International Journal of Obesity (2013) **37**, 1460–1466

www.nature.com/ijo

OPEN

ORIGINAL ARTICLE

Correlation between body mass index and gut concentrations of *Lactobacillus reuteri*, *Bifidobacterium animalis*, *Methanobrevibacter smithii* and *Escherichia coli*

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BACKGROUND: Genus and species level analysis is the best way to characterize alterations in the human gut microbiota that are associated with obesity, because the clustering of obese and lean microbiotas increases with the taxonomic depth of the analysis. *Bifidobacterium* genus members have been associated with a lean status, whereas different *Lactobacillus* species are associated both with a lean and an obese status.

OBJECTIVES AND METHODS: We analyzed the fecal concentrations of Bacteroidetes, Firmicutes, *Methanobrevibacter smithii*, the genus *Lactobacillus*, five other *Lactobacillus* species previously linked with lean or obese populations, *Escherichia coli* and *Bifidobacterium animalis* in 263 individuals, including 134 obese, 38 overweight, 76 lean and 15 anorexic subjects to test for the correlation between bacterial concentration and body mass index (BMI). Of these subjects, 137 were used in our previous study.

FINDINGS: Firmicutes were found in >98.5%, Bacteroidetes in 67%, *M. smithii* in 64%, *E. coli* in 51%, *Lactobacillus* species between 17 and 25% and *B. animalis* in 11% of individuals. The fecal concentration of *Lactobacillus reuteri* was positively correlated with BMI (coefficient = 0.85; 95% confidence interval (CI) 0.12–0.58; $P = 0.02$) in agreement with what was reported for *Lactobacillus sakei*. As reported, *B. animalis* (coefficient = −0.84; 95% CI −1.61 to −0.07; $P = 0.03$) and *M. smithii* (coefficient = −0.43, 95% CI −0.90 to 0.05; $P = 0.08$) were negatively associated with the BMI. Unexpectedly, *E. coli* was found here for the first time to negatively correlate with the BMI (coefficient = −1.05; 95% CI −1.60 to −0.50; $P < 0.001$).

CONCLUSION: Our findings confirm the specificity of the obese microbiota and emphasize the correlation between the concentration of certain *Lactobacillus* species and obesity.

International Journal of Obesity (2013) **37,** 1460–1466; doi:10.1038/ijo.2013.20; published online 5 March 2013

Keywords: body mass index; *Lactobacillus*; *Bifidobacterium*; probiotics; *Methanobrevibacter smithii*; *Escherichia coli*

INTRODUCTION

Obesity is defined by a body mass index (BMI) > 30 kg m^{-2} (ref. 1) and a massive expansion of fat and is associated with a significant increase in morbidity and mortality.[2,3] The frequency of obesity is rising among children, adolescents and adults and has doubled since 1980. According to the WHO, 65% of the world's population lives in countries where excess weight and obesity kills more people than underweight conditions, including all high-income and most middle-income countries (www.who.int).

The digestive microbiota is a complex ecosystem that consists of viruses, bacteria, archaea, fungi and parasites. Specific enterotypes have been identified regardless of ethnic or geographical origins.[4] They have been linked to diet,[5] and their antibiotic-mediated modulation can impact the metabolic profile of the host.[6] Because the gut is a 'hot spot' for horizontal gene transfer between an astronomical number of bacteria (>10^9 g^{-1}), archaea and viruses,[7] analysis at the gene level was found to be the best way to characterize gut microbiota alteration and its correlation with obesity.[8] Conversely, we and others have found that analysis on a taxonomic basis remains fully relevant, specifically at the species level.[9]

A decreased Bacteroidetes/Firmicutes ratio was initially shown to be associated with obesity,[10] but the discrimination between lean and obese gut microbiota is improved when the taxonomic depth of the analysis is increased.[11] For instance, the *Bifidobacterium* genus was associated with lean humans in a meta-analysis, including studies from Finland, Germany, Spain and China.[12] Conversely, we showed that among *Lactobacillus* species previously associated with obesity[13,14] and diabetes,[15] some have also been associated with weight gain[9,16] while others have more of a protective effect.[16–18] Other bacterial species, such as *Tropheryma whipplei*, have been associated with acquired obesity.[19] Finally, Karlsson *et al.*[20,21] linked the *Enterobacteriaceae* and specifically *Escherichia coli* to overweight and obesity.

