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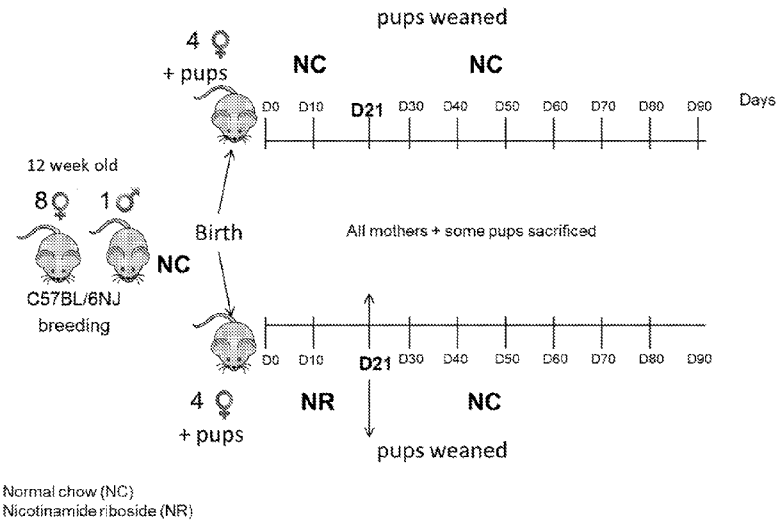
FIGURE 5



(57) Abstract: Certain embodiments of the invention provide a method for improving maternal and/or offspring health, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a female mammal (e.g., pregnant or lactating female mammal).

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USE OF NAD PRECURSORS FOR IMPROVING MATERNAL HEALTH AND/OR OFFSPRING HEALTH

RELATED APPLICATION

This application claims the benefit of priority of U.S. Provisional Application Serial No. 62/427,661 filed on November 29, 2016, which application is incorporated by reference herein.

BACKGROUND OF THE INVENTION

There are many challenges associated with maintaining proper maternal health during and after pregnancy. For example, mothers often have difficulties losing unwanted weight gained during pregnancy, sustaining sufficient milk production to feed newborns effectively and time-efficiently, and maintaining a suitable niacin status (Baker et al., *J. Am. Coll. Nutr.* 2002 Feb;21(1):33-7). Additionally, neonatal and child health and brain development are often limited by the availability of mother's milk, and the body composition, hypoglycemia and lack of mobility of neonates and young children. However, current methods and treatments for addressing these challenges are limited.

Thus, there is a need for new methods and therapies for improving maternal health and/or offspring health (e.g., neonatal, early childhood and/or adult health).

SUMMARY OF THE INVENTION

Accordingly, certain embodiments of the invention provide a method for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

Certain embodiments of the invention provide a NAD precursor for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy

associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

Certain embodiments of the invention also provide the use of a NAD precursor to prepare a medicament useful for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

Certain embodiments of the invention provide a composition (e.g., a pharmaceutical composition) comprising a NAD precursor and an acceptable carrier (e.g., a pharmaceutically acceptable carrier) for use in imparting a health benefit to a female mammal in need thereof and/or the offspring of the female mammal,

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

Certain embodiments of the invention also provide a kit comprising a NAD precursor and instructions for administering the NAD precursor to a female mammal in need thereof for imparting a health benefit to the female mammal and/or the offspring of the female mammal,

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

Certain embodiments of the invention provide a kit comprising 1) a composition (e.g., a pharmaceutical composition) comprising a NAD precursor and a carrier, wherein the composition is formulated for oral administration; and 2) instructions for orally administering the NAD precursor to a female mammal for imparting a health benefit to the female mammal and/or the offspring of the female mammal,

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

Certain embodiments of the invention also provide a method, compound, composition or kit as described herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Weight gain (gm) from mating to end of lactation (left panel) and percent weight gain from mating to end of lactation (right panel) in female mice fed NC or NC+nicotinamide riboside (NR).

Figure 2. Volume of milk collected from lactating female mice fed NC or NC+NR.

Figure 3. Fat mass (left panel) and brain mass (right panel) from mice raised by mothers fed NC or NC+NR.

Figure 4. Fasting glucose of mice raised by mothers fed NC or NC+NR.

Figure 5. Experimental design.

Figures 6A-D. NR-supplemented mouse mothers lose weight during the 21 day lactation period without diminished food consumption (day 7-21). Delta body mass refers to day 21 post-partum maternal weight minus day 0 post-partum weight (**Figure 6A**). Food consumption was day 7-21 (**Figure 6B**). NR-supplemented mothers tend to lose fat (**Figure 6C**) and maintain lean mass (**Figure 6D**). Body composition refers to day 21 post-partum maternal fat and lean mass minus day 0 post-partum maternal fat and lean mass.

Figure 7. NR-supplemented mothers produced more milk.

Figures 8A-C. Milk from NR-supplemented mothers had normal macronutrient density. Oxytocin-induced milk collection at indicated days. Protein (**Figure 8A**), carbohydrate (**Figure 8B**) and lipid concentration (**Figure 8C**) was not changed.

Figures 9A-B. Orally administered NR is not directly transmitted *via* milk. The concentration of nicotinamide and NR was slightly increased when mother was NR-supplemented. However, when mother was given oral NR with heavy atoms in the nicotinamide and ribose moieties of NR, mass spec analysis indicates that some of the milk nicotinamide is labeled but not of the milk NR is labeled. This indicates that oral NR to the mother has some somatic target in the mother that increases her milk production but that maternally ingested NR is not directly transmitted to the offspring.

Figure 10. NR supplemented mothers produce milk with higher brain-derived neurotrophic factor. Milk from NC and NR mothers was tested. NR-supplemented mothers have 2-fold higher levels of BDNF in the milk, suggesting a powerful mechanism by which NR supplementation supports neonatal development and health.

Figures 11A-D. Lactation but not NR boosts hepatic NAD biosynthesis but not accumulation of NAD metabolites in the liver, suggesting that lactation drives the liver to mobilize NAD metabolites elsewhere. Lactation boosts NAD biosynthetic genes (**Figure 11A**) and NAD levels (**Figure 11B**) in the liver. NR does not superinduce in the liver. **Figure 18C-D.** Lactation seems to depress levels of NR (**Figure 11C**) and Nam (**Figure 11D**) in the liver. NR supplementation depresses the accumulation of these metabolites in the liver even more. These data suggest that the liver may be working to mobilize NR and Nam to other tissues, like the mammary.

Figures 12A-D. Lactation and NR boost the mammary NAD program. **Figure 12A.** NR promotes expression of NAD biosynthetic genes in the mammary glands. **Figure 12B.** Lactating mammary gland has higher NAD+ levels and NR supplementation tends to further increase NAD+ levels. **Figure 12C.** Lactation boosts NMN in mammary. **Figure 12D.** Lactation and NR boost mammary NR in an additive manner.

Figures 13A-D. NR boosts prolactin circulation and mammary biosynthetic pathways for protein, fat and carbohydrate. **Figure 13A.** Prolactin levels in plasma from dam on NC or NR chow. **Figure 13B.** mTOR pathway is activated in NR-supplemented mammary tissue. **Figure 13C.** Genes involved in lipid biosynthesis are activated in NR-supplemented mammary tissue. **Figure 13D.** Genes involved in lactose synthesis pathway are activated in NR-supplemented mammary tissue.

Figures 14A-B. Postpartum NR supplementation promotes mammary gland development (n = 5 mice per group) (**Figure 14A**). NR increases heat loss by increasing expression of UCP1 (**Figure 14B**).

Figure 15. Maternal NR increases pup growth early in the neonatal period. Neonatal pups body weight from Day 0 to Day 14 (n = 5 litter per group).

Figures 16A-D. 21 day old pups of NR-supplemented mothers are bigger. Males and females were analyzed separately. The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 17A-C. 22 day old pups of NR-supplemented mothers have improved post-fasting glucose homoeostasis with higher gluconeogenic gene expression and higher hepatic glycogen storage. Not only are the offspring of NR-supplemented mothers a bit bigger, they also have better glycemic control. This is due to better gluconeogenic gene expression and higher glycogen storage. The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 18A-B. Mother's NR supplementation produces adventurous neonatal offspring: 15 day old pups of NR-supplemented mothers cover more distance in an open field test and males also spend more time in the center. The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 19A-D. 49 days after their mother's intervention, 70 day-old adults of NR-supplemented mothers cover more distance and move faster in an open field test with a tendency toward less immobile time. Males spend less time in the center. These adults were treated identically ever since weaning. The only difference is whether the mother was NR-supplemented for 21 days. They are faster and more adventurous. The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 20A-C. 54 days after their mother's intervention, 75 day-old female offspring of NR-supplemented mother spend more time in the light (e.g., have reduced anxiety and are less fearful). The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light

grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 21A-B. 64 days after their mother's intervention, 85-day-old male offspring of NR-supplemented mothers exhibit less anxiety on an elevated plus maze. Males are not afraid to "stick their head out". The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 22A-C. 64 days after their mother's intervention, 85-day-old adults of NR-supplemented mothers have better performance on a balance beam. They cross a beam balance faster (untrained); they slip fewer times; and they almost never grip the beam with their tail. Overall, these mice are more coordinated and are superior physical specimens. The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 23A-C. 69 days after their mother's intervention, 90 day-old adult offspring of NR-supplemented mothers have less fat mass. The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

Figures 24A-D. 69 days after their mother's intervention, the female offspring tend to have larger brains (statistically significant) (**Figure 24A**). Pups of NR-supplemented moms show advanced pruning of the caudate putamen at day 22 (**Figures 24B-C**); however, the caudate putamen in pups from NR-supplemented mothers grows to a normal size in adulthood (**Figure 24D**). The order of the bars from left to right are: males of normal chow (NC) mothers (white with light grey outline), followed by males of NR-supplemented mothers (filled light grey), followed by females of NC mothers (white with dark grey outline), followed by females of NR-supplemented mothers (filled dark grey).

DETAILED DESCRIPTION

Certain embodiments of the invention provide the use of NAD precursors, such as nicotinamide riboside (NR) and derivatives and analogs thereof, for improving maternal health

and the health of offspring (e.g, neonatal and early childhood health, as well as adult health). As described herein, a number of benefits of administering NAD precursors during and after pregnancy were surprisingly discovered (*see, e.g.,* the Examples). For example, a near total elimination of weight gain over the gestational and post-gestational interval was unexpectedly observed in pregnant/lactating mice administered NR. Additionally, a significant increase in the amount of milk produced by lactating females was observed. It was also surprisingly found that benefits of administering NR to pregnant/lactating mice were passed onto the offspring. For example, the following benefits to neonatal health were observed from the maternal administration of NR: 1) larger mass of neonates at the time of weaning; 2) larger brain mass of neonates at the time of weaning; 3) greater physical activity of neonates; and 4) correction of neonatal glycemic control from a slightly low level to an ideal level. Accordingly, an NAD precursor may be administered to a pregnant or lactating female mammal for, e.g., gestational and post-gestational weight management, to improve metabolic fitness during and post-pregnancy, to enhance lactation, to improve infant nutrition, to improve infant body composition and/or glycemic control, and/or to improve brain development, physical activity, cognitive and/or behavioral functions of offspring (e.g., babies). As described herein, an NAD precursor may also be administered to a pregnant or lactating female mammal to provide certain benefits to an offspring later in life (e.g., as an adult, despite cessation of consuming its mother's milk) (*see, e.g.,* the Examples).

Administration of an NAD precursor to a female mammal (e.g., a reproductively active female, a pregnant female or a nursing female) may improve maternal health and/or offspring health (e.g., neonatal, early-childhood and/or adult health) through multiple mechanisms, including, e.g., 1) decreased maternal adiposity and improved neonatal/child metabolism with some of the maternal benefit deriving from increased transmission of high quality calories from mother to baby; 2) increased milk volume and feeding efficiency, decreasing a baby's' stress during feeding time; 3) increased milk quality as evidenced by increased content of bioactive molecules such as brain derived neurotrophic factor, 4) reduced feeding time to satisfy the nutritional needs of a baby/improved mother-child bonding opportunities; 5) reduced emotional stress for mothers, who are better able to meet the nutritional needs of their baby; 6) freedom from neonatal hypoglycemia-associated sequelae, including jitteriness, cyanosis, apnea, hypothermia, poor body tone, poor feeding, lethargy and seizures; and/or 7) improved physical activity, cognitive and behavioral functions of offspring (e.g., babies).

Methods of the Invention

Accordingly, certain embodiments of the invention provide method for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal (e.g., in need thereof), comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss, increased milk production when lactating and/or increased milk quality; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

In certain embodiments, the health benefit is imparted to the female mammal. In certain embodiments, the NAD precursor is administered to the female mammal pre-pregnancy, during pregnancy and/or post-pregnancy. In certain embodiments, the NAD precursor is administered to the female mammal pre-pregnancy. In certain embodiments, the NAD precursor is administered to the female mammal during pregnancy. In certain embodiments, the NAD precursor is administered to the female mammal post-pregnancy. In certain embodiments, the NAD precursor is administered to the female mammal during pregnancy and post-pregnancy. In certain embodiments, the NAD precursor is administered to the female mammal pre-pregnancy, during pregnancy and post-pregnancy.

In certain embodiments, the health benefit is a reduction in pregnancy associated weight gain. Thus, certain embodiments of the invention provide a method for reducing pregnancy associated weight gain in a female mammal (e.g., a human) in need thereof, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal. In certain embodiments, pregnancy associated weight gain is reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the amount of weight gained during pregnancy by a female mammal not administered an NAD precursor). In certain embodiments, pregnancy associated weight gain is prevented (i.e., the mother's weight returns to her pre-pregnancy weight after giving birth).

As used herein, the term "in need thereof" refers to any mammal wanting/desiring to practice a method described herein for any purpose (e.g., for medical reasons or otherwise).

As used herein, the term "pregnancy associated weight gain" refers to weight gained by a female mammal during pregnancy, excluding the weight of the offspring, amniotic fluid and

other tissues/fluids expelled upon giving birth. Accordingly, "pregnancy associated weight gain" may be calculated by subtracting a female mammal's weight after giving birth (e.g., within 1 day, 1 week, 2 weeks, 1 month of giving birth, etc.) from the female mammal's pre-pregnancy weight.

In certain embodiments, the health benefit is promotion of post-pregnancy weight loss. Thus, certain embodiments of the invention provide a method for promoting post-pregnancy weight loss in a female mammal (e.g., a human) in need thereof, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal. In certain embodiments, weight loss is increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the amount of weight lost by a female mammal not administered an NAD precursor). In certain embodiments, milk production and/or mammary gland development is increased in the female mammal. In certain embodiments, the female mammal loses fat and maintains lean mass.

As used herein, the terms "post-pregnancy weight loss" refers to weight loss after giving birth (e.g., 1 day, 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, etc. after giving birth).

In certain embodiments, the health benefit is increased milk production. Thus, certain embodiments of the invention provide a method for increasing milk production in a lactating female mammal in need thereof, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal. In certain embodiments, milk production is increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the milk production prior to administration). Milk production may be measured using techniques known in the art, for example using a method as described in the examples, such as measuring the volume of collected milk.

In certain embodiments, the health benefit is an improvement in the quality of the milk. As used herein, the term "milk quality" refers to the level of nutrients and vitamins present in the milk. Thus, milk having improved quality may comprise increased levels of certain nutrients and/or vitamins (e.g., brain-derived neurotrophic factor (BDNF), *see also,* nutrients described in the Examples). Thus, certain embodiments of the invention provide a method for increasing the quality of milk from a lactating female mammal in need thereof, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal. In certain embodiments, the milk comprises increased levels of BDNF. In certain

embodiments, BDNF levels are increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the BDNF levels in milk from the female mammal prior to administration).

As discussed above, the maternal administration of a NAD precursor can result in a number of benefits being passed on to offspring. These benefits may be derived *in utero* (*i.e.*, a NAD precursor is administered to the mother during pregnancy) and/or through ingesting milk from the mother (*i.e.*, a NAD precursor is administered to a lactating mother). These benefits may be observed at the neonatal, adolescent and/or adult stages (e.g., throughout the life of the offspring, despite cessation of consuming milk from its mother).

Thus, in certain embodiments, the health benefit is imparted to the offspring of the female mammal (via maternal administration). In certain embodiments, an effective amount of the NAD precursor is administered to the female mammal while pregnant, thereby imparting the health benefit to the later born offspring. In certain embodiments, an effective amount of the NAD precursor is administered to the female mammal while lactating, wherein the offspring ingests milk from the lactating female mammal, thereby imparting the health benefit to the offspring.

In certain embodiments, the health benefit results when the offspring is a baby (e.g., for a human, e.g., less than 3 years of age). In certain embodiments, the health benefit results when the offspring is a child or adolescent (e.g., for a human, e.g., between ages 3 to 17). In certain embodiments, the health benefit results when the offspring is an adult (e.g., for a human, e.g., 18 years of age or older). In certain embodiments, the health benefit results throughout the life of the offspring.

In certain embodiments, the health benefit is improved metabolism in the offspring. Thus, certain embodiments of the invention provide a method for improving metabolism in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby improving metabolism in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby improving metabolism in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved metabolism in the

later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby improving metabolism in the offspring (e.g., as compared to the metabolism of offspring from a mother not administered an NAD precursor). Methods of measuring metabolism are known in the art, for example, using an assay described herein. In certain embodiments, the improved metabolism is experienced when the offspring is an adult. In certain embodiments, the improved metabolism is experienced throughout the life of the offspring. In certain embodiments, the improved metabolism is experienced when the offspring is a baby. Thus, certain embodiments of the invention also provide a method for improving metabolism in a mammalian baby, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby improving metabolism in the later born mammalian baby; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby improving metabolism in the mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved metabolism in the later born mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby causing improving metabolism in the mammalian baby (e.g., as compared to the metabolism of a baby from a mother not administered an NAD precursor). In certain embodiments, the mammalian baby has improved glycemic control. In certain embodiments, the mammalian baby has an increased storage of glycogen.

As used herein, the term "baby" refers to an infant or young child. In certain embodiments, the baby is an infant or young child that ingests milk from its mother. In certain embodiments, the baby is less than 3 years of age (e.g., less than 2 years of age, less than 18 months, less than 1 year, less than 6 months, etc.).

In certain embodiments, the health benefit is improved glycemic control. Thus, certain embodiments of the invention provide a method for improving glycemic control in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved glycemic control in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing improved glycemic control in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved glycemic control in the later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing improved glycemic control in the offspring. In certain embodiments, the improved glycemic control results when the offspring is a baby. Thus, certain embodiments of the invention provide a method for improving glycemic control in a mammalian baby, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved glycemic control in the later born mammalian baby; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby causing improved glycemic control in the mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved glycemic control in the later born mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby causing improved glycemic control in the baby.

In certain embodiments, glycemic control is improved by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% or more (e.g., as compared to a control, such as to the glycemic control of an offspring from a mother not administered an NAD precursor). In certain embodiments, blood glucose levels from the offspring (e.g., baby) are within a normal range.

