European Review for Medical and Pharmacological Sciences 2017; 21: 5827-5836

Effects of new probiotic mouthwash in patients with diabetes mellitus and cardiovascular diseases

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Abstract. – OBJECTIVE: To evaluate the impact of a new formulation of probiotic mouthwash (PM), using Biocult strong® dissolved in neutral mouthwash.

PATIENTS AND METHODS: Forty-two patients with cardiovascular disease (CVD) or type 1 and type 2 diabetes were enrolled. Plaque Control Record (PCR) and Bleeding on Probing (BOP) were assessed at baseline and after two weeks of PM or positive control treatment in intervention group (IG) and control group (CG). Food intake was estimated by 3-day diet record.

RESULTS: BOP was significantly reduced in all treatments and samples, except for IG in CVD sample (p=0.15). PCR decreased significantly in all treatments and samples (p<0.01). No significance was obtained for BOP and IP in the time x group interaction. Food intake was not significantly different between IG and CG in all samples. Nutrients such as fats and simple carbohydrates were correlated with BOP in patients who received positive control, rather than PM, indicating a lack of food influence on BOP and PCR in IG.

CONCLUSIONS: PM treatment was effective in relation to the reduction of PCR and BOP. Probiotics represent a good, but additional, tool for prophylaxis, because they cannot completely substitute the classic oral hygiene methods. Moreover, one week of treatment was not sufficient to draw firm conclusions about the efficacy of the treatment itself.

Key Words
Probiotic, Gingivitis, Plaque Control Record, Bleeding on probing, Mouthwash.

Introduction

According to World Health Organization (WHO), "probiotics" are defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host"[1]. Probiotics prevent the adhesion of pathogenic species, they inhibit bacterial growth, modulate cell proliferation and the mucosal immune system and improve the integrity of the intestinal barrier[2].

Literature data show that the mechanical removal of supragingival plaque is the most effective tool to prevent gingivitis[3]. However, as individuals often do not handle plaque accumulation[4], antimicrobial agents, like dentifrices or mouthwashes, have been tested for their additional ability to reduce plaque and gingivitis onset[5]. Another method is represented by the long-term use of antiseptic mouthwashes that, however, may be associated with unwanted side effects[5].

An alternative and preventive tool may be represented by the use of orally probiotics, which may increase the commensal flora, preventing the microbiological shift and colonization of pathogens associated with gingival inflammation[6].

Several studies have pointed out an improvement of oral cavity health and maintenance of its homeostasis. In particular, probiotics are able to reduce the amount of mutans streptococci in saliva and/or plaque[7-10], they have a positive effect on halitosis[11,12] and they fight oral infections caused by Candida.

Literature data demonstrate that the effects of probiotics are both species and strain specific[13]. Most studies on probiotics and oral health are intended to focus on measuring changes in mutans (MS) counts[14-15], even though high counts of MS do not necessarily imply an increased risk for the

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development of caries. In fact, decreasing MS without affecting the microbiota should decrease the plaque virulence.

Several studies have shifted their focus to *Lactobacillus reuteri*, a member of indigenous oral microbiota in humans. This strain has antibacterial properties because it converts glycerol into reuterin, a wide-spectrum antimicrobial substance. This antibacterial activity was demonstrated *in vitro* in non-oral pathogenic bacteria (*S. aureus, L. monocytogenes*) and in *Candida albicans*, without affecting the indigenous health-related microbiota[16].

Furthermore, a lot of studies have suggested that *L. reuteri* reduces counts of MS[17] even though contradictory results have been published[18].

Effects of probiotics on periodontal pathogens have received little interest so far although several studies have been conducted to evaluate the action of probiotics in the management of periodontal diseases[19-22].

Krasse et al[23] conducted a research where patients with gingivitis were given one of the two formulations containing 2 × 10 CFU/day of *L. reuteri*, or a corresponding placebo. In those patients, *L. reuteri* reduced plaque and gingivitis.

Other studies have aimed to find microorganisms with a potential probiotic effect that may prevent periodontal disease onset. *In vitro*, various oral strains of *lactobacilli, streptococci*[24-27], and *bifidobacteria*[28] are most effective against *mutans streptococci*[24,29,30], while others have been reported active against periodontal pathogens.

