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RESEARCH ARTICLE

Diet, anthropometric measurements, sleep quality and fecal levels of Akkermansia muciniphila: a cross-sectional study in older adults

Dieta, medidas antropométricas, calidad del sueño y niveles fecales de Akkermansia muciniphila: un estudio transversal en adultos mayores

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ABSTRACT

Introduction: *Akkermansia muciniphila* is a mucin-degrading bacterium that has been linked to metabolic and gut-barrier health, yet population-specific data are scarce in Latin America. No Peruvian study has examined how diet and other factors relate to its fecal levels. This study aimed to evaluate the association between dietary intake, anthropometric measurements, sleep quality, and fecal levels of *A. muciniphila* in a specific population of older Peruvian adults.

Methods: In this cross-sectional analytical study, 111 older adults (≥ 60 years) residing in Lima, Peru, were recruited. Absolute *A. muciniphila* levels (Log_{10} copies g^{-1} stool) were determined by real-time PCR. Dietary intake was assessed with a semi-quantitative food-frequency questionnaire covering 54 foods, grouped into four consumption categories (daily, weekly, monthly, none). Anthropometric measurements, sleep quality (Pittsburgh Sleep Quality Index, PSQI) and sociodemographic variables were also recorded. Group differences were tested with t-tests or one-way ANOVA followed by Tukey's HSD; effect sizes were expressed as Cohen's d or ω^2.

Results: *A. muciniphila* was quantifiable in 69 of the 111 participants (62%), with fecal abundance ranging across four logarithmic units (Log_{10} 4.3–9.4 copies g^{-1}). In the analysis restricted to participants positive for the bacterium (69/111), monthly consumption of dried split peas (*Pisum sativum*) and canary beans (*Phaseolus vulgaris*) was positively associated with higher *A. muciniphila* levels ($p < 0.05$; $\omega 2 = 0.11$), whereas weekly consumption or the absence of intake of these foods showed no association. Moreover, no significant associations were observed with body mass index (BMI), waist or calf circumference, sleep quality, medication use, or other sociodemographic variables.

Conclusions: In this first Peruvian study linking diet to *A. muciniphila*, monthly consumption of specific legumes was positively associated with its fecal levels. No associations were observed with anthropometric or sociodemographic factors. These findings highlight the potential dietary influence on beneficial gut microbes in older adults and underscore the need for longitudinal and metagenomic studies across broader Peruvian populations.

PALABRAS CLAVE

Dieta

Adultos mayores

Akkermansia

Índice de masa corporal

Calidad del sueño

RESUMEN

Introducción: *Akkermansia muciniphila* es una bacteria degradadora de mucina asociada a la salud metabólica y de la barrera intestinal, pero los datos poblacionales en América Latina son escasos. Ningún estudio peruano ha examinado cómo la dieta y otros factores se relacionan con sus niveles fecales. El objetivo de este estudio fue evaluar la asociación entre la ingesta dietética, las mediciones antropométricas, la calidad del sueño y los niveles fecales de *A. muciniphila* en una población específica de adultos mayores peruanos.

Métodos: En este estudio analítico transversal se reclutaron a 111 adultos mayores (≥ 60 años) residentes en Lima, Perú. Los niveles absolutos de *A. muciniphila* (Log_{10} copias g^{-1} de heces) se determinaron mediante PCR en tiempo real. La ingesta dietética se evaluó con un cuestionario semicuantitativo de frecuencia de alimentos que cubrió 54 ítems, agrupados en cuatro categorías de consumo (diario, semanal, mensual, ninguno). También se registraron mediciones antropométricas, calidad del sueño (Índice de Calidad del Sueño de Pittsburgh, PSQI) y variables sociodemográficas. Las diferencias entre grupos se analizaron con pruebas t o ANOVA de un factor, seguido de la prueba HSD de Tukey; los tamaños de efecto se expresaron como d de Cohen o ω^2.

