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The gut microbial community in metabolic syndrome patients is modified by diet

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Abstract

Intestinal microbiota changes may be involved in the development of metabolic syndrome (MetS), which is a multicomponent disorder frequently associated with obesity. The aim of this study was to test the effect of consuming two healthy diets: a Mediterranean diet and a low-fat high-carbohydrate diet, for 2 years in the gut microbiota of MetS patients and those in the control group. We analyzed the differences in the bacterial community structure between the groups after 2 years of dietary intervention (Mediterranean or low-fat diet) through quantitative polymerase chain reaction using primers, targeting specific bacterial taxa. We observed, at basal time, that the abundance of *Bacteroides, Eubacterium* and *Lactobacillus* genera is lower in the control group than in MetS patients, while *Bacteroides fragilis* group, *Parabacteroides distasonis, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Fusobacterium nucleatum, Bifidobacterium longum, Bifidobacterium adolescentis, Ruminococcus flavefaciens* subgroup and *Eubacterium rectale* are depleted in MetS patients (all *P* values <.05). Additionally, we found that long-term consumption of Mediterranean diet partially restores the population of *P. distasonis, B. thetaiotaomicron, F. prausnitzii, B. adolescentis* and *B. longum* in MetS patients (all *P* values <.05). Our results suggest that the Mediterranean diet could be a useful tool to restore potentially beneficial members of the gut microbiota, although the stability of these changes over time still remains to be assessed.

Keywords: Microbiota; Obesity; Metabolic syndrome; Mediterranean diet; Cardiovascular disease

1. Introduction

The microbial communities harbored in the human intestine are involved in innate and adaptative immunity, as well as in controlling energy balance; they act collectively as an organ that is fully integrated in the host metabolism [1]. Despite mounting evidence in animal models for the role of the gut microbiota in body weight and obesity [2–4], studies in humans are scarce and causality is yet to be established. While a balanced microbiota confers benefits to the host, microbial imbalances have been associated with metabolic disorders such as dyslipidemia, insulin resistance and type 2 diabetes [5,6]. In fact, some studies have suggested that changes in the intestinal microbiota may trigger pathogenic mechanisms that promote inflammation, insulin resistance and the development of metabolic syndrome (MetS) [7,8]. Moreover, the loss of immunological tolerance associated with changes in the Firmicutes/Bacteroidetes ratio seems to play a significant role in the development of obesity and eventually the initiation of MetS [7].

The shaping of the gut microbiome is currently considered as a therapeutic target, since specific changes in the gut microbial community might counteract the development of obesity and MetS [9]. Although the adult human gut microbiota community is relatively stable over long periods of time [10], dietary interventions can influence its composition and could potentially be used as therapeutic tools to alleviate and treat conditions triggered by microbial imbalances [11]. In fact, it has already been shown that the consumption of a high-fat high-protein diet increases levels of *Bacteroides* versus *Prevotella*, which is more abundant after high-carbohydrate diets [12]. Moreover, the inverse relationship between *Prevotella* and *Bacteroides* has been reproduced in studies comparing the microbiota of subjects from agrarian societies with those from industrialized societies [13,14]. In addition, the consumption of diets higher in fruit, vegetables and fiber is linked to increased microbial richness, at either the taxonomic level or the gene level [15]. The gut

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microbiome can even respond to short-term modifications of macronutrient content in the diet, although it quickly returns to baseline composition after the intervention ceases [16]. It is therefore hypothesized that only long-term dietary interventions can substantially impact the microbiota [11].

In this study, our aim was to evaluate the differences in the bacterial community structure of the intestinal microbiota between MetS patients and a group of individuals without MetS and to test the effect of the long-term consumption of two healthy diets: a Mediterranean diet and a low-fat high-carbohydrate diet, in restoring the gut microbiota composition.

2. Materials and methods

2.1. Study subjects

The current work was conducted in a subgroup of 239 patients within the CORDIOPREV study (Clinical Trials.gov.Identifier: NCT00924937), an ongoing prospective, randomized, opened and controlled trial in patients with coronary heart disease (CHD), who had their last coronary event over 6 months before enrolling in two different dietary models (Mediterranean and low-fat) over a period of 5 years, in addition to conventional treatment for CHD [17]. All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethics committees, following the Helsinki declaration and good clinical practice.

