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ORIGINAL ARTICLE

Influence of food consumption patterns and Galician lifestyle on human gut microbiota

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Abstract The proportion of different microbial populations in the human gut is an important factor that in recent years has been linked to obesity and numerous metabolic diseases. Because there are many factors that can affect the composition of human gut microbiota, it is of interest to have information about what is the composition of the gut microbiota in different populations in order to better understand the possibilities for improving nutritional management. A group of 31 volunteers were selected according to established inclusion and exclusion criteria and were asked about their diet history, lifestyle patterns, and adherence to the Southern European Atlantic Diet. Fecal samples were taken and subsequently analyzed by real-time PCR. The results indicated different dietary patterns for subjects who consumed a higher amount of fruits, vegetables, legumes, and fish and a lower amount of bakery foods and precooked foods and snacks compared to Spanish consumption data. Most participants showed intermediate or high adherence to Southern European Atlantic Diet, and an analysis of gut microbiota showed high numbers of total bacteria and Actinobacteria, as well as high amounts of bacteria belonging to the genera *Lactobacillus* spp. and *Bifidobacterium* spp. A subsequent statistical comparison also revealed differences in gut microbiota depending on the subject's body weight, age, or degree of adherence to the Southern European Atlantic Diet.

Keywords Gut microbiota · Actinobacteria · *Bifidobacterium* · *Lactobacillus* · Atlantic Diet · Southern European Atlantic Diet

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Introduction

The Atlantic Diet (AD) is a dietary pattern characterized by a high consumption of seafoods, derived from the major importance of the fishing industry in the Atlantic region [26]. Furthermore, red meat and dairy products are staple foods as a result of the high rainfall in the region and the abundance of good pastures that favor extensive cattle breeding [10, 22]. The meat/fish ratio consumption in the AD is near to neutral, and the AD is also characterized by a high intake of vegetables and legumes, soups, potatoes, and whole grain bread [22, 34]. The sauces used are low in calories, but high in nutritional quality. Olive oil is used as a dressing and for cooking, and eggs and wine consumption are moderate [7]. The AD is also characterized by a high intake of seasonal foods that are locally fresh and minimally processed and original food recipes that are simple but carefully prepared [22, 34]. With respect to cooking methods, foods are commonly steamed, boiled, baked, grilled, or stewed rather than fried [7]. The type of food associated with the traditional AD of Galicia has been stated in the AD pyramid [33].

There are variations in the dietary patterns of AD because the AD region includes large countries with very different climatology, such as Portugal, Ireland, the United Kingdom, Belgium, the Netherlands, Denmark, Norway, Iceland, and part of Spain and France [34]. Among these countries, Portugal and Galicia (northwestern Spain) are geographically,

climatically, and culturally similar because of their Celtic influence [7]. Their common eating habits have previously been defined such as the Southern European Atlantic Diet (SEAD) [11, 34].

Various scientific studies have already demonstrated the health benefits of the food commonly found in the SEAD. In particular, the consumption of fish and other seafood, vegetables, and low-alcohol-content beverages, all typical of the SEAD, have demonstrated protective effects against heart disease, metabolic diseases, and even in some cancers [34]. Vitamin B, n-3 fatty acids, and iodine are three components of the SEAD that may be associated with health benefits to consumers [34]. With regard to heart disease, Spain has one of the lowest mortality rates for ischemic heart disease in Europe. However, there is wide variation in such mortality is seen across the country; areas that typically rely on a diet based on the SEAD have been found to have an ischemic heart disease mortality rate that is up to 40% lower than the mean [7]. In a population-based case-control study in Porto, Portugal, it was reported that adherence to the traditional SEAD was associated with a lower probability of acute myocardial infarction [22]. Adherence to this diet has also been associated with lower serum concentrations of inflammation markers, triglycerides, and insulin; an improved insulin resistance index; and reduced systolic blood pressure [11]. Other studies have highlighted the benefits of food components typical of the SEAD, but they have not grouped those food components as a meal or aggregated them under the concept of the SEAD pattern [34].

