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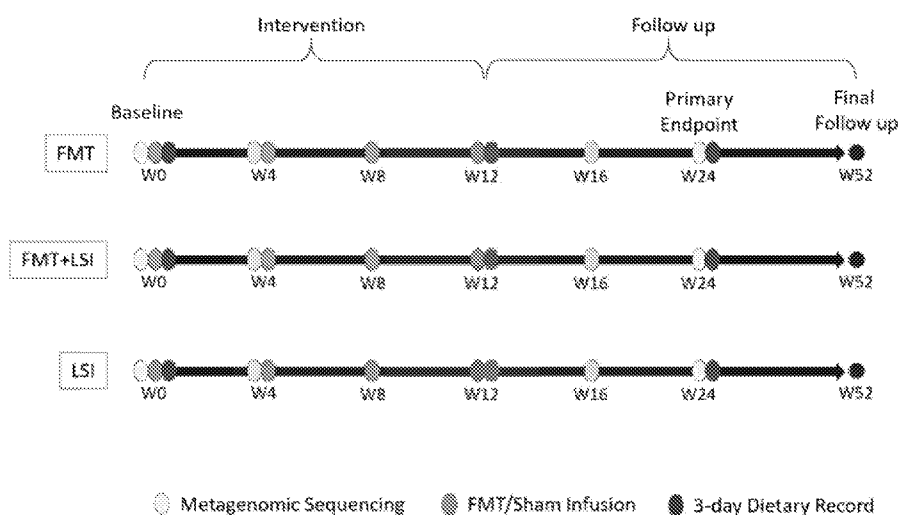
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(54) Title: USE OF BACTERIA IN BODYWEIGHT REGULATION

Figure 1



(57) Abstract: The presence and quantity of certain bacterial species is significantly altered in the gastrointestinal tract of overweight individuals, especially those who fail to achieve weight loss after receiving fecal microbiota transplantation (FMT) treatment. Thus, methods are provided for promoting weight loss by way of modulating recipients' gastrointestinal tract bacteria profile as well as for predicting the likelihood of success in achieving weight loss following FMT treatment in certain individuals. Also provided are kits and compositions for use in these methods.



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USE OF BACTERIA IN BODYWEIGHT REGULATION

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/067,217, filed August 18, 2020, and U.S. Provisional Patent Application No. 63/169,481, filed April 1, 2021, the contents of each of the above are hereby incorporated by reference in the entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] As living standards continue to improve globally, the number of individuals who are overweight or even obese is also rapidly increasing. Because of the serious health risks directly associated with excess body weight, this trend of an ever increasing proportion of the general population being overweight has led to a notably higher incidence of many diseases including type 2 diabetes (T2D), heart disease, hypertension, and stroke. For example, the World Health Organization (WHO) estimates that by 2030 the number of people living with diabetes will exceed 350 million worldwide. Due to the rising incidence of obesity-related diseases, their serious health implications, as well as their profound economic consequences, there exists an urgent need for new and effective means to treat individuals who are either already overweight or obese or at risk of becoming overweight or obese in order to help them reduce their bodyweight to, or maintain their bodyweight at, a lower and more healthful level, thus achieve or maintain normal cholesterol level (including low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels) and triglyceride level, and ultimately reduce or eliminate their risk of later suffering from serious illnesses such as diabetes and cardiovascular disease. The present invention fulfills this and other related needs by providing new methods and compositions that can effectively regulate a patient's bodyweight and are useful for treating or reducing risk of obesity or T2D.

BRIEF SUMMARY OF THE INVENTION

[0003] The invention relates to novel methods and compositions useful for facilitating weight loss in overweight or obese patients, for treating or reducing risk of obesity or T2D in patients, as well as for assessing a patient's likelihood of successfully achieving FMT-induced weight loss. In particular, the present inventors have discovered that certain microorganism species, especially certain bacteria, are present at distinctly different levels in

the gastrointestinal (GI) tract of individuals depending on whether or not they are able to successfully reduce their bodyweight after undergoing FMT procedure. Health benefits associated with bodyweight reduction such as improved blood glucose, triglyceride, and/or cholesterol level(s) and therefore reduced risks of serious medical conditions such as heart disease, hypertension, stroke, and diabetes can be achieved by modulating the level of pertinent microorganisms in patients' gut, for example, by fecal microbiota transplantation (FMT) treatment or oral administration of beneficial bacterial species. These findings also provide new methods assessing or predicting the prospect of individuals successfully achieving weight loss goals by FMT. Thus, in the first aspect, the present invention provides a novel method for bodyweight reduction, including reducing health risks associated with being overweight or obese, by increasing the level of one or more bacterial species named in Table 1 in the gastrointestinal tract of an overweight individual.

[0004] In some embodiments, the introducing step comprises oral administration to the subject a composition comprising an effective amount of the one or more of the bacterial species. In some embodiments, the introducing step comprises delivery to the small intestine, ileum, or large intestine of the subject a composition comprising an effective amount of the one or more of the bacterial species. In some embodiments, the introducing step comprises fecal microbiota transplantation (FMT). In some embodiments, the FMT comprises administration to the recipient a composition comprising processed donor fecal material. In some embodiments, the composition is orally administered; or the composition is directly deposited to the recipient's gastrointestinal tract. In some embodiments, the composition administered to the subject consists essentially of the one or more of the bacterial species and one or more of pharmaceutically acceptable excipients. In some embodiments, the level or relative abundance of the one or more of the bacterial species is determined in a first stool sample obtained from the recipient prior to the introducing step and in a second stool sample obtained from the recipient after the introducing step. In some embodiments, the level of the one or more of the bacterial species is determined by polymerase chain reaction (PCR), especially quantitative PCR. In some embodiments, the bacterial species given to the recipient includes one or more selected from *Anaerostipes hadrus*, *Collinsella tanakaei*, and *Roseburia hominis*.

[0005] In a second aspect, the present invention provides a kit for treating overweight or obese individuals for the purpose of facilitating their weight loss efforts. The kit comprises: a first container containing a first composition comprising an effective amount of a first one of

the bacterial species set forth in Table 1, and a second container containing a second composition comprising an effective amount of a second one of the bacterial species set forth in Table 1.

[0006] In some embodiments, the first composition comprises processed donor fecal material for FMT, for example, the material has been processed and formulated for oral administration, such as dried, frozen or lyophilized, and placed in a capsule suitable for oral ingestion. In some embodiments, the second composition is formulated for oral administration. In some embodiments, both the first and second compositions are formulated for oral administration. In some cases, the kit may include two or more compositions each comprising an effective amount of at least one, possibly two or even three, different species independently selected from those set forth in Table 1, namely *Anaerostipes hadrus*, *Collinsella tanakaei*, and *Roseburia hominis*. In some embodiments, each composition consists essentially of the one or more of the bacterial species and one or more of pharmaceutically acceptable excipients. The compositions in the kit may each comprise a physiologically acceptable carrier or excipient appropriate for the intended administration method (*e.g.*, oral ingestion or rectal suppository).

[0007] In a third aspect, a method is provided for determining the likelihood of bodyweight reduction by an FMT procedure to be performed on a subject. The method includes these steps: (1) determining, in a stool sample from the subject, the level or relative abundance of one or more of the bacterial species set forth in Table 2; (2) determining the level or relative abundance of the same bacterial species in a stool sample from a reference cohort comprising subjects who had FMT-induced weight loss after receiving an FMT procedure and subjects who had no FMT-induced weight loss after receiving an FMT procedure; (3) generating decision trees by random forest model using data obtained from step (2) and running the level or relative abundance of one or more of the bacterial species from step (1) down the decision trees to generate a score; and (4) determining the subject with a score greater than 0.5 as likely to achieve weight loss by an FMT procedure that is planned to be performed on the subject at a later time and determining the subject with a score no greater than 0.5 as unlikely to achieve weight loss by the to-be-performed FMT treatment. In some cases, the one or more bacterial species comprise any two or three of the bacterial species set forth in Table 2.

[0008] In a fourth aspect, a kit is provided for assessing the likelihood of weight reduction by an FMT procedure to be performed on a subject. The kit includes reagents for detecting one or more of the bacterial species set forth in Table 2. In some embodiments, the reagents

comprise a set of oligonucleotide primers for amplification of a polynucleotide sequence that is from any one of the bacterial species set forth in Table 2, preferably unique to the species and therefore would allow the detection of the bacterial species both qualitatively and quantitatively. In some cases, the amplification reaction is PCR, preferably quantitative PCR.

[0009] In some embodiments, the one or more bacterial species comprise or consist of one of *candidate division TM7 single-cell isolate TM7c*, *Bacteroides stercoris*, or *Bacteroides dorei*. In some embodiments, the one or more bacterial species comprise or consist of two species of *candidate division TM7 single-cell isolate TM7c*, *Bacteroides stercoris*, and *Bacteroides dorei*. In some embodiments, the one or more bacterial species comprise or consist of *candidate division TM7 single-cell isolate TM7c*, *Bacteroides stercoris*, and *Bacteroides dorei*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **Fig. 1** Study Scheme.

[0011] **Fig. 2** Body weight change of FMT responders and lifestyle responders post intervention. FMT responders had continues weight loss till week 52 (40 weeks after last intervention) while lifestyle responders regained weight after intervention stopped.

[0012] **Fig. 3** The relative abundance of (a) *Anaerostipes hadrus*, (b) *Collinsella tanakaei*, and (c) *Roseburia hominis* were significantly increased in FMT responders but not in lifestyle responders post intervention. Relative abundance were shown in percentage after logarithmic transformation.

[0013] **Fig. 4** a. Relative abundance of 3 bacterial species used for prediction of FMT response (weight loss at 24 weeks after first FMT compared with baseline) in FMT responders vs non-responders. b. ROC of the model trained to predict FMT response based on relative abundance of candidate division TM7 single-cell isolate TM7b+Bacteroides dorei+Bacteroides stercoris (light blue), Bacteroides dorei+Bacteroides stercoris (red), candidate division TM7 single-cell isolate TM7b+Bacteroides stercoris (green), candidate division TM7 single-cell isolate TM7b+Bacteroides dorei (dark blue) at baseline fecal microbiota composition is depicted.