The 239 patients were divided into two groups: the first group consisting of 138 MetS patients was selected according to the National Cholesterol Education Program's Adult Treatment Panel III criteria for MetS [18] with increased abdominal fat waist circumference (>102 cm for males and >88 cm for females), high triglycerides (TG; ≥150 mg/dl), low high-density lipoprotein cholesterol (HDL-C; <40 mg/dl for males and <50 mg/dl for females), high fasting glucose (≥100 mg/dl), systolic arterial blood pressure of ≥130 mmHg and/or diastolic arterial blood pressure of ≥85 mmHg. The other group consisted of 101 subjects without MetS. The baseline characteristics of the subjects in the study are shown in Supplemental Table 1.

2.2. Study design

The study design has been previously described [19]. Briefly, participants of each of the two groups were randomized to receive two diets: a Mediterranean diet and a low-fat diet. The composition was as follows: (a) low-fat high-carbohydrate diet: 28% fat (12% monounsaturated, 8% polyunsaturated and 8% saturated) and (b) Mediterranean diet: 35% fat (22% monounsaturated, 6% polyunsaturated and 7% saturated). Furthermore, to ensure that the main fat source of the Mediterranean diet (olive oil) was identical for all patients in this group, the olive oil was given to the participants by the research team. Food packs, including low-fat foods (cereals, biscuits, pasta, etc.) of similar cost, were provided for the patients who were randomized to the low-fat group. Diet assessment was performed using a validated 14-item questionnaire to assess adherence to the Mediterranean diet [20] and a similar 9-point score to assess adherence to low-fat diet at baseline before the start of the dietary intervention and yearly follow-up visits.

2.3. Clinical plasma parameters

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA at baseline before the start of the dietary intervention and yearly follow-up visits. The plasma was separated from the red cells by centrifugation at 1500*g* for 15 min at 4°C. Analytes determined in frozen samples were analyzed centrally by laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, who were unaware of the interventions. Lipid variables were assessed with a DDPPII Hitachi modular analyzer (Roche) using specific reagents (Boehringer Mannheim). Plasma TG and cholesterol concentrations were assayed by enzymatic procedures [21,22]. HDL-C was measured by the precipitation of a plasma aliquot with dextran sulfate-Mg^{2+}, as described by Warnick *et al.* [23]. Low-density lipoprotein cholesterol was calculated using the following formula: plasma cholesterol−(HDL-C+large TRL-C+small TRL-C). Therefore, glucose determination was performed by the hexokinase method.

2.4. DNA extraction from fecal samples

To collect the fecal samples, we gave the patients a box with carbonic snow and a sterile plastic bottle with a screw cap to keep the frozen sample. Once delivered to the laboratory staff, the sample was stored at −80°C until microbial DNA was extracted. Hence, this was performed using the QIAamp DNA Kit Stool Mini Kit Handbook (Qiagen, Hilden, Germany) following the manufacturer's instructions. This protocol was optimized for a 180- to 220-mg sample. Consequently, bacterial DNA was quantified using with a Nanodrop ND-1000 v3.5.2 spectrophotometer (Nanodrop Technology, Cambridge, UK); the samples were stored at −20°C.

2.5. Quantification of the bacterial composition by real-time quantitative polymerase chain reaction analysis

Specific primers for 16S rRNA gene in different bacterial species (Supplemental Table 2) were used to characterize the fecal microbiota using real-time quantitative polymerase chain reaction (PCR). We selected the bacterial species on the basis of finding specific primers and for being species with known functions. Each PCR reaction contained 5 ng of fecal DNA and 2 μl of each primer at a concentration of 5 pmol/μl using the iQ SYBR Green Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), in an iQ5 real-time PCR detection system thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The reaction was incubated at 95°C for 8 min, followed by 40 cycles of 1 min at 95°C, 30 s at 60°C and 20 s at 72°C.

In order to assess the specificity of the amplifications, PCR products were run on an agarose gel at 1.5% in a TBE buffer and the DNA bands were excised from the agarose gel for subsequent sequencing, which was performed at the Central Service for Research Support of the University of Cordoba. In addition, the nucleotide sequences were compared with known sequences in the GenBank database using the BLAST algorithm. Moreover, the specificity of PCR amplifications was checked in each PCR reaction by a melting curve program (60–95°C with a heating rate of 0.5°C/s and a continuous fluorescence measurement). The relative abundance of each bacterial species was calculated using the total bacterial abundance as a reference: the first two pairs of universal bacteria primer were used and then both were combined by the BestKeeper method to obtain an accurate reference value [relative abundance=$2^{-(Ct,\ target\ specie - Ct,\ reference)}$] [24].

