Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population

Carmen Haro, Miguel Montes-Borrego, Oriol A. Rangel-Zúñiga, Juan F. Alcalá-Díaz, Francisco Gómez-Delgado, Pablo Pérez-Martínez, Javier Delgado-Lista, Gracia M. Quintana-Navarro, Francisco J. Tinahones, Blanca B. Landa, José López-Miranda, Antonio Camargo,* and Francisco Pérez-Jiménez*

Lipids and Atherosclerosis Unit (C.H., O.A.R.-Z., J.F.A.-D., F.G.-D., P.P.-M., J.D.-L., G.M.Q.-N., J.L.-M., A.C., F.P.-J.), GC9 Nutrigenomics. IMIBIC/Reina Sofia University Hospital/University of Cordoba, 14004 Cordoba, Spain; CIBER Fisiopatología de la Obesidad y Nutrición (C.H., O.A.R.-Z., J.F.A.-D., F.G.-D., P.P.-M., J.D.-L., G.M.Q.-N., F.J.T., J.L.-M., A.C., F.P.-J.), Instituto de Salud Carlos III, 14004 Cordoba, Spain; Instituto de Agricultura Sostenible (M.M.-B., B.B.L.), Consejo Superior de Investigaciones Científicas, 14004 Cordoba, Spain; and Endocrinology and Nutrition Service (F.J.T.). Hospital Virgen de la Victoria, 29010 Málaga, Spain

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

Context: Gut microbiota, which acts collectively as a fully integrated organ in the host metabolism, can be shaped by long-term dietary interventions after a specific diet.

Objective: The aim was to study the changes in microbiota after 1 year's consumption of a Mediterranean diet (Med diet) or a low-fat, high-complex carbohydrate diet (LFHCC diet) in an obese population.

Design: Participants were randomized to receive the Med diet (35% fat, 22% monounsaturated) and the LFHCC diet (28% fat, 12% monounsaturated).

Setting and Participants: The study was conducted in 20 obese patients (men) within the Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention (CORDIOPREV) study, an ongoing prospective, randomized, opened, controlled trial in patients with coronary heart disease.

Main Outcome Measure: We evaluated the bacterial composition and its relationship with the whole fecal and plasma metabolome.

Results: The LFHCC diet increased the *Prevotella* and decreased the *Roseburia* genera, whereas the Med diet decreased the *Prevotella* and increased the *Roseburia* and *Oscillospira* genera ($P = .028$, .002, and .016, respectively). The abundance of *Parabacteroides distasonis* ($P = .025$) and *Faecalibacterium prausnitzii* ($P = .020$) increased after long-term consumption of the Med diet and the LFHCC diet, respectively. The changes in the abundance of 7 of 572 metabolites found in feces, including mainly amino acid, peptide, and sphingolipid metabolism, could be linked to the changes in the gut microbiota.

Conclusions: Our results suggest that long-term consumption of the Med and LFHCC diets exerts a protective effect on the development of type 2 diabetes by different specific changes in the gut microbiota, increasing the abundance of the *Roseburia* genus and *F. prausnitzii*, respectively. **(*J Clin Endocrinol Metab* 101: 233–242, 2016)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA
Copyright © 2016 by the Endocrine Society
Received September 4, 2015. Accepted October 21, 2015.
First Published Online October 27, 2015

* A.C. and F.P.-J. contributed equally to the study.

Abbreviations: CHD, coronary heart disease; CORDIOPREV, Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention; LFHCC diet, low-fat, high-complex carbohydrate diet; Med diet, Mediterranean diet; OGTT, oral glucose tolerance test; OTU, operational taxonomic unit; SCFA, short-chain fatty acid; T2D, type 2 diabetes.

Obesity was formerly thought to be caused only by a positive caloric balance when caloric intake exceeds caloric expenditure and the excess of energy is stored in adipose tissue (1). However, this simple idea has been altered for different reasons, because gut microbiota was proposed as an additional contributing factor to the pathophysiology of obesity—a link between gut microbial ecology and obesity (2). In fact, several studies have suggested that changes in the gut microbiota trigger pathogenic mechanisms to promote the development of obesity, type 2 diabetes (T2D), and metabolic syndrome (3, 4).

Gut microbiota, the complex, diverse, and vast microbial community harbored in the human intestine (5, 6), acts collectively as a fully integrated organ in the host metabolism, involved in extracting energy from nutrients, regulating innate and adaptive immunity, and helping to control the energy balance (7). Animal model studies have shown that obesity is associated with an increase in the Firmicutes/Bacteroidetes ratio, also known as "obese microbiota," which is transmissible between individuals (2). Moreover, the intestinal absorption of bacterial components, such as the endotoxin lipopolysaccharide, bacterial DNA, and flagellins, activate the Toll-like receptors, inducing inflammation, which favors insulin resistance (8), although it is not well established whether insulin resistance precedes the changes in the microbiota or vice versa (9). In addition, microbiota produces bioactive metabolites, such as short-chain fatty acids (SCFAs), originated by the fermentation of carbohydrates, which are involved in energy metabolism and appetite regulation, promoting healthy body weight, and secondary bile acid, which play a key role in glucose metabolism (10, 11).