Resultados: *A. muciniphila* fue cuantificable en 69 de los 111 participantes (62%), con una abundancia fecal que varió en un rango de cuatro unidades logarítmicas (Log_{10} 4,3–9,4 copias g^{-1}). En el análisis restringido a los participantes positivos para la bacteria (69/111), se observó que el consumo mensual de arvejas secas partidas (*Pisum sativum*) y frijol canario (*Phaseolus vulgaris*) se asoció de manera positiva con niveles más altos de *A. muciniphila*, ($p < 0,05$; $\omega^2 = 0,11$), mientras que el consumo semanal o la ausencia de ingesta de estos alimentos no mostró asociación. Además, no se observaron asociaciones significativas con el índice de masa corporal (IMC), circunferencia de cintura o pantorrilla, calidad del sueño, uso de medicamentos u otras variables sociodemográficas.

Conclusiones: En este primer estudio peruano que vincula la dieta con *A. muciniphila*, el consumo mensual de leguminosas específicas se asoció positivamente con sus niveles fecales. No se encontraron asociaciones con factores antropométricos ni sociodemográficos. Estos hallazgos resaltan la influencia potencial de la dieta sobre microbios intestinales beneficiosos en adultos mayores y subrayan la necesidad de estudios longitudinales y metagenómicos en poblaciones peruanas más amplias.

KEY MESSAGES

1. This is the first Peruvian study assessing the relationship between diet, anthropometry, sleep, and fecal *Akkermansia muciniphila* levels in older adults.
2. *A. muciniphila* was quantifiable in 62% of participants, with fecal abundance spanning four logarithmic units.
3. Monthly consumption of dried split peas and canary beans was positively associated with higher *A. muciniphila* levels.
4. No significant associations were observed with anthropometric measures, sleep quality, or sociodemographic factors, underscoring diet as a potential modulator.

CITATION

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INTRODUCTION

Environmental factors, including diet, physical activity, and sleep, play a crucial role in promoting healthy longevity, with their effects increasingly understood at mechanistic levels[1]. A key player in this interplay is the gut microbiota, which continuously influences systemic metabolism while being shaped by intrinsic and environmental factor[2]. Metagenomic analyses have revealed associations between specific microbial tax and diverse health outcomes[3], with some taxa exhibiting dual roles depending on the context. Among these, *Akkermansia muciniphila* has attracted significant attention for its role in modulating the intestinal physicochemical environment and its interactions with other members of the gut microbial community[4].

A. muciniphila is a Gram-negative, mucin-degrading bacterium and the predominant representative of the phylum Verrucomicrobiota in the human gut. It typically accounts for approximately 1–3% of the total gut microbiota in healthy individuals. Based on this relative abundance, one gram of fecal matter may contain between 10^8 and 10^9 *A. muciniphila* cells[5,6]. Dietary sources rich in indigestible carbohydrates, such as fruits, vegetables, cereals, and legumes, promote mucin turnover and thickening, thereby reinforcing gut barrier integrity and preventing the colonization of pathogenic microorganisms[7]. The degradation of mucin by *A. muciniphila* results in the production of short-chain fatty acids, which are essential for colonocyte energy metabolism and play a key role in modulating intestinal immune responses[8]. Given its mechanistic involvement in maintaining gut homeostasis, *A. muciniphila* has emerged as a promising next-generation probiotic with potential therapeutic applications in the prevention and management of various non-communicable diseases[9].

To ensure the efficacy and safety of *A. muciniphila*-based therapies, additional clinical and epidemiological studies are needed to clarify the influence of various factors that affect its abundance across different populations. Diet and its components, medication use, and age-related changes influence *A. muciniphila* levels[10]. Its dynamics across the lifespan are complex, and during aging, *A. muciniphila* has been implicated in maintaining gut barrier integrity and preventing the onset or progression of chronic diseases[11,12]. Age-related physiological changes and the high burden of multimorbidity and polypharmacy may disrupt gut homeostasis and contribute to lower *A. muciniphila* abundance[12,13]. Therefore, older adults represent a particularly relevant population for studying how intrinsic and extrinsic factors shape key taxa within the gut microbiota.

