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Are nutrition and physical activity associated with gut microbiota? A pilot study on a sample of healthy young adults

F. Valeriani[1], F. Gallè[2], M.S. Cattaruzza[3], M. Antinozzi[3], G. Gianfranceschi[1], N. Postiglione[2], V. Romano Spica[1], G. Liguori[2]

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Parole chiave: Microbiota Intestinale, Attività Fisica, Nutrizione

Abstract

Background. *The literature shows that gut microbiota composition is related with health, and a lot of individual and outer factors may determine its variability. In particular, nutrition and exercise seem to influence the presence in the gut of the two major bacterial phyla of Firmicutes and Bacteroidetes.*
Study design. *An ongoing cross-sectional investigation is aimed to explore these associations in humans.*
Methods. *Healthy Caucasian young adults were asked to provide a fecal sample in order to analyze their gut microbiome considering their Body Mass Index (BMI), adherence to Mediterranean diet and Physical Activity (PA) levels.*
Results. *A total of 59 participants (49.1% males, mean age 23.1 ± 3.14 years) were enrolled so far. Firmicutes (61.6±14.6) and Bacteroidetes (30.7 ± 13.3) showed the highest relative abundance in fecal samples. The Pearson's analysis showed a significant negative correlation between PA and Firmicutes ($r = -0.270$, $p = 0.03$). Linear regression confirmed a significant decrease of this phylum with the increase of PA ($R^2 = 0.07$, $p = 0.03$).*
Conclusions. *These preliminary results suggest the association between physical activity and gut microbiota composition in healthy humans.*

Introduction

Scientific evidence is increasing about the role that intestinal microbiota plays in contributing to health and disease (1-3).

Microbiota is responsible for mucosal homeostasis, biosynthesis and absorption of nutrients, maintenance of epithelial integrity, interaction with the immune system, competitive inhibition of possible invasion and colonization by pathogenic microorganisms (4, 5).

In the healthy adult population, two major bacterial phyla are predominant: the Gram-positive *Firmicutes* and the Gram-negative *Bacteroidetes*, nevertheless intestinal microbiota is different, in variety and quantity, from one person to another and it is influenced by constitutional and outer factors (6-9).

Relative proportions of *Bacteroidetes* and *Firmicutes* seem to influence health and are influenced by host characteristics. Indeed, these proportions have been

[1] *Public Health Unit, University of Rome "Foro Italico", Rome, Italy*
[2] *Department of Movement Sciences and Wellbeing, University "Parthenope", Naples, Italy*
[3] *Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy*

observed to change with age, gender and nutritional status (10-12). In obese people, in comparison with lean people, the relative proportion of *Bacteroidetes* is decreased and it was observed that, with weight loss, irrespective of diet type, it increases, while that of *Firmicutes* decreases (13, 14).

Exercise can determine changes in qualitative and quantitative microbial composition; however, the greatest part of the available evidence on this issue derives from studies on mice (7, 15, 16). Furthermore, some studies reported a greater biodiversity in the microbiota of athletes, while other investigations found an increase of selected bacteria of the phylum *Firmicutes* and a decrease of *Bacteroidetes* with overtraining (7, 15, 17, 18).

Thus, intestinal microbiota, nutrition and physical activity (PA) seem to interact with a complex relationship. Improving knowledge about their interactions can offer new opportunities to maintain health, reduce risk of diseases and develop therapeutic approaches.

The aim of this cross-sectional study report was to illustrate the preliminary results of an ongoing investigation exploring the qualitative and quantitative composition of bacterial gut microbiota in a sample of young Caucasian adults and its association with nutrition and PA levels.

Materials and methods

During the academic years 2017/18, students attending the degree courses on Movement Sciences and of Nursing at the Universities of Naples "Parthenope" and of Rome "La Sapienza" were invited to participate on a fixed day. According to the protocol exclusion criteria, students presenting chronic diseases, pregnancy, food intolerance, concomitant gastrointestinal infection, antibiotics assumption or gastrointestinal surgical procedure in the 3 months prior to the enrollment were asked not to participate.

