BRIEF REPORT

Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome

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Alterations in intestinal microbiota are associated with obesity and insulin resistance. We studied the effects of infusing intestinal microbiota from lean donors to male recipients with metabolic syndrome on the recipients' microbiota composition and glucose metabolism. Subjects were assigned randomly to groups that were given small intestinal infusions of allogenic or autologous microbiota. Six weeks after infusion of microbiota from lean donors, insulin sensitivity of recipients increased (median rate of glucose disappearance changed from 26.2 to 45.3 μmol/kg/min; $P < .05$) along with levels of butyrate-producing intestinal microbiota. Intestinal microbiota might be developed as therapeutic agents to increase insulin sensitivity in humans; www.trialregister.nl; registered at the Dutch Trial Register (NTR1776).

Keywords: Bacteria; Clinical Trial; Diabetes; Short-Chain Fatty Acids.

Accumulating data suggest that intestinal microbiota contributes to host metabolism.[1] In animal models obesity is associated with substantial changes in the composition and metabolic function of gut microbiota.[2,3] The colonic microbiota in obese mice shows a lower microbial diversity and is enriched in carbohydrate and lipid users.[2,3] However, in human beings consensus regarding the specific bacterial species involved and evidence for a causative role of the microbiota in host metabolism is lacking.[4,5] Most studies focus on the colonic microbiota, whereas the proximal intestine is crucial for carbohydrate and fat uptake, resulting in obesity and insulin resistance.[4] Furthermore, because small-intestinal sensing mechanisms exist to improve insulin sensitivity via neuronal circuits,[6] changes in small intestinal microbiota could be one of the stimuli driving this mechanism. We thus hypothesized that rebalancing the obesogenic microbiota by small intestinal infusion of gut microbiota from a lean donor would positively affect (host) energy metabolism and insulin sensitivity in subjects with metabolic syndrome.

A detailed description of patient selection and methods is available in the Supplementary Materials and Methods. Briefly, treatment-naive male subjects with metabolic syndrome underwent small-intestine biopsies and subsequent bowel lavage through a duodenal tube, followed by random assignment to either an allogenic (from lean male donors with a body mass index < 23 kg/m^2; n = 9) or autologous gut microbiota infusion (reinfusion of own collected feces; n = 9) (Supplementary Figure 1 and Supplementary Table 1). Insulin sensitivity was measured before and 6 weeks after gut microbiota infusion by means of a hyperinsulinemic euglycemic clamp using [6,6 $^2H_2$]-glucose to measure endogenous glucose production (EGP) and hepatic and peripheral insulin sensitivity (rate of disappearance [Rd]).[7] Large (fecal samples) and small- (duodenal biopsies) intestine gut microbiota composition and fecal short-chain fatty acids were measured at baseline and 6 weeks after infusion, as previously published.[8,9]

We found an improvement in peripheral insulin sensitivity 6 weeks after allogenic gut microbiota infusion (median rate of glucose disappearance, Rd: from 26.2 to 45.3 μmol/kg/min; $P < .05$) (Figure 1*A*). A trend toward improvement in hepatic insulin sensitivity, expressed as EGP suppression, was observed in the allogenic group (median EGP suppression, from 51.5% to 61.6%; $P = .08$) (Figure 1*B*). No differences in diet composition, resting energy expenditure (REE), or counter-regulatory hormones were found between all clamps (Supplementary Table 2). Fecal microbiota of obese subjects was characterized by lower microbial

Abbreviations used in this paper: BMI, body mass index; EGP, endogenous glucose production; Rd, rate of disposal; REE, resting energy expenditure.

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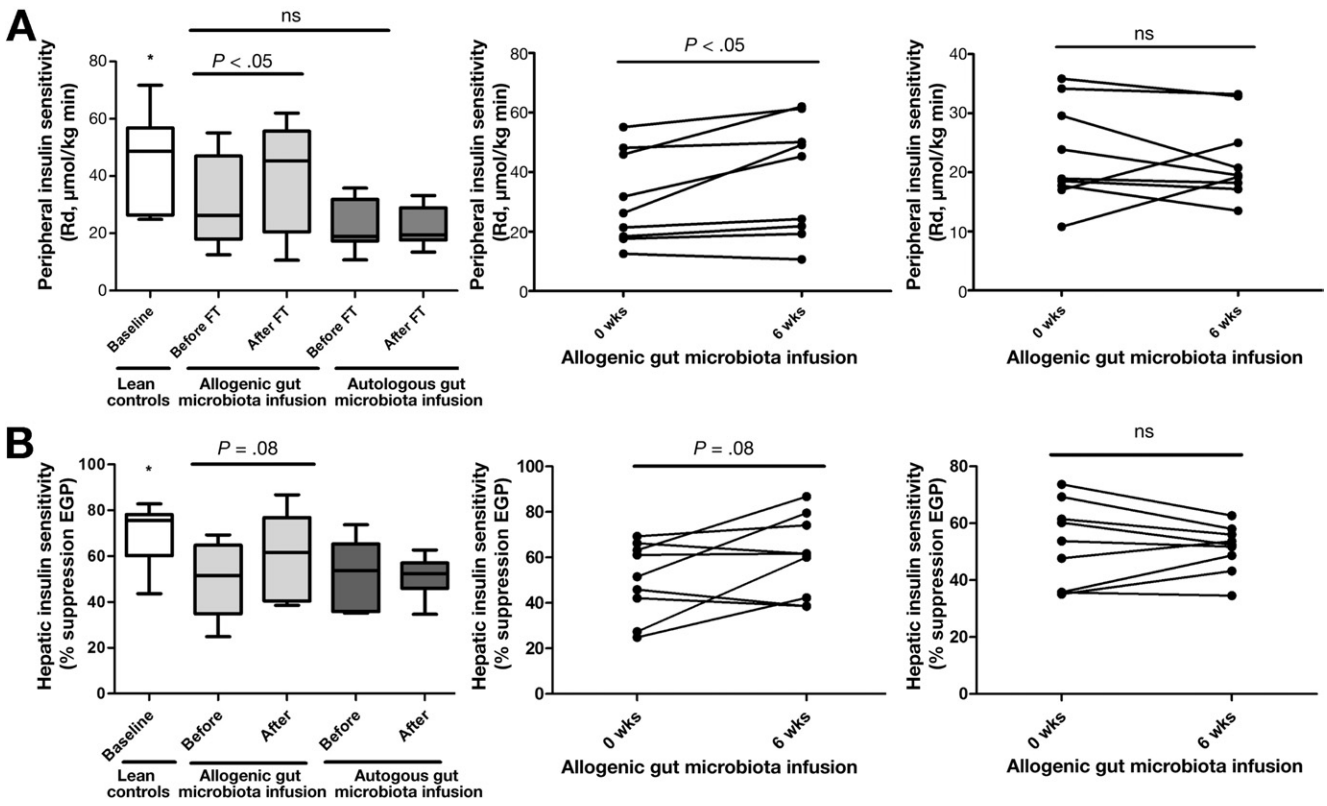