Studies in humans have shown that gut microbiota seem to have coevolved with the dietary habits of the population. For example, the microbiota of children in Burkina Faso is adapted to extract calories from the polysaccharide-rich (carbohydrates and fibers) diet consumed in that country (12). Thus, the human gut microbiota community seems to be very stable and more influenced by ambient and dietary factors than by genetic factors. However, there have been very few studies on the modification of microbiota composition by dietary intervention (13). Moreover, it has been shown that the consumption of a Western diet increases endotoxemia, which suggests a disruption of the intestinal barrier in addition to an increase in the Gram-negative bacterial content of the microbiota (14). In fact, a high-fat, high-carbohydrate meal induces comprehensive endotoxemia and inflammation, increasing the expression of Toll-like receptor-4, the specific receptor for endotoxin, and suppressor of cytokine signaling-3, a protein that interferes with insulin signal transduction (15), whereas a high-fruit, high-fiber meal or the intake of orange juice or a polyphenol preparation with resveratrol does not induce any of these effects (16, 17).

Although previous studies have pointed out that microbiota is individual specific and shows high stability and resistance to perturbations over time (18, 19), recent research indicates that changes in gut microbiota composition may occur after dietary interventions (14, 20–22). In addition, whereas short-term dietary interventions tend to induce only minor changes, long-term periods of consumption of a specific diet may affect the microbiota to a substantial degree (13). For example, it has been shown that the consumption of a high-fat, high-protein diet increases the abundance of Bacteroidetes vs *Prevotella*, which is more abundant after high-carbohydrate diets (21). In addition, we have shown that the consumption of a Mediterranean diet (Med diet) partially restores the alteration in the gut microbiota composition observed in patients with metabolic syndrome (22).

Our aim was to study the lasting changes in the microbiota after the long-term consumption (1 year) of a Med diet and a low-fat, high-complex carbohydrate diet (LFHCC diet) in an obese population with coronary heart disease (CHD), using the 16S sequencing method and analyzing the relationship between specific bacteria and the metabolomics profile found in feces and plasma.

Materials and Methods

Study subjects

The current work was conducted in a subgroup of 20 obese patients (men) within the Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention (CORDIOPREV) study (ClinicalTrials.gov registration no. NCT00924937), an ongoing prospective, randomized, opened, controlled trial in patients with CHD, who had their last coronary event more than 6 months before enrollment on 2 different dietary models (Med diet and LFHCC diet) over a period of 5 years, in addition to conventional treatment for CHD. CORDIOPREV inclusion and exclusion criteria are summarized as follows: patients were eligible if they were >20 but <75 years old, had established CHD without clinical events in the last 6 months, were thought to follow a long-term dietary intervention, and did not have severe diseases or an estimated life expectancy of <5 years (23). Antibiotic usage was included as an exclusion criterion for the current study, in addition to the general exclusion criteria defined in the CORDIOPREV study. All the subjects were receiving a standardized treatment for CHD. No differences were observed between groups. All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethic committees, following the Helsinki Declaration and good clinical practices. The baseline characteristics of the study subjects are shown in Table 1.

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

Table 1. Baseline Characteristics of the Study Population

Parameter	LFHCC Diet	Med Diet	P Value
Age, y	61.4 ± 2.6	65.2 ± 3.2	.362
Weight, kg	86.9 ± 1.9	88.3 ± 1.8	.606
Waist circumference, cm	105.9 ± 2.5	109.6 ± 2.2	.285
BMI, kg/m^2	31.6 ± 0.8	32.8 ± 0.5	.214
Glucose, mg/dL	94.2 ± 3.1	91.2 ± 2.7	.466
HbA$_{1c}$, %	6.3 ± 0.1	6.0 ± 0.1	.312
SBP, mm Hg	129.0 ± 9.4	136.0 ± 3.7	.495
DBP, mm Hg	72.7 ± 3.6	75.1 ± 3.5	.635
TG, mg/dL	102.2 ± 8.1	98.7 ± 7.6	.757
TC, mg/dL	149.8 ± 6.8	150.2 ± 7.0	.968
HDL, mg/dL	41.7 ± 2.5	42.1 ± 1.9	.900
LDL, mg/dL	83.7 ± 4.9	88.0 ± 6.4	.599

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HbA$_{1c}$, glycated hemoglobin A$_{1c}$; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides. Values are means ± SEM. One-way ANOVA *P* values.

Study design

The study design was described previously (23). In brief, participants were randomized to receive 2 diets: a Med diet and an LFHCC diet. The compositions were as follows: the LFHCC diet contained 28% fat (12% monounsaturated; 8% polyunsaturated and 8% saturated) and the Med diet contained 35% fat (22% monounsaturated; 6% polyunsaturated and 7% saturated). To ensure that the main fat source of the Med 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 (eg, cereals, biscuits, and pasta) of similar cost, were provided for the patients randomized to the low-fat group.

Clinical plasma parameters

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. The plasma was separated from red cells by centrifugation at 1500 × *g* for 15 minutes at 4°C. Analytes determined in frozen samples were studied centrally by laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, Córdoba, Spain, who were unaware of the interventions. An oral glucose tolerance test (OGTT) was performed (75 g of dextrose monohydrate in 250 mL of water) with sampling at 0, 30, 60, and 120 minutes to establish plasma glucose and insulin levels (for details, see Supplemental Materials and Methods).

DNA extraction from fecal samples

DNA extraction was performed using the QIAamp DNA Stool Mini Kit Handbook (QIAGEN), following the manufacturer's instructions, and quantified using a NanoDrop ND-1000 v3.5.2 spectrophotometer (NanoDrop Technology).

Microbiota analysis

A total of 40 fecal samples (20 basal and 20 after a year of dietary intervention, for each participant) were used for the microbial community analysis using the 454 Life Sciences (Roche) Junior platform, according to standard 454 platform protocols (see Supplemental Materials and Methods).