[0014] **Fig. 5** a. Risk score of an obese subject with T2DM compared to FMT recipients with no weight loss (n=18) and with weight loss (n=19) using 3 markers: candidate division TM7 single-cell isolate TM7b, Bacteroides dorei and Bacteroides stercoris. The subject was

deemed to be likely to lose weight after FMT. b. Risk score of an obese subject with T2DM compared to FMT recipients with no weight loss (n=18) and with weight loss (n=19) using 3 markers: candidate division TM7 single-cell isolate TM7b, *Bacteroides dorei* and *Bacteroides stercoris*. The subject was deemed to be unlikely to lose weight after FMT.

DEFINITIONS

[0015] The term “**fecal microbiota transplantation (FMT)**” or “stool transplant” refers to a medical procedure during which fecal matter containing live fecal microorganisms (bacteria, fungi, viruses, and the like) obtained from a healthy individual is transferred into the gastrointestinal tract of a recipient to restore healthy gut microflora that has been disrupted or destroyed by any one of a variety of medical conditions, for example, excess body weight or obesity and its related disorders. Typically, the fecal matter from a healthy donor is first processed into an appropriate form for the transplantation, which can be made through direct deposit into the lower gastrointestinal tract such as by colonoscopy, or by nasal intubation, or through oral ingestion of an encapsulated material containing processed (*e.g.*, dried and frozen or lyophilized) fecal material.

[0016] The term “**inhibiting**” or “**inhibition**,” as used herein, refers to any detectable negative effect on a target biological process, such as RNA/protein expression of a target gene, the biological activity of a target protein, cellular signal transduction, cell proliferation, and the like. Typically, an inhibition is reflected in a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater in the target process (*e.g.*, growth or proliferation of a microorganism of certain species, for example, one or more of the bacterial species shown in Table 2), or any one of the downstream parameters mentioned above, when compared to a control. “Inhibition” further includes a 100% reduction, *i.e.*, a complete elimination, prevention, or abolition of a target biological process or signal. The other relative terms such as “suppressing,” “suppression,” “reducing,” “reduction,” “decrease,” “decreasing,” “lower,” and “less” are used in a similar fashion in this disclosure to refer to decreases to different levels (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater decrease compared to a control level, *i.e.*, the level before suppression) up to complete elimination of a target biological process or signal. On the other hand, terms such as “activate,” “activating,” “activation,” “increase,” “increasing,” “promote,” “promoting,” “enhance,” “enhancing,” “enhancement,” “higher,” and “more” are used in this disclosure to encompass positive changes at different levels (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, or greater such as 3, 5, 8, 10, 20-fold increase compared to a

control level (before activation), for example, the control level of one or more of the bacterial species shown in Table 1) in a target process or signal. In contrast, the term "**substantially the same**" or "**substantially lack of change**" indicates little to no change in quantity from a comparison basis (such as a standard control value), typically within $\pm 10\%$ of the comparison basis, or within $\pm 5\%$, 4% , 3% , 2% , 1% , or even less variation from the comparison basis.

[0017] The term "**anti-bacterial agent**" refers to any substance that is capable of inhibiting, suppressing, or preventing the growth or proliferation of bacterial species, respectively, especially those of shown in Table 2. Known agents with anti-bacterial activity include various antibiotics that generally suppress the proliferation of a broad spectrum of bacterial species as well as agents such as antisense oligonucleotides, small inhibitory RNAs, and the like that can inhibit the proliferation of specific bacterial species. The term "anti-bacterial agent" is similarly defined to encompass both agents with broad spectrum activity of killing virtually all species of bacteria and agents that specifically suppress proliferation of target bacteria species. Such specific anti-bacterial agent may be short polynucleotide in nature (*e.g.*, a small inhibitory RNA, microRNA, miniRNA, lncRNA, or an antisense oligonucleotide) that is capable of disrupting the expression of a key gene in the life cycle of a target bacterial species and is therefore capable of specifically suppressing or eliminating the species only without substantially affecting other closely related bacterial species.

[0018] "**Percentage relative abundance**," when used in the context of describing the presence of a particular bacterial species (*e.g.*, any one of those shown in any one of Table 1 or 2) in relation to all bacterial species present in the same environment, refers to the relative amount of the bacterial species out of the amount of all bacterial species as expressed in a percentage form. For instance, the percentage relative abundance of one particular bacterial species can be determined by comparing the quantity of DNA specific for this species (*e.g.*, determined by quantitative polymerase chain reaction) in one given sample with the quantity of all bacterial DNA (*e.g.*, determined by quantitative polymerase chain reaction (PCR) and sequencing based on the 16s rRNA sequence) in the same sample.

[0019] "**Absolute abundance**," when used in the context of describing the presence of a particular bacterial species (*e.g.*, any one of those shown in Table 1 or 2) in the feces, refers to the amount of DNA derived from the bacterial species out of the amount of all DNA in a fecal sample. For instance, the absolute abundance of one bacterium can be determined by

comparing the quantity of DNA specific for this bacterial species (*e.g.*, determined by quantitative PCR) in one given sample with the quantity of all fecal DNA in the same sample.

[0020] “**Total bacterial load**” of a fecal sample, as used herein, refers to the amount of all bacterial DNA, respectively, out of the amount of all DNA in the fecal sample. For instance, the absolute abundance of bacteria can be determined by comparing the quantity of bacteria-specific DNA (*e.g.*, 16s rRNA determined by quantitative PCR) in one given sample with the quantity of all fecal DNA in the same sample.

[0021] The term “**overweight**” is used to describe a subject of excessive body weight and having a body mass index (BMI) greater than 25. Encompassed with this term is “**obese**” or “**obesity**,” which describes a condition in which the suffer has a BMI greater than 30.

[0022] The term “**treat**” or “**treating**,” as used in this application, describes an act that leads to the elimination, reduction, alleviation, reversal, prevention and/or delay of onset or recurrence of any symptom of a predetermined medical condition. In other words, “treating” a condition encompasses both therapeutic and prophylactic intervention against the condition, including facilitation of patient recovery from the condition.

[0023] The term “**effective amount**,” as used herein, refers to an amount of a substance that produces a desired effect (*e.g.*, an inhibitory or suppressive effect on the growth or proliferation of one or more detrimental bacterial species (*e.g.*, the bacterial species shown in Table 2) for which the substance (*e.g.*, an anti-bacterial agent) is used or administered. The effects include the prevention, inhibition, or delaying of any pertinent biological process during bacterial proliferation to any detectable extent. The exact amount will depend on the nature of the substance (the active agent), the manner of use/administration, and the purpose of the application, and will be ascertainable by one skilled in the art using known techniques as well as those described herein. In another context, when an “effective amount” of one or more beneficial or desirable bacterial species (*e.g.*, those listed in Table 1) are artificially introduced into a composition intended to be introduced into the gastrointestinal tract of a patient, *e.g.*, to be used in FMT, it is meant that the amount of the pertinent bacteria being introduced is sufficient to confer to the recipient health benefits such as reduced recovery time or reduced needs for therapeutic intervention for a pertinent disorder such as excessive body weight or obesity, including but not limited to medication (such as an appetite suppressant) and any of the variety of therapies such as behavior and communication therapy, educational therapy, family therapy, speech or physical therapy, and the like.

[0024] A "**pharmaceutically acceptable**" or "**pharmacologically acceptable**" excipient is a substance that is not biologically harmful or otherwise undesirable, *i.e.*, the excipient may be administered to an individual along with a bioactive agent without causing any undesirable biological effects. Neither would the excipient interact in a deleterious manner with any of the components of the composition in which it is contained.

[0025] The term "**excipient**" refers to any essentially accessory substance that may be present in the finished dosage form of the composition of this invention. For example, the term "excipient" includes vehicles, binders, disintegrants, fillers (diluents), lubricants, glidants (flow enhancers), compression aids, colors, sweeteners, preservatives, suspending/dispersing agents, film formers/coatings, flavors and printing inks.

[0026] The term "**consisting essentially of**," when used in the context of describing a composition containing an active ingredient or multiple active ingredients, refers to the fact that the composition does not contain other ingredients possessing any similar or relevant biological activity of the active ingredient(s) or capable of enhancing or suppressing the activity, whereas one or more inactive ingredients such as physiological or pharmaceutically acceptable excipients may be present in the composition. For example, a composition consisting essentially of active agents (for instance, one or more of *Anaerostipes hadrus*, *Collinsella tanakaei*, and *Roseburia hominis*) effective for treating or reducing the risk of obesity or type II diabetes (T2D) in a subject is a composition that does not contain any other agents that may have any detectable positive or negative effect on the same target process (*e.g.*, inhibition of weight gain or progression of T2D) or that may increase or decrease to any measurable extent of the disease severity among the receiving subjects.

[0027] As used herein, the term "**about**" denotes a range of value that is +/- 10% of a specified value. For instance, "about 10" denotes the value range of 9 to 11 (10 +/- 1).

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0028] The invention provides novel methods for achieving weight loss in individuals by modifying their bacteria profile in their gastrointestinal tract as well as for assessing the likelihood of achieving weight loss in individuals by way of fecal microbiota transplantation (FMT) treatment. During their studies, the present inventors discovered that the presence and relative abundance of certain bacterial species alter significantly in the gastrointestinal tract of overweight individuals who have failed to lose weight after receiving FMT treatment. For

example, the presence and abundance of bacterial species shown in Table 1 is found to be at an elevated level in the gastrointestinal tract of those who successfully lost weight as a result of FMT, whereas no such increase is observed in the gastrointestinal tract of FMT recipients who failed to lose weight. On the other hand, the level or relative abundance of certain bacterial species (such as those shown in Table 2) in individuals' stool samples has been observed to correlate with likelihood of successful weight loss following FMT. Thus, the results of this study provide useful tools for facilitating weight loss efforts in overweight individuals and for assessing the prospect of effective weight loss following FMT treatment among overweight individuals.

II. FMT Donor/Recipient Selection and Preparation

[0029] Overweight individuals suffer from a disrupted state of GI tract microflora are considered as recipients for FMT treatment in order to restore the normal healthy profile for microorganisms. As revealed by the present inventors, overweight individuals who have received FMT but failed to lose weight tend to have a depressed level of bacterial species such as those shown in Table 1 in their GI tract, a FMT donor whose fecal material contains an higher than average level of one or more of these bacterial species is favored as particularly advantageous for the purpose of a subsequent FMT therapy for bodyweight reduction. For example, a desirable donor may preferably have higher than about 0.01%, 0.02%, 0.05%, 0.10%, 0.20%, 0.40%, 0.50%, 0.60%, 0.80%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 8.5%, 9.0%, or higher of total bacteria in relative abundance for each of these bacterial species in his stool sample.

