Alterations in gut microbiota associated with a cafeteria diet and the physiological consequences in the host

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ABSTRACT

OBJECTIVE: Gut microbiota have been described as key factors in the pathophysiology of obesity and different components of metabolic syndrome (MetS). The cafeteria diet (CAF)-fed rat is a preclinical model that reproduces most of the alterations found in human MetS by simulating a palatable human unbalanced diet. Our objective was to assess the effects of CAF on gut microbiota and their associations with different components of MetS in Wistar rats.

METHODS: Animals were fed a standard diet or CAF for 12 weeks. A partial least square-based methodology was used to reveal associations between gut microbiota, characterized by 16S ribosomal DNA gene sequencing, and biochemical, nutritional and physiological parameters.

RESULTS: CAF feeding resulted in obesity, dyslipidemia, insulin resistance and hepatic steatosis. These changes were accompanied by a significant decrease in gut bacterial diversity, decreased Firmicutes and an increase in Actinobacteria and Proteobacteria abundances, which were concomitant with increased endotoxemia. Associations of different genera with the intake of lipids and carbohydrates were opposed from those associated with the intake of fiber. Changes in gut microbiota were also associated with the different physiological effects of CAF, mainly increased adiposity and altered levels of plasma leptin and glycerol, consistent with altered adipose tissue metabolism. Also hepatic lipid accretion was associated with changes in microbiota, highlighting the relevance of gut microbiota homeostasis in the adipose-liver axis.

CONCLUSIONS: Overall, our results suggest that CAF feeding has a profound impact on the gut microbiome and, in turn, that these changes may be associated with important features of MetS.

INTRODUCTION

The incidence of metabolic syndrome (MetS) is growing among developed and developing societies because of the increasing prevalence of obesity among all population groups.[1, 2] Different preclinical models have been developed in order to study the mechanisms involved in the progression of this complex condition, as well as to define effective treatments.[3, 4] In this regard, the cafeteria diet (CAF)-fed rodent has emerged as a valuable model because it develops the main features of MetS, that is, obesity, dyslipidemia, insulin resistance, chronic low-grade inflammation and hypertension.[5, 6] Although the exact components of CAF vary among experimenters, the main and common characteristic of this model is based on ad libitum access to a highly palatable diet consisting of a blend of commercially available processed food rich in saturated fats and simple carbohydrates with a low fiber content. These components are offered together with a commercial chow that contains all the nutritional requirements for the animals in terms of vitamins, minerals and oligo elements. The high palatability of CAF influences the mechanisms of satiety and the reward system,[7] blunting the natural homeostatic control of energy intake and inducing hyperphagia and therefore exacerbated food consumption, mimicking the mechanisms that lead to obesity in humans under the so-called Western diet.[8, 9] Therefore, the different effects of diet-induced MetS have been widely explored by means of the CAF rodent model, either at the physiological, psychological or molecular level, and the effects of different dietary interventions.[5, 10, 11]

During the last few years, a growing body of evidence has shown that the response to a given diet depends on the gut microbiome of the host among other factors such as the genetic background.[12, 13, 14, 15, 16] In fact, different studies have highlighted important differences that exist in the gut microbiota among subjects from around the world with extremely different diets.[15] The exact mechanisms are still under debate, but it has been widely demonstrated that the gut microbial community is shaped by diet and, at the same time, has a key role in the transformation and absorption of nutrients by the host, such as

fibers, fatty acids or amino-acid-derived molecules among others, as well as endogenous metabolites, such as bile acids.12, 17 Therefore, the microbiota determine how different key nutrients and bioactive molecules exert their signaling actions through modulation of hormones and other receptors. At the same time, the microbiota are a source of signaling metabolites, including lipopolysaccharide (LPS), a product of Gram-negative bacteria that has been described as a key trigger of inflammation and its derived alterations.12, 18 The gut microbiome has gained much attention in obesity research.12, 17, 19, 20, 21 Studies in both preclinical models and human volunteers have shown that obesity and MetS are associated with decreased gut microbial diversity and changes in the equilibrium of certain microbes at different taxonomic levels.16, 22 Thus, fluctuations in the population of four major phyla, that is, Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria, among others, have been strongly associated with obesity and its related alterations, such as insulin resistance, dyslipidemia or non-alcoholic hepatic steatosis. However, to date, no specific microbiome fingerprint for obesity has been identified.22

The clear effects of CAF on different components of MetS and the role of gut microbiota in obesity and its derived pathologies prompted us to hypothesize that CAF can alter the gut microbiome of healthy rats and that these changes may be associated with some physiological alterations related to MetS caused by this specific diet. Therefore, our objective was to assess the effects of CAF feeding on the gut microbiome and to describe the associations between these changes and alterations of lipid metabolism in the CAF-fed rat. To this aim, we fed Wistar rats with a standard diet (STD) or CAF for 12 weeks. The associations between gut microbiota and nutrient intake and biometric and biochemical parameters were analyzed by means of a sparse partial least square approach. We demonstrate that CAF impacts gut microbiota composition and that these changes may be associated with the intake of specific nutrients and parameters linked to the lipid metabolism of the adipose–liver axis.

MATERIALS AND METHODS

Animals and diets

The Animal Ethics Committee of the University Rovira i Virgili (Tarragona, Spain) approved all the procedures. All animals were housed individually at 22°C with a light/dark cycle of 12 h (lights on at 0900 hours) and were given free access to food and water. Individual housing was intended to allow an accurate estimation of food intake and to avoid crossed effects on microbiota because of the coprophagia that is usual in rats.

Six-week-old male Wistar rats (Harlan Laboratories, Barcelona, Spain) were randomly distributed to two experimental groups (n=8) depending on the diet received during 12 weeks: the STD group, which was fed standard chow (Teklad Global 18% Protein Rodent Diet 2018, Harlan, Barcelona, Spain), and the CAF group. Sample size and nutrient compositions of CAF and STD used herein were recently described by Cigarroa et al. Blinding was not done. The standard chow used for both diets is designed for young animals but was selected because of its higher content of vitamins and some minerals in order to guarantee a correct intake of micronutrients by the CAF group.