Here, we looked at the inter-relationships among *E. coli*, one of the main representatives of the *Enterobacteriaceae*, *Methanobrevibacter smithii*, a leading representative of the gut archaea,[22] *Bifidobacterium animalis* and 5 *Lactobacillus* species

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Received 24 October 2012; revised 14 January 2013; accepted 28 January 2013; published online 5 March 2013

(*Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*). All of the above species have been associated with weight in previous studies.[9,16] Based on our previous case-control study,[9] we have more than doubled the sample size, having included both anorexic and overweight patients in this study, and finally we have analyzed the correlations between the BMI and the considered taxa.

METHODS

Patients

This study was approved by the local ethics committee (accession number 10-002, 2010). Fecal samples were obtained from hospitalized patients and outpatients at the Nutrition Unit (Hopital La Timone, Marseille, France) who were overweight, obese or anorexic. The controls were healthy individuals recruited based on a snowball approach and included subjects of our previous study and outpatients who were not treated with antibiotics at the infectious disease unit (Hopital La Timone, Marseille, France). Anorexic subjects met the DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) for anorexia nervosa. The inclusion criteria were adults for whom the BMI value and a fecal sample were readily available. The exclusion criteria were patients <18 years of age, a history of colon cancer, the presence of an inflammatory bowel disease, an acute or a chronic diarrhea in the previous 4 weeks and an antibiotic administration <6 months before the fecal sampling. Clinical data (gender, date of birth, clinical history, weight, height and antibiotic use) were recorded using a standardized questionnaire. Other factors, such as yogurt (pro- and prebiotics) intake, vegetarian habits, ethnicity or familial obesity, were not taken into consideration in the analysis of the data. Four groups were identified as follows: group I: obese subjects (BMI $>30\,kg\,m^{-2}$), group II: overweight subjects (BMI >25 and $<30\,kg\,m^{-2}$), group III: lean subjects (BMI >19 and $<25\,kg\,m^{-2}$) and group IV: anorexic subjects (BMI $<19\,kg\,m^{-2}$). A total of 137 patients from our previous study[9] and 126 new subjects were included, of whom 15 were anorexic, 30 were lean controls, 21 were overweight and 60 were obese. Data from our previous study were also included, and most samples from that study were analyzed further for the presence of *E. coli*. All new samples were also analyzed for the presence of Bacteroidetes, Firmicutes, genus *Lactobacillus*, *E. coli*, *M. smithii*, *L. reuteri*, *L. plantarum*, *L. rhamnosus*, *L. fermentum* and *L. acidophilus*.

PCR

PCR analysis was performed as previously described[9] except for *E. coli*, for which the protocol was the same, but the primers and probes were the following: Forward, 5′-GCTGCGCGTGCAAATGCG-3′; Reverse, 5′-CATGGT CATCGCTTCGGTCT-3′; and probe, 5′-CATCAGAAACTGAACACCAC-3′. The primers for *L. reuteri* were evaluated in our previous study[9] and have a very high specificity at the species level with a low cross-reactivity (cycle threshold >35 for DNA extracted from pure culture) with *Lactobacillus oris* and *Lactobacillus pontis*. However, it cannot be excluded that the detection of *L. oris*, exceptionally present in the human gut,[23] could have yielded false-positive results. Conversely, *L. pontis* has never been reported in the human gut. The results in this study are depicted as log10 DNA copies ml^{-1}.

Statistical analysis

As an exploratory step, a principal component analysis was performed, including BMI and the concentrations of all taxa present at the phylum and species levels.

Initially, we tested whether the bacterial prevalence was different between each BMI group using the bilateral Pearson Chi-square test. A bilateral Barnard exact test[24] was used when the Pearson Chi-square test was not applicable. Because it is unknown whether overweight individuals should be considered as individuals with a disease or controls, all the groups were compared either with group I (obese subjects who were considered as cases) or with group III (lean subjects who were used as controls). A logistic regression using the ascendant maximum likelihood model was used to identify bacteria whose presence was associated with the BMI groups in a multivariate analysis. Three models were used as follows: considering age, sex, Bacteroidetes, Firmicutes and *M. smithii* (phylum level); considering age, sex and *Lactobacillus* (genus level); or considering age, sex, *M. smithii*, *E. coli*, *B. animalis*, *L. reuteri*, *L. plantarum*, *L. fermentum* and *L. rhamnosus* (species level).

