% by weight.

Multivalent forms of an immunomodulatory glycan can be generated using standard
methods. For example, the oligosaccharide portion of the glycan can be bound to a multivalent
carrier using techniques known in the art so as to produce a glycoconjugate in which more than
one individual molecule of the oligosaccharide is covalently attached to the multivalent carrier.
The multivalent carrier is sufficiently large to provide a multivalent molecule leaving from
between about 2-1,000, between about 2-200, between about 2-100, between about 10-100,
between about 2-50, between about 10-50, between about 20-50, between about 5-25, between
about 8-10, between about 8-12, between about 8-20, between about 8-25, between about 10-20,
or between about 20-25 molecules of the oligosaccharide portion may be bound to the
multivalent carrier. In some embodiments, the conjugates comprise about 10-11, about 12-13,
about 14-15, about 6-17, about 18-19, or about 20 or more sugars/conjugate. In some
embodiments, about 10, about 13, about 15, about 18, about 20, about 25, about 30, about 35,
about 40, about 45, about 50, about 100 or about 200 glycan molecules, such as, for example,
LNFPIII, are bound to the multivalent carrier.

In some embodiments a carrier molecule is between about 5,000 to about 100,000
daltons, between about 8,000 to about 80,000 daltons, between about 10,000 to about 50,000, or
between about 10,000 to about 40,000 daltons. In some embodiments a carrier molecule is about
10,000 daltons (10 kDa), about 20,000 daltons (20 kDa), about 30,000 daltons (30 kDa), about 40,000 daltons (40 kDa), or about 50,000 daltons (50 kDa).

For example, in some embodiments, a glycoconjugate includes LNFP III conjugates of about 8-12 LNFP III molecules conjugated per molecule of human serum albumin (HSA), 40kDa dextran, or other inert scaffolding (such as, for example, dendrimers). In some applications, at least 8 molecules of LNFP III, or a derivative thereof, are conjugated to a carrier. In some embodiments, a glycoconjugate includes about 8 to about 10 LNFP III molecules per dextran molecule. In some embodiments, a glycoconjugate includes about 12 LNFP III molecules per dextran molecule. In some embodiments, a carrier dextran molecule is about 10,000 to about 40,000 daltons. In some embodiments, a carrier dextran molecule is about 10,000 daltons (10 kDa), about 20,000 daltons (20 kDa), about 30,000 daltons (30 kDa), about 40,000 daltons (40 kDa), or about 50,000 daltons (50 kDa). Such glycoconjugates of LNFP III may be referred to as NeoGlycoConjugates (NFPIII-NGC). In some aspects, linkage may be via an APD linker.

Immunomodulatory glycans may be conjugated to a carrier molecule by any of many standard methods, for example using a chemical cross-linking agent. The chemistry necessary to create the multivalent molecule and to link the oligosaccharide to the multivalent carrier are well known in the field of linking chemistry. A wide variety of bifunctional or polyfunctional cross-linking reagents, both homo- and heterofunctional, are known in the art and are commercially available.

In some embodiments, a conjugate thereof includes a dendrimer. Dendrimers are highly branched, star-shaped macromolecules with nanometer-scale dimensions. A dendrimer is an artificially manufactured polymer or synthesized molecule built up from branched monomer units. A dendrimer is typically symmetric around the core, and often adopts a spherical three-dimensional morphology. Available dendrimer types include, for example, poly(amidoamine) (also known as PAMAM, or STARBURST® dendrimers), phosphorous dendrimers, polylysine dendrimers, and polypropylenimine (PPI) dendrimers (available, for example, from Sigma-Aldrich, USA). Dendrimers are constructed by the successive addition of layers of branching groups, with each new layer being called a generation. The final generation incorporates the surface molecules that give the dendrimer the desired function for pharmaceutical applications.

Methods for using dendrimers in drug delivery can include, for example, covalently attaching the drug (such as for example, an immunomodulatory glycan, a derivative, or a mimetic thereof,
including, but not limited to LNFPIII, a derivative thereof, or a mimetic thereof) to the periphery of the dendrimer to form dendrimer prodrugs, coordinating a drug to the outer functional groups via ionic interactions, and having the dendrimer act as a unimolecular micelle by encapsulating a pharmaceutical through the formation of a dendrimer-drug supramolecular assembly. See, for example, Kesharwani et al, 2014, *Progress in Polymer Science*; 39(2):268-307; and Madaan et al, 2014, *JPharm Bioallied Sci*; 6(3): 139-150.

The active agents of an immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate conjugate thereof, can be administered to a subject alone or in a pharmaceutical composition that includes the active agent and a pharmaceutically acceptable carrier. The active agent is administered to a patient, preferably a mammal, and more preferably a human, in an amount effective to produce the desired effect.

The administration of an immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate conjugate thereof to a subject may be by any of a variety of methods, including, but not limited to, orally, transdermally, parenterally, topically, intranasally, intraperitoneally, intravenously, intraarterially, sublingually, intramuscularly, rectally, transbuccally, liposomally, via inhalation, vaginally, intraocularly, subcutaneously, intraadiposally, intraarticularly, intrathecally, and/or local delivery by catheter or stent. Administration may be by injection, including for example, intramuscular, intradermal, or subcutaneously injection. Oral administration includes, for example, administration as a liquid suspension, tablet, a capsule, or a gel. Topical administration includes, for example, administration as an emulsion, cream, or ointment. Transdermal administration includes, for example, administration by a transdermal skin patch or a microneedle dermal patch. Administration may be as a long-lasting bolus, slow release dosage form, slow release biodegradable complex, nanoparticles, and/or a slow-release hydrogel preparation.

The formulations can be administered as a single dose or in multiple doses. Useful dosages of the active agents can be determined by comparing their in vitro activity and the in vivo activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art.

The active agent may be formulated in a pharmaceutical composition and then, in accordance with the method of the invention, administered to a mammal, such as a human subject, in any of a variety of forms adapted to the chosen route of administration. The
formulations include those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, and intravenous) administration.

The pharmaceutically acceptable carrier can include, for example, an excipient, a diluent, a solvent, an accessory ingredient, a stabilizer, a protein carrier, or a biological compound. In a preferred embodiment, the pharmaceutically acceptable carrier includes at least one compound that is not naturally occurring or a product of nature.

The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. Formulations of the present invention suitable for oral administration can be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. The tablets, troches, pills, capsules, and the like can also contain one or more of the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose, or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it can further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, sugar, and the like. A syrup or elixir can contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent can be incorporated into sustained-release preparations and devices.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Isotonic agents that can be included in the liquid preparation include sugars, buffers, and sodium chloride. Solutions of the active agent can
be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage.

The necessary fluidity can be achieved, for example, by using liposomes, by employing the appropriate particle size in the case of dispersions, or by using surfactants. Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze drying of the sterile injectable solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Nasal spray formulations include purified aqueous solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration can be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

Non-limiting ranges for therapeutically or prophylactically effective amounts of an immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate conjugate thereof, include about 0.01 nM to about 20 mM, about 0.1 nM to about 10 mM, or about 1 μM to about 1 mM. Alternatively, the compound can be used in vivo in an amount between about 1 μg to about 100 mg/kg body weight, about 10 μg to about 200 mg/kg body weight, about 20 μg to about 10 mg/kg body weight, or about 100 μg to about 10 mg/kg body weight. It is to be noted that dosage values may vary with the severity of the condition to be alleviated and according to factors such as the disease state, age, sex, and weight of the subject. It is to be further understood that for any particular subject, specific dosage regimens can be
adjusted over time to provide the optimum therapeutic response according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Alternatively, administration may be as a food product, food supplement, nutraceutical, or a dietary supplement. An immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate conjugate thereof, including, but not limited to LNFPIII, a mimetic thereof, a derivative thereof, or a conjugate thereof, can be packaged as a nutritional, health or dietary supplement, for example, in powder, pill, or capsule form. Additionally, an immunomodulatory glycan, a mimetic thereof, a derivative thereof, or a glycoconjugate conjugate thereof, can be added to a food product to yield what is commonly referred to as a "nutraceutical" food or "functional" food. Foods to which an immunomodulatory glycan, a mimetic, a derivative thereof, or a glycoconjugate conjugate thereof, can be added include, without limitation, bars, cereals, soups, and beverages.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein. All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

EXAMPLES

Example 1

Maternal Treatment with LNFPIII Conjugates Restores Physiologic Parameters in Offspring

This example demonstrates that administration of LNFPIII conjugates to HFD obese dams results in pups with restored gut-barrier functions, restored brain function, normalized inflammatory mediators and metabolic indicators, and reduced adiposity, supporting the therapeutic application of LNFPIII conjugate treatment for administration to obese women prior...
to and during gestation, resulting in reductions in children born of obese mothers that have altered gut-barrier functions, altered brain function, are obese, have a likelihood of developing diabetes and metabolic disease.

5 Materials and Methods

High Fat Diet (HFD). C57BL/6J mice at 8-10 weeks of age (obtained from The Jackson Laboratory) were placed on a high-fat, high-carbohydrate diet (F3282, Bio-Serv) or on normal chow or Low Fat Diet (LFD) chow for the duration of the experiments. Six weeks after high-fat feeding, mice were injected (intraperitoneally, i.p.) twice per week with 25 µg of dextran (40 kDa; vehicle) or LNFPIII conjugated to dextran (~8-10 LNFPIII per dextran or 0.168-0.2 10 µg LNFPIII per µg of dextran). Body weight and food intake were monitored weekly.

On adult HFD/LFD or normal chow fed mice, metabolic studies start 4 weeks after LNFPIII treatment and were conducted after 6 h of fasting.

GTT = glucose tolerance test. Mice are fasted overnight and injected with 1 g glucose per kg body weight into the peritoneum and measure blood glucose concentrations measured before and after injection at the time points indicated using the OneTouch glucose monitoring system (LifeScan).

ITT = Insulin Tolerance test. ITTs were conducted by injecting from 0.5 U to 1.0 U per kg body weight of insulin. In vivo insulin signaling was determined by injecting 5 U per kg body weight of insulin through the portal vein. Pieces of liver, epididymal fat and muscle were collected before and 10 min after insulin injection and rapidly stored the tissues in liquid nitrogen.

Serum levels of ALT, AST, and lipids. Serum and tissue lipids, ALT and AST were measured using commercial kits as described previously. For serum triglycerides mice were injected with Triton WR1339 (500 µg/gm body weight) and blood was drawn via tail bleeding at different time points. Serum and hepatic triglyceride, nonesterified fatty acid, total cholesterol, serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured using commercial kits (Wako Chemicals and ThermoDMA).

Insulin and adiponectin are measured using commercial ELISA Kits (Linco).

Gut microflora and gut permeability. At various time points on HFD, LFD or normal chow gut contents or feces are sampled. In addition, gut contents/feces are collected from pups
born of HFD, LFD, normal chow mothers treated with LNFPIII-conjugate or dextran control at various time points post-birth (PND). These samples are subject to gut microbiome analysis.

Gut permeability is determined by administering a 4 kDa FITC-Dextran (FITC is a fluorescein dye that is conjugated to the dextran so that how much Dextran crosses the gut-barrier and ends up in the serum can be quantified). Mice are bled one hour after oral administration of FITC-Dextran and the sera analyzed for FITC concentration.

Intestinal Alkaline Phosphatase Activity (IAP). This enzyme is measured from intestinal tissues (normally duodenum) using a commercially available kit.