Much of our understanding of how aging and dietary factors affect *A. muciniphila* stems from studies employing 16S rRNA gene sequencing, which have provided valuable insights into the role of the genus Akkermansia and the species *A. muciniphila*[14,15]. To complement these findings, quantitative PCR-based absolute quantification of specific microbial taxa, such as *A. muciniphila*, offers higher resolution for assessing microbial abundance and dietary associations[16]. However, studies using this approach in the context of human nutrition remain limited, particularly in Latin American countries such as Peru.

In this study, we conducted a cross-sectional analysis in a specific population of older adults residing in Lima, Peru, to investigate the associations between dietary intake, anthropometric measurements, and sleep quality with fecal levels of *A. muciniphila*. This line of research has clinical and nutritional relevance in older adults by helping to identify dietary and lifestyle factors associated with fecal *A. muciniphila* levels, a taxon proposed as a biomarker of healthy aging and a potential target for microbiota-based interventions.

METHODS

Participants

In this analytical cross-sectional study, participants were recruited through informational sessions conducted at the Center for Older Adults (CIAM) in the Los Olivos district of Lima, Peru. During the recruitment sessions, the following inclusion criteria were communicated to potential participants: provision of signed informed consent, formal enrollment at the center, age ≥60 years, and a psychological evaluation indicating no evidence of cognitive impairment. Exclusion criteria included: a history of diarrhea within the past 30 days due to intestinal infections or chronic conditions; history of major gastrointestinal surgery within the past three months; dependency on others for personal care; or the presence of a physical disability that would prevent accurate anthropometric assessments. A total of 111 participants who met all inclusion criteria and none of the exclusion criteria were enrolled in the study. The evaluation period took place between August and September 2024. After providing informed consent, participants were given instructions on how to collect, handle, and transport their stool samples. Each participant was then scheduled for a follow-up appointment within 1 to 3 days to complete anthropometric measurements and surveys. This study received approval from the Ethics Committee of Universidad Científica del Sur (Certificate No. 394-CIEI-Científica-2024).

Clinicodemographic Characteristics

In addition to age, sex, years of residence in Lima, and place of birth, information was collected on current illnesses (including hypertension, cancer, type 2 diabetes, renal insufficiency, dyslipidemia, arthritis, or others), history of previous surgeries (e.g., cesarean section, cholecystectomy, gastric or colorectal surgery, and others), family history of chronic disease (hypertension, cancer, type 2 diabetes, or cardiovascular disease), and current drug treatment (including antibiotics, antihypertensives, analgesics, cardiac medications, laxatives, immunosuppressants, hypnotics, and others).

Anthropometric Evaluation

Anthropometric measurements, including weight, height, waist and calf circumference, were obtained from each participant. Height was measured using a portable wooden stadiometer certified by CENAN-Ministry of Health (Diseños Flores SRL, Lima, Peru), weight by a digital scale (Seca robusta 813, Hamburg, Germany), and waist and calf circumference by a measuring tape (Cescorf, Porto Alegre, Brazil). The procedures adhered to the recommendations outlined in the Peruvian Technical Guide for Anthropometric Nutritional Assessment of Older Adults[17]. Body mass index (BMI) was calculated by dividing each participant's weight in kilograms by the square of their height in meters (kg/m^2). For older adults, the following BMI cutoffs were used: underweight (≤ 23.0 kg/m^2), normal (23.0 < BMI < 28.0 kg/m^2), overweight (28.0 ≤ BMI < 32.0 kg/m^2), and obesity (BMI ≥ 32.0 kg/m^2). Waist circumference thresholds were defined as follows: low risk (men: <94 cm; women: <80 cm), high risk (men: ≥94 cm; women: ≥80 cm), and very high risk (men: ≥102 cm; women: ≥88 cm). Calf circumference cutoffs were: normal (men: >34 cm; women: >33 cm), moderately reduced (men: ≤34 cm; women: ≤33 cm), and severely reduced (men: ≤32 cm; women: ≤31 cm)[18]. The data were recorded on an anthropometric data sheet, which also included information such as age, sex, current illnesses, surgical history, family history of disease, medication use and place of origin.