As a second step, we tested whether the bacterial concentrations were different according to the BMI groups. Because of a generally non-Gaussian distribution, comparisons were performed using the Kruskal–Wallis test.

Following that step, we tested for the correlation between each bacterial concentration and BMI. As most of the bacterial clades were present in a minority of individuals, a dose-dependent relationship (BMI vs bacterial load) was explored graphically, and the correlation was tested using the Spearman method only on patients harboring each of the clades considered (carriers). Linear regression was used to identify bacteria whose concentrations were correlated with BMI on the whole population. Three models were used as follows: considering age, sex, Bacteroidetes, Firmicutes and *M. smithii* (phylum level); considering age, sex and *Lactobacillus* (genus level); or considering age, sex, *M. smithii*, *E. coli*, *B. animalis*, *L. reuteri*, *L. plantarum*, *L. fermentum* and *L. rhamnosus* (species level).

M. smithii is the major representative of the gut archaeal phylum Euryarchaeota[21] and has been included in the analyses both at the phylum and at the species level. All the tests were bilateral and considered significant when $P<0.05$. The analyses were performed using the SPSS v20.0 (IBM, Paris, France), R version 2.14.0 (R-foundation, Vienna, Austria) and XLSTAT v12 (Addinsoft, Paris, France) software.

RESULTS

Of the 263 patients enrolled in this study, there were 134 obese, 38 overweight, 76 lean and 15 anorexic subjects (Table 1). The average age was $50 \pm$ s.d. 17 years, and 138 (52.5%) of them were males. As was expected, anorexic patients were more frequently found to be younger women. PCR detection and quantification was performed on 262 individuals to study the levels of Bacteroidetes, Firmicutes, *M. smithii* and the *Lactobacillus* genus; on 219 individuals to study each *Lactobacillus* species and *B. animalis*; and on 165 individuals to investigate the levels of *E. coli*.

The prevalences of each bacterial clade were heterogeneous. Firmicutes was found in all the individuals ($>98.5\%$), whereas Bacteroidetes was detected in only 67% (Supplementary Table S1). At the species level, *B. animalis* was found to be the rarest species (11%), whereas *M. smithii* (64%) was shown to be more prevalent than *E. coli* (51%). *Lactobacillus* genus was found in only one-third of the subjects (28%), with different species ranging from 17 to 25% in frequency. In agreement with our previous study,[8] *L. acidophilus* was not detected by our system in any sample, despite the positive amplification of the type strain *L. acidophilus* CIP7613 in our *in silico* study.

When present, the Firmicutes (10^9 DNA copies ml^{-1}) was the most abundant clade before the Bacteroidetes (10^8). At the

Table 1. Population characteristics

	Anorexic subjects (n = *15*)	*Lean subjects* (n = *76*)	*Overweight subjects* (n = *38*)	*Obese subjects* (n = *134*)	P-*value*[a]
Age (mean ± s.d.)	27.3 ± 10.8	49.5 ± 18.6[b]	54.1 ± 17.8	51.8 ± 14.7	<0.0001
Male sex (*n* (%))	1 (7%)	40 (57%)	32 (84%)	65 (49%)	<0.0001
BMI (median, IQR)	13.5 (11.7–14.6)	22.4 (20.7–23.7)	27.1 (25.9–28.6)	40.0 (36.4–46.8)	<0.0001

Abbreviations: BMI, body mass index; IQR, interquartile range. [a]Mann–Whitney *U* test for age and BMI, Pearson chi-square for sex. [b]Data unavailable for seven patients.

species level, when found, *E. coli* (10^7) was 10 times more abundant than *M. smithii* (10^6), whereas *Lactobacillus* species were present at much lower concentrations (10^4–10^5 DNA copies ml^{-1}; $P<0.0001$ when comparing *Lactobacillus* with *E. coli*).