2.6. Statistical analysis

We used PASW Statistics, Version 18 (Chicago, IL, USA) to perform the statistical analysis. The normal distribution of variables was assessed using the Kolmogorov–Smirnov test. When variables followed a normal distribution (metabolic variables), one-factor analysis of variance was used to compare the baseline metabolic variables between the MetS and the non-MetS groups. When variables did not follow a normal distribution, we used nonparametric methods. The Mann–Whitney *U* test analysis was used to compare the statistically significant differences in the relative abundance of the bacterial species between MetS patients and the group without MetS. The statistically significant microbiota changes by diet were assessed by the Wilcoxon signed-rank test. A study of the relationship among parameters was also carried out using Pearson's linear correlation coefficient. All data presented are expressed as mean±S.E.M. A *P* value <0.05 was considered significant.

3. Results

3.1. Baseline characteristic of the study participants

No significant differences in age were observed among the groups. As expected, the MetS group had higher waist circumference, TG, glucose and blood pressure and lower HDL-C plasma levels in metabolic variables than the non-MetS group. No significant differences were observed between the patients assigned to Mediterranean or low-fat diets for either MetS or non-MetS groups (Supplemental Table 1).

3.2. MetS and gut microbiota

Relative abundance of *Bacteroides*, *Eubacterium* and *Lactobacillus* genera at basal time was higher in the MetS patients than in the non-MetS group ($P<.05$). We also analyzed the differences in the relative abundance of 18 bacterial species belonging to the most abundant phyla and genera, known to be present in human gut intestinal microbiota in both groups (Fig. 1). We observed that the relative abundance of *Bacteroides fragilis* group, *Parabacteroides distasonis*, *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, *Fusobacterium nucleatum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Ruminococcus flavefaciens* subgroup and *Eubacterium rectale*, at basal time, was lower in MetS patients than in the control subjects ($P<.05$).

3.3. Relationship between MetS-related variables and the gut microbiota

We observed a negative relationship between the waist circumference and the relative abundance of *B. thetaiotaomicron*, *P. distasonis*, *F. prausnitzii*, *B. longum*, *R. flavefaciens* subgroup and *B. adolescentis* ($R=-0.162$, $P=.022$; $R=-0.213$, $P=.002$; $R=-0.294$, $P<.001$; $R=-0.297$, $P<.001$; $R=-0.176$, $P=.013$; $R=-0.211$, $P=.003$). We