Phylogenetic analysis of sequencing reads

The samples were processed and analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (version v1.8.0; http://qiime.org/) with default parameters unless otherwise noted (see Supplemental Materials and Methods).

Metabolomic analysis

Samples were sent to Metabolon and prepared using the automated MicroLab STAR system from Hamilton Company (see Supplemental Materials and Methods).

Statistical analysis

All of the data presented are expressed as means ± SEM. PASW statistical software, version 20.0 (IBM Inc) was used for the statistical analysis of individual data. We analyzed the changes in the abundance of bacterial genera and species when detected in at least 8 subjects per diet. The normal distribution of variables was assessed using the Kolmogorov-Smirnov test. The data were analyzed using ANOVA for repeated measures with time as the intrasubject factor and diet as the intersubject factor. Post hoc statistical analysis was performed by using the Bonferroni multiple comparison test. A study of the relationship among parameters was also performed using the Pearson linear correlation coefficient. A P value of $<.05$ was considered significant.

Results

Baseline characteristic of the study population

No significant differences ($P > .05$) were observed in the baseline characteristics of the 20 obese people participating in the dietary intervention (Table 1). In brief, the subjects (20 men) had an average age of 63.3 ± 2.0 years and an average body mass index of 32.2 ± 0.5 kg/m^2.

Effect of the dietary intervention on the main metabolic variables

No statistically significant differences ($P > .05$) were observed in the main metabolic variables of the 20 obese people after 1 year of dietary intervention (Table 2). However, we observed an increase in the insulin sensitivity index for both the LFHCC and Med diets ($P = .019$ and $P = .005$, respectively), when measured from an OGTT performed at basal time and after 1 year of dietary intervention.

Microbiota composition of the study population

Global pattern

For the bacterial community analyses of the 40 samples, after screening our data for poor quality sequences, we recovered 162 871 high-quality 16S rRNA gene sequences with an average of 4072 ± 2745 (SD) sequences per sample. From those, we obtained a total of 140 566 sequences (86% of the total), which could be classified

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

Table 2. Effect of the Dietary Intervention on the Main Metabolic Variables in the Population in Study

			P Value		
	LFHCC Diet	**Med Diet**	**Diet**	**Time**	**Interaction**
Glucose (mg/dL)					
Basal	94.2 ± 3.1	91.2 ± 2.8	.609	.059	.679
1 year	97.9 ± 3.6	96.8 ± 3.5			
HbA_{1c} (%)					
Basal	6.30 ± 0.17	6.06 ± 0.16	.520	.049	.115
1 year	6.06 ± 0.14	6.03 ± 0.13			
ISI					
Basal	3.07 ± 0.58	3.13 ± 0.58	.762	.001	.660
1 year	5.40 ± 1.13[a]	6.02 ± 1.13[a]			
TG (mg/dL)					
Basal	102.2 ± 7.9	98.7 ± 7.9	.350	.499	.313
1 year	104.8 ± 11.5	85.8 ± 11.5			
TC (mg/dL)					
Basal	149.8 ± 6.9	150.2 ± 6.9	.696	.254	.501
1 year	142.2 ± 5.8	148.2 ± 5.8			
HDL (mg/dL)					
Basal	40.3 ± 2.4	42.1 ± 2.4	.240	.459	.250
1 year	39.7 ± 2.0	44.8 ± 2.0			
LDL (mg/dL)					
Basal	83.7 ± 5.7	88.0 ± 5.7	.526	.410	.917
1 year	81.0 ± 5.1	85.9 ± 5.1			

Abbreviations: DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; HDL, high-density lipoprotein; ISI, insulin sensitivity index; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides. Values are means ± SEM. ANOVA for repeated-measures *P* values.

[a] $P < .05$, post hoc Bonferroni's multiple comparison tests between the values at 1 year and the values at baseline.

into operational taxonomic units (OTUs) with a mean of 3514 classifiable sequences per sample, that were used in the subsequent analyses (range, 945–13 788). Of these nonsingleton OTUs, 74 430 were found in the subjects that consumed the low-fat diet, and 68 136 were found in the subjects that consumed the Med diet.

The phylogenetic characterization of all the samples uncovered 3 main bacterial phyla in the following proportions: Bacteroidetes (57.3%), Firmicutes (39.6%), and Proteobacteria (1.8%). Less abundant bacterial phyla (<0.4%–0.001%) including Tenericutes, Actinobacteria, Fusobacteria, Verrucomicrobia, and Lentisphaerae were also present (Figure 1). Across all taxa, 116 genera and 10 480 OTUs, with an average of 768 observed OTUs per sample, were identified. The main taxa that accounted for 70.5% of the sequences were *Bacteroides* (33.0%), *Prevotella* (13.7%), unknown Lachnospiraceae (6.2%), *Faecalibacterium* (4.1%), unknown Clostridiales (3.1%), unknown Ruminococcaceae (3.0%), *Oscillospira* (2.7%), *Parabacteroides* (2.4%), and unknown Bacteroidales (2.3%). At the species level, 3 unknown *Bacteroides* (3.9%), 2 *Prevotella copri* (2.6%), *Bacteroides uniformis* (1.5%), *Bacteroides plebeius* (0.8%), and 1 unknown Lachnospiraceae (0.7%) represented the most abundant taxa (10.2%).