[0030] Fecal matter used in FMT is obtained from a healthy donor and then processed into appropriate forms for the intended means of delivery in the upcoming FMT procedure. While a healthy individual from the same family or household of the recipient often serves as donor, in practicing the present invention the donor microorganism profile is an important consideration and may favor the choice of an unrelated donor instead. The process of preparing donor material for transplant includes steps of drying, freezing or lyophilizing, and formulating or packaging, depending on the precise route of delivery to recipient, *e.g.*, by oral ingestion or by rectal deposit.

[0031] Various methods have been reported in the literature for determining the levels of all bacterial species in a sample, for example, amplification (*e.g.*, by PCR) and sequencing of bacterial polynucleotide sequence taking advantage of the sequence similarity in the

commonly shared 16S rRNA bacterial sequences. On the other hand, the level of any given bacterial species may be determined by amplification and sequencing of its unique genomic sequence. A percentage abundance is often used as a parameter to indicate the relative level of a bacterial species in a given environment.

III. Treatment Methods by Modulating Bacterial Level

[0032] The discovery by the present inventors reveals the direct correlation between an individual's prospect of bodyweight reduction and the presence and relative abundance of certain bacterial species (*e.g.*, those shown in Table 1 or 2) in the individual's GI tract. This revelation enables different methods for treating overweight/obese individuals for weight loss, for treating or reducing the risk of obesity or T2D, especially for aiding those who have already failed to achieve bodyweight reduction in one or more previous FMT efforts, by adjusting or modulating the level of these bacterial species in these individuals' GI tract via, *e.g.*, a subsequent FMT procedure or an alternative means, to deliver to the patients' GI tract an effective amount of one or more of the bacterial species of those shown in Table 1.

[0033] When a proposed FMT donor whose stool is tested and found to contain an insufficient level of one or more of the beneficial bacterial species such as those shown in Table 1 (*e.g.*, each is less than about 0.01%, 0.05%, 0.10%, 0.20%, 0.40%, 0.50%, 0.80%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, or 8.0% of total bacteria in the stool sample), the proposed donor is deemed as an unsuitable donor for FMT intended to treat overweight/obese individuals for the purpose of successful bodyweight control or reduction. Otherwise he may be disqualified as a donor in favor of another individual whose stool sample exhibits a more favorable bacterial profile, and his fecal material should not be immediately used for FMT due to the lack of prospect of conferring such beneficial health effects unless the stool material is adequately modified. In these cases of expected lack of weight loss benefits from FMT treatment can be readily improved in view of the inventors' discovery, for example, one or more of the bacterial species such as those shown in Table 1 may be introduced from an exogenous source into a donor fecal material so that the level of the bacterial species in the fecal material is increased (*e.g.*, to reach at least about 0.01%, 0.02%, 0.05%, 0.10%, 0.20%, 0.40%, 0.50%, 0.60%, 0.80%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 8.5%, 9.0%, or 10% of total bacteria in the fecal material) before it is processed for use in FMT for the treatment of overweight or obese individuals for the purpose of bodyweight reduction.

[0034] As an alternative, the beneficial bacterial species (one or more of those shown in Table 1) may be obtained from a bacterial culture in a sufficient quantity and then formulated into a suitable composition, which is without any fecal material taken from a donor, for delivery into an overweight/obese patient's gut. Similar to FMT, such composition can be introduced into a patient by oral, nasal, or rectal administration.

[0035] Immediately upon completion of the step of introducing an effective amount of the desired bacterial species into a patient's GI tract (*e.g.*, via an FMT procedure), the recipient may be further monitored by continuous testing of the level or relative abundance of the bacterial species in the stool samples on a daily basis for up to 5 days post-procedure while the patient's bodyweight as well as the general health status of the patient are also being monitored in order to assess treatment outcome and the corresponding levels of relevant bacteria in the recipient's GI tract: the level of bacterial species (one or more of those shown in Table 1) may be monitored in connection with observation of health benefits achieved in association with bodyweight reduction such as improvement in blood glucose, cholesterol, and triglyceride levels.

IV. Assessing Weight Loss Prospect and Corresponding Treatment

[0036] The present inventors also discovered that the altered level of certain bacterial species can indicate the prospect or likelihood of success in one's weight loss effort such as by way of FMT treatment: they revealed the correlation between increased level of certain bacterial species (*e.g.*, those shown in Table 1) in individuals' stool samples and FMT-induced weight loss in these patients. Further, the level or relative abundance of certain bacterial species (such as one or more of the species shown in Table 2) have been revealed to indicate an individual's prospect or likelihood for successful weight loss in a subsequent FMT treatment scheme when properly calculated using certain specified mathematic tools.

[0037] For example, when stool samples taken from two or more individuals, the level or relative abundance of bacterial species in Table 1 or 2 in the samples may be determined, for example, by PCR especially quantitative PCR. For the bacterial species listed in Table 1, a lower level found in a patient's stool sample indicates a lower likelihood for the patient to achieve FMT-induced weight loss; conversely, a higher level indicates a higher likelihood of FMT-induced weight loss in the individual. In the event that the level of multiple species (*e.g.*, those listed in Table 1 or 2) are measured and compared, the determination of the

likelihood of weight loss success is made based on the indication from the majority of the pertinent bacterial species measured.

[0038] Once the weight loss prospect assessment is made, for example, an overweight or obese individual is deemed to have a reasonable expectation of successfully achieve weight loss such as by FMT treatment, appropriate treatment steps can be taken as a measure to achieve this goal. For example, the patient may be given compositions that comprise an effective amount of one or more of the bacterial species listed in Table 1 either by FMT or by an alternative administration method, such that the bacterial profiled in the patient's GI tract will be modified to one that is favorable for the outcome of bodyweight reduction.

V. Kits and Compositions for Use in Bodyweight Control Treatment

[0039] The present invention also provides novel kits and compositions that can be used for facilitation of patient weight loss, for treating or reducing risk for obesity or T2D, or for assessing a patient's likelihood of successful weight loss by way of FMT treatment. For example, a kit is provided that comprises a first container containing a first composition comprising (i) an effective amount of one of the bacterial species set forth in Table 1, and (ii) an effective amount of another, different bacterial species set forth in Table 1. In some variations, the first and/or second composition may contain two of the bacterial species of Table 1.

[0040] In some cases, the first composition comprises a fecal material from a donor, which has been processed, formulated, and packaged to be in an appropriate form in accordance with the delivery means in the FMT procedure, which may be by direct deposit in the recipient's lower gastrointestinal track (*e.g.*, wet or semi-wet form) or by oral ingestion (*e.g.*, frozen, dried/lyophilized, encapsulated). Alternatively, the first composition may not contain any donor fecal material but is an artificially mix containing the preferred bacterial species, such as one or more of the bacterial species set forth in Table 1, at an appropriate ratio and quantity. The first composition is formulated and packaged in accordance with its intended means of delivery to the patient, for example, by oral ingestion, nasal delivery, or rectal deposit.

[0041] Similarly, the second composition may be formulated from donor fecal material or other non-fecal originated material for oral, nasal, or rectal delivery. Typically, the second composition contains a bacterial species or a combination of bacterial species different from

that comprised in the first composition. The first and second compositions may or may not be formulated for the same delivery method or route.

[0042] The first and second compositions are typically kept separately in two different containers in the kit. In some cases, the first and second compositions may be combined in a single composition so that they can be administered to the patient together, for example, by oral or local delivery, at the same time.

[0043] Lastly, a kit is provided for the quantitative detection of one or more bacterial species such as the bacterial species set forth in Table 1 or 2. The kit comprises reagents for quantitative detection of each of the bacterial species, for example, such reagents may comprise a set of oligonucleotide primers for the amplification, such as polymerase chain reaction (PCR) especially quantitative PCR, of a polynucleotide sequence derived from, and preferably unique to, each one of the pertinent bacterial species (such as any one or more of the bacterial species set forth in Tables 1-2).

EXAMPLES

[0044] The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially the same or similar results.

BACKGROUND

[0045] The purpose of this study is to determine how human gut bacteriome is associated with weight loss following fecal microbiota transplantation (FMT). The practical use of the invention includes improving human health and opposing disease risks associated with obesity by modulating human gut bacteriome. These measures may include FMT with optimized protocols, synthetic bacterial species supplementation, countermeasures for clearing microorganisms to modulate obese related diseases. These findings promote development microbial product and add up a set of criteria for establishment of stool bank and its derived products in diagnostics and therapeutics.

Example 1: Identification of potential probiotics in a randomized placebo-controlled trial of fecal microbiota transplantation in subjects with obesity and diabetes mellitus

METHODS

Study Design

[0046] A randomized placebo-controlled study of fecal microbiota transplant (FMT) was conducted in obese subjects with type 2 diabetes mellitus (NCT03127696). Consented subjects who fulfil eligibility criteria in Prince of Wales Hospital were recruited. Inclusion criteria include age between 18 to 70, BMI ≥ 28 kg/m² and < 45 kg/m²; and having a diagnosis of Type 2 diabetes mellitus for ≥ 3 months. Exclusion criteria include current pregnancy, use of any weight loss medications in the preceding 1 year, known history or concomitant significant gastrointestinal disorders (including Inflammatory Bowel Disease, current colorectal cancer, current GI infection), known history or concomitant significant food allergies, immunosuppressed subjects, known history of severe organ failure (including decompensated cirrhosis), inflammatory bowel disease, kidney failure, epilepsy, acquired immunodeficiency syndrome, current active sepsis, active malignant disease in recent 2 years, known contraindications to oesophago-gastro-duodenoscopy (OGD), use of probiotic or antibiotics in recent 3 months, on Sodium-glucose co-transporter-2 inhibitors or Glucagon-like peptide-1 receptor agonists at randomization, or on Proton-pump inhibitor at randomization. Subjects were randomized to 3 arms in 1:1:1 ratio (Arm 1: FMT and lifestyle intervention, Arm 2: FMT alone, Arm 3: Sham and lifestyle intervention) (Figure 1).