A questionnaire and a written informed consent in accordance with standards of the responsible committee on human experimentation and the Helsinki Declaration were administered to the students who agreed to participate. The questionnaire included questions regarding age, gender, weight and height, PA and diet. Body Mass Index was obtained dividing weight in kilograms by the square of height in meters to obtain a measure of nutritional status. Habitual PA was assessed through the short format of the International Physical Activity Questionnaire (IPAQ), which allows to quantify the total weekly energy expenditure expressed as Metabolic Equivalents (METs) per week by considering minutes spent on vigorous/moderate-intensity PA and walking (19). Dietary habits were assessed through the questionnaire developed by Martínez-González et al. (20), which allows to attribute a value of 1 to each reported dietary habit corresponding to the Mediterranean diet pattern, resulting in a maximum total score of 9. A fecal swab (Copan Italia S.P.A., Brescia, Italy) was given to each participant together with the instructions for the collection of their stool sample at home; participants were asked for delivering the swab on a planned day. Upon collection, samples were stored at 4-8°C and subsequently carried to the laboratory of the University of Rome "Foro Italico", where they were processed with a previously validated protocol for DNA extraction from fecal traces (21). Starting from the purified DNA, the libraries for NGS were prepared according to the 16S Metagenomic Sequencing Library Preparation guide (part# 15044223 rev A; Illumina, San Diego, CA). The PCR amplicons were obtained using primers (containing overhang adapters) previously employed (22). Libraries were quantified through PicoGreen dsDNA Quantitation Assay (ThermoFisher Scientific, Waltham,

MA) and validated on Bioanalyzer DNA 1000 chip (Agilent, Santa Clara, CA) for the presence of the expected amplicons. Sequencing was performed on a 16S rRNA by the MiSeq Desktop Sequencer (Illumina, San Diego, CA) and generated sequences were processed by the quantitative insights into QIIME2 package (https://qiime2.org). Sequences were clustered into Operational Taxonomic Units (OTUs) based on their sequence at 97% similarity (similar to species level). A representative sequence for each OTU was chosen for downstream analysis based on the most abundant sequence from each OTU.

Statistical analysis

A descriptive analysis was carried out on demographic information. Age, BMI and MD adherence score were expressed as mean±SD, median values and ranges; numbers and percentages of participants for each gender and BMI category on the basis of World Health Organization standards (http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi) were reported. The IPAQ total score was expressed in METs/week.

The comparisons between gender, age and BMI of participants and not participants were performed through chi-squared test and Student's *t* test. The relative abundance of bacterial community was determined on 29 phyla [% abundance = (number of sequences for phylum/total sequences for sample) x 100]. The subsequent mathematical analyses excluded the 24 rarest phyla, that is, those with a relative abundance below 0.1%. A Pearson's correlation analysis was carried out comparing BMI and PA levels with the presence of the bacterial phyla which showed the highest abundance. A linear regression model was performed for significant correlations, adjusting for age, gender and MD score. A value of $p < 0.05$ was assumed as significance level. Data were analyzed with IBM SPSS version 25 for Windows (SPSS, Chicago, IL, USA).

Results

On a total of 160 students invited during lessons time, 123 potentially eligible agreed to participate by signing the informed consent and completing the questionnaire, but only 59 of them gave back the fecal swab on the planned date.

Table 1 reports the main characteristics of the sample. The students who did not give back the fecal swab were statistically different from those who did neither in demographic variables, nor in BMI, MD adherence and habitual PA.

Out of 29 bacterial phyla detected in the fecal samples, the highest relative abundance was registered for *Firmicutes* (61.6 ± 14.6) and *Bacteroidetes* (30.7 ± 13.3) (Figure 1).

The results of the correlation analysis carried out considering these two mainly represented phyla show a positive, but not significant, correlation with BMI, especially for *Firmicutes* (Table 2). MD adherence seems to be positively related to *Firmicutes* and negatively associated with the *Bacteroidetes* amount; however, these correlations were not significant. Instead, a significant negative relation between *Firmicutes* content and habitual PA expressed in METs/week was found.

Figures 2 shows the linear regression plots for *Firmicutes* amount in relation to PA levels (METs/week) respectively. It is possible to observe the decrease of gut *Firmicutes* with the increase of habitual PA levels.

Discussion

This study is part of a multicenter investigation involving healthy young adults aimed to assess the role of lifestyle on the

Table 1 - Characteristics of not-participant and participant individuals enrolled in the study

	Not participants n=64	Participants n=59	*p*
Gender n (%)			
male	37 (57.8)	29 (49.1)	0.43[a]
female	27 (42.2)	30 (50.9)	
Age			
mean value ± sd (median; range)	23.1±2.83 (23, 19-34)	23.1±3.14 (22, 20-36)	0.91[b]
BMI			
mean value ± sd (median; range)	22.9±3.1 (22.8, 16.9-32.2)	22.2±2.6 (22, 16.6-29.7)	0.19[b]
BMI category n (%)			
underweight	5 (7.8)	1 (1.7)	0.13[a]
normal weight	43 (67.2)	49 (83.0)	
overweight	13 (20.3)	9 (15.2)	
obese	2 (3.1)	0 (0)	
MD score			
mean value ± sd (median; range)	5.4±1.4 (6; 1-9)	5.5±1.6 (6; 2-9)	0.71[a]
Habitual PA (METs/week)			
mean value ± sd (median; range)	3,847±3,218.5 (3,020; 150-12,802.1)	3,926.9±3,101.1 (3,420; 165-12,708)	0.89[a]

