LETTER TO THE EDITOR

Probiotics may modulate the impact of aging on adults

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To the Editor,

The ability to modulate the intestinal microbiota, exploiting the immunomodulatory properties of the "beneficial" bacteria, has led to the hypothesis of the use of probiotics in some pathological conditions that develop in the elderly, such as irritable bowel syndrome (modulating intestinal sensitivity), chronic inflammatory bowel diseases (through an anti-inflammatory action), but also in some secondary conditions that develop following inflammation such as cognitive deficits (1). Some probiotic bacteria of the genus *Lactobacillus* (*plantarum, rhamnosus GG* and *acidophilus*) are capable of exerting an anti-inflammatory action even *in vitro* and compare their action with that of a pathogenic strain (*Salmonella typhimurium*). In the elderly, the intestinal flora changes in relation to numerous phenomena including reduced gastric secretion, motility disorders and alterations in the diet, consequently these changes cause alteration of the intestinal barrier and therefore encourage the onset of inflammatory phenomena (2). Studies in the literature would seem to show that, in the elderly intestine, the production of pro-inflammatory cytokines, such as IL-6, corresponds to changes in the composition of the intestinal microbiota, which would encourage the onset of numerous inflammations and/or infections. By studying the effects of a mix of probiotic strains, frequently used in Great Britain, VSL # 3, a mixture of eight different bacterial strains, *B. longum DSM 24736*, *B. short DSM 24732, B. infantis DSM 24737, L. acidophilus DSM 24735, L. plantarum DSM 24730, L. paracasei DSM 24733, L. debrueckii subsp. Bulgaricus DSM 24734*, showed the anti-inflammatory effects on the intestines of elderly mice (Balb/c) with induced colitis that were given 4 mg of probiotic compound. Histological and metabolomic analyses showed that the treated mice started a healing process of colitis, with reduction of all inflammatory markers (5). This pilot study sheds light on the ability of specific probiotics stains (PRO-Bifido, Hyperbiotics, USA) on biochemical markers that affect adulthood.

MATERIALS AND METHODS

Participants

The present project was conducted at the American Stem Cell Hospital (AMEJSC), Health Centre (Vietnam), in collaboration with the University of Bari Aldo Moro, Italy and the School of Technical Medical Sciences, "A. Xhuvani" University, Elbasan, Albania. The Institutional Ethics Committee of the Faculty of Technical Medical

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Sciences of Elbasan "Aleksandër Xhuvani" approved the application to conduct the clinical trial in the Faculty. Title of the Protocol: Probiotics efficacy and safety in humans. Protocol Identification: INTL_ALITCOOP/Probiotics/INRES2019_w/a/c. The study was carried out according to the Helsinki Declaration and informed written consent was obtained from all the subjects.

Twenty male and female adults (aged 50 years and over) were enrolled in the present study. The participants had not taken antibiotics for ≥3 months prior to study participation. Nor had they taken medications or supplements (e.g. fibre supplements) that could influence the dependent variables of this study. All participants followed a standardized diet. Participants were allowed to consume 450 g/day (~16 fl. oz./day) caffeinated black coffee or unsweetened black tea if used to consuming them regularly. Furthermore, on the first visit, the participants were instructed on the objectives and methods of the clinical trials. Subjects who agreed to participate in the study, after having signed the informed consent, were randomly included and divided into two groups: a test group using probiotics, and a group without probiotics.

Experimental design

A randomized double-blind, placebo-controlled, parallel group design was used for this study. After completing baseline measures, participants were stratified by sex and randomized into one of two groups: Test Group, supplementation with PRO-Bifido, Hyperbiotics, USA (1 tablet daily), containing *Bifidobacterium infantis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis, Bifidobacterium longum, Lactobacillus plantarum* and *Lactobacillus acidophilus* or Placebo Group with a placebo for 7 weeks. The 20 enrolled subjects were divided as follows: A) Placebo Group: 4 women and 6 men; B) Test group: 6 women and 4 men.

Blood collection, glycaemic control, immunological and inflammatory markers

All measurements were registered between the hours of 7:00 and 11:00 a.m. Blood was collected at baseline (T0) and after 7 weeks (T1), for monitoring the glycaemic status and specific serum immunological (immunoglobulin A - IgA) as well as inflammatory markers (IL-6 and TNF-alpha). In addition, clinical signs were recorded.