A used herein, the term “glycemic control” refers to the ability to maintain blood glucose levels within a normal range (i.e., not hypo- or hyper-glycemic). Glucose levels may be

measured using techniques known within the art, for example, using an assay described in the Examples. In certain embodiments, glucose levels may be determined from a blood sample take from the offspring, such as a baby, (e.g., after fasting).

In certain embodiments, the health benefit is increased brain mass. Thus, certain embodiments of the invention provide a method for increasing brain mass in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased brain mass in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing increased brain mass in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased brain mass in the later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing increased brain mass in the offspring. In certain embodiments, the brain mass is increased by at least about 0.25%, 0.5%, 1%, 2%, 3%, 4%, 5% or more (e.g., as compared to a control, such as to the brain mass of offspring from a mother not administered an NAD precursor). In certain embodiments, the offspring's brain has more highly developed white matter. In certain embodiments, the offspring has improved cognitive and/or behavioral functions. In certain embodiments, the offspring is a male. In certain embodiments, the offspring is a female. Brain mass may be measured using techniques known in the art, for example, using brain scans. In certain embodiments, the increased brain mass occurs when the offspring is an adult. In certain embodiments, the increased brain mass occurs when the offspring is a baby. Thus, certain embodiments of the invention provide a method for increasing brain mass in a mammalian baby, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased brain mass in the later born mammalian baby; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the

lactating female mammal, thereby causing increased brain mass in the mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased brain mass in the later born mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby causing increased brain mass in the mammalian baby. In certain embodiments, the brain mass is increased by at least about 0.25%, 0.5%, 1%, 2%, 3%, 4%, 5% or more (e.g., as compared to a control, such as to the brain mass of a baby from a mother not administered an NAD precursor). In certain embodiments, the baby's brain has more highly developed white matter. In certain embodiments, the baby has improved cognitive and/or behavioral functions. In certain embodiments, the baby is a male. In certain embodiments, the baby is a female.

In certain embodiments, the health benefit is increase physical activity. Thus, certain embodiments of the invention provide a method for increasing physical activity in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased physical activity in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing increased physical activity in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased physical activity in the later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing increased physical activity in the offspring. In certain embodiments, physical activity is increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the physical activity of an offspring from a mother not administered an NAD precursor). In certain embodiments, the increase in physical activity is experienced when the offspring is an adult. In certain embodiments, the increase in physical activity is experienced throughout the life of the offspring. In certain embodiments, the

increase in physical activity is experienced when the offspring is a baby. Thus, certain embodiments of the invention provide a method for increasing physical activity in a mammalian baby, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased physical activity in the later born mammalian baby; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby causing increased physical activity in the mammalian baby.

In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing increased physical activity in the later born mammalian baby. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the mammalian baby ingests milk from the lactating female mammal, thereby causing increased physical activity in the mammalian baby. In certain embodiments, physical activity is increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the physical activity of a baby from a mother not administered an NAD precursor).

As used herein, the term "physical activity" refers to bodily movement. For example, physical activity could be assessed by measuring time spent moving versus time spent at rest. Other methods for measuring physical activity are known in the art, for example, using an assay described in the Examples.

In certain embodiments, the health benefit is improved development in the offspring. Thus, certain embodiments of the invention provide a method for improving development in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby improving development in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby improving development in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved development in the

later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing improved development in the offspring (e.g., as compared to the development of offspring from a mother not administered an NAD precursor). In certain embodiments, the improved development is experienced when the offspring is a baby. In certain embodiments, the improved development is experienced when the offspring is an adult. In certain embodiments, the improved development is experienced throughout the life of the offspring. In certain embodiments, the offspring has advanced brain and/or neurocognitive development and/or physical function (e.g., as compared to the development of offspring from a mother not administered an NAD precursor).

As used herein, the term "development" may refer to physical development or function, as well as cognitive development or function. In certain embodiments, the development is physical development (e.g., development of gross and/or fine motor skills). In certain embodiments, the development is cognitive development (e.g., development of information processing, conceptual resources, perceptual skill, and/or language learning). Methods for measuring physical and cognitive development are known in the art, for example, using an assay described in the Examples.

In certain embodiments, the health benefit is improved physical abilities (e.g., coordination) in the offspring. Thus, certain embodiments of the invention provide a method for improving physical abilities in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby improving physical abilities in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby improving physical abilities in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing improved physical abilities in the later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby improving physical abilities in the offspring (e.g., as compared to the physical abilities of offspring from a mother not administered an NAD precursor). In certain embodiments, the

improved physical abilities is experienced when the offspring is a baby. In certain embodiments, the improved physical abilities is experienced when the offspring is an adult. In certain embodiments, the improved physical abilities is experienced throughout the life of the offspring.

As used herein, the term "physical abilities" refers to the ability to perform a physical act. For example, physical abilities include those that influence, e.g., strength, endurance, flexibility, balance and coordination. Thus, in certain embodiments, the offspring may have improved strength, endurance, flexibility, balance and/or coordination. Methods for measuring improved physical abilities are known in the art, for example, as described in the Examples.

In certain embodiments, the health benefit is protection against age-induced weight gain. Thus, certain embodiments of the invention provide a method for protecting against age-induced weight gain in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby protecting against age-induced weight gain in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby protecting against age-induced weight gain in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby protecting against age-induced weight gain in the later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby protecting against age-induced weight gain in the offspring (e.g., as compared to the age-induced weight gain of offspring from a mother not administered an NAD precursor). In certain embodiments, age-induced weight gain is reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the amount of weight gained during a specified time period during adulthood by a female mammal not administered an NAD precursor).

As used herein, the term "age-induced weight gain" refers to weight gained due to changes in the body composition during aging. Thus, age-induced weight gain would be evaluated, e.g., over a specified period of time during the adult stage of life. In certain embodiments, a mammal's weight at the beginning of adulthood could be compared to the

mammal's weight later in life to determine the age-induced weight gain. Methods of evaluating age-induced weight gain are known in the art.

In certain embodiments, the health benefit is reduced anxiety. Thus, certain embodiments of the invention provide a method for reducing anxiety in the offspring of a female mammal, comprising

1) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing reduced anxiety in the later born offspring; and/or

2) administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing reduced anxiety in the offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a pregnant female mammal, thereby causing reduced anxiety in the later born offspring. In certain embodiments, the method comprises administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a lactating female mammal, wherein the offspring ingests milk from the lactating female mammal, thereby causing reduced anxiety in the offspring. In certain embodiments, anxiety is reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the anxiety of offspring from a mother not administered an NAD precursor). In certain embodiments, the reduced anxiety is experienced when the offspring is a baby. In certain embodiments, the reduced anxiety is experienced when the offspring is an adult. In certain embodiments, the reduced anxiety is experienced throughout the life of the offspring.

A used herein, the term "anxiety" is used to refer to various disorders that cause nervousness, fear, apprehension, and/or worrying. Thus, in certain embodiments, the offspring would be less nervous, have reduced fear, less apprehension and/or worry less.

Certain embodiments of the invention provide a method for increasing uncoupling protein 1 (UCP1) expression (e.g., mRNA levels or protein levels) in a cell in a mammal in need thereof, comprising contacting the cell with an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor. In certain embodiments, the cell is contacted by administering the NAD precursor to the mammal (e.g., orally or topically).

Certain embodiments of the invention provide a method of increasing heat loss in a mammal, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to a mammal in need thereof. In certain embodiments, uncoupling protein 1

(UCP1) expression (e.g., mRNA levels or protein levels) is increased (e.g., by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more (e.g., as compared to a control, such as to the expression level in a mammal that was not administered an NAD precursor)).

The term "mammal" refers to any mammalian species such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock (e.g., a cow, sheep, horse, pig, chicken, etc.), and the like. Accordingly, in certain embodiments, the mammal is a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit or livestock. In certain embodiments, the mammal is a human. In certain embodiments, the mammal is pregnant. In certain embodiments, the mammal has given birth within the last year, or within the last 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 month(s). In certain embodiments, the mammal is lactating.

In certain embodiments, the NAD precursor is administered to the female mammal once to three times daily.

In certain embodiments, the NAD precursor is administered to the female mammal prior to conception. In certain embodiments, the NAD precursor is administered to the female mammal starting, e.g., 1 day to 1 year prior to conception. In certain embodiments, the NAD precursor is administered to the female mammal starting, e.g., 1 day, 1 week, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 months prior to conception. In certain embodiments, the NAD precursor is administered to a pregnant female mammal. In certain embodiments, the NAD precursor is administered to a lactating female mammal. In certain embodiments, the NAD precursor is administered to a female mammal post-pregnancy. In certain embodiments, the NAD precursor is administered to a female mammal that has given birth within the last 3 years, 2 years, 1 year, or the last 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 month(s).

In certain embodiments, the NAD precursor is administered orally, transmucosally (e.g., nasally), via inhalation or topically. In certain embodiments, the NAD precursor is administered orally. In certain embodiments, the NAD precursor is administered via an injection. In certain embodiments, the NAD precursor is administered transdermally. In certain embodiments, the NAD precursor is formulated as a pill, a powder, a nasal spray or solution, or as an ointment or cream. In certain embodiments, the NAD precursor is in a lipophilic formulation.

In certain embodiments, the methods of the invention may further comprise the administration of a second biologically active agent. In certain embodiments, the second therapeutic agent is useful for modulating the absorption and/or distribution of the NAD precursor (e.g., improving the NAD precursor bioavailability). The second biologically active

agent may be administered either simultaneously or sequentially with the NAD precursor. In certain embodiments, the second biologically active agent is administered simultaneously with the NAD precursor. In certain embodiments, a pharmaceutical composition comprising the NAD precursor and the second biologically active agent is administered. In certain embodiments, the NAD precursor and the second biologically active agent are administered sequentially. In certain embodiments, the NAD precursor is administered first and the second biologically active agent is administered second. In certain embodiments, the second biologically active agent is administered first and NAD precursor is administered second.

Certain embodiments of the invention provide a NAD precursor for use in medical therapy.

Certain embodiments of the invention provide a NAD precursor for reducing pregnancy associated weight gain and/or to promote post-pregnancy weight loss in a female mammal (e.g., a human).

Certain embodiments of the invention provide the use of a NAD precursor to prepare a medicament for reducing pregnancy associated weight gain and/or to promote post-pregnancy weight loss in a female mammal (e.g., a human) in need thereof.

Certain embodiments of the invention provide a NAD precursor for increasing milk production in a lactating mammal (e.g., a human).

Certain embodiments of the invention provide a NAD precursor for increasing milk quality in a lactating mammal (e.g., a human).

Certain embodiments of the invention provide the use of a NAD precursor to prepare a medicament for increasing milk production in a lactating mammal (e.g., a human) in need thereof.

Certain embodiments of the invention provide a NAD precursor for improving metabolism in a mammal's baby (e.g., a human).

Certain embodiments of the invention provide the use of a NAD precursor to prepare a medicament for improving metabolism in a mammal's baby (e.g., a human) in need thereof.

Certain embodiments of the invention provide a NAD precursor for increasing glycemic control in a mammal's baby (e.g., a human).

Certain embodiments of the invention provide the use of a NAD precursor to prepare a medicament for increasing glycemic control in a mammal's baby.

Certain embodiments of the invention provide a NAD precursor for increasing brain mass in a mammal's baby (e.g., a human).

Certain embodiments of the invention provide the use of a NAD precursor to prepare a medicament for increasing brain mass in a mammal's baby.

Certain embodiments of the invention provide a NAD precursor for increasing physical activity in a mammal's baby (e.g., a human).

Certain embodiments of the invention provide the use of a NAD precursor to prepare a medicament for increasing physical activity in a mammal's baby.

Certain embodiments of the invention provide a composition (e.g., a pharmaceutical composition) for use in a method of the invention, comprising a NAD precursor, and an acceptable carrier (e.g., a pharmaceutically acceptable carrier).

Certain embodiments of the invention provide a composition (e.g., a pharmaceutical composition) for reducing pregnancy associated weight gain, for promoting post-pregnancy weight loss, for increasing milk production, for improving metabolism in the mammal's baby, for increasing glycemic control in the mammal's baby, for increasing brain mass in the mammal's baby and/or for increasing the physical activity of the mammal's baby, comprising a NAD precursor, and an acceptable carrier (e.g., a pharmaceutically acceptable carrier).

Certain embodiments of the invention provide a kit comprising a NAD precursor and instructions for administering the NAD precursor to a female mammal for reducing pregnancy associated weight gain, for promoting post-pregnancy weight loss, for increasing milk production, for improving metabolism in the mammal's baby, for increasing glycemic control in the mammal's baby, for increasing brain mass in the mammal's baby and/or for increasing the physical activity of the mammal's baby.

Certain embodiments of the invention provide a kit comprising 1) a composition (e.g., a pharmaceutical composition) comprising a NAD precursor and a carrier (e.g., a pharmaceutically acceptable carrier), wherein the composition is formulated for oral administration; and 2) instructions for orally administering the NAD precursor to a female mammal for reducing pregnancy associated weight gain, for promoting post-pregnancy weight loss, for increasing milk production, for improving metabolism in the mammal's baby, for increasing glycemic control in the mammal's baby, for increasing brain mass in the mammal's baby and/or for increasing the physical activity of the mammal's baby.

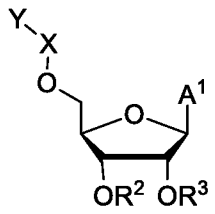
Thus, certain embodiments of the invention provide a NAD precursor, use or kit as described herein for imparting a health benefit to a female mammal in need thereof. In certain embodiments, the NAD precursor, use or kit described herein is for administration to the female mammal pre-pregnancy, during pregnancy and/or post-pregnancy.

Certain embodiments of the invention provide a NAD precursor, use or kit as described herein for imparting a health benefit to the offspring of the female mammal. In certain embodiments, the NAD precursor, use or kit described herein is for administration to the female mammal while pregnant, whereby the health benefit is imparted to the later born offspring. In certain embodiments, the NAD precursor, use or kit described herein is for administration to the female mammal while lactating, wherein the offspring ingests milk from the lactating female mammal, thereby imparting the health benefit to the offspring.

NAD Precursors

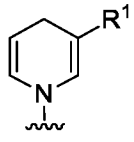
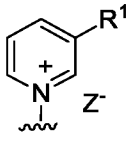
As used herein, the term "NAD precursor" refers to molecules that can be converted/synthesized *in vivo* into NAD. NAD precursors are known in the art and include, for example, NR and derivatives and analogs thereof (e.g., nicotinoyl ribosides), as well as molecules that can be converted/synthesized *in vivo* into NR. NR is a natural product, is currently produced under GMP, has achieved FDA new dietary ingredient status, and is generally regarded as safe (Bieganowski & Brenner, Cell (2004), 117(4), 495-502; Trammell, et al., Journal of Nutrition (2016), 146(5), 957-963). Additionally, certain NAD precursors are discussed in WO 2006/116322, WO 2015014722, WO 2015186114, WO 2015186068, WO 2016014927, WO 2016/149277, WO 2016049236, WO 2015066382, US 9,408,834, and Kulikova et al., Journal of Biological Chemistry (2015), 290(45), 27124-27137, which are incorporated by reference herein.

In certain embodiments, the NAD precursor is a compound of formula (I):



(I)

or a salt thereof (e.g., a pharmaceutically acceptable salt), wherein:

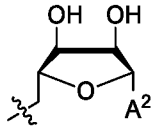
A^1 is  or  ;

R^1 is –COOH, –C(=O)NH_2 or –C(=O)OR^a;

R^2 is H or (C_1-C_3)alkanoyl;

R^3 is H or (C_1-C_3)alkanoyl;

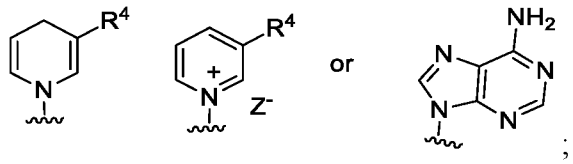
i) X is absent, -P(=O)(OW)(O-), or -P(=O)(OW)-O-P(=O)(OW)(O-); and

Y is W, or  ; or

ii) X is absent; and Y is (C_1-C_{10})alkanoyl;

R^a is (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl, or aryl, wherein each (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl and aryl is optionally substitutes with one or more groups independently selected from the group consisting of halo, hydroxyl, cyano, (C_1-C_3)alkoxy, (C_1-C_3)alkoxycarbonyl, aryl, and (C_1-C_3)alkanoyloxy;

each W is independently selected from the group consisting of H and pharmaceutically acceptable cations;

A^2 is  ;

Z is a pharmaceutically acceptable anion;

R^4 is –COOH, –C(=O)NH_2, or –C(=O)OR^b;

R^b is (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl, or aryl, wherein each (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl and aryl is optionally substitutes with one or more groups independently selected from the group consisting of halo, hydroxyl, cyano, (C_1-C_3)alkoxy, (C_1-C_3)alkoxycarbonyl, aryl, and (C_1-C_3)alkanoyloxy;.

In certain embodiments, R^1 is –COOH or –C(=O)OR^a;

In certain embodiments, R^1 is –COOH.

In certain embodiments, R^1 is–C(=O)NH_2.

In certain embodiments, R^1 is not –C(=O)NH_2.

In certain embodiments, R^1 is–C(=O)OR^a.

In certain embodiments, R^2 is H.

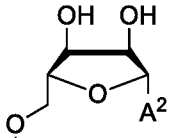
In certain embodiments, R^2 is (C_1-C_3)alkanoyl.

In certain embodiments, R^2 is acyl.

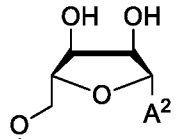
In certain embodiments, R^3 is (C_1-C_3)alkanoyl.

In certain embodiments, R^3 is acyl.

In certain embodiments, X is absent, -P(=O)(OW)(O-), or -P(=O)(OW)-O-P(=O)(OW)(O-); and



Y is W or .



In certain embodiments, Y is .

In certain embodiments, X is absent.

In certain embodiments, X is -P(=O)(OW)(O-), or -P(=O)(OW)-O-P(=O)(OW)(O-).

In certain embodiments, X is absent and Y is (C_1-C_{10})alkanoyl.

In certain embodiments, Y is acyl.

In certain embodiments, X is not absent and Y is not (C_1-C_{10})alkanoyl.

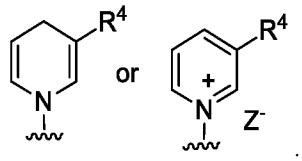
In certain embodiments, R^a is (C_1-C_{10})alkyl.

In certain embodiments, each W is H.

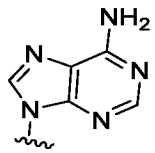
In certain embodiments, each W is independently selected from the group consisting pharmaceutically acceptable cations.

In certain embodiments, each W is independently selected from sodium and potassium.

In certain embodiments, A^2 is

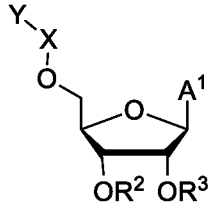


In certain embodiments, A^2 is



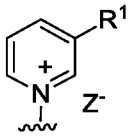
In certain embodiments, Z is chloride.