Type 1 and Type 2 diabetes are factors that exacerbate periodontal disease and gingival health, as reported in several studies, showing an aggravation of gingivitis and inflammatory indices in diabetic patients[31]. Probiotics seem to offer beneficial effects on diabetes, although more long-term studies are still needed to avoid any controversial result[32].

Furthermore, patients with cardiovascular disease, in therapy with anticoagulants, showed an increase of bleeding on probing (BOP) compared to placebo-treated patients[33].

Based on the health benefits reported by specific strains or probiotic combination, this study was carried out to evaluate the impact of a new formulation of probiotic mouthwash (PM), using Biocult strong® (HOMEOSYN, Rome, Italy) on type 1 and 2 diabetes or coronary disease, measured by plaque control record (PCR), and BOP.

The hypothesis is that the use of the present PM might be effective on the reduction of gingival inflammation in diabetic and cardiopathic patients with generalized gingivitis, in which the nutritional habitus was assessed by 3-day diet record.

Patients and Methods

Patients

A randomized, double-blinded, placebo-controlled, parallel-group clinical study was conducted between June 2017 and July 2017. Subjects were consecutively recruited within a program of routine medical check-up at the Simple Departmental Operative Unit for Diagnosis, Hygiene and Oral Prevention of "Tor Vergata Hospital", and the University of Rome "Tor Vergata".

Forty-two patients in hemodialysis treatment, 12 patients with cardiovascular disease (CVD) in therapy with proton pump-inhibitor (PPI) and 10 type 1 and type 2 diabetic patients, screened for eligibility at first medical visit, aged between 30 and 80 years old, were enrolled.

The subjects were assessed for PCR[34] and BOP[35], by single experienced investigator at baseline and after 1 week of treatment. The professional oral hygiene session was performed using ultrasound and manual courette. For home hygiene, patients were instructed to use the mouthwash only in the evening after brushing their teeth.

Eligible patients were randomly divided into two groups (1:1 ratio). A person not involved into the clinical trial carried out the randomization. The intervention group (IG) received the PM, and the positive control group (CG) a commercial mouthwash.

Each subject identified by a unique study number was instructed to use 5 ml of mouthwash daily. The study consisted of a two-week treatment. The PM and the commercial mouthwash were provided in identical packages and were identified with the study number. The codes were not broken until the end of the study.

Study design was clearly written in language for lay users and all participants recruited in the study authorized their participation by reading and signing the informed consent, conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. The design and protocol were reviewed and approved by the regional Ethical Committee. The participants received no financial compensation or gifts.

Outcomes

The primary outcome of this study was the evaluation of PM effects on patients with coronary diseases and diabetes type 2 or 1, measured by PCR and BOP.

The secondary outcome was to assess nutritional habitus according to 3-day diet record in order to verify a relationship between food intake and gingivitis.

Exclusion criteria

Exclusion criteria included nonsurgical and surgical periodontal therapy in the last 6 months, acute diseases, endocrine disorders, liver and kidney dysfunctions, history of chronic degenerative or infectious diseases, medication, antibiotic therapy until ten days before enrolment, smoke, drug or alcohol abuse, participation in another diet trial. No subjects with orthodontic and prosthodontics appliances were included in the study. Subjects could not have taken antibiotics or probiotics in the month before the study and were willing to avoid use of probiotics for the duration of the study.

Inclusion criteria

Inclusion criteria included subjects with diabetes type 2 and 1, cardiovascular diseases, non-smokers (never smokers or former smokers for at least 6 months), a dentition with ≥ 20 evaluable teeth (minimum of five teeth per quadrant) and a history of oral prophylaxis within 6 months previous to the study.

Clinical examination

Clinical variables were evaluated at baseline, and after 1 week. These variables included the PCR and BOP, as normally assessed in studies evaluating oral hygiene products.

1) Plaque control record (PCR): the PCR was assessed on individual tooth surfaces (mesial, distal, facial, lingual)[34]. After inserting a periodontal probe into the gingival groove, the operator signed 0 for no plaque on the probe and 1 for plaque presence on the probe.

The PCR was calculated according to the formula:

PCR =(number of plaque containing surfaces ÷ total number of available surfaces) ×100

2) Bleeding on probing (BOP): the BOP was used to clinically characterize the degree of gingival inflammation[35]. Each tooth present is gently probed with a periodontal probe at six sites (mesial, mid, and distal on both buccal and lingual surfaces).