Analysis of immune responses and metabolic genes. Having collected and stored tissues from adult mice and pups, the expression of various immune factors, signaling pathways and metabolic markers in these tissues is evaluated by staining sections of tissue and performing immunohistochemistry staining of the sections with antibodies specific for immune, signaling or metabolic proteins/factors. This can also be measured by performing Western blots, especially when looking at signaling pathways. By isolating RNA from the tissues using standard methods, RT-PCR can be used to measure expression of immune, signaling and metabolic genes.

Behavioral. Adult mice (n=8 per group) were subjected to behavioral tests after 5 weeks on LFD/HFD; a week later (6 weeks on diets, 48 h after the last behavioral test), these mice were sacrificed, brains were harvested, weighed, and quickly frozen at -80 °C for neurochemical analysis. The remaining behaviorally naïve mice (n=5-6 per group) were maintained on their respective diets for 11-12 weeks; at that time, brains were harvested and ex vivo hippocampal slices prepared for electrophysiology.

Behavioral tests were performed in succession over 3 days. Animals were naive to the testing ambience prior to testing initiation and all tests were performed by a treatment-blinded experimenter in a specially equipped behavioral testing room separate from the one where the mice were housed.

Open field. Mouse activity was monitored for a period of 30 min in an open field arena (25 cm x 25 cm x 40 cm; Coulbourn Instruments, Whitehall, PA) as described previously (Krishna et al, 2014, Arch Toxicol; 88(1): 47-64).

Parameters evaluated included: distance traveled (cm/5 min intervals) and number of crossings into the center of the arena per 100 cm distance analyzed for the 30 min and per 5 min
intervals (horizontal activity); and number of rearings during the first 5 min (vertical activity), counted using Limelight software (Actimetrics, Wilmette, IL).

Pole test. After 5 min resting period (following the open field test), mice were placed upright on a gauze-wrapped pole (1 × 55 cm; d × h) as in Lin et al. (Lin et al., 2013, Neurotoxicol Teratol; 39: 26-35). The maximum turning time allowed was 60 s and the total time per trial was 120 s. A total of four trials were completed with a 3-5 min intertrial interval. The average times to turn, to descend, and total time spent on the pole from the four trials were used for statistical analysis (Krishna et al., 2014, Arch Toxicol; 88(1): 47-64).

Grip strength. A strength gauge (Bioseb, France) with attached mouse-specific square wire grid (6 × 6 cm) was used to measure forelimb grip strength (10 min rest after the pole test) as previously described (Krishna et al., 2014, Arch Toxicol; 88(1): 47-64; and Lin et al., 2013, Neurotoxicol Teratol; 39: 26-35). The average maximum grip force (recorded in newtons [N]) of the four trials was used for statistical analysis.

Novel object recognition (NOR). The NOR was conducted at the beginning of day 2 of behavioral testing with the previous day open field test used as a habituation phase, as detailed in Lin et al (Lin et al., 2013, Neurotoxicol Teratol; 39: 26-35). Briefly, mice were allowed to explore two identical objects for five minutes. After one hour rest in their home cages, they were reintroduced into the arenas with one familiar and one novel object for 5 min. The number of approaches towards the familiar (Nf) or the novel object (Nn), as well as the time spent exploring the familiar (Tf) or the novel object (Tn), were extracted using the Limelight software. To determine novelty preference, the percent of time exploring the familiar vs. the novel object and the novelty preference indices (NPIs) based on the number of approaches (NPIA= [Nn - Nf]/fNn + Nf]) or time (NPIT = [Tn - Tf]/[Tn + Tf]) were calculated and analyzed (Cyrenne and Brown, 2011, Horm Behav; 60(5): 625-31).

Forced swim test (FST). Following the NOR test and a 1.5-h home cage rest period, FST was performed as in Krishna et al. (Krishna et al., 2014, Arch Toxicol; 88(1): 47-64). Mice were gently placed in a large cylindrical container (18 × 25 cm; d × h) filled approximately two-thirds with tap water (3 L, 29±1 °C) for 15 min. The total time spent swimming, climbing, or immobile, as well as the total distance swam, was scored using Limelight video tracking software.

Marble burying test (MBT). This test was performed on day 3 as described previously, but with some modifications (Gaikwad et al., 2010, Acta Pol Pharm; 67(5): 523-7). Mice were
individually placed in cages containing 4-5 cm-thick pine bedding (American Wood Fibers, Columbia, MD) for 10 min (habituation phase). After a 40-min home cage resting period, mice were reintroduced into the cages which now contained twenty glass marbles (diameter ~10 mm, Panacea Products Corp., Columbus, OH), overlaid on the bedding arranged in a 4x5 matrix for a 10 min testing phase. The number of marbles buried (>70%) was counted based on images collected at times 0 and 10 min of the testing phase (Gaikwad et al, 2010, *Acta Pol Pharm*; 67(5): 523-7).

Hippocampal electrophysiology. After 11 weeks and terminating before the end of 12 weeks on LFD or HFD diets, hippocampal electrophysiology was performed on vHIP slices as in Thompson et al. (Thompson et al, 2004, *Neuroscience*; 127(1): 177-85). The brain was removed quickly from C0₂ anesthetized mice and submerged in ice-cold, oxygenated (95% O₂ / 5% C0₂) dissection artificial cerebrospinal fluid (aCSF) containing: 120 mM NaCl, 3 mM KCl, 4 mM MgCl₂, 1 mM NaH₂PO₄, 26 mM NaHCO₃, and 10 mM glucose. 400-μm thick horizontal brain slices were harvested; slices were sub-dissected and the CA3 region removed, leaving the CA1 region intact (Keralapurath et al, 2014, *Hippocampus*; 24(5):577-90) and then incubated in a submersion chamber for 45 minutes at room temperature and an additional 45 minutes at 30°C continuously perfusing with oxygenated (95% O₂ / 5% C0₂) standard aCSF containing: 120 mM NaCl, 3 mM KCl, 1.5 mM MgCl₂, 1 mM NaH₂PO₄, 2.5 mM CaCl₂, 26 mM NaHCO₃, and 10 mM glucose. Using a bipolar stimulating electrode (Kopf Instruments, CA) Schaffer collaterals were stimulated at the CA3-end of CA1 region, and the field excitatory post-synaptic potentials (fEPSPs) generated at the stratum radiatum layer were recorded using a 1.0 MΩ tungsten recording electrode (WPI, Sarasota, FL). The initial slope of the fEPSPs was measured by fitting two straight lines to a 1 ms window immediately following the fiber volley. Paired-pulse responses with a 50 ms inter-stimulus interval were obtained, and paired-pulse ratio (PPR) was calculated using the formula, PPR = (initial slope of pulse 2)/(initial slope of pulse 1) (Thompson et al, 2004, *Neuroscience*; 127(1): 177-85).

For the long term potentiation (LTP) experiments, stable fEPSPs were recorded for at least 30 minutes at baseline frequency before stimulating the slices with a high frequency tetanic stimulation (HFS-3 trains of 100 pulses at 100 Hz, with 20s inter-train interval) (Keralapurath et al, 2014, *Hippocampus*; 24(5):577-90). fEPSPs were recorded at baseline frequency for additional 60 minutes post-tetanus. fEPSP slopes were normalized to the slopes of the last five
responses prior to the HFS and the LTP magnitude was assessed by comparing the average of normalized slopes from the last 5 responses of pre-tetanus and 55-60 min post-tetanus phases. Data were digitized at 10 kHz, low-pass filtered at 1 kHz, and analyzed with pCLAMP 10.2 software (Axon Instruments, Sunnyvale, CA). n-values (x[y]) indicate the number of mice (x) and the number of slices (y) assessed.

Following methods described in more detail in Bhargava et al., 2012, Nat Med; 18(11):1665-72, mice were placed on HFD for 6 weeks before mating. Treatment, 2X weekly by subcutaneous injection of LNFPIII-Dex conjugate or Dextran control, initiated after pregnancy was confirmed. Treatment was maintained throughout gestation and lactation. The impact of dam HFD obesity on pups was measured via behavioral studies, electrophysiology of brain function, gut permeability and inflammatory and metabolic mediators. Data were then separately analyzed as HFD vs LFD +/- LNFPIII or control, and as LFD/HFD males or females, +/- LNFPIII or control treated. Pups were analyzed 21, 35 and 90 days post-birth (PND).

High-fat diet consumption by female mice results in multiple metabolic, behavioral, neurochemical, and immunological perturbations even after 6-week dietary exposure and relatively modest weight gain. For example: six-week HFD consumption by female C57BL/6 mice leads to impaired glucose tolerance and insulin sensitivity; six-week HFD consumption by female C57BL/6 mice leads to increased activity in an open field test; six-week HFD consumption by female C57BL/6 mice leads to increased anxiety; and eleven-twelve week HFD consumption by female C57BL/6 mice leads to impaired long-term potentiation (LTP), an indicator of impaired learning. Dams exposed to HFD beginning six-weeks prior to mating and continuing through gestation and lactation have impaired glucose tolerance and insulin sensitivity. Both of these deficiencies are restored by LNFPIII (glycan) treatment during gestation and lactation. Thus, LNFPIII treatment during gestation and through lactation restores metabolic perturbations in dams fed HFD.

Analysis of mediators in female mice at 6 weeks post LFD or HFD showed elevation in liver levels of the mediators PPAR-α and PPAR-γ in HFD mice, with no significant change in levels of PPAR-δ. No significant differences in levels of TNF-α were detected between LFD and HFD mice. IL-6 was significantly elevated in livers of HFD mice. There were no significant differences in levels of liver IL-10 or MyD88.
No statistically significant differences of PPAR-α, PPAR-γ, PPAR-δ, TNF-α, IL-10, or HP were observed in livers of any of the groups of pups. However, there were definite trends. Analysis of liver tissue from PND 21 pups showed that PPAR-α, PPAR-γ, and PPAR-δ levels were reduced in pups born of HFD dams, compared to pups born of LFD dams. The levels were restored in pups born of HFD dams treated with LNFPIII. Similar findings were observed for liver levels of PPAR-γ and PPAR-δ. Levels of the microRNAs mir-34a and mir-155 were also measured. Mir-155 has been shown to regulate differentiation of brown and beige adipocytes, with inhibition of mir-155 leading to a brown adipocyte phenotype, which is preferred (Chen et al., 2013, *Nature Communications;* 4:1-13). Mir-34a is known to be upregulated in obese mice, particularly the liver. Mir-34a disrupts FGF19 signaling by targeting the co-receptor beta-Klotho causing metabolic disorder (Fu et al., 2012, *PNAS;* 109:16137). In addition, hepatic overexpression of miR-34a reduced NAMPT/NAD(+) levels, increased acetylation of the SIRT1 target transcriptional regulators, PGC-1α, SREBP-1c, FXR, and NF-κB, and resulted in obesity-mimetic outcomes (Choi et al., 2013, *Aging Cell;* 12:1062). Pups born of HFD dams treated with LNFPIII had mir-34a expression restored to normal in both males and females. Similar finding with mir-155, elevated in pups born of HFD dams, but normalized in pups of HFD dams treated with LNFPIII.