Preliminary analysis by principal component analysis and density plots suggested that some bacterial species or phyla were differentially distributed according to the BMI (Figure 1 and Supplementary Figure S1) and this was confirmed by further analyses (Figures 2 and 3).

Bacterial clades associated with obesity

Genus Lactobacillus. There was a trend towards a higher prevalence of *Lactobacillus* in obese compared with lean patients (32 vs 20%; $P=0.06$) and a higher frequency of *Lactobacillus* in patients with BMIs >25 vs BMIs <25 kg m^{-2} (32 vs 20.8%; $P=0.06$, Supplementary Table S1 and Supplementary Figure S2). In a logistic regression, the presence of *Lactobacillus* was not associated with any BMI group (Supplementary Table S2).

The *Lactobacillus* concentration was higher in obese patients compared with lean patients ($P<0.05$) and in individuals with BMIs >25 kg m^{-2} vs individuals with BMIs <25 kg m^{-2} ($P<0.05$, Figure 2). We also found a positive correlation between the concentration of *Lactobacillus* and BMI in the carriers (patients positive for the genus *Lactobacillus*, correlation coefficient 0.25; $P=0.03$). No significant result was found in a linear regression.

L. reuteri. There was a threefold increase in the *L. reuteri* occurrence in obese patients compared with lean subjects (22 vs 8%; $P=0.01$), a fourfold increase between overweight patients and lean subjects (34 vs 8%; $P=0.001$) and a threefold increase between individuals with BMIs >25 kg m^{-2} compared with individuals with BMIs <25 kg m^{-2} (20 vs 7%; $P=0.001$, Supplementary Figure S2). In a logistic regression, the presence of *L. reuteri* was associated with obesity (odds ratio (OR) $=5.31$; 95% confidence interval (CI) 1.04–27.1; $P=0.04$), overweight (OR $=2.8\times10^7$; 95% CI 6.9–10^{14}; $P=0.03$) or BMI >25 kg m^{-2} (OR $=8.07$; 95% CI 2.06–31.5; $P=0.003$).

The *L. reuteri* concentration was greater in obese vs lean individuals ($P<0.05$), in overweight vs lean individuals ($P<0.005$) and in individuals with BMIs >25 kg m^{-2} compared with individuals with BMIs <25 kg m^{-2} ($P<0.005$, Figure 3). Furthermore, we found a positive correlation between the concentration of *L. reuteri* and BMI (patients positive for *L. reuteri*, coefficient correlation 0.44; $P=0.004$, Figure 4). In a linear regression, a higher concentration of *L. reuteri* was associated with a higher BMI (Table 2).

Bacterial clades associated with lean status

Bacteroidetes. The difference in the occurrence of Bacteroidetes between the obese and the lean groups was not significant (60 vs 70%, respectively; $P=0.18$); however, we found a decreased frequency of Bacteroidetes in obese compared with non-obese individuals (60 vs 74%; $P=0.02$). Moreover, prevalence was increased in overweight compared with obese subjects (84 vs 60%; $P=0.008$, Supplementary Figure S2). In a logistic regression, the presence of Bacteroidetes was associated with the absence of obesity (OR $=0.51$; 95% CI 0.30–0.87; $P=0.01$) or overweight individuals when compared with obese population (OR $=0.28$; 0.11–0.74; $P=0.01$, Supplementary Table S2).

Finally, we found a trend towards decreased concentrations of Bacteroidetes in obese patients compared with lean controls ($P=0.054$), and this decrease in Bacteroidetes concentration was significant when comparing obese with non-obese ($P=0.01$) or with overweight individuals ($P=0.017$, Figure 2). No correlation was found between the Bacteroidetes concentration and BMI in the carrier subgroup. In a linear regression, Bacteroidetes concentration was not correlated with BMI.

M. smithii. There was a trend towards an increased prevalence of *M. smithii* in lean compared with obese individuals (72 vs 60%; $P=0.07$), and this frequency difference was significant when individuals with BMIs <25 kg m^{-2} were compared with individuals with BMIs >25 kg m^{-2} (72 vs 60%; $P=0.04$, Supplementary Figure S2). In a logistic regression, the presence of *M. smithii* was not associated with the absence of obesity but was associated with lean compared with overweight individuals (OR $=0.001$; 95% CI 0–0.98; $P=0.049$, Supplementary Table S2).