Dietary Assessment

On the same day the anthropometric measurements were taken, a structured questionnaire was administered to assess the frequency of food consumption over the previous month, focusing on major food groups such as legumes, cereals, fruits, vegetables, and meats and their derivatives. Although this instrument has been validated in other populations[19,20], it has not yet been formally validated in older adults in Peru. The food list was adapted based on the most commonly consumed foods in Lima, as reported by the National Center for Food and Nutrition (CENAN) through its educational strategy "La Mejor Receta" ("The Best Recipe")[21]. The questionnaire did not assess portion sizes but instead categorized food consumption frequency as follows: daily (1 to 3 times per day), weekly (1 to 6 times per week), monthly (1 to 3 times per month), or none. Trained researchers administered the questionnaire in a standardized manner to ensure consistency in data collection. On average, the survey required approximately 15 minutes to complete.

Sleep Quality

It was determined using the Pittsburgh Sleep Quality Index (PSQI), an instrument previously validated for an adult population in Peru[22]. Each component has assigned a score ranging from 0 to 3 points and the total scores are categorized as follows: good sleep quality (0–5 points) and poor sleep quality (6–21 points)[23]. Administering the instrument took an average of 5 minutes.

Fecal Samples and DNA Extraction

On the day of the survey and anthropometric evaluation, each participant provided a fecal sample. The sample was required to be from the first bowel movement of the day of their appointment. The samples were then transported to the Molecular Microbiology and Genomics Laboratory at the Universidad Científica del Sur, where they were assigned a code and stored at -80 °C until analysis. Bacterial DNA was extracted using an appropriate kit for fecal samples (QIAamp PowerFecal Pro DNA Kit, QIAGEN, Hilden, Germany). Before extraction, each sample was homogenized, and the amount of fecal matter used was on average 188 mg. The DNA concentration (ng/µL) of each sample was determined, and its quality was assessed based on the A260/A280 and A260/A230 ratios using a microplate spectrophotometer BioTek Epoch (Agilent, California, United States).

Quantification by Real-time PCR

We carried an absolute quantification using the Fluorescent Quantitative Detection System, Model FQD-48A (Hangzhou Bioer Technology Co., Ltd., Hangzhou, China). The final volume per reaction was 20 µL and we considered the following components: 10 µL of Genious 2X SYBR Green Fast qPCR Mix (ABclonal Technology, Woburn, MA, USA), 0.4 µL of 10 µM Forward/Reverse Primer set[24], 0.4 µL of ROX II, 2 µL of gDNA (25 ng/µL) and 6.8 µL molecular grade water. qPCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 58°C for 15 s, and 72°C for 15 s. All experiments were carried out by duplicate and with the respective standard curve using 5 serial 10-fold dilutions (10^1 to 10^6 copies/µL) of known genomic DNA (gDNA) of *A. muciniphila* strain Muc (BAA-835D-5™) obtained from ATCC (Manassas, VA). Melting curve analysis was performed in each run under the following conditions: initial denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute, followed by a gradual temperature increase of 0.2°C/s until reaching 95°C. To confirm the expected amplicon size of 126 base pairs[24,25], the PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with GoodView Nucleic Acid Stain (SBS Genetech, Beijing, China). Samples were considered positive for *A. muciniphila* if they met two criteria: 1) amplification with a cycle threshold (Ct) value lower than that of the lowest point on the standard curve, and 2) a melting temperature (Tm) consistent with the expected amplicon.

Statistical Analysis

Analyses were conducted using JASP version 0.19.3. Descriptive statistics, including percentages, means, and standard deviations (SD), were used to summarize the characteristics of the sample. To reduce skewness and allow for parametric testing, absolute quantification values (copies/µL) were converted to $\log_{10}$-transformed values and expressed as $\log_{10}$ copies per gram of stool. Descriptive

and association analyses were limited to the 69 participants with detectable *A. muciniphila* levels, defined as values above the lowest point of the standard curve. This restriction was applied to ensure analytical validity and to minimize bias arising from non-quantifiable measurements. To compare *A. muciniphila* levels between two groups of dichotomous variables, Student's t-test was used, as the data followed a normal distribution (confirmed by the Kolmogorov–Smirnov test). Differences in *A. muciniphila* levels across categories of food consumption frequency were evaluated using one-way ANOVA. Where significant differences were detected, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to identify specific group contrasts. A significance threshold of 95% ($p < 0.05$) was used in all inferential analyses.