Body weight and food intake were recorded weekly. For food intake estimation, the weight of chow and of CAF diet components were recorded before and 24 h after consumption. To do that, CAF components were confined in a container. After the feeding period, CAF remnants were manually recovered from rat cages and containers and weighted. Nutrient consumption was calculated according to the nutritional composition provided by the manufacturer of each component of the CAF diet or of the chow. For each dietary component, the area under the curve was calculated along the experimental period to report the total intake. Rats were killed after 6 h of fasting in order avoid interferences of the early postprandial state in plasma metabolites and under anesthesia (sodium pentobarbital, 80 mg kg-1 body weight). Blood was collected by cardiac puncture and serum was obtained by centrifugation and stored at -20°C until analysis. Cecum, liver, gastrocnemius and soleus muscles, interscapular brown adipose tissue and white

adipose tissue depots (retroperitoneal (RWAT), mesenteric (MWAT), epididymal (EWAT) and inguinal (IWAT)) were rapidly removed, weighed, frozen in liquid nitrogen and stored at -80°C. Adiposity index was computed as the sum of the EWAT, IWAT, MWAT and RWAT depot weights and expressed as a percentage of the total body weight, as previously described.

Serum analysis

Enzymatic colorimetric kits were used for the determination of serum total cholesterol, triglycerides, glucose and glycerol (QCA, Barcelona, Spain) and non-esterified free fatty acids (WAKO, Neuss, Germany). Circulating insulin, adiponectin and leptin levels were measured using rat ELISA kits (Merck, Madrid, Spain). Malondialdehyde was determined as previously described.

Lipid extraction and quantification

Lipids were extracted and quantified from the liver (100–120 mg) using methods described previously. Briefly, lipids were extracted with 1 ml of hexane/isopropanol (3:2, vol/vol), degassed with nitrogen before left overnight under orbital agitation at room temperature protected from light. After an extraction with 0.3 ml of Na2SO4 (0.47 M), the lipid phase was dried and total lipids quantified gravimetrically before emulsifying as described previously. Triglycerides, cholesterol and phospholipids were assayed with commercial enzymatic kits (QCA).

Microbiota

The genomic bacterial DNA was obtained from 700 to 1000 mg of cecal content of previously snap-frozen cecum with the QIAamp DNA stool kit (Qiagen, Hilden, Germany; cat. no. 51504) following the manufacturer's protocol. Partial 16S ribosomal RNA gene sequences were amplified from 20 ng of extracted DNA using three primer pairs (341F-532R, 515F-806R and 967F-1046R), which target the V3, V4 and V6 regions, respectively. Equimolar pools of each fragment were combined to create the DNA library, which was subjected to a clonal amplification by an emulsion PCR. After an Ion Sphere Particle enrichment process, samples were loaded onto 318 chips and sequenced using the Ion Torrent PGM (Life Technologies, Carlsbad, CA, USA). The individual sequence reads were filtered by the PGM software

(Life Technologies, Carlsbad, CA, USA) to remove low-quality and polyclonal sequences. Those reads were processed using QIIME, selecting only sequences with 150–200 bp and omitting homopolymers. 16S ribosomal RNA operational taxonomic units (OTUs) were assigned using uclust (>97% sequence homology) and a reference data set from GreenGenes (Lawrence Berkeley National Laboratory, Berkeley, CA, USA).

Analysis of plasma LPS

Internal standards, 3-hydroxydecanoic acid, 3-hydroxydodecanoic acid, 3-hydroxytetradecanoic acid and 3-hydroxyhexadecanoic acid (Larodan, Solna, Sweden) and 3-hydroxytetradecanoic acid-2,2,3,4,4-d5 (Sigma, Spain), were added to hydrolased (NaOH) and non-hydrolased samples to estimate total and free hydroxyfatty acids (3OHFA), respectively. After acidification (HCl) and liquid-liquid extraction with chloroform, sample compounds were derivatized with N,O-bis(Trimethylsilyl) trifluoroacetamide and 10% trimethylchlorosilane and 10% of pyridine. Samples were analyzed in a 7890A Series gas chromatograph coupled to a 7000 GCQqQ (Agilent Technologies, Santa Clara, CA, USA). Ionization was performed by electronic impact. LPS content was computed as the sum of nanomoles of individual esterified 3OHFAs (total minus free) divided by 4 to account for the four molecules of 3OHFAs assumed per molecule of LPS.

Statistical methods

Association studies were performed by applying a canonical sparse partial least square discriminant analysis using genus abundances as one of the matrices and four different data sets as the second data matrix in separate analyses using the Mixomics package for R following a previously described methodology. These four independent data sets were absolute values of macronutrient intake, biometric parameters as tissue weights and body weight itself, plasma biochemical parameters and liver biochemical parameters. Relevance networks were constructed with the software cytoscape 3.0 for each analysis following previously described methodologies and for the four analyses simultaneously using the genus identified in all the analyses as the link between networks. With the exception of the microbiome analysis described above, the rest of the statistical analyses were conducted with R commander software for

Windows.

RESULTS

Rats fed a CAF for 12 weeks developed obesity and MetS

As expected, CAF was associated with hyperphagia, resulting in a twofold increase in energy intake. This was mainly because of a fivefold and a twofold increase in lipid and carbohydrate intake, respectively. Fiber intake and protein intake were reduced by 28% and 33%, respectively. As a result, at the end of the experiment, CAF-fed animals presented a 30% increase in body weight because of a significant 78% increase in the adiposity index. CAF animals presented enlarged pale livers with a higher lipid content than their counterparts. Analysis of plasma parameters revealed a clear hypertriglyceridemia accompanied by significantly increased free fatty acids and a statistical tendency toward increased glycemia in CAF animals. It has to be taken into account that glycemia values were unusually high for both groups, likely due to anesthesia, and further results obtained using these values should be interpreted carefully. Both leptin and adiponectin were increased in the CAF group, suggesting alterations in adipose tissue metabolism. Altogether, these results indicated that CAF-induced obesity and hepatic steatosis were accompanied by altered lipid and glucose metabolism, resembling the human features of MetS.