α- and β-diversity and taxon representation patterns between diet samples

First, we used jackknifed hierarchical clustering (unweighted pair group method with arithmetic mean), based on weighted UniFrac distances, to investigate the relationships among the bacterial communities of the different samples according to the diet, after 1 year of intake. In an initial analysis, we examined the results from 40 samples and 2 sequencing runs in which the sampling yielded sufficient depth (>1000 sequences per samples, with the exception of 1 sample). With this analysis, most samples, with the exception of those for 2 individuals, were grouped according to the subject independently of the sampling period. In this way, samples from each patient at time zero (T0) and after 1 year of dietary intervention (T1) were grouped together. To test whether the outgrouping of those 2 subjects was due to amplification or sequencing errors, we performed a third run including 6 subjects at both sampling times (12 samples). The clustering of the samples from this new run paired them with the expected sample from previous runs, which supported the idea that a major change in gut microbiota occurred for those 2 subjects in 1 year. Finally, we combined all of the sequences from the 3 runs for each patient to have only 1 dataset per patient and sampling period for downstream analysis. Unweighted UniFrac analysis based on either a

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

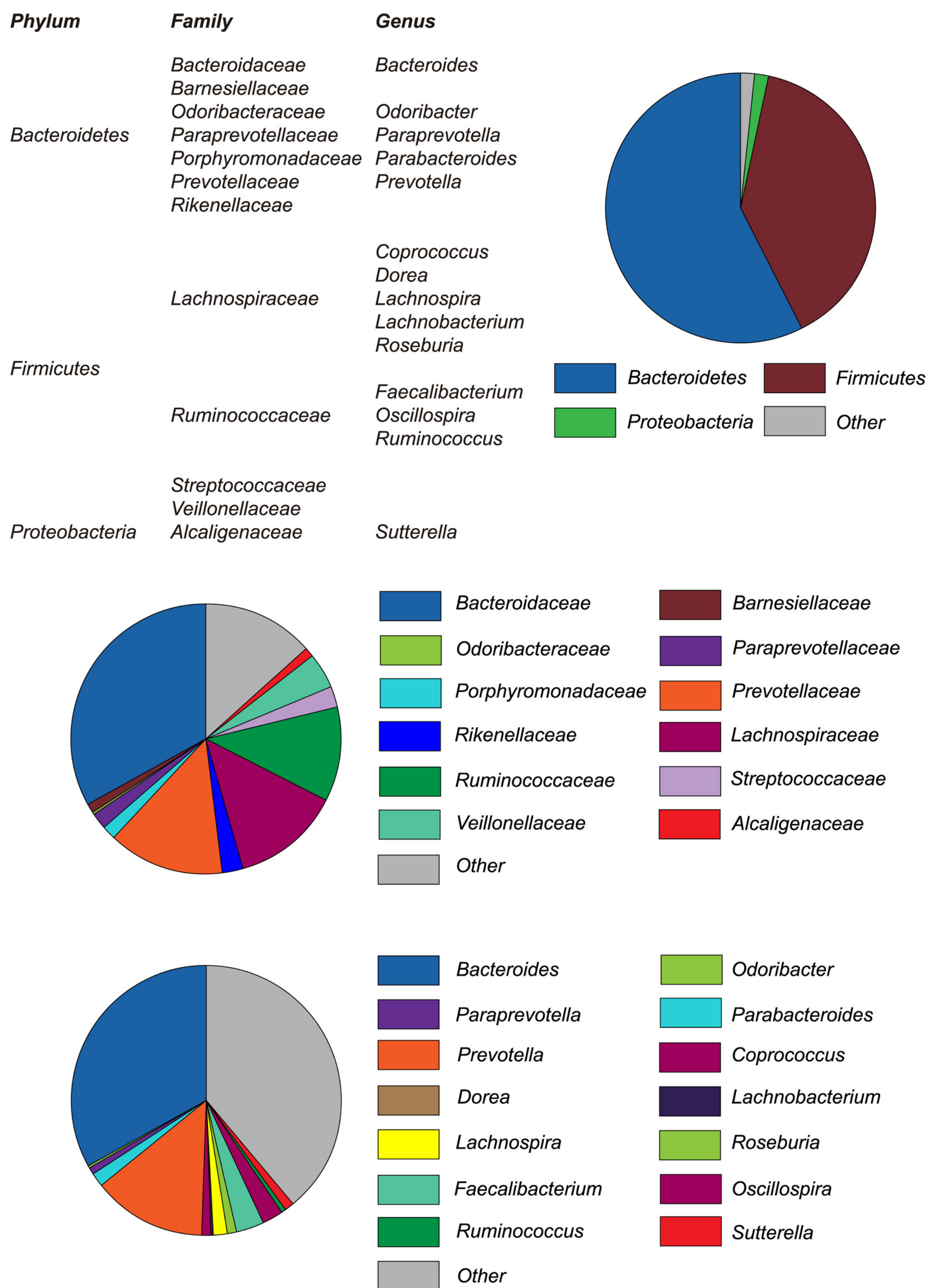

Figure 1. Microbiota composition of the study population.

hierarchical unweighted pair group method with arithmetic mean tree or principal coordinates analysis did not segregate the subject samples into different clusters according to the diet intervention or the sampling period, suggesting that the microbiota of the subjects was stable and similar in terms of proportion of the main taxonomic phyla. Thus, the global bacterial profile of feces samples from each individual cluster together and separately from those of other subjects (Supplemental Figure 1).