Table I. *Glycemic state and concentration of serum IgA, IL-6 and TNF-alpha in Placebo group at T0 and T1.*

BLOOD TESTS (T0) PLACEBO GROUP						**BLOOD TESTS (T1) PLACEBO GROUP**			
ID	***M / F***	***GLYCEMIA (mg/dl)***	***IL-6 (pg/ml)***	***TNF- α (pg/ml)***	***IgA (mg/dl)***	***GLYCEMIA (mg/dl)***	***IL-6 (pg/ml)***	***TNF- α (pg/ml)***	***IgA (mg/dl)***
DC	M	96	7.5	8.1	200	97	7.6	8	200
PS	F	90	6.7	7.7	205	90	6.7	7.8	204
CS	F	83.8	7	8	199	83.8	7.1	8	200
TG	F	95	6	7.1	158	94	6	7	158
MTP	M	92	5.9	6.4	169	92	5.9	6.4	170
LP	M	103.8	7.1	7.9	210	103	7	8	210
RD	F	80.9	6.8	6.9	207	81	6.5	6.8	206
MI	M	99.7	6.8	6	299	99.7	6.9	6	301
FA	M	102	7	7.8	256	102.9	6.7	7.7	255
SB	M	95.5	7.3	8.3	211	95	7.5	8.4	211

Statistical analyses

Outcome measures of the exploratory study were analysed with a *t*-test for paired samples for pre–post differences with time as the factor using Statistical Package for Social Sciences (SPSS for Windows, Version 11.5, Chicago, Ill) software, to detect significant differences between pre-test and post-test scores.

RESULTS

The data regarding the glycaemic status and concentration of serum IgA, IL-6 and TNF-alpha in the Placebo Test group at T0 and T1 obtained in the study, including the average values are shown in Tables I-III.

As seen in Fig. 1, the blood sugar levels changed in control *vs* placebo subjects. On the one hand from T0 to T1 there were no variations and glycemic levels were standard in group A. On the other hand, the same figure shows how blood sugar levels changed in the Test group B, that is after taking the probiotic. It is noted that the introduction of the probiotic in the normal diet improved the parameters by 2%.

Fig. 2 shows IL-6 and TNF-α levels in the Placebo *vs* Test group at T0 and T1. For IL-6 and TNF-α in the Placebo Group there was a similar trend variation of 0.2%, even if at T0 the values were almost high due to the age of the subjects. In the Test Group, who introduced the probiotic into their diet, IL-6 decreased by 10%, allowing us to underline how the use of probiotics can help reduce inflammatory processes. Moreover, in the TNF-α levels of the Test group, there was a 7% reduction from T0 to T1, in favor of the fact that taking probiotics in the diet for 7 weeks encourages an improvement in inflammation, thanks to an improvement in the immune system.

Finally, Fig. 3 shows the changes in IgA levels in

Table II. *Glycaemic state and concentration of serum IgA, IL-6 and TNF-alpha in Test group at T0 and T1*

BLOOD TESTS (T0) TEST GROUP						**BLOOD TESTS (T1) TEST GROUP**			
ID	***M / F***	***GLYCEMIA (mg/dl)***	***IL-6 (pg/ml)***	***TNF- α (pg/ml)***	***IgA (mg/dl)***	***GLYCEMIA (mg/dl)***	***IL-6 (pg/ml)***	***TNF-α (pg/ml)***	***IgA (mg/dl)***
ADS	M	97	5.9	8	278	95	5	7.5	280
SP	F	81	7	8.2	215	79.5	6	7.8	218
LR	F	99.5	6.8	7	199	98	5.8	6.2	201
MP	F	80	7.3	8	230	79	6.5	7.5	232
GS	M	92	7.1	7.6	256	90.1	6.5	7	255
RP	M	100	7	7.9	197	97.3	5.7	7.1	199
DA	F	84	6.5	6.7	188	83	5.5	6	190
FC	F	79.7	6.4	6.5	206	78.5	5.9	6	208
YM	M	102	7.2	7.6	233	101.2	6.4	7	235
RM	F	83.3	6.8	6	238	81	5.8	5.8	240