[a] chi-squared test
[b] Student's *t* test

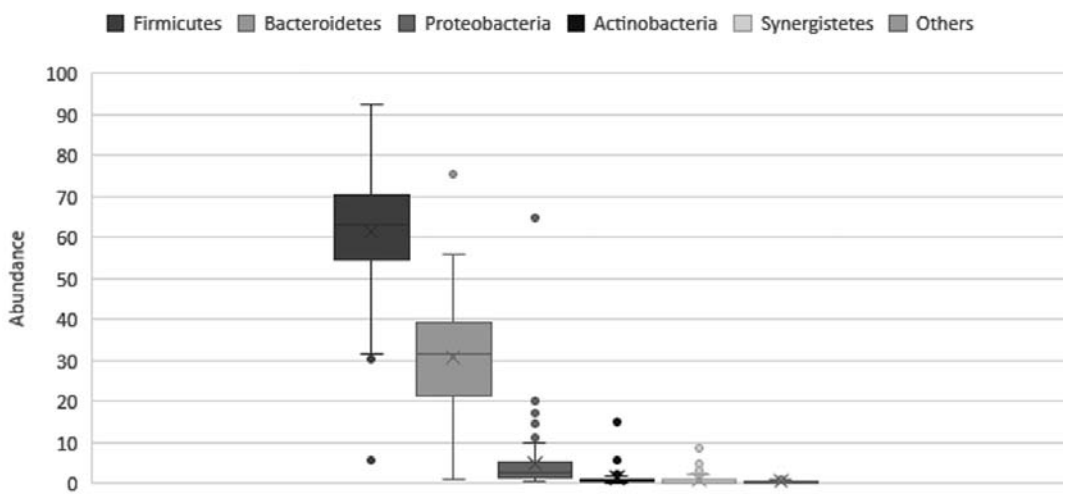

Figure 1 - Box plot showing the relative abundance for dominant phyla determined by Global Alignment Sequence Taxonomy assignments. Boxes are colored by their respective phylum (see legend). The boundary of the box closest to zero indicates the 25th percentile, the line within the box represents the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Phyla less than 1% of sequences were count as others.

Table 2 - Results from Pearson's correlation between *Firmicutes* and *Bacteroidetes* abundance and ratio, BMI, MD adherence and PA levels expressed as METs/week

	BMI	MD score	METs/week
Firmicutes			
r value	0.22	0.10	-0.27
p	0.08	0.43	0.03
Bacteroidetes			
r value	0.06	-0.09	-0.20
p	0.63	0.50	0.12
Firmicutes/Bacteroidetes ratio			
r value	0.11	0.07	0.01
p	0.38	0.62	0.94

human gut microbiota composition. Even if preliminary, the reported findings suggest some considerations for future research.

First of all, we found that *Firmicutes* and *Bacteroidetes* represent the two major bacterial phyla in the gut microbiota of the young adults included in the sample, confirming the findings of previous studies (6, 10, 13).

With regard to the two variables examined, our results confirm a positive relationship between BMI and *Firmicutes* proportion, even if this correlation was not statistically significant (11-13).