BOP bleeding was calculated as follows:

BOP =(number of sites where bleeding is recorded ÷ total number of available surface sites in the mouth) ×100

Dietary Assessments

The food intake before and during the clinical trial was assessed from 3-day diet record, considering two weekdays and one weekend day[36]. The subjects were instructed by a dietitian to record weight and/or measures of all foods and beverages consumed and to use product brand names when recording dietary intake. Photographs of food portion sizes were provided to better estimate the amount of food consumed. Diet records were reviewed as they were turned in, to confirm that all written food items were legible and to clarify the amounts of foods consumed. The estimated intake of calories and macronutrients were calculated by using the software Dietosystem®.

Probiotic mouthwash (PM) and positive control mouthwash

The patients in IG received a probiotic mouthwash. 3 g of Biocult strong® (HOMEOSYN, Rome, Italy), a probiotic dried mixture of total 13.5 $\times 10^{10}$ colony-forming unit (CFU)/ (1.5 $\times 10^{10}$ CFU)/strain) *of Streptococcus Thermophilus SGSt01*, *Bifidobacterium animalis subsp Lactis* SGB06, *Streptococcus thermophiles*, *Bifidobacterium Bifidum* SGB02, *Lactobacillus Delbrueckii spp Bulgaricus DSM, Lactococcus lactis subsp Lactis SGLc01, Lactobacillus Acidophilus SGL11, Lactobacillus Plantarum SGL07, Lactobacillus Reuteri SGL01*, were dissolved in 70 ml of commercial mouthwash Meridol® (Saninforma, Reggio Emilia Italy): 10 ml of this suspension were used at a dose of 1.93 $\times 10^{10}$ CFU/day.

The positive control mouthwash was Meridol® (Saninforma, Reggio Emilia, Italy), which is composed by water, castor oil, sodium, xylitol, PEG-40 hydrogenated castor oil, olaflur, sodium saccharin and stannous fluoride (250 ppm).

Statistical Analysis

The statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY, USA). Data are expressed as mean ± standard deviation (SD) and 95% confi-

dence interval. Categorical data, such as gender frequency, are expressed as absolute and percentage values and x^2 was performed. After the Shapiro-Wilk test, for checking normality of distribution, a paired *t*-test or a non-parametric Wilcoxon test was performed to evaluate differences between before and after PM or positive control. Interactions between time and group were analyzed through mixed-design ANOVA, where the within-subjects factor was defined as time (before and after treatment) and between-subjects factor was defined as group (IG and CG). To discuss variable changes after treatments, we used a ratio of the absolute variation to the baseline value (percent variation = Δ%). *t*-test or Mann-Whitney was used to compare the food intake between IG and CG during the treatment for independent samples. Correlations were performed between changes in gingival and periodontal conditions and nutrients intake during treatments using Person or Spearman coefficients. In all statistical tests performed, the null hypothesis (no effect) was rejected at the 0.05 level of probability.

Results

Of the forty-two subjects enrolled, two of them were excluded from the trial (one did not meet inclusion criteria, and another one declined to participate). Finally, forty patients completed the trial (Figure 1). No changes to trial outcomes after the trial commenced occurred. During the week of treatment, no side or adverse effects were reported.