RESULTS

Participants Characteristics

A total of 69 older adults aged 60–95 years (mean ± SD: 72.1 ± 7.6 years) were included in the analysis, and the sample was predominantly female (82.6%) (Table 1). On average, participants had lived in Lima for 52.8 ± 19.4 years, with 37.7% born in the Lima–Callao metropolitan area. Most participants reported at least one chronic condition (81.2%). The most frequently reported diagnoses were hypertension (33.3%), type 2 diabetes mellitus (20.3%), and arthritis (18.8%), whereas dyslipidemia (2.9%), cancer (2.9%), and renal insufficiency (1.5%) were reported less often. A history of prior surgery was common (81.2%); the most frequent procedures were cholecystectomy (24.6%) and cesarean section (13.0%). In addition, 62.3% of participants reported a family history of chronic disease, most commonly cancer (20.3%), hypertension (17.4%), and type 2 diabetes mellitus (15.9%). At the time of assessment, 78.3% were receiving pharmacological treatment, including antihypertensives (34.8%), analgesics (24.6%), antibiotics (11.6%), cardiac medications (11.6%), hypnotics (10.1%), and laxatives (4.3%).

Of the 111 older adults initially evaluated, only 69 were included in the characterization because *A. muciniphila* was detected in their fecal samples. This corresponds to a prevalence of 62.2% within the study population. Among these individuals, the fecal concentrations of *A. muciniphila* ranged from $\log_{10}$ 4.3 to $\log_{10}$ 9.4 copies per gram of stool.

With respect to nutritional status based on anthropometric measures, 30.4% of participants were classified as living with obesity, 29.0% as living with overweight, 34.8% as normal weight, and 5.8% as underweight, according to BMI cut-offs for older Peruvians. Central adiposity was highly prevalent: using the Peruvian waist-circumference thresholds adjusted for sex, 62.3% of individuals fell in the very-high cardiometabolic-risk category, 23.2% in the high-risk category and only 14.5% in the low-risk category. Consistent with this finding, 98.6% displayed a waist-to-height ratio ≥ 0.50, a widely accepted indicator of elevated cardiovascular risk[26]. Calf circumference screening, used here as a proxy for muscle mass, indicated normal values in 78.2% of participants; however, 8.7% showed moderately reduced measurements and 13.0% showed severely reduced measurements, patterns consistent with a heightened risk of sarcopenia. In Table 2, anthropometric measurements, *A. muciniphila* concentrations, and sleep quality variables were analyzed as numerical data.

Table 1. Characteristics of study participants (n=69)

Variables	Mean (SD)	n (%)
Age (years)	72.12 (7.63)	
60-69		27 (39.1)
70-79		31 (44.9)
80-89		9 (13.0)
90-99		2 (2.9)
Years of residence in the city of Lima	52.8 (19.4)	
Sex		
Female		57 (82.6)
Male		12 (17.4)
Lima & Callao birthplace		
Yes		26 (37.7)
No		43 (62.3)
Current illness		
Yes		56 (81.2)
No		13 (18.8)
History of surgery		
Yes		56 (81.2)
No		13 (18.8)
Family history of disease		
Yes		43 (62.3)
No		26 (37.7)
Drug treatment		
Yes		54 (78.3)
No		15 (21.7)
Sleep quality		
Good		21 (30.4)
Poor		48 (69.6)

*Lima and Callao are part of the Lima Metropolitan Area

Table 2. Characteristics of anthropometric, microbiological, and sleep quality variables in older adults (n=69).