When α-diversity indexes (Chao1, richness and PD estimation) for the samples at the level of 800 per sample were analyzed, the samples from the different diets after 1 year of intake showed similar numbers of the 3 estimated α-diversity indexes ($P > .180$) as determined by a non-

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

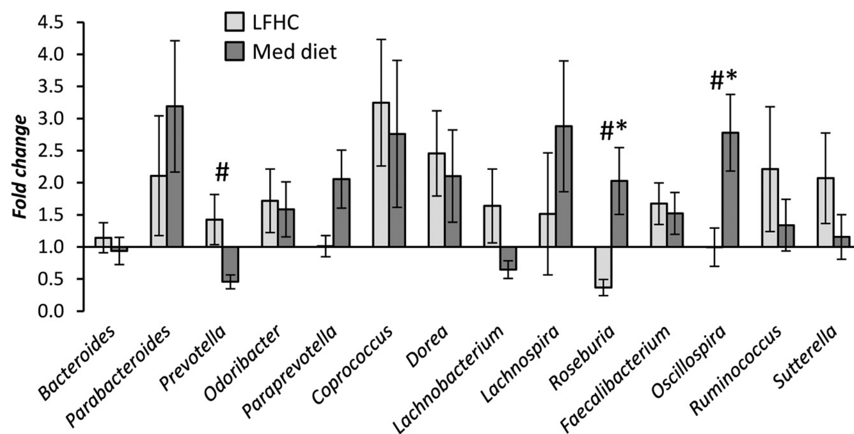

Figure 2. Microbiome changes by diet in the bacterial genus most commonly represented in our population. Values represent means ± SEM of the fold change after the consumption of LFHCC or Med diet. Fold change was calculated by dividing the bacterial abundance at 1 year by the abundance at baseline. #, $P < .05$ between sampling time by diet interaction in the ANOVA for repeated measures. *, $P < .05$ in the post hoc Bonferroni multiple comparison tests between the bacterial abundance at 1 year and the abundance at baseline.

parametric two-sample *t* test. The rarefaction α-diversity curves level off, showing that the sequencing effort was sufficient to detect most of the OTUs for all samples (Supplemental Figure 2).

Changes in specific bacterial taxa and their relationships with the consumption of different diets

To assess whether specific changes occurred in some bacterial taxa after the intake of each diet, we compared the frequency of occurrence of each taxa between both sampling times for each diet after intrasubject normalization. We observed an increase in the relative abundance of the *Prevotella* bacterial genus with the LFHCC diet, whereas a decrease was observed after the Med diet (time × diet interaction $P = .028$). Furthermore, the Med diet increased the relative abundance of *Roseburia* and *Oscillospira* bacterial genera ($P = .017$ and $P = .001$, respectively), whereas the LFHCC diet decreased the relative abundance of *Roseburia* without changing the abundance of the *Oscillospira* bacterial genera (time × diet interaction $P = .009$ and .016, respectively) (Figure 2). On the other hand, in terms of bacterial species, long-term consumption of the Med diet increased the relative abundance of *Parabacteroides distasonis* ($P = .025$), whereas the LFHCC diet increased *Faecalibacterium prausnitzii* ($P = .020$) (Table 3).

Relationship between diet-induced changes in metabolome and changes in microbiota

A total of 572 compounds were identified in feces. Analysis by ANOVA for repeated measures identified 37 compounds exhibiting a significant interaction between time and diet. The main diet-induced effects on the fecal metabolome were changes in amino acid, peptide, and sphingolipid metabolism. The plasma analysis identified 697 compounds. Analysis by ANOVA for repeated measures identified 6 compounds exhibiting significant interaction between time and diet, principally in amino acid and peptide metabolism. To analyze the relationship between the observed changes in both microbiota composition and fecal and plasma metabolome, we performed a correlation analysis between the individual fold changes in the bacterial genera and species whose abundance significantly changed by the consumption of either of the diets (6 bacterial genera and 2 bacterial species), and the individual fold changes of the metabolites with diet-induced statistically significant changes (43 metabolites: 37 metabolites in feces and 6 metabolites in plasma). This strategy allowed us to avoid interindividual variability, and in addition, search for relationships between fold changes (relationship in a dynamic system) instead of using the values obtained at basal or at the end of the intervention (relationship in a static system), thus strengthen-

Table 3. Microbiome Changes by Diet in the Bacterial Species Most Commonly Represented in Our Population

			P Value	
Bacterial Species	**LFHCC Diet**	**Med Diet**	**Time**	**Interaction**
Bacteroides ovatus	1.37 ± 0.42	0.78 ± 0.20	.734	.236
Bacteroides plebeius	1.75 ± 0.46	1.56 ± 0.67	.147	.836
Bacteroides uniformis	0.98 ± 0.23	0.83 ± 0.21	.556	.667
Parabacteroides distasonis	1.42 ± 0.40	2.32 ± 0.62[a]	.034	.296
Coprococcus eutactus	2.28 ± 0.88	1.51 ± 0.45	.104	.469
Faecalibacterium prausnitzii	1.91 ± 0.27[a]	1.53 ± 0.33	.007	.516

Means values ± SEM of the abundance of bacterial species; fold change was calculated by dividing the bacterial abundance at 1 year by the abundance at baseline. ANOVA for repeated-measures *P* values.

[a] $P < .05$, post hoc Bonferroni's multiple comparison tests between the abundance at 1 year and the abundance at baseline.