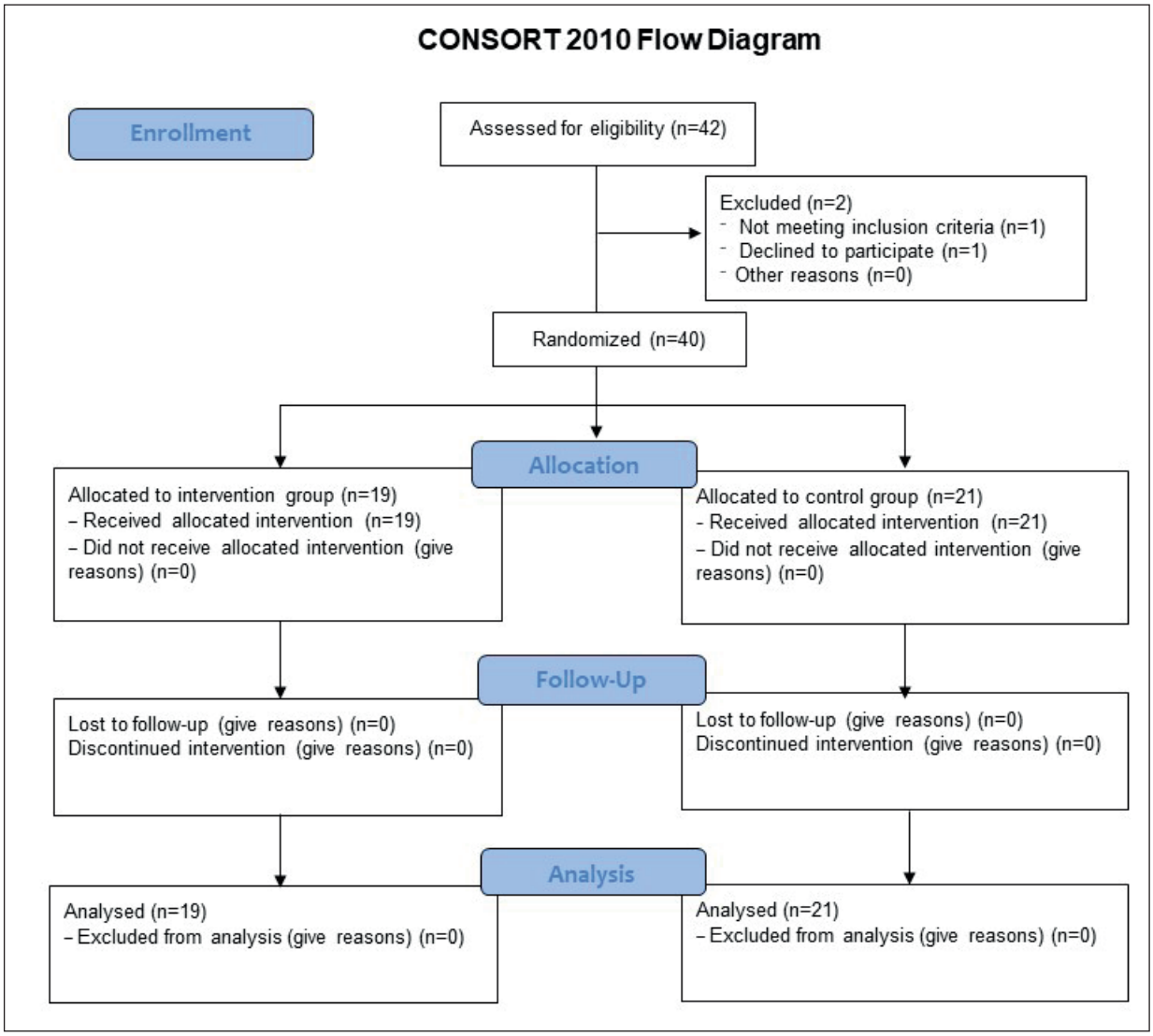

Figure 1. Study Flow Diagram according to Consort, 2010.

Table I. Bleeding on probing of IG and CG before and after treatments in the different samples.

	IG					CG					Interaction Time x Group
	T0		T1			T0		T1			
Sample	Mean ± SD	95% CI	Mean ± SD	95% CI	*p*	Mean ± SD	95% CI	Mean ± SD	95% CI	*p*	*p*
	n=19					n=19					
Total	53.16± 19.92	43.56-62.76	50.37± 19.63	40.91-59.83	0.00*	60.09± 18.47	51.69-68.51	56.62± 19.00	47.97-65.27	0.00*	0.56
	n=10					n=11					
Diabetes mellitus	56.00± 22.10	40.19-71.81	51.80± 21.60	36.34-67.25	0.00*	55.00± 17.54	43.21-66.79	51.73± 18.24	39.47-63.98	0.04*	0.60
	n=9					n=10					
CVD	50.00± 17.93	36.22-63.78	48.78± 18.34	34.68-62.88	0.15	65.70± 18.69	52.33-79.07	62.00± 19.27	48.21-75.79	0.01*	0.12

All results were expressed as mean ± standard deviation and 95% confidence interval. Statistical significance attributed to results with *p<0.05 between T0 and T1. BOP: bleeding on probing; IG: intervention group; CG: control group; T0: before treatments; T1: after treatments; CVD: cardiovascular diseases.