Despite the abovementioned advantages of the SEAD, Galicia records a paradoxical case: while its rates of mortality derived from heart diseases are clearly lower than those of the rest of Spain [7], Galicia has one the highest obesity and overweight rates in Spain, in both children [24] and adults [2], reaching an obesity rate of about 25% of the total population [2].

Several factors can influence in the obesity. Among them, one that has received more attention in recent years is the composition of the human gut microbiota (GM). It is well known that modulation of the GM can have beneficial effects in controlling obesity, and several mechanisms that may contribute to microbiota-induced susceptibility to obesity and metabolic diseases have been proposed [8, 35]. In addition to obesity, GM can play a key role in several other diseases [27, 29], such as diabetes [19], cancer [32], metabolic syndrome [8, 18] non-alcoholic fatty liver disease, and cirrosis [1, 27], and in several psychiatric disorders [6]. Consequently, GM plays a major role in health and disease in humans; it is sometimes referred to as our "forgotten organ" [27].

The main goal of this work was to characterize of the GM of people that live in Galicia. Additional goals were to identify factors, such as age or body mass index (BMI), that might play a role in the GM composition and to explore whether adherence to the SEAD was associated with GM composition in an adult sample with non-declared pathology. To the best of our knowledge, this was the first work to describe the GM composition of Galician people and its relationship to the SEAD.

Materials and methods

Participants

This study is part of an ongoing investigation into the associations between diet and GM. The study sample involved 31 Caucasian adults with non-declared pathology (22 females, 9 males; with a mean age of 28.3 ± 9.73 years old). Subjects were asked questions about any treatment received during the previous 6 months. Only individuals who were aged 18–65 years old who had not used antibiotics, pre- or probiotics, glucocorticoids, or immunosuppressive drugs were recruited for the study.

Qualified personnel took informed consent for the collection of anthropometric measures and extraction of fecal samples, and the use of the data followed the rules set forth in the Spanish legislation of personal data protection. The study was approved by the Clinical Research Ethics Committee of the Servicio Galego de Saúde (SERGAS, authorization number 119/2014).

Nutritional and lifestyle assessment

Diet was assessed with a validated SEAD adherence history [22]. SEAD adherence was measured using an index of nine food components (fresh fish, cod, red meat and pork products, dairy products, legumes and vegetables, vegetable soup, potatoes, wholegrain bread and wine) that ranged from 0 (lowest adherence) to 9 (highest adherence). For each participant, the scores (0 or 1) for the 9 food components of the SEAD were summed, which resulted in an index that ranged from 0 (lowest adherence to the SEAD) to 9 (highest adherence to the SEAD).

Food intake over the last 4 weeks of the study was assessed using a semi-quantitative food frequency questionnaire (FFQ). This questionnaire was structured using 13 food groups and it included 113 foods and drinks habitually consumed in Spain. Study participants completed the questionnaire in the presence of a nutritionist, and they were asked to record the mean daily, weekly, or monthly consumption of each food, bearing in mind serving size. During a personal interview, which took place prior to stool collection, subjects were asked by item how often and how much of the said product they consumed. The dietary history allows the collection of information on foods and uses sets of photographs to help quantify portion sizes [28]. Nutritional composition of

foods consumed, refers to average daily intake, and the nutrient requirements of the studied population were estimated by means of Easy diet® software version 1.0 (Biocentury, Barcelona, Spain). During the FFQ, participants were also asked about some factors related to their lifestyle, including physical activity, tobacco consumption, food allergies and intolerances, sleeping hours, and the hour that they go to bed.

Body weight and height were recorded by standard methods using Inbody 320 body composition analyzer (Biospace, Seoul, South Korea) and a BSM 170 stadiometer (Biospace), respectively, with the subjects in underwear and barefoot. Body weight was measured to the nearest 0.1 kg and body height to the nearest 0.1 cm. All measurements of participants weight and were made in triplicate. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared.