Gut microbiota were altered by CAF

Cecum content was obtained and the microbiome was characterized. After analysis, 15,962 OTUs were detected, and their changes between STD and CAF groups were quantified. Of these OTUs, 5,046 (31%) presented statistically significant differences when assessed by means of the false discovery rate, with q-values under 0.05. Analysis of the 27 phyla detected revealed that CAF induced a decrease in Firmicutes ($q=0.0003$) but not in Bacteroidetes ($q=0.11$) relative to the STD-fed animals. The ratio of Bacteroidetes to Firmicutes remained unaltered, though it tended to increase in CAF-fed animals (CAF=1.26±0.56 and STD=0.19±0.02; $q=0.08$). Moreover, a large increase in Actinobacteria ($q=0.03$) and Proteobacteria ($q=0.009$) was detected in the CAF group, although Actinobacteria was clearly increased only in five out of eight CAF-fed animals. Together with these four major phyla, we found a significant 10-fold increase

in Cyanobacteria (q=0.02) and decreased levels of Armatimonadetes (q=0.02), Deferribacteres (q=0.003), Fibrobacteres (q=0.04), Nitrospirae (q=0.01) SC4 (q=0.0001), TM7 (q=0.004) and Tenericutes (q=0.006). Despite the increase in predominant phyla in animals fed CAF, the alpha diversity, calculated either as the Shannon index, Simpson index or observed OTUs, was significantly lower (q=0.002) in the CAF group relative to the STD group. The estimation of beta diversity by Principal Coordinates Analysis revealed a clear and statistically significant separation between STD and CAF metagenomes (P=0.01). Moreover, a hierarchical clustering analysis of the 299 genera quantified further confirmed clear differences among metagenomes. Both analyses also show that the differences within CAF-fed animals are much greater than those within their STD counterparts.

Associations between dietary components and gut metagenome in rats fed STD and CAF

Values of genus abundances and dietary components intake were subjected to sparse partial least squares analysis. The associations with a R^2 coefficient over 0.7 were selected, resulting in 26 genera belonging to the following six phyla: Firmicutes (10 genus), Bacteroidetes (6 genus), Proteobacteria (7 genus), Chloroflexi (1 genus), Deferribacteres (1 genus) and SC4 (1 genus). Regarding dietary components, fiber was associated with 18 genera, carbohydrates with 20 genera, fatty acids and lipids with 22 genera and cholesterol with 23 genera. In turn, protein was only associated with two genera. Remarkably, all Firmicutes were positively associated with fiber intake. The details and directionality of the associations (positive or negative) are detailed in Supplementary Figure 1 and Figure 2. Codes for bacteria are described in Table 2.

Associations between physiological parameters and metagenome in rats fed STD and CAF

Selection of associations with R^2 over 0.7 revealed that, together with Firmicutes, only two more phyla presented negative associations, while the rest were positively associated with white adipose tissues and negatively associated with muscle weight. Proteobacteria were represented by seven genera. Again, associations with muscle were inverse to those shown by adipose tissues. Other represented phyla were Bacteroidetes, Chloroflexi, Lentisphaerae and SC4. IWAT, EWAT and MWAT were the adipose tissues with most associations, with 18, 17 and 16, respectively. RWAT and BAT were only associated with the

abundance of 10 genera. Together with the IWAT, the muscle was the most associated tissue with 18 associations.

Concerning the plasma parameters, those showing associations with phyla with a R^2 over 0.7 were glucose (three associations), insulin (four associations), triglycerides (seven associations), non-esterified free fatty acid (seven associations) and adiponectin (one association). Surprisingly, glycerol concentration was the biochemical parameter with more associations (18) followed by the hormone leptin (16). Regarding phyla, the most represented was again Firmicutes (nine genus), followed by Bacteroidetes (eight genus) and Proteobacteria (five genus). Other phyla found to be associated with plasma parameters were Chloroflexi (two), Lentisphaerae (one), SC14 (one) and Tenericutes (one). Associations showed a certain degree of specificity. Thus, both glucose and insulin showed positive associations with different phyla, whereas glycerol was negatively associated with Firmicutes and mostly positively (four out of five) associated with Bacteroidetes. Associations of non-esterified free fatty acids were all positive and reduced to Firmicutes except one with Chloroflexi. Most of the associations of leptin concentration were negative, independently of the phyla.

Liver lipids were mainly associated with the abundance of Firmicutes (seven) and Proteobacteria (four) and, to a lesser extent with Bacteroidetes (two), Chloroflexi (two) and SC14. As has been described for the weight of tissues, the combination of these associations with the metagenome-macronutrients network revealed associations between liver lipids and genera not associated with macronutrients.

The 40 genera that were found to be associated with nutrition or physiological parameters and summarized in Table 2 were used as a link to the different physiological parameters, producing the association network shown in Figure 2, which compiles the different results described in this section.

CAF increased endotoxemia

The increase in Proteobacteria prompted us to analyze the levels of endotoxemia in another cohort of STD and CAF-fed rats. As shown in Figure 3, all species of 3OHFAs were increased in the plasma of rats fed

CAF for 12 weeks, resulting in a statistically significant increase in plasma LPS concentration compared with rats fed STD.

DISCUSSION

Different works have shown that obesity involves a reduction in gut species richness in preclinical models and in humans.12, 13, 16, 32 In Danish subjects, lower gut bacterial diversity was associated with higher adiposity, insulin resistance and dyslipidemia, key components of MetS.32 In fact, loss of gut microbial diversity has been proposed as a hallmark of western societies when compared with rural areas around the world.12 This concept has been explored in mice, and research demonstrates that a Western-like diet with reduced fiber maintained over generations results in an eventual loss of bacterial diversity.13 Taken together, converging evidence indicates an association between gut microbial diversity and an obesity-prone phenotype. In agreement with this point of view, our results suggest that CAF-induced obesity leads to poorer alpha diversity.

A remarkable number of genera, 15 out of 40, associated with physiological and biochemical alterations in our study were changed by CAF in the direction described by previous works intended to identify the changes that obesity and its related alterations induce in gut microbial communities (summarized in Supplementary Table 2). These positive matches reinforce our findings and support the approach used herein to assess the cross-talk between the gut microbiome and host metabolism. Moreover, of the 40 genera included in the associations network, 25 have not been previously related to obesity to our knowledge. Nevertheless, since this is an association study, which is not addressed to underscore the cause of the associations found, more research assessing the relevance of these genera in the origin of metabolic alterations would shed light on the associations described herein.