Table III. *Average values of the markers in Placebo and Test groups.*

Marker	**Placebo**	**Test**
Glycemia (Average)	**±0.03**	**±1.60**
IL-6 (Average)	**±0.02**	**±0.90**
TNF- α (Average)	**±0.01**	**±0.60**
IgA (Average)	**±0.1**	**±1.80**

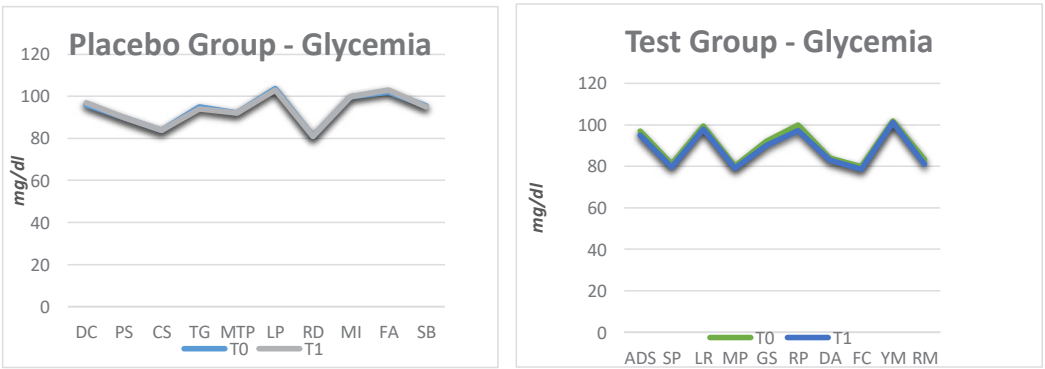

Fig. 1. *Glycemic values in Placebo vs Test group*

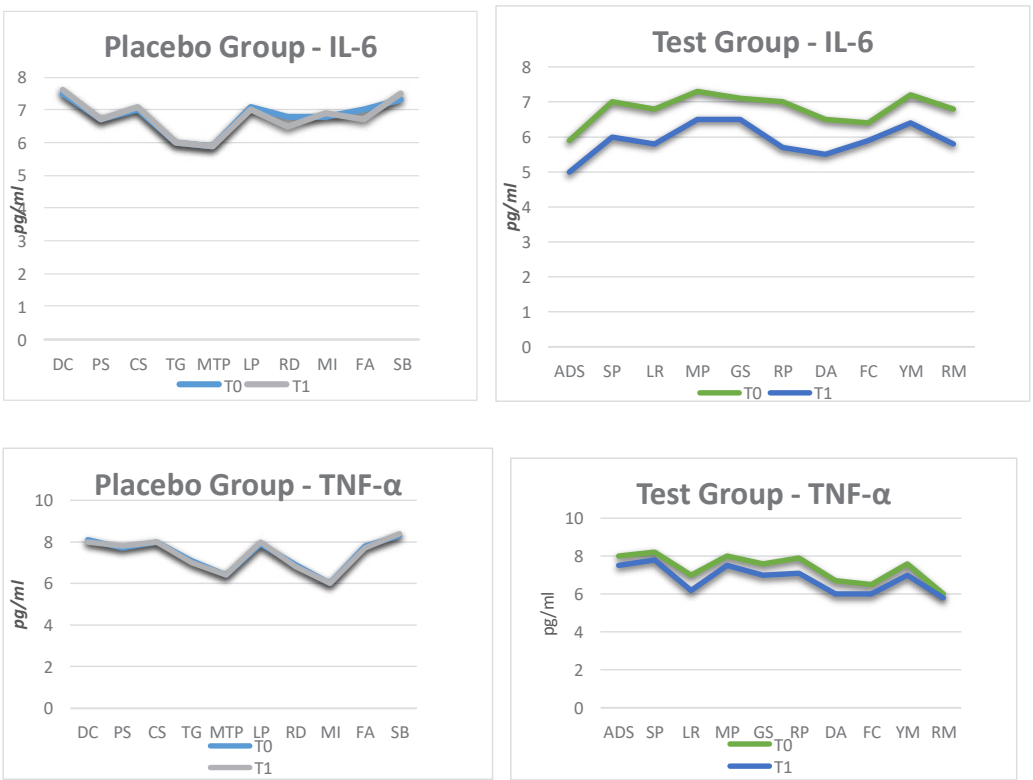

Fig. 2. *Inflammatory markers (IL-6 and TNF-α) in Placebo vs Test group*

the Placebo group *vs* Test Group. It is noted that at T0 the IgA levels are more or less low, demonstrating the fact that due to age, the subjects show a weakened immune system. The changes to T1 are irrelevant. In the Test Group, there was a 2% increase in Ig-A levels, given in favor of the fact that probiotics stimulate the production of immunoglobulins, improving the functionality of the immune system and reducing the inflammatory process.