Collection of stool samples and DNA extraction

Stool samples were collected in a sterile container (10–30 g per subject) using an anaerobiosis-generating system (GENbox, Biomérieux, Marcy L'Etoile, France) and were treated within 2 h of collection. In cases where this time range was not possible, the samples were kept frozen (−21 °C) until their processing. To prepare the samples for DNA extraction, the samples were combined with a 1:10 dilution of phosphate buffered saline (PBS) (Invitrogen, Paisley, UK) (adjusted to pH 7.2–7.4), and then homogenized in a sterile bag with a Masticator® (AES, Combourg, France) for 5 min. The homogenized samples were kept frozen until used. DNA extraction was performed using 1 mL of each sample in a commercial kit (Realpure Microspin, Durviz S., Valencia, Spain) using the manufacturer's instructions.

Bacterial quantification of fecal samples using real-time PCR

A real-time quantitative PCR (qPCR) procedure was used for the quantification of the main bacterial groups of human fecal samples by using specific primers, based on previously reported methods [21] (Table 1). Thus, the first step was a denaturation step at 95 °C for 10 min. The second step consisted of 45 denaturation cycles at 95 °C for 10 s per cycle and optimum ringing temperature for 1 min. The third and final step involved an analysis of the curves (0.05 °C per cycle) with a simultaneous measurement of the intensity of the Syber Green® fluorochrome (Applied Biosystems, Foster City, CA, USA). All reactions were carried out in triplicate, with the final volume being 20 mL and containing 2 mL of DNA and a concentration of 100 nM of each of the probes. From each fecal sample, PCR amplification was performed using an ABI PRISM 7000 (Applied Biosystems, Warrington, UK) equipped with ABI PRISM 7000 Software (Applied Biosystems). Quantifications were performed by comparing the amplifications against standard curves that were constructed for each experiment using serial tenfold dilutions of bacterial genomic DNA (of known concentration) from pure cultures [13]. The reference strains that were used were *Enterobacter cloacae* CECT 194, *Clostridium perfringens* CECT 376, *Bifidobacterium longum* CECT 4503, *Bacteroides vulgatus* LMG 17767, and *Lactobacillus reuteri* DSMZ 20016, (Table 1). All strains were grown in liquid medium under their optimal growth conditions and decimal dilutions were made for subsequent plate counting. Once counted, the DNA was extracted from the mother tube and the DNA was quantified by fluorescence using the Qubit Fluorometer (Invitrogen, Oregon, USA). Each of the curves was normalized to the copy number of the 16S rRNA gene for each of the species. Negative controls that contained all of the elements of the reaction mixture with the exception of template DNA were used in every analysis, and no product was ever detected. The data was presented as the mean values of duplicate real-time qPCR analyses. The amplification efficiency of the qPCR for all primer pairs was determined using the linear regression slope of a dilution series, which was calculated using the equation E = 10(−1/slope). The primer pairs demonstrated an efficiency in the range of 95% (E = 1.9) to 104% (E = 2.07) and slope values in the range of −3.59 to −3.16.

Statistical analysis

Average quantities of bacteria in the human fecal samples and their corresponding standard deviations were obtained. The results were expressed as $\log_{10}$ colony-forming units (CFU) per gram of feces. Bacterial data was compared between different groups for adherence to the SEAD, age, and BMI. Adherence to SEAD was considered high for values ≥6, medium for 3–6, and low for ≤3. For the determination of the relation of age with GM, subjects were stratified in three groups: ≤20 years, 20–30 years, and ≥30 years. For the determination of the relation of BMI on GM, subjects were stratified in three groups: ≤20, 20–35, and ≥30. The results obtained in the different populations studied were compared among the different population groups by means of analysis of variance (ANOVA). In all cases, the obtained differences were considered statistically significant if the *P* value was less than 0.05. All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