In one embodiment, the NAD precursor is a compound of formula (I):



(I)

or a salt thereof (e.g., a pharmaceutically acceptable salt), wherein:

A^1 is  ;

R^1 is –COOH, –C(=O)NH_2 or –C(=O)OR^a;

R^2 is (C_1-C_3)alkanoyl;

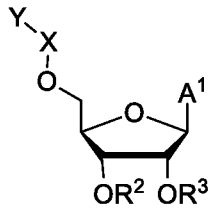
R^3 is (C_1-C_3)alkanoyl;

X is absent; and Y is (C_1-C_{10})alkanoyl;

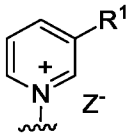
R^a is (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl, or aryl, wherein each (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl and aryl is optionally substitutes with one or more groups independently selected from the group consisting of halo, hydroxyl, cyano, (C_1-C_3)alkoxy, (C_1-C_3)alkoxycarbonyl, aryl, and (C_1-C_3)alkanoyloxy; and

Z is a pharmaceutically acceptable anion.

In one embodiment, the NAD precursor is a compound of formula (I):

 (I)

or a salt thereof (e.g., a pharmaceutically acceptable salt), wherein:

A^1 is  ;

R^1 is –C(=O)OR^a;

R^2 is H or (C_1-C_3)alkanoyl;

R^3 is H or (C_1-C_3)alkanoyl;

X is absent;

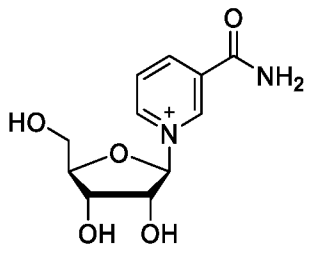
Y is W or (C_1-C_{10})alkanoyl;

R^a is (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl, or aryl, wherein each (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl and aryl is optionally substitutes with one or more groups independently selected from the group consisting of halo, hydroxyl, cyano, (C_1-C_3)alkoxy, (C_1-C_3)alkoxycarbonyl, aryl, and (C_1-C_3)alkanoyloxy;

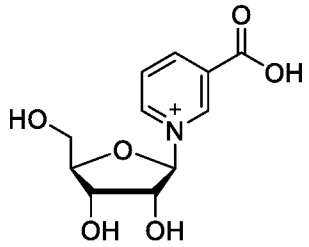
each W is independently selected from the group consisting of H and pharmaceutically acceptable cations; and

Z is a pharmaceutically acceptable anion.

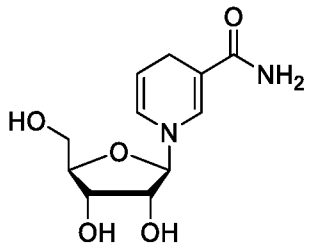
In certain embodiments, the compound of formula (I) is selected from the group consisting of:



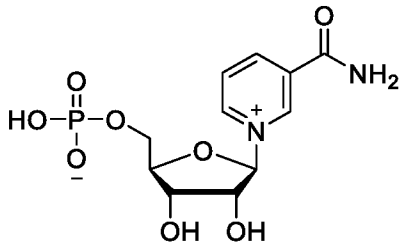
Nicotinamide riboside (NR)



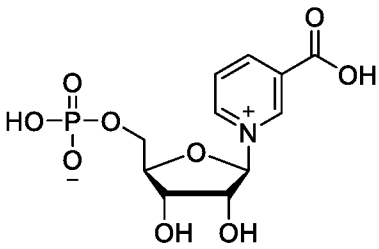
Nicotinic acid riboside (NAR)



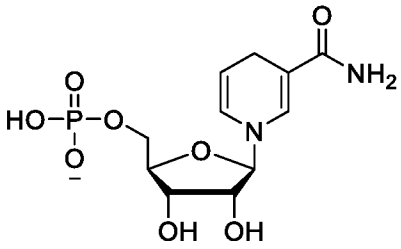
1,4-dihydronicotinamide riboside (NRH)



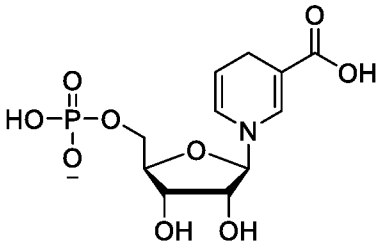
Nicotinamide mononucleotide (NMN)



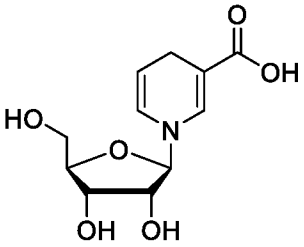
Nicotinic acid mononucleotide (NAMN)



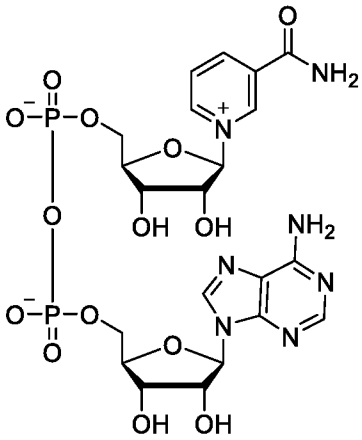
NMNH



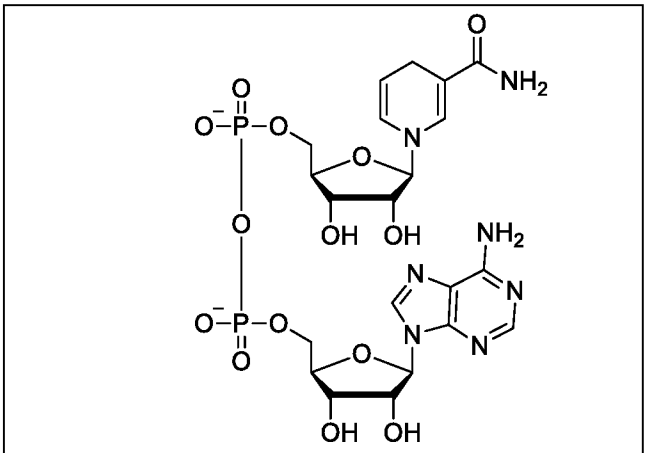
NAMNH



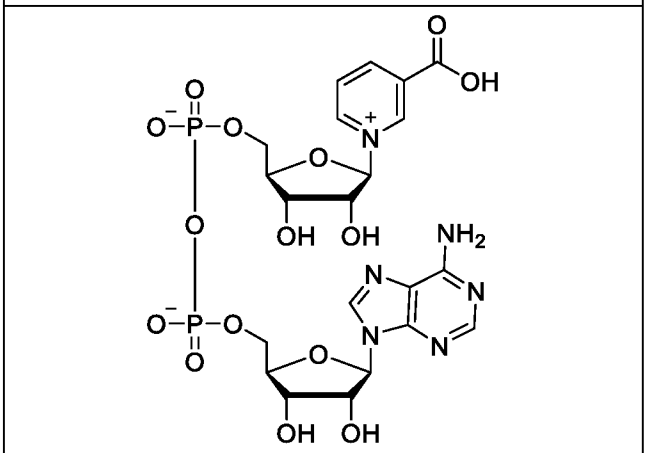
1, 4-dihydronicotinic acid riboside (NARH)



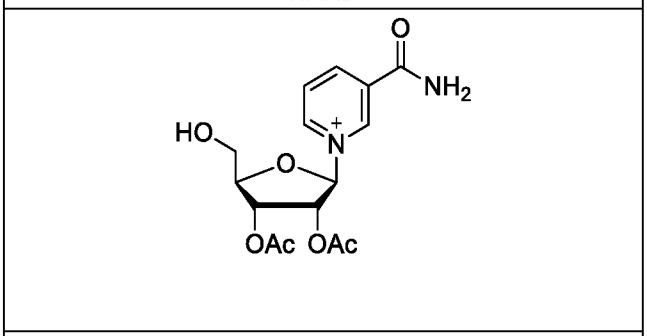
Nicotinamide Adenine Dinucleotide

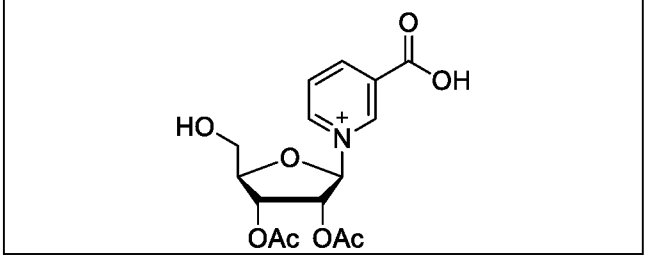


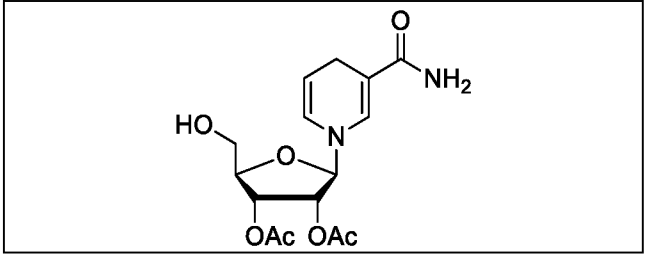
NADH

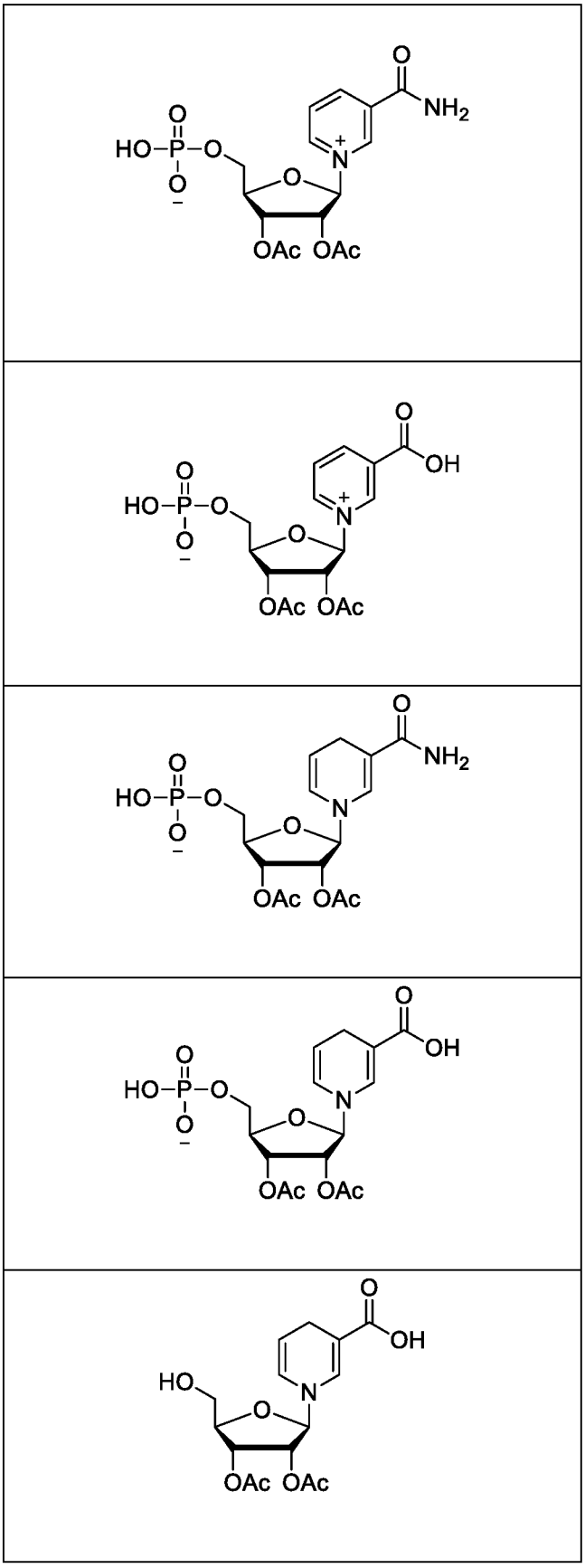


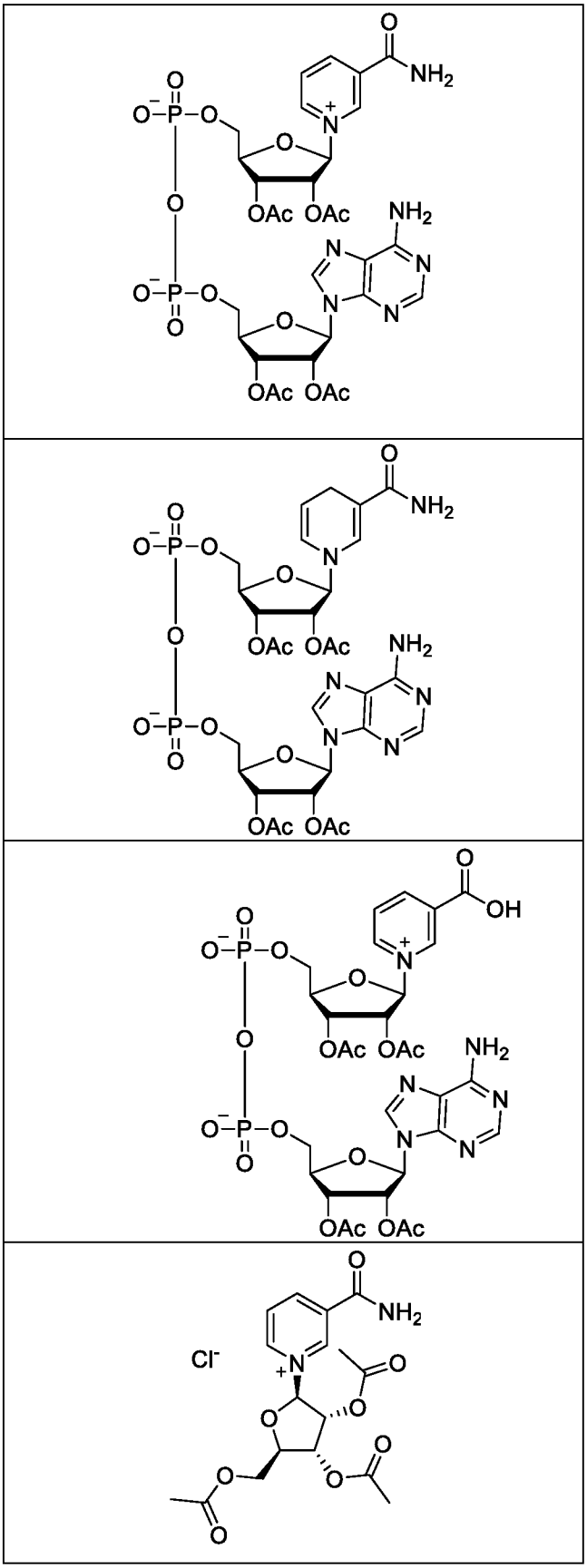
NAAD

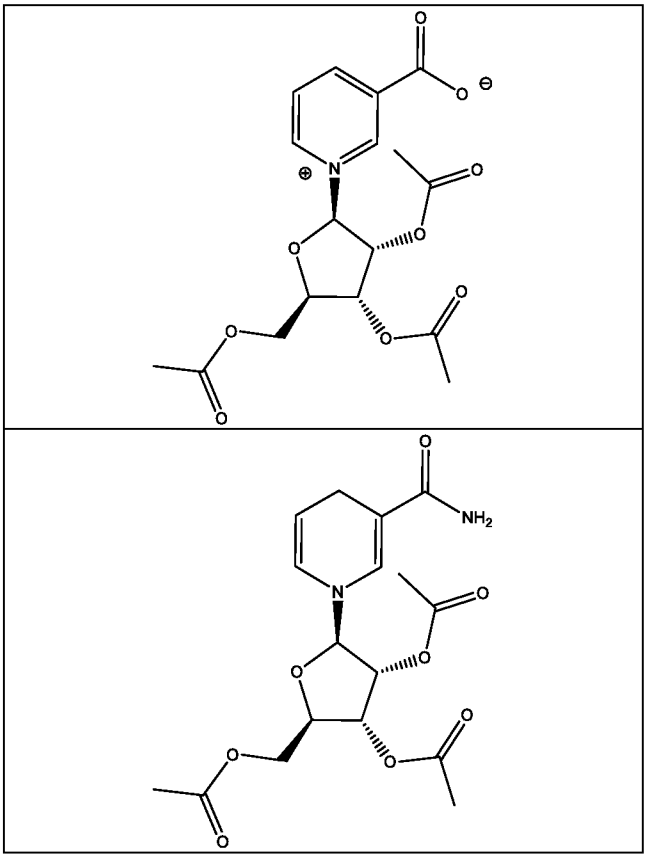






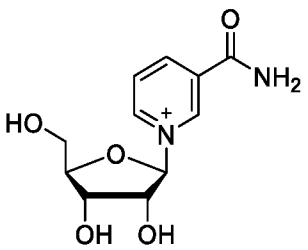






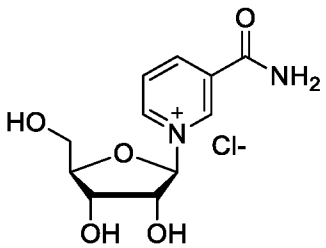
and salts thereof (e.g., pharmaceutically acceptable salts thereof).

In certain embodiments, the NAD precursor is NR:



or a salt thereof (e.g., a pharmaceutically acceptable salt thereof).

In certain embodiments, the NAD precursor is NR chloride:



or a salt thereof (e.g., a pharmaceutically acceptable salt thereof).

In certain embodiments, the NAD precursor is not NAMNH. In certain embodiments, the NAD precursor is not NARH.

In certain embodiments, the NAD precursor is NAMNH. In certain embodiments, the NAD precursor is NARH.

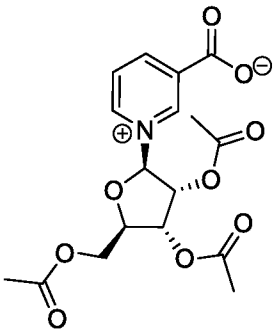
The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, and alkenyl etc. denote both straight and branched groups; but reference to an individual radical such as propyl embraces only the straight chain radical, a branched chain isomer such as isopropyl being specifically referred to. Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic.

Specific values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

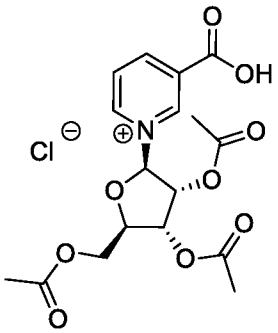
Specifically, (C_1-C_{10})alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, hexyl, heptyl, octyl, nonyl, or decyl; (C_1-C_3)alkoxy can be methoxy, ethoxy, or propoxy; (C_2-C_{10})alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1,-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1- hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl; 1-heptenyl, 1-octenyl, 1-nonenyl, or 1-decenyl; (C_1-C_3)alkoxycarbonyl can be methoxycarbonyl, ethoxycarbonyl, or propoxycarbonyl, (C_1-C_3)alkanoyloxy can be formyloxy, acetoxy, or propanoyloxy; and aryl can be phenyl, indenyl, or naphthyl.