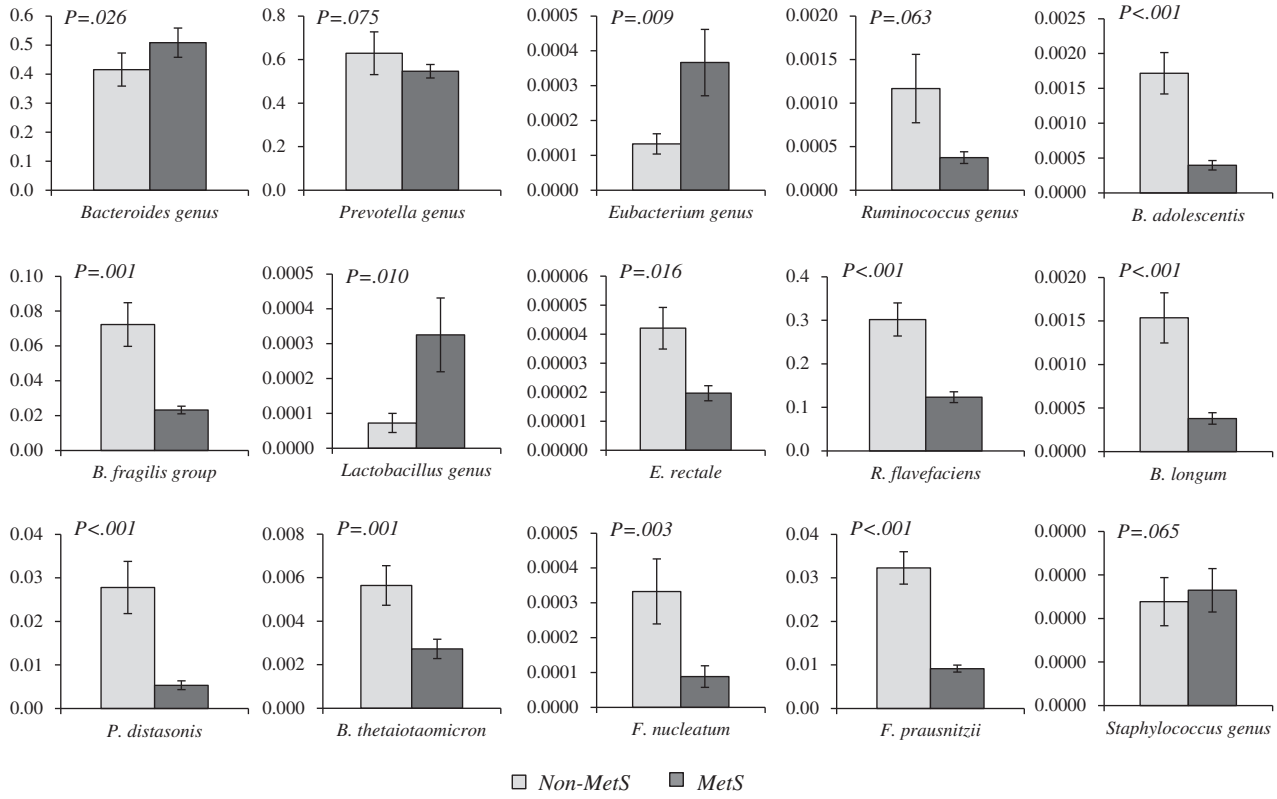

Fig. 1. Differences in the gut microbiota composition of MetS patients. Values are the mean±S.E.M. The statistically significant differences between each group were tested by the Mann–Whitney test.

also observed a positive relationship between the c-HDL plasma levels and the relative abundance of *B. fragilis* group, *B. thetaiotaomicron*, *F. prausnitzii*, *B. longum*, *R. flavefaciens* subgroup, *B. adolescentis* and *F. nucleatum* (R=0.146, P=.039; R=0.265, P<.001; R=0.296, P<.001; R=0.190, P=.007; R=0.163, P=.021; R=0.141, P=.046; R=0.140, P=.048). Furthermore, there was a negative relationship between the TG plasma levels and the relative abundance of *B. fragilis* group, *B. thetaiotaomicron*, *P. distasonis*, *F. prausnitzii*, *B. longum*, *R. flavefaciens* subgroup and *B. adolescentis* (R=−0.175, P=.013; R=−0.211, P=.003; R=−0.200, P=.005; R=−0.279, P<.001; R=−0.145, P=.040; R=−0.275, P<.001; R=−0.174, P=.014). In addition a negative relationship exists between the glucose plasma levels and the relative abundance of *P. distasonis* and *B. longum* (R=−0.161, P=.022; R=−0.145, P=.040). Finally, there was also a negative relationship between the systolic blood pressure and the relative abundance of *B. longum* (R=−0.167, P=.018) (Supplemental Fig. 1).

3.4. Mediterranean diet affects microbiota composition

In order to assess whether diet significantly impacts the microbiota profile of MetS patients, we analyzed bacterial composition after 2 years of consumption of a Mediterranean or a low-fat diet (Table 1). We observed that Mediterranean diet induced a statistically significant increase in the abundance of *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *B. adolescentis* and *B. longum* (P<.05) in the MetS, but not in the non-MetS group. By contrast, we observed a statistically significant increase in the abundance of *E. rectale* (P<.05) in the non-MetS, but not in the MetS group. Additionally, the consumption of the low-fat diet for 2 years decreased the abundance of *P. distasonis* (P<.05) in the non-MetS group, which remained unchanged in the MetS patients group.

We also observed a weak but significant relationship between the Mediterranean diet score after 2 years of dietary intervention and the abundance of *F. prausnitzii* (R=0.158, P=.028), as well as the changes in the abundance of *B. adolescentis* (R=0.147, P=.040).