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

Table 4. Relationship Between the Observed Changes in Both Microbiota Composition and Fecal and Plasma Metabolome

	FC LFHCC Diet	FC Med Diet	*P* Value Interaction	Bacterial Genera and Species	Pearson Coefficient	*P* Value for Pearson Correlation
Metabolite in feces						
N-Acetylaspartate	0.50	1.01	.020	*Roseburia*	0.734	<.001
Glutamate	1.25	0.69	.018	*Prevotella; Oscillospira*	0.498; −0.520	.025; .019
Arginylproline	1.78	0.85	.007	*Prevotella; Roseburia*	0.490; −0.467	.028; .044
Leucylvaline	1.87	0.79	.039	*Faecalibacterium prausnitzii*	0.470	.037
Pantothenate	1.56	0.69	.027	*Oscillospira*	−0.474	.035
cis-Vaccenate (18:1n7)	0.43	1.41	.010	*Oscillospira*	0.511	.021
3,7-Dimethylurate	2.48	0.29	.044	*Prevotella*	0.499	.025
Metabolite in plasma						
XHWESASXXR	0.02	1.72	.042	*Roseburia*	0.479	.038
Valylglutamine	0.26	0.99	.045	*Oscillospira*	0.563	.010
Glycocholenate sulfate	1.14	0.84	.027	*Bacteroides uniformis*	0.450	.049

Abbreviation: FC, fold change in metabolite abundance calculated by dividing the bacterial abundance at 1 year by the abundance at baseline. *P* value interaction: ANOVA for repeated-measures *P* value for time by diet interaction.

ing our findings. Our results showed that the changes in 7 of 572 metabolites in feces and 3 of 697 metabolites in plasma were related to the changes in 3 bacterial genera and 2 bacterial species (Table 4).

Discussion

Based on the jackknifed hierarchical clustering analysis, our data suggest that interindividual differences in the microbiota are greater than the changes undergone by the diet. However, after we normalized between individuals, several diet-induced changes were observed after 1 year of dietary intervention. The principal findings were an increase in relative abundance of *Prevotella* and a decrease in *Roseburia* genera after the LFHCC diet, whereas the Med diet significantly increased the abundance of the *Roseburia* and *Oscillospira* genera. Finally, a complete fecal metabolome analysis showed changes in the feces of 10 metabolites involved in amino acid, peptide, and sphingolipid metabolism, reflecting the changes in gut microbiota.

The increase in the *Prevotella* genus with the LFHCC diet might be expected, in agreement with the findings in the Burkina Faso population. It is known that *Prevotella* (together with other genera such as *Xylanibacter*, *Butyrivibrio*, and *Treponema*) may enhance the ability to extract calories from resistant starch and oligosaccharides, as well as carbohydrates that escape digestion in the small intestine and are fermented in the gut (24). In addition, another study showed the strong association of the *Prevotella*-rich enterotype with long-term diets rich in carbohydrates (21). In addition, an interesting finding was that the LFHCC diet increased the abundance of *F. prausnitzii*, a butyrate-producing bacteria whose abundance has been found to decrease in patients with T2D (25, 26) and that is negatively associated with inflammatory markers in T2D (27). Moreover, we have previously shown that Med diet consumption increases the abundance of several butyrate-producing bacteria including *F. prausnitzii* in patients with metabolic syndrome, whereas no changes were observed in this bacterial species after the consumption of an LFHCC diet (22), which suggests that the degree of dysbiosis associated with the pathophysiological conditions may be a determinant in the response to a specific therapeutic diet-based treatment. Although *Roseburia* spp. have been shown to decrease after high-fat feeding in animal models (28), in humans this does not seem to be the case, as Med diet consumption increased its abundance, despite it being a high-fat diet compared with the LFHCC diet. This butyrate-producing genus comprises a high proportion of the human bacteria and could play an important role in the maintenance of gut health (29), taking into account the fact that the abundance of this genus, known to be anti-inflammatory, has been found to be low in T2D patients (25, 26). This finding suggests a protective effect of Med diet consumption on the development of T2D, as evidenced from epidemiological studies (30, 31). Considering that *Roseburia* produces an inhibitory substance against *Bacillus subtilis* (28), this result suggests that some of the changes in the microbiota induced by the Med diet could be mediated by the antimicrobial effect of this genera, which modifies the microbial population in the colon. In addition, the Med diet increased the relative abundance of *Oscillospira*, a genus belonging to the Ruminococcaceae family, associated with the feeding of fresh forage in ruminants such as cattle and sheep, suggesting a microbiota adaptation to a vegetable-rich diet such as the Med diet (32). In agreement with that, Med diet consumption

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

has been associated with the low rate of cardiovascular mortality found in Mediterranean countries (33, 34).

In summary, the abundance of 2 bacterial populations related with T2D (25–27), the *Roseburia* genus and the bacterial species *F. prausnitzii*, was modulated by diet. On the one hand, the consumption of the Med diet increased the abundance of the *Roseburia* genus (found to be low in patients with T2D), in parallel with an increase in insulin sensitivity after the consumption of this diet for 1 year. On the other hand, LFHCC consumption decreased the abundance of this genus, while increasing the abundance of another diabetes-protective bacterial species, *F. prausnitzii* (found to be low in patients with T2D). These 2 changes could have a protective influence for the prevention of T2D, as suggested by the findings of an improvement in insulin sensitivity after the consumption of the LFHCC (evaluated with the OGTT). These data suggest that the consumption of the LFHC and Med diets could be a therapeutic and preventive tool for T2D, and this could open a new hypothesis to be tested in the future in bigger populations: on whether the consumption of healthy diets reduces the risk of T2D by influencing the microbiota profile.