Figure 1. Box plots and individual changes between baseline and after 6 weeks for (*A*) peripheral and (*B*) hepatic insulin sensitivity. *$P < .05$ lean controls vs obese subjects (Mann–Whitney test).

diversity, higher amounts of *Bacteroidetes*, and decreased amounts of *Clostridium* cluster XIVa as compared with lean donor healthy subjects. Quantitative polymerase chain reaction analysis showed no change in the total numbers of fecal bacteria (allogenic, from 10.8 ± 0.2 to 11.0 ± 0.4 vs autologous, from 11.6 ± 0.6 to 11.3 ± 0.4 $\log_{10}$ bacteria/g feces, non significant [ns]) and *Archaea* (allogenic, from 6.7 ± 0.9 to 6.8 ± 0.8 vs autologous, from 6.3 ± 0.4 to 6.7 ± 0.7 $\log_{10}$ bacteria/g feces, ns) after gut microbial infusion. Gut microbial diversity was increased significantly after allogenic gut microbiota transfer (from 178 ± 62 to 234 ± 40 species; $P < .05$), but was unchanged in the autologous group (from 184 ± 71 to 211 ± 50, ns). Sixteen bacterial groups increased significantly in the allogenic treatment group, including those related to the butyrate-producer *Roseburia intestinalis*, which showed a 2.5-fold increase. Moreover, a subset of 6 bacterial groups differed significantly between the allogenic and autologous treatment groups after 6 weeks, including the oxalate-converting *Oxalobacter formigenes* and other Firmicutes (Figure 2*A* and Supplementary Table 3). Also, fecal short-chain fatty acids decreased after allogenic gut microbiota infusion (median acetate from 49.5 to 37.6; $P < .05$; butyrate, from 14.1 to 8.9; $P < .05$; and propionate, from 18.2 to 16.3 mmol/kg feces; ns) compared with nonsignificant changes in the autologous group.

All small intestinal biopsy specimens showed normal histology. Total bacterial abundance in biopsy specimens showed no differences after either allogenic or autologous microbial fecal infusion (allogenic, from 7.0 ± 0.2 to 7.0 ± 0.3 vs autologous, from 7.1 ± 0.2 to 7.0 ± 0.2 $\log_{10}$ bacteria/cells per biopsy; ns). *Archaea* were below the detection limit in both groups. No significant differences were found with respect to microbial diversity in both treatment groups (allogenic, from 91.3 ± 54.7 to 68.1 ± 15.7; ns; autologous, from 73.6 ± 23.3 to 83.6 ± 52; ns). A total of 7 bacterial groups were altered within the allogenic treatment group after 6 weeks, including an increase of the butyrate-producer *Eubacterium hallii* (Supplementary Table 4). In contrast, *E hallii* showed an almost 2-fold reduction in the autologous treatment group. Three bacteria differed significantly between the allogenic and autologous treatment groups at 6 weeks (Figure 2*B*) including the nitric oxide producer *Alcaligenes faecalis* and the gram-negative *Escherichia coli*.

In conclusion, our data point toward a regulating role for butyrate derived from gut microbial metabolism leading to an improvement in insulin sensitivity. First, we observed a significant modification in intestinal microbiota composition in fecal samples after allogenic gut microbiota infusion, including a 2.5-fold increase in the number of bacteria related to the butyrate-producing *R intestinalis* (Figure 2*A*).[4] Second, we found that bacteria related to the similarly butyrate-producing *E hallii* (anaerobic, phylotype Clostridium cluster XIVa)[10] were increased in the small intestine mucosa after allogenic gut microbiota transfer (Figure 2*B*). Bu-