The *M. smithii* concentration was lower in obese compared with either lean ($P=0.008$) or non-obese individuals ($P=0.01$). Moreover, the *M. smithii* concentration was higher in patients having BMIs <25 kg m^{-2} compared with patients having BMIs >25 kg m^{-2} ($P=0.005$, Figure 2). We also found a negative correlation between the BMI values and *M. smithii* concentration in patients harboring *M. smithii* (correlation coefficient -0.20; $P=0.01$, Figure 4). In a linear regression, *M. smithii* was not associated with BMI when analyzed at the phylum level as the *M. smithii* phylum is the leading representative of the *Euryarchaeota* in the gut microbiota. Conversely, there was a trend towards a correlation between a higher BMI and a lower *M. smithii* concentration at the species level ($P=0.08$, Table 2).

B. animalis. The prevalence of *B. animalis* was very low in our population, between 6 and 15%, but there was a trend towards a significantly lower occurrence in obese compared with lean

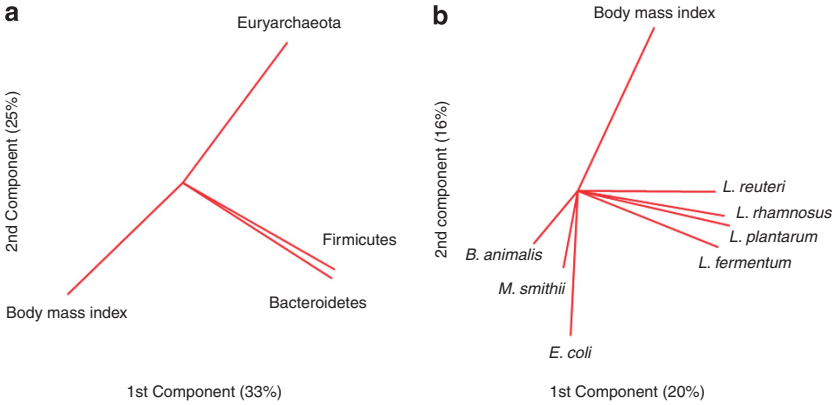

Figure 1. Primary component analysis associating the gut microbial phylum and species to the BMI. Principal component analysis, including (**a**) BMI and phylum or (**b**) species found in the gut microbiota (*Lactobacillus acidophilus* was not included because it was not found by our quantitative PCR system). The preliminary analyses shown in this figure were performed on the whole population.