The metabolic activity of the gut microbiota involves the anaerobic breakdown of dietary fiber, carbohydrates, protein, and peptides (35), producing SCFAs, which play an important role in maintaining intestinal health (36), and also at a systemic level (37). In line with this, the adaptation of the microbiota to the LFHCC diet, with an increase in the *Prevotella* genus and *F. prausnitzii*, facilitates the fermentation of the carbohydrates that escape intestinal digestion and increases the production of SCFAs (24). In contrast, the Med diet decreases the abundance of this genus but increases other SCFA-producing genera such as *Roseburia* (38). These apparently opposite changes in terms of bacterial populations seem to have a neutral net effect in terms of the production of SCFAs, based on the short- or medium-chain fatty acid levels in feces and plasma (7 and 10 fatty acids from 4 to 12 atoms of carbon in feces and plasma, respectively), which did not change after the dietary intervention. Furthermore, we did not observe any changes in other microbiota-derived metabolites, such as secondary bile acid (feces, 13 secondary bile acids; plasma, 15 secondary bile acids). These results suggest that the changes in gut microbiota composition may be functionally compensated for in terms of their capacity to metabolize or produce metabolites or bioactives, thus having a neutral net effect, as shown for SCFAs and secondary bile acid.

However, the changes in the abundance of 10 metabolites (7 in feces and 3 in plasma), mainly related with amino acid, peptide, and sphingolipid metabolism, can be explained by the changes in 3 bacterial genera and 2 bacterial species. In addition, the correlation analysis results suggest that the fecal metabolome changes observed after the dietary intervention may occur through changes in microbiota.

Finally, because dietary proteins are absorbed in the gastrointestinal tract after their degradation, the relationship between protein-related metabolites and microbiota, found in our study, explains how the composition of the intestinal microbial community may influence amino acid digestibility, a term that describes the proportion of consumed amino acid that is absorbed as a result of the interaction between the food and the individual eating it (39). In fact, it is known that the quantities of amino acids absorbed intact from the large intestine are very low, as the microbiota alters the amino acid composition (39, 40).

In conclusion, our results suggest that long-term consumption of both healthy diets, the Med diet and the LFHCC diet, exerts a protective effect on the development of T2D by different specific changes in the gut microbiota, which increase the abundance of the *Roseburia* genus and *F. prausnitzii*, respectively. In addition, the changes in the gut microbiota after 1 year's consumption of both diets may be linked to amino acid digestibility. Our study provides insight into microbial functions and their role in mediating the effect of diet when used as a therapeutic tool in human health; however, further studies are required to fully understand the potential role of gut microbiota modification by diet in the prevention of metabolic diseases.

Acknowledgments

The Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBEROBN) is an initiative of the Instituto de Salud Carlos III, Madrid, Spain. We thank Metabolon for manuscript revision, the Cordoba node of the Biobank of the Sistema Sanitario Publico de Andalucia (Andalucia, Spain) for providing the biological human samples, and the Escuela Andaluza de Salud Publica, Granada, Spain, for help in performing the randomization process for this study.

Address all correspondence and requests for reprints to: Antonio Camargo Garcia, Lipids and Atherosclerosis Research Unit, IMIBIC/Reina Sofia University Hospital/University of Cordoba, Avenue Menendez Pidal, s/n 14004 Cordoba, Spain. E-mail: antonio.camargo@imibic.org.

This study has been registered at ClinicalTrials.gov under registration no. NCT00924937.

Author contributions were as follows: J.L.-M. and F.P.-J. designed the clinical studies; C.H., M.M.-B., O.A.R.-Z., J.F.A.-D., F.G.-D., G.M.Q.-N., and A.C. performed laboratory experiments and clinical studies; P.P.-M., J.D.-L., F.J.T., and B.B.L. analyzed the statistics; C.H., M.M.-B., O.A.R.-Z. contributed to acquisition of data; F.J.T., B.B.L., J.L.-M., A.C., and F.P.-J. in-

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

terpreted the results and revised the article. All authors commented on the article and gave their final approval of the version of the article to be published.