In cases where compounds are sufficiently basic or acidic, a salt of a compound of formula I can be useful as an intermediate for isolating or purifying a compound of formula I. Additionally, administration of a compound of formula I as a pharmaceutically acceptable acid or base salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

It is understood that compounds of formula (I) that include both a positively charged nitrogen (e.g., a pyridinium nitrogen atom or quaternary nitrogen atom) and a carboxylic acid group may exist as a single molecule that possesses both a positive charge and a negative charge (e.g. a zwitterion). Accordingly, as used herein, the term "pharmaceutically acceptable salt" includes such zwitterions. For example, the compound **100** below represents a pharmaceutically acceptable salt of the compound **101**.



100



101

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

Pharmaceutically acceptable cations are well known in the art and include, sodium, potassium, magnesium and calcium.

Pharmaceutically acceptable anions are well known in the art and include, chloride, bromide, iodide, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate.

Administration

A compound described herein for use in the invention can be formulated as a composition (e.g., a pharmaceutical composition) and administered to a mammalian host, such as a woman intending to become pregnant, a pregnant woman or a lactating mother, in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical (e.g., transdermal, transmucosal), inhalation or subcutaneous routes.

Thus, the present compounds may be systemically administered, e.g., orally (e.g., added to drinking water), in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the compound. The percentage of the compositions and preparations

may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the compound may be incorporated into sustained-release preparations and devices.

The compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for

example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

The compounds may be conveniently formulated in unit dosage form. In one embodiment, the invention provides a composition comprising a compound formulated in such a unit dosage form.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

A NAD precursor can also be administered in combination with other therapeutic or biologically active agents, for example, other agents that are useful for modulating the absorption and/or distribution of the NAD precursor (e.g., improving the NAD precursor bioavailability, such as oral, nasal or topical bioavailability). Accordingly, in one embodiment the invention also provides a composition comprising an NAD precursor, at least one other therapeutic or biologically active agent, and a pharmaceutically acceptable diluent or carrier. The invention also provides a kit comprising a NAD precursor, at least one other therapeutic or biologically active agent, packaging material, and instructions for administering the NAD precursor and the other therapeutic/biologically active agent or agents to a female mammal to improve maternal, neonatal and/or child health.

The invention will now be illustrated by the following non-limiting Examples.

EXAMPLE 1

Mice are a widely used model of human metabolism because they are capable of recapitulating many aspects of human health. Specifically, female mice gain substantial body fat in the course of pregnancy and, despite best animal husbandry practices, the fasting glucose levels of weaned pups can be slightly to significantly hypoglycemic. Therefore, mice were used in a series of *in vivo* experiments to evaluate whether maternally supplemented NR has the ability to improve maternal and/or neonatal health and development. Specifically, it was asked whether addition of NR chloride (3 g/kg of chow) to the diet of female mice would alter their

health during and after pregnancy and whether this administration would produce any beneficial effects on the pups.

Methods

Female C57BL/6N mice were raised in 12 hour light:dark cycles on Teklad 2920X chow (NC). For 12 weeks prior to mating with a single C57BL/6N male, females were on either NC (n=4) or NC + NR (n=4). Females were maintained on the same diet through gestation and weaning, which was at 21 days after birth.

Results

As described herein, the provision of nicotinamide riboside (NR) in the diet of female mice resulted in 1) a near total elimination of weight gain over the gestational and post-gestational interval; 2) a significant increase in the amount of milk produced by lactating females; 3) leaner body mass of neonates at the time of weaning; 4) larger brain mass of neonates at the time of weaning; 5) greater physical activity of fasted neonates; and 6) correction of neonatal glycemic control from a slightly low level to an ideal level.

Specifically, as shown in Figure 1, females on NC gained nearly 15% of their body weight from the day of mating to the day of weaning, whereas females supplemented with NR were protected from this weight gain. Further, it was determined that at the time of weaning, NR-supplemented mothers tended to have lower body fat.

Figure 2 demonstrates that females supplemented with NR had a 2-3x increase in milk production. To determine the production of milk, we separated mothers were separated from pups for several hours and provided an injection of oxytocin on days 0, 7, 14 and 21 after partum. Milk was carefully collected by pipetting and the collected volume per mother was plotted. The galactogogue activity of NR was striking and entirely unexpected.

Figure 3 shows that mice raised by NR-supplemented mothers are leaner and tend toward larger brains at weaning. The improved body composition of mice from NR-supplemented mothers was entirely unexpected.

Figure 4 shows that mice raised by NR-supplemented mothers are better at maintaining glucose after an overnight fast. The mild hypoglycemia of mice on standard chow was not expected and the improvement in glycemic control by virtue of supplementing mothers was unexpected.

EXAMPLE 2

A series of *in vivo* murine experiments were performed to evaluate whether maternally supplemented NR has the ability to improve maternal and neonatal health and development. Note that in these and subsequent experiments and as shown in Figure 5, NR was only provided to mouse mothers after they had given birth. As shown in Figures 6A-D, mothers supplemented with NR ate as least as much as non-supplemented mothers but lost significant weight, specifically in fat mass, while they maintained their lean mass. These data confirm that NR supplementation increases post-gestational weight loss and that NR supplementation can begin post-partum in order to have this effect.

EXAMPLE 3

Nicotinamide riboside increases the quantity and quality of milk by stimulating lactation and stimulating the production of brain derived neurotrophic factor (BDNF).

A series of *in vivo* murine experiments were performed to evaluate the effects of nicotinamide riboside on milk production. As shown in Figure 7, the large increase in milk production was reproduced by virtue of supplementing post-gestational mothers. As shown in Figures 8A-C, the concentration of protein, carbohydrate and fat in the milk of NR-supplemented mothers was normal. Because there is approximately 2-fold more milk produced by NR-supplemented mothers, NR-supplemented mothers transmit more calories to their offspring, which thereby assists maternal weight loss and neonatal development. In Figure 9A, the concentration of NAD precursor vitamins in the milk from control mothers and NR-supplemented mothers was determined. It was discovered that the milk from NR-supplemented mothers has moderately higher nicotinamide (Nam) and NR by concentration than the milk from non-supplemented mothers. Coupled with the larger milk volume, NR-supplemented mothers transmit significantly more NAD precursor vitamins (total B3) than the non-supplemented mothers. As shown in Figure 9B, it was investigated whether oral NR is directly transmitted to offspring in milk or whether it has maternal targets. Oral NR was labeled with heavy atoms in the nicotinamide and ribose moieties of NR and this was provided to lactating mothers by gavage. The mothers released only single labeled nicotinamide and no double-labeled NR into their milk. These data indicate that the targets of maternal NR are in stimulation of her metabolic processes and are not simply confined to passing through oral NR to milk NR.

Based on the advanced brain and neurobehavioral development that was observed, it was investigated whether NR-supplemented mothers produced more BDNF in their milk.

Surprisingly, as shown in Figure 10, there was a 2-fold higher concentration of BDNF in the milk of NR-supplemented mothers than in non-supplemented mothers. Given that NR-supplemented mothers also produced more milk, NR-supplementation results in a large increase of maternally produced BDNF to aid neonatal development.

5

EXAMPLE 4

Lactation and maternal nicotinamide riboside mobilize NAD precursors from the liver to the mammary gland.

As shown in Figure 11A, the RNA expression of NAD biosynthetic genes in the liver of
10 female virgin mice versus the livers of lactating mice without or with NR supplementation was examined. Lactation greatly increased expression of NAD biosynthetic genes, especially NAMPT, NRK1, NMNAT1 and NADK. Lactation also increased expression of CD38, suggesting that the lactating liver is making more NAD from multiple precursors and then breaking it down for circulation to other tissues. NR supplementation did not further increase
15 hepatic NAD biosynthetic genes. As shown in Figure 11B, the lactating liver has a higher level of NAD than the nonlactating liver but it does not appear to hold on to a higher level of NR (Figure 11C) or Nam (Figure 11D), suggesting that lactation may lead to circulation of NAD precursors to the mammary.

As shown in Figure 12A, the lactating mammary in a mother supplemented with NR has
20 a very high level of expression of NAD biosynthetic genes. As shown in Figures 12B-12C, maternal NAD and NMN are elevated in the mammary of lactating mammary with respect to nonlactating mammary tissue. As shown in Figure 12D, maternal NR given to a lactating female mouse produces a large increase in mammary NR above the already elevated level of NR in the lactating female.

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EXAMPLE 5

Post-partum nicotinamide riboside boosts mammary biosynthetic programs.

As shown in Figure 13A, it was discovered that NR-supplemented mothers circulate very high levels of prolactin. Consistent with high level prolactin circulation and high level milk
30 production, as shown in Figure 13B, there is a higher level of phosph-mTOR in mammary of NR-supplemented mothers to drive higher protein synthesis. As shown in Figure 13C and 13D, mammary lipid biosynthetic genes and lactose biosynthetic genes are strongly induced by provision of NR in the diet. In addition, the size of the mammary tissue itself is greater in NR-

supplemented mothers than in non-supplemented mothers (Figure 14A). As shown in Figure 14B, the mammary tissue from NC and NR-supplemented mothers was probed and it was discovered that NR-supplementation produces an increase in expression of UCP1, indicating that NR supplemented mothers may have both an advantage in weight loss from increased transmission of calories from milk as well as increased thermogenesis.

EXAMPLE 6

Post-partum nicotinamide riboside increases neonatal health and development.

As shown in Figure 15, particularly at day 7 and 9, the mother's NR supplementation boosts neonatal size. As shown in Figure 16A-C, 21 day old weanlings of NR-supplemented mothers are larger in overall mass, fat mass and lean mass. As shown in Figure 16D, females tend to have larger brain weight.

Earlier it was shown that supplementation of females during their pregnancy and during the lactation period with NR produced pups with better glycemic control. Here again, it was found that 15 day old males offspring of non-supplemented mothers could not maintain their blood glucose with a 16 hour fast. Furthermore, it was discovered that males and females whose mothers were NR-supplemented had higher liver expression of the gluconeogenic bypass gene, PEPCK (Figure 17B), and stored more glycogen in their livers (Figure 17C). Particularly as NR is not directly transmitted to offspring of NR-supplemented mothers, none of these effects of maternally supplemented NR could have been anticipated.

EXAMPLE 7

Post-partum nicotinamide riboside produces profound, lasting effects on the activity, anti-anxiety, adventurousness and physical abilities of offspring.

At 15 days of age, it was observed that the offspring of NR-supplemented mothers were more physically active. In this experiment, the physical activity of male and female offspring of mothers supplemented or not with NR at 15 days of age was quantified. Particularly in the first 5 minutes in which they were introduced into the open field test, the offspring of NR-supplemented mothers were much more physically active than those of control 15 day old mice (Figure 18A). The males were also willing to spend a significant amount of time in the center of the open field test in the middle 5 minute epoch (Figure 18B), suggesting that they were not anxious.

At 21 days, the offspring were removed from their mothers and put on normal chow

(NR). While offspring of NR could have been put on NR chow, we aimed to determine if the 21 day intervention to their mothers was sufficient for a lasting effect on their neurocognitive and/or physical development. As shown in Figures 19A-D, 49 days after their mother's intervention, 70 day-old adults of NR-supplemented mothers cover more distance and move faster in an open field test with a tendency toward less immobile time. Males spend less time in the center.

It was considered interesting that males and female adults of NR-supplemented mothers maintain distinct physical advantages into adulthood on the basis of the mother's micronutrition. At 75 days of age—54 days after the mothers either had NR or not—the offspring of NR-supplemented mothers were more willing to spend time in the light chamber of a dark/light chamber (Figure 20). This could be interpreted either as a simple increase in activity and/or a less anxious state of being.

Just as the male adults of NR-supplemented mothers had a lasting and statistically significant advantage over genetically identical mice whose mothers were not supplemented, the female mice of NR-supplemented mothers showed other statistically significant demonstrable benefits.

At 64 days past the mother's intervention, the willingness of 85 day old adult offspring of mice from this experiment (NC or NR-supplemented) to put their heads out in an elevated plus maze was tested. In a clear indication of anti-anxiety, as shown in Figures 21A-B, the offspring of NR-supplemented mothers had a higher willingness to do this and the propensity to do this of the males of NR-supplemented mothers easily reached statistical significance. The clear indication is that maternal NR produces improvements in maternal behavior and/or qualitative and/or quantitative improvements in lactation sufficient to improve the development and health of offspring. The ability of the mother's 21 day micronutrition to produce beneficial effects on her offspring into adulthood was not at all anticipated.

The improved physical functions of adult offspring of NR-supplemented mothers were not confined to advantages in making less anxious choices about where to move in space, light and dark. At 85 days old, when mice were again 64 days past their mother's intervention—again all mice were on NC show for 64 days—their untrained ability to cross a balance beam was tested. As shown in Figures 22A-C, the male and female offspring of NR-supplemented mothers cross faster, with fewer slips and with virtually no use of their tails to grip the balance beam. This was not anticipated.

At 90 days old, body composition analysis was performed on the mice. As shown in

Figurse 23A-C, the male offspring of NR-supplemented mothers were slightly smaller. As shown in Figure 23B, males and females of NR-supplemented mothers have less adipose tissue 69 days after their mother's intervention. There is no effect on the total amount of lean mass (23C). Particularly because the maternal intervention produced somewhat bigger weanlings with more fat and lean mass, the protection against age-induced weight gain was unanticipated.

At 91 days, the mice were sacrificed and examined with respect to their brains. In Figure 24A, it was shown that female mice from NR-supplemented mothers have larger brains in adulthood. We hypothesized that the caudate putamen, which is the motor learning center of the brain, may have developed more rapidly in mice whose mothers had been NR-supplemented either due to the increased quality and/or quantity of milk they received and/or improved maternal care. The caudate putamen was slightly smaller for weanlings of NR-supplemented mothers at 21 days (Figure 24B) and showed clearly advanced pruning in females as evidenced by a smaller and more focused area of PSD95 staining (Figure 24C). As shown in Figure 24D, the caudate putamen in adults was not affected, indicating that the effect of NR-supplementation is to advance neonatal pruning and not to stunt brain growth.

Certain unanticipated activities of maternal NR supplementation on maternal and neonatal health are described below.

Maternal weight loss

Here, it is shown that NR has the unexpected property of a galactogogue, i.e., it stimulates lactation. As described herein, it was shown that NR boosts mammary biosynthetic programs and further that NR allows lactating females to produce larger mammary glands with a greater degree of UCP1 expression. As shown in Figures 1 and 6, mouse mothers on NR had a significant advantage in post-partum weight management with preservation of their lean mass. The observations and mechanisms of post-partum weight management were unexpected and possess significant commercial and translational potential.

Neonatal metabolic and whole body development of offspring from NR-supplemented mothers

Because of the remarkable ability of NR to promote maternal lactation, the ability of offspring of NR-supplemented mothers to maintain fasting euglycemia was examined. The data indicated that the mother's NR supplementation protects mice from low blood sugar after an overnight fast. Specifically, it was discovered that the offspring of NR-supplemented offspring grow somewhat faster, have better hepatic gluconeogenic gene expression, store more glucose, and can be somewhat larger at weaning. Because normal mouse chow is not deficient in any micronutrients, none of these effects could have been foreseen.

Neurocognitive and physical development of offspring from NR-supplemented mothers

At 15 days, mice are able to open their eyes. By 30 days, mice are generally considered adults. It was consistently found that the offspring of NR-supplemented mice were advanced, adventurous, non-anxious and physically superior specimens from 15 days all the way to adulthood at 3 months of age. None of these observations could have been anticipated based on known activities of NR. Moreover, the effects are not due to NR itself but rather the effect of NR on maternal milk production and/or behavior. It was shown that the mother's oral NR is not directly transmitted to her offspring. She does produce more milk than non-supplemented mothers and may also transmit more time or more quality maternal caretaking time on the basis of her NR supplementation. Strikingly, NR caused lactating mice to produce more BDNF. The increased volume of milk coupled with increased concentration of BDNF and potentially other neuroactive substances in the milk has the potential to promote advanced pruning in the caudate putamen, giving rise to greater physical prowess. We further propose that the quality of milk in NR-supplemented mothers promotes advanced brain development, anti-anxiety and increased physical performance that lasts into adulthood.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A method for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal, comprising administering an effective amount of a nicotinamide adenine dinucleotide (NAD) precursor to the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

2. The method of claim 1, wherein the health benefit is imparted to the female mammal.

3. The method of claim 2, wherein the NAD precursor is administered to the female mammal pre-pregnancy, during pregnancy and/or post-pregnancy.

4. The method of any one of claims 1-3, wherein the health benefit is a reduction in pregnancy associated weight gain.

5. The method of claim 4, wherein pregnancy associated weight gain is reduced by at least about 10%.

6. The method of claim 4, wherein pregnancy associated weight gain is reduced by at least about 30%.

7. The method any one of claims 1-3, wherein the health benefit is a promotion in post-pregnancy weight loss.

8. The method of claim 7, wherein weight loss is increased by at least about 10%.

9. The method of claim 7 or 8, wherein the female mammal has given birth within the last year.

10. The method of any one of claims 7-9, wherein milk production and/or mammary gland development is increased in the female mammal.

11. The method of any one of claims 1-3, wherein the health benefit is increased milk production.

12. The method of claim 11, wherein milk production is increased by at least about 10%.

13. The method of claim 11 or 12, wherein the quality of the milk is improved.

14. The method of claim 13, wherein the milk comprises increased levels of brain-derived neurotrophic factor (BDNF).

15. The method of claim 1, wherein the health benefit is imparted to the offspring of the female mammal.

16. The method of claim 15, wherein the NAD precursor is administered to the female mammal while pregnant, thereby imparting the health benefit to the later born offspring.

17. The method of claim 15 or 16, wherein the NAD precursor is administered to the female mammal while lactating, wherein the offspring ingests milk from the lactating female mammal, thereby imparting the health benefit to the offspring.

18. The method any one of claims 15-17, wherein the health benefit results when the offspring is a baby.

19. The method of any one of claims 15-17, wherein the health benefit results when the offspring is an adult.

20. The method of any one of claims 15-19, wherein the health benefit is improved metabolism.

21. The method of any one of claims 15-19, wherein the health benefit is improved glycemic control.

22. The method of claim 21, wherein glycemic control is improved by at least about 5%.

23. The method of claim 21, wherein glycemic control is improved by at least about 10%.

24. The method of any one of claims 15-19, wherein the health benefit is increased brain mass.

25. The method of claim 24, wherein the brain mass is increased by at least about 5%.

26. The method of claim 24 or 25, wherein the offspring has improved cognitive and/or behavioral functions.

27. The method of any one of claims 15-19, wherein the health benefit is increased physical activity.

28. The method of claim 27, wherein physical activity is increased by at least about 10%.

29. The method of any one of claims 15-19, wherein the health benefit is improved development.

30. The method of claim 29, wherein the improved development is improved physical development.

31. The method of claim 29, wherein the improved development is improved cognitive development.

32. The method of any one of claims 15-19, wherein the health benefit is improved physical abilities (e.g., coordination).