Variables	Mean	SD	Min	Max
Akkermansia muciniphila (log_{10} copies/g stool)	7.68	1.19	4.3	9.37
Weight (kg)	66.82	11.90	42.5	103.00
Height (m)	1.50	0.06	1.4	1.66
BMI (kg/m^2)	29.60	4.98	20.1	44.76
Waist circumference (cm)	94.51	10.39	67.7	117.30
Waist to height ratio	0.63	0.07	0.5	0.80
Calf circumference (cm)	35.53	3.21	27.5	43.00
PSQI score	7.51	3.36	1.0	15.00

SD: Standard deviation; Min: Minimum; Max: Maximum; BMI: Body mass index; PSQI: Pittsburgh sleep quality index.

Differences in Fecal *A. muciniphila levels* by Clinical and Demographic Factors

Table 3 presents the differences in fecal *A. muciniphila* levels (log_{10} copies/g stool) across clinical and demographic characteristics in older adults. No statistically significant differences were observed by sex, residence in Lima or Callao, current disease status, family history of disease, drug treatment, or sleep quality ($p > 0.05$ for all comparisons). Although the difference in *A. muciniphila* levels between participants with and without a history of surgery approached statistical significance ($p = 0.05$), it did not meet the predefined threshold, despite a moderate effect size (Cohen's d = –0.61).

Association between Food Consumption and A. muciniphila Levels

A detailed overview of the reported frequency of consumption of the 54 food items is provided in Supplementary Table S1. Overall, staple foods such as rice, bread, potatoes, chicken, and fresh fish were consumed most frequently (daily or weekly), whereas pulses, pseudocereals, and several fruits and vegetables were consumed less regularly, often on a weekly or monthly basis. Based on these patterns, we next explored whether the frequency of intake of specific foods was associated with fecal levels of *A. muciniphila*. Significant differences were identified for two legumes (Table 4). Participants who reported monthly consumption of dried split peas (*Pisum sativum*) had significantly higher *A. muciniphila* levels compared to those who consumed them weekly or not at all ($p < 0.01$; $\omega^2 = 0.11$). Similarly, individuals who consumed canary beans (*Phaseolus vulgaris*) monthly showed higher *A. muciniphila* levels than those with no consumption ($p = 0.01$; $\omega^2 = 0.11$).

DISCUSSION

The main finding of this study is that monthly consumption of canary beans and dried split peas was associated with higher fecal levels of *A. muciniphila*. *Pisum sativum* and *Phaseolus vulgaris* are widely consumed legumes in Latin America (including Peru) and in other regions, and are culturally relevant sources of fermentable substrates such as resistant starch, galacto-oligosaccharides, and phytochemicals[27,28]. These components have been shown to support the growth of *A. muciniphila* in animal models and, to a lesser extent, in human studies[7,29]. Still, the association with a

Table 3. Differences in *A. muciniphila* levels according to clinical and demographic characteristics in older adults.

Variables		N	Mean	SD	p*	Cohen's D
Sex	Female	57	7.69	1.13	0.86	0.06
	Male	12	7.63	1.52		
Lima & Callao Birthplace	Yes	26	7.79	0.88	0.56	-0.15
	No	43	7.61	1.35		
Current disease	Yes	56	7.64	1.23	0.56	0.18
	No	13	7.85	1.03		
Previous surgery	Yes	56	7.81	1.17	0.05	-0.61
	No	13	7.1	1.16		
Family history of disease	Yes	43	7.68	1.25	0.98	0.01
	No	26	7.69	1.13		
Drug treatments	Yes	54	7.59	1.22	0.23	0.35
	No	15	8.01	1.09		
Sleep quality	Good	21	7.56	1.52	0.59	-0.14
	Poor	48	7.73	1.04		

* Student's t

Table 4. Association between the frequency of food consumption and fecal levels of *A. muciniphila* in older adults.