Results

In global terms, the study population was considered as being between young and middle age (mean age 28.3 ± 9.73 years), which is on average of normal weight (average body weight of

Table 1 Bacterial species-specific primers, reference strains, and reference employed for real-time PCR

Target	Primer sequence (5′- 3′)	Reference strain	Culture conditions	Product size (bp)
Total microbiota	F: ACTCCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	*Clostridium perfringens* CECT 376	RCM, 37 °C for 48 h, anaerobically	200
Firmicutes	F: ATGTGGTTTAATTCGAAGCA R: AGCTGACGACAACCATGCAC	*Clostridium perfringens* CECT 376	RCM, 37 °C for 48 h, anaerobically	126
Bacteroidetes	F: CATGTGGTTTAATTCGATGAT R: AGCTGACGACAACCATGCAG	*Bacteroides vulgatus* LMG 17767	NB + Blood sheep, 37 °C for 24 h, anaerobically	126
Actinobacteria	F: GCGKCCTATCAGCTTGTT R: CCGCCTACGAGCYCTTTACGC	*Bifidobacterium longum* CECT 4503	MRS broth +0.05% Cysteine 37 °C for 48 h, anaerobically	333
Proteobacteria	F: CATGACGTTACCCGCAGAAGAAG R: CTCTACGAGACTCAAGCTTGC	*Enterobacter cloacae* CECT 194	NB, 30 °C for 24 h, aerobically	195
Bifidobacterium spp.	F: CTCCTGGAAACGGGTGG R: GGTGTTCTTCCCGATATCTACA	*Bifidobacterium longum* CECT 4503	MRS broth +0.05% Cys, 37 °C for 48 h, anaerobically	550
Lactobacillus spp.	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	*Lactobacillus reuteri* DSMZ *20016*	MRS broth, 37 °C for 24 h, aerobically	341

CECT Spanish Type Culture Collection (Valencia, Spain); DSMZ: German Collection of Microorganisms and Cell Cultures (Braunsxhweig, Germany); LMG: Bacteria Collection Laboratorium voor Microbiologie Universiteit Gent (Ghant, Belgium)

RCM reinforced clostridium medium, *NB* nutrient broth, *MRS* de Man. Rogosa and Sharpe.

68.39 ± 13.25 kg and an average height of 168 ± 8.91 cm), which gives a BMI of 23.3 ± 1.74). There were more females (22) than males (9). The number of reported allergies and intolerances reached a total rate of 25.8%, with intolerance to lactose being reported most often (12.9%). The population represented a low rate of smokers (12.9%) and the reported number of hours of sleep (7.12 ± 0.98) is below what is recommended for people who are considered middle aged (8 h a day). Additionally, participants usually went to bed at about half-past midnight. With regard to physical exercise, the subjects reported an average of 2.71 ± 1.76 h per week, which equates to 23.23 min per day.

The average daily intakes of different groups of foods are presented in Table 2. The food group with the highest intake was soft drinks. However, this drink consumption yields data of less than one L/person/day, which suggests that insights could have omitted or underestimated intake of some beverages. With regard to solid foods, fruit consumption (454.01 g/day), milk and dairy foods (348.89 g/day), and vegetables (329.50 g/day) were the food groups that were consumed most frequently. Cereal consumption (219.16 g/day), which traditionally occupies the first place among the different groups of foods that are consumed at the national level, was fourth place for the studied population. When other food groups were investigated, results showed that the ratio of meat to fish was 1.66 and there was a very low reported consumption of precooked foods and snacks (20.19 g/day) and eggs (19.93 g/day). With respect to macronutrients, the average number of total calories ingested was 2756 Kcal/day, being a 63.8% from carbohydrates, 24% from fats, and 12.2% from proteins. Results of adherence to the SEAD showed that seven subjects (22.6%) were categorized as showing high adherence, whereas most participants, 21 (67.4%), showed intermediate adherence and only 3 subjects (9.7%) showed low adherence (data not shown).