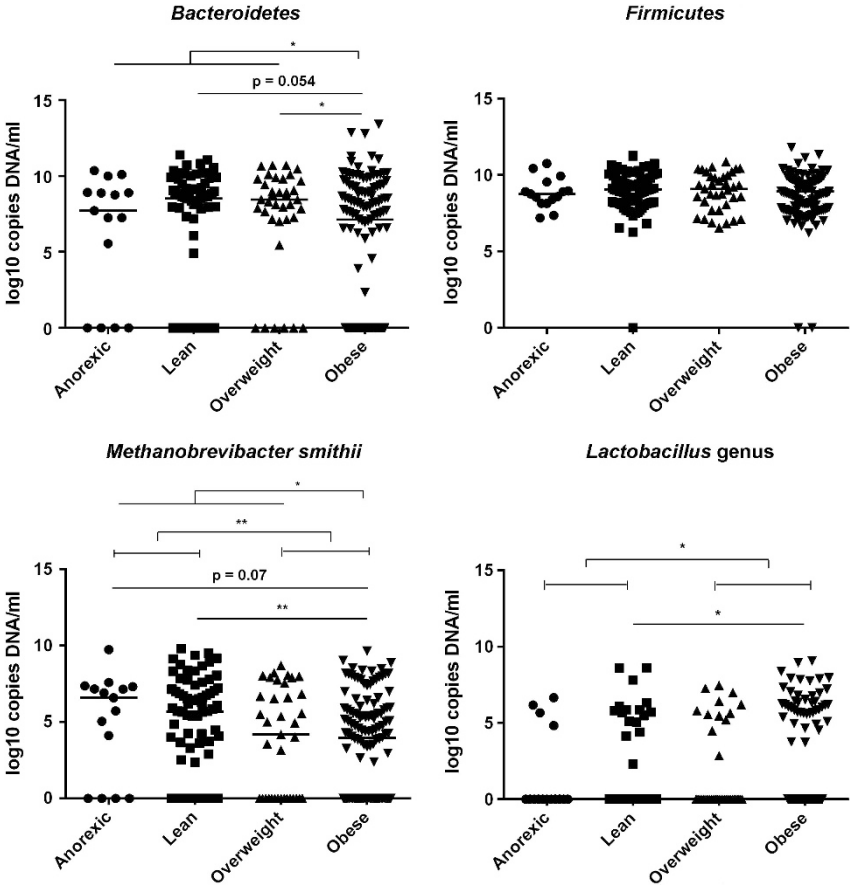

Figure 2. Scatter plots at the phylum and genus levels. *Methanobrevibacter smithii* is considered to be the leading representative of the *Euryarchaeota* phylum. $^{*}P<0.05$, $^{**}P<0.005$. The medians and the interquartile ranges are shown.

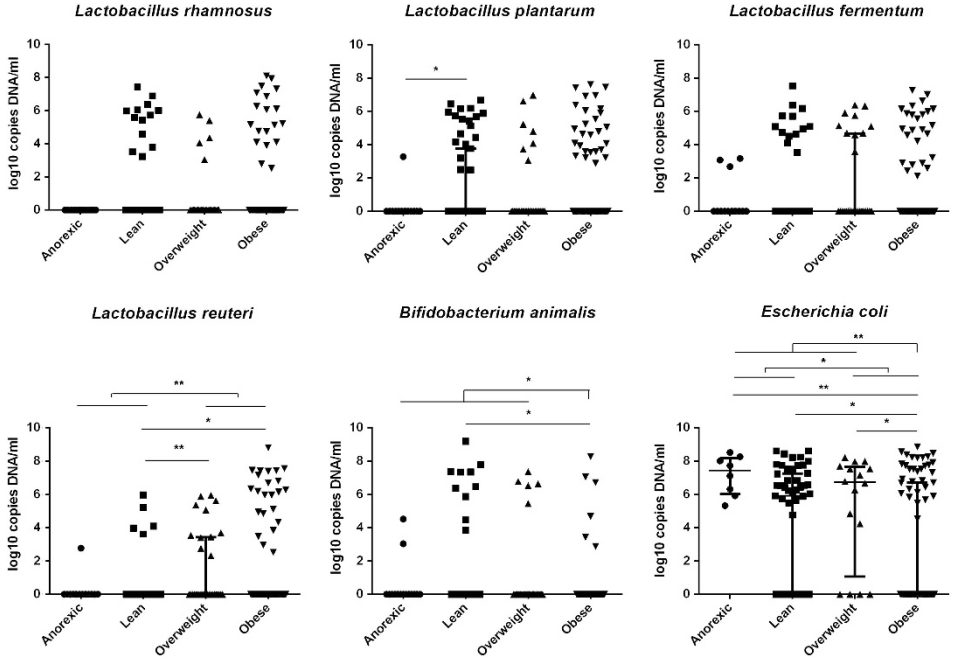

Figure 3. Scatter plots at the species level. $^{*}P<0.05$, $^{**}P<0.005$. The medians and the interquartile ranges are shown.