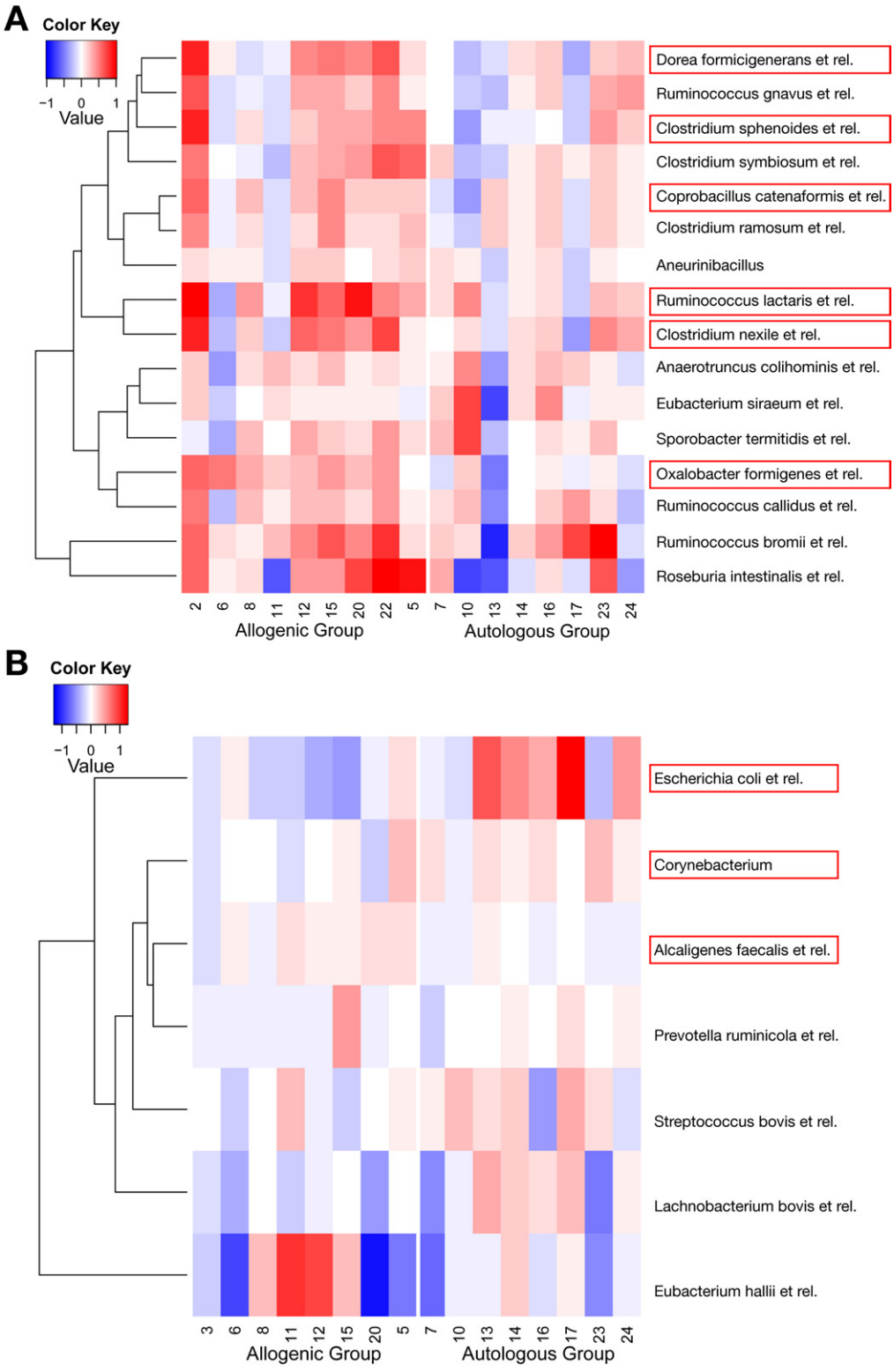

Figure 2. Heat maps of (*A*) fecal and (*B*) small intestinal gut microbiota with significant differences between both treatment groups depicted as a *red box*. The color value shows $\log_{10}$ fold changes.

tyrate is produced by microbiota both in the large and small intestines for energy and signaling purposes,[11,12] with orally administrated butyrate having a direct effect on glucose metabolism.[13]

Our data confirm that increased gut microbiota diversity also is associated with improved insulin resistance.[14] Whether diversity or changes in specific bacterial species contribute to this effect is unknown, although it has been suggested that butyrate produced by certain bacteria prevents translocation of endotoxic compounds derived from the gut microbiota,[14,15] which has been shown to drive insulin resistance.[14] Whether oral administration of identified microbiota can result in comparable effects on glucose metabolism is cur-

rently under study. If confirmed, this could offer a rationale for novel therapeutic interventions aimed at improving insulin sensitivity in humans.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.06.031.

References

1. Qin J, et al. Nature 2010;464:59–65.
2. Turnbaugh PJ, et al. Nature 2006;444:1027–1031.
3. Backhed F, et al. Proc Natl Acad Sci U S A 2004;101:15718–15723.
4. Kootte RS, et al. Diabetes Obes Metab 2012;14:112–120.
5. Duncan SH, et al. Int J Obes (Lond) 2008;32:1720–1724.
6. Wang P, et al. Nature 2008;452:1012–1016.
7. Langeveld M, et al. J Clin Endocrinol Metab 2008;93:845–851.
8. Arumugam M, et al. Nature 2011;473:174–180.
9. Wolever TM, et al. Br J Nutr 2000;84:57–61.
10. Munoz-Tamayo R, et al. FEMS Microbiol Ecol 2011;76:615–624.
11. Arvans DL, et al. Am J Physiol Gastrointest Liver Physiol 2005;288:696–704.
12. Zoetendal EG, et al. ISME J 2012;6:1415–1426.
13. Gao Z, et al. Diabetes 2009;58:1509–1517.
14. Cani PD, et al. Diabetes 2007;56:1761–1772.
15. Lewis K, et al. Inflamm Bowel Dis 2010;16:1138–1148.

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Conflicts of interest

The authors disclose no conflicts.