33. The method of any one of claims 15-19, wherein the health benefit is protection against age-induced weight gain.

34. The method of any one of claims 15-19, wherein the health benefit is reduced anxiety.

35. The method of any one of claims 1-34, wherein the female mammal is a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit or livestock.

36. The method of claim 35, wherein the female mammal is a human.

37. The method of claim 35 or 36, wherein the offspring is female.

38. The method of claim 35 or 36, wherein the offspring is male.

39. The method of any one of claims 1-38, wherein the NAD precursor is administered orally, transmucosally (e.g., nasally), via inhalation, via an injection or topically (e.g., transdermally).

40. The method of claim 39, wherein the NAD precursor is administered orally.

41. The method of any one of claims 1-40, further comprising administering a second biologically active agent.

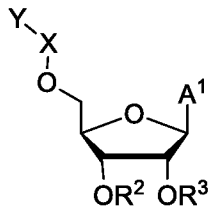
42. The method of claim 41, wherein the second biologically active agent is useful for modulating the absorption and/or distribution of the NAD precursor.

43. The method of claim 41 or 42, wherein the second biologically active agent and the NAD precursor are administered simultaneously.

44. The method of claim 41 or 42, wherein the second biologically active agent and the NAD precursor are administered sequentially.

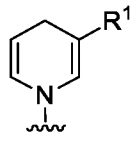
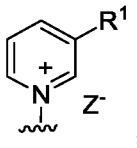
45. The method of any one of claims 1-44, wherein the NAD precursor is a compound that can be converted *in vivo* into NAD.

46. The method of any one of claims 1-44, wherein the NAD precursor is a compound of formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein:

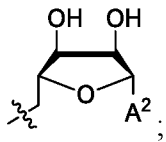
A^1 is  or  ;

R^1 is –COOH, –C(=O)NH_2 or –C(=O)OR^a;

R^2 is H or (C_1-C_3)alkanoyl;

R^3 is H or (C_1-C_3)alkanoyl;

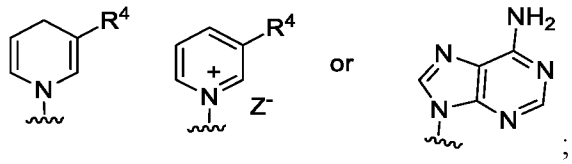
i) X is absent, -P(=O)(OW)(O-), or -P(=O)(OW)-O-P(=O)(OW)(O-); and

Y is W, or  ; or

ii) X is absent; and Y is (C_1-C_{10})alkanoyl;

R^a is (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl, or aryl, wherein each (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl and aryl is optionally substitutes with one or more groups independently selected from the group consisting of halo, hydroxyl, cyano, (C_1-C_3)alkoxy, (C_1-C_3)alkoxycarbonyl, aryl, and (C_1-C_3)alkanoyloxy;

each W is independently selected from the group consisting of H and pharmaceutically acceptable cations;

A^2 is  ;

Z is a pharmaceutically acceptable anion;

R^4 is –COOH, $–C(=O)NH_2$ or $–C(=O)OR^b$; and

R^b is $(C_1\text{-}C_{10})$alkyl, $(C_2\text{-}C_{10})$alkenyl, or aryl, wherein each $(C_1\text{-}C_{10})$alkyl, $(C_2\text{-}C_{10})$alkenyl and aryl is optionally substitutes with one or more groups independently selected from the group consisting of halo, hydroxyl, cyano, $(C_1\text{-}C_3)$alkoxy, $(C_1\text{-}C_3)$alkoxycarbonyl, aryl, and $(C_1\text{-}C_3)$alkanoyloxy.

47. The method of claim 46, wherein R^1 is –COOH.

48. The method of claim 46, wherein R^1 is–$C(=O)NH_2$.

49. The method of claim 46, wherein R^1 is–$C(=O)OR^a$.

50. The method of any one of claims 46-49, wherein R^2 is H.

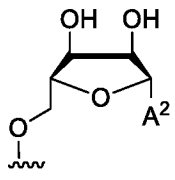
51. The method of any one of claims 46-49, wherein R^2 is $(C_1\text{-}C_3)$alkanoyl.

52. The method of any one of claims 46-49, wherein R^2 is acyl.

53. The method of any one of claims 46-52, wherein R^3 is $(C_1\text{-}C_3)$alkanoyl.

54. The method of any one of claims 46-52, wherein R^3 is acyl.

55. The method of any one of claims 46-54, wherein X is absent, -P(=O)(OW)(O-), or -P(=O)(OW)-O-P(=O)(OW)(O-); and

Y is W or  .

56. The method of claim 55, wherein X is absent.

57. The method of claim 55, wherein X is -P(=O)(OW)(O-), or -P(=O)(OW)-O-P(=O)(OW)(O-).

58. The method of any one of claims 46-54, wherein X is absent and Y is (C_1-C_{10})alkanoyl.

59. The method of claim 58, wherein Y is acyl.

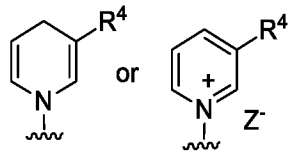
60. The method of any one of claims 46-59, wherein R^a is (C_1-C_{10})alkyl.

61. The method of any one of claims 46-55, wherein each W is H.

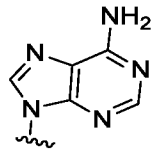
62. The method of any one of claims 46-55, wherein each W is independently selected from the group consisting pharmaceutically acceptable cations.

63. The method of any one of claims 46-55, wherein each W is independently selected from sodium and potassium.

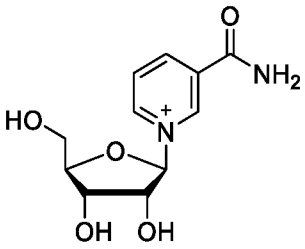
64. The method of claim 55, wherein A^2 is



65. The method of claim 55, wherein A^2 is



66. The method of claim 46, wherein the compound of formula (I) is selected from the group consisting of: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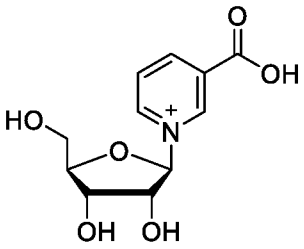
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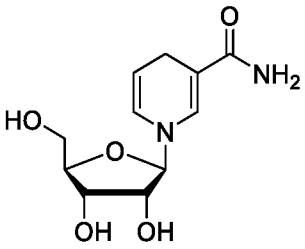
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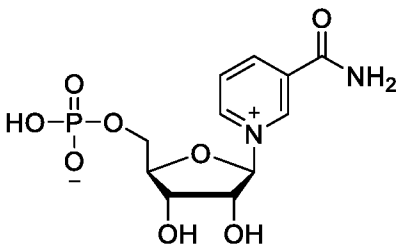
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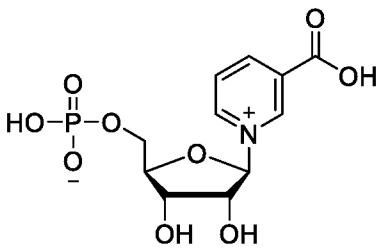
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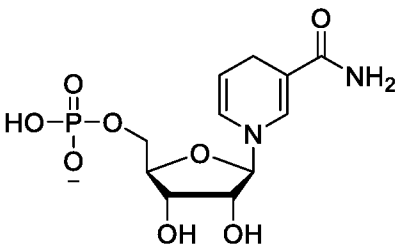
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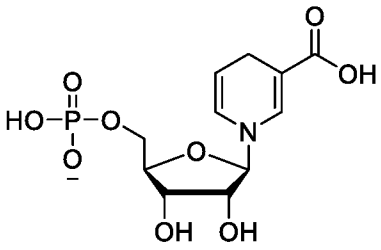
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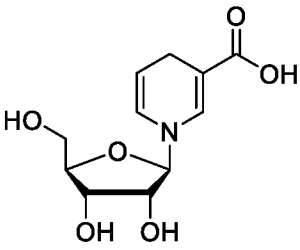
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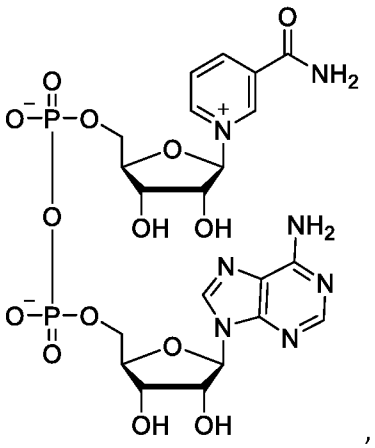
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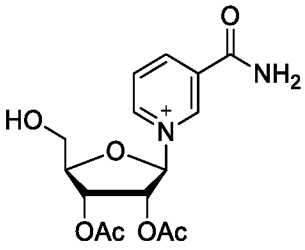
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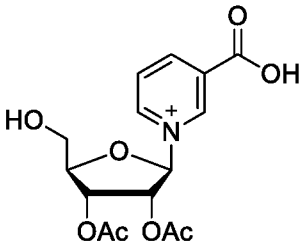
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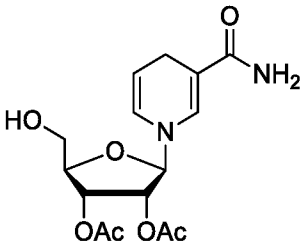
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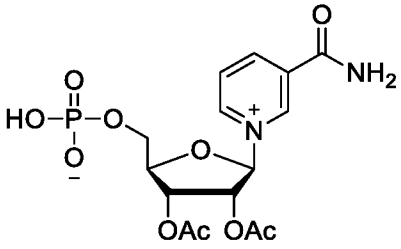
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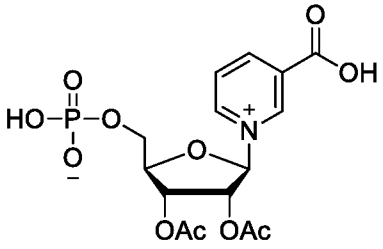
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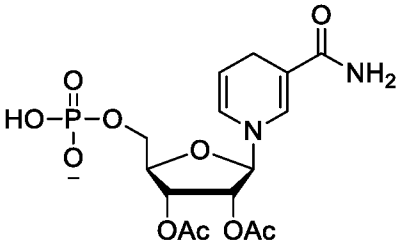
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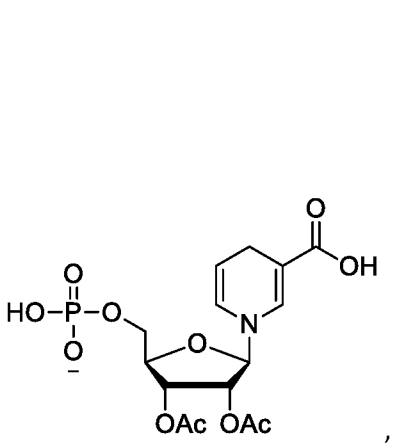
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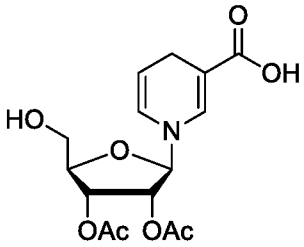
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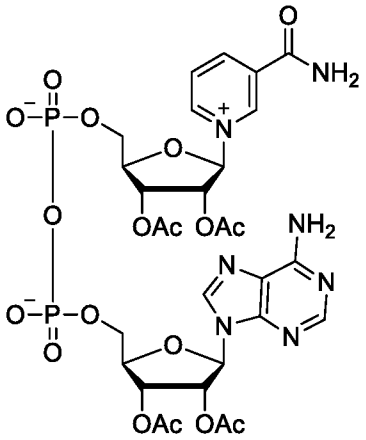
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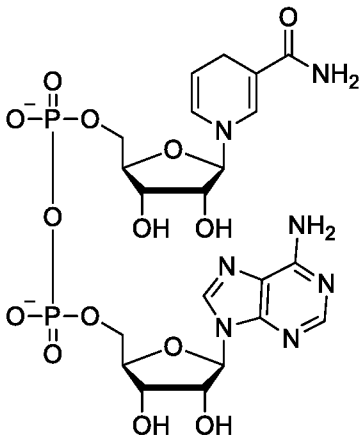
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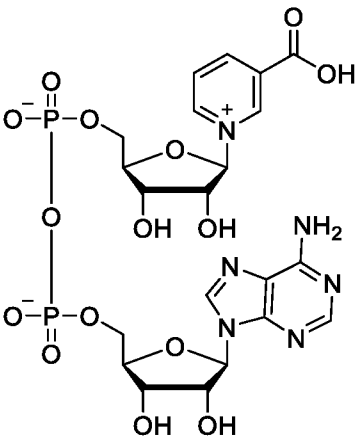
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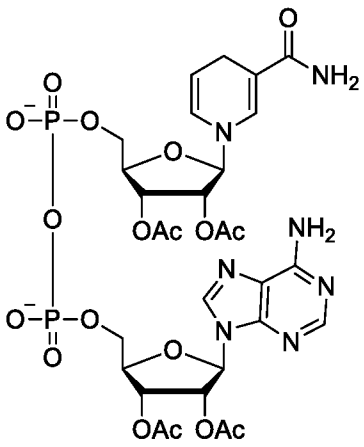
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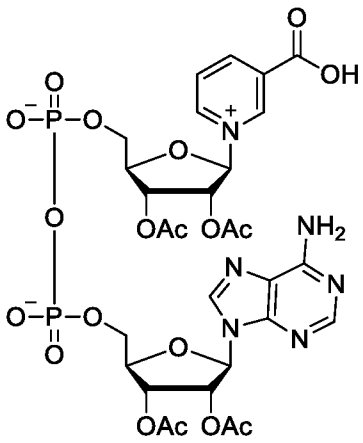
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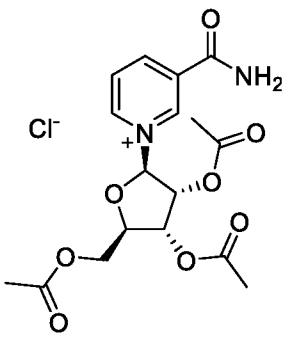
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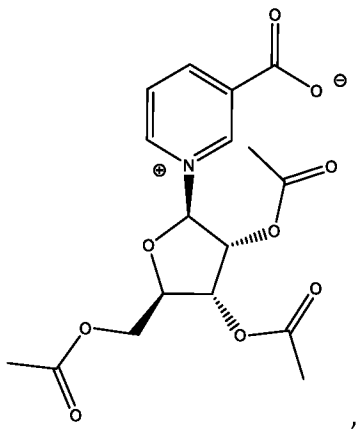
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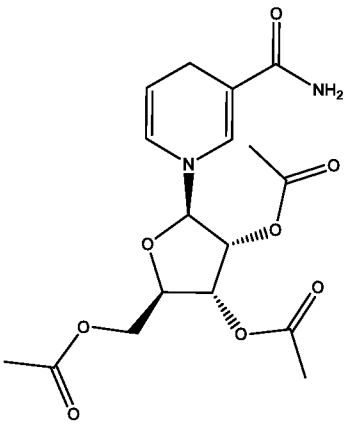
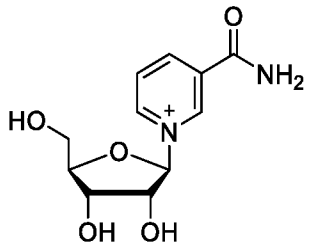

and

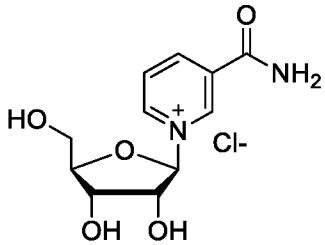
pharmaceutically acceptable salts thereof.

67. The method of claim 66, wherein the compound of formula (I) is:



or a pharmaceutically acceptable salt thereof.

68. The method of claim 46, wherein the compound of formula (I) is:



or a pharmaceutically acceptable salt thereof.

69. A NAD precursor for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

70. The use of a NAD precursor to prepare a medicament useful for imparting a health benefit to a female mammal in need thereof and/or to the offspring of the female mammal;

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

71. A composition (e.g., a pharmaceutical composition) comprising a NAD precursor and an acceptable carrier (e.g., a pharmaceutically acceptable carrier) for use in imparting a health benefit to a female mammal in need thereof and/or the offspring of the female mammal,

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

72. A kit comprising a NAD precursor and instructions for administering the NAD precursor to a female mammal in need thereof for imparting a health benefit to the female mammal and/or the offspring of the female mammal,

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development, improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

73. A kit comprising 1) a composition (e.g., a pharmaceutical composition) comprising a NAD precursor and a carrier, wherein the composition is formulated for oral administration; and 2) instructions for orally administering the NAD precursor to a female mammal for imparting a health benefit to the female mammal and/or the offspring of the female mammal,

wherein the health benefit imparted to the female mammal is a reduction in pregnancy associated weight gain, promotion of post-pregnancy weight loss and/or increased milk production when the female mammal is lactating; and

wherein the health benefit imparted to the offspring is improved metabolism, improved glycemic control, increased brain mass, increased physical activity, improved development,

improved physical abilities, protection against age-induced weight gain and/or reduced anxiety.

74. The NAD precursor, use or kit of any one of claims 69-73, for imparting a health benefit to a female mammal in need thereof.

75. The NAD precursor, use or kit of claim 74, for administration to the female mammal pre-pregnancy, during pregnancy and/or post-pregnancy.

76. The NAD precursor, use or kit of any one of claims 69-73, for imparting a health benefit to the offspring of the female mammal.

77. The NAD precursor, use or kit of claim 76, for administration to the female mammal while pregnant, whereby the health benefit is imparted to the later born offspring.

78. The NAD precursor, use or kit of claim 76, for administration to the female mammal while lactating, wherein the offspring ingests milk from the lactating female mammal, thereby imparting the health benefit to the offspring.