Additional data demonstrate that maternal high-fat diet (HFD) consumption (beginning 6 weeks prior to mating on though weaning), results in: (i) increased gut permeability, measured by FITC dextran, in postnatal day (PND) 21 offspring, (ii) neurochemical alterations indicative of altered monoamine homeostasis in the brains of PND 21 offspring, and (iii) compromised learning and memory in 3-4 month old offspring (measured electrophysiologically, more pronounced in females) that have been maintained on regular diet after weaning.

Postnatal day 21 (PND21) pups from dams exposed to HFD beginning six-weeks prior to mating and continuing through gestation and lactation have increased gut permeability, irrespective of pups’ sex. This permeability deficiency is restored by maternal-only LNFPIII (glycan) treatment during gestation and lactation.

Adult offspring (PND90) from dams exposed to HFD beginning six-weeks prior to mating and continuing through gestation and lactation have decreased LTP, which is far more prominent in female pups. This LTP deficit is restored by maternal-only LNFPIII (glycan)
treatment during gestation and lactation. Of note, after weaning (PND21), all offspring were raised on regular chow.

Maternal (gestational/lactational) exposure to HFD causes perturbation of the gut permeability of PND 21 offspring. This perturbation is restored back to normal by maternal treatment with the LNFPIII glycan.

Adult (>PND70) offspring maintained on regular chow after weaning but born to HFD-fed dams had metabolic and learning (LTP) deficits. These deficits were restored back to normal in the offspring born to the LNFPIII glycan treated dams.

LNFPIII treatment of dams during gestation and through lactation restores PPAR-a levels in livers of PND-21 mice pups, male and female; partially restores PPAR-d levels in livers of PND-21 mice pups, male and female; restores PPAR-g levels in livers of PND-21 mice pups, male and female; normalizes levels of IL-6 in livers of PND-21 mice pups, male and female; restores levels of liver HP in PND-21 mice pups, male and female; partially restores liver expression of mir-34a; and partially restores liver expression of mir.

Thus, LNFPIII treatment during gestation and through lactation restores and/or normalizes the expression of metabolic and inflammatory markers in pups compared to pups born of control treated HFP dams.

Pups born LNFPIII treated dams had lower levels of Mir-34a, indicative of restored metabolic pathway. Mir-34 is upregulated in obese mice and it disturbs the FGF19 pathway crucial for lipid metabolism. Similarly expression of Mir-155, which is a regulator of adipogenesis, was lowered by in pups born of LNFPIII treated dams. Therapeutic attempts to reduce expression of mir-155 have been considered as an approach to treat obesity.

Adult (>PND70) offspring maintained on regular chow after weaning but born to HFD-fed dams had metabolic and learning (LTP) deficits; these deficits were restored back to normal in the offspring born to the LNFPIII glycan treated dams.

Administration of LNFPIII conjugate reversed maternal HFD effects on offspring's gut permeability and on offspring's learning and memory; it also counteracted many of the neurochemical alterations in the PND 21 brains caused by maternal HFD.

This example demonstrates that the administration of LNFPIII conjugate to obese dams protects GI health and enteroendocrine cell signaling. Notably there was an increase in L enteroendocrine cells, an increase in CCK signaling, an increase in intestinal alkaline

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phosphatase (PND 21-90), and a decrease in GI permeability. Preliminary results with occludin may show a difference since there is a difference in GI permeability. Finally, there was a decrease in overall adiposity of pups born of dams treated with LNFPIII conjugates (PND 90). All other results are from PND 21.

In addition to these findings there may be differences in GI microflora composition, endocannabinoid tones, inflammatory tones, and/or insulin signaling. For example in a determination of response to CCK (PND 90), glycan seems to have a protective effect. In a determination of intestinal alkaline phosphatase (IAP) activity (PND 21), Maternal HF feeding leads to an increase in IAP activity (maybe the pups have started consuming foods), glycan treatment somewhat normalizes it. For PND 35: Animals weaned on chow, there is no difference between the maternal LF and maternal HF pups; however there is a significant (protective) elevation in IAP activity in the pups from the glycan treated dams. Elevation is maintained at PND 90 for pups maintained on LF diets. Both HF and HF-Glycan show a decrease in IAP when fed a HF diet.

In a determination of overall adiposity (PND 90), no effect of maternal diet, but a significant effect of offspring diet, high fat feeding leads to an increase in adiposity was observed. Maternal treatment with glycan seems to rescue HF feeding induced adiposity. Differences are marked for total WAT, retroperitoneal (visceral) and epididymal fat, not much of an effect on subcutaneous fat (inguinal). No effect on BAT.

An analysis of GI microflora composition included the following was observations.

Microflora Composition (Diversity). Trend towards an increase in diversity in pups from HF fed dams. This may be due to an earlier consumption of maternal diet.

Microflora Composition (Phyla). Pups from dams fed the LFD had a significantly lower proportion of Firmicutes and Verrucomicrobia and a significantly greater proportion of Bacteroidetes than pups from dams fed the HFD. This is consistent with higher carbs intake.

There were no differences between HFD+G and either LFD or HFD. There was a lot of variability in the HFD+G group.

Microflora Composition (Class). Five classes comprised the majority of microbiota. Actinobacteria made up a small fraction of total microbiota totaling in aggregate less than 1% Verrucomicrobiae was a significant component of HFD pup microbiota (10.2% ± 3.2%) but was less than 2% of the microbiota in pups from the other two groups.
Microflora Composition (Order). Glycan treatment limited appearance of verrucomicrobiales and desulfovibrionales (proteobacteria, gram neg, pro-inflammatory). Desulfovibrionales have been associated with ASD in mouse model and in humans.

Microflora Composition (Families). A total of 27 microbial families were identified. Of the majority families, 7 belonged to phyla Firmicutes, 4 to Bacteroidetes, 1 to Actinobacteria, 1 to Proteobacteria, and 1 to Verrucomicrobia. There were 5 significant differences overall, all between LFD and HFD. Those differences were for Porphyromonadaceae (59% vs. 24.5%, LFD vs. HFD, p < 0.006), Lachnospiraceae (9.8% vs. 34.3%, LFD vs. HFD, p < 0.0006), Ruminococcaceae (6.7% vs. 15.7%, LFD vs. HFD, p < 0.032), and Verrucomicrobiaceae (0.3% vs. 0.12%, LFD vs. HFD, p < 0.016). The fifth significant difference arose because no Desulfovibrionaceae were present in feces from pups from dams fed LFD and the presence of small fractional contributions of this family (0.104%) in feces of pups from dams fed HFD. Two of five pups in the HFD + G groups also had low levels of this family.

Microflora Composition (Genera) is shown in Table 1 below.

Table 1. Microflora Composition (Genera). Phylum (P): Bacteroidetes (B), Firmicutes (F), Proteobacteria (P), and Verrucomicrobia (V). Class (C): Bacteroidia (B), Clostridia (C), Deltaproteobacteria (D), and Verrucomicrobiae (V). Order (O): Bacteroidales (B), Clostridiales (C), Desulfovibrionales (D), and Verrucomicrobiales (V). Family (F): Clostridiaceae (C), Desulfovibrionaceae (D), Lachnospiraceae (L), Porphyromonadaceae Porphyromonadaceae (P), Prevotellaceae (Pr), Ruminococcaceae (R), Verrucomicrobiaceae (V).

<table>
<thead>
<tr>
<th>Genera</th>
<th>P, C, O, F</th>
<th>LFD</th>
<th>HFD</th>
<th>HFD+G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnesiella</td>
<td>B, B, B, P</td>
<td>4.20 ± 1.2</td>
<td>2.5 ± 0.7</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Prevotella</td>
<td>B, B, B, Pr</td>
<td>0.05 ± 0.009</td>
<td>0.03 ± 0.018</td>
<td>0.004 ± 0.01</td>
</tr>
<tr>
<td>Roseburia</td>
<td>F, C, C, L</td>
<td>6.2 ± 1.5</td>
<td>26.9 ± 4.0</td>
<td>13.3 ± 5.7</td>
</tr>
<tr>
<td>Pseudobutyribrio</td>
<td>F, C, C, L</td>
<td>0.7 ± 0.2</td>
<td>2.3 ± 0.6</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Ruminococcus</td>
<td>F, C, C, R</td>
<td>0.5 ± 0.2</td>
<td>2.0 ± 0.5</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Clostridium</td>
<td>F, C, C, C</td>
<td>0.2 ± 0.025</td>
<td>1.5 ± 0.50</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Marvinbryantia</td>
<td>F, C, C, L</td>
<td>Not present</td>
<td>0.3 ± 0.033</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Desulfovibrio</td>
<td>P, D, D, D</td>
<td>Not present</td>
<td>0.1 ± 0.033</td>
<td>0.018 ±0.018</td>
</tr>
<tr>
<td>Akkermansia</td>
<td>V, V, V, V</td>
<td>0.3 ± 0.2</td>
<td>10.2 ± 3.2</td>
<td>1.6 ± 1.2</td>
</tr>
</tbody>
</table>
In ongoing analyses, the effects of maternal HFD on multiple behavioral, neurochemical, metabolic, and molecular end points in offspring from several different ages are being characterized. The restorative/normalizing ability of the maternal-only LNFPIII glycan treatment on these multiple end points is also being evaluated. Experiments are in process to determine (i) behavioral alterations in the offspring (different ages) caused by maternal HFD consumption and potential ameliorative effects of the maternal-only LNFPIII treatment, (ii) Inflammatory effects in the dam and offspring, including in the offspring's brain, caused by maternal HFD and potential anti-inflammatory effects of maternal-only LNFPIII treatment, (iii) changes in the offspring's blood lipidome caused by maternal HFD consumption and potential reversal of these changes by maternal-only LNFPIII treatment.

Example 2

LNFPIII Conjugate Treatment

The prevalence of childhood obesity and diabetes has increased significantly in the United States and there is evidence that part of this increased prevalence of childhood obesity is in part, due to gestational exposure to a high fat environment. In the US, approximately 30% of women beginning their pregnancies are obese (Catalano, 2007, Obstetrics and Gynecology; 110:743-744). In humans, epidemiologic studies have demonstrated that maternal obesity and diabetes predispose their offspring towards developing metabolic disorders (Clausen et al, 2008, Diabetes Care; 31:340-346; Deierlein et al, 2011, Diabetes Care; 34:480-484; and Pedersen, 1971, Postgrad Med J; Suppl:66-67). Little is understood regarding the phenomenon of maternal metabolic programming of progeny, however, animal models support human epidemiologic studies, showing that maternal high fat diet/obesity negatively influences metabolism in offspring (Horvath and Bruning, 2006, Nat Med; 12:52-53; Hales and Barker, 2001, Br Med Bull; 60:5-20; Vogt et al, 2014, Cell; 156:495-509; Nathanielsz et al, 2007, Clinics in Perinatology; 34:515-526; McMillen and Robinson, 2005, Physiological Reviews; 85:571-633; Plagemann and Harder, 2005, Metab Syndr Relat Disord; 3:222-232; Plagemann, 2005, Physiol Behav; 86:661-668; Bouret, 2009, J Pediatr Gastroenterol Nutr; 48(Suppl 1):S3 1-38; Grayson et al, 2010, Frontiers in Neuroendocrinology; 31:16-31; and Carmody et al, 2011, 31
In addition to metabolic disease, many studies suggest a link between maternal obesity and increased prevalence of negative, neural-behavioral outcomes such as impaired memory function or development of Autism spectral disease in offspring (Vogt et al, 2014, Cell; 156:495-509; Bouret, 2009, J Pediatr Gastroenterol Nutr; 48(Suppl 1):S31-38; Grayson et al, 2010, Frontiers in Neuroendocrinology; 31:16-31; Carmody et al, 2011, Obesity (Silver Spring); 19:492-499; and White et al, 2009, Am J Physiol Regul Integr Comp Physiol; 296:R1464-1472).