At the phylum level, we found that CAF resulted in a clear reduction in Firmicutes. Despite the

observation that initial obesity was related to increased Firmicutes abundance and decreased Bacteroidetes to Firmicutes ratio,[20, 21, 22] further studies have been unable to detect such changes in different human cohorts.[19, 22, 33, 34] Moreover, our results agree with a recent study describing a decrease in Firmicutes phylum in rats with obesity induced by different obesogenic diets.[35] Similarly, obesity has been associated with decreased Bacteroidetes,[36, 37] but, again, we have not found evidence consistent with this previous observation. Despite these contrasting findings, we show other results that agree with previous works. Thus, we observed a marked increase in Actinobacteria in CAF animals relative to their STD counterparts, and an abundance of this phylum has been previously associated with obesity,[17, 19, 22] although some genera within this phylum, such as Bifidobacterium, might present the opposite behavior.[38] Among the different Actinobacteria detected, only the genus Propionibacterium was positively associated with plasma concentrations of insulin and glucose. In agreement with our observations, a study in obese women found similar results, indicating a positive correlation between Propionibacterium and glucose homeostasis parameters.[39]

Together with the changes indicated above for Bacteroidetes, Firmicutes and Actinobacteria, Proteobacteria is another predominant phylum in the gut microbiome that was markedly increased by CAF. The positive association between these species and obesity or other diseases has been widely described in different studies, in both preclinical models and human subjects.[20, 40, 41, 42] Moreover, different families of Proteobacteria have been described as important LPS producers, such as the Enterobacteriaceae family,[43] which was one of the Proteobacteria increased by CAF in the present work. Bacterial LPS has been proposed as a trigger of different metabolic disorders, including increased adiposity and insulin resistance.[44, 45] In the present study, we found a large increase in plasma LPS that completely agrees with the role of endotoxemia in the metabolic effects of diet-induced obesity. Moreover, together with the major phyla described above, we found changes in the abundance of other phyla previously associated with obesity, such as Cyanobacteria,[46] Deferribacteres,[18, 47]

Fibrobacteres,48 Tenericutes 37, 46 and TM7.49 Other phyla, such as Nitrospirae and SC4, were also altered between CAF and STD groups; however, to our knowledge, little is known regarding the role of these phyla in obesity.

Our results strongly suggest that the balance among fiber, simple carbohydrate and fat intake has a key role in the effects of diet on gut microbial populations. This result agrees with the relevance of fiber in shaping the gut microbiome.12, 15 In this sense, we found positive associations between fiber intake and a phylum of well-known fermenters, such as Firmicutes. Fermentative bacteria are important for the breakdown of non-digestible carbohydrates leading to the production of short chain fatty acids (SCFAs) that are proposed as key factors in the development and progression of obesity and related alterations, although their exact role is not yet fully understood. On the one hand, it has been proposed that SCFA might represent a salvage pathway of energy derived from non-digestible carbohydrates, as SCFA can be used as precursors of fatty acids by different tissues.50 Although in our experiment CAF animals presented a decrease in Firmicutes, a reduction in SCFA derived from fermentative bacteria would not have a relevant impact on energy balance, as the energy provided by these metabolites is irrelevant when compared with the exacerbated energy intake induced by the hyperphagia associated with CAF. Contrary to the putative role of SCFA in energy salvage, different studies have demonstrated that these metabolites present a wide array of beneficial actions, increasing fatty acid oxidation and energy expenditure by different mechanisms and leading to reduced adiposity, ectopic deposition of hepatic fat and insulin resistance.51 Thus, SCFA can signal directly to different tissues after absorption, activating peroxisome proliferator activated receptors and AMP activated kinase in liver and adipose tissues inducing fatty acid catabolic pathways and reducing body fat content.51, 52 In our case, Firmicutes showed an important number of negative associations with adipose tissue weights and hepatic lipids and, at the same time, positive associations with the intake of fiber. Therefore, it is plausible to hypothesize that the reduced intake of fiber observed in CAF animals resulted in a decrease in fermentative bacteria leading to a

reduced production of metabolites, such as SCFA. Indeed, in a recent experiment of our laboratory, we analyzed the levels of SCFA in feces of Wistar rats fed STD and CAF diets for 9 weeks, observing a threefold decrease of SFCA expressed as the sum of acetic, propionic and butyric acids, resulting in a P-value under 0.001 when analyzed by Student's t-test (data not shown). These results would support our hypothesis. If these changes were also found in blood, could contribute to suboptimal activation of catabolic pathways in the host and, together with other factors such as increased metabolic endotoxemia and exacerbated fat and glucose absorption, could also contribute to increased fat deposition in adipose tissues and liver. In fact, Vrieze et al. found that treatment of MetS patients with microbiota from lean subjects results in ameliorated insulin resistance accompanied by an increased population of Firmicutes.53 Therefore, the associations found between microbiota and tissues such as adipose, liver or muscle might reflect the cross talk between microbiota and the host metabolism mediated by different metabolites and hormones.

Among the different plasma components analyzed, both glycerol and leptin clearly showed the highest number of associations with cecum bacteria, mainly Firmicutes, Bacteroidetes and Proteobacteria. Both glycerol and leptin are highly related to adipose tissue. Thus, leptin is commonly used as a biomarker of adipose tissue status, whereas glycerol is a well-accepted biomarker of adipose tissue lipolysis.54, 55 As has been discussed above, the host microbiota affect different tissues by different mechanisms, with adipose tissue metabolism being a clear target of this cross talk.12 Together with the high number of associations found between gut bacteria and adiposity-related parameters, such as adiposity index or the weight of different adipose depots, our results agree with the accepted hypothesis that gut microbiota modulate, in part, adipose tissue physiology. In addition, the association between the microbiome and liver lipids found in this work is remarkable, and previous studies have shown that the gut microbiome can influence liver health via different mechanisms, involving a wide array of microbial metabolites, such as SCFA, bile acids, choline or ethanol, as well as by increasing LPS producing bacteria and consequently

endotoxemia.36, 40 In agreement with our work, results from a recent study suggest that analysis of microbiota in human stool samples can be used as a biomarker of non alcoholic fatty liver disease progression.56 In that work, differences at the phylum level between advanced and mild non alcoholic fatty liver disease were decreased Firmicutes and increased Proteobacteria, changes that have been also identified by our analysis when comparing rats with normal and steatosis livers. Taken together, these results may be related to the known link between adipose tissue remodeling and liver health, as the hepatic metabolism of lipids is highly interconnected with the metabolism of the adipose tissue in order to maintain the homeostasis of lipid metabolism.57, 58 Our association study highlights the relevance of the microbiota on this adipose–liver axis, a putative link that has been explored by other authors.40 Nevertheless, more research is needed to clearly define the extent to which the microbiota affect this complex system.