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Supplementary Materials and Methods

Obese Subjects

Male Caucasian obese subjects were recruited by newspaper advertisements and screened for characteristics of the metabolic syndrome, specifically with a body mass index > 30 kg/m^2 or waist circumference > 102 cm and a fasting plasma glucose level > 5.6 mmol/L (Supplementary Table 1).[1] Because animal studies have shown that gut microbiota composition is affected by several factors including bile acid composition, subjects with a history of cholecystectomy were excluded, as well as subjects who used any medication, probiotics, and/or antibiotics in the past 3 months.[2] Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board and conducted at the Academic Medical Center in accordance with the Declaration of Helsinki (updated version 2008). The study was registered at the Dutch Trial Register (NTR1776).

Screening of Lean Donors

Lean healthy Caucasian males (body mass index < 23 kg/m^2) also were recruited by newspaper advertisements and matched for age and sex. They completed questionnaires regarding diet and bowel habits, travel history, comorbidity including (family history of) diabetes mellitus, and lack of medication use. They were screened for the presence of infectious diseases according to an adapted version of the Dutch Red Cross donor questionnaire.[3] Blood was screened for the presence of antibodies to human immunodeficiency virus; human T-lymphotropic virus; hepatitis A, B, and C; cytomegalovirus; Epstein–Barr virus; *Strongyloides*; and amebiasis. Donors also were excluded if screening of their feces revealed the presence of parasites (eg, *Blastocystis hominis* or *Dientamoeba fragilis*), *Clostridium difficile*, or other pathogenic bacteria (*Shigella, Campylobacter, Yersinia, Salmonella*).

Experimental Design

On the first study day a hyperinsulinemic clamp was applied in all study subjects to measure insulin sensitivity (see Hyperinsulinemic–Euglycemic Clamp section). On the second day, after an overnight fast, study subjects and donors brought freshly produced morning stool for processing; study subjects were randomized in a double-blind fashion to either allogenic (from lean male donors) or autologous (from own collected feces) gut microbial infusion via a gastroduodenal tube (see Gut Microbiota Transfer Procedure section). A gastroduodenoscopy without sedation was performed and small intestinal biopsy specimens were taken. Biopsy samples immediately were collected in sterile tubes, snap-frozen in liquid nitrogen, and processed as described.[4] A duodenal tube was positioned and bowel lavage with polyethylene glycol solution (Macrogol or Klean-Prep, Norgine BV, Amsterdam the Netherlands) was performed over 5 hours to clean out endogenous fecal contamination followed by gut microbial infusion. Gastroduodenoscopy-assisted biopsies and the hyperinsulinemic euglycemic clamp were repeated after 6 weeks. The experimental study design is shown in Supplementary Figure 1. Of note, the main reasons for choosing infusion of intestinal microbiota into the small rather than the large intestine were as follows: (1) metabolic role of the small intestine as compared with the colon and (2) in our experience the nasoduodenal route provides better access and tolerability in patients while being equally effective in administrating large amounts of intestinal microbiota.[5]

Hyperinsulinemic–Euglycemic Clamp

Glucose metabolism was measured in the basal state and during a 2-step hyperinsulinemic euglycemic clamp using [6,6 ^{2}H2]-glucose to measure EGP and hepatic and peripheral insulin sensitivity (Rd).[6] Body fat composition was measured using bioimpedance analysis. The REE was measured using indirect calorimetry. Participants were allowed to follow their own diet, but were asked to keep a weekly online nutritional diary (www.dieetinzicht.nl) to monitor caloric intake including amount of dietary carbohydrate, fat, protein, and fiber. Details of the hyperinsulinemic euglycemic clamp are shown in Supplementary Table 2.

Subjects refrained from heavy physical exercise for 24 hours before the clamp. After a 12-hour fast, a catheter was inserted into an antecubital vein for infusion of stable-isotope tracer [6,6-$^2H_2$]glucose (Cambridge Isotopes, Andover, MA), insulin, and glucose. A second catheter was inserted retrogradely in the contralateral hand vein and kept in a thermoregulated (60°C) clear plastic box for sampling of arterialized venous blood. Saline was infused as 0.9% NaCl at a rate of 50 mL/h to keep the catheters patent. At t = 0 hours (0800), blood samples were drawn for determination of background enrichments. Then, a primed continuous infusion of isotope [6,6-$^2H_2$]glucose was started (prime, 8.8 μmol/kg; continuous, 0.11 μmol/kg^{-1}/min^{-1}) and continued until the end of the clamp. After a 2-hour equilibration period, blood samples were drawn for isotope enrichments and samples for glucoregulatory hormones and free fatty acids. Thereafter (t = 2.0 h), a 2-step hyperinsulinemic euglycemic clamp was started: step 1 included an infusion of insulin at a rate of 20 mU/m^{-2}/min^{-1} (Actrapid 200 IU/mL; Novo Nordisk Farma BV, Alphen aan den Rijn, The Netherlands) to assess hepatic insulin sensitivity. Glucose 20% was started to maintain a plasma glucose concentration of 5 mmol/L. Plasma glucose concentrations were measured every 5 minutes at the bedside using a Beckman glucose meter (Beckman, Fullerton, CA). After 2 hours (t = 4 h), blood samples were drawn at 5-minute intervals for the measurement of glucose concentrations and isotopic enrichments. Another blood sample was drawn for measurement of glucoregulatory

hormones and free fatty acids. Hereafter, insulin infusion was increased to a rate of 60 $mU/m^{-2}/min^{-1}$ (step 2) to assess peripheral insulin sensitivity. After another 2 h ($t = 6$ h), blood sampling was repeated.

Body composition was measured at baseline and after 6 weeks with bioelectrical impedance analysis (Maltron BF906; Maltron, Rayleigh, UK). Oxygen consumption and CO_2 production were measured continuously during the final 20 minutes of both the basal state and the hyperinsulinemic euglycemic clamp by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, CA). REE including carbohydrate oxidation and fatty acid oxidation rates were calculated from oxygen consumption and carbon dioxide production. Rate of appearance and Rd of glucose were calculated using the modified form of the Steele equations for non–steady-state measurements as described previously.[7] EGP was calculated as the difference between the rate of appearance of glucose and the glucose infusion rate. Both peripheral (Rd) and hepatic insulin sensitivity (suppression of EGP) were calculated and expressed as the median with a range.