Taken together, maternal obesity during gestation increases the risk of children being born with, or developing metabolic disease(s) and/or negative neural physiology. Thus, children born of obese mothers will have increased prevalence of Autism Spectral Disease, fatty liver disease and/or diabetes. Importantly for diabetes and fatty liver disease, children born of obese mothers may develop these diseases in the first two decades of life rather than in their 30s and 40s, significantly increasing the public health burden and health care costs due to obesity.

Currently, there are no therapies/interventions that can be given to obese women during gestation and/or lactation that reduce or prevent adverse health events in children. Thus, the present invention explored whether LNFPIII conjugate therapy administered to obese pregnant mice could prevent adverse maternal metabolic programming in offspring.

This example shows that LNFPIII conjugate therapy of obese dams during pregnancy and lactation prevented adverse health outcome in offspring, specifically leading to normalized gut-permeability function and microbiome and importantly, restoring neural function of offspring to normal. These results suggest that LNFPIII conjugate treatment of obese dams through gestation and lactation is likely to also prevent adverse metabolic and immune function in offspring, making them less likely to develop NAFLD, diabetes or cardiovascular disease as a result of maternal metabolic programming.

Based on the results of this example, it is predicted that LNFPIII conjugate treatment of obese mice during gestation/lactation will normalize metabolic, neural and immune parameters in pups compared to pups born of control treated obese dams.

HF feeding and/or obesity during pregnancy can affect post-natal development of the GI tract leading to enhanced intestinal permeability (White et al., 2009, Am J Physiol Regul Integr Comp Physiol; 296:R1464-1472); increased GI permeability in the offspring is associated with weight gain (Fak et al, 2012, IntJObes (Lond); 36:744-751). In this example (Obesity Initiative
Pilot Study), female C57B1/6 mice were placed on low fat or high-fat diets for 6 weeks. Beginning in week 7, cohorts of HFD females were allocated to LNFPIII conjugate or dextran control groups. LNFPIII conjugates are comprised of LNFPIII (obtained from Dextra Inc., purified from human milk, >99% purity) that is conjugated to a 40kDa dextran (clinical grade) molecule using spacer-linker technology such that on average, there are 8-10 LNFPIII residues per molecule of dextran. Mice are treated as described in Bhargava et al. (Bhargava et al., 2012, Nat Med; 18:1665-1672), 2x a week subcutaneous injections with 25 μg of LNFPIII conjugate or dextran. Also in week 7, female mice in all three groups are paired with male mice to generate pregnant mice. Twice weekly treatment with LNFPIII conjugate or dextran is continued along with HFD for duration of gestation/lactation. 21 days post-birth (PND21) pups were collected for gut permeability studies, levels of intestinal alkaline phosphatase (LAP) and determination of gut microflora composition. Gut permeability was quantified by measuring plasma fluorescent dextran as described in de La Serre et al. (de La Serre et al., 2010, Am J Physiol Gastrointest Liver Physiol; 299:G440-448).

Figure 1 Panel A shows that HFD during gestation/lactation alters gut permeability in offspring, making guts more "leaky" and that treatment with LNFPIII conjugate during gestation/lactation normalizes gut-permeability in offspring to levels seen in pups born of LFD dams. Intestinal alkaline phosphatase is a duodenal enzyme with anti-inflammatory properties. Further, IAP can detoxify gut lipopolysaccharide (Bates et al, 2007, Cell Host Microbe; 2:371-382; Beumer et al, 2003, J Pharmacol Exp Ther; 307:737-744; and Koyama et al, 2002, Clin Biochem; 35:455-461). IAP activity was measured in duodena from PND 21 and day 90 offspring using a commercially available kit. Figure 1 Panel B shows that similar to gut permeability, IAP activity was significantly elevated in the duodena of offspring from control treated HFD dams, with statistical significance of LF vs. HF, p<0.05. Pups born of HFD dams treated with LNFPIII conjugate were partially normalized at PND 21, and fully normalized at PND90, at which offspring from HF fed dams had significantly lower levels of IAP activity than the offspring from the LF and HF- Glycan mothers (one way anova, LF vs. HF p<0.05, HF- Glycan vs. HF, p<0.01). Lower IAP levels are associated with likelihood of being obese (de La Serre et al, 2010, Am J Physiol Gastrointest Liver Physiol; 299:G440-448).

Figure 1 Panel C depicts the number of different gut microflora genera found in the various offspring cohorts, with offspring of LFD dams having the fewest genera, being
completely disregulated in guts of pups born of control treated HFD dams. As with the other gut parameters measured here, pups born of LNFPIII conjugate treated HFD dams had normalized gut microflora. These data support the concept that LNFPIII conjugate treatment of obese dams can prevent adverse health outcomes in pups. The normalized IAP levels in PND 90 offspring shows the influence of LNFPIII treatment of obese dams are long-lived and therefore, may be due to epigenetic changes. One other area the Obesity Initiative Pilot focused on was pup neurophysiology and behavior. Many studies suggest a link between maternal obesity and increased prevalence of negative, neural-behavioral outcomes in offspring (Vogt et al, 2014, Cell; 156:495-509; Bouret, 2009, J Pediatr Gastroenterol Nutr; 48(Suppl 1):S31-38; Grayson et al, 2010, Frontiers in Neuroendocrinology; 31:16-31; Carmody et al., 2011, Obesity (Silver Spring); 19:492-499; and White et al, 2009, Am J Physiol Regul Integr Comp Physiol; 296:R1464-1472).

Figure 2 shows the data from long-term potentiation measurements of the hippocampal regions of adult offspring (PND90) clearly demonstrating that PND90 offspring born of control treated HFD dams have decreased hippocampal LTP (long-term potentiation, a measure of synaptic plasticity, that is associated with learning and memory), which is more prominent in female pups. This LTP deficit was restored by LNFPIII conjugate treatment of HFD Dams during gestation/lactation. Thus, in addition to normalization of gut permeability and function, LNFPIII conjugate treatment of HFD dams, also normalizes synaptic plasticity associated with learning and memory in pups.

Lastly, liver expression of microRNAs was examining in regards to inflammation. In this example, it is proposed that innate, receptor based M1 and M2 pathways regulate expression of microRNAs, like mir-155 and mir-34a, and that mir-155 and mir-34a in turn target signaling cascades that regulate macrophage M1/M2 polarization. These two microRNAs were selected from array analysis of M1 and M2 polarized macrophages. For the purpose of this example, elevated levels of mirl55 would promote an anti-inflammatory phenotype. Figure 3 shows that PND 21 pups born of control treated HFD dams have significantly reduced expression of mirl55 compared to pups born of LFD dams. Similar to results for gut permeability/function and neural function in Figures 1 and 2, LNFPIII conjugate treatment of HFD dams normalizes expression of mirl55 in livers of PND 21 pups.

Taken together, LNFPIII conjugate treatment of obese dams during gestation/lactation was able to "normalize" three different aspects of pup development. This included gut-
permeability and function, neurologic function and liver microRNA expression. For each parameter, this example demonstrated that pups born of control treated dams had significant, negatively altered functions compared to pups born of LFD dams. Importantly for the proposed studies, LNFPIII conjugate treatment of obese dams was able to normalize each of these parameters, and some were at PND 90, suggesting that the influence of LNFPIII conjugate treatment is long-lived in offspring.

Experimental Approach. Cohorts of n=12, naïve female C57BL/6 mice will be placed on a high fat HFD (F3282) or low fat diet for the duration of the experiment. This number of female mice will insure that there are sufficient numbers of pups in each cohort to carry out the proposed metabolic and immune parameter analyses. Baseline controls will be cohorts of normal chow fed aged-matched mice. After six weeks on HFD, mice will be placed into control (dextran carrier) or LNFPIII-conjugate treated groups by 2X weekly injection of 25 μg control or LNFPIII conjugate as described (Bhargava et al., 2012, Nat Med; 18:1665-1672). Mouse body weight will be monitored at each injection. Mice will be bled at day -1 of treatment and weeks 2, 4 and 6 for collection of serum.

After 6 weeks HFD or LFD, 1 normal chow male will be mated with 2 females (LFD or HFD) to begin timed pregnancies. Once pregnancies are confirmed, female mice will be placed in cages with LFD and HFD diets continued throughout gestation/lactation as well as continuation of 2X weekly injections of LNFPIII-conjugate or dextran carrier control. Pups will be weaned at postnatal day 21, and placed into male and female groups. One cohort of male and female pups from each group will be used for metabolic studies that will be conducted 6 hrs post-fasting.

Glucose tolerance test (GTT) will be performed by injecting 1g glucose/kg body weight into the peritoneum. Blood glucose will be measured every 20 minutes from 0-120 minutes using the OneTouch glucose monitoring system (LifeScan). Insulin tolerance test (ITT) will be conducted similarly by injecting IU/kg body weight of insulin. Insulin-stimulated Akt phosphorylation will also be measured in WAT from vehicle- and LNFPIII-treated mice (from four individual mice per treatment) by Western blot and ELISA as described (Bhargava et al., 2012, Nat Med; 18:1665-1672). In HFD obese mice, M1 Macs represent between 50-60% of the cellular population in adipose tissue compared to 10-15% M2 Macs (Odegaard and Chawla, 2011, Ann Rev Pathol; 6: 275-297).
Obese human subjects also display defective M2 macrophages compared to healthy individuals (Bories et al., 2012, *Diabetes & Vascular Disease Research*; 9:189-195). In contrast, M1 Macs via CD14 or TLR4 dependent pathways promote insulin resistance and glucose tolerance in mice (Fernandez-Real et al., 2011, *Diabetes*; 60:2179-2186; and Chawla et al., *Nat Rev Immunol*; 11:738-749). This suggests that similar pathogen sensing signaling pathways take part in regulation of insulin sensitivity via macrophage M1/M2 regulation (Ricardo-Gonzalez et al, 2010, *Proc Natl Acad Sci USA*; 107: 22617-22622). It will be determined if LNFPIII conjugate therapy of HFD obese dams normalizes M1/M2 balance in tissues of pups, helping restore immune and metabolic functions. For this, adipose tissues and livers will be subjected to collagenase treatment and single cell suspensions stained with antibodies for surface markers for neutrophils (Ly6G/C), macrophages (CD1lb/F4/80), eosinophils (CD1 lb/SiglecF) and T-cells (CD4/CD8/CD3). Stained cells will be analyzed by flow cytometry as recently described (Tundup et al, 2014, *Infect Immun*; 82(8):3240-3251).

In addition, some of the stained cells will be permeabilized and then intracellularly stained using antibodies against iNOS (M1 marker) and RELM a/ Ym1 (M2 markers) as described (Tundup et al, 2014, *Infect Immun*; 82(8):3240-3251). Cytokine production in T-cells and macrophages recruited in the tissues of PND 21 pups will also be determined from the different cohorts by intracellularly staining with antibodies against IL-10, IL-4, IL-13 and IFN. Results obtained from flow cytometry will be analyzed using Flowjo Software. Serum markers of inflammation and acute phase markers will also be measured in PND21 pups by ELISA, specifically sCD14, LPS binding proteins, TNF a, serum amyloid A1 and haptaglobin.