The average age of subjects was 66.07 ± 11.73 years, 52.50% female and 47.50% male; 52.50% was affected by diabetes type 1 or 2 and 47.50% were cardiopathic patients in therapy with PPI. In groups divided by treatment, there was no significant difference between age (p=0.40), IG aged 67.74 ± 10.39 years old and CG aged 64.57 ± 13.02. Moreover, there was no sex difference between IG (52.60% female and 47.40% male) and CG (52.40% female and 47.60% male) (p=0.99).

Gingival and periodontal conditions were assessed (Tables I-II). BOP was seen to be signifi-

Table II. Plaque index of IG and CG before and after treatments in the different samples.

	IG					CG					Interaction Time x Group
	T0		T1			T0		T1			
Sample	Mean ± SD	95% CI	Mean ± SD	95% CI	*p*	Mean ± SD	95% CI	Mean ± SD	95% CI	*p*	*p*
	n=19					n=19					
Total	54.05± 21.11	43.88-64.23	50.63± 21.07	40.47-60.79	0.00*	61.24± 20.99	51.68-70.79	56.95± 20.78	47.49-66.41	0.00*	0.16
	n=10					n=11					
Diabetes mellitus	52.20± 20.85	37.29-67.11	48.90± 20.90	33.94-63.85	0.00*	58.18± 19.89	44.82-71.55	53.82± 20.33	40.16-67.48	0.00*	0.14
	n=9					n=10					
CVD	56.11± 22.47	38.84-73.38	52.55± 22.35	35.37-69.74	0.00*	64.60± 22.70	48.36-80.83	60.40± 21.780	44.81-75.99	0.00*	0.56

All results were expressed as mean ± standard deviation and 95% confidence interval. Statistical significance attributed to results with *p<0.05 between T0 and T1. PCR: plaque control record; IG: intervention group; CG: control group; T0: before treatment; T1: after treatment; CVD: cardiovascular diseases.

cantly reduced in all treatments and samples, except for IG in CVD sample (p=0.15). In addition, PCR decreased significantly in all treatments and samples (p<0.01).

No significance was obtained for BOP and IP in the time x group interaction. Both PM and positive control had the same mean changes (p>0.05) (Tables I-II).

During the protocol, food intake was not significantly different between IG and CG in all samples (Table III). Associations between changes in oral health and food intake, independently of the sample, indicated significant correlations in IG but not in CG. Nutrients, such as fats and simple carbohydrates, were seen to be significantly correlated with BOP in patients who received positive control, rather than PM, indicating a lack of food influence on BOP and PCR in IG (Table IV).

Discussion

Gingival and periodontal conditions and inflammatory markers in gingival crevicular fluid[17] have been described to improve with the use of probiotic preparations (tablets, mouth rinse, lozenges etc.)[24,37]. In fact, it has been highlighted the beneficial effect in oral health of probiotics

Table III. Food intake during treatment with PM or positive control, in the different samples.

Food Intake	Total (n=40)			Diabetes (n=21)			CVD (n=19)		
	IG (n=19)	CG (n=21)	p	IG (n=10)	CG (n=11)	p	IG (n=9)	CG (n=10)	p
	Mean± SD	Mean± SD		Mean± SD	Mean± SD		Mean± SD	Mean± SD	
Kcal/day	894.76± 115.43	861.48 ±97.42	1.00	915.90± 150.00	870.91± 131.66	0.43	871.28± 166.93	851.10± 40.03	0.45
Protein (g/day)	39.64± 7.67	37.88± 6.55	0.29	40.67± 6.30	39.28± 7.05	0.65	38.50± 9.21	5.91 36.33±	0.36
Total Fat (g/day)	36.52± 6.23	36.28± 5.74	0.98	37.51± 5.97	36.57± 6.22	0.76	35.42± 6.68	35.96± 5.47	0.91
SFA (g/day)	8.57± 3.22	8.24± 2.09	0.92	9.01± 3.82	8.58± 2.27	0.86	8.07± 2.52	7.86± 1.91	1.00
MUFA (g/day)	21.35± 3.54	21.62± 3.78	1.00	21.62± 2.48	21.52± 3.61	0.65	21.06± 4.59	21.73± 4.14	0.78
PUFA (g/day)	3.99± 0.73	3.84± 0.67	0.31	4.12± 0.58	3.86± 0.68	0.11	3.85± 0.88	3.81± 0.69	0.91
Total Carbohydrate (g/day)	108.76± 32.89	102.33± 28.88	0.65	110.91± 27.65	102.65± 35.59	0.35	106.37± 39.52	101.97± 21.12	0.66
Complex Carbohydrate (g/day)	83.06± 22.65	79.71± 22.50	0.69	84.19± 17.21	78.28± 26.36	0.28 81.80±	28.59 81.29±	18.66	
Simple Carbohydrate (g/day)	17.40± 10.13	14.64± 7.50	0.81	18.31± 10.15	16.55± 9.48	1.00	16.40± 10.62	12.55± 3.97	0.84
Fibre (g/day)	5.98±2.41 5.22±1.36	0.66	5.46± 1.89	0.73	5.82± 2.38		5.68± 2.31	0.97	6.16 ±2.57