Gut Microbiota Transfer Procedure

At the day of infusion the patient and donor delivered feces produced within 6 hours before use.[5] Fecal samples were taken before and after processing to study procedural effects on microbial composition. Immediately after delivery, the feces was covered with sterile saline (500 mL 0.9% NaCl) to reduce exposure to oxygen, transferred to a blender, and mixed for 10 minutes. The homogenized solution then was filtered twice through a clean metal sieve. Subsequently, the filtrate was transferred to a 1000-mL sterile glass bottle and stored at room temperature until the patient had finished the bowel lavage. Finally, the gut microbiota solution was infused gradually through the duodenal tube in approximately 30 minutes.

Gut Microbiota Analysis

Donor and study subjects collected a morning stool sample at baseline and after 6 weeks to determine the microbiota composition. Samples were collected in 2 plastic containers, immediately frozen at −20°C, and transferred to −80°C within a week. The microbiota composition of the small intestinal biopsy specimens and fecal samples was determined by analyzing RNA signatures using the Human Intestinal Tract Chip (HITChip), a custom-made Agilent microarray (Agilent Technologies, Palo Alto, CA) containing approximately 5500 specific oligonucleotide probes that cover more than 1000 intestinal phylotypes.[8,9]

DNA was isolated from fecal samples and purified using the repeated bead-beating plus column method as described previously.[10] For DNA isolation of the biopsy specimens we used a different bead beating protocol.[11] In short, 0.5 g (wet weight) of feces was suspended in lysis buffer (500 mmol/L NaCl, 50 mmol/L Tris-HCl, pH 8, 50 mmol/L EDTA, 4% sodium dodecyl sulfate) plus Zirconia beads and glass beads (Biospec Products, Battlesville, OK). The tube was shaken with Fastprep (MPBio, Santa Ana, CA) at 5.5ms for 3 minutes at 4°C, followed by incubation at 95°C for 15 minutes. The DNA in the supernatant was precipitated with ammonium acetate and isopropanol, washed with 70% ethanol, and afterward treated with proteinase K and DNase-free RNase. Finally, the DNA was purified on a QIAamp spin column (Qiagen, Foster City, CA) according to the manufacturer's instructions. DNA concentration was quantified using the NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Quantification of total bacteria and methanogens in fecal and small intestinal biopsy specimens was performed with the same bacterial DNA used for HITChip analysis. Quantitative polymerase chain reaction was performed with a real-time polymerase chain reaction system (PRISM 7900HT; Applied Biosystems, Foster City, CA). All reactions were performed at least in duplicate. To facilitate comparison with other bacterial quantification methods, the number of detected molecules (DNA) was converted to cell equivalents. A bacterial culture of the stated reference strain (grown in the appropriate media and collected at the stationary phase) was used to generate a standard curve-relating cycle threshold to bacterial cell number (determined microscopically with 4′,6-diamidino-2-phenylindole staining from a dilution series of the reference strains). Standard curves of DNA from *Bifidobacterium longum* and *Methanobrevibacter smithii* were created using 10^6 to 10^{10} cells. Samples were analyzed in a 25-μL reaction mix consisting of 12.5 μL SYBR Premix (50 mmol/L KCl, 20 mmol/L Tris-HCl, pH 8.4, 0.2 mmol/L of deoxynucleoside triphosphate, 0.625 U TaKaRa Taq (Clonetech, Mountain View, CA), 3 mmol/L $MgCl_2$, and 10 nmol/L fluorescein), 0.2 μmol/L of each primer, and 5 μL of DNA. For detection of total bacteria and methanogens in feces and small intestine, serial dilutions (100–1000) of the extracted DNA were subjected to quantitative polymerase chain reaction using Uni331F (5′-TCCTACGGGAGGCAGCAGT −3′) and Uni797R (5′-GGACTACCAGGGTATCTAATCCTGTT-3′).[11] For methanogenic *Archaea*, primers targeting the methanobacteriales order Mtb857F (5′-CGWAGGGAAGCTGTTAAGT-3′) and Mtb1196R (5′-TACCGTCGTCCACTCCTT-3′) were used as described earlier.[12] The following conditions of quantitative polymerase chain reaction used were as follows: 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing temperature of 60°C for 20 seconds, extension at 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. A melting curve was performed at the end of each run to verify the specificity of the polymerase chain reaction amplicons by slowly heating the final reaction

mix to 95°C (0.5°C per cycle). Data analysis was performed using the Sequence Detection System (version 2.3) software (Applied Biosystems). By using cycle threshold values in the linear range of the assay, bacterial equivalents were interpolated from a standard curve generated in the same experiment and then weight-corrected to yield a value in bacterial cell equivalents/g feces.

The HITChip, a phylogenetic microarray, was used for the high-throughput profiling of the microbiota in feces and small intestinal biopsy specimens as described previously.[8] The advantage of the HITChip method is that phylogenetic profiling of microbiota goes beyond the depth of canonical pyrosequencing.[13,14] The disadvantage is that 16S ribosomal RNA gene profiling only provides insight into population dynamics, not into microbial activity.