As presented in Tables 3, 4, and 5, for the category of the total microbiota, the results obtained show high $\log_{10}$ CFU/g counts, ranging from 12 to 13. With regard to the phyla composition of the GM, it was found that Firmicutes were the most abundant phylum in the samples obtained from the study subjects. With regard to Bacteroidetes phylum, their counts were lower than Firmicutes, but adding both groups together accounted for most of the total microbiota. For the other two phyla investigated, the results showed that the counts of Actinobacteria were higher compared to Proteobacteria. Additionally, two genera relevant to human health, such as *Lactobacillus* spp. and *Bifidobacterium* spp., were found in high quantities (8–9 $\log_{10}$ CFU/g).

Gut microbiota composition in subjects with different BMI

With respect to the BMI, the results obtained can be seen in Table 3. In global terms, no significant differences were found for most bacterial groups investigated. However, it was found that Bacteroidetes were significantly higher in overweight subjects than those in underweight subjects, whereas Actinobacteria phyla were higher in overweight subjects than those in under- and normal-weight subjects.

Table 2 Average daily intake (g) of different food groups from participants

Food groups	Amount
Soft drinks	909.33 ± 268.84
Fruits	454.01 ± 157.97
Milk and dairy foods	348.89 ± 161.32
Vegetables	329.50 ± 185.36
Cereals	219.16 ± 101.59
Meat and meat products	136.12 ± 104.98
Alcoholic beverages	84.21 ± 86.32
Fish and fishery products	82.13 ± 13.56
Legumes	63.61 ± 48.50
Oils and fats	39.15 ± 30.14
Bakery foods	39.39 ± 43.80
Precooked foods and snacks	20.19 ± 16.22
Eggs	18.93 ± 9.23
Nutrients	Amount
Energy (Kcal/d)	2756.22 ± 413.56
Total protein (g/day)	82.57 ± 12.14
Animal protein	53.1 ± 11.34
Vegetal protein	29.5 ± 17.13
Total fats (g/day)	72.14 ± 21.78
Saturated fatty acids	17.0 ± 4.12
Monounsaturated fatty acids	27.2 ± 18.32
Polyunsaturated fatty acids	10.8 ± 3.42
Total carbohydrates (g/day)	431.05 ± 64.28
Sugars (g/day)	286.0 ± 42.28
Polysaccharides	145.0 ± 28.18
Fiber (g/day)	27.31 ± 7.13
Ethanol (g/day)	6.8 ± 2.13
Sodium (mg/day)	3520.3 ± 1523.74

With regard to the genera studied, it should be noted that *Bifidobacterium* spp. showed significantly higher amounts in the subjects with low body weight, and their presence decreases gradually as the BMI increases.

Table 3 Bacterial groups quantified in human fecal samples in subjects with different body mass index (BMI)

Target	BMI <20 (n = 5)	BMI 20–25 (n = 15)	BMI >25 (n = 11)
Total microbiota	13.05 ± 0.13	12.56 ± 1.40	12.71 ± 0.42
Firmicutes	12.24 ± 0.27	11.94 ± 0.51	11.68 ± 0.46
Bacteroidetes	9.71 ± 0.49^{b}	10.06 ± 0.53^{a,b}	10.34 ± 0.72^{a}
Actinobacteria	9.34 ± 0.27^{b}	9.82 ± 0.93^{b}	11.12 ± 0.32^{a}
Proteobacteria	6.46 ± 0.25	6.18 ± 0.46	5.95 ± 0.43
Bifidobacterium spp.	10.35 ± 0.19^{a}	9.33 ± 0.93^{b}	8.67 ± 0.71^{b}
Lactobacillus spp.	8.03 ± 0.02	8.15 ± 1.13	7.96 ± 1.67

Results are expressed as $\log_{10}$ CFU/g ± standard deviation. Values in the same row with different letters are significantly different.