4. Discussion

Our data show that the *Bacteroides*, *Eubacterium* and *Lactobacillus* genera were significantly increased, while *B. fragilis* group, *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *F. nucleatum*, *B. longum*, *B. adolescentis*, *R. flavefaciens* subgroup and *E. rectale* were decreased significantly in MetS patients compared with the non-MetS group. More interestingly, our results suggest that long-term consumption of Mediterranean diet increases the abundance of *P. distasonis*,

Table 1
Diet-induced relative abundance fold change in microbiota composition in the panel of bacterial species.

Experimental group	Non-MetS group		MetS group	
Bacterial species/diet	Low fat	Mediterranean	Low fat	Mediterranean
P. distasonis	**0.87±0.29** *	1.70±0.23	1.79±0.22	**1.75±0.22** *
B. thetaiotaomicron	1.38±0.25	1.37±0.20	1.29±0.19	**1.58±0.19** *
F. prausnitzii	1.47±0.28	1.63±0.23	1.29±0.21	**1.78±0.22** *
B. adolescentis	1.11±0.34	1.50±0.28	1.40±0.26	**2.26±0.27** *
B. longum	1.54±0.33	1.16±0.27	1.58±0.25	**2.01±0.25** *
E. rectale	1.08±0.25	**1.90±0.21** *	1.39±0.19	1.23±0.20
B. fragilis group	1.51±0.26	1.65±0.21	1.17±0.20	1.37±0.20
R. flavefaciens subgroup	1.32±0.29	1.64±0.24	1.10±0.22	1.63±0.22
F. nucleatum	1.32±0.27	1.50±0.22	1.10±0.21	1.04±0.21

Fold change normalized versus relative abundance values at baseline. The statistically significant microbiota changes by diet were assessed by the Wilcoxon signed-rank test.
* P<.05.

B. thetaiotaomicron, *F. prausnitzii*, *B. adolescentis* and *B. longum* in the MetS patients, although MetS persists.

Several studies have shown evidence that alterations in gut microbiota may lead to obesity and MetS, directly or as a consequence of the disturbances in the gut microbiota that causes the "low-grade" inflammation that may promote the development of MetS [25,26]. In this regard, we note that our results show a negative correlation between the abundance of *B. fragilis* group, *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *F. nucleatum*, *B. longum*, *B. adolescentis*, *R. flavefaciens* subgroup and *E. rectale* with plasma levels of glucose and TG and a positive correlation with plasma levels of HDL. These results further support the idea that gut microbiota acts collectively as a fully integrated organ in the host metabolism [1]. However, it also modulates host energy and lipid metabolism [4]. Thus, changes in the intestinal microbiota may trigger pathogenic mechanisms once obesity is established; this promotes insulin resistance and the development of MetS [7–9].

Moreover, the observed reduction in MetS patients in the abundance of several bacterial species within the *Bacteroides* and *Ruminococcus* genera with important saccharolytic activity, such as *B. fragilis* group, *P. distasonis*, *B. thetaiotaomicron* and the *R. flavefaciens* subgroup [27–29], suggests a reduction in carbohydrate degradation capacity in MetS patients, which may also cause a reduction in propionate and acetate production [30,31]. The latter point is particularly relevant in this context, as a reduction of acetate levels in the gut may also reduce the abundance of beneficial bacteria (as observed in our study) such as *F. prausnitzii* and *E. rectale*. Hence, this bacteria consume acetate and produce butyrate [32,33], in addition to the decrease in *E. rectale*, *F. nucleatum* and *F. prausnitzii*, which directly degrade carbohydrate to produce butyrate.

Although previous studies have described an individual-specific microbiota with high stability over time [10] and resistance to perturbations [34,35], recent research indicates that changes in the gut microbiota composition may occur after dietary interventions [12,16,36,37] and that long-term periods following a specific diet can affect the microbiota in a substantial way [11]. Our results further strengthen this hypothesis, as the consumption of a Mediterranean diet over 2 years resulted in a significant modification of the gut microbiota composition of MetS patients.

