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Original article

Yogurt in the diet of the elderly: a preliminary investigation into its effect on the gut ecosystem and lipid metabolism

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Abstract – The effect of yogurt as a dietary supplement was investigated with regard to the gut ecosystem and lipid metabolism of 12 healthy, elderly people (78.3 ± 9.8 years, body mass index 23.6 ± 5.3 kg·m^{-2}, mean ± SD). Commercial yogurt with homogenized fruit was prepared by fermenting milk with yogurt specific cultures *Lactobacillus delbrueckii* ssp. *bulgaricus* (strain AY/CSL) and *Streptococcus thermophilus* (strain 9Y/CSL). The subjects consumed their usual diet (equal to 6279–6698 kJ·d^{-1}) over a 2-week baseline period (baseline start to end) and then were supplemented for 4 weeks with 250 g·d^{-1} of fruit yogurt. The yogurt was administered in 125 g portions twice per day: at breakfast in substitution of milk and in the afternoon in substitution of tea with milk (test). At the end of the 4-week period the volunteers returned to their usual diet for a further 4 weeks (follow-up). At the end of each trial period no changes were observed in faecal water content, pH, bile acid concentration or cytolytic activity of the faecal water. Throughout the study there was significant variation neither in dietary intake of macro- and micronutrients, nor in the plasma lipids and, during the experimental period, in the counts of the total anaerobic microorganisms, bifidobacteria, lactobacilli, coliforms or enterococci. The only significant difference was observed in the clostridia counts, that decreased ($P < 0.05$) after the consumption of yogurt. Moreover, this effect was still evident at the end of the follow-up period. Since this last result can be considered a positive modification of the colon ecosystem, as clostridia are involved in the production of putrefactive compounds, it is possible that a yogurt-supplemented diet can maintain and/or improve the intestinal microbiota of elderly subjects.

Yogurt / faecal microflora / bile acid / cytolytic activity / plasma lipid

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Résumé – **Le yoghourt dans le régime alimentaire des personnes âgées : enquête préliminaire de son effet sur l'écosystème intestinal et le métabolisme lipidique.** Le but de l'expérimentation a été de vérifier l'acceptabilité de la part de 12 sujets sains âgés (âge : 78,3 ± 9,8 ans ; indice de masse corporelle : 23,6 ± 5,3 $kg \cdot m^{-2}$; moyenne ± écart-type) de l'apport de yoghourt à leur régime alimentaire habituel (6279–6698 $kJ \cdot j^{-1}$) et de mesurer l'effet de cette administration sur l'écosystème intestinal et le métabolisme lipidique. Du yoghourt commercial contenant des fruits homogénéisés a été préparé en utilisant les ferments lactiques spécifiques du yoghourt, *Lactobacillus delbrueckii* ssp. *bulgaricus* (souche AY/CSL) et *Streptococcus thermophilus* (souche 9Y/CSL). Durant une période de deux semaines précédant l'administration de yoghourt (baseline start to end), les individus étaient alimentés selon leur régime alimentaire habituel. Durant la période de traitement de quatre semaines (test) 250 g par jour de yoghourt aux fruits ont été ajoutés au régime alimentaire, répartis en deux portions de 125 g en substitution du lait au petit-déjeuner et du thé au lait dans l'après-midi. À l'issue du traitement, les sujets reprenaient leur régime alimentaire pendant quatre semaines (follow-up). Durant la période de l'étude, l'alimentation avec apport de yoghourt n'a pas modifié les habitudes alimentaires des sujets considérés ; le contenu de l'eau fécale, le taux d'humidité, le pH, la concentration d'acides biliaires et l'activité cytolytique de l'eau fécale, ainsi que les lipides plasmatiques et le nombre de microorganismes de la flore totale anaérobie, des bifidobactéries, des lactobacilles, des coliformes et des entérocoques n'ont pas varié de façon statistiquement significative. Au contraire, on a constaté une diminution significative des clostridies ($P < 0,05$) pendant toute la période de follow-up, résultats pouvant avoir un effet bénéfique sur la santé, puisque les clostridies génèrent des produits de putréfaction potentiellement toxiques pour la muqueuse colique. La consommation de yoghourt pourrait donc améliorer l'équilibre écologique intestinal des sujets âgés.