In short, 10 ng DNA was used to amplify the 16S ribosomal RNA genes using the *T7prom*-Bact-27-for and Uni-1492-rev primers followed by in vitro transcription and labeling with Cy3 and Cy5, respectively, for fecal samples. The primer Prok-1369-rev was used as reverse primer for the biopsy samples because Uni-1492-rev was majorly targeting the overabundant human DNA, resulting in its depletion for efficient bacterial 16S ribosomal RNA gene amplification (data not shown). Equal molar mixes of Cy3-/Cy5-labeled 16S ribosomal RNA targets were fragmented and subsequently hybridized on the microarrays at 62.5°C for 16 hours in a rotation oven (Agilent Technologies, Amstelveen, The Netherlands), followed by washing and drying of the slides. Samples were arrayed in duplex for technical replication, which resulted in exclusion of 1 fecal sample in the autologous treatment group as well as 1 biopsy sample in each treatment group. After scanning the slides, the data were extracted from the microarray images using the Agilent Feature Extraction software (versions 7.5–9.1; available: http://www.agilent.com). Subsequently, the microarray data were minimum–maximum normalized and further analyzed using a set of R-based scripts (http://www.r-project.org/) in combination with a custom-designed relational database that runs under the MySQL database management system (http://www.mysql.com). Hierarchical clustering of probe profiles was performed using Pearson correlation-based distance and complete linkage method. Gut microbial diversity was determined by the Simpson reciprocal index as previously described.[15]

Determination of Fecal and Plasma Short Chain Fatty Acids

In short, 0.2–0.5 g feces was weighed and resuspended in phosphate-buffered saline so that a 10× dilution was obtained. A total of 350 μL of homogenized feces was mixed with 200 μL formic acid (5% by volume), 100 μL 2-ethylbutyric acid (1.25 g/L; Sigma–Aldrich, Zwijndrecht, the Netherlands), and 350 μL MilliQ. Fecal samples were centrifuged for 5 minutes at 15,000 × *g* to remove large particles, filtered, and protein-free samples were subjected to vacuum distillation after the addition of an internal standard solution containing 1:1 mmol/L methylbutyric acid and 110 mmol/L [13C]-formic acid.[16] Short chain fatty acids acetate, proprionate and butyrate were determined quantitatively using a Shimadzu GC2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector.

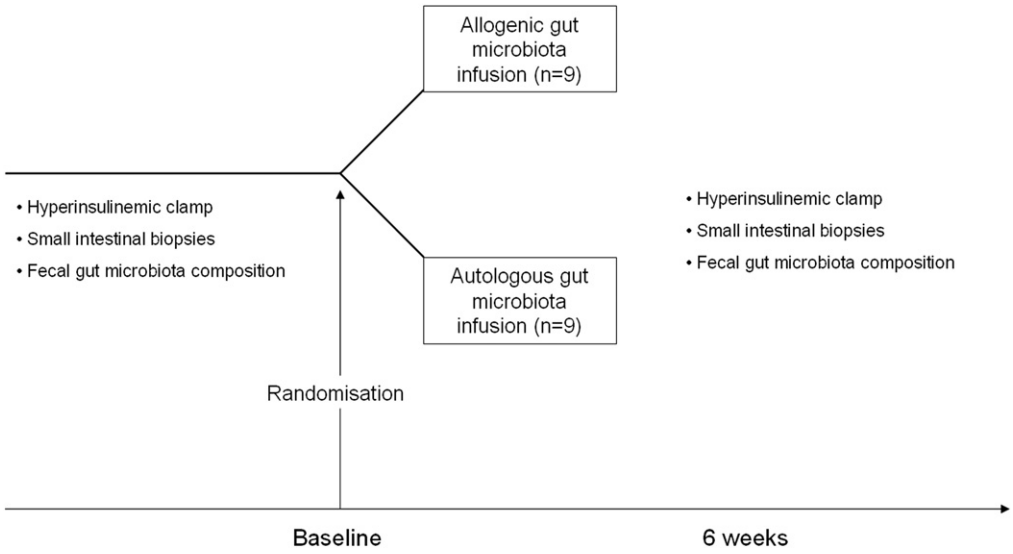
Statistics

Sample size calculations showed that to detect 30% difference in peripheral insulin sensitivity upon allogenic treatment, compared with 5% difference after autologous treatment with 80% power, the sample size in each group was estimated to be 9. Accepting a 10% drop-out rate per group, we enrolled 20 patients in total. Two subjects (1 in each treatment arm) were excluded from analyses because of antibiotic use during the trial unrelated to the microbial infusion. Statistical analyses were performed with SPSS software (version 16; IBM, Chicago, IL). Depending on the distribution of the data, data are expressed as mean ± standard error of the mean or median with range. The primary outcome was effect of lean donor gut microbiota infusion on insulin sensitivity after 6 weeks. The secondary outcome was the change in specific small- and large-gut microbiota as well as produced fecal short chain fatty acids. For significant changes between and within treatment groups, clinical parameters were tested with the (paired) Student *t* test or the Mann–Whitney test (differences between groups) and Wilcoxon signed-rank test (differences within treatment group), depending on distribution of the data**.** Expression analysis for HITChip was performed with non linear mixed effects (nlme) package in R.[17] Differences between treatment groups at 6 weeks for both fecal samples and small intestinal biopsy samples were computed using a linear mixed model, and the *P* values then were corrected for multiple comparisons by the q-value package.[18,19] To detect differences within each treatment group in these samples, a multivariate mixed model taking into account the effects of repeated measurements, treatment group, and individual temporal stability of gut microbiota was constructed. Moreover, because of low small-intestinal-gut microbiota diversity we could not detect differences between treatment groups using models mentioned earlier; we thus used Random Forests multivariate analyses to detect differences in small intestinal bacteria within treatment groups after 6 weeks.[20]

Supplementary References

1. Alberti KG, et al. Circulation 2009;120:1640–1645.
2. Kootte RS, et al. Diabetes Obes Metab 2012;14:112–120.
3. Van der Bij AK, et al. Transfusion 2006;46:1729–1736.
4. Dethlefsen L, et al. PLoS Biol 2008;6:2383–2400.
5. Nieuwdorp M, et al. Ned Tijdschr Geneeskd 2008;152:1927–1932.
6. Langeveld M, et al. J Clin Endocrinol Metab 2008;93:845–851.