Table 4 Bacterial groups quantified in human fecal samples in subjects with different ages

Target	<20 years (n = 5)	20–30 years (n = 15)	>30 year (n = 11)
Total microbiota	12.83 ± 1.72	12.57 ± 1.46	12.66 ± 0.59
Firmicutes	11.88 ± 0.63	11.97 ± 0.65	11.73 ± 0.65
Bacteroidetes	9.87 ± 0.80	9.89 ± 0.57	10.12 ± 1.03
Actinobacteria	11.70 ± 1.71	11.60 ± 0.76	10.12 ± 0.25
Proteobacteria	5.29 ± 0.29^{b}	6.54 ± 0.67^{a}	6.68 ± 0.52^{a}
Bifidobacterium spp.	9.74 ± 1.11	9.08 ± 1.52	9.37 ± 0.55
Lactobacillus spp.	8.30 ± 0.68^{a,b}	7.82 ± 1.22^{b}	9.44 ± 0.24^{a}

Results are expressed as $\log_{10}$ CFU/g ± standard deviation. Values in the same row with different letters are significantly different

Gut microbiota composition in subjects of different ages

Table 4 presents the amounts of different bacteria identified in study participants by age. There were no significant differences between the different age groups, except in some specific cases. With regard to the phyla studied, only statistically significant differences were found for Bacteroidetes and Proteobacteria in the age group of >40 years compared to <20 years. With regard to genera, *Lactobacillus* spp. was significantly higher in the age group >40 years compared to subjects in the intermediate age group; there were no significant differences when comparisons were made to the younger age groups.

Gut microbiota composition in subjects with different SEAD adherence

As presented in Table 5, there are no major differences in the GM of subjects with different adherences to SEAD. There was a slight reduction in the number of Firmicutes and a slight increase in Proteobacteria, although these changes do not reach levels of statistical significance. The only bacterial group with results that were statistically significant differences was the genus *Bifidobacterium* spp., which was found in higher quantities in subjects with greater adherence to the SEAD.

Discussion

Anthropometric and lifestyle data of subjects investigated showed that their average BMI for the subjects in our study was in the normal weight range, but 11 subjects (35.48%) were categorized as overweight or obese (BMI >25). This result is consistent with recently reported data, in which an obesity rate

Table 5 Bacterial groups quantified in human fecal samples in subjects with low, intermediate, and high adherence to Southern European Atlantic Diet

Target	Low adherence (n = 3)	Intermediate adherence (n = 21)	High adherence (n = 7)
Total microbiota	13.11 ± 0.38	12.44 ± 1.30	12.89 ± 0.10
Firmicutes	12.36 ± 0.38	11.80 ± 0.49	11.85 ± 0.29
Bacteroidetes	10.17 ± 0.68	10.85 ± 0.74	10.20 ± 0.22
Actinobacteria	11.09 ± 0.95	10.93 ± 1.13	11.56 ± 0.62
Proteobacteria	6.18 ± 0.14	6.22 ± 0.56	6.32 ± 0.53
Bifidobacterium spp.	8.92 ± 0.92^{b}	9.29 ± 1.10^{a,b}	9.92 ± 0.43^{a}
Lactobacillus spp.	8.43 ± 1.10	8.17 ± 1.26	8.37 ± 0.44

Results are expressed as $\log_{10}$ CFU/g ± standard deviation. Values in the same row with different letters are significantly different

of about 25% was found in Galicia [2]. The number of reported food allergies and intolerances was also at a high level (25.8%). Of those intolerances, lactose intolerance was the most common (12.9%), which is lower than recent reports of lactose intolerance for all Spain (about 30%) [4]. There was a low rate of smoking in the study population (12.9%) compared to current rates across Spanish (nearly 30%) [14].

The fact that the target population reported going to sleep at a late hour is a serious drawback for the long-term maintenance of correct body weight, taking into account reports that demonstrate a greater predisposition to gain weight in the nocturnal hours described in recent works [10]. With regard to physical exercise, the daily average was lower than what has been recommended by Spanish Society of Community Nutrition (SENC) [30]; guidelines suggest that a minimum of 30 min of daily exercise are necessary for the prevention of weight gain that occurs with the decline in basal metabolism as people age.