Levels of cytokines and other cell markers (TNF a, IL-12, IL-6, IL-10, IFN γ, IL-4, MglL, Caspl, IL18, Nlrp3, ILlb) will be quantitated in white adipose tissue (WAT) and liver tissues by qRT-PCR as described (Bhargava et al, 2012, *Nat Med*; 18:1665-1672). Data for each parameter will be minimally from n=5 mice. Any changes associated with immune cell activation and phenotypes associated with protection will be measured.

In addition to measuring liver and adipose tissue macrophage and T cell phenotypes, cytokine and inflammasome markers, metabolic analysis of these tissues will be performed, including measurement of liver triglyceride content and circulating levels of AST and ALT to assess liver function. Relative levels of expression of insulin signaling pathway (InsR, Irs2, Cebpa, Glut4 and Pckl), lipogenesis (Fas, Acc, Acc2), B-oxidation (Acosl, Acadm), and fatty
acid uptake (CD36, Fabp4), nuclear receptors (Nrlh3, Fxr-al/2, Fxr-a3/4), Fxr target genes 
(Shp, Oatpl,Pltp, Bsep) will be measured by qRT-PCR as described (Bhargava et al, 2012, Nat 
Med; 18:1665-1672). Data for each parameter will be minimally from n=5 mice.

Example 3
Effect of an Immunomodulatory Glycan on Early Hepatic Metabolic Function 
in Male and Female Offspring of Obese Dams

Maternal obesity and over nutrition can imprint an increased risk of metabolic diseases, 
including non-alcoholic fatty liver disease, in the offspring. Emerging data has indicated that 
metabolic differences may also be affected by sex and placental transfer of inflammatory 
cytokines. Thus, this example investigated the effects of an immunomodulatory glycan 
(LNFPIII) on programmable hepatic metabolic pathways in both sexes of offspring from obese, 
high fat-fed dams. Livers were taken from postnatal day 21 C57BL/6 mice from dams fed 
either a low fat (4.3%) or a high fat diet (HFD) (35%). A subset of HFD dams were given a 
biweekly injection of the glycan (n=22). Histological analysis and hepatic triglyceride assays 
confirmed increased hepatic lipid deposition in offspring from HFD-fed dams (p<0.02). HFD 
caused increased SREBF1 and PPARy expression in both sexes of offspring (p<0.02). Females 
from LFD-fed dams had a higher expression of SLC24A compared to all groups (p<0.03). 
Additionally in females HFD resulted in an increase in IRS1 expression (p<0.02), which was not 
observed in males. While HFD failed to increase hepatic GPT2 and spliced XBP1 mRNA, 
glycan treatment caused reductions in both genes in females only (p<0.05). The data from this 
example presents evidence that glycan LNFPIII protects female offspring from obese dams from 
metabolic injury due to early sex differences in insulin signaling and hepatic stress.

Maternal high fat diets have been shown to influence the risk of obesity and metabolic 
diseases, specifically non-alcoholic fatty liver disease (NALFD), in the offspring of various 
species (Tamashiro and Moran, 2010, Physiology and Behavior; 100:560-566; Bruce et al, 
NALFD is the third most prevalent obesity co-morbidity affecting 20-30% of adults in 
Westernized societies (Preiss and Sattar, 2008, Clinical Science; 115:141-150). What is more
concerning is the increasing trend of NAFLD in children. Autopsy reports suggest that up to 9% of US children may have NAFLD, with 27% of these children experiencing non-alcoholic steatohepatitis and 9% experiencing fibrosis (Schwimmer et al., 2006, *Pediatrics*; 118:1388-1393). Obese children are more likely to become obese adults; therefore children diagnosed with NAFLD will most likely have worsening liver conditions throughout their lifespan. Research has indicated that maternal programming of the offspring may influence the risk of early metabolic diseases, however little information is known on the mechanisms behind this programming.

The pathophysiology of NAFLD has been described as a 2 hit response that starts with increased hepatic lipid deposition (hit 1) and subsequent inflammation (hit 2), which causes downstream hepatic damage, fibrosis, and death. While it may be difficult to regulate the first hit, particularly in the offspring of obese mothers, it may be possible to defend against the pro-inflammatory hit 2 that can cause damage. The immunomodulatory glycans (LNFPIII) has already demonstrated the ability to reduce hepatic steatosis in diet-induced obese mice through modulating both hepatic lipogenesis and inflammation (Bhargava et al., 2012, *Nature Medicine*; 18:1665-1672). LNFPIII increases the anti-inflammatory cytokine IL-10 capable of blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) and can inhibit the pro-inflammatory response to bacterial LPS. A relationship between IL-10 and hepatic steatosis has been previously established in various rodent models and within certain human populations (den Boer et al., 2006, *Endocrinology*; 147:4553-4558; and Zahran et al, 2013, *Indian Journal of Clinical Biochemistry*; IJCB; 28:141-146).

Breastfed infants have lower rates of NAFLD compared to their formula-fed counterparts (Nobili et al, 2009, *Archives of Disease in Childhood*; 94:801-805). LNFPIII is present in human milk and considering its anti-inflammatory properties may serve as an extremely important modulator of later life metabolic outcomes in children (Erney et al., 2001, *Advances in Experimental Medicine and Biology*; 501:285-297). Delivery of this compound to obese mothers may not only reduce their pro-inflammatory state, but may also prevent hepatic dysfunction by acting specifically in the offspring.

Lastly, a sex difference in childhood NAFLD occurrence has been observed, with boys showing higher incidences than in girls (Barshop et al, 2008, *Alimentary Pharmacology and Therapeutics*; 28:13-24). It is possible that females are less sensitive to lipotoxicity and
inflammatory stress than males, leading to a reduced risk of NAFLD in female offspring of dams fed a high fat diet. The example assessed whether sex differences exist in the efficacy of the aforementioned glycan in the risk of NAFLD development in offspring of obese dams.

Methods

Animals and Housing. Female C57BL/6 mice (6-7 weeks old) were purchased from Harlan Labs (Indianapolis, IN). Mice were housed 4-5 per cage and were given a 1 week acclimation period before entering into the study. After 1 week, mice were randomly assigned to either a low-fat diet (LFD; 4.3% fat, D12405J, Research Diets, Inc., New Brunswick, NJ; n=22) or a high-fat diet (HFD; 35% fat, D12492 Research Diets, Inc.; n=42) (Table 2). Mice were maintained on their respective diets and provided ad libitum access to food and water. The University of Georgia Institutional Animal Care and Use Committee approved all protocols prior to the start of this experiment.

Table 2 presents diet composition. Adult female mice were randomly assigned to either a LFD (D12450J) or a HFD (D12492) (Research Diets, Inc.) throughout the duration of the study. Post-natal day 21 offspring of obese and non-obese dams were used in this study.

Table 2. Diet Composition. Abbreviations: low-fat diet (LFD) and high-fat diet (HFD).

<table>
<thead>
<tr>
<th></th>
<th>LFD</th>
<th>HFD</th>
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<tbody>
<tr>
<td><strong>Energy (%)</strong></td>
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<td></td>
</tr>
<tr>
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</table>
Glycan Treatment. After 6 weeks of feeding, mice were mated with control C57BL/6 males maintained on standard laboratory chow. Plug-positive females were individually housed during the duration of pregnancy and lactation, continuing to be fed their assigned LFD or HFD. Furthermore, at 6 weeks, female mice in the HFD group were randomly assigned to receive treatment of the immunomodulatory glycan (LNFPIII-25 μg) or its dextran vehicle (Jadhao et al, 2004, Hepatology; 39:1297-1302). Glycan and vehicle treatments, given to both the control HFD and LFD mice, were delivered subcutaneously twice per week, beginning the day before initial mating and continuing through weaning. One male and one female mouse was randomly selected from each liter to obtain experimental groups (n=7-12 per group).

Hepatic Gene Expression. At post-natal day 21, the offspring of the dams were euthanized and tissue samples were weighed and snap frozen in liquid nitrogen following dissection. Isolation of mRNA was performed using the Qiagen MiniKit (Valencia, CA) following manufacturer's instructions. The concentration of the isolate was determined using the NanoDrop 8000 spectrophotometer (Wilmington, DE). cDNA synthesis was performed using the High Capacity cDNA Reverse Transcription kit by Applied Biosystems (Grand Island, NY) using 2 micrograms of the isolated RNA. Quantitative PCR was run to determine differences in selected genes using the Applied Biosystems Sybr Green master mix on the 7500 Prism system (Applied Biosystems, Grand Island, NY). Primers were designed using PubMed databases and purchased from Integrated DNA Technologies (San Diego, CA).

The primers used for gene expression assays were designed using PubMed databases and purchased from IDT, Inc. Abbreviations: beta actin (ACTS), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), DNA damage-inducible transcript 3 (DDIT3), X-box binding protein 1- spliced variant (XBPls), DNA-methyltransferase 1 (DNMTI), glutamic pyruvate transaminase 2 (GPT2), actin alpha 2 (ACTA2), insulin receptor (INSR), insulin receptor substrate (IRS1), solute carrier family 2 (facilitated glucose transporter) member 4 (SLC2A4), acetyl-CoA carboxylase alpha (ACACA), fatty acid synthase (FASN), peroxisome proliferator-activated receptor gamma (PPAR γ), sterol regulator element binding factor 1 (SREBF1).

Hepatic Protein and Alanine Aminotransferase (ALT) Assay. Frozen hepatic tissue was homogenized in modified RIPA Buffer containing 10% protease inhibitor (Sigma-Aldrich, St. Louis, MO). Briefly, the homogenate was twice spun at 1,200 rpm for 10 minutes and the supernatant was collected and stored at -20°C. The BCA Protein Assay Kit (Thermo Scientific,
Rockford, IL) was used to quantify total protein using manufacturer's instructions. Hepatic ALT concentrations from the protein isolate were quantified using Point Scientific ALT Reagent (Canton, MI). Absorbance was read on 96-well plates using the FlexStation 3 (Molecular Devices, Sunnvale, CA). Final ALT concentration was adjusted to the total protein content of the sample.

Hepatic Triglyceride Accumulation. The total lipid fraction from whole tissue was isolated using the Folch Method as described previously (Folch et al., 1957, JBC; 226:497-509). Briefly, a 15-20 mg liver sample was homogenized in a 2:1 chloroform and methanol solution. Samples were incubated overnight at 4°C. The following day, samples were centrifuged at 2,000 rpm for 15 minutes. The supernatant was pipetted into a fresh microcentrifuge tube and washed with 1% NaCl. The mixture was vortexed and centrifuged at 2,000 rpm for 10 minutes. The subsequent top layer was discarded and the bottom layer was dehydrated and resuspended in 1% Triton X-100. Isolated triglycerides were quantified using the colorimetric Triglyceride (TAG) Reagent (Pointe Scientific, Canton, MI). Absorbance was read using the FlexStation 3 system (Molecular Devices, Sunnyvale, CA) and final TAG concentrations were determined using manufacturer's instructions.

Histology. Hepatic samples were frozen on dry ice at the time of sacrifice and stored at -80°C until use. Hepatic sectioning was performed using the Leica 3050M cryostat-microtome at 6 μm. The liver samples were stained using Oil Red O with hematoxylin as a counter nuclear stain. Hematoxylin and eosin (H&E) staining was performed at the UGA College of Veterinary Medicine Pathology Laboratory.