7. Steele R. Ann N Y Acad Sci 1959;82:420–430.
8. Rajilic-Stojanovic M, et al. Environ Microbiol 2009;11:1736–1751.
9. Arumugam M, et al. Nature 2011;473:174–180.
10. Zoetendal EG, et al. Syst Appl Microbiol 2001;24:405–410.
11. Nadkarni MA, et al. Microbiology 2002;148:257–266.
12. Yu Y, et al. Biotechnol Bioeng 2005;89:670–679.
13. van den Bogert B, et al. Appl Environ Microbiol 2011;77:2071–2080.
14. Claesson MJ, et al. Gut Microbes 2010;1:277–278.
15. Zilliox MJ, et al. Nat Methods 2007;4:911–913.
16. Wolever TM, et al. Br J Nutr 2000;84:57–61.
17. Pinheiro JC, et al. Springer 2000.
18. Bretz F, et al. Boca Raton: CRC Press, 2010.
19. Storey JA, et al. J R Stat Soc Series B Stat Methodol 2010;64:479–498.
20. Breiman L. Machine Learning 1996;24:123–140.



Supplementary Figure 1. Overview of study scheme.

Supplementary Table 1. Characteristics of Study Subjects at Baseline and After 6 Weeks

	Allogenic group (N = 9)		Autologic group (N = 9)	
	Baseline	6 weeks	Baseline	6 weeks
Age, *y*	47 ± 4		53 ± 3	
Length, *cm*	185 ± 2		178 ± 2	
Weight, *kg*	123 ± 6	122 ± 6	113 ± 7	113 ± 7
Body mass index, *kg/m²*	35.7 ± 1.5	35.6 ± 1.4	35.6 ± 1.5	35.7 ± 1.6
Body fat mass, *%*	40 ± 1	40 ± 1	39 ± 2	39 ± 1
Fasting plasma glucose, *mmol/L*	5.7 ± 0.2	5.7 ± 0.2	5.7 ± 0.2	5.7 ± 0.2
Glycated hemoglobin, *mmol/mol*	39 ± 1.1	38 ± 1.2	40 ± 1.5	39 ± 3
Cholesterol, *mmol/L*	4.5 ± 0.4	4.6 ± 0.4	4.8 ± 0.3	4.8 ± 0.2
HDLc	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
LDLc	3.1 ± 0.4	3.0 ± 0.3	2.9 ± 0.2	2.9 ± 0.2
TG	1.4 ± 0.3	1.5 ± 0.4	1.6 ± 0.3	1.8 ± 0.4
Plasma free fatty acid, *mmol/L*	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
Systolic blood pressure, *mm Hg*	138 ± 3	132 ± 6	140 ± 2	142 ± 8
Diastolic blood pressure, *mm Hg*	85 ± 2	83 ± 5	84 ± 2	86 ± 6

NOTE. Values are expressed as mean ± standard error of the mean. The body mass index is the weight in kilograms divided by the square of the height in meters. No significant differences in clinical variables were found between baseline and 6 weeks in both treatment groups.
HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; TG, triglycerides.

Supplementary Table 2. Glucose Kinetics and Glucoregulatory Hormones in the Allogenic (Donor) and Autologous Gut Microbiota Infusion Group

	Basal state		Clamp (step 1)		Clamp (step 2)	
	Allogenic	Autologous	Allogenic	Autologous	Allogenic	Autologous
First clamp: baseline						
Glucose level, *mmol/L*	5.4 (5.0–6.9)	5.4 (4.8–6.6)	5.2 (4.7–5.4)	5.2 (4.7–5.5)	4.9 (4.2–5.3)	5.1 (4.6–5.8)
Insulin level, *pmol/L*	74 (40–230)	135 (26–220)	217 (127–390)	280 (230–400)	626 (405–900)	750 (620–880)
Glucagon level, *ng/L*	60 (44–90)	65 (38–150)	52 (35–76)	68 (29–78)	33 (26–71)	46 (23–68)
Free fatty acid level, *mmol/L*	0.5 (0.3–1.0)	0.6 (0.3–2.0)	0.1 (0.1–0.4)	0.2 (0.1–0.3)	[a]	[a]
Cortisol level, *nmol/L*	239 (118–320)	230 (203–363)	230 (100–475)	175 (135–466)	217 (101–348)	238 (156–406)
EGP level, *μmol/kg/min*	10.0 (8.9–13.0)	10.0 (8.2–12.1)	3.8 (2.9–9.8)	4.6 (2.6–12.1)	[b]	[b]
Rd, *μmol/kg/min*	—	—	11.6 (8.4–19.5)	10.5 (7.7–14.3)	26.2 (12.6–55.1)	18.9 (10.8–35.9)
Caloric intake, *kcal/day*	2100 (1460–2750)	1970 (1500–2800)	—	—	—	—
REE, *kcal/day*	2168 (1990–2473)	1937 (1796–2237)	—	—	2230 (1810–2487)	1989 (1670–2183)
Second clamp: 6 weeks after fecal transplant						
Glucose level, *mmol/L*	5.4 (4.8–6.6)	5.5 (5.3–6.9)	5.0 (4.8–5.6)	5.0 (4.9–5.3)	5.0 (4.3–5.8)	5.0 (4.7–5.3)
Insulin level, *pmol/L*	77 (18–250)	140 (30–287)	220 (150–350)	306 (190–370)	561 (415–900)	760 (650–890)
Glucagon level, *ng/L*	69 (44–100)	76 (50–115)	52 (35–76)	68 (46–68)	39 (21–61)	46 (23–68)
Free fatty acid level, *mmol/L*	0.4 (0.3–0.8)	0.5 (0.3–0.8)	0.1 (0.0–0.3)	0.2 (0.1–0.5)	[a]	[a]
Cortisol level, *nmol/L*	230 (100–475)	175 (135–466)	217 (109–466)	193 (78–364)	207 (56–322)	216 (137–450)
EGP level, *μmol/kg/min*	9.8 (9.4–12.5)	10.3 (8.8–12.5)	3.8 (1.2–7.8)	4.8 (3.9–12.5)	[b]	[b]
Rd, *μmol/kg/min*	—	—	13.6 (7.9–19.9)	10.3 (8.7–13.9)	45.3(10.6–62.0)	19.5(13.5–33.2)
Caloric intake, *kcal/day*	1990 (1440–4020)	2000 (1566–2603)	—	—	—	—
REE, *kcal/day*	2113 (1875–2419)	1905 (1600–2580)	—	—	2165 (1834–2790)	1836 (1600–2280)