All results were expressed as mean ± standard deviation. Statistical significance attributed to results with *p<0.05 between IG and CG, during treatments. PM: probiotic mouthwash; IG: intervention group; CG: control group; CVD: cardiovascular diseases. SFA: saturated fatty acids; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acids.

Table IV. Correlations of changes in gingival and periodontal conditions with nutrients intake during treatments.

Parameters		Total							
		IG (n=19)				CG (n=21)			
		PCR (%)	Total Fat (g/day)	MUFA (g/day)	Simple CHO (g/day)	PCR (%)	Total Fat (g/day)	MUFA (g/day)	Simple CHO (g/day)
BOP (%)	*r*	0.35	-0.16	-0.06	-0.12	-0.01	0.53*	0.52*	0.16
	p	0.14	0.50	0.81	0.61	0.95	0.01	0.01	0.48
PCR (%)	*r*		-0.20	-0.11	-0.02		0.07	0.07	0.15
	p		0.40	0.65	0.93		0.76	0.76	0.52
Total Fat (g/day)	*r*			0.85*	0.57*			0.93*	0.13
	p			0.00	0.01			0.00	0.56
MUFA (g/day)	*r*				0.43				0.18
	p				0.06				0.42
		Diabetes							
		IG (n=10)				CG (n=11)			
BOP (%)	*r*	0.50	0.11	-0.06	-0.33	0.46	0.58	0.67*	0.84*
	p	0.14	0.77	0.87	0.34	0.15	0.06	0.02	0.00
PCR (%)	*r*		-0.03	0.13	-0.06	0.46	0.33	0.39	0.11
	p		0.93	0.72	0.88	0.15	0.31	0.23	0.75
Total Fat (g/day)	*r*			0.72*	0.44			0.94*	0.33
	p			0.01	0.20			0.00	0.33
MUFA (g/day)	*r*				0.28				0.41
	p				0.42				0.21
		CVD							
		IG (n=9)				CG (n=10)			
BOP (%)	*r*	0.43	-0.27	-0.07	0.21	-0.48	0.43	0.41	-0.64*
	p	0.25	0.48	0.86	0.59	0.16	0.22	0.24	0.04
PCR (%)	*r*		-0.50	-0.41	0.03		-0.20	-0.08	0.19
	p		0.17	0.27	0.93		0.58	0.82	0.59
Total Fat (g/day)	*r*			0.93*	0.45			0.93*	-0.07
	p			0.00	0.22			0.00	0.85
MUFA (g/day)	*r*				0.38				0.03
	p				0.31				0.93

All results were expressed as *r* and *p* values. Statistical significance attributed to results with *$p<0.05$ between variables. IG: intervention group; CG: control group; CVD: cardiovascular diseases; BOP: bleeding on probing; PCR: plaque control record; MUFA: monounsaturated fatty acids; CHO: carbohydrates.

administered orally. This result is related to the prevention and modulation of harmful bacteria growth, and mucosal immunity, respectively, in the oral cavity[38]. Several clinical studies have examined the effect of different strains of probiotics on gingival inflammation, demonstrating that although a specific strain may exert a beneficial effect for general health, not all the probiotics may be useful in gingivitis management[19,20,39,40].

Sabatini et al[31] analyzed the efficacy of probiotics on periodontal health in diabetes patients and gingivitis. The study has demon-

strated, thanks to the use of probiotics, an improvement in plaque and BOP index[31].