Statistics. Statistics were performed using STATISTICA software (version 7.0; Tulsa, OK). Two 2-way analysis of variance tests were used to determine significance of differences between main effects of diet and sex, and between sex and glycan treatments in the HFD mice. Significance between individual treatments was determined using Fisher's Least Significant Difference test.

Results

Figure 4 shows the impact of glycan LNPFIII on hepatic steatosis. Hepatic tissue of 21-day old offspring from dams fed either a LFD diet or HFD diet was isolated. A subset of dams from the HFD group were given a biweekly injection of the immunomodulatory glycan LNPFIII.
Panel (A) represents LFD versus HFD. Panel (B) represents HFD versus glycan. Pre-natal HFD exposure caused an increase in Oil Red O staining and lipid droplet formation observed in the H&E stain. Isolated lipid from hepatic tissue was resuspended in Triton-X and assayed using a colorimetric assay for triglycerides. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter are statistically significant at p<0.05.

Figure 5 shows lipogenic gene expression.

Figure 6 shows the impact of glycan LNFPIII on insulin signaling. Hepatic mRNA was isolated from post-natal day 21 offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). Panel (A) represents SLC2A4 gene expression for LFD versus HFD. Panel (B) represents SLC2A4 gene expression for HFD versus glycan. Panel (C) represents IRS1 gene expression for LFD versus HFD. Panel (D) represents IRS1 gene expression for HFD versus glycan. Panel (E) represents INSR gene expression for LFD versus HFD. Panel (F) represents INSR gene expression for HFD versus glycan. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05.

Figure 7 shows the impact of glycan LNFPIII on endoplasmic reticulum stress gene expression. Hepatic mRNA was isolated from post-natal day 21 offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). Panel (A) represents DDIT3 gene expression for LFD versus HFD. Panel (B) represents DDIT3 gene expression for HFD versus glycan. Panel (C) represents XBP1 spliced mRNA for LFD versus HFD. Panel (D) represents XBP1 spliced mRNA for HFD versus glycan. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05.

Figure 8 shows the impact of glycan LNFPIII on the expression of epigenetic regulator DNA methyltransferases 1. Hepatic mRNA was isolated from post-natal day 21 offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). Panel (A) represents DNMT1 gene expression for LFD versus HFD. Panel (B) represents DNMT1 gene expression for HFD versus glycan. Data were
normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD. Means that do not share a common letter indicate statistical difference at p<0.05.

Figure 9 shows the impact of glycan LNFPIII on hepatic function.

Panels (A) and (C) represent ACTA2 gene expression for LFD versus HFD. Panels (B) and (D) represents ACTA2 gene expression for HFD versus glycan. In Panels (A)-(D), hepatic mRNA was isolated from post-natal day 21 offspring from dams fed either HFD or LFD. A subset of HFD-fed dams were given a biweekly injection of an immunomodulatory glycan (LNFPIII). In Panels (E) and (F) total protein was isolated from frozen livers. Total protein concentration and ALT concentration was performed using manufacturer's protocols. Data were normalized to GAPDH. Statistics were performed using ANOVA and Fisher LSD.

Offspring Characteristics. Diet had a significant impact on body weight at post-natal day 21 (p<0.02; F(7.40, 1)). Male offspring of HFD-fed dams had a higher body weight than their LFD controls (p<0.02) (Table 3). Interestingly this increase was not observed in the HFD female offspring, as LFD and HFD female offspring were not significantly different. There was no significant effect of glycan treatment in the offspring, thus, body weights were similar to those of the offspring from HFD-fed control dams.

A significant sex effect was found in hepatic tissue weight between offspring from LFD and HFD-fed dams (p<0.03; F(5.20, 1)). Females had lower hepatic tissue weight compared to males. A significant glycan effect was found between HFD offspring (p<0.03;F(5.23,1)). Also in males from dams fed HFD, glycan treatment of the dams led to a reduced hepatic weight compared to male offspring from HFD-fed control dams (p<0.03) (Table 3).

Table 3 shows offspring characteristics of female C57BL/6 mice fed either a LFD or a HFD. After 6 weeks of feeding a subset of the HFD mice were given an immunomodulatory glycan (LNFPIII). Male and female offspring of all treatment groups were euthanized at post-natal day 21 and included in the analysis. Data are presented as mean ± SEM values. Statistics were performed using ANOVA and Fisher LSD was used for post-hoc analysis. Means that do not share a common letter are statistically significant at p<0.05. Abbreviations: adjusted (Adj), low-fat diet (LFD), high-fat diet (HFD).
Table 3. Offspring characteristics of Female C57BL/6 mice fed either a LFD or a HFD.

<table>
<thead>
<tr>
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<th>HFD Males</th>
<th>LFD Females</th>
<th>HFD Females</th>
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<tr>
<td>Body weight (g)</td>
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<td>8.37 ± 0.45</td>
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<tr>
<td>Liver weight (g)</td>
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<td>0.096 ± 0.00</td>
<td>0.093 ± 0.00</td>
<td>0.091 ± 0.00</td>
</tr>
<tr>
<td>Adj. liver weight</td>
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<td>0.0106 ± 0.00</td>
<td>0.01028 ± 0.00</td>
<td>0.01016 ± 0.00</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>HFD Males</td>
<td>9.66 ± 0.32</td>
<td>9.05 ± 0.28</td>
<td>9.32 ± 0.41</td>
<td>9.25 ± 0.22</td>
</tr>
<tr>
<td>Liver weight (g)</td>
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<td>0.089 ± 0.00</td>
<td>0.091 ± 0.00</td>
<td>0.090 ± 0.00</td>
</tr>
<tr>
<td>Adj. liver weight</td>
<td>0.0106 ± 0.00</td>
<td>0.0099 ± 0.00</td>
<td>0.01016 ± 0.00</td>
<td>0.0099 ± 0.00</td>
</tr>
</tbody>
</table>

Hepatic Steatosis. Oil Red O staining of representative hepatic samples showed a large increase in lipid accumulation caused by a HFD. The treatment of glycan in the HFD-fed dams was unable to prevent this deposition in the offspring. Furthermore, lipid droplets can be identified within the H&E stains of the HFD and glycan mice. To confirm histological analysis, hepatic TAG were isolated and quantified. A significant diet effect was measured (p<0.00; F(19.14, 1)). Both sexes of offspring from dams fed a HFD had increased hepatic TAG content compared to the LFD offspring (p<0.03) (Figure 4 Panels (A) and (B)). Similar to the histological assay, glycan failed to reverse the increase in TAG in either sex.

Lipogenic Gene Expression. Hepatic mRNA was isolated and gene expression assayed using qPCR. Changes in ACACA mRNA neared significance for diet effect at p=0.10, however a significant interaction was found for sex by glycan treatment (p<0.04;F(4.85,1)). There was a trend (p<0.06) for glycan treatment of dams decreased ACACA expression in female offspring but not male offspring (Figure 5, Panel A). A significant sex by diet interaction was observed for FASN expression (p<0.02; F(6.92, 1)). HFD in dams increased FASN expression in female offspring from HFD compared to female offspring from LFD fed dams (p<0.05) (Figure 5, Panel...
B). A sex by glycan treatment interaction was also measured (p<0.05; F(4.22,1)); however, no individual group differences reached significance.

A significant effect of diet was measured for the upstream regulator of lipogenesis, PPARγ (p<0.04; F(5.04, 1)), with PPARγ mRNA levels being higher in offspring from dams fed the HFD (Figure 5, Panel C). No effect of the glycan treatment was observed. A HFD effect was additionally observed in SREBF1 expression (p<0.00; F(20.16,1)). Both male and female offspring from HFD-fed dams had an increase in SREBF1 mRNA compared to their representative LFD controls (p<0.03) (Figure 5, Panel D). Glycan treatment had no effect and thus is not presented.

Insulin Signaling Gene Expression. For SLC2A4 (GLUT4) RNA expression, both sex and diet effects were observed, (p<0.04; F(5.08,1)) and (p<0.02; F(6.87,1)) respectively. Female offspring from LFD fed dams had a higher level of SLC2A4 mRNA compared to female offspring of HFD fed dams (p<0.04), while there was no difference between male offspring groups (Figure 6, Panels A and B). In addition, females had higher SLC2A4 mRNA levels compared to males, but it was mainly due to the large difference between male and female offspring from LFD fed dams. Furthermore, an interaction for sex and glycan treatment was also seen (p<0.02; F(7.38,1)). However individual group differences within sex didn't reach significance.

A sex by diet interaction was found in IRS1 expression (p<0.02; F(6.19,1)). Interestingly, HFD in the dams led to an increase in IRS1 expression in females only (p<0.03); no other differences were observed (Figure 6, Panels C and D). A significant sex effect was observed in the glycan treatment analysis (p<0.05; F(4.23,1)); the only difference being between males from glycan treated dams and females from HFD vehicle-treated dams (p<0.05). Lastly, a significant sex by diet interaction was observed in INSR expression (p<0.02; F(6.06,1)). Males from HFD fed dams had lower INSR expression compared to males from LFD fed dams; there was no difference in females (Figure 6, Panels E and F). An effect of the glycan treatment was found (p<0.05; F(4.32,1)). In males from glycan treated dams, INSR mRNA was higher compared to males from HFD vehicle-treated dams (p<0.05).

Endoplasmic Reticulum Stress Gene Expression. There were no diet or sex effects on DDIT3 (CHOP) expression, however there was a significant glycan treatment effect.
Males from glycan-treated dams had a greater level of DDIT3 mRNA compared to the females from glycan-treated dams (p<0.02) (Figure 7, Panels A and B).

The spliced variant of XBPI (XBPls) was assayed to determine activation of the unfolded protein response arm of endoplasmic reticulum stress. A significant diet x sex effect was seen in XBPls mRNA (p<0.04;F(5.03,1)). Females from LFD fed dams had lower levels of XBPls mRNA than males from LFD fed dams. There was no difference between males and females from HFD fed dams. (Figure 7, Panels C and D). A significant sex by glycan treatment interaction was also observed (p<0.01;F(8.40,1)). Female offspring from glycan-treated dams had reduced XBPls compared to females from control dams (p<0.01), whereas glycan treatment of dams had no effect in males (Figure 7, Panels C and D).

Expression of a regulator of DNA methylation. Because of the widely hypothesized relationship between transfer of maternal stress onto the offspring epigenome, DNMT1 (DNA (cytosine-5)-methyltransferases 1) was a chosen marker for alterations in epigenetic control. A significant ANOVA failed to be found in either the dietary analysis or glycan treatment analysis (Figure 8 Panels A and B).

Hepatic Function Assays. ACTA2 RNA expression was measured due to its role in tissue fibrosis, however no significant effects of sex, diet, or glycan treatment were found (Figure 9, Panels A and B). A significant diet effect was observed in GPT2 expression (p<0.05;F(4.31,1)). Male offspring from HFD-fed dams had reduced expression of GPT2 compared to offspring from LFD-fed dams (Figure 9, Panels C and D). A significant sex by glycan treatment was also measured in GPT2 expression (p<0.00;F(19.43,1)). Glycan treatment of HFD fed dams increased GPT2 expression in male offspring (p<0.02), whereas, the opposite effect occurred in female offspring (p<0.02).