Prohibited Medications

[0047] No antibiotics, probiotic or prebiotic preparations, Sodium-glucose co-transporter-2 (SGLT2) inhibitors, Glucagon-like peptide-1 (GLP-1) receptor agonists or Proton-pump inhibitor (PPI) were permitted during the study.

[0048] Subjects with intake of prohibited medication during study period were allowed to remain in study and outcome were assessed. The reported intake of prohibited medication were recorded and documented.

Fecal microbiota transplant (FMT) / sham infusion

[0049] Subjects received FMT / Sham infusion 4 times at week 0, 4, 8, and 12 and were followed up until week 52. Each time, 100-200ml of FMT / sham solution were infused over 2-3 minutes into the distal duodenum or jejunum via OGD. FMT and sham solution were prepared as follow.

[0050] FMT: Frozen stool from donors of stool bank were used. For each FMT, FMT solution were infused using stool from single donor or mixing of stool from multiple donors. FMT solution were prepared by diluting feces with sterile saline (0.9%). This solution were blended and strained with filter. The resulting supernatant were then stored as frozen FMT solution for later use.

[0051] Sham: Sterile saline (0.9%) were used as sham.

Stool donors of stool bank of the Chinese University of Hong Kong

[0052] Donors (BMI < 23kg/m²) were volunteers from general population including spouses or partners, first-degree relatives, other relatives, friends and others who are known or unknown to the potential subjects that met eligibility criteria will be invited for screening laboratory test. A series of laboratory tests for infectious diseases and interviews were done. Stool from the eligible donors were used in this study. Subjects may receive stools from single or multiple donors whose identity may not be made available to the subjects.

Lifestyle Intervention

[0053] Subjects, randomized into either lifestyle intervention (LSI) or combined FMT and lifestyle intervention arms, received seven individual dietitian-led consultation sessions in 12-week treatment period. They were scheduled to attend the sessions at Prince of Wales Hospital at baseline, week 1, 2, 4, 6, 8 and 12.

[0054] The lifestyle intervention principally targeted at body weight loss via decreasing calorie intake while enhancing energy expenditure. In addition to diet, it emphasized on lifestyle and behavioral changes facilitating sustainable weight loss effect. During the first consultation session at baseline, an approximately 1 hour comprehensive assessment regarding medical history, dietary and lifestyle habits and behaviors, knowledge on diet-disease relationships, motivation of lifestyle changes was conducted by the dietitian who then discussed with subjects the weight goals for the treatment period, and individualized dietary and lifestyle advice to attain the goals. During subsequent 20-minute follow-up sessions, dietitian reviewed the compliance of dietary and lifestyle advice negotiated previously and provided further recommendation accordingly.

[0055] A personalized diet plan was given to each subject at the first session, based on the recommendations of the American Dietetic Association [1]. The diet plan was nutritionally balanced with emphasis on adequate fruits and vegetables, moderate carbohydrate, low fat

and glycemic index (GI) food options. A set of 2 booklets containing the diet plan, food portion exchanges, general tips for eating out was given to each subject for reference.

[0056] Other than practical dietary and lifestyle advice, multiple approaches were employed to foster sustainable healthier behavioral changes during the intervention period. Subjects were advised to record their diet and physical activity daily prior to each review session to develop self-monitoring behavior, while negotiated strategies to deal with ‘at-risk’ scenarios such as stress situations, travelling, festive and party eating. Recipes with simple instructions were also given to subjects where appropriate to encourage healthy cooking.

[0057] The adherence of lifestyle intervention was revealed by the percentage attendance of dietitian sessions in 12-week treatment period and the weekly dietary and lifestyle record at each follow-up session.

Fecal DNA extraction and DNA sequencing

[0058] Fecal DNA was extracted by using Maxwell® RSC PureFood GMO and Authentication Kit (Promega) with modifications to increase the yield of fungal DNA. Approximately 100 mg from each stool sample was prewashed with 1 ml ddH₂O and pelleted by centrifugation at 13,000×g for 1 min. The pellet was resuspended in 800 µL TE buffer (pH 7.5), supplemented with 1.6 µl 2-mercaptoethanol and 500 U lyticase (Sigma), and incubated at 37 °C for 60 min. The sample was then centrifuged at 13,000×g for 2 min and the supernatant was discarded. After this pretreatment, DNA was subsequently extracted from the pellet using a Maxwell® RSC PureFood GMO and Authentication Kit (Promega) following manufacturer’s instructions. Briefly, 1 ml of CTAB buffer was added to the pellet and vortexed for 30 s, then the solution heated at 95°C for 5 min. After that, samples were vortexed thoroughly with beads (Biospec, 0.5mm for fungi and 0.1mm for bacteria, 1:1) at maximum speed for 15 min. Following this, 40 µl proteinase K and 20 µl RNase A were added and the mixture Incubated at 70°C for 10 min. The supernatant was then obtained by centrifuging at 13,000×g for 5 min and placed in a Maxwell® RSC instrument for DNA extraction. The extracted fecal DNA was used for ultra-deep metagenomics sequencing via Illumina Novoseq 6000 (Novogen, Beijing, China). An average of 52 ± 6.3 million reads (12G clean data) per sample were obtained.

Quality trimming of raw sequences

[0059] Raw sequence reads were filtered and quality-trimmed using *Trimmomatic v0.36* [2] as follows: 1) Trimming low quality base (quality score < 20); 2) Removing reads shorter

than 50bp; 3) removing sequences less than 50 bp long; 3) Tracing and cutting off sequencing adapters. Contaminating human reads were filtering using *Kneaddata* (Reference database: *GRCh38 p12*) with default parameters.

Profiling of the bacterial microbiome

[0060] Profiling of bacterial microbiome (bacteriome) was performed via *MetaPhlAn2* by mapping reads to clade-specific markers [3] and annotation of species pangenomes through *Bowtie2* [4]. The result bacterial species abundance table was used to correlate to the blood parameters. Pearson correlation and spearman correlations and *P* values were calculated using *cor* and *cor.test* functions in R and visualized using the *ggplot2*.

Clinical Laboratory Test

[0061] Fasting blood samples were collected before FMT and at week 4, 12, 16, 20 and 24, which were tested complete blood count (CBC), renal function test (RFT), liver function test (LFT), C-reactive protein (CRP), magnesium, glucose, insulin (only before FMT and week 24), total cholesterol, low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol, triglycerides and haemoglobin A1c (HbA1C).

Statistical Methods

[0062] The statistical analyses were done using SPSS. Repeated measure ANOVA was used to determine the difference between baseline, week 12, week 16, week 20, and week 24 follow-up within the three treatment arms. The difference between baseline and each time points was calculated. Post-hoc analysis was done using Bonferroni test. *p*-value of <0.05 was considered significant.

RESULTS

Bacterial species associated with FMT-induced weight loss

[0063] 61 subjects were recruited and randomly assigned to FMT plus lifestyle intervention (FMT plus LSI; n=21), FMT alone (n=20) and sham and lifestyle intervention (LSI; n=20). Four subjects withdrew from the study.

[0064] While there was no significant change in bacterial diversity between treatment groups, bacterial richness significantly increased in FMT plus LSI arm at week 4 and week 16 after intervention (*p*<0.01 and *p*=0.039, Wilcoxon signed-rank test). Both viral diversity and richness increased in FMT alone and FMT plus LSI groups after treatment (*p*<0.05, Wilcoxon signed-rank test). No significant alteration in bacterial and viral richness was seen

in the sham plus LSI group. Bacterial and viral richness in the FMT groups were significantly higher than sham plus LSI group after treatment ($p < 0.0001$, repeated measures ANOVA). The euclidean distance between baseline and post-treatment samples in FMT alone and FMT plus LSI groups were significantly higher than that of sham plus LSI group for both bacteriome and virome ($p < 0.001$ and $p < 0.05$, respectively, Wilcoxon rank sum test), indicating FMT had a greater impact on microbiota than lifestyle intervention. Concurrently, the relative abundance of 27, 26 and 6 bacterial species were significantly altered in FMT plus LSI group, FMT alone and sham plus LSI alone group post intervention, respectively (LDA > 2 , adjusted $p < 0.05$). Subjects in both FMT groups had a significant increase in several **butyrate-producing bacteria**, including *Faecalibacterium prausnitzii*, *Roseburia hominis*, *Collinsella tanakaei*, and *Prevotella copri*, and reduction of *Flavonifractor plautii*, *Clostridiales bacterium1_7_47FAA*, *Clostridium clostridioforme* and *Fusobacterium ulcerans* which was not seen in the sham plus LSI group. Following treatment, the butyrate producing bacteriome profile in FMT groups resembled that of the donors while sham plus LSI group showed no obvious shift in bacteria. The relative abundance of several microbiota functional pathways was increased after FMT, compared with their respective baseline samples. Fecal microbiota in the FMT alone and FMT plus LSI groups showed decreased level of amino acid degradation, secondary metabolite degradation, increased level of secondary metabolite biosynthesis, non-carbon nutrients degradation, L-Ornithine biosynthesis, increased level of carbohydrates degradation, vitamin biosynthesis, nucleotide degradation and amino acid biosynthesis, compared with their baseline fecal samples (LDA > 2 , adjusted $p < 0.05$). In the sham plus LSI group, no significant changes in microbiota functional pathways was observed.

[0065] Although 20% (4 out of 20) of the subjects receiving placebo infusion with lifestyle intervention had over 5% weight loss at week 24, all of them regained weight after intervention stopped. None of them had over 10% weight loss, while 13.5% (5 out of 37) recipients achieved over 10% weight loss during the 52-week study period (**Figure 2**). This indicates that the effect of FMT induced weight loss is more persistent than lifestyle intervention. Therefore, change of gut microbiome played a role in the FMT-induced weight loss. Shot gun metagenomic sequencing revealed that in FMT responders this post-FMT compositional change was mostly dominated by an expansion of butyrate-producing bacteria (BPB) including *Anaerostipes hadrus*, *Collinsella tanakaei*, *Roseburia hominis*, while no

significant change in LSI arm (**Figure 3**), indicating that these species played a role in the FMT-induced weight loss.