Yoghourt / microflore fécale / acide biliaire / activité cytolytique / lipide plasmatique

1. INTRODUCTION

The human intestinal tract is a complex ecosystem harboring a microbial community of more than 400 bacterial species, and the biochemical activity of such species influences, to a very marked extent, the health of the host [10, 37]. Nowadays the possibility of modulating the composition of intestinal microbiota is of great interest as such "manipulation" could allow the stimulation of the growth and/or metabolic activity of some microbial groups considered "beneficial to health", mostly lactic acid bacteria and bifidobacteria, and could hinder the growth of harmful species such as clostridia [13].

A major factor in influencing the composition and/or metabolic activity of intestinal microbiota is the subject's diet, and the most common dietary treatment aimed at ameliorating it is the consumption of probiotics. According to Fuller [12], a probiotic is "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Probiotics for human consumption are mostly available as fermented dairy products containing viable bacteria, such as yogurt obtained by milk fermentation through the action of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus.* Yogurt is consumed on a large scale and its nutritional value is well known. Previous studies [5–7, 35] have already demonstrated that yogurt administration positively influences the equilibrium of the intestinal microbiota and the various metabolic activities related to the physiopathology of the host. Various effects have been observed in both man and animals following the ingestion of yogurt bacteria: increased numbers of indigenous lactic acid bacteria and bifidobacteria, alleviation of lactose maldigestion and stimulation of both immunity and antitumoral activity [2, 8, 22, 31].

Little is known about the "throughout a lifetime" evolution of human intestinal

microflora, despite the fact that some authors have observed a decrease in bifidobacteria and an increase in lactobacilli, enterococci, enterobacteria and clostridia in the elderly [15, 20, 29].

Owing to the increasing number of elderly people in the western world it is important to prevent and treat disease and maximize the quality of life. Therefore, knowledge about possible alterations in the intestinal microbiota composition and biochemical activity linked to aging are of great interest. Moreover, there are no available data relevant to the intestinal microflora of the elderly concerning the effects of the consumption of fermented milk products containing viable microorganisms which, through intestinal microbial balance improvement, are claimed to be beneficial to the host [12]. Furthermore, in some western countries, such as Italy, the inclusion of yogurt into dietary habits has occurred only recently; consequently, very few elderly people have been habitual consumers of yogurt during their lifetime.

The aim of this research was to study the mid-term influence of daily consumption of fruit yogurt on the intestinal ecosystem of the elderly, evaluating the effect on microflora composition and some of the microflora biochemical activities, as already investigated in children and adults [2, 8, 17]. The investigation concerned the effect of yogurt in modulating colonic microflora and in influencing some metabolic pathways with colonic location (such as the conversion of primary to secondary bile acids, faecal bile acids and cytotoxicity of faecal water), parameters known to affect the health of colonic mucosa [25, 32, 34]. Finally, given the contrasting results reported for the hypocholesterolemic activity of yogurt [3, 21, 29, 36], we investigated the effects of this fermented milk product on plasma lipids.

2. MATERIALS AND METHODS

2.1. Volunteers and experimental design

Twelve healthy elderly subjects (11 females and 1 male, 78.3 ± 9.8 years old, mean ± SD), residents of the Pio Albergo Trivulzio Institute (an old people's residency) in Milan, Italy, participated in the study after informed consent. Given the difficulty in finding really healthy elderly people compliant with such a long experiment with fixed deadlines, it was impossible to recruit a higher number of volunteers and equal numbers of male and female subjects. At the beginning of the study, the health of volunteers was assessed by the physician (a geriatric specialist) of the residency.

Exclusion criteria included: pathologies or surgery of the gastrointestinal tract, and regular or current use of antibiotics or medications able to influence lipid metabolism. Table I shows the anthropometric parameters of the subjects. The body mass index was calculated as the weight (kg) divided by the height squared (m^2) and provided an estimate of obesity.

The experimental protocol was approved by the Ethical Committee of the Pio Albergo Trivulzio Institute, Milan, Italy.