Lastly, hepatic ALT concentrations were determined from extracts of total hepatic protein. A significant effect of diet was observed (p<0.000, F(15.86, 1)). HFD in the dams resulted in an increase in hepatic ALT in offspring of both sexes, when compared to LFD-fed dams (p<0.05) (Figure 9, Panels E and F). In HFD fed dams glycan treatment had no effect in either male or female offspring.
Discussion

Maternal over nutrition causes increased hepatic lipid deposition in the offspring (Bayol et al, 2010, *Endocrinology*; 151:2577-2589; McCurdy et al, 2009, *Journal Clinical Investigation*; 119:323-335; and Shankar et al, 2010, *Endocrinology*; 151:2577-2589). Consistent with previous studies, livers from HFD-offspring in our study had increased lipid accumulation and hepatic triglycerides. Similarly to other studies, the expression of major regulators of lipogenesis, including SREBF1, were disturbed with maternal HFD exposure (Shankar et al., 2010, *Endocrinology*; 151:2577-2589). Fatty acid oxidation was not investigated in this example. However epigenetic reductions in PPAR gamma coactivator 1 alpha (PGC1α) expression have been previously documented in a primate model of maternal over nutrition (McCurdy et al, 2009, *Journal Clinical Investigation*; 119:323-335). Deficiencies in PGC1α impair mitochondrial biogenesis and fatty acid oxidation (Chambers et al., 2012, *PloS One*; 7:e52645). Therefore it is possible that reductions in genes related to lipid utilization also occurred in our study. Furthermore, future work should also determine how sex can regulate these pathways, as recent work has indicated this possibility (Mischke et al, 2013, *PloS One*; 8:e78623).

In a 2007 study, investigators reported reductions in serum triglycerides and insulin in female offspring of HFD-fed dams compared to males (Ferezou-Viala et al., 2007, *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*; 293 :R1056-1062). A sexual dimorphic insulin concentration was also seen by Sun et al. (Sun et al, 2012, *Diabetes*; 61:2833-2841); however, statistical analysis was not performed between sexes. Findings from these two studies suggest a sexual dimorphism in insulin sensitivity and signaling. In this example, females, particularly ones from LFD-fed dams, had a greater SLC2A4 expression compared to the males. Furthermore, IRS1 expression was increased in the female offspring from HFD-fed dams. In combination with potential differences seen in serum insulin in the aforementioned studies, it is possible that females may be protected from metabolic diseases including NAFLD through a beneficial insulin profile.

Sex specific differences in metabolic diseases have long been identified and attributed to the female hormone 17P-estradiol. Emerging research has demonstrated an inherent dimorphism in early metabolic diseases that is seen prior to puberty, thus removing a causative role of estrogens in this protection (Mischke et al, 2013, *PloS One*; 8:e78623; Chechi et al, 2009,
American Journal of Physiology, Regulatory, Integrative and Comparative Physiology; 296:R1029-1040; and Elahi et al, 2009, The British Journal of Nutrition; 102:5 14-5 19). It has been hypothesized that epigenetics may regulate both inherent sex differences in utero and early infancy, in addition to dimorphic responses to disease promoters. Interestingly, the placenta is highly adaptable to the maternal environment in a sexually dimorphic manner and may provide a mechanism behind early epigenetic modifications in the offspring (Gabory et al, 2013, Biology of Sex Differences; 4:5). While no differences in DNMT1 expression were observed in this example, this does not negate the possibility of epigenetic regulation of the metabolic outcomes that were measured.

An immunomodulating, anti-inflammatory glycan was used in this example to determine whether maternal HFD-induced inflammatory programming in the offspring could be prevented. A similar sexual dimorphic response was measured with maternal glycan treatment. The most significant contribution of the glycan was on the activation of endoplasmic reticulum stress. While in general females had less XBPI splicing compared to males, females from glycan-treated dams had lower splicing females from vehicle treated dams (p<0.05). This is not surprising as endoplasmic reticulum stress is highly related to inflammation and lipotoxicity, and thus may indicate reduced hepatic stress in this group (Wang et al, 2006, Endocrinology; 147:943-95 l; and Wei et al., 2006, American Journal of Physiology Endocrinology and Metabolism; 291:E275-281). Furthermore, hepatic GPT2 expression was also downregulated in the female offspring of glycan-treated dams. Interestingly, the enzymatic ALT activity did not follow the same pattern as GPT2 expression. It is possible that the discrepancy between these two data points could be related to assay sensitivity. Enzymatic assays for ALT measures both isoforms of ALT, whereas the gene expression data of this example reflects the isoform more associated with NAFLD (Jadhao et al, 2004, Hepatology; 39:1297-1302). While a functional assay to determine hepatic ALT concentrations found no reduction with glycan treatment, the reduced GPT2 mRNA may be an indicator of reduced hepatic damage in this group.

Maternal obesity may impair the offspring's ability to regulate energy in our current obesogenic environment, further exaggerating metabolic disease in adulthood (Bruce et al, 2009, Hepatology; 50:1796-1808; and Pruis et al, 2014, Acta Physiologica; 210:215-227). Therefore it is necessary to determine mechanisms by which maternal transfer occurs and how it may be prevented. This example provides evidence for a sex-specific response in an early programming
From these data, we conclude that females may be at a reduced risk of childhood NAFLD due to enhanced insulin sensitivity and reduced hepatic stress compared to their male counterparts. Furthermore, supplementation of an immunomodulatory glycan during gestation produced a sex-specific response in offspring suggesting a need for further research in this area.

Example 4
Microbiota Data

This example provides microbiota data (phyla and class, PND 21, 35 and 90, males and females). Direct and transgenerational impacts of glycan LNFP III on gut microbiota composition were determined. Briefly, at weaning (6 weeks after LNFP III treatment started), cecal contents are sampled from dams on LF and HF diets. In addition, cecal contents are collected from pups born of HFD, LFD, and HFD mothers treated with LNFP III-conjugate or dextran control at various time points post-birth (PND). These samples are subject to gut microbiome analysis. Bacterial DNA was extracted using a commercial kit (Zymo) and 16S microbiome was analyzed using Illumina sequencing.

As shown in Figure 10, an analysis of dam's phyla demonstrates a significant glycan effect on Firmicutes (decrease) and Verrucomicrobia (increase). Materials and methods as described in the previous examples were used. As shown in Figure 11, an analysis of PND 21 phyla demonstrates overall a significant glycan effect on Verrucomicrobia (decrease, opposite as the one seen in dams). In males the glycan effect is on bacteriodetes. As shown in Figure 12, an analysis of PND 35 Phyla demonstrates an effect of glycan on bacteriodetes (increase) (P=0.09). PND 21 and 35 combined demonstrates an effect of glycan on bacteriodetes (P=0.05). In females, significant glycan effect on Firmicutes and Bacteriodetes. As shown in Figure 13, an analysis of PND 21 Class demonstrates no overall effect of the glycan. In males there was a significant effect on Clostridia (Firmicutes) and Bacteriodia (Bacteriodetes). Figure 14 presents PND 21 PCA. Figure 15 presents PND 90 Phyla. For Phyla, there was a significant interaction of glycan X diet (postnatal) on firmicutes and bacteriodetes. A significant overall effect of the glycan at the class level was observed on Clostridia (Firmicutes), Alphaproteobacteria (proteobacteria), and cOd2 (Cyanobacteria). Figure 16 presents PND 90 Class.
Example 5
Prevention of Maternal Diet-Induced Neurochemical Dysregulation

This example demonstrates the ability of maternal glycan treatment to prevent maternal diet-induced neurochemical dysregulation in the still developing offspring brains. Materials and methods as described in the previous examples were used. Figure 17 shows the effects of maternal high-fat diet (HFD) consumption and maternal administration of the LNFPIII glycan on dopamine (DA) homeostasis in the dorsal (dHIPP) (Figure 17 Panel A) and ventral (vHIPP) (Figure 17 Panel B) hippocampus, key brain structure for learning and memory. Female C57BL/6 mice were fed low-fat (LFD) or high-fat (HFD) beginning 6 weeks prior to mating until weaning of the offspring, which occurred on postnatal day (PND) 21. Subset of the dams on HFD were given the glycan (G) twice per week beginning the day before mating until weaning; the LFD and HFD dams were give the dextran (D) vehicle. On PND 10, which is a critical neurodevelopmental window, brains from male and female offspring (n=7 each) from each maternal treatment were collected and levels of several neurotransmitters and their major metabolites, including dopamine (DA), were determined in several brain regions by HPLC with electrochemical detection. One major effect of maternal HFD consumption was increased DA in both the dorsal and ventral sectors of the hippocampus. This effect, which was not gender-specific, was reversed by maternal glycan administration. Thus, maternal glycan treatment prevents the dyshomeostasis of DA in the still developing offspring brain that was caused by maternal HFD consumption.
This example summarizes those genes that were found to be significantly influenced in the pups born of mothers treated with LNFPIII-Dex during pregnancy. Using materials and methods as described in the previous examples, hippocampus tissues were analyzed. Effects of maternal HFD and the addition of LNFPIII treatment on dorsal and ventral hippocampal mRNA transcript levels in male and female postnatal day 21 and postnatal day 35 offspring are summarized below:

Hippocampal (HIP) postnatal day 21 (PND21) Brain-derived Neurotrophic Factor (BDNF). Treatment with the LNFPIII in addition to HFD decreased transcript levels of the neurotrophin BDNF in the dorsal hippocampus of PND21 males, but increased BDNF in both the dorsal and ventral hippocampus of PND21 females.

HIP PND21 Insulin Receptor (INSR). Treatment with LNFPIII in addition to HFD increased transcript levels of insulin receptor in the dorsal and ventral hippocampus of PND21 females.

HIP PND21 Glucocorticoid Receptor (GR). Treatment with LNFPIII in addition to HFD increased transcript levels of glucocorticoid receptor in the dorsal and ventral hippocampus of PND21 females.

HIP PND21 5-hydroxytryptamine receptor 1A (5HT1a). Treatment with LNFPIII in addition to HFD increased transcript levels of the serotonin receptor 5HT1a in the dorsal hippocampus of PND21 females.

HIP PND21 Interleukin-10 (IL10). IL10. Treatment with LNFPIII in addition to HFD increased transcript levels of the anti-inflammatory factor interleukin-10 in the dorsal hippocampus of PND21 males.

HIP postnatal day 35 (PND35) BDNF. Treatment with LNFPIII in addition to HFD increased transcript levels of the neurotrophin BDNF in the dorsal hippocampus of PND35 males. This is interesting since the same treatment showed the opposite effect at the earlier time point.
HIP PND35 INSR. Treatment with LNFP III in addition to HFD increased transcript levels of insulin receptor in the dorsal hippocampus of PND35 males.

HIP PND35 Glucose Transporter Type 4 (GLUT4). Treatment with LNFP III in addition to HFD increased transcript levels of glucose transporter 4 in the dorsal and ventral hippocampus of PND35 males.

HIP PND35 Insulin receptor substrate 1 (IRS1). Treatment with LNFP III in addition to HFD increased transcript levels of insulin receptor substrate 1 in the dorsal hippocampus of PND35 males.

HIP PND35 Leptin receptor, long form (OBRB). Treatment with LNFP III in addition to HFD increased transcript levels of leptin receptor in the dorsal and ventral hippocampus of PND35 males.

HIP PND35 GR. Treatment with LNFP III in addition to HFD increased transcript levels of glucocorticoid receptor in the dorsal hippocampus of PND35 males.