Table 1 Bacterial species associated with FMT-induced weight loss regardless of calories intake

Bacterial Species	NCBI:txid
<i>Anaerostipes hadrus</i>	649756
<i>Collinsella tanakaei</i>	626935
<i>Roseburia hominis</i>	301301

[0066] As such, the bacterial species listed in Table 1 can be administered to subjects for weight reduction.

Example 2: Machine Learning Model to Predict Success Rate of FMT on Weight Reduction

METHODS

Machine Learning Model

[0067] Random forest (RF) was chosen to build prediction model of successful FMT on weight reduction using fecal microbes because of its superior performance for classification with binary features. Random Forest [5] is one of the most popular approaches in metagenomic data analysis to identify the discriminative features and build prediction models. As a widely used ensemble learning algorithm, Random Forest consists of a series of classification and regression trees (CARTs) to form a strong classifier. A subset of data randomly sampled from the original dataset with replacement is known as bootstrap sampling, applying to build the trees. When the training dataset for the current tree is drawn by the bootstrap method, $1 - \left(1 - \frac{1}{N}\right)^N$ observations are left out from the overall dataset. With infinite N, there are 36.8% data not occurred in the training samples called out-of-bag (OOB) observations, which would not be used for constructing the trees. In addition, extra randomness introduced to the random forest as each decision tree splits nodes based on a random subset of features selected from the overall features. The features with the least Gini (Gini are used to evaluate the purity of the node) would be utilized to split the nodes in each iteration to generate the trees. With different subsets of data and features, the algorithm is able to train different trees and obtain the final classification by averaging the result from the tree models. In addition to the prediction model, Random Forest has the capability to assess the importance of variables [6]. The OOB observations are used to estimate the classification error for each tree in the forest. To measure the importance of a given variable, the values of

the variable in the OOB data are randomly altered, and then the changed OOB data is used to generate new predictions. The difference of the error rate between the altered and the original OOB observations divided by the standard error is calculated as the importance of a variable. To classify a new sample, the features of the sample passed down to each tree to estimate the probability for classification. The Random Forest used the average probability of all trees to determine the final result of the classification.

[0068] The model was built with a group with no weight loss (n=18) and a group with weight loss (n=19) between baseline and week 24. The importance value of each species to the classification model was evaluated by recursive feature elimination. According to descending importance value, the selected species were added one by one to the random forest model if its Pearson correlation value with any already existing probe in the model was <0.7. Each time a new feature was added to the model, the performance of the model was re-evaluated using 10-fold cross-validation. These models were compared in terms of binary classifiers with Area Under the Curve (AUC) in Receiver Operating Characteristic (ROC) curves. The final model was chosen when best accuracy and kappa were achieved. These analysis was done using R packages randomForest v4.6-14 [5] and pROC v1.15.3 [7].

RESULTS

[0069] The microbiome composition at baseline was assessed between subjects who had weight loss (FMT responders; n=19) and those without weight loss (FMT non responders; n=18) at week 24 compared to baseline. A random forest model based on baseline relative abundance of 5 species (*Desulfovibrio desulfuricans*, candidate division TM single-cell isolate TM7b, *Bacteroides stercoris*, *Weissella cibaria* and *Bacteroides dorei*), all more abundant in FMT responders (**Figure 4a**) achieved a good prediction with receiver operating characteristic [ROC] AUC 0.912 in predicting response upon lean donor FMT (**Figure 4b**). Notably, all 5 subjects with over 10% weight loss during the 52-week study period were classified as “responder” by the prediction model.

Table 2 Bacterial species included in the machine learning model for prediction of FMT success rate of weight reduction

Bacterial Species	NCBI:txid
candidate division TM7 single-cell isolate TM7b	447455
<i>Bacteroides stercoris</i>	46506
<i>Bacteroides dorei</i>	357276

[0070] Thus, to determine the FMT success rate of weight reduction in a subject, the following steps will be carried out:

1. Obtain a set of training data by determine the relative abundance of species (*e.g.*, any 2 or 3 of the species) selected from Table 2 in a cohort of FMT recipients with no weight loss and with weight loss at baseline before receiving FMT;
2. Determine the relative abundance of these species in the subject whose FMT success rate is to be determined;
3. Compare the relative abundance of these species in the subject with the training data using random forest model; and
4. Decision trees will be generated by random forest from the training data. The relative abundances will be run down the decision trees and generate a risk score. If more than 50% trees in the model consider the subject will have weight loss after FMT, the outcome will be “the subject being tested is deemed to have a high success rate of weight loss after receiving FMT”. If less than 50% trees in the model consider the subject as no weight loss after FMT, the outcome will be “the subject being tested is deemed to have a low success rate of weight loss after receiving FMT”.

[0071] Species selected from Table 2 may be any 2 or 3 of the species, for example:

1. candidate division TM7 single-cell isolate TM7b + *Bacteroides dorei* + *Bacteroides stercoris* (AUC: 93%, light blue; Figure 4)
2. *Bacteroides dorei* + *Bacteroides stercoris* (AUC: 73.7%, red; Figure 4)
3. candidate division TM7 single-cell isolate TM7b + *Bacteroides stercoris* (AUC: 78.4%, green; Figure 4)
4. candidate division TM7 single-cell isolate TM7b + *Bacteroides dorei* (AUC: 81.3%, dark blue; Figure 4)

Study I

[0072] The relative abundance of 3 species listed in Table 2 from FMT recipients with no weight loss (n=18) and with weight loss (n=19) was determined by metagenomics sequencing and taxonomy assigned as described in methods (relative abundance listed in Table 3).

Decision trees were generated by random forest from data in Table 3 with parameter: trees=801, mtry=2.

[0073] The likelihood of another obese subject with T2DM was determined. The relative abundance of the 5 species listed in Table 3 in fecal sample of this subject was determined by metagenomics sequencing and taxonomy assigned as described in method (Table 4). The relative abundances were run down the decision trees and a risk score was generated. The score of the subject was 0.57 (Figure 5a), and therefore the subject was deemed to be likely to lose weight after FMT.

Study II

[0074] The relative abundance of 3 species listed in Table 2 from FMT recipients with no weight loss (n=18) and with weight loss (n=19) was determined by metagenomics sequencing and taxonomy assigned as described in methods (relative abundance listed in Table 3). Decision trees were generated by random forest from data in Table 3 with parameter: trees=801, mtry=2.

[0075] The likelihood of another obese subject with T2DM was determined. The relative abundance of the 5 species listed in Table 3 in fecal sample of this subject was determined by metagenomics sequencing and taxonomy assigned as described in method (Table 4). The relative abundances were run down the decision trees and a risk score was generated. The score of the subject was 0.11 (Figure 5b), and therefore the subject was deemed to be likely to lose weight after FMT.

Table 3 Relative abundance of species listed in Table 2 in FMT recipients with no weight loss (n=18) and with weight loss (n=19)

group	candidate division TM7 single-cell isolate TM7b	Bacteroides stercoris	Bacteroides dorei
responder	0	0.2151	1.71892
responder	0	0.44262	0.00157
non_responder	0	0	0
responder	0	0.63074	2.92262
non_responder	0	0.19493	0.02882
responder	0	0.14545	0.05869
responder	0	1.03775	0
non_responder	0	0.12171	0
non_responder	0	0.32889	0.02335
responder	0.00122	0.02669	0.00795

non_responder	0	0.26171	0.0928
responder	0.00356	0	1.62989
responder	0.00171	0.014	0.1802
non_responder	0	0.24599	0
responder	0.00157	2.15287	9.15962
non_responder	0	0	0.00246
non_responder	0	0.0277	0
non_responder	0	0.05174	0.13443
non_responder	0	0.12214	0.00608
responder	0	0.67459	1.42752
responder	0	0.15941	0.29319
non_responder	0	0.00224	0.00041
responder	0.00305	0.01253	0
non_responder	0	0	0.13803
responder	0	0.43893	0.02083
non_responder	0	0.00491	0
non_responder	0	0.04157	6.15639
responder	0	0	0.95711
non_responder	0	0.01345	0.06015
responder	0.00508	0.91856	0
responder	0.00737	0.1094	0.28737
responder	0	2.35214	0.02574
non_responder	0	0.32019	0
responder	0.00541	0	0.00112
non_responder	0	0.17835	0
responder	0	0.01597	0.3372
non_responder	0	0.13342	0.00379

Table 4 Relative abundance of species listed in Table 2 in FMT recipients with no weight loss (n=18) and with weight loss (n=19)

group	candidate division TM7 single-cell isolate TM7b	Bacteroides stercoris	Bacteroides dorei
Study I	0	0	0.406
Study II	0	0.00161	0.00597

[0076] All patents, patent applications, and other publications, including GenBank Accession Numbers and the like, cited in this application are incorporated by reference in the entirety for all purposes.

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WHAT IS CLAIMED IS:

1. A method for reducing bodyweight in a subject, comprising introducing into the subject's gastrointestinal tract an effective amount of one or more bacterial species of *Anaerostipes hadrus*, *Collinsella tanakaei*, and *Roseburia hominis*.
2. The method of claim 1, wherein the introducing step comprises oral administration to the subject a composition comprising an effective amount of the one or more of the bacterial species.
3. The method of claim 1, wherein the introducing step comprises delivery to the small intestine, ileum, or large intestine of the subject a composition comprising an effective amount of the one or more of the bacterial species.
4. The method of claim 1, wherein the introducing step comprises fecal microbiota transplantation (FMT).
5. The method of claim 4, wherein the FMT comprises administration to the subject a composition comprising processed donor fecal material.
6. The method of claim 2, wherein the composition is orally administered.
7. The method of claim 2, wherein the composition is directly deposited to the subject's gastrointestinal tract.
8. The method of claim 1, wherein the level or relative abundance of the one or more of the bacterial species is determined in a first stool sample obtained from the subject prior to the introducing step and in a second stool sample obtained from the subject after the introducing step.
9. The method of claim 8, wherein the level of the one or more of the bacterial species is determined by quantitative polymerase chain reaction (PCR).
10. A kit for reducing bodyweight, comprising: a first container containing a first composition comprising an effective amount of one of the bacterial species of *Anaerostipes hadrus*, *Collinsella tanakaei*, or *Roseburia hominis*, and a second container

containing a second composition comprising an effective amount of another one of *Anaerostipes hadrus*, *Collinsella tanakaei*, or *Roseburia hominis*.