As presented in Table 2, the data obtained from our study did not fully correlate with the national consumption averages that are published annually by the Consumer Panel of the Ministry of Agriculture, Fisheries, Food, and Environment [20]. First, drink consumption (considering that the survey includes both alcoholic and non-alcoholic beverages) yielded data showing less than 1 l per person per day, which suggests that people omitted their consumption of water or may have underestimated their intake of some beverages. With regard to the rest of the components, there was enough deviation from the national estimates to suggest the presence of a different food pattern of food consumption. Thus, the most striking differences with respect to Spanish food consumption were higher consumption of fruits (454.01 vs 271.5 g/day respectively), vegetables (329.5 vs 260.9 g/day), legumes (63.61 vs 8.39 g/day), and fish (82.13 vs 70.96 g/day). In contrast, our study found lower consumption of bakery foods (39.39 vs 96.3 g/day) and precooked foods and snacks (20.19 vs 73.32 g/day) compared to national estimates [12, 20].

Study subjects consumed a diet with an average energy content of 2756.22 Kcal; the energy was derived from carbohydrates (63.8%), fats (24%), and proteins (12.2%). This distribution is considered to be low in fats and high in carbohydrates with respect to the objectives stated by SENC for Spanish people. This pattern is related to the influence of SEAD dietary profile. With respect to adherence to the SEAD, there were seven subjects (22.6%) that were considered to have high adherence, 21 subjects (67.4%) with intermediate adherence, and three subjects (9.7%) showed low adherence. The study subjects needed an average diet of 2767 Kcal per day, which was calculated based on their age, anthropometric conditions, and physical activity. In general, the caloric intake of the subjects was adequate and did not justify the high percentage of subjects in this geographic region who were overweight or obese [2, 24].

Tables 3, 4, and 5 show that the total quantity of GM is higher than what has been published routinely by some authors [13]. We took into account that fact that the results were an extrapolation from DNA copies, which does not correspond in a direct and exact way with CFUs. Other authors have recently reported that total GM counts have been found in the distal portion of the colon at around 10^{11} CFU/g [1, 5, 25]. Our results demonstrate that the quantity of total GM counts is higher than what has been reported by other authors. This may be due to a different age distribution of the subjects used in our study, as is known that the GM varies in quantity and composition during the person's life [9]. Because a multitude of dietary factors influence the composition and quantity of the colonic microbiota [25]; the high counts found may also be the result of individual differences of the diets ingested by each subject. In any case, despite the fact that there is currently no consensus on the composition of an "ideal" GM, it is accepted that the larger amounts and variety of GM are indicative of good intestinal health [5].

With regard to the phyla composition identified in our study subjects, it was found that Firmicutes was the most abundant phylum in the GM. The predominance of Firmicutes agrees with findings reported by other authors [5, 18, 25, 27] and is a representative of an individual who

consumes a type of Western diet that is rich in foods with fat that are of anima origin [18, 25, 35]. With respect to the Bacteroidetes phylum, previous published reports have documented counts that are usually lower than Firmicutes; however, adding both groups accounted for almost 90% of the total microbiota [18, 27, 39]. In our work, other phyla, specifically Actinobacteria, showed similar counts to those of Bacteroidetes and were significantly higher than what has been reported by other authors [5, 17]. The presence of a high quantity of Actinobacteria could be related to the high level of obesity in Galician people, as some research has demonstrated that this phylum is particularly abundant in obese people [5]. On the other hand, our finding of a low level of Proteobacteria was a positive finding, as this phylum includes most of the known human digestive pathogenic bacteria [16, 17]. Therefore, we interpret a low presence of this phylum to denote a low risk of gastrointestinal infectious diseases.

