Eat2beNICE

Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours

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1. Deliverable report

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Introduction

Lifestyle interventions are of paramount importance for older adults[1] for healthy aging. In a previous work, in the frame of PREvención con DIeta MEDiterránea (PREDIMED)-Plus, a trial designed to evaluate the long-term effectiveness of an intensive weight loss lifestyle intervention on primary cardiovascular prevention[2] , demonstrated that the intervention resulted in effective increase in daily Physical Activity (PA) in older adults[3]. However, despite the plethora of benefits associated with PA, a great increase in sedentary behaviors and physical inactivity have irrupted in the last decades, particularly in older adults, which is associated with weight gain, lower cognitive performance and an increased incidence of obesity and obesity-related comorbidities.

Recent findings pointed out that PA can determine changes in the gut microbiota composition, playing a positive role in energy regulation[4]. PA can enrich microbial biodiversity and enhance the number of beneficial microbial species[4], like the phylum *Verrucomicrobia*, which includes bacteria related to a better body composition and improved metabolic health[6].

We hypothesized that the increase of PA as part of a lifestyle intervention could affect differently to gut microbiota. Thus, the objective of the current study is to identify how an increase in the total PA, within a context of a Mediterranean lifestyle, affect metabolic parameters and gut microbiota in older individuals with overweight/obesity and metabolic syndrome.

Methods

Participants

This study was performed in the context of the PREDIMED-Plus trial, an ongoing multicenter, parallel-group, randomized trial conducted in Spain, to assess the effect of a weight-loss intervention program based on an energy-restricted traditional Mediterranean diet, PA promotion and behavioral support, in comparison with a usual care intervention only with the energy-unrestricted Mediterranean diet (see http://predimedplus.com for details).

In the present sub-study with the data corresponding to baseline and the first year of the PREDIMED-Plus study, participants were included. These participants were chosen from two PREDIMED-Plus study centers, Malaga and Reus (Spain) and randomized based on age, sex, BMI and with stool samples available at baseline and after 12-month of intervention as well as available PA information.

Type of activity, frequency and duration (minutes/day) of PA were self-reported using the validated REGICOR Short Physical Activity Questionnaire 14. PA was defined as the sum of total minutes and hours daily of 6 types of activities performed during a month (brisk walking, walking at a slow/normal place, walking in the countryside, climbing stairs, working in the garden, exercise or play sports at home, outdoors or in a gym). Then, total PA was determined as the sum of the total activities according to the Compendium of Physical Activities 15.

The observational study was performed stratifying the participants by tertiles of change in PA-related energy expenditure after 12-month intervention, with the following tertile groups T1 (n=99), T2 (n=99), and T3 (n=99). The changes in the variable total PA-related energy expenditure were expressed as percentages and were calculated between basal and the 12-months differences, and divided by the basal.

Measures

At baseline and 12-month follow-up visits, waist circumference (midway between the lowest rib and the iliac crest using an anthropometric tape), weight (using high-quality electronic calibrated scales), and height (using a wall-mounted stadiometer) were measured. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times using a validated semiautomatic oscillometer (Omron HEM-705CP, Kyoto, Japan) and the mean value was recorded.

Fecal samples were collected at baseline and 12-month timepoint in a sterile hermetic flask and bring it to the study center (within 12 hours of excretion) in a cooled condition (with a provided ice pack or stored at -20°C). Stool samples were then aliquoted and 164 stored at −80 ◦C, for subsequent analysis. DNA extraction from stools was performed using the QIAamp DNA stool Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The Ion 16S Metagenomics Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to amplify the ribosomal 16S rRNA gene region from stool DNA, with two primer sets (V2–4–8 and V3–6, 7–9) covering the most of the hypervariable regions of the 16S rRNA region in bacteria.

Torrent Suite™ Server software (Thermo Fisher Scientific), version 4.0, with default parameters for the 16S Target Sequencing (bead loading ≤30, key signal ≤30, and usable sequences ≤30) was used to base calling and run demultiplexing. Alpha diversity indexes were calculated and a pairwise comparison using Wilcoxon rank sum test was performed to evaluate differences in microbial diversity between groups. Weighted and Unweighted Unifrac distance matrices were calculated and permutational multivariate analysis of variance (PERMANOVA) was used looking to look for differences in group

compositions. Taxonomic analysis assessment was performed through clustering with VSEARCH and the reference base Greengenes version 13_8 at 97% of identity.