We have demonstrated that CAF modifies markedly the host microbiome. Recent literature suggests that dietary habits have a profound impact on gut microbiome diversity and microbial populations. Our results strongly suggest that the balance between fiber, simple carbohydrates and fats determines the abundance of different genera, which, in turn, can be associated with physiological outcomes. In this sense, we describe clear associations between the alteration of gut microbiota and the physiology of adipose tissues and liver health. Our results reveal that the CAF-fed rat is a useful model for studying diet-induced dysbiosis and may provide further evidence supporting a role of the gut microbiota in obesity and its derived pathologies, such as non alcoholic fatty liver disease or insulin resistance. The associations between specific bacterial genera and physiology require further investigation to assess the relevance of each association and whether these results can be used to design new strategies based on microbiome modulation for improving metabolic dysfunctions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE CAPTIONS

Figure 1. Changes in the metagenome of rats fed a CAF or a STD were assessed by (a) phyla abundance, (b) differences in alpha diversity between CAF- and STD-fed animals calculated by the Shannon, observed OTUs and Simpson indexes; (c) analysis of beta diversity represented by scores of each CAF- and STD-fed animals after principal coordinates analysis; (d) hierarchical clustering analysis for the 16 animals colorized as indicated and the corresponding heatmap depicting the fold-change with respect to

511 the average abundance of each genus. * Denotes a $P < 0.05$ between the highlighted groups by T
512 statistics.

513 **Figure 2.** Network of associations between genus and nutritional, physiological and biochemical
514 parameters. Associations with an $R^2 > 0.7$ between depicted genus and parameters as reported by sparse
515 partial least squares analysis are represented as the edges of a network. Red edges depict positive
516 associations and green edges indicate negative associations between the connected nodes. Increased
517 intensity denotes higher R^2. The size of each node is proportional to the number of connected edges.
518 Genera are coded in Table 2. The form of the node for each genus represents its phylum as diamonds
519 (Firmicutes), squares (Bacteroidetes), triangles (Chloroflexi), hexagons (proteobacteria) and octagons
520 (SC4). Actinobacteria, Deferribacteres and Tenericutes are represented by a circle. SFA, saturated fatty
521 acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; total FA, total fatty acids;
522 TG, triglycerides, TC, total cholesterol, PL, phospholipids, NEFA, non-esterified fatty acids; RWAT,
523 retroperitoneal white adipose tissue; EWAT, epididymal adipose tissue; IWAT, inguinal adipose tissue;
524 MWAT, mesenteric adipose tissue; MUS, muscle; BAT, brown adipose tissue.

525 **Figure 3.** Analysis of LPSs in plasma of rats fed a CAF or a STD. Plasma concentrations of hydroxyl-
526 fatty acids of 8, 10, 12, 14, 16 and 18 carbons were determined and the concentration of total LPS was
527 calculated from their concentration as reported in the Materials and methods section. The results are
528 represented as the mean ± s.e.m. of each group. * Denotes a $P < 0.05$ between the CAF and STD groups
529 by T statistics.

530

TABLES

Table 1. Macronutrient intake during 12 weeks, tissue weights and plasma and liver biochemistry in rats fed a STD or a CAF.

	STD	**CAF**	**P-value**
Macronutrient intake			
Energy intake (kJ day^{-1})	182 ± 5	410 ± 8	6.E-09
Proteins (g day^{-1})	3.3 ± 0.1	2.4 ± 0.2	7.E-04
Lipids (g day^{-1})	0.59 ± 0.01	3.6 ± 0.2	3.E-09
Carbohydrates (g day^{-1})	6.9 ± 0.2	15.4 ± 0.7	1.E-08
Fiber (g day^{-1})	0.41 ± 0.01	0.14 ± 0.02	4.E-08
Saturated fatty acids (g day^{-1})	0.20 ± 0.01	1.8 ± 0.1	3.E-10
Monounsaturated fatty acids (g day^{-1})	0.23 ± 0.01	1.11 ± 0.08	2.E-08
Polyunsaturated fatty	0.14 ± 0.01	0.44 ± 0.01	1.E-06

acids (g day^{-1})			
Total fatty acids (g day^{-1})	0.45 ± 0.01	3.0 ± 0.2	2.E-09
Cholesterol (g day^{-1})	0.0027 ± 7.E-05	0.014 ± 9.E-04	1.E-08
Water (g day^{-1})	34 ± 2	60 ± 3	4.E-06
Tissue weights (absolute values)			
Body weight (g)	453 ± 11	598 ± 20	2.E-05
Liver (g)	12 ± 1	16 ± 1	1.E-04
RWAT (g)	17 ± 1	36 ± 2	8.E-07
EWAT (g)	16 ± 1	31 ± 3	9.E-05
IWAT (g)	23 ± 1	63 ± 7	9.E-05
MWAT (g)	10 ± 1	21 ± 2	1.E-04
BAT (g)	0.62 ± 0.01	1.26 ± 0.13	5.E-04
Gastrocnemius and soleus (g)	2.63 ± 0.05	2.56 ± 0.05	3.E-01
Tissue weights (relative to body			

weight)			
Liver (%)	2.68 ± 0.06	2.76 ± 0.12	6.E-01
RWAT (%)	3.7 ± 0.2	5.9 ± 0.2	4.E-06
EWAT (%)	3.5 ± 0.2	5.1 ± 0.3	3.E-04
IWAT (%)	5.0 ± 0.2	10.4 ± 0.9	5.E-05
MWAT (%)	2.2 ± 0.1	3.5 ± 0.2	8.E-05
Adiposity index	14.3 ± 0.5	25.0 ± 1.4	5.E-06
BAT (%)	0.14 ± 0.01	0.21 ± 0.02	4.E-03
Gastrocnemius and soleus (%)	0.58 ± 0.02	0.43 ± 0.02	7.E-05
Plasma parameters			
Triglycerides (mM)	1.7 ± 0.2	3.3 ± 0.3	5.E-04
Cholesterol (mM)	2.5 ± 0.2	2.6 ± 0.1	5.E-01
Glucose (mM)	11.2 ± 0.7	13.4 ± 0.8	7.E-02
Malondialdehyde (nM)	257 ± 8	404 ± 82	1.E-01
Insulin (nM)	6.6 ± 0.6	8.4 ± 0.6	1.E-01