DISCUSSION

Maintaining health in old age depends on the correct function of the homeostatic systems i.e. nervous,

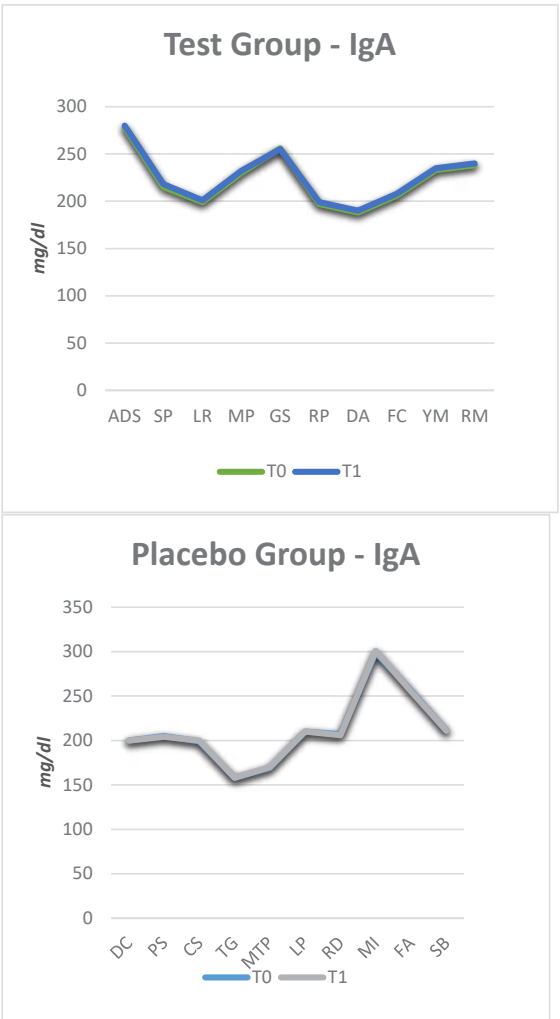

Fig. 3. *Immunomodulatory markers (IgA) in Placebo vs Test group*

endocrine and immune systems, with sequentially correct interactions between these systems and the intestinal microbiota (6). However, in the elderly, there is a reduction in the body's functional capacity, which, in turn, can evolve towards "inflammation". Probiotics can have a particular application in elderly populations, especially in terms of protection against infections and perhaps also in the prevention of various age-related diseases (7). Current evidence shows a correlation between the composition of the gut microbiota and cognitive performance, frailty and comorbidity in the elderly. Studies conducted over the years on animals have shown a benefit of guided therapy with probiotic supplement on the microbiota in reducing chronic low-grade inflammation in the elderly; however, this remains controversial in clinical trials. A correct diet with the addition of probiotics reduces reactive protein C and tumour necrosis factor, modulating the release of cytokines (8, 9). However, some studies say otherwise, therefore suggesting that the use of probiotics in the elderly does not affect the inflammatory state and therefore does not improve life expectancy (10-12). In our study, we demonstrated a close correlation with the trend of inflammatory markers and the use of PRO-Bifido Hyperbiotics, USA. The role of probiotics in maintaining well-being and health is confirmed, as mentioned, on the control of low-grade systemic inflammation in the elderly, a common risk factor for the development of chronic, cardiometabolic diseases (type 2 diabetes, obesity, metabolic syndrome, cardiovascular disease) and liver disease, developing during aging. The use of PRO-Bifido Hyperbiotics, USA, may also have preventive effects on the onset of age-related obesity. In fact, blood sugar values were decreased by 2%. In our study, the additional use of PRO-Bifido Hyperbiotics, USA, improved the serum concentration of IgA, thereby promoting better immune defense in the elderly. However, further clarification is needed to better understand the correlation between the gut microbiota, the aging process and the degenerative diseases typical of the elderly.

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