Analisys

Quantitative variables were expressed as mean ± standard deviation (SD) for normally distributed data and as median ± interquartile range (IQR) for non-normally distributed data and percentages for categorical variables. The bivariate analysis was performed using paired Student's tests for continuous data or Wilcoxon test for non-normally distributed data. Differences across groups were evaluated through one-way analysis of variance (ANOVA) for continuous data or Kruskal–Wallis's test for non-normally distributed data. Categorical data were analyzed using Pearson's chi-square test.

Results

The anthropometric and clinical characteristics, laboratory parameters, and blood pressure at

baseline and 12 months of intervention are shown in Table 1. After 12 months of intervention, a significant decrease in weight and waist circumference was observed in all three groups. After 12-months of intervention the percentage of changes of total PA were significantly greater in the T3 group compared to the other T2 and T1 groups ($p<0.001$) , due to the highest change of total PA of the T3 234 group (273.64±221.42) versus T1 and T2 group (-44.83±24.94; 28.96±23.33, 235 respectively) (Table 2).

Changes at 12 months in anthropometric and laboratory and blood pressure are shown in Table 2. Concerning the anthropometric variables, participants from the three groups decreased an average of -2,85±4,24 kg of body weight (-2,04±3,75; -2,57±4,48; -3,95±4,51, for T1, T2, and T3, respectively), with a greater weight loss in T3 participants ($p=0.005$). BMI, waist circumference, hip circumference changes decreased in the three groups, but significant differences in changes were observed between T2 and T3 compared to T1 in hip circumference ($p=0.002$). T3 decreased significantly their waist circumference and BMI with respect to T1 participants ($p<0.001$ and $p=0.020$, respectively).

Gut microbiota populations changed within each group according to weighted UniFrac distances: T1 group (p=0.042; Fig. 1A), T2 group (p=0.009; Fig. 2A), and T3 group (p=0.046; Fig. 3A), although this trend was not observed in the unweighted version.

- The T1 group was characterized by a decrease in the level of Verrucomicrobia (p<0.001; q=0.004), Verrucomicrobiaceae (p<0.001; q=0.018), Akkermansia (p<0.001; q=0.019), as well as its species A. muciniphila (p=0.003; q=0.059). While, Christensenellaceae (p=0.003; q=0.044) and Bifidobacteriaceae (=0.020, q=0.173) were significantly expanded at 12-month time point.
- The T2 group increased its levels of Lentisphaerae (p< 0.001; q=0.001) and decreased in Verrucomicrobia 270 (p=0.0017; q=0.005) and Proteobacteria (p=0.035; q=0.071).
- The T3 participants increased their levels of Lachnospira (p=0.042; q=0.203), Desulfovibrio (p=0.008; q=0.129) and Akkermansia (p=0.025; q=0.174) at 12-month timepoint. On the other hand, T3 group decreased their levels of Anaerostipes (p< 0.001; q=0.003), Blautia (p=0.022; q=0.174), and Collinsella (p=0.030; q=0.174) through the time.

Correlation studies demonstrated that the changes in Verrucomicrobia, its family Verrucomicrobiaceae and its genus Akkermansia, were negatively associated with changes in waist circumference.

Discussion

We have observed multiple benefits associated with an increase in PA in an older adult population. Moreover, the increasing PA was associated with specific changes in gut microbiota profile and its potential functionality.

Any increase in PA is well received by metabolic health, and recent research has demonstrated that exercise-related physiological remodeling might be extended to gut microbiota[6]. Through weighted UniFrac distances, our study has shown that the gut microbiota population changed differently with PA over a period of 12 months. Additionally, others have reported that exercise might increase α-diversity[7], although others did not observe this alteration[8]. Our results were in line with works in which α-diversity remained unchanged but the overall community composition (β-diversity) changed after PA intervention[9]. Despite these controversial results, PA has been proposed as a therapeutic approach for obesity and/or hypertension plus behavioral readouts through the modulation of gut microbiota.

Our study has multiple strengths like the fact of being built under the PREDIMED-Plus with complete phenotyping of the volunteers, it also has some limitations that should be pointed out. First, its multifaceted intervention strategy involving a limitation on the inference of results that cannot be associated entirely to a single component of the intervention and may be influenced by others, such as diet in this case, or the fact that activity was calculated from a self-reported questionnaire, which although validated, might over- or under-estimate the values. However, these results point out an implication of the gut microbiome in the improvements associated with an increase of PA that deserves to be further investigated with cohorts specially chosen for this hypothesis.