Two genera with a relevant function for human health are *Lactobacillus* spp. and *Bifidobacterium* spp. They are both important for physiologic functions, such as the development of the host immune response [9], and several species belonging to these genera are considered probiotics [8]. The high quantities of *Lactobacillus* spp. and *Bifidobacterium* spp. identified in the GM of participants are thought to have a protective effect on their health.

In general, when it was investigated, the relationship between BMI and GM composition, there were no significant differences for most bacterial groups. However, it was found that the quantity of Actinobacteria was higher in overweight subjects compared to the other subjects, and also higher with respect to other data published to date [5, 15]. Additionally, it was also found that the quantity of Bacteroidetes was higher if we stratify subjects in increasing weight. This result is according with those published by other authors, who have found that high quantities of Bacteroidetes are traditionally associated with a high consumption of proteins and animal fats [18]. It is not strange, therefore, that those people with high consumption of animal fats consume a more caloric diet and are therefore more overweight. On the other hand, *Bifidobacterium* spp., a genus traditionally related to the concept of healthy microbiota [3], was present in significantly higher amounts in the subjects with low body weight, and their presence decreased gradually as the subjects' BMI increased.

One of the factors that can affect GM composition is the age of a person [15, 16, 31]. In the present work, Proteobacteria phylum was significantly higher in the subjects who were older than 40 years old compared to the subjects who were less than 20 years old. This result is consistent if we consider that Proteobacteria contain the highest number of antibiotic-resistant genes of all groups of microbiota [23]. Logically, the older an individual, the greater likelihood that he or she has been subjected to antibiotic treatments that influence his or her microbiota and microbiome. It is also important to note that this group includes the family Enterobacteriaceae, which in turn includes most of the pathogens of intestinal origin for humans and is an indirect marker of food intake of animal origin [8, 9, 18, 27].

The presence of *Lactobacillus* spp. in the subject's GM was also remarkable. There was a significantly higher quantity in older subjects compared to subjects in the intermediate age group, and there were no differences between the older group or the intermediate group and the younger age groups. As a probiotic, it is one of the most commonly used bacterial genera [9, 16], and older people consume more functional foods than younger people.

With respect to differences based on adherence to the SEAD, the only bacterial group that reached statistical significance was the genus *Bifidobacterium*, which was present in higher quantities in the subjects with greater adhesion to the SEAD. As previously mentioned, the genus *Bifidobacterium* spp. is related to the concept of healthy microbiota [3]. It is also besides being closely related to dairy consumption, which is one of the ten points in the decalogue of the Atlantic Diet.

In conclusion, in this work we observed a different dietary pattern in Galician compared to other Spanish people. The Galician dietary pattern was characterized by a higher consumption of fruits, vegetables, legumes, and fish and a lower consumption of bakery and precooked foods and snacks. Analyses of the GM demonstrated high total counts and differences between some bacterial groups depending on BMI, age, and adherence to SEAD. The most surprising finding was the high levels Actinobacteria quantities in the GM of the participants. Subjects with a higher BMI showed higher counts of Bacteroidetes and Actinobacteria phyla in their GM, and lower counts of *Bifidobacterium* spp. The GM of older participants contained higher levels of Proteobacteria and *Lactobacillus* spp. compared to the GM of younger subjects. Taking into account the dietary, climatic, and lifestyle peculiarities of the SEAD area, it should be not surprising that the bacterial groups forming the GM were not similar to those obtained by other authors in distant geographic locations. However, adherence to SEAD only was found associated with higher counts of *Bifidobacterium* spp. than the subjects with low adherence to SEAD.

Strength and limitations

The sample size was the main limitation of this study. Nevertheless, the main strength was the fact that this is the first approximation of GM composition of Galician people and, furthermore, in an area with an AD pattern. Other interesting aspect of this study is that we were able to determine the entire gut microbiota of the subjects the genetic expression of the bacteria, which was determined by gut microbiome. For these reasons, additional studies are needed to determinate the

potential specificities of GM in a larger population from this geographic area.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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