11. The kit of claim **10**, wherein the first composition comprises processed donor fecal material for FMT.

12. The kit of claim **10** or **11**, wherein the first composition is formulated for oral administration.

13. The kit of claim **10**, wherein the second composition is formulated for oral administration.

14. The kit of claim **11**, wherein both the first and second compositions are formulated for oral ingestion.

15. A method for determining likelihood of weight reduction by FMT in a subject, comprising:

(1) determining, in a stool sample from the subject, the level or relative abundance of one or more of the bacterial species set forth in Table 2;

(2) determining the level or relative abundance of the same bacterial species in a stool sample from a reference cohort comprising subjects who had weight loss after FMT and subject with no weight loss after FMT;

(3) generating decision trees by random forest model using data obtained from step (2) and running the level or relative abundance of one or more of the bacterial species from step (1) down the decision trees to generate a score; and

(4) determining the subject with a score greater than 0.5 as likely to achieve weight loss by FMT and determining the subject with a score no greater than 0.5 as unlikely to achieve weight loss by FMT.

16. The method of claim **15**, wherein the one or more bacterial species comprise any two or three bacterial species set forth in Table 2.

17. A kit for assessing likelihood of weight reduction by FMT in a subject, comprising reagents for detecting one or more of the bacterial species set forth in Table 2.

18. The kit of claim **17**, wherein the reagents comprise a set of oligonucleotide primers for amplification of a polynucleotide sequence from any one of the bacterial species set forth in Table 2.

19. The kit of claim **18**, wherein the amplification is PCR.

20. The kit of claim **19**, wherein the PCR is quantitative PCR.

21. A method for reducing the risk of obesity and type 2 diabetes (T2D) or treating obesity and T2D in a subject, comprising introducing into the subject's gastrointestinal tract an effective amount of *Anaerostipes hadrus* or *Roseburia hominis*.

22. The method of claim **21**, wherein the introducing step comprises oral administration to the subject a composition comprising an effective amount of *Anaerostipes hadrus* or *Roseburia hominis*.

23. The method of claim **21**, wherein the introducing step comprises delivery to the small intestine, ileum, or large intestine of the subject a composition comprising an effective amount of *Anaerostipes hadrus* or *Roseburia hominis*.

24. The method of claim **21**, wherein the introducing step comprises fecal microbiota transplantation (FMT).

25. The method of claim **24**, wherein the FMT comprises administration to the subject a composition comprising processed donor fecal material.

26. The method of claim **21**, wherein the composition is orally administered.

27. The method of claim **22**, wherein the composition is directly deposited to the subject's gastrointestinal tract.

28. The method of claim **21**, wherein the level or relative abundance of *Anaerostipes hadrus* or *Roseburia hominis* is determined in a first stool sample obtained from the subject prior to the introducing step and in a second stool sample obtained from the subject after the introducing step.

29. The method of claim **28**, wherein the level of *Anaerostipes hadrus* or *Roseburia hominis* is determined by polymerase chain reaction (PCR), preferably quantitative polymerase chain reaction (qPCR).

30. A kit for assessing risk of obesity and type 2 diabetes (T2D) in a subject or for assessing whether a subject has microbiome-dependent obesity and T2D, comprising reagents for detecting one or more of the bacterial species *Anaerostipes hadrus* and *Roseburia hominis*.

31. The kit of claim **30**, wherein the reagents comprise a set of oligonucleotide primers for amplification of a polynucleotide sequence from any one of the bacterial species *Anaerostipes hadrus* and *Roseburia hominis*.