Taken together our results have demonstrated that PA such as lifestyle and Mediterranean diet induces a shift of the gut microbiota. Moreover, this study identifies that the modulation suffered by the gut microbiome populations (and their metabolic capacities) could have a role in healthy aging including improved mental helath. Although further research is needed for a complete understanding of the PA metabolic outcomes through gut microbiota, this study adds evidence to continue investigating this line.

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2. Tables and other supporting documents where applicable and necessary

Table 1. Clinical and laboratory characteristics at baseline and 12-months of intervention according to study total physical activity tertile groups.

	T1 (n=99)		T2 (n=99)		T3 (n=99)		T1 vs. T2 vs. T3 *p*	
Variables	Baseline	12-months of intervention	Baseline	12-months of intervention	Baseline	12-months of intervention	Baseline	12-months of intervention
Age (years)	65.00 ± 5.02		64.45 ± 4.64		64.42 ± 5.50		0.740	
Sex (male/female)	45/54		51/48		56/43		0.293	
Weight (Kg), mean±SD	85.77±12.21	84.03±12.41*	88.55±12.06	86.20±11.87*	89.69±12.67	86.14±12.87*	0.072	0.435
Waist circumference (cm), mean±SD	107.70±9.72	106.25±10.48*	109.86±9.34	107.30±9.78*	110.12±9.12	106.11±10.16*	0.140	0.688
Hip circumference (cm), median (IQR)	109.0 (103.9-115.3)	108.0 (102.5-114.3) *	109.0 (103.5-114.5)	108.9 (103.0-114.5)	110.5 (104.0-116.0)	107.0 (102.0-113.0) *	0.711	0.333
Waist-Hip ratio, mean±SD	0.98±0.07	0.98±0.07	1.00±0.07	0.98±0.07*	0.99±0.07	0.98±0.07*	0.159	0.628
BMI (Kg/m^2), median (IQR)	32.3 (29.6-35.1)	31.5 (29.5-34.7) *	32.8 (30.2-35.4)	32.1 (29.4-34.5)	32.7 (31.1-35.9)	31.7 (29.5-35.0) *	0.205	0.883
Glucose (mg/dL), median (IQR)	103.0 (93.0-116.0)	101.0 (94.0-114.0)	101.0 (91.0-118.0)	101.0 (91.0-119.0)	104.0 (95.0-117.0)	101.0 (91.0-113.0) *	0.561	0.841
Triglycerides (mg/dL), median (IQR)	162.0 (117.0-232.0)	153.0 (109.0-203.0)	151.0 (103.0-196.0)	135.0 (103.7-177.5)	152.0 (118.0-210.0)	137.0 (109.0-184.0) *	0.345	0.323
Total cholesterol (mg/dL), median (IQR)	197.0 (174.0-225.0)	195.0 (174.0-223.0)	199.0 (172.0-223.0)	202.0 (167.7-228.0)	197.0 (178.0-223.0)	192.0 (175.0-219.0)	0.760	0.853
HDL (mg/dL), median (IQR)	47.0 (40.0-52.0)	49.0 (41.0-58.0) *	48.0 (42.0-57.0)	49.0 (42.0-59.0)	46.0 (41.0-55.0)	51.0 (44.0-58.0) *	0.372	0.601
LDL (mg/dL), median (IQR)	111.0 (97.0-130.0)	113.0 (91.0-135.0)	114.0 (91.7-140.2)	118.5 (96.5-145.2)	117.0 (102.0-138.0)	113.0 (99.0-131.0)	0.333	0.488
HbA1c (%)	6.07±1.10	5.95±0.89	6.10±0.85	6.07±1.11	6.02±0.68	5.91±0.64 *	0.206	0.298
SBP (mm Hg), median (IQR)	139.0 (130.0-149.7)	136.3 (124.3-146.3) *	138.3 (128.0-146.3)	136.7 (125.7-146.7)	138.3 (128.3-149.3)	133.7 (125.3-146.0)	0.526	0.961
DBP (mm Hg), mean±SD	79.00±9.94	76.72±9.18*	80.11±8.60	78.13±8.14*	80.17±10.78	78.34±10.72*	0.642	0.440
17-point Mediterranean adherence score, median (IQR)	8.0 (6.0-9.0)	12.0 (10.0-14.0) *	8.0 (6.0-10.0)	13.0 (10.0-15.0) *	8.0 (6.0-10.0)	14.0 (11.0-16.0) *	0.785	0.002
Total physical activity (METs-min/week), median (IQR)	3338.0 (1766.9-5048.9)	1524.4 (671.3-2564.1) *	2125.8 (1165.5-3356.6)	2937.0 (1342.6-4510.5) *	1021.9 (524.4-1874.1)	3416.3 (1958.0-5272.7) *	<0.001	<0.001