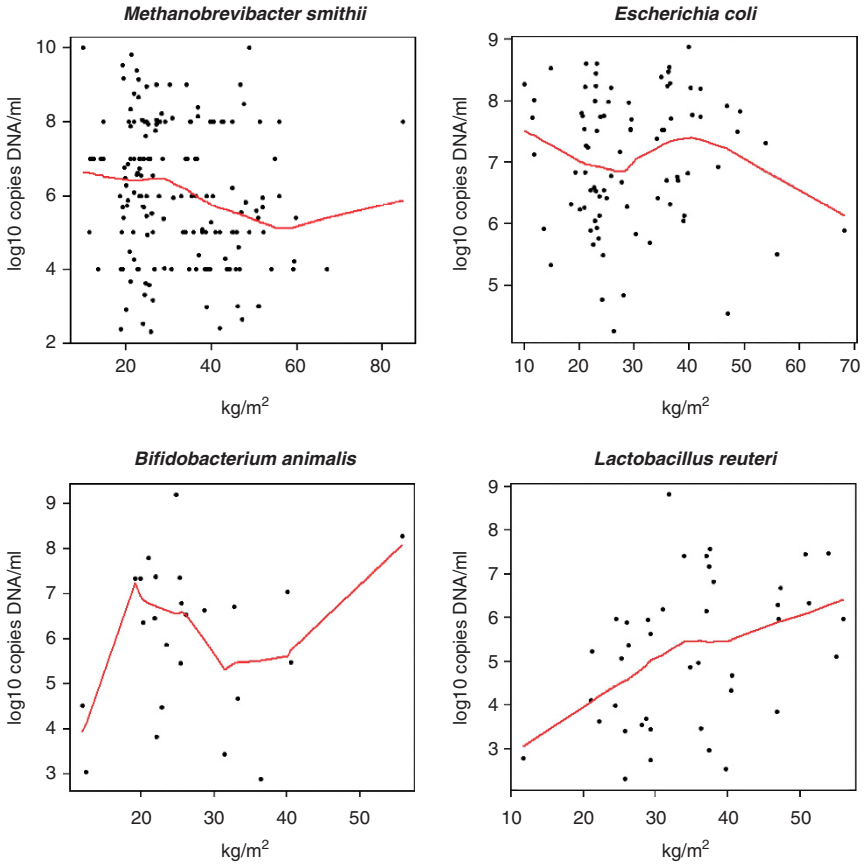

Figure 4. Correlation between the BMI and specific bacterial clades. Plots represent analyses performed only on the carriers for each bacterial clade studied. Spearman correlation test: *Methanobrevibacter smithii* $r = -0.20$, $P = 0.01$. *Lactobacillus reuteri* $r = 0.44$, $P = 0.004$. No correlation was found in the patients positive for *E. coli* ($P = 0.80$) or *Bifidobacterium animalis* ($P = 0.99$).

Table 2. BMI linear regression according to each bacterial clade

Species[a]	*Coefficient (95% CI)*	P-*value*
Methanobrevibacter smithii	−0.43 (−0.90 to 0.05)	0.08
Escherichia coli[b]	−1.05 (−1.60 to −0.50)	<0.001
Bifidobacterium animalis	−0.84 (−1.61 to −0.07)	0.03
Lactobacillus reuteri	0.85 (0.12 to 1.58)	0.02

Abbreviations: BMI, body mass index; CI, confidence interval. [a]Linear regression, adjusted by age and sex, was performed on 218 patients for whom data for *M. smithii*, *B. animalis*, *L. reuteri*, *L. plantarum*, *L. fermentum* and *L. rhamnosus* were available. [b]*E. coli* concentration was available only for 133 of these patients and was replaced by the mean for the 85 lacking data.

individuals (6 vs 15%; $P = 0.052$) and a significant decrease in the incidence of *B. animalis* in obese compared with non-obese individuals (6 vs 15%; $P = 0.04$, Supplementary Figure S2). Using a logistic regression, there was a trend towards an association between the presence of *B. animalis* and lean compared with obese individuals (OR = 0.22; 95% CI 0.05–1.03; $P = 0.054$). Furthermore, the presence of *B. animalis* was associated with lean individuals when compared with overweight subjects (OR = 0; 95% CI 0–0.76; $P = 0.045$).

The concentration of *B. animalis* was significantly lower in obese population compared with lean ($P = 0.045$) and non-obese populations ($P = 0.03$, Figure 3) but no correlation was found between the *B. animalis* concentration and BMI when we performed a univariate analysis (Figure 4). In a linear regression, a higher concentration of *B. animalis* was associated with a lower BMI ($P = 0.03$, Table 2).

E. coli. The prevalence of *E. coli* was lower in obese compared with lean (36 vs 60%; $P = 0.006$), overweight (36 vs 75%; $P = 0.004$) and non-obese individuals (36 vs 47%; $P < 0.001$, Supplementary Figure S2). The prevalence was also significantly lower in individuals with BMIs > 25 kg m^{-2} compared with those with BMIs < 25 kg m^{-2} (31 vs 51%; $P = 0.004$). In a logistic regression, the presence of *E. coli* was associated with the absence of obesity (OR = 0.25; 95% CI 0.1–0.5; $P < 0.001$, Supplementary Table S2), with lean when compared with obese individuals (OR = 0.3; 95% CI 0.1–0.8; $P = 0.01$), with overweight when compared with obese individuals (OR = 0.15; 95% CI 0.03–0.9; $P = 0.01$) and with individuals with BMIs < 25 kg m^{-2} vs individuals with BMIs > 25 kg m^{-2} (OR = 0.3; 95% CI 0.1–0.6; $P = 0.002$).