FIGURE 1

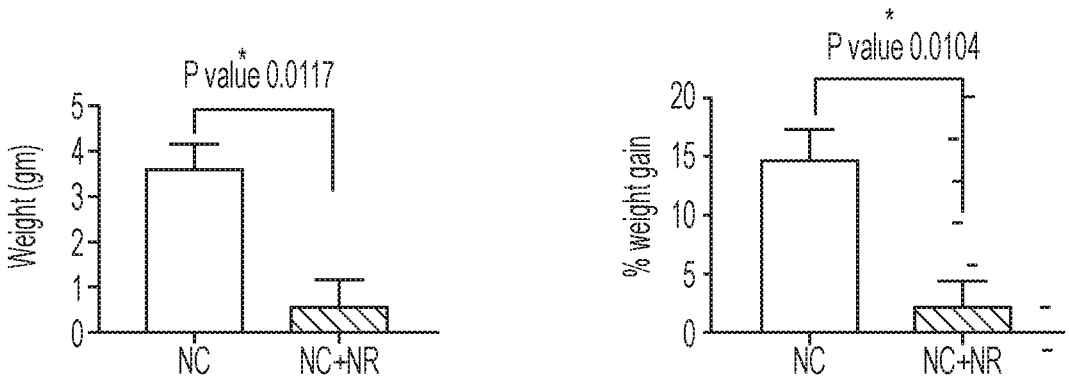


FIGURE 2

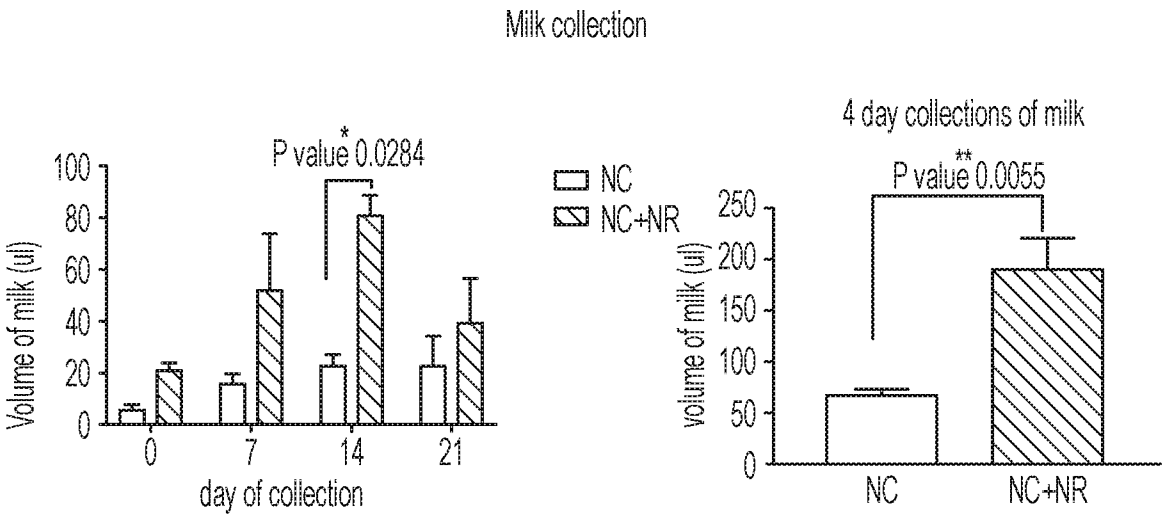


FIGURE 3

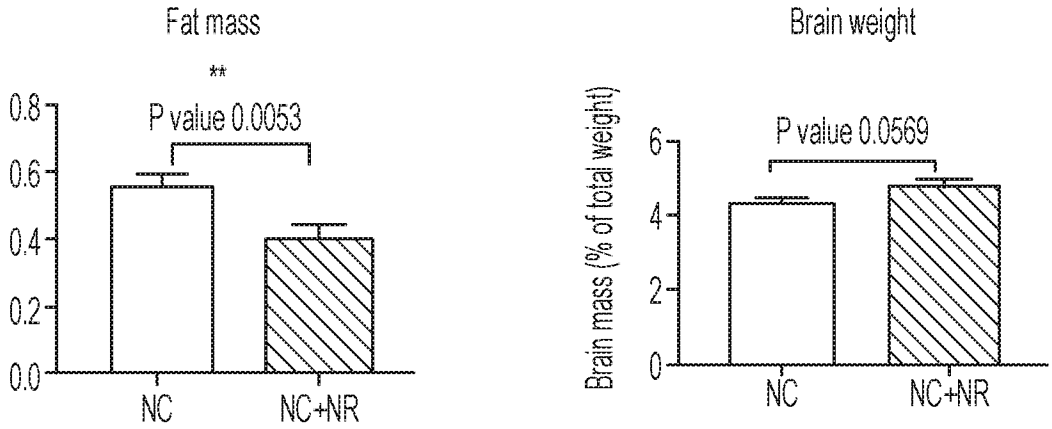


FIGURE 4

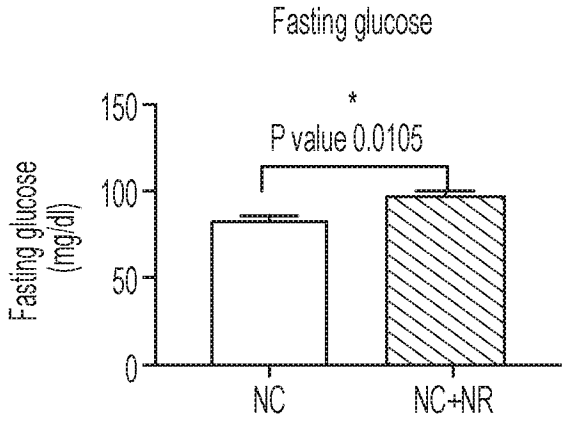
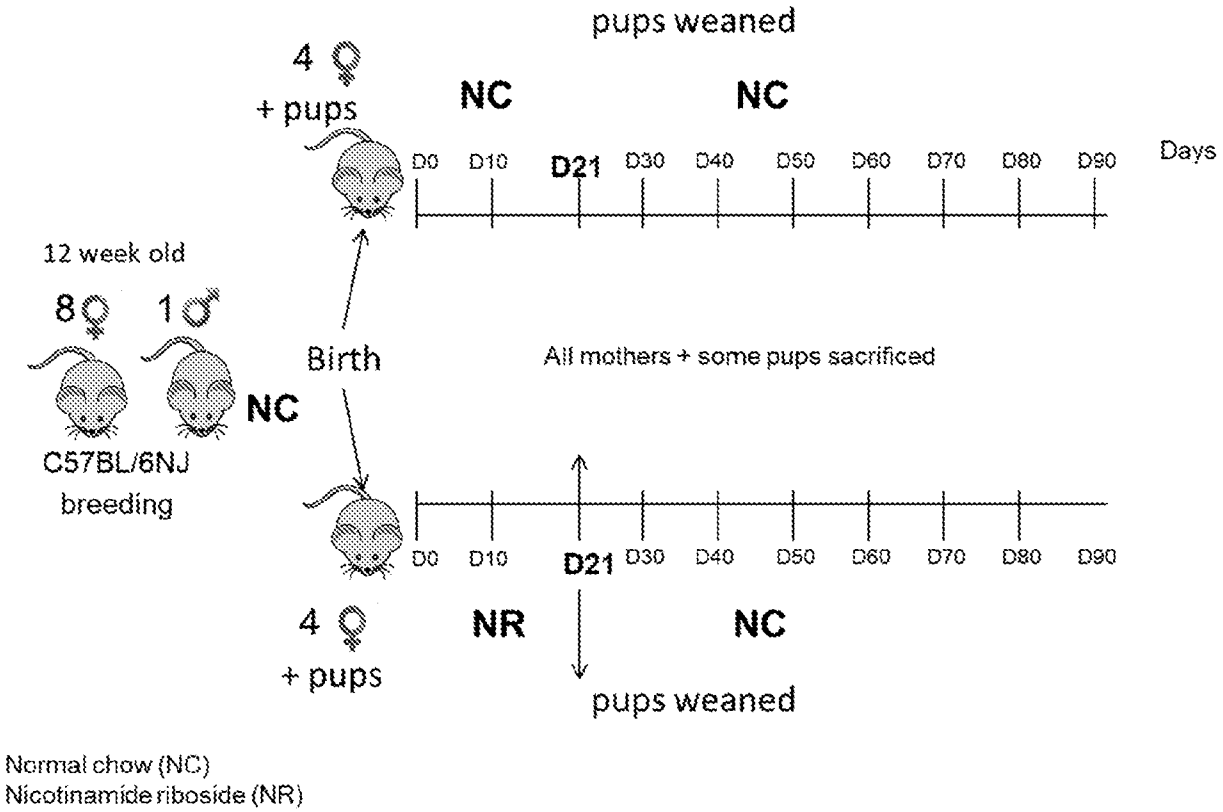


FIGURE 5



Normal chow (NC)
Nicotinamide riboside (NR)