Iniesta et al[20] showed a decrease in the prevalence of mouth bacteria known as pathogens of periodontal disease after treatment with probiotics tablets containing *Lactobacillus reuteri*. Probiotic species generally belong to genera *Lactobacillus* and *Bifidobacterium*. Studies *in vitro* have showed that probiotic *Lactobacillus* can inhibit or hamper growth of pathogens associated with periodontal disease. Moreover, some strains such as *L. rhamnosus*, *Lactobacillus. casei*, *Lactobacillus reuteri*, or a mix of *Lactobacilli* are related to the reduction of oral inflammation, due to a decrease of pro-inflammatory cytokines production[41].

As recent data highlighted[42] that a new formulation of probiotics, Biocult strong®, has potential as a therapeutic strategy for prevention and/or treatment of certain eating behaviour disorders and anxiety, in our study we tested the influence of daily administered probiotic mouthwash formulated with the same bacterial strains of Biocult strong® on inflammatory reactions in the gingiva.

In this randomized, double-blinded, placebo-controlled, parallel-group clinical trial, we tested the efficacy of this new PM formulation on plaque and bleeding in patients with diabetes or cardiovascular diseases.

The rationale is based on previous data that show positive effects of different probiotics on these parameters, having two main purposes: 1) to check whether it could change all the examined parameters, with the possibility to decrease the inflammatory status of gingivitis in patients with CVD or diabetes type 2 and 1; 2) to investigate the correlation between food habits and PCR before and after treatments.

It has been postulated that the oral cavity may be a potential source of bacteria associated with increased cardiovascular and peripheral artery disease, due to atherogenic properties of oral bacteria[43]. Menon et al[44] demonstrated the presence of the five major phyla which constitute the salivary microbiome (*Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes* and *Fusobacteria*), in patients with coronary artery disease which are similar in patients with dental caries.

In the present work, we were able to verify the good potential of a probiotic mouthwash as an antiplaque agent and its efficacy in reducing gingivitis according to BOP and PCR results.

In fact, after PM treatment, positive effects on PCR (Δ%= -7.35) and BOP (Δ%= -8.00) were observed. However, according to Hallström et al[45], no significant differences in PCR and BOP were displayed between test and positive control.

Shimauchi et al[46] did not reveal significant differences between the probiotic and placebo groups at the end of the 8-week placebo-controlled trial with Lactobacillus salivarius WB21 on periodontal conditions. Instead, we observed some efficacy of the positive control in BOP (Δ%= -6.77) and after PM (Δ%= -5.48).

In diabetes patients, the reduction of BOP after PM was of -7.69%, even if comparable results were obtained after positive control (Δ%= -6.69). Similarly, a significant reduction in BOP was observed in patients with cardiovascular risk, both after PM (Δ%= -3.02) and positive control (Δ%= -6.87).

As previously reported[47], although the composition of the oral microbioma is stable over time, there are different events able to affect the permanence in the oral cavity of some bacterial strains. Among these, it is important to take in account the effects of the diet, as nutrients can interfere with oral microbioma. In particular, foods like legumes, fruits and vegetables are considered as prebiotics[48], but others, like sugary foods, can change microbioma and increase the risk of oral and systemic disease[49].

From a nutritional point of view, no difference in the estimated food intake between IG and CG patients was observed during the treatment, in both diseases and total sample. Moreover, the changes of BOP during treatments were significantly correlated with fats and simple carbohydrates consumed, in the same period, only for CG. Therefore, although both groups have had similar consumption, patients who received PM had their BOP condition improved without any association with food consumption. Thus, we could suggest that patients who received the commercial mouthwash had their improvement related with carbohydrate and fats intake, mainly MUFA, in all samples. In spite of limited studies in humans and conflicting results, lipids and sugar have been seen, indeed, to affect gingival and periodontal conditions[50,51].

Conclusions

These results reinforce the efficacy of PM treatment, which would be advisable in relation to the reduction of PCR and bleeding. However, one week of treatment, chosen in order to ensure complete adhesion of volunteers to clinical trial, was not sufficient to draw firm conclusions about the efficacy of the treatment itself. The limits of this research were the small number of enrolled sub-

jects and the short duration of treatment. Therefore, further clinical trials are needed on a larger population and over a longer period to obtain conclusive data. Moreover, as previously suggested[46], probiotics represent a good, but additional, tool for prophylaxis, because they cannot completely substitute the classic oral hygiene methods.

Conflict of Interest

The authors declared no conflict of interest.

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