	Basal state	Clamp (step 1)	Clamp (step 2)
Healthy lean male volunteers			
Glucose level, *mmol/L*	5.0 (4.0–5.5)	4.9 (4.6–5.1)	4.9 (4.8–5.3)
Insulin level, *pmol/L*	24 (8–83)	162 (132–194)	504 (426–712)
Glucagon level, *ng/L*	51 (38–86)	35 (30–54)	37 (22–44)
Free fatty acid level, *mmol/L*	0.4 (0.3–0.8)	0.1 (0.0–0.2)	[a]
Cortisol level, *nmol/L*	215 (128–422)	214 (164–474)	191 (113–298)
EGP level, *μmol/kg/min*	13.0 (11.4–14.0)	2.2 (1.3–6.4)	[b]
Rd, *μmol/kg/min*	—	22.5 (18.8–42.9)	65.0 (44.3–82.9)
REE, *kcal/day*	1951 (1598–2514)	—	1990 (1670–2488)

NOTE. All values are medians (minimum–maximum) (n = 9).

[1]Free fatty acids were below the detection limit.

[2]EGP was completely suppressed during step 2 clamp.

Supplementary Table 3. Gut Microbiota in Fecal Samples

Phylum level	Bacterial taxa	Fold-change after/before allogenic infusion	*q value after/before allogenic infusion*	Fold-change after/before autologous infusion	*q value after/before autologous infusion*
Firmicutes	*Dorea formicigenerans* et rel.[a]	1.92	*0.02*	1.4	*0.26*
Firmicutes	*Ruminococcus gnavus* et rel.	1.74	*0.02*	1.22	*0.33*
Firmicutes	*Clostridium sphenoides* et rel.[a]	1.95	*0.02*	1.29	*0.32*
Firmicutes	*Clostridium symbiosum* et rel.	1.71	*0.05*	1.45	*0.18*
Firmicutes	*Coprobacillus catenaformis* et rel.[a]	1.65	*0.02*	1.18	*0.33*
Firmicutes	*Clostridium ramosum* et rel.	1.51	*0.02*	1.13	*0.3*
Firmicutes	*Aneurinibacillus*	1.26	*0.04*	1.1	*0.33*
Firmicutes	*Ruminococcus lactaris* et rel.[a]	2.47	*0.02*	1.47	*0.3*
Firmicutes	*Clostridium nexile* et rel.[a]	2.09	*0.03*	1.38	*0.32*
Firmicutes	*Anaerotruncus colihominis* et rel.	1.49	*0.01*	1.14	*0.31*
Firmicutes	*Eubacterium siraeum* et rel.	1.56	*0.02*	1.00	*0.6*
Firmicutes	*Sporobacter termitidis* et rel.	1.39	*0.05*	1.31	*0.18*
Proteobacteria	*O formigenes* et rel.[a]	1.70	*0.02*	1.27	*0.24*
Firmicutes	*Ruminococcus callidus* et rel.	1.63	*0.02*	1.15	*0.42*
Firmicutes	*Ruminococcus bromii* et rel.	2.49	*0.02*	1.65	*0.2*
Firmicutes	*R intestinalis* et rel.	2.45	*0.05*	1.22	*0.52*

NOTE. Significant changes in 16 fecal gut microbiota within the allogenic lean donor gut microbiota treatment group after 6 weeks are shown, whereas no significant change was seen after autologous gut microbiota treatment. Moreover, a total of 6 different gut microbiota were identified between allogenic and autologous treatment groups ([a]$P < .05$; for description of statistical models see the Supplementary Materials and Methods section).

Supplementary Table 4. Gut Microbiota in Small Intestinal Biopsy Specimens

Phylum level	Bacterial group	Fold-change after/before allogenic infusion	Fold-change after/before autologous infusion
Proteobacteria	*E coli* et rel.[a]	0.58	2.21
Actinobacteria	*Corynebacterium* spp.[a]	0.87	1.34
Proteobacteria	*A faecalis* et rel.[a]	1.18	0.97
Bacteroidetes	*Prevotella ruminicola* et rel.	0.99	1.01
Firmicutes	*Streptococcus bovis* et rel.	0.89	1.23
Firmicutes	*Lachnobacterium bovis* et rel.	0.63	0.98
Firmicutes	*E hallii* et rel.	1.09	0.61

NOTE. Seven groups of small intestinal gut microbiota were changed within the allogenic lean donor gut microbiota treatment group after 6 weeks. Moreover, a total of 3 different gut microbiota were identified between allogenic and autologous treatment groups ([a]$P < .05$; for description of statistical models see the Supplementary Materials and Methods section).