Food consumption frequency	n	Mean	SD	p*	ω²	Group comparison (Tukey's HSD)	Difference of means	p (adjusted)a
Dried split peas								
Monthly	20	8.38	0.95	0.01	0.11	Weekly	1.02	0.01
						No consumption	0.94	0.02
Weekly	25	7.36	1.37			No consumption	-0.08	0.97
No consumption	24	7.44	0.96					
Canary beans								
Monthly	24	8.24	0.87	0.01	0.11	Weekly	0.73	0.05
						No consumption	1.25	0.01
Weekly	34	7.51	1.26			No consumption	0.52	0.38
No consumption	11	6.99	1.19					

*ANOVA p<0.05; aPost hoc test (p Tukey)

sporadic frequency of legume consumption was unexpected, as current scientific literature and dietary guidelines in various parts of the world recommend legumes on a daily or near-daily basis[30]. It is also noteworthy that most of the foods evaluated did not show a significant association with *A. muciniphila* levels in the post hoc analysis. This lack of association with members of the genus Akkermansia is not uncommon in studies exploring the relationship between diet and the gut microbiota[31]. For instance, Salazar et al[16]. reported that levels of the genus Akkermansia were not correlated with macro- or micronutrient intake.

Although the limited number of studies quantifying fecal levels of *A. muciniphila* in relation to dietary patterns assessed through food frequency questionnaires (FFQs) restricts direct comparisons, we hypothesize that several factors may explain the lack of significant associations observed for most food groups in this study. First, the gut microbiota is intrinsically complex and influenced by multiple interacting variables. Second, there was considerable variability in the frequency of food consumption within the study population, which may have limited the ability to detect consistent dietary patterns. Third, other intrinsic or extrinsic factors such as host genetics, medication use, or environmental exposures may have had a stronger influence on the microbiota composition than individual dietary items. These dynamics are difficult to disentangle in a cross-sectional design, which captures only a single time point and does not account for long-term dietary habits or microbiota stability over time.

In this study, we found a prevalence of *A. muciniphila* of 62% in a sample of older adults living in Lima, Peru. Moreover, there was substantial variability in fecal levels of *A. muciniphila* across participants, and these levels were not associated with age. As other many members of gut microbiota, this microbe is highly variable among individuals because of intrinsic and environmental factors[32]. In the context of aging, studies have indicated that *A. muciniphila* levels tend to decline, potentially contributing to age-related metabolic and inflammatory disorders[12]. However, some research suggests increment of this bacterium in older adults, emphasizing a complex relationship between the abundance of genera *Akkermansia* and healthy aging[16,33]. For instance, using a quantitative approach comparable to ours, Salazar et al[16]. reported a statistically significant increase in the abundance of the genus *Akkermansia* in individuals over 80 years of age compared to younger individuals. The reported values ranged from 4 to 9 $\log_{10}$ cells/g of stool in the oldest group; ranges comparable to those observed in our study. The authors suggest a potential link between this bacterium and increased longevity. Notably, the reported protective effects appeared to be more pronounced at higher levels of Akkermansia, and no adverse outcomes were observed in individuals with elevated abundance[15]. Nevertheless, future studies should investigate the optimal abundance of *A. muciniphila* within the gut microbiota, as excessive colonization has been reported to negatively impact intestinal homeostasis[34].

Unlike diet, neither adiposity nor sleep appeared to shape *A. muciniphila* in our cohort. Absolute counts did not differ across BMI categories, waist or calf-circumference strata, even though 59.4% of participants were living with overweight or obesity. In humans, the relationship between the abundance of the genus Akkermansia, particularly *A. muciniphila*, and obesity or overweight remains inconclusive, with studies reporting mixed results[14,15,35,36]. In our study, the observed discrepancies may be partly explained by the distinct physiological and metabolic characteristics of older adults. Aging is associated with changes in body composition, including sarcopenia and fat redistribution, that may not be fully captured by traditional anthropometric measures such as BMI or waist circumference[37]. Moreover, the gut microbiota in older individuals is influenced by a broader range of factors, including polypharmacy, comorbidities, and dietary heterogeneity, that may attenuate the relationship between microbial composition and adiposity seen in younger or middle-aged populations. It is also possible that in later life, the responsiveness of *A. muciniphila* to metabolic status becomes

less pronounced due to age-related immune and mucosal changes that alter its ecological niche[14]. Given the cross-sectional nature of our study, we cannot exclude reverse causality or unmeasured confounding factors, and longitudinal studies in elderly populations are needed to clarify whether *A. muciniphila* retains its role as a metabolic biomarker beyond midlife.