32. The kit of claim **31**, wherein the amplification is PCR.

33. The kit of claim **32**, wherein the PCR is quantitative PCR.

34. The kit of claim **30**, wherein the bacterial species is *Roseburia hominis*.

Figure 1

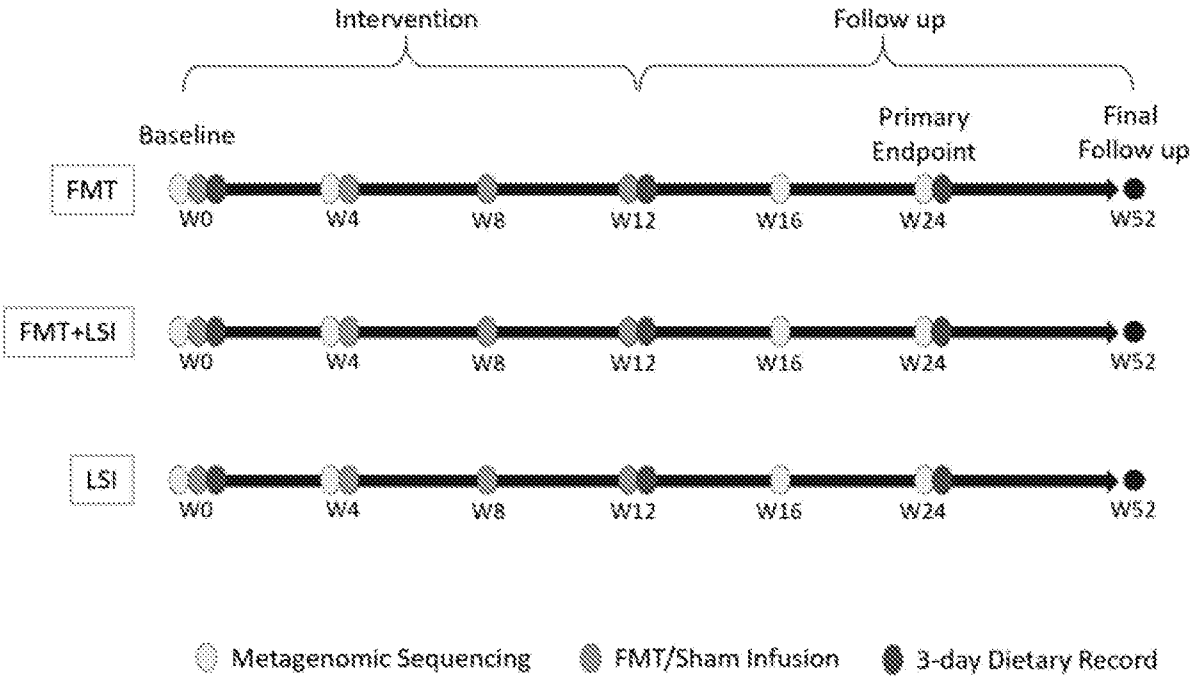


Figure 2

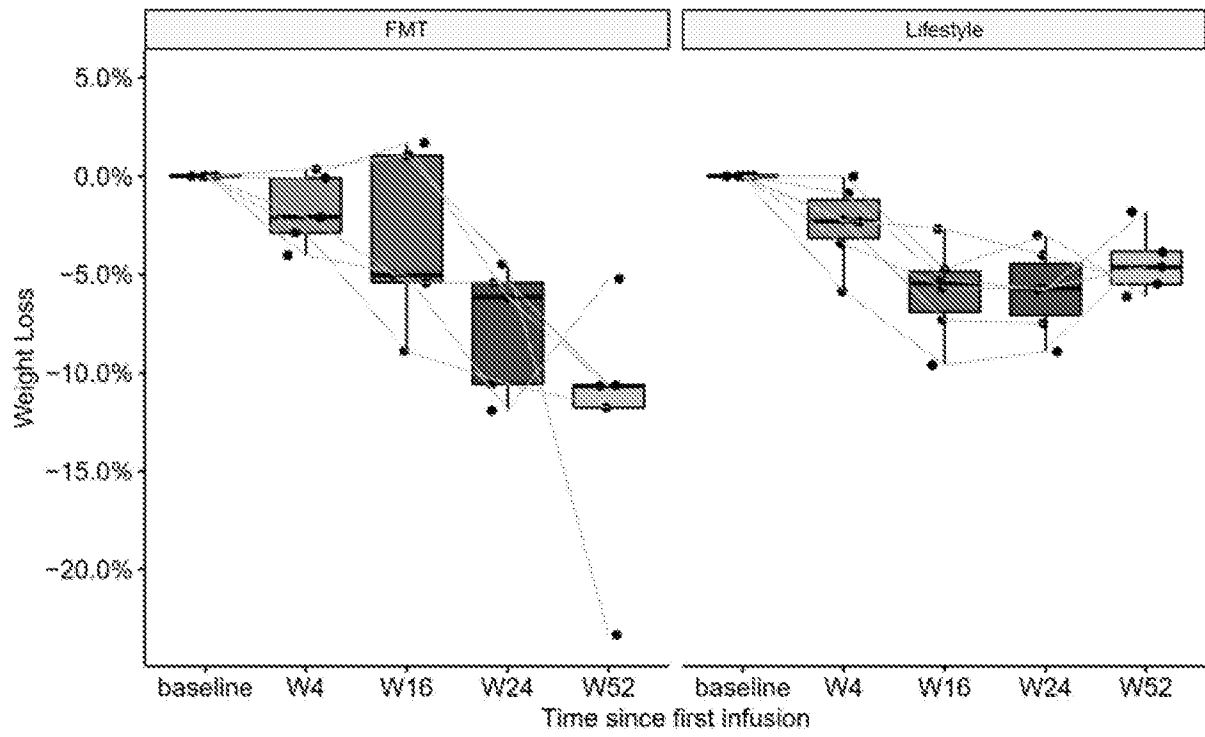


Figure 3a

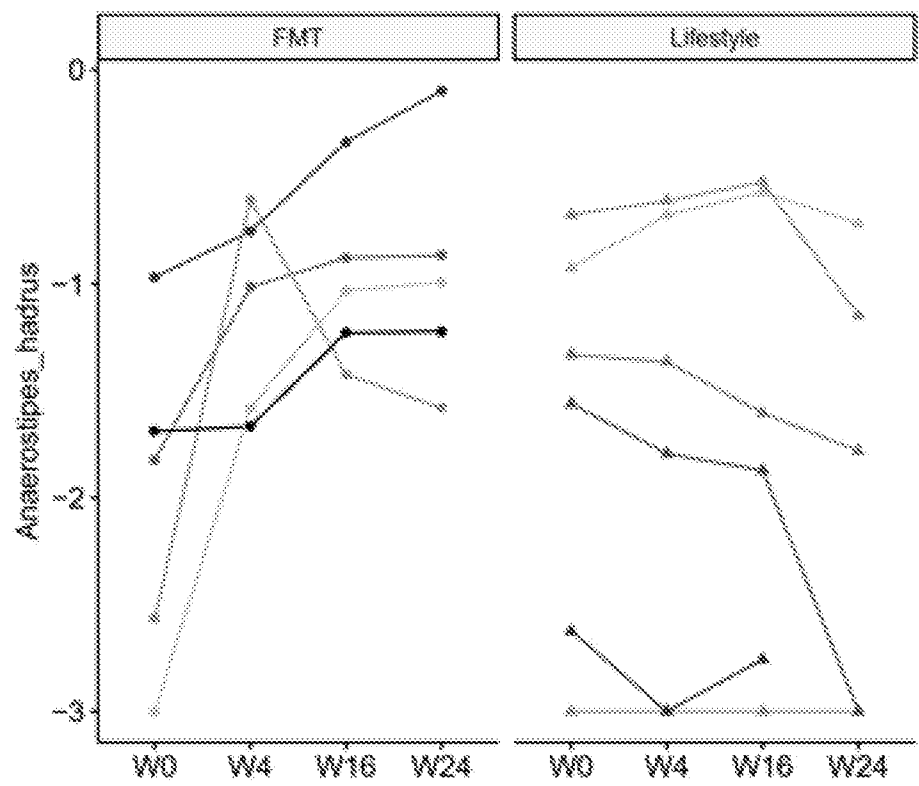


Figure 3b

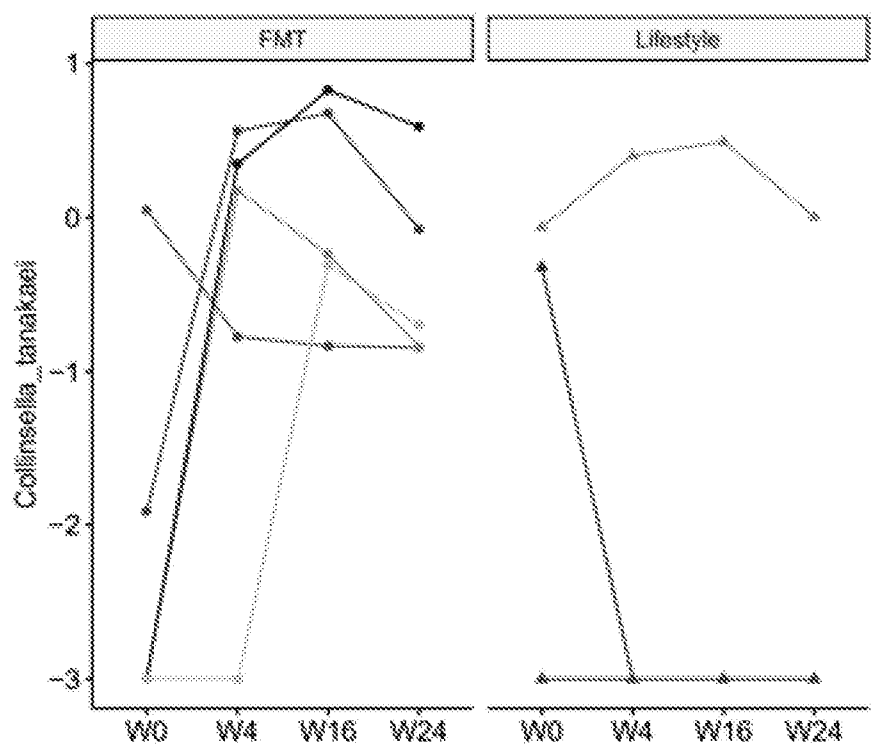


Figure 3c

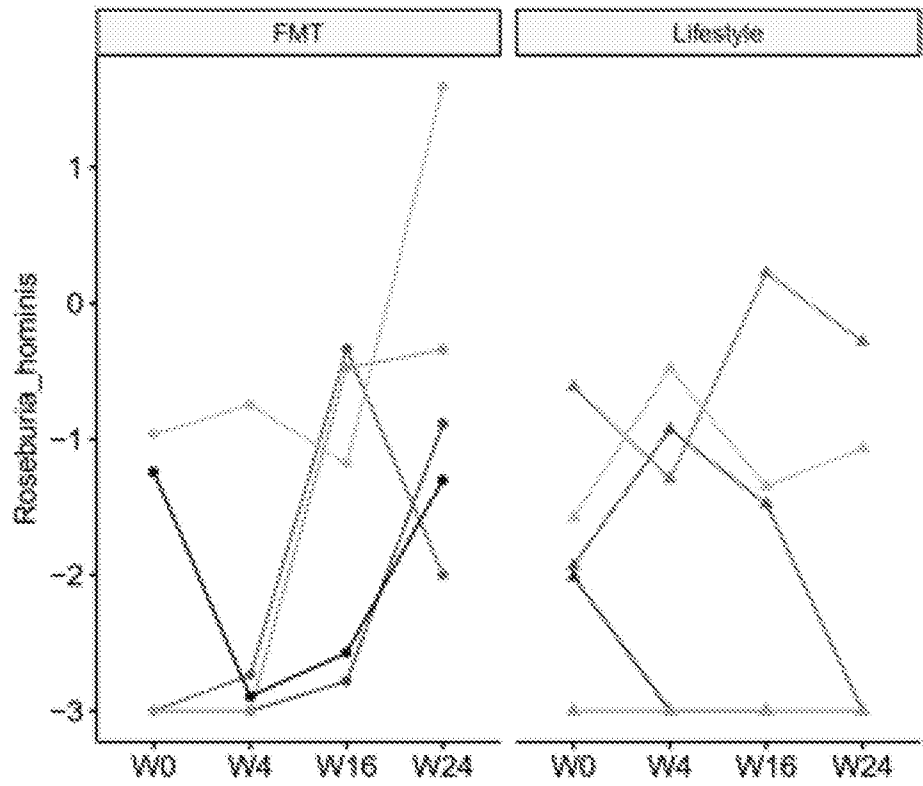


Figure 4a

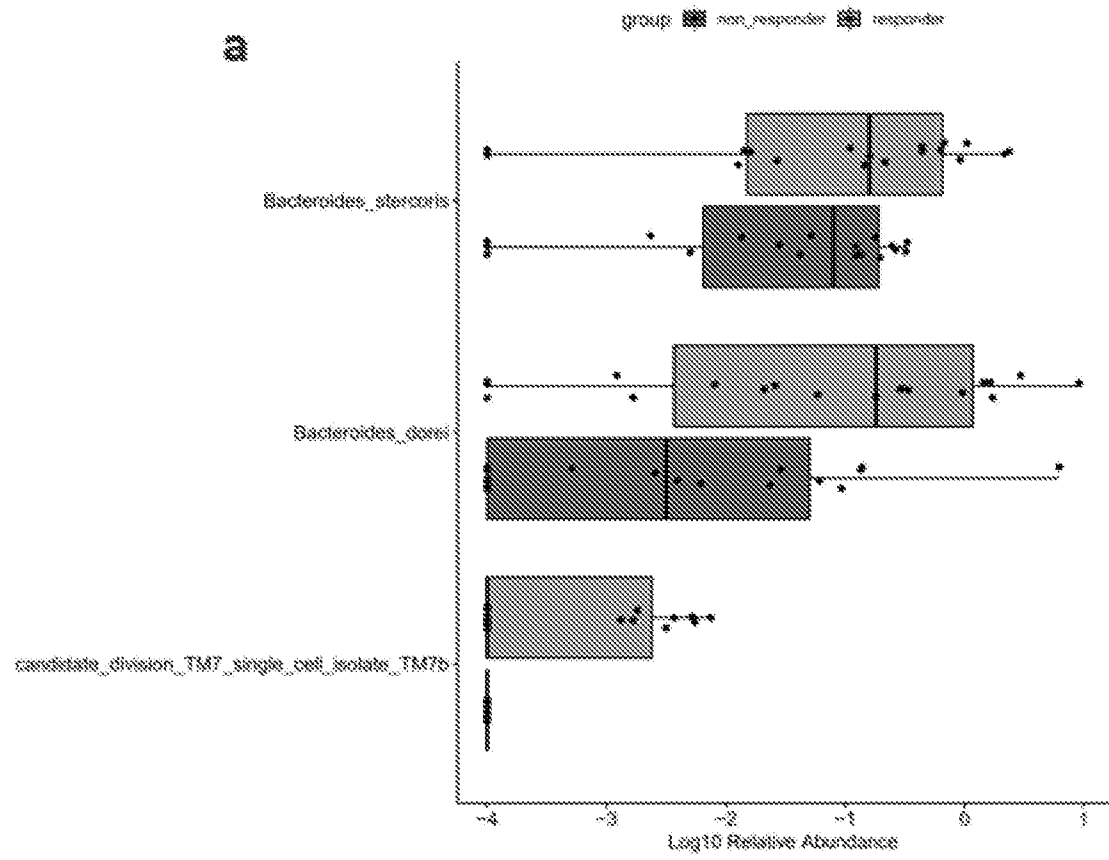


Figure 4b

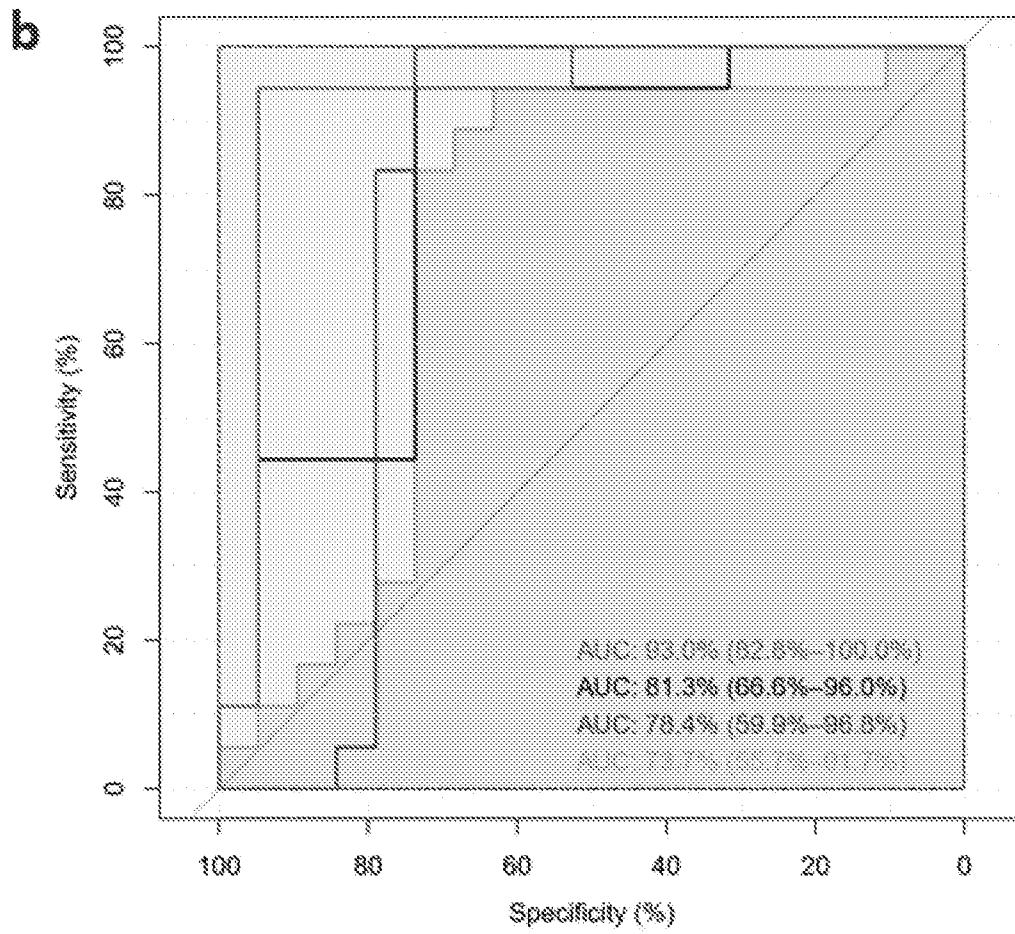


Figure 5a

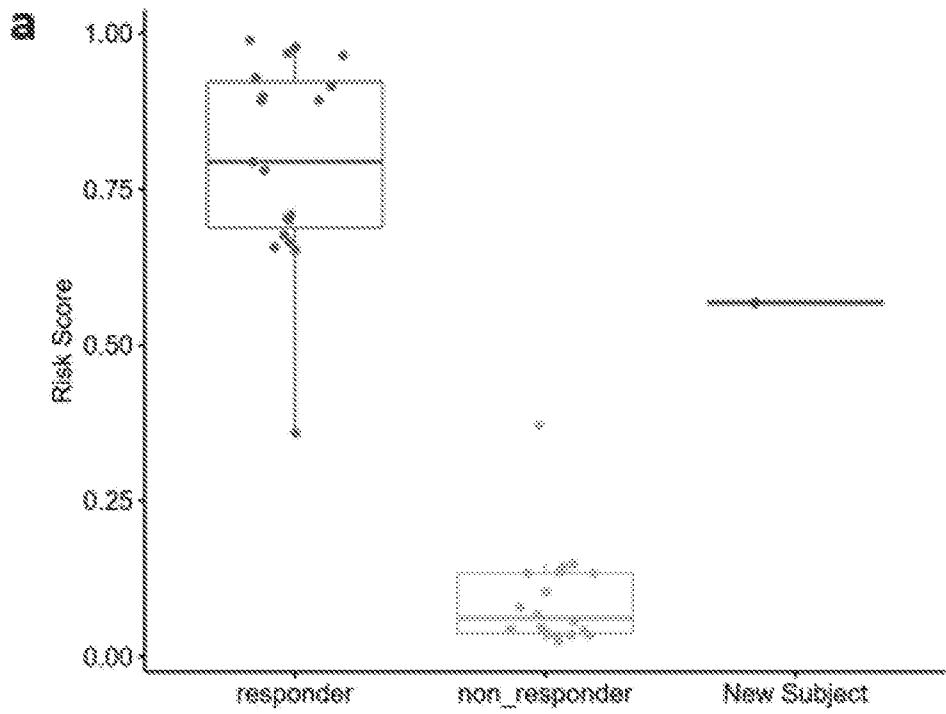
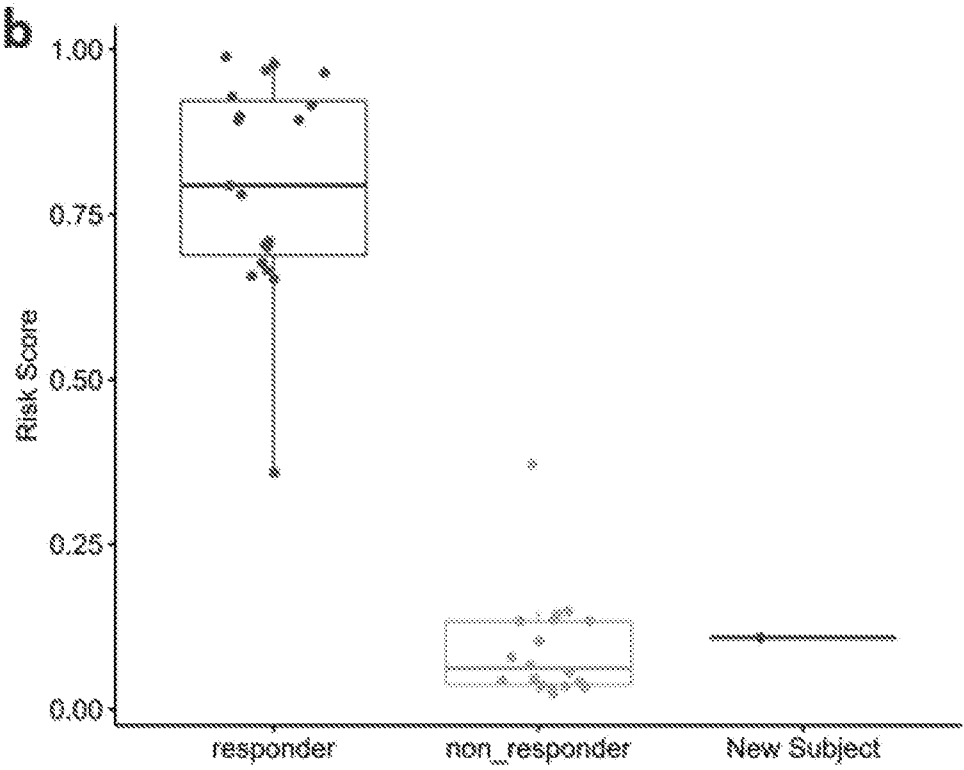


Figure 5b



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/113213

A. CLASSIFICATION OF SUBJECT MATTER

C12N 1/20(2006.01)i; A61P 3/04(2006.01)i; A61P 3/10(2006.01)i; A61K 35/74(2015.01)i; A61K 38/00(2006.01)i

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)

C12N; A61P; A61K

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

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

CNTXT,CNABS,VEN,WOTXT,EPTXT,USTXT,PUBMED,BAIDU,ISI Web of Knowledge,CNKI:THE CHINESE UNIVERSITY OF HONG KONG,reduce,bodyweight, probiotic,fat,gastro,intestinal,Anaerostipes hadrus,Collinsella tanakaei, Roseburia hominis,fecal microbiota transplantation,FMT.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014227227 A1 (BGI SHENZHEN CO., LIMITED) 14 August 2014 (2014-08-14) claim 11	1-9, 15-34
Y	US 2014227227 A1 (BGI SHENZHEN CO., LIMITED) 14 August 2014 (2014-08-14) claim 11	1-34
X	CN 110060778 A (PERFECT SHANGHAI HEALTH TECHNOLOGY CO., LTD., et al.) 26 July 2019 (2019-07-26) claims 1-5, description paragraph 11	1-9, 15-34
Y	CN 110060778 A (PERFECT SHANGHAI HEALTH TECHNOLOGY CO., LTD., et al.) 26 July 2019 (2019-07-26) claims 1-5, description paragraph 11	1-34
X	US 2015246081 A1 (Shayne Kenneth Morris) 03 September 2015 (2015-09-03) claims 1-5	1-9, 15-34
Y	US 2015246081 A1 (Shayne Kenneth Morris) 03 September 2015 (2015-09-03) claims 1-5	1-34