Adiponectin (μg ml^{-1})	3.3 ± 0.2	5.0 ± 0.3	5.E-04
Glycerol (mM)	0.14 ± 0.02	0.32 ± 0.02	4.E-05
NEFA (mM)	0.23 ± 0.02	0.33 ± 0.02	7.E-04
Leptin (ng ml^{-1})	19 ± 1	106 ± 11	1.E-06
Liver biochemistry			
Total lipids (mg g^{-1})	42 ± 3	78 ± 6	6.E-05
Triglycerides (mg g^{-1})	5.4 ± 0.4	11.7 ± 0.9	3.E-05
Total cholesterol (mg g^{-1})	2.4 ± 0.2	4.7 ± 0.4	4.E-05
Phospholipids (mg g^{-1})	10.9 ± 0.9	14.6 ± 0.7	6.E-03

Abbreviations: BAT, brown adipose tissue; CAF, cafeteria diet; EWAT, epididymal adipose tissue; IWAT, inguinal adipose tissue; MWAT, mesenteric adipose tissue; NEFA, non-esterified fatty acids; RWAT, retroperitoneal white adipose tissue; STD, standard diet. Values are represented as mean ± s.e.m.

Table 2. Bacteria highly associated with physiological parameters in rats fed a STD and a CAF for 12 weeks.

Code	**Fold-change**	**P-value**	**Phylum**	**Class**	**Order**	**Family**	**Genus**

bact18	99.79	2.E-02	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium
bact35	7.78	2.E-04	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
bact36	Up	1.E-02	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Candidatus Azobacteroides
bact37	13.70	4.E-02	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter
bact38	16.12	9.E-04	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides
bact41	0.05	1.E-05	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	-
bact42	0.07	5.E-04	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
bact46	111.80	2.E-03	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes

bact47	0.38	5.E-04	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	-
bact50	4.65	8.E-02	Bacteroidetes	Bacteroidia	Bacteroidales	(Paraprevotellaceae)	-
bact60	0.01	3.E-06	Chloroflexi	Anaerolineae	50208	-	-
bact62	0.26	3.E-02	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerofilum
bact69	0.00	3.E-03	Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Mucispirillum
bact72	Down	9.E-05	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	-
bact89	0.10	2.E-04	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia
bact96	25.24	1.E-02	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
bact103	0.04	1.E-10	Firmicutes	Clostridia	Clostridiales	-	-

bact111	0.05	2.E-08	Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	Dehalobacterium
bact120	Up	9.E-03	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium
bact135	0.17	5.E-06	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	-
bact136	12.99	1.E-02	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Bilophila
bact138	34.01	5.E-03	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Clostridium
bact140	0.11	5.E-07	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira
bact142	0.18	3.E-06	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus
bact152	74.66	9.E-03	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Phascolarctobacterium
bact164	0.03	8.E-04	Firmicutes	Clostridia	MBA08	-	-

bact180	15.11	8.E-03	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	-
bact206	20.70	1.E-04	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella
bact211	0.00	7.E-05	Proteobacteria	Deltaproteobacteria	-	-	-
bact216	Up	5.E-02	Lentisphaerae	(Lentisphaeria)	Lentisphaerales	Lentisphaeraceae	Lentisphaera
bact220	0.01	6.E-04	Proteobacteria	Deltaproteobacteria	Myxococcales	0319-6G20	-
bact224	0.02	3.E-06	Proteobacteria	Deltaproteobacteria	Thermodesulfobacteriales	-	-
bact229	81.72	7.E-02	Proteobacteria	Gammaproteobacteria	Aeromonadales	-	-
bact231	Up	2.E-01	Proteoba	Gammap	Aeromo	Aeromo	Zobellell

			cteria	roteobacteria	nadales	nadaceae	a
bact238	Up	5.E-02	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	FFCH10602
bact244	3.94	7.E-02	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Morganella
bact261	25.31	7.E-03	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
bact271	33.23	3.E-02	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas
bact273	Down	1.E-04	SC4	-	-	-	-
bact285	0.10	6.E-03	Tenericutes	Mollicutes	RF39	-	-