Previous studies have shown that specific foods consumed in the traditional Mediterranean diet have an influence on the gut microbiota composition [38,39]. Antioxidant phenolic compounds are consumed in the Mediterranean diet through different products such as fresh fruit, vegetables, red wine and olive oil. In fact, it has been shown that red wine consumption increases the growth of *Enterococcus*, *Prevotella*, *Bacteroides* and *Bifidobacterium* genera abundance in healthy humans [37]. In addition, a study performed using culture fermentation systems reflective of the distal region of the human large intestine showed that a pomegranate product significantly enhances the growth of *Bifidobacteria* and *Lactobacilli* [40], suggesting that the fruit, another source of antioxidants in Mediterranean diet, may also influence gut microbiota composition.

In line with this, our study showed that the consumption of a Mediterranean diet, containing phenolic-compound-rich foods such as fresh fruit, vegetables, red wine and olive oil, is more effective in increasing the levels of bacterial species found to be lower in MetS patients, such as *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *B. adolescentis* and *B. longum*. Consequently, the consumption of a low-fat diet that is more abundant in whole grains significantly lowers in sources of phenolic compounds and lowers in fiber intake than the Mediterranean diet, which did not result in a similar increase in the abundance of these bacteria. Hence, this was evidenced by the nutritional assessment of the diet compliance by surveys. Moreover, the positive correlation between Mediterranean diet score and the abundance of *F. prausnitzii* and *B. adolescentis* further supports the hypothesis that Mediterranean diet induces significant changes in gut microbiota composition.

Additionally, in terms of fat percentage, our study supports the idea that the consumption of diet with a high percentage of fat as Mediterranean diet in comparison with the low-fat diet administered increases the abundance of bile resistance taxa such as *Bacteroides* [16]. However, this is because the intake of fat increases the secretion of bile acids [41].

Moreover, it is particularly important due to the fact that the consumption of Mediterranean diet increased the abundance of the *Bacteroides* genus member *B. thetaiotaomicron* and *F. prausnitzii*, which suggest that the consumption of this diet may increase or maintain a microbiota with antiinflammatory capability [42]. Thus, this is in agreement with the antiinflammatory effects associated with the consumption of Mediterranean diet consumption [43]. Overall, our study showed that the consumption of Mediterranean diet influenced the gut microbiota composition mainly in the MetS patients. This was presumed by the dysbiosis observed in this population compared to the non-MetS, suggesting that its consumption may help in maintaining the gut microbiota homeostasis, which is particularly important in conditions of an alteration of microbiota such as obesity and MetS. Hence, this could contribute to explaining the low rates of cardiovascular mortality found in Southern European Mediterranean countries, in comparison with other Western populations [44].

However, our study has the limitation that the relationship between cardiovascular risk factors and microbiota species, although significant, was low. Moreover, the long-term consumption of a Med diet partially restores the alteration in the gut microbiota composition observed in MetS patients, without the disappearing of the syndrome, which suggests that longer periods of Med diet consumption may be needed.

In conclusion, our results suggest that Mediterranean diet could be a useful tool in manipulating the gut microbiota. Thus, further studies will be required to fully understand the effect of the Mediterranean diet in shaping gut microbiota and its effect on human health.

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Conflict of Interest

None of the authors has any conflict of interest that could affect the performance of the work or the interpretation of the data.