Y	CN 109266766 A (302 MILITARY HOSPITAL OF CHINA) 25 January 2019 (2019-01-25) description paragraph 37	1-34



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

04 November 2021

Date of mailing of the international search report

18 November 2021

Name and mailing address of the ISA/CN

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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.: **21-29**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] The subject-matter of claims 21-29 relates to a method for reducing the risk of obesity and type 2 diabetes(T2D) or treating obesity and T2D in a subject, therefore does not warrant an international search according to the criteria set out in Rule 39.1(iv). However, the search has also been carried out based on the use of an effective amount of Anaerostipes hadrus or Roseburia hominis for the manufacturing of a medicament for reducing the risk of obesity and type 2 diabetes (T2D) or treating obesity and T2D in a subject.
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.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/113213

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIAO, W.Y., et al. "Potencial function of probiotics in obesity and associated metabolic disorder" <i>Journal of Northeast Agricultural University</i> , Vol. 42, No. 8, 31 August 2011 (2011-08-31), Pages159-164	1-34
A	ELSON, C.O., et al. "Host-microbiota interactions in inflammatory bowel disease" <i>Gut Microbes</i> , Vol. 3, No. 4, 31 August 2012 (2012-08-31), Pages332-344	1-34

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2021/113213

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2014227227	A1	14 August 2014	DK	2774616	T3	22 June 2020
				CN	103082292	A	08 May 2013
				CN	103082292	B	04 March 2015
				US	2015037286	A1	05 February 2015
				HK	1182905	A1	13 December 2013
				WO	2013063849	A1	10 May 2013
				EP	2774616	A1	10 September 2014
				EP	2774616	A4	15 April 2015
				EP	2774616	B1	29 April 2020
CN	110060778	A	26 July 2019	None			
US	2015246081	A1	03 September 2015	None			
CN	109266766	A	25 January 2019	None			