FIGURE 6A

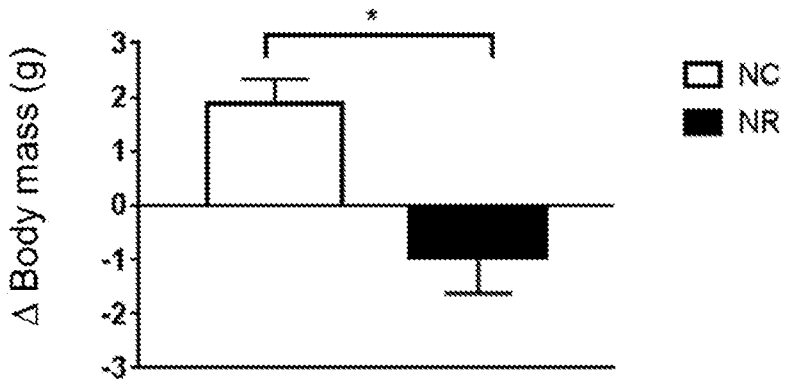


FIGURE 6B

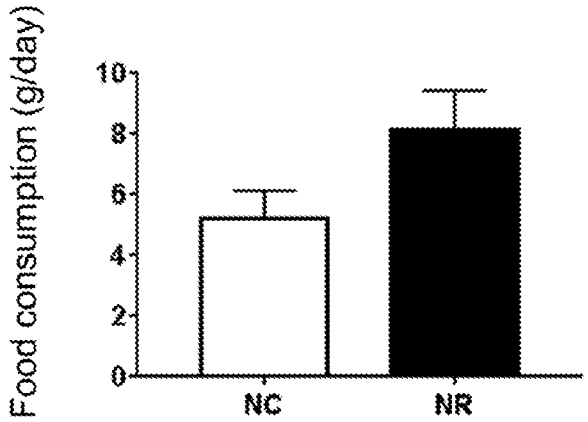


FIGURE 6C

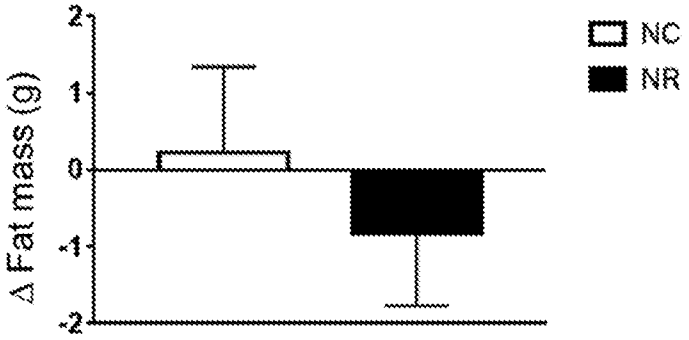


FIGURE 6D

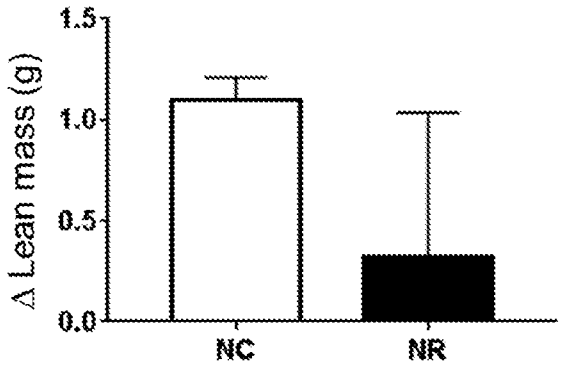


FIGURE 7

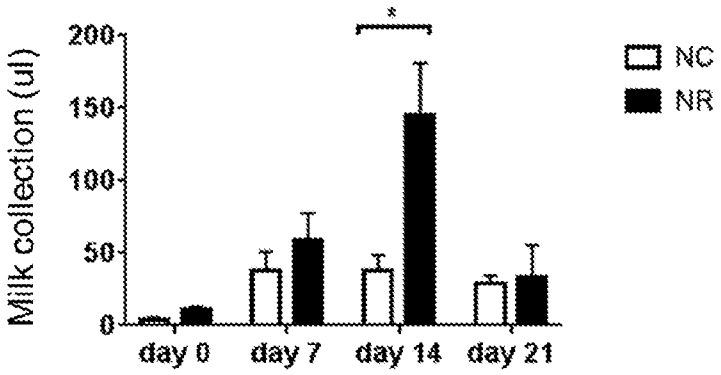


FIGURE 8A

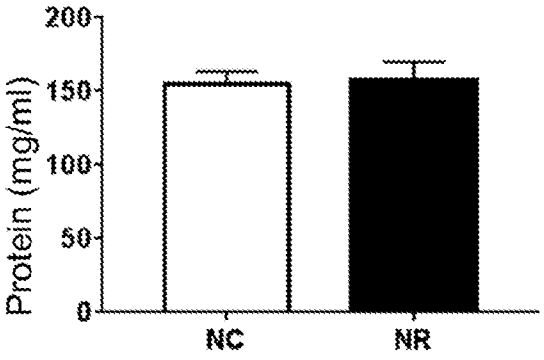


FIGURE 8B

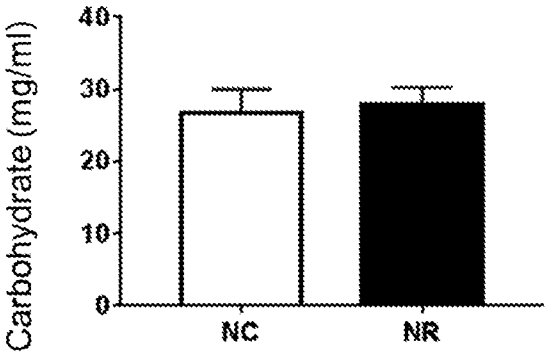


FIGURE 8C

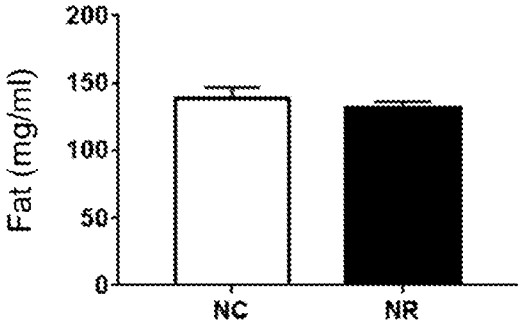


FIGURE 9A

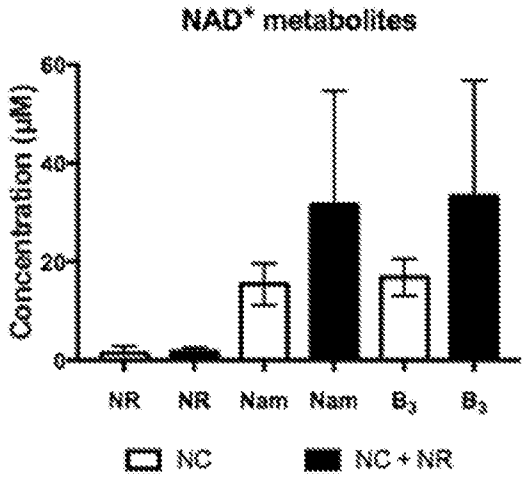


FIGURE 9B

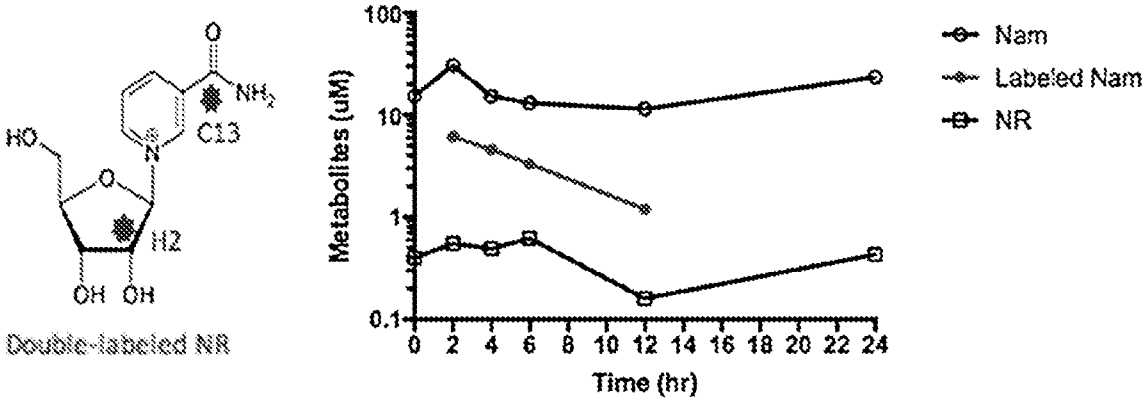


FIGURE 10

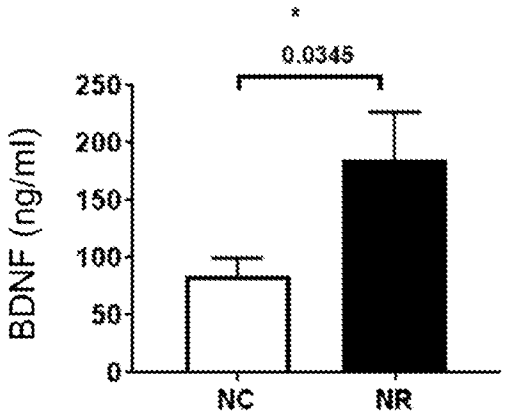


FIGURE 11A

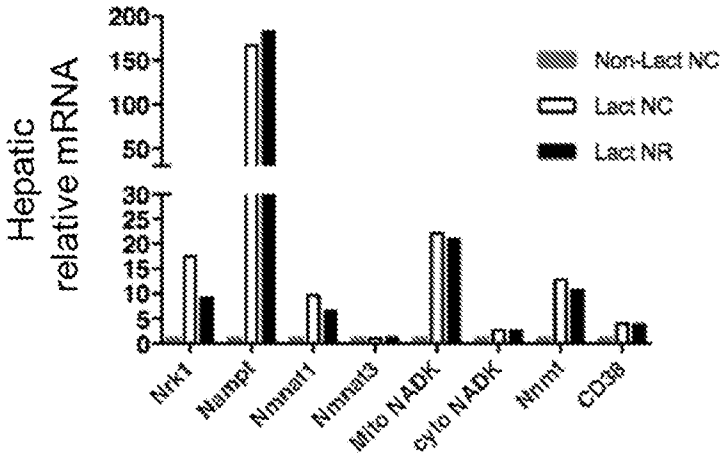


FIGURE 11B

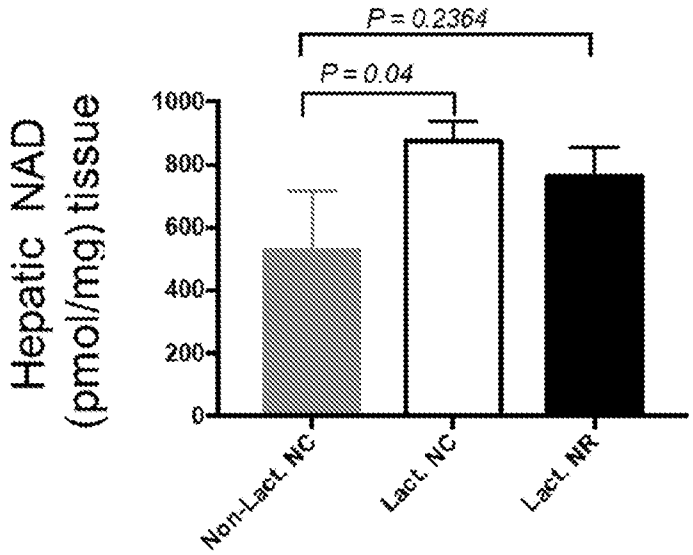


FIGURE 11C

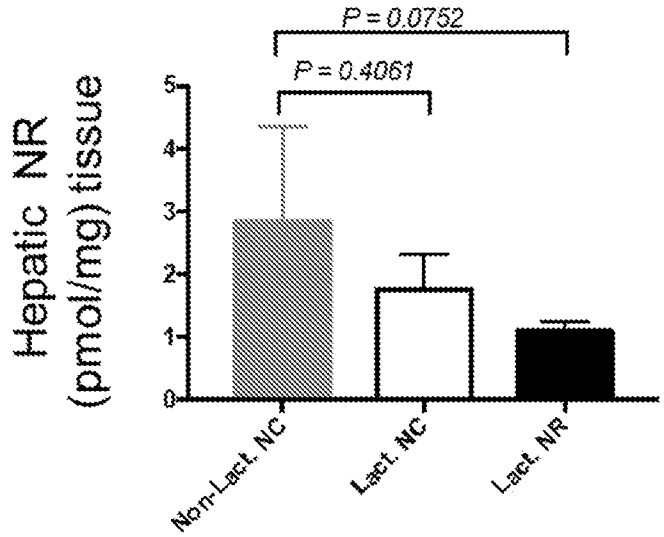


FIGURE 11D

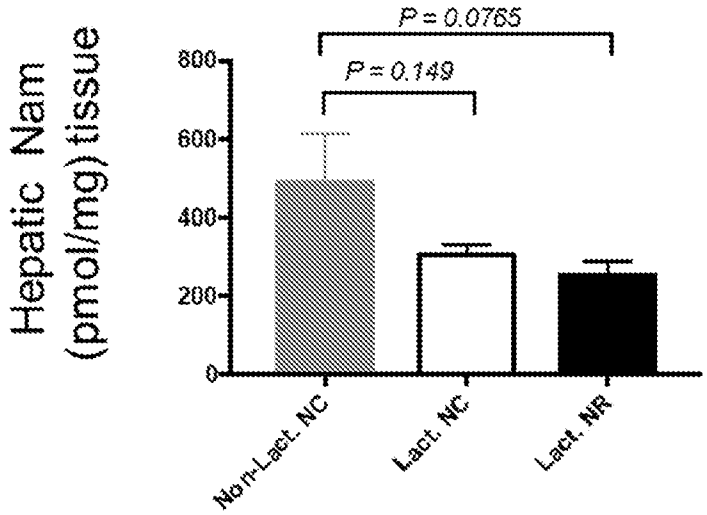


FIGURE 12A

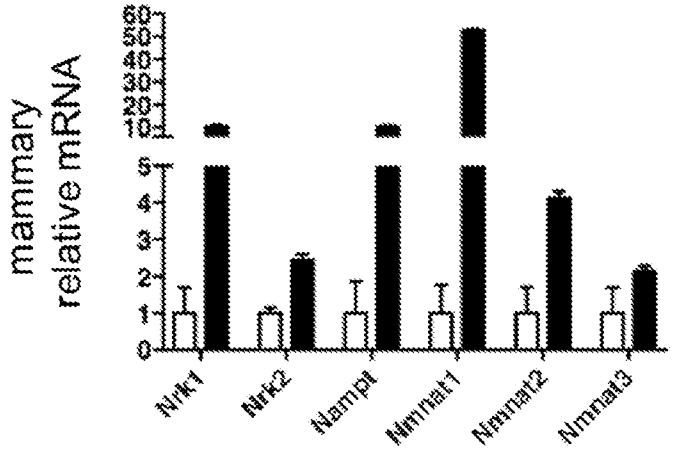


FIGURE 12B

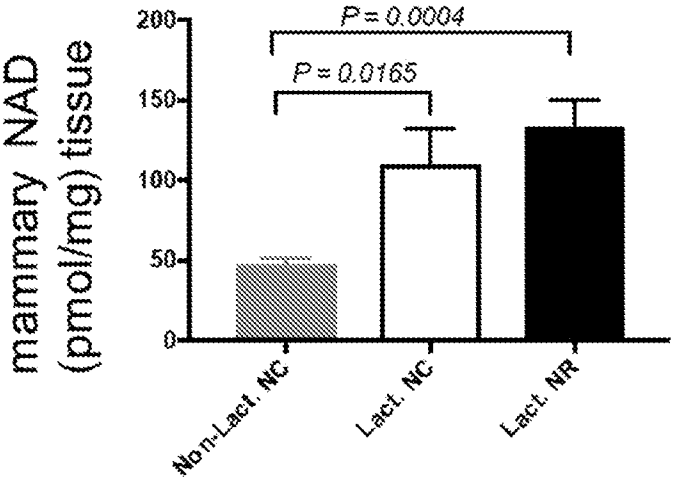


FIGURE 12C

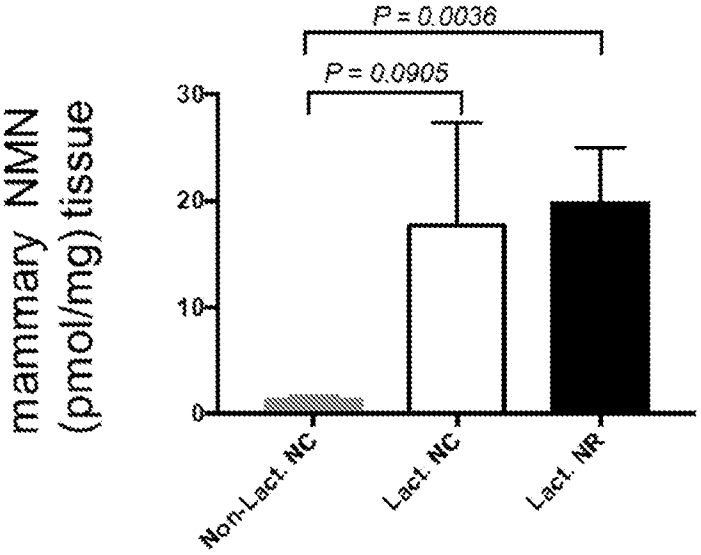


FIGURE 12D

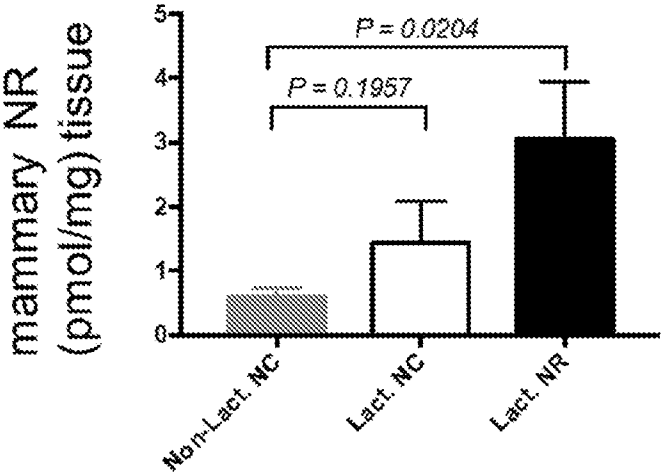


FIGURE 13A

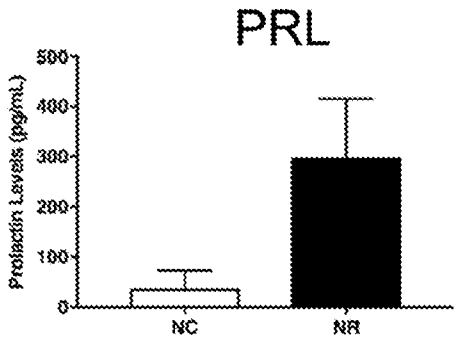


FIGURE 13B

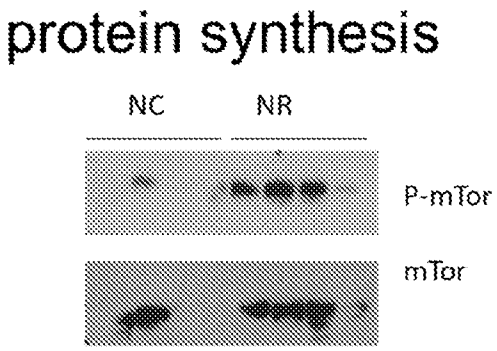


FIGURE 13C

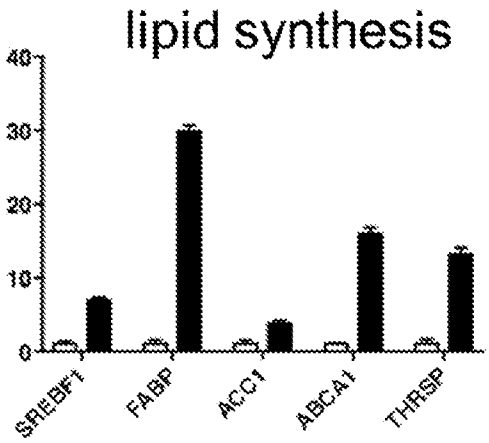


FIGURE 13D

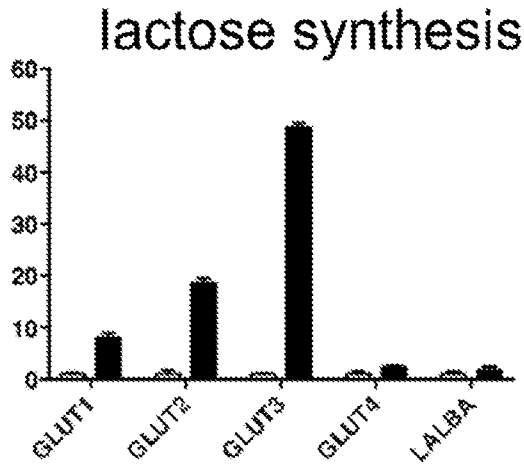


FIGURE 14A

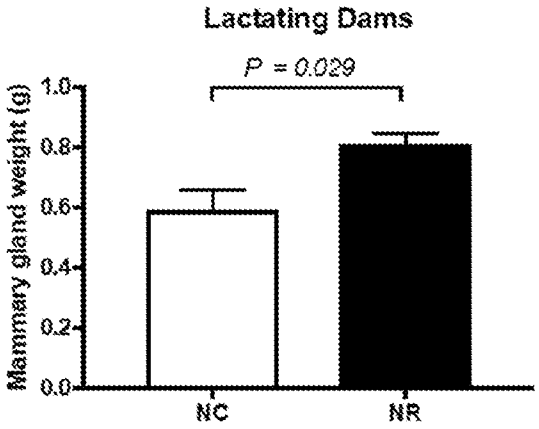


FIGURE 14B

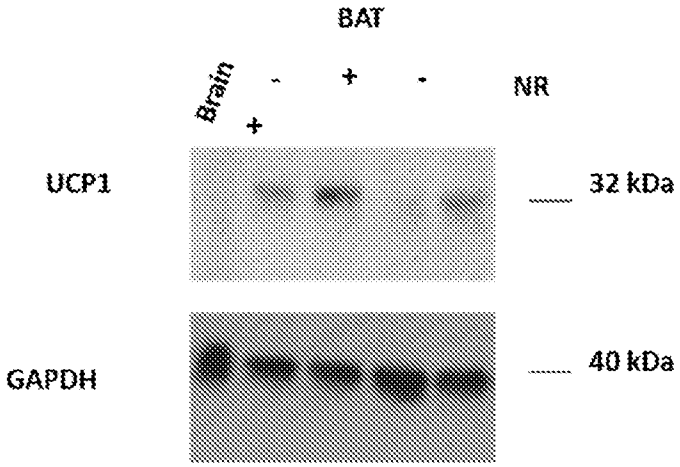


FIGURE 15

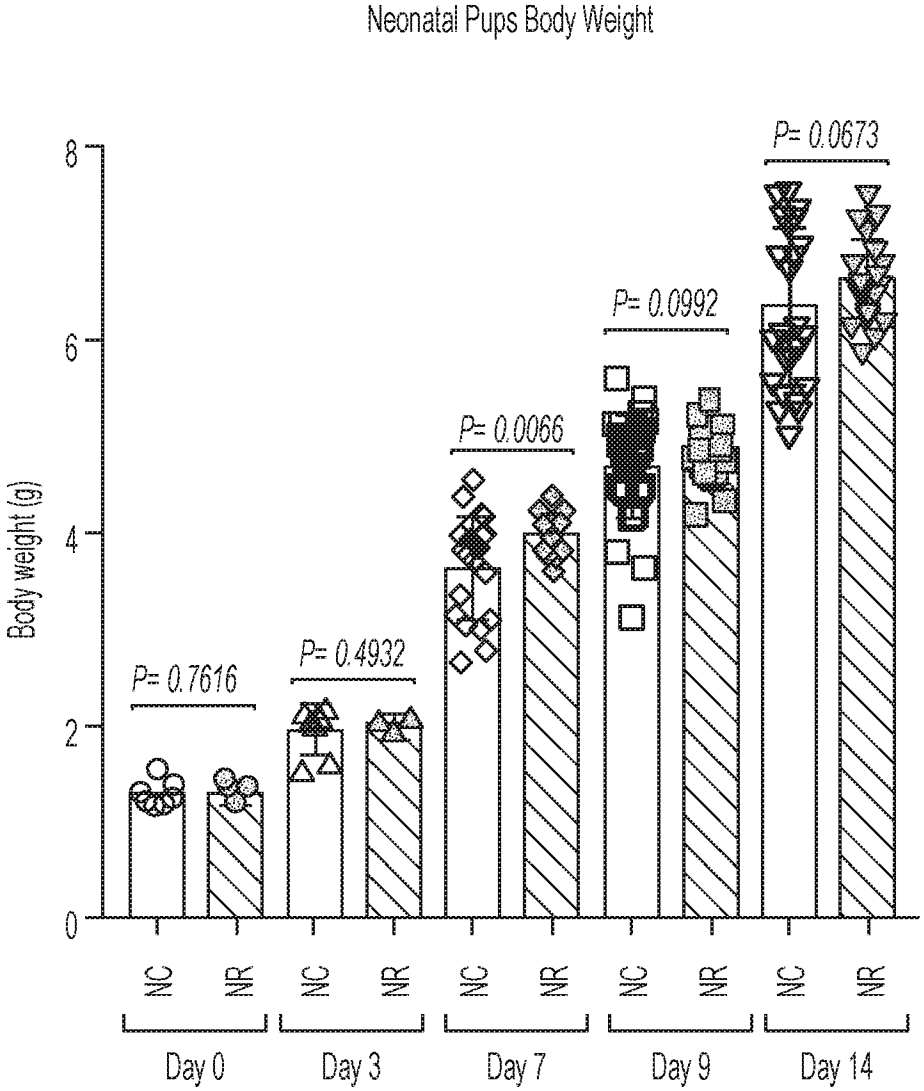

Neonatal Pups Body Weight
Body weight (g)
0
2
4
6
8
P= 0.7616
P= 0.4932
P= 0.0066
P= 0.0992
P= 0.0673
NC
NR
Day 0
Day 3
Day 7
Day 9
Day 14