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References

[1] Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489:242–9.
[2] Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–103.
[3] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31.
[4] Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, et al. The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res 2010;51:1101–12.
[5] Caesar R, Fak F, Backhed F. Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism. J Intern Med 2010;268:320–8.
[6] Diamant M, Blaak EE, de Vos WM. Do nutrient-gut-microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes? Obes Rev 2011;12:272–81.
[7] Bajzer M, Seeley RJ. Physiology: obesity and gut flora. Nature 2006;444:1009–10.
[8] Iyer A, Fairlie DP, Prins JB, Hammock BD, Brown L. Inflammatory lipid mediators in adipocyte function and obesity. Nat Rev 2010;6:71–82.
[9] Cani PD, Delzenne NM. The gut microbiome as therapeutic target. Pharmacol Ther 2011;130:202–12.
[10] Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, et al. The long-term stability of the human gut microbiota. Science 2013;341:1237439.
[11] Moschen AR, Wieser V, Tilg H. Dietary factors: major regulators of the gut's microbiota. Gut Liver 2012;6:411–6.
[12] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–8.
[13] Lin A, Bik EM, Costello EK, Dethlefsen L, Haque R, Relman DA, et al. Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. PLoS One 2013;8:e53838.
[14] Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222–7.
[15] Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 2014;146:1564–72.
[16] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559–63.
[17] Alcala-Diaz JF, Delgado-Lista J, Perez-Martinez P, Garcia-Rios A, Marin C, Quintana-Navarro GM, et al. Hypertriglyceridemia influences the degree of postprandial lipemic response in patients with metabolic syndrome and coronary artery disease: from the CORDIOPREV study. PLoS One 2014;9:e96297.
[18] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Curr Opin Cardiol 2006;21:1–6.
[19] Perez-Martinez P, Alcala-Diaz JF, Delgado-Lista J, Garcia-Rios A, Gomez-Delgado F, Marin-Hinojosa C, et al. Metabolic phenotypes of obesity influence triglyceride and inflammation homoeostasis. European Journal of Clinical Investigation 2014;44:1053–64.
[20] Schroder H, Fito M, Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr 2011;141:1140–5.
[21] Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–5.
[22] Paisley EA, Park EI, Swartz DA, Mangian HJ, Visek WJ, Kaput J. Temporal-regulation of serum lipids and stearoyl CoA desaturase and lipoprotein lipase mRNA in BALB/cHnn mice. J Nutr 1996;126:2730–7.
[23] Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379–88.
[24] Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper — Excel-based tool using pair-wise correlations. Biotechnol Lett 2004;26:509–15.
[25] Chassaing B, Gewirtz AT. Gut microbiota, low-grade inflammation, and metabolic syndrome. Toxicol Pathol 2014;42:49–53.
[26] Zupancic ML, Cantarel BL, Liu Z, Drabek EF, Ryan KA, Cirimotich S, et al. Analysis of the gut microbiota in the old order Amish and its relation to the metabolic syndrome. PLoS One 2012;7:e43052.
[27] Bayer EA, Lamed R, White BA, Flint HJ. From cellulosomes to cellulosomics. Chem Rec 2008;8:364–77.
[28] Salyers AA, Vercellotti JR, West SE, Wilkins TD. Fermentation of mucin and plant polysaccharides by strains of Bacteroides from the human colon. Appl Environ Microbiol 1977;33:319–22.
[29] Salyers AA, West SE, Vercellotti JR, Wilkins TD. Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. Appl Environ Microbiol 1977;34:529–33.
[30] Falony G, Vlachou A, Verbrugghe K, De Vuyst L. Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. Appl Environ Microbiol 2006;72:7835–41.
[31] Wrzosek L, Miquel S, Noordine ML, Bouet S, Joncquel Chevalier-Curt M, Robert V, et al. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. BMC Biol 2013;11:61.
[32] Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol 2007;73:1073–8.
[33] Hold GL, Schwiertz A, Aminov RI, Blaut M, Flint HJ. Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. Appl Environ Microbiol 2003;69:4320–4.
[34] Vanhoutte T, De Preter V, De Brandt E, Verbeke K, Swings J, Huys G. Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and Saccharomyces boulardii. Appl Environ Microbiol 2006;72:5990–7.
[35] Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microbiol 1998;64:3854–9.
[36] Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology 2012;142:1100–1.
[37] Queipo-Ortuno MI, Boto-Ordonez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, et al. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Am J Clin Nutr 2012;95:1323–34.
[38] Del Chierico F, Vernocchi P, Dallapiccola B, Putignani L. Mediterranean diet and health: food effects on gut microbiota and disease control. Int J Mol Sci 2014;15:11678–99.
[39] Lopez-Legarrea P, Fuller NR, Zulet MA, Martinez JA, Caterson ID. The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. Asia Pac J Clin Nutr 2014;23:360–8.
[40] Bialonska D, Ramnani P, Kasimsetty SG, Muntha KR, Gibson GR, Ferreira D. The influence of pomegranate by-product and punicalagins on selected groups of human intestinal microbiota. Int J Food Microbiol 2010;140:175–82.
[41] Reddy BS. Diet and excretion of bile acids. Cancer Res 1981;41:3766–8.
[42] Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008;105:16731–6.
[43] Estruch R. Anti-inflammatory effects of the Mediterranean diet: the experience of the PREDIMED study. Proc Nutr Soc 2010;69:333–40.
[44] Perez-Jimenez F, Alvarez de Cienfuegos G, Badimon L, Barja G, Battino M, Blanco A, et al. International conference on the healthy effect of virgin olive oil. Eur J Clin Investig 2005;35:421–4.