539 *Abbreviations: CAF, cafeteria diet; STD, standard diet.*

540

FIGURES

Figure 1

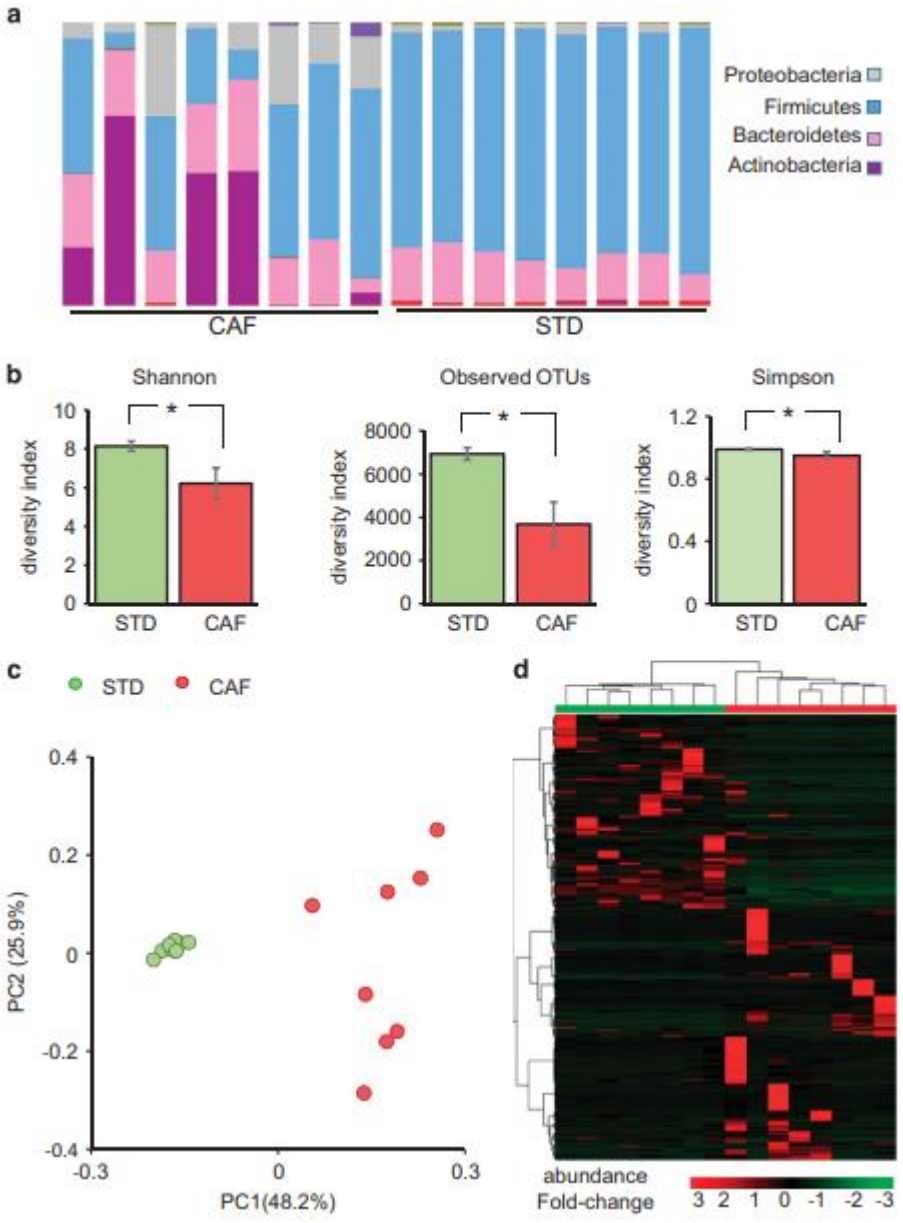
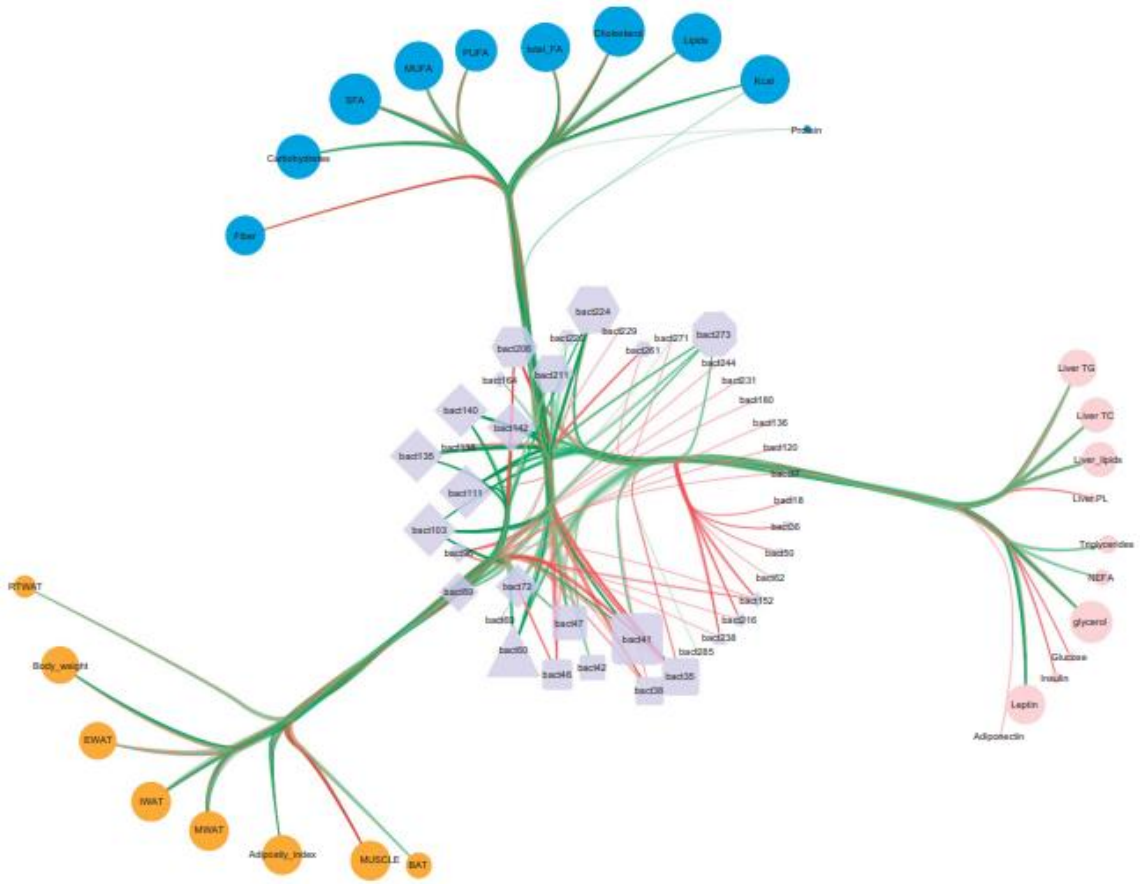


Figure 2



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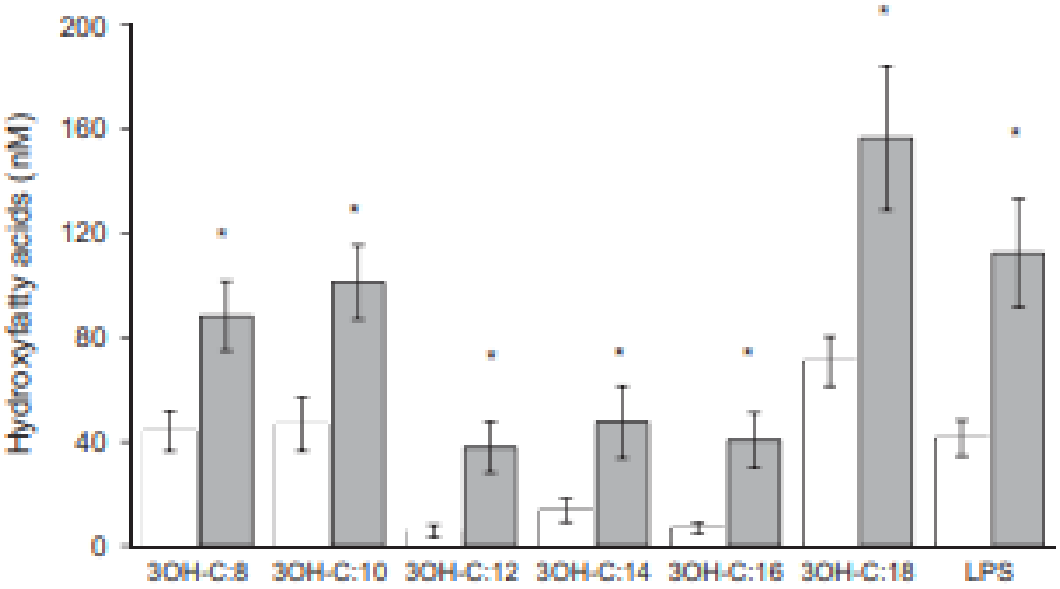
548 Figure 3