FIGURE 16A

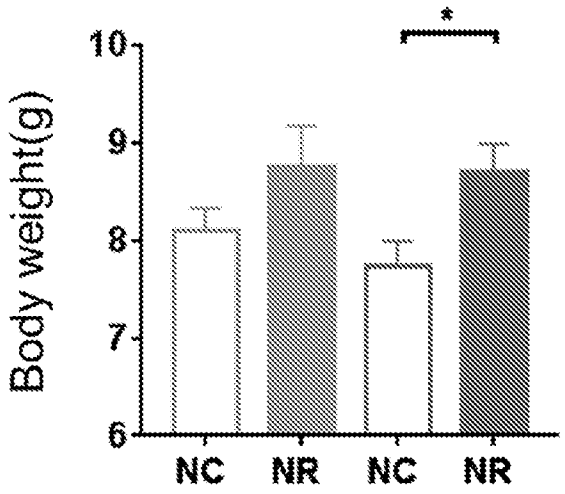


FIGURE 16B

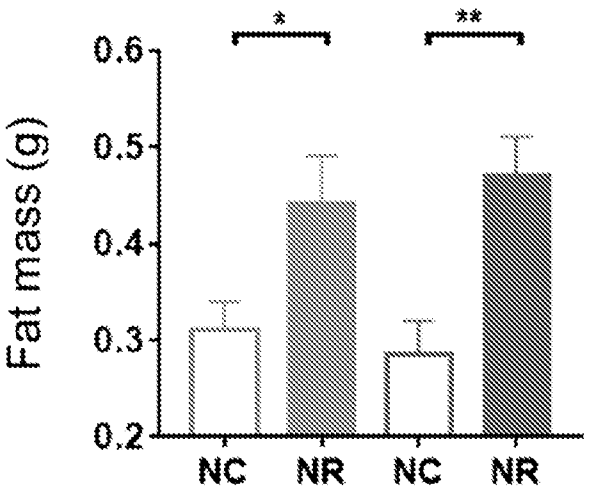


FIGURE 16C

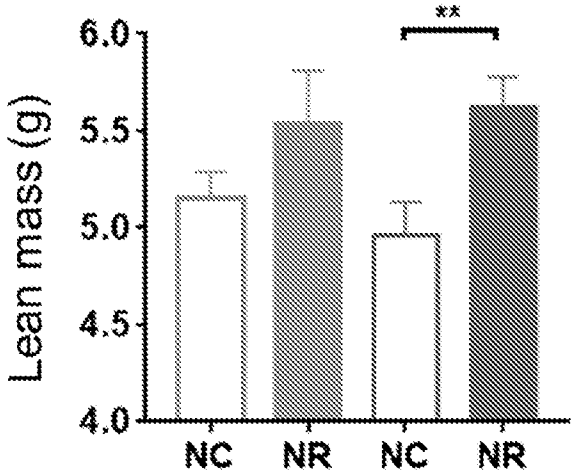


FIGURE 16D

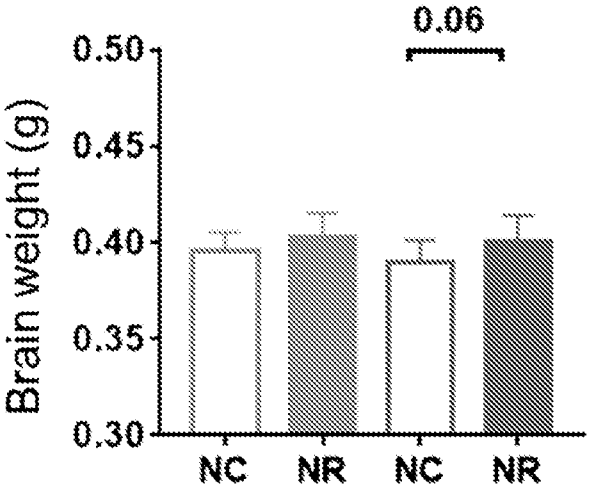


FIGURE 17A

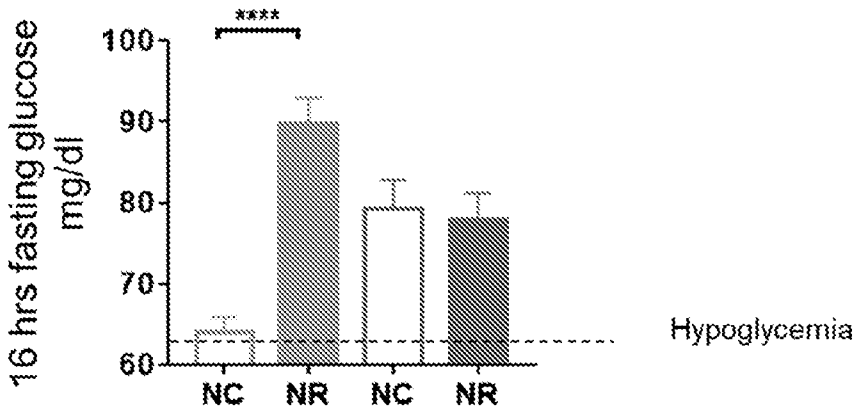


FIGURE 17B

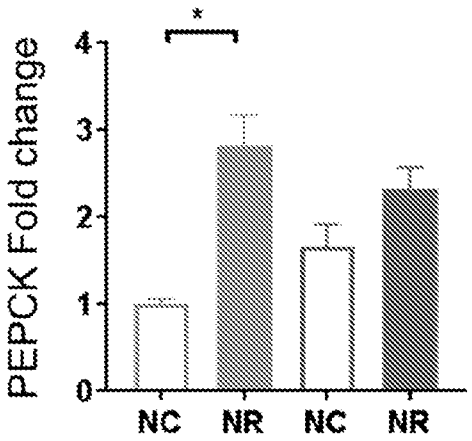


FIGURE 17C

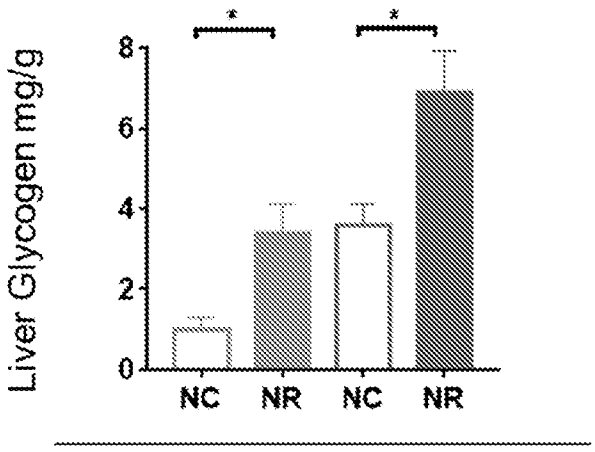


FIGURE 18A

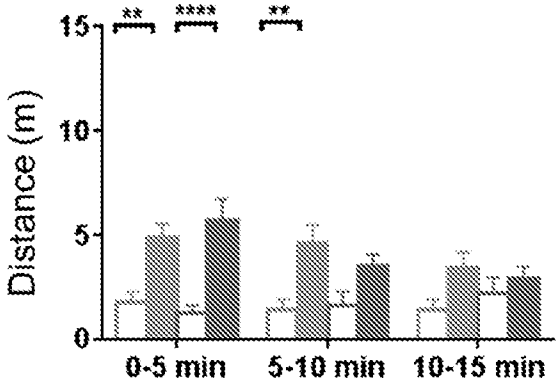


FIGURE 18B

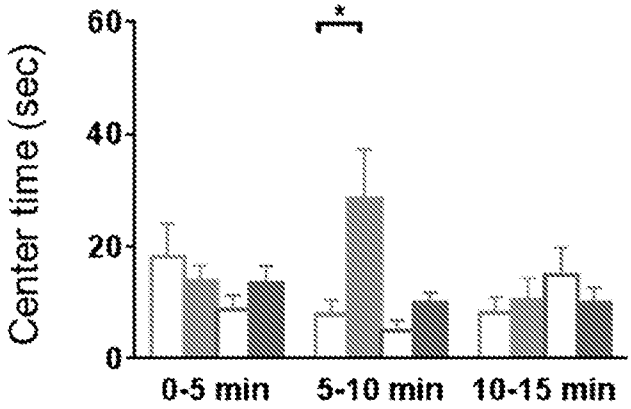


FIGURE 19A

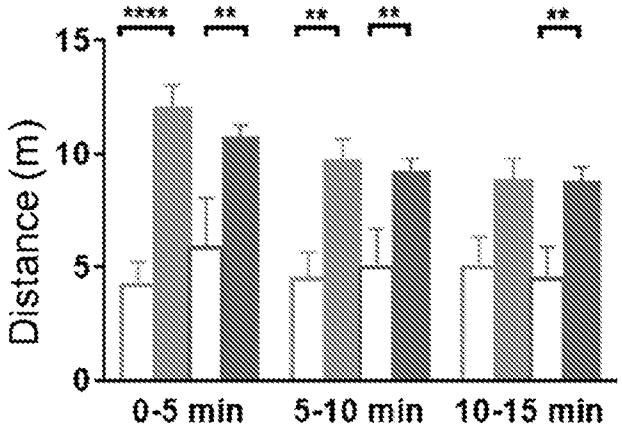


FIGURE 19B

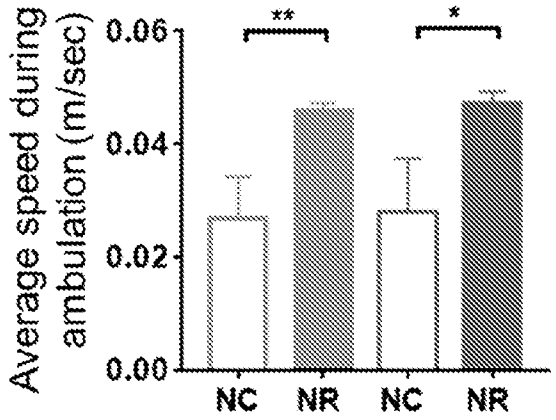


FIGURE 19C

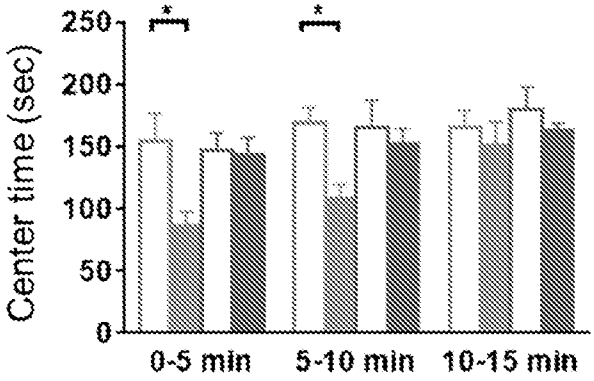


FIGURE 19D

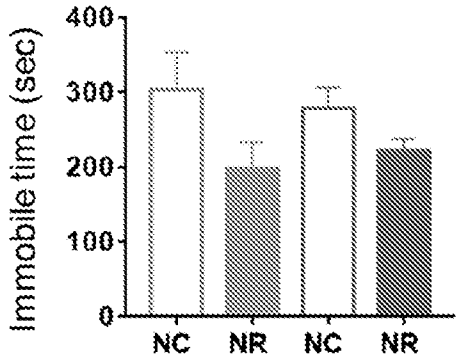


FIGURE 20A

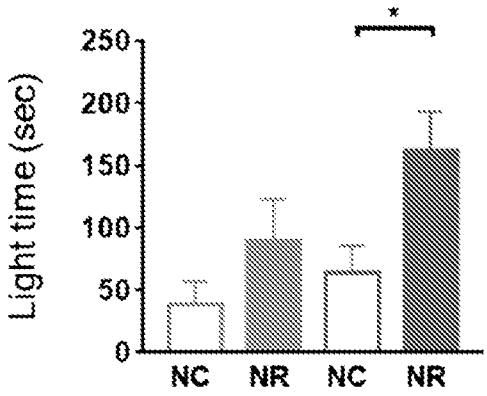


FIGURE 20B

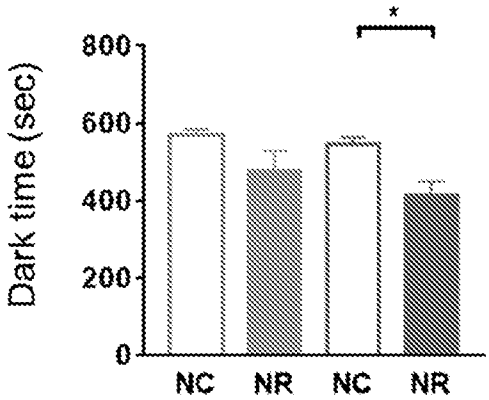


FIGURE 20C

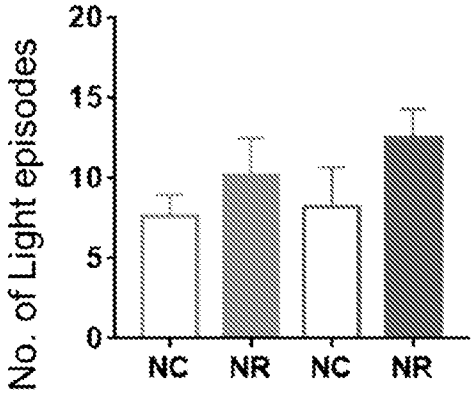


FIGURE 21A

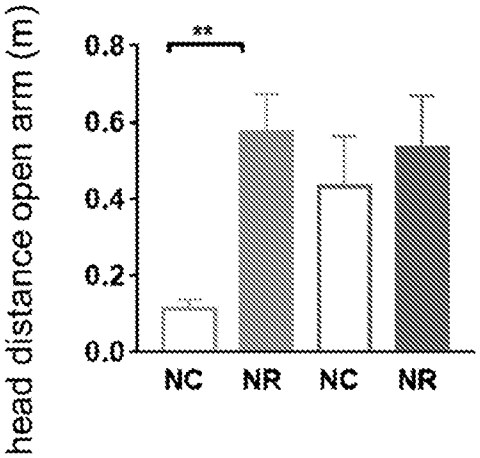


FIGURE 21B

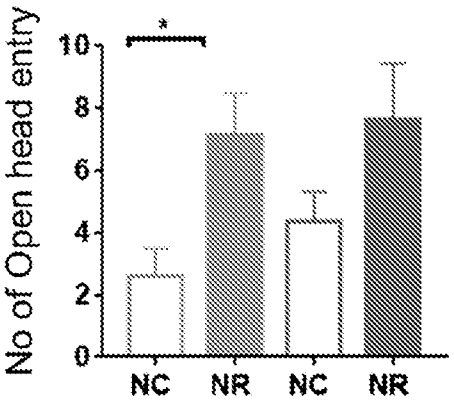


FIGURE 22A

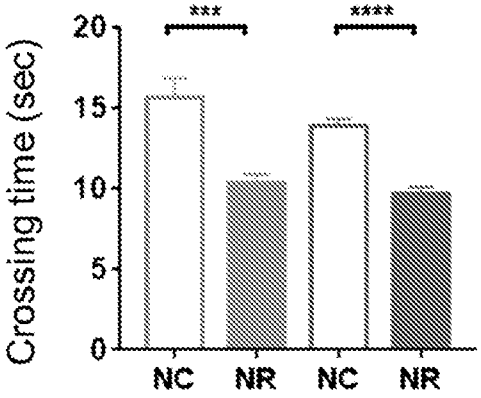


FIGURE 22B

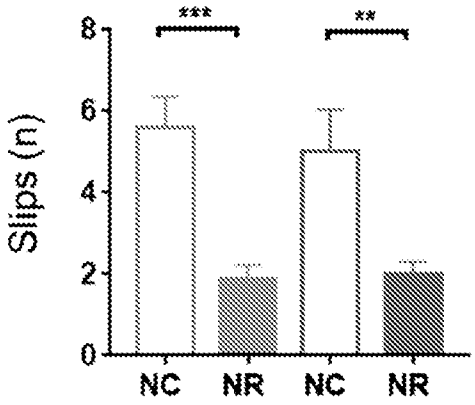


FIGURE 22C

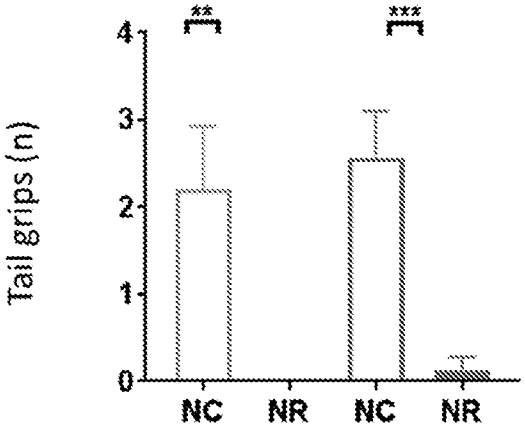


FIGURE 23A

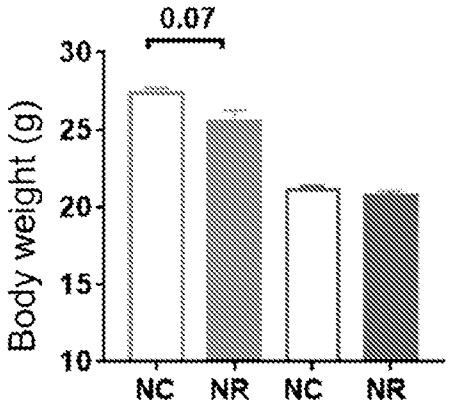


FIGURE 23B

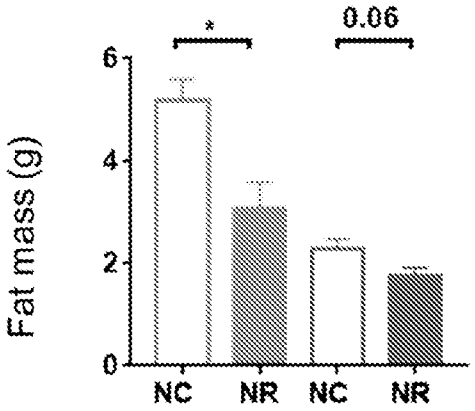


FIGURE 23C

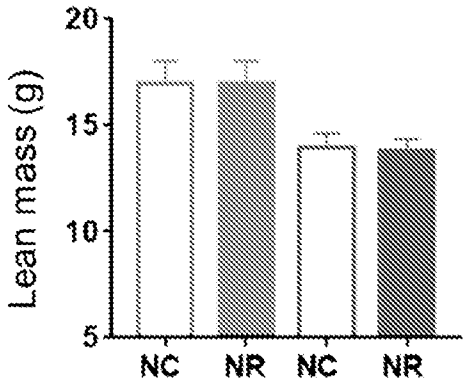


FIGURE 24A

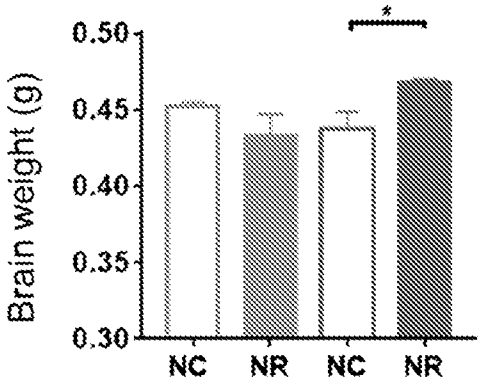


FIGURE 24B

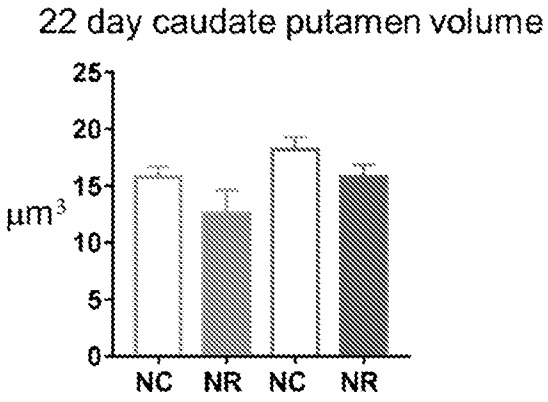


FIGURE 24C

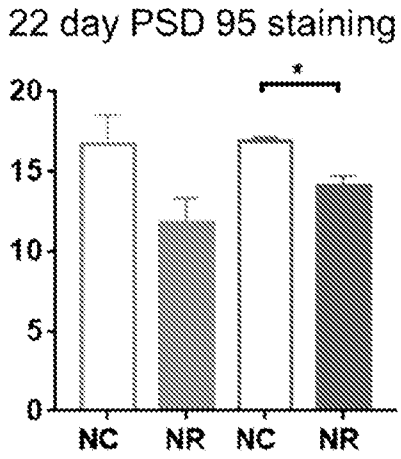
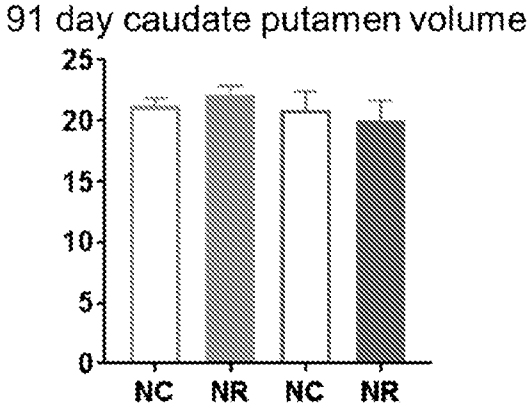


FIGURE 24D



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/063733

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.: 9, 10, 13, 14, 18-68
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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/063733

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 33/15; A61K 31/455; A61K 31/51; A61P 3/02; A61P 3/04 (2018.01)

CPC - A23V 2250/7046; A61K 9/20; A61K 9/48; A61K 31/455; A61K 33/00; A61P 3/02 (2018.01)

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)

See Search History document

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

USPC - 424/400; 514/356; 514/277 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2013/0011377 A1 (PERRIN et al) 10 January 2013 (10.01.2013) entire document	1-3, 15-17, 69-71 ---------- 4-8, 11, 12
X	WO 2014/059031 A2 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 17 April 2014 (17.04.2014) entire document	72-78
Y	US 2016/0000745 A1 (NAN GLOBAL, LLC) 07 January 2016 (07.01.2016) entire document	4-8
Y	WO 2015/016829 A1 (BENEMILK OY) 05 February 2015 (05.02.2015) entire document	11, 12
A	US 2014/0113928 A1 (ALBAGHDADI et al) 24 April 2014 (24.04.2014) entire document	1-8, 11, 12, 15-17, 69-78
A	WO 2016/111992 A1 (CORNELL UNIVERSITY) 14 July 2016 (14.07.2016) entire document	1-8, 11, 12, 15-17, 69-78
A	US 5,055,460 A (FRIEDLANDER) 08 October 1991 (08.10.1991) entire document	1-8, 11, 12, 15-17, 69-78
A	US 2009/0270503 A1 (HERMELIN et al) 29 October 2009 (29.10.2009) entire document	1-8, 11, 12, 15-17, 69-78

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* 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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2018	27 FEB 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774