A lower concentration of *E. coli* was found in obese vs anorexic ($P = 0.001$), lean ($P = 0.02$), overweight individuals ($P = 0.012$) and in individuals with BMIs > 25 vs < 25 kg m^{-2} ($P = 0.02$). Moreover, a lower concentration of *E. coli* was found when comparing obese with non-obese individuals ($P = 0.001$, Figure 3). No correlation was found in the subgroup of individuals positive for *E. coli* (correlation coefficient 0.03, $P = 0.8$, Figure 4). In a linear regression, a higher concentration of *E. coli* was associated with a lower BMI (Table 2).

DISCUSSION

In this study, we found a relatively low prevalence of *Lactobacillus* species because it was detected in only 30% of the individuals, but

L. reuteri was detected in 20% of the study population with occurrence increasing along with BMI values (7, 8, 34 and 22% for anorexic, lean, overweight and obese individuals, respectively). *Lactobacillus* species, and specifically *L. reuteri*, have been previously associated with obesity as it has been reported in our previous case-control studies.[9,13] However, this is the first time that a correlation between the bacterial loads of this species and BMI is reported. To our knowledge, only one other previous study identified a correlation between the *Lactobacillus* species, and specifically *Lactobacillus sakei*, and BMI.[14]

Other prokaryotes have been associated with a lower BMI, as has been previously reported in other publications, such as Bacteroidetes,[5,10,13,25] *B. animalis*[9,12,26,27] and the archeal species *M. smithii*.[28] In contrast to previous studies,[29] we found a lower frequency and lower bacterial loads for *E. coli* in obese individuals with a strong statistical significance. This finding demands a word of caution and requires further confirmation. Moreover, our results suggest a 'dose-dependent' relationship between certain species of bacteria and archaea in the human gut and BMI.

A limitation of our study, as it is for most studies, is that the analysis of the digestive microbiota associated with obesity was performed by analyzing stool samples.[5,25] However, as 95% of fat is absorbed before the cecum,[30] the proximal gut microbiota may be critical for the analysis of factors associated with obesity and diabetes.[31–33] The analysis of the fecal microbiota reflects only indirectly the upper intestinal flora. Indeed, several studies have shown a significant difference in the gut microbiota composition according to the gut section in animals[34] and humans[35] with a proximal (small bowel) enrichment in aerobic Firmicutes (*Streptococcaceae* and *Lactobacillaceae*) and Actinobacteria.[34,35]

Finally, obesity is a multifactorial disease. The causes that drive obesity appear to be influenced by a mixture of environmental, genetic, neural and endocrine factors along with microbes that are also thought to have a role in weight gain.[12,36] Accumulating data has shown that the gut microbiota is associated with both obesity and diet, and there is evidence that modulation of the gut flora by antibiotics,[6,37] during pregnancy[38] or by probiotics[16,36] causes weight gain. The repertoire of bacteria, and especially *Lactobacillus* species, that protect or result in weight gain should be determined at the strain level as the genomic variations within a single species of *Lactobacillus* may be dramatic (only 64% of protein genes are common between *Lactobacillus johnsonii* FI9785 and *L. johnsonii* NCC 533[39]).

CONCLUSION

This work confirms the link between the microbiota and obesity. This link appears to be the result of both diet[5] and the cause of the weight gain as demonstrated by microbiota transplantation from obese individuals or pregnant women to axenic animals.[8,38]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all the volunteers because without them, this study would not have been possible. The sponsors took no part in the design of the study, data collection and analysis, decision to publish or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

DR conceived and designed the experiments. MM, EA, MM, RV, BV and DR performed the clinical study. MM, EA and MH performed the experiments. MM and RG analyzed the data. MM, EA and DR wrote the manuscript.

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