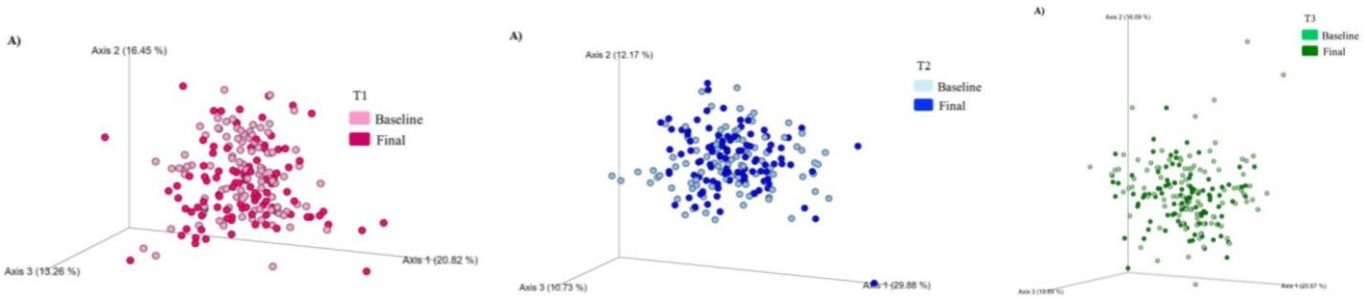
Participants were divided by tertiles of 12-month change in total PA-related energy expenditure, between-98.77 and -10.24 for T1, between -10.25 and 69.10 for T2, and between 69.11 and 1099.99 for T3. * $p < 0.05$ baseline *vs.* 12-month of intervention value, according to paired Student's tests or Wilcoxon tests. BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; METs, metabolic equivalent of tasks; SBP, systolic blood pressure.

Table 2. Changes of the clinical and laboratory variables according to tertiles of change in total physical activity after the 12-month intervention.

	T1 (99)	T2 (99)	T3 (99)	*p*
Weight change (%)	-2.04 ± 3.75	-2.57 ± 4.48	-3.95 ± 4.51*†	0.005
Waist circumference change (%)	-1.34 ± 4.19	-2.27 ± 4.84	-3.60 ± 5.30*	<0.001
Hip circumference change (%)	-0.72 ± 4.01	-0.20 ± 4.53	-2.40 ± 4.19*†	0.002
Waist-hip ratio change	-0.53 ± 4.39	-1.99 ± 4.49	-1.17 ± 4.82	0.055
BMI change (%)	-1.99 ± 3.48	-2.50 ± 4.64	-3.68 ± 4.42*	0.020
Glucose change (%)	-0.94 ± 13.35	0.83 ± 16.62	-2.06 ± 17.95	0.192
Triglycerides change (%)	2.34 ± 44.74	-2.20 ± 32.98	0.76 ± 68.39	0.441
Total cholesterol change (%)	1.02 ± 17.92	0.66 ± 15.83	-0.98 ± 14.62	0.354
HDL cholesterol change (%)	5.68 ± 12.00	2.48 ± 18.97	8.69 ± 17.71	0.093
LDL cholesterol change (%)	3.23 ± 30.38	4.05 ± 21.45	-1.40 ± 22.86	0.051
HbA1c change (%)	-2.23 ± 13.93	-1.40 ± 14.27	-1.38 ± 6.73	0.496
SBP change (%)	-2.98 ± 9.94	-1.27 ± 11.18	-1.90 ± 10.85	0.523
DBP change (%)	-2.29 ± 10.24	-1.79 ± 11.13	-1.82 ± 9.90	0.930
Difference in 17-point Mediterranean adherence score (points)	3.93 ± 3.03	4.14 ± 3.63	5.27 ± 3.48*	0.005
Total physical activity change (%)	-44.83 ± 24.94	28.96 ± 23.33*	273.64 ± 221.42*†	<0.001

Participants were divided by tertiles of 12-month change in total PA-related energy expenditure, between-98.77 and -10.24 for T1, between -10.25 and 69.10 for T2, and between 69.11 and 1099.99 for T3. One-way ANOVA or Kruskal–Wallis's tests. Bonferroni adjusted post-hoc analysis results are shown as *p <0.05 *vs.* T1; †p <0.05 *vs.* T2. Values showed as mean±SD. BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; METs, metabolic equivalent of tasks; SBP, systolic blood pressure.

Figure 1-3 A. Microbiota group differences at three time points



3. Acknowledgement and Disclaimer

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