Table I. Anthropometric parameters of involved subjects.

N = 12 (11 females, 1 male)	Means ± S.D.	Range
Age (years)	78.3 ± 9.8	65–93
Weight (kg)	62 ± 15.2	42–77
Height (m)	1.62 ± 0.05	1.55–1.70
Body Mass Index ($kg \cdot m^{-2}$)	23.6 ± 5.3	17–35

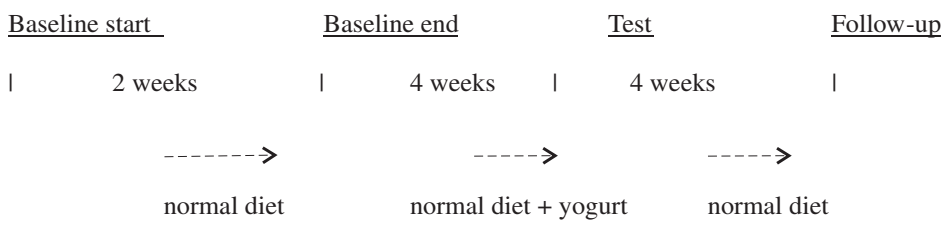

Figure 1. Experimental design.

The volunteers were studied for a total of ten weeks (Fig. 1). During the first two weeks (from baseline start to baseline end) they consumed their habitual diet. During the following four weeks (test period) they were asked to consume a realistic intake (250 $g \cdot d^{-1}$), of fruit yogurt in two portions of 125 g, one at breakfast in substitution of milk and the other in the afternoon instead of the usual tea served with milk. There were no other dietary restrictions. During the test period yogurt consumption was checked by trained personnel to assess compliance. In the last four-week period (follow-up period) the subjects returned to their usual diet. The dietary intake of each subject was controlled, both during the test and follow-up periods, and estimated on the basis of a 7-day food diary [9] filled in by a trained dietitian.

At the beginning of the trial, and at the end of each period, faecal microbiological analyses were performed. At the end of the baseline, test and follow-up periods, pH, bile acid concentration and cytolytic activity were determined on faecal water. Meanwhile, blood samples (20 mL) were taken by venipuncture for lipid analysis.

2.2. Yogurt

A standard commercial yogurt containing homogenized fruit (banana, strawberry, apricot), prepared by fermenting milk with the traditional yogurt cultures *L. delbrueckii* ssp. *bulgaricus* strain AY/CSL (BCCM-LMG P-17224) and *S. thermophilus* strain 9Y/CSL (BCCM-LMG P-17225) (with a ratio cocci: rods around 1:1 and a total count of 6×10^8 $CFU \cdot g^{-1}$), was obtained by YOMO S.p.A. (Milan, Italy). Yogurt prepared with these strains has been proven to produce a probiotic effect (e.g. with the equilibrium of the gut ecosystem) in both healthy adults and children, and in dyspeptic babies [6]. The yogurt, kept at 4 °C, was administered within 10–15 days from production, as preliminary tests had shown no significant change in microbial counts during this storage. Table II shows the proximate composition of the yogurt and its microbiological characteristics obtained according to IDF standard methods [21].

2.3. Faecal specimen collection

During the experimental periods, the normal use of lactulose (Laevolac, Boehringer, Milan, Italy), vegetable extracts (Pursennid, Novartis, Origgio, VA, Italy) and enemas with a phosphate base (Clisma Fleet, Bergamon, Rome, Italy) was substituted, when necessary, with a mix of two oils (olive 50% and vaseline 50%) prepared by the Pharmacy of the Pio Albergo Trivulzio Institute. When it was necessary to stimulate evacuation on collection days, the subject in need was also given a 200 mL enema of physiological solution containing 500 mg of pantotenic acid (Bepanten, Roche, Milan, Italy) or 0.5 mg of neostigmine (Prostigmina, Roche, Milan, Italy).

To avoid prolonged contact with urine, all the faeces of each evacuation were

Table II. Chemical and microbiological composition of yogurt during shelf-life (15 days).