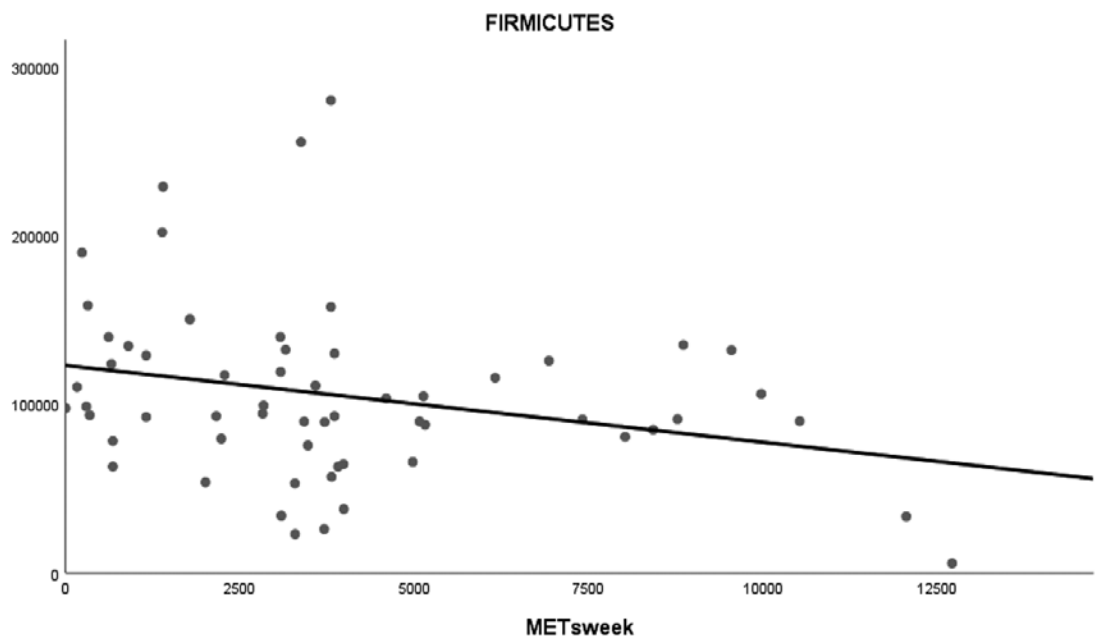

Figure 2 - Scatter plot of the linear regression for gut *Firmicutes* content in relation to habitual PA expressed as METs/week ($R^2 = 0.07$, $p = 0.03$)

Moreover, an inverse relationship was found between PA and gut *Firmicutes* content. These first results allow to hypothesize the role of low PA in favouring *Firmicutes* rather than other gut bacterial phyla, which may represent another way by which inactive lifestyle acts on human health. However, it should be noted that this finding is in contrast with the study by Lambert et al., which showed an increase of selected *Firmicutes* species with exercise, even if their results refer to mice and were obtained from cecal matter instead of fecal swabs (17). Among the few studies carried out in healthy humans so far, that of Clarke et al. showed a higher biodiversity in professional athletes, while Mahnic et al. did not find differences related to PA in the intestinal bacterial community of healthy humans but only in the fungal one (11, 15).

Further investigations are needed to clarify the effects of PA on the human gut microbiota composition beyond of what murine models show.

It is also important to consider the source of the samples, with respect to mucosal sample versus fecal material and to the different compartment of the gut from which the samples come from (23).

One of the main limitations of this study is the low proportion of students who gave back the fecal swab, which reduced the initial sample size. However, the students who did not deliver the swab did not statistically differ from those who did. We think that the lack of delivery of the samples on the planned day was probably due to forgetfulness or unavailability of the stool sample. The small number of participants did not allow to evaluate the possible differences in gut microbiota composition among subgroups defined by BMI category. In particular, the number of obese subjects was too small. Furthermore, the small sample size hindered the comparison among PA levels and the analysis of potential confounders, as well as that of microbiome composition at genus or species level. Therefore, it's not possible to generalize the findings of this pilot study. However, these preliminary results represent a first contribution to the characterization of gut microbiota composition in Italian young adults. The ongoing enrollment of further participants will allow to explore in-depth these preliminary findings.

Riassunto

Esiste un'associazione tra dieta, attività fisica e microbiota intestinale? Uno studio pilota su di un campione di giovani adulti sani

Premessa. La letteratura scientifica mostra come la composizione del microbiota intestinale sia correlata con la salute e come molti fattori individuali o estrinseci possano determinarne la variabilità. In particolare, lo stato nutrizionale e l'esercizio fisico sembrano influire sulla presenza dei due maggiori phyla batterici *Firmicutes* e *Bacteroidetes* nell'intestino.

Disegno dello studio. Questa indagine trasversale è finalizzata ad esplorare tali associazioni nell'uomo.

Metodi. Giovani adulti sani di etnia Caucasica sono stati invitati a fornire un campione fecale per analizzarne la composizione microbica in riferimento a Indice di Massa Corporea (IMC), aderenza alla dieta Mediterranea e livelli di Attività Fisica (AF).

Risultati. Un totale di 59 partecipanti (49.1% males, mean age 23.1 ± 3.14 years) è stato finora arruolato. *Firmicutes* (61.6 ± 14.6) e *Bacteroidetes* (30.7 ± 13.3) hanno mostrato la maggiore abbondanza relativa nei campioni fecali. Il test di Pearson ha rivelato una correlazione negativa tra firmicuti e AF (r = -0.270, p = 0.03). L'analisi di regressione lineare ha confermato la diminuzione significativa di questo phylum all'aumentare dei livelli di AF (R^2 = 0.07, p = 0.03).

Conclusioni. Questi risultati preliminari suggeriscono l'associazione tra attività fisica e composizione del microbiota intestinale negli adulti sani.

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Corresponding Author: Francesca Gallè, Department of Movement Sciences and Wellbeing, University "Parthenope", Via Medina 40, 80133 Naples, Italy
e-mail francesca.galle@uniparthenope.it