Sleep quality, assessed with the PSQI, was likewise unrelated to bacterial load. Current evidence linking sleep and *A. muciniphila* remains limited and inconsistent. One previous study employing the same assessment tool reported an association between poor sleep quality and a reduced proportion of the phylum Verrucomicrobia in the gut microbiota of older adults[23]. Meanwhile, other cross-sectional analyses have implicated different genera rather than *A. muciniphila* itself[38]. Our null finding is consistent with the current ambiguity in the literature, and we hypothesize that any association between sleep and *A. muciniphila* may be subtle or obscured by comorbidities and polypharmacy commonly present in elderly populations.

This study provides one of the first insights into the fecal abundance of *A. muciniphila* in older adults in Lima-Peru, using absolute quantification by qPCR, a method that offers greater resolution and accuracy compared to relative abundance metrics from 16S rRNA sequencing. The use of standardized anthropometric measurements, validated sleep quality assessments, and a detailed FFQ allowed for a multidimensional analysis of host and lifestyle factors potentially associated with *A. muciniphila*. Additionally, participant recruitment was efficient and engagement was high, likely due to the setting within an active aging center, where health-related programs are routinely integrated into daily life.

Nonetheless, several limitations should be acknowledged. First, the cross-sectional design limits causal inference and may be subject to unmeasured confounding. Second, the sample was drawn from a single institution located in Los Olivos, a middle-income, urban district of Lima, where participants are regularly exposed to health education and structured physical activity programs. This recruitment context may have introduced selection bias toward more health-conscious and functionally active older adults. Thus, the generalizability of our findings to the broader elderly population in Peru may be limited. Third, FFQ used to assess dietary intake was validated in Peruvian adults but not specifically in the elderly population. This may introduce potential measurement bias in estimating food consumption patterns. Fourth, the observed associations with specific foods should be interpreted with caution and treated as exploratory, as the FFQ did not quantify portion sizes or capture preparation methods (e.g., boiling, frying, soaking), both of which can influence the availability of microbial-accessible substrates such as resistant starch or polyphenols[39,40]. Residual confounding from overall dietary patterns not fully characterized may also have influenced the findings[41]. For instance, we did not assess the use of probiotics, prebiotics, fiber supplements, or the habitual consumption of fermented foods, all of which may meaningfully modulate the abundance of *A. muciniphila*. Fifth, while we quantified *A. muciniphila* at the species level, emerging evidence indicates that different strains exhibit distinct functional properties and host interactions, which could not be addressed in this study[42]. Future research using shotgun metagenomics or strain-resolved approaches will be essential to explore these functional differences in aging populations. In addition, longitudinal designs and broader geographic sampling are needed to enhance generalizability and to investigate temporal relationships between *A. muciniphila* levels and modifiable lifestyle factors[16].

CONCLUSION

We observed substantial variability in fecal *A. muciniphila* levels among older adults residing in Lima, Peru. No significant associations were found between *A. muciniphila* abundance and anthropometric measurements, sleep quality, or clinical variables. However, monthly consumption of specific legumes, namely dried split peas and canary beans, was positively associated with higher bacterial levels. Given that food quantity, preparation methods, and traditional dietary patterns can influence the availability of microbial-accessible substrates, and that different *A. muciniphila* strains may exert distinct functional effects, future research should investigate gut microbiota composition and its determinants across diverse Peruvian populations. Longitudinal and strain-resolved studies are warranted to clarify the ecological and functional roles of *A. muciniphila* in aging populations.

AUTHOR CONTRIBUTIONS

The authors' contributions were as follows: FT, EPG, VM, JR: conceptualization and funding acquisition; FT, EPG, FR: data collection; FT, EPG, VM, JR: data analysis; FT, EPG, VM, FR, JR: writing, review and editing. All authors read and approved the final version of the paper submitted to the journal.

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