Constituents	% by weight
Lipids	4.1 ± 1
Proteins	2.8 ±1
Total carbohydrates	13 ±1
Lactose	3.5
Viable count	CFU·g^{-1}
S. thermophilus	3×10^8
L. delbrueckii subsp. *bulgaricus*	2×10^8
Salmonella	Absent
Listeria monocytogenes	Absent
Yeasts	< 10
Moulds	< 10
Enterococci	< 10
Coliforms	< 10
Staphylococci	< 10

CFU = colony forming unit.

gathered in either a liquid-absorbing padded panty, or a sieve-like container placed over the toilet bowl.

Immediately after collection, the faecal samples for microbiological analyses were put into an anaerobic bag (Generbag Anaer., Biomerieux, Marcy l'Étoile, France), kept at +4 °C, and delivered to the laboratory within 6 h from defecation.

The faecal samples for chemical analyses were stored at –80 °C.

2.4. Microbiological analyses

The microbiological analyses were performed under strict anaerobic conditions in an anaerobic cabinet (Forma Scientific, Marietta, OH, USA) under N_2:H_2:CO_2 atmosphere (85/10/5, v/v). A sub-sample of about 4 g was suspended in a pre-reduced dilution blank [19] (final volume of 40 mL) and homogenized. Subsequently, serial tenfold dilutions up to 10^{-10} were performed in the same dilution blank. To evaluate any possible shift in the intestinal microbiota we carried out a total anaerobic microorganism count, as a marker of microflora balance. Lactobacilli and bifidobacteria counts were also performed in order to assess whether there was any stimulation in their growth after the administration of yogurt. Furthermore, clostridia, enterococci and coliforms were enumerated as, being proteolytic activity microorganisms, they could be inhibited by the acidifying bacteria. Duplicate plates were inoculated with 0.1 mL samples and incubated aerobically or anaerobically, as appropriate, at 37 °C. The microbial analyses were performed using selective growth media: total anaerobes on blood agar [19], coliforms on Difco MacConkey agar, clostridia on sulphite-polymixin-milk agar [28] after ethanol treatment of the dilutions, enterococci on Difco KF *Streptococcus* agar, lactobacilli on Lamvab medium [18] and bifidobacteria on Beerens medium [4]. Ten colonies were picked at random from countable plates containing about 100 colonies. Single colonies were isolated in peptone yeast glucose (PYG) [19]. The microorganisms were classified only to the genus level on the basis of Gram reaction, spore formation, cell morphology and metabolites from glucose assessed by gas-chromatographic procedures [19, 30]. The dilution blank and all the media used for anaerobic microorganism cultivation were reduced in an anaerobic cabinet for 48 h before use. The microbial counts were reported in relation to the dry faecal weight, determined by placing 3–4 g aliquots of the faecal sample in weighing bottles and drying them in an oven at 100 °C until they reached a constant weight.

2.5. Faecal water preparation

Due to the impossibility of obtaining faecal water by direct centrifugation of

fresh faeces, faecal water was obtained, according to Govers et al. [16], on freeze-dried faeces, as proposed by the same authors. Freeze-dried faeces were reconstituted with 154 mmol·L^{-1} NaCl solution to the original amount of water and incubated for 2 h at 37 °C in a shaking water bath. The samples were then centrifuged for 2 h at 37 °C at 12 000 × *g* (Sorvall RC 5 Plus, GMI, Clearwater, MN, USA). The faecal water was carefully aspirated and assayed for pH, (PHM 93 Reference pH-meter, Radiometer, Copenhagen, Denmark), cytolytic activity and bile acid content.

2.5.1. Faecal bile acid analyses

Bile acids in faecal water were assessed according to the method by Korpela et al. [23] slightly modified as follows: 500 µL faecal water, with cholanic acid (5-β-cholanic acid) (Sigma C7628 Ursocholanic Acid, Milan, Italy) added as internal standard, were applied to Sephadex DEAE A-25, 5 mm × 5 cm column (Pharmacia, Uppsala, Sweden). After washing with 7 mL of chloroform –70% aqueous methanol (1.5:10, v/v), the free bile acids were eluted with 7 mL of 0.2 mol·L^{-1} acetic acid in 70% aqueous methanol. This fraction was evaporated to dryness with N_2 and bile acids were quantified by gas-liquid chromatography (GLC) using a flame ionization detector (GLC-FID, Varian 3300, Palo Alto, CA, USA) after trimethylsilylester derivatization.