HIP PND35 5HT1a. Treatment with LNFP III in addition to HFD increased transcript levels of the serotonin receptor 5HT1a in the dorsal hippocampus of PND35 males and in the ventral hippocampus of PND35 females.

HIP PND35 IL10. Treatment with LNFP III in addition to HFD decreased transcript levels of the anti-inflammatory factor interleukin-10 in the ventral hippocampus of PND35 males.

HIP PND35 X-box binding protein 1 spliced (XBPls). Treatment with LNFP III in addition to HFD increased transcript levels of the spliced form of the transcription factor XBPl in the dorsal and ventral hippocampus of PND35 males, and in the ventral hippocampus of PND35 females.

HIP PND35 B-cell lymphoma 2-like protein 11 (Bcl2-1 1). Treatment with LNFP III in addition to HFD decreased transcript levels of the apoptosis-regulator Bcl2-1 1 in the ventral hippocampus of PND35 males.
These results are also summarized in Tables 4 and 5 below.

Table 4. PND21.

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Table 5. PND35.

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</table>
Maternal HFD in addition to treatment with LNFPIII had no effect on transcript levels in PND21 offspring for the following gene targets: Leptin receptor, long form (OBRB); Peroxisome proliferator-activated receptor alpha (PPARa); Insulin receptor substrate 1 (IRS1); 5-hydroxytryptamine receptor IB (5HT1b); 5-hydroxytryptamine receptor 2B (5HT2b); B-cell lymphoma 2-like protein 11 (Bcl2-11); and X-box binding protein 1 spliced (XBPls).

Maternal HFD in addition to treatment with LNFPIII had no effect on transcript levels in PND35 offspring for the following gene targets: Peroxisome proliferator-activated receptor alpha (PPARa); 5-hydroxytryptamine receptor IB (5HT1b); 5-hydroxytryptamine receptor 2B (5HT2b); and Interleukin 6 (IL-6).

In summary, for HFD vs LFD (Statistically significant (p<0.05)):
- Increase in glucocorticoid receptor in the dorsal hippocampus of PND21 males;
- Decrease in 5HT2b in ventral hippocampus of PND35 males; and
- Decrease in PPARalpha in the ventral hippocampus of PND35 females.

For HFD vs LFD (Statistical trends (p<0.10)):
- Increases in BDNF and insulin receptor in dorsal hippocampus of PND21 males;
- Increase in insulin receptor substrate in dorsal hippocampus of PND35 males;
- Increase in 5HT1a in dorsal hippocampus of PND35 females;
- Decrease in 5HT1b in ventral hippocampus of PND35 males;
- Decrease in IL-10 in ventral hippocampus of PND35 males; and
- Decrease in Bcl2-11 (apoptosis marker) in ventral hippocampus of PND35 males.

For effects of Glycan + HFD (Statistically significant (p<0.05)):
- Decrease in BDNF in the dorsal hippocampus of PND21 males;
- Increase in PPARalpha in the dorsal hippocampus of PND21 males;
- Increase in glucocorticoid receptor in the dorsal hippocampus of PND21 males;
- Increase in BDNF in both dorsal and ventral hippocampus of PND21 females;
- Increase in insulin receptor in both dorsal and ventral hippocampus of PND21 females;
- Increase in glucocorticoid receptor in both dorsal and ventral hippocampus of PND21 females;
- Increase in BDNF in the dorsal hippocampus of PND35 males;
Increase in leptin receptor in both dorsal and ventral hippocampus of PND35 males;
Increase in glucocorticoid receptor in the dorsal hippocampus of PND35 males;
Increase in insulin receptor in the dorsal hippocampus of PND35 males;
Increase in GLUT4 in both the dorsal and ventral hippocampus of PND35 males;
Increase in 5HT1a receptor substrate in the dorsal hippocampus of PND35 males;
Decrease in 5HT2b in the ventral hippocampus of PND35 males;
Decrease in IL-10 in the ventral hippocampus of PND35 males;
Increase in XBPls in both dorsal and ventral hippocampus of PND35 males;
Decrease in Bcl2-1 in the ventral hippocampus of PND35 males;
Increase in 5HT1a in the ventral hippocampus of PND35 females; and
Increase in XBPls in the ventral hippocampus of PND35 females.

For effects of Glycan + HFD (Statistical trends (p<0.10)):
Increase in leptin receptor in dorsal hippocampus of PND21 females;
Increase in 5HT1a in the ventral hippocampus of PND21 females;
Decrease in BDNF in dorsal hippocampus of PND35 females;
Decrease in IL-6 in the ventral hippocampus of PND35 males; and
Decrease in Bcl2-1 in the ventral hippocampus of PND35 females.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.
What is claimed is:

1. A method of reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject’s mother, the method comprising the maternal administration of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, a mimetic thereof, or a conjugate thereof, to the subject's mother.

2. A method of reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject's mother, the method comprising the maternal administration of an immunomodulatory glycan or a glycoconjugate thereof to the subject's mother.

3. The method of claim 2, wherein the immunomodulatory glycan comprises lacto-N-fucopentaose III (LNFPIII), LacdiNAc, fucosylated LacdiNac, or omega-1.

4. The method of claim 1 or 2, comprising administration to the subject's mother prior to pregnancy, prior to lactation, during pregnancy, and/or during lactation.

5. The method of claim 1 or 2, comprising administration to the subject's mother prior to gestation of the subject.

6. The method of claim 1 or 2, comprising administration to the subject's mother during gestation of the subject.

7. The method of claim 1 or 2, comprising administration to the subject's mother prior to the initiation of breast feeding of the subject by the mother.

8. The method of claim 1 or 2, comprising administration to the subject's mother during breast feeding of the subject by the mother.

9. The method of claim 1 or 2, wherein maternal administration restores gut, metabolic, brain, neurologic and/or immune functions to normal in the offspring.
10. The method of claim 1 or 2, wherein administration comprises by injection, orally, transdermally, topically, or intranasally.

11. The method of claim 10, wherein administration by injection comprises intramuscular, intradermal, or subcutaneous injection.

12. The method of claim 10, wherein administration by injection comprises formulation as a slow release biodegradable complex, nanoparticles, and/or a hydrogel.

13. The method of claim 10, wherein oral administration comprises administration as a liquid suspension, tablet, a capsule, or a gel.

14. The method of claim 10, wherein oral administration comprises formulation as a food item, nutraceutical, or a dietary supplement.

15. The method of claim 10, wherein topical administration comprises administration as an emulsion, cream, or ointment.

16. The method of claim 10, wherein transdermal administration comprises administration by a transdermal skin patch or a microneedle patch.

17. The method of any one of claims 1 to 16, wherein a glycoconjugate thereof comprises a BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer.

18. The method of claim 1, wherein the LNFPIII glycan conjugate comprises a BSA, human serum albumin (HSA), dextran conjugate, or a dendrimer.

19. The method of claim 18, wherein the LNFPIII glycan conjugate comprises about 8 to about 10 LNFPIII per dextran.
20. Use of an immunomodulatory glycan or a glycoconjugate thereof as a medicament for maternal administration for reducing one or more of the effects of maternal high fat diet (HFD) obesity in the offspring.

21. Use of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof, as a medicament for maternal administration for reducing one or more of the effects of maternal high fat diet (HFD) obesity in the offspring.

22. Use of an immunomodulatory glycan or a glycoconjugate thereof for reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject's mother.

23. Use of a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof, for reducing in a subject one or more of the effects in the subject of maternal high fat diet (HFD) obesity in the subject's mother.

24. The use of claim 21 or 23, wherein the lacto-N-fucopentaose III (LNFPIII) glycan, derivative thereof, or conjugate thereof, comprises a LNPIII dextran conjugate comprising about 8 to about 10 LNFPIII per dextran.

25. The use of any one of claims 20 to 24 wherein the maternal administration comprises prior to pregnancy, prior to lactating, during pregnancy, and/or during lactating.

26. A nutraceutical or dietary supplement for consumption by a pregnant and/or lactation woman, the nutraceutical or dietary supplement comprising an immunomodulatory glycan or a glycoconjugate thereof.

27. A nutraceutical or dietary supplement for consumption by a pregnant and/or lactation woman, the nutraceutical or dietary supplement comprising a lacto-N-fucopentaose III (LNFPIII) glycan, a derivative thereof, or a conjugate thereof.
**Figure 1**

**A**
Gastrointestinal permeability
PND 21 (n = 14-21/Group)

**B**
IAP activity PND 21
(n = 7/Group)

**C**
Microflora composition-genera

- AKKERMANSIA
- DESULFOVIBRIO
- MARVINBRYANTIA
- CLOSTRIDIUM
- RUMINOCOCUS
- PSEUDOBUTYRVIBRIO
- ROSEBURIA
- PREVOTELLA

% Microbial sequence-genera

- 45.0
- 40.0
- 35.0
- 30.0
- 25.0
- 20.0
- 15.0
- 10.0
- 5.0
- 0.0

LF, HF, HF-GLYCAN
Figure 2
Figure 3
Figure 4

A

HEPATIC TAG (mg/g)

LFD V. HFD

B

HEPATIC TAG (mg/g)

HFD V. GLYCAN

LFD MALES
HFD MALES
LFD FEMALES
HFD FEMALES

GLYCAN MALES
GLYCAN FEMALES
Figure 5

RA VALUES RELATIVE TO MALE LF MICE

A

ACACA GENE EXPRESSION

0.00 0.75 1.50

AB

LFD MALES HFD MALES LFD FEMALES HFD FEMALES

B

FASN GENE EXPRESSION

0.00 0.75 1.50

AB

LFD MALES HFD MALES LFD FEMALES HFD FEMALES

C

PPARY GENE EXPRESSION

0.00 0.75 1.50 2.25 3.00

AB

LFD MALES HFD MALES LFD FEMALES HFD FEMALES

D

SREBF1 GENE EXPRESSION

0.00 0.75 1.50 2.25 3.00

A

LFD MALES HFD MALES LFD FEMALES HFD FEMALES
Figure 6
Figure 7
Figure 9
Figure 11
Figure 12
Figure 15
### INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/US 15/43212

#### A. CLASSIFICATION OF SUBJECT MATTER

**IPC(8)** - A61K39/00 (2015.01)

**CPC** - A61K 38/00; A61K 2039/505; C07K 2317/622

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC(8)** - A61K 39/00 (2015.01)

**CPC** - A61K 38/00; A61K 2039/505; C07K 2317/622

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

UC - 424/135.1, 424/141.1, 514/789, 424/130.1

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 2013/0044044 A2 (Lee et al.) 28 March 2013 (28.03.2013) abstract; page 3, lines 30-33; (page 17, lines 10-30; page 23, lines 10-15)</td>
<td>1-16, 18-24, 26, 27</td>
</tr>
<tr>
<td>Y</td>
<td>US 2013/0259879 A1 (Baumhof et al) 03 October 2013 (03.10.2013) para [0298], [0309]</td>
<td>15</td>
</tr>
</tbody>
</table>

* Further documents are listed in the continuation of Box C.

- **A**: Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

- **T**: later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- **X**: document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

- **Y**: document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

- **K**: document member of the same patent family

#### Date of the actual completion of the international search

05 October 2015 (05.10.2015)

#### Date of mailing of the international search report

03 NOV 2015

#### Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4380
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)
INTERNATIONAL SEARCH REPORT

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos. 17, 25
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

Remark on Protest

☐ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)