549

Genus	Study	Subjects	Relation with diet or obesity	Agreement with the present study
Propionibacterium	Dewulf, 2013[1]	Obese women	Positively correlated with changes in body composition and glucose homeostasis	Yes
Bacteroides	Caesar, 2015[2]	Mice	Increased by lard feeding	Yes
	Everard, 2014[3]	Mice	Increased by high-fat feeding	Yes
	Kaakoush, 2017[4]	Rats	Increased by a CAF diet	Yes
	Hamilton, 2015[5]	Rats	Increased by a high-fat diet	Yes
	Lecomte, 2015[6]	Rats	Increased by a high-fat diet	Yes
	Liu, 2016[7]	Rats	Decreased by a high-fat diet	No
	Parnell, 2012[8]	Lean and obese rats	Decreased in obese rats. Negatively correlated with body weight, body fat and energy intake. Increased with high fiber intake.	No
	David, 2013[9]	Men and women (BMI 19-32)	Increased by an animal-based diet (rich in fat and protein and low in fiber)	Yes
	Wu, 2011[10]	Healthy men and women	Associated with protein and animal fat	Yes
	Boursier, 2016[11]	Men and women with NAFDL	Increased in NASH patients	Yes
Parabacteroides	Everard, 2014[3]	Mice	Increased by high-fat feeding	Yes
	Lecomte, 2015[6]	Rats	Increased by a high-fat diet	Yes
	Liu, 2016[7]	Rats	Decreased by a high-fat diet	No
	Wong, 2013[12]	Healthy men and women or with NASH	Increased in NASH patients	Yes
Prevotella	Everard, 2014[3]	Mice	Decreased by a high-fat diet	Yes
	Hamilton, 2015[5]	Rats	Decreased by a high-fat diet	Yes
	Wu, 2011[10]	Healthy men and women	Associated with high-carbohydrate intake. Enriched in vegetarians.	Yes

Genus	Study	Subjects	Relation with diet or obesity	Agreement with the present study
	Ou, 2013[13]	Healthy men and women from America or Africa	Increased in native Africans (high fiber intake) compared to African Americans (high fat intake)	Yes
	Boursier, 2016[11]	Men and women with NAFDL	Decreased in NASH patients	Yes
Alistipes	Daniel, 2014[14]	Mice	Increased by high-fat feeding	Yes
	David, 2013[9]	Men and women (BMI 19-32)	Increased by an animal-based diet (rich in fat and protein and low in fiber)	Yes
	Zhu, 2013[15]	Healthy, obese and NASH adolescents	Decreased in obese and NASH adolescents	No
Mucispirillum	Ravussin, 2012[16]	Mice	Increased by high-fat feeding. Positively correlated with plasma leptin.	No
	Everard, 2014[3]	Mice	Increased by high-fat feeding	No
	Hamilton, 2015[5]	Rats	Increased by high-fat feeding	No
Dehalobacterium	Fu, 2015[17]	Men and women from LifeLines-DEEP cohort	Negatively associated with BMI	Yes
	Chan, 2015[18]	ApoE$^{-/-}$ mice	Decreased by a high-fat diet-induced atherosclerosis	Yes
Bilophila	Everard, 2014[3]	Mice	Increased by high-fat feeding	Yes
	Caesar, 2015[2]	Mice	Increased by lard feeding	Yes
	David, 2013[9]	Men and women (BMI 19-32)	Increased by an animal-based diet (rich in fat and protein and low in fiber)	Yes
Clostridium	Chan, 2015[18]	ApoE$^{-/-}$ mice	Increased in high-fat diet-induced atherosclerosis. Positively correlated with cholesterol levels	Yes
Oscillospira	Chan, 2015[18]	ApoE$^{-/-}$ mice	Decreased in high-fat diet-induced atherosclerosis. Correlated with saturated fat intake.	Yes
	Liu, 2016[7]	Rats	Increased by high-fat feeding	No
	Ou, 2013[13]	Healthy men and women from America or Africa	Decreased in Americans (high fat intake) compared to Africans (high fiber intake)	Yes

Genus	Study	Subjects	Relation with diet or obesity	Agreement with the present study
	Zhu, 2013[15]	Healthy, obese and NASH adolescents	Decreased in obese and NASH adolescents	Yes
	Goodrich, 2014	Normal weight and obese twin pairs	Enriched in lean subjects	Yes
	Escobar, 2014	Normal-weight and obese adults	Decreased in obese subjects	Yes
Ruminococcus	Kaakoush, 2017[4]	Rats	Increased by a CAF diet	No
	Zhu, 2013[15]	Healthy, obese and NASH adolescents	Decreased in obese and NASH adolescents	Yes
	Boursier, 2016[11]	Men and women with NAFDL	Increased in fibrosis	-
Phascolarctobacterium	Lecomte, 2015[6]	Rats	Increased by a high-fat diet	Yes
Sutterella	Liu, 2016[7]	Rats	Increased by a restrictive high-sugar diet and by a high-protein diet	Yes
	Mandal, 2016[19]	Pregnant women	Inversely associated with fiber intake	Yes
Morganella	Lecomte, 2015[6]	Rats	Increased by a high-fat diet	Yes
Acinetobacter	Ou, 2013[13]	Healthy men and women from America or Africa	Increased in Americans (high fat intake) compared to Africans (high fiber intake)	Yes
	Chiu, 2014[20]	Normal-weight and obese adults	Positively associated with obesity	Yes

NASH, non-alcoholic steatohepatitis

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