2.5.2. Cytolytic activity assay

The human erythrocytes employed in the assay were provided by a local blood bank (AVIS, Milan, Italy) in a single 500 mL batch. The incubation mixture contained 40, 80, 120 and 160 µL faecal water, and 154 mmol·L^{-1} NaCl to a total volume of 160 µL, and 40 µL erythrocytes (final hematocrit 5%). In each assay, faecal water without erythrocytes was used to correct for the natural iron content of faecal water, and erythrocytes in double-distilled water (=100% lysis) and erythrocytes in 154 mmol·L^{-1} NaCl (0% lysis) were incubated simultaneously. The samples were incubated for 2 h at 37 °C in a Dubnoff bath (60 strokes per minute), then appropriately diluted with 154 mmol·L^{-1} NaCl and centrifuged for 10 min at 1 000 × *g*. The iron content in the supernatants was measured using atomic absorption spectrophotometry (IL-551, Instrumentation Laboratory, Lexington, MA, USA). The cytolytic activity of each faecal water sample was quantified as the area under the lytic curve. The cytolytic activity was expressed as the percentage of the maximal area, which implies 100% lysis, for each dilution of faecal water [24].

2.6. Plasma lipids

Fasting total cholesterol, HDL-cholesterol and total triacylglycerols were determined using a dedicated clinical analyzer (Kone Specific Selective Chemistry Analyzer, Kone Instruments S.A., Evry, France). LDL-cholesterol was derived by the Friedewald formula [11].

2.7. Statistical analysis

The results are expressed as mean ± SD. Data were submitted to Repeated Measures Analysis of Variance (RM-ANOVA). Specific differences between treatments were evaluated by applying the Tukey's Honestly Significant Differences post-hoc test. The analyses were performed using the StatSoft Statistica Package for Windows (release 4.5, Statsoft Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

Only 10 subjects out of 12 completed all the experimental periods, the remaining two subjects completed the period of yogurt administration but not the last follow-up phase.

Table III. Dietary intake (means ±SD) recorded over one week for both periods of the study (test and follow-up).

	Test	Follow-up
kJ·d^{-1}	6752 ±912	6564 ±912
Protein (g·d^{-1})	67.3 ±7.9	67.2 ±7.1
Carbohydrates (g·d^{-1})	212.3 ±41.5	204.9 ±41.3
Lipids (g·d^{-1})	55 ±5.8	53.3 ±6.5

Even though yogurt is not a common food product in the everyday diet of the subjects studied, its consumption was well accepted during the experimental period. One subject did not like the yogurt but continued the experiment until the end of the study; two were indifferent to the dietary trial, whereas the other nine subjects greatly appreciated the yogurt and would have liked to avoid returning to their usual diet during the follow-up period. At the end of the trial three volunteers spontaneously introduced yogurt into their dietary habits.

Food consumption analysis revealed no differences in the average dietary intake of energy, protein, fat and carbohydrates during the test and follow-up periods (Tab. III).

Table IV summarizes the microbial counts in the stool specimens collected at the beginning of the trial and at the end of each experimental period. Results on the faecal microflora composition before and after yogurt administration are comparable with those reported by some authors on the faecal microflora of healthy individuals [10, 36]. However, our results did not confirm a decrease in bifidobacteria and an increase in lactobacilli, enterococci, enterobacteria and clostridia, as reported by other authors [15, 29]; this discrepancy could be ascribed to the considerable variation existing among individuals of the same age, and to different analytical microbial procedures, as well as to the moderate but realistic daily amount of yogurt administered in our study.

A significant ($P < 0.05$) reduction in clostridia counts was recorded after yogurt consumption and at the end of the follow-up period, compared with the end of the baseline period. This result is particularly interesting since clostridia are involved in the fermentation of amino acids and in the

Table IV. Means values ±SD (range) of microbial counts (log CFU·g^{-1} dry weight) in faecal samples at the different periods of the study.