The CORDIOPREV study is supported by the Fundación Patrimonio Comunal Olivarero, Junta de Andalucía (Consejería de Salud, Consejería de Agricultura y Pesca, Consejería de Innovación, and Ciencia y Empresa), Diputaciones de Jaén y Córdoba, Centro de Excelencia en Investigación sobre Aceite de Oliva y Salud and Ministerio de Medio Ambiente, Medio Rural y Marino, and Gobierno de España. In addition, it was partly supported by research grants from the Ministerio de Ciencia e Innovación (AGL2009–122270 to J.L.-M., FIS PI10/01041 to P.P.-M., and PI10/02412 to F.P.-J.), Ministerio de Economia y Competitividad (AGL2012/39615, PIE14/00005, and PIE14/00031 to J.L.-M.; PI13/00619 to F.P.-J.), Consejería de Economía, Innovación y Ciencia, Proyectos de Investigación de Excelencia, Junta de Andalucía (AGR922 to F.P.-J.), Consejería de Salud, Junta de Andalucía (PI0193/09 to J.L.-M., PI-0252/09 to J.D.-L., and PI-0058/10 to P.P.-M.), Consejería de Innovación Ciencia y Empresa (CVI-7450 to J.L.-M.), and Fondo Europeo de Desarrollo Regional. A.C. is supported by an Instituto de Salud Carlos III research contract (Programa Miguel-Servet CP14/00114). J.F.A.-D. is supported by an Instituto de Salud Carlos III research contract (Programa Rio-Hortega).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Bays HE, González-Campoy JM, Bray GA, et al.** Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Rev Cardiovasc Ther*. 2008;6:343–368.
2. **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;444:1027–1031.
3. **Cani PD, Delzenne NM, Amar J, Burcelin R.** Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding. *Pathol Biol (Paris)*. 2008;56:305–309.
4. **Ley RE.** Obesity and the human microbiome. *Curr Opin Gastroenterol*. 2010;26:5–11.
5. **Blaser MJ, Falkow S.** What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol*. 2009;7:887–894.
6. **Neish AS.** Microbes in gastrointestinal health and disease. *Gastroenterology*. 2009;136:65–80.
7. **Tremaroli V, Bäckhed F.** Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012;489:242–249.
8. **Caesar R, Fåk F, Bäckhed F.** Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism. *J Intern Med*. 2010;268:320–328.
9. **Johnson AM, Olefsky JM.** The origins and drivers of insulin resistance. *Cell*. 2013;152:673–684.
10. **Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S.** Host-gut microbiota metabolic interactions. *Science*. 2012;336:1262–1267.
11. **Shen J, Obin MS, Zhao L.** The gut microbiota, obesity and insulin resistance. *Mol Aspects Med*. 2013;34:39–58.
12. **De Filippo C, Cavalieri D, Di Paola M, et al.** Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA*. 2010;107:14691–14696.
13. **Moschen AR, Wieser V, Tilg H.** Dietary factors: major regulators of the gut's microbiota. *Gut Liver*. 2012;6:411–416.
14. **Pendyala S, Walker JM, Holt PR.** A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology*. 2012;142:1100–1101.e2.
15. **Ghanim H, Abuaysheh S, Sia CL, et al.** Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes Care*. 2009;32:2281–2287.
16. **Ghanim H, Sia CL, Korzeniewski K, et al.** A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. *J Clin Endocrinol Metab*. 2011;96:1409–1414.
17. **Ghanim H, Sia CL, Upadhyay M, et al.** Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. *Am J Clin Nutr*. 2010;91:940–949.
18. **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;72:5990–5997.
19. **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;64:3854–3859.
20. **Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, et al.** Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am J Clin Nutr*. 2012;95:1323–1334.
21. **Wu GD, Chen J, Hoffmann C, et al.** Linking long-term dietary patterns with gut microbial enterotypes. *Science*. 2011;334:105–108.
22. **Haro C, Garcia-Carpintero S, Alcala-Diaz JF, et al.** The gut microbial community in metabolic syndrome patients is modified by diet [published online ahead of print August 20, 2015]. *J Nutr Biochem*. doi:10.1016/j.jnutbio.2015.08.011.
23. **Garcia-Rios A, Gomez-Delgado FJ, Garaulet M, et al.** Beneficial effect of CLOCK gene polymorphism rs1801260 in combination with low-fat diet on insulin metabolism in the patients with metabolic syndrome. *Chronobiol Int*. 2014;31:401–408.
24. **Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA.** Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol*. 2008;6:121–131.
25. **Karlsson FH, Tremaroli V, Nookaew I, et al.** Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*. 2013;498:99–103.
26. **Qin J, Li Y, Cai Z, et al.** A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490:55–60.
27. **Furet JP, Kong LC, Tap J, et al.** Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes*. 2010;59:3049–3057.
28. **Hatziioanou D, Mayer MJ, Duncan SH, Flint HJ, Narbad A.** A representative of the dominant human colonic Firmicutes, *Roseburia faecis* M72/1, forms a novel bacteriocin-like substance. *Anaerobe*. 2013;23:5–8.
29. **Aminov RI, Walker AW, Duncan SH, Harmsen HJ, Welling GW, Flint HJ.** Molecular diversity, cultivation, and improved detection by fluorescent in situ hybridization of a dominant group of human gut bacteria related to *Roseburia* spp. or *Eubacterium rectale*. *Appl Environ Microbiol*. 2006;72:6371–6376.
30. **Kastorini CM, Panagiotakos DB.** Dietary patterns and prevention of type 2 diabetes: from research to clinical practice; a systematic review. *Curr Diabetes Rev*. 2009;5:221–227.
31. **Schröder H.** Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. *J Nutr Biochem*. 2007;18:149–160.
32. **Mackie RI, Aminov RI, Hu W, et al.** Ecology of uncultivated *Oscillospira* species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. *Appl Environ Microbiol*. 2003;69:6808–6815.
33. **De Lorgeril M, Salen P, Martin JL, Mamelle N, Monjaud I, Touboul**

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022

P, Delaye J. Effect of a Mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the cardioprotective effect of certain nutriments. *J Am Coll Cardiol*. 1996;28:1103–1108.
34. **Mena MP, Sacanella E, Vazquez-Agell M, et al.** Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. *Am J Clin Nutr*. 2009;89:248–256.
35. **Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TM, Comelli EM.** Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutr Diabetes*. 2014;4:e121.
36. **Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ.** Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol*. 2006;40:235–243.
37. **Ichimura A, Hirasawa A, Hara T, Tsujimoto G.** Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. *Prostaglandins Other Lipid Mediat*. 2009;89:82–88.
38. **Duncan SH, Aminov RI, Scott KP, Louis P, Stanton TB, Flint HJ.** Proposal of *Roseburia faecis* sp. nov., *Roseburia hominis* sp. nov. and *Roseburia inulinivorans* sp. nov., based on isolates from human faeces. *Int J Syst Evol Microbiol*. 2006;56:2437–2441.
39. **Fuller M.** Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. *Br J Nutr*. 2012;108(suppl 2):S238–S246.
40. **Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CF, Committee on Terminology to Report AA Bioavailability and Digestibility.** Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *J Anim Sci*. 2007;85:172–180.

Downloaded from https://academic.oup.com/jcem/article/101/1/233/2806797 by guest on 20 August 2022