	Baseline (start)	Baseline (end)	Test	Follow-up
Anaerobes	10.73 ±0.52 (9.75–11.36)	10.68 ±0.64 (9.24–11.76)	10.35 ±0.73 (9.16–11.4)	10.46 ±0.55 (9.35–11.17)
Lactobacilli	5.35 ±1.74 (2.8–8.61)	5.37 ±1.99 (2.97–8.7)	5.02 ±1.89 (2.73–8.62)	5.06 ±2.19 (2.55–8.67)
Bifidobacteria	9.75 ±0.82 (8.25–10.84)	9.28 ±1.81 (4–10.51)	9.00 ±2.09 (3.29–11.12)	8.88 ±2.12 (3.73–10.24)
Enterococci	6.91 ±1.85 (4.64–10.29)	6.59 ±1.77 (4.08–9.99)	6.54 ±2.00 (3.38–10.41)	6.21 ±1.66 (4.29–10.03)
Coliforms	8.16 ±1.27 (6.34–10.19)	8.28 ±1.32 (6.53–10.34)	8.21 ±1.02 (7.05–10.37)	8.14 ±1.23 (6–10.47)
Clostridia[a]	5.63 ±1.99 (2.89–9.3)	5.59 ±1.70 (3.81–9.3)	4.82 ±1.90 (2.43–8.46)	4.61 ±2.04 (2.97–9.07)

[a] Follow-up and test differ from baseline (end) $P < 0.05$.

production of toxic metabolites, such as NH_3, indoles and phenols, related to intestinal chronic pathologies [26]. In a study in which yogurt was administered to 10–18 month-old healthy children, a reduced faecal concentration of branched fatty acids, resulting from bacterial protein degradation, were observed [17]. Our data tend to support these results, as clostridia are the main proteolytic microorganisms in the colon. On the other hand, our findings revealed that yogurt administration did not significantly influence the counts of the total anaerobic microorganisms, lactobacilli, bifidobacteria, enterococci and coliforms. Other authors have observed an increase in the counts of bifidobacteria in children and young adults [2, 8] and of enterococci [17] in children, after yogurt administration.

Table V shows the percentage of faecal moisture and the parameters measured on faecal water. The moisture content of faeces was similar across experimental periods. Therefore, it would seem that yogurt influences neither the intestinal bulk nor the intestinal habits. The effectiveness of yogurt in constipation, a disorder often associated with old age, was reported only when the yogurt was administered combined with fibers, such as guar gum and wheat bran [33], most likely due to the bulk effect of dietary fiber.

Our data suggest that yogurt did not alter faecal pH or influence the concentration of the major bile acids and the cytotoxicity of faecal water. It is possible that freeze-drying and reconstitution of dried faeces with water for 2 h at 37 °C could have modified faecal water biochemical parameters and activities: the pH may have changed due to loss of volatile fatty acids during freeze-drying and secondary bile acids may have formed during the two-hour incubation. However, we thought that these changes, if they occurred, can be considered of limited effect, and it is more likely due to an overall lack of effect of yogurt on the colon environment at the doses of intake used in this work.

No significant modification of the faecal pH, neutral sterol or bile acid excretions after prolonged yogurt consumption has ever been demonstrated by other authors, suggesting great stability in the human ecosystem to this kind of dietary intervention [2]. However, significant variations in these parameters have been assessed following drastic dietary modifications, such

Table V. Means values ±SD (range) for fecal humidity, pH, cytolytic activity and bile acids concentration in faecal water at the different periods of the study.

	Baseline (end)	Test	Follow-up
% Fecal humidity	67.6 ±7.8 (57.2–82.6)	70.0 ±9.3 (51.8–83.1)	67.4 ±7.5 (53.3–79.5)
pH	7.16 ±0.62 (6.30–8.09)	7.23 ±0.61 (6.26–7.95)	7.04 ±0.70 (6.29–8.15)
% Cytolytic activity	22.2 ±21.4 (0.2–54.4)	17.7 ±27.4 (0.4–85.0)	33.5 ±34.0 (1.1–97.8)
LCA ($\mu mol \cdot L^{-1}$)	33.3 ±25.4 (0.0–91.0)	43.0 ±52.7 (0.0–150.0)	59.3 ±79.7 (0.0–238.0)
DCA ($\mu mol \cdot L^{-1}$)	92.8 ±57.0 (49.0–240.0)	98.6 ±67.9 (36.0–273.0)	93.3 ±40.3 (44.0–163.0)

LCA = lithocholic acid;
DCA = deoxycholic acid.

as a shift from a dairy product-rich to a dairy product-free diet [14], or when a ten-fold increase in calcium intake from dairy products was considered [16]. In our study the calcium in the diet in the different phases of the experiment was not evaluated; on the other hand, as the yogurt was consumed in place of milk or milky tea, we hypothesized that the consumption of yogurt would not significantly influence the faecal calcium concentration which could promote the faecal buffer capacity and faecal excretion of bile acids.

Table VI shows the results of the lipid study for all the volunteers. No significant differences were observed in the blood lipids during the three periods of study. With regard to blood cholesterol only 3 subjects showed reduced levels after yogurt consumption (baseline values: 236, 221, 260 $mg \cdot dL^{-1}$ vs. test values: 199, 178 and 220 $mg \cdot dL^{-1}$, subjects 4, 5 and 7, respectively) while in the others there was no observed change. In addition, two of the subjects (subjects 5 and 7) were also observed to have a reduction in the triacylglycerols (baseline values: 116 and 137 $mg \cdot dL^{-1}$ vs. test values: 65 and 68 $mg \cdot dL^{-1}$, subjects 5 and 7, respectively). The literature data regarding the influence of yogurt on lipid metabolism in human subjects are discordant [3, 38]. Moreover, generally speaking, studies have been carried out on normolipidemic subjects for whom it is very difficult to induce alterations in the lipid metabolism. Our volunteers had quite high cholesterol values but, in relation to their age, they can be considered normolipidemic [1]. Since we observed reduced blood cholesterol levels in those elderly subjects who had the most elevated lipid values at the baseline, it could be hypothesized that there is a potentiality for yogurt as a hypocholesterolemic food for subjects with altered lipid metabolism. However, further research is needed to assess such an effect in elderly hypercholesterolemic subjects.

In conclusion, the study has shown good acceptability of yogurt as a dietary supplement on the part of the studied elderly volunteers, and the results have revealed a substantial stability of the subjects' faecal flora after this type of dietary intervention. Moreover, the reduction in clostridia counts observed following yogurt consumption can be considered a positive modification of the colonic ecosystem, as clostridia are involved in the formation of putrefactive compounds that are potentially toxic to the host.

However, given the limited number of subjects studied (due to the difficulty in recruiting such elderly subjects), the results must be considered preliminary. Furthermore, the effects on faecal microflora composition, evaluated by classical culture-dependent techniques, require in-depth studies by molecular tools that, being less time-consuming and more accurate, would

Table VI. Means values ± SD (range) of plasma lipids at the different periods of the study.

($mg \cdot dL^{-1}$)	Baseline (start)	Baseline (end)	Test	Follow-up
Total cholesterol	216.1 ± 40.1 (140–270)	211.8 ± 31.9 (160–260)	202.7 ± 29.6 (162–247)	202.4 ± 41.2 (162–246)
LDL-cholesterol	131.4 ± 38.8 (66–187)	129.2 ± 26.9 (77–159)	125.7 ± 29.1 (77–174)	128.5 ± 31.8 (59–174)
HDL-cholesterol	59.0 ±16.4 (36–92)	58.7 ± 13.9 (40–82)	54.3 ± 15.0 (37–83)	51.7 ± 19.3 (22–82)
Triacylglycerols	111.8 ± 44.2 (66–207)	119.6 ± 42.2 (48–214)	113.3 ± 51.3 (57–207)	110.8 ± 45.3 (48–201)

allow the monitoring of the distribution of other microbial populations, also giving a rapid characterization of isolates at the level of species or strains [27].

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